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P01

CANCER GENETICS

P01.001.A Hypoxia-mimetic agent DFO inhibits cancer cell proliferation and induces DNA damage

David Diez-Castro[1;2], *Luis Perez-Romasanta*[3], *Rogelio González-Sarmiento*[1;2;4], *Ana-Belén Herrero*[1;2;4]

[1]*Institute of Biomedical Research of Salamanca, Institute of Biomedical Research of Salamanca, Salamanca;* [2]*Faculty of Medicine, Departament of Medicine, Salamanca;* [3]*Hospital University of Salamanca, Oncology service, Salamanca;* [4]*Institute of Molecular and Cellular Biology of Cancer (IBMCC), University-CSIC, Salamanca*

Background/Objectives: Hypoxia is common in solid tumour microenvironments and has been associated with radiotherapy resistance and poor clinical outcomes. Replicating hypoxic conditions in vitro requires incubation at 1% O_2, 5% CO_2 and 94% N_2. This nitrogen-induced hypoxia is costly and time-consuming since it needs 24 hours to establish hypoxia. An alternative technique to replicate hypoxic conditions is the use of mimetics like deferoxamine (DFO). This molecule is an iron chelating agent that facilitates the accumulation of hypoxia-inducible factors and has been shown to induce apoptosis in breast tumor cell lines[1].

Methods: MCF7 (breast), VCAP (prostate), CAL33 (head and neck) and OVCAR-8 (ovarian) cancer cell lines were used to test the effect of DFO on proliferation using MTT and annexin-V/propidium iodide assays. DNA damage, specifically double strand break (DSB) formation, was tested by γH2AX foci quantification via immunofluorescence assays.

Results: DFO inhibited cell proliferation and apoptosis in a dose- and time-dependent manner. These effects were more marked than the one achieved through nitrogen-induced hypoxia. Additionally, DFO induces DNA damage through DSB formation.

Conclusion: Although DFO is usually used as a hypoxia-mimetic agent because it is cheaper and less time-consuming than nitrogen-induced hypoxia, our results show that it decreases cell proliferation and induces DNA damage through DSB formation. Therefore, its antitumoral effect should be further explored.

Grants: Study was financed by Gerencia Regional de Salud, JCyL (GRS2171/A/2020)

1. Lynn, J. V. et al. The Role of Deferoxamine in Irradiated Breast Reconstruction: A Study of Oncologic Safety. Plast. Reconstr. Surg. 143, 1666–1676 (2019).

Conflict of Interest: None declared

P01.002.B Mapping of Splicing Regulatory Elements-rich intervals and identification of spliceogenic variants in ATM exon 7

Inés Llinares-Burguet[1], *Lara Sanoguera-Miralles*[1], *Elena Bueno-Martínez*[1], *Alicia García-Álvarez*[1], *Eladio A. Velasco-Sampedro*[1]

[1]*Instituto de Biomedicina y Genética Molecular de Valladolid (IBGM), Consejo Superior de Investigaciones Científicas – Universidad de*

Valladolid (CSIC-UVa), Splicing and Genetic Susceptibility to Cancer Laboratory, Valladolid, Spain

Background/Objectives: Alternative splicing regulates gene expression patterns and is controlled by splicing regulatory elements (SRE). Mutations in SREs might affect splicing and be involved in disease susceptibility. Our purpose was to study the alternatively spliced exon 7 of the breast cancer susceptibility gene *ATM*.

Methods: A minigene with exons 4 to 9 of *ATM* (MG_ATM_4-9) was used in this work. The enhancer/silencer profile in *ATM* exon 7 was bioinformatically analysed by HexPlorer to identify the potential regions to study. Selected sequences were deleted in MG_ATM_4-9 by site-directed mutagenesis. In the region with more impact on exon 7 inclusion, we introduced 3 internal overlapping microdeletions. Additionally, we tested all possible variants localised in critical SRE intervals and variants with HexPlorer score below -40 were selected. Thus, 19 variants were introduced into the minigene and functionally tested.

Results: We identified two minimal regions (12/13-nt) involved in exon recognition, located in the 3'-end exon [△(E7): 50-75%]. Of the 19 variants analysed, seventeen (89%) impaired splicing, seven of which had strong impact on splicing, producing exon 7 skipping (59-69%) and <30% of the minigene full-length transcript.

Conclusion: The functional SRE mapping by exonic microdeletions was a useful approach to identify enhancer-rich sequences and SRE variants that impair splicing. The identification of spliceogenic variants is a helpful tool in the genetic counselling consultation, facilitating the early detection and diagnostic of diseases.

Grants: Predoctoral fellowship from the *Junta de Castilla y León* (2022-2025); ISCIII (PI20/00225 and PI23/00047).

Conflict of Interest: None declared

P01.003.C Rare Germline Variants in glioma: A genomic analysis of 125 individuals from Northern Sweden

Adam Rosenbaum[1], *Anna Dahlin*[1], *Carl Wibom*[1], *Beatrice Melin*[1]

[1]*Umeå universitet, Department of Diagnostics and Intervention, Umeå, Sweden*

Background/Objectives: The etiology of glioma, the most common malignant CNS tumor, remains inadequately understood. Although germline predisposition, including common and rare variants, have been studied, the role of rare germline variants to glioma predisposition remains insufficiently explored.

Methods: We conducted whole genome sequencing on germline samples from 125 individuals from Northern Sweden, diagnosed with various subtypes of glioma. Of these, 46 were diagnosed before 40 years of age, and 12 familial gliomas from 5 families. We studied rare variants in established cancer predisposition genes, as well as a broader investigation of other genes with rare likely pathogenic variants.

Results: Our study reveals 20 rare coding variants in 18 genes, including *TP53*. Several are likely pathogenic and reside within known cancer predisposition genes, suggesting a link to glioma development. We identified rare variants in genes not previously associated with glioma, providing possible new insights into the etiology of glioma. Comparing the frequency of rare coding mutation among our cases and 300 individuals of similar lineage 7 genes have a significantly increased burden of rare coding variants. These genes are awaiting validation.

Conclusion: Our findings show that the germline genetic predisposition to glioma still holds some explanatory potential to the development of the disease. Continuing studying this aspect of predisposition may unlock new understanding of glioma development.

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Umeå University Hospital Grant (7003839 to BM)
Northern Sweden Cancer Foundation (AMP 23-1141 to AR)

Conflict of Interest: None declared

P01.007.C Large genomic rearrangement: tandem duplication and triplication in *BRCA1* gene causative for hereditary breast and ovarian cancer

Bernardus Aldrige Allister[1], *Jonathan Lühmann*[1], *Lena Wendeburg*[1], *Tim Ripperger*[1], *Bernd Auber*[1], *Frank Dechend*[2], *Carmela Beger*[3], *Stefanie Tölle*[3], *Julia von Ehr*[4], *Nataliya Di Donato*[1], *Doris Steinemann*[1]

[1]*Hannover medical school (MHH), Department of Human Genetics, Hannover, Germany;* [2]*MVZ Reproduction Medicine and Human Genetics, Hildesheim, Germany;* [3]*MVZ Labor Krone GbR, Bad Salzuflen / Bielefeld, Germany;* [4]*Frauenärzte am Schloss, Wolfenbüttel, Germany*

Background/Objectives: Large genomic rearrangements (LGRs) include copy number variations like duplications or triplications of coding or non-coding genomic regions within the human genome. Here, we report on two LGRs in two families with the suspicion of hereditary breast and/or ovarian cancer (HBOC) syndrome, a pathogenic *BRCA1* tandem duplication targeting exon 18-19 and a for the first time a pathogenic *BRCA1* tandem triplication of exon 2.

Methods: MLPA, Optical Genome Mapping (OGM), cDNA analysis and Sanger sequencing have been performed to identify these rearrangements and to characterize their localization and orientation as well as to predict their pathogenicity for HBOC development.

Results: We show that the duplication of exon 18-19 in *BRCA1*, is a tandem duplication: ogm[GRCh38] dup(17)(q21.31q21.31)(43057052_43063373). Validation by sanger sequencing of the cDNA shows that this tandem duplication of exon 18-19 generate a premature termination codon at the second codon of the duplicated exon 18. In the second index patient, four copies of the *BRCA1* promotor region, exon 1a,1b and exon 2 were detected via MLPA and OGM enabled us to resolve this LGR as a *BRCA1* exon 2 tandem triplication: ogm[GRCh38] trip(17)(q21.31q21.31)(43117155_43124115). cDNA analysis shows that this LGR causes an inclusion of a portion of intron 2 sequence and provokes a premature termination codon exactly at the junction between exon 2 and the intronic sequence of intron 2.

Conclusion: Due to a predicted premature termination codon we classify both LGRs as pathogenic variants. This signifies that LGRs such as tandem duplications and triplications can be causative for HBOC.

Grants:

Conflict of Interest: None declared

P01.008.D Atypical cancer risk profile in carriers of Italian founder *BRCA1* variant p.His1673del: implications for classification and clinical management

Giovanni Innella[1,2], *Cristina Fortuno*[3], *Laura Caleca*[4], *Bing-Jian Feng*[5], *Courtney Carroll*[5], *Michael T. Parsons*[3], *Sara Miccoli*[2], *Marco Montagna*[6], *Daniele Calistri*[7], *Laura Cortesi*[8], *Barbara Pasini*[9], *Siranoush Manoukian*[10], *Daniela Giachino*[11], *Laura Matricardi*[6], *Maria Cristina Foti*[6], *Valentina Zampiga*[7], *Claudia Piombino*[8], *Elena Barbieri*[8], *Francesca Vignolo*[9], *Jacopo Azzolini*[10], *Rita Danesi*[12], *Valentina Arcangeli*[12], *Sandrine Caputo*[13], *Nadia Boutry-Kryza*[14],

Vincent Goussot[15], Susan Hiraki[16], Marcy Richardson[17], Simona Ferrari[2], Paolo Radice[4], Amanda B. Spurdle[3], Daniela Turchetti[1;2]

[1]Università di Bologna, Dipartimento di Scienze Mediche e Chirurgiche, Bologna, Italy; [2]2.IRCCS Azienda Ospedaliero-Universitaria di Bologna, Medical Genetics Unit, Bologna, Italy; [3]QIMR Berghofer Medical Research Institute, Population Health, Brisbane, Australia; [4]Fondazione IRCCS Istituto Nazionale dei Tumori, Unit of Predictive Medicine: Molecular Bases of Genetic Risk, Department of Experimental Oncology, Milano, Italy; [5]University of Utah, Salt Lake City, United States; [6]Veneto Institute of Oncology IOV - IRCCS, Immunology and Molecular Oncology Unit, Padova, Italy; [7]IRCCS Istituto Romagnolo per lo Studio dei Tumori "Dino Amadori" IRST, SC Laboratorio di Bioscienze, Meldola, Italy; [8]Centro Oncologico Modenese, Azienda Ospedaliero-Universitaria Policlinico di Modena, Dipartimento di Oncologia ed Ematologia, Modena, Italy; [9]Azienda Ospedaliero-Universitaria Città della Salute e della Scienza di Torino, SC Genetica Medica U, Torino, Italy; [10]Fondazione IRCCS Istituto Nazionale dei Tumori, Unit of Medical Genetics, Department of Medical Oncology and Hematology, Milano, Italy; [11]Azienda Ospedaliero-Universitaria San Luigi Gonzaga, Regione Gonzole, Counselling Genetico, Orbassano, Italy; [12]IRCCS Istituto Romagnolo per lo Studio dei Tumori "Dino Amadori" IRST, SC Epidemiologia Clinica e Sperimentale, Meldola, Italy; [13], Institut Curie, Paris, France and Paris Sciences Lettres Research University, Department of Genetics, Paris, France; [14]Hospices Civils de Lyon, Service de génétique, Lyon, France; [15]Centre de Lutte Contre le Cancer Georges François Leclerc, Département de Biologie et Pathologie des Tumeurs, Dijon, France; [16]GeneDx, Gaithersburg, United States; [17]Ambry Genetics, Aliso Viejo, United States

Background/Objectives: *BRCA1*:c.5017_5019del(p.His1673del) is a founder variant relatively frequent in Northern Italy. Despite previous suggestion of pathogenicity, variant classification in public databases is still conflicting, needing additional evidence.

Methods: Maximum likelihood penetrance of breast/ovarian and other cancer types was estimated using full pedigree data from 53 informative Italian families. The effect of the variant on BRCA1-ABRAXAS1 interaction was assessed using a GFP-fragment reassembly-based PPI assay. Results were combined with additional data from multiple sources to classify the variant according to ACMG/AMP classification rules specified for *BRCA1/2*.

Results: Variant-carriers displayed increased risk for ovarian cancer (HR = 33.0, 95%CI = 7.0-155.0; cumulative risk at age 70 = 27.6%, 95%CI = 12.6-40.0%) but not for breast cancer (HR = 0.7, 95%CI = 0.2-2.2). An increased risk of uterine cancer (HR = 8.0, 95%CI = 1.03-61.6) emerged, warranting further evaluation. Likelihood ratio in favor of pathogenicity was 98898642.82 under assumption of standard *BRCA1* breast and ovarian penetrance, and 104240832.84 after excluding breast cancer diagnoses (based on penetrance results). Functional analysis demonstrated that the variant abrogates the BRCA1-ABRAXAS1 binding, supporting the PS3 code assignment within the ACMG/AMP rule-based model. Collectively, these findings allowed to classify the variant as pathogenic.

Conclusion: Pathogenicity of *BRCA1*:c.5017_5019del(p.His1673del) has been confirmed; however, breast cancer risk in Italian families is not increased, unlike in families from other countries and in carriers of most *BRCA1* pathogenic variants. Haplotype analysis is underway to explore the hypothesis of potential in-cis modifiers. Nevertheless, the knowledge of atypical risk profiles for this and other variants will pave the way for personalized management based on specific genotype.

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P01.009.A Further elucidating the genetic landscape of glioma predisposition in a European familial glioma cohort

Frank Brand[1], *Amir H. Akbarzadeh[1], Christine A. M. Weber[1], Lily S. Rose[1], Robert Geffers[2], Gunnar Schmidt[1], Bernd Auber[1], Michael Friese[3], Mareike Müller[4], Michael Lalk[5], Isabel Eckert[6], Arya Nabavi[5], Paul Kremer[7], Amir Samii[8], Guido Reifenberger[9;10], Joachim K. Krauss[11], Bettina Wiese[6;11], Christian Hartmann[12], Ruthild G. Weber[1]*

[1]Hannover Medical School, Department of Human Genetics, Hannover, Germany; [2]Helmholtz Centre for Infection Research, Genome Analytics Research Group, Braunschweig, Germany; [3]Asklepios Klinik Nord - Heidberg, Department of Pathology and Neuropathology, Hamburg, Germany; [4]Heinrich Heine University, Medical Faculty, Department of Neurosurgery, Düsseldorf, Germany; [5]KRH Klinikum Nordstadt, Department of Neurosurgery, Hannover, Germany; [6]Diakovere Krankenhaus gGmbH, Henriettenstift, Department of Neurology, Hannover, Germany; [7]Asklepios Klinik Nord - Heidberg, Department of Neurosurgery, Hamburg, Germany; [8]International Neuroscience Institute, Department of Neurosurgery, Hannover, Germany; [9]Heinrich Heine University, Medical Faculty, Institute of Neuropathology, Düsseldorf, Germany; [10]German Cancer Consortium (DKTK), Partner Site Essen/Düsseldorf and German Cancer Research Center (DKFZ), Heidelberg, Germany; [11]Hannover Medical School, Department of Neurosurgery, Hannover, Germany; [12]Hannover Medical School, Institute of Pathology, Department of Neuropathology, Hannover, Germany

Background/Objectives: Familial occurrence of glioma, the most common type of malignant brain tumor, is observed in about 5% of cases. Studies on the genetic landscape of glioma predisposition are still scarce.

Methods: Leukocyte DNA of 122 glioma patients from 115 tumor families with at least one glioma patient each were analyzed by whole-exome sequencing. Data were analyzed using two approaches: (1) variants in established cancer predisposition genes (CPGs) or suspected glioma risk genes (n = 154) were extracted and classified, (2) the frequency of rare (MAF < 0.01%) or deleterious variants in all genes in the glioma cohort was compared with that in a control cohort (n = 257 families). Exome data of >70 additional patients are pending and will be included in our analysis.

Results: Both approaches associated *BRCA2* variants, including the pathogenic variants c.316+5G > C, p.(K944*), p.(I1470Kfs*11), most strongly with familial glioma. *BRCA2* variants were significantly more frequent in the glioma than in the control cohort ($P = 0.0088$). In addition, rare deleterious variants in *APC, ATM*, and *EGFR* were recurrently observed in the glioma cohort. In agreement with Choi et al., Sci. Adv., 2023, our familial glioma

cases carried rare deleterious variants in known CPGs, e.g. *ATM*, *PMS2*, and *POLE*, and novel CPGs, e.g. *SLC4A7* and *WDR7*. Furthermore, we identified 31 genes, not previously associated with cancer predisposition, that were recurrently affected in the glioma but not at all in the control cohort.

Conclusion: Our study on a large European familial glioma cohort implicates known CPGs, particularly *BRCA2* and *ATM*, and novel CPGs in glioma risk.

Conflict of Interest: None declared

P01.010.B Analysis of aberrant splicing in capture RNA-seq data as a supplement to DNA germline testing to increase diagnostic yield

*Florentine Scharf[1], Thomas Keßler[1], **Evgenia Vibe**[1], Oliver Klaas[1], Jonas Ingermann[1], Caroline Heintz[1], Anna Benet-Pages[1;2], Sabrina Angerbauer[1], Martin Wendlandt[1], Tobias Wohlfrom[1], Verena Steinke-Lange[1;3], Andreas Laner[1], Ariane Hallermayr[1;3], Julia Romic-Pickl[1;3], Morghan Lucas[1;3], Elke Holinski-Feder[1;3]*

[1]MGZ – Medizinisch Genetisches Zentrum, Munich, Germany; [2]Institute of Neurogenomics, Helmholtz Research Center, München, Germany; [3]Medizinische Klinik und Poliklinik IV, Campus Innenstadt, Klinikum der Universität München

Background/Objectives: Hereditary tumor syndromes comprise 5-10% of cancer cases, necessitating accurate identification of causal genetic variants for effective patient management. The diagnostic yield of whole-exome sequencing (WES), the gold standard for molecular diagnostics, ranges from 15-25% in these syndromes. High-throughput functional studies, such as RNA sequencing (RNA-seq), play a crucial role in reclassifying variants of uncertain significance (VUS) and unveiling pathomechanisms like aberrant splicing, particularly when initial variant detection fails.

Methods: We developed a cost-efficient, high-throughput RNA-seq approach to analyze RNA phenotypes where we complement polyA mRNA or hexamer capture with enrichment of 49 cancer-associated genes from PAXgene-derived RNA samples.

Results: We achieved ultra-high coverage sequencing data of ~3,500X mean target coverage and, on average, 80% of exons covered with >50 read depth using hexamer capture and target enrichment. In almost 80% of positive controls for aberrant splicing, we detected a difference of >20% in the PSI (percent-spliced in) score compared to negative controls.

Conclusion: Our workflow provides high-quality RNA-seq data, which allows the assessment of splicing events. Our data on the comparisons between positive and negative controls substantiate the possibility of an automated high throughput pipeline. This work represents the basis for implementing targeted RNA-seq in cancer diagnostics, in which we want to establish the parallel analysis of WES and RNA-seq to increase the diagnostic yield and turnaround time for patients with hereditary tumor syndromes.

Grants: N/A

Conflict of Interest: None declared

P01.011.C Identification of shared genetic variants among different cancers and between adenomyosis and cancer utilizing members of the same family

Sevcan Aydın[1], *nura fitnat topbas selcuki[2], pinar yalcin bahat[3], engin oral[4], Feyza Tuncer[5]*

[1]Istanbul University, Graduate School of Health Sciences, Istanbul, Türkyie; [2]University of Health Sciences Turkey, Istanbul Sisli Hamidiye Etfal Training and Research Hospital, Obstetrics and Gynecology, Istanbul, Türkyie; [3]University of Health Sciences Turkey, Istanbul Kanuni Sultan Suleyman Training and Research Hospital, Department, Obstetrics and Gynecology, Istanbul, Türkyie; [4]Biruni University Faculty of Medicine, Obstetrics and Gynecology, Istanbul, Türkyie; [5]Istanbul University, Aziz Sancar Institute of Experimental Medicine, Genetics, Istanbul, Türkyie

Background/Objectıves: Adenomyosis is a benign uterine condition characterized by the localization of endometrial-like tissue in myometrium. Its pathogenesis remains elusive, but it carries cancer-like characteristics. We aimed to identify shared genetic variants both between different cancers and adenomyosis and cancer, utilizing a family having women affected by both conditions.

Methods: Five women from the same family were subjected to gynecological examinations via transvaginal ultrasonography, amongst two receieved pathologically confirmed diagnosis of ovarian mucinous adenocarcinoma and clear cell renal cell carcinoma (ccRCC). Whole exome sequencing was performed on all women utilizing Illumina NextSeq550. Bioinformatic analyses were performed on Genomize SEQ and Pairend platforms, MAF < %1 was applied to identify rare and novel variants. Cases with cancer versus all cases were evaluated in parallel, for which one member was utilized as a control, who manifested neither of the conditions.

Result: Pathological evaluations excluded adenomyosis in the case diagnosed with ovarian mucinous adenocarcinoma, while the case with ccRCC received adenomyosis diagnosis. A novel genetic variant in *NTN1* gene was identified across all cases, in addition to rare variants in 10 genes shared among cases with cancers.

Conclusıon: *NTN1* gene might be one of the many links between the pathogenesis of cancer and adenomyosis, fitting in with its attributed roles in cell migration and angiogenesis. The impact of the novel variant in *NTN1* merits clarification functionally. Further studies are in need to elucidate the diversifying mechanisms among two similar conditions evolving towards either malignity or benignity.

Grant Reference:Istanbul University Scientific Research Projects Coordination Unit(TSA-2023-38950)

Conflict of Interest: None declared

P01.012.D Reproductive factors and sex hormones levels on differentiated thyroid cancer risk: A Mendelian Randomization study

See Hyun PARK[1;2], *Mojgan Karimi[2], Cloe Domenighetti[2], Thérèse Truong[2]*

[1]Paris Saclay Universite, Gif-sur-Yvette, France; [2]INSERM, Exposome and Heredity, Villejuif

Consortium: EPITHYR consortium, and The European Prospective Investigation into Cancer and Nutrition (EPIC-Europe)

Background: Differentiated thyroid carcinoma (DTC) is the most common type of thyroid cancer, occurring three times more frequently in women than in men. However, the underlying biological mechanisms driving this sex-specific discrepancy remain poorly understood. To analyze the causal role of sex hormones and reproductive factors in the risk of DTC, we implemented a two-sample Mendelian Randomization (MR) analysis using genome-wide association studies (GWAS) summary statistics.

Methods: GWAS on DTC were derived from a meta-analysis of 6 studies including 7,705 cases and 963,612 controls of European population, while GWAS summary statistics on sex hormones, reproductive factors, and gynecological factors related to hysterectomy were retrieved from publicly available literature. We used the inverse-variance weighted method to estimate odds ratio,

with additional multiple sensitivity analyses to ensure the suitability of the MR analysis, and conducted multivariable MR to account for potential confounding variables.

Results: We showed that endometrial cancer was associated with DTC (OR = 1.15, $p = 9 \times 10^{-3}$). We also observed an association between SHBG levels and increased risk of DTC, however this association lost significance upon adjustment for obesity-related factors. Putative causal associations was observed with uterine fibroids; nonetheless, this association was not supported by additional sensitivity analyses. No significant associations were found for levels of sex hormones, age at menopause, menarche and first birth, nor with endometriosis.

Conclusion: Using the largest GWAS on DTC to date, our findings do not support a significant influence of sex hormones and reproductive factors in the observed differences in DTC risk between women and men.

Grants: Funded by doctoral school of Université Paris-Saclay

Conflict of Interest: None declared

P01.013.A Evolution of genomic profiles in primary and recurrent astrocytic gliomas

Libuse Lizcova[1], *Halka Lhotska*[1], *Karolina Janeckova*[1], *Lucie Hodanova*[1], *Karla Svobodova*[1], *Hana Cechova*[2], *Tatiana Aghova*[1], *Sarka Ransdorfova*[2], *Lenka Pavlistova*[1], *Filip Kramar*[3], *David Netuka*[3], *Zuzana Zemanova*[1]

[1]*General University Hospital and First Faculty of Medicine, Charles University in Prague, Center of Oncocytogenomics,Institute of Medical Biochemistry and Laboratory Diagnostics, Prague, Czech Republic;* [2]*Institute of Hematology and Blood Transfusion, Prague, Czech Republic;* [3]*First Faculty of Medicine of Charles University and Military University Hospital Prague, Department of Neurosurgery and Neurooncology, Prague*

Background/Objectives: Astrocytic gliomas, including grades 2-4 astrocytoma and grade 4 glioblastoma, are characterized by diverse biological behaviors and recurrent lesions. During disease progression, these tumors undergo genomic evolution with newly acquired genetic properties. However, the mechanisms and evolutionary dynamics underlying tumor progression and relapse remain poorly understood.

Methods: Genomic profiles of 35 paired glioma samples (primary and recurrent; 15x astrocytoma, 20x glioblastoma) were analyzed using combination of cytogenomic methods: I-FISH (Abbott Molecular, MetaSystems), aCGH/SNP (Agilent), MLPA, MS-MLPA (MRC-Holland) and targeted NGS panel (Invitae).

Results: Primary tumors displayed common aberrations like *IDH1/IDH2* and *TERT* mutations, CNAs of chromosome 7, 10, and *CDKN2A/2B* and *EGFR* genes, persisting in recurrent lesions. All but two patients experienced recurrences with newly acquired genetic/epigenetic changes with a high frequency of CNAs leading to complex rearrangements. Divergent clonal evolution was observed in 22 cases, while 11 cases displayed linear evolution. Aberrations of chromosomal regions 4p, 6p, 8q, 9q, 11p and 11q were recurrently identified in recurrences. cnLOH was proved in 22 cases, mainly on 7p and 17p. Disease progression occurred in 28 patients.

Conclusions: This study highlights heterogeneity in tumor evolution driven by genomic/microenvironmental imbalances or treatment. Recurrence may arise from major tumor clone or multiple subclones within the primary tumor. Cytogenomic analyses of recurrent tissues contribute to understanding these processes and identification of alterations associated with glioma progression. These biomarkers could subsequently serve as resource for precision oncology targeting cancer dynamics in astrocytic gliomas.

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Conflict of Interest: None declared

P01.014.B PAX5 alterations in a consecutive childhood B-ALL cohort treated on ALL IC-BFM 2009 protocol

Klementina Črepinšek[1;2], *Janez Jazbec*[2;3], *Marko Kavčič*[2;3], *Tomaž Prelog*[3], *Tine Tesovnik*[1;2], *Barbara Jenko Bizjan*[1;2], *Robert Sket*[1;2], *Jernej Kovač*[1;2], *Marusa Debeljak*[1;2]

[1]*University Children's Hospital, UMC Ljubljana, Clinical Institute for Special Laboratory Diagnostics, Ljubljana, Slovenia;* [2]*University of Ljubljana, Faculty of Medicine, Ljubljana;* [3]*University Children's Hospital, UMC Ljubljana, Department of Oncology and Haematology, Ljubljana*

Background/Objectives: In this study, we aimed to identify patients within our B-ALL cohort with altered *PAX5*. Our objective was to characterize the types of genetic changes, determine their origin (somatic/germline), and analyze the clinical outcomes associated with them.

Methods: A consecutive cohort of 99 patients with B-ALL treated at the Children's Hospital of the UMC Ljubljana according to the ALL IC-BFM 2009 protocol was included in our study. We used RNA sequencing data for gene expression analysis, fusion gene detection and variant identification, multiplex-ligation dependent probe amplification for copy number variation assessment, and Sanger sequencing to detect germline variants.

Results: *PAX5* was impacted in a 33.3% of our patients, with the genetic alterations ranging from CNVs and rearrangements to SNVs. The most common were CNVs, which were found in 33.7% of patients, followed by point mutations in 5.2% and gene rearrangements in 4.1%. We identified 8 patients with a *PAX5*-associated genetic subtypes that were previously classified as "B-other", and they showed intermediate outcomes. We showed a trend of poorer survival in hyperdiploid cases carrying duplications in *PAX5* compared to other hyperdiploid cases. We also report an interesting case of a patient with *PAX5::FKBP15* and a pathogenic variant in *PTPN11* who underwent an early relapse with a monocytic switch.

Conclusion: In conclusion, this study provides valuable insights into the presence, frequency, and prognostic significance of diverse *PAX5* alterations in B-ALL patients, highlighting the complexity of genetic factors and their impact on patient outcomes.

Grants: Young Research Fellowship SRA#54654, UMC-tertiary grants: TP20230039

Conflict of Interest: None declared

P01.015.C Rare mutations in FH and POLE genes in a patient with Ewing sarcoma and familial cancer burden

Dimitar Bakalov[1], *Svilen Maslyankov*[2], *Kalina Belemezova*[3], *Radka Tafradjiiska-Hadjiolova*[1], *Zafer Sabit*[1], *Ivanka Dimova*[4]

[1]*Medical University of Sofia, Department of Physiology and Pathophysiology, Sofia, Bulgaria;* [2]*Medical University of Sofia, Department of Surgery, Sofia, Bulgaria;* [3]*Medical University of Sofia, Department of Medical Biology, Sofia, Bulgaria;* [4]*Medical University of Sofia, Department of Medical Genetics, Sofia, Bulgaria*

Background/objectives: Ewing sarcoma is a rare and aggressive form of bone and soft tissue cancer, predominantly affecting young individuals. We explore the underlying genetic factors contributing to Ewing sarcoma in a 22-year-old patient, having familial cancer burden (colon, kidney, breast, bladder, melanoma).

Methods: Whole exome sequencing was performed on genomic DNA from the affected individual. Comprehensive bioinformatic analyses were employed to prioritize and validate variants, with a particular focus on genes implicated in cancer susceptibility.

Results: The analysis revealed two very rare heterozygous missense variants of unknown significance: c.809 A > G (p.Tyr270Cys) in the *FH* gene (frequency of 3e-5) and c.1015 G > A (p.Asp339Asn) in *POLE* gene (frequency of 8e-5) in the patient's germline. *FH* encodes fumarate hydratase, a key enzyme in the tricarboxylic acid cycle, which is associated with autosomal dominant "Leiomyomatosis and renal cancer" phenotype. *POLE* is essential for DNA replication and repair, and is associated with autosomal dominant "Colorectal cancer, succeptibility to, 12" phenotype.

Conclusion: This study contributes to the growing body of knowledge surrounding the genetic landscape of Ewing sarcoma, highlighting the role of *FH* and *POLE* gene variants. Additionally, the severe family history of colon, urothelial, breast and skin cancers in first- and second-degree relatives raises intriguing questions about shared genetic predisposition. Further investigations into the potential synergistic effects of these variants and their implications for familial cancer risk are warranted. This case underscores the importance of genetic counseling in individuals with Ewing sarcoma and multidisciplinary approach to personalized cancer care.

Grant: Project № BG-RRP-2.004-0004-C01

Conflict of Interest: None declared

P01.016.D Liquid biopsy integrating machine learning for prostate cancer detection

Ondrej Pös[1;2], *Zuzana Hanzlikova*[2;3], *Jaroslav Budiš*[1;2;4], *Jakub Styk*[1;2], *Matej Hrnčiar*[2], *Werner Krampl*[1;2;5], *Tomáš Sládeček*[1;2], *Jozef Sitarcik*[1;2], *Pavol Misenko*[2], *Silvia Bokorova*[1;2], *Diana Rusnakova*[1;2], *Lydia Lukyova*[1;5], *Monika Kubanova*[1], *Terezia Duranova*[1], *Tatiana Sedlackova*[1;2], *Tomas Szemes*[1;2;5]

[1]*Comenius University Science Park, Bratislava, Slovakia;* [2]*Geneton Ltd., Bratislava, Slovakia;* [3]*Faculty of Informatics and Information Technologies, Slovak University of Technology, Bratislava, Slovakia;* [4]*Slovak Center of Scientific and Technical Information, Bratislava, Slovakia;* [5]*Faculty of Natural Sciences, Comenius University, Department of Molecular Biology, Bratislava, Slovakia*

Background/Objectives: Early-stage cancer manifests with minimal or no noticeable symptoms, leading to a diagnostic delay and subsequent less effective treatment. Since analysis of cell-free DNA (cfDNA) may serve to identify early neoplastic changes, liquid biopsy-based tests hold potential for non-invasive cancer screening.

Methods: The plasma of 60 prostate cancer patients and 369 control individuals were collected to analyze cfDNA by low-coverage whole-genome next-generation sequencing. Data were processed using an in-house bioinformatics pipeline to detect genetic variability and characterize qualitative and quantitative sequencing metrics. A total of 379 samples (49 patients, 330 controls) were used to train the model from decision trees using gradient boosting to facilitate prostate cancer prediction. Subsequently, a testing set of 50 samples (11 patients, 39 control) was used to assess the accuracy of the model.

Results: The robust combination and evaluation of 671 attributes, including genomic variability and sequencing metrics, by ensemble learning model, have shown a sensitivity of 81.8% and specificity of 100.0% on the testing dataset. The ROC AUC of 97.4% suggests a high prediction capability of the test.

Conclusion: Here, we emphasize the decent potential of liquid biopsy test enhanced by artificial intelligence to evaluate an extensive set of cfDNA sequencing metrics and genomic attributes for non-invasive prostate cancer screening.

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Conflict of Interest: Ondrej Pös The author is an employee of Geneton Ltd. and is involved in numerous research and development efforts to adapt novel approaches to liquid biopsy screening applications. However, the employee does not hold any financial interest in the company., Zuzana Hanzlikova The author is an employee of Geneton Ltd. and is involved in numerous research and development efforts to adapt novel approaches to liquid biopsy screening applications. However, the employee does not hold any financial interest in the company., Jaroslav Budiš The author is an employee of Geneton Ltd. and is involved in numerous research and development efforts to adapt novel approaches to liquid biopsy screening applications. However, the employee does not hold any financial interest in the company., Jakub Styk The author is an employee of Geneton Ltd. and is involved in numerous research and development efforts to adapt novel approaches to liquid biopsy screening applications. However, the employee does not hold any financial interest in the company., Matej Hrnčiar The author is an employee of Geneton Ltd. and is involved in numerous research and development efforts to adapt novel approaches to liquid biopsy screening applications. However, the employee does not hold any financial interest in the company., Werner Krampl The author is an employee of Geneton Ltd. and is involved in numerous research and development efforts to adapt novel approaches to liquid biopsy screening applications. However, the employee does not hold any financial interest in the company., Tomáš Sládeček The author is an employee of Geneton Ltd. and is involved in numerous research and development efforts to adapt novel approaches to liquid biopsy screening applications. However, the employee does not hold any financial interest in the company., Jozef Sitarcik The author is an employee of Geneton Ltd. and is involved in numerous research and development efforts to adapt novel approaches to liquid biopsy screening applications. However, the employee does not hold any financial interest in the company., Pavol Misenko The author is an employee of Geneton Ltd. and is involved in numerous research and development efforts to adapt novel approaches to liquid biopsy screening applications. However, the employee does not hold any financial interest in the company., Silvia Bokorova The author is an employee of Geneton Ltd. and is involved in numerous research and development efforts to adapt novel approaches to liquid biopsy screening applications. However, the employee does not hold any financial interest in the company., Diana Rusnakova The author is an employee of Geneton Ltd. and is involved in numerous research and development efforts to adapt novel approaches to liquid biopsy screening applications. However, the employee does not hold any financial interest in the company., Lydia Lukyova: None declared, Monika Kubanova: None declared, Terezia Duranova: None declared, Tatiana Sedlackova The author is an employee of Geneton Ltd. and is involved in numerous research and development efforts to adapt novel approaches to liquid biopsy screening applications. However, the employee does not hold any financial interest in the company., Tomas Szemes The author is an employee of Geneton Ltd. and is involved in numerous research and development efforts to adapt novel approaches to liquid biopsy screening applications. However, the employee does not hold any financial interest in the company.

P01.017.A Development and validation of an expanded comprehensive genomic profiling assay with enhanced variant sensitivity for tumor biopsies

Stephanie Constantinou[1], *Alexia Eliades*[1], *Kyriakos Tsangaras*[1], *Chrysa Soteriou*[1], *chrystalla lazarou*[1], *Achilleas Achilleos*[1], *charalambos loizides*[1], *Christos Lemesios*[1], *Chrystalla Havadjia*[1], *sarah barbour*[1], *aris pallaris*[1], *chrysovalando soteriou*[1], *Louisa Constantinou*[1], *Haris Kkoufou*[1], *Michalis Spyrou*[1], *antonis antoniou*[1], *Antonia Matsentidou*[1], *Melina Vaki*[1], *george manoli*[1], *Christos Prokopi*[1], *Styliana Georgiou*[1], *Elena Kypri*[1], *Marios Ioannides*[1], *George Koumbaris*[1], *Philippos Patsalis*[1;2]

[1]*Medicover Genetics, Nicosia, Cyprus;* [2]*University of Nicosia Medical School, Department of Basic and Clinical Sciences, Nicosia, Cyprus*

Background/Objectives: Comprehensive genomic profiling using next-generation sequencing (NGS) has become increasingly important in the classification and management of cancer, enabling a growing number of patients to benefit from targeted therapies. Such approved therapies include ones developed for less prevalent gene alterations, thus emphasising the need for assessing a broader panel of genes for multiple alteration classes with an enhanced sensitivity. Herein, we describe the development of an expanded 392-gene panel, offering high analytical performance.

Methods: DNA isolated from a set of formalin-fixed, paraffin-embedded (FFPE) tissue samples comprising reference materials, non-malignant and tumor samples was subjected to library preparation and hybrid capture enrichment with our expanded 392-gene panel. Enriched libraries were subsequently sequenced by NGS on a NovaSeq platform and data was analysed using proprietary bioinformatic pipelines.

Results: A set of FFPE samples comprising benign, malignant and reference material samples, was used to evaluate the assay's analytical performance. The assay demonstrated high sensitivity and specificity for all classes of genomic alterations, including single nucleotide variants (SNVs), small insertions and deletions (Indels), copy number alterations (CNAs), translocations, as well as the immuno-oncology biomarkers, microsatellite instability (MSI) and tumor mutational burden (TMB). Notably, the developed assay can detect mutations down to 1% variant allele frequency (VAF).

Conclusion: Validation results demonstrate the development of a tissue-based NGS assay for the assessment of an expanded panel of genomic alterations and therapy-associated biomarkers that can detect less prevalent variants with enhanced sensitivity.

Conflict of Interest: Stephanie Constantinou Author employed by Medicover Genetics, Alexia Eliades Author employed by Medicover Genetics, Kyriakos Tsangaras Author employed by Medicover Genetics, Chrysa Soteriou Author employed by Medicover Genetics, chrystalla lazarou Author employed by Medicover Genetics, Achilleas Achilleos Author employed by Medicover Genetics, charalambos loizides Author employed by Medicover Genetics, Christos Lemesios Author employed by Medicover Genetics, Chrystalla Havadjia Author employed by Medicover Genetics, sarah barbour Author employed by Medicover Genetics, aris pallaris Author employed by Medicover Genetics, chrysovalando soteriou Author employed by Medicover Genetics, Louisa Constantinou Author employed by Medicover Genetics, Haris Kkoufou Author employed by Medicover Genetics, Michalis Spyrou Author employed by Medicover Genetics, antonis antoniou Author employed by Medicover Genetics, Antonia Matsentidou Author employed by Medicover Genetics, Melina Vaki Author employed by Medicover Genetics, george manoli Author employed by Medicover Genetics, Christos Prokopi Author employed by Medicover Genetics, Styliana Georgiou Author employed by Medicover Genetics, Elena Kypri Author employed by Medicover Genetics, Marios Ioannides Author employed by Medicover Genetics, George Koumbaris Author employed by Medicover Genetics, Philippos Patsalis Author employed by Medicover Genetics

P01.019.C Increased prevalence of pathogenic and likely pathogenic *CHEK2* variants in the Balearic Islands Hereditary Cancer cohorts.

Maria Antònia Caro-Miró[1], *Catalina Lladó-Sampol*[2], *Antònia Perelló-Martorell*[2], *Evelin Horvath*[2], *Paloma de la Torre-Rubio*[3], *DAMIAN HEINE SUÑER*[1], *Victor Asensio-Landa*[1], *Laura Torres-Juan*[1], *María Carmen Prado-Farnos*[1], *Susana Renee Avella-Klaassen*[1], *Iciar Martínez*[1], *Jesús Alarcón Company*[2], *Antonia Obrador-Hevia*[1]

[1]*Hospital Universitari Son Espases, Molecular Diagnosis and Clinical Genetics Unit, Palma, Spain;* [2]*Hospital Universitari Son Espases, Oncology, Palma, Spain;* [3]*Hospital Universitari Son Espases, Digestology, Palma, Spain*

Background/Objectives: *CHEK2* has been identified as an intermediate-risk gene associated with breast, prostate, and colon cancer (OMIM: 609265).

In this study, we examined the frequency and mutational spectrum of *CHEK2* variants within the Balearic Islands' hereditary cancer (HC) cohort, comparing it to the Catalonian population databases in order to investigate potential founder mutations.

Methods: The research assessed the prevalence of pathogenic/likely pathogenic (P/LP) *CHEK2* variants and included two Balearic Island cohorts: 1559 patients from the HC cohort sequenced by the TruSight Hereditary Cancer panel (Illumina®) between 2016 and 2022, and 3352 non-HC patients sequenced via Whole Exome Sequencing (WES) (Illumina®). Frequencies from the genetically closest population, Catalonia, were used for comparison.

Results:

Group	Patients	Patients (%) with P/LP variants in *CHEK2**
Breast, ovarian and prostate (BOP) cancer	1101	24 (2.2%)
Colorectal cancer	368	9 (2.4%)
Other cancer conditions	130	2 (1.5%)
Total Balearic Islands HC	1599	35 (2.2%)
Non-HC	3352	20 (0.6%)
Catalonian HC (Vargas-Parra 2022)	1848	14 (0.8%)

*Variants analysed (NM_007194): c.1427C > T, c.279G > A, c.349A > G, c.433C > T, c.1100del, c.591del, c.319+2T > A.

CHEK2 variants	% BOP cancer	% Colorectal cancer	% Other cancer conditions	% Non-HC
c.1427C > T	0.82	2.17	0.00	0.39
c.279G > A	0.73	0.27	0.77	0.21

Conclusion: The prevalence of P/LP variants in the HC cohort was found to be 2.2% (2.2% in BOP cancer patients and 2.4% in colorectal cancer patients), significantly higher than the genetically closest population of Catalonia. This possibly suggests the

presence of a founder effect of c.1427C > T and c.279G > A variants, enriched in Mallorca and Ibiza patients, respectively.

Grants:

Conflict of Interest: None declared

P01.020.D Deciphering the Gene Expression Signature of High-Grade B-cell Lymphomas with 11q Aberrations

Emil Chteinberg[1], *Gioia Di Stefano*[2], *Henry Löffler-Wirth*[3], *Annette M. Staiger*[4,5], *Sina Hillebrecht*[1], *Rabea Wagener*[1], *Susanne Bens*[1], *Kathrin Oehl-Huber*[1], *Sonja Dahlum*[1], *Dmitriy Abramov*[6], *Birgit Burkhardt*[7], *Abner Louissaint,Jr.*[8], *Katrin Kurz*[5], *Heike Horn*[4], *Anja Mottok*[1,9], *Raffaella Santi*[2], *Ilske Oschlies*[10], *Wolfram Klapper*[10], *Kristian Schafernak*[11], *Yanming Zhang*[12], *Andreas Rosenwald*[9], *Hans Binder*[3], *Megan Limm*[12], *German Ott*[5], *Lorenzo Leoncini*[13], *Sebastien Hergalant*[14], *Coral DelVal*[1,15,16], *Reiner Siebert*[1]

[1]*Ulm University and Ulm University Medical Center, Institute of human genetics, Ulm, Germany;* [2]*University of Florence, Careggi University Hospital and Department of Health Sciences, 2 Pathology Section, Florence, Italy;* [3]*Leipzig University, Interdisciplinary Centre for Bioinformatics (IZBI), Leipzig, Germany;* [4]*University of Tübingen, Dr. Margarete Fischer-Bosch Institute of Clinical Pharmacology, Stuttgart, Germany;* [5]*Robert-Bosch-Hospital, Department of Clinical Pathology, Stuttgart, Germany;* [6]*Dmitry Rogachev National Medical Research Center of Pediatric Hematology, Department of Pathology, Moscow, Russian Federation;* [7]*Münster University Hospital, Pediatric Hematology and Oncology, Münster, Germany;* [8]*Massachusetts General Hospital, Department of Pathology, Boston, United States;* [9]*Julius-Maximilians-University Würzburg, Department of Pathology, Würzburg, Germany;* [10]*University of Kiel, Department of Pathology, Kiel, Germany;* [11]*Phoenix Children's Hospital, Department of Pathology and Laboratory Medicine, Phoenix, United States;* [12]*Memorial Sloan Kettering Cancer Center, Department of Pathology and Laboratory Medicine, New York, United States;* [13]*University of Siena, Department of Medical Biotechnologies, Section of Pathology, Siena, Italy;* [14]*University De Lorraine, Inserm UMR 1256 Nutrition-Génétique et Exposition aux Risques Environnementaux (NGERE), Nancy, France;* [15]*University of Granada, Andalusian Research Institute in Data Science and Computational Intelligence, Department of Computer Science and Artificial Intelligence, Granada, Spain;* [16]*Instituto de Investigación Biosanitaria ibs.GRANADA, Granada, Spain*

Consortium: ICGC-MMML-Seq Consortium

Background/Objectives: The 5th edition of the WHO Classification of Haematolymphoid Tumours describe disease entities defined by genetic abnormalities. For instance, the diagnosis of Burkitt lymphoma (BL) requires an *IG::MYC* juxtaposition, e.g. due to t(8;14)(q24;q32). Some high-grade B-cell lymphomas (HGBL) lacking the *MYC* translocation exhibit immunophenotypic similarities to BL but share similarities at the mutational level with diffuse large B-cell lymphomas (DLBCL). A subset of these HGBL (HGBL-11q) show characteristic chromosomal aberration defined by gains in 11q23.2-11q23.3 and losses in the 11q24.1-qter region. Here, we identify differential transcriptomic features of such HGBL-11q relative to BL and DLBCL.

Methods: Gene expression profiling was conducted using the HTG EdgeSeq Pan B-Cell Lymphoma Panel using RNA obtained from formal-fixed paraffin-embedded (FFPE) tissues of 7 BL, 26 HGBL-11q, 132 DLBCL, and two reactive tonsils. The panel was verified against RNAseq data available from overlapping samples of the ICGC-MMML-Seq cohort, leaving 206 genes for subsequent clustering and DESeq2 analyses. A two-step cumulative gene expression classifier was developed to segregate HGBL-11q from BL and DLBCL.

Results: Using unsupervised UMAP analysis of the 206 genes, we observed that HGBL-11q clustered apart from BLs and DLBCLs. DESeq2 revealed 48 and 38 genes differentially expressed between HGBL-11q and DLBCL or BL, respectively. A two-step classifier was trained to distinguish HGBL-11q from DLBCLs and BLs, respectively. PostHoc genomic analyses of cases classified as HGBL-11q showed two cases not yet assigned to this entity to carry typical 11q aberrations.

Conclusion: Gene expression profiling of FFPE tissues was effective in identifying HGBL-11q among other mature aggressive lymphomas.

Grants: None

Conflict of Interest: None declared

P01.021.A Germline ESR1 variants may be causative of juvenile papillomatosis of the breast

Zahra Firoozfar[1], *Shahryar Alavi*[1], *Mohammadreza Dehghani*[2], *Mohammad Yahya Vahidi Mehrjardi*[3]

[1]*Palindrome, Isfahan, Iran;* [2]*Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran;* [3]*Diabetes Research Center, Shahid Sadoughi University of Medical Sciences, Yazd, Iran*

Objectives: Germline variants are causative of 10% of cancer incidences. Juvenile papillomatosis of the breast (JPB), also known as Swiss cheese disease, is a benign condition occurs in individuals with a family history of breast carcinoma and its incidence in males is rare. Here, we report a case with several affected males by JPB or gynecomastia, harbouring a significant germline mutation in the ESR1 gene.

Methods: WES was performed on 200 Iranian individuals with hereditary cancers. Sequence raw data was analysed using GATK, and GRCh38 as reference genome. We also called copy number variations using PalinDepth, our proprietary algorithm. Variants were filtered based on the ACMG guideline, then were manually curated to find the best match with the patients' phenotype.

Results: Germline ESR1 D538G mutation (chr6:g.152098791A > G) was detected in heterozygous state in a 5 years old boy suffering from JPB, with several males in the pedigree are affected with gynecomastia. No other significant exome variant was detected. Sanger sequencing showed that another affected family member is heterozygous for the ESR1 variant.

Conclusion: ESR1 D538G mutant is well described in sporadic breast cancer where ESR1 somatic mutations lead to malignancies. Recently it has been shown that ESR1 variants could increase the risk of hereditary breast cancer. Interestingly, we detected D538G mutation in a patient with familial breast anomalies in males. Further experiments could validate plausible effects of germline D538G mutation, investigating if this could trigger hereditary breast cancer in females.

Grants:

Conflict of Interest: None declared

P01.022.B Cascade testing for hereditary breast cancer: what is achievable in a South African clinical service?

Tabitha Osler[1], *Mardelle Schoeman*[2], *Jennifer Edge*[3], *Willem Pretorius*[2], *Felix Rabe*[4], *Christopher Mathew*[5], **Michael Urban**[6,7]

[1]*Sydney Brenner Institute for Molecular Bioscience, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa;* [2]*Division of Molecular Biology and Human Genetics, University of Stellenbosch, Cape Town, South Africa;* [3]*Department of Surgery, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa;* [4]*Faculty of Medicine and Health*

Sciences, University of Stellenbosch, Cape Town, South Africa; [5]Sydney Brenner Institute for Molecular Bioscience, University of the Witwatersrand, Johannesburg, South Africa; [6]Division of Human Genetics, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa; [7]Department of Obstetrics and Gynaecology, Faculty of Medicine and Health Sciences, University of Stellenbosch, Cape Town, South Africa

Background/Objectives: A main reason for genetic counselling and testing people with possible hereditary breast cancer is to identify probands and their asymptomatic family members who carry pathogenic variants, by the process of cascade testing. This allows prevention by radiographic screening and risk-reducing surgery, especially in female relatives. We investigated uptake in a South African regional breast cancer service.

Methods: Pedigrees and laboratory electronic records in 83 consecutive female breast cancer patients with pathogenic variants associated with high or moderate penetrance of hereditary breast cancer were used to assess family member testing. Electronic appointment, clinical and radiology records were used in test-positive relatives to identify preventive interventions conducted by a year later.

Results: 1173 family members were considered eligible for testing, 55% female. Relatives of 39/83 (47%) probands took up testing, with 111 tested and 40 testing positive (3 in ATM, 17 in BRCA1, 17 in BRCA2, 3 in PALB2), including 31 females. Family member testing was associated with gender and degree of relationship. In female relatives, the proportion tested dropped from 25% in first degree to 8% in second degree relatives, and in males from 14% to 0.8%. Prophylactic measures undertaken in guideline-eligible asymptomatic female relatives included radiographic screening in 5/20 (25%), risk-reducing mastectomy in 9/19 (47%), and bilateral salpingo-oophorectomy in 3/13 (23%).

Conclusion: Compared to international evidence uptake of presymptomatic testing is low; in test-positive female relatives, uptake of screening is low but of risk-reducing surgery appears similar. We will explore further with a qualitative study.

Grants: Cancer Association of South Africa Research Grant 2022

Conflict of Interest: None declared

P01.023.C Piperine exerts antitumor effects in head and neck cancer

Juliana Prado Gusson-Zanetoni[1], Luana Cardoso[1], Stefanie Oliveira de Sousa[1], Laura Luciana De Melo Moreira Silva[1], Tiago Henrique[2], Eloiza Tajara[2], Claudia Regina Bonini Domingos[1], Sonia Maria Oliani[1], Eny Maria Goloni-Bertollo[2], **Flavia Cristina Rodrigues-Lisoni**[1]

[1]*São Paulo State University (UNESP), Institute of Biosciences, Humanities and Exact Science, Department of Biological Science, São José do Rio Preto; [2]School of Medicine of São José do Rio Preto (FAMERP), Molecular Biology Department, São José do Rio Preto, Brazil*

Background/Objectives: Head and neck cancer (HNC) is promoted by genomic instability that affects cell growth, metabolism and inflammation. Treatments for patients with this cancer are generally very invasive and the use of natural products has been used as an adjuvant. Among these, piperine (1-piperoylpiperidine) stands out, which can be isolated from the fruits or roots of black pepper (*Piper nigrum*), as it has pharmacological effects such as antioxidants, anti-inflammatory and immunomodulatory. Therefore, the aim of the present study was to investigate the molecular mechanisms of action of piperine in relation to its potential antitumor effect on head and neck squamous cells carcinoma (HNSCC).

Methods: Parameters related to neoplastic potential including proliferation, cytotoxicity, migration, apoptosis, cell cycle, clonogenicity, genotoxicity were verified in HNSCC (HEp-2 and SCC-25) treated with piperine. Furthermore, molecular analysis of the cytokines, proteins and genes were investigated to understand how piperine modulates the inflammatory pathways.

Results: The results of the tests indicated that piperine modified the morphology, inhibited growth/viability and the formation of cell colonies, promoted genotoxicity by triggering apoptosis and cell cycle arrest in G2/M and S. The decrease in cell migration also was observed, probably due to the decrease in the expression of *MMP2/9* genes. Furthermore, piperine reduced the expression of molecules related to inflammation such as PTGS2, PTGER4, ERK, p38 and cytokines.

Conclusion: These results suggest that piperine exerts antitumor effects on HNSCC and may be a promising natural treatment by regulating signaling pathways associated with inflammation and cancer.

Grants: Fapesp, CNPq.

Conflict of Interest: None declared

P01.024.D Germline Pathogenic Variants In Pakistani Patients Evaluated At A Hereditary Breast Cancer Clinic

Fizza Akbar[1], *Shamila Ladak[2], aushna saleem[3], alizeh fatimi[2], bassim zahid[2], zahraa siddiqui[2], uzair ansari[4], Salman Kirmani[1]*

[1]*Aga Khan University Hospital, Division of women and child health, Karachi, Pakistan; [2]Aga Khan University Hospital, Medical College, Karachi, Pakistan; [3]Albert Einstein College of Medicine, United States; [4]Aga Khan University Hospital, Paediatrics and Child Health, Karachi, Pakistan*

Background/Objectives: Hereditary breast cancer (HBC) accounts for 5-15% of all breast cancer cases. These are attributed to pathogenic (P) and likely pathogenic (LP) germline variants in breast cancer predisposition genes. This study describes the spectrum of germline variants causing HBC in Pakistan and explores clinical correlations with P/LP variants.

Methods: Retrospective chart review of 751 patients from HBC clinic who proceeded with multi-gene panel testing between 2017 and 2023. Descriptive and inferential statistical analyses were done.

Results: Eighteen percent (137/751) tested positive, 36% (273/751) had a VUS and 45% (341/751) had a negative result. Sixty percent of positive patients had P/LP variants in *BRCA1/2*, while the other 40% were attributed to other genes. Six recurrent *BRCA1* variants reported were in 20 unrelated patients, with *BRCA1* c.1961dup (p.Y655Vfs*18) being the most common, present in eight patients. *CHEK2 (5.4%), FANCM (5.4%), PALB2 (4.7%)* were the next most prominent genes. Patients with positive results had a younger age at diagnosis at 42, IQR (34-50). Having a positive result showed a positive association with triple-negative breast cancer (TNBC). Of the patients who tested positive for *BRCA1/2*, 84% had a diagnosis of TNBC. Of all patients with positive results, 12% of patients had a negative family history of cancer.

Conclusion: Our findings give unique insights into the genetic factors causing HBC in the Pakistani population. As more patients are tested and protocol-driven referrals are made across the country, our insight into the genetic architecture of hereditary breast cancer in our population will continue to increase.

Grants: Not-funded

Conflict of Interest: None declared

P01.025.A Recurrent variant in the 3'UTR of the MSH6 gene in four unrelated Italian families with Lynch syndrome phenotype

Domizia Pasquetti[1], *Federica Francesca L'Erario*[2], *Roberta Pietrobono*[1], *Maria Grazia Pomponi*[2], *Arianna Panfili*[3], *Emanuela Lucci Cordisco*[1;2], *Maurizio Genuardi*[1;2]

[1]*Section of Genomic Medicine, Department of Life Sciences and Public Health, Catholic University of Sacred Heart and Unit of Medical, Rome, Italy;* [2]*UOC Genetica Medica, Fondazione Policlinico Universitario "A.Gemelli" IRCCS, Rome, Italy;* [3]*Direzione Scientifica, Fondazione Policlinico Universitario "A.Gemelli" IRCCS, Roma*

Background/Objectives: Pathogenic variants in MSH6 account for 12%-35% of Lynch syndrome (LS). Despite classification guidelines, variants of uncertain significance (VUS) in MMR genes represents a diagnostic dilemma. We discuss here the c.*23_*26dup *MSH6* variant, that is reported as a VUS in ClinVar but considered as pathogenic in a previous published study.

Methods: Genetic counseling involved four unrelated Italian families, with germline NGS analysis performed in four subjects and targeted variant analysis in two. IHC of MMR proteins was conducted on four endometrial tumors (EC) and one colorectal cancer (CRC); MSI was performed on one of two CRCs.

Results: All the six individuals were heterozygotes for *MSH6* c.*23_*26dup. EC was diagnosed in all four female carriers (median age: 45.7 years), showing no MSH2 and MSH6 protein expression. CRC occurred in two male carriers at ages 62 and 32; IHC and MSI revealed no MSH2 and MSH6 expression, with MSI-H status in one tested CRC.

Conclusion: The clinical phenotypes of c.*23_*26dup *MSH6* carriers are highly suggestive of LS, with significantly high penetrance for early-onset gynecological tumors. The *MSH6* variant could be pathogenic and cause LS in these families. However, the lack of MSH2 expression suggests involvement of the *MSH2* gene. Under this assumption, the *MSH6* c.*23_*26dup variant could be a linked marker of an undetected *MSH2* alteration. The previously reported families were from Northern Italy, while our families are from Central Italy. The geographic origin and the phenotypic similarities across the families suggest that they might derive from a common founder.

Conflict of Interest: None declared

P01.026.B A case of *MYC* and *BCL2*- positive high-grade B-cell lymphoma with concurrent 11q gain-loss aberration

Gulleyla Kilic[1], *Olga Meltem Akay*[2], *Emre Osmanbasoglu*[2], *Dicle Ballikaya*[1], *Tuba Akan*[3], *İbrahim Öner Doğan*[4], *Hülya Kayserili*[1;5]

[1]*Koc University Hospital, Genetic Diseases Evaluation Center, Istanbul, Türkyie;* [2]*Koc University Medical School, Hematology, Istanbul, Türkyie;* [3]*Koc University Hospital, Flow Cytometry, Istanbul, Türkyie;* [4]*Koc University Medical School, Pathology, Istanbul, Türkyie;* [5]*Koc University Medical School, Medical Genetics, Istanbul, Türkyie*

Background/Objectives: High-grade B-cell lymphoma (HGBCL, previously known as double-hit lymphoma) is an aggressive type of B-cell non-Hodgkin lymphoma characterized by rearrangements in *MYC* and *BCL2*, or/and less commonly, *BCL6* gene. Rare cases of HGBCL with concurrent *MYC* rearrangement and 11q aberrations have been described[1]. In this study we provide further evidence that 11q aberrations can also be found in HGBCL with *MYC* and *BCL2* rearrangements (HGBCL-MYC-BCL2-11q)[2]. Clinical, immunohistochemical and detailed cytogenetic characterization of the case is discussed.

Methods:A 36-year-old woman with three-month history of cervical lymphadenopathy,

pancytopenia and elevated LDH was referred to our hospital. The diagnosis of HGBCL-MYC-BCL2-11q was established based on histopathologic/immunohistochemical examination of the bone marrow sample with flow cytometry, classical cytogenetic and interphase/metaphase fluorescence in situ hybridization analyses.

Results: A bone marrow examination showed diffuse infiltration of neoplastic B-cells with CD19 +, HLA-DR +, CD10 +, CD38 +, CD20 +, CD22 +, TdT- and kappa light chain expression. iFISH analysis showed abnormal *BCL2*, *cMYC*, *IGH*, *MLL (KMT2A*) and *TEL/AML1* signals. Conventional cytogenetic analysis defined a highly complex karyotype with a derivative chromosome 1, trisomy 7, t(8;14), triplication 11q, deletion 11q, partial trisomy 12p, t(14;18), trisomy 18 and monosomy X. The complex karyotype was further clarified by additional selected FISH probes for chromosomes 1,8,11,14,18 and X. She received GMALL-B-ALL 2002 protocol treatment and expired 7 months after the diagnosis.

Conclusion: Our findings highlight the importance of clarifying the complex karyotype in HGBCL patients. Future studies are needed to define the prognosis, treatment response and survival of *MYC* and *BCL2*-positive HGBCL patients with 11q aberrations.

Grants:-

Conflict of Interest:-

References:[1]Shestakova et al.,2023;PMID:36997031.[2]Gebauer et.al.,2021;PMID:34500469.

Conflict of Interest: None declared

P01.027.C A pilocytic astrocytoma with wovel *ATG16L1::NTRK2* fusion responsive to larotrectinib: A case report with genomic and functional analysis

Lily Deland[1;2], *Simon Keane*[2], *Thomas Olsson Bontell*[3;4], *Tomas Sjöberg Bexelius*[5;6], *Esther De La Cuesta*[7], *Inga Gudinaviciene*[8], *Jonas A Nilsson*[9;10], *Helena Carén*[11], *Helena Mörse*[12], *Frida Abel*[1;2]

[1]*Sahlgrenska University Hospital, Clinical Genetics & Genomics, Center for Medical Genomics, Gothenburg, Sweden;* [2]*Gothenburg University, Institute of Bimedicine, Laboratory Medicine, Gothenburg, Sweden;* [3]*Sahlgrenska University Hospital, Dept. of Clinical Pathology, Dept. of Clinical Pathology, Gothenburg, Sweden;* [4]*Gothenburg University, Institute of Neuroscience & Physiology, Sahlgrenska Academy, Gothenburg, Sweden;* [5]*Karolinska University Hospital, Section for Pediatric Oncology, Highly Specialized Pediatric Medicine 2, Astrid Lindgren's Children's Hospital, Stockholm, Sweden;* [6]*Karolinska Institute, Department of Women's and Children's Health, Stockholm, Sweden;* [7]*Bayer Pharmaceuticals U.S., Global Medical Affairs – Oncology, Whippany, United States;* [8]*Laboratory Medicine Region Skåne, Department of Genetics and Pathology, Lund, Sweden;* [9]*Gothenburg University, Sahlgrenska Center for Cancer Research, Institute of Clinical Sciences, Department of Surgery, Gothenburg, Sweden;* [10]*Harry Perkins Institute of Medical Research, Perth, Australia;* [11]*Gothenburg University, Institute of Biomedicine, Sahlgrenska Center for Cancer Research, Department of medical biochemistry and cell biology, Gothenburg, Sweden;* [12]*Skåne University Hospital, Pediatric Cancer Center, Lund, Sweden*

Background/Objectives: The outcome of pilocytic astrocytoma depends heavily on the success of surgery. In the cases where surgery alone is not curative, next generation sequencing approaches can be used to identify treatment targets for precision medicine. Here we report a pediatric pilocytic astrocytoma case that underwent incomplete surgical resection due to the location of the tumor.

Clinical genetics analyses demonstrated that the tumor did not carry a *BRAF* fusion or a *BRAF* mutation. After post-operative surveillance according to the low-grade glioma protocol, recurrent tumor progressions resulted in multiple chemotherapy regimens.

Methods: RNA sequencing by an open-ended targeted approach was performed and detected the presence of a novel *ATG16L1::NTRK2* fusion gene. The rearrangement was subsequently confirmed by interphase fluorescent in situ hybridization

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in tumor cells. Functional analysis of the fusion was performed by in-vitro transient transfection of HEK293 cells. The ATG16L1::TRKB fusion protein was found to activate both the MAPK-pathway, and the PI3K-pathway, through increased phosphorylation of ERK, AKT and S6.

Results: As a result of the identification of a NTRK fusion, the patient was enrolled in a phase I/II clinical trial of the nonselective TRK inhibitor larotrectinib. The patient responded well without significant side-effects. Eight months after start of treatment, tumor lesions were no longer detectable consistent with complete response. Presently, 14 months later, complete remission is sustained.

Conclusion: Our findings highlight the importance of screening for other MAPK-drivers in *BRAF*-negative low-grade gliomas, since rare fusion genes may serve as targets for precision oncology therapy.

Conflict of Interest: Lily Deland: None declared, Simon Keane: None declared, Thomas Olsson Bontell: None declared, Tomas Sjöberg Bexelius: None declared, Esther De La Cuesta Medical doctor at Bayer Oncology, clinical trials with larotrectinib., Inga Gudinaviciene: None declared, Jonas A Nilsson: None declared, Helena Carén: None declared, Helena Mörse: None declared, Frida Abel: None declared

P01.028.D The neurofibromin-lysosome axis in breast cancer

Antonella Delicato[1;2], Pilar Chacon Millan[1;2], Bruno Achutti Duso[3], Alessia Castiglioni[3], Eleonora Messuti[3], Sara Galavotti[3], Diego Medina[2;4], Luca Mazzarella[3], **Manuela Morleo**[1;2]

[1]University of Campania "Luigi Vanvitelli", Department of Precision Medicine, Naples, Italy; [2]Telethon Institute of Genetics and Medicine (TIGEM), Pozzuoli, Italy; [3]IEO European Institute of Oncology - IRCCS, Department of Experimental Oncology, Milan, Italy; [4]University of Naples 'Federico II', Department of Medical and Translational Science, Naples, Italy

Background/Objectives: Breast cancer is the most frequent cancer in women. Neurofibromin (NF1) loss-of-function mutations are associated with poor breast cancer survival. Loss of NF1 (a negative regulator of the Ras-MAPK pathway) leads to constitutive mTORC1 signaling by activation of the Ras-MAPK pathway contributing to neoplastic transformation. NF1 interacts with LAMTOR1, a lysosomal protein crucial for mTORC1 activation on lysosomes, while changes in lysosome function and spatial distribution are closely related to cancer development and progression. We hypothesize that neurofibromin has a direct functional role in lysosomal metabolism and mTOR activation in breast cancer.

Methods: We generated NF1 knockout (NF1-KO) human breast cancer cells (SKBR3, BT474, HCC1954) using CRISPR-Cas technology and developed cell-based assays by high-content imaging approaches for lysosomal phenotype screening. mTOR signaling was analyzed by Western blot upon nutrient deprivation and then upon refeeding.

Results: mTOR activity decreases under nutrient deprivation and is reactivated under nutrient refeeding in the three breast cancer cell lines. However, loss of NF1 abrogates the decrease in mTOR signaling under nutrient deprivation. Moreover, lysosomes appear more condensed and increase in number in NF1-KO cells.

Conclusions: These results demonstrate that mTOR is constitutively active and lysosomal activity is altered in three different NF1-KO breast cancer cell models. In-depth investigation of specific lysosomal changes and NF1-dependent molecular mechanisms will provide a deeper understanding of the dynamic changes in cancer development, providing more opportunities for cancer diagnosis and treatment.

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Conflict of Interest: None declared

P01.029.A Treatment-related clonal competition and disrupted molecular processes in chronic lymphocytic leukemia

Kristyna Zavacka[1;2], *Jakub Porc[1;3], Petr Taus[1], Karol Pál[1], Sarka Pavlova[1;2;3], Jitka Malcikova[1;2;3], Kamila Stranska[1;2;3], Marcela Zenatova[2], Boris Tichy[1], Anna Panovska[2], Michael Doubek[1;2;3], Sarka Pospisilova[1;2;3], Karla Plevova[1;2;3]*

[1]Central European Institute of Technology, Masaryk University, Center of Molecular Medicine, Brno, Czech Republic; [2]University Hospital Brno, Department of Internal Medicine – Hematology and Oncology, Brno, Czech Republic; [3]Medical faculty, Masaryk University, Institute of Medical Genetics and Genomics, Brno, Czech Republic

Background/Objectives: Chronic lymphocytic leukemia (CLL), the most common adult leukemia in Western countries, is characterized by a highly variable clinical course, which is closely related to dynamic changes in leukemic cell clones over time. The expansion of subclones with different gene mutations, many of which are non-recurrent, makes CLL challenging to treat. We aimed to describe the clonal evolution in CLL, focusing on specific treatments and different stages of the disease. We particularly studied factors influencing the clonal development of *TP53*-mutated subclones, as defects in the *TP53* gene are the strongest predictive and prognostic marker in CLL and drive chemoimmunotherapy resistance.

Methods: We investigated a cohort of 62 CLL patients with detailed clinical characterization and known patterns of *TP53* mutation evolution. Whole-exome sequencing was employed to identify somatic variants at two to six different timepoints during the disease course of each patient. Subsequently, we analyzed abnormal molecular pathways and mutational signatures in defined patient groups and tracked their changes over time.

Results: Our analysis revealed striking competition among co-existing CLL subclones of individual patients. We observed progressive evolution of specific gene mutations, highlighting both their mutual exclusivity and co-occurrence. Moreover, we observed several impaired pathways and mutational signatures associated with different disease stages, treatment outcomes, and *TP53* status.

Conclusion: Our study provides insights into the molecular aspects underlying clonal evolution in CLL. These findings enhance our understanding of distinct disease phenotypes and suggest potential targets for personalized treatment strategies in CLL.

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Conflict of Interest: None declared

P01.030.B Joint data analysis of more than 29,000 individuals with HBOC as part of the NASGE initiative

*Jan Henkel[1], **Andreas Laner**[1;2], Melanie Locher[1;2], Tobias Wohlfrom[1], Birgitta Neitzel[1], Kerstin Becker[1;2], Teresa Neuhann[1;2], Angela Abicht[1;2;3], Verena Steinke-Lange[1;2;4], Birgit Eichhorn[5], Winfried Schmidt[6], Daniel Berner[7], Anna Teubert[2;8], Anne Holtorf[9], Sarah Heinrich[9], Gabriele Wildhardt[10], Martin Schulze[11], Laura von der Heyden[2;7], Daniela Steinberger[10], Saskia Kleier[2;6], Peter Lorenz[2;5], Ralf Glaubitz[2;8], Saskia Biskup[2;11], Elke Holinski-Feder[1;2;4]*

[1]MGZ - Medical Genetics Center, München, Germany; [2]Nationale Allianz für seltene genetische Erkrankungen; [3]Neurologische Klinik und Poliklinik mit Friedrich-Baur-Institut, München, Germany; [4]Medizinische Klinik und Poliklinik IV, Campus Innenstadt, Klinikum der Universität München, München, Germany; [5]MVZ Mitteldeutscher Praxisverbund Humangenetik, Dresden, Germany; [6]Gemeinschaftspraxis für Humangenetik und Genetische Labore - DNA Diagnostik Hamburg, Hamburg, Germany; [7]MVZ genetikum GmbH, Neu-Ulm, Germany; [8]AMEDES Medizinische Dienstleistungen GmbH, Germany; [9]Medicover Genetics, MVZ Martinsried GmbH, Planegg, Germany; [10]MVZ diagnosticum Frankfurt, Zentrum für Humangenetik, Frankfurt am Main, Germany; [11]Zentrum für Humangenetik Tübingen, Tübingen, Germany

Consortium: NASGE - Nationale Allianz für seltene genetische Erkrankungen

Background/Objectives: Although there are recommendations at national and international level as to which genes should be analyzed in the context of a hereditary breast and ovarian cancer (HBOC), the individual composition of the corresponding gene panels varies, resulting in a different diagnostic yield.

Methods: We performed a retrospective NGS dataset analysis of suspected HBOC patients who had been tested at different German diagnostic laboratories that are part of the NASGE network. These results were compared with the most common panel recommendations and an internal HBOC core gene panel. Additionally, we analyzed the data concerning other potential tumor risk syndromes (TRS) not caused by pathogenic variants in the core panel genes.

Results: We collected 29,317 HBOC datasets and at least one pathogenic variant (class 4/5) was reported in about 4250 datasets, resulting in an overall diagnostic yield of 14.5%. The diagnostic yield of pathogenic variants (class 4/5) varied depending on the analyzed panel (between 5 and 26 genes) from 9.0% to 13.8% with the internal panel having a yield of 12.7%. Notably, in about 1% of cases, a pathogenic variant outside the established HBOC core genes was identified, potentially indicating the presence of other TRS.

Conclusion: These results are consistent with previous observations that a significant proportion of patients with HBOC predisposition were not detected by the guideline-based gene panels and suggest that expanded diagnostics compared to currently recommended multigene panels may identify additional patients at high risk for developing cancer.

Grants:

Conflict of Interest: None declared

P01.031.C RNA-seq of a cohort of cancer patients demonstrates its capacity to reclassify uncertain variants by unveiling aberrant splicing events

*Florentine Scharf[1], Thomas Keßler[1], Evgenia Vibe[1], Oliver Klaas[1], Jonas Ingermann[1], Caroline Heintz[1], Sabrina Angerbauer[1], Tobias Wohlfram[1], Andreas Laner[1], Ariane Hallermayr[1;2], Julia Romic-Pickl[1;2], Morghan Lucas[1;2], **Verena Steinke-Lange**[1;2], Elke Holinski-Feder[1;2]*

[1]MGZ – Medizinisch Genetisches Zentrum, Munich, Germany; [2]Medizinische Klinik und Poliklinik IV, Campus Innenstadt, Klinikum der Universität München, Munich, Germany

Background/Objectives: Around 5-10% of cancers are caused by hereditary tumor syndromes. For the current state-of-the-art method of gene panel testing via whole-exome sequencing (WES), the diagnostic yield ranges between 15-25% for these syndromes. Additional high-throughput methods are therefore required to increase the diagnostic yield. RNA-seq can guide the reclassification of variants of unclear significance (VUS) and detect previously unknown causative variants by revealing aberrant splicing at the RNA level.

Methods: We developed a cost-efficient, high-throughput targeted RNA-seq approach to analyze aberrant splicing events.

Results: Over 100 cases underwent RNA-seq, previously examined by NGS exome sequencing without revealing a clear pathogenic variant. Of these, 144 had a VUS, while 4 showed no initial concerning variants. RNA-seq led to the reclassification of 4 variants as likely benign and 8 as (likely) pathogenic, an increase in diagnostic yield by 8%. Of the 8 (likely) pathogenic reclassified variants, 2 were in *BRCA*1/2 genes. Among reclassified cases, 9 variants affected an intronic splice site, and 3 were outside of obvious splice-relevant regions. For one case with no initially reported variants, RNA-seq identified the use of a cryptic exon in the *APC* gene, guiding subsequent intronic region analysis.

Conclusion: RNA-seq offers clinically relevant insights on VUS and cases where no variant was detected, aiding clear diagnoses for unresolved cases. Overall, ~8% of cases previously unsolved by DNA exome sequencing were successfully reclassified by our RNA-seq approach, significantly improving diagnostic yield and optimizing patient care for hereditary cancer syndromes.

Grants: N/A

Conflict of Interest: None declared

P01.032.D The role of liquid biopsy by rapid NGS in cancer management

Mustafa Ozen[1], *Serhat Seyhan[2], Emre Ilhan[2], Rabia Aksoy[2]*

[1]Istanbul University-Cerrahpasa, Medical Genetics, Istanbul, Türkyie; [2]Medroyal Genetic Assessment Center, Medical Genetics, Istanbul, Türkyie

Background/Objectives: Liquid biopsy (LB) is a promising technique for early detecting and monitoring cancer; however, there are still room to improve in sensitivity and duration of the test. Here, we report data in 20 cancer patients referred to us for LB. We propose that LB by rapid NGS technique is crucial for cancer management.

Methods: At least 10 ml of whole blood is collected in EDTA containing vacutainers. Plasma is seperated immediately and stored in -80 until further use. Total nucleic acid (TNA) is isolated from the plasma samples using MagMAX ™ Cell-Free TNA isolation kit. Total of 10 ng TNA is subjected to Genexus NGS platform where library preperation and sequencing are done automatically in 24 hours by using Oncomine Precision assay. This assay detects actionable mutations in 50 genes in DNA and RNA level. The data is analysed initially by Genexus platform and further analysed by Oncomine software.

Results: Out of 20 patients analysed, 10 patients are dignosed with Lung cancer, 3 had suspicion for lung cancer, 2 had pancreas cancer, 1 patient in each cancer type of breast, bronchial, hepatocellular and colon cancers. We were able to detect

actionable mutations in 70 % of our patients. Most of the mutations were SNV in the following genes analyzed: EGFR, p53, KRAS, PTEN and BRAF. One patient had EML4-ALK fusion and one AR amplification.

Conclusion: LB by rapid NGS is an important tool for cancer management. İt is critical to include DNA and RNA in the analysis to precisely detect mutations.

Grants: N/A

Conflict of Interest: None declared

P01.033.A Meta-analysis of *GRIN2A* variants contradicts a role in tumorigenesis

Robin-Tobias Jauss[1], *Johannes Lemke*[1;2], *Vincent Strehlow*[1]

[1]*Institute of Human Genetics, University of Leipzig Medical Center, Leipzig;* [2]*Center for Rare Diseases, University of Leipzig Medical Center, Leipzig*

Background/Objectives: *GRIN2A* has previously been identified as frequently mutated in tumor samples, leading to a hypothesised involvement of *GRIN2A* in tumorigenesis. Pathogenic *GRIN2A* germline variants on the other hand lead to neurodevelopmental disorders, and extensive phenotyping of affected individuals revealed no evidence for tumor burden.

Methods: All publicly available *GRIN2A* variants were obtained from ClinVar, Cosmic and gnomAD to account for germline variation, somatic variation and variants identified in the general population, respectively. Functional consequences, mutational hotspots and gene expression were assessed for each dataset to draw conclusions on potential pathomechanisms of tumorigenesis.

Results: Pathogenic germline variants in *GRIN2A* expose a genotype-phenotype correlation with distinct clustering in functionally relevant domains. Somatic *GRIN2A* variants are indeed unique when compared to pathogenic germline variants, but exhibit a homogeneous distribution and no enriched functional consequences in specific domains of *GRIN2A*. Expression of *GRIN2A* is lower in tumor samples compared to non-diseased tissues.

Conclusion: The homogeneous distribution and low expression of somatic variants implies a lack of mutational clustering and functional relevance, which refutes the hypothesised major role of *GRIN2A* in tumorigenesis.

Grants: None

Conflict of Interest: None declared

P01.036.D First mutational analysis of TERT promoter and CTNNB1 in hepatocellular carcinoma, cirrhotic, and non-cirrhotic liver tissues from Brazilian patients.

Mariana Leonardo Terra[1], *José Junior Barros*[1], *Antônio Hugo José Fróes Marques Campos*[2;3], *Vera Pannain*[4], **Natalia Araujo**[1]

[1]*Oswaldo Cruz Institute - FIOCRUZ, Laboratory of Molecular Virology and Parasitology, Rio de Janeiro, Brazil;* [2]*A.C.Camargo Cancer Center, Department of Anatomic Pathology, São Paulo, Brazil;* [3]*Rede D'Or São Luiz, Division of Pathology, São Paulo, Brazil;* [4]*Federal University of Rio de Janeiro, Clementino Fraga Filho University Hospital, Rio de Janeiro, Brazil*

Background/Objectives: Hepatocellular carcinoma (HCC) is a major global health burden, with poor prognosis especially at later stages. HCC often arises in cirrhotic livers due to specific risk factors. Its development involves complex genetic mutations. TERT and CTNNB1 somatic mutations are prevalent in HCC but unexplored in Brazilian patients. This study aims to investigate the association of TERT promoter mutations (C228T and C250T) and CTNNB1 exon 3 mutations with HCC development in Brazil.

Methods: Eighty-five liver tissue samples, comprising 26 tumor/surrounding tissue pairs, and 16 HCC, 9 cirrhotic and 8 non-cirrhotic unpaired samples, were analyzed. DNA extracted from cryopreserved or formalin-fixed tissues underwent mutation analysis through Sanger sequencing of PCR fragments.

Results: C250T frequency in HCC tissues was notably low (2.4%) and absent in cirrhotic and non-cirrhotic tissues. C228T was more prevalent in HCC (45.2%) and cirrhotic tissues (19%) than non-cirrhotic (0%) tissues, exhibiting a gradual increase with liver disease progression. Significantly different C228T frequencies were observed in HCC vs. non-cirrhotic ($P = 0.0001$) and cirrhotic vs. non-cirrhotic ($P = 0.0485$) tissues. Paired analysis demonstrated exclusive C228T presence in HCC in 46.2% of pairs. While C228T showed higher prevalence in HCCs linked to HCV infection (66.7%), statistical significance was not reached. Preliminary CTNNB1 analysis showed mutations occurred more frequently in HCC (23.8%) than cirrhotic (9.1%) and non-cirrhotic (0%) tissues, with concordance observed with C228T mutation in 66.7% of cases.

Conclusion: TERT promoter mutation C228T, and potentially CTNNB1 mutations, emerge as promising biomarkers for HCC in Brazilian patients, facilitating early detection, risk stratification, and offering potential therapeutic targets for this aggressive cancer.

Conflict of Interest: None declared

P01.039.C Importance of detecting mosaic variants in a case suggestive of Gardner's syndrome.

Jesús Cabanes Madrid[1], *Blanca Luis Sánchez*[2], **José Manuel Sánchez Zapardiel**[1], *Beatriz Hidalgo Calero*[1], *Montserrat de Miguel*[1], *Ricardo Blázquez Martín*[1], *Marina Ibáñez Vizcaíno*[1], *Francisca Luengo Sainz de Baranda*[1], *Victoria Carrero Blázquez*[1], *Luis Robles Díaz*[3]

[1]*Hereditary Cancer Unit, Hospital 12 de Octubre. Madrid, Department of Clinical Analysis-Clinical Biochemestry, Madrid, Spain;* [2]*Hospital Puerta del Mar, Cádiz, Department of Clinical Analysis-Clinical Biochemestry, Cádiz;* [3]*Familiar Cancer Unit, Hospital 12 de Octubre, Madrid, Department of Medical Oncology, Madrid, Spain*

Background/Objectives: A 19-year-old male was found to have 14 adenomatous polyps and was diagnosed with multiple osteomas in the left ethmoid sinus and frontal sinus. These findings pointed to Gardner's syndrome and the patient was referred to the Family Cancer Unit.

Methods: Next Generation sequencing (Illumina) was carried out using the Hereditary Cancer Solution kit (Sophia Genetics). The results were analyzed with Sophia-DDM and the variant allele frequency (VAF) detected were confirmed from different tissues by high depth NGS and Sanger sequencing.The segregation was carried out in both parents.

Results: The variant c.4537G > T p.(Glu1513*) in the *APC* gene are located on mutation cluster region (MCR) and generates a premature stop codon which causes a truncated protein. According to American College of Medical Genetics (ACMG) criteria it was classified as pathogenic. The variant has been identified by high depth NGS in blood and buccal swab at a VAF 10% an 9% respectively, and by sanger sequencing in different sections of colon biopsy at a VAF mean of 10-30%. The results obtained confirm the presence of this variant in mosaicism and "de novo" origin.

Conclusion: Unusual percentage of allele frequencies may be due to artefacts or false positives from sequencing errors. However, mosaic variants are also found in this range and it's important screening them, paying special attention to genes

related to the patient's phenotype. Otherwise, it would result in inadequate genetic counselling for the patient and their relatives.

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Conflict of Interest: None declared

P01.040.D Germline predisposition in paediatric melanoma patients undergoing matched tumor-normal sequencing

Alexandra Liebmann[1], *Jakob Admard*[1], *Hannah Wild*[2], *Michael Abele*[2], *Irina Bonzheim*[3], *Ewa Bien*[4], *Stephan Forchhammer*[5], *Dominik Schneider*[6], *Christopher Schroeder*[1], *Stephan Ossowski*[1], *Ines Brecht*[2]

[1]*University Hospital Tübingen, Institute of Medical Genetics and Applied Genomics, Tübingen, Germany;* [2]*University Children's Hospital Tübingen, Paediatric Hematology and Oncology, Tübingen, Germany;* [3]*University Hospital Tübingen, Institute of Pathology and Neuropathology, Tübingen, Germany;* [4]*Medical University of Gdansk, Poland, Department of Paediatrics, Hematology, Oncology, Gdansk, Poland;* [5]*University Hospital Tübingen, Center for Dermatooncology, Department of Dermatology, Tübingen, Germany;* [6]*Dortmund Municipal Hospital, Dortmund, Clinic of Paediatrics, Dortmund, Germany*

Background/Objectives: Genomic characterisation has led to an improved understanding of adult melanoma. However, the aetiology of melanoma in children is still unclear. The aim of this study was to further characterize paediatric melanoma and to identify putative causative germline alterations possibly relevant for prevention and surveillance.

Methods: The cohort of 26 paediatric melanoma patients was grouped into different categories: spitzoid melanoma (SM), conventional melanoma (CM) and others (OT). Tumor-normal exome sequencing was performed and analyzed using an in-house bioinformatics pipeline (megSAP). Germline variants were called with freebayes and annotated with VEP.

Results: No (likely) pathogenic variants were found in the high-risk melanoma genes *CDK4* and *CDKN2A*. In all patients with CM (n = 10) germline variants were found in low to moderate melanoma risk genes: 8 CM patients carried at least one *MC1R* variant, one patient carried the *MITF* E318K variant, another patient carried variants in *PTEN* (c.800del, p.Lys267Arg*9) and in *BRCA2* (c.9253dup, p.Thr3085Asn*26). In the SM group (n = 12), 41.7% (n = 5) patients carried at least one *MC1R* variant. In the OT (n = 4) group, 2 patients carried a *MC1R* variant. The *MC1R* variants detected were V92M (n = 6), R151C (n = 5), R160W (n = 4), V60L (n = 4), D84E (n = 1), R163Q (n = 1), K278E (n = 1).

Conclusion: Genetic risk factors may play a more important role in paediatric melanoma than previously anticipated. It can be envisioned to develop combinatorial risk models including genetic and environmental factors to identify individuals at risk and to establish prevention measures.

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Conflict of Interest: None declared

P01.041.A Validation of the BOADICEA model for epithelial tubo-ovarian cancer risk prediction in the UK Biobank

Xin Yang[1], *yujia wu*[1], *Lorenzo Ficorella*[1], *naomi wilcox*[1], *Joe Dennis*[1], *jonathan tyrer*[1], *Timothy Carver*[1], *nora pashayan*[1], *marc tischkowitz*[2], *paul pharoah*[3], *Douglas F. Easton*[1;4], *Antonis C. Antoniou*[1]

[1]*University of Cambridge, Department of Public Health and Primary Care, Cambridge, United Kingdom;* [2]*University of Cambridge, Department of Medical Genetics, Cambridge, United Kingdom;* [3]*Cedars-Sinai Medical Center, Department of Computational Biomedicine, Los Angeles, United States;* [4]*University of Cambridge, Department of Oncology, Cambridge, United Kingdom*

Background/Objectives: To assess the clinical validity of the multifactorial BOADICEA model for predicting 10-year epithelial tubo-ovarian cancer (EOC) risk in a large, independent prospective cohort.

Methods: We evaluated the model discrimination and calibration in the prospective UK Biobank cohort comprising 199,429 women (733 incident EOCs) of European ancestry without previous cancer history. We predicted 10-year EOC risk incorporating data on questionnaire-based risk factors (QRFs), family history, a 36-SNP polygenic risk score (PRS) and pathogenic variants (PV) in six EOC susceptibility genes (*BRCA1*, *BRCA2*, *RAD51C*, *RAD51D*, *BRIP1* and *PALB2*).

Results: Individually, QRFs, the PRS and PVs (each combined with age), gave similar discrimination (AUC~0.64-0.65). Discrimination was considerably greater using the multifactorial model that included the PRS, QRFs, FH and PV status (AUC = 0.68, 95%CI:0.66-0.70). This model was well calibrated in deciles of predicted risk with calibration slope = 0.99 (95%CI:0.98-1.01). The model had similar discriminative ability in women younger or older than 60 years. The AUC was higher when analyses were restricted to PV carriers (0.76, 95%CI:0.69-0.82); the model remained well calibrated in all risk groups. Using relative risk (RR) thresholds, the full model classified 97.7%, 1.7%, 0.4% and 0.2% women in the RR < 2.0, 2.0≤ RR < 2.9, 2.9 ≤ RR < 6.0 and RR ≥ 6.0 categories respectively, identifying 9.1% of incident EOC among those with RR ≥ 2.0.

Conclusion: BOADICEA, implemented in CanRisk (www.canrisk.org), provides a valid individualized 10-year EOC risks which can facilitate decision making in clinical EOC risk management.

Grants: Cancer Research UK (PPRPGM-Nov20\100002).

Conflict of Interest: None declared

P01.043.C Histopathological phenotyping of cancers in PTEN Hamartoma Tumor Syndrome for improved recognition

Ane Jordan Schei-Andersen[1], *Linda Hendricks*[1], *Rachel van der Post*[2], *Arjen Mensenkamp*[1], *Jolanda Schieving*[3], *Muriel A Adank*[4;5], *Floor Duijkers*[5;6], *Mirjam de Jong*[5;7], *Marjolijn Jongmans*[5;8], *Liselotte P van Hest*[5;9], *Yvette Van Ierland*[5;10], *Tjitske Kleefstra*[1;5], *Edward Leter*[5;11], *Maartje Nielsen*[5;12], *Janneke Schuurs-Hoeijmakers*[1], *Nicoline Hoogerbrugge*[1], *Janet Vos*[1]

[1]*Radboud University Medical Center, Department of Human Genetics, Nijmegen, Netherlands;* [2]*Radboud University Medical Center, Department of Pathology, Nijmegen, Netherlands;* [3]*Radboud University Medical Center, Department of Pediatric Neurology, Nijmegen, Netherlands;* [4]*The Netherlands Cancer Institute (NKI), Department of Clinical Genetics, Amsterdam, Netherlands;* [5]*PTEN Study Group, Netherlands;* [6]*Amsterdam UMC, locatie AMC, Department of Human Genetics, Amsterdam, Netherlands;* [7]*University Medical Center Groningen, Department of Genetics, Groningen, Netherlands;* [8]*UMC Utrecht, Princess Máxima Center for Pediatric Oncology and Department of Genetics, Utrecht, Netherlands;* [9]*Vrije Universiteit Amsterdam, Department of Clinical Genetics, Amsterdam, Netherlands;* [10]*Erasmus MC, Department of Clinical Genetics, Rotterdam, Netherlands;* [11]*Maastricht UMC+, Department of Clinical Genetics, Maastricht, Netherlands;* [12]*Leiden University Medical Center (LUMC), Department of Clinical Genetics, Leiden, Netherlands*

Consortium: PTEN Study Group

Background/Objectives: PTEN Hamartoma Tumor Syndrome (PHTS) has a broad clinical spectrum including benign and malignant tumors with varying age of onset. Many patients remain unrecognized, unaware of their increased cancer risk. We assessed the cancer spectrum and histopathological cancer characteristics to determine whether this could improve PHTS recognition.

Methods: Genetic testing results and pathology reports were collected for patients tested for germline *PTEN* variants between 1997-2020 from the diagnostic laboratory and the Dutch nationwide pathology databank (Palga). Cancer spectrum and age of onset were assessed in patients with (PTENpos) and without (PTENneg) a germline *PTEN* variant. Histopathological cancer characteristics were assessed in a nested cohort.

Results: 341 PTENpos patients (56% females) and 2,882 PTENneg patients (66% females) were included. PTENpos presented mostly with female breast (BC, 30%), endometrial (EC, 6%), thyroid (TC, 4%) or colorectal cancer (4%). Besides PHTS-related cancers, PTENpos had non-serous ovarian cancer (3%). PTENpos were significantly younger at cancer onset (43 vs. 47years) and developed more often (46% vs. 18%) a second BC. *PTEN* detection rates were highest for BC < 40 years (9%), TC < 20 years (15%) and EC < 50 years (28%). Histopathological characteristics were similar between groups.

Conclusion: No histopathological cancer characteristics were distinctive for PHTS. However, PTENpos were significantly younger at cancer onset. Therefore early-onset BC, EC or TC warrants consideration of PHTS diagnostics either by pre-screening for other PHTS features or direct germline testing.

Grants: This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement No955534

Conflict of Interest: None declared

P01.044.D Evaluation of the results of cytogenetic, molecular cytogenetic and molecular analysis in non-Hodgkin lymphoma

Can Berk Leblebici[1], *Nüket Yürür Kutlay*[1], *Arzu Vicdan*[1], *Sadiye Ekinci*[1], *Hatice Ilgın Ruhi*[1], *Timur Tuncalı*[1], *Halil Gürhan Karabulut*[1], *Şule Altıner*[1], *Ezgi Gökpınar İli*[1]

[1]*Ankara University Medical School, Medical Genetics, Turkiye*

Background/Objectives: Non-Hodgkin lymphomas (NHL) are the most common lymphoma type in adults. In this study, the results of cytogenetic, fluorescence in situ hybridization (FISH) and next generation sequencing (NGS) analysis of patients diagnosed with NHL is presented.

Methods: The results of genetic analysis performed on BM or PB samples of patients with NHL in our department between 2022-2023 were evaluated retrospectively.

Results: Sixty patients (28 women 32 men), their average age was 58, were included in the study. There were a total of 12 types of NHL and the most frequent one was diffuse large B-cell lymphoma. Loss-of-function changes in the *DNMT3A* gene in B-cell-derived lymphomas and gain-of-function changes in *STAT5B* and *STAT3* genes in T-cell-derived lymphomas were detected as the most common mutations.

The number of detected pathological findings by the method used is summarized in the table below:

Method	DLBCL[1]	MZL[2]	FL[3]	TCL[4]	MCL[5]	T-LGL[6]
NGS	8	7	5	3	4	2
Cytogenetics	4	1	0	2	2	0
FISH	0	1	1	1	1	0

[1]Diffuse large B-cell, [2]Marginal zone, [3]Follicular, [4]T-cell and [5]Mantle cell lymphoma. [6]T-cell large granular lymphocytic lymphoma/leukemia

Conclusion: Lymphoma pathogenesis is complex. Genetic test results make important contributions to the evaluation of parameters related to diagnosis, treatment and prognosis in cases with NHL. Pathological NGS findings may be related to the pathogenesis of lymphoma but the interpretation may be difficult. In this study, evaluation will be made regarding the genetic test results of NHL cases.

References:
PMID:35732829
PMID:37672206

Grants: No

Conflict of Interest: None declared

P01.045.A Utility of whole body MRI surveillance in children and adults with cancer predisposition syndromes

Frances Victoria Que[1], *Shao-Tzu Li*[1], *Jeanette Yuen*[1], *Tarryn Shaw*[1], *Hui Xuan Goh*[1], *Nur Diana Binte Ishak*[1], *Zewen Zhang*[1], *Jianbang Chiang*[1], *Lee Lian Chew*[2], *Choon Hua Thng*[2], *Joanne Ngeow*[1;3;4]

[1]*National Cancer Centre Singapore, Cancer Genetics Service, Division of Medical Oncology, Singapore, Singapore;* [2]*National Cancer Centre Singapore, Division of Oncologic Imaging, Singapore, Singapore;* [3]*Duke-NUS Medical School, Oncology Academic Clinical Program, Singapore, Singapore;* [4]*Lee Kong Chian School of Medicine, Nanyang Technological University, Singapore*

Background/Objectives: Surveillance improves outcomes by detecting cancer early in individuals with cancer predisposition syndromes (CPS). Whole body MRI (WB-MRI) provides head-to-thigh imaging in one sitting without radiation exposure and is recommended for individuals with CPS associated with increased risk of multi-focal tumors/cancers and/or susceptibility to radiation. This study evaluated the diagnostic performance of WB-MRI as a screening tool for Li-Fraumeni syndrome, Constitutional Mismatch Repair Deficiency syndrome, Hereditary Paraganglioma-Pheochromocytoma syndrome and other CPS.

Methods: A review of patients with CPS seen at the Cancer Genetics Service, National Cancer Center Singapore was conducted. Patients who underwent screening WB-MRI from 2014 to 2022 were identified to determine the sensitivity and specificity of WB-MRI in detecting cancer early.

Results: Of 103 patients with CPS who were indicated to have WB-MRI surveillance, 54 underwent the procedure (52% uptake rate): 28 (52%) were female, median age was 41 years (range 1-65 years); 21 (39%) had Li-Fraumeni syndrome, six (11%) had Hereditary Paraganglioma-Pheochromocytoma syndrome, and seven (13%) had Von Hippel Lindau syndrome. WB-MRI screening led to a diagnosis of cancer in seven (13%) patients (renal cell carcinoma, prostate adenocarcinoma, osteosarcoma, neuroendocrine tumor); four of these seven patients (57%) were treated with curative intent. Twelve (22%) patients required additional investigations, with eight (15%) having benign findings. The sensitivity and specificity of WB-MRI in our cohort was 64% and 81% respectively, with a false-positive rate of 19% and false-negative rate of 36%.

Conclusion: WB-MRI is an effective screening tool in patients with specific CPS, demonstrating a high specificity and low false positive rate.

Grants: none

Conflict of Interest: None declared

P01.046.B Targeted transcriptomics validates metastasis-relevant genes from case-case GWAS risk variants and functional annotation in sporadic colorectal carcinomas.

Peh Yean Cheah[1], *Kuen Kuen Lam*[1], *Lai Fun Thean*[1], *Michelle Su Mei Lo*[1], *Emile Tan*[1], *Choong Leong Tang*[1], *Iain Bee Huat Tan*[2]

[1]*Singapore General Hospital;* [2]*National Cancer Centre Singapore, Singapore*

Background/Objectives: Colorectal cancer (CRC) is the third highest incidence cancer and leading cause of cancer mortality worldwide. Metastasis to distal organ is the major cause of cancer mortality. Currently, the underlying genetic factors are unclear. This study aims to identify metastasis-relevant genes and pathways for better management of metastasis-prone patients.

Methods: A case-case genome-wide association study comprising 2677 sporadic Chinese CRC cases with clinically determined metastasis status (1282 metastasis-positive vs 1395 metastasis-negative) was performed using the Human SNP6 microarray platform and analyzed with the correlation/trend test based on the additive model. 647,545 single nucleotide polymorphism (SNP) variants with association testing -log10p-value ≥ 5 were imported into Functional Mapping and Annotation (FUMA) and Ingenuity (IPA) software for functional annotation and pathway analysis.

Results: About 196 candidate genes were identified for both overall and disease subtypes stratified by gender, tumor stage, site, and metastasis organs. Targeted high throughput transcriptomics was performed on 200 (100 metastasis-positive vs 100 metastasis-negative) left-sided stage III tumors using the Fluidigm platform. About a third of the candidate genes identified by the combined case-case GWAS and in silico analysis were validated; half of these are significant in the female subset only. This reaffirms that there is female-specific metastasis-relevant genes contributing to sexual dimorphism in CRC metastasis.

Conclusion: Combining genome-wide association testing with in silico functional annotation and wet-bench validation identified metastasis-relevant genes that could serve as features to develop subtype-specific metastasis-risk signatures for tailored management of CRC patients.

Grants:

Conflict of Interest: None declared

P01.047.C Exploring Germline Variations and Predisposition in Childhood Lymphoma

TUĞÇE SUDUTAN[1;2], *Khusan Khıdzhaev*[1;2], *Yucel Erbilgin*[2], *didem altındirek*[1;2], *sumeyye kocaağa*[1;2], *merve sarıtaş*[1;2], *süheyla ocak*[3], *ayça kıykım*[4], *deniz tuğcu*[5], *hikmet gülşah tanyıldız*[5], *rejin kebudi*[6], *tülin tiraje celkan*[7], **Muge Sayitoglu**[2]

[1]*Istanbul University Institute of Graduate Studies in Health Sciences, Istanbul, Türkyie;* [2]*Istanbul University Aziz Sancar Experimental Medicine Research Institute, Department of Genetics, Istanbul, Türkyie;* [3]*Istanbul University-Cerrahpasa, Cerrahpasa Medical Faculty, Department of Pediatric Hematology-Oncology,Department of Children's Health and Disease, Istanbul, Türkyie;* [4]*Istanbul University-Cerrahpasa, Cerrahpasa Medical Faculty, Department of Pediatric Allergy and Immunology, Department of Children's Health and Disease, Istanbul, Türkyie;* [5]*Istanbul University Istanbul Medical Faculty, Department of Pediatric Hematology-Oncology, Istanbul, Türkyie;* [6]*Istanbul University Oncology Institute, Department of Pediatric Hematology-Oncology, Istanbul, Türkyie;* [7]*İstinye University, İstinye Medical Faculty, Department of Pediatric Hematology-Oncology, Istanbul, Türkyie*

Background/Objectives: Approximately 10% of cancer patients harbour germline variations predisposing to cancer. Increased lymphoma development risk has been reported in several syndromes and patients with familial history. We aimed to investigate germline predisposition genes in paediatric lymphoma cases/families with at least one indication for genetic counselling.

Methods: Whole exome sequencing was performed in 22 paediatric lymphoma cases (Hodgkin's lymphoma (n = 13), diffuse large B-cell lymphoma (n = 3), Burkitt's lymphoma (n = 3), T-cell anaplastic lymphoma (n = 2), lymphoproliferation (n = 1)) with indications for genetic counselling. Segregation analyses were performed for candidate genes.

Results: Almost 60% of cases were associated with immunodeficiency without differential clinical diagnosis, and germline variations in genes related to primary immunodeficiency such as *CD70, RAG2, LRBA, NBN, ITK* and *TNFSF9*. In other lymphoma families, germline variations in inherited cancer susceptibility genes like *PRF1, BRIP1, SMAD4, KDR, ALK* have been observed. In addition to the major predisposition genes, each case was found to carry numerous heterozygous variants of germline cancer genes (*NOTCH1, FLT3, BCR, JAK2, CCDN1*,etc.). Two new candidate genes/variants thought to be responsible for germline predisposition to lymphoma have also been identified.

Conclusion: Implementation of next-generation sequencing has recently enabled discovering rare genetic variants predisposing paediatric patients to lymphoid neoplasms. It is valuable for identifying individuals at risk in families for early diagnosis, genetic counselling and follow-up. It is also important to evaluate patients for indications for genetic counselling in order not to miss rare hereditary cases in the clinic.

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Conflict of Interest: None declared

P01.048.D Barriers and facilitators to sustainment of the Tumor-First workflow for hereditary epithelial ovarian cancer detection

Vera M. Witjes[1], *Yvonne H.C.M. Smolders*[1], *Joanne A. De Hullu*[2], *Margreet G.E.M. Ausems*[3], *Marian J.E. Mourits*[4], *Marjolijn Ligtenberg*[1;5], *Nicoline Hoogerbrugge*[1], *Julie E.M. Swillens*[6]

[1]*Radboud university medical center, Department of Human Genetics, Nijmegen, Netherlands;* [2]*Radboud university medical center, Department of Obstetrics and Gynecology, Nijmegen, Netherlands;* [3]*University Medical Center Utrecht, Division Laboratories, Pharmacy and Biomedical Genetics, Department of Genetics, Utrecht, Netherlands;* [4]*University of Groningen, University Medical Center Groningen, Department of Obstetrics and Gynecology, Groningen, Netherlands;* [5]*Radboud university medical center, Department of Pathology, Nijmegen, Netherlands;* [6]*Radboud university medical center, Scientific Center for Quality of Healthcare (IQ Healthcare), Nijmegen, Netherlands*

Background/Objectives: Around 10-15% of women with epithelial ovarian cancer (OC) have a genetic predisposition, making genetic testing essential. To optimize detection of heredity, we have implemented the "Tumor-First" workflow nationwide. This workflow implies that OC tumor DNA is examined for presence of

pathogenic variants (e.g. in *BRCA1/2*) serving to stratify germline testing and to provide information on the effectiveness of PARP-inhibitors. Implementation was successful with the uptake of tumor DNA testing exceeding 80% and positive experiences. However, with the everchanging field of genetic testing, we aimed to determine the sustainability of the Tumor-First workflow.

Methods: We used a qualitative study design. 30 healthcare professionals from 7 medical disciplines were interviewed to discuss factors important for sustainment of the Tumor-First workflow. Barriers and facilitators of sustainment were analyzed using the framework of Flottorp.

Results: Main facilitators of sustainment were support, motivation, and awareness amongst healthcare professionals, presence of leading figures in the field, practical advantages of the workflow and (financial) embeddedness within the healthcare system. Main barriers were concerns about sustainment of nationwide uniformity considering ongoing developments in the field and the nearing end of project-related nationwide coordination. Without coordination, developments in tumor sequencing techniques and the rise of homologous-recombination-deficiency testing might lead to heterogeneity amongst laboratories in procedures and workflows.

Conclusion: As the field of genetic testing undergoes rapid developments, nationwide coordination is needed to sustain a uniform workflow. These results provide guidance on implementation and sustainment of innovations in the field of genetic testing.

Grants: Dutch Cancer Society (12732)

Conflict of Interest: None declared

P01.049.A Risk reducing surgery, cancer incidence and mortality in the Swedish BRCA cohort

Anna Rosén[1], Annika Idahl[2], Ylva Bengtsson[3;4], Åke Borg[3], Hans Ehrencrona[5;6], Linda Hartman[7], Per Karlsson[8], Ylva Karlsson[9], Ekaterina Kuchinskaya[10;11], Svetlana Bajlica Lagercrantz[12], Malin Sund[13], Anna von Wachenfeldt Väppling[14], Rebecka Wiberg[13], Anna Öfverholm[8], Barbro Numan Hellquist[1], **Niklas Loman**[3;4]

[1]Umeå University, Diagnostics and Intervention, Oncology, Umeå, Sweden; [2]Umeå University, Clinical Sciences, Obstretics and Gynecology, Umeå, Sweden; [3]Lund University, Division of Oncology, Department of Clinical Sciences, Lund, Sweden; [4]Skåne University Hospital, Dept of Hematology, Oncology and Radiation physics, Lund, Sweden; [5]Lund University, Laboratory Medicine, Clinical Genetics, Lund, Sweden; [6]Skåne University Hospital, Office of Medical services, Pathology and Molecular Diagnostics, Clinical genetics, Lund, Sweden; [7]Lund University, Centre of Mathematical Sciences, Lund, Sweden; [8]Sahlgrenska Academy, University of Gothenburg, Clinical sciences, Oncology, Gothenburg, Sweden; [9]Uppsala University Hospital, Immunology, Genetics and Pathology, Uppsala, Sweden; [10]Linköping University, Clinical Genetics, Linköping, Sweden; [11]Linköping University, Biomedical and Clinical Sciences, Linköping, Sweden; [12]Karolinska Institutet, Oncology-Pathology, Stockholm, Sweden; [13]Umeå University, Diagnostics and Intervention, Surgery, Umeå, Sweden; [14]Södersjukhuset, Breast Center, Stockholm, Sweden

Background/objectives: Women carrying germline pathogenic variants in *BRCA1* and *BRCA2* are offered surveillance, risk-reducing bilateral mastectomy (RRBM) and salpingoophorectomy (RRSO) to reduce cancer morbidity and mortality. The aim of this study is to evaluate cancer incidence and mortality in relation to risk-reducing surgery in a nationwide cohort of healthy *BRCA*-carriers.

Methods: The Swedish *BRCA*-cohort includes Swedish *BRCA*-carriers identified 1994-2018. Their data was linked with nationwide Cancer -, Patient-, and Cause of Death registers. Cancer-free women were followed from the date of testing, until death or end of follow-up (Aug 2022).

Results: The 837 women cumulated to 9329 person years, whereof 4972 before/never RRBM and 4357 after RRBM, 6102 before/never RRSO and 3227 after RRSO. Preliminary results indicate that at the end of follow-up, 74 (16%) developed breast cancer (median age 47, range 24-72), 16 (4%) ovarian cancer (median age 54, range 30-73), and 44 (median age 66, range 29-97) had died. In total, 386 carriers (46%) had undergone RRBM (median age 39, range 20-72) and 400 (48%) RRSO (median age 44, range 21-77).

Conclusions: This study will summarize cancer incidence and mortality and the effect of risk reducing interventions, on a national level in Sweden during the first 20 years of clinical *BRCA1* and *BRCA2* -testing. Updated data on age-dependent cancer incidence as well as the effect of risk reducing surgery on mortality will be presented.

Funding: The Swedish Breast Cancer Association

Conflict of Interest: Anna Rosén: None declared, Annika Idahl: None declared, Ylva Bengtsson: None declared, Åke Borg: None declared, Hans Ehrencrona: None declared, Linda Hartman: None declared, Per Karlsson Coinventor Prelude DX and Exact Sciences (not related to the current study), Astrazeneca, Novartis, Seagen, Ylva Karlsson: None declared, Ekaterina Kuchinskaya: None declared, Svetlana Bajlica Lagercrantz: None declared, Malin Sund: None declared, Anna von Wachenfeldt Väppling: None declared, Rebecka Wiberg: None declared, Anna Öfverholm Lecture for oncology health care professionals on request from Pfizer and Astra, Barbro Numan Hellquist: None declared, Niklas Loman: None declared

P01.050.B Expression analysis of ultraconserved regions in childhood acute lymphoblastic leukemia

Maria Augusta Poersch[1], Gabriela Canalli Kretzschmar[2], Saloe Bispo[2], Daniela Fiori Gradia[1], Roberto Rosati[2], **Jaqueline Carvalho de Oliveira**[1]

[1]Federal University of Parana, Genetics, Curitiba - PR, Brazil; [2]Pelé Pequeno Príncipe Research Institute, Curitiba - PR, Brazil

Background/Objectives: The ultraconserved regions (UCRs) are genomic elements that exhibit complete sequence conservation across human, rat, and mouse genomes and are implicated in various cancers. However, their role in childhood acute lymphoblastic leukemia (ALL) remains underexplored. The present study aims to identify differentially expressed transcripts associated with UCRs across ALL subtypes.

Methods: Using RNA sequencing data from St. Jude Research Hospital (804 subjects aged 0-15), differential expression analysis was conducted with DESeq2, including 240 annotated transcripts. Comparisons spanned T-ALL versus B-ALL, high-risk versus low-risk subtypes, and distinct expression profiles of hypodiploid, high hyperdiploid, and recurrent genetic abnormalities: ETV6-RUNX1, rearrangement of KMT2A, iAMP21, BCR-ABL1, and TCF3-PBX.

Results: The differential expression analyses revealed 40 significantly deregulated transcripts, with the T-ALL vs. B-ALL comparison displaying the highest diversity (16 transcripts). Noteworthy oncogenes, including ZNF521, EBF1, EBF3, PBX1, and EPHA7, exhibit subtype-specific expression patterns. ZNF521 is more expressed in high-risk ALL, while EBF1 was found to be overexpressed in B-ALL. PBX1 shows increased expression in the TCF3-PBX1 subtype, and EPHA7 demonstrates higher expression in B vs. T ALL. Furthermore, regulatory genes such as HOXA2, HOXA3, and HOXA5 were found to be downregulated in BCR-ABL1 positive patients and upregulated in the KMT2A-rearrangement.

Conclusion: This comprehensive analysis identifies UCR-related transcripts deregulated in ALL subtypes, including critical oncogenes. In conclusion, our study sheds light on the crucial role of T-UCR-associated transcripts in childhood ALL, improving the understanding of the disease and offering valuable insights for personalized treatment approaches in the future.

Grants: CNPq, CAPES, Fundação Araucária, PRPPG-UFPR.

Conflict of Interest: None declared

P01.051.C Enhanced cytogenomic analysis of complex karyotype in myelodysplastic syndrome using optical genome mapping

Andriana Valkama[1], *Sandra Vorimo*[1], *Anna Tervasmäki*[1], *Hannele Räsänen*[2], *Eeva-Riitta Savolainen*[2], *Katri Pylkäs*[1;2], *Tuomo Mantere*[1]

[1]*Laboratory of Cancer Genetics and Tumor Biology, Translational Medicine Research Unit, Medical Research Center Oulu and Biocenter Oulu, University of Oulu, Oulu, Finland;* [2]*Northern Finland Laboratory Centre Nordlab, Oulu, Finland*

Background/Objectives: Myelodysplastic syndrome with complex karyotype (CK-MDS) is a clinically challenging disease. In the clinical workup of MDS, cytogenetic analyses detecting structural variants (SVs) are crucial for prognostication and guiding treatment decisions. However, the current standard of care (SOC) testing, relying on karyotyping, often yields ambiguous results in cases with a complex karyotype.

Methods: We evaluated the SV detection by optical genome mapping (OGM) for 15 CK-MDS cases compared with SOC and explored the use of OGM as a tool for novel discoveries in combination with exome and transcriptome sequencing.

Results: Abnormalities were reported in 73 chromosomes within the cohort using karyotyping. The concordance with OGM, defined as the presence of large SVs in the same chromosomes, was 89% (65/73). The concordance between OGM and FISH was 100% (20/20). Importantly, OGM provided additional important information for all the analyzed CK-MDS cases. This included the detection of SVs >5Mb in chromosomes that were not specified by karyotyping to harbor abnormalities (93% of cases) and smaller SVs overlapping with leukemia-associated genes (53% of cases). Our analysis also confirmed the presence of three in-frame gene-fusions, including *NUP98::PRRX2*, and two novel fusions.

Conclusion: This study supports the application of OGM for CK-MDS cases to enhance SV detection and acquiring gene-level information. OGM could be effective as either a first-tier test or in combination with karyotyping. Furthermore, our results underscore the utility of OGM as a research tool in cancer samples with complex genomic rearrangements.

Grants: Sigrid Juselius Foundation [220111], Academy of Finland [338374].

Conflict of Interest: None declared

P01.052.D Developing the UK infrastructure for the identification and management of patients with germline predisposition to haematological malignancy.

Olga Tsoulaki[1], *Beverley Speight*[2], *James Drummond*[2], *Clair Engelbrecht*[3], *Jude Fitzgibbon*[4], *Shreyans Gandhi*[3], *Angela Hamblin*[5], *Helen Hanson*[6;7], *Steven Hardy*[8], *Jamshid Khorashad*[9], *Austin Kulasekararaj*[3], *Joanna Large*[3], *Paula Page*[10], *Nicholas Parkin*[3], *Ana Rio-Machin*[4], *Polly Talley*[11], *Kate Tatton-Brown*[12], *Roochi Trikha*[3], *beth torr*[13], *Clare Turnbull*[13], *Sarah Westbury*[14], *Christopher Wragg*[15], *Terri McVeigh*[9], **Katie Snape**[1]

[1]*St George's University of London, London, United Kingdom;* [2]*Addenbrooke's Hospital, East Anglian Medical Genetics Service, Cambridge, United Kingdom;* [3]*Kings College Hospital NHS Foundation Trust, Dept of Haematology, London;* [4]*Barts Cancer Institute, Queen Mary University, Centre for Haemato-Oncology, London;* [5]*Oxford University Hospitals NHS Foundation Trust, Dept of Haematology, Oxford;* [6]; [7]*Royal Devon University Healthcare NHS Foundation Trust, Department of Clinical Genetics, Exeter, United Kingdom;* [8]*Genomics and Rare Disease, National Disease Registration Service, Leeds;* [9]*Royal Marsden NHS Foundation Trust, Centre for Molecular Pathology, London, United Kingdom;* [10]*Birmingham Women's and Children's NHS Foundation Trust, West Midlands Regional genetics Laboratory, Birmingham, United Kingdom;* [11]*St James's University Hospital, North East and Yorkshire Genomic Laboratory Hub, United Kingdom;* [12]*NHS England, National Genomics Education, Birmingham;* [13]*Institute of Cancer Research, Division of Genetics and Epidemiology, London;* [14]*University of Bristol, School of Cellular and Molecular Medicine, Bristol, United Kingdom;* [15]*North Bristol NHS Trust, Bristol Genetics Laboratory, Bristol*

Background/Objectives: Use of genomic technologies in the investigation of haematological malignancies has led to an increase in the identification of disease associated genetic variants which may also confer inherited susceptibility. We report a collaborative approach supported by the CanGene-CanVar research programme and UK Cancer Genetics Group to develop a clinical and educational infrastructure to improve clinical pathways for the assessment and management of germline predisposition to haematological malignancies across the UK National Health Service (NHS).

Methods:

1. An online National consensus group meeting was convened with stakeholders across clincial and laboratory services alongside policy makers from NHS England. Stakeholders completed pre-meeting questionnaires, followed by in-meeting polling on consensus statements. Consensus was deemed to be reached when ≥80% respondents selected 'Agree/Strongly Agree' or 'Yes' in response to the statement posed.
2. An online survey based gap and training needs analysis (TNA) was undertaken across the relevant healthcare workforce to identify and analyse related training needs mapped to clinical pathways.

Results: 146 stakeholders registered for participation in the consensus meeting. Consensus was reached on 28 statements across the whole clinical-laboratory pathway. 272 healthcare workers participated in the TNA. This showed significant variation across the workforce in knowledge and skills required to deliver these clinical pathways.

Conclusion: A national clinical/scientific collaboration enabled the publication of consensus guidelines. Identification of training needs highlighted a requirement for educational resources. We present updates on the UK national educational and clinical infrastructure to iteratively improve clinical care pathways for patients with germline predisposition to haematological malignancies.

Grants: CanGene-CanVar (C61296/A27223)

Conflict of Interest: None declared

P01.053.A Identifying genetic vulnerabilities and trascriptional programs in TERT-promoter-mutant glioblastoma

Kevin Tu[1;2], *Connor Stewart*[2;3], *Peter Hendrickson*[2], *Joshua Regal*[2;4], *So Young Kim*[2], *David Ashley*[2], *Matthew Waitkus*[2], *Zachary Reitman*[2]

[1]*Cancer Research UK Cambridge Institute, United Kingdom;* [2]*Duke University, Durham, United States;* [3]*Emory University, Atlanta, United States;* [4]*Roswell Park Comprehensive Cancer Center, Buffalo, United States*

Background/Objectives: Mutations in the promoter of the telomerase reverse transcriptase (TERT) gene are early somatic mutations that occur in >80% of glioblastomas (GBMs). TERT promoter mutations (TPMs) create a neomorphic E-twenty-six (ETS) transcription factor binding site which reactivates TERT expression and drives GBM immortality. Directly targeting TERT and transcription factors has historically proven challenging. Here, we seek to identify potential targets upstream of TPMs using pooled genetic screens.

Methods: We generated T98G-TERT-ON, a TPM + GBM cell line with inducible TERT expression. We performed a minature pooled CRISPR/Cas9 screen in T98G-TERT-ON against TERT and GABPB1L, a critical ETS subunit that binds to TPM+ promoters. We annotated TPM status for >400 cell lines and 500 primary GBMs, then conducted comparative analyses between TPM status using genetic dependency data and/or gene expression profiles.

Results: We did not detect a dependency for TERT or GABPB1L in the CRISPR/Cas9 screen in T98G-TERT-ON. Similarly, TERT was not a dependency in TPM + GBM cell lines within the computational cohort. The ETS subunits GABPB1 and GABPA were dependencies within both TPM+ and TPM- cell lines. TPM+ cell line expression profiles were regulated by multiple ETS transcription factors. Moreover, in primary GBMs, TERT expression was strongly associated with the expression of ETS transcription factors ELF5, ETV1, ETV5, ERF, ETV4, ETV2, ETS2, GABPB1, SPI1.

Conclusion: Our findings suggest multiple ETS-factor regulate TERT interchangeably and/or redundantly in a cellular context-dependent manner, posing challenges in targeting TPMs through upstream regulators. Approaches to target other aspects of TPM biology merit attention.

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Conflict of Interest: Kevin Tu: None declared, Connor Stewart: None declared, Peter Hendrickson: None declared, Joshua Regal: None declared, So Young Kim: None declared, David Ashley: None declared, Matthew Waitkus: None declared, Zachary Reitman ZJR is listed as an inventor for intellectual property related to genetic testing for TERT and other alterations in brain tumors that is managed by Duke Office of Licensing and Ventures and has been licensed to Genetron Health.

P01.054.B First case of congenital diffuse colonic hamartomatous polyposis, genetically confirmed as Peutz-Jeghers syndrome

Didac Casas-Alba[1], *Diana Salinas-Chaparro*[1], *Antonio Federico Martínez-Monseny*[1], *Loreto Martorell Sampol*[1], *Jordi Genoves*[1], *Daniel Castillo*[1], *Laura Martí*[1], *Adrián Alcalá San Martín*[1], *Cristina Hernando-Davalillo*[1], *Moira Garraus Oneca*[2], *Juan Pablo Muñoz*[2], *Victor Vila Miravet*[3], *Silvia Planas Roman*[4], *Miguel Bejarano Serrano*[5], *Pedro Palazon Bellver*[5], *Ruth del Rio Florentino*[6], *Lluisa Hernandez Platero*[7], *Sara Bobillo Perez*[7], *Marina Pons Espinal*[8], *Aina Martinez Planas*[8], *Francesc Palau*[1], *Hector Salvador*[2]

[1]*Sant Joan de Déu Barcelona Hospital, Genetics Department, Esplugues de Llobregat, Spain;* [2]*Sant Joan de Déu Barcelona Hospital, Department of Oncology and Hematology, Esplugues de Llobregat, Spain;* [3]*Sant Joan de Déu Barcelona Hospital, Pediatric Gastroenterology Hepatology and Nutrition Unit, Esplugues de Llobregat, Spain;* [4]*Sant Joan de Déu Barcelona Hospital, Department of Pathology, Esplugues de Llobregat, Spain;* [5]*Sant Joan de Déu Barcelona Hospital, Department of Pediatric Surgery, Esplugues de Llobregat, Spain;* [6]*Sant Joan de Déu Barcelona Hospital, Neonatal Unit, Esplugues de Llobregat, Spain;* [7]*Sant Joan de Déu Barcelona Hospital, Pediatric Intensive Care Unit Service, Esplugues de Llobregat, Spain;* [8]*Sant Joan de Déu Barcelona Hospital, Paediatrics Department, Esplugues de Llobregat, Spain*

Background: Pathogenic variants in the *STK11* gene cause Peutz-Jeghers syndrome (PJS) (OMIM#175200). PJS-type hamartomatous polyps have been reported in only three newborns without genetic confirmation, with the youngest confirmed case documented at 6 months. Infant cases involved either the small bowel or stomach.

Methods: We present a 5-month-old male, second child of non-consanguineous parents, with an unremarkable familial history. Prenatal ultrasounds revealed an arachnoidal cyst and polyhydramnios. At birth, he presented marked abdominal distension. An abdominal ultrasound revealed diffuse thickening of the colon, a hepatic arteriovenous malformation, and multiple hepatic lesions suggesting hemangiomas. No mucocutaneous pigmentation was observed. After inconclusive studies, the condition worsened with subocclusion episodes, leading to subtotal colectomy at 2 months. Pathological study revealed diffuse colon involvement with hamartomatous polyposis.

Results: Clinical exome sequencing identified two heterozygous variants in *STK11*. First, an exon 1 deletion, further confirmed by MLPA, spanning at least 1300 bp and including the promoter region and start codon. This deletion, previously reported in PJS, was classified as pathogenic following ACMG criteria. Second, a missense variant of unknown significance (NM_000455.5:c.525G > C/p.Lys175Asn). Digital PCR and direct sequencing validated the patient's results and showed that the variants were absent in both parents. Microsatellite marker genotyping further supported declared paternity and maternity. Long-range PCR revealed a cis configuration. Array-CGH was normal.

Conclusion: This is the first case of congenital presentation of diffuse colonic hamartomatous polyposis, which was genetically confirmed as PJS. The codominant trans configuration hypothesis was ruled out, leaving other explanations for early onset severity under consideration.

Conflict of Interest: None declared

P01.055.C Genetic and Epigenetic characterization of B-cell neoplasms with *IG* enhancer hijacking

Cosima Drewes[1], *Cristina López*[2], *Nnamdi Okeke*[1], *Sina Hillebrecht*[1], *Petra Schuetz*[1], *Anja Fischer*[1], *Anja Mottok*[1], *Susanne Bens*[1], *Christof Schneider*[3], *Stephan Stilgenbauer*[3], *Eugen Tausch*[3], *Reiner Siebert*[1]

[1]*Institute of Human Genetics, Ulm University and Ulm University Medical Center, Ulm, Germany;* [2]*Institut d'Investigaciones Biomèdiques August Pi i Sunyer (IDIBAPS), Barcelona, Spain;* [3]*Division of CLL, Department of Internal Medicine III, Ulm University Medical Center, Ulm, Germany*

Background/Objectives: Oncogene translocations leading to enhancer hijacking from one of the immunoglobulin (*IG*) loci, such as *IGH::MYC*, *IGH::BCL2*, or *IGH::CCND1*, are pathognomonic aberrations of several lymphoma entities. In Chronic Lymphocytic Leukemia (CLL), e.g. *BCL2* and *BCL3* are oncogenes recurrently targeted by *IG* translocations. We here aimed to comprehensively characterize CLL with *IG* translocations at the genetic and epigenetic level.

Methods: B-cell neoplasms with *IG* translocations and respective partner oncogenes as well as recurrent imbalances were

identified by FISH (n = 325). DNA methylation and CNV analyses were performed using Infinium® MethylationEPIC (EPIC) BeadChip arrays (n = 313). The IGHV mutation status was determined by Sanger Sequencing (n = 284).

Results: We investigated 313 B-cell neoplasms, including 305 CLLs, with *IG* translocation affecting various partners. (*IGH*::*BCL2*: n = 43, *IG*::*BCL3*: n = 89, *IG*::*MYC*: n = 41, *IGH*::unknown partner: n = 140). From the *IGH*::*BCL2*-translocated CLLs, 87% (33/38) carried a mutated IGHV, 37% (16/43) trisomy 12 and 35% (15/43) 13q deletion. In contrast, *IG*::*BCL3*-translocated CLLs exhibited in 96% (72/75) unmutated IGHV and in 59% (50/84) trisomy 12, whereas 13q deletion was rare (12%, 11/84). In unsupervised DNA methylation analyses, IGHV mutation status segregated subgroups of CLLs with best discrimination applying a threshold of ≥99% for unmutated IGHV. Moreover, despite confounding by the IGHV mutation status, also *IGH*::*BCL2* and *IG*::*BCL3*-translocated CLLs segregated between them and also from other CLLs. *IGH*::*MYC*-translocated CLL and *IGH*::unknown partner CLL built no specific groups.

Conclusions: Our genetic and epigenetic analyses suggest that *IGH*::*BCL2* and *IG*::*BCL3*-translocated CLLs form two distinct subgroups of CLL based on DNA methylation, IGHV mutation status, and CNVs.

Grants: SFB1074 B10

Conflict of Interest: None declared

P01.056.D Exome sequencing identified rare recurrent copy number variants and hereditary breast cancer susceptibility

Tuomo Mantere[1], *Timo Kumpula*[1], *Sandra Vorimo*[1], *Taneli Mattila*[2], *Luke O'Gorman*[3], *Galuh Astuti*[3], *Anna Tervasmäki*[1], *Susanna Koivuluoma*[1], *Tiina Mattila*[1], *Mervi Grip*[4], *Robert Winqvist*[1], *Outi Kuismin*[5], *Jukka Moilanen*[5], *Alexander Hoischen*[3], *Christian Gilissen*[3], *Katri Pylkäs*[1]

[1]*Laboratory of Cancer Genetics and Tumor Biology, Research Unit of Translational Medicine and Biocenter Oulu, University of Oulu, Oulu, Finland;* [2]*Department of Pathology, Oulu University Hospital and University of Oulu, Oulu, Finland;* [3]*Department of Human Genetics and Radboud Institute of Medical Life Sciences, Radboud University Medical Center, Nijmegen, Netherlands;* [4]*Department of Surgery, Oulu University Hospital and University of Oulu, Oulu, Finland;* [5]*Department of Clinical Genetics, Medical Research Center Oulu and PEDEGO Research Unit, Oulu University Hospital and University of Oulu, Oulu, Finland*

Background/Objectives: Copy number variants (CNVs) are a major source of genetic variation and can disrupt genes or affect gene dosage. They are known to cause or contribute to the predisposition to various diseases. However, the role of CNVs in inherited breast cancer (BC) susceptibility has not been thoroughly investigated.

Methods: We performed a whole-exome sequencing-based analysis of rare CNVs in 98 high-risk Northern Finnish BC cases. After filtering, selected candidate alleles were validated and characterized using a combination of orthogonal methods, including optical genome mapping and long-read sequencing. The variants were genotyped in geographically matched cases and controls, comprising 278 hereditary and 1983 unselected BC cases, as well as 1229 controls.

Results: Analysis revealed three recurrent alterations: a 31 kb deletion co-occurring with a retrotransposon insertion (delins) in *RAD52*, a 13.4 kb deletion in *HSD17B14* and a 64 kb partial duplication of *RAD51C*. The *RAD52* delins and *HSD17B14* deletion both showed significant enrichment among cases with indications of hereditary disease susceptibility. *RAD52* delins was identified in 7/278 cases (P = 0.034, OR = 2.86) and *HSD17B14* deletion in 8/278 cases (P = 0.014, OR = 3.28), the frequency of both variants in the controls being 11/1229. The *RAD51C* duplication was identified in 2/278 of hereditary cases and 2/1229 controls (P = 0.157, OR = 4.45).

Conclusion: This study suggests a role for *RAD52* and *HSD17B14* in hereditary BC susceptibility, highlighting the importance of studying CNVs alongside single nucleotide variants in the search for genetic factors underlying hereditary disease predisposition.

Grants: Academy of Finland [307808], Cancer Foundation of Finland and Sigrid Jusélius foundation.

Conflict of Interest: None declared

P01.057.A Identification of genetic tumor risk syndromes in individuals with non-small cell lung cancer within the German National Network Genomic Medicine Lung Cancer (nNGM)

Rebecca Grüneis[1;2;3;4;5;6], *Marie Arlt*[1;2;3;4;5;6], *Oliver Kutz*[1;2;3;4;5;6], *Judith Böthig*[1;2;3;4;5;6], *Sarah Wölffling*[1;2;3;4;5;6], *Tim Hutschenreiter*[1;2;3;4;5;6], *Karl Hackmann*[1;2;3;4;5;6], *Doreen William*[1;2;3;4;5;6], *André Reis*[7], *Arndt Hartmann*[8;9], *Stefan Gattenlöhner*[10], *Axel Hillmer*[11;12;13], *Arne Jahn*[1;2;3;4;5;6], *Reinhard Büttner*[11;12;14], *Evelin Schröck*[1;2;3;4;5;6]

[1]*Institute for Clinical Genetics, University Hospital Carl Gustav Carus at TUD Dresden University of Technology and Faculty of Medicine of TUD Dresden University of Technology, Dresden, Germany;* [2]*ERN GENTURIS, Hereditary Cancer Syndrome Center Dresden, Germany;* [3]*National Center for Tumor Diseases (NCT), NCT/UCC Dresden, a partnership between German Cancer Research Center (DKFZ), Faculty of Medicine and University Hospital Carl Gustav Carus, TUD Dresden University of Technology and Helmholtz-Zentrum Dresden-Rossendorf (HZDR), Germany;* [4]*German Cancer Consortium (DKTK), Dresden, Germany;* [5]*German Cancer Research Center (DKFZ), Heidelberg, Germany;* [6]*Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany;* [7]*Institute of Human Genetics, Friedrich-Alexander University Erlangen-Nürnberg, Erlangen, Germany;* [8]*Institute of Pathology, Friedrich-Alexander University Erlangen-Nürnberg, Erlangen, Germany;* [9]*Comprehensive Cancer Center Erlangen-EMN (CCC ER-EMN), Universitätsklinikum Erlangen, FAU Erlangen-Nürnberg, Erlangen, Germany;* [10]*Institute for Pathology, Justus Liebig University, Gießen, Germany;* [11]*Institute of Pathology, University Hospital and Medical Faculty, University of Cologne, Cologne, Germany;* [12]*Network Genomic Medicine, Cologne, Germany;* [13]*Center for Molecular Medicine Cologne, University of Cologne, Cologne, Germany;* [14]*Lung Cancer Group Cologne, Department I for Internal Medicine, University Hospital Cologne, Cologne, Germany*

Background/Objectives: Very recent studies reported pathogenic germline variants in up to 10% of non-small cell lung cancer (NSCLC) patients. Possible implications are (experimental) targeted therapy or interventions, adjusted cancer surveillance, and predictive testing for family members. The "national Network Genomic Medicine (nNGM) Lung Cancer" offers multicentric somatic gene panel testing for patients with advanced NSCLC throughout Germany. Within this network the Task Force 5 "Genetic Tumor Risk" investigates pathogenic germline variants and further oncogenic somatic alterations by parallel whole-exome/ whole-genome sequencing.

Methods: Inclusion criteria favor never-smokers, young age of onset, multiple cancer diseases and a family cancer history. Whole-genome sequencing of control samples (University Hospital Dresden) and whole-exome sequencing of formalin-fixed paraffin-embedded lung cancer tissues (University Hospital Cologne) were performed. Germline variants were assessed in 141 cancer predisposition genes and somatic variants were evaluated to identify the relevance of germline variants for tumorigenesis and

alternative drivers. Clinical information and follow-up information were collected.

Results: 103 individuals have been included. Median age of onset was 57.5 years (IQR: 47.8;67.5), 30% (n = 31) were never-smokers, 43% (n = 44) had multiple tumor diseases, and 73% (n = 75) a positive family cancer history. Prospective germline sequencing of 72 individuals revealed 10 pathogenic heterozygous variants in two dominant (*BRCA2*, *RAD51D*) and six recessive cancer predisposition genes.

Conclusion: This ongoing prospective, multicentric study identifies pathogenic germline variants in selected NSCLC patients and investigates associations with clinical and molecular parameters. An increased cohort size and extended bioinformatic analysis will allow assessment of pathogenic germline variant yields and their clinical implications for NSCLC patients.

Grants: DKH 13006795

Conflict of Interest: None declared

P01.058.B Whole exome sequencing revealed GATA2 rare mutations in Bulgarian patients with personal/family history of breast cancer

Svilen Maslyankov[1], *Kalina Belemezova*[2], *Tsvetan Popov*[2;3], *Dimitar Bakalov*[2], *Radka Tafradzhyiska*[2], *Irina Miteva*[3], *Ivanka Dimova*[2]

[1]*Second surgery clinic, Department of Surgery, Medical Faculty, Medical University Sofia, Sofia, Bulgaria;* [2]*Medical University Sofia;* [3]*Second surgery clinic, Department of Surgery, Medical Faculty, Medical University Sofia*

Background/Objectives: The significance of GATA2 mutations in cancer risk is an area of ongoing research, and our understanding of the role of this gene in cancer continues to evolve. While GATA2 mutations are associated with an increased risk of certain hematological malignancies, they are not as commonly implicated in solid tumors, such as breast or ovarian cancers. We aimed to characterize the GATA2 genetic variants, revealed in BRCA1/2 negative Bulgarian women with strong indications for familial breast cancer.

Methods: After DNA isolation from peripheral venous blood, whole exome sequencing was performed using Illumina technology.

Results: In two women rare genetic variants in GATA2 gene were discovered. The variant c.829A > G (p.Ser277Gly) was revealed in a woman with strong family history for breast cancer. The variant c.669G > A (p.Met223Ile) was found in 42-years old woman affected by bilateral invasive breast cancer from non-specific type (NST), which was Her2-positive; the woman had grand-mother and aunt affected by gynecological cancer. Both variants have been reported in patients with leukemia syndromes.

Conclusion: A comprehensive evaluation of the individual's personal and family history is essential in determining the most appropriate genetic testing strategy. The landscape of genetic testing for hereditary cancer is continually evolving, and new genes associated with breast cancer risk may be identified over time. GATA2 is a key transcriptional regulator of hematopoiesis involved in the development of monocytes, mast cells, NK cells, and megakaryocytes. Its deficiency could be predisposing factor to breast cancer development as well.

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Conflict of Interest: None declared

P01.059.C Comprehensive splicing analysis of the alternatively spliced *CHEK2* exons 8 and 10 by hybrid minigenes

Lara Sanoguera-Miralles[1], *Inés Llinares-Burguet*[1], *Elena Bueno-Martínez*[1], *Lobna Ramadane-Morchadi*[2], *Cristiana Stuani*[3], *Alberto Valenzuela-Palomo*[1], *Alicia García-Álvarez*[1], *Pedro Pérez-Segura*[2], *Emanuele Buratti*[3], *Miguel de la Hoya*[2], *Eladio A. Velasco-Sampedro*[1]

[1]*Instituto de Biomedicina y Genética Molecular de Valladolid (IBGM), Consejo Superior de Investigaciones Científicas – Universidad de Valladolid (CSIC-UVa), Splicing and Genetic Susceptibility to Cancer Laboratory, Valladolid, Spain;* [2]*Instituto de Investigación Sanitaria del Hospital Clínico San Carlos (IdISSC), Molecular Oncology Laboratory, Madrid, Spain;* [3]*International Centre of Genetic Engineering and Biotechnology (ICGEB), Molecular Pathology Laboratory, Trieste, Italy*

Background/Objectives: Exon recognition is controlled by a large set of splicing regulatory elements (SREs), such as splicing enhancers and silencers. Variants at these motifs may impair splicing and lead to disease-associated loss-of-function transcripts. Our aim was to investigate the alternatively spliced exons 8 and 10 of the breast cancer susceptibility gene *CHEK2* with a view to identifying spliceogenic SRE variants.

Methods: We used a *CHEK2* minigene with exons 6–10 that produced the expected minigene full-length transcript and replicated naturally occurring events, in particular the skipping of exons 8 and 10. By site-directed mutagenesis, 12 internal microdeletions were incorporated in order to map SRE-rich intervals of both exons by splicing assays in MCF-7 cells. Then, 87 variants located in critical SRE intervals were introduced into the wild-type minigene and functionally tested.

Results: We identified three minimal regions (10-, 11- and 15-nt) essential for exon recognition: c.863_877del [ex8, Δ(E8): 75%] and c.1073_1083del and c.1083_1092del [ex10, Δ(E10): 97% and 62%, respectively]. Of the 87 variants tested in these intervals, thirty-eight (44%) impaired splicing, four of which (c.883G > A, c.883G > T, c.884A > T, and c.1080G>T) induced negligible amounts (<5%) of the minigene full-length transcript.

Conclusion: Any exonic change is capable of disrupting splicing, since thirty-three out of the 38 spliceogenic variants were annotated as missense, three as nonsense and two as synonymous. Moreover, c.883G>A, c.883G>T and c.884A>T were classified as pathogenic/likely pathogenic variants according to ACMG/AMP-based criteria.

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Conflict of Interest: None declared

P01.060.D Molecular cytogenetic characterization of Polyploid Giant Cancer Cells (PGCCs) in cytarabine-resistant AML cells

Pei-Yi Chen[1;2], *Yu-Xuan Lin*[2], *Chia-Ling Wu*[1], *Jui-Hung Yen*[2]

[1]*Laboratory of Medical Genetics, Genetic counseling center, Hualien Tzu Chi Hospital, Hualien, Taiwan;* [2]*Department of Molecular Biology and Human Genetics, Tzu Chi University, Hualien, Taiwan*

Background/Objectives: Polyploid giant cancer cells (PGCCs) are cells with multiple nuclei or a single giant nucleus containing multiple sets of chromosomes. These cells exhibit cancer stem cell characteristics, regulate the cancer microenvironment, and are highly associated with drug resistance, metastasis, and cancer relapse. While PGCCs have been extensively studied in solid tumors, their role in liquid tumors remains unclear. We generated two AML PGCC cell lines derived from MV4-11 and MOLM13 cells through prolonged exposure to cytarabine. This study aims to

further investigate the cytogenetic characteristics of these AML PGCCs.

Methods: Cell viability was assessed using the MTT assay. Cell morphology was observed through cytospin/Giemsa staining and immunofluorescence analysis. The chromosomal polyploidy level was measured using karyotyping, FISH, and flow cytometry. Molecular characteristics of the PGCCs were assessed using microarray analysis, RT-q-PCR, and western blot analysis.

Results: Drug resistance was confirmed by a pronounced increase in cell viability in the derived cells after cytarabine treatment. Immunofluorescence staining revealed that a portion of the resistant cells displayed multiple nuclei and giant cell size, resembling the so-called PGCCs. Interphase FISH was conducted to demonstrate a significant increase in the polyploid cell population detected in these PGCC cell lines. Microarray analysis showed differential gene expression and signaling pathways associated with the PGCCs compared to the parent cells.

Conclusion: We successfully established AML PGCCs and assessed their biological features. Our data provide information to elucidate the characteristics of PGCCs in liquid tumors, including phenotypes, genotypes, and crucial molecular mechanisms.

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Conflict of Interest: None declared

P01.061.A Importance of a bioinformatics pipeline for the detection of Alu in the NGS panel of hereditary cancer genes

Ricardo Blázquez Martín[1], *José Manuel Sánchez Zapardiel*[1], *Celia Amil Manjón*[1], *Pilar Duarte García*[1], *Marina Ibáñez Vizcaíno*[1], *Francisca Luengo Sainz de Baranda*[1], *Victoria Carrero Blázquez*[1], *Beatriz Hidalgo Calero*[1], *Montserrat de Miguel*[1], *Juan de Dios García Díaz*[2]

[1]*Hereditary Cancer Unit, Hospital 12 de Octubre. Madrid, Department of Clinical Analysis-Clinical Biochemestry, Madrid, Spain;* [2]*Clinical Genetic Unit, Hospital Principe de Asturias, Madrid, Department Internal Medicine, Alcalá de Henares, Spain*

Background/Objectives: Alu elements are the most abundant transposable elements, they are short interspersed elements (SINEs) ~300 nucleotides in length. Transposable elements are rare sequences of DNA that can move themselves to new positions within the genome and may be linked to genetic diseases and susceptibility to certain disorders, including cancers. In our case, it is a case of a family with a multiple history of breast, ovarian, pancreatic and prostate cancer at an early age. After a non-informative study, it was reprocessed with the multigene panel we are currently working with.

Methods: Next-generation sequencing was performed using the Hereditary Cancer Solution kit (Sophia Genetics) and sequencing on the NextSeq platform (Illumina). Results were analyzed using Sophia-DDM software.

Results: The bioinformatic pipeline detected an Alu element in exon 5 of *PALB2* gene, likely resulting in a premature stop codon and subsequent production of a truncated or ausent protein by NMD mechanism. According to American College of Medical Genetics (ACMG) criteria it was classified as pathogenic. However, the exact location, the complete sequence and the generated break points of this Alu element could not be determined and an orthogonal method is required.

Conclusion: It is important to have a bioinformatics pipeline capable of identifying Alu elements in families with a long history of cancer and at very young ages that have been previously uninformative. An accurate characterisation of these elements and a correct genetic counselling is crucial for a correct clinical management for patients and their families.

Conflict of Interest: None declared

P01.062.B Rare genetic phenomena in *TP53* can lead to a misdiagnosis of Li-Fraumeni Syndrome

Vasiliki Dellatola[1], *Paraskevi Apostolou*[1], *Jan Traeger-Synodinos*[2], *MARIA TZETIS*[2], *DRAKOULIS YANNOUKAKOS*[1], *Irene Konstantopoulou*[1], *Florentia Fostira*[1]

[1]*Human Molecular Genetics Laboratory, Institute of Nuclear & Radiological Sciences and Technology, Energy & Safety, National Center for Scientific Research "Demokritos", Athens, Greece;* [2]*Laboratory of Medical Genetics, St. Sophia's Children's Hospital, Medical School, National and Kapodistrian University of Athens, Athens, Greece*

Background/Objectives: Next-generation sequencing of germline DNA extracted from whole blood can reveal *TP53* Pathogenic/Likely Pathogenic (P/LP) variants in low Variant Allele Frequencies (VAF ≤30%). These might be attributed to mosaicism, circulating tumor DNA, or clonal hematopoiesis, a process induced by advanced age and chemotherapy. Herein, we aimed to differentiate these rare phenomena in a selected group of patients with cancer diagnoses focusing on association to Li-Fraumeni Syndrome (LFS).

Methods: This study included 14 patients with *TP53* P/LP variants of low VAF (7-30%) and a diagnosis of breast (5), ovarian (4), colorectal (3), endometrial (1), and prostate cancer (1). The mean age at diagnosis was 58.6 years (range:28-78 years). Re-sampling of blood/saliva (mesoderm) and re-analysis was performed >3 months after initial testing. If the *TP53* variant was still evident, DNA from hair follicles (ectoderm) was analyzed through Sanger sequencing. Parameters considered involved patient age, time at genetic testing, personal history, and disease burden.

Results: Through re-sampling, the *TP53* variant under investigation was not present in 85.7% (12/14) of patients, indicating either clonal hematopoiesis or the presence of circulating tumor DNA. Two patients (14.3%) proved to have the underlying *TP53* variant in mosaicism, predisposing for LFS, upon validation in both mesodermal and ectodermal tissues. Notably, both individuals had breast cancer and one met the genetic testing criteria for LFS.

Conclusion: Accurate delineation of *TP53* variants origin and their association with LFS is imperative for tailored clinical interventions. This is of high importance in regions lacking systematized genetic centers, such as Greece.

Conflict of Interest: Vasiliki Dellatola: None declared, Paraskevi Apostolou: None declared, Jan Traeger-Synodinos: None declared, MARIA TZETIS: None declared, DRAKOULIS YANNOUKAKOS: None declared, Irene Konstantopoulou: None declared, Florentia Fostira AstraZeneca, Roche Pharmaceuticals, Takeda, GSK and AstraZeneca

P01.063.C Surveillance in children with PTEN Hamartoma Tumor Syndrome: the yield of yearly thyroid ultrasound in a Dutch expertise centre

Esther Bormans[1], **Janneke Schuurs-Hoeijmakers**[2], *Petra van Setten*[3], *Linda Hendricks*[2;4], *Meggie Drissen*[2;4], *Martin Gotthardt*[5], *Hedi Claahsen*[3], *Nicoline Hoogerbrugge*[2;4], *Jolanda Schieving*[1]

[1]*Radboud University Medical Centre, Amalia Children's Hospital, Department of Pediatric Neurology, Nijmegen, Netherlands;* [2]*Radboud University Medical Centre, Department of Human Genetics, Expertise centre for PHTS, Nijmegen, Netherlands;* [3]*Radboud University Medical Centre, Amalia Children's Hospital, Department*

of Pediatric Endocrinology, Nijmegen, Netherlands; [4]Radboud University Medical Centre, Radboud Institute for Medical Innovation, Nijmegen, Netherlands; [5]Radboud University Medical Centre, Department of Medical Imaging, Nuclear Medicine, Nijmegen, Netherlands

Consortium: -

Background/Objectives: Children with PTEN hamartoma tumor syndrome (PHTS) are at increased risk for developing thyroid abnormalities, including differentiated thyroid carcinoma (DTC). The Dutch PHTS guideline recommends ultrasound surveillance starting from age 18. The literature describes PHTS patients who developed DTC before age 18, the Dutch PHTS expertise centre has initiated annual ultrasound surveillance starting from age 12. We describe the yield of thyroid ultrasound surveillance in these children with PHTS.

Methods: A retrospective single centre cohort study was conducted. Pediatric PHTS patients who received thyroid ultrasound surveillance before age 18 between 2016–2023 were included. Patients' medical records have been reviewed. Primary outcomes included prevalence and time to develop thyroid nodules ≥10mm, nodular growth, goiter, thyroiditis and DTC. Descriptive statistics and Kaplan-Meier analyses were performed.

Results: Forty-three patients were included. Two patients (5%) were diagnosed with DTC at ages 12 and 17. Both DTCs were identified as minimally invasive follicular carcinoma at stages pT3NxMx and pT1NxMx. A total of 84% were diagnosed with thyroid abnormalities at a median age of 12 years (range 9-18, table 1). Most common findings were benign, including nodular disease (74%), goiter (30%) and autoimmune thyroiditis (12%). Nodular growth was observed in 14 patients (33%) resulting in (hemi)thyroidectomy in 7 patients (16%).

Conclusion: Thyroid ultrasound surveillance resulted in the detection of DTC in 2/43 PHTS patients before age 18. These findings support the recommendation to initiate thyroid ultrasound surveillance in children from age 10 – 12 onwards, preferably within an expertise centre.

Grants: None.

Conflict of Interest: None declared

P01.064.D Chloroquine-induced DNA damage synergizes with DNA repair inhibition to cause head and neck cancer cell death

Diego Iglesias-Corral[1;2;3], *marta lópez alonso*[2], *Lucía garcía collado*[2], *Nuria Arroyo-Garrapucho*[1;2;3], *Juan-Luis García*[1;3], *Rogelio González-Sarmiento*[1;2;3], *Ana-Belén Herrero*[1;2;3]

[1]Biomedical Research Institute of Salamanca (IBSAL), IIMD-07, Salamanca, Spain; [2]University of Salamanca, Faculty of Medicine, Department of Medicine, Unit of Molecular Medicine, Salamanca, Spain; [3]Institute of Molecular and Cellular Biology of Cancer (IBMCC), University of Salamanca-CSIC, Salamanca, Spain

Background/Objectives: Head and neck cancer (HNC) is a heterogeneous group of malignancies that accounts for 450.000 deaths annually. Tumor heterogenicity, along with the emergence of resistances to conventional treatments highlight the necessity for new therapies. Chloroquine (CQ) is a well-known autophagy inhibitor that induces DNA double-strand breaks (DSBs) through reactive oxygen species (ROS) production. We have previously described in in vitro studies that the combination of CQ with inhibitors of homologous recombination (HR) and nonhomologous end-joining (NHEJ) DNA repair pathways is a promising approach for the treatment of different tumors.

Methods: HNC cell lines 32816 (generated in our laboratory) and CAL-33 were used. The combination of CQ with the HR inhibitor, Panobinostat (LBH) or NHEJ inhibitors (KU-57788, NU-7026) were tested. Antitumoral activity was evaluated by proliferation and apoptosis assays and synergistic interaction between the drugs was calculated using the Chou-Talalay method. DSB induction was tested by γH2AX foci quantification via immunofluorescence assays. To evaluate HR efficiency, cells were transfected with a GFP-reporter construct, then treated with LBH for 48 h. HR proficient cells (GFP +) were quantified by flow cytometry.

Results: All drugs tested inhibited cell proliferation and induced apoptosis in the studied cell lines. The combination of CQ with either HR or NHEJ inhibitors exhibited a synergistic effect on apoptosis induction that was largely prevented by antioxidant addition.

Conclusion: Our studies suggest that the combination of CQ and inhibitors of DNA-repair pathways could be a new therapeutic approach against HNC.

Grants: Study funded by PI20/01569.

Conflict of Interest: None declared

P01.065.A A Canadian Lab's Experience Using Optical Genome Mapping To Clinically Genotype Hematological Neoplasms

Zeid Hamadeh[1;2], *Eric McGinnis*[1;2], *Tara Spence*[1;2]

[1]The University of British Columbia, Medicine, Vancouver, Canada; [2]Vancouver General Hospital, Cytogenomics, Vancouver, Canada

Background/Objectives: Diagnosis and management of hematologic malignancies relies on detection of acquired genetic abnormalities in cancer cells. We report the added yield of optical genome mapping (OGM) observed in our retrospectively selected clinical validation cohort of hematologic neoplasms.

Methods: We comprehensively assessed 83 patients by both standard-of-care (SOC) techniques and OGM to validate OGM as a routine clinical test for acute myeloid leukemia, myelodysplastic syndrome, acute lymphoblastic leukemia, and chronic lymphocytic leukemia. Samples included 61 bone marrow aspirates (BMAs) and 22 peripheral bloods received by our laboratory for routine karyotyping and/or FISH.

Results: We observed high concordance with SOC methods with a sensitivity of 97.6%, specificity of 100%, and accuracy of 98.6%. OGM identified or clarified findings either cryptic or not resolvable using SOC, decreasing the proportion of with normal karyotype by 23% of BMAs, identifying chromothripsis in 13% of all cases, identifying cytogenetically visible or recurring variants in 46% of BMAs, and generating prognostically or diagnostically relevant findings in 42% of BMAs. Additional findings included, but were not limited to, three KMT2A rearrangements, a KMT2A partial tandem duplication, a RUNX1::MECOM rearrangement, a NUP214::ABL1 fusion, and two submicroscopic TP53 deletions.

Conclusion: OGM shows significant power in detecting or resolving abnormalities previously inadequately characterized by SOC testing, permitting refinement of genomics-based diagnosis and risk stratification and potentially altering treatment and prognostication for a portion of patients. Future assessment will be needed in a prospective unselected cohort to definitively establish clinical utility of OGM.

Conflict of Interest: None declared

P01.066.B Role of *ATG7* loss in taxane-resistant head and neck cancer

Nerea Gestoso-Uzal[1;2;3], *Laura Molinero-Sicilia*[2;3], *Mercedes León-López*[2;3], *Ana-Belén Herrero*[1;2;3], *Juan-Luis García*[2;3], *Juan Jesús Cruz-Hernández*[1;2;3], *Rogelio González-Sarmiento*[1;2;3]

[1]*Molecular Medicine Unit, Department of Medicine, University of Salamanca, Salamanca, Spain;* [2]*Biomedical Research Institute of Salamanca (IBSAL), University Hospital of Salamanca-USAL- CSIC, Salamanca, Spain;* [3]*Institute of Molecular and Cellular Biology of Cancer (IBMCC), University of Salamanca-CSIC, Salamanca, Spain*

Background/Objective: Head and neck squamous cell carcinomas (HNSCC) include heterogeneous tumours in the cervicofacial area. Combating therapy resistance is one of the main challenges in HNSCC. The main aim of this work was to characterize the molecular mechanisms underlying taxane resistance in HNSCC.

Methods: Taxane resistance was induced in two HNSCC cell lines, 32816 and 32860, by gradually increasing paclitaxel concentration. Cell lines were characterized by aCGH and expression microarrays. Autophagy was analyzed by western blot. CRISPR-Cas9 technology was used to generate *ATG7* knockout cell lines. The efficacy of its design was tested by Sanger sequencing, RT-qPCR and western blot.

Results: A 32816 taxane-resistant stable cell line was established, while 32860 cells remained sensitive to taxanes after twenty cycles of treatment. The aCGH study showed a differential deletion in 3p26.6-3p21.1 in 32860 cell line. Expression microarrays identified a significant underexpression of *ATG7*, a gene located in this region and involved in autophagy. Moreover, the study of the autophagy signalling pathway suggested a blockade of autophagy in 32860 cells, that may be responsible for the 32860 response to taxane resistance induction. To get a deeper understanding of the role of *ATG7*, we generated *ATG7* knockout cell lines from 32816 and confirmed the blockade of autophagy caused by *ATG7* loss in them. Further studies are being performed to establish the response of *ATG7* knockout cells to taxane resistance induction.

Conclusion: The loss of *ATG7* causes a blockade of autophagy in HNSCC that may be associated with the therapeutic response to taxanes.

Grants: This project was funded by FIS-FEDER:PI18/01476.

Conflict of Interest: None declared

P01.067.C digitalMLPA: Multiplex NGS-based assays to detect a wide range of structural variants implicated in hereditary cancer predisposition syndromes

Sebastiaan Lamers[1], *Maria Giovanna Maturo*[2], *Janina Luitz*[2], *Jonas de Groot*[3], *Martin Loden*[4], *Lisette Stolk*[1]

[1]*MRC Holland, Cytogenetics, Amsterdam, Netherlands;* [2]*MRC Holland, IVD Development, Amsterdam, Netherlands;* [3]*MRC Holland, Genetic Disorders, Amsterdam, Netherlands;* [4]*MRC Holland, Oncogenetics, Amsterdam, Netherlands*

Background/Objectives: Hereditary cancer predisposition syndromes (HCPS) account for 5-10% of all cancers. Among these syndromes is Lynch syndrome, a disorder that predisposes carriers of DNA mismatch repair (MMR) gene sequence mutations and copy number variants (CNVs) to early-onset cancer. A recently identified paracentric inversion involving MMR gene *MSH2* exon 2-6 may account for a subset of unexplained Lynch syndrome cases.

Methods: An updated version of SALSA® digitalMLPA™ Probemix D001 Hereditary Cancer Panel 1 and an extended assay, SALSA® digitalMLPA™ Probemix D002 Hereditary Cancer Panel 2 are in development. The assays incorporate probes for the identification of CNVs and multiple targeted mutations in more than 50 genes linked to HCPS, of which 28 are targeted by both panels. Specifically, two probes were integrated in both panels detecting the 3′ and 5′ breakpoints of the *MSH2* exon 2-6 inversion. These probemixes were tested on more than 50 positive samples carrying aberrations in different target regions, including a sample carrying the *MSH2* exon 2-6 inversion.

Results: Both assays successfully identified CNVs and targeted mutations in all target regions in the tested samples. Additionally, the *MSH2* exon 2-6 inversion was detected with the two inversion-specific probes.

Conclusion: These assays are suitable for detection of a wide range of CNVs involved in HCPS. Furthermore, the integration of probes targeting mutations like the *MSH2* exon 2-6 inversion in SALSA® digitalMLPA™ assays underscores the potential of the digitalMLPA technique in uncovering a broad spectrum of genetic factors involved in HCPS.

Grants:

Conflict of Interest: Sebastiaan Lamers MRC Holland, Maria Giovanna Maturo MRC Holland, Janina Luitz MRC Holland, Jonas de Groot MRC Holland, Martin Loden MRC Holland, Lisette Stolk MRC Holland

P01.068.D Comprehensive molecular profiling of breast cancer subtypes in a Turkish population: Insights from next-generation sequencing analysis

Drenushe Zhuri[1], *Engin Atli*[1], *Hazal Sezginer Guler*[1], *Fulya Dusenkalkan*[1], *Nilay Ozupek*[1], *Selma Demir*[1], *Sinem YALÇINTEPE*[1], *HAKAN GURKAN*[1]

[1]*Trakya University, Medical Genetics, Edirne, Türkyie*

Background/Objectives: Breast cancer is a heterogeneous disease with diverse molecular subtypes that have implications for prognosis and treatment. The most commonly recognized immunohistochemical classification of breast cancer is based on the expression of the hormone receptors estrogen (ER), progesterone (PR), and human epidermal growth factor (HER2). As a result, the following four breast cancer subtypes are commonly recognized as luminal A, luminal B, HER2-positive, and triple-negative. This study presents the findings from a single-center experience of next-generation sequencing analysis of breast cancer subtypes in the Thrace region.

Methods: We retrospectively analyzed the NGS data of 397 patients with breast cancer who applied to our center between 2020 January-2023 December. After the isolation of DNA, the NextSeq550-Illumina system and the Qiaseq Targeted (Qiagen) Amplicon panel kit were used for molecular analysis. *BRCA1/BRCA2* deletion/duplications were evaluated with the Multiplex ligation-dependent probe amplification method.

Results: As a result of analysis for ninety-three targeted cancer genes, we detected eighty-three pathogenic/likely pathogenic (P/LP) variations in seventy-four cancer cases. Twenty-one P/LP variants (25%) in nineteen Luminal A breast cancer cases. Forty-four P/LP variants (53%) in thirty-nine Luminal B breast cancer cases and nineteen P/LP variants (22%) in HER2-positive cases. BRCA1/BRCA2 gene deletion was detected only in one HER2-positive case.

Conclusion: Clinical and pathological characteristics were correlated with molecular subtypes identified by NGS. The diagnosis rate was 18.63% in this study. Our results shed light on the distribution of breast cancer subtypes in the Thrace region and provide insights into the genomic landscape of these tumors in this population.

Conflict of Interest: None declared

P01.069.A Prevalence and consequences of APC mosaicism in patients with colorectal adenomas

Diantha Terlouw[1], Manon Suerink[2], Monique van Leerdam[3], Frederik Hes[4], Demi van Egmond[2], Dina Ruano[2], Anja Wagner[5], Floris Groenendijk[6], Sanne ten Broeke[7], Arjen Mensenkamp[8], Iris Nagtegaal[9], Carli Tops[10], Alexandra Langers[3], Tom van Wezel[2], Hans Morreau[2], **Maartje Nielsen**[10]

[1]Lumc, Clinical genetics, Leiden, Netherlands; [2]Lumc, Pathology, Leiden, Netherlands; [3]Lumc, Gastroenterology and Hepatology, Leiden, Netherlands; [4]UZ Brussel, Clinical Genetics, Jette, Belgium; [5]Erasmus University Medical Center, Clinical Genetics, Rotterdam, Netherlands; [6]Erasmus University Medical Center, Pathology, Rotterdam, Netherlands; [7]University Medical Center Groningen, Clinical genetics, Groningen, Netherlands; [8]Radboud University Medical Center, Molecular genetics, Nijmegen, Netherlands; [9]Radboud University Medical Center, Pathology, Nijmegen, Netherlands; [10]Lumc, Clinical Genetics, Leiden, Netherlands*

Background/Objectives: Although germline pathogenic *APC* variants are detected in up to 80% of adenomatous polyposis patients, a substantial proportion remains unexplained

Methods: APC mosaicism was analyzed in 458 patients with a broad spectrum of phenotypes in three university medical centers in the Netherlands.

Results: The mosaicism detection rate was 11.1% (51 out of 458) in the entire cohort. This rate was 17.1% (46 out of 269) in patients with ≥10 adenomas before 60 years or with ≥20 adenomas before 70 years and 2.6% (5 out of 189) in patients falling outside Dutch polyposis testing guidelines. Overall, the odds of finding *APC* mosaicism increased significantly with adenoma count and a younger age at diagnosis. Interestingly, 28% (9 out of 32) of mosaic patients undergoing an esophagogastroduodenoscopy were diagnosed with gastroduodenal adenomas. Moreover, none of the children tested inherited the mosaic variant. In one patient without children, the mosaic variant was detected in semen. Patients having a recurrent colibactin-associated c.835-8A>G variant in some but not all lesions were phenotypically similar to non-mosaic patients.

Conclusion: APC mosaicism was found in over 10% of unexplained polyposis patients. We recommend testing in -at least- all patients with negative germline results with (1) multiple adenomas before 50 years, (2) ≥20 adenomas before 60 years, or (3) ≥30 adenomas before 70 years. Regular colonoscopy and at least one gastroduodenoscopy should be offered; the frequency of follow-up should depend on the findings. Furthermore, consider germline testing for offspring, especially when mosaicism exceeds the colon.

Grants: Dutch Cancer Society (11292).

Conflict of Interest: Diantha Terlouw Dutch Cancer Society Project number: 11292, Manon Suerink: None declared, Monique van Leerdam: None declared, Frederik Hes: None declared, Demi van Egmond: None declared, Dina Ruano: None declared, Anja Wagner: None declared, Floris Groenendijk: None declared, Sanne ten Broeke: None declared, Arjen Mensenkamp: None declared, Iris Nagtegaal: None declared, Carli Tops: None declared, Alexandra Langers: None declared, Tom van Wezel: None declared, Hans Morreau: None declared, Maartje Nielsen: None declared

P01.070.B A Recurrent POT1 Founder Germline Variant Associated with Early Onset Melanoma, Various Malignancies and High Tumor Burden

Aasem Abu Shtaya[1,2], *Inbal Kedar[2], Lily Bazak[2], lina basel-salmon[2], Sarit Farage-Barhom[2], Ori Segol[1], Michal Naftali[3], Marina Eskin-Schwartz[4], Ohad Shmuel Birk[4], Shirley Polager-Modan[5], Nitzan Keidar[6], Gili Reznick Levi[7], Ahmad Mahamid[8], Noy Azulay[2], Yael Goldberg[2]*

[1]Carmel Medical Centre, Unit of Gastroenterology, Haifa, Israel; [2]Rabin Medical Center, Recanati Genetics Institute, Petah Tikva, Israel; [3]Clalit Genomic Center, Petach Tikba, Israel; [4]Soroka Medical Center, Genetics Institute, Be'er Sheva, Israel; [5]Carmel Medical Centre, Genetics Institute, Haifa, Israel; [6]Schneider- Children's Medical Center, Pediatric Genetic Unit, Petah Tikva, Israel; [7]Rambam Health Care Campus, Genetics Institute, Haifa, Israel; [8]Carmel Medical Centre, Department of Surgery, Haifa, Israel*

Background/Objectives: POT1 is a key component of the shelterin complex that plays a critical role in telomere protection and length regulation. Germline variants in the *POT1* gene have been implicated in predisposition primarily to malignant melanoma and chronic lymphocytic leukemia. This study reports the identification of *POT1* p.I78T, a founder variant among Ashkenazi Jews, and describes a unique clinical landscape in carriers.

Methods: A directed database search was conducted for individuals referred for genetic counselling in 2018 to 2023 who were found to carry the *POT1*(NM_015450.3) c.233T>C:p.(I78T) germline variant. Demographic, clinical, genetic, and pathology data of patients and family members were analyzed.

Results: Ten carriers of the *POT1*:p.(I78T) variant aged 25 to 67 years from ten unrelated families of AJ descent were identified. Carriers had a total of 28 primary malignancies (range 1-6); eight carriers aged 25-63 years had recurrent malignant melanoma, three carriers (30%) had desmoid tumors; three (30%) had Papillary thyroid cancer, and four women (57% of female carriers) had breast cancer. Additional tumors included CLL, sarcoma, endocrine tumors and colonic polyps. Review of a local genome database yielded an allelic frequency of the variant of 0.06% among all ethnicities and of 0.25% in Ashkenazi Jews. A shared haplotype was found in all probands carrying the variant, indicating that a common founder was likely in these families.

Conclusion: *POT1*:p.(I78T) is a recurrent, founder disease-causing variant associated with early-onset melanoma in addition to other solid malignancies, with a high tumor burden. Genetic testing of *POT1* should be included in germline panels for various cancer types.

Conflict of Interest: None declared

P01.071.C Analysis of molecular genetic testing yields for hereditary forms of cancer 2020-2023

Ilona Pirushka[1], *Baiba Lace[2], Ieva Mičule[3;4], Arvids Irmejs[5], Peteris Loža[5], jelena maksimenko[5]*

[1]Children's Clinical University Hospital, Clinic of Medical Genetics and Prenatal Diagnosis, Riga, Latvia; [2]Riga East Clinical University Hospital, Rīga, Latvia; [3]Riga Stradins University, Rīga, Latvia; [4]Children's Clinical University Hospital, Rīga, Latvia; [5]Pauls Stradiņš Clinical University Hospital, Rīga, Latvia*

Objectives: The aim of this study was to determine the clinical and molecular characteristics of patients from 2020 to 2023 who underwent hereditary cancer multigene panel testing.

Methods: The study enrolled 247 adults: 5% (n = 13) men and 95% (n = 234) women with: breast cancer 89% (n = 221), ovarian cancer 6% (n = 14), colorectal cancer 3% (n = 8), testicular cancer 0,8% (n = 2), melanoma 0,8% (n = 2). 17 adults underwent cascade testing. Patients with cancer (n = 247) were tested by gene panels, predictive testing for patients' relatives (n = 17) - by Sanger sequencing. All breast and ovarian cancer patients had initial testing for BRCA1/2 founder mutations before NGS testing.

Results: The mean age at the time of NGS was 46 years. Genetic testing identified a pathogenic/likely pathogenic variant in 32 % of patients ($n = 78$). Test result was negative in 64% ($n = 158$) of patients. 11 VUS were identified in 4% ($n = 9$) of patients.

The most identified germline mutations were in *BRCA1* ($n = 36$; 46%) *BRCA2*, ($n = 17$; 22%), *CHEK2*($n = 9$;12%). Pathogenic/likely pathogenic variants in *ATM*, *PALB2*, *RAD51C*, *FANCM*, *TP53*,*MLH1*, *PMS2* were also identified. 33% (n = 15) from all positive *BRCA1/2* variants were founder mutations. The cascade screening for the asymptomatic persons was positive in 88% (n = 15), negative in 12% (n = 2).

Conclusion: We propose that genetic testing in Latvia, utilizing a panel of 11 *BRCA1/BRCA2* gene founder mutations, provides an inexpensive, efficient platform for cancer prevention/treatment. This panel may be expanded or replaced by multigene hereditary cancer panel in the future.

Conflict of Interest: None declared

P01.072.D Genome sequencing in patients with hereditary breast and ovarian cancer

Ulrike Faust[1], *Dennis Witt*[1], *Antje Stäbler*[1], *Silja Gauß*[1], *Marc Sturm*[1], *Benita Menden*[1], *Olga Kelemen*[1], *Leon Schütz*[1], *Ines Gruber*[2], *Kristin Bosse*[1], *Stephan Ossowski*[1], *Tobias Haack*[1], *Olaf Riess*[1], *Christopher Schroeder*[1]

[1]*Institute of Medical Genetics and Applied Genomics, Tübingen, Germany;* [2]*Department for Women's Health, Tübingen, Germany*

Background/Objectives: Multi-gene panel or exome sequencing are the current standard for hereditary breast and ovarian cancer (HBOC) testing. However, a pathogenic variant in the HBOC genes is only found in 15% to 25% of high-risk families. The implementation of genome sequencing (GS) has the potential to increase the diagnostic sensitivity through detection of structural variants, alterations in regulatory regions, as well as the integration of polygenic-risk-scores (PRS) and analysis of actionable genes.

Methods: The study involved 819 patients with breast and/or ovarian cancer, fulfilling criteria for HBOC testing and 4810 healthy controls. GS was performed and analyzed in our accredited diagnostic laboratory following standard procedures. Our pipeline was designed to incorporate the Breast Cancer PRS 313 (Mavaddat et al., 2019) and Ovarian Cancer PRS (OCAC 36 by canrisk.org).

Results: Causative variants were found in 12.8% of cases across 9 HBOC genes. The in-depth characterization of a complex *BARD1*-structural variant confirmed the advantages of GS over other methods. The raw PRS of our breast cancer cohort as well as of our ovarian cancer cohort was significantly higher than in our local controls. Additionally, 20 pathogenic variants in actionable genes were found in 18 patients.

Conclusion: GS offers advantages over current standard multi-gene panel or exome sequencing. We were able to benefit from additional structural variants and increased process standardization. PRS might explain up to an additional 20% of breast cancer cases. Finally, GS has a higher flexibility regarding re-analysis of candidate genes, regulatory regions, and structural variants.

Grants:

Conflict of Interest: Ulrike Faust: None declared, Dennis Witt: None declared, Antje Stäbler: None declared, Silja Gauß: None declared, Marc Sturm: None declared, Benita Menden: None declared, Olga Kelemen: None declared, Leon Schütz: None declared, Ines Gruber: None declared, Kristin Bosse: None declared, Stephan Ossowski S.O. received reimbursement for travel expenses and payment for conference presentations from Illumina Inc. and Oxford Nanopore Technologies., S.O. received reimbursement for travel expenses and payment for conference presentations from Illumina Inc. and Oxford Nanopore Technologies., Tobias Haack: None declared, Olaf Riess: None declared, Christopher Schroeder Illumina Inc. Institutional grant for the GE-MED subproject, medical writing

P01.073.A Unique mutation spectrum in the Israeli Arab population suspected of Hereditary Breast and Ovarian Cancer or MUTYH Associated Polyposis syndrome

Gili Reznick Levi[1], *Elizabeth E Half*[2;3], *Tamar Paperna*[1], *Hanna Segev*[1], *Yael Goldberg*[4;5], *Karin Weiss*[1;3]

[1]*Rambam Health Care Campus, The Genetics Institute, Haifa, Israel;* [2]*Rambam Health Care Campus, Institute of Gastroenterology, Haifa, Israel;* [3]*The Rappaport Faculty of Medicine, Technion, Haifa, Israel;* [4]*Raphael Recanati Genetic Institute, Rabin Medical Center, Petach Tikva, Israel;* [5]*Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel*

Background/Objectives: Most of the Founder Pathogenic Variants (PVs) in genes related to cancer predisposition syndromes in Israel were described in the Jewish population.

This work aims to describe the mutation spectrum of Hereditary Breast and Ovarian Cancer (HBOC) in Christian Arabs and *MUTYH* associated polyposis (MAP) in Israeli Arabs.

Methods: Electronic records of all Arab patients presenting to the Oncogenetic clinic at Rambam Health Care Campus between 2013- 2020 were reviewed for personal or family history suspected of HBOC or polyposis syndromes.

Results: 35 unrelated Christian Arab patients, with a personal or family history of breast and\or ovarian cancer, underwent *BRCA1/BRCA2* (14/35) testing or multi-gene panel testing (21/35). A recurrent *BRCA2* exon 5-11 duplication was found in 5/33 (15.2%) patients for whom copy number variant (CNVs) analysis was performed. 37 Arab polyposis patients from 30 unrelated families underwent *APC* and *MUTYH* testing; 8(26.6%) carried bi-allelic *MUTYH* PVs. The major variant detected was p.(Glu452del) seen in 75% of positive cases. Interestingly, Druze and Muslims shared the same haplotype.

Conclusions: We identified a high prevalence of a *BRCA2* duplication among Christian Arabs with personal/ family history of HBOC in Israel, emphasizing the importance of CNV analysis in this sub-population. MAP is quite frequent (27%) among Arab polyposis cases in Northern Israel. PV spectrum is unique, with high frequency of the founder variant p.(Glu452del). Our results impact the genetic testing strategy in the Israeli Arab population suspected of HBOC or MAP.

Conflict of Interest: None declared

P01.074.B Compilation and reclassification of *BRCA2* VUS found in the Genetic Counselling Unit of Salamanca in the last 23 years

Paloma Martín-Bejarano Soto[1;2;3], *Eva María Sánchez-Tapia*[1;2;3], *Paula García-Vallés*[1;2;3], *Mercedes León-López*[1;2;3], *Teresa Martín-Gómez*[4], *emilio fonseca sánchez*[4], *Rogelio González-Sarmiento*[1;2;3], *Ana-Belén Herrero*[1;2;5]

[1]*Molecular Medicine Unit, Department of Medicine, University of Salamanca, Salamanca, Spain;* [2]*Biomedical Research Institute of Salamanca (IBSAL), Salamanca, Spain;* [3]*Institute of Molecular and Cellular Biology of Cancer (IBMCC), University of Salamanca-CSIC, Salamanc, Spain;* [4]*Medical Oncology Service, University Hospital of Salamanca, Salamanca, Spain;* [5]*Institute of Molecular and Cellular Biology of Cancer (IBMCC), University of Salamanca-CSIC, Salamanca, Spain*

Background/Objectives: BRCA1/2 genetic testing is key for improving clinical management and for patients with hereditary breast/ovarian cancer. Variants found in these genetic tests are classified according to their impact on the protein function and thus on the risk of developing breast cancer. However, the identification of variants of unknown significance (VUS) limits the clinical utility of genetic testing as the impact on protein function is unknown. VUS are mainly missense variants that cannot be classified due to insufficient experimental and clinical data.

Methods: The variants identified in the Genetic Counselling Unit (GCU) of the University Hospital of Salamanca between 2000-2023 have been compiled. They were searched for re-evaluation in the aggregators Varsome and ClinVar, which are based on the use of the classification guidelines specified by the ACMG/AMP.

Results: Of the 79 VUS detected in over 2000 studies performed in the last 23 years, 62 (76.4 %) were reclassified according to Varsome and 23 (26.6%) according to ClinVar (Table 1). Of the 79 variants, most (91.1%) are missense variants and belong to 94 families.

Table 1. Updated classification of the 79 VUS according to Varsome and ClinVar.

Clasification	Varsome	ClinVar
Bening	17	17
Likely bening	41	1
VUS	16	55
Likely pathogenic	1	0
Pathogenic	3	3
Not described	1	3

Conclusion: This study demonstrates the need for updating VUS detected in GCU due to their important clinical impact. Furthermore, it highlights the need to generate methods that allow the reclassification of these VUS as a priority.

Grants: This project was funded by IP20.

Conflict of Interest: None declared

P01.075.C Investigation of variant distribution and frequency in triple negative breast cancer patients living in the Trakya Region of Turkey: A Single Center Experience

HAKAN GURKAN[1], *Engin Atli*[1], *Nilay Ozupek*[1], *Bilge Nihan Satkın*[2], *Drenusha Zhuri*[1], *Hazal Sezginer Guler*[1], *Selma Demir*[1], *Sernaz Topaloğlu*[3], *Atakan Sezer*[4], *Ebru Taştekin*[5], *Nermin Tunçbilek*[6]

[1]Trakya University Faculty of Medicine, Department of Medical Genetics, Edirne, Türkyie; [2]Trakya University Faculty of Engineering, Department of Genetics and Bioengineering, Edirne, Türkyie; [3]Trakya University Faculty of Medicine, Department of Medical Oncology, Edirne, Türkyie; [4]Trakya University Faculty of Medicine, Department of General Surgery, Edirne, Türkyie; [5]Trakya University Faculty of Medicine, Department of Medical Pathology, Edirne, Türkyie; [6]Trakya University Faculty of Medicine, Department of Radiology, Edirne, Türkyie

Background/Objectives: Approximately 15% to 20% of all breast cancers are triple negative breast cancers (TNBCs). TNBC is most common in premenopausal women aged < 40 years and is highly aggressive, and poorer clinical outcome than those of hormone receptor positive and HER2-enriched breast cancers. In fact, >85% of breast cancers developing in the context of BRCA1 germline pathogenic variant carriers display a triple-negative phenotype, and 11% to 19% of patients with triple-negative disease harbor BRCA1 germline or somatic mutations.

Methods: The aim of our study is to investigate the distribution and frequency of variants in TNBC patients living in the Trakya Region of Turkey. 45 patients who applied to our Genetic Diseases Evaluation Center with a diagnosis of TNBC between October 2019 and October 2023 were included in the study. The average age of the patients is 53.27 years. Targeted Breast NGS panel (94 genes) and BRCA1/2 MLPA were studied in patients using the NGS method.

Results: Pathogenic/likely pathogenic (P/LP) variant was detected in 16 patients (35.5%), and VUS variant was detected in 16 patients (35.5%). 10 of the 16 P/LP variants (62.5%) were located in BRCA1. NM_007294.3(BRCA1):c.5266dupC (p.Gln1756Profs) variant was detected in four patients (40%). Other P/LP variants were detected in the BRCA2 (2), CHEK2 (1), MUTYH (1), PALB2 (1) and MUC16 (1) genes.

Conclusion: Unlike the literature, we detected a higher rate (62.5%) of P/LP variants in the BRCA1 gene in our patients with triple-negative phenotype. This result may be related to ethnic stratification and/or variants with a founder effect.

Grants: No grants

Conflict of Interest: None declared

P01.077.A Addressing the 'leaky pipe' in colorectal cancer genetics referrals: The critical need for optimization

Nur Diana Binte Ishak[1], *Shao-Tzu Li*[1], *jianglei wu*[1], *Jianbang Chiang*[1], *Joanne Ngeow*[1;2]

[1]National Cancer Centre Singapore, Cancer Genetics Service; [2]Lee Kong Chian School of Medicine, Nanyang Technological University

Background/Objectives: Colorectal cancer significantly burdens Singapore, with over 1,865 cases diagnosed annually. Lynch syndrome, the most common hereditary colorectal cancer, affects 1 in 300 Singaporeans. Despite high prevalence, referrals to Cancer Genetics Service (CGS) remain low, highlighting a gap in Lynch syndrome detection. This study delves into referral discrepancies and barriers to timely hereditary cancer syndrome diagnosis among colorectal cancer patients in Singapore.

Methods: We evaluated patients seen at the National Cancer Centre Singapore from 2017 to 2021, identifying 1,716 colorectal cancer cases diagnosed under age 50 or with a second Lynch syndrome-related cancer. Appropriate CGS referrals were determined based on age at cancer diagnosis, deficient mismatch repair protein expression, polyp pathology (adenomatous, hamartomatous, juvenile polyps), and presence of desmoid tumours.

Results: We found that only 15% of the 878 colorectal cancer patients under the age of 50 were referred to CGS, while the referral rate for patients with a second Lynch syndrome-associated cancer was even lower at 9.8%. While most non-referred cases had MMR evaluation, 20% were not referred in spite of tumour pathology, indicating missed opportunities for hereditary cancer risk detection.

Conclusion: This gap in referrals underscores the urgent need for increased awareness and improved referral practices among medical professionals. Prioritizing early detection through timely referrals is essential not only for initiating preventive strategies and enhancing patient outcomes but also for identifying at-risk relatives and substantially reducing the community's cancer burden. Proactively addressing these gaps is crucial, ensuring effective management of hereditary colorectal cancer syndromes towards a more preventive healthcare model.

Grants:NA

Conflict of Interest: None declared

P01.078.B An interdisciplinary approach to counsel children with cancer predisposition and their families for genetic testing and surveillance

Nicola Dikow[1], *Anna Lisa Nitschke*[1;2], *Steffen Hirsch*[1;2;3], *Joachim Kunz*[2;4;5], *Kerstin Grund*[1], *Peggy Lüttich*[6], *Christian Sutter*[1], *Katrin Hinderhofer*[1], *Cornelis M. van Tilburg*[2;4;6], *Olaf Witt*[2;4;6], *Andreas Kulozik*[2;4;6], *David Jones*[2;5], *Maja Hempel*[1], *Till Milde*[2;4;6], *Christian Schaaf*[1], *Stefan Pfister*[2;4;6], *Kristian Pajtler*[2;4;6]

[1]*Heidelberg University, Institute of Human Genetics, Heidelberg, Germany;* [2]*Hopp Children's Cancer Center Heidelberg (KiTZ), and National Center for Tumor Diseases (NCT), Heidelberg, Germany;* [3]*German Cancer Consortium (DKTK), German Cancer Research Center (DKFZ), Division of Pediatric Neurooncology, Heidelberg, Germany;* [4]*German Cancer Research Center (DKFZ) and German Cancer Consortium (DKTK), Heidelberg, Germany, Clinical Cooperation Unit Pediatric Oncology, Heidelberg;* [5]*German Cancer Consortium (DKTK), German Cancer Research Center (DKFZ), Heidelberg, Division of Pediatric Glioma Research, Heidelberg;* [6]*Heidelberg University Hospital, Heidelberg, Germany, Department of Pediatric Oncology, Hematology, Immunology & Pulmonology*

Background: The knowledge of cancer predisposition syndromes (CPS) is the prerequisite for surveillance programs, that have proven benefits in an increasing number of CPS. Our interdisciplinary expert panel discusses, evaluates and counsels children with suspected CPS and their families. Here, we report the diagnostic rates and surveillance recommendations.

Patients and Methods: All individuals seen in the interdisciplinary consultation (2018 - 2022) were included for retrospective analysis. Data on medical and family history, physical examination, molecular and histological tumor characteristics, diagnosis and surveillance recommendations were retrieved from medical records.

Results: Of 144 individuals (median age 9 years), 99 had symptoms of CPS (including 52 with a tumor diagnosis); 45 were clinically unaffected and presented for predictive genetic testing of familial genetic variants. A CPS was diagnosed prior to consultation in 33/99 patients and unknown in 66/99. For 57/66 patients, molecular analysis was performed. A CPS was detected in 26 patients (45,5%), and in 8/10 (80%) of those with more than one type of cancer. The most frequently affected genes were *NF1*, *TP53*, *MEN1*, *APC* and *PTEN*.

Surveillance recommendations could be given for almost all individuals with a CPS diagnosis.

Conclusion: Our interdisciplinary approach contributes to the identification of CPS in pediatric patients and families at risk and highlights the need for personalized surveillance recommendations established by an expert panel, especially in rare CPS. Our approach is intended to lay the foundation for more systematic CPS assessment and the establishment of standardized surveillance concepts.

Conflict of Interest: None declared

P01.079.C Hematological malignancies as a secondary finding on diagnostic exome sequencing: should we consider reporting them?

Gaber Bergant[1], *Aleš Maver*[1], *Borut Peterlin*[1]

[1]*University Medical Center Ljubljana, Clinical Institute of Genomic Medicine, Ljubljana, Slovenia*

Background/Objectives: The detection of somatic secondary findings (SFs) during diagnostic next-generation sequencing presents significant challenges in the context of patients with suspected rare disorders with no existing international guidelines. The differentiation between somatic and germline variants is a complex aspect of genetic analysis, with implications for diagnostic accuracy and patient care.

Methods: We conducted a detailed analysis of four cases where patients underwent diagnostic exome sequencing for suspected rare disorders in which we detected somatic secondary findings associated with hematological malignancies.

Results: Our investigation revealed the presence of somatic SFs in the patient samples, which were associated with hematological malignancies. We found that these somatic SFs could potentially influence the primary diagnostic interpretation, underscoring the difficulty in distinguishing them from germline variants. The analysis highlights the complexity of interpreting exome sequencing data when somatic variants are present.

Conclusion: Our findings underscore the urgent need for clear guidelines on how to analyze and report somatic secondary findings in diagnostic exome sequencing. This work emphasizes the importance of increased awareness and a systematic approach to the reporting of SFs that are unrelated to germline genomic variations. The presence of somatic SFs has significant implications for patient care, and addressing this challenge is crucial for the advancement of precision medicine.

Grants: None

Conflict of Interest: None declared

P01.080.D Differences in polygenic score distributions in European ancestry populations: implications for breast cancer risk prediction

Kristia Yiangou[1], *Nasim Mavaddat*[2], *Joe Dennis*[2], *Maria Zanti*[1], *Jacques Simard*[3], *Antonis C. Antoniou*[2], *Douglas F. Easton*[2;4], *Kyriaki Michailidou*[1]

[1]*The Cyprus Institute of Neurology and Genetics, Biostatistics Unit, Nicosia, Cyprus;* [2]*University of Cambridge, Centre for Cancer Genetic Epidemiology, Department of Public Health and Primary Care, Cambridge, United Kingdom;* [3]*Centre Hospitalier Universitaire de Québec – Université Laval Research Center, Genomics Center, Québec City, Québec, Canada;* [4]*University of Cambridge, Centre for Cancer Genetic Epidemiology, Department of Oncology, Cambridge, United Kingdom*

Consortium: Breast Cancer Association Consortium (BCAC)

Background/Objectives: Polygenic risk scores (PRSs) provide a promising tool for personalized breast cancer (BC) risk prediction. A 313-variant PRS (PRS-313) has been constructed and is being used in several clinical trials of risk-based screening. However, specific evaluation of its distribution across different European populations has not been performed which could be potentially important for accurate risk estimation.

Methods: We explored the distribution of PRS-313 across European ancestry populations, using data from 94,072 females without BC from 21 countries participating in the Breast Cancer Association Consortium (BCAC) and from 225,105 white females without BC participating in the UK Biobank. We calculated the mean PRS by country in BCAC and by country of birth in UK Biobank. We investigated the implications of PRS distribution variability across the countries in BC risk prediction and potential approaches to account for the observed distribution variability.

Results: The mean PRS differed markedly by country in both BCAC and UK Biobank; being highest in the south-east (e.g. Cyprus, Greece, Italy) and lowest in the north-west (e.g. Ireland). Using the PRS distribution to define risk categories leads to risks over- or underestimation in a proportion of individuals from south-eastern and north-western countries, respectively.

Adjustment for the leading ancestry informative principal components explained the differences in PRS distribution in both datasets.

Conclusion: PRS distributions differ across European ancestry populations. Country-specific distributions are required to provide well calibrated risk estimates. These approaches could also be used for the evaluation of PRSs for other diseases.

Grants: None

Conflict of Interest: None declared

P01.081.A Stress granules: from rare disorder to potential novel cancer treatment

Johannes Lederbauer[1], *Davor Lessel*[1], *Prof. Dr. rer. nat. Hans-Jürgen Kreienkamp*[2]

[1]*University Hospital Salzburg, Institute of Human Genetics, Salzburg, Austria;* [2]*University Hospital Hamburg-Eppendorf, Institute of Human genetics, Hamburg, Germany*

Background/Objectives: Stress granules (SGs) are cytoplasmic aggregates that assemble upon stress to block the translation and thereby provide a protective mechanism. However, hyper-assembly of SGs is observed in many cancers and thought to cause chemotherapy resistance. Recently, we linked the RNA helicase DHX30 to a rare, neurodevelopmental syndrome, and found that underlying, heterozygous missense variants cause an increased propensity to trigger SG formation.

Methods: We utilized DHX30-deficient cellular and animal models, different SG inducing agents including different chemotherapeutics and analysis on protein and RNA level to further investigate the role of DHX30 in SG assembly.

Results: Our in depth analyses identified *DHX30* as the first essential gene for general SG assembly. Mechanistic analyses revealed that DHX30 interacts via RNA with two main nucleators of SGs, G3BP1 and Caprin1. Notably, expression of both proteins was significantly reduced in *DHX30* deficient models upon several stressors. Overexpression of both proteins in DHX30 deficient cells led to formation of SGs thus providing a mechanistic understanding. In addition, highly prevalent, somatic, *KRAS* missense variants (p.Gly12/Asp/Cys/Val) have been shown to enhance SG formation in pancreatic tumours. Interestingly, *DHX30* silencing mitigated SG hyper-assembly in *KRAS*-mutant cancer cells. Similar results were obtained when *KRAS*-mutant plasmid were overexpressed in *DHX30* deficient cell lines.

Conclusion: By analysing a rare Mendelian disorder, we identified *DHX30* as the first essential SG assembly gene. Furthermore, we show that molecular findings from such rare disorders can be translated to common diseases. Indeed, we identified a potential novel molecular target to treat *KRAS*-mutated cancers.

Grants: Fritz Thyssen Stiftung

Conflict of Interest: None declared

P01.083.C How does polygenic risk score (PRS) modify breast cancer (BC) risk estimation in carriers versus non-carriers of a moderate pathogenic variant (PV)?

Laura Duran-Lozano[1], *Alejandra Rezqallah*[1;2], *Adrià López-Fernández*[1;2], *Mònica Pardo*[3], *Eduard Perez*[1;2], *Esther Darder*[4], *Rosa Alfonso*[5], *Ana Raquel Jimenez-Macedo*[6], *Mireia Cartro*[7], *Mara Cruellas*[1;2], *Estela Carrasco López*[1;2], *Adriana Bareas*[1], *Maite Torres*[8], *Anna Vallmajo*[9], *Victor Navarro*[10], *Ariadna Roque*[8], *Lidia Feliubadaló*[8], *Conxi Lazaro*[8], *Noemi Tuset*[9], *Martín Espinosa-Bravo*[2], *Teresa Ramon y cajal*[5], *Joan Brunet*[8], *Judith Balmaña*[1;2]

[1]*Vall d'Hebron Institute of Oncology (VHIO), Hereditary Cancer Genetics Group;* [2]*Vall d'Hebron Hospital, Medical Oncology, Barcelona, Spain;* [3]*Hospital del Mar, Medical oncology service. Genetic Counselling Unit, Barcelona, Spain;* [4]*Catalan Institute of Oncology, Genetic Counselling Unit. Hereditary Cancer Program, Spain;* [5]*Hospital de Sant Pau, Familial Cancer Clinic. Medical Oncology, Barcelona, Spain;* [6]*Consorci Sanitari de Terrassa, Genetic Counselling Unit, Spain;* [7]*Corporació Sanitària Parc Taulí, Genetic Counselling Unit, Spain;* [8]*Catalan Institute of Oncology, Genetic Counselling Unit. Hereditary Cancer Program., Spain;* [9]*Hospital Universitari Arnau de Vilanova, Genetic Counselling in Familial Cancer Unit. Medical Oncology Service., Lleida, Spain;* [10]*Vall d'Hebron Institute of Oncology (VHIO), Oncology Data Science (ODySey) Group, Spain*

Consortium: PRiSma

Background/Objectives: BC risk is a multifactorial phenotype, and incorporation of PRS in risk estimation can lead to higher accuracy accounting for a bigger proportion of the genetic risk fraction. We aimed to explore the risk distribution in different populations of women at risk and investigate the effect of incorporating PRS in their risk estimation.

Methods: PRS genotyping was performed using the $PRISMA_{268}$ array. BC risk was estimated with the BOADICEA.v6 model using personal and family history, breast density (BD) and then compared to estimations including PRS in the same model.

We estimated the 10-year risk in two populations of healthy women: (1) 133 women carrying a germline PV in *ATM, BARD1, CHEK2, PALB2 or RAD51C/D* and (2) 593 women with a BC family history and negative genetic test. Patients were classified in average, moderate, or high risk following the NICE guidelines. t-test and X2 were used to assess differences in BC risk estimation between carriers and non-carriers and the change of distribution when adding PRS.

Results: There were no statistically significant differences in 10-year BC risk between carriers and non-carriers (7.43% vs 4.10%, $p = 0.55$). Adding PRS led to re-classification of 28% and 22% of carriers and non-carriers, respectively($p = 0.16$). Overall, 11-15% increased their risk group and 11-13% decreased their risk group.

Conclusion: Risk stratification for carriers of moderate penetrance variants is comparable to non-carriers with family history. Incorporation of PRS_{268} leads to change in risk group in 20-30% of healthy individuals with a family history, regardless of their carrier status.

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Conflict of Interest: None declared

P01.084.D Cytotoxic effect of thymoquinone with tamoxifen on hormone-positive breast cancer cells

Vildan Betul Yenigun[1], *Ebru Kanimdan*[1], *Ayfer Sendul*[2], *Abdurrahim Kocyigit*[2]

[1]*Bezmialem Vakıf University, Vocational School of Health Services, Istanbul, Türkyie;* [2]*Bezmialem Vakıf University, Medical Biochemistry, Istanbul, Türkyie*

Background/Objectives: Breast cancer is the most common cancer among women. Hormonal therapy is frequently used for hormone receptor-expressing breast cancers which is the most common form. Tamoxifen (TAM) is an estrogen receptor antagonist which competes with estrogen to bind to its receptor. Thymoquinone (TQ) is the main bioactive component of black cumin essential oil with anti-cancer properties. Recent studies have shown that using TQ alone or with other chemotherapeutics is effective on cancer cells. Therefore, this study aimed to examine the possible synergistic effect of the combined use of TAM and TQ on breast cancer.

Methods: ER+ breast cancer cells (MCF-7) were used in this study to investigate the combination effect of TAM and TQ. MTT cell viability test was used to calculate both drugs' half-maximal inhibitory concentrations (IC50) at 24 h and observe the cell viability after they were co-administered. Apoptosis was detected by Annexin V/PI in addition to Acridine Orange/Ethidium Bromide (AO/EB) staining.

Results: Statistical difference in cell death was observed starting from 25 µM TQ with an IC_{50} value of 74,7 µM in 24 hours. TAM induced statistical cell death starting from 10 µM concentration. Combining TQ with TAM showed apparent synergism, decreasing the IC_{50} value of TAM. Combination index analysis showed additive effect with CI values of 1,07. Apoptosis was also increased with a non-toxic dose of TAM (5 µM) and TQ combination.

Conclusion: The results of this study showed that TQ could synergistically increase the efficacy of TAM on ER+ breast cancer cells.

Conflict of Interest: None declared

P01.085.A Towards characterizing the DNA methylome of classic Hodgkin lymphoma

Nadine Matscheko[1], *Anja Fischer*[1], *Selina Glaser*[1], *Cosima Drewes*[1], *Björn Brändl*[2;3], *Franz-Josef Müller*[3], *Helene Kretzmer*[2], *Ole Ammerpohl*[1], *Reiner Siebert*[1]

[1]*Institute of Human Genetics, Ulm University and Ulm University Medical Center, Ulm, Germany;* [2]*Max Planck Institute for Molecular Genetics, Department of Genome Regulation, Berlin, Germany;* [3]*Zentrum für Integrative Psychiatrie gGmbH, Universitätsklinikum Schleswig-Holstein Campus Kiel, Kiel, Germany*

Background/Objectives: Classic Hodgkin's lymphoma (cHL) is a B-cell tumor characterized by B-cell identity loss, chromosomal instability, and aberrant long tandem repeat expression. Malignant mono-nucleated Hodgkin and multi-nucleated Reed-Sternberg cells (HRS) constitute only minorities in tumor samples, making (epi-)genetic alteration screening challenging. Here, cHL cell lines offer promising alternatives and were examined for HRS-specific DNA methylation compared to B-cell lymphoma cell lines (BCL) and normal B-cell populations (BCs).

Methods: Bisulfite-converted DNA from 10 cHL, 45 BCL cell lines, and 110 BCs was investigated using Illumina HumanMethylation450K and EPIC BeadChips. Significantly differentially methylated cytosines (DMCs/CpGs) in cHL cell lines were identified (FDR ≤ 0.001, $\Delta\beta_{mean} \geq 0.4$). Moreover, 6 cHL and 10 BCL cell lines were sequenced with Oxford Nanopore (ONT), data was processed with ONT softwares dorado and modkit, and DMCs were identified (FDR ≤ 0.001).

Results: Array data-based analysis identified 4493 hyper- and 264 hypomethylated CpGs in cHL cell lines compared to BCL cell lines and BCs. Associated genes were enriched for B-cell receptor signaling, transcription, and cytoskeleton functions. Comparing ONT data of cHL and BCL cell lines, the former revealed 40,432 hypermethylated and 17,192 hypomethylated DMCs, with 10.08% and 1.22% overlap, respectively, with array-identified DMCs. Thereby, hypermethylated sites were enriched for promoter-sequences.

Conclusion: Compared to BCs and BCL cell lines, cHL cell lines predominantly exhibit hypermethylated, promoter-localized DMCs. However, the contribution of differentially methylated B-cell receptor signaling genes to B-cell identity loss is still unclear. Furthermore, investigating affected cytoskeleton functions would be worthwhile as they may affect cytokinesis, potentially leading to multi-nucleated HRS.

Grants: None.

Conflict of Interest: None declared

P01.086.B Assessment of pathogenic germline variants in patients with luminal B subtype breast cancer.

Andressa Almeida[1], *Karina Miranda Santiago*[1], *Fabiana Makdissi*[1], *José Rocha*[1], *Giovana Torrezan*[1], *Dirce Carraro*[1]

[1]*A.C. Camargo Cancer Center, Brazil*

Background/Objectives: This study aimed to examine the prevalence of germline pathogenic variants (GPVs) in luminal B breast cancer and correlate these with patient clinical data.

Methods:A retrospective study was conducted on an unselected series of 69 Brazilian women diagnosed with luminal B breast cancer, with samples available at the biobank of the A.C.Camargo Cancer Center. Participants underwent genetic sequencing of a 112-gene panel related to hereditary cancer predisposition, with clinical data from medical records review..

Results: Of the 69 patients, 20% (14/69) presented GPVs in the genes: *CHEK2* (4 patients), *BRCA2* (2), *TP53* (2), *ATM* (1*), BARD1 (*1), *BLM* (1), *BRIP1* (1), *LZTR1* (1), *MUTYH* (1) and *RAD51C* (1). GPVs carriers showed an earlier cancer diagnosis compared to non-carriers (p-value 0,021), ranging between 31 and 72 years, median of 39.5 years. 57% (8/14), were diagnosed before 45 years of age, 21% (3/14) between 46 and 60 years, and 14% (2/14) after 60 years. HER2 overexpression was observed in 64% (9/14) of cases, and the ductal histological subtype was predominant in 85% (12/14) of cases.

Conclusion: We identified a high incidence of GPVs in luminal B breast cancer, and an earlier onset of cancer in these patients.The research revealed genetic diversity, including a low incidence of variants in the *BRCA1* and *BRCA2* genes.Despite a small sample, variants in other genes and high HER2 overexpression offer new insights, highlighting the need for research on less studied breast cancer subtypes.Future research should delve into how genetic variants, HER2 overexpression, and histological subtypes interrelate to clarify hereditary breast cancer's origins.

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Conflict of Interest: None declared

P01.087.C miRNA Profiling Identifies Potential Risk Biomarkers in Familial Prostate Cancer Cases

Lucía Chica Redecillas[1;2], *Juan Miguel Guerrero-González*[2], *Sergio Cuenca López*[1], *Elena Arance*[2], *Fernando Marín-Benesiu*[1;2], *Carmen María Morales-Álvarez*[1;2], *Maria Jesus Alvarez Cubero*[1;2;3], *Luis Javier Martínez-González*[1;2]

[1]*Faculty of Medicine - University of Granada, Department of Biochemistry and Molecular Biology III and Immunology, Faculty of Medicine, Granada, Spain;* [2]*Centre for Genomics and Oncological Research: Pfizer, University of Granada, Andalusian Regional Government (GENYO), Granada, Spain;* [3]*Biosanitary Research Institute (ibs. GRANADA), University of Granada, Spain*

Background/Objectives: About 5-10% of prostate cancer (PC) cases have a hereditary component. Men with a family history of this disease face a twofold increased risk, with a tendency for more aggressive cancer. Current guidelines for early diagnosis and patient stratification are inadequate. Present study aims to identify potential miRNA biomarkers for the detection and stratification of high-risk familial PC.

Methods: We analysed miRNA expression in two PC multicase families. We used the edgeR package to study the transcriptome and only considered results with FDR < 0.05 as significant. To

ensure the validity of the results, we compared them with The Cancer Genome Atlas (TCGA) database.

Results: Eight miRNAs significantly expressed in hereditary PC patients were identified and validated by TCGA: hsa-miR-205-5p, hsa-miR-197-3p, hsa-miR-144-5p, hsa-miR-423-3p, hsa-miR-101-3p, hsa-miR-501-5p, hsa-miR-671-3p and hsa-miR-744-5p. Enrichment analysis revealed the involvement of target genes of identified miRNAs, such as *SOX9, ZEB2, FOXO3* and *LRRK2*, in crucial pathways related to cancer development, highlighting the Wnt signaling pathway. Construction of miRNA-mRNA network also revealed a high interconnection among identified miRNAs with genes involved in the Wnt pathway.

Conclusion: miRNAs have the potential to be significant cancer biomarkers. Specifically, hsa-miR-205-5p, hsa-miR-101-3p and hsa-miR-144-5p play important roles in crucial pathways in PC. Our study underscores the importance of the Wnt signaling pathway in the development of hereditary PC. These findings are consistent with our previous research on this disease.

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Conflict of Interest: None declared

P01.088.D Germline variants of homology-directed repair or mismatch repair genes in cervical cancer

Lara Kokemüller[1], *Dhanya Ramachandran[1], Peter Schürmann[1], Geffers Robert[2], Gerd Böhmer[3], Hans Georg Strauss[4], Christine Hirchenhain[5], Schmidmayr Monika[6], Florian Müller[7], Peter Fasching[8], Norman Häfner[9], Alexander Luyten[10;11], Matthias Jentschke[1], Peter Hillemanns[1], Thilo Dörk[1]*

[1]Hannover Medical School, Department of Gynaecology, Hannover, Germany; [2]Helmholtz Centre for Infection Research, Braunschweig, Germany; [3]IZD Ärztliche Partnerschaft Böhmer & Partner, Hannover, Germany; [4]Martin-Luther-University Halle-Wittenberg, Gynecology Department, Halle (Saale), Germany; [5]University Hospital Carl Gustav Carus Dresden, Gynecology Department, Dresden, Germany; [6]Technical University of Munich, Gynecology Department, München, Germany; [7]Martin Luther Hospital, Berlin, Germany; [8]University of Erlangen-Nuremberg, Department of Gynecology and Obstetrics, Erlangen, Germany; [9]Friedrich Schiller University Jena, Department of Gynecology, Jena, Germany; [10]MARE Klinikum, Dysplasia Unit, Department of Gynecology and Obstetrics, Kronshagen, Germany; [11]Hospital of the city of Wolfsburg, Wolfsburg, Germany

Consortium: Cervigen consortium

Background/Objectives: While cervical cancer is associated with HPV infection, it appears to be influenced by genomic risk factors which have remained largely obscure. Pathogenic variants in genes of homology-directed repair (HDR) or mismatch repair (MMR) are known to predispose to diverse malignancies including breast and ovarian cancer (HDR) or colon and endometrial cancer (MMR). We here investigate the spectrum of HDR and MMR germline variants in cervical cancer, stratified by HPV status and histological subgroups.

Methods: We performed targeted next-generation sequencing of eleven HDR genes and five MMR genes on genomic DNA samples from 555 German patients with cervical dysplasia or invasive cancer. Variants were classified as pathogenic or likely pathogenic based on ClinVar categories and additional ESM1b and AlphaMissense prediction.

Results: 5 % of our patients carried a pathogenic or likely pathogenic germline variant. These included eight patients (1.4%) with truncating variants in BRCA1, BRCA2, BARD1 or BRIP1. While one patient with HPV-negative adenocarcinoma carried a pathogenic MMR gene variant (in MSH6), HDR germline variants were associated with HPV-positive squamous invasive cancer. Age at diagnosis of invasive cancer tended to be lower in HDR variant carriers (38.8 ys) compared to non-carriers (46.3 ys).

Conclusion: Our study indicates a potential risk-modifying role of HDR germline variants in a small fraction of cervical cancer patients but no association with HPV-negative status.

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P01.089.C Comparative sequencing study of mismatch repair and homology-directed repair genes in endometrial cancer and breast cancer patients from Kazakhstan

Ying Zheng[1], **Natalia Vdovichenko**[1], *Peter Schürmann[1], Dhanya Ramachandran[1], Geffers Robert[2], Lisa-Marie Speith[1], Natalia Bogdanova[1;3], Julia Enßen[1], Natalia Dubrowinskaja[1], Tatyana Yugai[4], Zura Berkutovna Yessimsiitova[5], Nurzhan Turmanov[1], Peter Hillemanns[1], Thilo Dörk[1]*

[1]Hannover Medical School, Gynaecology Research Unit, Hannover; [2]Helmholtz Institute for Infection Research, Genome Analytics, Braunschweig; [3]Hannover Medical School, Radiation Oncology Research Unit, Hannover; [4]Rahat Clinics, Almaty, Kazakhstan; [5]al-Farabi Kazakh National University, Department of Biodiversity and Bioresources, Almaty, Kazakhstan

Background/Objectives: Endometrial cancer has been associated with pathogenic variants in mismatch repair (MMR) genes, especially in the context of the hereditary Lynch Syndrome. More recently, pathogenic variants in genes of homology-directed repair (HDR) have also been suggested to contribute to a subset of endometrial cancers although their causal role is debated.

Methods: In the presented targeted sequencing study, we investigated the relative distribution of pathogenic MMR or HDR gene variants in a hospital-based series of 351 endometrial cancer patients from the Oncology Clinic in Almaty, Kazakhstan. In a direct comparison, we also sequenced 179 breast cancer patients from the same population with the same panel of 16 genes. Identified variants were classified according to ClinVar, ESM1b and AlphaMissense prediction tools.

Results: We found 10 endometrial cancer patients (2.8 %) carrying pathogenic or likely pathogenic variants in MMR genes while 14 endometrial cancer patients (4.0 %) carried pathogenic variants in HDR genes. In the breast cancer series, we found nine carriers (4.8%) of pathogenic or likely pathogenic variants in MMR genes while 14 patients (7.4%) harbored pathogenic or likely pathogenic HDR gene variants. One patient who developed breast cancer first and endometrial cancer later carried a novel frameshift variant in *MSH6*.

Conclusion: Our results indicate that pathogenic MMR and HDR gene variants occur at substantial frequencies in both breast and endometrial cancer patients from the Kazakh population.

Grants: Funded by the Wilhelm Sander Foundation.

Conflict of Interest: None declared

P01.090.A Genomic Risk Variants and HPV Infection Modulate Gene Expression at the Human Leukocyte Antigen Locus in Cervical Cancer

Rieke Eisenblätter[1], *Finja Seifert*[1], *Theresa Beckhaus*[1], *Peter Schürmann*[1], *Gerd Böhmer*[2], *Hans Georg Strauss*[3], *Christine Hirchenhain*[4], *Schmidmayr Monika*[5], *Florian Müller*[6], *Peter Fasching*[7], *Norman Häfner*[8], *Alexander Luyten*[9;10], *Matthias Jentschke*[1], *Peter Hillemanns*[1], *Thilo Dörk*[1], *Dhanya Ramachandran*[1]

[1]*Hannover Medical School, Department of Gynecology, Hannover, Germany;* [2]*IZD Ärztliche Partnerschaft Böhmer & Partner, Hannover, Germany;* [3]*Martin-Luther-University Halle-Wittenberg, Department of Gynaecology, Halle (Saale), Germany;* [4]*University Hospital Carl Gustav Carus Dresden, Department of Gynaecology, Dresden, Germany;* [5]*Technical University of Munich, Department of Gynaecology, München, Germany;* [6]*Martin Luther Hospital, Berlin, Germany;* [7]*University of Erlangen-Nuremberg, Department of Gynecology and Obstetrics, Erlangen, Germany;* [8]*Friedrich Schiller University Jena, Department of Gynecology, Jena, Germany;* [9]*MARE Klinikum, Dysplasia Unit, Department of Gynecology and Obstetrics, Kronshagen, Germany;* [10]*Hospital of the city of Wolfsburg, Wolfsburg, Germany*

Consortium: Cervigen consortium

Background/Objectives: Human papillomavirus (HPV) infection triggers cervical cancer development, and genome-wide association studies have identified multiple genomic variants at the human leukocyte antigen (HLA) locus (6p21.32-33). The targets and mode of action at this locus are not well understood. We here aim to refine these associations in an independent case-control series and investigate their functional relevance by eQTL analysis in cervical specimens.

Methods: We genotyped candidate variants at the HLA locus in a German series of invasive cervical cancers, dysplasias, and healthy controls (n > 1,000 in each group). We then used stepwise conditional logistic regression analyses to identify independent signals and tested these variants for their impact on 36 HLA gene transcripts in 200 cervical tissue samples.

Results: The HLA region contained at least four independently associated risk variants for cervical cancer. In addition to previous findings from our cohort, rs17190106 associated with overall cervical disease and invasive cancer, and rs535777 associated with adenocarcinoma. We identified mRNA transcripts upregulated in HPV-positive samples. One variant, rs9272117, affected the expression of multiple genes across the HLA region.

Conclusion: We confirm the presence of independent cervical cancer risk signals at 6p21 in our hospital-based cohort. We identify genomic variants that modulate gene transcript levels together with HPV, indicating that highly controlled gene regulation underlies cervical cancer susceptibility at the HLA locus. These genes may act in concert to modulate the host immune response towards HPV infection.

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P01.091.B Germline PTEN pathogenic variant in a patient with Kaposiform haemangioendothelioma

Hannah Massey[1], *Jennie Murray*[1], *larry hayward*[1]

[1]*Western General Hospital, United Kingdom*

Background/Objectives: Kaposiform haemangioendothelioma (KHE) is a rare vascular neoplasm usually presenting in childhood. Its high morbidity and mortality is secondary to compression, invasion, and coagulopathy. The genetics of KHE remain poorly understood. Studies have detected somatic variants in GNA14 and germline variants in PIKC3A contributing to tumorigenesis via the Ras and mTOR pathways respectively. We present the first case of a germline change in PTEN identified in the context of Kaposiform haemangioendothelioma.

Methods: A 28-year-old female with metastatic KHE was enrolled in the IMAGINE study (Integrating Medically Actionable Genomics Into Early Phase Trials).

Results: A PTEN heterozygous c.277C>Tp.(His93Tyr) pathogenic variant was detected initially in tumour and subsequently saliva indicating a constitutional genetic change. Reverse phenotyping revealed the patient had a large head circumference, palmar keratosis and papillomas on the hand, feet, and gums, in keeping with PTEN-Hamartoma tumour syndrome (PHTS).

Conclusion: We report the first case of a constitutional PTEN pathogenic variant in association with a KHE. PHTS is a well characterised tumour predisposition syndrome with an 85% lifetime tumour risk. While over 50% of patients with pathogenic PTEN variants have vascular anomalies no malignant vascular tumours have previously been reported. Pathogenic variants in PTEN lead to loss of protein function with a subsequent upregulation of mTOR signalling. Upregulation of mTOR signalling is commonly observed in cancer. This raises the question as to whether mTOR inhibitors could be used in the treatment of KHE.

Grants: None

Conflict of Interest: None declared

P01.092.C Genomic risk loci for cervical cancer: Associations with disease severity and HPV type

Theresa Beckhaus[1], *Linda Kachuri*[2], *Finja Seifert*[1], *Rieke Eisenblätter*[1], *Dandan Liao*[1], *Peter Schürmann*[1], *Gerd Böhmer*[3], *Hans Georg Strauss*[4], *Christine Hirchenhain*[5], *Schmidmayr Monika*[6], *Florian Müller*[7], *Peter Fasching*[8], *Norman Häfner*[9], *Alexander Luyten*[10;11], *Matthias Jentschke*[1], *Peter Hillemanns*[1], *Francis Stephen*[12], *John Witte*[2], *Thilo Dörk*[1], *Dhanya Ramachandran*[1]

[1]*Hannover Medical School, Department of Gynecology, Hannover, Germany;* [2]*Stanford University, Department of Epidemiology and Population Health, Stanford, United States;* [3]*IZD Ärztliche Partnerschaft Böhmer & Partner, Hannover, Germany;* [4]*Martin-Luther-University Halle-Wittenberg, Department of Gynaecology, Halle (Saale), Germany;* [5]*University Hospital Carl Gustav Carus Dresden, Department of Gynaecology, Dresden, Germany;* [6]*Technische Universität München, Department of Gynaecology, München, Germany;* [7]*Martin Luther Hospital, Berlin, Germany;* [8]*Friedrich-Alexander University Erlangen-Nuremberg Department of Law (Juridicum), Department of Gynecology and Obstetrics, Erlangen, Germany;* [9]*Friedrich Schiller University Jena, Department of Gynecology, Jena, Germany;* [10]*MARE Klinikum, Dysplasia Unit, Department of Gynecology and Obstetrics, Kronshagen, Germany;* [11]*Hospital of the city of Wolfsburg, Wolfsburg, Germany;* [12]*University of California, San Francisco, Department of Neurological Surgery, San Francisco, United States*

Consortium: Cervigen consortium

Background/Objectives: Persistent infection by high-risk human papillomavirus triggers the development of cervical cancer and dysplasia. Genome-wide association studies for cervical cancer have so far identified multiple genomic risk variants at the human leukocyte antigen (HLA) locus (6p21.32-33), as well as variants on chromosome 2 (PAX8), 5 (near CLPTM1L) and 17 (GSDMB).

Methods: We directly genotyped reported variants, as well as 15 novel variants derived from a HPV seropositivity GWAS, in a German case-control series of invasive cervical cancer, dysplasia, and healthy controls (N > 1,000 in each group). We performed unadjusted logistic regression analyses stratified by histology and HPV type. Selected variants were further investigated for their impact on RNA transcript levels in cervical specimens.

Results: We replicated risk loci in the HLA region as well as at PAX8 and GSDMB at $p < 0.05$ in our independent case-control series, although the GSDMB locus was only associated with cervical dysplasia. We found evidence that HPV16 and HPV18 seropositivity variants at chromosome 6 and 14 associate with cervical cancer risk in an HPV type-specific manner. rs9357152 may exert its effect through inducing HLA-DRB1 in the presence of HPV. Genotyping of further suggestive HPV-type specific signals from the seropositivity GWAS provided evidence of association at $p < 0.05$ for seven additional cervical cancer risk regions.

Conclusion: Our results support the view that several genomic risk loci exist for cervical dysplasia and cancer, and some of them may be HPV-type specific.

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P01.093.D Precision medicine from the renal cancer genome

Yasser Riazalhosseini[1]

[1]*McGill University, Human Genetics, Montreal, Canada*

Background/Objectives: Renal cell carcinomas (RCC) are characterized by their inherent resistance to chemotherapy and radiation and their largely diverse clinical outcomes. Leveraging genomic diversity among individual tumors, our research during the past decade has concentrated on developing personalized medicine approaches for the most common and the most aggressive type of RCC, clear cell renal carcinoma (ccRCC).

Methods: We generated the largest dataset of ccRCC including somatic genomic and clinical annotations for over 940 ccRCC patients, and developed a genomic classifier, based on mutational status of 12 RCC-relevant genes, which is able to stratify patients according to their risk of relapse after nephrectomy and/or death due to RCC.

Results: The performance of this classifier, which is independent from tumor stage and patient age, is validated for 75% of ccRCC patients whose tumors are affected by mutations of *VHL* gene. We have expanded the application of our 12-gene RCC assay to liquid biopsy analysis, aiming at developing a non-invasive approach for the application of our classifier, and establishing early detection of relapse in high-risk patients. Additionally, we observe a different genomic evolution trajectory of disease progression in ccRCCs that do not harbor VHL mutations. Our ongoing research focuses on understanding the distinct biology of these tumors using single-cell and spatial genomics, and our cohort of patient-derived organoid models of aggressive ccRCCs to identify novel therapeutic targets.

Conclusion: At the conference, I will present this decade-long endeavor and will discuss how our Pan-Canadian 'Precision RCC' program leverages this legacy to establish personalized medicine in RCC.

Grants:

Conflict of Interest: None declared

P01.094.A Predicting mutation risk at nucleotide scale using transformer neural networks

Marcell Veiner[1,2], *Iván Galván-Femenía*[1], *Daniel Naro*[1,3], *Fran Supek*[1]

[1]*IRB Barcelona - Institute for Research in Biomedicine, Genome Data Science, Barcelona, Spain;* [2]*Universitat de Barcelona, Faculty of Medicine and Health Sciences, Barcelona, Spain;* [3]*Universitat Politècnica de Catalunya · Barcelona Tech - UPC, Barcelona, Spain*

Background/Objectives: Somatic mutation rates in cancer vary across the human genome at different scales. At the megabase- and gene-scale variation is well-studied, the mutation rate heterogeneity at sub-gene scales remains less explored. The aim of this contribution is to evaluate somatic mutation rates of Single Nucleotide Variants (SNV) in the human genome at base-pair resolution (1-300bp), and associate DNA sequence features to each mutational process.

Methods: We separated ~230M SNVs obtained from 12,166 pan-cancer whole-genome sequences into de novo mutational signatures using non-negative matrix factorisation, and fit to known COSMIC signatures. Subsequently, we trained a transformer neural network called MutFormer to differentiate between mutated vs trinucleotide-matched non-mutated sites on 18 SBS signatures, and to guide the discovery process of novel DNA determinants.

Results: Our models were validated on an independent cohort, achieving AUCs ranging from 0.55 on flat signatures (SBS5) to 0.86 on signatures dominated by a few mutation types (SBS17a/b), suggesting wider sequence context for these signatures (5-10bp on each side of the mutation). We found several DNA features shaping the mutational landscape, some as a wider sequence context (e.g. AAAKAATAAAW, SBS10a), while some by being in the vicinity of the mutation (CCWGG, SBS17b). Our method was also applied to model the germline mutation rate, achieving similarly high AUCs.

Conclusion: Overall, our transformers provide a state-of-the-art method for inferring the risk of SNVs occurring given the nearby DNA sequence, with implications to disease gene discovery and evolutionary biology.

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Conflict of Interest: None declared

P01.095.B Dyskeratosis congenita segregation in a large Middle Eastern family reveals a novel pathogenic *TERC* variant

Michal Barzily Rokni[1], *Chezi Ganzel*[2;3], *Malka BenUziyahu*[1], *Adi Zisman*[1], *Rachel Beeri*[1], *Ephrat Levy-Lahad*[1;3]

[1]*Shaare Zedek Medical Center, Medical Genetics Institute, Jerusalem, Israel;* [2]*Shaare Zedek Medical Center, Department of Hematology, Jerusalem, Israel;* [3]*Hebrew University of Jerusalem, Faculty of Medicine, Jerusalem, Israel*

Background: Dyskeratosis congenita (DC) is a rare, heterogeneous genetic disorder caused by defective telomere maintenance resulting in shortened telomeres. *TERC* pathogenic variants (PVs) (the telomerase RNA component) gene cause autosomal dominant DC. The highly variable expression of the disease, even in the same family, makes diagnosis and surveillance recommendations a challenge.

Objectives: To determine the pathogenicity of a *TERC* variant of unknown significance (VUS), using segregation in an extensive Muslim Arab family (over 100 members).

Results: A 50-year-old man with myelodysplastic syndrome (MDS) reported family history of four brothers and a sister who died of leukemia. Exome-based panel testing identified a *TERC* gene variant, n.97T>G (rs1777963567). This variant has been reported once in ClinVar, as a VUS. It is not present in any database, and is located within the template/pseudoknot domain of the *TERC* RNA component, which is required for RNA or RNP stability. We reclassified the VUS as a novel, likely pathogenic variant after: (1) Identifying clinical features of DC in the patient, including: dysplastic nails, oral leukoplakia, pulmonary fibrosis, prematurely gray hair and liver disease. (2) Determining shortened telomere length in the patient's blood. (3) Segregation analysis revealing all affected siblings (four) as heterozygous for the variant and non-affected siblings (five) as negative for the variant. Genetic counseling services and surveillance recommendations were offered to all at risk family members, of which only a few responded.

Conclusion: We report n.97T>G as a novel PV in the *TERC* gene causing DC, in order to further aid identification of and treatment for DC families.

Conflict of Interest: None declared

P01.096.C A rare homozygous germline missense variant of *TP53* causing Li-Fraumeni syndrome

Fuad Chowdhury[1], *Melanie Finkbeiner*[2], *Katie Girgulis*[2], *Ryan Lamont*[3], *Jillian Parboosingh*[3], *Lucie Pecheux*[4], *Renee Perrier*[1]

[1]*University of Calgary and Alberta Children's Hospital, Department of Medical Genetics, Calgary, Canada;* [2]*University of Calgary and Alberta Children's Hospital, Department of Pediatric Hematology-Oncology, Calgary, Canada;* [3]*Molecular Diagnostic Laboratory, Department of Medical Genetics, Calgary, Canada;* [4]*University of Alberta and Stollery Children's Hospital, Department of Pediatric Hematology-Oncology, Edmonton, Canada*

Background: Li-Fraumeni syndrome (LFS) is a cancer predisposition syndrome caused by heterozygous variants in *TP53* often through loss-of-function mechanisms. Subsequent somatic loss-of-heterozygosity is frequently observed in tumour samples of many cancer types, while bi-allelic germline variants are extremely rare in individuals with LFS. Most of these individuals are homozygous for the lowly-penetrant R337H founder variant.

Methods: Clinical panel or single-gene sequencing was performed on proband peripheral blood DNA to identify *TP53* variants. Subsequently, first-degree relatives were tested for inheritance of the *TP53* variant, when possible.

Results: We report two previously healthy and unrelated girls diagnosed with adrenocortical carcinoma (ACC). Both presented with several months of virilization and clinical evidence of androgen excess. The first patient is a 5-year-old girl of Pakistani ancestry with consanguineous parents who is homozygous for a NM_000546.6(*TP53*):c.328C>T (p.R110C) germline variant of uncertain significance (VUS). The second is a 2-year-old girl presenting additionally with hypercortisolism and she is heterozygous for the same *TP53* variant. Neither family had a history of LFS-spectrum cancers.

Conclusions: The presence of a germline R110C variant in two unrelated girls with a rare ACC provides strong clinical evidence that *TP53* p.R110C can cause LFS. Pathogenic variants affecting the same residue have been described. Furthermore, previous in vitro functional characterization of p53-R110C demonstrated reduced transcriptional activity, induction of apoptosis, and altered subcellular localization. Moreover, this report provides further clinical evidence of the pathogenicity of hypomorphic germline *TP53* variants when homozygous and supports surveillance for relatives with at least one R110C variant.

Grants: None.

Conflict of Interest: None declared

P01.097.D Systematic application of ClinGen InSiGHT APC-specific ACMG/AMP variant classification criteria alleviates the burden of variants of uncertain significance in ClinVar and LOVD databases

Xiaoyu Yin[1;2;3], *Marcy Richardson*[4], *Andreas Laner*[5], *Xuemei Shi*[6], *Elisabet Ognedal*[7], *Valeria Vasta*[8], *Thomas van Overeem Hansen*[9;10], *Marta Pineda*[11;12;13], *Deborah Ritter*[14;15], *Johan T. den Dunnen*[16], *Emadeldin Hassanin*[17;18], *Lyman Lin*[19], *Ester Borras*[20], *Karl Krahn*[21], *Margareta Nordling*[22;23], *Alexandra Martins*[24], *Khalid Mahmood*[25], *Emily Nadeau*[26], *Victoria Beshay*[27], *Carli Tops*[16], *Maurizio Genuardi*[28], *Tina Pesaran*[4], *Ian M. Frayling*[29;30;31], *Gabriel Capellá*[11;12;13], *Andrew Latchford*[29;32], *Sean V. Tavtigian*[33;34], *Carlo Maj*[17;35], *Sharon E Plon*[14;15], *Marc Greenblatt*[26], *Finlay A. Macrae*[1;2], **Isabel Spier**[3;11;36], *Stefan Aretz*[3;11;36]

[1]*Department of Colorectal Medicine and Genetics, Royal Melbourne Hospital, Parkville, Australia;* [2]*Department of Medicine, University of Melbourne, Parkville, Australia;* [3]*Institute of Human Genetics, Medical Faculty, University of Bonn, Bonn, Germany;* [4]*Ambry Genetics, Aliso Viejo, California, USA;* [5]*Medical Genetics Center Munich, MGZ Munich, Germany;* [6]*Greenwood Genetic Center, Greenwood, South Carolina, USA;* [7]*Western Norway Familial Cancer Center, Haukeland University Hospital, Bergen, Norway;* [8]*Northwest Genomics Center, Department of Genome Sciences, University of Washington, Seattle;* [9]*Department of Clinical Genetics, Rigshospitalet, Copenhagen University Hospital, Copenhagen, Denmark;* [10]*Department of Clinical Medicine, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark;* [11]*European Reference Network on Genetic Tumour Risk Syndromes (ERN GENTURIS) – Project ID No 739547;* [12]*Hereditary Cancer Program, Catalan Institute of Oncology – ONCOBELL, IDIBELL, Barcelona, Spain;* [13]*Centro de Investigación Biomédica en Red de Cáncer (CIBERONC), Instituto Salud Carlos III, Madrid, Spain;* [14]*Baylor College of Medicine, Houston, Texas, USA;* [15]*Texas Children's Cancer Center, Texas Children's Hospital, Houston, Texas, USA;* [16]*Department of Human Genetics, Leiden University Medical Center, Leiden, Netherlands;* [17]*Institute for Genomic Statistics and Bioinformatics, University Hospital Bonn, Bonn, Germany;* [18]*Luxembourg Centre for Systems Biomedicine, University of Luxembourg, Esch-sur-Alzette, Luxembourg;* [19]*St Vincents Hospital Melbourne, East Melbourne, Australia;* [20]*Invitae Corporation, San Francisco, California, USA;* [21]*GeneDx, Gaithersburg, Maryland, USA;* [22]*Department of Biomedical and Clinical Sciences, Linköping University, Linköping, Sweden;* [23]*Department of Clinical Genetics, Linköping University Hospital, Linköping, Sweden;* [24]*Univ Rouen Normandie, Inserm U1245, F-76000 Rouen, France;* [25]*Colorectal*

Oncogenomics Group, Department of Clinical Pathology, University of Melbourne, Melbourne, Australia; [26]Department of Medicine, Larner College of Medicine, University of Vermont, Burlington, Vermont, USA; [27]Peter MacCallum Cancer Centre, Melbourne, Australia; [28]Fondazione Policlinico Universitario A. Gemelli IRCCS, and Dipartimento di Scienze della Vita e Sanità Pubblica, Università Cattolica del Sacro Cuore, Rome, Italy; [29]Polyposis Registry, St Mark's Hospital, London, UK; [30]Inherited Tumour Syndromes Research Group, Institute of Cancer & Genetics, Cardiff University, UK; [31]National Centre for Colorectal Disease, St Vincent's University Hospital, Dublin, Ireland; [32]Department of Surgery and Cancer, Imperial College, London UK; [33]Huntsman Cancer Institute, University of Utah, Salt Lake City, Utah, USA; [34]Department of Oncological Sciences, School of Medicine, University of Utah, Salt Lake City, Utah, USA; [35]Centre for Human Genetics, University of Marburg, Germany.; [36]National Center for Hereditary Tumor Syndromes, University Hospital Bonn, Bonn, Germany

Consortium: ClinGen InSiGHT Hereditary Colorectal Cancer/Polyposis Variant Curation Expert Panel

Background/Objectives: To resolve the interpretative challenges of variants of uncertain significance (VUS), gene-specific ACMG/AMP variant classification criteria were developed and validated for the APC gene by the ClinGen-InSiGHT Hereditary Colorectal Cancer/Polyposis Variant Curation Expert Panel (VCEP) (https://cspec.genome.network/cspec/ui/svi/doc/GN089).

Methods: A streamlined algorithm using the APC-specific criteria was developed and applied to reassess all APC variants in ClinVar and the reference APC gene variant database in the Leiden Open Variation Database (LOVD).

Results: A total of 10,228 unique APC variants were analysed. 94% and 96% of the ClinVar and LOVD variants, respectively, with an initial classification of (Likely) Benign or (Likely) Pathogenic remained in their original categories. 41% of ClinVar and 61% of LOVD VUS were reclassified into clinically actionable classes, the vast majority as (Likely) Benign. The total number of VUS was reduced by 37% from 6142 to 3865. For 36 promising APC variants that remained VUS despite evidence for pathogenicity, a data mining-driven work-up was undertaken to curate additional evidence which allowed the further reclassification of 18 VUS as (Likely) Pathogenic.

Conclusion: The application of APC-specific classification criteria substantially reduced the number of VUS in ClinVar and LOVD. The study also demonstrates the feasibility of a systematic approach to variant classification with large datasets, which serves as a generalisable model for other gene-/disease-specific variant interpretation initiatives. It also allows for the prioritization of VUS that will benefit from in-depth evidence collection. The reclassified promising APC variants will be subjected to VCEP approval and made publicly available through ClinVar and LOVD for widespread clinical use.

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P01.098.A Using the homologous recombination deficiency mutational signature to look for the missing heritability in breast cancer

Jose Camacho Valenzuela[1;2], *nancy hamel[2;3], Thibaut Matis[4;5], paz polak[6], Carla Daniela Robles Espinoza[7;8], William Foulkes[1;2;3]*

[1]McGill University, Human Genetics, Montréal, Canada; [2]MUHC Research Institute, Cancer Research Program, Montréal, Canada; [3]Lady Davis Institute at the Jewish General Hospital, Montréal, Canada; [4]Institute Bergonié, Cancer genetics unit, Bordeaux, France; [5]Bric Bordeaux Institute Of Oncology - Inserm U1312 University Bordeaux - Institute D'oncologie De Bordeaux., Bordeaux, France; [6]Icahn School of Medicine at Mount Sinai, Oncological sciences, New York, United States; [7]Laboratorio Internacional de Investigación sobre el Genoma Humano (LIIGH) UNAM, Juriquilla, Mexico; [8]Wellcome Sanger Institute, Experimental cancer genetics, Hinxton, United Kingdom

Background/Objectives: Approximately 50% of hereditary breast cancer is explained by germline variants in well-known Breast Cancer Susceptibility Genes (BCSGs), predominantly *BRCA1/2*. The remaining 50% remains unexplained. We hypothesized that analyzing DNA from normal tissue and breast tumor from the same patient would allow us to identify other Homologous Recombination Repair (HRD)-related BCSGs, should they exist.

Methods: Focusing on matched normal/tumor exome data of breast cancer patients from The Cancer Genome Atlas, we developed a combined multidimensional pipeline to correlate the presence of Mutational Signature 3 (Sig3) with germline cancer predisposition variants, accounting for secondary tumor events. We evaluated genes enriched with variants in patients with Sig3 in their tumors, including non-cancer genes.

Results: We confirmed a significant association of *BRCA1/2* with Sig3 ($p < 0.05$). We also observed an association for some HRD genes, specifically *RAD51C* ($p < 0.05$), *PALB2* and *RAD51D*, though the last two did not reach significance. We did not identify positive correlation with Sig3 for previously unrelated genes. Somatic biallelic loss of *BRCA1/2* (~70%) and methylation in *BRCA1/RAD51C* (~20%) explain most cases lacking germline *BRCA1/2* variants. *BARD1* and *PALB2* germline variants with somatic second hits explained some of the remaining non-*BRCA1/2* cases. We are further investigating the non-BRCA cases with Sig3.

Conclusion: Somatic and epigenetic events in well-known HRD genes appear to explain approximately 90% of HRD cases without *BRCA1/2* germline variants. Other non-*BRCA1/2* genes likely explain only a small proportion of cases. An integrated germline/tumor approach is a powerful genomic strategy to understand non-*BRCA1/2* cases.

Grants: CIHR (FDN-148390).

Conflict of Interest: None declared

P01.099.B Landscape of Germline Variants in Cases of Ovarian Cancer: Insights from a Single-Center Study

Ece ÇİÇEK[1], *Drenushe Zhuri[1], Nilay Ozupek[1], Hazal Sezginer Guler[1], umut çiçek[1], HAKAN GURKAN[1]*

[1]Trakya University, Medical Genetics, Edirne, Türkyie

Background/Objectives: Ovarian cancer stands as one of the predominant gynecological malignancies globally. Diverse risk

factors have been elucidated in connection with ovarian cancer, encompassing advanced age, genetic predisposition, familial history, nulliparity, and others. Existing research underscores that over 20% of ovarian cancer cases exhibit a hereditary genetic basis, with approximately 70% of these genetic anomalies manifesting as germline mutations in the *BRCA*1/2 genes. In this study, we aimed to investigate the mutational spectrum of ovarian cancer in a single-center patient group.

Methods: Between January 2022 and September 2023, our study enrolled 60 patients diagnosed with ovarian cancer who applied to our Genetic Diseases Evaluation Center. DNA samples extracted from the peripheral blood of patients underwent analysis utilizing the The QIAseq Human Breast Cancer Panel (93 genes, DHS-001Z; Qiagen).

Results: In fifteen cases, comprising 25% of the study cohort, sixteen pathogenic/likely pathogenic (P/LP) variants were identified, with one of them being novel. Additionally, seventeen cases presented 22 variants of unknown clinical significance (VUS). These cases were categorized into two groups: (1) carriers of *BRCA1/2* variant and (2) non-carriers of *BRCA1/2* variant, with 50% of the P/LP variants detected in *BRCA1/2* genes, primarily within group1.

Conclusion: The mutations most frequently observed in cases of ovarian cancer are typically found in the *BRCA* genes. However, our study's findings indicate a lack of correlation with previously reported mutation rates in genes other than *BRCA1/2*. This observation suggests the potential to offer novel insights into the mutational spectrum of ovarian cancer.

Conflict of Interest: None declared

P01.100.C Mutational landscape of glioblastoma tissue and derived tumoroid exomes

Vita Rovite[1], *Sigita Hasnere*[2], *Raitis Pečulis*[1], *Aija Gerina*[2], *Olesja Rogoza*[1], *Omkar Suhas Vinchure*[3], *Vaibhav Jadhav*[3], *Jay Gopalakrishnan*[3]

[1]*Latvian Biomedical Research and Study centre, Molecular and Functional Genomics, Riga, Latvia;* [2]*P. Stradins Clinical University Hospital, Oncology, Riga, Latvia;* [3]*Institute for Human Genetics Heinrich-Heine-Universität Universitätsklinikum Düsseldorf, Laboratory for Centrosome and Cytoskeleton Biology, Düsseldorf, Germany*

Background/Objectives: Glioblastoma (GB) is the most frequent malignant brain tumor. GB patients' median survival is <24 months, and there is no cure. Glioma-PerMed project is consortium based effort to establish rapid and efficient glioma invasion assays and machine learning algorithms for predicting the GB invasion in ex-vivo, personalized diagnostics, drug discovery, and patient follow-up in the clinics. Aim of this study was to charcterize mutational landscape of GB tissue and derived tumoroid models.

Methods: Five GB tissue and seven tumoroids was obtained at Jay Gopalakrishnan lab in University of Dusseldorf, Germany. Sequencing of the obtained biological samples was done at Latvian Biomedical Research and Study cente using MGIEasy Exome Capture V4 Probe Set and MGIEasy Exome Universal Library Prep Set on DNBSEQ-G400 sequencer. Exome sequencing reads were trimmed using fastp v0.23.4, afterwards reads were aligned to human genome reference GRCh38.p14 using bwa mem v0.7.17. Tumor tissue - tumoroid sample pair variant differences were detected with Strelka v2.9.10.

Results: GB tissue samples contained from 149124 to 209524 variants while tumoroids from 18169 to 186502 variants. Specifically, we assessed the presence of mutations in 79 genes realted to GB pathogenesis. In tumour tissue we found mutations in 34-30 genes, while tumoroids this varied from 16 - 45 genes.

Conclusion: In our sample set tumour tissues most often we detected mutations in known glioma associted genes, for example, TP53, PDGFRA, ATR, and less number of cases IDH1, EGFR and PTEN mutations.

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Conflict of Interest: None declared

P01.102.A Germline mutations in *HIC1* are associated with serrated polyposis syndrome

Xavier Domínguez-Rovira[1], *Coral Arnau-Collell*[1], *Gemma Gonfaus*[1], *Gemma Llargués-Sistac*[1], *Jenifer Muñoz*[1], *Ainara Llopis*[1], *Yasmin Soares de Lima*[1], *Cristina Herrera-Pariente*[1], *Leticia Moreira*[1], *Teresa Ocaña*[1], *Marcos Díaz-Gay*[2], *Miriam Cuatrecasas*[3], *Sabela Carballal*[1], *Anael Lopez-Novo*[4], *Guerau Fernandez*[5], *Antoni Castells*[1], *Luis Bujanda*[6], *Gabriel Capellá*[7], *Joaquin Cubiella*[8], *Daniel Rodriguez-Alcade*[9], *Laura Valle*[7], *Francesc Balaguer*[1], *Clara Ruiz-Ponte*[4], *Laia Bonjoch*[1], *Sergi Castellví-Bel*[1]

[1]*Hospital Clínic, FRCB-IDIBAPS, CIBEREHD, Gastroenterology, Barcelona, Spain;* [2]*Moores Cancer Center at UC San Diego, La Jolla, San Diego, United States;* [3]*Hospital Clínic de Barcelona, Pathology, Barcelona, Spain;* [4]*Fundación galega de medicina xenómica, Grupo de Medicina Xenómica-USC, IDIS, CIBERER, Santiago de Compostela, Spain;* [5]*Sant Joan de Déu Barcelona Hospital, Barcelona, Spain;* [6]*Donostia Hospital, CIBEREHD, Gastroenterology, San Sebastián, Spain;* [7]*Catalan Institute of Oncology, Barcelona, Spain;* [8]*Complexo Hospitalario Universitario de Ourense - CHUO, Gastroenterology, Ourense, Spain;* [9]*Hospital Universitario de Móstoles, Digestive diseases section, Móstoles, Spain*

Background/Objectives: Serrated polyposis syndrome (SPS) is characterized by multiple and/or large serrated polyps and colorectal cancer (CRC) risk. *HIC1* (*Hypermethylated in cancer 1*) is a transcriptional repressor that functions as a tumor suppressor gene, previously suggested as a new candidate gene to germline predisposition to SPS (1). We aimed to provide functional evidence to evaluate *HIC1* as a new hereditary gene for SPS.

Methods: Gene-panel sequencing of 211 SPS patients was performed to validate the findings in a discovery cohort of 39 patients from 16 SPS families. Two cellular models for *HIC1*, a CRISPR/Cas9 knock-out model and an overexpression model, were produced. We assessed the DNA damage response and the *SIRT1* regulatory pathway upon *HIC1* genetic variation.

Results: Three predicted pathogenic variants were identified within the *HIC1* gene (p.Ala37Val, p.Gln432Arg and p.Gly471Arg) in the discovery and validation cohorts. Functional characterization revealed higher p-H2AX levels (a marker for ongoing DNA damage) and impaired *HIC1* binding to the *SIRT1* promoter in cellular models carrying the identified genetic variants when compared with the wild-type sequence.

Conclusion: Our results indicate that *HIC1* genetic variants located in the zinc finger region can lead to disruption of protein function. Our results support *HIC1* as a new gene associated with SPS predisposition, although additional cohorts should be evaluated to further investigate its hereditary role.

References:

(1) Soares de Lima Y et al. Cancers 2021; 13(4):1–21.

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Conflict of Interest: None declared

P01.103.B Breast Cancer Risk Polygenic Score (PGS) optimisation through a novel SNP selection algorithm

Carla Debernardi[1], *ANGELO SAVOCA*[1], *Elisabetta Casalone*[1], *Kristina Zguro*[2], *Giulia Brunelli*[2], *Zhiyu Yang*[3], *Brooke Wolford*[4], *Alessia Russo*[1], *Samuli Ripatti*[5], *Giuseppe Matullo*[1]

[1]*University of Turin, Department of Medical Sciences, Turin, Italy;* [2]*University of Siena, Department of Medical Biotechnologies, Med Biotech Hub and Competence Center, Siena, Italy;* [3]*University of Helsinki, Helsinki;* [4]*HUNT Center for Molecular and Clinical Epidemiology, Department of Public Health and Nursing, NTNU, Norwegian University of Science and Technology, Trondheim, Norway;* [5]*Institute for Molecular Medicine Finland, FIMM, HiLIFE, University of Helsinki, Helsinki, Finland; Clinicum, Department of Public Health, University of Helsinki, Helsinki, Finland; Broad Institute of MIT and Harvard,Cambridge, MA, USA., Finland*

Background/Objectives: Many Polygenic Scores (PGS) have been described for Breast Cancer (BC), differing by number and selection of SNPs. This variability leads to a wide range of estimated PGS in the same individual and therefore to inconsistent risk stratification. The aim of this study is to optimise the computation of BC risk scores by the selection of the variants involved.

Methods: Starting from the 100 PGSs available for BC on the PGS Catalog, we aligned all their variants to obtain the most represented SNPs associated to disease risk. TheSNPs selection approach was based on the association with Breast Cancer and on the concordance between the different PGSs. Standardization and embedding operations were performed on the effect weights to create a new PGS.

Results: The newly created PGS has 4,606 SNPs. We tested this PGS on Genomic England, UK Biobank and FinnGen data. In all the data sets tested the combined approach to estimate the effect weights shows the best performance, distributions between BC patients and controls were significantly different (Kolmogorov–Smirnov test p-value < 0.0001). Across cohorts, women in the highest 1% of the score had a two to three-fold increased risk of develop BC compared with women in the middle quintile Lifetime risk of BC for women in the lowest and highest quintiles of the PRS was 10% and more than 25%, respectively.

Conclusion: These finding and optimization approach strengthen the standardisation of PGS that could help clinicians to better stratify patients risk of developing BC.

Grants: INTERVENE, HORIZON Project 2020 n.101016775

Conflict of Interest: None declared

P01.104.C Innovative Prostate Cancer Classifier: Validating an Explainable Artificial Intelligence Tool

Patricia Porras-Quesada[1;2], *Alberto Ramírez-Mena*[2], *Verónica Arenas-Rodríguez*[1;2], *Beatriz Álvarez-González*[3], *Jesús Alcalá-Fdez*[4], *Luis Javier Martínez-González*[1;2], *Maria Jesus Alvarez Cubero*[1;2;5]

[1]*Faculty of Medicine - University of Granada, Biochemistry and Molecular Biology III and Immunology Department, Granada, Spain;* [2]*Pfizer-University of Granada-Junta de Andalucía Centre for Genomics and Oncological Research (GENYO), Granada, Spain;* [3]*Faculty of Medicine - University of Granada, Legal Medicine and Toxicology Department, Granada, Spain;* [4]*E.T.S. of Computer and Telecommunication Engineering, Computer Science and Artificial Intelligence Department. Andalusian Research Institute in Data Science and Computational Intelligence (DaSCI). University of Granada, Granada, Spain;* [5]*IBS, Biosanitary Research Institute (ibs. GRANADA), University of Granada, Granada, Spain*

Background/Objectives: Although prostate cancer (PC) is the second most frequent tumour worldwide, its diagnosis focuses on inconclusive or not necessarily cancer-specific strategies. Therefore, a classifier is proposed to predict the occurrence of PC and to provide a tool to assist the pathologist in the decision-making process.

Methods: Different machine learning methods based on eXplainable Artificial Intelligence were trained using gene expression data from 550 samples available on 'The Cancer Genome Atlas' repository. Our model was validated in 463 samples from four external cohorts. Additionally, a set of SHapley Additive exPlanations was provided to help clinicians to understand each prediction. To analyze reliability and feasibility of applying the classifier in clinical practice, a gene expression analysis by qPCR was performed in 60 fresh prostate tissue samples (20 control individuals and 40 PC patients stratified by tumor aggressiveness).

Results: An in-depth analysis showed that the Random Forest algorithm combined with undersampling procedure was the best performing approach with robust statistical significance (sensitivity:0.90; specificity:0.8; AUC:0.84) across all databases. Classifier validation in prostate tissue samples revealed an increase in *DLX1, TDRD1, AMACR, HPN, HOXC6* and *OR51E2* expression when comparing high vs. low aggressive PC patients and PC patients vs. controls.

Conclusion: Proposed tool has shown good performance in 4 independent external cohorts of different ancestries and the explanations provided are consistent with the results obtained in the experimental validation with biological samples. In the near future, highlighted genes expression, in combination with our model, could be applied to liquid biopsy to improve PC screening.

Grants: Regional Government of Andalusia (PIP-0043-2022).

Conflict of Interest: None declared

P01.105.D Risk management for breast cancer in BRCA mutation carriers; compliance to the recommended surveillance.

Toine Werker[1], *Simone Salemink*[1], *Wendy Van Zelst-Stams*[1], *Arjen Mensenkamp*[1], *Angela van Remortele*[1], *Maaike Haadsma*[1]

[1]*Radboud University Medical Center, Human Genetics, Nijmegen, Netherlands*

Background/Objectives: Due to their highly increased risk of breast cancer, women with a pathogenic mutation in the BRCA1- or BRCA2-gene are eligible for preventive mastectomy or specific breast cancer surveillance protocols to warrant early detection. This study investigates the extent to which BRCA mutation carriers follow the Dutch breast cancer guideline recommendations.

Methods: We performed a cross-sectional study at the department of Human Genetics of the Radboud University Medical Center, Nijmegen, The Netherlands, among presymptomatic women aged 25 to 75 with a pathogenic mutation in the BRCA1- or BRCA2-gene, identified between 2012 and 2021. Women were asked about their current surveillance status through a survey.

Results: 256 out of 709 (36,1%) women responded and the 200 women without a history of breast cancer were included. In total, 86% (172/200) of them were compliant to the recommended guidelines. Of these women, 85 (42.5%) underwent preventive mastectomy. In the 115 other women, eligible for surveillance, in 75.7% (87/115) breast screening was performed according to the guideline. Women non-compliant to guideline surveillance were more often BRCA1 mutation carriers ($p = 0.045$), of older age ($p = 0.019$), and had a longer time since mutation detection ($p = 0.031$).

Conclusion: This study, not previously conducted among Dutch BRCA mutation carriers, reveals that the majority of women were compliant with recommendations concerning their breast cancer risk. However, nearly one-fourth of those eligible for surveillance

do not receive guideline-compliant breast screening. We believe that guideline compliance can be improved by closer collaboration between involved healthcare providers.

Grants: Not applicable.

Conflict of Interest: None declared

P01.106.A Clinical and Molecular Characterization of Fumarate Hydratase (FH) germline carriers in Omani families.

Abeer Alsaegh[1], *Reem Abdulrahim*[1], *Chantel Van Wyk*[2], *Ilse Crous*[2], *Sara Al Kiyumi*[2], *Amira Al Balushi*[2], *Suad Al Malki*[2], *Bushra Al Muhairi*[2]

[1]*Sultan Qaboos Comprehensive Cancer Care and Research Center, Genomics, Oman;* [2]*Sultan Qaboos Comprehensive Cancer Care and Research Center*

Backgroud/Objecties: Although there are several families described with FH mutations associated with Hereditary Leiomyomatosis and Renal Cell Cancer, the prevalence and full spectrum of the phenotype is still unknown. The objective of this study is to collect and describe the clinical and molecular phenotype in Omani families with pathogenic mutations in FH gene.

Methods: Retrospective hospital chart review of individuals referred to clinical genetics service from 2021 to 2023 to Sultan Qaboos Comprehensive Cancer Care and Research Center.

Results: A total of 30 FH mutation carriers were identified in this cohort. The majority were diagnosed with renal cell carcinoma (19/30). There was also (2/30) patients diagnosed with Uterine leiomyosarcoma, (5/30) diagnosed with pre- menopausal young onset breast cancer, (2/30) diagnosed with bladder carcinoma and only 2/30 with the typical skin lesions for cutaneous leiomyomas. Uterine fibroids were only reported in (4/30).

Conclusion: In our cohort we seem to identify more carriers diagnosed with breast and bladder cancer and all have the common founder mutation in FH gene (c.698G>T). In view of these results, patients with this mutation may benefit from early breast cancer screening in addition to other common cancers associated with this syndrome.

Grant: no grant was obtained to do this study

Conflict of Interest: None declared

P01.107.B *ATM* c.7475T>G, p.(Leu2492Arg) variant confers a moderate risk for breast cancer

Ivana Maleva Kostovska[1], *Predrag Noveski*[1], *Sanja Kiprijanovska*[1], *Simona Jakovchevska*[1], *Dijana Plaseska-Karanfilska*[1]

[1]*Macedonian Academy of Sciences & Arts, Research Center for Genetic Engineering and Biotechnology "Georgi D. Efremov", Skopje, Macedonia*

Background/Objectives: Case-control and family-based studies have identified *ATM* as a moderate breast cancer (BC) susceptibility gene. While germline protein truncating variants (PTVs) in *ATM* are linked to a 2-4 fold elevated BC risk, the risk posed by missense variants remains uncertain, with the majority classified as variants of uncertain significance (VUS). Our study aimed to comprehensively evaluate the prevalence of *ATM* variants among BC cases and general population.

Methods: Sequencing of BC cases ($n = 971$) was performed using TruSight Cancer NGS sequencing panel, while *ATM* variants in the control samples ($n = 871$) were derived from WES conducted in our laboratory. Additionally, 280 BC cases were screened for the *ATM* c.7475T>G variant with allele specific PCR.

Results: The observed *ATM* mutation rate was 1.9% (18/971) in BC cases and 0.5% (4/871) in the control cohort, with the majority of pathogenic variants (80%, 12/15) being PTVs. VUS variants were identified in 53 BC cases (5.5%) and 41 controls (4.7%). The most common VUS c.7475T>G, p.(Leu2492Arg) was identified in 1.6% of the BC patients and 0.6% of the controls (20/1251 vs. 5/871), thus carrying a moderate increased risk for BC (OR 2.8; 95%CI 1.05-7.52, $p = 0.03$). This variant has higher allele frequency in our population compared to gnomAD (0.59% vs. 0.01%), is located in the FAT domain, has CADD score of 26.7 and is classified as VUS in ClinVar.

Conclusion: This is the first study to demonstrate moderate BC risk in *ATM* c.7475T>G carriers, emphasizing the importance of studying *ATM* VUS in different populations where some VUS exhibit higher frequencies.

Conflict of Interest: None declared

P01.108.C Investigating the Impact of IDH1 Mutation on Monocyte Differentiation Transcriptome in Early Glioma Development

Ebru BAKIR[1], *Yavuz Oktay*[1]

[1]*izmir Biomedicine and Genome Center, Molecular Biology and Genetics, İzmir, Türkyie*

Background/Objectives: Gliomas, the most prevalent primary brain tumors, often harbor IDH1 mutations, notably the R132H alteration, leading to the production of the oncometabolite 2-hydroxyglutarate (2-HG). This study aims to investigate the effects of secreted factors from astrocytes mimicking early IDH1 R132H gliomas on monocyte differentiation, shedding light on the molecular mechanisms underlying glioma development.

Methods: Immortal human astrocytes expressing IDH1 R132H were utilized to generate conditioned media (CM) mimicking early glioma stages. Primary monocytes were exposed to these CM during differentiation to macrophages. Transcriptome-wide analysis of differentiated macrophages was conducted via RNA sequencing to identify differentially expressed genes in response to the IDH1 mutation.

Results: RNA sequencing revealed 124 differentially expressed genes associated with the IDH1 mutation. Gene ontology analysis indicated interference with the p53 signaling pathway and elevated signatures related to negative regulation of complement activation in IDH1 R132H-exposed cases. Additionally, pathways associated with Th1 and Th2 cell differentiation, as well as cytotoxic T cell homeostasis, were significantly enriched.

Conclusion: Secreted factors from astrocytes in early glioma formation significantly impact monocyte differentiation transcriptome, potentially shaping the tumor microenvironment. The findings underscore the role of IDH1 mutation in modulating immune responses and highlight potential therapeutic targets for glioma treatment.

Grants: TÜBİTAK 1001 Project no:214S097

Conflict of Interest: None declared

P01.109.D Characterization of the transgenic animal models *Danio Rerio* and *Caenorhabditis Elegans* to examine pathophysiology associated with SMARCB1 variants

Elena Tacchetto[1;2], *Lisa Buson*[2], *valeria morbidoni*[1], *Cristina Cerqua*[1], *Valerio Magnaghi*[3], *Laura Papi*[4], **Eva Trevisson**[1;2]

[1]*IRP – Istituto di Ricerca Pediatrica Città della Speranza, Padova, Italy;* [2]*Università degli Studi di Padova, Salute della Donna e del Bambino, Roma, Italy;* [3]*Università degli Studi di Milano, Dipartimento di scienze farmaceutiche e molecolari, Milano, Italy;* [4]*Università di Firenze, Department of Experimental and Clinical Biomedical Sciences "Mario Serio", Firenze, Italy*

Background/ Objectives: SMARCB1 is a nuclear protein and core subunit of the BAF chromatin-remodeling complex. SMARCB1 variants are associated with tumor predisposition syndromes and developmental disorders. We developed and characterized two transgenic models, *C.elegans* and zebrafish, to examine the pathophysiology associated with SMARCB1.

Methods: We analyzed the expression of SMARCB1 orthologues. In *C.elegans*, *snfc-5* was analyzed by q-RT PCR. In *D.rerio* there are two paralogs: *smarcb1a* and *smarcb1b*, whose spatial and temporal expression was analyzed. We employed CRISPR/Cas9 to obtain our transgenic animals: we generated *smarcb1a-/-*, *smarcb1b-/-* and *smarcb1ab* + /- lines in *D. rerio*. In *C. elegans*, we modeled mutations associated with distinct human phenotypes. We performed a series of experiments to characterize the transgenic lines, as brood size and longevity and we are studying nociception. In zebrafish, we are performing phenotypical observation, survival curves and histological analysis.

Results: In zebrafish the two isoforms were detected from the first stages of embryonic development and ubiquitously. With an in-vivo analysis, we observed that *smarcb1* reaches the highest levels of expression in the central nervous system. In *C. elegans*, the single isoform *snfc-5* is expressed from the egg stage to the adulthood. Our data suggest that *smarcb1* is a maternal determinant. The phenotypical analysis of our transgenic lines, suggest that SMARCB1 plays an important role in survival, embryonic development and tissue maintenance.

Conclusion: Our findings indicate that SMARCB1 plays key roles in different pathways and developmental stages. The integration of this multidisciplinary approach could help to identify novel therapeutic targets.

Grants: PRINN2017

Conflict of Interest: Elena Tacchetto PRINN2017, Lisa Buson: None declared, valeria morbidoni: None declared, Cristina Cerqua: None declared, Valerio Magnaghi PRINN2017, Laura Papi PRINN2017, Eva Trevisson PRINN2017

P01.110.A KMT2C mutations associated with colorectal and lung cancer development

Dimitar Dimitrov[1], *Nelly Miteva-Marcheva*[1], *Hristo Ivanov*[1], *Aleksandar Linev*[1], *Vili Stoyanova*[1]

[1]*Medical University of Plovdiv, Department of Pediatrics and Medical Genetics, Plovdiv, Bulgaria*

Background: Worldwide, cancer is a leading cause of death. We conducted a study which aim was to investigate mutations associated with tumorigenesis in patients with colorectal and lung cancer.

Methods: A total of 31 cancer patients (12 with lung cancer, 19 with colorectal carcinoma) were enrolled, from whom ctDNA samples were collected by liquid biopsy and subjected to targeted sequencing with a panel of 484 cancer-related genes followed by bioinformatic analysis.

Results: Sequencing revealed diverse genetic variants, and pathogenic or likely pathogenic variant were found in the KMT2C gene in 100% of the patients (KMT2C c.2961C>G, KMT2C c.8390del, KMT2C c.2578C>T, KMT2C c.2573G>T and KMT2C c.2917A>G).

Discussion: The KMT2C gene is part of the KMT2 family. Dysregulation or mutation of KMT2 family have been observed frequently in various types of human cancers including lung and colorectal cancers. Most KMT2C mutations lead to protein reduction, promoting tumorigenesis by dysregulating enhancer activity. Similar to our study, a research by Chatarina Larsson et al. found that the KMT2C variant c.8390del occurs in 25-48% of MSI CRC cases, also this variant leads to inactivation of KMT2C, promoting the development of colorectal cancer through transcriptional dysregulation in several pathways known to be involved to cancer.

Conclusion: Our findings underscore the potential of KMT2C mutations as significant contributors to cancer development and as promising targets for future therapeutic strategies. Further research is needed to fully understand the implications of these mutations in cancer progression and treatment.

Grants: Project № BG-RRP-2.004-0007-C01 Strategic Research and Innovation Program for the Development of MU - PLOVDIV–(SRIPD-MUP)

Conflict of Interest: None declared

P01.111.B Parafibromin immunohistochemical staining of tumors from patients with hyperparathyroidism-jaw tumor syndrome

Ana Blatnik[1], *Olga Blatnik*[2], *Primož Drev*[2], *Vita Setrajcic Dragos*[3], *Anja Zagožen Klasinc*[3], *Iva Opalič*[1], *Srdjan Novaković*[3], *Mateja Krajc*[1]

[1]*Institute of Oncology Ljubljana, Department of Clinical Cancer Genetics, Ljubljana, Slovenia;* [2]*Institute of Oncology Ljubljana, Department of Pathology, Ljubljana, Slovenia;* [3]*Institute of Oncology Ljubljana, Department of Molecular Diagnostics, Ljubljana, Slovenia*

Background/Objectives: Germline pathogenic variants (PV) in CDC73 gene are associated with hyperparathyroidism-jaw tumor syndrome (HPT-JT), characterized by parathyroid adenomas/carcinomas, ossifying fibromas of the maxilla/mandible, renal lesions, and benign and malignant uterine tumors. CDC73 encodes a putative tumor suppressor parafibromin. In our study, we assessed the results of parafibromin immunostaining of tumors from HPT-JT patients.

Methods: We identified carriers of CDC73 PVs in the Slovenian National Registry of Tested Individuals from Families with Hereditary Cancer and obtained their tumor specimens. Nuclear expression of parafibromin was assessed in tumor tissue sections using a rabbit monoclonal antibody, according to standard operating procedures.

Results: Of 3283 cancer predisposition carriers, 6 (5 female, 1 male) were carriers of *CDC73* PVs. One was asymptomatic, 3 developed parathyroid adenomas, 4 had uterine tumors, and 2 were treated for breast ductal carcinoma in situ (DCIS). Notwithstanding technical difficulties, immunostaining was successful in 1 parathyroid adenoma, 3 uterine tumors and both DSICs. Loss of parafibromin was demonstrated in the parathyroid adenoma. All 3 uterine tumors (2 fibroepithelial polyps and 1 atypical adenomyoma/adenosarcoma) showed loss of parafibromin in the mesenchymal component and normal staining in the epithelial component. Both DCIS samples had normal parafibromin expression.

Conclusion: Our parafibromin immunostaining results are compatible with what is currently known about HPT-JT tumor spectrum. Breast cancers in our patients do not appear to be associated with germline *CDC73* variants. Epithelial-mesenchymal uterine lesions displayed loss of parafibromin in the stromal component which metastasized to the lungs and proved to be fatal for one patient.

Conflict of Interest: None declared

P01.112.C Functional analysis of genetic variants in genes involved in cancer helps establish pathogenicity and improves diagnostics for familial tumour syndromes.

Rick van Minkelen[1], *Mark Nellist*[1], *Marianne Hoogeveen-Westerveld*[1], *Hannie Douben*[1], *Leontine M A van Unen*[1], *Esmee Kasteleijn*[1], *Evelien Kroon*[1], *Heidi de Gruyter*[1], *Guido Breedveld*[1],

Margreethe van Vliet[1], Yvette Van Ierland[1], Berna Beverloo[1], Anja Wagner[1], Tjakko Van Ham[1]

[1]Erasmus Medical Centre, Clinical Genetics, Rotterdam, Netherlands

Background/Objectives: The identification of a pathogenic DNA variant in an individual with a genetic predisposition to cancer helps establish a diagnosis, enabling appropriate treatment and monitoring, and facilitating informed reproductive decision-making. However, in many individuals, variants of uncertain clinical significance (VUS) are identified. In these cases the genetic specialists are unable to provide the patient with certainty regarding their affection and/or carrier status.

Methods: To improve variant classification we apply in vitro functional testing to interrogate the effects of diagnostically relevant genetic variants on the expression and activity of components of the MTOR and RAS signalling pathways. Defects in MTOR or RAS signalling are common features of many sporadic tumours, and are associated with a diverse group of Mendelian disorders, particularly familial tumour syndromes such as tuberous sclerosis complex (*TSC1*, *TSC2*) and neurofibromatosis (*NF1*).

Results: Using our functional approach, we have so far been able to test >700 variants in genes involved in MTOR (AKT3, DEPDC5, MTOR, NPRL2, NPRL3, PIK3CA, PIK3R2, PTEN, TSC1 and TSC2) and RAS signalling (HRAS, NF1, SPRED1). Evidence to support pathogenicity was obtained for 73/114 clinically relevant variants in *NF1*, 63/197 in *TSC1* and 291/543 in *TSC2*.

Conclusion: Our work demonstrates that a range of specific biochemical assays can be applied in a diagnostic setting to help establish variant pathogenicity, and provide an accurate molecular diagnosis.

Grants: None

Conflict of Interest: None declared

P01.114.A Pathogenic insertion of a mitochondrial DNA fragment in the *PALB2* gene associated with hereditary breast and ovarian cancer syndrome.

Alejandro Moles-Fernandez[1;2], *Miriam Masas[1;2], Jordi Leno[1;2], Eduard Perez[3;4], Berta Campos[1;2], Judith Balmaña[3;4], Orland Diez[1], Elena García-Arumí[1;2;5], Eduardo Tizzano[1;2]*

[1]Vall d'Hebron Barcelona Hospital Campus, Vall d'Hebron Hospital Universitari, Department of Clinical and Molecular Genetics, Barcelona, Spain; [2]Vall d'Hebron Institut de Recerca (VHIR), Vall d'Hebron Barcelona Hospital Campus, Vall d'Hebron Hospital Universitari, Medicine Genetics Group, Barcelona, Spain; [3]Vall d'Hebron University Hospital and Vall d'Hebron Institute of Oncology, Medical Oncology Department, Barcelona, Spain; [4]Vall d'Hebron Institute of Oncology, Vall d'Hebron Barcelona Hospital Campus, Hereditary Cancer Genetics Group, Barcelona, Spain; [5]Vall d'Hebron Institut de Recerca (VHIR), Vall d'Hebron Barcelona Hospital Campus, Vall d'Hebron Hospital Universitari, Research Group on Neuromuscular and Mitochondrial Disorders, Barcelona, Spain

Background/Objectives: Hereditary breast and ovarian cancer syndrome (HBOCS) has been related to deleterious alterations in genes involved in homologous recombination, such as *BRCA1*, *BRCA2*, and *PALB2*. Point, copy number variants, and even the insertion of transposable elements, such as *Alu* sequences, have been identified as mechanisms of alteration of these, and others genes related to monogenic diseases. However, in very few cases, the insertion of mitochondrial DNA fragments (mtDNA) in coding sequences of disease-related nuclear genes has been described.

Methods: Massive parallel sequencing (MPS) of a gene panel associated with HBOCS in germline DNA was performed. The variant found was confirmed by PCR and sequencing of the DNA fragment of interest. The sequence was aligned with the nuclear and mitochondrial genome.

Results: The patient, with a family history compatible with suspected hereditary cancer, was diagnosed with invasive ductal carcinoma of the left breast at the age of 55 years. The pathogenic variant c.2515_2516ins70, p.(Thr839Ilefs*14), in the *PALB2* gene was detected in heterozygosity. This 70-nucleotide insertion was localized into exon 6 of *PALB2* and 65 nucleotides of this, were coincident with an mtDNA fragment comprising the sequence of the mitochondrial *MT-TA* gene, mitochondrial alanine tRNA.

No other pathogenic variants or variants of unknown significance were identified in the patient. Following the *ACMG* variant classification guidelines, this variant was classified as pathogenic.

Conclusion: To our knowledge, this is the first germline pathogenic variant caused by an mtDNA insertion in an HBOCS susceptibility gene.

Grants: FIS PI19/ 01772. La Marató TV3-Foundation (590/C/2020).

Conflict of Interest: None declared

P01.115.B *DDX41* germline predisposition to Myeloid Neoplasms: two siblings with the same double-hit germline/somatic non-canonical genomic constellation

Chiara Minotti[1;2], *Carmelo Gurnari[3], Giorgia Silvestrini[3], Enrico Attardi[1], Giorgia Ranucci[3], Camilla Page[3], Valentina Ferradini[1;2], Giuseppe Novelli[1;2], Maria Teresa Voso[3], Federica Sangiuolo[1;2], Enrica Marchionni[1;2]*

[1]Tor Vergata University of Rome, Medical Genetics Section, Dep. of Biomedicine and Prevention, Rome, Italy; [2]Tor Vergata Hospital, Medical Genetics Unit, Rome, Italy; [3]Tor Vergata University of Rome, Dep. of Biomedicine and Prevention, Rome, Italy

Up to 15% of Acute Myeloid Leukemia (AML) and Myelodysplastic Syndrome/Neoplasms (MDS) patients harbor inherited predisposition traits. Germline mutations of *DDX41* (MIM*608170), encoding for an RNA helicase, accounts for about 80% of MDS/AML with a documented germline predisposition. General disease ontogenesis encompasses the acquisition of a second somatic hit on the contralateral allele (most typically p.R525H). *DDX41*-mutant MDS/AML are characterized by male predominance, late onset, hypoplastic bone marrow, and indolent disease course.

We report on a 68-year old patient with an initial diagnosis of hypoplastic MDS (2% blasts). At the age of 76, he received a diagnosis of MDS-IB2 (10.5% blasts). At that time, a 94 myeloid genes NGS panel on his bone marrow aspirate detected the c.1547A>G [p.Tyr516Cys] heterozygous pathogenic variant in *DDX41* (NM_016222.4) at a Variant Allele Frequency (VAF) of 48%. Given the suspicion of a germline origin, Sanger sequencing confirmed the presence of this variants on fingernail DNA.

Notably, the patient's older brother received a diagnosis of MDS at 69 years of age. Segregation analysis, performed on DNA extracted from CD3+ lymphocytes used as a germline reference, disclosed the presence of the known heterozygous variant.

Interestingly, both siblings progressed to MDS upon acquisition of the same non-canonical *DDX41* somatic variant p.Thr227Met (VAF of 10% and 3.5%), whose functional characterization is ongoing.

Recognition of germline predisposition in MDS/AML is crucial for disease surveillance/therapy, and donor selection for hematopoietic stem cell transplantation. Genetic counselling of affected patients and their unaffected relatives is advisable to establish a proper multidisciplinary management.

Conflict of Interest: None declared

P01.117.D Clinical utility of RNA-based targeted next-generation sequencing for the molecular diagnostics of non-small cell lung cancer

Magdalena Pelc[1], *Urszula Lechowicz*[1], *Joanna Moes-Sosnowska*[1], *Adam Szpechcinski*[1], *Paulina Skronska*[1], *Aneta Zdral*[1], *Adriana Rozy*[1], *Emil Wojda*[2], *Mateusz Polaczek*[2], *Małgorzata Szołkowska*[3], *Tadeusz Orłowski*[4], *Pawel Sliwinski*[5], *Reanata Langfort*[3], *Joanna Chorostowska-Wynimko*[1]

[1]*Institute of Tuberculosis and Lung Diseases, Department of Genetics and Clinical Immunology, Warsaw, Poland;* [2]*Institute of Tuberculosis and Lung Diseases, III Department of Lung Diseases and Oncology, Warsaw, Poland;* [3]*Institute of Tuberculosis and Lung Diseases, Department of Pathology, Warsaw, Poland;* [4]*Institute of Tuberculosis and Lung Diseases, Department of Surgery, Warsaw, Poland;* [5]*Institute of Tuberculosis and Lung Diseases, II Department of Lung Diseases, Warsaw, Poland*

Background/Objectives: In recent years, the number of druggable somatic alterations with relevant targeted therapeutic agents has significantly increased in non-small cell lung cancer (NSCLC). Therefore, a robust, time- and cost-effective testing methods should be applied in molecular diagnostics of NSCLC. We evaluated the utility of RNA-based targeted next-generation sequencing (NGS) for the detection of clinically significant genetic variants in NSCLC patients.

Methods: RNA-based targeted NGS (FusionPlex_Lung assay, Archer) was performed in 312 tumor specimens (~75% adenocarcinoma; mainly advanced) from 309 NSCLC patients (mean age of 71 ± 8.4 years) in search of pathogenic single nucleotide molecular variants (SNVs, including indels) and/or actionable gene fusions.

Results: Genetic changes were detected in 171 NSCLC samples (~55%) from 169 patients. Sequencing results from 14 samples (~4,5%) did not meet quality criteria for further analysis. Pathogenic SNVs were detected in 160 tumor specimens from 158 patients, including single nucleotide changes or small deletions/insertions in *KRAS* (n = 106 samples, 105 patients), *EGFR* (n = 31 samples, 30 patients), *BRAF* (n = 14), *MET* (n = 1), *RET* (n = 1) genes and splicing mutations resulting in *MET* exon 14 skipping (n = 8). In four cases a co-occurrence of two molecular variants was noted. Actionable gene fusions occurred in 11 patients, comprising *EML4::ALK* (n = 7), *CD74::ROS1* (n = 2), *SDC4::ROS1* (n = 1), *KIF5B::RET* (n = 1).

Conclusion: Targeted RNA-based NGS assay enables robust, specific and sensitive detection of the clinically relevant molecular alterations essential to predict the potential benefit of NSCLC patients from targeted therapies, as it simultaneously tests for both SNVs and gene fusions within single analysis.

Grants: IGICHP 3.3/22

Conflict of Interest: None declared

P01.118.A Validation of RNA-based targeted next-generation sequencing assay for molecular variants detection in non-small cell lung cancer

Urszula Lechowicz[1], *Magdalena Pelc*[1], *Adam Szpechcinski*[1], *Joanna Moes-Sosnowska*[1], *Paulina Skronska*[1], *Adriana Rozy*[1], *Aneta Zdral*[1], *Mateusz Polaczek*[2], *Emil Wojda*[2], *Małgorzata Szołkowska*[3], *Tadeusz Orłowski*[4], *Pawel Sliwinski*[5], *Reanata Langfort*[3], *Joanna Chorostowska-Wynimko*[1]

[1]*The Institute of Tuberculosis and Lung Diseases, Department of Genetics and Clinical Immunology, Warsaw, Poland;* [2]*The Institute of Tuberculosis and Lung Diseases, III Department of Lung Diseases and Oncology, Warsaw, Poland;* [3]*The Institute of Tuberculosis and Lung Diseases, Department of Pathology, Warsaw, Poland;* [4]*The Institute of Tuberculosis and Lung Diseases, Department of Surgery, Warsaw, Poland;* [5]*The Institute of Tuberculosis and Lung Diseases, II Department of Lung Diseases, Warsaw, Poland*

Background/Objectives: The accurate molecular characterization of non-small cell lung cancer (NSCLC) is mandatory for the optimal care of patients. We have validated RNA-based targeted next-generation sequencing (NGS) assay for the detection of important genetic variants in different types of NSCLC specimens.

Methods: 379 tumor specimens from 372 patients were analyzed using the Archer FusionPlexLung (AFPL) with reference to conventional IVD qPCR and fluorescence in situ hybridization (FISH) tests and DNA-based targeted NGS assay (TruSight_Tumor15, Illumina). A minimum required number of reads per sample was 0.5 million and an Internal Sample Quality Control index ≥10.

Results: Valid molecular results were obtained in 312 NSCLC samples; 17.8% specimens did not meet the acceptance criteria either for NGS procedure or data analysis. Actionable variants were detected in 171 (54.8%) samples. Concentrations ≥10ng of RNA and Cp at PreSeq <29 were established as borderline values allowing a sample to be qualified for further NGS analyses. The validation process allowed to set the reporting thresholds for SNVs at the lowest allele frequency of ≥4,9%, with at least 10 altered reads at ≥100 reads coverage. The reporting a gene fusion at ≥10% fusion site reads and supporting reads ≥5, with unique origins ≥3 in NGS resulted in very good concordance with FISH and qPCR. The median values for Total Fragments, RNA QC and RNA fragment length were 1371234, 194 and 123, respectively.

Conclusion: RNA-based NGS offers a diagnostic solution ensuring precise identification of variants across numerous genes that hold therapeutic significance in NSCLC.

Grants: IGICHP_3.3/22

Conflict of Interest: None declared

P01.119.B POU5F1B is a human-restricted ROCK inhibitor-sensitive oncoprotein that restructures membrane nanodomains to increase cell adhesion.

Laia Simó Riudalbas[1], *Sandra Offner*[2], *Abrami Laurence*[2], *Eduard Unterauer*[3], *Julien Duc*[2], *Evarist Planet*[2], *Ralf Jungmann*[3], *Alexandre Reymond*[1], *Gisou van der Goot*[2], *Didier Trono*[2]

[1]*Center for Integrative Genomics, Lausanne, Switzerland;* [2]*École polytechnique fédérale de Lausanne, Lausanne, Switzerland;* [3]*Max Planck Institute of Biochemistry, Planegg, Germany*

Background/Objectives: *POU5F1B* arose by retrotransposition of *POU5F1/OCT4* in the last common ancestor of modern hominidae. In human the encoded protein is endowed with oncogenic properties. It promotes colorectal cancer growth and metastasis. Here, we aim at investigating the molecular mechanisms by which *POU5F1B* exerts its pro-oncogenic effect.

Methods: Detergent resistant membranes (DRMs) were isolated from colorectal cancer cell lines to study cholesterol-rich nanodomains. Acyl-RAC capture assay and radiolabeling 3H-palmitic acid incorporation were used to study protein S-acylation. We used LC-MS/MS and high-resolution microscopy to investigate protein composition and cell focal adhesions, respectively.

Results: We found that the oncogenic action of POU5F1B requires ubiquitination of lysine residues present only in the human isoform. This post-translational modification is essential for POU5F1B to localize to the cytoplasm and become S-acylated by the DHHC17 S-acyltransferase. This leads to POU5F1B association with DRMs, where it triggers the accumulation of signaling molecules and integrins, thereby stimulating cell focal adhesion

and modifying the mechanic properties of cancer cells and their interactions with the extracellular matrix. The stability of POU5F1B depends on Rho-associated protein kinase (ROCK), a regulator of cytoskeletal dynamics and cell motility. Consistently, we identified ROCK inhibitors as POU5F1B destabilizers in a high-throughput drug screening.

Conclusion: We identified POU5F1B as the first ever described human-lineage specific cancer-promoting protein. It acts by an unprecedented mechanism, the remodeling of membrane nanodomains. Its action can be blocked by ROCK inhibitors. These results have interesting evolutionary and therapeutic implications.

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Conflict of Interest: Laia Simó Riudalbas 2 patent applications: EP23154002.2

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P01.121.D DNA methylation biomarkers of thymomas and thymic carcinomas

Vanessa Nicoli[1], Marianna Giangreco[1], Eleonora Pardini[1], Iacopo Petrini[1], Diana Bacchin[2], Vittorio Aprile[2], Franca Melfi[3], Marco Lucchi[2], Roberta Ricciardi[4], michelangelo maestri[4], Andrea Stoccoro[1], Lucia Migliore[1], **Fabio Coppedè**[1]

[1]University of Pisa, Translational Research and of New Surgical and Medical Technologies, Pisa, Italy; [2]University of Pisa, Division of Thoracic Surgery, Dept. of Surgical, Medical and Molecular Pathology and Critical Care Medicine, Italy; [3]University Hospital of Pisa, Minimally Invasive and Robotic Thoracic Surgery, Robotic Multispecialty Center of Surgery, Italy; [4]University of Pisa, Neurology Unit., Dept. of Clinical and Experimental Medicine, Italy

Background/object: Thymic epithelial tumors (TETs) are the most frequent neoplasms of the anterior mediastinum and include thymomas (TMs), thymic carcinomas (TCs), and rare neuroendocrine tumors. Epigenetic modifications are increasingly observed in these tumors, indicating the need for further characterization of their distribution among different subtypes, stages and histotypes.

Methods: We reviewed the literature related to DNA methylation studies in TETs, including candidate gene association studies and genome-wide investigations. We then designed a panel of genes among the most promising epigenetic biomarkers of TETs and evaluated their methylation levels in 71 bioptic TET samples consecutively collected over a 3-year period, including 64 TMs and 7 TCs. Blood samples were collected at thymectomy from each patient and screened for peripheral epigenetic biomarkers of TET subtypes.

Results: TCs showed hypermethylation of *GHSR* and *ELF3* genes and reduced *IL1RN* methylation levels compared to TMs. We also observed a significant hypomethylation of the *RAG1* promoter region in TMs, but not in TCs, compared to healthy thymic tissues. No difference in the methylation levels of the investigated genes was seen among TM stages and subtypes. No changes in the blood methylation levels of the investigated genes were seen among TET subtypes.

Conclusion: The present study revealed TET methylation biomarkers and a set of genes that can discriminate between TMs and TCs.

Grant References: THYMOGENE project, Bando Salute Tuscany Region.

Conflict of Interest: None declared

P01.123.B Identification and validation of new DNA methylation panels for the differentiation between hepatocellular carcinoma and cholangiocarcinoma

Tina Draškovič[1], *Nina Hauptman[1]*

[1]Institute of Pathology, Faculty of Medicine, University of Ljubljana, Department of Molecular Genetics, Ljubljana, Slovenia

Background/Objectives: Differentiating between hepatocellular carcinoma (HCC) and cholangiocarcinoma (CCA) is sometimes challenging. The aim of our study was to identify and validate novel DNA methylation biomarkers and to develop panels that can differentiate between HCC, CCA and their paired normal tissue.

Methods: Using bioinformatics, we identified new differentially methylated promoter regions and designed panels for the diagnosis and differentiation between HCC and CCA. DNA was isolated from formalin-fixed, paraffin-embedded tissue samples of 15 HCC, 15 CCA and their paired normal tissue. After DNA isolation and DNA bisulfite conversion, the methylation status of the selected regions was determined by dPCR. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated based on the hyper- or hypomethylation of each sample.

Results: The design panel for HCC includes the promoter regions of the genes RNF135 and EFNB2, which successfully differentiate HCC from CCA with a sensitivity of 87%, a specificity of 100%, a PPV of 1 and an NPV of 0.88. The design panel for CCA includes the promoter regions of the genes RNF125, HOXC4 and ABLIM1, which differentiate CCA from HCC with a sensitivity of 67%, a specificity of 100%, a PPV of 1 and an NPV of 0.75. Both panels are 100% cancer specific.

Conclusion: Our results show that the DNA methylation status of the selected promoter regions and the designed panels successfully diagnose and differentiate HCC and CCA with high sensitivity, specificity, PV and NPV and are cancer specific.

Grants: Slovenian Research and Innovation Agency (P3-0054, J3-3070 and PhD research funding).

Conflict of Interest: None declared

P01.124.C Genetic landscape of hereditary hematological malignancies (HHM) in adults

Dora Csaban[1], *Livia Varga[1], Zoltan Orfi[1], Lenke Tanko[1], Nora Kondor[1], Andras Bors[1], Jozsef Harasztdombi[1], János Dolgos[1], Judit Reichardt[1], Beata Koller[1], János Fábián[1], Andrea Varkonyi[1], Viktor Lakatos[1], Laszlo Gopcsa[1], Valyi-Nagy Istvan[1], Peter Remenyi[1], Hajnalka Andrikovics[1]*

[1]Central Hospital of Southern Pest, National Institute of Hematology and Infectious Diseases, Budapest, Hungary

Background: Due to advancements in sequencing technologies, more insights are gained in the background of hereditary hematological malignancies (HHM), which were recently recognized as novel clinical entity in WHO and European LeukemiaNet Classifications.

Methods: Peripheral blood or bone marrow samples were used for isolation of tumour derived DNA from 455 patients with haematological malignancy (ALL, AML, MDS, MPN). Next generation sequencing (NGS) targeted panel contained genes associated with somatic and germline hematologic malignancies. The

presumed germline pathogenic variants were confirmed by Sanger sequencing from independent samples (hair follicles or potential hematopoietic stem cell donor family members).

Results: In our study with 'tumour only' testing strategy, 58 variants were presumed to be hereditary pathogenic alterations, of which were confirmed in 9 cases (1.98%): DDX41 (n = 3), RUNX1 (n = 3), TP53 (n = 1), TERT (n = 1), PTPN11 (n = 1). With the exception of DDX41 (age at diagnosis: 60-69 year), HMM patients were younger than average patients with haematological malignancies (17-49 years). All patients showed unobtrusive personal and family history without any malignancies. Cascade screening of family members identified carrier status in 16 out of 50 cases, and no clinical symptoms were observed in several older relatives.

Conclusion: Our results highlight that HHM occurs not only in young individuals. The prevalence of HMM might be underestimated due to its low penetrance. Germline genetic information influence the choice of targeted therapy and the selection of hematopoietic stem cell donor from the family. Additionally, it draws attention to the inclusion of asymptomatic carrier relatives in hematological/oncological care.

Conflict of Interest: None declared

P01.125.D A Novel Association Of *CHEK2* Splice Site Mutation With Early Onset Gastric Cancer

AHMED AL-OMAR[1], Süleyman Alıcı[2], **İlknur Sunar**[1]*, Ayse Busra Akalin[3], Ibrahim Akalin[1]*

[1]Metagentech Center for Genetic Diseases, istanbul, Türkyie; [2]Hisar Intercontinental Hospital, Medical Oncology, istanbul, Türkyie; [3]Bahcesehir University, Faculty of Medicine, istanbul, Türkyie

Background/Objectives: *CHEK2* is a tumor suppressor gene that arrests cell cycles and leads to apoptosis after DNA damage. Germline mutations in *CHEK2* gene increase risk for breast and other types of cancer. There are different types of pathogenic variants such as short insertions/deletions, single nucleotide variations, large genomic rearrangements, and splicing variants.

Methods: A 31-year-old female patient with a history of gastric cancer was admitted to the hospital and operated for total gastrectomy. Pathological evaluation revealed T4N3 MSI stabile, lymph node and extra peritoneal metastatic adenocarcinoma. Biochemistry laboratory results showed elevated levels of ALT, AST, GGT and alkaline phosphatase. The patient received 6 cycle FLOT chemotherapy. External radiotherapy was applied with capecitabine. The patient was referred for genetic testing for hereditary cancer genes.

Result: Exome sequencing analysis revealed unknown significance of c.-185-6del heterozygous splice site mutation in *CHEK2* gene, responsible for autosomal dominantly inherited "Cancer Predisposition Syndrome, *CHEK2*-related, Li-Fraumeni Syndrome 2, AD(?) [MIM#609265]".

Conclusion: The mutation was identified in gnomAD database and splice site *CHEK2* mutations were attributed to be responsible for gastric cancer development under 55-year-old patients. Here we for the first time report a *CHEK2* splice site variation (c.-185-6del) supposed to be pathogenic in gastric cancer patients up to date.

Grants:

Conflict of Interest: None declared

P01.126.A Harnessing cDNA analysis to enhance variant classification in cancer predisposition genes

Liesbeth Claeys[1;2]*, Suzanne Vanhauwaert[1;2;3], Annelies De Jaegher[1;2], Kim De Leeneer[1;2;3], Bram Parton[1;2], Ilse Coene[1;2], Martine De Bleeckere[1;2], Katia De Pauw[1;2], Arne De Visscher[1;2], Elke D'Haese[1;2], Tjoïlina Reyniers[1;2], Hélène Verhaeghe[1;2], Bruce Poppe[1;2;3], Robin de Putter[1;2;3], Kathleen Claes[1;2;3]*

[1]Ghent University Hospital, Center for Medical Genetics Ghent, Ghent, Belgium; [2]Ghent University, Department of Biomolecular Medicine, Ghent, Belgium; [3]Ghent University and Ghent University Hospital, Cancer Research Institute Ghent (CRIG), Ghent, Belgium

Background/Objectives: In clinical practice a panel of 107 genes linked to familial cancer syndromes, comprising various subpanels tailored to clinical requests, is routinely employed. This study aims to assess the utility of cDNA analysis in enhancing molecular diagnostics.

Methods: SeqCap EZ HyperCap technology was applied for target enrichment, followed by NovaSeq6000 sequencing. Digital MLPA and ExomeDepth were utilized for exon-level CNV detection. RNA extraction from short term lymphocyte cultures, supplemented with puromycin as an NMD inhibitor, enabled RT-PCR and Sanger sequencing. A total of 44 cases spanning 16 genes were investigated.

Results: Among 9871 patients screened, (likely) pathogenic variants (class 4/5) were detected in 10% (n = 1038), with 6% (n = 585) harboring variants of unknown clinical significance (VUS, class 3).

Algorithms predicted aberrant splicing for 25 variants, with 12 corroborated by cDNA-analysis, upgrading them to class 4. Nine variants were downgraded to class 2 while 4 others remained class 3. Notably, an aberrant transcript was confirmed in 4 cases with an SVA element insertion in *PMS2*. Twelve exon CNVs were further investigated by cDNA testing. Only one of five affecting PMS2 exons 13-15 was confirmed. Four exon-CNVs in other genes were confirmed. In three other cases cDNA analysis discerned disease relevant from pseudogene artifacts.

Conclusion: We demonstrate the multifaceted benefits of cDNA analysis in familial cancer syndrome diagnostics: it enhances variant classification, validates exon CNVs, and distinguishes gene sequences from (non-expressed) pseudogenes. The integration of cDNA analysis enriches the molecular diagnostic arsenal, refining patient management strategies.

Conflict of Interest: None declared

P01.127.B Structural variant in *LRRFIP2* detected by whole-genome sequencing as the cause of an unsolved Lynch-like syndrome family

Gemma Llargués-Sistac[1]*, Laia Bonjoch[1], Jenifer Muñoz[1], xavier domínguez-rovira[1], Teresa Ocaña[1], Maria Isabel Alvarez[2], Celia Badenas[2], Miriam Cuatrecasas[3], Antoni Castells[1], Francesc Balaguer[1], Leticia Moreira[1], Guerau Fernandez[4], Sergi Castellví-Bel[1]*

[1]Hospital Clínic, FRCB-IDIBAPS, CIBEREHD, Gastroenterology, Barcelona, Spain; [2]Hospital Clinic, FRCB-IDIBAPS, CIBERER, Biochemistry and Molecular Genetics, Barcelona, Spain; [3]Hospital Clínic, IDIBAPS, Pathology, Barcelona; [4]Hospital San Joan de Déu, CIBERER, Barcelona, Spain

Background/Objectives: Lynch Syndrome is the most common form of hereditary colon cancer, caused by germline mutations in four genes of the DNA-mismatch repair pathway (*MLH1*, *MSH2*, *MSH6* and *PMS2*). Current diagnostic approaches identify causative genetic variants in less than 50% of cases. We present an unsolved case from an Equatorian family with Lynch-like syndrome, with tumor loss of *MLH1* and *PMS2* expression and *BRAF* wild-type, and a familial history of colorectal and stomach cancer, but no underlying genetic variants detected through standard diagnostic tests.

Methods: We performed germline whole-genome sequencing (WGS) of DNA from blood samples of two siblings to identify the causative mutational event. To further underpin the molecular mechanism involved, subsequent transcriptome sequencing (RNA-seq) and quantitative PCR in one of the siblings was performed.

Results: In both siblings, a tandem duplication of 37 kilobases was found in the *LRRFIP2* gene, contiguous to *MLH1*. RNA-seq data confirmed an altered expression of *LRRFIP2* and *MLH1*. Results were validated with a quantitative PCR for both genes.

Conclusions: WGS allowed the identification of a duplication in the *LRRFIP2* gene that affects the expression of *MLH1*, explaining the clinical presentation of the patient and their family. This case highlights the added value of using WGS and RNA-seq to current standard diagnostic approaches to help solve cases that present with complex genomic rearrangements that otherwise would have not been detected with current genetic testing.

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Conflict of Interest: None declared

P01.128.C Liquid Biopsy in Glioblastoma Management – preliminary results

domingos roda[1], pedro veiga[2], Luís Miguel Pires[2], alexandra mascarenhas[2], Francisco Caramelo[3;4], Clara Romero[5], inês pinto[6], Tomás Dinis[6], Pedro Abreu[5], Pedro Teles[5], José Luís Alves[7], Joana Barbosa de Melo[2;4;8], Isabel Marques Carreira[2;4;8], **Ilda Patrícia Ribeiro**[2;4;8]

[1]Joaquim Chaves Saúde (JCS), Algarve Radiation Oncology Unit, Faro, Portugal; [2]University of Coimbra, Cytogenetics and Genomics Laboratory, Institute of Cellular and Molecular Biology, Faculty of Medicine, Coimbra, Portugal; [3]Laboratory of Biostatistics and Medical Informatics, iCBR - Faculty of Medicine, University of Coimbra, Coimbra, Portugal, Coimbra, Portugal; [4]University of Coimbra, Coimbra Institute for Clinical and Biomedical Research (iCBR) and Center of Investigation on Environment Genetics and Oncobiology (CIMAGO), Faculty of Medicine, Coimbra, Portugal., Coimbra, Portugal; [5]Centro Hospitalar e Universitário do Algarve, CHUA, Serviço de Neurocirurgia, Faro, Portugal; [6]Centro Hospital e Universitário de Coimbra, Serviço de Radioterapia, Coimbra, Portugal; [7]Centro Hospitalar e Universitário de Coimbra, CHUC, Serviço de Neurocirurgia, Coimbra, Portugal; [8]University of Coimbra, Center for Innovative Biomedicine and Biotechnology (CIBB), Coimbra, Portugal and Clinical Academic Center of Coimbra (CACC), Coimbra, Portugal., Coimbra, Portugal

Background/Objectives: Glioblastoma (GBM) represents the most aggressive form of nervous system cancer. The objectives of this pilot study were to characterize the genomic profile of GBM tumor tissue samples and to investigate the potential of liquid biopsies for diagnosing and monitoring patients.

Methods: A total of 110 samples of plasma were collected from 45 GBM patients before and during treatment. Plasma samples from 10 controls were also collected. After isolating cfDNA, concentrations were determined, compared between patients and controls, and monitored throughout the patients' clinical course. Tumor tissue samples were analyzed using array Comparative Genomic Hybridization (aCGH).

Results: aCGH analysis showed frequent gains and losses of chromosomes 1, 4, 5, 6, 7, 8, 9, 10, 12, 15, 16, 17, 20, 21, 22, and Y. Amplifications involving the *EGFR* and *MET* genes and deletions of *CDKN2A*, *PTEN*, *RB1* and *NF1* were common alterations observed in our cohort. The levels of plasma cfDNA revealed that GBM patients have higher plasma baseline cfDNA levels than healthy controls. In some of the patients a substantial increase in plasma cfDNA levels was observed during follow up, which is being correlating with clinical events.

Conclusion: Our findings demonstrate the feasibility of isolating cfDNA from the plasma of GBM patients. Furthermore, monitoring cfDNA levels during treatment may provide valuable insights into disease progression and treatment response. Integrating the analysis of liquid and tissue biopsies enables a comprehensive characterization of the tumor profile. A larger cohort and an extended follow-up period are required for further validation.

Conflict of Interest: None declared

P01.129.D Rare variants modulate phenotype in NF1 carriers'

Elena Pasquinelli[1;2], **Chiara Fallerini**[2;3]*, Samantha Minetto[2;3], Giulia Rollo[2;3], Margherita Baldassarri[2;3;4], Alessandra Renieri[2;3;4]*

[1]Medical Genetics, University of Siena, Italy, Department of Medical Biotechnologies, Siena; [2]Med Biotech Hub and Competence Center, Department of Medical Biotechnologies, University of Siena, Siena, Italy, Department of Medical Biotechnologies, Siena, Italy; [3]Medical Genetics, University of Siena, Italy, Department of Medical Biotechnologies, Siena, Italy; [4]Genetica Medica, Azienda Ospedaliera Universitaria Senese, Siena, Italy, Siena, Italy

Background/Objectives: Neurofibromatosis type 1, is a rare genetic disorder predisposing to a pleiotropic phenotype ranging from psychosocial issues, congenital malformations, benign tumors of the nervous systems or aggressive solid cancer. We hypothesize that this phenotypic variability is due to additional rare variants in other genes, acting as an oligogenic threshold model.

Methods: Clinical and Exome sequencing data were retrieved in a retrospective nested-study from 32 patients with pathogenic/like pathogenic NF1 variants.

Results: NF1 carriers with solid cancers show a mean of 4.12 variants in cancer driver genes per subject while NF1 carriers without solid cancers have 2.16 variants per subject with Mann-Whitney test significant at $p < 0.05$. Furthermore, we present that less frequently (macrocephaly or cognitive disorder) or rarely (connective disorder or polydactyly) ancillary characteristics could be linked to rare variants in additional genes. NF1 carriers with cognitive impairment have a mean of 2.7 additional variants, with at least one of them being a P/LP variant in a well known dominant neurodevelopmental disorder gene. In inherited cases, we detect in probands that NF1 inherited variant combined with other rare variants inherited from both parents act reinforcing the NF1 susceptibility with an additive trend.

Conclusion: In conclusion, we have highlighted that rare variants may modulate phenotypic outcome in neurofibromatosis. These results are relevant for both clinical policy and clinical practice: we propose to use the rare variant capture for amelioration screening program in NF1 carriers and identification of specific gene cards for personalized treatment.

Grants: INTERVENE, HORIZON Project 2020 n.101016775

Conflict of Interest: None declared

P01.130.A Germline testing for patients with pancreatic adenocarcinoma: a retrospective study in the department of genetics at Amiens University Hospital, France

Luana Giovannangeli[1]*, Emilie Lacot-Leriche[1], Gilles Morin[1], Bénédicte Bonte[1], Florence Amram[1], Florence Jobic[1], Emma Lachaier[1]*

[1]CHU Amiens-Picardie, Genetics, Amiens, France

Background/Objectives: In 2019, the American Guidelines recommended universal germline testing for patients with Pancreatic Cancer (PC). Literature reports pathogenic variations in 10-15% of PC. In France, due to limited access to oncogenetics, germline testing is usually restricted to patients with familial history, age under 50 years or somatic alteration in *BRCA* which can lead to PARP-inhibitor therapy. In our department, we applied universal testing. This study aimed to determine the benefit of universal germline testing compared to French practices.

Methods: We conducted a retrospective study (2022-2023) in our department of genetics in Amiens, France. Patients with pancreatic adenocarcinoma (locally advanced or metastatic) regardless of familial history or age were included. The analyzed genes were, at least: *EPCAM, ATM, BRCA1, BRCA2, TP53, PALB2, CDKN2A/p14, CDKN2A/p16*, and MMR ones.

Results: Among 45 patients, one was excluded (neuroendocrine cancer). According to American Guidelines: 5 had pathogenic variations (1 *BRCA1*, 3 *BRCA2, 1 ATM*), 11 had variants of unknown significance (VUS) and 28 had negative testing. According to French practices, 8 had criteria for germline testing. Among them, 1 had pathogenic variation (*BRCA2*), 3 VUS, and 4 negative results.

Conclusion: According to French practices, 4 pathogenic variations would not have been highlighted. Despite a restricted panel of genes with follow-up recommendation compared to the American panel, the rates of pathogenic variants and VUS remain high. Our study confirms the relevance of universal testing for PC. Universal testing improves analytical performance but also increase consultation activity, approximatively 20 per year in our department.

Conflict of Interest: None declared

P01.132.C The advantage of targeted next-generation sequencing over qPCR in testing for druggable *EGFR* variants in non-small cell lung cancer

Adam Szpechcinski[1], *Joanna Moes-Sosnowska[1], Urszula Lechowicz[1], Paulina Skronska[1], Magdalena Pelc[1], Małgorzata Szołkowska[2], Emil Wojda[3], Piotr Rudzinski[4], Krystyna Maszkowska-Kopij[5], Mateusz Polaczek[3], Renata Langfort[2], Pawel Sliwinski[6], Tadeusz Orłowski[4], Joanna Chorostowska-Wynimko[1]*

[1]The Institute of Tuberculosis and Lung Diseases, Department of Genetics and Clinical Immunology, Warszawa, Poland; [2]The Institute of Tuberculosis and Lung Diseases, Department of Pathology, Warszawa, Poland; [3]The Institute of Tuberculosis and Lung Diseases, III Department of Lung Diseases and Oncology, Warszawa, Poland; [4]The Institute of Tuberculosis and Lung Diseases, Department of Thoracic Surgery, Warszawa, Poland; [5]The Institute of Tuberculosis and Lung Diseases, Outpatient Clinic, Warszawa, Poland; [6]The Institute of Tuberculosis and Lung Diseases, II Department of Lung Diseases, Warszawa, Poland

Background/Objectives: Targeted therapies in non-small cell lung cancer (NSCLC) are dedicated to patients with tumors exhibiting features of oncogene addiction, including activation of epidermal growth factor receptor (EGFR) by SNVs/indels in exons 18-21 of the gene. Nowadays, diagnostic techniques based on qPCR and direct DNA sequencing are being replaced by next-generation sequencing (NGS) for actionable variants detection. In this study, we evaluated the diagnostic usefulness of targeted NGS panel for druggable EGFR variants testing in clinical NSCLC material previously analyzed by IVD-certified qPCR test with respect to DNA reference material.

Methods: The *EGFR* variants were tested in tumor tissue specimens from 59 NSCLC patients by the NGS 'TruSight Tumor 15' assay (Illumina) and the qPCR 'Cobas EGFR mutation test' (Roche Diagnostics). The sensitivity and specificity of NGS assay were evaluated using the biosynthetic and biological DNA reference material with known allelic frequencies (VAF) of *EGFR* variants.

Results: In DNA reference material, NGS assay demonstrated sufficient lower detection limit for diagnostic applications (VAF < 5%); all *EGFR* variants were correctly identified. NGS showed high repeatability of VAF assessment between runs (CV% from 0.02 to 3.98). In clinical material, the overall concordance between NGS and qPCR was 59%. The majority of discordant results concerned false-positive detection of *EGFR* exon 20 insertions by qPCR. *TP53* was frequently co-mutated gene in *EGFR*-positive NSCLC patients.

Conclusion: The NGS showed a number of superior features over the qPCR in *EGFR* variant detection (exact identification of *EGFR* variants, calculation of allelic frequency, high analytical sensitivity) which might enhance the basic diagnostic report.

Grants: IGIChP_3.11/23

Conflict of Interest: None declared

P01.134.A Exome sequencing identifies candidate genes for platinum response in ovarian cancer patients

Lisa-Marie Speith[1], *Lara Thomys[1], Donna-Lucil Sperber[1], Peter Schürmann[1], Dhanya Ramachandran[1], Robert Geffers[2], Britta Wieland[1], Mu Yang[1], Kristine Bousset[1], Clemens Liebrich[3], Tjoung-Won Park-Simon[1], Peter Hillemanns[1], Thilo Dörk[1]*

[1]Hannover Medical School, Gynaecology Research Unit, Hannover; [2]Helmholtz Institute for Infection Research, Genome Analytics, Braunschweig, Germany; [3]Wolfsburg Clinics, Gynaecology and Obstetrics, Wolfsburg, Germany

Background/Objectives: Carriers of pathogenic germline variants in BRCA1 or BRCA2 have an improved response after standard platinum treatment for ovarian cancer. In the present study we used genomic DNA from long-term survivors to identify and characterize additional genes that may be involved in the development of an extraordinary platinum response.

Methods: We performed exome sequencing in 30 selected patients with high-grade serous ovarian cancer who responded particularly well to carboplatin chemotherapy and were alive and tumor-free more than two years after the end of therapy. Candidate genes harboring truncating variants were ranked through pathway enrichment analyses and literature search. Selected candidate genes were further investigated through silencing or inhibitor studies in different ovarian cell lines using flow cytometry and survival assays.

Results: We identified truncating germline variants in nine genes associated with DNA replication in 7/30 patients (23%). Four of the nine genes were also known to be involved in R-loop suppression. Analyses of large case-control sequencing data sets indicated that none of them was associated with overall risk for ovarian cancer. In functional studies using a pair of platinum-sensitive and platinum-resistant A2780 cell lines, the inhibition or down-regulation of two of the candidate genes modulated platinum response and differentially induced cell death.

Conclusion: Germline variants in replication-associated genes may contribute to the platinum sensitivity of tumors in long-survivors. Inhibition of such genes may open an avenue for improving platinum therapy in patients with resistant tumors.

Grants: Supported by the German Research Foundation (Do761/15-1).

Conflict of Interest: None declared

P01.135.B Whole Exome Sequencing (WES), SNP-array and Shallow Whole Genome Sequencing (sWGS) identifies recurrent genomic alterations and molecular heterogeneity in multiple myeloma.

Federica Cannas[1;2], *Baran Bayindir*[1], *Alessia Mascia*[3], *Chiara Cocco*[1], *Laura Serventi*[1], *Roberta Murru*[4], *Ludovica Martorana*[4], *Annalisa Azzena*[4], *Maria Valentina Licheri*[4], *Stefano Mocci*[1;2], *Celeste Sanna*[1], *Michela Murgia*[1], *Meropi Plousiou*[1], *Giulia Nutile*[1], *Federico Marongiu*[1], *Maximilian Frick*[1], *Daniele Derudas*[5], *Erika Giuressi*[4], *Andrea Perra*[3], *Sabrina Giglio*[1;2;4]

[1]*University of Cagliari, Medical Genetics, Department of Medical Sciences and Public Health, University of Cagliari, Cagliari, Italy;* [2]*University of Cagliari, Centre for Research University Services (CeSAR, Centro Servizi di Ateneo per la Ricerca), University of Cagliari, Monserrato (CA), Italy;* [3]*University of Cagliari, Unit of Oncology and Molecular Pathology, Department of Biomedical Sciences, University of Cagliari, Cagliari, Italy;* [4]*Medical Genetics, R. Binaghi Hospital, Local Public Health and Social Care Unit (ASSL) of Cagliari, Cagliari, Italy;* [5]*UO of Hematology and Bone Marrow Transplant Centre, A. Businco Hospital, Cagliari, Italy*

Background/Objectives: multiple myeloma (MM) is a hematologic disease caused by clonal proliferation of plasma cells. We examined the genetic aspects of MM, including sequence-related genetic alterations and genomic instability, to demonstrate the superiority of sWGS, a high-throughput technology, as a valid alternative compared to classical cytogenetics. Indeed, although FISH is the current gold standard to detect cytogenetic events in MM, it is costly, labor-intensive and does not show the exact genomic background of this disorder.

Methods: we analyzed 100 patients at various stages of MM. We performed SNP-array and FISH to detection of copy number aberrations (CNA) and balanced translocations, respectively. Additionally, we performed sWGS (0.1X) and WES on each sample.

Results: SNP-arrays suggested genomic instability in approximately 30% of patients, often associated with gain-of-function variants in several oncogenes (*NRAS, KRAS, BRAF*) and loss-of-function variants in *TP53*, directly related to the risk of progression, survival, and resistance to therapy. Moreover, we detected variants in *DNMT3A* gene correlated with tumor progression. Furthermore, sWGS detected CNA and LOH with superior sensitivity compared to array and, with a specific bioinformatic pipeline, was able to identify balanced translocations, demonstrating sWGS is superior to FISH, which is not able to identify all CNA and LOH.

Conclusion: sWGS has proven to be a superior method for identifying biallelic events and rearrangements with heterogeneous breakpoints. WES and sWGS, to be used as a first diagnostic approach, can be helpful in addressing various aspects related to targeted therapy, therapy failure and relapse, providing crucial information on clinical decision-making.

Conflict of Interest: None declared

P01.136.C Whole Exome Sequencing (WES) and Shallow Whole Genome Sequencing (sWGS) of tissue and Cell-Free DNA (cf-DNA) for the assessment of Homologous Recombination Deficiency (HRD) in Ovarian Cancer

Giulia Nutile[1], *Baran Bayindir*[1], *Federica Cannas*[1;2], *Chiara Cocco*[1], *Laura Serventi*[1], *Roberta Murru*[3], *Ludovica Martorana*[3], *Mirella Casula*[3], *Gaia Maria Tosone*[1], *Michela Lorrai*[1], *Caterina Mereu*[1], *Stefano Mocci*[1;2], *Nicola Orrù*[3], *Federico Marongiu*[1], *Maximilian Frick*[1], *Erika Giuressi*[3], *Andrea Perra*[4], *Daniela Fanni*[1], *Sabrina Giglio*[1;2;3]

[1]*University of Cagliari, Medical Genetics, Department of Medical Sciences and Public Health, University of Cagliari, Cagliari, Italy;* [2]*University of Cagliari, Centre for Research University Services (CeSAR, Centro Servizi di Ateneo per la Ricerca), University of Cagliari, Monserrato (CA), Italy;* [3]*Medical Genetics, R. Binaghi Hospital, Local Public Health and Social Care Unit (ASSL) of Cagliari, Cagliari, Italy;* [4]*Unit of Oncology and Molecular Pathology, Department of Biomedical Sciences, University of Cagliari, Cagliari, Italy*

Background/Objectives: pathogenetic variants in the genes *BRCA1/BRCA2* have a significant role in developing ovarian cancer (OC) and are essential for genome integrity by repairing double-stranded DNA breaks via the homologous recombination repair (HRR) pathway. Our aim is to identify whether the HR disruption correlates with oncogene alterations and, ideally, with therapy responses.

Methods: a total of 96 women affected by OC (13 in paclitaxel-platinum with or without bevacizumab, 3 in PARP-inhibitors) were enrolled. We performed: on 66 samples a multigene panel on FFPE tissue and HRS-test to identify HRD; on 30 samples WES (germinal and somatic) and SNP-array; on all 96 samples shallow whole-genome sequencing (sWGS), which results were compared to the reference assay SOPHiA. Furthermore, sWGS was also performed on cf-DNA in 50 cases.

Results: we found germinal variants in *BRCA1/BRCA2* on 5.21% and 6.25% of subjects, respectively. Moreover, we detected several variants in suppressor genes and oncogenes, including mismatch repair (MMR) genes, likewise causative variants in HRR-related genes like *BARD1* (2,08%), *ATM* (2,08%), *CHEK2* (1,04%). Somatic pathogenic variants were: 71,88% in *TP53*, 16,67% in *BRCA1* and 11,46% in *BRCA2*. Almost all cases (98%) presented genomic instability highlighted both on tissue and cf-DNA.

Conclusion: *BRCA1/BRCA2* mutation status is the main genetic biomarker of HRD but is not sufficient to describe the total HRD status, which can be driven by somatic events and further genetic alterations. Therefore, a bioinformatics pipeline to evaluate the HRR system status in OC has been set, based on sWGS, to support therapeutics and follow-up strategies.

Conflict of Interest: None declared

P01.137.D Rare Capillary Malformation-Arteriovenous Malformation Syndrome (CM-AVM): Intrafamilial phenotypic heterogeneity

stavroula psoni[1], *STYLIANI AMENTA*[2], *Emmanouel Manolakos*[3], **Eirini Tsoutsou**[4], *Christina Kanaka-Gantenbein*[5], *HELEN FRYSSIRA*[6]

[1]*Pediatrician-Medical Geneticist, Private Practice;* [2]*MITERA Maternity Hospital, Clinical Genetics;* [3]*ATG-Access to Genome Labs, Athens, Greece;* [4]*General Hospital of Athens "G. Gennimatas", Thalassemia Unit;* [5]*"Aghia Sofia" Children's Hospital, Medical School, National and Kapodistrian University of Athens, Athens, Greece, Pediatrics-Pediatric Endocrinology, First Pediatric Clinic;* [6]*Medical School, National and Kapodistrian University of Athens, Greece, Medical Genetics*

Background/Objectives: Capillary Malformation-Arteriovenous Malformation Syndrome (CM-AVM) (OMIM:608354) syndrome is a rare autosomal dominant genetic disease characterized by broad phenotypic variability and caused by mutations in the *RASA1* gene (p210RasGAP-protein-activator-1). CM-AVM comprises a wide phenotypic spectrum with atypical capillary and fast-flow vascular malformations being the most prominent clinical features. The CMs are usually multifocal and surrounded by pale halo with central red dot and can be observed in the brain, face or extremities. We describe a new CM-AVM case.

Methods: The 3.5 year-old female proband was offspring of non-consaguineous parents and presented with short stature, lordosis, genu valgum, macrocephaly and multiple nodular macule like café-au-lait skin lesions on the trunk and extremities. The family history revealed propensity for malignancies on the maternal ancestors. Hormonal, biochemical testing and internal organs imaging were normal. The karyotype was 46, XX.

Whole Exome Sequencing (WES) was subsequently performed on DNA extracted from the proband and her parents after obtaining signed informed consent, using standard procedures.

Results: WES identified in heterozygous state the *RASA1* mutation c.261_262del (p.Gly89ArgfsTer22) which comprises a 2bp deletion at exon-1 leading to frameshift and premature stop codon. The proband's mother was a carrier, although not presenting any skin lesions.

Conclusion: Upregulation of the Ras/MAPK signaling pathway, caused by haploinsufficiency of the Ras21-protein-activator-1, disrupts the normal growth regulation, cell differentiation and proliferation which in turn leads to angiogenesis overdrive. Given the CM-AVM phenotypic variability, clinical genetic evaluation and testing is essential for the diagnosis, follow-up and counseling of the patients and their families.

Grants: None

Conflict of Interest: None declared

P01.138.A Deciphering the molecular landscape of diffuse gastric cancers in truncating *CTNNA1* germline variant carrier patients

Silvana Lobo[1;2;3], *Alexandre Dias*[1;2;3], *Marta Ferreira*[1;2;4], *Nelson Martins*[1], *Rita Barbosa-Matos*[1;2;3], *João Fonseca*[1;2;5], *J. Garcia-Pelaez*[1;2;5], *Ana Maria Pedro*[1], *Irene Gullo*[1;6], *André Oliveira*[1], *Celina São José*[1], *Chrystelle Colas*[7;8], *Robert Hüneburg*[8;9;10], *Jacob Nattermann*[8;9;10], *Lise Boussemart*[11;12], *Liselotte P van Hest*[13], *Leticia Moreira*[14;15;16], *Carolyn Horton*[17], *Dana Farengo Clark*[18], *Sigrid Tinschert*[19;20], *Golmard Lisa*[7;8], *Isabel Spier*[8;9;21], *Adrià López-Fernández*[8;22], *Daniela Oliveira*[23;24;25], *Magali Svrcek*[26;27], *Pierre Bourgoin*[27], *Helene Delhomelle*[7;8], *Jeremy Davis*[28], *Birthe Zäncker*[29], *Conxi Lazaro*[8;30;31], *Joana Guerra*[3;32], *Manuel Teixeira*[3;8;32], *Kasmintan Schrader*[33;34], *Verena Steinke-Lange*[8;35;36], *Sérgio Sousa*[23;24;25], *Manuela Balsinha*[6], *Stefan Aretz*[8;9;21], *Judith Balmaña*[8;22], *Melyssa Aronson*[37], *Augusto Perazzolo Antoniazzi*[38], *Edenir Palmero*[39;40], *Lizet Van der Kolk*[41], *Annemieke Cats*[42], *Jolanda M van Dieren*[42], *Sergi Castellvi-Bel*[14;15;16], *Bryson Katona*[18], *Rachid Karam*[17], *Florence Coulet*[43], *Paulo Pereira*[1;44], *Patrick Benusiglio*[45;46], *carla oliveira*[1;5;8]

[1]*i3S – Instituto de Investigação e Inovação em Saúde, Porto, Portugal;* [2]*IPATIMUP – Institute of Molecular Pathology and Immunology of the University of Porto, Porto, Portugal;* [3]*Institute of Biomedical Sciences Abel Salazar of the University of Porto, Porto, Portugal;* [4]*Faculty of Science of the University of Porto, Porto, Portugal;* [5]*Faculty of Medicine of the University of Porto, Porto, Portugal;* [6]*Centro Hospitalar Universitário São João, Porto, Portugal;* [7]*Institut Curie, University Paris Sciences Lettres, Department of Genetics, Paris, France;* [8]*Full Member of the European Reference Network on Genetic Tumor Risk Syndromes (ERN GENTURIS) – Project ID No 739547;* [9]*National Center for Hereditary Tumor Syndromes, University Hospital Bonn, Bonn, Germany;* [10]*University Hospital Bonn, Department of Internal Medicine I, Bonn, Germany;* [11]*Nantes Université, Univ Angers, CHU Nantes, INSERM, Immunology and New Concepts in ImmunoTherapy (INCIT), Nantes, France;* [12]*Nantes University Hospital, Department of Dermatology, Nantes, France;* [13]*Amsterdam UMC, Vrije Universiteit Amsterdam, Amsterdam, Netherlands;* [14]*Hospital Clínic Barcelona, Department of Gastroenterology, Barcelona, Spain;* [15]*Centro de Investigación Biomédica en Red en Enfermedades Hepáticas y Digestivas (CIBEREHD), Barcelona, Spain;* [16]*Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), University of Barcelona, Barcelona, Spain;* [17]*Ambry Genetics, Aliso Viejo, California, United States;* [18]*University of Pennsylvania Perelman School of Medicine, Division of Gastroenterology and Hepatology, Philadelphia, Pennsylvania, United States;* [19]*Medical University Innsbruck, Division of Human Genetics, Innsbruck, Austria;* [20]*IFLb Laboratoriumsmedizin Berlin GmbH, Windscheidstraße, Berlin-Charlottenburg, Berlin, Germany;* [21]*Institute of Human Genetics, Medical Faculty, University of Bonn, Bonn, Germany;* [22]*Hereditary Cancer Group, Medical Oncology Department Hospital Vall d'Hebron, and Vall d'Hebron Institute of Oncology, Barcelona, Spain;* [23]*Paediatric Hospital, Coimbra Hospital and University Centre, Medical Genetics Unit, Coimbra, Portugal;* [24]*Faculty of Medicine, University of Coimbra, University Clinic of Genetics, Coimbra, Portugal;* [25]*Clinical Academic Centre of Coimbra, Coimbra, Portugal;* [26]*Sorbonne Université, UPMC Univ Paris 06, INSERM, UMRS 938, SIRIC CURAMUS, Equipe Instabilité Des Microsatellites Et Cancer, Equipe Labellisée Par La Ligue Contre Le Cancer, Centre de Recherche Saint Antoine, Paris, France;* [27]*Sorbonne Université, Laboratoire D'anatomie Et Cytologie Pathologiques, Hôpital Saint-Antoine, AP-HP, Paris, France;* [28]*National Cancer Institute, National Institutes of Health, Surgical Oncology Program, Maryland, United States;* [29]*Institut für Klinische Genetik, Universitätsklinikum Carl Gustav Carus Dresden, Dresden, Germany;* [30]*Hereditary Cancer Program, Catalan Institute of Oncology, Bellvitge Institute for Biomedical Research, Barcelona, Spain;* [31]*Centro de Investigación Biomédica en Red de Cáncer (CIBERONC), Madrid, Spain;* [32]*Portuguese Oncology Institute of Porto, Department of Laboratory Genetics, Porto, Portugal;* [33]*Faculty of Medicine, University of British Columbia, Department of Medical Genetics, Vancouver, Canada;* [34]*BC Cancer, Department of Molecular Oncology, Vancouver, Canada;* [35]*Medizinische Klinik und Poliklinik IV, Klinikum der Universität München, Munich, Germany;* [36]*Medizinisch Genetisches Zentrum, Munich, Germany;* [37]*Zane Cohen Centre, Sinai Health System, Toronto, Canada;* [38]*Barretos Cancer Hospital, Cancer Genetics Departament, Barretos, Brazil;* [39]*Barretos Cancer Hospital, Medical Genetics Unit, Barretos, Brazil;* [40]*National Cancer Institute, Department of Genetics, Brazil;* [41]*Netherlands Cancer Institute, Department of Clinical Genetics, Amsterdam, Netherlands;* [42]*Netherlands Cancer Institute, Department of Gastrointestinal Oncology, Amsterdam, Netherlands;* [43]*Unité fonctionnelle d'Onco-angiogénétique et génomique des tumeurs solides, Département de Génétique médicale, Hôpital Pitié-Salpêtrière, AP-HP, Sorbonne Université, Paris, France;* [44]*IBMC – Instituto de Biologia Molecular e Celular, Porto, Portugal;* [45]*Unité fonctionnelle d'Oncogénétique clinique, Département de Génétique, Groupe Hospitalier Pitié-Salpêtrière, AP-HP, Sorbonne Université, Paris, France;* [46]*Chirurgie générale et digestive, Hôpital Saint-Antoine, AP-HP, Sorbonne Université, Paris, France*

Background/Objectives: We previously demonstrated that carriers of *CTNNA1*-truncating variants present increased lifetime-risk of developing diffuse gastric cancer (DGC). These carriers are 14-fold more likely to develop DGC than carriers of *CTNNA1*-missense variants. Herein, we aim at understanding if *CTNNA1* germline transcripts bearing premature termination codons (PTC) are targeted for nonsense-mediated mRNA decay (NMD), and to disclose the transcriptomic profile of CTNNA1-deficient tumors.

Methods: We CRISPR/Cas9 edited gastric cancer cell lines mimicking CTNNA1 truncating variants (CTNNA1-PTC) and assessed whether NMD is triggered, and obtained the transcriptomic profile of CTNNA1-deficient DGC tumors using RNA-seq from CTNNA1 germline carriers.

Results: CTNNA1-PTC cells lose CTNNA1/ αE-catenin mRNA/ protein expression. NMD blockade significantly increased (~13-fold) their CTNNA1 mRNA expression, compared to untreated cells. As CTNNA1 expression recovered wild-type mRNA levels, NMD is likely the mechanism responsible for CTNNA1 mRNA decay. Immunohistochemistry showed αE-catenin protein loss in DGC tumors, but not in normal tissue. Transcriptome analysis of 11 DGC tumors revealed 14 significantly downregulated and 940 upregulated genes in comparison to matched normal tissue ($p < 0.01$; differential expression: 2-fold increase/decrease in tumors *vs* normal). Upregulated signaling pathways involve cell adhesion, actin cytoskeleton, cell migration, angiogenesis and

immune response, as well as JAK/STAT, EGFR or TGF-β oncogenic pathways.

Conclusion: We demonstrated that NMD acts over CTNNA1 PTC-bearing transcripts, being responsible for mRNA decay of germline mutant alleles. CTNNA1-mutant tumors present a transcriptional landscape recapitulating the behavior of poorly cohesive, invasive, proliferative and migratory cancers. *CTNNA1*-deficient DGC overexpress molecules and pathways worth exploring as therapy targets.

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P01.139.B Challenges of multigene panel testing of breast cancer patients in Turkey

Deniz Agirbasli[1], *Mehmet Erinc Onal*[2], *Didem Gulhan*[2], *Aysel Kalayci*[1]

[1]*Istanbul University-Cerrahpasa, Cerrahpasa Faculty of Medicine, Medical Genetics, Istanbul, Türkyie;* [2]*Istanbul University-Cerrahpasa, Cerrahpasa Faculty of Medicine, Istanbul, Türkyie*

Background/Objectives: Hereditary breast cancer represents 5-10% of all breast cancer cases. Germline pathogenic/likely pathogenic (P/LP) variants in *BRCA1/2* genes are responsible for only 15% of the familial cases. Next-generation sequencing (NGS) based-multigene panels for detecting germline variants have emerged as a valuable tool in genetic assessment.

Methods: The multigene NGS panel results of 207 women with breast cancer who applied to our center between 2021-2022 were analyzed retrospectively.

Results: The mean age of cases at diagnosis was 45,93 ± 9,98. Out of 180 patients, the most common histologic types were ductal (n = 150, 83%) and lobular carcinoma, respectively (n = 15, 8,3%), followed by mixed type (n = 8, 4,4%), tubular (n = 4, 2,2%), mucinous (n = 2, 1,1%), metastatic spindle cell carcinoma (n = 1, 0,5%). No identifiable variants were detected in 110 patients (53%). Number of pathogenic variants detected were 24 (11,5%), variants of uncertain significance (VUS) were 61 (29,5%), and likely pathogenic variants were 14 (%6,7). 19 patients carried multiple variants (9,1%). Most common P/LP mutations were detected in *CHEK2* (n = 8, 3.8%), *BRCA1* (n = 6, 2,8%), *BRCA2* (n = 6, 2.8%), *ATM* (n = 4, 1.9%), *PALB2* (n = 3, 1,4%) genes followed by *BARD1, BRIP1, XRCC2, RAD50, NBN, BLM*.

Conclusion: The results of multigene panels will diversify genetic counseling as well as prevention and treatment selection. Although no clinical decisions can be made on the basis of variants of uncertain significance, genetic re-evaluation should be performed at regular intervals, and functional studies should be performed. Current family history risk assessment tools must be improved for patients with a strong family history of cancer as in our cohort.

Conflict of Interest: None declared

P01.140.C Prevalence and impact of CDKN2A/2B Gene Deletions in Pediatric Acute Lymphoblastic Leukemia: A SNPa study.

Luís Miguel Pires[1], *Pedro Veiga*[1], *Mariana Val*[1], *Susana Isabel Ferreira*[1], *Nuno Lavoura*[1], *Marta Pinto*[1], *Ana Jardim*[1], *Paula Gameiro*[2], *Maria João Martins*[2], *Margarida Coucelo*[3], *Sónia Silva*[4], *Manuel Brito*[4], *Ximo Duarte*[5], *Ilda Patrícia Ribeiro*[1;6;7], *Joana Barbosa de Melo*[1;6;7], *Isabel Marques Carreira*[1;6;7]

[1]*Laboratory of Cytogenetics and Genomics, Faculty of Medicine, University of Coimbra, Coimbra, Portugal;* [2]*Laboratório de Hemato-Oncologia, Instituto Português de Oncologia de Lisboa, Lisboa, Portugal;* [3]*Unidade Funcional de Hematologia Molecular, Centro Hospitalar e Universitário de Coimbra EPE, Hospital Pediátrico, Coimbra, Portugal;* [4]*Oncologia Pediátrica, Centro Hospitalar e Universitário de Coimbra EPE, Hospital Pediátrico, Coimbra, Portugal;* [5]*Serviço de Pediatria e do Adolescente, Instituto Português de Oncologia de Lisboa, Lisboa, Portugal;* [6]*Center for Innovative Biomedicine and Biotechnology (CIBB), University of Coimbra, Coimbra, Portugal;* [7]*Institute for Clinical and Biomedical Research (iCBR) and Center of Investigation on Environment Genetics and Oncobiology (CIMAGO), Faculty of Medicine, University of Coimbra, Coimbra, Portugal*

Background/Objectives: The *CDKN2A/2B* gene is a tumor suppressor and its deletion is one of the most frequent events in acute lymphoblastic leukemia (ALL), acting as a secondary cooperating factor and playing a critical role in cell cycle regulation and chemosensitivity. *CDKN2A/B* deletions are detected in approximately 20-25% of pediatric B-cell precursor ALL cases and 40-50% of T-cell precursor ALL patients. It is indicative of poor prognosis in pediatric and adult B-ALL. We present the incidence of deletions in *CDKN2A/2B* and other specific genetic alterations in ALL key-genes in a Portuguese cohort of pediatric ALL used for risk stratification.

Methods: 105 ALL samples were analyzed by Array Comparative Genomic Hybridization combined with Single Nucleotide Polymorphism array (SNPa). The analysis included the evaluation of ploidy, whole-genome alterations, loss of heterozygosity and copy number alterations affecting ALL critical genes, including *CDKN2A/B*.

Results: The results of our study demonstrate that the most frequent genetic alteration was the deletion of the *CDKN2A/B* gene, present in 34% of the samples, followed by deletions in *ETV6*, *PAX5* and *EGR*. *CDKN2A/B* homozygous deletion is more prevalent than heterozygous deletion present in 23% of the cases. On the other hand, is relevant that in 64% of the cases in which the *CDKN2A/B* deletion was found, another genetic alteration was present (*PAX5* or *ETV6* deletion).

Conclusion: The presence of *CDKN2A/2B* homozygous deletions can represent a new ALL subgroup, with different outcomes. Risk stratification considering new sub-groups of genetic alterations, can improve survival rates and therapeutic response.

Conflict of Interest: None declared

P01.141.D Next generation sequencing results in acute myeloid leukemia diagnostics

Zoltan Orfi[1], Dora Csaban[1], Andras Bors[1], Livia Varga[1], Nora Meggyesi[1], Lenke Tanko[1], Adrienn Borsy[1], Andras Kozma[1], Nora Kondor[1], János Dolgos[1], Laszlo Gopcsa[1], Jozsef Harasztdombi[1], Viktor Lakatos[1], Gabor Mikala[1], Judit Reichardt[1], János Fábián[1], Peter Remenyi[1], Valyi-Nagy Istvan[1], Hajnalka Andrikovics[1]

[1]Central Hospital of Southern Pest, National Institute of Hematology and Infectious Diseases, Budapest, Hungary

Background/Objectives: Acute myeloid leukemia (AML) is a genetically heterogeneous clonal disorder of myeloid stem cells. In favor of increasing the precision of diagnosis, prognosis and in order to select most suitable targeted therapy, a comprehensive genetic mutation screening for acquired genetic abnormalities is necessary. Massively parallel sequencing effectively enables the identification of measurable residual disease marker(s). According to international recommendations (ELN, NCCN) more than 30 genes should be screened besides recurrent cytogenetic abnormalities.

Methods: At our institute, 175 bone marrow samples from AML patients were analyzed by cytogenetics and next-generation sequencing (NGS targeted panel of 23-68 genes or WES).

Results: Recurrent cytogenetic abnormalities were detected in 20 cases [t(15;17)(q24.1;q21.2)/PML::RARA; t(8;21)(q22;q22.1)/RUNX1::RUNX1T1; inv(16)(p13.1q22)/CBFB::MYH11; t(11q23;v)/KMT2A; inv(3)(q21.3q26.2)/MECOM]. Recurrent WHO defining genetic mutations were found in CEBPA (n = 6; 3%), NPM1 (n = 67; 38%) in normal karyotype AML. Mutations in TP53 (n = 10; 6%), in MDS related mutations (n = 48; 27%) or MDS type cytogenetics (n = 4; 2%) were also observed. No WHO defining category were identified in 20 cases (11%). In the NPM1 group the following frequent mutation associations were observed: DNMT3A, FLT3, IDH1/2, NRAS, PTPN11, RAD21 and WT1.

Conclusion: The frequency of identified gene mutations in our study group is in agreement with the international literature. Genetic profiling with NGS facilitates and complements the diagnosis, prognosis of complex disease clusters and provides valuable information about targeted therapeutic possibilities and significantly enhances the identification of hereditary malignancies as well. Thus, NGS combined with cytogenetics is now an indispensable tool for patients with curative myeloid malignancies.

Conflict of Interest: None declared

P01.142.A BRG1 and HIF1A functional crosstalk defines transcription of ABC transporters in paclitaxel resistantcancer cells

Karolina Gronkowska[1], Sylwia Michlewska[2], Tomasz Płoszaj[3], Kinga Kołacz[1], Agnieszka Robaszkiewicz[1]

[1]University of Lodz, Department of General Biophysics, Lodz; [2]University of Lodz, Laboratory of Microscopic Imaging and Specialized Biological Techniques, Lodz; [3]Medical University of Lodz, Department of Clinical Genetics, Lodz

Background/Objectives: Cancer drug irresponsiveness to chemotherapy is followed by substantial chromatin remodeling at the regulatory elements of genes, which confer drug resistance. Based on TCGA database we delineated strong correlation between mRNA level of SWI/SNF subunit BRG1 encoded by frequently mutated SMARCA4 and ABC transporters, which remove or sequester anticancer drugs inside cancer cell organelles. Therefore, we asked if overexpression of BRG1 is responsible for the limited drug responsiveness of cancers, which developed resistance during paclitaxel therapy.

Methods: ChIP- and RNA-Seq samples were sequenced with Illumina550, data analysis and integration were performed in Galaxy, gene mutations were detected with Sanger sequencing, genes were silenced with siRNA, co-immunoprecitpiated proteins were detected by western blot, colocalization was quantified by confocal microscopy.

Results: The tested *SMARCA4*$^{+/+}$ TNBC samples showed redistribution of BRG1 around TSS of ABC genes responsible for anticancer drug detoxication in paclitaxel-resistant phenotypes without substantial enrichment of the enzyme, whereas mutations gained during the therapy reverted BRG1 truncation caused by pre-existed deletions in *SMARCA4*$^{del/del}$ in non-small lung cancer cells, thereby causing recruitment of BRG1 to ABCC5 and ABCC10 gene promoters. Motif spacing analysis of BRG1 binding sites allowed to identify inter alia HIF1A as possible co-factor of BRG1-based SWI/SNF complex. This transcription factor co-occurred in the nucleus and formed immunoprecipitable complex with BRG1 and p300 acetyltransferase in paclitaxel resistant cancer phenotypes. The deficiency of BRG1 or HIF1A declined ABC gene overexpression and restored paclitaxel-resistant cancer cell responsiveness to numerous chemotherapeutics.

Conclusion: HIF1A defines BRG1-SWI/SNF activity in paclitaxel-resistant cancer cells.

Grants: LIDER/22/0122/L-10/18/NCBR/2019.

Conflict of Interest: None declared

P01.143.B Analysis of aberrantly expressed microRNAs in advanced laryngeal carcinoma showed most targetable pathways

Silva Kyurkchiyan[1], Veronika Petkova[1], Gergana Stancheva[1], Diana Popova[2], Radka Kaneva[1], Todor Popov[2]

[1]Medical University - Sofia, Molecular Medicine Center, Department of Medical Chemistry and Biochemistry, Sofia, Bulgaria; [2]Medical University - Sofia, UMHAT "Tsaritsa Yoanna – ISUL", Department of ENT, Sofia, Bulgaria

Background/Objectives: Advance laryngeal squamous cell carcinoma (LSCC) is still tumor burden, associated with poor survival. miRNAs were known regulatory molecules. They were suggested as biomarkers to seek new treatments. The aim of our study is to evaluate miRNAs, which were deeply and significantly involved in deregulation of major pathways in advanced LSCC.

Methods: In total 131 patients, diagnosed with advanced LSCC were enrolled. The patients were separated in two: training (n = 60) and validation (n = 71) groups. Global microRNA profiling was done, and most significantly miRNAs were analyzed additionally in patient cohort via RT-qPCR. Promising miRNA results were used in in silico, validation and correlation analysis. SPSSv.27 and data bases were used in statistical and bioinformatic analysis. P-value <0.05 was taken as appropriate.

Results: Several miRNAs were most often co-expressed, namely miR-93-5p, miR-145-5p and miR-210-3p. Their expressions were validated in in vivo advanced LSCC samples and further used in bioinformatic and enrichment analysis. Our results highlight the significant roles played by miR-93-5p, miR-145-5p, and miR-210-3p in the processes, regulating pathways linked to cell cycle, hypoxia,

metabolism, apoptosis, angiogenesis, and metastasis. miR-93-5p/miR-145-5p/miR-210-3p appear to correlate with biomarkers of those pathways: three HIFα isoforms, VEGFA, VEGFR1, VEGFR2, TP53, BCL-2. Moreover, these miRNAs are actively involved in regulating processes that affect currently used chemo- and target therapies.

Conclusion: Our findings suggest that investigating key regulators of hypoxia, apoptosis, autophagy, and metabolic deregulations could be essential in the management of LSCC.

Grants: European Union-Next Generation EU/National Recovery and Resilience Plan of the Republic of Bulgaria/project №BG-RRP-2.004-0004-C01/group3.1.5 and project D01-302/17.12.2021 by MES, Bulgaria.

Conflict of Interest: None declared

P01.144.C Incidental detection of acquired cryptic chromosomal rearrangements (CCRs) in patients who underwent germline genomic testing.

Moneeb Othman[1], *Peter Bauer*[1], *Boodor Al-Kawlani*[1], *Uros Hladnik*[1], *Omid Paknia*[1]

[1]*Centogene AG, Rostock, Germany*

Background/Objectives: Acquired genetic changes contributing to cancer, such as complex genomic rearrangements and cryptic copy number abnormalities (CNAs) can be incidentally detected by NGS-based copy number variant (CNV) analysis. We report here two cases referred to our laboratory. First case is a 13-year-old girl with abnormalities in bone marrow cell morphology, acute leukemia, and pancytopenia. The second case is a 12-year-old boy abnormalities in multiple cell lineages in the bone marrow, hepatosplenomegaly, leukocytosis, eosinophilia, monocytosis and myelofibrosis.

Methods:WES-based CNV analysis was performed for both cases, and the results were confirmed by chromosomal microarray analysis.

Results: In the first case, a terminal pathogenic gain of 7.3 Mb within the 9q34.12q34.3 chromosomal cytobands, encompassing the *ABL1* gene and a proximal pathogenic gain of 7.5 Mb within the 22q11.1q11.23 chromosomal cytobands, encompassing the *BCR* gene, have been detected. The unbalanced rearrangement results in the gain of a second copy of the Philadelphia chromosome and is predicted to lead to an abnormal *BCR-ABL1* fusion. In the second case, an interstitial deletion of 876 kb encompassing partially *FIP1L1* and *PDGFRA* genes and the entire *CHIC2* gene was identified within the 4q12 chromosomal cytoband was detected. The deletion was predicted to result in an abnormal *FIP1L1-PDGFRA* fusion. A *FIP1L1-PDGFRA* fusion gene usually results from a cryptic interstitial deletion in 4q12.

Conclusion: The incidental detection of acquired genetic alterations in patients undergoing germline genomic testing highlights the potential of NGS-based CNV analysis in uncovering unsuspected genomic rearrangements. Therefore, detecting gene fusions in leukemia is significantly valuable for diagnosis, prognosis and potential treatment strategies.

Conflict of Interest: None declared

P01.145.D RNA-based gene alteration and expression analysis in NSCLC with known KRAS G12C status

Joanna Moes-Sosnowska[1;1], *Urszula Lechowicz*[1], *Magdalena Pelc*[1], *Adam Szpechcinski*[1], *Paulina Skronska*[1], *Adriana Rozy*[1], *Aneta Zdral*[1], *Mateusz Polaczek*[2], *Emil Wojda*[2], *Małgorzata Szołkowska*[3], *Tadeusz Orłowski*[4], *Pawel Sliwinski*[5], *Renata Langfort*[3], *Joanna Chorostowska-Wynimko*[1]

[1]*The Institute of Tuberculosis and Lung Diseases, Department of Genetics and Clinical Immunology, Warsaw, Poland;* [2]*The Institute of Tuberculosis and Lung Diseases, III Department of Lung Diseases and Oncology, Warsaw, Poland;* [3]*The Institute of Tuberculosis and Lung Diseases, Department of Pathology, Warsaw, Poland;* [4]*The Institute of Tuberculosis and Lung Diseases, Department of Surgery, Warsaw, Poland;* [5]*The Institute of Tuberculosis and Lung Diseases, II Department of Lung Diseases, Warsaw, Poland*

Background/Objectives: The *KRAS* c.34G>T, p.(Gly12Cys) (G12C) variant is frequently present in non-small cell lung cancer (NSCLC) targeted with KRAS inhibitors. However, the reliability of that treatment is not satisfactory. To better understand NSCLC biology and identify potential biomarkers, it is important to analyze the prevalence of KRAS variants and their relationship to other oncogenesis-related genes. Therefore, we aimed to compare the expression of 14genes among *KRAS*-positive and negative tumors using RNA-based next-generation sequencing (NGS).

Methods: *KRAS* variants and expression levels of 14genes (*ALK*,*BRAF*,*EGFR*,*FGFR1-3*,*KRAS*,*MET*,*NRG1*,*NTRK1-3*,*RET*, and *ROS1*) were evaluated in 160 tumors (formalin-fixed, paraffin-embedded(FFPE) biopsies and surgically resected tissues) including 126(79%) adenocarcinomas, 24(15%) large cell lung cancer and 10(6%) not otherwise specified (NOS) with the use of RNA-FusionPlex Lung panel(Archer).

Results: Overall, 58(17.4%) of the 160 samples harbored 58 clinically significant *KRAS* variants, of which 95% were found in adenocarcinomas. The G12C was the most frequent variant among all *KRAS*-positive tumors(36%). *KRAS*-negative tumors characterized with variants: *EGFR* ($n = 13$); *BRAF*($n = 7$); CD74::ROS1($n = 1$); EML4::ALK($n = 3$) and METex14 skipping($n = 6$). The gene expression analysis revealed significantly higher median expression of the *NTRK2*(*TrkB*) in tumors with the G12C variant ($p = 0.016$) and significantly lower median expression of the *KRAS* in tumors carrying the c.37G>T, p.(Gly13Cys) variant ($p = 0.011$).

Conclusion: This study adds new data to the genomic characterization of *KRAS*-positive tumors, especially those with G12C variants. Our study revealed higher levels of *NTRK2* in G12C-positive tumors which can be associated with unfavorable prognosis since *NTRK2* signaling may contribute to metastasis, and is associated with intratumoral hypoxia. Further research is needed to confirm these observations.

Grants: project 3.9/2022

Conflict of Interest: None declared

P01.146.A deepHRD, a clinically validated NGS- and AI-based method for detection of genomic instability in ovarian cancer

Juliette Albuisson[1], *Gwladys Le Dain*[2], *Sandy Chevrier*[1], *Anthony Comte*[1], *Romain Boidot*[1], *Valentin Derangère*[1], *Vincent Goussot*[1], *Caroline Truntzer*[2]

[1]*Centre Georges François Leclerc, Département de Biologie et Pathologie des Tumeurs, DIJON, France;* [2]*Centre Georges François Leclerc, Plateforme de transfert en Biologie Cancérologique, DIJON, France*

Homologous Recombination Repair Deficiency (HRD) leads to tumor genomic instability (GIS) and sensitivity to PARP inhibitors in ovarian cancers (OC), and the identification of HRD status is considered a major therapeutic issue.

We developed an NGS- and AI-based HRD detection tool that allows the detection of GIS in tumor samples. First, sequencing of a SNP and CNV containing genomic backbone (2.8 Mb size) is performed in a standard Illumina NGS platform. Second an AI-based algorithm analyzes NGS results in tumor sample is applied.

We performed several steps of validation: technical validation was done comparing Myriad Mychoice GIS score/class and our own GIS score/class in 177 tumor samples analyzed in our Centre. Second, technical sensitivity was assessed using a tumor sample with GIS score just above threshold and decreasing tumor purity. Third, clinical validation was done in 360 tumor samples from PAOLA-2 study.

Our method showed very good technical performance (sensitivity and specificity 0.94 and 0.95 respectively). Clinical performance was good (Sensitivity 0.93, specificity 0.84, using combined BRCA sequencing results and deepHRD scoring, PFS 54.3 [40.2-60.6] vs 18.7 [14.3-24.7] in GIS+ vs GIS- respectively). In all 43 Myriad Mychoice inconclusive results, deepHRD showed a similar performance. Technical sensitivity could be confirmed with GIS detection in samples with as less as 20% of tumor purity.

Technology transfer is currently under progress in our centre and will be soon available either through collaboration or commercial use.

Conflict of Interest: Juliette Albuisson Grant Astra-Zeneca, Gwladys Le Dain: None declared, Sandy Chevrier: None declared, Anthony Comte: None declared, Romain Boidot: None declared, Valentin Derangère: None declared, Vincent Goussot: None declared, Caroline Truntzer: None declared

P01.148.C Impact of rare and low-frequency genetic variants in pleural mesothelioma

Elisabetta Casalone[1], *elton jalis herman*[1], *Cecilia Di Primio*[1], *Carla Debernardi*[1], *Rebecca Filomena*[1], *ANGELO SAVOCA*[1], *Miriam Rosselli*[1], *Dario Mirabelli*[2], *Giuseppe Matullo*[1;3]

[1]*University of Turin, Medical Sciences, Turin, Italy;* [2]*University-Hospital and Center for Cancer Prevention, Cancer Epidemiology, Turin, Italy;* [3]*AOU Città della Salute e della Scienza, Medical Genetics, Turin, Italy*

Consortium: EPIC network

Background: Previous Genome Wide Association Studies (GWASs) in pleural mesothelioma (PM) identified low risk genetic variants conferring a limited increase risk of the diseases and showing evidence of interaction with asbestos exposure. Other sequencing studies on cancer gene panels in PM cases identified 5-10% of germline mutations increasing susceptibility to PM even at low levels of asbestos exposure. Here, we conducted a whole exome sequencing (WES) analysis aimed at identifying genes with rare variants contributing to PM risk.

Methods: WES screening was performed on DNA isolated from blood of 129 pre-clinical PM and 129 non-cancer individuals. Alignment of the sequencing reads, variant calling and annotation were performed using JuliaOmixTM software (GenomeUp, Italy). SNVs with a read depth > 20X and a m.a.f. < 0.01 in GnomAD database, were taken into account for downstream analyses.

Results: In the group of cases we identified pathogenic rare variants in cancer predisposing genes such as *ACVR1B, B2M, DNMT3A, HRAS, NPM1, PTPN11, STAG2*. Sequence Kernel association test performed in about 1 million of rare variants across 17758 genes showed a significant enrichment in genes involved in DNA methylation, chromosome replication and segregation.

Conclusion: WES sequencing technologies allowed us to identify rare germline mutations in novel genes potentially contributing, together with asbestos exposure, to increase risk of PM development. Functional analysis will be performed to test pathogenic effects and the potential impact of the mutational processes in somatic cells.

Grants: AIRC under IG 2018—[ID.21390 project—P.I. GM]

Conflict of Interest: None declared

P01.149.D Major impact on modifiable colo-rectal cancer risk factors on at risk individuals through personalised prevention in clinical genetics practice

marie-gabrielle delorme guinand[1], *anna serova-erard*[1], *laury nicolas*[1], *noemie demare*[2], *simon rey*[1], *martine duclos*[3], **François Cornélis**[1]

[1]*CHU Clermont-Ferrand, 63000, Clermont-Ferrand, France;* [2]*Avicenne Hopital, Assistance Publique des Hopitaux de Paris, Paris, France;* [3]*CHU Observatoire National de l'Activité Physique et de la Sédentarité, 63000, Clermont-Ferrand, France*

Introduction : In EU-27 countries in 2020, colorectal cancer (CCR) was the 2nd cause of cancer death. Major modifiable risk factors are the lack of physical activity (PA) and of dietary fiber intake (DFI). The majority of outpatients in clinical genetics showing no germline pathogenic variants are affected with a multifactorial disease, for which correction of risk factors is essential. Our aim was to evaluate the impact of personalised advice following standardized evaluation of PA and DFI.

Patients & Methods : Genetics adult outpatients were systematically evaluated using the International PA Questionnaire short form and a in-house questionnaire for DFI, taking 5 minutes on average. At high CCR risk individuals had personal or familial history of CCR or colon adenomas.

Results : Evaluation of PA and FDI revealed 51.3% (n = 559, since 2018) and 74.5% (n = 212, since 2021), respectively, of individuals below the OMS recommendation, who were given personalised advice. At the second evaluation, 9 months later (in mean, range 3-12 months), out of those, 42.7% and 24.1%, respectively, had corrected to meet the OMS target. For the subgroup of individuals at high CCR risk (n = 92), 48.9% and 81.6% were below the targets at the first evaluation and, out of those, 35.6% and 24.1%, respectively, had corrected at the second evaluation.

Conclusion : Systematic personalised prevention in clinical genetics practice has a major impact on modifiable colo-rectal cancer risk factors such as PA and, to a lesser extents, DFI, with a similar magnitude on individuals at high or low CCR risk.

Conflict of Interest: None declared

P01.150.A Assessment of cancer predisposition syndromes in a pediatric oncology center from Midwest-Brazil

Flávia Delgado Martins[1], *Renata Sandoval*[2]

[1]*Hospital da Criança de Brasília José Alencar, Pediatric oncology, Brasília;* [2]*Hospital da Criança de Brasília José Alencar, Genetic service, Brasília*

Background/Objectives: Pediatric cancer can be a sentinel for cancer predisposition syndromes (CPS). Understanding the association of specific tumor subtypes with recognizable clinical patterns may help to identify carriers, and to inform care. Here we provide descriptive data from pediatric cancer patients with referral for genetic evaluation.

Methods: We retrospectively reviewed medical charts of childhood cancer patients who were referred to the oncogenetics

service between January 2018 and July 2023. Cases without pathology and/or imaging reports supporting a malignancy diagnosis were excluded. Referral indications, clinical data and germline genetic tests results (when available) were assessed.

Results: Among 129 patients who were referred to genetic evaluation, 96 met study criteria. A suspicious family history of cancer was a recurrent referral indication (n = 47). Phenotypic-driven diagnosis was possible for 37 cases (38.5%): 14 Neurofibromatosis type 1, 12 Tuberous Sclerosis Complex and 11 polyposis syndromes. Among patients who performed germline testing, 85% (17/21) harbored a pathogenic/likely pathogenic germline variant in a CPS gene.

Conclusion: In this highly selected cohort we found almost 40% of CPSs identified by a phenotype-driven approach and among those who performed germline testing 85% of positive results. This preliminary data allowed the creation of an institutional registry of CPSs and future prospective studies.

Conflict of Interest: None declared

P01.151.B Reflex CRC Initiative - Advancing Colorectal Cancer Care through Comprehensive Genetic Testing

Christoforos Pappas[1], *Hannes Olauson*[2], *Felix Haglund*[3], *Johan Lindberg*[4], *Caroline Beergrehn*[5], *Emma Tham*[1], *Annika Sjövall*[1]

[1]*Karolinska Institute, Department of Molecular Medicine and Surgery, Stockholm, Sweden;* [2]*Karolinska Institute, Department of Laboratory Medicine, Stockholm, Sweden;* [3]*Karolinska Institute, Department of Oncology-Pathology, Stockholm, Sweden;* [4]*Karolinska Institute, Department of Medical Epidemiology and Biostatistics, Stockholm, Sweden;* [5]*Capio St Göran Hospital, Department of Surgery, Stockholm, Sweden*

Background/Objectives: Colorectal cancer management is evolving towards personalized medicine, with genetic testing playing a crucial role in treatment decisions. The Reflex Testing for Colorectal Cancer initiative aimed to integrate comprehensive genetic testing into routine care. Specifically, the aim was to promptly determine treatment predictive genetic factors and hereditary cancer risk syndromes that would lead to potential prophylactic surgery as well as identify DPYD deficiency that affects oncological treatment.

Methods: The study, conducted from October 2021 to December 2023, included 370 patients diagnosed with colorectal cancer. Genetic analysis was performed on DNA extracted from blood and from tumor tissue taken from biopsy during endoscopy, using a gene panel of 385 genes (GMCK panel) designed to identify variants relevant to treatment prediction, prognosis, and heredity for all solid tumors.

Results: In the first 100 cases (48 females, 52 males), 7% were diagnosed before 50 years and 18% between 50 and 60 years of age. The tumor localization varied, with 23% in the right colon, 9% in the transverse colon, 27% in the left colon and 41% in the rectum.15% showed high microsatellite instability. Additionally, four patients (4%) were identified with Lynch syndrome (disease-associated variants in *MLH1*(x2) or *MSH6*(x2)). Regarding treatment-predictive biomarkers, pathogenic variants were found in *KRAS* in 44% and in *BRAF* in 20%.

Conclusion: The analysis of paired tumor and blood data can identify both somatic and germline aberrations that are of clinical significance and thus enable personalized therapeutic decisions at diagnosis.

Grants: Regional Cancer Center Stockholm-Gotland funded this project.

Conflict of Interest: Christoforos Pappas Karolinska University Hospital, Hannes Olauson Karolinska University Hospital, Felix Haglund Illumina (reagents), ROCHE Global (reagents), Cancerfonden, Stockholm Cancer Society, Merck, Swedish Sarcoma Society, Karolinska University Hospital and Karolinska Institutet, Johan Lindberg Karolinska University Hospital, Caroline Beergrehn Capio Saint Göran Hospital, Emma Tham Cancerfonden, Barncancerfonden, Region Stockholm, Japan-Sweden Foundation, Karolinska University Hospital, Annika Sjövall Karolinska University Hospital

P01.153.D Recessive effect of human polymerase delta proofreading deficiency discovered through mutational analysis of POLD1-mutated normal and cancer cells

Maria A. Andrianova[1], *Vladimir B. Seplyarskiy*[2;3], *Mariona Terradas*[4], *Ana Beatriz Sánchez-Heras*[5;6], *Pilar Mur*[4], *José Luis Soto*[5;7], *Gemma Aiza*[4], *Emma Borràs Angosto*[8], *Fyodor A. Kondrashov*[9;10], *Alexey S. Kondrashov*[11], *Georgii A. Bazykin*[2;3], **Laura Valle**[4;12]

[1]*Institute for Research in Biomedicine (IRB Barcelona), the Barcelona Institute of Science and Technology, Barcelona, Spain;* [2]*Harvard Medical School, Department of Biomedical Informatics, Boston (MA), United States;* [3]*Brigham and Women's Hospital, Harvard Medical School, Division of Genetics, Boston (MA), United States;* [4]*Catalan Institute of Oncology, IDIBELL, Hereditary Cancer Group, Oncobell Program, Hospitalet de Llobregat (Barcelona), Spain;* [5]*Foundation for the Promotion of Health and Biomedical Research of Valencia Region (FISABIO), Elche Health Department, Elche, Spain;* [6]*Elche University Hospital, Medical Oncology Department, Cancer Genetic Counseling Unit, Elche, Spain;* [7]*Elche University Hospital, Molecular Genetics Unit, Elche, Spain;* [8]*Consorci Sanitari de Terrassa, Molecular Genetics Unit, Terrassa (Barcelona), Spain;* [9]*Institute of Science and Technology Austria, Klosterneuburg, Austria;* [10]*Okinawa Institute of Science and Technology Graduate University, Evolutionary and Synthetic Biology Unit, Okinawa, Japan;* [11]*University of Michigan, Department of Ecology and Evolutionary Biology, Ann Arbor (MI), United States;* [12]*Centro de Investigación Biomédica en Red de Cáncer (CIBERONC), Madrid, Spain*

Background: Constitutional heterozygous pathogenic variants in the exonuclease domain of *POLE* and *POLD1*, which affect the proofreading activity of corresponding polymerases, cause a cancer predisposition syndrome (polymerase proofreading-associated polyposis), characterized by increased risk of gastrointestinal polyposis, colorectal cancer, endometrial cancer and other tumour types. The connection between the disruption of polymerase proofreading activity and cancer development is through an increase in the somatic mutation rate.

Methods: We studied an extended family with multiple members heterozygous for the pathogenic *POLD1* variant c.1421T>C p.(Leu474Pro). Genome sequencing was performed to evaluate mutational patterns of patient-derived fibroblasts colonies and of de novo mutations obtained by parent-offspring comparisons. Exome sequencing data from tumours developed by patients with the hereditary cancer syndrome were also analysed.

Results: Heterozygous *POLD1* L474P just subtly increases somatic and germline mutation burden in non-tumour tissues. In contrast, tumours developed in individuals with a heterozygous mutation of the exonuclease domain of *POLD1*, including L474P, have extremely high mutation rates (>100 mut/Mb) associated with tumour mutational signature SBS10d. To explain these observations, also common to other *POLD1* exonuclease domain pathogenic variants, we show, for the first time, that *POLD1* proofreading deficient tumours developed in the context of the hereditary cancer syndrome require somatic inactivation of the wildtype allele in the target tissue (e.g. colon or endometrial epithelium), usually through loss of heterozygosity, resulting in the elimination of all exonuclease-proficient copies of the gene.

Conclusion: These results provide strong evidence that *POLD1* pathogenic variants have a recessive effect on mutation rate in somatic cells.

Conflict of Interest: None declared

P01.154.A Germline testing in routine care of ovarian cancer patients in Estonia 2009-2023

Mikk Tooming[1;2], *Kadri Rekker*[2], *Kadri Toome*[2], *Olga Fjodorova*[2], *Hanno Roomere*[2], *Ülle Murumets*[2], *Piret Laidre*[2], *Katrin Ounap*[1;2], *Tiina Kahre*[1;2]

[1]*Institute of Clinical Medicine, Department of Clinical Genetics, Tartu, Estonia;* [2]*Genetics and Personalized Medicine Clinic, Tartu University Hospital, Tartu, Estonia*

Background/Objectives: Germline testing in routine care of ovarian cancer (OC) patients in Estonia has evolved from single-gene testing to targeted NGS gene panels during the years 2009 to 2023. This study aimed to investigate the prevalence of (likely) pathogenic variants (PV) in Estonian OC patients.

Methods: The study included 761 OC patients referred from tertiary hospitals between 2009 and 2023 to the Genetics and Personalized Medicine Clinic at Tartu University Hospital for genetic testing. Individuals were analyzed using either APEX microarray, *BRCA1/2* Sanger sequencing, *BRCA1/2* multiplex ligation-dependent probe amplification analysis or Illumina TruSight Cancer/Hereditary Cancer (94 or 113 genes) gene panels.

Results: Most common histological OC type was serous carcinoma 559/761 (73%). The mean age of OC onset was 56 years (4-91y), and the mean age of testing was 60 years (10-91y). Bilateral OC occurred in 42/761 (5.5%) patients. Overall, 207/761 (27%) OC patients carried PV. *BRCA1/2* PV was found in 158/761 (21%) patients. Additionally, 49/761 (6.4%) patients had PV in non-BRCA genes associated with OC, including *ATM*, *BRIP1*, *DICER1*, *PALB2*, *PMS2*, *RAD51C* 25/761 (3.3%), and secondary findings in *BARD1*, *CHEK2*, *FANCM*, *MITF*, *MUTYH*, *PPM1D*, *TP53* 24/761 (3.1%). *BRCA1* was the most prevalently mutated gene, PV detected in 123/207 (59%) of patients, followed by *BRCA2* 35/207 (17%), *CHEK2* 13/207 (6.2%), and *BRIP1* 11/207 (5.3%).

Conclusion: The diagnostic efficacy of OC patients in Estonia was ~27%. The detection rate with the NGS gene panel is ~6% higher than focusing on *BRCA1/2* gene testing alone.

Grants: Estonian Research Council grant PRG471/PRG2040

Conflict of Interest: None declared

P01.155.B The co-regulation of circular RNAs and messenger RNAs in colorectal cancer: the fate of an unbalanced couple?

Corentin Levacher[1], *Philippe Ruminy*[2], *Camille Charbonnier*[3], *Joanna Delfosse*[1], *Edwige Kasper*[4], *Charbonnier Françoise*[1], *Mathieu Viennot*[2], *Jacques Mauillon*[5], *Nathalie Parodi*[5], *Stéphanie Baert-Desurmont*[4], **Claude Houdayer**[4]

[1]*Univ Rouen Normandie, Inserm U1245, Normandie Univ, F-76000 Rouen, France;* [2]*Univ Rouen Normandie, Inserm U1245, Normandie Univ, Centre Henri Becquerel, F-76000 Rouen, France;* [3]*Univ Rouen Normandie, Inserm U1245, Normandie Univ, CHU Rouen, Department of Biostatitics, F-76000 Rouen, France;* [4]*Univ Rouen Normandie, Inserm U1245, Normandie Univ, CHU Rouen, Department of Genetics, F-76000 Rouen, France;* [5]*Univ Rouen Normandie, Normandie Univ, CHU Rouen, Department of Genetics, F-76000 Rouen, France*

Background/Objectives: Circular RNAs (circRNAs) are emerging players in human diseases, with plausible function as part of competing endogenous networks. We hypothesized that circRNAs and mRNAs are co-regulated with a mandatory balance which, when broken, may favor disease development.

Methods: This hypothesis was tested in colorectal cancer (CRC) patients, thanks to the French multicenter Oligogenic Determinism of Colorectal Cancer collection (Baert-Desurmont et al., 2016) which contains whole blood RNA samples from 716 patients suspected of CRC predisposition excluding Lynch syndromes and polyposis, and 249 matched controls. The circRNA-mRNA couple was studied by SEALigHTS (Splice and Expression Analyses by exon Ligation and High-Throughput Sequencing) (Levacher et al., 2023) using a panel of 23 genes involved in CRC predisposition, i.e. 935 probes designed at exon ends, enabling the exploration of all exon-exon junctions. Following reverse transcription and probing on cDNA, nearby probes are ligated and the number of ligations quantified using unique molecular identifiers and sequencing.

Results: We described the landscape of circRNAs for these 23 genes i.e. 258 circular junctions, including 64 novel ones. The circRNA/mRNA ratios were calculated for each gene and ranged from 0.5% to 17% for *MLH1* and *POLD1*, respectively. The circRNA/mRNA ratio was 1.5-fold higher in patients compared to controls ($p < 2 \times 10\text{-}16$), irrespective of age of cancer onset or gender. This increase was mainly driven by *POLD1* ($p = 5.2 \times 10\text{-}8$) and did not reflect a competitive mechanism.

Conclusion: Overall, the unbalanced circRNA/mRNA couple observed in this large cohort opens new avenues in circRNA importance and CRC genetics.

Grants: RIN RECHERCHE

Conflict of Interest: None declared

P01.156.C Empowering Hereditary Cancer risk assessment: an unforeseen local geographic pattern revealed by Copy Number Variation analysis

Lia Bonamici[1], *Marco Marino*[2], *Lucia Artuso*[2], *Enrico Tagliafico*[2;3], *Elena Tenedini*[2;3]

[1]*University of Modena and Reggio Emilia, Department of Biomedical, Metabolic and Neural Sciences, Modena, Italy;* [2]*Modena University Hospital, Department of Laboratory Medicine and Pathology, Modena, Italy;* [3]*University of Modena and Reggio Emilia, Department of Medical and Surgical Sciences, Modena, Italy*

Background/Objectives: Next Generation Sequencing (NGS) multi-gene panels (MGP) are extensively employed in molecular oncology and recommended for individuals with personal or familial criteria for hereditary cancer testing. Analysis algorithms for NGS data, which allow the simultaneous detection of sequence (SNV) and copy number variants (CNVs), have significantly increased the detection rates and cost-effectiveness of the tests, while maintaining high accuracy and reliability. CNVs, consisting of large deletions or duplications, potentially occur in all the genes currently examined for SNVs and may play an important role in tumor development. Despite this, they remain less characterized.

Methods: In this study, we analyzed results from 2989 patients affected mainly by breast or ovarian cancer, who underwent testing with a 26-gene panel for hereditary cancer.

Results: The cohort carried either variants of undetermined significance (42%) or Pathogenic/Likely Pathogenic (13.3%). According to previous studies, SNVs were ubiquitous across all tested genes, with pathogenic variants being more frequent in *BRCA1*, *BRCA2*, *CHEK2*, and *MUTYH*. Conversely, CNVs, observed in 1.8% of the patients, affected only ten genes. Among these, *PALB2* showed over 50% of the total variants. Moreover, the pathogenic deletion of exon 11, accounted for the 85.7% of the *PALB2* CNVs. The identification of a common place of origin for patients with exon 11 deletion, suggests a potential geographic effect contributing to this phenomenon.

Conclusion: These data provide insights into the relevance of CNVs detection, which can reveal valuable information in variant-associated cancer risk assessment and reveal unforeseen prevalence and distributions of variants across different regions and ethnic groups.

Grants:

Conflict of Interest: None declared

P01.157.D Homozygous substitution of threonine 191 by proline in polymerase η causes Xeroderma pigmentosum variant

Roberto Ricciardiello[1], Giulia Forleo[1], Lina Cipolla[1], Geraldine Van Winckel[2], Caterina Marconi[2], Thierry Nouspikel[2], Thanos Halazonetis[3], **Omar Zgheib**[2]*, Simone Sabbioneda[1]*

[1]Istituto di Genetica Molecolare "Luigi Luca Cavalli-Sforza", CNR, Pavia, Italy; [2]Geneva University Hospitals, Division of Genetic Medicine, Geneva, Switzerland; [3]University of Geneva, Department of Molecular and Cellular Biology, Geneva, Switzerland

Consortium: The submitted abstract has not yet been presented at a conference, but was recently published in:

Ricciardiello, R., Forleo, G., Cipolla, L. et al. Homozygous substitution of threonine 191 by proline in polymerase η causes Xeroderma pigmentosum variant. Sci Rep 14, 1117 (2024). https://doi.org/10.1038/s41598-023-51120-1

Background/Objectives: DNA polymerase eta (Polη) is the only translesion synthesis polymerase capable of error-free bypass of UV-induced cyclobutane pyrimidine dimers. A deficiency in Polη function is associated with the human disease Xeroderma pigmentosum variant (XPV). To date, no missense variant has been reported as being pathogenic or likely pathogenic, owing to the lack of functional studies adhering to ACMG guidelines for variant interpretation.

Methods: We hereby report the case of a 60-year-old woman known for XPV and carrying a Polη Thr191Pro variant in homozygosity. We further characterize the variant in vitro by primer extension assays and in vivo by UV survival assays and cell cycle analysis.

Results: We provide evidence that the substitution abrogates polymerase activity and results in UV sensitivity through deficient damage bypass. This is the first functional molecular characterization of a missense variant of Polη, whose reported pathogenic variants have thus far been loss of function truncation or frameshift mutations.

Conclusion: Our work allows the upgrading of Polη Thr191Pro from 'variant of uncertain significance' to 'likely pathogenic mutant', bearing direct impact on molecular diagnosis and genetic counseling. Furthermore, we have established a robust experimental approach that will allow a precise molecular analysis of further missense mutations possibly linked to XPV. Finally, our work provides insight into critical Polη residues that may be targeted to develop small molecule inhibitors for cancer therapeutics.

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Conflict of Interest: None declared

P01.158.A CRISPR/Cas9-mediated Generation of reference iPSCs expressing the Philadelphia translocation

Md Faruq Hossain[1]*, Lisa Hagenau[1], Lars Jensen[1], Anna-Maria Pielka[2], Susanne Thümecke[2], Björn Nowack[2], Andreas Kuß[1]*

[1]Interfaculty Institute for Genetics and Functional Genomics, Department of Functional Genomics, Greifswald, Germany; [2]SensID GmbH, Rostock, Germany

Background/Objectives: Next Generation Sequencing (NGS), particularly RNA-sequencing (RNAseq), has become vital for research and diagnostics. Reliable reference materials are essential for ensuring comparability in results. Here CRISPR/Cas9 technology was used to develop reference cell lines, including a human iPSC line, expressing the well-studied BCR-ABL1 fusion transcript. This fusion arises from a chromosomal translocation involving the ABL and BCR proto-oncogenes.

Methods: CRISPR/Cas9 technology was employed to create a fusion gene in different cell types including human iPSCs, blood and bladder cancer cells by targeting the breakpoint region of two introns associated with the rearrangement. Co-transfection of CRISPR guide RNAs and Cas9 induced double-strand breaks within both genes, leading to DNA misrepair and subsequent gene fusion.

Results: The BCR-ABL1 fusion gene was successfully incorporated into the wildtype human iPSC line MHHi001-A, confirmed by sequencing. Notably, the pluripotency and differentiation capabilities of the genetically modified iPSCs into the three germ layers remained uncompromised with no observed genomic aberrations or off-target modifications. Further cell lines that were modified in this fashion include the Jurkat cell line and human bladder cancer cells with a pre-existing FGFR3-TACC3 fusion on chromosome 4. The latter results in a cell line with two distinct fusion transcripts per cell.

Conclusions: The newly created genetically modified cell lines, particularly the human iPSC featuring the BCR-ABL1 fusion gene, can serve as valuable reference materials for NGS-based RNA analysis. Their utilization significantly improves the precision and reliability of NGS applications, addressing a crucial need in research and diagnostics.

Grants: TBI-V-1-423-VBW-144 (Mecklenburg Vorpommern).

Conflict of Interest: None declared

P01.159.B Systematic review of multiple primary renal tumours (MPRT)

Huairen Zhang[1]*, Avgi Andreou[1], Rupesh Bhatt[2], James Whitworth[1], Bryndis Yngvadottir[1], Eamonn Maher[1;3]*

[1]School of Clinical Medicine, University of Cambridge, Department of Medical Genetics, Cambridge, United Kingdom; [2]Queen Elizabeth Hospital Birmingham, Department of Urology, Birmingham, United Kingdom; [3]Aston Medical School, Birmingham, United Kingdom

Background: In a subset of patients with renal tumours, multiple primary lesions may occur. Predisposition to multiple primary renal tumours (MPRT) is a well-recognised feature of some inherited renal cancer syndromes. The diagnosis of MPRT should therefore provoke a thorough assessment for clinical and genetic evidence of disorders associated with predisposition to renal tumourigenesis.

Method: To define the clinical and genetic characteristics of MPRT, a systematic literature review was undertaken.

Result: A total of 7562 patients from 448 articles were identified with MPRT. Compared to all patients with renal cell carcinoma (RCC), people with MPRT were more likely to be male (63% versus 71.7%) and have an earlier age at diagnosis (< 46 years, 19%

versus 34.5%). In 60.1% of cases MPRT were synchronous, and MPRT lesions had similar histology in 78.9% of individuals enrichment of multiple papillary RCC (16.8%). 10.2% of patients with MPRT had a family history of cancer or were diagnosed with a hereditary RCC associated syndrome with von Hippel Lindau (VHL) disease being the most common one (71.3%), followed by Birt Hogg Dube syndrome (14.6%). Individuals with a known or likely genetic cause were, on average, younger (43.3 years versus 56.7 years). In rare cases intrarenal metastatic RCC can phenocopy MPRT.

Conclusion: Based on these findings we suggest an investigative pathway for individuals with MPRT and approaches to cases in which routine germline genetic testing does not provide a diagnosis.

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Conflict of Interest: None declared

P01.160.C Deciphering copy number variations within the complex genomic region of the *PMS2* gene using Optical Genome Mapping

*Anne Holtorf[1], **Jasmin Maier**[1], Wilena Telman[1], Charlotte Singruen[1], Sarah Heinrich[1], Nina Wohlrab[1], Sophie Stoesslein[1], Siegel Corinna[1], Konstanze Hörtnagel[1]*

[1]Medicover Genetics, MVZ Martinsried GmbH, Martinsried, Germany

Background/Objectives: The *PMS2* gene is one of four DNA mismatch repair (MMR) genes and is critical for correcting errors after DNA replication and homologous recombination. Inactivation of *PMS2* leads to the accumulation of mutations, predisposing carriers to cancer and causing constitutional mismatch repair deficiency syndrome (CMMRDS). However, *PMS2* sequence analysis is complicated because the gene is located within a complex genomic region on chromosome 7, which contains a pseudogene with almost 100% sequence homology (*PMS2CL*). Particularly challenging is the identification of copy number variations (CNVs) within this genomic region, with the added complexity of the inability to distinguish between *PMS2* and *PMS2CL* using conventional laboratory techniques such as MLPA. Here we report on the possibility of using Optical Genome Mapping (OGM) as a new tool for detecting CNVs in this complex region of the genome.

Methods: One colon cancer patient (MSI-H, loss of PMS2) consented to germline testing. Next-generation sequencing was performed, including CNV analysis and additional MLPA. Subsequently, OGM was done to follow up on MLPA results.

Results: MLPA results suggested a deletion on chromosome 7, possibly encompassing *PMS2* exons 12-15. However, further investigation using long-range PCR to determine whether this deletion was in *PMS2* or its pseudogene remained inconclusive. Fortunately, OGM results revealed a heterozygous deletion of 77 kb including exons 12-15 of the coding *PMS2* gene, confirming Lynch syndrome.

Conclusion: OGM is a promising tool for identifying and deciphering structural variants in complex genomic regions, enabling diagnosis for patients and their families.

Grants: -

Conflict of Interest: None declared

P01.161.D DNA methylation patterns in families with rhabdoid tumor predisposition syndrome (RTPS)

Matej Boros[1], *Karolina Nemes[2;3], Sebastian Bühner[2;3], Susanne Bens[1], Nnamdi Okeke[1], Sonja Dahlum[1], Selina Glaser[1], Nadine Matscheko[1], Anja Fischer[1], Britt König[1], Ole Ammerpohl[1], Pascal Johann[2;3], Martin Hasselblatt[4], Michael C. Frühwald[2;3], Reiner Siebert[1]*

[1]Institute of Human Genetics, Ulm University & Ulm University Medical Center, Ulm, Germany, Ulm, Germany; [2]Pediatrics and Adolescent Medicine, Swabian Children's Cancer Center, University Medical Center Augsburg, Augsburg, Germany; [3]Bavarian Cancer Research Center (BZKF), Augsburg, Germany; [4]Institute of Neuropathology, University Hospital Münster, Münster, Germany

Background/Objectives: Rhabdoid Tumor Predisposition Syndrome (RTPS) is due to germline *SMARCB1* or *SMARCA4* pathogenic variants. It confers a high risk for malignant rhabdoid tumors in early childhood. Despite a grim prognosis with 5-year overall survival rates of around 40%, the molecular mechanisms underlying mutation origin, tumor penetrance,or unfavorable prognosis are incompletely understood. We investigated familial DNA methylation patterns in RTPS.

Methods: 90 children with RTPS from 16 countries were identified in the EU-RHAB database (01/2004 - 01/2021). We analyzed a subcohort of 49 well-characterized RTPS1 and 2 RTPS2 patients, along with 44 mutation-negative parents and 4 mutation-negative siblings. DNA methylation was assessed in blood DNA using the Infinium MethylationEPIC v1.0 array and analyzed using ANOVA (limma R package) as well as UMAP- and PCA-based clustering.

Results: Comparing patients with parents or siblings group identified 12,238 CpGs (4,662 genes) and 355 CpGs (214 genes) differentially methylated CpGs, respectively, with 128 CpGs and 72 genes shared among these analyses. Only 1 CpG was differently methylated between parent and sibling controls. Sibling samples clustered with RTPS patients and both segregated apart from parents. Age was a predominant driver of variability between the observed clusters whereas sex, type of *SMARCB1/SMARCA4* mutation, or tumor DNA methylation subtype were unrelated to the observed clusters.

Conclusion: DNA methylation analyses of patients and relatives did not show a specific blood-born epigenotype of RTPS patients. DNA methylation heterogeneity in the families was strongly confounded by age, emphasizing the need for age-matched control populations.

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P01.162.A Cancer prognosis and treatment results in patients with PTEN Hamartoma Tumor Syndrome (PHTS) – a European cohort study

*Linda Hendricks[1;2;3], **Katja Verbeek**[1;2;3], Janneke Schuurs-Hoeijmakers[1;3], Robin de Putter[4], Hilde Brems[5], Sien van Daele[5], Violetta Anastasiadou[6], Lenka Foretova[7], Patrick Benusiglio[8], Anna Gerasimenko[9;10], Chrystelle Colas[11;12], Marie-Charlotte Villy[11], Claude Houdayer[13], Maud Brauchaud[13], Robert Hüneburg[14;15], Stefan Aretz[14;16], Arne Jahn[17;18;19], Verena Steinke-Lange[20], Giovanni Innella[21;22], Daniela Turchetti[21;22], Valeria Barili[23], Maurizio Genuardi[24;25], Arianna Panfili[24], Margherita Baldassarri[26;27;28], Arvids Irmejs[29;30], Mirjam de Jong[31], Thera Links[32], Edward Leter[33], Daniëlle Bosch[34], Stephany Donze[34], Rachel van der Post[35], Arjen*

1286

Mensenkamp[1;2;3], harm westdorp[36], Hildegunn Høberg Vetti[37;38], Marianne Tveit Haavind[37], Kjersti Jørgensen[39], Lovise Maehle[39], Siri Briskemyr[40], Juliette Dupont[41], Ana Blatnik[42], Judith Balmaña[43], Maite Torres[43], Joan Brunet[44], Roser Lleuger-Pujol[44], Emma Tham[45;46], Marc Tischkowitz[47], D Gareth Evans[48], Zerin Hyder[48], Nicoline Hoogerbrugge[1;2;3], Janet Vos[1;2;3]

[1]Radboud university medical center, Radboudumc expert center for PHTS, Nijmegen, Netherlands; [2]Radboud university medical center, Radboud Institute for Medical Innovation, Nijmegen, Netherlands; [3]Radboud university medical center, Human Genetics, Nijmegen, Netherlands; [4]Ghent University Hospital, Center for Medical Genetics, Ghent, Belgium; [5]University of Leuven, Human Genetics, Leuven, Belgium; [6]Archbishop Makarios III Children's Hospital, Karaiskakio Foundation, Nicosia, Cyprus; [7]Masaryk Memorial Cancer Institute, Cancer Epidemiology and Genetics, Brno, Czech Republic; [8]Hôspital Pitié-Salpêtrière, AP-HP, Sorbonne Université, UF d'oncogénétique Clinique, department de Génétique, Paris, France; [9]APHP Sorbonne Université, GH Pitié Salpêtrière et Trousseau, Département de Génétique, Centre de référence "déficiences intellectuelles de causes rares", Paris, France; [10]Sorbonne Université, Hôpital de la Pitié Salpêtrière, Institut du Cerveau - Paris Brain Institute - ICM, Inserm, CNRS, Paris, France; [11]Institut Curie, Service de Génétique, Paris, France; [12]Inserm U830, DNA Repair and Uveal Melanoma (D.R.U.M.), Equipe Labellisée Par la Ligue Nationale Contre le Cancer, Paris, France; [13]Univ Rouen Normandie, Inserm U1245 and CHU Rouen, Department of Genetics, Rouen, France; [14]University Hospital of Bonn, Center for Hereditary Tumor Syndromes, Bonn, Germany; [15]University Hospital Bonn, Department of Internal Medicine I, Bonn, Germany; [16]University of Bonn, Institute of Human Genetics, Medical Faculty, Bonn, Germany; [17]Technische Universität Dresden, Institute for Clinical Genetics, Faculty of Medicine Carl Gustav Carus, Dresden, Germany; [18]Hereditary Cancer Syndrome Center Dresden, ERN-GENTURIS, Dresden, Germany; [19]German Cancer Consortium (DKTK) and National Center for Tumor Diseases (NCT), Dresden, Germany; [20]Klinikum der Universität München, Medical Genetics Center, Germany; Arbeitsgruppe Erbliche Gastrointestinale Tumore, Medizinische Klinik und Poliklinik IV - Campus Innenstadt, München, Germany; [21]University of Bologna, Department of Medical and Surgical Sciences, Center for Studies on Hereditary Cancer, Bologna, Italy; [22]IRCCS Azienda Ospedaliero-Universitaria di Bologna, Unit of Medical Genetics, Bologna, Italy; [23]University of Parma, Medical Genetics, Department of Medicine and Surgery, Parma, Italy; [24]Fondazione Policlinico Universitario A. Gemelli IRCCS, Department of Laboratory and Infectious Diseases, Rome, Italy; [25]Università Cattolica del Sacro Cuore, Medical Genetics Section, Department of Life Sciences and Public Health, Rome, Italy; [26]University of Siena, Medical Genetics, Siena, Italy; [27]University of Siena, Med Biotech Hub and Competence Center, Department of Medical Biotechnologies, Siena, Italy; [28]Azienda Ospedaliero-Universitaria Senese, Genetica Medica, Siena, Italy; [29]Riga Stradins University, Institute of Oncology, Riga, Latvia; [30]Pauls Stradins Clinical University Hospital, Breast Unit, Riga, Latvia; [31]University of Groningen, University Medical Center Groningen, Department of Genetics, Groningen, Netherlands; [32]University of Groningen, University Medical Center Groningen, Department of Endocrinology, Groningen, Netherlands; [33]Maastricht University Medical Center, Department of Clinical Genetics, Maastricht, Netherlands; [34]Erasmus MC Rotterdam, Department of Clinical Genetics, Rotterdam, Netherlands; [35]Radboud university medical center, Department of Pathology, Nijmegen, Netherlands; [36]Radboud university medical center, Department of Medical Oncology, Nijmegen, Netherlands; [37]Haukeland University Hospital, Western Norway Familial Cancer Center, Department of Medical Genetics, Bergen, Norway; [38]VID Specialized University, Faculty of Health Studies, Bergen, Norway; [39]Oslo University Hospital, Department of Medical Genetics, Oslo, Norway; [40]University Hospital of North Norway, Department of Medical Genetics, Tromsø, Norway; [41]Centro Hospitalar Universitário Lisboa Norte, Lisbon, Portugal; [42]Institute of Oncology Ljubljana, Department of Clinical Cancer Genetics, Ljubljana, Slovenia; [43]Vall d'Hebron Hospital Universitari, Medical Oncology Department, Barcelona, Spain; [44]Catalan Institute of Oncology, IDIBELL-IDIBGI, Hereditary Cancer Program, Barcelona-Girona, Spain; [45]Karolinska University Hospital, Department of Clinical Genetics, Stockholm, Sweden; [46]Karolinska Institutet, Department of Molecular Medicine and Surgery, Stockholm, Sweden; [47]University of Cambridge, Department of Medical Genetics, National Institute for Health Research Cambridge Biomedical Research Centre, Cambridge, United Kingdom; [48]University of Manchester, Manchester Centre for Genomic Medicine, St Mary's Hospital, Division of Evolution and Genomic Sciences, School of Biological Sciences, Manchester, United Kingdom

Background/Objectives: PTEN Hamartoma Tumor Syndrome (PHTS) patients have hereditary cancer risks reaching 76% for breast cancer (BC), comparable to *BRCA1/2*, 22% for endometrial cancer (EC), and 21% for thyroid cancer (TC). Cancer prognosis in PHTS is unknown, and we aimed to provide this for PHTS-associated cancers.

Methods: This European cohort study included adult PHTS patients with data from medical files, registries, and/or questionnaires. Survival and risk factors were assessed using Kaplan-Meier and Cox regression analyses and were compared with sporadic cancer and population mortality using standardized mortality (SMR) and relative survival rates (RSR). Survival bias was addressed using left-truncation.

Results: Overall, 147 female BC patients were included (73% PHTS-indexes), median age of 41 years(IQR:35-49). The 10y-OS was 77%(95%CI:66-90), decreasing per increasing stages 0-IV: 90%(95% CI:73-100), 83%(95%CI:64-100), 74%(95%CI:48-100), 51%(95% CI:26-100) and 0%(95%CI:0-0). Relative survival after BC was comparable to sporadic BC until 2 years (2y-RSR = 1.1;95% CI = 1.1-1.1), gradually increasing to 5y-RSR = 1.7(95%CI = 1.6-1.7). For TC (N = 56, median age of 37(IQR:26-46)) and EC patients (N = 35, median age of 46(IQR:36-55)), the 10y-OS was 87%(95% CI:74-100) and 64%(95%CI:38-100). Overall and cancer-specific mortality in female patients was increased compared to the general population (SMR = 3.7;95%CI:2.6-5.0 and SMR = 2.7;95% CI:1.6-4.4).

Conclusion: This study showed comparable cancer prognosis in PHTS to the general population. The increased overall mortality in the total PHTS population was presumably related to higher cancer incidence. These results, together with the high survival observed in early-stage cancer, emphasize the importance of early PHTS recognition for the potential benefit of early cancer detection in patients with high hereditary cancer risks.

Grants: PTEN Research Foundation

Conflict of Interest: Linda Hendricks: None declared, Katja Verbeek: None declared, Janneke Schuurs-Hoeijmakers: None declared, Robin de Putter: None declared, Hilde Brems: None declared, Sien van Daele: None declared, Violetta Anastasiadou: None declared, Lenka Foretova: None declared, Patrick Benusiglio: None declared, Anna Gerasimenko: None declared, Chrystelle Colas: None declared, Marie-Charlotte Villy: None declared, Claude Houdayer: None declared, Maud Brauchaud: None declared, Robert Hüneburg: None declared, Stefan Aretz: None declared, Arne Jahn: None declared, Verena Steinke-Lange: None declared, Giovanni Innella: None declared, Daniela Turchetti: None declared, Valeria Barili: None declared, Maurizio Genuardi: None declared, Arianna Panfili: None declared, Margherita Baldassarri: None declared, Arvids Irmejs: None declared, Mirjam de Jong: None declared, Thera Links: None declared, Edward Leter: None declared, Daniëlle Bosch: None declared, Stephany Donze: None declared, Rachel van der Post: None declared, Arjen Mensenkamp AstraZeneca, harm westdorp: None declared, Hildegunn Høberg

Vetti: None declared, Marianne Tveit Haavind: None declared, Kjersti Jørgensen: None declared, Lovise Maehle: None declared, Siri Briskemyr: None declared, Juliette Dupont: None declared, Ana Blatnik: None declared, Judith Balmaña: None declared, Maite Torres: None declared, Joan Brunet GlaxoSmithKline, Roser Lleuger-Pujol: None declared, Emma Tham: None declared, Marc Tischkowitz: None declared, D Gareth Evans: None declared, Zerin Hyder: None declared, Nicoline Hoogerbrugge: None declared, Janet Vos: None declared

P01.163.B Deciphering the molecular complexity of the IKZF1plus profile using Optical Genome Mapping

Jonathan Lühmann[1], *Winfried Hofmann*[1], *Anke Katharina Bergmann*[1], *Anja Möricke*[2], *Gunnar Cario*[2], *Martin Schrappe*[2], *Brigitte Schlegelberger*[1], *Martin Zimmermann*[3], *Martin Stanulla*[3], *Doris Steinemann*[1]

[1]*Hannover Medical School, Department of Human Genetics, Hannover, Germany;* [2]*ALL-BFM Study Group, Christian-Albrechts University Kiel and University Medical Center Schleswig-Holstein, Department of Pediatrics, Kiel, Germany;* [3]*Hannover Medical School, Pediatric Hematology and Oncology, Hannover, Germany*

Background/Objectives: Acute lymphoblastic leukemia (ALL), the most frequent pediatric cancer, has witnessed improved survival rates exceeding 90% through genetic marker-based stratification and novel therapies. The IKZF1^{plus} profile, characterized by *IKZF1* deletion (IKZF1^{del}) alongside additional deletions, emerged as a poor prognostic marker in retrospective analyses of AIEOP-BFM ALL 2000/2009 trials and is used for high-risk stratification in the ongoing AIEOP-BFM ALL 2017 trial. A deeper genome-wide characterization of leukemic cells is necessary for a better understanding of the molecular response.

Methods: In total, 142 patients with *IKZF1* deletion from the AIEOP-BFM ALL 2000/2009 trial were re-analyzed by means of Optical Genome Mapping (OGM) enabling the simultaneous detection of structural variants and aneuploidies. Genomic profiles were correlated with patient outcome with respect to event-free survival (EFS), cumulative incidence of relapse (CIR) and overall survival (OS).

Results: About half of the patients in this study exhibited well-known and new genomic alterations in addition to IKZF1$^{del/plus}$. In 53 patients gene fusions (*ABL*-class, *CRLF2*, *PAX5*, *JAK2*, *ZNF384*) have been detected. Eighteen patients displayed established prognostic markers namely high hyperdiploidy, *ETV6::RUNX1*, or iAMP21. Our data demonstrate the genomic complexity and heterogeneity of the IKZF1$^{del/plus}$ patient group. In this cohort EFS, CIR and OS are similar in IKZF1^{del} and IKZF1^{plus} patients and mainly dependent on the accompanying good or poor prognostic markers.

Conclusion: Through the utilization of OGM, we reveal the complex genomic landscape in ALL and demonstrate that the significance of the IKZF1^{plus} profile is not as prominent as previously anticipated.

Grants: Deutsche Kinderkrebsstiftung (German Childhood Cancer Foundation)

Conflict of Interest: None declared

P01.164.C Functional assessment of variants of uncertain significance in *PTEN* with a predicted effect on splicing

Elke van Veen[1], *Arjen Mensenkamp*[1], *Janneke Schuurs-Hoeijmakers*[1], *Nicoline Hoogerbrugge*[1], *Richarda de Voer*[1]

[1]*Radboud University Medical Center, Department of Human Genetics, Nijmegen, Netherlands*

Background: Pathogenic variants in *PTEN* are associated with PTEN hamartoma tumour syndrome (PHTS). However, in a number of patients variants of uncertain significance (VUSs) are identified. To assign these VUSs a meaningful pathogenicity classification, the functional effect of these variants needs to be assessed. Here, we selected *PTEN* VUSs identified in PHTS-like patients with a predicted effect on splicing to assess their functional effect.

Methods: VUSs were identified in an ERN-GENTURIS PHTS cohort ($n = 510$). All variants were classified as a VUS using the *PTEN*-specific ACMG guidelines and had a spliceAI score of >0.1, with no published functional data to date were included. Amplicons spanning one or two exons that harboured the identified VUSs were designed and cloned into a minigene vector using gateway cloning. Site-directed mutagenesis was used to generate the variants of interest and splice assays with mutant and wild type constructs were performed.

Results: Eight *PTEN* VUSs were identified for further functional testing. We identified five missense variants (c.77C>A, p.(Thr26Asn); c.89C>A, p.(Pro30Gln); c.94A>T, p.(Ile32Phe); c.801G>T, p.(Lys267Asn); c.929A>G, p.(Asp310Gly)) and three intronic variants (c.80-1G>C; c.165-18T>A; c.253+5G>C). One additional variant (c.334C>G, p.(Leu112Val)) was included in the assay as a positive control. These nine variants were distributed over seven exon-intron regions, captured in five amplicons ranging between ~2.5-5.2 kb in size.

Conclusion: Upon assessment, coding variants may also exert an effect on splicing and thus impact the function of PTEN. Splice effect analysis will help to further classify the pathogenicity of *PTEN* VUSs.

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Conflict of Interest: None declared

P01.165.D Complex translocation leading to13q interstitial deletion in a Moroccan child with retinoblastoma and intellectual disability

ZHOUR EL AMRANI[1], *siham chafai elalaouia*[2], *Wafaa Jdioui*[3], *aziza sbiti*[4], *ilham ratbi*[5], *Thomas Liehr*[6], *abdelaziz sefiani*[7], *Abdelhafid Natiq*[8]

[1]*Research team in genomics and molecular epidemiology of genetic diseases, Genomics Center of Human Pathologies, Faculty of Medicine and Pharmacy, Medical Genetics, RABAT, Morocco;* [2]*Faculty Of Medicine And Pharmacy Of Rabat, Medical Genetics, Rabat, Morocco;* [3]*Centre des consultations et des explorations externes, Hopital d'enfants, CHU Ibn SinaConsultation de génétique, Rabat, Medical Genetics, Morocco;* [4]*National Institute D'hygiène Du Maroc, Medical Genetics, Rabat, Morocco;* [5]*Faculty Of Medicine And Pharmacy Of Rabat, Rabat, Morocco;* [6]*Institute of Human Genetics, Jena University Hospital, Friedrich Schiller University, Germany;* [7]*National Institute D'hygiène, Medical Genetics, Morocco;* [8]*Faculty Of Medicine And Pharmacy Of Rabat, Medical Genetics, Morocco*

Background/Objectives: Retinoblastoma (RB) is the most common malignant intraocular tumor in children; it affects their eyes often even prenatally. RB may be sporadic or familial, due to germinal mutation in *RB1* gene or by abnormal chromosomal abnormalities involving *RB1* gene, located in 13q14. Monosomy of subband 13q14 as a partial deletion can also be responsible for RB with additional symptoms. The latter may be RB associated with psychomotor retardation, macrocephaly, broad forehead, thick earlobes, and bulbous nose.

Methods: We present here the case of a boy from a consanguineous marriage with bilateral retinoblastoma, intellectual disability and facial dysmorphic features. Classical and molecular cytogenetics(FISH) were used to recognize genotype-phenotype association.

Results: The karyotype showed a three way translocation involving chromosomes 5, 12 and 13. Further molecular cytogenetics analysis revealed a deletion of 13q14 involving the tumor suppressor gene *RB1*.

Conclusion: This case highlights the impact of classical and molecular cytogenetics in diagnosis of rare genetic syndromes and for the genetic counselling of patients and their families. Pure molecular karyotyping analyses would miss the underlying chromosomal mechanism leading to the rearrangement.

Grants: this work does not receive any funding, and I would like to apply for a fellowship to attend the European Human Genetics Conference.

Conflict of Interest: None declared

P01.166.A Deep intronic insertions in patients at high risk for hereditary breast and ovarian cancer

*Bernardus Aldrige Allister[1], **Winfried Hofmann**[1], Jonathan Lühmann[1], Bernd Auber[1], Nataliya Di Donato[1], Monika M. Golas[2;2;3], Doris Steinemann[1]*

[1]Hannover medical school (MHH), Department of Human Genetics, Hannover, Germany; [2]University of Augsburg, Human Genetics, Faculty of Medicine, Augsburg, Germany; [3]University Hospital Augsburg, Comprehensive Cancer Center Augsburg, Augsburg, Germany

Background/Objectives: 13 core genes (*ATM*, *BARD1*, *BRCA1*, *BRCA2*, *BRIP1*, *CDH1*, *CHEK2*, *PALB2*, *PTEN*, *RAD51C*, *RAD51D*, *STK11*, and *TP53*) are routinely tested in patients with the suspicion of the hereditary breast and ovarian cancer (HBOC). Non-coding variants such as Alu-elements, the most prevalent mobile-element insertion (MEI) in the human genome are increasingly recognized as a cause of the genetic disorders. However, their relevance in HBOC predisposition may be underestimated.

Methods: We perform whole genome and whole transcriptome sequencing on 134 high risk HBOC patients (Asian:9, European:125), who are tested negative for pathogenic variants in HBOC core genes. The data was analyzed using an in-house-NGS-megSAP-pipeline coupled with the MEI detection tool Mobster.

Results: We detected eight deep intronic Alu-element insertions in eight different European patients: 7 in *BRCA2*, 1 in *PTEN*. The Alu-element insertion in *BRCA2* intron 13 (NC_000013.11:g.32348295_32348296insAluYa5) was annotated in seven unrelated patients by Mobster. This Alu-element insertion is present within the International Genome Sample Resource with different population frequencies: European:4/628 = 0,64%, Asian:56/1172 = 4,78%, African:12/885 = 1,36%, American:13/485 = 2,68%. cDNA analysis, showed that this AluYa5 insertion causes *BRCA2* in-frame exon 12 skipping.

Conclusion: High population frequency of the insertion suggests that the possible hypomorphic-effect of exon 12 deletion requires additional analysis of its pathogenicity. However, our findings suggested that deep intronic Alu-element insertion could play a role in causing disease in unresolved cases of HBOC.

Grants: Deutsche Forschungsgemeinschaft (DFG)

Conflict of Interest: None declared

P02

REPRODUCTIVE GENETICS

P02.001.C Heterozygote missense variations in *PROKR2* present partial penetrance for causing Hypogonadotropic Hypogonadism

Florence Abou[1;2], *moran gershoni[3], Netanel Waldenberg[4], Naama Steiner[5], Noga Fuchs Weizman[6], Shimi Barda[6], Sandra Kleiman[6], Shlomi Barak[4;7], Ruti Parvari[1;2]*

[1]Ben-Gurion University of the Negev, National Institute of Biotechnology in the Negev, Be'er sheva, Israel; [2]Ben-Gurion University of the Negev, The Shraga Segal Department of Microbiology, Immunology and Genetics Faculty of Health Sciences, Be'er sheva, Israel; [3]Institute of Animal Science, Agricultural Research Organization, Volcani Center, Rishon LeZion, Israel; [4]The Multidisciplinary Center for Female and Male Infertility, Male fertility, Tel Aviv, Israel; [5]Soroka University Medical Center, Fertility and IVF Unit, Department of Obstetrics and Gynecology, Be'er Sheva, Israel; [6], Male Fertility Clinic and Sperm Bank, Lis Maternity Hospital, Tel Aviv Sourasky Medical Center, affiliated with the Sackler Faculty of Medicine, Tel Aviv, Israel; [7]Assuta Ashdod University Hospital, Reproductive Services, Ashdod, Israel

Background: Hypogonadotropic hypogonadism (HH), with a prevalence of 1–10 per 100,000 individuals, is characterized by reduced function of the gonads due to insufficient stimulation by the gonadotropin-releasing hormone (GnRH) from the hypothalamus. Kallman syndrome is a form of HH characterized by delayed or absent puberty and an impaired sense of smell.

Heterozygote mutations in the *PROKR2* (prokineticin receptor-2) gene were associated with HH. PROKR2 and its ligand PROK2 have a role in the cellular migration of both GnRH neurons and the Olfactory interneurons.

Objective: Identify the genetic causes of infertility in males.

Methods: Whole Exome Sequencing of peripheral blood DNA and bioinformatic analysis.

Results: In one infertile man not presenting HH, we identified a previously reported heterozygote variant in *PROKR2* (p.L173R) associated with Kallman syndrome, which was suggested to be a founder mutation. The variation occurs in up to 0.5% in various control populations (gnomAD). In a cohort of 83 individuals, none present with HH, we identified two additional missense variations (p.R286C and p.R85C) reported in the literature, each in a different infertile patient. The population frequencies of these variants are up to 4.6% and 0.27% respectively in specific control populations. Overall, 23 variations in *PROKR2* were reported to be associated with Kallman syndrome or HH, and six additional pathogenic variations appear in Clinvar. Most are more common in the population than the prevalence of HH.

Conclusion: Clinical manifestations of p.L173R, p.R286C, and p.R85C, together with other reported variations in *PROKR2* suggest complex inheritance and low penetrance in the disease phenotype.

Grants: ISF 2844/21.

Conflict of Interest: None declared

P02.002.D Detection and interpretation of mosaicism in PGT-A programs

*Julia Gontar[1], Nadiya Kazachkova[1], Nataliia Buderatska[2], Ihor Ilyin[3], Olga Parnytska[2], Yuriy Herevych[3], Eduard Kapustin[3], Sergiy Lavrynenko[2], Yana Lakhno[1], **Olena Fedota**[4]*

[1]LLC "Medical Center IGR", Diagnostic Laboratory, Kyiv, Ukraine; [2]LLC "Medical Center IGR", Embriologic Laboratory, Kyiv, Ukraine; [3]LLC "Medical Center IGR", IVF Deparment, Kyiv, Ukraine; [4]LLC "AMS", Research Department, Kharkiv, Ukraine

Background/Objectives: Mosaicism interpretation is a PGT-A problem. Comprehensive chromosome screening methods differ in the results information. Genetic analysis methodology depends on the tasks, economic expediency and family features. The study

of approaches to optimizing mosaicism detection in PGT-A is relevant.

Methods: The study involved 3382 embryos samples obtained from 668 women (33.7 ± 5.4 years) treated with IVF/ICSI cycles. For PGT-A a trophectoderm biopsy was performed on the post-fertilization hours 120 or 144. 1134 samples were diagnosed by NGS, 2248 samples - using FISH in chromosomes 9, 13, 15, 16, 17, 18, 21, 22, X, Y, 70 samples - by both methods.

Results: According to NGS or FISH, variants were established: norma – 43.7% and 43.5% ($p > 0.05$), aneuploidy – 53.7% and 26.4%, mosaicism – 1.7% and 25.8%, polyploidy – 0 and 4.3%. Thus, the proportions of embryos with abnormalities show a difference ($p < 0.001$). After FISH-detected mosaicism, such combinations were established: diploidy/polyploidy – 13,4%, disomy/monosomy/trisomy – 1,6%, trisomy/monosomy – 1,2% on the same chromosome. These mosaicisms cannot be identified by NGS, but may have clinical implications. Disomy/monosomy and disomy/trisomy were detected by NGS – 1,1% and 0,6% and FISH – 5,7% and 4,0%.

Using both NGS/FISH, the same result was obtained for 78.6% of embryos: true match – 64.3%, N/N – 45.7%, An/An – 18.6%; formal – aneuploidy on different chromosomes, An/An – 7.1%, various combinations – An/mos 4.3%, An or mos/An+mos – 2.9%. 21.4% of samples were different: An/N – 8.6%, N/An – 1.4%, N/mos – 4.3%, N/mos or An – 5.7%, mos or An/N – 1.4%.

Conclusion: Optimizing of PGT-A, especially for families with biological limitations, may require a combination of several methods.

Grants: Absent

Conflict of Interest: None declared

P02.003.A Characterisation of novel variants for endometriosis risk identified in participants within the genomics England database

*Elpida Fragouli[1], **Amelia Warren**[1], Demetra Andreou[1], Anna Mantzouratou[1]*

[1]Bournemouth University, Life and Environmental Sciences, Bournemouth, United Kingdom

Background/Objectives: Endometriosis, altough multifactorial, tends to affect women of the same family with heritability between 47-51%. Studies have identified 40+ single nucleotide polymorphisms (SNPs) mostly associated with advanced endometriosis. To date no variants have been associated with earlier endometriosis stages. Genomic England database (GED) cantains whole genomes information and medical records from thousands of participants. The aim of this is to identify endometriosis risk variants in selected populations within GED and investigate the pathways these variants could act on.

Methods: Previous studies identified genomic areas associated with an increased endometriosis risk. Of these, 5 genes were selected for furher invesigation, IDO1, IL6, CNR1, TACR3 and KISS1R. Participant data with endometriosis were extractred from selected participant panels, filtered according to specific inclusion criteria and classified to stages according to the degree of medical interventions presented in the clinical data. A database was created and populated with targeted genomic similarity queries within our participant cohorts.

Results: Fifty-seven patients were identified as appropriate candidates for the study. Deferential variant frequency in the GED endometriosis cohort was seen in 10 SNPs compared with that in the general GED population. Two variants were significanly increased in frequency in the endometriosis cohort and assosciatied with IDO1 and CNR1 genes. Both variants are not yet characterised, one is novel, and not previously associated with the condittion.

Conclusion: Targeted investigations in GED identified new variations for IDO1 and CNR1 genes with significantly increased frequency in endometriosis patients. Their further characterisation could reveal novel pathways about the onset of endometriosis.

Grants: Bournemouth University

Conflict of Interest: None declared

P02.004.B The "Goldilocks" panel: Determining the optimal number of genes to include for equitable, pan-ancestry Carrier Screening.

Mia Gruzin[1;2], Matthew Hobbs[1], Sarah Poll[3], Jaysen Knezovich[4], Swaroop Aradhya[3], Leslie Burnett[1;2;5]

[1]Garvan Institute of Medical Research, Darlinghurst, Australia; [2]School of Clinical Medicine, UNSW Medicine and Health, St Vincent's Clinical Healthcare Campus, Darlinghurst, Australia; [3]Invitae Corporation, San Francisco, United States; [4]Blue Jay Consulting Services, Brisbane, Australia; [5]Northern Clinical School, Faculty of Medicine and Health, University of Sydney, St Leonards, Australia

Background/Objectives: Clinical utility of reproductive carrier screening varies across diverse populations because of the preponderance of genomic data from European individuals in public databases, and the heterogeneity of available carrier tests. We have defined the size and composition of an optimal ("Goldilocks") carrier panel with clinical utility across diverse populations, regardless of ancestry.

Methods: Using gnomAD (v4.0) and ClinVar data for ~1,300 serious, childhood-onset autosomal recessive (AR) and X-linked (XL) monogenic conditions, we modelled various in silico screening panels, validating with real-world data from >380,000 individuals. To compare targeted "hotspot" variant analysis against full gene sequencing, we studied differences in carrier yield between specific ancestry groups for *CFTR*.

Results: A gene panel of ~500 prioritised genes ("Goldilocks") achieved 99% of maximum possible carrier yield, irrespective of genetic ancestry. Smaller panels provided diminished carrier yield and inequitable benefit to specific ancestry groups. Real-world carrier screening data from >75 countries validated our model. Using *CFTR* as an example, compared to full gene sequencing, "hotspot" analysis provided unequal yield between specific ancestry groups, missing >10% of the most prevalent pathogenic variants for patients with self-reported Asian, Hispanic, and Middle Eastern ancestry.

Conclusion: An optimally-sized, globally equitable, pan-ancestry Goldilocks panel will identify carrier risk for 99% of most-severe AR/XL conditions. This panel may be analysed virtually from exome or genome sequencing, allowing for ongoing refinement based on emerging evidence. To maximise clinical utility across diverse ancestry groups, full gene sequencing approaches are preferred over targeted "hotspot" variant analyses.

Grants: Not applicable.

Conflict of Interest: Mia Gruzin Former contractor at Invitae Australia., Matthew Hobbs: None declared, Sarah Poll Shareholder at Invitae (NVTA)., Current employee at Invitae Corp., Jaysen Knezovich Former employee at Invitae Australia., Swaroop Aradhya Shareholder at Invitae (NVTA)., Current employee at Invitae Corp., Leslie Burnett Shareholder at Invitae (NVTA)., Former employee of Invitae Australia., 1. Member, Community Genetics Advisory Board, Wolper Jewish Hospital - Honorary; 2. Consultant, Virtus Health Specialist Diagnostics; 3. Consultant, 23 Strands.

P02.005.C Copy number variation sequencing for the products of conception: what is the optimal testing strategy

1290

Shuyuan Li[1], *yiyao chen*[1]

[1]*Hengshan Road, Shanghai, China*

Background/Objectives: Copy number variation sequencing (CNV-seq) is crucial in prenatal diagnosis, but its limitations in detecting polyploidy, maternal cell contamination (MCC), and uniparental disomy (UPD) restrict its application in the analysis of products of conception (POCs). This study aimed to investigate an optimal genetic testing strategy for POCs in the era of CNV-seq.

Methods: CNV-seq and quantitative fluorescent polymerase chain reaction (QF-PCR) were performed in all 4,211 spontaneous miscarriage cases. Different testing strategies were compared and the optimal testing strategies were proposed.

Results: Of the 4211 cases, 2561 (60.82%) exhibited clinically significant chromosomal abnormalities. CNV-seq alone, without QF-PCR, might misdiagnose 311 (7.39%) cases, including 278 polyploidy, 13 UPD, and 20 MCC. In 20 MCC cases identified by QF-PCR, CNV-seq successfully pinpointed the cause of miscarriage in 13 cases. Furthermore, in cases where QF-PCR suggested polyploidy, CNV-seq improved the diagnostic accuracy in 54 (1.28%) hypo/hypertriploidy cases. After comparing four different strategies, the sequential approach (initiating with CNV-seq followed by QF-PCR if necessary) emerged as advantageous, reducing approximately 70% of the cost associated with QF-PCR while maintaining result accuracy.

Conclusion: We propose an initial CNV-seq followed by QF-PCR if needed—an efficient and cost-effective strategy for the genetic analysis of POCs.

Grants: This work was supported by the Shanghai Municipal Science and Technology Commission (22Y11906700) and Clinical Research Plan of IPMCH (IPMCH2022CR1-02).

Conflict of Interest: None declared

P02.007.A A homozygous *HIPK4* variant causes a familial form of male infertility

Sophie Adina Koser[1], *Avinash S. Gaikwad*[1], *Andela Kovacevic*[2], *Isabella Aprea*[3], *Claudia Krallmann*[4], *Birgit Stallmeyer*[1], *Johanna Raidt*[3], *Heymut Omran*[3], *Sabine Kliesch*[4], *Hubert Schorle*[2], *Corinna Friedrich*[1], *Frank Tüttelmann*[1]

[1]*Institute of Reproductive Genetics (IRG), University of Münster, Münster, Germany;* [2]*Institute of Pathology, Department of Developmental Pathology, University of Bonn, Bonn, Germany;* [3]*Department of General Paediatrics, University of Münster, Münster, Germany;* [4]*Centre of Reproductive Medicine and Andrology (CeRA), Department of Clinical and Surgical Andrology, University of Münster, Münster, Germany*

Background/Objectives: Oligoasthenoteratozoospermia (OAT) is a highly variable phenotype of male infertility that is characterised by reduced sperm count and motility, and abnormal sperm morphology. Its genetic basis is not well understood. Exome sequencing in familial cases offers the opportunity to discover novel candidate genes also for this infertility phenotype.

Methods: Four infertile brothers with varying OAT were recruited at the CeRA. Exome sequencing including the parents and a fertile brother enabled segregation analysis expecting a shared autosomal recessive or X-linked cause. Candidate variants were validated by Sanger sequencing and further analysed via heterologous expression in HEK293T cells.

Results: Three infertile brothers were homozygous for a start loss variant (c.1A>G) in *HIPK4*, which is highly expressed in spermatids. Heterologous expression demonstrated that a downstream start codon in frame was used, likely resulting in an N-terminally truncated protein disrupting a part of HIPK4's kinase domain. Interestingly, the fourth brother carried compound heterozygous variants in *DNAH17* (c.[1076_1077dup];[7752 + 2T>A]) known to cause multiple morphological abnormalities of the sperm flagella (MMAF).

Conclusion: In contrast to previous studies reporting *HIPK4* as associated with azoospermia, we identify *HIPK4* as likely cause for familial OAT. Importantly, published male knockout mice mimic the patients' OAT phenotype with predominantly abnormal sperm heads in mice and men. Our work highlights the utility of exome-based segregation analysis and illustrates the possibility of different monogenic causes within the same family.

Grants: This work was supported by the DFG Clinical Research Unit 326 'Male Germ Cells' (CRU326) and the Medical Faculty Münster's 'CareerS' programme.

Conflict of Interest: None declared

P02.008.B Towards a diagnostic gene panel for impaired sperm morphology and motility causing male infertility

Clara Barkhaus[1], *Johanna Kuß*[1], *Birgit Stallmeyer*[1], *Frank Tüttelmann*[1]

[1]*Institute of Reproductive Genetics (IRG), University of Münster, Münster, Germany*

Background/Objectives: Male infertility is a clinically and genetically extremely heterogeneous disease affecting approximately 7% of men. Morphological defects of spermatozoa (teratozoospermia), often associated with impaired sperm motility (asthenozoospermia), are a common clinical phenotype. Several candidate genes have been described to cause the disorder. To establish a diagnostic gene panel for affected men, it is necessary to separate validated disease genes from genes with limited evidence.

Methods: To identify candidate genes for impaired sperm motility and morphology, we performed a Pubmed search using the terms, 'asthenozoospermia', 'teratozoospermia', 'multiple abnormalities of the sperm flagella' and 'disease gene', followed by a standardised evaluation of all described candidate genes based on the ClinGen gene-disease clinical validity curation.

Results: We identified 128 genes in which genetic variants were associated with teratozoospermia, asthenozoospermia or a combination of both. Of these, 50 genes were reported with autosomal recessive inheritance. For 41 genes, a gene-disease relationship was described in at least 2 independent reports and/or for more than 3 different variants. According to the ClinGen framework, more than 30 of these genes reached sufficient evidence to be included in a diagnostic gene panel.

Conclusion: Male infertility due to impaired sperm motility and/or morphology is a common and heterogeneous disease. Analysis of genes with a validated gene-disease correlation should be implemented in the diagnostic workflow of affected men.

Grants: This work was supported by the DFG Clinical Research Unit 326 'Male Germ Cells' (CRU326).

Conflict of Interest: None declared

P02.010.D Expanded carrier screening for monogenic disorders as part of the preconception care

Martina Hajduskova[1], *Martina Hruba*[1], *Lucie Solcova*[1], *Anna Rykovska*[1], *Monika Pittrova*[1], *Petr Losan*[1]

[1]*Genetika Plzen, s.r.o., Laboratory of reproductive genetics, Plzeň*

Background/Objectives: Clinical genetics aims to minimize the risk of inheriting monogenic recessive disorders (MRDs). This is achieved by screening for multiple MRDs and identifying

asymptomatic carriers in a population. Such pan-ethnic expanded carrier screening (ECS) with follow-up care may improve preventive preconception measures.

Methods: A new ECS at Genetika Plzen targets 128 autosomal and 23 X-linked MRDs (103 genes), reflecting the international guidelines (ESHG, ACOG, ACMG). Our ECS employs an NGS panel with a hybridization enrichment using custom probes (Agilent Technologies), the MiSeq V3 Platform (Illumina), and the Varsome Clinical software (Saphetor SA). For all genes, the entire coding sequence including UTRs and exon-intron junctions are examined, both SNVs and CNVs are assessed. Only variants of pathogenicity classes 4 and 5 are reported.

Results: During 2021-2023, we screened 406 subjects, 198 couples and 10 individuals, mostly living in the Czech Republic. 238 individuals (59%) carried at least one class-4/-5 gene variant. We identified 16 couples (8%) with increased reproductive risk, i.e., either the female partner (for X-linked MRDs) or both partners (autosomal MRDs) carrying a clinically significant variant in the affected gene. As follow-up care, they were offered preimplantation/prenatal diagnostics.

Conclusion: Within preconception care, we have successfully established ECS to significantly reduce the risk of having a child suffering from a severe MRD (the ECS detection rate of up to 99% is achieved for most genes). Our results show that ECS detects significant percentage of couples at reproductive risk even in a population without high prevalence of particular MRDs.

Conflict of Interest: None declared

P02.011.A Association of IL-6 polymorphism -174G>C with the Premature Ovarian Insufficiency in Bulgarian patients

*Ivelina Oprova[1], **Eliana Dimova**[2], Kalina Belemezova[2], Mariela Hristova-Savova[2], Petya Andreeva[1], Ivanka Dimova[3]*

[1]Shterev Hospital, Department of Infertility and IVF, Sofia, Bulgaria; [2]Shterev Hospital, Department of Medical genetics, Sofia, Bulgaria; [3]Medical University Sofia, Department of Medical genetics, Sofia, Bulgaria

Background/Objectives: Primary premature ovarian insufficiency (POI) represents the premature exhaustion of the ovarian reserve in women under the age of 40. Interleukin-6 is implicated in the pathophysiological characteristics associated with this condition. We explored the frequency in IL6 gene polymorphism -174 G>C among patients with POI, compared to non-selected European individuals (controls), with the aim of establishing a correlation between this genetic factor and the disease.

Methods: Twenty-four patients with POI (median age of 33.5 years) were subjected to our survey. IL 6 gene polymorphism -174 G>C was examined by Real-Time PCR Genotyping kit (DNA Technology LLC). The allele and genotype frequencies were compared to the data from 503 controls (data collected from ensembl.org). For the purpose of our study, we used the Chi-Square Test ($p < 0.05$ was considered significant).

Results: The genotype frequencies observed among the 24 cases and 503 controls were as follows: *G/G* - 62.5 % and 36 %, *G/C* - 33.3 % and 44,9 %, and *C/C* - 4.2 % and 19,1 %. The *G* and *C* allele frequencies were 79.2 % (cases) and 58,4 % (controls), and 20.8 % and 41,6 %, respectively. The genotype G/G and G allele distribution revealed significant differences between POI patients and controls (p values < 0.05).

Conclusion: We discovered an association of IL 6 –174 G/G genotype and G allele with POI in Bulgarian patients. IL-6 is involved in inflammation and apoptosis and dysregulation of these processes could contribute to the accelerated loss of ovarian follicles seen in POI.

Conflict of Interest: None declared

P02.012.B Fractonation of high-quality spermatozoa promotes peripherial positioning of sex chromosomes

Zuzanna Graczyk[1], *Jagoda Kostyk[1], Julia Pospieszna[1,2], Zuzanna Myślicka[1], Marzena Kamieniczna[1], Marta Olszewska[1], Maciej Kurpisz[1]*

[1]Institute of Human Genetics, Polish Academy of Sciences, Poznan, Poland; [2]Poznan University of Medical Sciences, Department of Toxicology, Collegium Pharmaceuticum, Poznan, Poland

Background/Objectives: Sperm chromosomes are non-randomly organized in three-dimensional nuclear space. This nuclear organization is one of the epigenetic mechanisms that can regulate genome functioning. After fertilization, chromosomes undergo chromatin remodeling, transcriptional activation and replication.

The aim of this study was to determine whether selection of spermatozoa with good motility or proper chromatin density, defines specific positioning of chromosomes.

Methods: Semen samples from 5 normozoospermic males were collected and processed for fractionation *via* swim up (to select viable and motile spermatozoa) or Percoll gradient (to select sperm with properly packed chromatin). Fluorescence in situ hybridization (FISH) was applied to analyse the positioning of chromosomes 4, 7, 8, 9, 18, X and Y. At least $n \geq 100$ of spermatozoa per subpopulation/chromosome were analysed (Leica DM5500 microscope, filter set: DAPI, SpG, SpO, SpAq, 630x magnification, motorized stage, LASX Navigator software).

Results: In spermatozoa with good motility or proper chromatin density, chromosome 4 moves towards the acrosome, compared to non-fractionated sperm. In motile sperm fraction chromosomes 18, X, and Y shift to the nuclear periphery, while in sperm with condensed chromatin, chromosomes 8, 9, and Y move to the nuclear periphery, compared to non-fractionated sperm.

Conclusion: High-quality sperm selectioning promotes repositioning of sex chromosomes towards nuclear periphery, which is the first region that interacts with the ooplasm during fertilization. Then, sex chromosomes must undergo remodelling of chromatin due to Meiotic Sex Chromosome Inactivation, which is crucial in the early stages of embryo development.

Grants: National Science Centre 2020/38/E/NZ2/00134

Conflict of Interest: None declared

P02.013.C Genetic and functional evidence for *TDRD6* as male infertility-associated gene

Farah Ghieh[1], *Erik Schüftan[1], Sabine Kliesch[2], Frank Tüttelmann[1], Birgit Stallmeyer[1]*

[1]Institute of Reproductive Genetics (IRG), University of Münster, Münster, Germany; [2]Center of Reproductive Medicine and Andrology (CeRA), University Hospital Münster, Münster, Germany

Background/Objectives: Tudor domain-containing protein 6 (TDRD6) is essential for spermatogenesis and male fertility in mice. It localises to the chromatoid body (CB), a subcellular structure in round spermatids, and interacts with key proteins of the piRNA pathway. The function of TDRD6 in humans is unexplored warranting investigations whether *TDRD6* is also a disease gene for male infertility.

Methods: Exome/genome data of 2,362 infertile men with severe oligo- or azoospermia from the Male Reproductive Genomics (MERGE) study were screened for rare (MAF <0.01 in gnomAD) biallelic high impact variants in *TDRD6* (loss-of-function or missense with CADD >20). Compound heterozygosity was

established by segregation analysis or long-read sequencing. Testicular phenotypes and the impact of TDRD6 on the chromatoid body architecture were determined by immunohistochemical staining of marker proteins.

Results: We identified five infertile men with biallelic variants in *TDRD6* who shared a common reproductive phenotype of oligoasthenoteratozoospermia (OAT). One patient carried a homozygous missense variant and four patients were compound heterozygous for a loss-of-function variant and a missense variant. Two missense variants were predicted to destabilise TDRD6 protein structure. In two variant carriers, the subcellular localisation of CB marker protein DDX4 was impaired while the expression profile of piRNA pathway factors PIWIL1 and MAEL was unaffected.

Conclusion: This study establishes *TDRD6* as candidate gene for OAT-related human male infertility and provides functional evidence for a role of TDRD6 in CB formation during late spermatogenesis.

Grants: This work was supported by the IZKF Münster (Tüt4/011/23).

Conflict of Interest: None declared

P02.014.D Gennet Endometrium Receptivity Test

Štěpán Chvojka[1], *Lucie Dohnalova*[1], *Romana Vlckova*[1], *Simona Suhajova*[1], *Hana Dvorackova*[1], *Martina Bittoova*[1], *Monika Koudová*[1]

[1]*GNTlabs by GENNET, Czech Republic*

Introduction: Nowadays in vitro fertilization treatment is a complex process which thanks to new knowledge and technological development is constantly being expanded to include additional examinations with one common goal - to increase the success of this treatment and thus enable the couple to have a healthy child. One of them is the examination of endometrial receptivity.

Methods: The endometrium is normally examined using ultrasound, with the development of Next Generation Sequencing (NGS) methods it is possible to assess the endometrium from another point of view, that of molecular genetics. The endometrium becomes receptive to the embryo only during a short period of time in the menstrual cycle. NGS techniques in combination with prediction algorithms make it possible to determine this period and determine the "implantation window", the time when the endometrium is most receptive to embryo transfer. This should ideally be between 19-21 day of the cycle for every fertile woman. However according to studies, up to 30% of women have a shifted implantation window.

Results: In cooperation with the Yikon company we have implemented the GERT (Gennet Endometrium Receptivity Test) in our laboratory which evaluates a total of 175 genes involved in the receptivity. Examination is indicated in women with repeated failure of euploid embryo implantation (after PGT-A). The result of the test is to determine whether the patient's endometrium is in the pre-, post- or receptive phase.

Conclusion: Based on the results the optimal time of the personalized embryo transfer is then recommended to the patients.

Conflict of Interest: None declared

P02.015.A Investigating the role of solute carrier family of genes in adenomyosis pathogenesis utilizing whole exome sequencing in a sporadic patient cohort

Nimet Eser[1], *nura fitnat topbas selcuki*[2], *Ipek Yildiz Ozaydin*[3], *engin oral*[4], **Feyza Tuncer**[5]

[1]*Istanbul University, Graduate School of Health Sciences, Istanbul, Türkyie;* [2]*Istanbul Sisli Hamidiye Etfal Training and Research Hospital, Department of Obstetrics and Gynecology, Istanbul, Türkyie;* [3]*Istanbul Kanuni Sultan Suleyman Training and Research Hospital, Department of Pathology, Istanbul, Türkyie;* [4]*Biruni University Faculty of Medicine, Department of Obstetrics and Gynaecology, Istanbul, Türkyie;* [5]*Istanbul University, Aziz Sancar Institute of Experimental Medicine, Department of Genetics, Istanbul, Türkyie*

Background/Objectives: Adenomyosis and endometriosis are gynecological benign conditions characterized by the ectopic localization of endometrial tissue and stroma, which manifest with similar symptomology. Definite diagnosis is accomplished through histopathological confirmation of disease with surgical material. So far, genetic studies focused on endometriosis with limited attention to adenomyosis. We aimed to identify shared genetic variants among sporadic adenomyosis patients utilizing whole exome sequencing (WES).

Methods: Ten women, who received histopathological diagnosis of adenomyosis were recruited. Genomic DNA isolated from peripheral blood was subjected to WES on Illumina NovaSeq 6000 system. Pairend NGS Cloud platform was utilized in bioinformatic analysis, followed by filtering for MAF < 1%. Variants were prioritized in accordance with functional relevance and impact determined by in silico prediction tools.

Results: Remarkable accumulation of rare genetic variants in the members of the solute carrier family of genes (SLCs) were detected in our cohort. Among them, three of our patients carried rare variants of SLC12A1 and SLC12A2 which were prominent due to abnormal expression levels determined in a previous study. Moreover, nine of our patients harbored rare variants in genes (*SIX4, BSND, WNK2, SGK1, SLC12A9)* shown to influence the expression of *SLC12A1* and *SLC12A2*.

Conclusion: This is the first study to assert the importance of SLCs in the pathogenesis of adenomyosis through WES analysis. Prospective work includes assessing the expressions of *SLC12A1* and *SLC12A2* utilizing the genomic materials recovered from these patients' formalin-fixed paraffin-embedded hysterectomy tissues.

Grants: Istanbul University Scientific Research Projects Coordination Unit(TYL-2023-40298)

Conflict of Interest: None declared

P02.016.B Estimation of carrier frequencies of autosomal recessive genetic conditions based on gnomAD v4.0 data

Ronja Hotakainen[1], *Timo Järvinen*[1], *Kaisa Kettunen*[1], *Anna-Kaisa Anttonen*[1;2;3], *Eveliina Salminen*[2;3]

[1]*University of Helsinki and Helsinki University Hospital, Laboratory of Genetics, Helsinki, Finland;* [2]*Helsinki University Hospital, Department of Clinical Genetics;* [3]*University of Helsinki, Department of Medical and Clinical Genetics, Helsinki, Finland*

Background/Objectives: A comprehensive strategy in the screening and prevention of rare diseases would benefit the current healthcare system. While monogenic rare diseases are relatively rare on their own, they contribute to around 10% of infant deaths and 20% of pediatric hospitalizations. The American College of Medical Genetics and Genomics recommends screening of autosomal recessive and X-linked conditions with a population frequency of ≥ 1/200, for all pregnant patients and those planning a pregnancy. Previous studies have used gnomAD v2.1.1 and v3.1.2 to estimate the gene carrier frequencies (GCF). In November 2023 gnomAD v4.0 was released, being nearly 5x larger than previous versions v2 and v3. The new 4.0 release provides added global diversity since it includes around 138 000 individuals of non-European ancestry.

Methods: We utilized the newest gnomAD v4.0 to calculate the GCF for gnomAD v4.0 populations. The analysis was performed for OMIM autosomal recessive genes utilizing ClinVar P/LP and

Conflicting variants with > 80% P and/or LP classifications. The gnomAD v4.0 data is available for download separately as exomes and genomes. We combined these data to calculate the combined GCF and at-risk couple rates (ACR) per population.

Results: The number of genes with GCF ≥ 1/200 varied greatly per population, whereas the at-risk couple rates were more similar between populations, Ashkenazi Jewish having the highest ACR of 6.16%.

Conclusion: This work highlights the effects of increased samples sizes in gnomAD v4.0 on GCFs.

Grants: This work was supported by Helsinki University Hospital Diagnostic Center Research fund.

Conflict of Interest: None declared

P02.017.C Success rate of mosaic embryos in ART cycles

Jose A. Ortiz[1;1], Ruth Morales[1], Belen Lledó[1], Alba Cascales[1], Adoracion Rodriguez-Arnedo[2], Andrea Bernabeu[3], Rafael Bernabeu[3]

[1]Instituto Bernabeu, Molecular Biology and Genetics, Alicante; [2]Instituto Bernabeu, Reproductive Biology, Alicante; [3]Instituto Bernabeu, Reproductive Medicine, Alicante

Background/Objectives: After embryo biopsy and PGT-A analysis, embryos can be classified as euploid, aneuploid or mosaic in which normal and chromosomally altered cells are combined. The transfer of mosaic embryos has been controversial, but currently the recommendation of international scientific societies is to transfer them as they have been shown to result in healthy children and the risk of congenital anomaly is similar to spontaneous pregnancies. The aim of the study is to analyse the clinical outcomes of mosaic embryos compared to euploid embryos.

Methods: Observational and retrospective study. A total of 9385 embryos from 2899 PGT-A cycles were included (January-2017 to October-2023). The trophectoderm biopsies were analysed by NGS. Clinical data from euploid and mosaic embryos were compared using binary-logistic regression (Rv.4.3.1).

Results: The analysis of embryos through PGT-A revealed that 54.17% were aneuploid, and 9.97% showed mosaicism. A total of 2166 embryos were transferred, of which 274 showed mosaicisms. No differences were observed in the clinical outcomes between euploid and mosaic groups. The pregnancy, biochemical and clinical-pregnancy loss and ongoing-pregnancy rates were 54.3 vs. 51.8, 9.0 vs. 8.8, 14.0 vs. 11.2 and 38.8 vs. 37.9 percent, respectively. Furthermore, when data are analysed according to the percentage or type of mosaicism (segmental vs. whole chromosome) or the number of chromosomes involved, no differences in clinical outcomes are observed.

Conclusion: Mosaic embryos have a high implantation potential, comparable to that of euploid embryos. Non-transfer of these embryos would reduce the chances of successful ART.

Grants: Not applicable.

Conflict of Interest: None declared

P02.018.D Possible exacerbation of semen phenotype in infertile men by an additive contribution of 2 genetic factors

Sandra Kleiman[1;2], Noga Weizman[1], Shimi Barda[1], Ofer Lehavi[1], Eli Arama[3], Shmuel Pietrokovski[3], moran gershoni[4]

[1]Tel Aviv Sourasky Medical Center; [2]Sackler Faculty of Medicine, Tel Aviv University; [3]Weizmann Institute of Science; [4]Institute of Animal Science, ARO-The Volcani Center

Background:

Patients with **c**omplete microdeletions of the **az**oospermia **f**actor-**c** region (c-AZFc) present with severe oligozoospermia (sO) or azoospermia. c-AZFc azoospermic men have a 75% chance of locating sperm cells in testicular extraction but have poorer ICSI-related outcomes than oligozoospermic men.

Objectives: To assess the involvement of additive genetic factors in the phenotype heterogeneity of c-AZFc patients.

Methods: Whole exome sequence analysis on three pairs of related male patients, Sanger sequencing, RFLP screening, scRNA-seq data analysis, microsatellites analysis, Y-chromosome micro-deletions, and karyotyping.

Results: In two brothers from a consanguineous family, one was azoospermic with c-AZFc microdeletion, and the other presented sO with no microdeletion; we identified a novel and likely pathogenic homozygous rare variant in a gene previously annotated in sO in humans and mice. The variant was absent in our cohort of fertile and infertile men.

The second pair was of a father and his son from a consanguineous family. The father has sO, and his son is azoospermic with a c-AZFc microdeletion and testicular maturation arrest. Six candidate pathogenic variants (CPV) are currently under assessment.

Lastly, a CPV in a testis exclusively expressed gene was detected in two remotely related infertile men. One was sO, and the second was azoospermic with c-AZFc microdeletion. In our cohort, this allele was detected only in infertile men in a heterozygote state.

Conclusion: An additive effect of two genetic factors on the semen phenotype is demonstrated for the first time. Additional CPV-causing sO that are likely exacerbated by c-AZFc microdeletion are currently being assessed.

Grants: ISF-2844/21

Conflict of Interest: None declared

P02.019.A Whole-genome sequencing for infertile couples: Moscow experience

Madina Kaplanova[1], Aleksandra Galaktionova[1], Alexander Potapov[1], Olesya Sagaidak[1], Ekaterina Kuznetsova[1], Maxim Belenikin[1], Anton Olenev[2]

[1]LLC Evogen, Moscow, Russian Federation; [2]Moscow State Budgetary Institution of Healthcare "G. M. Savelevoi City Clinical Hospital No.31", Moscow, Russian Federation

Background/Objectives: Infertility is defined by the failure to achieve a pregnancy after 1 year or more of regular unprotected sexual intercourse, it is affects an estimated 15% of couples globally. Whole-genome sequencing (WGS) may identify previously unknown infertility's causes and could be an effective method of identifying genetic variants causing reproductive loss.

Methods: WGS was performed for 192 infertile couples (384 persons: 192 males and 192 females) at Evogen lab using DNBseq-T7/G400 (MGI, China). The sequencing data were bioinformatically processed and interpreted by experts, the genetic variants were validated using Sanger sequencing.

Results: According to the results of sequencing 49 (12,8%) samples 71 genetic variants were defined, which could be considered as a cause of reproductive losses, including: 18 (25,4%) pathogenic variants, 9 (12,7%) likely pathogenic variants, 39 (54,9%) variants of uncertain significance and 5 (7,0%) structural anomalies. Among 192 males with diagnosis of infertility 20 (10,4%) variants were identified associated with spermatogenesis disorder. Among 192 females diagnosis of infertility 29 (15,1 %) variants were identified associated with pregnancy loss. Thus, among infertility couples 200 (10,4%) males and 29 (15,1%) females were found to have variants that were the probable cause of reproductive failure.

Conclusion: Pathogenic, likely pathogenic and variants of uncertain significance that could have been the cause of

reproductive failures were identified by WGS. These results helped couples and doctors to plan future pregnancies with the use of assisted reproductive technology.

Grants: The work was supported by the Moscow Department of Health grant.

Conflict of Interest: None declared

P02.020.B Analysis of mitochondrial DNA variant segregation - a tool for implementing Preimplantation Genetic Testing for mitochondrial DNA-related disorders at the blastocyst stage

Paula Rubens[1;2], *Kalliopi Chatzovoulou*[1;2], *Anne Mayeur*[3;4], *Nadine Gigarel*[5], *sophie monnot*[5], *Agnès Rötig*[1;2], *nelly achour*[3;4], *Julie Steffann*[1;2;5]

[1]*Imagine Institute, Genetics of mitochondrial diseases, Paris, France;* [2]*Université Paris Cité, Paris, France;* [3]*Antoine Béclère Hospital, Reproductive Biology Unit CECOS, Clamart, France;* [4]*Université Paris-Saclay, Paris, France;* [5]*Necker Sick Children's Hospital, Genomic Medicine Unit, Paris, France*

Background/Objectives: Mitochondrial DNA (mtDNA) related diseases are severe, maternally inherited disorders. Their clinical heterogeneity is attributed to the coexistence of mutant and wild-type mtDNA molecules in the same cell, called heteroplasmy. Preimplantation genetic testing for mtDNA disorders (PGTmt) is challenging because it requires that the mutant load remain stable among the different cells and during development. Because data is scarce concerning mtDNA segregation at the blastocyst stage, we aimed to assess the reliability of trophectodem (TE) biopsy for PGTmt, by comparing it to the traditional blastomere results.

Methods: Among the 43 embryos studied, 28 carried pathogenic mtDNA variants (m.8344A>G n = 12, m.3243A>G n = 8, m.8993T>G n = 4, m.8993T>C n = 2, m.13094T>C n = 2) and 15 had mtDNA polymorphism (HV2). Heteroplasmy levels were determined by semiquantitative fluorescent PCR as described. Approval was granted by the French Biomedical Agency.

Results: A strong correlation was found between heteroplasmy levels of: i) blastomere and TE biopsies for both mutant load ($R^2 = 0.93$, $p < 0.0001$,$n = 14$) and the prevalent HV2 allele ($R^2 = 0.91$, $p < 0.0001$,$n = 20$); ii) TE biopsies and the rest of the blastocyst ($R^2 = 0.97$, $p < 0.0001$,$n = 12$), and iii) TE and inner cell mass samples ($R^2 = 0.93$, $p < 0.0004$,$n = 7$). A variation of up to 18% of the HV2 heteroplasmy level was observed among TE single-cells (n = 38) isolated from 6 embryos (mean SD = 2.6).

Conclusion: The strong correlation of mtDNA heteroplasmy levels between cleavage and blastocyst stage contributes to a potential shift to the desirably less invasive trophectoderm biopsy but must be considered with caution due to variability found among single TE cells.

Grants: Funding by AFM and EUR G.E.N.E.(#ANR-17-EURE-0013)

Conflict of Interest: None declared

P02.021.C Large copy number variants in infertile men

Triin Kikas[1], **Rain Inno**[1], *Avirup Dutta*[1], *Kristjan Pomm*[2], *Margus Punab*[2], *Maris Laan*[1]

[1]*Institute of Biomedicine and Translational Medicine, Chair of Human Genetics, Tartu, Estonia;* [2]*Tartu University Hospital, Andrology Clinic, Tartu, Estonia*

Background/Objectives: Spermatogenic failure (SPGF) affects ~10% of men. Our microarray based study in 215 SPGF patients identified a two-fold higher representation of > 1 Mb copy number variants (CNVs) in cases compared to fertile controls (13% vs 6.5%) (Kikas et al Sci Rep 2023). This study aimed to exploit whole exome (WES) data to map large CNVs and their clinical consequence in infertile men.

Methods: The study group comprised of 428 idiopathic azoo- or oligozoospermia patients recruited to the Estonian Andrology (ESTAND) cohort. WES was generated for DNA extracted from the whole blood using the FIMM NGS Sequencing service (Helsinki, Finland). Primary sequencing analysis and CNV calling were performed using the Illumina DRAGEN Bio-IT Platform (v3.9 and v3.10, GRCh38 assembly). CNV dataset was filtered for deletions or duplications > 500kb and a frequency of <1% in our study sample. All large CNVs were evaluated using VEP v105 to determine the likelihood of their pathogenicity. Prioritized CNVs were externally experimentally validated by arrayCGH or whole-genome sequencing (WGS).

Results: In total, 83 large CNVs with high confidence were identified in 64 patients. CNVs linked to known microdeletion and microduplication syndromes were identified in nine previously undiagnosed individuals (2.1%). Additional six large CNVs (0.5-2.5 Mb) included several haplo- and triplosensitive genes, some of which are implicated in genetic conditions and/or testicular function.

Conclusion: Among severe SPGF patients, 15% carried >500 kb CNVs. Clinically valid microdeletions or -duplications (15/428, 3.5%) were overrepresented compared to the general population.

Grants: Estonian Research Council PRG1021

Conflict of Interest: None declared

P02.022.D Reverse phenotyping informing variant pathogenicity in reproductive carrier screening

Cara Beck[1], *Alison Archibald*[1;2;3], *Edwin Kirk*[4;5;6], *Nigel Laing*[7;8;9], *Martin Delatycki*[1;3;10]

[1]*Victorian Clinical Genetics Services, Parkville, Australia;* [2]*Murdoch Children's Research Institute, Parkville, Australia;* [3]*Department of Paediatrics, The University of Melbourne, Parkville, Australia;* [4]*School of Women's and Children's Health, University of New South Wales, Randwick, Australia;* [5]*NSW Health Pathology Randwick Genomics Laboratory, Randwick, Australia;* [6]*Centre for Clinical Genetics, Sydney Children's Hospital, Randwick, Australia;* [7]*Department of Diagnostic Genomics, PathWest Laboratory Medicine, Nedlands, Australia;* [8]*Centre for Medical Research, University of Western Australia, Nedlands, Australia;* [9]*Harry Perkins Institute of Medical Research, Nedlands, Australia;* [10]*Bruce Lefroy Centre, Murdoch Children's Research Institute, Parkville, Australia*

Consortium: The Mackenzie's Mission Study Team

Background/Objectives: A particular challenge of variant classification in reproductive carrier screening is the need to assess variants in the absence of a proband with a phenotype. Familial segregation can provide additional evidence for or against pathogenicity, particularly for X-linked variants. The Australian Reproductive Genetic Carrier Screening Project (Mackenzie's Mission) screened 9107 couples for >1300 genes that underlie ~750 serious childhood onset conditions. We report on three X-linked variants, initially classified as Likely Pathogenic, that were found to segregate in unaffected males.

Methods: Mackenzie's Mission methods and gene selection processes have been described previously. When a likely pathogenic variant was identified in an X-linked gene, genetic counselling included a discussion around testing male family members. If a male was identified with the variant, phenotypic assessment, including biomarker analysis where possible, was undertaken.

Results: Likely Pathogenic X-linked variants *IDS*, *COL4A5* and *F8* were identified in multiple couples. Males in the families were tested and some were found to carry the variant without a

significant phenotype. The male carrying the *IDS* variant had reduced iduronate-2-sulphatase levels with no clinical evidence of Hunter syndrome. Biomarker evaluation of males who carried the *COL4A5* and *F8* variants was normal, therefore ruling out Alport syndrome and haemophilia respectively.

Conclusion: Segregation and reverse phenotyping provided additional information, and in some cases, reproductive reassurance to the couples. This approach will become more important as increased numbers of genes are included in expanded reproductive carrier screening.

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P02.023.A Polymorphisms of *ACE* and thrombophilic genes: risk for recurrent pregnancy loss

Olivera Miljanovic[1;2], *Vesna Ilic*[3], *Dragan Likic*[4], *Sladjana Teofilov*[1], *Bojana Cikota-Aleksić*[5], *Zvonko Magic*[6], *Jelena Jovanovic*[1]

[1]*Clinical Centre of Montenegro, Center for Medical Genetic and Immunology, Podgorica, Montenegro;* [2]*University of Montenegro, Faculty of Medicine, Podgorica, Montenegro;* [3]*Military Medical Academy, Institute of Medical Research, Belgrade, Serbia;* [4]*Institute for Public Health of Montenegro, Podgorica, Montenegro;* [5]*Military Medical Academy, Beograd, Serbia, Center of Clinical Pharmacology, Belgrade, Serbia;* [6]*Serbian Medical Society, Academy of Medical Sciences, Belgrade, Serbia*

Background/Objectives: The etiology of recurrent pregnancy loss (RPL) remains unclear in about 50% of cases. Polymorphisms in genes affecting coagulation and fibrinolysis have been postulated as a risk factor for RPL. We report the association between *SERPINE1* and *ACE* gene polymorphisms, and RPL in Montenegrin women.

Methods: Five gene polymorphisms, *F2* 20210G>A (rs1799963), *F5* 1691G>A (rs6025), *MTHFR* 677C>T (rs1801133), *SERPINE1* −675 4G/5G (rs1799762) and *ACE* I/D (rs1799752) were investigated in total of 224 women: 129 women with ≥2 early RPL or ≥1 late pregnancy loss, and 95 women with at least two normal life births and no history of pregnancy loss. Gene polymorphisms were genotyped by PCR-based methods.

Results: *SERPINE1* 4G/4G and *ACE* D/D gene polymorphisms are found significantly more prevalent among women with RPL ($p < 0.001$ both, OR 2.91 and 3.02, respectively). No association was found between *F2* 20210G>A, *F5* 1691G>A and *MTHFR* 677C>T polymorphisms and risk for RPL. A combination of *SERPINE1* 4G/4G + *ACE* D/D homozygotes was highly associated with RPL (Cochran-Armitage test, $p < 0.001$), and their strong independent association with RPL was confirmed by logistic regression analysis (both p values <0.001, OR 3.35 and 3.43, respectively).

Conclusion: This study demonstrated that impaired fibrinolysis, caused by the hypofibrinolytic polymorphisms in the *SERPINE*1 and *ACE* genes, may be a significant risk for RPL. The findings may add to the data on hereditary thrombophilia role in the pathogenesis of RPL and may address whether *SERPINE1* and *ACE* gene polymorphisms should be included in the diagnostic work-up of women with RPL.

PMID: 37977651

Conflict of Interest: None declared

P02.024.B Maternal whole blood transcriptomic signatures across 4 time points during pregnancy in 1,300 women

Sophie Hoffman[1], *Katie L Burnham*[1], *Emma Cook*[2], *D. Steve Charnock-Jones*[2;3], *Gordon Smith*[2;3], *Emma Davenport*[1]

[1]*Wellcome Sanger Institute, Hinxton, United Kingdom;* [2]*University of Cambridge, Department of Obstetrics and Gynaecology, Cambridge, United Kingdom;* [3]*University of Cambridge, Centre for Trophoblast Research, Department of Physiology, Development and Neuroscience, Cambridge, United Kingdom*

Background/Objectives: Pregnancy is a unique condition involving physiological and immunological changes in women throughout the three trimesters. Previously, transcriptomic studies performed on small cohorts have identified genes that are differentially expressed throughout pregnancy. However, lack of comprehensive descriptions of normal change in the maternal immune system during pregnancy precludes the identification and interpretation of abnormal changes and the subsequent development of measures to mitigate or prevent pregnancy complications.

Methods: In the Pregnancy Outcome Prediction Study (POPS2), we collected whole blood from pregnant women for genotyping ($n = 1,086$) and bulk RNA-seq ($n = 1,308$) at up to 4 time points (12, 20, 28 and 36 weeks). The resulting 3,781 RNA-seq samples make this the largest such cohort to date, with 887 individuals having ≥ 3 RNA-seq samples.

Results: Dream identifies differentially expressed genes (DEGs) between time points, both recapitulating findings from previous pregnancy transcriptome studies and introducing novel DEGs. DEG quantification (table) highlights time points between which the maternal immune system changes the most (12 vs. 36 weeks, $n = 3947$) and least (20 vs. 28 weeks, $n = 605$). CIBERSORTx analysis reveals imputed immune cell proportions vary by individual and time point.

Conclusion: Characterisation of normal variation in the maternal immune system during pregnancy will ultimately enable the identification and interpretation of biomarkers predictive of complications. Future work will incorporate genotyping data to identify regulatory variants contributing to transcriptomic variation in pregnancy.

Number of DEGs between timepoints	20wks.	28wks.	36wks.
12wks.	787	1875	3947
20wks.		605	2317
28wks.			654

Conflict of Interest: None declared

P02.025.C Contribution of *HLA-G* 14bp insertion/deletion polymorphism in the genesis recurrent pregnancy loss

Kateryna Sosnina[1], *Danuta Zastavna*[1], *Oresta Terpyliak*[1], *Bohdan Tretiak*[1]

[1]*State Institution «Institute of Hereditary Pathology of the National Academy of Medical Sciences of Ukraine», Diagnostics of hereditary pathology, Lviv, Ukraine*

Background/Objectives: HLA-G is a non-classical human leukocyte antigen expressed primarily in fetal tissues at the maternal–fetal interface and be involved in interactions that are critical in establishing and maintaining pregnancy. The specific polymorphisms found in coding and regulatory parts of *HLA-G* gene and associated with its different expression, might be important for pregnancy prolongation. In this study, we focused our attention on polymorphism insertion/deletion 14 base pairs at 3`UTR region of *HLA-G* gene, since it is known that this polymorphism directly affects the level of gene expression. The **aim** of our study was to analyze of the distribution of *HLA-G 14bp insertion/deletion* polymorphism among the woman with recurrent pregnancy loss (RPL) and in spontaneously aborted embryos.

Methods: DNA extraction from peripheral blood cells and chorionic villi, PCR, gel electrophoresis.

Results: Genotyping of *HLA-G 14bp insertion/deletion* polymorphism was performed in 86 women with RPL, 220 spontaneously aborted embryos and 55 control. The significantly higher *HLA-G 14bp insertion/insertion* genotype frequency was established in women with RPL and in spontaneously aborted embryos ($P < 0.05$) compared to the control. Calculation of odds ratio showed that the presence *14 bp insertion/insertion genotype* of the *HLA-G* gene in the embryo or in the woman increases the risk of recurrent pregnancy loss more than twice (OR = 2.34 and 2.65).

Conclusion: Considering the obtained results regarding the association of the homozygous genotype of 14 bp insertion of the *HLA-G* gene, we anticipate a specific role of the non-classical antigen HLA-G in the prolongation of human pregnancy.

Grants:

Conflict of Interest: None declared

P02.026.D Decoding the genetic links between endometriosis and its comorbidities: implications for disease risk and aetiology

Isabelle McGrath[1], *Grant W. Montgomery*[1], *Sally Mortlock*[1]

[1]*Institute for Molecular Bioscience, Saint Lucia, Australia*

Background/Objectives: Endometriosis is a poorly understood gynaecological disease with prolonged diagnostic delays, invasive diagnosis and ineffective treatment. The disease has diverse symptoms and there are multiple comorbidities. We aim to comprehensively characterise the comorbidities of endometriosis.

Methods: Comorbidities were identified by correlation with both endometriosis diagnosis and polygenic risk scores (PRS-PheWAS) in 5,432 endometriosis cases and 92,344 controls from the UK Biobank. Shared genetic architecture was explored with genetic correlation and causality investigated with Mendelian randomisation. The interplay between endometriosis PRS and comorbidities on endometriosis risk was examined.

Results: Endometriosis diagnosis correlated with 292 ICD10 codes, spanning gynaecological, immune, pain, psychiatric, gastrointestinal and urinary systems. PRS-PheWAS in males underscored the importance of female-specific pathways in the comorbid relationships. Most comorbidities could be attributed to shared genetics, with one novel causal effect discovered for testosterone levels. While cases harboured more comorbidities than controls, their comorbidity burden inversely correlated with PRS, indicating the contribution of comorbidities in addition to genetics for diagnosis. Furthermore, the impact of certain traits on endometriosis risk varied by endometriosis PRS, emphasising the nuanced relationship between comorbidities and genetic predisposition.

Conclusion: Endometriosis shares a genetic architecture with conditions across diverse biological systems. Despite limited evidence of individual traits causing endometriosis, overlapping biological pathways and the cumulative burden of conditions influence endometriosis risk. These findings offer valuable insights into the endometriosis aetiology and hold clinical implications for risk ascertainment.

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Conflict of Interest: None declared

P02.027.A Endometrial Fluid: a promising liquid biopsy to search for (epi)genomic-based diagnostic biomarkers of gynecological diseases

Bárbara P. González-García[1;2], *Aintzane Rabanal*[1;2;3], *Olaia Farto-Juaristi*[1], *Sofia g. Castresana*[1], *Elena Urquijo*[3], *Alba Hernangomez-Laderas*[1;2], *Ariadna Cilleros-Portet*[1;2], *Sergi Marí*[1;2], *Nora Fernandez-Jimenez*[1;2], *Amaia Irizar*[1;4;5], *Santiago Díez*[2;3], *Roberto Matorras*[1;2;3], *Jose Ramon Bilbao*[1;2;6], **Iraia García-Santisteban**[1;2]

[1]*University of the Basque Country (UPV/EHU), Leioa, Spain;* [2]*Biobizkaia Health Research Institute, Barakaldo, Spain;* [3]*Cruces University Hospital, Barakaldo, Spain;* [4]*Biogipuzkoa Health Research Institute, Donostia, Spain;* [5]*Center for Biomedical Research in Epidemiology and Public Health Network (CIBERESP), Madrid, Spain;* [6]*Center for Biomedical Research in Diabetes and Associated Metabolic Disorders (CIBERDEM), Madrid, Spain*

Background/Objectives: Gynecological diseases are posing a great burden on healthcare systems, due, in part, to the lack of minimally invasive diagnostic biomarkers. Endometrial fluid, a type of liquid biopsy that can be obtained in routine gynecological examinations, has proven promising for biomarker identification. Considering that part of the susceptibility to complex gynecological diseases is mediated by genetic variants that regulate DNA methylation (methylation Quantitative Trait *Loci*, mQTL), here we aim to identify (epi)genomic diagnostic biomarkers in endometrial fluid.

Methods: Endometrial fluid was collected from more than 200 women of reproductive age, together with more than 30 clinical parameters. DNA was isolated in order to obtain genotype and methylation data, using the Illumina GSA and EPIC arrays, respectively. After quality control (QC), (epi)genomics data were combined with TensorQTL to calculate endometrial fluid mQTLs. Finally, Summary data-based Mendelian Randomization (SMR) was used to integrate the identified mQTLs and public summary statistics of GWAS from several gynecological disease.

Results: In the first batch of DNA isolated from endometrial fluid, 103 samples passed genotyping and methylation QC, and were used to calculate 564,734 mQTL on endometrial fluid ($p < 5 \times 10^{-8}$). Integration of mQTL and GWAS of endometriosis, uterine leiomyoma, and ovarian, endometrial and cervical cancers, pinpointed several CpG sites that might be mediating disease etiology, and could serve as diagnostic biomarkers upon validation in independent case-control cohorts.

Conclusion: The combination of endometrial fluid mQTL with GWAS data enabled the identification of potential diagnostic biomarkers for gynecological disorders.

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Conflict of Interest: None declared

P02.028.B M1AP in meiotic recombination: genotype-specific differences in meiotic arrest

Nadja Rotte[1], Jessica Dunleavy[2], Michelle Diane Runkel[1], D. Fietz[3], Adrian Pilatz[4], Johanna Kuß[1], Dicke Ann-Kristin[1], Sofia Boeg Winge[5], Sara di Persio[6], Christian Ruckert[7], Verena Nordhoff[6], Hans-Christian Schuppe[4], Kristian Almstrup[5;8], Sabine Kliesch[6], Nina Neuhaus[6], Birgit Stallmeyer[1], Moira O'Bryan[2], Frank Tüttelmann[1], **Corinna Friedrich**[1]

[1]*Institute of Reproductive Genetics, University of Münster, Münster, Germany;* [2]*School of BioSciences and Bio21 Molecular Sciences and Biotechnology Institute, Faculty of Science, Melbourne, Australia;* [3]*Institute of Veterinary Anatomy, Histology and Embryology, University of Gießen, Gießen, Germany;* [4]*Clinic and Polyclinic for Urology, Paediatric Urology and Andrology, University Hospital Gießen, Gießen, Germany;* [5]*Department of Growth and Reproduction, University Hospital Copenhagen, Copenhagen, Denmark;* [6]*Centre of Reproductive Medicine and Andrology (CeRA), University of Münster, Münster, Germany;* [7]*Department of Medical Genetics, University Hospital Münster, Münster, Germany;* [8]*Department of Cellular and Molecular Medicine, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark*

Background/Objectives: Meiosis is an indispensable process for generating spermatozoa. The meiosis-specific protein M1AP interacts with the ZZS (Zip2-Zip4-Spo16) proteins SHOC1-TEX11-SPO16 in mice, which are essential for meiotic recombination, especially crossover formation. *M1AP*, *TEX11*, and *SHOC1* represent validated disease genes for male infertility. However, little is known about the interplay of M1AP and ZZS in humans.

Methods: Infertile men with rare (MAF <0.01), biallelic/hemizygous loss-of-function (LoF) variants in *M1AP*, *SHOC1*, *TEX11*, and *SPO16* were queried from exome/genome data from the Male Reproductive Genomics (MERGE) study. Testicular phenotypes were determined by histological staining of marker proteins for different meiotic sub-stages. Using immunofluorescence staining of spermatocyte spreads, the impact on meiotic recombination in affected men was analysed.

Results: We identified the first homozygous LoF variant in *SPO16* in a man with complete meiotic arrest (MeiA). Men with assumed ZZS deficiency shared an early MeiA (*SHOC1* N = 3, *TEX11* N = 7, *SPO16* N = 1). In contrast, in men with *M1AP* LoF variants, haploid round (10 men) or elongated (6 men) spermatids were concordantly detected in testicular sections. Asynapsis of homologous chromosomes and unrepaired DNA double-strand breaks were specifically observed when the ZZS complex was disturbed, while impaired M1AP lead to decreased crossovers. Medically-assisted reproduction in a man homozygous for a *M1AP* LoF variant led to the birth of a healthy child.

Conclusion: While the ZZS proteins seem mandatory for meiotic recombination, it remains possible despite loss of M1AP. Future studies will elucidate the consequences for the offspring.

Grants: This work was supported by the DFG Clinical Research Unit 326 'Male Germ Cells'.

Conflict of Interest: None declared

P02.029.C Children conceived using assisted reproductive technologies compared to spontaneous conception show sex differences in DNA methylation

Dana Kristjansson[1;2], *Yunsung Lee[1], Christian Magnus Page[1;3], Maria Christine Magnus[1], Astanand Jugessur[1], Robert Lyle[4], Siri Håberg[1]*

[1]*Norwegian Institute of Public Health, Center of Fertility and Health;* [2]*Norwegian Institute of Public Health, Department of Genetics and Bioinformatics;* [3]*Norwegian Institute of Public Health, Department of Physical Health and Ageing, Center of Fertility and Health;* [4]*Oslo University Hospital*

Background/Objectives: Previous studies have indicated differences in cord blood DNA methylation patterns according to conception by assisted reproductive technology (ART). Furthermore, studies have also shown sex differences in birth outcomes of infants conceived by ART. However, the extent and specific underpinnings of these differences require further exploration through comprehensive research.

Methods: We investigated sex differences in cord blood DNA methylation using the Illumina EPIC platform among 456 ART-conceived versus 507 naturally conceived girls and 503 ART-conceived and 473 naturally conceived boys.

Results: We found widespread differences in DNA methylation levels in ART-conceived newborns compared to those conceived naturally. Notably, we identified 37 differentially methylated CpGs for ART-conceived girls (FDR < 0.01), and 70 differentially methylated CpGs for ART-conceived boys, when compared to same sex naturally-conceived counterparts. Within these, ten CpGs were differentially methylated among the ART-conceived for both sexes.

Conclusions: These sex-specific epigenetic distinctions underscore the nuanced impact of ART on the fetal epigenome and prompt further exploration into the potential implications for developmental trajectories and health outcomes in ART-conceived individuals.

Grants: Norwegian Cancer Association, project number 244291, and Research Council of Norway through its Centres of Excellence funding scheme, project number 262700

Conflict of Interest: None declared

P02.030.D Celiac disease genetic markers in men as a genetic component of idiopathic infertility

Oresta Terpyliak[1], *Danuta Zastavna[1], Kateryna Sosnina[1]*

[1]*State Institution «Institute of Hereditary Pathology of the National Academy of Medical Sciences of Ukraine», Diagnostics of hereditary pathology, Lviv, Ukraine*

Background/Objectives: Celiac disease (CD) is an autoimmune disease triggered by dietary gluten and has been associated with several conditions influencing female and male reproduction. CD genetic components are *HLA-II* class genes known as *HLA-DQ2* and *DQ8*. The aim of study was to investigate distribution of *HLA-DQ2.5* (*HLA-DQA1*05:01* + *HLA-DQB1*02*) and *HLA-DQ8* (*HLA-DQB1*03:02*) genotypes among men from couples with idiopathic infertility (CII)

Methods: SSP-PCR for distribution of *HLA-DQ2.5* and *HLA-DQ8* genotypes. Pooled odds ratio (OR) and prevalence, with 95% confidence intervals were calculated.

Results: We did not establish a significant difference in the frequency of high (*DQ2.5* + *DQ8*) and low risk (*DQ8*) celiac genotypes (P > 0.05) in men from the CII group compared to the control group. As for the medium-high risk genotype (*DQ2.5*), statistical calculations showed a significant (P < 0.01) increase of the *DQ2.5*-genotype frequency in this group compared to the control, and the OR proves 5 times increases of idiopathic infertility risk. We did not establish a significant difference in the

presence of celiac markers in men with CII compared to women from the same couples, which may indicate the equal contribution of men with the celiac genotype as a possible cause of idiopathic infertility.

Conclusion: An increased risk of idiopathic infertility is associated with men carrying *DQ2.5*, a genotype predisposing to CD ($P < 0.01$). The OR proves 5 times increases of idiopathic infertility risk in the presence of the *DQ2.5* genotype in men. Our results indicate the equal contribution both men with celiac genotype and women as a possible cause of idiopathic infertility.

Grants:

Conflict of Interest: None declared

P02.031.A The effect of VitD on testicular tissue gene expression in metabolic syndrome induced endoplasmic reticulum stress

MERYEM SARIKAYA[1;2], *Betül Zorkaya*[2], *Tugce Ozbilenler*[3], *Ayse Evrim Bayrak*[1], *Fatma Kaya Dagistanli*[4], *Bilge Ozsait Selcuk*[1]

[1]*Istanbul Faculty of Medicine, Istanbul University, Medical Genetics, Istanbul, Türkyie;* [2]*Institute of Health Science, Istanbul University, Istanbul, Türkyie;* [3]*Institute of Graduate Studies, Istanbul University-Cerrahpasa, Istanbul, Türkyie;* [4]*Cerrahpasa Faculty of Medicine, Istanbul University-Cerrahpasa, Medical Biology, Istanbul, Türkyie*

Background/Objectives: Metabolic syndrome (MetS) is one of the prominent risk factors leading to cardiovascular diseases. In recent studies, MetS was associated with testicular dysfunction and male infertility. MetS induced endoplasmic reticulum stress (ERS) and cell death was also reported in testicular tissue. This study aims to investigate the possible effect of Vitamin D (VitD) on MetS induced ERS in testicular tissue.

Methods: A Sprague-Dawley rat MetS model was formed by a special diet. The study groups were consisted of control group ($n = 9$), VitD administrated (170 IU/week, orally) group (VitD; $n = 8$), MetS group ($n = 9$), and MetS+VitD group ($n = 8$). After 15 weeks, testicular tissues of the sacrificed rats were dissected. The gene expressions of ERS pathway *related PERK, IRE1, ATF6, ATF4, GRP78* and *CHOP*; VitD related *VDR* and *ERp57*; and male fertility related aromatase (*CYP19A1*) were analyzed qRT-PCR. Immunohistochemical analyses were performed for GRP78 and CHOP proteins. The statistical significance was analyzed by Student-T test and one-way ANOVA.

Results: The gene expressions of *ERP57* ($p = 0.004$) and *GRP78* ($p = 0.005$) was almost two fold higher in MetS than MetS+VitD group. *VDR* and *CYP19A1* expression was higher in MetS+VitD than MetS group ($p = 0.024$ and $p = 0.018$, respectively). The protein levels of GRP78 ($p < 0.0001$) and CHOP ($p < 0.0001$) was increased in MetS group when compared to other study groups.

Conclusion: The findings of this study suggest that MetS induced ERS pathway play an important role in testicular tissue and can be regulated by VitD.

Grants: This study was supported by the Scientific Research Projects Coordination Unit of Istanbul University (Project No: TYL-2022-39216).

Conflict of Interest: None declared

P02.032.B Gender peculiarity of the material of reproductive losses

Iryna Tkach[1;2], *Nataliya Huleyuk*[1], *Danuta Zastavna*[1], *Thomas Liehr*[3], *Halyna Bezkorovaina*[1], *Oksana Lototska-Savchak*[1], *Olga Benko*[4]

[1]*State Institution Institute of Hereditary Pathology of the National Academy of Medical Sciences of Ukraine, Lviv, Ukraine;* [2]*Scientific Medical Genetic Center "LeoGENE", Lviv, Ukraine;* [3]*Institute of Human Genetics, Jena University Hospital, Jena, Germany, Jena, Germany;* [4]*Municipal non-profit enterprise Third Lviv territorial medical association, department of gynecology, Lviv, Ukraine*

Background/Objectives: The prognosis of the reproductive function of women with pregnancy loss is complex and partly based on the results of karyotyping of material of reproductive loss. According to the literature, during conception are formed the same number of male and female fetuses, but more boys are born. The purpose of this study was to evaluate the gender ratio and incidence of chromosomal anomalies in the products of conception (POC).

Methods: Banding cytogenetic and interphase mFISH with the centromeric probe panel for chromosomes 13, 14, 15, 16, 17, 18, 21, 22, X and Y were used.

Results: Were examined 2554 cases of material POC. Among all samples of POC, female karyotype was established in 1330 cases (52.1%), and male karyotype in 1224 cases (47.9%). Gender ratio of female to male in the POC, regardless of presence or absence of chromosomal anomalies (CA), is 1.09:1. Normal karyotype was established in 1638 cases (64.2%), abnormal karyotype in 916 (35.9%). Among the samples of POC with a normal karyotype, the female gender was determined in 801 cases (48.9%), and the male gender in 837 cases (51.1%). In samples with an abnormal karyotype, the female gender was established in 529 cases (57.8%), and the male gender in 387 cases (42.2%). The prevalence of female in POC with CA is due to monosomy X(18.4% of all pathology)

Conclusion: Depending on presence or absence of chromosomal anomalies in POC, the gender ratio differs: with a normal karyotype, female-to-male ratio is 1:1.05, and with an abnormal karyotype, it is 1.37:1 respectively.

Grants:

Conflict of Interest: None declared

P02.035.A Role of MUTYH gene variants in the etiology of recurrent pregnancy loss

Ceren Gezik[1;2], *Durmuş Durmaz*[1], *Cagri Gulec*[1], *Güven Toksoy*[1], *Ezgi Gizem Berkay*[3], *Seher Basaran*[1], *Bilge Ozsait Selcuk*[1]

[1]*Istanbul Faculty of Medicine, Istanbul University, Department of Medical Genetics, Istanbul, Türkyie;* [2]*Istanbul University Institute of Health Sciences, Department of Genetics, Istanbul, Türkyie;* [3]*Istanbul Kent University Taksim Campus, Department of Medical Biology and Genetics, Istanbul, Türkyie*

Background/Objectives: Recurrent pregnancy loss (RPL), is defined as the loss of two or more pregnancies before 24 weeks of gestation that affects 1-2% of pregnancies worldwide. The cause of RPL cannot be explained in about half of the cases. Recent studies suggest that mitochondrial dyshomeostasis and oxidative stress may play a role in RPL. The mutY DNA glycosylase *(MUTYH)* is a novel candidate gene in RPL involved in repair of oxidative stress induced DNA damage. This study aimed to investigate the association of *MUTYH* gene variants and RPL.

Methods: Variant analysis was performed on whole exome sequencing data from 10 couples and an additional 25 female patients examined due to the RPL in the first trimester.

Results: All patients had normal karyotypes and had no other causing factor related to infertility. In three female patients (8%), heterozygous pathogenic missense (rs143353451 and rs374950566) and nonsense (rs121908380) variants were detected in *MUTYH* gene. Protein modeling of these variants was performed by UniProt automated modeling and showed that mutated

MUTYH residues are located within conserved motifs at the surface of the molecule.

Conclusion: Findings of this study suggest that pathogenic variants of *MUTYH* gene might be crucial in the trigger of oxidative stress in human placenta and thus contribute to RPL etiology. Additive interaction of other heterozygous variants in RPL related genes should be explored and further functional studies should be conducted to explain the role of these variants in female infertility.

Grants: This study was supported by Turkish Health Institutes Presidency(No:28416) and Istanbul University Scientific Research Projects Unit(No:TYL-2022-39278).

Conflict of Interest: None declared

P02.036.B The molecular riddle of Endometriosis (EM) disorder: filling in the blanks between genetics, diet, and microbiome.

Aurora Santin[1], *Beatrice Spedicati*[1;2], *Anna Morgan*[2], *Stefania Lenarduzzi*[2], *Paola Tesolin*[2], *Alessandro Pecori*[2], *Giuseppe Giovanni Nardone*[1], *Silvia Camarda*[1], *Harry Stevens*[1], *Giulia Pianigiani*[2], *Lara Emily Rosso*[1], *Cristina Bon*[2], *Daniela Mazzà*[2], *Elena Vinerbi*[3], *Giovanni Di Lorenzo*[2], *Federico Romano*[2], *Francesca Buonomo*[2], *Alessandro Mangogna*[2], *Giuseppina Campisciano*[2], *Gabriella Zito*[2], *Serena Sanna*[3;4], *Maria Pina Concas*[2], *Giuseppe Ricci*[1;2], *Giorgia Girotto*[1;2]

[1]*University of Trieste, Department of Medicine, Surgery and Health Sciences, Trieste, Italy;* [2]*Institute for Maternal and Child Health – I.R.C.C.S. "Burlo Garofolo", Trieste, Italy;* [3]*Institute of Genetic and Biomedical Research (IRGB), National Research Council (CNR), Monserrato, Italy;* [4]*University Medical Center Groningen, Department of Genetics, Groningen, Netherlands*

Background/Objectives: EM is a multifactorial gynaecological disease, constituting the leading cause of infertility in affected patients. Little is known about EM aetiopathogenesis; however, genetics, diet and dysbiosis are involved in determining EM onset.

Methods: One-hundred and ten EM patients were enrolled at IRCCS "Burlo Garofolo" (Italy). Patients underwent a careful clinical examination; data regarding EM-symptoms severity, food preferences, and vaginal/rectal microbiome were collected. Whole-Exome Sequencing (WES) was conducted to identify rare damaging variants within a list of 47 EM-associated genes and new candidates. Regression models to investigate relationships between EM-symptoms, food preferences, microbiome, and logistic models to deepen EM-risk factors employing a control cohort (Women4Health) were performed.

Results: WES analysis detected >80 rare predicted damaging heterozygous variants within 26 genes, involved in cellular proliferation and immune response. A significant association between high-FODMAP food preference and dysmenorrhea severity ($p = 0.038$) was detected, in line with the known role of high-FODMAP foods in determining pain and inflammation (PMID:36424920). Moreover, gluten-containing foods preference was identified as an EM-risk factor ($p = 0.042$); indeed, 12 months of gluten-free diet have been proven to relieve EM-symptoms (PMID:23334113). Preliminary microbiome data confirmed vaginal and rectal dysbiosis; further, EM stage I-II was associated with an increased rectal colonization of *Prevotella* bacterium and dysmenorrhea.

Conclusion: This study provided novel glimpses into the role of genetics in EM development, the relation between EM-symptoms and food preferences, dysbiosis and EM-symptoms, thus supporting the hypothesis that genetics, diet, and microbiome act together in EM. Further analyses will deepen the relationships between all these causal factors.

Grants:

Conflict of Interest: None declared

P02.037.C Medicover Carrier Screening according to current guidelines

Melanie Isau[1], *Konstanze Hörtnagel*[2], *Elisabeth Gödde*[3], *Markus Stumm*[3]

[1]*Medicover Humangenetik Berlin-Lichtenberg MVZ, Molecularge-netics, Berlin, Germany;* [2]*Medicover MVZ Martinsried GmbH, Humangenetics, Martinsried, Germany;* [3]*Medicover Humangenetik Berlin-Mitte, Humangenetics, Berlin, Germany*

Background:

Carrier screening provides prospective parents information about their carrier status for autosomal recessive and X-linked conditions. If couples prove to be carriers, they have a 1 in 4 risk in each pregnancy of having a child with an autosomal recessive condition. For X-linked recessive conditions, there is a 50% risk for male offspring to be affected if the woman is found to be a carrier. It is estimated that approximately 1 in 100/125 couples have a risk of having a child affected with a serious recessive condition [1].

Methods: Based on ACMG guideline we established a tiered approach with which CS can be offered to all couples planning a pregnancy [2]. Our strategy supports a multi-sample CS workflow and is able to compare variants between samples and identify genes in which clinically relevant variants are present in both partners. We generate a combined report for the couple that includes reproductive risk.

Results: CS is a valuable tool in genetics and reproductive medicine that helps families to make informed choices about family planning and reduce the risk of passing autosomal recessive or X-linked genetic disorders to their children.

Conclusion: Since NGS offers an affordable, high-throughput solution, CS has become common practice in healthcare systems. The standardization of the screening approach will facilitate testing consistency and further it will be important that ACMG reevaluates the genes listed for screening and considers the need to modify criteria used to include and exclude genes.

Grants:

[1] Fridman H et al. 2021
[2] Gregg AR et al. 2021

Conflict of Interest: None declared

P02.038.D Insights in the carrier screening for *CFTR* gene in the field of reproductive medicine

Andrea Domingo Gallego[1], **Sara Reyes**[1], *Lydia Madrid Cortés*[1], *Marta Carreño*[1], *Anna Borgia*[1], *Patricia Karrera*[1], *Paula Coleto*[1], *Raquel Muñoz Siles*[1], *Ester Olivé*[1], *Raquel Garcia*[1], *Lidia Carreño*[1], *Maria Segura-Puimedon*[1], *LLuís Armengol*[1]

[1]*qGenomics, Dry Lab, Esplugues de Llobregat*

Background/Objectives: Pathogenic variants in the *CFTR* gene are causative of classic cystic fibrosis (CF), as well as *CFTR*–related disorders. In 2023, the ACMG recommended a minimal set of 100 disease-causing variants to be studied. However, owing to the great diversity of variants in the *CFTR* gene, the effective detection of carriers with this approach is limited.

Methods: Observational retrospective study in 20,853 samples from gamete donors and patients undergoing assisted reproduction treatments referred to our laboratory from 2020 to 2023 for carrier screening using a NGS panel.

Results: Our data indicate that 27% (5,563/20,853) of the individuals were carriers of a disease-causing variant in the *CFTR* gene, with 143 different pathogenic variants detected. 73% of the

variants (105/143) were not included in the list proposed by ACMG. The top five most frequently identified were c.1210-34TG(12)T(5) (n = 648), p.Gly576Ala(;)Arg668Cys (n = 487), p.Phe508del (n = 385), p.Leu997Phe (n = 358) and p.Val754Met (n = 135). Only the p.Phe508del is considered to be causative of classic CF by CFTR2 database. A coincidence of variants in the *CFTR* gene has been identified in 6% (56/972) of the couples, of whom 57% (32/56) had an increased reproductive risk of having offspring with CF or *CFTR*-related disease.

Conclusion: This study provides data on the application of carrier screening of the *CFTR* gene in the field of assisted reproduction and highlights the relevance of performing broader NGS studies on all patients with reproductive desire.

Grants: not applicable.

Conflict of Interest: None declared

P02.039.A GDF-15: A promising biomarker for identifying mitochondrial dysfunction in insulin resistance, polycystic ovary syndrome, and associated infertility

Vera Várhegyi[1;2], *Anna Módos*[1;2], *Domonkos Trager*[2], *Eszter Mária Horváth*[3], *Miklós Sipos*[1], *Maria Judit Molnár*[2], *Szabolcs Várbíró*[1;4], *Aniko Gal*[2]

[1]*Semmelweis University, Department of Obstetrics and Gynecology, Budapest, Hungary;* [2]*Semmelweis University, Institute of Genomic Medicine and Rare Disorders, Budapest, Hungary;* [3]*Semmelweis University, Department of Physiology, Budapest, Hungary;* [4]*University of Szeged, Albert Szent-Györgyi Health Centre, Department of Obstetrics and Gynecology, Szeged*

Background/Objectives: Recent research indicates that mitochondrial dysfunction plays a significant role in the underlying mechanisms of insulin resistance, polycystic ovary syndrome (PCOS), and associated infertility. GDF-15 (growth differentiation factor 15) is a stress-induced cytokine, with elevated plasma levels linked to mitochondrial dysfunction and various pathologies. This study aimed to investigate the role of GDF-15 as a biomarker in diagnosing IR and PCOS.

Methods: 81 female patients (mean age 35.43 ± 7.86 years) diagnosed with IR or PCOS and undergoing infertility treatment, displaying clinical features suggestive of mitochondrial dysfunction, were included. DNA isolation was performed from blood and urine epithelial cells. GDF-15 levels were measured using an ELISA kit, while mitochondrial DNA (mtDNA) deletions were assessed via long-range PCR.

Results: GDF-15 levels in the study cohort were slightly elevated compared to healthy controls (1213.58 vs. 818.68; Reference <2000). Abnormal GDF-15 levels were observed in 12 patients, with 11 having BMI values exceeding 25. Nine patients were undergoing metformin therapy. MtDNA deletion was confirmed in 8 cases (66.6%).

Conclusion: Elevated levels of GDF-15 were detected in 14.8% of patients, with 91.6% of this subgroup having a high BMI. MtDNA deletion was identified in 58% of cases, with 75% of them also undergoing metformin therapy. The degree of carbohydrate metabolism disturbance was more pronounced in high GDF-15 cases than in normal GDF-15 patients. These findings suggest that GDF-15 may be a predictive biomarker of abnormal metabolic processes in the background of IR, PCOS, and associated infertility.

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OTKA-2021 grants.

Conflict of Interest: None declared

P02.040.B Set of structural rearrangements of chromosome Y and their impact on male fertility

Lucie Liskova[1], *Vjaceslav Harmas*[1], *Vlasta Cejnova*[1], *Lenka Vancová*[1], *Lilly Dejová*[1], *Monika Stará*[2], *Marek Broul*[2;3], *Jana Lastuvkova*[1]

[1]*Krajská zdravotní, a.s. - Masaryk Hospital in Ústí nad Labem, o.z., Department of Medical Genetics, Ústí nad Labem, Czech Republic;* [2]*Krajská zdravotní, a.s. - Masaryk Hospital in Ústí nad Labem, o.z., Department of Sexology, Ústí nad Labem, Czech Republic;* [3]*Jan Evangelista Purkyně University, Faculty of Health Studies, Ústí nad Labem, Czech Republic*

Background/Objectives: Chromosome Y plays an important role in process of fertilization and development for males because it holds the SRY gene determining sex and numerous spermatogenesis genes, e.g. DAZ, AZF1/2. Various abnormalities can affect the Y chromosome; these are divided into numerical or structural abnormalities. Around 6% of infertile males have structural Y rearrangements (duplication, deletion, inversions, dicentrics, rings), in males with azoospermia, this number rises to 15% (Jiang, 2017).

Methods: Molecular diagnosis of Y-chromosomal microdeletions, microarray, karyotyping, FISH.

Results: We present 7 cases with structural rearrangements of chromosome Y detected by routine karyotyping and FISH analysis, further characterized by molecular diagnosis of Y microdeletion, and by microarray in some cases. We determinated three cases of partial deletion of Yq chromosome, mostly prenatally, when fertility of the male was not interrupted and the deleted Y chromosome was transmitted from father to the son. Two cases of isodicentric chromosomes were revealed in mosaic forms 45,X/46,X,idic(Y); the first in normal male with azoospermia, the second prenatally in amniotic fluid. One case of isochromosome i(Y)(p10) in nonmosaic form was detected in azoospermic male. The last case represents an inversion of Y(p11.2q11.2) in male with OAT syndrome.

Conclusion: The impact of genotype on male fertility is discussed, and depends on Y chromosome breakpoints, deleted genes and degree of 45,X line mosaicism. Cytogenetic analysis and molecular characterization of infertile men is very important to clarify a possible cause of infertility, and to establish the suitable subsequent medical therapy.

Conflict of Interest: Lucie Liskova: None declared, Vjaceslav Harmas: None declared, Vlasta Cejnova: None declared, Lenka Vancová full time, Lilly Dejová: None declared, Monika Stará: None declared, Marek Broul: None declared, Jana Lastuvkova: None declared

P02.041.C Cumulus cells DNA damage assessed by alkaline comet assay reveals distinct oocyte maturation stages

Vanessa Sousa[1;2;3;4], *Bárbara Rodrigues*[1;2;3;4], *Emídio Vale-Fernandes*[2;3;5], *Daniela Sousa*[5], *Raquel Brandão*[5], *Carla Leal*[5], *Isabel Gaivão*[6], *Filipa Esteves*[3;7;8], *Solange Costa*[3;7;8], *Joana Pires*[3;7;8], *João Paulo Teixeira*[3;7;8], *António J A Nogueira*[9], *Paula Jorge*[1;2;3]

[1]*Laboratório de Genética Molecular, Serviço de Genética Laboratorial, Clínica de Genética e Patologia, Unidade Local de Saúde de Santo António (ULSSA), Porto, Portugal;* [2]*UMIB - Unit for Multidisciplinary Research in Biomedicine, ICBAS - School of Medicine and Biomedical Sciences, University of Porto, Porto, Portugal;* [3]*ITR - Laboratory for Integrative and Translational Research in Population Health, Porto, Portugal;* [4]*equal contributors;* [5]*Centre for Medically Assisted Procreation/ Public Gamete Bank, Gynaecology Department, Centro Materno-Infantil do Norte Dr. Albino Aroso (CMIN), Unidade Local de Saúde de Santo António (ULSSA), Porto, Portugal;* [6]*CECAV - Veterinary and Animal Research Centre and Department of Genetics and Biotechnology, University of Trás-os-Montes and Alto Douro*

(UTAD), Vila Real, Portugal; [7]Environmental Health Department, National Institute of Health Dr Ricardo Jorge, Porto, Portugal; [8]EPIUnit - Instituto de Saúde Pública, Universidade do Porto, Porto, Portugal; [9]CESAM - Center for Environmental and Marine Studies, Department of Biology, University of Aveiro, Aveiro, Portugal

Background/Objectives: Cumulus cells (CCs) surround the oocyte and play an important role in oocyte development and maturation. CCs DNA damage has been associated with oocyte status; however, the existing studies present contradictory results. Considering the physical proximity to the oocyte, the aim of this study is to understand if CCs DNA damage, may serve as a biomarker for oocyte quality.

Methods: The alkaline comet assay was conducted on CCs obtained from 42 females undergoing intracytoplasmic sperm injection (ICSI). Among these, 20 exhibited ovulatory dysfunctions, while 22 were experiencing male-factor-related fertility issues. ICSI is an assisted reproductive technology which involves the injection of a single sperm into the cytoplasm of the oocyte. Prior to injection, the oocyte undergoes denudation, and CCs are usually rejected. Several reproductive parameters collected in the infertility context were evaluated.

Results: CCs DNA damage levels were significantly higher in females with ovulatory dysfunction when compared to those experiencing male-factor-related infertility ($p = 0.034$). A significant correlation was found between CCs DNA damage levels and the number of oocytes in 2PN stage ($p = 0.026$). Furthermore, females with a fertilization percentage above 50% exhibited increased levels of CCs DNA damage, greater number of oocytes in 2PN stage and a higher number of embryos obtained when compared to females with lower fertilization.

Conclusion: Overall, our findings led us to propose that higher CCs DNA damage may reflect the complete oocyte maturation (nuclear and cytoplasmatic), indicating an ideal stage for sperm reception, fecundation and, consequently, increased ICSI success.

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Conflict of Interest: None declared

P02.042.D Variant curation of the largest compendium of FOXL2 coding and non-coding sequence and structural variants to improve precision medicine in BPES

Charlotte Matton[1;2], *Julie Van De Velde[2], Miriam Bauwens[1;2], Eva D'haene[1;2], Sally Hooghe[2], Dave Bunyan[3;4], Hannah Verdin[2], Elfride De Baere[1;2]*

[1]Ghent University, Biomolecular medicine, Ghent, Belgium; [2]Ghent University Hospital, Center for Medical Genetics Ghent, Ghent, Belgium; [3]University of Southampton, Faculty of Medicine, Southampton, United Kingdom; [4]Salisbury District Hospital, Wessex Regional Genetics Laboratory, Wiltshire, United Kingdom

Background/Objectives: Monoallelic *FOXL2* coding and non-coding sequence and structural variants (SVs) lead to BPES (OMIM 10100), a rare, autosomal dominant developmental disorder characterized by a completely penetrant eyelid malformation and incompletely penetrant primary ovarian insufficiency (POI). As most variants have not been collected and classified systematically, we aimed to curate all reported and in-house constitutional *FOXL2* variants.

Methods: Here, we collected variants from our in-house database, generated via clinical genetic testing and downstream research testing in the Center for Medical Genetics Ghent, Belgium (2001-2024), and via literature and other resources in the same period. All retrieved variants were categorized using ACMG/AMP classifications to increase the knowledge of pathogenicity.

Results: We collected 405 unique genetic defects of the *FOXL2* region in 876 index patients. Of these, 88% are coding *FOXL2* variants, 52 of which are novel. The polyalanine tract is a known mutational hotspot of *FOXL2*, illustrated by the high percentage of pathogenic polyalanine expansions (27%). Furthermore, the molecular spectrum is characterized by 9% coding copy number variants (CNVs) and 2% non-coding CNVs, all but one located upstream of *FOXL2*. The remaining 1% are translocations along with chromosomal rearrangements of 3q23. In total, 98.4% variants were categorized as (Likely) Pathogenic, whereas 1.6% variants were categorized as Variant of Unknown Significance. All variants and corresponding classification will be uploaded in LOVD.

Conclusion: This uniform and structured reclassification, incorporating the largest dataset of variants implicated in *FOXL2*-associated disease so far, will improve both the diagnosis as well as genetic counselling for individuals with BPES.

Grants: FWO/11Q3D24N, FWO/1802220N

Conflict of Interest: None declared

P02.044.B Maternal genetic risk factors for spontaneous preterm birth: a systematic review and meta-analysis

Tea Mladenić[1], *ANITA BARIŠIĆ[2], Nina Pereza[1], Saša Ostojić[1], Borut Peterlin[3], Sanja Dević Pavlić[1]*

[1]Faculty of Medicine, University of Rijeka, Medial Biology and Genetics, Rijeka, Croatia; [2]University Hospital Rijeka, Gynecology and Obstetrics, Rijeka, Croatia; [3]Ljubljana, Clinical Institute of Medical Genetics, Ljubljana, Slovenia

Background/Objectives: A spontaneous preterm birth (sPTB) is any live birth completed before 37 weeks of gestation caused by the spontaneous onset of preterm labor. As there is ample evidence that the maternal genome plays a role in the occurrence of sPTB, we conducted a comprehensive systematic review of studies analysing the association between maternal genetic variants and sPTB using either a hypothesis-based or a hypothesis-free approach.

Methods: A literature search was conducted in PubMed, Scopus and PHGKB databases on the association between maternal genetic variants and sPTB. All included studies were categorised into two categories: hypothesis-based and hypothesis-free.

Results: The systematic review included 81 studies, of which 72 used a hypothesis-based approach and 9 used a hypothesis-free approach. Using additional criteria for hypothesis-based studies, a meta-analysis of 34 studies was performed for 133 variants in 49 genes. A significant association between the maternal genetic variant and sPTB was only found in the TNF-α (rs1800629) gene for both genotype ($P = 0.025$) and allele ($P = 0.038$). Moreover, in the hypothesis-free approach, 7 variants in 5 genes (EBF1, EESEC, HSPA1L, ASTN1, MAST1) reached global significance ($P < 5 \times 10\text{-}8$).

Conclusion: Despite numerous studies using both approaches, no specific genes or variants were clearly associated with the risk of sPTB. Due to the large differences in the definition of sPTB and the selection criteria for participants, standardization is required. Therefore, future genetic association studies should use a more precise description of subject groups and outcomes measured.

Grants: None.

Conflict of Interest: None declared

P02.045.C Characterization of somatic variants in placentas from pregnancies complicated by preeclampsia and other adverse outcomes

Gordon Ye[1], *Nathaniel Anderson*[1], *Thomas Oliver*[1;2], *Omar El-Tokhy*[1], *D. Steve Charnock-Jones*[3;4], *Gordon Smith*[2;3;4], *Sam Behjati*[1;2;5]

[1]*Wellcome Sanger Institute, Hinxton, United Kingdom;* [2]*Cambridge University Hospitals, United Kingdom;* [3]*NIHR Cambridge Biomedical Research Centre, Department of Obstetrics and Gynaecology, United Kingdom;* [4]*Centre for Trophoblast Research, Department of Physiology, Development and Neuroscience, Cambridge, United Kingdom;* [5]*University of Cambridge, Department of Paediatrics, United Kingdom*

Background/Objectives: The placenta is implicated in many adverse pregnancy outcomes. Past work has characterized the high mutation burden of normal placental tissue while identifying preliminary evidence of genomic signatures unique to those in pregnancies that experienced adverse outcomes including preeclampsia.

Methods: The Pregnancy Outcome Predicting Study was a prospective cohort study enrolling nulliparous women with ultrasound scans performed at the Rosie Hospital between January 2008 and July 2012. This work focuses on 150 pregnancies with maternal blood samples, clinical data, and placenta and cord tissue collected following birth. Samples underwent whole-genome sequencing and data were sorted into following groups based on clinical data: 1) healthy control; 2) preeclampsia 3) preeclampsia with other adverse pregnancy outcomes; and 4) other adverse pregnancy outcomes. Following sequencing quality-control, data from 453 placental samples and 149 cord samples (representing 149 pregnancies) were included for analysis. Somatic single nucleotide variants (SNV), copy number variants, structural variants, and insertion-deletion variants were called by CaVEMan, ASCAT/Battenberg, BRASS, and Pindel, respectively. Umbilical cord tissue served as the matched normal for our placental samples at the level of each pregnancy.

Results: Preliminary results suggest SNV burden does not differ substantially across our clinical groups, while tandem-duplications were observed more frequently in placentas from pregnancies experiencing preeclampsia (28.6% of preeclampsia cases vs. 13.6% of healthy controls).

Conclusion: We observe a potential relationship between structural variants and preeclampsia, particularly an enrichment of tandem duplications.

Grants: Gordon Ye: The Winston Churchill Foundation of the United States. The POPS cohort was supported by the NIHR Cambridge Biomedical Research Centre.

Conflict of Interest: None declared

P02.046.D Use of array-CGH and NGS in etiological definition of idiopathic premature ovarian insufficiency: our recent experience in a sample of Italian women

Gaia Visani[1], *Silvia Kalantari*[1], *Laura Cucinella*[2], *Elena Rossi*[1;3], *Elisa Giorgio*[1], *Enza Maria Valente*[1;4], *Rossella Nappi*[2;5], *Fabio Sirchia*[1;6]

[1]*University of Pavia, Department of Molecular Medicine, Pavia, Italy;* [2]*IRCCS Policlinico San Matteo Foundation, Research Center for Reproductive Medicine and Gynecological Endocrinology - Menopause Unit, Pavia, Italy;* [3]*IRCCS Mondino Foundation, Neurogenetics Research Center, Pavia, Italy;* [4]*IRCCS Mondino Foundation, Medical Genetics Unit, Pavia, Italy;* [5]*University of Pavia, Department of Clinical, Surgical, Diagnostic and Pediatric Sciences, Pavia, Italy;* [6]*IRCCS Policlinico San Matteo Foundation, Department of Diagnostic Medicine, Medical Genetics Unit, Pavia, Italy*

Background/Objectives: Premature ovarian insufficiency (POI) has a strong genetic component, but etiology remains often unidentified. Historically, karyotype and *FMR1* analyses were the mainstay and sole genetic tests performed for POI patients. However, it was recently demonstrated that copy number (CNVs) and single nucleotide variants (SNVs) can contribute to the genetic landscape of the disease. Our objective is to perform array-CGH and Whole exome sequencing (WES) in women that had negative baseline genetic testing.

Methods: We included 63 women referred to the Menopause Clinic at our University Hospital for diagnosis and treatment of idiopathic POI during the last three years.

Results: The mean age at POI diagnosis in the cohort was 26.9 years (range 11-40 years), with 15 patients (22.7%) reporting primary amenorrhea. All women had normal karyotype, and *FMR1* gene testing resulted normal in all patients with secondary amenorrhea.

Array-CGH and WES identified a genetic cause of POI in 8 patients overall.

In particular, a genetic cause of POI was identified through Array-CGH and WES in 3 and 5 patients, respectively. Variants of unknown significance (VUS) were identified in 3 patients.

Conclusion: A genetic cause of POI was found thanks to Array-CGH and WES in 17.4% of our patients, playing a crucial role in the diagnosis of POI and providing relevant prognostic information to affected individuals and their families.

Grants:

Conflict of Interest: None declared

P02.047.A Analysis of human sperm DNA after cryopreservation

Sofia Coelho[1;2;3;4], *Carolina Almeida*[1], *Margarida Fonseca Cardoso*[3;5], *Sofia Lobo Xavier*[6], *Alberto Barros*[1;4;7], *Mário Sousa*[2;8], *Joana Marques*[1;4]

[1]*FMUP - Faculdade de Medicina, Universidade do Porto, Genetics Unit, Department of Patology, Porto, Portugal;* [2]*UMIB-Unit for Multidisciplinary Research in Biomedicine / ITR-Laboratory for Integrative and Translational Research in Population Health, Porto, Portugal;* [3]*ICBAS-Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Laboratory of Cell Biology, Department of Microscopy, Porto, Portugal;* [4]*RISE-Health, Porto, Portugal;* [5]*CIIMAR - Centro Interdisciplinar de Investigação Marinha e Ambiental, Universidade do Porto, Porto, Portugal;* [6]*Centro de Responsabilidade Integrada de Medicina da Reprodução, Unidade Local de Saúde de São João, Porto, Portugal;* [7]*Centro de Genética da Reprodução Professor Alberto Barros, Porto, Portugal;* [8]*ICBAS-Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Porto, Portugal*

Background/Objectives: Cryopreservation of human spermatozoa is widely used for fertility preservation, being generally considered a safe technique. However, a detailed analysis of DNA fragmentation and epigenetic modifications after cryopreservation is lacking.

Methods: Human spermatozoa samples (11) were collected from normozoospermic (NZ) individuals, after written informed consent, and were analysed before and after cryopreservation during 1 month. The TUNEL technique was used to evaluate sperm DNA fragmentation (SDF) and bisulphite-sequencing to analyse sperm DNA methylation (SDM) at two imprinted regions.

Results: Results evidenced a small but significant increase in SDF after sperm cryopreservation in 36.4% of the samples. The remaining 63.6% samples showed no significant changes. Regarding SDM, results showed no relevant changes in the methylation of the imprinted regions in either *H19* or *MEST/PEG1* genes. Correct imprinting marks, with no changes in the expected normal *H19* gene hypermethylation and *MEST* gene hypomethylation, were observed.

Conclusion: The present data gives important information regarding ensuring the safety of sperm cryopreservation in relation to imprinting marks. Especially because it was previously shown that imprinting errors occur in sperm from infertile patients, and reported an increased risk of imprinting syndromes, such as Beckwith-Wiedemann and Silver-Russell syndromes, in children born after assisted reproductive treatments. Although SDF was increased in a small fraction of samples, cryopreservation did not result in altered SDM patterns. To confirm these results, we are analysing longer periods of cryopreservation, with a higher number of NZ and non-NZ individuals.

Grants: FCT – Fundação para a Ciência e Tecnologia (2021.08252.BD, CEECIND/0037/2017 2022.09829.PTDC) (UIDB/00215/2020; UIDP/00215/2020;LA/P/0064/2020).

Conflict of Interest: None declared

P02.048.B A large deletion of the androgen receptor (AR) gene in a patient with androgen insensitivity syndrome.

Leona Morožin Pohovski[1], *Adriana Bobinec*[1], *Ana-Maria Meašić*[1], *Ljubica Odak*[1,2]

[1]*Children's Hospital Zagreb, University of Zagreb, School of Medicine, Zagreb, Department of Medical and Laboratory Genetics, Endocrinology and Diabetology with daily care unit, Zagreb, Croatia;* [2]*European Reference Network –Intellectual disability, Tele Health, Autism and Congenital Anomalies*

Background/Objectives: Different genomic alterations affecting the androgen receptor gene (AR) located on chromosme Xq11-12 are associated with Androgen insensitivity syndrome (AIS). The AR is an important steroid hormone receptor that plays a critical role in male sexual differentiation, and development of secondary male characteristics. The most prominent molecular defects in the AR gene associated with AIS are missense variants. A eighteen-year-old girl was referred to our genetics clinic due to primary amenorrhea, absent uterus and ovaries, short and blind-ending vagina and presence of testis tissue in right ingvinal canal. External genitalia showed sparse pubic hair and normal breast development.

Methods: Chromosome analyses were performed by conventional highresolution cytogenetic technology. Clinical exome sequencing (CES) was performed using TruSightOne sequencing kit and NextSeq machine (Illumina, USA). Data analysis was carried out with BaseSpace Variant Interpreter (Illumina, USA) and curated online databases. Comparative genomic hybridization (CMA) was conducted using 8×60K oligonucleotide array (Agilent, Santa Clara, CA, USA).

Results: Chromosome analysis showed 46,XY karyotype. CES did not reveal any variants in genes known to be associated with proband's phenotype. Nevertheless, analysis in Integrative Genome Viewer (Broad Institute) showed a partial deletion of AR gene. Array-CGH revealed a 96 kb partial deletion of the AR gene, encompassing exons 2-8/8 (NM_001011645).

Conclusion: To our knowledge, this is the first patient described in the literature with large deletion in AG gene. The presented case emphasizes the use of molecular cytogenetics as an additional diagnostic tool for the detection of rare intragenic abnormalities.

Grants: None.

Conflict of Interest: None declared

P02.049.C Novel *STARD9* gene mutations identified in a patient with 46,XY gonadal dysgenesis, osteoporosis, and heart deficiency lend support to this gene as a syndromic DSD candidate

Dmytro Sirokha[1], *Alexey Rayevsky*[1], *Vitalii Kalynovskyi*[2], *Oksana Samson*[3], *Olexandra Gorodna*[1], *Jadwiga Jaruzelska*[4], *Kamila Kusz-Zamelczyk*[4], *Serge Nef*[5], *Ludmila Livshits*[1]

[1]*Institute of Molecular Biology and Genetics, National Academy of Sciences of Ukraine, Laboratory of Human Genomics;* [2]*Taras Shevchenko Kyiv National University, Kyiv, Ukraine;* [3]*Shupyk National Healthcare University of Ukraine, Department of Pediatric Endocrine Pathology of the Komisarenko Institute of Endocrinology and Metabolism, Endocrine Department;* [4]*Institute of Human Genetics, Polish Academy of Sciences, Poznan, Poland;* [5]*University of Geneva, Department of Genetic Medicine and Development, Faculty of Medicine, Geneva, Switzerland*

Objectives: Investigating gene mutations that affect gonad development is crucial for understanding the mechanisms behind Differences/Disorders in Sex Development (DSD). This research aims to identify novel DSD genetic variants using whole exome sequencing (WES) and genotype-phenotype correlation analysis.

Methods: WES was performed on a 46,XY SRY-positive patient with gonadal dysgenesis, osteoporosis, and heart deficiency. Bioinformatic analysis and simulation of mutant protein were conducted.

Results: Two different mutations in a compound heterozygous state were identified in the *STARD9* gene: NM_020759.3 c.5585_5590del (p.Ser1862_Thr1863del) (rs528276071) – an in-frame deletion (MAF = 0.0019); combined with NM_020759.3 c.3514 C>T (p.Arg1172Cys) (rs12594837) – a missense mutation (MAF = 0.00837). The patient's genetic background analysis did not reveal any pathogenic variants implicated in the DSD. Detected mutant variants were inherited from healthy parents — heterozygous carriers and were not previously implicated in the pathogenesis of any disease. It was shown that Stard9 is strongly expressed in Sertoli cells, some granulosa cells, late male interstitial progenitors, meiotic female germ cells, endothelium, and immune cells of mice. Bioinformatics analysis showed that STARD9 mutant protein's regions are conserved in primates only and have a disordered structure that probably affects some regulatory patterns responsible for the PPI interactions/interaction with specific interfaces.

Conclusion: Based on the results obtained in this study, previous reports concerning the expression pattern of the *STARD9* gene, and its cellular functions we suppose that *STARD9* can be considered as a candidate gene causing 46,XY syndromal DSD.

Grants: "Molecular-Genetic Mechanisms of Human Sexual Development Disorders" National Academy of Sciences of Ukraine [0121U110054].

Conflict of Interest: None declared

P02.051.A Genomic newborn screening, first clinical experience in Spain

Vincenzo Cirigliano[1;1], *Elena Ordonez*[1], *Bibiana Palao*[2], *Miriam Leon*[2], *Laura Garcia*[1], *Nerea Lobato*[2], *Irene Perea*[2], *Raquel Bernad*[2], *Enrique Fernandez*[2], *Mireia Lechuga*[1], *Isabel Castilla*[1], *Maria Moreno*[2], *Luis Izquierdo*[2]

[1]*Veritas Intercontinental, Barcelona, Spain;* [2]*Veritas Intercontinental, Madrid, Spain*

Background/Objectives: The advances in genomic sequencing and the growing knowledge in variant interpretation is facilitating the implementation of genomic screening in different medical areas. Genomic newborn screening is becoming a priority, with several countries implementing programs to evaluate the cost-effectiveness of integrating this determination. Here we present

the results of the first 800 newborn genomic screening test performed by a Spanish private laboratory.

Methods: Saliva, cord blood or venous blood sample was obtained from the newborn. Extracted genomic DNA is processed by a capture-based assay and sequenced on a next-generation sequencer (illumina). Exome sequencing data was processed using a proprietary bio-pipeline designed for this intended use. Only childhood onset diseases were analyzed.

Results: Around 8.6% of newborns presented variants in any of the genes included on the panel. The variants detected were related to hematological conditions (68.1%), syndromic (5.8%), Neurologic (5.8%), Cancer (5.8%), Musculoskeletal (4.4%), Metabolic (2.9%), Cardiovascular (2.9%), Immunological (1.5%), Endocrine (1.5%) and Urogenital (1.5%). More than 80% of the infants were newborns (0 months), the mean age was 3 month.

Conclusion: Even though the conditions included in the analysis are considered rare, the subset of pathologies tested affected more than 8% of newborns. Early detection lead to an adapted medical management that may involve symptoms prevention or the reduction of the impact of the condition in the child. Besides, rare conditions can take years before being diagnosed, this approach not only enables prevention in most of the cases, but also avoid diagnostic odyssey.

Grants: None significant to the purpose of this research

Conflict of Interest: Vincenzo Cirigliano full time, Elena Ordonez full time, Bibiana Palao full time, Miriam Leon full time, Laura Garcia full time, Nerea Lobato full time, Irene Perea full time, Raquel Bernad full time, Enrique Fernandez full time, Mireia Lechuga full time, Isabel Castilla full time, Maria Moreno full time, Luis Izquierdo full time

P02.056.B Loss-of-function variants in *NXT2* lead to male infertility due to non-obstructive azoospermia

Ann-Kristin Dicke[1], *Claudia Krallmann*[2], *Godfried van der Heijden*[3], *Miguel Xavier*[4], *Joris A. Veltman*[4], *Sabine Kliesch*[2], *Frank Tüttelmann*[1], *Birgit Stallmeyer*[1]

[1]*Institute of Reproductive Genetics, University of Münster, Münster, Germany;* [2]*Centre of Reproductive Medicine and Andrology, University Hospital Münster, Münster, Germany;* [3]*Department of Obstetrics and Gynecology, Radboud University Medical Center, Nijmegen, Netherlands;* [4]*Biosciences Institute, Newcastle University, Newcastle Upon Tyne, United Kingdom*

Background/Objectives: The nuclear export protein NXT1 ubiquitously facilitates the transport of a diverse spectrum of RNAs from the nucleus to the cytoplasm. In humans, the NXT1 paralogue NXT2 is specifically expressed in the testis, but little is known about its function.

Methods: From the Male Reproductive Genomics (MERGE) study, exome/genome data of >2,300 infertile men with severe oligo- or azoospermia was screened for rare (MAF <0.01 in gnomAD) variants in *NXT2*. Prioritised variants were validated and segregation analyses were performed. Reproductive phenotypes of variant carriers were evaluated and effects on subcellular localisation of NXT2 in patients' testicular tissue were determined.

Results: In a family with three infertile brothers, the hemizygous *NXT2* loss-of-function (LoF) variant c.354dup p.(Asp119*) co-segregates with the disease. The identification of a de novo deletion of the entire *NXT2* gene in an independent infertile man supports the association of LoF variants in *NXT2* with male infertility. Histological analysis of two patients' testicular tissue revealed a Sertoli cell-only phenotype (SCO) explaining the azoospermia. In testicular tissue with normal spermatogenesis, NXT2 is predominantly expressed in Sertoli cells according to immunohistochemical staining. In contrast, expression was lost in both *NXT2* variant carriers.

Conclusion: *NXT2* is a novel candidate gene for X-linked non-obstructive azoospermia. The concordant testicular phenotype of SCO points towards a role of NXT2 in germ cell formation or early maturation. Further studies need to clarify the pathomechanism of NXT2-related male infertility.

Grants: This work was supported by the DFG Clinical Research Unit 326 'Male Germ Cells'.

Conflict of Interest: None declared

P02.057.C A novel digitalMLPA carrier screening assay for simultaneous detection of copy number variations and selected point mutations in challenging genomic regions

Romy Kuilboer[1], *Silvia Dyson Coral*[2], *Lisette Stolk*[1]

[1]*MRC Holland, Cytogenetics, Amsterdam, Netherlands;* [2]*MRC Holland, IVD Development, Amsterdam, Netherlands*

Background/Objectives: Carrier screening is crucial to identify those at risk of having children affected by genetic disorders. We present a novel multiplex carrier screening assay, designed for the simultaneous detection of copy number variations (CNVs) and selected point mutations in challenging genomic regions, including *CYP21A2*, *HBA1/HBA2*, *SMN1*, *CFTR*, *DMD*, *HBB*, *CLN3*, *CTNS*, and *GJB2/GJB6*.

Methods: A novel multiplex carrier screening assay is being developed that employs a targeted next-generation sequencing approach and is suitable for high throughput routine use: SALSA® digitalMLPA™ Probemix D028 Carrier Screening. Over 65 positive samples, for which independent confirmation was available, were tested with this assay.

Results: The results demonstrate the efficacy of the assay in accurately identifying aberrations in all targeted regions across a diverse sample set including 31 deletions, 6 duplications, 28 point mutations and 3 cases with both a CNV and a point mutation. Additionally, inclusion of a novel probe type allows the assay to detect not only *CYP21A2* deletions, but also a considerable percentage of the point mutations, showcasing its capability to navigate the challenges presented by complex genomic regions.

Conclusion: The development of this assay represents a significant advancement in addressing the challenges posed by complex genomic regions in carrier screening. By effectively detecting both CNVs and selected point mutations, this robust SALSA® digitalMLPA™ assay can complement sequence analysis for informed reproductive decision-making.

Grants: None.

Conflict of Interest: Romy Kuilboer MRC Holland, Silvia Dyson Coral MRC Holland, Lisette Stolk MRC Holland

P02.058.D 'Everyone is a carrier': A frame and content analysis of preconception expanded carrier screening (ECS) on social media

Kriss Fearon[1], *Cathy Herbrand*[1], *Lucina Wilde*[1], *Nicky Hudson*[1]

[1]*De Montfort University, Centre for Reproduction Research, Leicester, United Kingdom*

Background/Objectives: ECS is a preconception genetic test which informs prospective parents about recessive conditions they might transmit to their future children. Laboratories are increasingly marketing genetic testing through social media (Rose and Wick, 2018), yet no studies have analysed social media content on ECS, which is currently unregulated. Our study aims to

understand how this technology is framed on social media and the messages conveyed.

Methods: A systematic search strategy identified relevant posts across six social media platforms: X/Twitter, Instagram, Facebook, TikTok, Reddit and YouTube. 365 posts were analysed using Frame Analysis (Entman 1993) and inductive content analysis (Vears and Gillam, 2022), focussing on the origin of the post and the framing of ECS's aims, limitations and transmission risks.

Results: Most posters were test providers, fertility clinics, and clinicians with fewer posts from test takers. The content analysis highlighted five major themes: (1) informing about ECS and reproductive risks, (2) promoting ECS, (3) personal experiences of ECS, (4) pro-ECS advocacy, and (5) challenges and limitations of ECS. Commercial providers promoted uptake with frames like 'everyone is a carrier'. Test takers shared experiences and provided advice; they were broadly positive, framing ECS as 'science gives reassurance' and 'reproductive responsibility'.

Conclusion: Social media is used to raise awareness and provide information, as well as encourage wider adoption of ECS. This enables prospective users access to information, but the lack of regulation presents potential challenges for test takers to distinguish what constitutes reliable information.

Grants: Economic and Social Research Council [ES/W012456/1]

Conflict of Interest: None declared

P03

PRENATAL GENETICS

P03.001.A Droplet digital PCR application for rapid aneuploidy and maternal cell contamination testing of invasive prenatal samples

Olga Mikhaylichenko[1], *Endre Hegedus*[2], *Vanessa Felice*[2], *Chenyu Li*[1], *Nathan Hendel*[1], *Eric Hall*[1], *Nyari Dzvova*[1], *Monica Herrera*[1], *Richard Dannebaum*[1], *Severine Margeridon*[1], *Ronald Wapner*[3], *Vaidehi Jobanputra*[2]

[1]*Bio Rad Laboratories;* [2]*New York Genome Center;* [3]*Columbia University Irving Medical Center*

Background/Objectives: Efficient aneuploidy screening and maternal cell contamination (MCC) control are essential in invasive sample analysis workflows, eliminating costs of whole genome sequencing for contaminated or aneuploid samples. This study introduces rapid aneuploidy screening using droplet digital PCR (ddPCR) on genomic DNA (gDNA) from fetal amniocytes or chorionic villi, requiring only 5 ng of gDNA per sample. The method reduces analysis time to 9 hours for a batch of 46 samples, eliminating the need for imaging or capillary electrophoresis. MCC estimation is performed alongside aneuploidy analysis in one test to assess sample quality and aneuploidy status.

Methods: gDNA from various sources underwent extraction using a standard column-based protocol. Multiplexed ddPCR assays targeted chromosomes 18, 13, 21, 3, X, and Y for aneuploidy detection, while the MCC assay focused on differentially methylated genomic regions between placental tissue and maternal cfDNA. Aneuploidy and MCC readouts were compared against QF PCR and NGS results.

Results: Evaluation involved 62 gDNA samples, demonstrating 100% concordance with QF PCR for fetal aneuploidy detection. MCC assay performance showed high reproducibility ($R2 = 0.98$) and correlation with QF PCR readout ($R2 = 0.78$) for amniotic fluid samples, with recommended optimization for chorionic villi.

Conclusion: In conclusion, ddPCR emerges as a fast, low-input, and cost-efficient alternative for rapid aneuploidy screening in invasive prenatal samples. Combining aneuploidy detection with MCC quality control in the same reaction plate using a methylation-based ddPCR approach reduces turnaround time and instrument costs, presenting a promising approach for invasive testing.

Conflict of Interest: Olga Mikhaylichenko: None declared, Endre Hegedus: None declared, Vanessa Felice: None declared, Chenyu Li: None declared, Nathan Hendel: None declared, Eric Hall: None declared, Nyari Dzvova: None declared, Monica Herrera: None declared, Richard Dannebaum: None declared, Severine Margeridon: None declared, Ronald Wapner: None declared, Vaidehi Jobanputra NYGC received instrument, reagents and assays from Bio-Rad Laboratories to conduct experiments described.

P03.002.B Optimized non-invasive prenatal diagnosis (NIPD) of monogenic disorders by mutation exclusion in real-life experience.

Nathalie Couque[1], *Ines Defer*[1], *Alexia Kinoo*[1], *Cédric Vignal*[1], *Hélène Cavé*[1], **severine drunat**[1]

[1]*CHU Robert-Debré, Molecular Genetics Unit, Paris, France*

Background/Objectives: The development of NIPD for monogenic disorders has increased the demand for prenatal diagnosis in families with a de novo mutation in a previous child. Importantly, the amount of circulating fetal DNA (cffDNA) analysed must be sufficient for reliable mutation exclusion, which requires accurate quantification.

Method: NIPD was performed using a variant-specific digital droplet PCR (ddPCR) assay. DNA was extracted from 10 ml plasma. Indel markers (DigitalTRACE®, Bionobis) were used for cffDNA quantification. Analyses were performed in four replicates.

Results: From mid 2022 to 2023, 61 NIPD (10-31 weeks of gestation; median:13.5 weeks) for 46 genes were performed. All assays were validated within a mean of 26 ± 8 days. The mean limit of detection (LOD) was 4.98 ± 0.9 droplet. Linearity assessment showed that for an average variant concentration of 0.85 copies/ul, the mean count of positive droplets was 13.8 ± 2.4. Accordingly, minimum levels for reliable reporting of negative results were defined as 1 copy/ul of cffDNA, i.e. 40 genomic equivalents (GE) of fetal DNA and 50 copies/ul of total DNA (i.e. 500 GE of DNA). The mean cffDNA concentration in each of the four replicate was 1.81 ± 1.32 copies/uL. Most NIPD (59/61) were related to previous occurrences of de novo variants. A positive result was found in 1 case, revealing *KIF1A* germinal mosaicism in an asymptomatic parent

Conclusion: The use of a panel of highly informative paternal indel polymorphic markers allowed accurate quantification of cffDNA. Furthermore, our data highlight the importance of offering NIPD in families with previous 'de novo' mutation

Conflict of Interest: None declared

P03.004.D Using low-coverage non-invasive prenatal screening data for polygenic risk score calculation

Sara Becelaere[1], *Simone Andrea Biagini*[1], *Ludovica Molinaro*[1], *Isabelle Cleynen*[1], *Toomas Kivisild*[1]

[1]*KU Leuven, Department of Human Genetics, Belgium*

Background/Objectives: Non-invasive prenatal screening (NIPS) samples derived from maternal blood contain cell free DNA (cfDNA) from fetus and mother. Investigating the maternal cfDNA provides opportunities for genetic analyses. However, calling genotypes directly from the low-coverage NIPS data is

challenging. We investigated the efficiency of using imputed low-coverage sequencing data from NIPS for polygenic risk score (PRS) calculations for height, preeclampsia and gestational diabetes mellitus (GDM).

Methods: NIPS samples were collected at UZ Leuven and imputed with QUILT. PRS for height were calculated for 2,057 NIPS samples using PRS-CS based on GWAS by Yengo (2022). The PRS for preeclampsia was calculated for 177 cases and 2,049 controls (Honigberg_2023). For GDM, GDM PRS and type-2 diabetes (T2D) PRS were calculated for 555 cases and 1,945 controls (Pervjakova_2022, and Mahajan_2022, respectively).

Results: The phenotypic variance in height explained by the PRS was 0.28, compared to 0.44 estimated from high quality genotype data. The variance in preeclampsia explained by the PRS was 0.029 (OR = 1.51(1.30-1.78);p-value = 2.23e-07) and the model had an area under the receiver operator curve (AUC) of 0.61(0.57-0.65), which is rather low but not unexpected given the low number of cases and the limited GWAS size. The phenotypic variance in GDM explained by the GDM PRS was 0.018 (OR = 1.32(1.19-1.46);p-value = 5.87e-08;AUC = 0.57(0.54-0.60)) and by the T2D PRS was 0.047 (OR = 1.55(1.40-1.71);p-value = 6.89e-18;AUC = 0.60(0.57-0.63)).

Conclusion: Height results indicate that imputed NIPS can be used for estimating PRS with sufficient accuracy. We show that PRS effectiveness in stratifying pregnancy-related complex diseases relies on the underlying genetic architecture and GWAS cohort sizes, expected to improve.

Grants: FWO-SBO/E grant: "MICADO"-project

Conflict of Interest: None declared

P03.005.A Outcome of genetic testing in fetal echogenic bowel

Ruxandra Nagy[1], *Ilse Feenstra*[2], *Dineke Westra*[3]

[1]*Maastricht UMC+, Clinical Genetics, Maastricht, Netherlands;* [2]*Radboudumc, Clinical Genetics, Nijmegen, Netherlands;* [3]*Radboudumc, Genetics, Nijmegen, Netherlands*

Consortium: n/a.

Background/Objectives: Fetal echogenic bowel (FEB) is detected in 0.2-1.8% of pregnancies during the second trimester. FEB is associated with fetal abnormality, including chromosomal anomalies, cystic fibrosis (CF), infection, and intra-amniotic haemorrhage. In the present study, we provide an overview of genetic testing in FEB.

Methods: We performed a retrospective observational study of all pregnancies with FEB referred to our center for genetic counseling between 2017 and 2022. Invasive genetic testing was performed via amniocentesis, including *CFTR* gene analysis, QF-PCR, copy number variant (CNV) analysis and whole exome sequencing. If parents opted out for invasive testing, parental *CFTR* carrier screening and noninvasive prenatal testing (NIPT) (if not previously executed) were performed.

Results: 268 pregnancies were included of which 183 (68.3%) with isolated FEB. NIPT excluded trisomy 13, 18 and 21 in 201 pregnancies (of which 104 isolated FEB). Invasive testing was performed in 49 fetuses [45 *CFTR* analysis, 28 CNV analysis, 11 SNV analysis (all with additional ultrasound findings)]. Parental *CFTR* carrier screening performed in 454 cases detected 27 carriers (5.9%), of which two couples. One fetus (isolated FEB; 0.4% of all pregnancies) had CF (homozygous pathogenic *CFTR* variant).

Conclusion: Genetic testing for FEB revealed a higher risk for CF than in the general Dutch population. This risk was, nonetheless, lower than previously reported in literature. The higher frequency of CF carriers in our cohort may be the result of a selection bias or could potentially be associated with FEB.

Grants: None.

Conflict of Interest: None declared

P03.006.B Diagnostic yield of prenatal genetic testing in pregnancies with cleft lip, alveolus and/or palate on fetal ultrasound

Maria Albers[1], *Marie-José Van den Boogaard*[1], *Judith Ummels*[2], *Aebele Barber Mink van der Molen*[3], *Mireille Bekker*[2], *Maarten Massink*[1], *Guus Lachmeijer*[1]

[1]*University Medical Center Utrecht, Department of Genetics, Utrecht, Netherlands;* [2]*University Medical Center Utrecht, Department of Obstetrics, Utrecht, Netherlands;* [3]*University Medical Center Utrecht, Department of Pediatric Plastic Surgery, Utrecht, Netherlands*

Background/Objectives: Differentiating prenatally between syndromic and non-syndromic cases of cleft lip, alveolus and/or palate (CLA/P) can be difficult since additional anomalies or developmental problems may only be recognizable after birth. A prenatal genetic diagnosis can aid in this distinction and improve prenatal counseling. The purpose of this study was to assess the yield of prenatal genetic testing in a retrospective cohort of fetuses with CLA/P detected by ultrasound imaging.

Methods: Between November 2020 and November 2022, 37 fetuses with CLA/P were prenatally detected by ultrasound, of which 11 with and 26 without additional anomalies. Prenatal genetic testing, including QF-PCR, SNP array or CNV analysis, gene panel testing of genes associated with CLA/P and/or trio whole exome sequencing (WES), was performed in 10 (38,5%) fetuses with CLA/P without additional anomalies and in all fetuses with CLA/P and additional anomalies. The outcomes were evaluated retrospectively.

Results: In 1 fetus (10%) with presumed isolated CLA/P a genetic diagnosis was identified with gene panel testing (Kallman syndrome, FGFR1). In 8 fetuses (72,7%) with CLA/P and additional anomalies a genetic diagnosis was established, of which 1 with QF-PCR (trisomy 13), 1 with chromosomal array (unbalanced translocation), 4 with gene panel testing (Kallman (FGFR1), Hartsfield (FGFR1), Baraitser-Winter (ACTG1) and CHARGE (CHD7) syndrome) and 2 with trio WES analysis (LMNB1-primary microcephaly and Neu-Laxova syndrome (PSPH)).

Conclusion: The results of this retrospective study suggest that prenatal gene panel and open exome testing increase the diagnostic yield in fetuses with cleft lip, alveolus and/or palate, both with or without additional anomalies.

Grants:

Conflict of Interest: None declared

P03.007.C Prenatal Diagnosis of SCN2A-related severe developmental and epileptic encephalopathy 11 by trio exome analysis

Uwe Ahting[1], *Siegel Corinna*[1], *Konrad Platzer*[2], *Johannes Lemke*[2;3], *Sophia Stoecklein*[4], *Christoph Hübener*[5], *Konstanze Hörtnagel*[1]

[1]*Medicover Genetics, MVZ Martinsried GmbH, Martinsried, Germany;* [2]*University of Leipzig Medical Center, Institute of Human Genetics, Leipzig, Germany;* [3]*University of Leipzig Medical Center, Center for Rare Diseases, Leipzig, Germany;* [4]*LMU University Hospital, Department of Radiology, München, Germany;* [5]*LMU University Hospital, Department of Obstetrics and Gynecology, München, Germany*

Background: Prenatal trio exome analysis can meet at least two major challenges: It can help find the correct genetic explanation

of fetal sonographic abnormalities and it can expand the prenatally manifesting phenotypic spectrum of diseases.

Method: Prenatal trio exome analysis.

Result: The fetus presented at 27 weeks of a so far uneventful pregnancy with polyhydramnios, small stomach bubble and fixed joints indicative of fetal arthrogryposis.

Trio exome analysis demonstrated a pathogenic heterozygous de novo missense variant in *SCN2A:* p.(Ala1329Asp). Pathogenic variants in *SCN2A* are associated with autosomal dominant neurologic diseases, ranging from benign familial seizures to severe developmental and epileptic encephalopathy 11 (EIEE11). The spectrum of symptoms primarily includes postnatal manifestations; little is known about prenatal onset. The found variant is not published as pathogenic yet. We classified the variant in accordance with ACMG-criteria to PS1, PM1, PM2, PM5, PP2, PP3 as pathogenic. However, the analog AS-change in evolutionary and functionally comparable SCN1A (Ala1339Asp) is associated with congenital arthrogryposis and perinatal seizures, so we suggested EIEE11 with prenatal onset in this case.

No relevant structural abnormalities of the brain were detected in the fetal MRI. After premature rupture of membranes the child was born at 29 weeks of gestation with fixed limb positions. The newborn showed seizures aggravating into status epilepticus, confirming the diagnosis of EIEE11 showing the severe end of the spectrum of this disease.

Conclusion: This case clearly exemplifies how prenatal trio exome analysis can help find the correct diagnosis and expand the prenatal phenotypic spectrum of diseases.

Conflict of Interest: None declared

P03.008.D Assessment of AION, an AI-based variant interpretation tool, for prenatal diagnostics in cases with fetal abnormalities

Rocio Acuna Hidalgo[1], *Maayke de Koning*[2], *Kristina Ibáñez Garikano*[1], *Andrea Bertana*[1], *Max Schelker*[1], *Ilona Lehtinen*[1], *Lourdes Rosano Gonzalez*[1], *Mariette Hoffer*[2], *Manon Suerink*[2], *Gijs Santen*[2]

[1]*Nostos Genomics GmbH, Computational Genomics, Berlin, Germany;* [2]*Leiden University Medical Center (LUMC), Department of Clinical Genetics, Leiden, Netherlands*

Background/Objectives: Interpretation of genetic variants using limited phenotypic data while maintaining a quick turnaround time for reporting complicates prenatal genetic testing. AION is an AI-based medical device software designed to classify and prioritize genetic variants for automated variant interpretation. This study retrospectively evaluates AION's clinical performance in identifying causative genetic variants in prenatal whole exome sequencing data, as well as in dealing with variants of unknown significance (VUS).

Methods: We analyzed 35 prenatal diagnostic cases with fetal abnormalities (including 10 positive cases and 25 with VUS). All cases were processed on AION, using trio SNV/indel VCF files and clinical phenotype information as input to generate a list of prioritized variants based on potential for disease causality. An exhaustive search to identify additional cases from published scientific literature supplemented our validation dataset.

Results: AION accurately identified the causative variants in all 10 positive cases, ranking 9 of these as the top variant. Among the 25 VUS cases, interpretation was complex due to incomplete or contradicting evidence. Here, the VUS was included in the prioritized list in 16 cases, with 12 identified as strong follow-up candidates based on pathogenicity evidence. The remaining 9 VUS cases were considered less likely to be disease-causing due to phenotype/segregation mismatches or limited pathogenicity evidence.

Conclusion: AION effectively prioritized confirmed disease-causing variants in all positive cases. AION also effectively distinguished strong follow-up candidates in VUS cases from those with weaker or conflicting evidence. The study demonstrates AION's potential in enhancing prenatal diagnostic accuracy and efficiency.

Grants: NA

Conflict of Interest: Rocio Acuna Hidalgo Nostos Genomics GmbH, Nostos Genomics GmbH, Maayke de Koning: None declared, Kristina Ibáñez Garikano Nostos Genomics GmbH, Andrea Bertana Nostos Genomics GmbH, Nostos Genomics GmbH, Max Schelker Nostos Genomics GmbH, Nostos Genomics GmbH, Ilona Lehtinen Nostos Genomics GmbH, Nostos Genomics GmbH, Lourdes Rosano Gonzalez Nostos Genomics GmbH, Nostos Genomics GmbH, Mariette Hoffer: None declared, Manon Suerink: None declared, Gijs Santen: None declared

P03.009.A BASHer (BiAllelic SNP Haplotyper) Software: Streamlining SNP Haplotyping in Pre-Implantation Genetic Testing for Monogenic Disorders (PGT-M)

Graeme Smith[1], **Rebecca Haines**[1], *Heema Hewitson*[1;2], *Elaine Ronaldson*[1;2], *Pamela Renwick*[1;2]

[1]*Synnovis LLP, Genomics Laboratory, Guy's Hospital, London, United Kingdom;* [2]*Centre for Preimplantation Genetic Diagnosis, Genetics Department, Guys Hospital, London, United Kingdom*

Background/Objectives: For couples where one or both partners carry, or are affected by a genetic condition, pre-implantation genetic testing for monogenic disorders (PGT-M) can identify risk haplotypes inherited in embryos. These predict the genetic status, allowing embryos unaffected by the condition to be considered for transfer. Following the withdrawal of a previous genome wide next generation sequencing based assay and analysis platform, it was necessary to develop a solution using an existing in-house SNP array platform to enable the continued delivery of PGT-M for new conditions for our patients.

Methods: Biallelic SNP Haplotyper (BASHer) uses SNP array data (Thermo Fisher Scientific HT-CMA) from both partners and a related reference to automate the identification of SNPs associated with high or low risk haplotypes. The tool generates HTML reports with interactive tables and plots to assist users in identifying the risk haplotypes inherited in embryos. BASHer reports were validated by comparison with manual SNP case analysis where a spreadsheet filters SNPs using logic for the different inheritance modes.

Results: BASHer has been used to deliver the PGT-M service for the majority of new tests (80/104 cases). BASHer is enabling informative reporting to support decision making for clinicians and couples going through PGT-M. Clinical Scientist analysts report that it is a user-friendly service.

Conclusion: BASHer software provides a method for analysis of SNP array data to enable the reporting of PGT-M cases using existing laboratory workflows, and within required turn-around times.

Grants:

Conflict of Interest: None declared

P03.010.B Novel digital PCR assay utilizing in-droplet methylation-sensitive digestion for estimation of fetal cfDNA from plasma

Chenyu Li[1], *Olga Mikhaylichenko*[1], **Eric Hall**[1], *Thea Riel*[1], *Madhumita Ramesh*[1], *Maria Gencoglu*[1], *Nathan Hendel*[1], *Richard Dannebaum*[1],

Severine Margeridon[1], Monica Herrera[1], Xinhua Lin[2], Nazeeh Hanna[2], Martin Chavez[2]

[1]Bio Rad Laboratories, Hercules, United States; [2]NYU Langone Hospital Long Island, Mineola, United States

Background/Objectives: Accurate quantification of fetal fraction in cell-free DNA (cfDNA) is crucial for obtaining reliable non-invasive prenatal screening (NIPS) results. While next-generation sequencing (NGS) is presently the predominant NIPS method, its high cost and turnaround time impede universal and equitable NIPS implementation. We present a methylation-based approach for quantifying fetal fraction via droplet digital PCR (ddPCR) and methylation-sensitive restriction enzyme (MSRE) digestion, and demonstrate ddPCR as an attractive alternative to NGS for fetal fraction determination.

Methods: Assays for fetal DNA targeted regions with high levels of methylation in fetal cfDNA; assays for maternal DNA targeted regions with high levels of methylation in maternal cfDNA; universally hypo- or hypermethylated regions were included as controls. Proof of concept in-droplet MSRE-ddPCR workflow was established on Bio-Rad QX600 instrument. The ddPCR-derived fetal fraction values were compared against chromosome Y concentration values for male pregnancies, and against NGS annotated values for all pregnancies regardless of fetal sex.

Results: Fetal fraction assay multiplex was tested on a total of 130 prospective euploid (n = 125) and aneuploid (n = 5) clinical plasma samples from pregnant individuals. Methylation-based fetal fraction quantification demonstrated high concordance with NGS results and chromosome Y based calculation for male samples. Run to run reproducibility analysis demonstrated lower variability than previously reported for NGS (median SD 0.61% versus 1.3-3.4%).

Conclusion: Utilizing ddPCR allows for obtaining accurate fetal fraction readout in a more time- and cost-effective manner compared to NGS. By integrating methylation-based biomarker identification with ddPCR, we pave the way for compelling alternatives in a wide array of NIPS diagnostic applications.

Conflict of Interest: Chenyu Li: None declared, Olga Mikhaylichenko: None declared, Eric Hall: None declared, Thea Riel: None declared, Madhumita Ramesh: None declared, Maria Gencoglu: None declared, Nathan Hendel: None declared, Richard Dannebaum: None declared, Severine Margeridon: None declared, Monica Herrera: None declared, Xinhua Lin: None declared, Nazeeh Hanna Dr. Nazeeh Hanna and Dr. Xinhua Lin conducted experiments under collaboration agreement with Bio-Rad Laboratories and received instrumentation and reagents from Bio-Rad Laboratories., Martin Chavez Bio-Rad Laboratories obtained plasma samples from Dr. Martin Chavez to conduct experiments described.

P03.011.C Determining the origin of genome aberrations improves the positive predictive value of NIPT for 22q11.2 deletion syndrome

Jiale Xiang[1;2], Xiangzhong Sun[1], Jiguang Peng[1], Hongfu Zhang[1], Jiangkun Shen[1], Jingrou Li[1], Hongyu Li[2], Lanping Hu[2], Jingjing Zhang[2], Shihao Zhou[2], Sihu Xu[1], Yun Yang[1], Jun He[2], **Zhiyu Peng**[1;2]

[1]BGI Genomics, Shenzhen, China; [2]Changsha Hospital for Maternal & Child Health Care Affiliated to Hunan Normal University, Hunan Provincial Key Laboratory of Regional Hereditary Birth Defects Prevention and Control, Changsha, China

Background/Objectives: Non-invasive prenatal testing (NIPT) has been endorsed by the American College of Medical Genetics and Genomics as the preferred method for screening fetal 22q11.2 deletion syndrome (22q11.2 DS). Maternal genomic aberrations represent a significant source of false positives in NIPT, and there are currently no solutions that effectively address this challenge.

Methods: We have devised an innovative NIPT bioinformatics pipeline designed to discern the origins of copy number variations (CNVs). Then, we recruited a cohort of 22q11.2 DS cases to validate the effectiveness of our methodology. Follow-up tests including amniocentesis and genome sequencing of maternal leukocytes were conducted.

Results: Leveraging a dataset of over 900 CNVs, we developed a new pipeline that classifies CNVs into those of fetal, maternal, and maternal-fetal origin based on NIPT data. The use of our pipeline led to a notable increase in the positive predictive value of NIPT for detecting 22q11.2 DS from 87% (34/39) to 94% (34/36). Furthermore, our approach has the potential to reduce the number of invasive tests by 8% (3/39).

Conclusion: Our innovative and reliable bioinformatics pipeline has enabled the accurate differentiation of CNV origin into fetal, maternal, and maternal-fetal categories. Incorporating this pipeline into the analytical workflow could reduce false positives in NIPT results and minimize the need for invasive prenatal diagnoses.

Conflict of Interest: Jiale Xiang Jiale Xiang is employed by the BGI Genomics, Xiangzhong Sun Xiangzhong Sun is employed by the BGI Genomics, Jiguang Peng Jiguang Peng is employed by the BGI Genomics, Hongfu Zhang Hongfu Zhang is employed by the BGI Genomics, Jiangkun Shen Jiangkun Shen is employed by the BGI Genomics, Jingrou Li Jingrou Li is employed by the BGI Genomics, Hongyu Li: None declared, Lanping Hu: None declared, Jingjing Zhang: None declared, Shihao Zhou: None declared, Sihu Xu Sihu Xu is employed by the BGI Genomics, Yun Yang Yun Yang is employed by the BGI Genomics, Jun He: None declared, Zhiyu Peng Zhiyu Peng is employed by the BGI Genomics

P03.012.D Whole exome sequencing in recurrent cases of fetal sonographic anomalies

julia grinshpun-cohen[1], *Lena Sagi-Dain[2], Amihood Singer[1]*

[1]Israel Ministry of Health, Community Genetics, Ramat Gan, Israel; [2]Carmel Medical Center, Genetic Institute, Haifa, Israel

Background/Objectives: The evidence exploring the yield of whole exome sequencing (WES) in pregnancies with recurrent sonographic anomalies is scarce. In this study, we examine the yield of exome sequencing in a relatively large cohort.

Methods: in 2020, the Israeli Ministry of Health (MOH) introduced public funding of exome sequencing for cases of recurrent fetal sonographic anomalies. Pregnancies with recurrent (two or more pregnancies) anomaly in a specific anatomic system undergone trio exome sequencing following genetic counseling and normal microarray analysis. We retrospectively retrieved clinical data for this study from MOH electronic medical records.

Results: Until October 2023, 117 cases of WES due to recurrent similar sonographic anomalies were included in the study. Most commonly affected system was brain (37.6%), followed by hydrops (12%), KACUT (10.3%), cardiac anomalies (9.4%), and skeletal anomalies (8.5%). We detected pathogenic and likely pathogenic WES findings related to the phenotype in 33 cases (28.2%). The highest rates of abnormal genetic findings (P/LP) were for skeletal anomalies (40.0%), hydrops (35.7%), KACUT (33.3%), multiple anomalies (30.0%), brain anomalies (29.5%) and cardiac anomalies (27.3%).

Conclusions: The yield of WES in pregnancies with recurrent sonographic anomalies is appreciable (over 25% for most indications), reaching 40% in skeletal anomalies. Establishment of a specific genetic diagnosis in pregnancies with fetal anatomic

anomalies is of importance for exact estimation of prognosis and recurrence risk, allowing for prenatal or preimplantation genetic testing in future pregnancies. Thus, WES analysis in pregnancies with sonographic abnormalities is beneficial, especially in cases with recurrent anomalies.

Conflict of Interest: None declared

P03.013.A Extending the clinical spectrum of X-linked Tonne-Kalscheuer syndrome (TOKAS): new insights from the fetal perspective

*Silvestre Cuinat[1], Chloe Quelin[1;2], Claire Effray[1], Christele Dubourg[3], Gwenaelle Le Bouar[4], Anne-Sophie Cabaret-Dufour[4], Philippe Loget[2], Maia Proisy[5], Fanny Sauvestre[6], Mélie Sarreau[6], Sophie Martin-Berenguer[6], Claire Beneteau[7], Sophie Naudion[7], Vincent MIchaud[7;8], Benoit Arveiler[7;8], Aurélien Trimouille[7;8], Pierre Macé[9], Sabine Sigaudy[10], Olga Glazunova[10], Julia Torrents[11], Laure Raymond[12], Marie-Hélène Saint-Frison[13], Tania Attie-Bitach[14], Mathilde Lefèbvre[15], YLINE CAPRI[16], Nicolas Bourgon[17;18], Christel Thauvin-Robinet[18;19;20], Frédéric Tran Mau-Them[21;22], Ange-Line Bruel[21;22], Antonio Vitobello[21;22], Anne-Sophie Denommé-Pichon[21;22], Laurence Faivre[20;21], Anne-Claire Brehin[23], Alice Goldenberg[24], Sophie Patrier Sallebert[25], alexandre perani[26], Benjamin Dauriat[26], Sylvie Bourthoumieu[26;27], Catherine Yardin[26;27], Valentin Marquet[26], Marion Barnique[26], Maryse Fiorenza-Gasq[28], Isabelle Marey[29], Danielle Tournadre[30], Raïa Doumit[31], Frédérique Nugues[31], Stefan Barakat[32;33;34], Francisco Bustos[35;36], Sylvie Jaillard[37;38], Erika Launay[38], Laurent Pasquier[1;39;40], **Sylvie Odent**[1;39;40]*

[1]Service de Génétique Clinique, CRMR anomalies du développement CLAD-Ouest, CHU Rennes; [2]Service d'Anatomie et Cytologie Pathologiques, Hôpital Pontchaillou, CHU Rennes; [3]Laboratoire de Génétique Moléculaire, CHU Pontchaillou, UMR 6290 CNRS, IGDR, Faculté de Médecine, Université de Rennes 1; [4]Unité de Médecine fœtale, Service de Gynécologie-Obstétrique, CHU Rennes; [5]CHU Brest, Radiology Department, Brest University; [6]Unité de Pathologie Fœto-placentaire, Service de Pathologie, CHU de Bordeaux; [7]Service de Génétique Médicale, CHU de Bordeaux; [8]INSERM U1211, Maladies Rares, Génétique et Métabolisme, Université de Bordeaux; [9]Institut méditerranéen d'imagerie médicale appliquée à la gynécologie, la grossesse et l'enfance IMAGE2, 6, rue Rocca, 13008 Marseille; [10]Département de Génétique Médicale, Hôpital Timone Enfant, Marseille; [11]Department of Pathology and Neuropathology, La Timone Hospital, Aix Marseille University, Marseille, France; [12]Genetics Department, Laboratoire Eurofins Biomnis, Lyon; [13]Foetopathology Unit, AP-HP Nord, Hôpital Robert Debré, Paris, France; [14]Université Paris Cité, INSERM UMR 1163, Imagine Institute, Paris; Service de Médecine Génomique des Maladies Rares, Hopital Universitaire Necker-Enfants Malades, Paris; [15]APHP Service de Pathologie fœtale, Hôpital Universitaire Armand Trousseau, Paris; [16]Département de Génétique, Assistance Publique des Hôpitaux de Paris (AP-HP) Hôpital Robert Debré, 75019 Paris; [17]Service d'Obstétrique-Maternité Chirurgie, Médecine et Imagerie foetales, AP-HP.Centre, Hôpital Necker Enfants Malades, Paris; [18]UMR1231 GAD, INSERM - Université Bourgogne-Franche Comté, Dijon, France; [19]Unité Fonctionnelle Innovation en Diagnostic Génomique des Maladies Rares, FHU-TRANSLAD, CHU Dijon Bourgogne, Dijon, France; [20]Centre de référence Anomalies du Développement et Syndromes Malformatifs, Fédération Hospitalo-Universitaire TRANSLAD, CHU Dijon; [21]UMR1231 GAD, INSERM - Université Bourgogne-Franche Comté, Dijon; [22]Unité Fonctionnelle Innovation en Diagnostic Génomique des Maladies Rares, FHU-TRANSLAD, CHU Dijon Bourgogne, Dijon; [23]Univ Rouen Normandie, Normandie Univ, Inserm U1245 and CHU Rouen, department of Pathology, department of Genetics and reference center for developmental abnormalities, F-76000 Rouen; [24]Univ Rouen Normandie, Normandie Univ, Inserm U1245 and CHU Rouen, department of Genetics and reference center for developmental abnormalities, F-76000 Rouen; [25]Service de Fœtopathologie, CHU de Rouen; [26]Cytogenetic, medical genetic and reproductive biology department, Hôpital de la Mère et de l'Enfant, CHU Dupuytren, Limoges; [27]UMR 7252, CNRS, XLIM, F-87000, Limoges University, Limoges; [28]Limoges Regional University Hospital, Department of Gynaecology and Obstetrics, Mother and Children's Hospital, 8 Avenue Dominique Larrey, 87000 Limoges; [29]Université Grenoble Alpes, INSERM, U1209, CHU Grenoble Alpes, Institute for Advanced Bioscience, Grenoble; [30]CPDPN de Grenoble, Echographie obstétricale dépistage et diagnostic, CHU de Grenoble; [31]Service d'Imagerie Pédiatrique, Centre Hospitalier Universitaire Grenoble Alpes, Grenoble; [32]Department of Clinical Genetics, Erasmus MC University Medical Center, Rotterdam, The Netherlands; [33]ENCORE Expertise Center for Neurodevelopmental Disorders, Erasmus MC University Medical Center, Rotterdam, The Netherlands; [34]Discovery Unit, Department of Clinical Genetics, Erasmus MC University Medical Center, Rotterdam, The Netherlands; [35]Pediatrics and Rare Diseases Group, Sanford Research, Sioux Falls, SD, USA; [36]Department of Pediatrics, Sanford School of Medicine, University of South Dakota, Sioux Falls, SD, USA; [37]INSERM, EHESP, IRSET-UMR_S, 1085, Université Rennes 1, Rennes, France; [38]Service de Cytogénétique et Biologie Cellulaire, CHU Rennes, Rennes, France; [39]FHU GenoMeds, ERN ITHACA, CHU Rennes, Rennes, France; [40]Univ Rennes, CNRS, INSERM, IGDR (Institut de Génétique et Développement de Rennes) - UMR 6290, ERL U1305, F-35000 Rennes

Background/Objectives: Tonne-Kalscheuer syndrome (TOKAS) is a recessive X-linked multiple congenital anomaly disorder caused by *RLIM* variations. Of the 41 patients reported, only 7 antenatal cases were described.

Methods: After the antenatal diagnosis of TOKAS by exome analysis in a family that was followed for over 35 years because of multiple congenital anomalies in five male fetuses, a call for collaboration was made, resulting in a cohort of 11 previously unpublished cases.

Results: We present a TOKAS antenatal cohort, describing 11 new cases in 6 French families. We report a high frequency of diaphragmatic hernia (9/11), differences in sex development (10/11), and various visceral malformations. We report some recurrent dysmorphic features, but also pontocerebellar hypoplasia, preauricular skin tags and olfactory bulb abnormalities previously unreported in the literature. Although no clear genotype-phenotype correlation has yet emerged, we show that a recurrent p.(Arg611Cys) variant accounts for 66% of fetal TOKAS cases. We also report 2 new likely-pathogenic variants in *RLIM*, outside of the two previously known mutational hotspots.

Conclusion: Overall, we present the first fetal cohort of TOKAS, describe the clinical features that made it a recognizable syndrome at fetopathological examination, extend the phenotypic spectrum and the known genotype of this rare disorder.

Grants:

Conflict of Interest: None declared

P03.014.B Evaluation of PCR-based and isothermal whole genome amplification strategies for preimplantation genetic testing of repeat expansion and gene deletion disorders in combination with aneuploidy screening

*Vivienne Tan[1], Ying Liang[2], Arnold Tan[1], Simin Wong[3], Mulias Lian[3], Caroline Lee[4], Mahesh Choolani[3;5], Truong Dang[6], **Samuel Chong**[1;3;5;7]*

[1]National University of Singapore, Paediatrics, Singapore, Singapore; [2]Shijiazhuang Obstetrics and Gynaecology Hospital, Reproductive Medical Center, Shijiazhuang, China; [3]National University Health System, National University Centre for Women and Children, Singapore, Singapore; [4]National University of Singapore,

Biochemistry, Singapore, Singapore; [5]National University of Singapore, Obstetrics & Gynaecology, Singapore, Singapore; [6]Vietnam Military Medical University, Anatomy, Hanoi, Viet Nam; [7]National University Hospital, Laboratory Medicine, Singapore, Singapore

Background/Objectives: The isothermal multiple displacement amplification (MDA) whole genome amplification (WGA) method is commonly used for preimplantation genetic testing of monogenic disorders (PGT-M) utilizing short tandem repeat (STR) haplotyping. In contrast, PCR-based WGA methods such as multiple annealing and looping-based amplification cycles (MALBAC) are ideal for preimplantation genetic testing for aneuploidy or structural rearrangements (PGT-A/-SR). We compared the MDA and MALBAC WGA methods for PGT-M of repeat expansion or gene deletion disorders in conjunction with PGT-A aneuploidy screening.

Methods: Replicates of five-cell aliquots from cell lines of a Huntington disease (HD) case-parent trio and a spinal muscular atrophy (SMA) case-parent trio were subjected to MDA and MALBAC WGA. WGA products of the HD trio underwent fluorescent triplet-primed PCR (TP-PCR) of the *HTT* CAG repeat, while WGA products of the SMA trio underwent multiplex-PCR of three *SMN* gene fragments containing single nucleotide differences between *SMN1* and *SMN2*, followed by minisequencing. Amplified and minisequenced products were analysed using capillary electrophoresis.

Results: TP-PCR failed from MALBAC-amplified samples, regardless of WGA product input amount. Triplex PCR and minisequencing succeeded from MALBAC-amplified samples, but two of three amplicons consistently under-amplified. In contrast, both HD TP-PCR and SMN triplex PCR and minisequencing consistently succeeded from MDA WGA product. Both MDA and MALBAC products could also be used for PGT-A to detect whole chromosome or segmental aneuploidy.

Conclusion: Isothermal WGA is superior to PCR-based WGA when used for PGT-M of repeat expansion disorders and gene deletion disorders in conjunction with PGT-A aneuploidy screening.

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Conflict of Interest: Vivienne Tan: None declared, Ying Liang: None declared, Arnold Tan: None declared, Simin Wong: None declared, Mulias Lian: None declared, Caroline Lee: None declared, Mahesh Choolani: None declared, Truong Dang: None declared, Samuel Chong MOH-000644-01

P03.015.C Transcriptional networks in placenta and fetal brain reveal extensive sex differences in a mouse model of polychlorinated biphenyl mixture exposure and folate supplementation potentially counteracts the effects of prenatal PCB exposure

Kelly Chau[1], *Kari Neier*[1], *Anthony Valenzuela*[1], *Rebecca Schmidt*[1], *Pamela Lein*[1], *Janine LaSalle*[1]

[1]*UC Davis, Davis, United States*

Background: In a human study "Markers of Autism Risk in Babies: Learning Early Signs", detectable concentrations of polychlorinated biphenyls (PCB) in maternal blood were associated with NDDs and alterations to methylation patterns of neurodevelopmental genes. In a mouse model of prenatal exposure to this PCB mixture, there were enhanced repetitive behavior and deficits in sociability and vocalizations at the lowest dose (0.1 mg/kg) specifically in males.

Methods: To investigate sexual dimorphism, dosage effects, and nutrient supplementation, we exposed C57Bl/6J females to varying doses (0, 0.1, 1, 6 mg/kg) of the PCB mixture supplemented with folate prior to timed mating. At embryonic day 18, brain and placenta were collected for RNA-sequencing and Whole Genome Bisulfite Sequencing (WGBS).

Results: Differential expressed genes analysis showed sex-specific dose responses and non-monotonic responses at the 0.1 mg/kg dose. Weighted gene co-expression network analysis was applied to investigate groups of highly correlated genes affected by PCBs and revealed extensive sex differences, with male brains impacted more than female brains. Broad pathways are disrupted including DNA repair and folate supplementation counteracts the effects of PCBs in brain and placenta.

Conclusion: A weighted region correlation network analysis of WGBS data is in progress to identify comethylated regions correlated with PCB exposures. Integration of gene expression and DNA methylation will be performed to identify epigenomic signatures in placenta that will be compared to human NDDs for their predictive value.

Grants/Fellowships: NIH grant R01ES029213; National Institute of Environmental Health Sciences Training Grant

Conflict of Interest: None declared

P03.016.D The increased nuchal translucency paradox

Tanja Richter[1], *Ann-Kathrin Tschürtz*[1], *Karl Mehnert*[1]

[1]*MVZ genetikum, Neu-Ulm*

Background/Objectives: Nuchal translucency (NT) is defined as sonographic appearance of subcutaneous accumulation of liquid in the back of the fetal neck at 11-14 gestational week. Increased NT is defined as measurement above 99th percentil. Besides the association with chromosomal abnormalities, it is also known to be caused by monogenic conditions. Currently, increased NT is mostly associated with Noonan syndrome (NS). However, prenatal Whole Exome Sequencing (WES) reveals an increasingly number of monogenic syndromes which may also be associated with increased NT. Besides increased NT can be a variation without pathological significance.

Methods: In our laboratory we performe Trio-WES combined with a Noonan-Plus-panel. Alongside known NS genes, the panel includes RASopathy genes and genes reported with increased NT and/or hydrops fetalis. Furthermore it includes e. g. genes for Kabuki syndrome. We performed data analysis of prenatal WES with either isolated increased NT or combined with one or more of the following abnormalities: cystic hygroma, hydrops fetalis, ductus venosus abnormalities, congenital heart defects.

Results: Pathogenic variants were mostly detected in PTPN11 followed by LZTR1, BRAF, RIT1, SOS1, RAF1. With the Trio-WES apporach we also detected variants in genes not yet associated with increased NT like ARID1A, H1-4, KANSL1, NFIX.

Conclusion: Our results give rise to the question, if increased NT is caused by the underlying syndrome or if the detected syndrome may be an incidental finding based on a benign variation on ultrasound examination. To solve this „Increased nuchal translucency paradox"further evaluation of increased NT and its correlation with monogenic syndroms is needed.

Grants:

Conflict of Interest: None declared

P03.017.A Whole genome sequencing in prenatally detected congenital malformations

Eini Westenius[1;2], *Peter Conner*[3;4], *Maria Pettersson*[1;2], *Ellika Sahlin*[1;2], *Nikos Papadogiannakis*[5;6], *Anna Lindstrand*[1;2], *Erik Iwarsson*[1;2]

[1]*Karolinska Institutet, Department of Molecular Medicine and Surgery, Stockholm, Sweden;* [2]*Karolinska University Hospital,*

Department of Clinical Genetics and Genomics, Stockholm, Sweden; [3]Karolinska Institutet, Department of Women's and Children's Health, Stockholm, Sweden; [4]Karolinska University Hospital, Center for Fetal Medicine, Stockholm, Sweden; [5]Karolinska Institutet, Department of Laboratory Medicine, Stockholm, Sweden; [6]Karolinska University Hospital, Department of Pathology, Stockholm, Sweden

Background/Objectives: This prospective cohort study investigated the diagnostic yield of short-read whole genome sequencing (WGS) in prenatally detected congenital malformations.

Methods: In this prospective study, 85 fetuses with different congenital malformations, negative for trisomies and causative copy number variants, were further analyzed with fetal-parental trio WGS analysis.

Results: A molecular diagnosis was achieved in 23/85 (27%) of cases. Causative variants were identified in 21 genes: *FGFR3* (n = 2), *L1CAM* (n = 2), *ACTA1* (n = 1), *CDH2* (n = 1), *CHD7* (n = 1), *COL1A1* (n = 1), *COL1A2* (n = 1), *DHCR7* (n = 1), *EYA1* (n = 1), *FBXO11* (n = 1), *FRAS1* (n = 1), *NIPBL* (n = 1), *OFD1* (n = 1), *PDHA1* (n = 1), *POMGNT1* (n = 1), *PTPN11* (n = 1), *RIT1* (n = 1), *SMARCC1* (n = 1), *SMC3* (n = 1), *SOX9* (n = 1) and *STAG2* (n = 1). The phenotypes of the cases were divided into different groups with the following diagnostic yields: multisystem 8/19 (42%), CNS 8/21 (38%), skeletal 4/12 (33%), thorax 2/16 (13%), abdominal 1/11 (9%) and other isolated anomalies 0/6 (0%). Additionally, five cases carried variants that were considered potentially clinically relevant.

Conclusion: We demonstrate a diagnostic yield of 27% with WGS in prenatally detected congenital malformations. Our study emphasizes the benefits that WGS can bring to the diagnosis of fetal structural anomalies. It is noteworthy that causative chromosome aberrations were excluded from our cohort before WGS. As chromosome aberrations are a well-known cause of prenatally detected congenital malformations, future studies using WGS as a primary diagnostic test, including assessment of chromosome aberrations, will most likely show that the detection rate will exceed the diagnostic yield of this study.

Grants: -

Conflict of Interest: Eini Westenius Karolinska University Hospital, Illumina invited speaker compensated to speak at the industry sponsored symposium at the annual (virtual) COGEN meeting 2022., Peter Conner Karolinska University Hospital, Maria Pettersson Karolinska University Hospital, Ellika Sahlin Karolinska University Hospital, Nikos Papadogiannakis Karolinska University Hospital, Anna Lindstrand Karolinska University Hospital, Erik Iwarsson Karolinska University Hospital, Illumina invited speaker compensated to speak and reimbursed travel expenses at ISPD 2023 conference.

P03.018.B Report of PRKAG2-related lethal congenital glycogen storage disease of the heart as an ultra-rare cause of foetal hydrops mimicking RASopathies.

Alexandre White-Brown[1], Marilyn Richard[1], **Isabelle De Bie**[1]

[1]MUHC - McGill University Health Centre (Glen Site), Specialized Medicine, Division of Medical Genetics, Montréal, Canada

Background/Objectives: Non immune foetal hydrops (NIFH) has been associated with over 200 chromosomal and single gene conditions, RASopathies representing about a quarter of the underlying monogenic aetiologies. Lethal Congenital Glycogen Storage Disease of the Heart (LCGSDH) is an ultra-rare entity with less than 10 cases reported in the literature so far, for which prenatal features significantly overlap those of RASopathies. The majority of reported cases of LCGSDH harbor the recurring *PRKAG2* pathogenic variant NM_016203.4:c.1592G>A (p.Arg531Gln).

We report the clinical features of a pregnancy with Lethal Congenital Glycogen Storage Disease of the Heart (LCGSDH) associated with a novel likely pathogenic de novo *PRKAG2* variant.

Methods: Pregnancy was uneventful until 28 weeks gestation, where foetal bradycardia was noted. Follow up ultrasound at 32 weeks revealed bradycardia, hydrops, moderate biventricular hypertrophy, prominent tongue and polyhydramnios. The newborn, delivered prematurely at 35 weeks, passed away at 17 hours of life due to respiratory insufficiency.

Results: Trio exome sequencing uncovered a novel likely pathogenic de novo missense variant in *PRKAG2*, NM_016203.3:c.1150A>G, p.(Arg384Gly). Pathological examination revealed extensive cardiac glycogenosis and severe pulmonary hypoplasia.

Conclusion: This report expands on the phenotypic spectrum of *PRKAG2*-related LCGSDH and further supports the usefulness of genome-wide molecular studies in the clinical evaluation of NIFH.

Grants: None

Conflict of Interest: None declared

P03.019.C Incidental findings of microdeletions/microduplications of mother and fetus through non-invasive prenatal testing (NIPT).

Minyeon Go[1,2], *Hyunjin Kim[1], Ji Eun Park[1], Kyung Min Kang[1], Hee Yeon Jang[1,2], So Hyun Yang[1], Jong Chul Kim[1], Dong Hyun Cha[1,3], Sung Han Shim[1,2]*

[1]Center for Genome Diagnostics, CHA Biotech Inc., Molecular Genetics Laboratory, Seoul, Korea, Rep. of South; [2]College of Life Science, CHA University, Department of Biomedical Science, Gyeonggi-do, Korea, Rep. of South; [3]CHA Gangnam Medical Center, CHA University, Department of Obstetrics and Gynecology, Seoul, Korea, Rep. of South

Background/Objectives: Non-invasive prenatal testing (NIPT) is a screening test with high sensitivity and specificity in confirming trisomy 21,18, and 13. Recently, NIPT has been expanded to the microdeletion/microduplication syndromes (MMSs). In this study, we described two incidental finding cases of MMSs found in NIPT.

Methods: NIPT and Chromosome microarray analysis (CMA) were conducted.

Results: A 37-year-old woman (G3P0) underwent NIPT at 11 + 1 weeks of gestation, and the results revealed a microdeletion of 1.42Mb in the 17p12 region. Karyotyping and CMA test were conducted. The karyotype analysis results showed that both mother and fetus have a balanced translocation between chromosome 6 and 16, 46,XXt(6;16)(q25.1;q22). As a result of CMA analysis, the1.4Mb deletion was confirmed in both mother and fetus. The deleted region contains the *PMP22* gene that known to be related to CMT1A. The mother did not show any symptoms and decided to maintain the pregnancy and is currently 33 weeks of gestation.

A 37-year-old woman (G2P1) with a natural pregnancy underwent NIPT at 14 + 0 weeks of gestation. As a result, a 33.9 Mb duplication on 7pter(p14.3p22.3) and a 22.1Mb deletion on Xqter (q26.3q28) were identified. Both genomic variations were confirmed in the fetal CMA result. Those variations are suspected in one of the parents so genetic counseling was conducted, but further follow-up was refused.

Conclusion: This study shows that NIPT can be a tool to reliably detect MMS, and if MMS is found in NIPT results, confirmation through diagnostic testing should be considered.

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Conflict of Interest: None declared

P03.021.A Antenatal phenotype associated with 7q11.23 recurrent copy number variation in 44 fetuses

Cécile Courdier[1], *John Boudjarane@ap-hm.fr*[2], *VALÉRIE MALAN*[3], *Christine Muti*[4], *Brian Sperelakis-Beedham*[4], *Sylvie Odent*[5], *Sylvie Jaillard*[6], *Chloe Quelin*[5], *Cedric Le Caignec*[7], *Olivier Patat*[7], *Charlotte Dubucs*[7], *Sophie Julia*[7], *Caroline Schluth-Bolard@chu-strasbourg.fr*[8;9], *Carole Goumy*[10], *Sylvia Quemener*[11], *Jean-Baptiste Gaillard*[12], *Céline Dupont*[13], *Guillaume Cogan*[13], *François Vialard*[14], *Rodolphe Dard*[14], *Guillaume Jedraszak*[15], *Florence Jobic*[16], *Mathilde Lefebvre*[17], *Geneviève Quenum*[18], *Saori Inai*[19], *melanie rama*[20], *Fanny Sauvestre*[21], *Frédéric Coatleven*[22], *Julie Thomas*[23], *Caroline Rooryck-Thambo*[1;24]

[1]*Bordeaux University hospital, Genetics, Bordeaux, France;* [2]*Timone Children hospital, Cytogenetics laboratory, Marseille, France;* [3]*Necker Children hospital, Genetics, Paris, France;* [4]*Versailles hospital, Genetics, Versailles, France;* [5]*Rennes University hospital, Genetics, Rennes, France;* [6]*Rennes University hospital, Cytogenetics and Cell biology laboratory, Rennes, France;* [7]*Toulouse University hospital, Genetics, Toulouse, France;* [8]*Hospices Civils de Lyon, Genetics, Bron, France;* [9]*Strasbourg University hospital, Genetics laboratory, Strasbourg, France;* [10]*Estaing University hospital, Cytogenetics, Clermont-Ferrand, France;* [11]*Morvan University hospital, Molecular laboratory, Brest, France;* [12]*Montpellier University hospital, Genetics, Montpellier, France;* [13]*Robert Debré hospital AP-HP, Cytogenetics, Paris, France;* [14]*Poissy/Saint-Germain-en-Laye hospital, Genetics, Poissy, France;* [15]*Amiens University hospital, Genetics laboratory, Amiens, France;* [16]*Amiens University hospital, Genetics and Oncogenetics, Amiens, France;* [17]*Armand Trousseau hospital, Foetopathology, Paris, France;* [18]*Armand Trousseau hospital, Cytogenetics laboratory, Paris, France;* [19]*Libourne hospital, Gynecology, Libourne, France;* [20]*Jeanne de Flandre hospital, Lille University hospital, Genetics, Lille, France;* [21]*Bordeaux University hospital, Foetopathology, Bordeaux, France;* [22]*Bordeaux University hospital, Obstetrics and Gynecology, Bordeaux, France;* [23]*Bordeaux University hospital, Congenital Heart disease, Bordeaux, France;* [24]*INSERM U1211, Maladies Rares : Génétique et Métabolisme (MRGM), Bordeaux, France*

Consortium: the AChro-puce Consortium

Background/Objectives: Williams-Beuren syndrome (WBS) is a well known disorder often diagnosed before 6 years of life. However, its prenatal diagnosis remains challenging with only 30 scattered fetuses described to date. We aimed to gather fetal cases carrying a 7q11.23 copy number variation (CNV) and collect precise clinical data to broaden knowledge of antenatal features in these syndromes.

Methods: We retrospectively recruited unrelated cases with 7q11.23 deletion, known as WBS, or 7q11.23 duplication who had prenatal ultrasound findings. We collected laboratory and clinical data, fetal ultrasound and fetal autopsy reports from 18 prenatal diagnostic centers throughout France.

Results: Forty fetuses with WBS were collected and the most common features were intra-uterine growth retardation (IUGR) (70.0%, 28/40), cardiovascular defects (30.0%, 12/40), polyhydramnios (17.5%, 7/40) and protruding tongue (15.0%, 6/40). Cardiovascular defects were mostly associated with extra-cardiac features (83,3%, 10/12). Fetal autopsy reports were available for 11 cases and confirmed all features identified by prenatal ultrasound. Four fetuses with 7q11.23 microduplication were collected and prenatal ultrasound signs were moderate and often isolated.

Conclusion: This work strengthens the fact that 7q11.23 CNVs are associated with a broad spectrum of antenatal presentations. Early IUGR and cardiovascular defects were the most frequent ultrasound signs. Protruding tongue and premature ductus arteriosus closure could be emergent WBS specific prenatal signs. By reporting the biggest series of antenatal WBS, we aim to better delineate distinctive signs in fetuses with 7q11.23 CNVs. We also broaden the spectrum of 7q11.23 duplication as we describe milder phenotype than previously reported.

Grants: None

Conflict of Interest: None declared

P03.022.B Diagnosis of tuberous sclerosis in the prenatal period: a retrospective study of 240 cases and review of the literature

Vincent Milon[1], *Marie-Claire Malinge*[1], *Maud Blanluet*[1], *Marine Tessarech*[1;2], *Clarisse Battault*[1], *Sarah Prestwich*[1], *Béatrice Vary*[1], *Pierre Gueracher*[1], *Louis Legoff*[1;2], *Magalie Barth*[1;2], *Clara Houdayer*[1;2], *Agnes Guichet*[1;2], *Audrey Rousseau*[3], *Dominique Bonneau*[1;2], *Vincent Procaccio*[1;2], *Céline Bris*[1;2], *Estelle Colin*[1;2]

[1]*Angers University Hospital Center, Genetics department, Angers, France;* [2]*Univ Angers, [CHU Angers], Inserm, CNRS, MITOVASC, Équipe CARME ou Equipe MITOLAB, SFR ICAT, Angers, France;* [3]*Angers University Hospital Center, Tissular and Cellular Pathology Department, Angers, France*

Background/Objectives: Tuberous sclerosis complex (TSC) is a rare multisystemic disorder caused by a pathogenic variant in the *TSC1* or *TSC2* gene. During foetal life, TSC may be suspected by detecting cardiac rhabdomyomas (CRs), cortical tubers, or subependymal nodules. Multiple CRs are associated with a very high risk of TSC, but the CR could be single and isolated. Few data exist to estimate the risk of TSC in these cases.

Methods: We assessed the clinical and genetic diagnosis of TSC in a retrospective study of 240 fetuses tested for suggestive antenatal signs. We provide a review of the literature to specify the probability of TSC in case of detection of single or multiple CRs.

Results: In this series, a definite diagnosis was confirmed in 50% (41/82) of foetuses initially presenting with a single CR, and 80.3% (127/158) in case of multiple CRs. We detected 3 cases of parental germinal mosaicism and estimated the risk at 2.6% (3/115).

Conclusion: The initial detection of an isolated single CR was associated with a proven TSC in half of the cases. TSC's great phenotypic variability raises challenges for genetic counseling and variant interpretation.

Conflict of Interest: None declared

P03.023.C A comprehensive analysis of rare chromosomal abnormalities for individual chromosomes based on 582,996 non-invasive prenatal test (NIPT) results: a systematic review and meta-analysis

Marton Konya[1;2;3], *Agnes Czimbalmos*[1], *Lotti Loczi*[1;4], *Tamas Koi*[1], *Caner Turan*[1;5], *Rita Nagy*[1;6], *Péter Hegyi*[1;7], *Szabolcs Varbiro*[4;8;9], **Aniko Gal**[2;3]

[1]*Semmelweis University, Centre for Translational Medicine, Budapest, Hungary;* [2]*Semmelweis University, Institute of Genomic Medicine and Rare Disorders, Budapest, Hungary;* [3]*Czeizel Institute, Budapest, Hungary;* [4]*Semmelweis University, Department of Obstetrics and Gynecology, Budapest, Hungary;* [5]*Semmelweis University, Department of Anesthesiology and Intensive Therapy, Budapest, Hungary;* [6]*Heim Pál National Pediatric Institute, Budapest, Hungary;* [7]*Semmelweis University, Institute of Pancreatic Diseases, Budapest, Hungary;* [8]*University of Szeged, Department of Obstetrics and Gynecology, Szeged, Hungary;* [9]*Semmelweis University, Workgroup for Science Management, Doctoral School, Budapest, Hungary*

Background/Objectives: Genome-Wide Non-Invasive Prenatal Testing (GW-NIPT) can provide positive results not only for common autosomal aneuploidies but also for rare autosomal trisomies (RATs) and structural chromosomal abnormalities (SCAs). Due to their rarity, there is currently insufficient information on the positive predictive values (PPV) of RAT and SCA-positive cases in the literature.

Methods: In this study, the screening accuracy and pregnancy outcomes of cases positive for RATs were examined based on publications where GW-NIPT testing was performed. True positive cases were determined using two different methodologies. One was a confirmed methodology where only cases validated by genetic testing were considered true positives, the other is an extended methodology in which, in addition to cases confirmed by genetic testing, intrauterine foetal death and abortion due to an ultrasound-confirmed abnormality were considered true positive cases where no diagnosis was made but the foetus was likely to be affected.

Results: 14 studies were analyzed, with a total GW-NIPT population of 582,996. Of these, 1,364 were RAT positive. Using a confirmed method, we found the highest rates of true positives in T16, followed by T22, and T2, with extended method, the highest rate of true positives in T22, followed by T16, and T15.

Conclusion: This is the first meta-analysis to determine with high precision the frequency of rare chromosomal abnormalities, test-positive rates, and the PPV of each chromosomal abnormality. Our results could help pre- and post-test genetic counseling and can help patients and clinicians in their decision-making.

Grants: NKFIH_FK_132812

Conflict of Interest: None declared

P03.024.D Prenatal Exome Sequencing Implementation in Anomalous Fetuses; First Report from Turkey

*Ezgi Susam[1], **Beyhan Durak Aras**[1], Sinem Kocagil[1], Zafer Bütün[2], Melih Velipasaoglu[1], Hüseyin Mete Tanır[1], Sevilhan Artan[1]*

[1]Eskisehir Osmangazi University, Meselik Campus, Türkyie; [2]Eskişehir City Hospital, Eskişehir, Türkyie

Background/Objectives: Prenatal WES started to take place more frequently in the literature since 2016 and has been studied in cases with different numbers of participants and different phenotypic findings. The diagnostic yield ranges from 15% to 42% in several studies. Unlike other literature, our study was comprised of 20 cases, 50% of whom had parental consanguineous marriage and 25% had a positive family history due to population characteristics.

Methods: After excluding chromosomal aberrations by microarray analysis, WES (Twist Human Core Exome capture kit, MGI DNBSEQ-G40) analyses were studied in 20 fetuses with major or structural anomalies.

Results: In 9 of 20 cases, genetic etiology was found to explain the fetal phenotype, and the diagnosis yield was found as 45%. The highest diagnostic rate was obtained in fetuses with fetal akinesia (66%), central nervous system anomalies (50%), and skeletal system (40%) involvement, lowest yields were obtained from cardiac anomaly group (10%) and genitourinary anomalies (20%). It was thought that the diagnosis rate was higher than the literature, due to the high rate of inter-parental consanguinity and similarly affected individuals in families. In addition, in contrast to the high sporadic rates in the literature, it has been shown that all encephalocele cases are syndromic and caused by monogenic genetic etiologies.

Conclusion: Our study is one of the very first examples in the literature with high consanguinity rates and in which postnatal evaluations of the family members are made. The diagnostic yield was demonstrated with the results, and its applicability in our society has been questioned.

Conflict of Interest: None declared

P03.025.A Characterization of a complex rearrangement between three chromosomes in a fetus with skeletal anomalies by optical genome mapping

Uwe Heinrich[1], *Wilena Telman[1], Aysegül Klapperich[2], Jenny Schiller[1], Konstanze Hörtnagel[1]*

[1]MVZ Martinsried GmbH, Planegg, Germany; [2]MVZ Humangenetik Köln GmbH, Köln, Germany

Background/Objectives: We present a prenatal case involving a 29-week-old fetus exhibiting ultrasound anomalies, notably hallux valgus on both sides, mild micromelia, and polysyndactyly. Previous chromosome, FISH, and microarray analyses initially showed a seemingly balanced complex rearrangement encompassing chromosomes 2, 7, and 14. This resulted in three derivative chromosomes with approximately five breakpoints.

Methods: Seeking further elucidation, we conducted optical genome mapping (OGM, Bionano Genomics) on ultrahigh molecular DNA extracted from amniotic fluid cells, revealing a more complex rearrangement than initially perceived.

Results: The refined characterization delineates 13 breakpoint regions with partially inverted chromosomal fragments, impacting a total of four protein-coding genes. Among the identified breakpoints, a significant finding emerged within an 8.24 kb region on chromosome 7 in band p14.1, specifically within intron 3 of the *GLI3* gene (gli-kruppel family member 3). Notably, loss-of-function variants in *GLI3* are causative for the autosomal-dominant "Greig cephalopolysyndactyly syndrome" (GCPS). GCPS is primarily characterized by finger duplication, polydactyly, syndactyly, and/or hyperphalangy. Postnatally, manifestations such as macrocephaly, hypertelorism, and developmental delay were reported with variable expressivity.

Conclusion: The implication of the disrupted *GLI3* gene as the potential genetic cause of the fetal ultrasound abnormalities is noteworthy. This case serves as a compelling illustration of the diagnostic efficacy of optical genome mapping in unraveling the genetic underpinnings behind diagnostic anomalies. The detailed insights gained from this approach not only contribute to our understanding of complex rearrangements but also hold significant implications for genetic counseling and prognosis in cases of congenital anomalies in the prenatal as well as postnatal setting.

Grants: -

Conflict of Interest: None declared

P03.026.B Prenatal manifestation of a severe form of Mitochondrial Short-Chain Enoyl-CoA Hydratase 1 Deficiency

Jana Lastuvkova[1], *Marketa Tesarova[2], Blanka Vavrinkova[3], Pavel Gerych[3], Patrik Hitka[4], Mikulas Zich[1], Vlasta Cejnova[1]*

[1]Masaryk Hospital in Ústí nad Labem, o.z., Krajská zdravotní, a.s., Department od Medical Genetics, Ustí nad Labem, Czech Republic; [2]First Faculty of Medicine, Charles University and General University Hospital in Prague, Department of Paediatrics and Inherited Metabolic Disorders, Prague, Czech Republic; [3]Jan Evangelista Purkyne University and Masaryk Hospital in Ústí nad Labem, o.z., Krajská zdravotní, a.s., Department of Gynecology and Obstetrics, Ustí nad Labem, Czech Republic; [4]Jan Evangelista Purkyne University and Masaryk Hospital in Ústí nad Labem, o.z., Krajská zdravotní, a.s., Department of Neonatology, Ustí nad Labem, Czech Republic

Background/Objectives: Mitochondrial Short-Chain Enoyl-CoA Hydratase 1 Deficiency (ECHS1D) is a very rare autosomal recessive neurodegenerative disorder with a wide phenotypic spectrum. The patients most commonly present with severe encephalopathy and lactic acidosis in the neonatal period; later manifestations in infancy are rarer. So far, the only described prenatal signs have been intrauterine growth restriction and/or oligohydramnios.

Methods: Prenatal ultrasound, Sanger sequencing

Results: We present 3rd trimester prenatal ultrasound findings of foetal hydrocephalus in two consecutive pregnancies of a healthy young woman of Mongolian origin. In the 1st pregnancy, hydrocephalus and oligohydramnios were detected at the 39th week. After birth, the male newborn was hypotonic, had elevated serum lactate levels, developed metabolic acidosis and died 39 hours postpartum. During the 2nd pregnancy, the 20-week ultrasound finding was normal, the 33-week ultrasound showed a mild dilatation of cerebral ventricles and at the 35th week foetal hydrocephalus was confirmed. After birth, the female newborn had similar symptoms as her sibling and died 41 hours postpartum. A DNA analysis in both children revealed 2 heterozygous pathogenic variants of the *ECHS1* gene (NM_004092.4): c.712G>C(p.Ala238Pro) and c.814C>T(p.Arg272Trp). Each of the variants was subsequently detected in one of the parents.

Conclusion: To our knowledge, this is the first report of prenatal hydrocephalus in ECHS1D. An ultrasound finding of foetal hydrocephalus in the 3rd trimester can be the first manifestation of this severe metabolic disease. The aforementioned parents are planning another pregnancy with the use of preimplantation genetic testing.

Grants:

Conflict of Interest: None declared

P03.027.C Cell-based noninvasive prenatal test (cbNIPT) using whole-genome sequencing (WGS-cbNIPT)

*Maria Bach Laursen[1], **Maibritt Nørgaard Lauridsen**[1], Katarina Ravn[1], Line Dahl Jeppesen[1], Anne-Marie Bruun[1], Ripudaman Singh[1], Lars Henning Pedersen[2;3;4], Ida Vogel[2;5], Lotte Hatt[1]*

[1]Arcedi Biotech, Arcedi Biotech, Aarhus N, Denmark; [2]Aarhus University Hospital, Department of Gynecology and Obstetrics, Aarhus N; [3]Aarhus University, Department of Clinical Medicine, Aarhus N; [4]Aarhus University, Department of Biomedicine, Aarhus C; [5]Aarhus University, Center for Fetal Diagnostics, Aarhus N

Background/Objectives: We aimed to investigate the performance of cell-based noninvasive prenatal test (cbNIPT) using whole-genome sequencing (WGS-cbNIPT) for detection of genome-wide copy number variants (CNV) as compared to chromosomal microarray analysis on fetal cells (CMA-cbNIPT), and on chorionic villus sampling (CMA-CVS).

Methods: Pregnant women (N = 102) scheduled for CVS were recruited (gestational week 10 + 0-14 + 6). Intact fetal cells were isolated from peripheral blood and whole-genome amplified fetal DNA was used for WGS-cbNIPT. WGS (mean coverage: 5x) and CNV analysis (VarSeq) was performed on single cells or pools (2-3 cells). For 92 women, matching CMA-cbNIPT data was available.

Results: From a cohort of 102 pregnant women, WGS-cbNIPT results were compared to CMA-CVS (55 normals/47 anomalies). Detected aberrations included twenty aneuploidies (T21/T18/T13: 16/3/1), three cases with mosaicism (T16M/T8M: 2/1), and four cases with sex-chromosome anomalies. The WGS-cbNIPT detection rate for aneuploidies was 100% (27/27). In twenty pregnancies with clinically relevant non-mosaic CNVs, WGS-cbNIPT correctly identified all pathogenic deletions (range: 0.5–33Mb. 4/4 ≥5Mb, 5/5 ≤ 5Mb). For duplications ≤5Mb, 3/6 pathogenic duplications (range: 0.4–2.8Mb) and 5/5 duplications of unknown significance (range: 0.4–1.4Mb) were detected by WGS-cbNIPT. In total, 44/47 aberrations were detected by WGS-cbNIPT compared by CMA-CVS. All normal cases were correctly identified (55/55). The performance of WGS-cbNIPT was comparable to CMA-cbNIPT, however more sensitive in detecting duplications ≤5Mb with detection rates of 100% vs 25%, respectively.

Conclusion: WGS-cbNIPT is a comprehensive screening method showing results on-par-with CMA-CVS in 44/47 samples. The performance of WGS-cbNIPT is superior to CMA-cbNIPT for detection of duplications ≤5Mb.

Conflict of Interest: Maria Bach Laursen Employed by Arcedi Biotech who holds patents of cbNIPT, Full-time at Arcedi Biotech, Maibritt Nørgaard Lauridsen Employed by Arcedi Biotech who holds patents of cbNIPT, Full-time at Arcedi Biotech, Katarina Ravn Employed by Arcedi Biotech who holds patents of cbNIPT, Full-time at Arcedi Biotech, Line Dahl Jeppesen Employed by Arcedi Biotech who holds patents of cbNIPT, Full-time at Arcedi Biotech, Anne-Marie Bruun Employed by Arcedi Biotech who holds patents of cbNIPT, Full-time at Arcedi Biotech, Ripudaman Singh Employed by Arcedi Biotech who holds patents of cbNIPT, Full-time at Arcedi Biotech, Lars Henning Pedersen: None declared, Ida Vogel: None declared, Lotte Hatt Employed by Arcedi Biotech who holds patents of cbNIPT, Full-time at Arcedi Biotech

P03.028.D The implementation of Whole Exome Sequencing in the molecular diagnosis of fetal structural anomalies: the experience of a referral Italian hospital

__Stefania Zampieri__[1], Agnese Feresin[2], Anna Morgan[1], Elisa Paccagnella[1], Elisa Rubinato[1], Daniela Mazzà[1], federica perino[2], Anna Samarin[2], Tamara Stampalija[2;3], Maurizio Pinamonti[4], Giovanni Turchetto[1], Rossana Bussani[2;4], Beatrice Spedicati[1;2], Flora Maria Murru[2;5], Giorgia Girotto[1;2]

[1]IRCCS Burlo Garofolo, Trieste, Italy, SC Genetica Medica, Trieste; [2]University of Trieste, Trieste, Italy, Department of Medicine, Surgery and Health Sciences, trieste, Italy; [3]IRCCS Burlo Garofolo, Trieste, Italy, Fetal Medicine and Prenatal Diagnosis Unit, Trieste; [4]Azienda Sanitaria Universitaria Giuliano-Isontina and University of Trieste, Pathology Department, Trieste, Italy; [5]IRCCS Burlo Garofolo, Trieste, Italy, Paediatric Radiology, Trieste, Italy

Background/Objectives: Fetal structural anomalies are responsible for ~3% of pregnancy complications, which are usually identified in the second trimester. Whole Exome Sequencing (WES) is recommended as a second-tier analysis after normal karyotype or chromosomal microarray analyses in the case of fetuses with a major malformation or multiple anomalies. This work aims to highlight the value of WES as an essential diagnostic technology in the prenatal setting by studying a deeply characterized cohort of fetuses with malformations.

Methods: A cohort of 68 fetuses with structural anomalies from 62 families, without aneuploidies or pathogenic copy number variants, and with a fulfilled post-mortem autopsy was selected for WES analysis. For all the fetuses, detailed phenotypic information (i.e., dysmorphology examination, histology, and imaging) and fetal tissue were available.

Results: In 24 cases (39.7%), we identified "likely pathogenic variants" or suggestive "variants of unknown significance" in genes with an already established genotype/phenotype association. Interestingly, in some cases, we also identified already-known pathogenic variants previously reported only in postnatal conditions, expanding their phenotypical spectrum in the prenatal setting. As an example, a female fetus with severe skeletal dysplasia carried a pathogenic variant in the *EBP* gene (c.364G>A,

p.(Glu122Lys)), which was first described in an adult woman affected by a rare X–linked dominant condition known as Conradi–Hünermann–Happle syndrome.

Conclusion: Our study demonstrated that WES can provide clinically relevant information for fetuses with malformation, allowing couples counseling, the estimation of recurrence risk, and the description of novel fetal genotype-phenotype associations.

Grants:.

Conflict of Interest: None declared

P03.029.A Residual risks for chromosomal aberrations in patients with normal NIPS (non-invasive prenatal screening) – a retrospective study

Adriana Iglesias[1], *Diane Van Opstal*[1], *Florentine Thurik*[1], *Mark Drost*[1], *Marjolein Weerts*[1], *Marieke Joosten*[1], *Karin Diderich*[1], *Vyne van der Schoot*[1], *Myrthe van den Born*[1], *Robert-Jan Galjaard*[1], *Sabina de Weerd*[2], *Anneke Dijkman*[3], *Dimitri Papatsonis*[4], *Jerome Cornette*[5], *Sander Galjaard*[5], *Maarten Knapen*[5], *Krista Prinsen*[5], *Attie Go*[5], *Kyra Stuurman*[1], *Malgorzata Srebniak*[1]

[1]*Erasmus MC Sophia Children's Hospital, Clinical Genetics, Rotterdam, Netherlands;* [2]*Albert Schweitzer Hospital, Obstetrics and Gynecology, Dordrecht, Netherlands;* [3]*Reinier de Graaf Gasthuis Hospital, Obstetrics and Gynecology, Delft, Netherlands;* [4]*Amphia Hospital, Obstetrics and Gynecology, Breda, Netherlands;* [5]*Erasmus MC, Obstetrics and Gynecology, Rotterdam, Netherlands*

Background/Objective: Systematic follow-up of pregnancies with low-risk NIPS results in the general obstetric population is currently lacking. Here, we evaluated pregnancies with low-risk NIPS results which were later referred for chromosomal microarray analysis (CMA) testing. We aimed to assess the residual risk for chromosomal aberrations in pregnancies with normal NIPS results and to indicate potential causes of discordant negative NIPS results.

Methods: Between April 2017 and March 2021, 46,004 women received a low-risk NIPS result. Cytogenomic follow-up included prenatal (primarily due to ultrasound anomalies) or postnatal CMA data. The cohort was subdivided into women receiving NIPS results for only chromosomes 13, 18, and 21 (targeted NIPS = tNIPS) and for all autosomes (genome-wide NIPS = gwNIPS).

Results: 1.7% (789/46,004) of women with normal NIPS results were referred for CMA (146/9619 tNIPS and 643/36,388 gwNIPS). The residual risk for a pathogenic CNV (excluding susceptibility CNV) in the studied general population was 1:641 (0.20%) in the tNIPS group, and 1:888 (0.15%) in gwNIPS. Between 1% (tNIPS) and 1.1% (gwNIPS) of the fetuses were referred for CMA due to ultrasound anomalies. The residual risk for a pathogenic CNV in a fetus with ultrasound anomalies (excluding susceptibility CNV) was 1:8 (13%) in tNIPS group and 1:13 (7.7%) in gwNIPS group.

Conclusions: The residual risk for a pathogenic CNV after normal NIPS result is low in the general obstetric population. However, in pregnancies affected with ultrasound anomalies, the residual risk is notably increased justifying offering invasive testing. These findings are important for pre-and post-test counseling for NIPS and invasive testing.

Conflict of Interest: Adriana Iglesias: None declared, Diane Van Opstal Will present this work as invited Speaker at The 11th World Congress on Controversies in Preconception, Preimplantation and Prenatal Genetic Diagnosis (CoGEN). May 16-17, 2024. Paris, France, Florentine Thurik: None declared, Mark Drost: None declared, Marjolein Weerts: None declared, Marieke Joosten: None declared, Karin Diderich: None declared, Vyne van der Schoot: None declared, Myrthe van den Born: None declared, Robert-Jan Galjaard: None declared, Sabina de Weerd: None declared, Anneke Dijkman: None declared, Dimitri Papatsonis: None declared, Jerome Cornette: None declared, Sander Galjaard: None declared, Maarten Knapen: None declared, Krista Prinsen: None declared, Attie Go: None declared, Kyra Stuurman: None declared, Malgorzata Srebniak: None declared

P03.030.B Effect of Polygenic Scores of Telomere Length Alleles on Telomere Length in Newborns and Parents

Yunsung Lee[1], *Astanand Jugessur*[1;2], *Haakon Gjessing*[1;2], *Jennifer Ruth Harris*[1], *Ezra Susser*[3;4], *Per Magnus*[1], *Abraham Aviv*[5]

[1]*Norwegian Institute of Public Health, Centre for Fertility and Health, Oslo, Norway;* [2]*University of Bergen, Department of Global Public Health and Primary Care, Bergen, Norway;* [3]*Columbia University, Mailman School of Public Health, New York, United States;* [4]*New York State Psychiatric Institute, New York, United States;* [5]*Rutgers University, Center of Human Development and Aging, New Jersey Medical School, Newark, United States*

Background/Objectives: In adults, polygenic scores (PGSs) of telomere length (TL) alleles explain only about 4.5% of the variance in TL, as measured by quantitative polymerase chain reaction (qPCR). Yet, these PGSs infer a causal role of telomeres in aging-related diseases. TL is primarily established by birth, making it crucial to understand how these PGSs explain TL in newborns.

Methods: This study investigates the effect of PGSs on TL in both newborns and their parents, with TL measured by Southern blotting and expressed in base-pairs (bp), in the Norwegian Mother, Father, and Child Cohort Study. Additionally, it explores the impact of PGSs of transmitted or non-transmitted alleles on TL in newborns.

Results: For parents and newborns, the PGS effects on TL were 172 bp ($p = 2.03 \times 10^{-15}$) and 161 bp ($p = 3.06 \times 10^{-8}$), explaining 6.5% and 4.9% of the TL variance, respectively. The strongest PGS effect was observed from maternally transmitted alleles in newborn girls, amounting to 214 bp ($p = 3.77 \times 10^{-6}$) and explaining 7.2% of the TL variance. The PGS effect of non-transmitted alleles was 56 bp ($p = 0.0593$) explaining 0.4% of the TL variance.

Conclusion: Our findings emphasize the importance of TL genetics in understanding early-life determinants of TL. They point to the potential utility of PGSs composed of TL alleles in identifying susceptibility to aging-related diseases from birth and reveal the presence of sexual dimorphism in the effect of TL alleles on TL in newborns.

Grants: The National Institutes of Health (R01 HL134840) and the Research Council of Norway (project number 262043 and 262700).

Conflict of Interest: Yunsung Lee National Institutes of Health (R01 HL134840) and the Research Council of Norway (project number 262043 and 262700), Astanand Jugessur National Institutes of Health (R01 HL134840) and the Research Council of Norway (project number 262043 and 262700), Haakon Gjessing National Institutes of Health (R01 HL134840) and the Research Council of Norway (project number 262043 and 262700), Jennifer Ruth Harris National Institutes of Health (R01 HL134840) and the Research Council of Norway (project number 262043 and 262700), Ezra Susser: None declared, Per Magnus National Institutes of Health (R01 HL134840) and the Research Council of Norway (project number 262043 and 262700), Abraham Aviv National Institutes of Health (R01 HL134840) and the Research Council of Norway (project number 262043)

P03.031.C Development of a non-invasive prenatal diagnostic test for rare monogenic disorders.

Styliana Philippou[1], *Skevi Kyriakou[1], Michaella Georgiadou[1], Kyriakos Tsangaras[1], Achilleas Achilleos[1], charalambos loizides[1], Christos Lemesios[1], aris pallaris[1], Chrystalla Havadjia[1], sarah barbour[1], Gaetan Billioud[1], chrysovalando soteriou[1], Louisa Constantinou[1], Lygia Ioannou[1], Antonia Matsentidou[1], Christos Prokopi[1], Melina Vaki[1], Styliana Georgiou[1], george manoli[1], Elena Kypri[1], Marios Ioannides[1], George Koumbaris[1], Philippos Patsalis[1,2]*

[1]Medicover Genetics, Nicosia, Cyprus; [2]University of Nicosia Medical School, Department of Basic and Clinical Science, Nicosia, Cyprus

Background/Objectives: Non-invasive prenatal testing for monogenic disorders is of great clinical need. While nearly 75% of dominant single gene disorders arise from de novo mutations, limited next-generation sequencing (NGS) based tests are currently available for their detection. We aim to employ our proprietary targeted capture enrichment technology in developing a non-invasive prenatal test designed to identify the most severe de novo disorders.

Methods: A 34-gene panel was developed focused on the selection of genes leading to severe autosomal dominant or X-linked conditions. A total of 1900 probes were designed to encompass all coding regions of the genes of interest. DNA samples from both pregnant and non-pregnant women, as well as artificial samples were utilized. These samples were barcoded with unique molecular identifiers (UMIs), were subjected to a novel enrichment method, and were analyzed using NGS for the specific targeted regions.

Results: The assay yielded a high raw read depth per sample, facilitating efficient UMI error correction. Accurate detection of fetal fraction (FF) was achieved, and a background substitution-based error model was trained enabling a highly accurate fetal-specific variant calling in all tested samples. More than 97% of all targeted regions were covered at 200x read depth.

Conclusion: This work establishes the groundwork for the detection of monogenic disorders caused by de novo mutations. Future work will focus on the development of an accurate non-invasive screening test for rare monogenic disorders.

Grants: Research and Innovation foundation, RESTART 2016-2020 Program for Research, Technological Development and Innovation (INNOVATE/0719(B)/0021).

Conflict of Interest: Styliana Philippou Author employed by Medicover Genetics, Skevi Kyriakou Author employed by Medicover Genetics, Michaella Georgiadou Author employed by Medicover Genetics, Kyriakos Tsangaras Author employed by Medicover Genetics, Achilleas Achilleos Author employed by Medicover Genetics, charalambos loizides Author employed by Medicover Genetics, Christos Lemesios Author employed by Medicover Genetics, aris pallaris Author employed by Medicover Genetics, Chrystalla Havadjia Author employed by Medicover Genetics, sarah barbour Author employed by Medicover Genetics, Gaetan Billioud Author employed by Medicover Genetics, chrysovalando soteriou Author employed by Medicover Genetics, Louisa Constantinou Author employed by Medicover Genetics, Lygia Ioannou Author employed by Medicover Genetics, Antonia Matsentidou Author employed by Medicover Genetics, Christos Prokopi Author employed by Medicover Genetics, Melina Vaki Author employed by Medicover Genetics, Styliana Georgiou Author employed by Medicover Genetics, george manoli Author employed by Medicover Genetics, Elena Kypri Author employed by Medicover Genetics, Marios Ioannides Author employed by Medicover Genetics, George Koumbaris Author employed by Medicover Genetics, Philippos Patsalis INNOVATE/0719(B)/0021, Author employed by Medicover Genetics

P03.032.D Prenatal Exome sequencing: results from 411 cases in Switzerland

Emmanuelle Ranza[1], *Xavier Blanc[1], Federico Santoni[1], Claire Redin[1], Marion Patxot[1], Maria Teresa Carminho[1], Guerry Frederic[2], Bernard Conrad[2], Cecile Tissot[3], Cecile Deluen[4], Tanguy Araud[4], Loïc Baerlocher[4], Audrey Méleu[2], Yasmine Sayegh-Martin[5], Anne-Claude Müller-Brochut[6], Christian Bisch[7], Katayoun Afshar[1], Marta Cotado[4], Nadia Berkane[3], Marco Belfiore[2], Fabienne Marcelli[2], Heidi Fodstad[2], Marion Krueger[2], Frédérique Bena[4], Lina Quteineh[1], Federica Capanna[8], Jean-Marie Pellegrinelli[9], Marta Carrasco[10], Nathalie Beurret[11], Chloé Barraud[11], Thibaud Quibel[12], Sylvie Lacroix[12], Nicole Jastrow Meyer[13], Wawrzyniec Rieder[7], Isabelle Eperon[7], Romaine Robyr Susini[12], Stylianos Antonarakis[1]*

[1]Medigenome, Geneva, Switzerland; [2]Genesupport, Lausanne, Switzerland; [3]Hirslanden Clinique des Grangettes, Geneva, Switzerland; [4]Genesupport, Geneva, Switzerland; [5]Centre Jean-Violette, Geneva, Switzerland; [6]Centre GynEcho, Fribourg, Switzerland; [7]Dianecho, Geneva, Switzerland; [8]GynoUs, Geneva, Switzerland; [9]University Hospitals of Geneva, Geneva, Switzerland; [10]Cabinet de Gynecologie, Nyon, Switzerland; [11]Imagerie du Flon, Lausanne, Switzerland; [12]EchoFemme, Geneva, Switzerland; [13]Hôpital La Tour, Geneva, Switzerland

Background/Objectives: Exome sequencing (ES) is increasingly used in prenatal diagnosis. We report our experience with 411 consecutive prenatal cases through ES.

Methods: Step 1 of the computational analysis was limited to gene panels according to the fetal phenotype; step 2 consisted of examining all known mendelian genes (OMIM, CGD, and literature updates).

Results: After performing a-CGH, 30 cases had solo and 381 trio ES. In 56 cases (13.6%), we have identified a variant likely causative of the fetal phenotype. A total of 17 of 56 cases were solved in Step 2 analysis. In 54 of the solved cases, the pathogenic variants were SNVs, while 2 were structural variants (SV). There were 3 cases of uniparental disomies UPD6, UPD16 and UPD17, all including isodisomic segments with likely causative recessive genes. The diagnostic yield of the various gene panels ranged from 24% for Brain Malformations to 9% for Congenital Heart Disease. Only VUS+ with a substantial likelihood of contributing to the phenotype were disclosed (24 cases, 5.84%).

Conclusion: ES is a powerful method for diagnosing causative variants prenatally; trio sequencing reduces the turnaround time (median 16 days during 2023) and increases the diagnostic yield. The fetal phenotypes of many of postnatal diagnostic entities are still to be defined. The VUS remain a challenge and guidelines are needed to assist in the interpretation and disclosure of the results.

The discovery of novel mendelian genes and additional laboratory (long-read sequencing, RNA sequencing, and improvement of SV detection) and computational methods may further increase the diagnostic yield.

Conflict of Interest: Emmanuelle Ranza Medigenome, Medigenome, Xavier Blanc Medigenome, Federico Santoni Medigenome, Claire Redin Medigenome, Marion Patxot Medigenome, Maria Teresa Carminho Medigenome, Guerry Frederic Genesupport, Bernard Conrad Genesupport, Cecile Tissot Hirslanden, Cecile Deluen Genesupport, Tanguy Araud Genesupport, Loïc Baerlocher Genesupport, Audrey Méleu Genesupport, Yasmine Sayegh-Martin Centre Jean Violette Geneva, Anne-Claude Müller-Brochut Centre GynEcho, Christian Bisch Dianecho, Katayoun Afshar CHUV, Lausanne, Marta Cotado Genesupport, Nadia Berkane Hirslanden, Marco Belfiore Genesupport, Fabienne Marcelli Genesupport, Heidi Fodstad Genesupport, Marion Krueger Genesupport, Frédérique Bena Genesupport, Lina Quteineh Medigenome, Federica Capanna GynoUs Geneva, Jean-Marie Pellegrinelli University Hospital of Geneva, Marta Carrasco Nyon Hospital, Nathalie Beurret Imagerie du Flon, Chloé Barraud Imagerie du Flon,

Thibaud Quibel Echofemme, Sylvie Lacroix Echofemme, Nicole Jastrow Meyer La Tour Hospital, Wawrzyniec Rieder Dianecho, Isabelle Eperon hirslanden, Romaine Robyr Susini Echofemme, Echofemme, Stylianos Antonarakis Medigenome, Medigenome, Necker Hospital Imagine Institute, Paris

P03.033.A A novel mechanism for the origin of androgenetic/biparental chimeras with two biparental cell lines leading to placental mesenchymal dysplasia with Beckwith-Wiedemann syndrome

Mar Xunclà[1], *Natàlia Rey*[1], *Maria Ángeles Sánchez Duran*[2], *María Serrano*[1], *Lourdes Trobo*[1], *Pedro Antonio Martínez*[1], *Neus Castells*[1], *Alberto Plaja*[1], *Jessica Camacho Soriano*[3], *Maria Àngels Rigola*[4], *Elena García-Arumí*[1], *Eduardo Tizzano*[1]

[1]*Hospital Universitari Vall d'Hebron, Department of Clinical and Molecular Genetics, Barcelona, Spain;* [2]*Hospital Universitari Vall d'Hebron, Department of Prenatal Diagnostics, Barcelona, Spain;* [3]*Hospital Universitari Vall d'Hebron, Pathology Department, Barcelona, Spain;* [4]*Universitat Autònoma de Barcelona, Cellular Biology and Medical Genetics, Spain*

Background: Placental mesenchymal dysplasia (PMD) is often related to genome-wide paternal uniparental disomy and Beckwith-Wiedemann Syndrome (BWS), mostly due to the presence of androgenetic/biparental mosaics/chimeras.

Methods: G-band cytogenetic analysis, QF-PCR STR analysis (Devyser).

Results: An amniocentesis was performed on a 15w pregnant woman suspected of PMD, who underwent termination of pregnancy at 20w. PMD and BWS fetus were confirmed by fetal autopsy. Short tandem repeat (STR) analysis of amniotic fluid detected one maternal and two different paternal chromosomal endowments. Cytogenetic analysis found three different cell lines: 45,XX,der(15;22)(q10;q10)mat, 45,XY,der(15;22)(q10;q10)mat and 46,XY. Maternal contamination was ruled out. STR and chromosomal heteromorphisms analyses indicated that all three cell lines were of fetal origin. Cell lines with the Robertsonian translocation were biparental with the same maternal but different paternal haplotype; the 46,XY cell line was androgenetic showing uniparental paternal heterodisomy. Therefore, we conclude that the fertilization leading to this fetus involved one haploid ovum and two different haploid sperms. DPM with heterodisomic androgenetic cell line has been rarely described and the proposed mechanism is a concatenation of errors including nondisjunction events, resulting in androgenetic/biparental chimeras. To our knowledge, this is the first case of PMD with two different biparental and one androgenetic cell lines.

Conclusion: We propose a new, simpler mechanism in which a three pronuclei zygote through a heterogoneic division divides directly into three cells with different haplotype that will end up in the three different cell lines. We believe this mechanism could also explain other previously described cases of PMD.

Conflict of Interest: None declared

P03.034.B Exome sequencing in 20 fetuses with structural anomalies: expanding genotype phenotype correlations.

Elodie Javey[1], *Sarah Ponthus*[2], *Audrey Schalk*[1], *Claire Feger*[1], *Manuela Antin*[1], *Emmanuelle Ginglinger*[3], *Amandine Zampa*[3], *Anne-Sophie Weingertner*[4], *Audrey Labalme*[5], *Louis Januel*[5], *Damien Sanlaville*[2;5], *Audrey Putoux*[5;6], *Jerome Massardier*[7], *Chloe Quelin*[8], *Sophie Collardeau-frachon*[9], *Salima EL CHEHADEH*[10], *Bénédicte Gerard*[1], *Cristina Antal*[11], *Nicolas Chatron*[2;5], *Elise Schaefer*[10], **Caroline Schluth-Bolard**[1;5;12]

[1]*Hôpitaux Universitaires de Strasbourg, Laboratoire de Diagnostic Génétique, Strabourg, France;* [2]*NeuroMyoGene Insititute, Laboratoire Physiopathologie et Génétique du Neurone et du Muscle, Lyon, France;* [3]*Groupement Hospitalier Régional Mulhouse Sud Alsace, Laboratoire de Génétique, Mulhouse, France;* [4]*Hôpitaux Universitaires de Strasbourg, Centre Pluridisciplinaire de Diagnostic Prénatal, Schiltigheim, France;* [5]*Hospices Civils de Lyon, Service de Génétique, Bron, France;* [6]*Centre de Recherche en Neurosciences de Lyon, GENDEV team, Bron, France;* [7]*Hospices Civils de Lyon, Centre Pluridisciplinaire de Diagnostic Prénatal, Bron, France;* [8]*CHU de Rennes, Service de Génétique, Rennes, France;* [9]*Hospices Civils de Lyon, Service d'anatomocytopathologie, Bron, France;* [10]*Hôpitaux Universitaires de Strasbourg, Institut de Génétique Médicale d'Alsace, Strabourg, France;* [11]*Hôpitaux Universitaires de Strasbourg, Service d'anatomocytopathologie, Strasbourg, France;* [12]*Institut de Génétique Médicale d'Alsace, INSERM UMRS_1112, Université de Strasbourg, Strasbourg, France*

Background/Objectives: Multiple congenital anomalies account for around 3% of live births and 20% of stillbirths. After standard genetic investigations, including karyotype, and/or chromosomal microarray analysis, greater than 60% of pregnancies do not have a genomic diagnosis.

Methods: In this French collaborative study, we performed trio-exome sequencing in deceased fetuses presenting structural anomalies of at least two systems documented by fetal autopsy, without chromosomal abnormality.

Results: We included 20 fetuses (16 termination of pregnancy, 3 intra-uterine fetal death and 1 neonatal death) aged between 11 to 39 weeks' gestation. We observed an overall diagnostic yield of 35% (7/20). Causative molecular variants were identified in 7 fetuses, involving *MYH7*, *NRAS*, *USP9X*, *KMT2D*, *DYRK1A*, *ACTN2* and *EPHB4 genes*. The variants were de novo in all but one case, in which we detected a biallelic dominant variant in *ACTN2*, each inherited from a healthy parent. Two variants (*ACTN2* and *EPHB4)* involved splicing sites and their pathogenicity was confirmed by functional studies (minigene, RNA sequencing). Two fetuses presented atypical features compared to the phenotype classically associated to the genes (*MYH7*, *NRAS*). In two fetuses (10%), variants of unknown significance involving *PKD1* were detected. For one fetus, *UBR5* was identified as a new candidate gene. Ten fetuses (50%) remained without diagnosis.

Conclusion: exome sequencing allowed to make a diagnosis in 35% of cases, contributing to guide future care and genetic counseling. Data obtained from fetal autopsy helped to conclude to the variants pathogenicity.

Grants: young researcher grants 2019 of HCL and CREGEMES.

Conflict of Interest: None declared

P03.035.C Application of the ClinGen Framework for gene-disease relationship evaluation in prenatal genetic testing

Alejandra Reyes[1], *Mariana Ferreira*[1], *Emir Zonic*[1], *Catarina Pereira*[1], *Ligia Almeida*[1], *fatima torres*[1], *Joaquin Ferzuli*[1], *Douglas deFaria*[1], *Nayla Leon Carlos*[1], *Jorge Pinto Basto*[1], *Peter Bauer*[1], *Aida Bertoli-Avella*[1]

[1]*CENTOGENE GmbH, Rostock, Germany*

Background/Objectives: Prenatal testing involving genes with limited or unclear gene-disease relationships (GDR) necessitates careful evaluation. Here we describe the approach followed in our laboratory when prenatal testing is requested or considered after a potentially relevant variant is identified in a family. Our objective

was to assess the impact of applying the ClinGen framework for GDR evaluation in prenatal testing.

Methods: We evaluated targeted prenatal testing requests received at our laboratory from January to December 2023. Requests involving genes with limited or unclear GDR, as reported in databases (e.g., OMIM, ClinGen), were selected. Acceptance criteria for performing the genetic test were primarily based on GDR evaluation using the ClinGen framework, variant classification, and clinical information.

Results: We received 18 requests for targeted testing in genes with previously limited or unclear GDR. The associated phenotypes varied but could be grouped into neurological disorders and genetic syndromes. Following ClinGen framework evaluation, two genes were upgraded to strong, nine to moderate, and seven remained with limited association. Test requests involving genes with strong or moderate associations were accepted for prenatal testing. Only two requests (2/6) from the limited association group were accepted based on specific clinical overlap with described phenotypes, additional patients in our Biodatabank, and variant strength.

Conclusion: Seventy-two percent (13/18) of test requests were accepted based on supporting GDR evidence. This is very relevant for the families/couples with genetic disorders as it impacts the clinical management of ongoing pregnancies. The 'upgrade' of 11 genes broadens our knowledge of genetic diseases and benefits future genetic diagnoses.

Conflict of Interest: Alejandra Reyes CENTOGENE, Mariana Ferreira CENTOGENE, Emir Zonic CENTOGENE, Catarina Pereira CENTOGENE, Ligia Almeida CENTOGENE, fatima torres CENTOGENE, Joaquin Ferzuli CENTOGENE, Douglas deFaria CENTOGENE, Nayla Leon Carlos CENTOGENE, Jorge Pinto Basto CENTOGENE, Peter Bauer CENTOGENE, Aida Bertoli-Avella CENTOGENE

P03.036.D DNA methylation episignatures in fetal DNA: Preliminary study on CHARGE syndrome

Nicolas Bourgon[1;2], *Amale Achaiaa*[3], *Badreddine Mohand Oumoussa*[4], *Hélène Madry*[4], *Karim Labreche*[4], *Lucile Boutaud*[2;3], *Antonio RAUSELL*[2;3], *Veronique Pingault*[2;3], *Tania Attie-Bitach*[2;3]

[1]*Necker Enfants Malades University Hospital, Obstetric and Fetal Medicine, Paris, France;* [2]*Imagine Institute, INSERM UMR1163, Paris, France;* [3]*Necker Enfants Malades University Hospital, Genomic Medicine for Rare Diseases, Paris, France;* [4]*Sorbonne University, P3S Platform - INSERM UMS 37 PASS, Paris, France*

Background/Objectives: Some Mendelian disorders have been associated with DNA methylation episignatures (DME), a specific pattern of DNA methylation. In a postnatal setting, DME performed on blood samples have been suggested as candidate biomarkers, essentially to reclassify VUS identified by genome-wide sequencing. However, their application in a prenatal setting may be challenged by DNA methylation variation across fetal development and tissues. Validation of DME in DNA extracted from fetal samples is mandatory for exploring their application in fetal disorders.

Methods: We conducted a preliminary study in a cohort of 36 individuals, including 15 patients with *CHD7* variant (7 children and 8 fetuses) and 21 controls (6 children and 15 fetuses). DNA was extracted from blood for children, and fetal DNA was extracted from lung and amniotic fluid (uncultured and cultured). DNA methylation was analysed using EPICv2 array. Raw data (iDAT) were analysed using EpigenCentral and a local pipeline. The predictive abilities of published DME were assessed for both children and fetuses.

Results: The mean detection rate of CpG dinucleotides was 99.93%. Sensitivity was 85.7%, specificity was 100% for postnatal cases, but dropped to 50.0% and 53.3%, respectively, for fetal samples. DNA methylation profiles varied significantly depending on the fetal tissue. Bioinformatics analysis are ongoing to potentially identify specific fetal DME and compare them with postnatal DME.

Conclusion: Our results confirm the validity of published episignature in a postnatal setting for *CHD7*-related disorders. However, the postnatal episignature is not applicable in a fetal setting. Further studies are needed to identify and validate specific fetal episignature.

Grants: AP-HP

Conflict of Interest: Nicolas Bourgon Investigator CYMEVALIII-2 RCT (2020-002924-35), Masterclas BioMarin prenatal diagnosis of ACH, Amale Achaiaa: None declared, Badreddine Mohand Oumoussa: None declared, Hélène Madry: None declared, Karim Labreche: None declared, Lucile Boutaud: None declared, Antonio RAUSELL: None declared, Veronique Pingault: None declared, Tania Attie-Bitach: None declared

P03.037.A New homozygous mutation c.32G>A in TMEM107 gene revealed in a fetus with a posterior fossa abnormality

Rada Staneva[1;2], **Ivelina Dimitrova**[2], *Kunka Kamenarova*[2], *Kalina Mihova*[3], *Ivanka Dimova*[3]

[1]*MBAL Nadezhda, Sofia, Bulgaria;* [2]*Medical University Sofia, Sofia, Bulgaria;* [3]*Medical University Sofia, Molecular Medicine Centre, Sofia, Bulgaria*

Background/objectives: The systematic evaluation of the brain anatomy of the fetus is an essential part of every ultrasound assessment during the pregnancy.

One of the most frequently detected congenital abnormalities of the brain are those of the posterior fossa with the incidence of 1:5000 livebirths. Furthermore, the presence of an abnormal posterior fossa of the fetus always requires to carry out an invasive testing to exclude aneuploidies or genetic syndromes.

We present the case of two consecutive pregnancies of a non-consanguineous couple, in which were detected similar abnormalities of the fetuses during the early ultrasound anatomy assessment (11 + 2 - 13 + 6 g.w.) - abnormal posterior fossa, shortening of the long bones with abnormal position of the wrists and feet, postaxial polydactyly in all four limbs.

Method: Whole exome sequencing of isolated fetal DNA was performed to detect possible genetic aberrations.

Resuts: A novel genetic variant - c.32G>A (p.Arg11His) in *TMEM107* gene in homozygous condition was detected in the fetus and classified as likely pathogenic based on the established relation to the phenotype and in silico predicted pathogenicity. The parents were found to be heterozygous carriers of the variant. Mutations in *TMEM107* gene are associated with Meckel-Gruber syndrome (MGS).

Conclusions: MGS is an autosomal-recessive condition and presents with occipital encephalocele or other CNS abnormalities, polydactily and polycystic kidneys and has a lethal outcome in utero or shortly after birth. We report a novel genetic variant detected in non-consanguineous parents. Meckel-Gruber syndrome should be suspected in the presence of posterior fossa abnormalities.

Conflict of Interest: None declared

P03.038.B Biallelic *ITGA9* variants related to chylothorax and nonimmune hydrops fetalis in three patients

Tugba Kalayci[1], *Michael E. March*[2], *Tugba Sarac-Sivrikoz*[3], *Zehra Oya Uyguner*[1], *Gulandam Bagirova*[1], *Hakon Hakonarson*[2], *Yoav Dori*[2], *Dong Li*[2]

[1]*Istanbul University, Istanbul Faculty of Medicine, Department of Medical Genetics, Istanbul, Türkyie;* [2]*The Children's Hospital of Philadelphia, Philadelphia, United States;* [3]*Istanbul University, Istanbul Faculty of Medicine, Division of Maternal-Fetal Medicine, Department of Obstetrics and Gynecology, Istanbul, Türkyie*

Background: The Integrin Alpha 9 (*ITGA9*), a member of the integrin family, plays a pivotal role in cell-cell adhesion and interactions between cells and the extracellular matrix. In murine models, biallelic null variants of Itga9 have been linked to fatal chylothorax. Morever, heterozygous missense variants reported in humans have been associated with central congenital lymphatic anomalies (CCLA). In this study, we present three patients exhibiting biallelic novel variants in the *ITGA9*, presenting varying degrees of CCLA.

Methods: Comprehensive prenatal and postmortem clinical examinations were conducted in all patients, with whole exome sequencing employed to identify disease-causing variants.

Results: The first patient, a female, exhibited prenatal-onset pleural effusion, postnatally diagnosed as chylothorax, along with labial and lower extremity lymphedema and umbilical hernia. She was found to have a splice site(c.1236+4A>G) and a missense[c.289T>G, p.(Cys97Gly)] variant in *ITGA9* inherited from each parent. In vitro assay demonstrated that the splicing variant led to exon skipping[c.1142_1236del, p.(Asp381Glufs*23)]. The other patients involved dizygotic twins with nonimmune hydrops fetalis detected prenatally, and medically terminated at 18 gestational weeks. Both fetuses had homozygous frameshift[c.818delG, p.(Gly273Alafs*17)] variants.

Conclusion: Our findings establish the first instances of biallelic *ITGA9* variants, confirming the association of *ITGA9* and CCLA. The null variants, as evidenced by the homozygous frameshift variant in our cohort, may contribute to a more severe phenotype. This study enhances our understanding of CCLA genetics and emphasizes the clinical relevance of *ITGA9*.

Grants: This study was funded by the Research Fund of Istanbul University (project no:TDK-2023-39690) to TK.

Conflict of Interest: None declared

P03.039.C Diagnostic efficiency of clinical exome sequencing in fetuses with congenital anomalies

Candy Kumps[1], *Machteld Baetens*[1], *Sofie Symoens*[1], *Ellen Roets*[2], *Noortje Van Oostrum*[2], *Olivier Vanakker*[1], *Bert Callewaert*[1], *Björn Menten*[1], *Sandra Janssens*[1]

[1]*Center for Medical Genetics, Molecular Medicine, Gent, Belgium;* [2]*Prenatal diagnostics, Gynaecology, Gent, Belgium*

Background/Objectives: Structural fetal abnormalities are detected by prenatal ultrasound in ~3% of pregnancies. Identifying a genetic cause in prenatal or fetal context is impactful, enabling prognosis for current as well as risk assessment and reproductive options for subsequent pregnancies. In addition to routine tests, i.e. NIPT and CNV sequencing with diagnostic uptake rates up to 15%, exome sequencing (ES) based analysis has become feasible in clinical practice. We assessed the diagnostic value of ES-based virtual gene panel analysis, in fetuses with congenital abnormalities.

Methods: This retrospective study (2020-2022) reports on (trio) ES-based analyses of 99 fetuses with ultrasound abnormalities, pregnancy terminations or mors in utero with neither a clear environmental etiology, nor explanatory NIPT or CNV sequencing result.

Results: ES-based analysis obtained a molecular diagnosis in 25.3% of cases, i.e. in 27.3% of cases with multiple organ (n = 55) and 22.7% of cases with single organ (n = 44) anomalies. In particular, skeletal dysplasia (50%), multiple congenital anomalies (26.2%), congenital heart disease (16.7%) and nephrology (10%) virtual gene panels, including 522, 4878, 471 and 343 genes, harbored the highest number of diagnoses, comprising 52% inherited and 48% de novo variants with a limited number of incidental findings (n = 1).

Conclusion: Generally, ~15% of fetuses with ultrasound abnormalities have an abnormal karyotype or submicroscopic CNV confirming diagnosis. ES-based virtual gene panel analyses offer a significant additional diagnostic value to routine analyses, increasing the overall diagnostic yield to an estimated 40% in our cohort. The approach limits incidental findings, while offering a systematic laboratory workflow.

Grants:NA

Conflict of Interest: None declared

P03.040.D *RMRP* and *RNU4ATAC* reportable variants in prenatal exome sequencing: the value of including non-coding genes in exome enrichments.

Louise McClelland[1], *Rebecca Keelagher*[1], *Harjeet Rai*[1], *Deirdre Cilliers*[2], *Samuel Clokie*[3], *Stephanie Allen*[1]

[1]*West Midlands Regional Genetics Laboratory, Birmingham, United Kingdom;* [2]*Oxford Centre for Genomic Medicine, Oxford, United Kingdom;* [3]*Nonacus, Birmingham, United Kingdom*

Background/Objectives: Prenatal exome sequencing (pES) for fetuses with structural abnormalities has been offered within the NHS since October 2020. Most whole exome sequencing (WES) enrichments only capture protein-coding exons with limited coverage of UTRs or non-coding genes (PMID: 37082142). Here we summarise three years of pES service and demonstrate the utility of including clinically-relevant non-coding genes within WES capture.

Methods: We report results from trio pES using the Nonacus ExomeCG WES enrichment and filtered for a panel of genes associated with fetal anomalies. These results represent the first 3 years of service from October 2020 to November 2023.

Results: During this time period, 667 pregnancies were tested, with diagnoses made in 29% of cases (194/667). Diagnoses were in 117 different genes, with recurrence in 31 genes. Most common were *PTPN11* (n = 11; Noonan syndrome), *CHD7* (n = 7; CHARGE syndrome), *COL2A1* (n = 7), *FGFR3* (n = 6), *COL1A1* (n = 5), *PIEZO1* (n = 5), *COL1A2* (n = 4), *DHCR7* (n = 4), *DYNC2H1* (n = 4), *RAF1* (n = 4). Three confirmed diagnoses and one probable diagnosis were identified in non-coding genes, representing 2.1% of the positive diagnostic yield (*RMRP* n = 3; *RNU4ATAC* n = 1). These cases will be presented.

Conclusion: Use of a whole exome approach allows rapid reporting in a prenatal setting with a high diagnostic yield in fetuses with structural anomalies. Exome enrichments focus on protein-coding regions and therefore miss detection of variants in non-coding genes. Inclusion of clinically-relevant non-coding genes within exome enrichments enhances diagnostic yield and negates the need to proceed with further testing such as WGS in these cases.

Grants: N/A

Conflict of Interest: None declared

P03.041.A Evaluation of the clinical utility of using SNP-array chromosomal analysis in prenatal diagnosis

Timothée Moyret[1], *Massymissa Braik*[1], *Lucie Guilbaud*[2], *Nicolas Rive Le Gouard*[1], *Sarah Grotto*[3], *Mikaël Tassin*[4], *Daphné Lehalle*[3], *Anne Gael Cordier*[4], *Solveig Heide*[5], *Sandra Chantot-Bastaraud*[1], *Marc Dommergues*[6], *Valentine Faure*[6], *Delphine Heron*[3;5], *Jean-Marie Jouannic*[2], *Laïla El Khattabi*[1;7]

[1]*Chromosomal Genomics, Department of medical genetics, Armand Trousseau hospital, AP-HP Sorbonne Université, Paris, France;* [2]*Fetal medicine, DMU ORIGYNE, Armand Trousseau hospital, AP-HP Sorbonne Université, Paris, France;* [3]*Clinical Genetics, Armand Trousseau hospital, AP-HP Sorbonne Université, Paris, France;* [4]*Obstetrics and Gynecology, Tenon hospital, AP-HP Sorbonne Université, Paris, France;* [5]*Clinical Genetics, Department of medical genetics, Pitié-Salpêtrière hospital, AP-HP Sorbonne Université, Paris, France;* [6]*Obstetrics and Gynecology, Pitié-Salpêtrière hospital, AP-HP Sorbonne Université, Paris, France;* [7]*Brain development team, ICM - Paris Brain Institute – Pitié-Salpêtrière, Paris, France*

Background/Objectives: DNA-microarray analysis has become widely used for prenatal diagnosis in high-risk pregnancies. Current recommendations suggest their use at least in cases of elevated nuchal translucency, severe intrauterine growth restriction (IUGR) or ultrasound (US) abnormalities. Comparative genomic hybridization is the most used approach although SNP-arrays offer an added-value of detecting uniparental disomy. This advantage has been evaluated only in few studies. We, retrospectively, evaluated the diagnosis rate, including uniparental disomy, when using SNP-arrays in our prenatal population over 3 years.

Methods: In this retrospective cohort study, we collected results from SNP-array analysis using the Affymetrix platform in a context of prenatal diagnosis in fetuses presenting mostly with US abnormalities. We calculated the diagnosis rate of copy number variations or uniparental disomy, according to the category of US aberrations. Trio analysis was preferentially performed. We compared our results to published data using CGH-microarray.

Results: Out of 745 pregnancies included, we identified 61 chromosomal anomalies (8.2%), among which 5 uniparental disomies (0.7%) (2 upd(15), 1 upd(16) and 2 upd(22)) all of which were associated with IUGR. Among the published cohorts, 27631 fetuses were investigated using SNP-arrays, 15 uniparental disomies were identified (0.05%), with high variability between the different studies.

Conclusion: The rate of uniparental disomy identified by SNP-arrays in prenatal diagnosis is low, but their identification can significantly impact the pregnancy management. This is particularly interesting in case of IUGR. This approach provides also additional information, compared to CGH, such as the identification of triploidies and maternal contamination.

Conflict of Interest: None declared

P03.042.B Enhancing non-invasive prenatal screening: a comprehensive analysis of Trisomy test performance, clinical implications, and future directions

Michaela Hyblova[1], *Andrej Gnip*[1], *Dominik Hadzega*[1], *Oliver Petrovic*[1], *Gabriel Minarik*[1]

[1]*Trisomy s.r.o., Nitra, Slovakia*

Background/Objectives: Since its launch in 2016, the Slovak Trisomy test has evolved significantly from detecting whole-chromosome aneuploidies to identifying subchromosomal aberrations and advancing towards nucleotide-level analysis. The test is noted for its highly competitive sensitivity and specificity across all chromosomal aberations, including those involving sex chromosomes. It differentiates between fetal and maternal aberrations, significantly enhancing screening quality and expanding the range of screening options

Methods: We analysed more than 30,000 plasma samples from pregnant women, utilizing low-coverage whole-genome sequencing for comprehensive screening. The methodology for circulating cell-free DNA isolation and DNA library preparation adheres to previously established protocols. We targeted 5 million reads for whole-chromosome aneuploidy detection, while copy number variants required 20 million reads.

Results: The positive predictive values (PPV) for trisomy 21, 18, 13, X0, XXY, XYY, XXX, rare autosomal trisomies (RAT), and copy number variations (CNV) are 99.09%, 97.96%, 86.21%, 38%, 67%, 70.9%, 57.5%, 15%, and 75%, respectively. The overall rate of non-informative results after the second collection, defined as a fetal fraction below 4%, is significantly below 1%.

Conclusion: Non-invasive prenatal testing has evolved significantly beyond its initial scope as a screening tool for assessing fetal health. With its high PPV, it is now considered not just a screening test but also a diagnostic tool for maternal genetic findings and markers that could impact both current pregnancy outcomes and future fetal health. The use of non-invasive testing as a liquid biopsy is continually advancing, promising to revolutionize genetic screening and care in unprecedented ways

Grants: none

Conflict of Interest: Michaela Hyblova part-time, Andrej Gnip consultant, Dominik Hadzega part time, Oliver Petrovic part-time, Gabriel Minarik part-time

P03.043.C Exome and genome sequencing as time effective methods for prenatal diagnosis: overview of 679 patients

Catarina Pereira[1], *Ligia Almeida*[1], *Alejandra Reyes*[1], *Omid Paknia*[1], *Aida Bertoli-Avella*[1], *Jorge Pinto Basto*[1], *Peter Bauer*[1]

[1]*CENTOGENE GmbH, Rostock, Germany*

Background/objectives: Prenatal exome (ES) and genome sequencing (GS) have the potential to enable a more comprehensive and time effective genetic diagnosis in fetuses with sonographic abnormalities for single-nucleotide variants and copy number variations (CNVs). We present our experience using prenatal ES/GS solo or trio.

Methods: The study cohort comprised referred cases within a two-year period (January 2022-January 2024). ES/GS was performed for 130 trio and 545 solo cases of ongoing or terminated pregnancies with fetal abnormalities or positive family history of severe early onset disorders.

Results: From 679 cases evaluated, a 30% (202 cases) diagnostic yield with a clear genetic diagnosis including SNV and CNVs was achieved. For 1.8% (12 cases), analysis could not be performed due to sample quality related issues. For the trio analysis, the simultaneous study of the parents allowed the identification and reporting of de novo variants in 8/27 positive cases. A 27% (54/202 cases) diagnostic rate with CNVs was achieved (from single exon deletions to whole chromosome aneuploidies). The analysis of these cases is performed in a seamless and straightforward workflow allowing a turnaround time (TAT) of less than 15 days.

Conclusion: Prenatal ES and GS contributed to the clinical management in a single step analysis with a diagnostic yield of 30% in fetuses mostly presenting with structural anomalies. The short TAT enables the identification of early onset and severe diseases which would have not been diagnosed by other technologies in the same timeframe. This data suggests an actionable impact for the clinical management and genetic counseling of families.

Conflict of Interest: None declared

P03.045.A RhD variants: when serology reaches its limits - observation in the determination of the fetal rhesus factor from maternal blood

Sofia Isabell Rupp[1], *Thomas Hackenbeck*[1], *Gudrun Göhring*[1]

[1]*amedes genetics, Hannover*

Background/Objectives: The blood group including rhesus factor is determined by serologically at the latest during pregnancy. If the rhesus factor is negative, pregnant women are able to analyse fetal rhesus factor of their unborn child molecularly from maternal blood as a health insurance benefit (Germany) since 01.07.2021 in order to enable targeted anti-D prophylaxis.

Methods: A commercial kit was used to determine the fetal rhesus factor (ingenetix GmbH, Hüttenbrennerg 5, 1030 Wien). The Ct-values generated or not detected indicate whether the unborn child is positive or negative. The raw data were analysed in regard to the presence of a previously unkown RhD variant.

Results: The methodology used has revealed the presence of an unexpected high percentage of previously unknown RhD variants in analysed pregnant women. In 2022, 473 suspected RhD variants were detected (samples received: 24599). We were able to identify two main groups: RhD variants that occur in the mother herself and mask the analysis or RhD variants that were found in the unborn child.

Conclusion: The data collected could not yet be examined with further molecular analyses. However, the initial results indicate that there is an increase in RhD variants in Germany. If they remain undetected and no anti-D-prophylaxis is given, RhD variants could have serious consequences and, in the worst case, lead to haemolytic neonatal disease. The results demonstrate how important additional molecular analyses are for blood group and rhesus determination as there are many RhD variants that cannot be detected serologically.

Grants: None.

Conflict of Interest: None declared

P03.046.B Comparative analysis of dPCR and NGS-based NIPT methods for fetal aneuploidy detection

Veronika Korenika[1], *Samanta Strojeva*[1], *Sandra Martinsone*[1], *Ludmila Volozonoka*[2], *Mikus Gavars*[1]

[1]*E. Gulbis Laboratory, Riga;* [2]*Riga Stradinš University, Scientific Laboratory of Molecular Genetics, Rīga*

Background/Objectives: Non-invasive prenatal testing (NIPT) has become a widely adopted approach for screening fetal aneuploidies. While next-generation sequencing (NGS) is the predominant technology in NIPT, digital PCR (dPCR) has recently emerged as an alternative method for absolute target quantification.

Methods: A total of 100 samples, with an average gestational week of 14 (±4.3 SD), were assessed. Cell-free plasma DNA from pregnant women was subjected to NIPT using both NGS and dPCR methods. The evaluation included analysis for chromosome 13, 18, 21, and sex chromosomes.

Results: Comparative analysis revealed no difference in sex determination between the two methods. On average, dPCR-based NIPT exhibited reduced fetal fraction estimation by 3.5 percentage points or 29% ($p < 0.001$) for male and 3.5 percentage points or 34% ($p < 0.001$) for female fetuses. The specificity of dPCR NIPT for chromosome 21, 18, 13, and X was 97.9%, 93.9%, 100%, and 88.5%, respectively. An investigation was conducted on a case involving a confirmed vanishing twin, wherein the developing fetus was female. NGS-based NIPT indicated no aneuploidy and suggested a male fetus gender. In contrast, dPCR NIPT revealed a general fetal fraction of 6.2%, while the fetal fraction based on Y chromosome count was 1.8%.

Conclusion: dPCR-based NIPT stands out as a fast (~ 24h turnover from sample receipt to report) and economically efficient screening technology when compared to NGS-based NIPT. Its promising capabilities position dPCR NIPT as a tool capable of surmounting the challenges associated with NGS-based NIPT.

Conflict of Interest: None declared

P03.047.C Hemoglobin screening and genetic counseling in prenatal diagnosis for the prevention of hemoglobinopathies in Albania

Suela Leli[1], *Merita Xhetani*[2], *Eliona Demaliaj*[3], *Irena Korita*[4]

[1]*University Hospital Obstetrics - Gynecologic "Mbreteresha Geraldine", Center for Molecular Diagnostics and Genetic Research, Tirane, Albania;* [2]*University of Tirana, Faculty of Natural Sciences, Department of Biology, Tirane, Albania;* [3]*University of Medicine, University Hospital Obstetrics Gynecologic, Department of Obstetrics gynecologic, Tirane, Albania;* [4]*University of Medicine and University Hospital "Mother Tereza", Department of Clinic laboratory, Tirana, Albania*

Consortium: NanoAlb

Background/Objectives: Screening for carriers of hemoglobinopathies has been included in the routine protocols of examinations for all pregnant women. Here we present data from pregnant women diagnosed with a combine screening and molecular diagnosis from our center within one year.

Methods: We analyzed 50 pregnant women hospitalized in the Department of Pregnancy Pathology at UHOG in the 1st trimester of pregnanc. Whole blood with K3 EDTA was used for HGB electrophoresis by the Mini Lite device; cell counter for blood parameters and for DNA extraction in samples with abnormalities in the percentage of Hb fractions. Molecular diagnosis was done by PCR Reverse dot blot using Strip Assay®.

Results: Of the 50 pregnant women analyzed, 10 (20%) showed abnormalities in the percentage of Hb fractions. Out of 10 women, 4 with sickle cell anemia and 6 carriers of thalassemia minor with these HbA fraction values were 58.1%; HbS 37.8%; HbA2 4.1%, HbA 62.2%; HbS 34.5%; HbA2 3.3%, HbA 55.6%; HbS 40.8%; HbA2 3.6%, HbA 57.2%; HbS 38.8%; HbA2 4%. Molecular diagnosis report heterozygous mutation in codon 6 [A>T] HbS in individuals suspect sickle cell anemia and IVS-I-110 (G->A), Codon 39 (C->T) and IVS-I-6 (T ->C) in heterozygous in β-thalassemia minor suspect individuals. Genetic counseling for the couples was done, and 2 prenatal diagnosis was performed.

Conclusion: Hereditary anemia is evident in a Mediterranean country like Albania (our result of 20% out of 50 shows this). The only way of prevention is informing the population, screening, genetic counseling and prenatal diagnosis if both parents are carriers.

Conflict of Interest: None declared

P03.048.D Enhancing Fetal Outcomes in GCK-MODY Pregnancies: A Precision Medicine Approach via Non-Invasive Prenatal GCK Mutation Detection

Valerie Schwitzgebel[1], *Jean-Louis Blouin*[2], *Barbara Dehos*[3], *Bettina Kohler-Ballan*[4], *Jardena Puder*[5], *Claudine Rieubland*[6], *Maria Triantafyllidou*[7], *Anne Zanchi*[8], *Marc Abramowicz*[2], **Thierry Nouspikel**[2]

[1]*Geneva University Hospitals, Pediatric Endocrine and Diabetes Unit, Genève;* [2]*Geneva University Hospitals, Genetic Medicine, Genève;* [3]*Spital Grabs, Division of Endocrinology and Diabetes, Grabs, Switzerland;* [4]*Geneva University Hospitals, Department of Infectious*

Diseases, Genève; [5]Lausanne University Hospital, Department Women-Mother-Child, Lausanne, Switzerland; [6]Hospital of the Valais, Department of Medical Genetics, Sion, Switzerland; [7]Luzerner Kantonsspital, Division of Endocrinology, Diabetes and Clinical Nutritio, Lucerne, Switzerland; [8]Lausanne University Hospital, Service of Endocrinology, Diabetes and Metabolism, Lausanne, Switzerland

Background/Objectives: Mutations in the *GCK* gene cause Maturity Onset Diabetes of the Young (MODY-GCK, MODY2) by impairing glucose-sensing in pancreatic beta cells. Diabetes management during pregnancy depends on fetal genotype: Fetuses carrying a *GCK* mutation can benefit from moderate maternal hyperglycemia, stimulating insulin secretion in fetal islets, whereas this may cause macrosomia in wild-type fetuses. Modulating maternal glycemia is thus a form of personalized prenatal therapy, highly beneficial but not justifying the risk of invasive testing. We therefore developed a monogenic non-invasive prenatal diagnostic (NIPD-M) test to detect the transmission of known maternal *GCK* mutations to the fetus.

Methods: Small amounts of fetal circulating cell-free DNA are present in maternal plasma but cannot be distinguished from maternal cell-free DNA. Determining transmission of a maternal mutation to the fetus thus implies sequencing adjacent polymorphisms to determine the balance of maternal haplotypes, the transmitted haplotype being over-represented.

Results: We present a series of such tests in which fetal genotype was successfully determined and show that it can be used to guide therapeutic decisions during pregnancy, improving the outcome for the offspring. We discuss the principal hurdles inherent to the haplotyping approach, and strategies to overcome these. We also introduce a metric to detect several potentially confounding situations, like placental mosaicism or vanishing twins.

Conclusion: Our NIPD-M test allows reliable determination of the presence of a maternal *GCK* mutation in the fetus, thereby enabling personalized in-utero therapy by modulating maternal glycemia, without incurring the risk of miscarriage inherent to invasive testing.

Grants: None

Conflict of Interest: None declared

P03.049.A Whole Exome Sequencing- first tier test for fetuses with severe central nervous system anomalies

Natalia Salgueiro[1], *Lisandra Castro[1], Ariana Conceição[1], Elsa Garcia[1], Claudia Alves[1], Carolina Dias[2], Maria Lopes Almeida[3], Fabiana Ramos[4], Nuno Pereira[2], Lina Ramos[4], Margarida Reis-Lima[1]*

[1]SynlabHealth Genética Médica, Porto, Molecular Genetics Department, Porto, Portugal; [2]Viseu Hospital, Obstetrics Gynaecology, Fetal Medicine unit, Viseu, Portugal; [3]Guimarães Hospital, Medical Genetics Department, Guimaraes, Portugal; [4]Coimbra Pediatric Hospital, Medical Genetics Department, Coimbra, Portugal

Background/Objectives: Next–generation sequencing technology, such as targeted multigene panels or Whole Exome sequencing (WES) is sometimes used as a second–or third–option diagnostic test in selected cases of central nervous system (CNS) fetal anomalies. Recent studies have shown that WES provides an additional rate of 5–57% over that of chromosomal microarray analysis (CMA) in prenatal setting.

We retrospectively studied 21 cases referred to our center for genetic evaluation due to CNS anomalies. Five of these cases were evaluated after termination of pregnancies (TOP) and 19 were ongoing pregnancies.

Methods: WES analysis were performed in all cases. Bioinformatic analysis was focused on our 60 gene panel for congenital anomalies of nervous system and mainly on the targeted phenotype. In all cases the bioinformatic analysis included copy number variants (CNVs).

Results: In 28,7% (6/21) of the cases, our study identified a genetic aetiology for the fetus phenotype. Of these 6 diagnosed cases, 2 were "de novo" autosomal dominant (AD) condition, 3 were X linked diseases, and the last one was an inherited autosomal recessive (AR) condition.

Conclusion: In our study, WES provided a high diagnostic rate (around 30%) in fetuses with CNS anomalies. These results demonstrated that an accurate identification of the ultrasound detected anomalies is crucial for a successful diagnosis. These data suggest that WES may be considered a major tool for the prenatal diagnosis of fetal CNS anomalies. This is of particular importance given the time constraints of an ongoing pregnancy and the risk of recurrence in future pregnancies.

Conflict of Interest: None declared

P03.050.B Size Selection impact on Fetal Fraction estimates by SeqFF for late Gestational Age in NIPS

seyedeh saideh daryabari[1], *Sylvie Giroux[1], andre caron[1], francois rousseau[1,2]*

[1]Université Laval, Centre de recherche du CHU de Québec-, Québec, Canada; [2]Université Laval, Department of Molecular Biology, Medical Biochemistry and Pathology, Faculty of Medicine, Quebec, Canada

Background/Objectives: Noninvasive prenatal screening (NIPS) uses cell-free DNA (cfDNA) from maternal plasma to detect fetal abnormalities. Fetal fraction estimation is influenced by factors like gestational age (GA), prompting a need for comprehensive assessment to optimize screening protocols. This study investigated GA's impact on fetal fraction and SeqFF precision in samples with advanced gestational ages.

Methods: Peripheral blood samples were collected from 24 pregnant women across two distinct gestational age groups (>25 weeks and <16 weeks). In-vitro and in-silico size selection techniques were applied to increase smaller cfDNA fragments abundance. FF estimation was conducted using SeqFF and RAPIDR algorithms after paired-end sequencing.

Results: Results revealed that size selection significantly increased fetal fraction, in samples <16 weeks, SeqFF results were consistent with RAPIDR results. However, SeqFF unexpectedly resulted in a decrease in FF in samples >25 weeks, which diverged from RAPIDR estimates. In silico size selection confirmed this trend, suggesting that SeqFF may lose crucial fragments in samples from late GA.

Conclusion: Smaller fragment selection improves FF, sensitivity, and specificity of NIPS, yet SeqFF is no longer working for samples over 25 weeks. This observation suggests that some genomic locations used by SeqFF to estimate FF might not be filled by fragments after size selection. Without size selection, fragments are preserved for both groups of GA. Further research is needed to explore cfDNA fragment size dynamics during pregnancy to advance our understanding of cfDNA biology.

Grants: Genome Canada Project ID 13527

Conflict of Interest: None declared

P03.051.C Prenatal phenotype of Coffin-Siris Syndrome: Insights from a case series

Giulia Vitetta[1], *Vera Uliana[2], Enrico Ambrosini[2], Mina Grippa[3], Valeria Barili[2], Sofia Cesarini[2], Giulia Lanzoni[1], Giulia Severi[1], Antonio Percesepe[2]*

[1]IRCCS AOU Policlinico S. Orsola, Medical Genetics, Bologna, Italy; [2]University of Parma - Hospital, Medical Genetics, Parma, Italy; [3]AOU Policlinico di Modena, Medical Genetics, Modena, Italy

Background: Coffin-Siris syndrome (CSS) is an autosomal dominant disorder characterized by developmental delay, intellectual disability, dysmorphic features, and congenital anomalies. Its aetiology involves mutations in various genes, leading to a broad phenotypic spectrum.

Methods: We conducted a retrospective collection of prenatal CSS cases, employing detailed morphological ultrasound examinations and genetic testing, including exome sequencing and targeted gene panel analysis.

Results: Three out of six patients in our cohort exhibited a likely gene-disrupting variant in the ARID1B gene; each of them presented brain anomalies (colpocephaly, hydrocephalus, partial corpus callosum agenesis) associated with other minor malformations. In Case 4, a terminated pregnancy revealed hypoplasia of the cerebellar vermis, bilateral clubfoot, and a conotruncal heart defect upon morphological ultrasound. Exome sequencing identified a de novo truncating pathogenic variant (c.1991C>G,p.Ser664Ter) in the ARID1A gene. Case 5 involved an infant who passed away at six months of age due to a severe syndromic condition, displaying prenatal agenesis of the corpus callosum, colpocephaly, and polyhydramnios. Postnatal exome analysis detected a de novo pathogenic variant (c.1121G>A,p.Arg374Gln) in the SMARCB1 gene. Case 6 followed up on a prenatal diagnosis marked by IUGR, cystic hygroma and partial corpus callosum agenesis. Upon re-evaluation at six years of age, a panel of CSS genes revealed a de novo pathogenic variant, c.3641T>G (p.Ile1214Ser), in the SMARCA4 gene.

Conclusions: We present six unreported prenatal CSS cases outlining the distinct phenotypes and outcomes observed based on the identified genes, providing new insight into the spectrum of prenatal diagnostic signs linked to this condition.

Conflict of Interest: None declared

P03.052.D Prenatal Genome-Wide Sequencing analysis in Central Nervous System Anomalies: systematic review and meta-analysis

Enrica Marchionni[1], *Daniele Guadagnolo*[1], *Gioia Mastromoro*[1], *Antonio Pizzuti*[1]

[1]*Sapienza University of Rome, Department of Experimental Medicine, Rome, Italy*

Background: Prenatal Genome-Wide Sequencing analysis showed a significant incremental diagnostic yield over karyotype and chromosomal microarray analysis (CMA) in fetal structural anomalies.

Presently, Prenatal Exome Sequencing (pES) is the predominant sequencing approach in prenatal clinical practice, however prenatal genome sequencing (pGS) studies are emerging. The aim of this study was to assess the incremental diagnostic yield of Prenatal Genome-Wide Sequencing analysis (pES or pGS) in fetuses affected by apparently isolated and non-isolated Central Nervous System (CNS) anomalies.

Methods: A systematic review on antenatal diagnosed CNS anomalies was performed according to PRISMA guidelines, including $n = 12$ paper, accounting for 428 fetuses. Results were pooled in a meta–analysis fitting a logistic random mixed-effect model. The primary effect of interest was the incremental diagnostic rate of pES over karyotype/CMA in detecting likely pathogenic/pathogenic Single Nucleotide Variants (SNVs). A further meta-analysis adding the available pGS studies (including diagnostic coding SNVs only) and submeta-analysis on three CNS subcategories were also performed.

Results: The pooled incremental diagnostic yield estimate of pES studies was 38% (95% C.I.: [29%;47%]) and 36% (95% C.I.: [28%;45%]) when including diagnostic SNVs from pGS studies. The point estimate of the effect resulted 22% (95% C.I.: [15%;31%] in apparently isolated anomalies, 33% (95% C.I.: [22%;46%]) in CNS-only related anomalies (≥1) and 46% (95% C.I.: [38%;55%]) in non-isolated anomalies (either ≥ 2 anomalies in CNS, or CNS and extra-CNS).

Conclusion: Meta-analysis and sub meta-analysis showed a substantial diagnostic improvement in performing Prenatal Genome-Wide Sequencing analysis over karyotype and CMA in CNS anomalies.

Conflict of Interest: None declared

P03.053.A Tortuous arteries : a new prenatal sign of Kabuki 2 ?

Alice BOURGES[1;2], *Françoise Boussion*[3], *Agnes Guichet*[1;2], *Florence Biquard*[3], *Mathilde Becmeur-Lefebvre*[4], *Clara Houdayer*[1;2], *Vincent Procaccio*[1;2], *Frederic Tran Mau Them*[5], *Antonio Vitobello*[5], *Estelle Colin*[1;2]

[1]*University Hospital of Angers, Genetics, Angers, France;* [2]*University of Angers, Mitovasc Unit, UMR CNRS 6015 INSERM 1083, Angers, France;* [3]*University Hospital of Angers, Gynecology and Obstetrics, Angers, France;* [4]*Hospital of Orléans, Genetics, Orleans, France;* [5]*University Hospital of Dijon, Genetics, Dijon, France*

Background/Objectives: Kabuki Syndrome (KS), a rare genetic disorder, is caused by pathogenic variations in *KMT2D* and *KDM6A*. There is a significant lack in the literature regarding the description of signs during the prenatal stage. Reports indicate the presence of organ malformations in KS during pregnancy, especially cardiac anomalies. These include aortic insufficiency and stenosis, hypoplastic left heart syndrome, valve-related problems, and coarctation of the aorta.

Methods: A woman was referred to our center at 22 weeks of gestation (WG) because of vascular abnormalities. These findings were subsequently confirmed at 25 WG + 2, revealing a tortuous aortic arch, a dolicho-aorta, sinuosity of the right common carotid artery and IUGR. The first hypothesis raised was the syndrome of tortuous arteries.

Results: The array performed on amniotic fluid was normal. Trio exome sequencing evidenced a heterozygous pathogenic variant in *KDM6A* inherited from her healthy mother, who carries it at a low mosaic level. No pathogenic variant has been identified in *SLC2A10*. The fetal pathology examination confirmed the morphological anomalies. Even though we know that *KDM6A* has an essential function in the differentiation of embryonic stem cells and the development of tissues, including cardiac tissues, no comparable phenotype had been described until now.

Conclusion: We report the first case of prenatal tortuous aortic arch in KS. Although this case is unusual, clinicians should be aware of the possibility of prenatal tortuous arteries in patients with KS.

Grants: none

Conflict of Interest: None declared

P03.054.B NGS based analysis in fetuses with isolated brain anomalies

Susanne Leidescher[1], *Moneef Shoukier*[1], *Birgit Anwald*[1]

[1]*Eurofins Humangenetik und Pränatal-Medizin MVZ GmbH, München, Germany*

Background/Objectives: Identifying the genetic cause of fetal brain anomalies is a diagnostic challenge. We present the results of NGS based analysis of fetuses with isolated brain malformations analyzed from January 2018 until November 2023.

Methods: 344 prenatal cases with isolated brain anomalies were analyzed by targeted NGS of the coding regions of selected genes in a custom designed panel analysis. When necessary, pathogenicity assessment of variants was performed by familial co-segregation analysis. Variants were categorized into classes 1 to 5 (ACMG-AMP guidelines (2015)). Fetal karyotypes were normal.

Results: Likely pathogenic (class 4) and pathogenic (class 5) variants were identified in 62/344 (18%) cases (autosomal dominant: 29/62 (47%), autosomal recessive: 17/62 (27%), X-linked dominant: 1/62 (2%), X-linked recessive: 7/62 (11%). The mutations were found in 35 different genes. In eight cases copy number variations were detected (8/62 (13%)). Variants of uncertain significance (class 3) were detected in 31 cases (9%) (in 29 different genes). No class 3-5 variants were identified in 251/344 fetuses (73%). Furthermore, we specify phenotypical subgroups and assess diagnostic yield as well as causative mutations within these groups.

Conclusion: Genetic causes can often be detected in isolated fetal brain malformations. It is therefore advisable to carry out a genetic analysis for corresponding indications, particularly with regard to a possible risk of recurrence in the event of a further desire to have children. The expansion of genetic analyses including comprehensive exome sequencing in a trio setting is expected to increase the diagnostic yield in the future.

Grants:

Conflict of Interest: Susanne Leidescher Eurofins Humangenetik und Pränatal-Medizin MVZ GmbH, Moneef Shoukier Eurofins Humangenetik und Pränatal-Medizin MVZ GmbH, Birgit Anwald Eurofins Humangenetik und Pränatal-Medizin MVZ GmbH

P03.055.C Beyond prenatal exome sequencing. Lessons learned from postnatal follow up of patients with inconclusive prenatal testing

Irene Valenzuela Palafoll[1], *Anna Abulí*[1], *Amaia Lasa-Aranzasti*[1], *Laura Trujillano*[1], *Eulàlia Rovira-Moreno*[1], *Marta Codina*[1], *Barbara Masotto*[1], *Núria Martínez-Gil*[1], *Patricia Muñoz-Cabello*[1], *Mar Costa-Roger*[1], *Alejandro Moles-Fernandez*[1], *Berta Campos*[1], *Paula Fernandez-Alvarez*[1], *Jordi Leno*[1], *Anna Maria Cueto-González*[1], *Elena García-Arumí*[1], *Eduardo Tizzano*[1]

[1]*Hospital vall d'hebron, Clinical and Molecular Genetics, Spain*

Prenatal exome sequencing (PES) increases genetic diagnoses in fetuses with ultrasound structural abnormalities and a normal microarray. Published diagnostic yields from PES differ between studies and may be influenced by sample size, case selection and fetal phenotype.

This study aims to assess the evolution of patients born with inconclusive prenatal exome results. Our cohort includes 35 children born between 2018-2023. Postnatal follow up in this cohort ranged from 6 months to 4 years.

Fifteen children showed adequate postnatal development in the last review. Most indications for PES in these children included minor anomalies of the central nervous system and intrauterine growth retardation. In the remaining 20 cases, suspicion of an unexplained genetic entity persisted. Postnatal exome re-analysis was conducted in 18, including new postnatal phenotypic manifestations. Molecular diagnosis was achieved in two (11%). One of the children corresponded to orofaciodigital syndrome (homozygous variant in a novel gene) and the other to a Rubinstein-Taybi with a likely pathogenic variant in *EP300*. In 1/20 (5%), an alternative study leads to the diagnosis (Beckwith-Wiedemann). In another child, postnatal diagnosis of prenatal Caffey disease was made (5%). Five children are pending of genome sequencing (25%) due to suspicion of an unexplained genetic entity.

Postnatal follow-up of children born after inconclusive PES is relevant not only to achieve unexplained diagnosis but also to better define guidelines to improve indications for PES. Re-analysis of exome should be recommended in case of new postnatal clinical findings, together with the consideration of other alternative studies.

Conflict of Interest: None declared

P03.056.D Non-invasive prenatal screening (NIPS) for Trisomy 21, 18 and 13: empiric conclusions after 10,000 samples

Ana Rita Pereira[1], *Inês Moura Tavares*[1], *luena pitrez*[1], *Joaquim Sá*[1;2], *rita cerqueira*[1], **Alexandra Lopes**[1]

[1]*Unilabs, CGC Genetics, Portugal;* [2]*ULS Coimbra, Serviço de Genética, Portugal*

Background: Our private diagnostic laboratory in Porto has been offering NIPS screening from ten weeks gestation since 2015. Screening is strongly recommended for all pregnant women with singleton and twin pregnancies by international guidelines [PMID: 36524989] but the Portuguese national health system doesn't make this test available to the vast majority of women. In Portugal's public health system, the availability of screening is dependent on clinical information allowing the risk to be assessed as high for trisomies 21, 18 or 13.

Methods: Evaluation of the rates of inconclusive results and of each of these trisomies in the total number of samples processed; categorisation of clinical information when screening indicates trisomy 21.

Results: In 10,068 samples, *no call* occurred in 43 (0.4%), trisomy 21 in 107 (1.1%), trisomy 18 in 23 (0.2%), trisomy 13 in 15 (0.1%). In 64% (69/107) of the samples with trisomy 21 result the pregnancy was classified as high risk.

Conclusion: In our sample, around 1/3 of pregnant women with NIPS indicative of trisomy 21 would have their trisomy 21 diagnosis delegated to an amniocentesis to be carried out in the event that the ultrasound scan between 20-22 weeks identified anomalies. These empirical data corroborate the validity of current recommendations, revealing the importance of this screening in public health.

Conflict of Interest: None declared

P03.057.A Diagnostic yield of SNP-array and NGS-based testing in fetal ultrasound anomalies: results from 220 cases

Hilal Pırıl Saraçoğlu[1], *Umut Altunoglu*[2;3], *Esra Börklü*[3], *Şahin Avcı*[3], *Serpil Eraslan*[3], *Mert Turğal*[4], *Ebru Çelik*[4], *Recep Has*[5], *Atıl Yüksel*[5], *Hülya Kayserili*[2;3]

[1]*Graduate School of Health Sciences, Koç University, İstanbul, Türkyie;* [2]*School of Medicine, Koç University, Medical Genetics Department, İstanbul, Türkyie;* [3]*Genetic Diseases Evaluation Center, Koç University Hospital, İstanbul, Türkyie;* [4]*Obstetrics and Gynecology Department, Koç University Hospital, İstanbul, Türkyie;* [5]*Division of Perinatology, Department of Obstetrics and Gynecology, İstanbul Faculty of Medicine, Istanbul University, İstanbul, Türkyie*

Background/Objectives: The aim of this retrospective study is to evaluate the diagnostic yield of SNP-array and next generation sequencing (NGS) analyses in fetal anomalies at a tertiary health center.

Methods: In an eight-year period (2016-2023), 220 fetuses were consulted at the Medical Genetics Department of Koç University School of Medicine due to positive ultrasound (USG) findings. All fetuses underwent SNP-array analysis, and 62 underwent NGS comprising clinical exome sequencing (CES) and prenatal exome

sequencing (pES). The diagnostic yield of SNP-array and NGS was analyzed, along with the impact of diagnosis on pregnancy management and outcome.

Results: SNP-array showed likely pathogenic/pathogenic (LP/P) variants in 13/220 (5.9%) of samples, while the general diagnostic yield for NGS was 12/62 (19.4%). Most common referrals were due to multisystem anomalies (n = 77), followed by gastrointestinal (n = 28) and cardiovascular (n = 24) anomalies. Diagnostic yield of SNP-array was 10.4% (8/77) for multiple ultrasound abnormalities, and diagnostic yield of NGS was 27.3% (6/22) for multiple fetal structural anomalies.

Conclusion: Our retrospective fetal anomaly cohort further supports the additional diagnostic yield of SNP-array and NGS-based testing in the prenatal setting, and contributes to existing literature on genetic variants in association with specific prenatal phenotypes.

Grant: -

Conflict of Interest: None declared

P03.058.B Single-cell Analysis Reveals the Spatial-temporal Expression of Genes Associated with Esophageal Malformations

Antonia Eberts[1], *Carlo Maj*[1], *Johannes Schumacher*[1], *Pouria Dasmeh*[1]

[1]*Center for Human Genetics, Marburg University and Marburg University Hospital, Marburg, Germany*

Background/Objectives: Esophageal malformations (EM) are congenital diseases that arise due to the abnormal development of the esophagus. We know little about the molecular mechanisms of such congenital diseases as they primarily occur within specific developmental stages. This knowledge helps us identify disease-relevant genes for enhanced diagnosis and risk assessment as well as the development of potential therapeutic strategies.

Methods: Here, we analyzed the single-cell preferential expression of the genes known to be associated with EM from genetic studies including GWAS, genes implicated in foregut anomalies in animal models, and genes identified from exome analysis of patients. We then calculated single-cell disease-relevant risk scores to detect cells with a significant excess expression of EM-associated genes across large-scale single-cell atlases in mouse, and human embryos as well as single cells from 24 human organs (approximately 800,000 cells).

Results: EM-associated genes showed a significant preferential expression in mesodermal cell types and a significantly higher expression at early stages of development. The expression of *FOXF1*, *PTCH1* and *SOX2* contributed the most to the preferential expression of EM-associated genes. In the human adult cells, subpopulations of epithelial cells, and fibroblasts showed the most significant preferential expression of EM-associated genes.

Conclusion: Our observations demonstrate that genes associated with esophageal malformations show their highest cell-type specific expression in lateral plate mesoderm cells and at the developmental stage of E8.75-E9.0 days. Overall, our findings provide insights into the temporal and cellular contexts contributing to esophageal malformations.

Conflict of Interest: None declared

P03.059.C Exploring the fetal presentation of 3M syndrome: clinical, imaging, and molecular insights for prenatal recognition of a rare constitutional bone disease

Claire Beneteau[1], *Sabine Sigaudy*[2], *Anne Dieux-Coeslier*[3], *Gijs Santen*[4], *Elena Mansilla*[5], *Natalija Krasovskaja*[6], *Julie Desir*[7], *Julien Thevenon*[8], *Maayke de Koning*[4], *Sophie Patrier*[9], *alicia cauder*[8], *marie denis.musquer*[10], *William Dufour*[11], *Audrey Putoux*[11], *fabienne allias-montmayeur*[11], *Fe Amalia García Santiago*[12], *Valérie CORMIER-DAIRE*[13], *Caroline Rooryck-Thambo*[1]

[1]*CHU de Bordeaux, Département de génétique, Bordeaux;* [2]*CHU de Marseille, Marseille, France;* [3]*CHU de Lille, génétique, Lille, France;* [4]*Leiden University Medical Center, Department of Clinical Genetics;* [5]*La Paz University Hospital, INGEMM, Institute of Medical and Molecular Genetics;* [6]*Vilnius University Hospital;* [7]*Hôpital Erasme, Cliniques Universitaires de Bruxelles;* [8]*CHU de Grenoble, Grenoble, France;* [9]*CHU de Rouen, Rouen, France;* [10]*CHU de Nantes, Nantes, France;* [11]*CHU de Lyon, Lyon, France;* [12]*La Paz University Hospital;* [13]*APHP - Necker, Paris, France*

Background/Objectives: 3M syndrome, a rare constitutional bone disorder, is characterized by severe pre- and postnatal growth retardation, distinctive facial features, and normal intelligence, with an average final height of around 125 cm. Pathogenic variants in *CUL7*, *OBSL1*, or *CCDC8* are predominantly identified postnatally, leaving limited clinical data for the prenatal period. This study seeks to unravel the fetal presentation of 3M syndrome, aiding clinicians in recognition and supporting biologists in interpreting variants from exome/genome sequencing.

Methods: collaboratively through ERN-ITHACA, a data collection initiative for prenatal and molecular data was launched for individuals molecularly diagnosed with 3M syndrome.

Results: our fetal cohort included 18 fetuses from 14 families with pathogenic variants in *CUL7* and *OBSL1*. Eight pregnancies were terminated, one resulted in intrauterine demise, and nine continued. Initial signs emerged between 17 and 28 weeks of gestation, revealing severe intrauterine growth restriction (IUGR), relative macrocephaly, thin long bones without fractures, and preserved fetal movements. Autopsies unveiled suggestive dysmorphia, prominent heels, short arms and thin long bones. Families exhibited *CUL7* and *OBSL1* variants, predominantly truncating variants yet unreported. Amid approximately 450 skeletal dysplasias, precise diagnosis in fetal medicine remains challenging. Severe IUGR without fractures or curvatures may suggest Silver-Russell syndrome, Dubowitz, Mulibrey, fetal alcohol syndromes, or 3M syndrome in cases of extreme IUGR.

Conclusion: this study unveils the inaugural fetal cohort of 3M syndrome, presenting crucial clinical, imaging, and molecular insights for its recognition. Emphasis is placed on slender long bones, beyond fetal akinesia context, guiding prenatal and fetal genetic diagnosis of 3M syndrome.

Grants : none

Conflict of Interest: None declared

P03.060.D Outcome of 2 years of prenatal parent-fetus trio exome sequencing in 107 pregnancies with multiple fetal anomalies

Stefanie Beck-Wödl[1;2], *Andreas Dufke*[1;2], *Ulrike Mau-Holzmann*[1;2], *Stephanie Spranger*[3], *Marc Sturm*[1;2], *Tizia Matthäus*[1;2], *Miriam Bertrand*[1;2], *Luise Hackenbruch*[1;2], *Markus Hoopmann*[4], *Karl Oliver Kagan*[4], *Ute Grasshoff*[1;2], *Olaf Riess*[1;2], *Tobias Haack*[1;2]

[1]*University Hospital Tübingen, Institute of Medical Genetics and Applied Genomics, Tübingen, Germany;* [2]*Rare Disease Center, ZSE, Tübingen;* [3]*Limbach genetics, Bremen, Germany;* [4]*University Hospital Tübingen, Department for Women's Health, Tübingen, Germany*

Background/Objectives: Here we report on the results of 2 years of fast track trio exome-based diagnostics in a cohort of 107 pregnancies with fetal ultrasound abnormalities. The fetuses presented with fetal akinesia, increased nuchal translucency, ascites, hydrops fetalis, renal agenesis or multiple congenital

anomalies. Cytogenetic analyses performed prior to the trio exome analyses revealed a normal karyotype in all cases.

Methods: Trio exome sequencing was done on DNA from chorionic villi samples, amniocentesis and parental blood. We performed diagnostic sequencing on a NovaSeq6000 platform (Illumina) using the Twist Bioscience (Twist Custom Exome IMGAG V2) on 107 patient-parent trios. Prioritization of clinically relevant genomic variants was performed according to in-house standard procedures.

Results: Clinically relevant pathogenic sequence variants were identified in 44% of cases, including copy number variations in more than 10%. The causative variants detected occurred de novo in 38% of cases and were thus easily identified with the trio approach.

Conclusions: In summary, our trio exome-based approach in pregnancies with various fetal anomalies proved to be a fast and highly efficient diagnostic tool in detecting disease-causing single nucleotide and copy number variants. The current study confirms our previous published observations (PMID: 35574990) and highlights the clinical utility of trio-based prenatal exome diagnostics.

Conflict of Interest: None declared

P03.061.A A novel likely pathogenic variant in the non-coding gene *RNU4ATAC* causing MOPD1

Valerie Krauth[1], *Matthias Vockel*[1], *Judit Horvath*[1], *Frank Tüttelmann*[1], *Mareike Moellers*[2], *Katja Masjosthusmann*[3], *Andreas Busche*[1]

[1]*Department of Medical Genetics, University Hospital Münster, Münster, Germany;* [2]*Department of Gynaecology and Obstetrics, University Hospital Münster, Münster, Germany;* [3]*Department of Paediatrics, University Hospital Münster, Münster, Germany*

Background/Objectives: Biallelic pathogenic variants in *RNU4ATAC* cause the rare autosomal recessive disorder Microcephalic Osteodysplastic Primordial Dwarfism, type I (MOPD1). This condition is characterised by small stature, microcephaly, immunodeficiency and cognitive impairment. *RNU4ATAC* is encoding the small nuclear RNA (snRNA) U4atac, which is a part of the minor spliceosome complex responsible for removing minor introns.

Methods: Prenatal trio-exome sequencing and analysis of genes potentially related to the prenatal phenotype led to a comprehensive review of rare variants in non-coding RNA genes in combination with the clinical phenotype.

Results: We present a 7-month-old girl whose mother was referred to us in the 27th week of gestation. Prenatal ultrasound showed intrauterine growth restriction as well as brain and facial anomalies. Exome sequencing on DNA extracted from amniotic fluid revealed compound-heterozygosity for two variants in *RNU4ATAC*. While the maternally inherited variant n.51G>A has been published as pathogenic before, the paternally inherited nucleotide substitution n.38A>T had not been described yet. This variant is affecting a highly conserved nucleotide in the 5′ Stem-Loop of the U4atac-snRNA and was classified as likely pathogenic according to ACMG guidelines (class 4: PM1, PM2, PM3, PP5). After birth, the girl showed typical symptoms of MOPD1, including skeletal dysplasia, dysgenesis of the corpus callosum and agammaglobulinaemia.

Conclusion: The description of the aforementioned novel nucleotide substitution is extending the spectrum of pathogenic variants in *RNU4ATAC*. Genetic and detailed clinical data of a patient with MOPD1 will further delineate the phenotypic and genotypic spectrum of this rare condition.

Grants: None.

Conflict of Interest: None declared

P04

SENSORY DISORDERS (EYE, EAR, PAIN)

P04.001.B Functional characterization of *ABCA4* missense variants aids variant interpretation and phenotype prediction in patients with ABCA4-retinal dystrophies

Ingvild Aukrust[1;2], *Sigrid Aslaksen*[1;2], *Laurie Molday*[3], *Josephine Prener Holtan*[4], *Ragnhild Wivestad Jansson*[5], *Siren Berland*[1], *Eyvind Rødahl*[5;6], *Cecilie Bredrup*[5;6], *Ragnheiður Bragadóttir*[4;7], *Eirik Bratland*[1;2], *Robert S. Molday*[3;8], *Per Morten Knappskog*[1;2]

[1]*Haukeland University Hospital, Department of Medical Genetics, Bergen, Norway;* [2]*University of Bergen, Department of Clinical Science, Bergen, Norway;* [3]*University of British Columbia, Department of Biochemistry and Molecular Biology, Vancouver, Canada;* [4]*Oslo University Hospital, Department of Ophthalmology, Oslo, Norway;* [5]*Haukeland University Hospital, Department of Ophthalmology, Bergen, Norway;* [6]*University of Bergen, Department of Clinical Medicine, Bergen, Norway;* [7]*University of Oslo, Institute of Clinical Medicine, Oslo, Norway;* [8]*University of British Columbia, Department of Ophthalmology and Visual Sciences, Vancouver, Canada*

Background/Objectives: Biallelic pathogenic variants in the gene encoding the ATP-binding cassette transporter ABCA4 are the leading cause of irreversible vision loss in inherited retinal dystrophies. Interpretation of *ABCA4* variants is challenging, due to cis-modifying and hypomorphic variants. We have previously detected 10 missense variants of unknown significance (VUSs) in patients with suspected ABCA4-retinal dystrophies (ABCA4-RDs) in Norway. In this study, we functionally characterized the VUSs to aid interpretation of the variants and to determine if they are associated with the disease.

Methods: The *ABCA4* VUSs were expressed in HEK293T cells and the ABCA4 expression and ATPase activity were determined and correlated with the patients' phenotype. The functional data was further used for reclassification of the VUSs following the ACMG guidelines.

Results: Two variants, Cys205Phe and Asn415Thr, were categorized as functionally severe. The age at presentation in the two patients carrying these variants was divergent and seemed to be driven by the patients' second pathogenic variants. Three variants, Val643Gly, Pro799Leu and Val1433Ile, were categorized as functionally moderate, and were found in patients with intermediate/late age at presentation. The remaining five variants were categorized as functionally normal/mild. Based on our data, Cys205Phe, Asn415Thr and Pro799Leu, were reclassified to (likely) pathogenic, while four of the functionally normal/mild variants could be reclassified to likely benign.

Conclusion: Functional analyses of ABCA4 variants are a helpful tool in variant classification and enable us to better predict the disease severity in patients with ABCA4-RDs.

Grants: Norwegian RP Association and Norwegian Association of the Blind and Partially Sighted.

Conflict of Interest: None declared

P04.002.C HiFi long-read genome sequencing uncovers a large novel *RD3* deletion representing a founder allele in Afghan patients with Leber congenital amaurosis

Monika Gawai[1], **Ariane Kröll-Hermi**[1], *Milan Hiersche*[1], *Christian Decker*[1], *Johannes Birtel*[2], *Anna Lentzsch*[3], *Christoph Friedburg*[4], *Markus Preising*[4], *Christian Betz*[1], *Hanno Bolz*[1;5]

[1]*Bioscientia Institute for Medical Diagnostics GmbH, Human Genetics, Ingelheim am Rhein, Germany;* [2]*University Medical Center*

Hamburg-Eppendorf, Ophthalmology, Hamburg, Germany; [3]University Hospital of Cologne, Ophthalmology, Köln, Germany; [4]Justus-Liebig-University Giessen, Ophthalmology, Gießen, Germany; [5]University Hospital of Cologne, Institute of Human Genetics, Köln, Germany

Background/Objectives: Leber congenital amaurosis (LCA) is an inherited congenital retinal dystrophy associated with severe visual impairment. More than 30 LCA genes are currently known. Loss-of-function (LoF) variants in the retinal degeneration 3 gene (*RD3)* cause LCA12. We carried out in-depth genetic analyses of three apparently unrelated LCA patients (P1-3) from Afghanistan.

Methods: Genomic DNA was extracted from peripheral blood leukocytes and analyzed by short-read whole-exome sequencing (SR-WES) and PacBio HiFi long-read whole-genome sequencing (LR-WGS). Variants were called with in-house CNV and SNV pipelines.

Results: We identified a novel deletion encompassing *RD3* exon 3, (c.296+641_*606del (p.(?)), in homozygous state in P1 and P2, while P3 carried the deletion and a novel nonsense *RD3* variant (c.394G>T, p.(Glu132*)), both in heterozygous state. SR-WES indicated the deletion, but not the exact breakpoints, and in P3, the nonsense variant was initially called as homozygous. LR-WGS accurately determined the deletion's breakpoints and size (2050 bp) and correctly annotated the nonsense variant as hemizygous. The three patients shared identical breakpoints and haplotypes, qualifying this *RD3* deletion as a novel founder mutation.

Conclusion: Particularly in heterozygous state, this founder deletion may escape detection in SR-WES and could represent an underrated cause of LCA in Central Asia. In contrast, HiFi LR-WGS precisely detects such structural variants. Because difficult regions (e.g. disease genes with pseudogene backgrounds or highly repetitive elements, such as *RPGR-ORF15*) become readable as well, the clinical application of this comprehensive technology will increase the diagnostic yield – for retinal dystrophies and beyond.

Conflict of Interest: None declared

P04.003.D Streamlined CRISPR-Cas9-mediated gene knockout in zebrafish for swift assessment of auditory developmental and behavioral anomalies.

Daniel Bengl[1], *Julia Doll*[2], *Daniel Liedtke*[1], *Michaela AH Hofrichter*[1], *Thomas Haaf*[1]

[1]*Institute of Human Genetics at the University of Würzburg, Würzburg, Germany;* [2]*Universität Würzburg Institut für Pathologie, Würzburg, Germany*

Background/Objectives: Hearing loss (HL), a prevalent sensory disorder, affects up to two newborns per 1,000, with 50-80% of cases attributed to genetic factors. Advances in molecular methods enable the rapid identification of novel disease-causing candidate genes. Studying the pathophysiology of these genes is essential for understanding the complexity of auditory development. For this purpose, the zebrafish serves as an exemplary model organism due to the similarities of its auditory system to humans, the ease of manipulation, and the repertoire of modern molecular biological tools. This study aims to validate potential HL candidate genes and understand their impact on zebrafish auditory development by using a CRISPR Cas9-mediated knockout screen.

Methods: Injecting multiple guide RNAs per genetic target alongside the Cas9 protein into zebrafish embryos allows the reliable generation of a statistically significant number of knockout individuals. Headloop PCR is employed to quantify the knockout efficiency. Five days post-fertilization, larvae are examined for their innate acousto-vestibular response by using an imaging setup with a high-speed camera. Behavior analysis, extraction of kinetic data, and quantification of behavioral changes are performed using the open-source software ZebraZoom.

Results: We were able to validate the entire experimental pipeline by a proof-of-principle experiment involving the zebrafish ortholog of the known HL gene *MYO6*. Our experiments replicated published results for a myo6b mutant line and furthermore enables the differentiation of individual behavioral phenotypes.

Conclusion: We present a rapid and reliable screening pipeline to quantify changes in the zebrafish acousto-vestibular escape response after knockout of potentially disease-causing HL candidate genes.

Grants:

Conflict of Interest: None declared

P04.004.A Modeling genetic forms of hearing loss with Inner Ear Organoids

Giulia Pianigiani[1], *Anna Morgan*[1], *Mabel Troian*[1], *Giovanni Turchetto*[1], *Beatrice Spedicati*[1,2], *Marta Roccio*[3,4], *Giorgia Girotto*[1,2]

[1]*Institute for Maternal and Child Health – I.R.C.C.S. "Burlo Garofolo", Trieste, Italy;* [2]*University of Trieste, Department of Medicine, Surgery and Health Sciences, Trieste, Italy;* [3]*University Hospital Zurich (USZ), Zurich, Switzerland;* [4]*University of Zurich (UZH), Zurich, Switzerland*

Background/Objectives: iPSC-derived inner ear organoids (IEOs) have recently emerged as new tools to model inherited inner ear diseases. Here, we used IEOs to assess the expression and function of *MYO5C* as a candidate gene for HL. Indeed, a novel homozygous missense variant in *MYO5C* was identified in a Pendred syndrome-like patient, negative for mutations in the *SLC26A4* gene and presenting with a severe-to-profound HL and inner ear malformations.

Methods: MYO5C expression was analyzed in fetal human and early postnatal mouse inner ear tissues. MYO5C knockout (KO) and patient-identified variant (NM_018728.4, c.3592C>T, p.(Arg1198Cys)) knock-in (KI) iPSCs were generated with CRISPR/Cas9 technology.

Results: scRNA-seq and immunofluorescence analysis show that at day-30 of differentiation, MYO5C is expressed in otic progenitors and other epithelial cells. At day-60, when sensory epithelia appear in IEOs, MYO5C becomes visible at the developing hair bundle in hair cells. A similar expression is observed in vivo in the sensory epithelia of the utricle, saccule (week-10) and cochlea (week-12) primary tissues. Genome-edited iPSCs and the isogenic control line were induced to differentiate in IEOs following a stepwise induction protocol. We did not observe differentiation defects towards the otic lineage so far at the histological level. Ongoing work is focused on the analysis of IEOs derived from the engineered iPSCs by transcriptional profiling.

Conclusion: this study will not only allow to investigate MYO5C potential role in sensory organ development, but also provide the basis for a broader application of IEOs as a tool to probe disease-causing mutation.

Conflict of Interest: None declared

P04.005.B An integrated approach to uncover the genetic architecture of inherited blindness in a consanguineous Iranian cohort

Lieselot Vincke[1], *Kristof Van Schil*[2], *Marieke De Bruyne*[1], *Quinten Mahieu*[1], *Marta del Pozo Valero*[1], *Mattias Van Heetvelde*[1], *Toon*

Rosseel[1], Ebrahim Al Hajj[3], Astrid Rombaut[1], Sarah Van Malderen[1], Reza Maroofian[4], Fatemeh Suri[5], Miriam Bauwens[1], Elfride De Baere[1]

[1]Center for Medical Genetics Ghent, Ghent University and Ghent University Hospital, Ghent, Belgium; [2]Center for Medical Genetics Antwerp, Antwerp University Hospital, Antwerp, Belgium; [3]Free University of Brussels, Brussels, Belgium; [4]Institute of Neurology, Department of Neuromuscular Diseases, University College London, London, United Kingdom; [5]Ophthalmic Research Center, Research Institute for Ophthalmology and Vision Science, Shahid Beheshti University of Medical Sciences, Tehran, Iran

Objectives: This study aimed to elucidate the genetic architecture of inherited retinal disease (IRD) in 192 unrelated Iranian families using an integrated approach combining whole exome sequencing (WES) and autozygosity mapping.

Methods: WES was conducted on 192 Iranian IRD patients, predominantly originating from a consanguineous background (76%). Data-analysis was performed using the in-house Seqplorer tool. Copy number variants (CNVs) were assessed via ExomeDepth, while AutoMap identified runs of homozygosity (ROHs). Variants were validated and classified (ACMG/ACGS guidelines).

Results: Interrogating 290 IRD genes via WES yielded a molecular diagnosis for 73% of the cohort, revealing 208 variants in 77 IRD-associated genes. Of these variants, 82% were unique, and 53% were novel. Most variants were homozygous (69%) and 83% of variants found in consanguineous families were located in a ROH. In addition, the importance of structural variation (SV) in IRD was demonstrated, with CNVs identified in 6% of the cohort, including novel CNVs in *ABCA4, CDH3, CDHR1, CHM, CNGA1* and *RD3*. Locus heterogeneity was observed in two families, in which multiple genotypes segregated with IRD.

Conclusion: This integrated study, utilizing WES and comprehensive variant assessment, provided genetic diagnoses for 73% of IRD patients. The molecular spectrum of IRD in Iran was expanded with the identification of both known and novel variants, offering valuable insights for family counselling. The study contributes to understanding the genetic architecture of IRD in the understudied Iranian population and presents unsolved cases as a unique cohort for identifying novel candidate IRD genes.

Grants: FWO11PS324N; FWO1802220N

Conflict of Interest: None declared

P04.006.C Evaluation of the efficacy of multigene panel tests in the largest Bardet Biedl Syndrome cohort in Turkey

Şenol Demir[1], *esra arslan ateş[2], cekdar kapazan[1], AYBERK TURKYILMAZ[3], Hamza Polat[4], Bilgen Bilge Geçkinli[1]*

[1]Marmara University School of Medicine, Department of Medical Genetics, Istanbul, Türkyie; [2]Istanbul University-Cerrahpaşa Cerrahpaşa Faculty of Medicine, Department of Medical Genetics, Istanbul, Türkyie; [3]Karadeniz Technical University School of Medicine, Department of Medical Genetics, Trabzon, Türkyie; [4]Gazi Yasargil Training and Research Hospital, Department of Medical Genetics, Diyarbakır, Türkyie

Background/Objectives: Bardet-Biedl Syndrome (BBS) is a ciliopathy characterized by retinitis pigmentosa, polydactyly, obesity, renal malformation, cognitive impairment, and hypogonadism. The syndrome's prevalence is estimated to be 1 in 100,000 live births. BBS is a genetically heterogeneous syndrome which is associated with more than 20 genes. This study aims to determine the effectiveness of multigene panel testing in patients who have BBS clinical features and to reveal the genetic etiology of the BBS patients.

Methods: Thirty patients having specific examination findings related to BBS were included in this study. After evaluation of clinical and family histories, probands were screened using a custom-designed Bardet Biedl syndrome panel including 24 genes via next-generation sequencing (NGS). The family members of the probands carrying pathogenic variations were screened via Sanger Sequencing.

Results: Among 30 cases major clinical findings were polydactyly (n = 22)(73%), obesity (n = 16)(53%), and retinal dystrophy (n = 13)(43%). We detected in 21 (70%) patients biallelic pathogenic variations, of which 5 were novel, in BBS1(n = 4), BBS9(n = 4), BBS10(n = 4), BBS4(n = 3), MKKS(n = 3), BBS7(n = 2), ARL6(n = 1) genes.

Conclusion: The detection of 5 novel pathogenic variations has contributed to the expansion of the mutation spectrum. Also, the confirmation of clinical diagnosis in 70% of the patients with molecular genetic testing supports that the screening related genes via NGS panel is an effective diagnostic method for BBS. Defining the molecular etiology of BBS is important in terms of screening at-risk family members and providing appropriate genetic counseling for preimplantation genetic diagnosis opportunities.

Grants: None

Conflict of Interest: None declared

P04.007.D Pacbio HiFi long-read genomes offer better exomes by unlocking retinal disease variants missed by short-read sequencing

Kadin Karakaya[1], *Ariane Kröll-Hermi[1], Milan Hiersche[2], Christian Decker[2], Sandra Liakopoulos[3;4], Markus Preising[5], Klaus Rohrschneider[6], Christian Betz[1], Hanno Bolz[1]*

[1]Bioscientia Institute for Medical Diagnostics GmbH, Bioscientia Human Genetics, Ingelheim, Germany; [2]Bioscientia Institute for Medical Diagnostics GmbH, Bioscientia Bioinformatics, Ingelheim, Germany; [3]Goethe University, Department of Ophthalmology, Frankfurt am Main, Germany; [4]Cologne Image Reading Center, University of Cologne, Department of Ophthalmology, Köln, Germany; [5]Justus-Liebig-University, Department of Ophthalmology, Gießen, Germany; [6]University of Heidelberg, Department of Ophthalmology, Heidelberg, Germany

Background/Objectives: Next-generation sequencing enables rapid, comprehensive and cost-effective analysis of disease-associated genes. Short-read (SR) technologies currently predominate, but face several limitations: For example, regions with high repeat content or pseudogene background may be inaccessible, and large structural variations may escape detection.

Methods: We investigated 3 patients with inherited retinal disease (IRD) who had remained unsolved after short-read whole-exome sequencing (SR-WES), and a patient with a known *RPGR-ORF15* mutation, with Pacbio HiFi long-read genome sequencing (LR-WGS).

Results: Patient 1 with a known monoallelic pathogenic variant in *USH2A*, c.14926G>A p.(Gly4976Ser), was found to carry a previously undetected *Alu* element insertion in exon 45 which, of note, had been missed despite high coverage in WES. In Patient 2, a WES-derived seemingly pathogenic *PDE6B* variant (c.169_239dup p.(Leu83Cysfs*91)) turned out homozygous in LRS. In Patient 3, LRS showed that *EYS* exon 33 was duplicated on both alleles instead of one and precisely identified the breakpoints. Patient 4 has a known variant in the highly repetitive *ORF15* of X-linked *RPGR*, a mutational hotspot that is only accessible via special adaptations of SR-WES and bioinformatics. LRS unlocks this formerly difficult-to-sequence major IRD locus.

Conclusion: Pacbio LR-WGS provided a clear genetic diagnosis in IRD patients whose causative exonic variants had not or only partially been detected by SR-WES. In addition to resolving difficult regions of the genome, LR-WGS provides detailed information on phasing and structural variations and should soon become a major diagnostic technology for the comprehensive analysis of human genetic disorders.

Conflict of Interest: None declared

P04.008.A Identification of expression and splicing Quantitative Trait Loci in the retina and retinal pigment epithelium implicates novel regulatory regions and divergence in genetic regulation across diverse human populations

Jacob Sampson[1;2], *Steve Haynes*[1], *Siddharth Banka*[1;2], *Panagiotis Sergouniotis*[1;2], *Graeme Black*[1;2], *Jamie Ellingford*[1;2;3]

[1]*University of Manchester, Division of Evolution, Infection and Genomic Sciences, School of Biological Sciences, Faculty of Biology, Medicines and Health, Manchester, United Kingdom;* [2]*St Mary's Hospital, Manchester University NHS Foundation Trust, Manchester Centre for Genomic Medicine, Manchester, United Kingdom;* [3]*Genomics England, United Kingdom*

Background/Objectives: Interpreting the functional impact of non-coding variants is important for rare and common ophthalmic disorders. Molecular Quantitative Trait loci (molQTL) mapping detects genetic variation significantly associated with gene expression and/or splicing. We present a novel molQTL resource that advances our understanding of the regulatory landscape of the human adult retina and retinal pigment epithelium (RPE).

Methods: Whole genome sequencing (WGS) and bulk RNA-sequencing of retina and RPE was performed in 206 post-mortem eyes. Expression and splicing QTLs (eQTL/sQTL) in retina and RPE were detected using a linear regression model.

Results: We identified 1.4 million genetic variants (allele frequency >2.5%) significantly associated (FDR < 5%) with expression (n = 544,345), splicing (n = 412,571) or both expression and splicing (n = 422,005) of 12,076 genes in retina or RPE (including 333 known ophthalmic disease-associated genes). eQTL variants (eVariants) were enriched in previously annotated cis-regulatory elements (CREs) compared to non-eQTL variants (p-value < 2.2e-16). Moreover, the relative enrichment in retina-specific CREs was significantly stronger than in other tissue-specific CREs (p-value < 2.2e-16). Despite this, 84% of all eVariants and 77% of the top hits do not overlap with known CREs in any tissue (99% of eVariants when including only retina CREs). Further, we observe significant variability in the allelic frequency of molQTLs across diverse human populations.

Conclusion: This resource facilitates the discovery of novel retina-specific regulatory mechanisms and provides insight into non-coding variants that regulate ophthalmic disease-related genes. These findings have broad applications to the discovery and diagnosis of ophthalmic genomic disorders.

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Conflict of Interest: None declared

P04.009.B Using the pSPL3 exon trapping vector to unmask splicing effects of otoferlin coding variants

Friederike Jahnke[1;2], *Barbara Vona*[1;2], *Daniel Owrang*[1;2], *Zijin Zhang*[3]

[1]*University Medical Center Göttingen, Institute of Human Genetics, Göttingen, Germany;* [2]*University Medical Center Göttingen, Institute for Auditory Neuroscience and InnerEarLab, Göttingen, Germany;* [3]*University Medical Center Frankfurt, Pediatric and Experimental Hematology, Frankfurt, Germany*

Background/Objectives: Otoferlin (OTOF) is an essential protein with at least three distinct functions in the synaptic signaling of inner hair cells. Genetic variations in otoferlin contribute to a clinically recognizable autosomal recessive auditory synaptopathy known as DFNB9 or AUNB1. An overlooked yet significant consequence of missense and synonymous variants encompasses the potential profound influence on the contextual landscape of splice signals. These signals are crucial for coordinating proper splicing orchestrated by the spliceosome.

Methods: All documented missense variants were gathered from literature sources and clinical databases, and then subjected to comprehensive analysis using various splice prediction tools. Similarly, synonymous variants listed in the Deafness Variation Database underwent parallel scrutiny. A prioritized catalog, comprising 8 missense and 15 synonymous variants based on potential splice effects, was compiled for further investigation through minigene assays.

Results: Out of the 23 minigenes, only four exhibited normal splicing patterns, while the remaining ones displayed evidence of aberrant splicing. The most prevalent anomalies included frameshift effects and indications of incomplete or "leaky" splicing, resulting in multiple splice effects.

Conclusion: This study functionally characterizes the splicing effects of missense variants, often misclassified as pure non-synonymous substitutions, and synonymous variants, typically regarded as lacking deleterious effects. This is a crucial step in understanding the molecular mechanisms of coding variants in *OTOF*, which is vital for successful genotype-phenotype correlation studies and for identifying potential sources of variability in gene therapy clinical trials.

Grants: DFG funding: VO2138/7-1 #469177153 (B.V.)

Conflict of Interest: None declared

P04.010.C The discovery of the second family with Clarin-2-related autosomal recessive hearing loss

Nele Christophersen[1;2], *Barbara Vona*[1;2], *Neda Mazaheri*[3], *Mina Zamani*[3], *Aboulfazl Rad*[4], *Reza Maroofian*[5], *Hamid Galehdari*[3]

[1]*University Medical Center Göttingen, Institute of Human Genetics, Göttingen, Germany;* [2]*University Medical Center Göttingen, Institute for Auditory Neuroscience and InnerEarLab, Göttingen, Germany;* [3]*Shahid Chamran University of Ahvaz, Department of Biology, Faculty of Science, Ahvaz, Iran;* [4]*Eberhard Karls University Tübingen, Department of Otolaryngology—Head and Neck Surgery, Tübingen Hearing Research Centre, Tübingen, Germany;* [5]*UCL Queen Square Institute of Neurology, University College London, Department of Neuromuscular Diseases, London, United Kingdom*

Background/Objectives: The recently discovered deafness-associated gene Clarin-2 (*CLRN2*, DFNB117) encodes a tetraspanin-like protein essential for maintaining auditory hair cell bundles. A homozygous c.494C>A, p.Thr165Lys (NM_001079827.2) variant in *CLRN2* has been previously linked to autosomal recessive non-syndromic hearing loss that manifests post-lingually and shows progression in a single family. Continued work led to the discovery of a second family whose autosomal recessive deafness is caused by the same variant in *CLRN2*, strengthening the association between *CLRN2* and hearing loss. In response to this discovery, we sought to aggregate audiometry and clinical reports from the only two identified *CLRN2* families to date.

Methods: DNA samples of a multi-ethnic patient cohort were exome sequenced. Exome data from the two *CLRN2* families were analysed including haplotype analysis and autozygosity mapping.

Sanger sequencing was used for validation and segregation analysis. Minigene studies explored aberrant splicing. Clinical information such as audiometry was compared in the two *CLRN2* families.

Results: Exome analysis and Sanger sequencing confirmed a homozygous *CLRN2* c.494C>A, p.Thr165Lys variant in a newly recruited family. The variant causes aberrant splicing through exonic splice enhancer activation. Individuals in both families describe hearing impairment onset range from 7-10 and 9-10 years of age, respectively. Audiometry of the new family shows a progression to moderate-to-severe sensorineural hearing loss.

Conclusion: The pathogenic c.494C>A variant in *CLRN2* is associated with autosomal recessive, non-syndromic, post-lingual, progressive hearing loss in humans.

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Conflict of Interest: None declared

P04.011.D A combined approach of innovative DNA- and RNA-technologies reveals hidden LINE-1/ERV insertion in *IQCB1* as causative variant for Senior Løken syndrome

Suzanne E. de Bruijn[1], *L. Ingeborgh van den Born*[2], *Ronny Derks*[1], *Luke O'Gorman*[1], *Ronald van Beek*[1], *Alexander Hoischen*[1,3], *Susanne Roosing*[1], *Kornelia Neveling*[1]

[1]*Radboud University Medical Center, Department of Human Genetics, Nijmegen, Netherlands;* [2]*The Rotterdam Eye Hospital, Rotterdam, Netherlands;* [3]*Radboud University Medical Center, Department of Internal Medicine and Radboud Center for Infectious Diseases (RCI), Nijmegen, Netherlands*

Background/Objectives: Senior Løken syndrome is a rare ciliopathy characterized by retinal degeneration and nephronophthisis (chronic kidney disease). The recessively inherited disease can be caused by pathogenic variants in several genes including *IQCB1*. Previously, short-read genome sequencing failed to identify a second causative allele in a Senior Løken patient harboring a monoallelic *IQCB1* defect. By combining innovative DNA- and RNA-technologies we aimed to find a genetic explanation for this patient.

Methods: Optical genome mapping was performed to identify structural variants potentially missed by short-read sequencing. Long-read sequencing (LRS) of DNA was performed to validate and determine the breakpoints of the candidate variant. Functional consequences of the variant were explored by targeted LRS using blood-derived RNA.

Results: Optical genome mapping revealed an intronic 6.2kb-insertion in *IQCB1*. LRS determined the variant constituted a LINE-1/ERV class-I element insertion. Targeted RNA-sequencing revealed that the insertion results in a complex splice-defect including exon 6 skipping and partial inclusion of the LINE-1 element as a pseudoexon. This splice defect leads to the introduction of a premature stop codon and the variant has been classified as pathogenic.

Conclusion: We identified a novel causative *IQCB1* variant by combining long-read DNA and RNA-approaches. This finding suggests that mobile element insertions still represent a underestimated variant class that are difficult to detect using short-read sequencing. Recent studies have shown that a combination of different mobile element insertion callers can help to resolve this problem, and therefore should be considered in standard diagnostic pipelines to increase diagnostic yields of Mendelian diseases.

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Conflict of Interest: None declared

P04.012.A Sequencing 105 macula disease-associated genes in an AMD cohort highlights the involvement of *PRPH2*, *BEST1* and *RP1* in AMD-mimicking diseases.

Rebekkah Hitti-Malin[1], *Anna Tracewska*[1], *Carel Hoyng*[2], *Caroline Klaver*[2], *Yara Lechanteur*[2], *Anneke den Hollander*[2], *Björn Bakker*[1], *Erica Boonen*[1], *Susanne Roosing*[1], *Frans P.M. Cremers*[1]

[1]*Radboud University Medical Center, Department of Human Genetics, Nijmegen, Netherlands;* [2]*Radboud University Medical Center, Department of Ophthalmology, Nijmegen, Netherlands*

Background/Objectives: Inherited macular dystrophies (iMDs) and age-related macular degeneration (AMD) often present with similar clinical features, complicating an accurate diagnosis. Genetic screening plays a crucial role in distinguishing between AMD and AMD-mimicking diseases, guiding appropriate treatment decisions.

Methods: We conducted targeted sequencing of 105 genes associated with iMD and AMD, intronic disease-associated loci, AMD-associated risk variants and the mitochondrial genome in 1,115 AMD cases and 841 controls from the Netherlands and Germany. Variants were analysed using the Franklin by Genoox platform, with a focus on ACMG-classified variants of uncertain significance (VUS), likely pathogenic, and pathogenic variants. Copy number variant analysis was performed using an in-house pipeline.

Results: Sequencing revealed potentially explanatory variants in 3.0% of AMD cases ($n = 34$) implicating iMD-associated genes. Notably, *PRPH2* variants were found in 27.3% of these cases ($n = 9$), including the previously reported variant of reduced penetrance c.424C>T; p.(Arg142Trp), which was absent in controls. Six probands (18.2%) harbour variants in *BEST1* and six probands (18.2%) carry *RP1* variants. Additionally, an intergenic SNV linked to North Carolina macula dystrophy was found in an AMD patient and a control.

Conclusion: These findings highlight the importance of genetic screening in refining an iMD or AMD diagnosis. Ophthalmologic reassessment of individuals will elucidate the clinical implications of these genetic findings. Genetic analysis can aid in distinguishing AMD-mimicking dystrophies from AMD to achieve the best outcome for patients that are eligible for treatment.

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Conflict of Interest: Rebekkah Hitti-Malin: None declared, Anna Tracewska: None declared, Carel Hoyng Astherna, Caroline Klaver: None declared, Yara Lechanteur: None declared, Anneke den Hollander AbbVie, Björn Bakker: None declared, Erica Boonen: None declared, Susanne Roosing: None declared, Frans P.M. Cremers: None declared

P04.013.B Correlation of CAD-related disorder (EIEE-50) with cortical visual impairment (CVI).

Sarah Thurman[1], *Callie Fischer*[2], *Julie Guerin*[3], *Michael Brodsky*[4], *Ralitza Gavrilova*[1]

[1]*Mayo Clinic, Clinical Genomics, Rochester, United States;* [2]*Mayo Clinic, Pediatrics, Rochester, United States;* [3]*Mayo Clinic, Diagnostic Radiology, Rochester, United States;* [4]*Mayo Clinic, Ophthalmology, Rochester, United States*

Background/Objectives: To document the association of *CAD*-related disorder (EIEE-50) with cortical visual impairment (CVI).

Methods: Carbamoyl phosphate synthetase/aspartate transcarbamylase/dihydroorotase *(CAD)* enzyme deficiency(OMIM #114010), first described in 2015, also referred to as Early Infantile Epileptic Encephalopathy (EIEE50, OMIM 3616457), is characterized by seizures, language delay, difficulty with fine motor skills, wide based gait, and anemia. Case reports indicate benefit of uridine supplementation that results in decreased seizure activity, improved development and resolution of anemia. Uridine supplementation bypasses the intrinsic pyrimidine synthetic pathway defect in CAD-related disorders, working through the salvage pathway to provide uridine that the body needs for DNA and RNA synthesis. We describe profound cortical visual impairment (CVI) impairment in an infant with *CAD*-related disorder.

Results: An 8 month old Caucasian male presented for work up and evaluation of seizures, microcephaly, hyperreflexia, hypotonia, anemia, and severe CVI. MRI brain noted thickened cortical gray matter along the right calcarine fissure as well as radiographic changes suggesting malformation of cortical development. Trio WGS testing confirmed two CAD variants in trans: one variant of uncertain significance CAD:c.4837T>C and another likely pathogenic variant (CAD:c.3016G>A). Empiric uridine therapy (100mg/kg/d) provided notable improvement in seizure activity and hypotonia with concomitant resolution of his anemia. EEG at 19 months notes no seizure activity.

Conclusion: *CAD*-related disorder is treatable and may affect visual cortical development causing severe secondary CVI, a newly described clinical manifestation.

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P04.015.D A multi-omic approach to characterize the corneal epithelium in patients with post-refractive ectasia

Katarzyna Jaskiewicz[1], *Alicja Wysocka*[1], *Eliza Matuszewska*[2], *Magdalena Maleszka-Kurpiel*[3;4], *Jakub Wozniak*[5;6], *Rafał Płoski*[7], *Jan Matysiak*[2], *Malgorzata Rydzanicz*[7], *Marzena Gajecka*[1;5]

[1]*Institute of Human Genetics, Polish Academy of Sciences, Poznan, Poland;* [2]*Poznan University of Medical Sciences, Department of Inorganic and Analytical Chemistry, Poznan, Poland;* [3]*Poznan University of Medical Sciences, Chair of Ophthalmology and Optometry, Poznan, Poland;* [4]*Optegra Eye Health Care Clinic in Poznan, Poznan, Poland;* [5]*Poznan University of Medical Sciences, Chair and Department of Genetics and Pharmaceutical Microbiology, Poznan, Poland;* [6]*Poznan University of Life Sciences, Department of Genetics and Animal Breeding, Poznan, Poland;* [7]*Medical University of Warsaw, Department of Medical Genetics, Poznan, Poland*

Background/Objectives: Post-refractive ectasia (PRE) is a sight-threatening complication of 0.09-0.66% of corneal refractive surgeries. As the mechanism of the PRE as well as genetic predisposition/susceptibility remain unknown, we applied a multi-omic approach to characterize the corneal epithelium (CE) of PRE patients and screen for molecular biomarkers.

Methods: The 11 PRE patients (7 males/4 females) undergoing cross-linking procedure and 8 control-mild myopia patients (4 males/4 females) undergoing refractive error correction were ascertained. The RNA and proteins were extracted using RNA/DNA/Protein PurificationPlus MicroKit (Norgen Biotek) from the *central*, *middle*, and *peripheral* CE *topographic* regions of each CE sample (a total of 57 experimental samples). Next, NGS libraries were prepared using TruSeq Stranded TotalRNA LibraryPrep Gold (Illumina) and sequenced on Novaseq platform (60 mln read pairs/sample). MALDI-TOF/TOF Tandem Mass Spectrometry was performed after tryptic digestion, with an UltrafleXtreme mass spectrometer (Bruker Daltonics) in the mass-to-charge range of 700 to 3500. The obtained data were interpreted regarding our previous keratoconus data (https://doi.org/10.1167/iovs.64.2.22).

Results: In *central* and *peripheral* CE regions differentially expressed genes and proteins contributing to the JAK-STAT pathway via interferon-gamma and interleukin 12 signaling were identified. The main disrupted pathway, downregulated across the corneal surface, was keratinization (including downregulated expression of *KRT3*, *KRT7*, *KRT12*, and *KRT76*). The upregulated level of TBC1D4 protein was limited to the *central* region of CE.

Conclusion: These results, validated by future studies of ectasia-free fellow eyes, may contribute to the development of preoperative molecular tests to identify patients who may develop ectasia.

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Conflict of Interest: None declared

P04.016.A Demographic, genetic and phenotypic association of Fuchs endothelial corneal dystrophy in a large, multi-centre, molecularly and clinically characterised cohort

Siyin Liu[1;2], *Amanda Sadan*[1], *Anita Szabo*[1], *Nihar Bhattacharyya*[1], *Christina Zarouchlioti*[1], *Marcos AbreuCosta*[1], *Nathaniel Hafford-Tear*[1], *Lubica Dudakova*[3], *Marc Ciosi*[1], *Ismail Moghul*[1], *Pavlina Skalicka*[3;4], *Mark Wilkins*[2], *Bruce Allan*[2], *Alison Hardcastle*[1;2], *Nikolas Pontikos*[1;2], *Catey Bunce*[5], *Darren Monckton*[6], *Kirithika Muthusamy*[1;2], *Petra Liskova*[1;3], *Stephen Tuft*[1;2], *Alice Davidson*[1;2]

[1]*UCL, Institute of Ophthalmology, London, United Kingdom;* [2]*Moorfields Eye Hospital NHS Foundation Trust, London, United Kingdom;* [3]*Charles University, Department of Paediatrics and Inherited Metabolic Disorders, Czech Republic;* [4]*General University Hospital in Prague, Department of Ophthalmology, Czech Republic;* [5]*National Institute for Health and Care Research (NIHR) BRC at the Royal Marsden NHS Foundation Trust and the Institute of Cancer Research, National Institute for Health and Care Research (NIHR) BRC at the Royal Marsden NHS Foundation Trust and the Institute of Cancer Research, United Kingdom;* [6]*University of Glasgow, School of Molecular Biosciences, United Kingdom*

Background/Objectives: Fuchs endothelial corneal dystrophy (FECD) is commonly associated with an intronic CTG repeat expansion (CTG18.1) in *TCF4*. Rare pathogenic variants in other genes have also been reported. This large multi-centre study probes links between genotype, demographic factors, and age-at-first corneal transplant (a surrogate for severity) in a genetically characterized FECD cohort.

Methods: Blood DNA was collected from participants at Moorfields Eye Hospital, London($n = 569$) and the General University Hospital, Prague($n = 353$). Ethnicity was determined by Principal Component Analysis. CTG18.1 was genotyped by capillary electrophoresis of PCR amplicons. Cases with ≥1 expanded allele (≥50 CTG repeats) were classified as expansion-positive. Biallelic expansion-negative cases were analysed by exome/genome sequencing.

Results: A CTG18.1 expansion was detected in 672/836(80.4%) of European cases, conferring >77-fold increased odds for FECD($OR = 77.3$, CI:49.51-120.69, $P < 0.0001$). CTG18.1 expansions were significantly less common in non-European cases(23/65[35.38%], $P < 0.0001$). In the expansion-positive group, the CTG18.1 length was inversely correlated with age-at-first keratoplasty($r = -0.0859$, $P = 0.011$), but independent of sex and ethnicity. Females were enriched in the total cohort(58.25%), with

a more marked difference in the expansion-negative group(76.1%). Biallelic expansion-positive cases in European patients were enriched compared to ethnicity-matched controls(Chi-square = 218.62, P < 0.00001). Furthermore, biallelic expansion-positive cases had surgery at a younger mean age(66 vs 69 years, P = 0.02). Candidate pathogenic variants (MAF < 0.01; CADD >10) in FECD-associated genes were identified in 6.4% of expansion-negative cases.

Conclusion: Our results demonstrates that CTG18.1 length is a modifier of FECD severity, that female preponderance is mainly driven by CTG18.1 independent factors, and that allelic dosage increases both disease penetrance and severity.

Grants: MR/X006271/1_MR/S031820/1_

Conflict of Interest: None declared

P04.017.B Identification of genetic modifiers for *RIPOR2*-associated hearing loss type DFNA21

Janine Reurink[1], *Hedwig Velde*[1], *Suzanne E. de Bruijn*[2], *Jordi Corominas Galbany*[2], *Christian Gilissen*[2], *Ronald Pennings*[1], *Hannie Kremer*[1;2]

[1]*Radboud University Medical Center, Otorhinolaryngology, Nijmegen, Netherlands;* [2]*Radboud University Medical Center, Human Genetics, Nijmegen, Netherlands*

Background/Objectives: DFNA21 is a dominantly inherited, mainly adult-onset type of hearing impairment caused by a 12-nucleotide deletion in *RIPOR2*. A remarkable feature of DFNA21 is its high variability in (self-reported) age of onset. We hypothesize that genetic modifiers may underlie this high variability in age of onset.

Methods: Whole genome sequencing was performed for 16 cases with a relatively early onset (≤20 years of age) and 15 cases with a relatively late onset of hearing loss (≥40 years), derived from nine families. We assessed variants in and surrounding *RIPOR2*, genes encoding interaction partners of RIPOR2, 37 genes that encode stereocilia proteins and that are associated with hearing loss and 48 risk variants associated with age-related hearing impairment[1].

Results: Four haplotypes, located in or upstream of *RIPOR2*, were found to be associated with the onset of hearing loss. Genotyping of target SNPs of the haplotypes in 61 additional DFNA21 subjects from 10 families confirmed the association of one of these haplotypes with an earlier onset of disease (p = 0.0017). This haplotype locates on the wildtype allele and two of its 28 variants are in a cluster of predicted binding sites of the hair cell transcription factor POU4F3.

Conclusion: We identified a haplotype upstream of *RIPOR2* that is associated with an earlier onset of DFNA21. A potential effect of two variants on POU4F3-mediated gene regulation is being assessed.

Grants: The Heinsius Houbolt Foundation

1. Trpchevska N, et al. Am J Hum Genet 2022;109:1077–91.

Conflict of Interest: None declared

P04.018.C A novel *RPE65*-related dominant retinopathy caused by founder variant c.1555G>A p.(Glu519Lys) enriched in Belgian patients with a recognizable pattern dystrophy spectrum

Eline Van Vooren[1;2], *Filip Van den Broeck*[3;4], *Eline Geens*[1;2], *Marieke De Bruyne*[2], *Stijn Van de Sompele*[2], *Quinten Mahieu*[1;2], *Sheetal Uppal*[5], *Eugenia Poliakov*[5], *Michael T. Redmond*[5], *Julie De Zaeytijd*[3;4], *Bart Leroy*[3;4;6], *Miriam Bauwens*[1;2], *Elfride De Baere*[1;2]

[1]*Biomolecular Medicine, Ghent University, Ghent, Belgium;* [2]*Center for Medical Genetics, Ghent University Hospital, Ghent, Belgium;* [3]*Department of Head and Skin, Ghent University, Ghent, Belgium;* [4]*Department of Ophthalmology, Ghent University Hospital, Ghent, Belgium;* [5]*Laboratory of Retinal Cell and Molecular Biology, National Eye Institute, NIH, Bethesda, United States;* [6]*Division of Ophthalmology & Center for Cellular & Molecular Therapeutics, Children's Hospital of Philadelphia, Philadelphia, PA, United States*

Consortium: AD-RPE65

Background/Objectives: Recessive *RPE65*-retinopathy is a well-known target for gene therapy (Luxturna). Dominant *RPE65*-retinopathy however has been reported only once, caused by the 'Irish' variant c.1430A>G p.(Asp477Gly). Here, we discovered a novel dominant *RPE65*-retinopathy caused by variant c.1555G>A p.(Glu519Lys), enriched in Belgian patients with pattern dystrophy.

Methods: The c.1555G>A variant was identified in genome sequencing (GS) data (n = 3), RetNet filtered exome data (n = 27) and targeted NGS data of unsolved inherited retinal disease (IRD) cases (n = 40) with pattern dystrophy/maternally-inherited diabetes and deafness (MIDD)-like phenotypes. Haplotype phasing was done by long-read GS (n = 4), followed by broader SNV and microsatellite analysis. The variant was functionally assessed using an enzymatic RPE65 assay, Western blotting, co-immunoprecipitation, cellular thermal shift assay (CETSA), minigene assays and protein modelling (AlphaFold). Affected cases were clinically reassessed.

Results: We identified 70 index patients and family members with the rare and highly conserved c.1555G>A *RPE65* variant (gnomAD v4, 5x). Haplotyping revealed a shared region of 200 kb, supporting a founder effect. Segregation analysis showed complete penetrance with extensive variability, from subtle mottling to macular pattern dystrophy resembling the one found in MIDD. Enzymatic activity of Glu519Lys was lowered to ~56%, similar to the Irish Asp477Gly. Immunoblotting showed reduced mutant protein expression. Protein modelling and CETSA support a potential shift in protein stability and binding. Unlike Asp477Gly no splice-effect was observed.

Conclusion: We report a novel dominant *RPE65*-IRD in Belgian patients with a recognizable retinal phenotype, reducing the diagnostic gap in dominant IRD and opening new therapeutic perspectives.

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Conflict of Interest: Eline Van Vooren: None declared, Filip Van den Broeck: None declared, Eline Geens: None declared, Marieke De Bruyne: None declared, Stijn Van de Sompele: None declared, Quinten Mahieu: None declared, Sheetal Uppal: None declared, Eugenia Poliakov: None declared, Michael T. Redmond: None declared, Julie De Zaeytijd: None declared, Bart Leroy Novartis, Spark Therapeutics, Miriam Bauwens: None declared, Elfride De Baere: None declared

P04.019.D Closing the gap: overcoming the challenges of *RPGR* ORF15 sequencing, a target for gene therapy of X-linked retinitis pigmentosa

Stijn Van de Sompele[1;2], *Marieke De Bruyne*[1;2], *Mattias Van Heetvelde*[1;2], *Toon Rosseel*[1;2], *Bart Leroy*[1;3;4], *Julie De Zaeytijd*[3], *Brecht Guillemyn*[1;2], *Kathleen Claes*[1;2], *Kim De Leeneer*[1;2], *Miriam Bauwens*[1;2], *Elfride De Baere*[1;2]

[1]*Ghent University, Department of Biomolecular Medicine, Ghent, Belgium;* [2]*Ghent University Hospital, Center for Medical Genetics Ghent, Ghent, Belgium;* [3]*Ghent University Hospital, Department of*

Ophthalmology, Ghent, Belgium; [4]The Children's Hospital of Philadelphia, Division of Ophthalmology & Center for Cellular & Molecular Therapeutics, Philadelphia, United States

Background/Objectives: Retinitis pigmentosa (RP) is a group of inherited retinal diseases (IRD) causing vision impairment in 2 million people worldwide. X-linked RP (XLRP), comprising 10% of cases, is the most severe, leading to earlier onset and rapid progression. *RPGR* is the major XLRP gene, harboring approximately 80% of pathogenic variants, primarily in ORF15, a retina-specific exon. Gene therapy trials aim to halt *RPGR*-IRD degeneration, requiring a precise molecular diagnosis. However, ORF15's repetitive and purine-rich nature presents technical challenges for genetic testing, including PCR artifacts, inadequate coverage, misalignments, and variant calling inaccuracies.

Methods: To overcome these challenges, the performance (coverage and variant calling) of different sequencing technologies were evaluated for ORF15. In particular, we compared targeted PCR- and probe-based approaches, whole exome sequencing (WES) using two types of probes, and short- and long-read whole genome sequencing (WGS).

Results: WES and short-read WGS provide a minimum coverage of <20X for ORF15, limiting their variant calling performance. Long-read WGS demonstrated drastic improvements in the coverage uniformity for ORF15, albeit at a significantly higher cost and suboptimal variant calling performance. Our custom KAPA HyperCap probes for the enrichment of 400kb clinically relevant regions, provides a minimum coverage of >120X for ORF15. Moreover, this low-cost technology allowed the accurate detection of variants in ORF15 and a broad selection of other IRD.

Conclusion: The targeted probe-based approach allows sufficient coverage and reliable variant calling in ORF15, which will accelerate molecular diagnoses of *RPGR*-IRD in a cost-effective way, thereby improving patients' eligibility for therapeutic interventions.

Grants: Ghent University Hospital Innovation Fund NucleUZ

Conflict of Interest: None declared

P04.020.A Worldwide multicenter study (EyeConic) identifies patients eligible for cone-based optogenetics therapy, underlying specific genetic determinants

Dhryata Kamdar[1;2], *Lucas Janeschitz-Kriegl[1;2], Mathieu Quinodoz[1;2], Jacque L. Duncan[3], Stefanie Duret[3], Michel Michaelides[4], Michalis Georgiou[4], Zoltan Nagy[5], Viktória Szabó[5], Ditta Zobor[5], Jose Sahel[6], Boris Rosin[6], Eyal Banin[7], Itay Chowers[7], Samer Khateb[7], Byron L. Lam[8], Dr. Carlos Mendoza-Santiesteban[8], Potyra Rosa[8], Tamara Juvier[8], Katarina Stingl[9], Melanie Kempf[9], Giacomo Calzetti[1;2;10], Zi-Bing Jin[11], Ren-Juan Shen[11], Kaoru Fujinami[12], Karsten Borgwardt[13], Michael Adamer[13], Bence György[1;2], Hendrik Scholl[1;2], Carlo Rivolta[1;2;14]*

[1]IOB - Institute of Molecular and Clinical Ophthalmology Basel, Ophthalmic Genetics group, Basel, Switzerland; [2]Universität Basel, Department of Ophthalmology, Basel, Switzerland; [3]University of California San Francisco Parnassus Campus, Department of Ophthalmology, San Francisco, United States; [4]Moorfields Eye Hospital, London, United Kingdom; [5]Semmelweis University, Department of Ophthalmology, Budapest, Hungary; [6]University of Pittsburgh, Department of Ophthalmology, Pittsburgh, United States; [7]The Hebrew University of Jerusalem, Jerusalem, Israel; [8]Bascom Palmer Eye Institute, Miami, United States; [9]University of Tuebingen, University Eye Hospital, Center for Ophthalmology, Tübingen, Germany; [10]Pad.9 - Centro Senologico/Ambulatori Oculistica - Ospedale Maggiore di Parma, Parma, Italy; [11]Beijing Institute of Ophthalmology, Beijing, China; [12]National Hospital Organization Tokyo Medical Center, National Institute of Sensory Organs, Meguro City, Japan; [13]ETH Zürich - Department of Biosystems Science and Engineering ETH, Basel, Switzerland; [14]University of Leicester, Department of Genetics and Genome Biology, Leicester, United Kingdom

Consortium: the EyeConic Study Group

Objectives: The EyeConic study is a multicenter study, aiming at estimating the proportion of low-vision patients with preserved foveal cone photoreceptors. Previous studies have shown that in inherited retinal degenerations (IRDs) cones may lose their outer segments, involved in light sensing, while keeping their cell bodies intact. Thus, this dormant state creates an opportunity to re-sensitize cone cell bodies with optogenetic tools to restore vision in blind patients.

Methods: We enrolled 446 eyes from 291 low-vision patients with generalized IRDs (study patients) from 11 centers, in addition to eyes from 31 individuals with Stargardt disease (atrophy controls), and from 93 healthy controls. Machine-learning algorithm segmented Optical Coherence Tomography scans to calculate corrected rod-free volume (cRFV) in the central part of the macula, excluding hyperreflective material for accurate cone volume estimation. Genotype data was manually annotated and classified using ACMG classification and ClinVar. Linear mixed model analysis was performed to identify genetic association with foveal preservation.

Results: Mean (±s.d.) cRFV values were $8.7 \pm 4.4 \times 10^6$ μm^3, $4.2 \pm 1.7 \times 10^6$ μm^3, and $1.29 \pm 1.97 \times 10^7$ μm^3, for study patients, atrophy controls, and healthy controls, respectively. In total, 235 study patients had informative genotype-phenotype data, with causal mutations identified in 53 known IRDs genes. Only ABCA4 was found to have a significant effect on foveal preservation.

Conclusions: Mean foveal volumes in the EyeConic cohort are significantly higher than in the atrophy control group. Still, genotype was found only to have a limited effect on the presence of foveal preservation.

Conflict of Interest: None declared

P04.021.B Autosomal recessive loss-of-function variants in POC5 cause a syndrome with retinitis pigmentosa and lipodystrophy and impair ciliary function

Anneke Vulto-van Silfhout[1;2], *Lonneke Haer-Wigman[2], Suzanne Yzer[3], Ronald van Beek[2], Ingrid Jazet[4], Charlotte Brasch-Andersen[5], Simon Frost[5], Miriam Bauwens[6], Elfride De Baere[6], Irina Balikova[7], Filip Van den Broeck[8], Monika Weisz Hubshman[9], Pascal Joset[10], Peter Miny[10], Isabel Filges[10], S. Demirdas[11], Tjakko Van Ham[11], Elisabeth F.C. van Rossum[12], A. Thiadens[13], Susanne Kohl[14], Laura Kühlewein[15], Jan-Philipp Bodenbender[15], Tobias Haack[16], Karin Schaeferhoff[16], Susanne Roosing[2], Machteld Oud[2]*

[1]Maastricht University Medical Center, Department of Clinical Genetics, Maastricht, Netherlands; [2]Radboud University Medical Centre, Department of Human Genetics, Nijmegen, Netherlands; [3]Radboud University Medical Center, Department of Ophthalmology, Nijmegen, Netherlands; [4]Leiden University Medical Center, Department of Medicine, Div. Endocrinology, Leiden, Netherlands; [5]Odense University Hospital, Department of Clinical Genetics, Odense, Denmark; [6]Ghent University and Ghent University Hospital, Center for Medical Genetics, Ghent, Belgium; [7]University Hospital Leuven, Department of Ophthalmology, Leuven, Belgium; [8]Ghent University Hospital, Department of Ophthalmology, Ghent, Belgium; [9]Baylor College of Medicine, Department of Molecular and Human Genetics, Houston, United States; [10]University Hospital Basel, Institute of Medical Genetics and Pathology, Basel, Switzerland; [11]Erasmus University Medical Center, Department of Clinical Genetics, Rotterdam, Netherlands; [12]Erasmus University Medical Center, Department

of Internal Medicine, Division of Endocrinology, Rotterdam, Netherlands; [13]Erasmus University Medical Center, Department of Ophthalmology, Rotterdam, Netherlands; [14]University of Tuebingen, Molecular Genetics Laboratory, Institute for Ophthalmic Research, Center for Ophthalmology, Tuebingen, Germany; [15]Eberhard Karls University, University Eye Hospital, Department for Ophthalmology, Tuebingen, Germany; [16]University of Tuebingen, Institute for Medical Genetics and Applied Genomics, Tuebingen, Germany

Background/Objectives: A homozygous loss-of-function (LoF) variant in *POC5* was previously described as a novel cause for retinitis pigmentosa.[1] This patient, however, was also reported to have short stature, microcephaly and recurrent glomerulonephritis. We studied a cohort of patients with bi-allelic *POC5* LoF variants.

Methods: We identified eight patients from seven families with homozygous LoF *POC5* variants and performed detailed phenotype analysis. POC5 localization studies were performed in three patient-derived fibroblast cell lines.

Results: In depth phenotyping of patients with *POC5* variant showed not only retinitis pigmentosa but also variable other organ involvement including lipodystrophy with diabetes and liver disease, kidney disease, and dysmorphic features.

The POC5 protein localizes to the centrioles and plays an essential role during the cell cycle and cilium formation. Ciliopathies are a clinically heterogeneous group of disorders that can affect almost any organ of the human body. The phenotypes of the eight patients in this study fit well in the ciliopathy phenotypic spectrum, except for lipodystrophy which is not common in ciliopathies.

Interestingly, POC5 localization studies in three patient-derived fibroblast cell lines show aberrant localization indicating a ciliary defect in these patients. This further supports the hypothesis that defective POC5 results in a ciliary disorder.

Conclusion: In conclusion, we describe a novel syndrome caused by autosomal recessive LoF variants in *POC5* that includes retinitis pigmentosa, lipodystrophy, and dysmorphic features and provide further evidence that this is a novel ciliopathy. Further studies are required to elucidate the role of POC5 in lipodystrophies.

Conflict of Interest: None declared

P04.022.C The establishment of a patient registry for Otoferlin-related hearing loss

Barbara Vona[1;2], Nicola Strenzke[2;3], Tobias Moser[2;4;5], **Bernd Wollnik**[1;4]

[1]University Medical Center Göttingen, Institute of Human Genetics, Göttingen, Germany; [2]University Medical Center Göttingen, Institute for Auditory Neuroscience and InnerEarLab, Göttingen, Germany; [3]University Medical Center Göttingen, Department of Otolaryngology, Göttingen; [4]University Medical Center Göttingen, Cluster of Excellence "Multiscale Bioimaging: from Molecular Machines to Networks of Excitable Cells" (MBExC), Göttingen; [5]Max Planck Institute for Multidisciplinary Sciences, Auditory Neuroscience and Synaptic Nanophysiology Group, Göttingen

Background/Objectives: Among the hundreds of genes responsible for isolated hearing impairment, otoferlin (*OTOF*, DFNB9, AUNB1) ranks among the prevalent forms. Biallelic otoferlin variants cause auditory synaptopathy, frequently appearing as congenital/early onset deafness with absent auditory brainstem responses but preserved otoacoustic emissions. In response to promising pre-clinical and early in-human trial results, we sought to develop the first global registry for otoferlin patients.

Methods: REDCap software guided database development. An interdisciplinary team was consulted to shape data fields and consent forms. Fields were created for entry of phenotype, genotype and inheritance patterns in pseudonymized form, following digital consent. A data security concept was devised and approved.

Results: The registry provides an overview of the frequency and manifestations of *OTOF* and of the success of various treatment strategies. It supports patient-driven clinical and translational research. The registry, available in English and German languages, was approved by the Ethics Committee of the University Medical Center Göttingen and is registered under the ClinicalTrials.org ID NCT05946057.

Further information and link to consent forms can be found via the Institute of Auditory Neuroscience: http://www.auditory-neuroscience.uni-goettingen.de/otoferlin_registry_en.html

Conclusion: We foresee this registry as essential infrastructure for conducting longitudinal natural history studies on a broad spectrum of patients, monitoring outcomes of future gene therapies, and prioritizing patient-driven research. We have since extended a collaborative invitation to the facebook "Otoferlin Gene Mutation" patient group. We invite patients and clinicians to participate.

Grants: DFG funding: VO2138/7-1 #469177153 (B.V.); Heisenberg program #406266759 (N.S.); Multiscale Bioimaging Cluster of Excellence EXC 2067/1-390729940 (T.M., B.W.).

Conflict of Interest: None declared

P04.023.D Elucidation of the *OTOA* paralogous sequence in non-syndromic deafness

Tine Tesovnik[1;2], *Katarina Trebušak Podkrajšek[1;3], Robert Sket[1;2], Marusa Debeljak[1;4], Saba Battelino[5;6], Jernej Kovač[1;2], Barbara Jenko Bizjan[1;2]*

[1]University Medical Centre Ljubljana, Clinical Institute of Special Laboratory Diagnostics, Ljubljana, Slovenia; [2]Faculty of Medicine, University Ljubljana, Department of Paediatrics, Ljubljana, Slovenia; [3]Faculty of Medicine, University Ljubljana, Institute of Biochemistry, Ljubljana, Slovenia; [4]Faculty of Medicine, University Ljubljana, Institute of Cell Biology, Ljubljana, Slovenia; [5]University Medical Centre Ljubljana, Department of Otorhinolaryngology and Cervicofacial Surgery, Ljubljana, Slovenia; [6]Faculty of Medicine, University Ljubljana, Department of Otorhinolaringology, Ljubljana, Slovenia

Background/Objectives: Otoancorin is a protein that plays a crucial role in the development and function of the inner ear, specifically in auditory and vestibular function. *OTOA* genetic aberrations cause autosomal recessive deafness, which manifests as a severe to profound sensorineural non-syndromic hearing impairment affecting all frequencies, manifesting in the prelingual phase. The impact of *OTOA*-related hearing loss may be underestimated due to the limitations of NGS sequencing technology and the paralog-specific region.

Methods: Our study involved 328 individuals referred for genetic testing due to hearing impairment and without other syndromic features. All subjects underwent WES and WGS was performed in cases with significant discrepancies in the *OTOA* paralog-specific region. Long-range PCR with long-read sequencing was used to characterize all participants, and additional long-read sequencing of chromosome 16 was conducted to confirm *OTOA*-associated deafness.

Results: The WES analysis did not identify known pathogenic or likely pathogenic SNV or CNV. However, the CNV analysis revealed a significant variability representation of reads in the *OTOA* paralogous region, which could not be resolved by WGS. To clarify the NGS results, we performed long-range PCR and long-read

sequencing. This revealed *OTOA* paralogous sequence variants as the cause of hearing loss in over 1% of subjects.

Conclusion: Our study revealed *OTOA* paralogous deafness in a non-negligible proportion of subjects with non-syndromic deafness. These results emphasize the importance of integrating long-read sequencing into clinical diagnostics to enhance the understanding of complex, repetitive, and paralogous sequences in the human genome.

Grants: ARIS-programme: P3-0343

Conflict of Interest: None declared

P04.024.A The diagnostic chameleon of Hereditary Hearing Loss (HHL): a genetic investigation on Non-syndromic Mimics (NSMs)

Beatrice Spedicati[1;2], *Anna Morgan*[2], *Stefania Lenarduzzi*[2], *Elisa Rubinato*[2], *Gabriele Zucca*[1], *Mabel Troian*[2], *Dario Marangoni*[3], *Giorgia Girotto*[1;2]

[1]*University of Trieste, Department of Medicine, Surgery and Health Sciences, Trieste, Italy;* [2]*Institute for Maternal and Child Health – I.R.C.C.S. "Burlo Garofolo", Trieste, Italy;* [3]*University of Trieste, Department of Medicine, Surgery and Health Sciences, Eye Clinic, Ophthalmology Clinic, Trieste, Italy*

Background: HHL is characterised by extensive clinical and genetic heterogeneity and its molecular diagnosis is further complicated by peculiar and understudied clinical conditions known as NSMs.

Methods: In the last 18 months, 73 apparently non-syndromic Italian HHL patients, negative to *GJB2* and *STRC* analyses, underwent Whole Exome Sequencing to identify NSMs.

Results: The genetic cause of HHL was identified in 36/73 patients (49.3%). Among them, 12 subjects were found to be affected by NSMs, which may be divided in two groups: 1) two patients with subtle additional signs that were missed during the initial clinical evaluation; 2) ten patients whose further clinical features are expected to develop later in life. In group 1, we identified pathogenic variants in *MITF* and *GATA3* genes, associated with Waardenburg and Barakat syndromes, respectively. In group 2, the identified genes were *CDH23* (one patient) *USH2A* and *ADGRV1* (six and three patients, respectively). These results highlight how 75% of all NSMs are represented by Usher syndrome type 2 and are in line with our previous findings, that confirmed the high prevalence of Usher syndrome type 2 carriers in the Italian population (1:70).

Conclusion: Group 1 NSMs provide crucial insight on very mild forms of syndromic deafness, thus helping clinicians to a critical re-evaluation of the diagnostic criteria of each condition. Conversely, group 2 NSM patients need to be assessed by a multidisciplinary team to provide a tailored management plan, with the final goal of identifying a personalized follow-up and specific preventive strategies.

Grants: NA

Conflict of Interest: None declared

P04.025.B Retrospective analysis over 20 years of DNA diagnostics in 7,017 index patients with inherited retinal dystrophies (IRDs)

Christina Kiel[1], *Heidi Stöhr*[1], *Kristin Geiger*[1], *Helmut Roth*[1], *Ulrike Friedrich*[1], *Fabiola Biasella*[1], *Bernhard Weber*[1;2]

[1]*University of Regensburg, Institute of Human Genetics, Regensburg, Germany;* [2]*University Hospital Regensburg, Institute of Clinical Human Genetics, Regensburg, Germany*

Background/Objectives: Inherited retinal dystrophies (IRDs) are a diverse group of rare retinopathies ultimately culminating in vision impairment of varying degrees. High clinical and genetic heterogeneity makes DNA diagnostics in IRDs a challenging task. Our retrospective study reports one of the largest IRD cohorts with over 7,000 index patients representing a wide spectrum of IRD phenotypes.

Methods: Genetic data of 7,017 index patients with clinically distinct IRD conditions collected over 20 years were considered retrospectively. Patients were analyzed by either targeted next-generation sequencing (NGS), DNA microarray or Sanger sequencing. The obtained data were standardized and a characterization of the entire cohort was performed.

Results: Overall, 47 % of patients (3,300 out of 7,017) obtained a fully informative DNA diagnosis, whereby the diagnostic yield strongly varied between disease entities. The majority of solved cases displayed autosomal-recessive (54.1 %), followed by autosomal-dominant (28.7 %), X-linked (15.7 %) and mitochondrial (1.5 %) inheritance. In total, 145 genes were found to be associated with disease in at least one patient. The most common disease-causing genes were *ABCA4* (26.1 %), *BEST1* (7.8 %), *PRPH2* (5.7 %), *RPGR* (5.5 %) and *RS1* (5.5 %). Out of 5,378 genetic variants identified, 61.9 % were not listed in HGMD, of which 28.6 % were classified as (likely) pathogenic.

Conclusion: The results of genetic testing in our large cohort provide important insight into the spectrum of mutated alleles in the IRDs, which is a crucial basis for future clinical trials and therapeutic options.

Grants: no

Conflict of Interest: None declared

P04.026.C Functional analysis of *CNGA3* missense variants associated with autosomal recessive achromatopsia allows confirmation and reclassification of variants

Susanne Kohl[1], *Maria Solaki*[1], *Bernd Wissinger*[1], *Peggy Reuter*[1]

[1]*Institute for Ophthalmic Research, Centre for Ophthalmology, Tübingen, Germany*

Background/Objectives: Achromatopsia (ACHM) is a rare congenital retinal disorder due to impaired cone photoreceptor function. CNGA3, responsible for up to 40% of ACHM cases, encodes the A3 subunit of the cyclic nucleotide-gated (CNG) channel. Most missense variants in CNGA3 are functionally uncharacterized, often leading to uncertain significance (VUS) classification. To address this, we developed a medium-throughput aequorin-based calcium influx assay for assessing the functional impact of uncharacterized CNGA3 missense variants, aiding in clinical variant interpretation.

Methods: Wild-type or variant homomeric CNGA3 channels were expressed in HEK293 cells stably expressing aequorin. Activation of CNG channels was achieved through 8-Br-cGMP application. CNG channel-mediated Ca^{2+} influx was recorded via aequorin-mediated luminescence, correlating with channel functionality. Dot blot analysis of whole cell lysates confirmed CNGA3 protein expression.

Results: The bioassay validation included testing 9 benign and 15 extensively studied pathogenic CNGA3 missense variants. Thresholds for "functionally normal" and "functionally abnormal" were determined through ROC analysis. Out of 150 uncharacterized missense variants, 55 were previously classified as VUS. Among the variant channels, 80 showed no function, while 70 displayed luminescence signals. Of these, 45 indicated altered but residual channel function, and 25 exhibited wild-type-like

behavior. The functional assessment led to the re-classification of 33 VUS as (likely) pathogenic and 19 VUS as (likely) benign.

Conclusion: Our bioassay is a crucial instrument for assessing the influence of CNGA3 missense variants on the functionality of homomeric CNGA3 channels, ultimately contributing to the reclassification of 94.5% of VUS.

Grants: Deutsche Forschungsgemeinschaft (DFG) project number: KO 2176/3-1, 398539671.

Conflict of Interest: Susanne Kohl Deutsche Forschungsgemeinschaft (DFG) project number: KO 2176/3-1, 398539671, University Hospital Tübingen, Maria Solaki: None declared, Bernd Wissinger University Hospital Tübingen, Peggy Reuter: None declared

P04.027.D Expanding the Enhanced S cone Syndrome (ESCS) phenotype-genotype correlation the role of the developmental gene NRL

John Grigg[1;2], *Elisa Cornish*[1;2], *Stephanie Rozos*[1], *Vanessa Leung*[1], *Chris Ovens*[1], *Marium Raza*[1], *Benjamin Nash*[2;3], *Julie McGaughran*[4], *Robyn Jamieson*[1;2]

[1]*Save Sight Institute, Faculty of Medicine and Health, The University of Sydney, Sydney, Australia;* [2]*Eye Genetics Research Unit, Children's Medical Research Institute, The Children's Hospital at Westmead, Westmead, Australia;* [3]*Sydney Genome Diagnostics, Western Sydney Genetics Program, Sydney Children's Hospitals Network, Westmead, Sydney, Australia;* [4]*Genetic Health Queensland, Royal Brisbane and Women's Hospital Metro North health Service District, Herston, Australia*

Background/Objectives: We describe the ocular phenotype in three patients from two families with novel mutations in Neural retina leucine zipper (NRL) resulting in an enhanced S-cone phenotype (ESCS). Visual electrophysiology recordings in ESCS have traditionally been identified as being specific for the gene NR2E3. Recently cases of autosomal recessive NRL mutations have been described resulting in an ESCS phenotype.

Methods: Investigations including full-field electroretinogram (ffERG), best corrected visual acuity (BCVA), optical coherence tomorgraphy (OCT) and ultra-wide field autofluorescence (UWAF) and colour imaging are reported.

Results: Family 1 I1 (Male) initially with symptoms from age 5 years. BCVA was 6/120 in both eyes with nystagmus, UWAF, OCT and ffERG were consistent with the classic features of ESCS. Genetic testing identified a novel homozygous likely pathogenic NRL variant c.256G>T; p.Glu86*

Family 2 II1 and II2 (Female aged 4.5 and Male 3 years) presented with nyctalopia. BCVA 3/6 Kay pictures both eyes. ffERG, retinal curvilinear yellow glistening stripes and hyperfluorescent dots throughout the fundus were recorded representing an atypical ESCS phenotype. NRL pathogenic variants c.223dup p.Leu75Profs* and c.16del p.Ser6Alfs*13 were identified.

Conclusion: Biallelic NRL genetic variants in these two families expand the geneotype association with the Enhanced S cone Syndrome phenotype.

NRL binds directly to the promoter of NR2E3 and thus acts upstream of this transcription factor to guide the developing photoreceptor into the rod lineage. It is this interaction that contributes to the clincal phenotype of ESCS for NRL in addition to NR2E3.

Grants:

Conflict of Interest: John Grigg Janssen
Belite Bio, Belite Bio
Janssen
Novartis, Elisa Cornish: None declared, Stephanie Rozos: None declared, Vanessa Leung: None declared, Chris Ovens: None declared, Marium Raza: None declared, Benjamin Nash: None declared, Julie McGaughran: None declared, Robyn Jamieson Janssen, Novartis, Janssen, Novartis

P04.028.A Virtual, national ocular genomic multidisciplinary team (OcularGen MDT) for variant reclassification, and functional genomic and therapy eligibility in inherited retinal disorders

Robyn Jamieson[1;2], *Benjamin Nash*[1;3], *Alan Ma*[1;2], *Emma Hackett*[3], *Elisa Cornish*[4], *John Grigg*[1;3], *Bruce Bennetts*[3]

[1]*University of Sydney, Children's Medical Research Institute & Save Sight Institute, Eye Genetics Research Unit, Sydney, Australia;* [2]*Sydney Children's Hospitals Network, Department of Clinical Genetics, Sydney, Australia;* [3]*Sydney Children's Hospitals Network, Sydney Genome Diagnostics;* [4]*University of Sydney, Save Sight Institute*

Background/Objectives: The growing number of emerging genetic therapies/clinical trials for patients with inherited retinal disorders (IRDs), leads to increased demand for accurate molecular diagnosis to fulfill eligibility criteria. Variants of uncertain significance (VUS) need resolution, which requires detailed review and assessment for functional genomic studies where indicated.

Methods: A virtual, ocular genomic multidisciplinary team (OcularGen-MDT) approach was implemented, with lead clinical genetic, ophthalmic, genetic counsellor, diagnostic scientist and IRD researcher contributions. Referring clinical teams presented history, pedigree and ophthalmic information. The OcularGen MDT diagnostic scientists independently evaluated variant curation using ACMG criteria. Cases were reviewed in context with ophthalmic and pedigree information.

Results: Over 120 cases with VUS or other pathogenicity queries were referred from across Australia between 2019-2023. Overall, 227 variants in 94 genes were reviewed. 42% (42/99 variants) of VUS were able to be resolved through a combination of detailed ophthalmic analysis, literature review, family segregation studies and review of ACMG criteria. Cases with *RPE65* variants were assessed to meet therapy eligibility. In almost all referred cases, clinically significant information was returned.

Conclusion: The OcularGen-MDT provides referring clinical teams an avenue for expert review of unresolved cases in a regular, virtual format. The combined expertise led to VUS resolution, or other recommendations for clinical management or functional genomic studies in almost all cases. The virtual format facilitated access for rural and regional cases. This study highlights the benefits of a cross-discipline structured MDT, especially in assessing suitability for emerging clinical trials and therapies.

Grants:

Conflict of Interest: Robyn Jamieson Novartis, Janssen, Janssen, Benjamin Nash: None declared, Alan Ma: None declared, Emma Hackett: None declared, Elisa Cornish: None declared, John Grigg Janssen, Janssen, Novartis, Bruce Bennetts: None declared

P04.030.C Exome variant prioritization in a large cohort of hearing-impaired individuals indicates *IKZF2* to be associated with non-syndromic hearing loss and guides future research for unsolved cases

Hedwig Velde[1;2], *Maryam Vaseghi-Shanjani*[3], *Gayatri Ramakrishnan*[4], *Jeroen Smits*[5], *Jaap Oostrik*[1], *Helger Yntema*[6], *Lonneke Haer-Wigman*[6], *Martijn Huynen*[4], *Anna Lehman*[7], *Stuart Turvey*[3], *Ronald Pennings*[1], **Hannie Kremer**[1;6]

[1]*Radboud university medical center, Otorhinolaryngology, Nijmegen, Netherlands;* [2]*Radboud university medical center, Institute for Brain*

Cognition and Behaviour, Nijmegen, Netherlands; [3]The University of British Columbia and BC Children's Hospital, Pediatrics, Vancouver, Canada; [4]Radboud university medical center, Medical Biosciences, Nijmegen, Netherlands; [5]University Medical Center Utrecht, Medical Genetics, Utrecht, Netherlands; [6]Radboud university medical center, Human Genetics, Nijmegen, Netherlands; [7]The University of British Columbia, Medical Genetics, Vancouver, Canada

Consortium: the DOOFNL consortium

Background/Objectives: Non-syndromic hereditary hearing loss (HL) is genetically highly heterogeneous, yet at least half of the cases remain unexplained in medical genetic testing. One of several reasons for this is that pathogenic variants are located in "unknown" deafness genes. With a variant prioritization approach, we aimed to identify novel (candidate) genes for HL.

Methods: Exome-wide sequencing data were collected for subjects with (presumed) genetic HL that remained unexplained in medical genetic testing (gene-panel analysis). Cases in group AD had presumed autosomal dominantly inherited HL (n = 124) and in group AR presumed autosomal recessive HL (n = 337). Variants in known or candidate deafness genes (e.g. preferential inner ear expression, mouse deafness genes) were prioritized based on allele frequencies and predicted effects (e.g. truncating). In silico tools were employed to predict effects on transcript splicing or deleteriousness of missense variants. Selected variants were tested for their co-segregation with HL.

Results: We associated *IKZF2* with dominantly inherited non-syndromic HL in three families. Missense variants specifically affected Cysteines or Histidines of C2H2 Zn-finger motifs 2 and 3 of the encoded protein Helios. Our findings indicate a complex genotype-phenotype correlation for defects in *IKZF2* since this gene was previously associated with ICHAD syndrome or non-syndromic dysfunction of the immune system. Only two cases were solved by variants in recently identified deafness genes.

Conclusion: Variants of *IKZF2* can underlie non-syndromic HL. The large number of candidate genes for HL stresses the importance of inclusion of family members (e.g. for trio analysis) for variant prioritization.

Grants: the Heinsius Houbolt Foundation

Conflict of Interest: None declared

P04.031.D Biallelic variants in ADIPOR1 cause intellectual disability with retinitis pigmentosa.

Adam Jackson[1;2], *Francesca Cancellieri[3;4], Tobias Bartolomaeus[5], Ji Hoon Han[3;4], Mathieu Quinodoz[3;4;6], Louiza Koutroumanou[7], Georgios Papadakis[7], Miltiadis Tsilimbaris[7], Iro Boura[8], Cleanthe Spanaki[8], Balázs Varsányi[9], Viktória Szabó[9], Rahat Perveen[1;2], Jacob Sampson[1;2], Eva Lenassi[1;2], Rami Abou Jamra[5], Carlo Rivolta[3;4;6], Siddharth Banka[1;2]*

[1]Manchester Centre for Genomic Medicine, St Mary's Hospital, Manchester University NHS Foundation Trust, Health Innovation Manchester, Manchester, UK., Manchester, United Kingdom; [2]Division of Evolution, Infection and Genomics, School of Biological Sciences, Faculty of Biology, Medicine and Health, University of Manchester, Manchester, UK., Manchester, United Kingdom; [3]IOB - Institute of Molecular and Clinical Ophthalmology Basel, Basel, Switzerland; [4]Universität Basel, Basel, Switzerland; [5]Leipzig University, Leipzig, Germany; [6]Department of Genetics and Genome Biology, University of Leicester, Leicester, UK; [7]University of Crete Medical School, Department of Ophthalmology; [8]University of Crete, Medical School, Department of Neurology; [9]Semmelweis University Budapest, Dept. of Ophthalmology, Budapest, Hungary

Background/Objectives: ADIPOR1 is one of two receptors for Adiponectin, a well-known hormone produced by adipose tissue and implicated in metabolic dysfunction. ADIPOR1 is also essential for lipid homeostasis in photoreceptors. Thus far, two families with *ADIPOR1* variants and retinitis pigmentosa have been described. The extra-retinal consequences of *ADIPOR1* variants are poorly understood.

Methods: We genetically and clinically characterised individuals with biallelic *ADIPOR1* variants, performed RT-QPCR on patient samples, undertook haplotype analysis and examined tissue expression datasets.

Results: We identified 4 individuals from 2 families of Pakistani origin with an identical homozygous frameshift *ADIPOR1* variant and showed that the transcript undergoes nonsense-mediated decay. Haplotype analysis showed that the affected individuals from the two families likely shared an ancestor ~200 years ago. We identified 6 additional individuals from three families with rare biallelic predicted deleterious missense *ADIPOR1* variants. In total, 7/10 individuals displayed retinal pigmentary abnormalities. In two families, retinal changes were identified following reverse phenotyping, whilst in two families visual symptoms were the presenting feature and in one family retinal phenotyping was not possible. Importantly, 9/10 individuals also had significant developmental delay and intellectual disability. Analysis of gene expression in retinal tissue demonstrated high expression of *ADIPOR1* but negligible expression of its close paralog, *ADIPOR2*.

Conclusion: *ADIPOR1* variants cause a recessive syndrome of intellectual disability and retinitis pigmentosa. ADIPOR1 likely transduces both Adiponectin-dependent and Adiponectin-independent signalling that may result in the neurodevelopmental and retinal phenotypes, respectively.

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Conflict of Interest: None declared

P04.032.A A novel hypothesis on Choroideremia manifesting female carriers: could *CHM* in-frame variants exert a dominant negative effect? A case report

Niccolò Di Giosaffatte[1;2], *Michele Valiante[1], Stefano Tricarico[3], Giulia Parise[1;2], Anna Maria De Negri[4], Guido Ricciotti[5], Florean Lara[1], Alessandro Paiardini[6], Irene Bottillo[1;7], Paola Grammatico[1;2]*

[1]San Camillo Forlanini Hospital, Laboratory of Medical Genetics, Roma, Italy; [2]Sapienza University of Rome, Department of Experimental Medicine, Roma, Italy; [3]ASL Roma C, Roma, Italy; [4]San Camillo Forlanini Hospital, Neuro-Ophthalmology Clinic, Roma, Italy; [5]University of Parma - Hospital, Department of Ophthalmology, Parma, Italy; [6]Sapienza University of Rome, Department of Biochemical Sciences "A. Rossi Fanelli", Roma, Italy; [7]Sapienza University of Rome, Department of Molecular Medicine, Roma, Italy

Background/Objectives: Choroideremia is a X-linked recessive condition presenting in males with progressive retinal degeneration and visual loss. It is associated with variants in *CHM* gene that lead to loss of REP-1 expression/function which is required for Rab prenylation by Rab geranylgeranyl transferase (GGTA). Even though female carriers are predicted to be not affected by the disease, a wide phenotypic spectrum has been reported.

Methods: We describe a family with a novel CHM pathogenic splicing variant. The variant, initially found in a 63 female proband with impaired visual acuity and severe retinal degeneration, segregated in the 31 years old daughter and the 37 years old son, both presenting with fundus anomalies.

Results: mRNA studies revealed a shorter *CHM* isoform lacking exon 10 without alteration of the reading frame. Molecular modelling of the ternary REP-1/Rab/GGTA protein complex, predicted significant impairing of REP-1/Rab binding without alteration of REP-1/GGTA interaction.

Conclusion: While chromosome X inactivation (XCI) skewing has been proposed as putative mechanism for choroideremia manifestation in female carriers, some evidence suggest that *CHM* could variably escape from XCI. We hypothesize that, in the two females, the biallelic expression of *CHM* could have led to the production of both the mutant and wild type REP-1. The mutant isoform, sequestrating GGTA, could have reduced its availability for prenylation of Rab proteins, thus exerting a dominant-negative effect which contributed to disease manifestation. More studies regarding the escape of *CHM* from XCI are warranted as this could open new possibilities in understanding clinical heterogeneity in female carriers.

Grants: None

Conflict of Interest: None declared

P04.033.B Functional evaluation of *CNGB3* variants associated with Achromatopsia for their potential to affect mRNA splicing

Peggy Reuter[1], *Katharina Rawnsley*[1], *Bernd Wissinger*[1], *Susanne Kohl*[1]

[1]*Institute for Ophthalmic Research, Molecular Genetics Laboratory, Tübingen, Germany*

Background/Objectives: Achromatopsia is a rare congenital retinal disorder caused by impaired cone photoreceptor function. *CNGB3* accounts for the majority of ACHM cases and encodes the modulatory B3 subunit of the cyclic nucleotide-gated channel. Several variants have been described that are located at or in proximity to exon-intron junctions but do not affect the canonical splice sites essential for mRNA splicing. Such variants are often of uncertain significance. To understand their impact on *CNGB3* splicing, in vitro minigene splice assays were conducted.

Methods: Preferentially multi- but also single-exon minigenes were created by amplifying wild-type *CNGB3* exons and flanking intronic regions, followed by cloning into the pSPL3 exon trapping vector. Mutant constructs were generated by in vitro mutagenesis. Wild-type or mutant minigenes were transfected into HEK293T cells. After RNA isolation and cDNA synthesis, PCR amplified splice products were analyzed by direct sequencing or sequencing of subcloned fragments.

Results: Of the 20 variants examined, 80% were initially classified as VUS. Minigene splice assays showed no effect on splicing for 3 variants. For the remaining 17 variants exon skipping, intron retention and/or the use of cryptic splice sites could be confirmed. Using these minigene data, 13 VUS were re-classified as (likely) pathogenic, and 2 were deemed likely benign. One variant was confirmed as pathogenic, while 3 variants initially classified as likely pathogenic were re-categorized as pathogenic.

Conclusion: Using the in vitro Minigene splice assay, 94% of the uncertain variants were successfully reclassified.

Grants: Deutsche Forschungsgemeinschaft (DFG) project number: KO 2176/3-2, 398539671.

Conflict of Interest: None declared

P04.034.C Identification of novel candidate genes for inherited retinal disorders by analyzing consanguineous families from Pakistan and Iran

Helen Nabiryo Frederiksen[1], *Asad Munir*[2], *Ijaz Anwar*[1], *Sergey Oreshkov*[1], *Ainee Zafar*[3], *Abdur Rashid*[1], *Mukhtar Ullah*[4;5], *Asad Umar*[2], *Salma Afsar*[2], *Fatima Javed*[6], *Anum Murtaza*[6], *Kanza Ashfaq*[6], *Sana Rehman Khan Khakwani*[6], *Samra Javed*[7], *Abida Arshad*[3], *Shumaila Zulfiqar*[6], *Humera Kausar*[6], *Fatemeh Suri*[8], *Sabika Firasat*[9], *Atta Ur Rehman*[2], *Stylianos Antonarakis*[10;11], **Muhammad Ansar**[1;12]

[1]*Department of Ophthalmology, University of Lausanne, Jules Gonin Eye Hospital, Fondation Asile Des Aveugles, Lausanne, Switzerland;* [2]*Department of Zoology, Faculty of Biological and Health Sciences, Hazara University, Mansehra, Pakistan;* [3]*Department of Biology, PMAS-Arid Agriculture University, Shamsabad, Rawalpindi, Pakistan;* [4]*Institute of Molecular and Clinical Ophthalmology Basel, Basel, Switzerland;* [5]*Department of Ophthalmology, University of Basel, Basel, Switzerland;* [6]*Department of Biotechnology, Kinnaird College for Women, Lahore, Pakistan;* [7]*Department of Biotechnology, Lahore College for Women University, Lahore, Pakistan;* [8]*Opthalmic Research Center, Research Institute for Ophthalmology and Vision Science, Shahid University of Medical Sciences, Tehran, Iran;* [9]*Department of Zoology, Faculty of Biological Sciences, Quaid-i-Azam University, Islamabad, Pakistan;* [10]*Department of Genetic Medicine and Development, University of Geneva, Geneva, Switzerland;* [11]*iGE3 Institute of Genetics and Genomics of Geneva, Geneva, Switzerland;* [12]*Advanced Molecular Genetics and Genomics Disease Research and Treatment Centre, Dow University of Health Sciences, Sindh, Pakistan*

Background: Consanguinity offers a unique opportunity to unveil novel gene-phenotype correlations, as children of consanguineous parents exhibit significantly higher genomic homozygosity, providing an ideal setting for identifying high-impact genomic variants associated with autosomal recessive disorders. Pakistan and Iran, characterized by high rates of consanguineous marriages (up to 70% and 38.6%, respectively), serve as valuable populations for such investigations.

Methods: This study encompasses the genetic analysis of 244 consanguineous families (913 individuals) from Pakistan and Iran, with two or more offspring with Retinal Disorders. Whole exome sequencing (WES) was conducted on affected individuals, followed by genotyping of potential candidate variants in all family members, including parents and siblings.

Results: Among the families with known Inherited Retinal Disorder (IRD) genes, the diagnostic yield was 71% (174 out of 244 families). In the 70 undiagnosed families, several novel candidate genes were identified including, *MOB1A* (NM_018221:c.503C>G:p.Ser168Cys), *CCDC180* (NM_020893:c.1969_1970del: p.Asn657ArgfsTer10), *PLA2G3* (NM_015715:c.601C>T:p.Arg201Ter), *ATP8A1* (NM_006095:c.3043C>T:p.Pro1015Ser), and *MPHOSPH6* (NM_005792:c.414T>G: p.Tyr138Ter). Notably, *PLA2G3* and *CCDC180* have been associated with ciliary dysfunction which has previously been linked to various ocular diseases. A $Ccdc180^{-/-}$ mouse model was shown to exhibit pronounced ocular phenotypes. MOB1A, a regulator of YAP1 activity, has implications in IRDs. Lastly, while *ATP8A1* and *MPHOSPH6* are expressed in the eye, their roles in visual function remain unclear.

Conclusions: This study underscores the effectiveness of WES in unraveling the genetic basis of IRDs in consanguineous families. The identification of novel candidate genes enhances our understanding of molecular pathways involved in these disorders, paving the way for future therapeutic interventions.

Conflict of Interest: None declared

P04.035.D Novel cases with biallelic variants in *Wnt ligand secretion mediator (WLS)* further support its role in structural eye anomalies

Fabiola Ceroni[1], *Lidiya Talbot*[1], *Yesim Kesim*[2;3], *Hande Tunbak*[1;4], *Dorine Bax*[1], *Zena Lam*[5], *Joanna Jarvis*[5], *Gaia Gestri*[4], *Nicola Ragge*[1;5]

[1]*Oxford Brookes University, Department of Biological and Medical Sciences, Oxford, United Kingdom;* [2]*Wellcome Centre for Human*

Genetics, University of Oxford, Oxford, United Kingdom; [3]NIHR Biomedical Research Centre, Oxford, United Kingdom; [4]University College London, Department of Cell and Developmental Biology, London, United Kingdom; [5]West Midlands Regional Clinical Genetics Service and Birmingham Health Partners, Birmingham Women's and Children's NHS Foundation Trust, Birmingham, United Kingdom

Consortium: Genomics England Research Consortium

Background/Objectives: Anophthalmia, microphthalmia and coloboma (AMC) form a spectrum of rare congenital eye anomalies; >50% of cases exhibit associated systemic features. AMC is genetically heterogenous: >130 genes are routinely tested for these disorders, including members of the Wnt signalling pathway. Essential to the secretion of all Wnt proteins is the transmembrane receptor Wntless (WLS), recently implicated in AMC in three out of seven families with Zaki syndrome.

Methods: To determine the role of *WLS* in AMC, we analysed whole exome/genome sequencing data of individuals with AMC from 174 UK families, the Deciphering Developmental Disorders (DDD) study (CAP69) and the 100,000 Genomes Project, and then generated a CRISPR-Cas9 zebrafish *wls* knockout model.

Results: We identified two individuals with rare biallelic *WLS* variants inherited from unaffected parents and predicted damaging. Individual 1 presented with bilateral microcornea, colobomas, microcephaly, ear and renal anomalies, and developmental delay. She carried a maternal stop-gain variant (c.G443A;p.W148X) and a paternal missense variant (c.G473A;p.R158Q). Individual 2 presented with bilateral microphthalmia, colobomas, cleft lip, preauricular tags, renal anomalies, cryptorchidism and developmental delay, and carried a homozygous missense variant (c.C1261T;p.R421W). Our zebrafish knockout models exhibited a truncated body axis, consistent with the short stature observed in patients with Zaki syndrome, but no AMC phenotypes. However, coloboma was observed in *wls* knockouts performed in AMC-sensitized genetic backgrounds.

Conclusion: Our new cases support AMC as part of the phenotypic spectrum associated with human *WLS* variants. Furthermore, the zebrafish data indicate important gene-gene interactions underlying developmental eye phenotypes.

Grants: Baillie Gifford

Conflict of Interest: None declared

P04.036.A IHH enhancer within neighboring *NHEJ1* intron acts in eye development and its variant causes microphthalmia, anophthalmia and coloboma

Ohad Wormser[1], *yonathan perez[1], Vadim Dolgin[1], Bahman Kamali[2], Jared Tangeman[3], Iiba gradstein[4;5], Yuval Yogev[1], Noam Hadar[1], ofek freund[1], Max Drabkin[1], Daniel Halperin[1], Inbar Irron[6], Erika Grajales-Esquivel[3], Katia Del Rio-Tsonis[3], Ramon Birnbaum[6], Gidon Akler[7;8], Ohad Shmuel Birk[1;9]*

[1]Ben Gurion University of the Negev, The Morris Kahn Laboratory of Human Genetics, National Institute for Biotechnology in the Negev and Faculty of Health Sciences, Be'er Sheva, Israel; [2]Shaare Shalom - Mashadi Jewish Center, Medical Advisory Committee, Great Neck, United States; [3]Miami University, Department of Biology and Center for Visual Sciences, Oxford, United States; [4]Soroka Medical Center, Department of Ophthalmology, Be'er Sheva, Israel; [5]Ben Gurion University of the Negev, Be'er Sheva, Israel; [6]Ben Gurion University of the Negev, Department of Life Sciences, Be'er Sheva, Israel; [7]Precision Medicine Insights, GREAT NCK PLZ, United States; [8]TOVANA Health, Houston, United States; [9]Soroka Medical Center, Genetics Institute, Be'er Sheva, Israel

Background/Objectives: Microphthalmia, anophthalmia and ocular coloboma (MAC) constitute a phenotypic continuum, resulting from incomplete choroid fissure fusion. We set out to unravel the molecular mechanism of autosomal recessive isolated MAC in inbred Iranian Jewish kindreds.

Methods: Homozygosity mapping, whole genome sequencing, segregation analysis and population screening, ATAC-seq library preparation and sequencing, mouse and chicken embryonic studies, enhancer reporter assays.

Results: Genetic studies identified a 0.5Mb disease-associated chromosome 2q35 locus (maximal LOD score 6.8) harboring a single-nucleotide intronic founder variant in *NHEJ1*, not predicted to affect NHEJ1. Notably, a nearby intronic deletion in the canine *NHEJ1* ortholog is related to a coloboma-like phenotype called "Collie eye anomaly". The human *NHEJ1* intronic variant lies in the core of PAX6's predicted binding site. Furthermore, it lies within a known limb-development enhancer in mice, affecting the neighboring gene, Indian hedgehog (Ihh). Extracellular signaling through Ihh was previously shown to be involved in eye development in mice, and haploinsufficiency of Ihh in chickens causes the "Creeper" phenotype with its microphthalmic and colobomatous eyes. Through extensive mouse and chicken molecular embryonic studies, we demonstrated that the *Ihh* enhancer drives gene expression in the developing eye, and that the variant identified affects this enhancer activity.

Conclusion: We delineate an Ihh enhancer active in mammalian eye development whose mutation causes human MAC. We highlight disease causation by an intronic mutation affecting the expression of a neighboring gene, unraveling novel molecular pathways of eye development.

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Conflict of Interest: Ohad Wormser: None declared, yonathan perez: None declared, Vadim Dolgin: None declared, Bahman Kamali: None declared, Jared Tangeman: None declared, liba gradstein: None declared, Yuval Yogev: None declared, Noam Hadar: None declared, ofek freund: None declared, Max Drabkin: None declared, Daniel Halperin: None declared, Inbar Irron: None declared, Erika Grajales-Esquivel: None declared, Katia Del Rio-Tsonis: None declared, Ramon Birnbaum: None declared, Gidon Akler: None declared, Ohad Shmuel Birk The studies were supported by the Morris Kahn Family Foundation; by the Israel Science Foundation grant 2463/23 to OSB; by the Israeli ministry of health grant to OSB, and the National Knowledge Center for Rare/Orphan Diseases of the Israel Ministry of Science, Technology and Space, at Ben-Gurion University of the Negev and Soroka Medical Center, Beer-Sheva, Israel.

P04.037.B Matching patients with ultra-rare inherited retinal diseases using deep retinal phenotype representations from Eye2Gene

Alexander Hustinx[1], *William Woof[2], Kristina Pfau[3;4], Larissa Mattern[5], Tibor Karl Lohmann[6], Miriam Elbracht[5], Frank G. Holz[3], Peter Krawitz[1], Nikolas Pontikos[2], Behnam Javanmardi[1]*

[1]University Hospital Bonn, Institute for Genomic Statistics and Bioinformatics, Bonn, Germany; [2]University College London, Institute of Ophthalmology, London, United Kingdom; [3]University Hospital Bonn, Department of Ophthalmology, Bonn, Germany; [4]University Hospital Basel, Department of Ophthalmology, Basel, Switzerland; [5]University Hospital RWTH Aachen, Institute for Human Genetics and Genomic Medicine, Aachen, Germany; [6]University Hospital RWTH Aachen, Department of Ophthalmology, Aachen, Germany

Background/Objectives: Inherited retinal diseases (IRDs) are a group of rare monogenic conditions affecting about 1 in 3000 people. Most IRDs exhibit distinctive retinal phenotypes observable through various imaging modalities. Utilizing artificial

intelligence, trained on large image datasets linking retinal phenotypes to the corresponding syndromes, ophthalmologists can be assisted in identifying these patterns. Eye2Gene's deep learning algorithm, trained on the largest available imaging dataset of IRD patients using three different imaging modalities (fundus autofluorescence, infrared reflectance, and spectral-domain optical coherence tomography), achieves expert-level accuracy in genetic diagnosis for the 36 most prevalent IRDs (Pontikos et al. 2022). However, this 'supervised' method requires including the to-diagnose IRD in the training set, which for ultra-rare conditions can be difficult.

Methods: To enable the identification of ultra-rare IRDs, we adopt a strategy similar to GestaltMatcher (Hsieh et al. 2022). We construct a high-dimensional feature space, referred to as the clinical retinal phenotype space (CRPS), using the penultimate layer of Eye2Gene's deep learning architecture. Different disorders occupy different regions in this 2048-dimensional CRPS.

Results: We show that the inter-case distances within CRPS establish syndromic similarity, providing a quantitative means for matching patients with identical molecular diagnoses. We also show that the distances in CRPS can be used to match cases even if their IRDs were absent from Eye2Gene's training set.

Conclusion: We present Eye2Gene's CRPS, the first high-dimensional phenotype space for IRDs. The CRPS not only accelerates clinical diagnosis for patients with ultra-rare IRDs but also facilitates the delineation of novel retinal phenotypes.

Grants:

Conflict of Interest: Alexander Hustinx: None declared, William Woof: None declared, Kristina Pfau: None declared, Larissa Mattern: None declared, Tibor Karl Lohmann: None declared, Miriam Elbracht: None declared, Frank G. Holz: None declared, Peter Krawitz: None declared, Nikolas Pontikos Eye2Gene, Phenopolis limited, Behnam Javanmardi: None declared

P04.038.C Non-canonical splicing variants as genetic cause of Nance-Horan syndrome

Carolina Ruiz-Sanchez[1], *Alejandra Damián*[1;2], *Alba Martín*[1], *Carmen Ayuso*[1;2], *María José Trujillo Tiebas*[1;2], *Berta Almoguera Castillo*[1;2], *Marta Corton*[1;2]

[1]*Health Research Institute - Fundación Jiménez Díaz (IIS-FJD), Genetics Department, Madrid, Spain;* [2]*Center for Biomedical Network Research on Rare Diseases (CIBERER), ISCIII, Madrid, Spain*

Background/Objectives: The X-linked *NHS* gene codes for an essential protein in embryonic lens development and is involved in the congenital cataract (CC) related Nance-Horan syndrome. In this work, we studied the implication of several non-canonical variants in *NHS* detected in male probands with syndromic CC.

Methods: Clinical exome sequencing and/or functional splicing assays were performed. RNA assays were carried out from blood and/or in vitro minigene constructs using RT-PCR and nanopore sequencing to validate the potential splicing deregulation of synonymous and non-canonical *NHS* variants.

Results: First, we identified the synonymous variant NM_198270.4: c.1008A>G in exon 4 of *NHS*, previously unreported, in a male proband referred with a clinical suspicion of Nance-Horan syndrome. This variant arose de novo in the asymptomatic heterozygous mother and showed. In silico analysis suggested that this variant could activate an exonic cryptic donor. Similar in silico effects were predicted for synonymous and non-canonical splicing variants initially reported as benign in ClinVar. Splicing assays showed the presence of an aberrant 37-nt skipping of *NHS* exon 4, confirming the potential pathogenicity of both synonymous variants.

Conclusion: This work helped us to reclassify 2 splicing synonymous variants altering the *NHS* splicing as pathogenic. Variant c.1008A>G showed an allele-specific expression in female carriers, possibly associated with skewed X chromosome inactivation. Abnormal skipping of this exon constitutes a new pathogenic mechanism associated with *NHS*, thus synonymous variants can be potentially pathogenic and should not be overlooked in routine genetic studies.

Grants: Spanish Health Institute Carlos III (PI17_01164 and PI20_00851) and ONCE.

Conflict of Interest: None declared

P04.039.D Metabolic biomarkers for earlier diagnosis of retinal disease Macular Telangiectasia Type 2 (MacTel)

Ajda Kunčič[1], *Elmar Zügner*[2], *Christoph Magnes*[2], *Eva Maria Prugger*[2], *Mojca Urbančič*[3], *Darja Dobovšek Divjak*[3], *Petra Hudler*[1], *Nataša Debeljak*[1]

[1]*University of Ljubljana, Faculty of Medicine, Institute of Biochemistry and Molecular Genetics, Ljubljana, Slovenia;* [2]*JOANNEUM RESEARCH HEALTH, Graz, Austria;* [3]*University Medical Center Ljubljana, Department of Ophthalmology, Ljubljana, Slovenia*

Background/Objectives: Macular telangiectasia type 2 (MacTel) is clinically and genetically intricate bilateral retinal condition. Loss of glial cells in the macular area and degeneration of photoreceptors lead to central vision loss. Although a genetic predisposition to MacTel is suspected based on relatives' studies, the exact cause of the disease remains elusive. There is a great need to improve the current multimodal imaging diagnostics to identify the disease at earlier stages. Our pilot study aimed to identify potential disease-associated biomarkers in the liquid biopsies of MacTel patients.

Methods: Plasma and tear fluid samples were collected from patients and healthy controls (age, gender, and BMI-matched) at the Department of Ophthalmology, UMC Ljubljana. Samples were extracted using 80% methanol (v/v) and analysed using UHPLC-HRMS. Metabolites were identified based on reference lists with accurate mass and retention time. Data was analysed using PCA and ANOVA with pairwise post hoc tests.

Results: A total of 375 known metabolites in plasma and 306 in tear fluid were detected. Notably, a trend of elevated free fatty acids and lipids was observed in plasma samples from MacTel patients. Furthermore, a significant alteration in plasma serine levels ($p = 0.0325$) was identified, and a similar reduction was found in tear fluid.

Conclusion: MacTel patients showed significant alternations in plasma and tear fluid metabolic profiles. Non-invasive collection of tear fluid makes it a potential source of diagnostic biomarkers. These preliminary findings lay the groundwork for developing a biomarker analysis protocol for earlier disease diagnosis.

Grants: ARIS grants P1-0390 and young researcher funding to the presenting author.

Conflict of Interest: None declared

P04.040.A RIPOR2: a new gene of non-syndromic cochleovestibular dysfunction, discrepancy between human pathology and animal models

Godelieve Morel[1], *sylvain ernest*[1], **Margaux Serey Gaut**[1;2], *Laurence Jonard*[2], *Ralyath Balogoun*[2], *Marine Parodi*[2], *Natalie Loundon*[2], *Sophie ACHARD*[2], *Sandrine MARLIN*[1;2;3;4]

[1]*Institute Imagine, UMR-1163 INSERM, Paris, France;* [2]*Hôpital Necker-Enfants Malades, crmr genetic hearing loss, Paris, France;* [3]*Hôpital*

Necker-Enfants Malades, Centre de recherche en audiologie, Paris, France; [4]ern cranio

Background/Objectives: Vestibular and cochlear hair cells share the same embryologic origins and show physiological mechanism similarities.

The prevalence of vestibular impairment is high in children and adults with HL. For the vast majority of genetic forms for which VD is associated to HL in human, VD is also present in the animal models and responsible for motor and swimming abnormalities.

RIPOR2 was associated to both congenital profound recessive non syndromic sensorineural HL and late onset dominant HL.

Methods: Whole exome sequencing in 3 tunisian siblings suffering from congenital bilateral severe to profound hearing loss associated with vestibular dysfunction Using Crispr/Cas9 genome editing, we generated zebrafish models of the human variant.

Results: An homozygote pathogenic variation c.1561C>T (p.R521*) in RIPOR2 (RHO family interacting cell polarization regulator 2) has been identified by whole exome sequencing in the 3 siblings.

We have generated three independent zebrafish lines with mutations leading to a truncated Ripor2 protein in exon 14. Exon14 nonsense homozygous F2 larva did not exhibit a different hearing and balance behaviour from wild-type or heterozygous larva at 5dpf.

Conclusion: We describe a discrepancy between the presence of a severe VD in our patients presenting with a homozygous *RIPOR2* stop mutation and the absence of evidence for vestibular abnormality in animal models (*Ripor2* KO mouse and our Zebrafish model).

For success of future therapies, a deep phenotyping with complete vestibular tests in patients and animal models is mandatory to improve the knowledge regarding the physio-pathology and natural history of the vestibular impairments.

Grants: none

Conflict of Interest: None declared

P04.041.B Success of Targeted Sequencing in the Search for Genetic Causes of Usher Syndrome Type 2

Dominika Ozieblo[1], *Janine Reurink*[2], *Natalia Baldyga*[1], *Henryk Skarzynski*[3], *Hannie Kremer*[2;4], *Monika Oldak*[1]

[1]*Institute of Physiology and Pathology of Hearing, Department of Genetics, Warsaw, Poland;* [2]*Radboud University Medical Center, Department of Human Genetics, Nijmagen, Netherlands;* [3]*Institute of Physiology and Pathology of Hearing, Oto-Rhino-Laryngology Surgery Clinic, Warsaw, Poland;* [4]*Radboud University Medical Center, Department of Otorhinolaryngology, Nijmagen, Netherlands*

Background/Objectives: Usher syndrome is one of the most common rare diseases in which both hearing impairment and retinitis pigmentosa coexist. Currently, four types of Usher syndrome are known and they are genetically and clinically heterogenous. The aim of the study was to characterize the genetic background of Usher syndrome type 2 (USH2) in a group of Polish patients.

Methods: A total of 55 patients with a clinical diagnosis of USH2 were recruited to the study. The DNA was isolated from blood and genetic testing was performed using three different methods: RT-PCR genotyping, targeted sequencing of the *USH2A* gene, and a multigene panel (237 genes). Segregation analysis was performed using Sanger sequencing and RT-qPCR.

Results: The cause of USH2 was identified in all patients. In 98% (54/55) of the individuals, causative variants were located in the *USH2A* gene. In one patient (2%; 1/55), a new homozygous terminating variant in the *ADGRV1* gene was identified. In *USH2A* gene, 42 different genetic variants were identified (28 known and 14 novel). A total of 74% (31/42) of the variants were deleterious. The most frequently identified genetic cause of USH2 was c.11864G>A (p.Trp3955Ter) (29 alleles). Deletions of exons 22-24 (17 alleles) and 10-11 (8 alleles) of the *USH2A* gene also contributed frequently to USH2 development.

Conclusion: Genetic testing of USH2 patients should be based on high-throughput tests that enable simultaneous identification of SNVs and CNVs. The gathered data can serve as a starting point for further genotype-phenotype association analyses.

Grant: 2020/37/N/NZ5/02800 National Science Centre, Poland

Conflict of Interest: None declared

P04.042.C Apparently isolated early onset hearing loss: diagnosis of syndromic forms by whole genome sequencing

Margaux Serey Gaut[1;2], *Ralyath Balogoun*[3;4], *Sophie ACHARD*[5], *Isabelle Mosnier*[6], *Isabelle Rouillon*[5], *Marine Parodi*[5], *Natalie Loundon*[5], *Laurence Jonard*[4], *Pierre Blanc*[3], *Sandrine MARLIN*[2;7]

[1]*Hôpital Necker, AP-HP.CUP, Centre de Recherche en Audiologie, PARIS, France;* [2]*Hôpital Necker, AP-HP.CUP, CRMR Surdités Génétiques, Service Médecine Génomique des maladies rares, UF Développement et Morphogenèse, Fédération de Génétique et de médecine génomique, PARIS, France;* [3]*SeqOIA, Laboratoire SeqOIA (PFMG2025), PARIS, France;* [4]*Hôpital Necker, AP-HP.CUP, Laboratoire de Génétique Moléculaire, Service Médecine Génomique des maladies rares, UF Développement et Morphogenèse, Fédération de Génétique et de médecine génomique, PARIS, France;* [5]*Hôpital Necker, AP-HP.CUP, Service d'ORL, PARIS, France;* [6]*Hôpital de la Pitié-Salpêtrière, APHP, Service d'ORL, PARIS, France;* [7]*Institut Imagine, Unité INSERM UMR1163, PARIS, France*

Background/Objectives: Congenital hearing loss have a genetic origin in 80% of the cases and 10% of them are syndromic. Neonatal audiologic screening is done in many European countries and an early genetic consultation is recommended by the European societies in the standard care.

Methods: In France, patients presenting with early onset isolated or syndromic hearing loss have access to whole genome sequencing through the Plan France Medecine Genomique 2025 sequencing platforms. From 2020 to 2023, almost 500 families had whole genome sequencing for this indication on the SeqOIA platform only.

Results: Among patients assessed in our Reference Center for Genetic Deafness and presenting at the moment of the genetic consultation with isolated early onset hearing loss, whole genome sequencing identified pathogenic variations in a gene responsible for syndromic hearing loss in 4 patients: *PEX6*, *NARS2*, *BCAP31* and *GATA3*.

Conclusion: Diagnostic whole genome sequencing enables the diagnosis of rare or ultra-rare syndromic forms of genetic hearing loss before the onset of the additional clinical signs. For the patients, the diagnosis of a syndromic form allows for better care and for parents to have an accurate genetic counselling. However, "presymtomatic" diagnosis of a syndromic hearing loss with a variable prognosis can also be distressing, requiring appropriated conditions to deliver and follow those diagnoses.

Conflict of Interest: None declared

P04.043.D Genetic background of hearing loss in patients with inner ear malformations

Natalia Baldyga[1;2], *Dominika Ozieblo*[1], *Nina Gan*[1;2], *Mariusz Furmanek*[3], *Marcin Leja*[1], *Henryk Skarzynski*[4], *Monika Oldak*[1]

[1]*Institute of Physiology and Pathology of Hearing, Department of Genetics, Warsaw, Poland;* [2]*Medical Centre of Postgraduate*

Education, Doctoral School of Translational Medicine, Warsaw, Poland; [3]Institute of Physiology and Pathology of Hearing, Bioimaging Research Center, Warsaw, Poland; [4]Institute of Physiology and Pathology of Hearing, Oto-Rhino-Laryngology Surgery Clinic, Warsaw, Poland

Background/Objectives: Inner ear malformations (IEMs) can occur in an isolated manner or in genetically determined syndromes. Enlarged vestibular aqueduct (EVA) is the most frequently observed IEM often accompanied with incomplete partition type 2 (IP2) of the cochlea and a dilated vestibule. *SLC26A4* is the major IEMs gene with 25% of EVA/IP2 patients carrying two *SLC26A4* pathogenic variants. In 2017, CEVA haplotype was identified upstream of *SLC26A4* but its role remains unknown.

Methods: DNA was isolated from blood samples of 23 hearing loss (HL) patients with bilateral IEMs. Sequencing was performed using custom multigene panel (237 genes) and the TruSight One clinical exome. CEVA haplotype and segregation of variants were tested with Sanger sequencing. Minigene assay was used to evaluate the impact of novel synonymous variant on splicing.

Results: Genetic testing identified the cause of EVA/IP2 in 74% (17/23) of individuals. Two pathogenic variants in the *SLC26A4* gene were identified in 8 of them (35%). CEVA haplotype was identified in 86% (6/7) of *SLC26A4* monoallelic patients. In two individuals, cochlear hypoplasia resulted from *EYA1* mutations. In one patient, a novel *CHD7* variant was detected. Minigene assay revealed that *SLC26A4* transcripts containing synonymous c.162C>T variant produce shortened proteins.

Conclusion: Mutations in *SLC26A4* and the CEVA haplotype account for more than half of EVA/IP2 cases. Syndromic forms of HL should also be considered in patients with EVA/IP2. There is a need to look for pathogenic variants in noncoding regions of known HL genes or in novel genes.

Grants: 2021/41/B/NZ5/04390 National Science Centre, Poland

Conflict of Interest: None declared

P04.044.A New insights on the genetic basis underlying hereditary high myopia in a cohort of more than 100 Spanish families

Xènia Ferrer-Cortès[1], *Sheila Ruiz-Nogales[1], Yasmin Soares de Lima[1], Oscar Recasens[1], Pilar Mendez[1], Laura González[1], Esther Pomares[1]*

[1]IMO Grupo Miranza, Genetica, Barcelona, Spain

Background/Objectives: High myopia occurs when the spherical refractive error exceeds -6.00 diopters or the axial length of the eye is greater than 26mm. Genetic factors play a crucial role in its development, since it has an estimated heritability in more than 70% of cases, according to the literature. This study focuses on the identification of new mutations and novel genes associated with hereditary high myopia, in order to determine the most prevalent variants in the Spanish population.

Methods: DNA samples from 347 patients were analysed by Whole Exome Sequencing. In silico analyses were carried out prioritising a custom-designed panel of 549 genes related to myopia.

Results: Genetic analysis was performed in 102 families affected by high myopia. In more than 20% of them, a clear genetic cause was identified in genes already involved with high myopia. Additionally, potentially pathogenic variants in the myopia-related genes were identified in about 25% of the cases, and in 15% of the families, we identified likely pathogenic variants in candidate genes that have not yet been directly associated to myopia. These evidences lead us to estimate the genetic diagnosis in approximately 50% of the families. As we expected, most of the cases presented mutations in genes involved in the extracellular matrix's organisation (*COL9A3, COL11A1, COL11A2, COL12A1* and *FBN1*).

Conclusion: We were able to point out a possible genetic cause in about 50% of the families. Furthermore, we target new genes that could be involved in the molecular basis of hereditary high myopia, expanding the knowledge of this condition.

Conflict of Interest: None declared

P04.045.B Autosomal dominant macular dystrophy associated to *THRB* gene: identification of new families and variants

Lidia Fernández-Caballero[1;2], *Fiona Blanco-Kelly[1;2], Inmaculada Martin Merida[1;2], Pablo Mínguez[1;2;3], Saoud Tahsin-Swafiri[1;2], Ester Carreño Salas[4], María Pilar Martin Gutierrez[4], Blanca García Sandoval[4], Marta Corton[1;2], Carmen Ayuso[1;2]*

[1]Department of Genetics & Genomics, Instituto de Investigación Sanitaria-Fundación Jiménez Díaz University Hospital, Universidad Autónoma de Madrid (IIS-FJD, UAM), Madrid, Spain; [2]Center for Biomedical Network Research on Rare Diseases (CIBERER), Instituto de Salud Carlos III, Madrid, Spain; [3]Bioinformatics Unit, Instituto de Investigación Sanitaria-Fundación Jiménez Díaz University Hospital, Universidad Autónoma de Madrid (IIS-FJD, UAM), Madrid, Spain; [4]Department of Ophthalmology, Fundación Jiménez Díaz University Hospital, Madrid, Spain

Background/Objectives: Inherited retinal dystrophies (IRD) are a group of rare diseases that affects the photoreceptors and retinal pigment epithelium cells. *THRB* gene encodes thyroid hormone receptor β which produces two human isoforms (TRβ1 and TRβ2) by alternative splicing. Recently, the first *THRB* variant associated with autosomal dominant macular dystrophy, c.283+1G>A, has been described. This study aims to refine the ophthalmologic phenotype and describe a novel variant in the gene *THRB*.

Methods: *THRB* variants were identified by re-analysis of next-generation sequencing data from the FJD database (6,688 both IRD and pseudocontrols). Clinical data were collected from a self-reported ophthalmic history recorded from questionnaires and ophthalmic exam data.

Results: We identified 12 patients from 3 new families carrying variants in *THRB*. Two of them carry the variant c.283+1G>A. In a third family, we identified the novel variant c.283G>A (p.Gly95Arg), with a predicted similar splicing consequence. Patients were diagnosed with macular dystrophy or cone/cone-rod dystrophy. Most patients showed common fundus and OCT findings with disruption of subfoveal ellipsoid layers. However, onset of symptoms ranged from the first to the seventh decade, with visual acuity loss or dyschromatopsia.

Conclusion: Our results confirm a common autosomal dominant macular dystrophy phenotype associated with *THRB* with high intra-familial variability, which could be due to modifiers. Additionally, c.283+1G>A shows a likely founder effect in the Spanish population. This study confirms that *THRB* is an IRD-associate gene, therefore it should be considered in diagnostic NGS panels.

Grants: CIBERER, ISCIII (PI19/00321-PI22/00321), IIS-FJD_Biobank, University Chair UAM-IIS-FJD Genomic Medicine, ONCE.

Conflict of Interest: None declared

P04.046.C SMARCA4 Mutation Causes Human Otosclerosis in Humans and Mice

Matan M. Jean[1], *Max Drabkin[1], yael noy[2], Daniel Halperin[1], Yuval Yogev[1], Ohad Wormser[1], Regina Proskorovski-Ohayon[1], Vadim*

Dolgin[1], noam levaot[3], vlad brumfeld[4], shira ovadia[5], mor kishner[5], udi Kazenell[6], keren b avraham[2], Ilan Shelef[7], Ohad Shmuel Birk[1;8]

[1]Ben-Gurion University, The Morris Kahn Laboratory of Human Genetics, Faculty of Health Sciences, Beer Sheva, Israel; [2]Tel Aviv University, Department of Human Molecular Genetics and Biochemistry, Faculty of Medicine and Sagol School of Neuroscience, Tel Aviv, Israel; [3]Ben-Gurion University, Department of Physiology and Cell Biology, Beer-Sheva, Israel; [4]Weizmann Institute of Science, Rehovot, Department of Chemical Research Support, Rehovot, Israel; [5]Ben-Gurion University, Faculty of Health Sciences, beer sheva, Israel; [6]Kaplan Medical Center, Department of Otolaryngology, Rehovot, Israel; [7]Soroka Medical Center and Faculty of Health Sciences, Ben-Gurion University, Department of Radiology, Beer-Sheva, Israel; [8]Soroka University Medical Center, Genetics Institute, Beer-Sheva, Israel

Background/Objectives: Otosclerosis is a common cause of hearing loss in adults, with known familial predilection. The molecular pathogenesis of otosclerosis is mostly unknown.

Methods: Linkage analysis and whole-exome sequencing studies of an affected kindred, followed by bioinformatics data analysis and segregation studies using Sanger sequencing; Generation of CRISPR-Cas9 mutant mice harboring the human mutation in the mouse ortholog. The genetically modified mice underwent comprehensive auditory function assessments, including acoustic startle responses and auditory brainstem response tests, alongside micro-CT imaging to evaluate changes in bone structure within the otic capsule.

Results: Through combined linkage analysis and whole exome sequencing studies of seven individuals of a single kindred affected with apparently autosomal dominant heredity of otosclerosis, we identified a disease-causing variant in SMARCA4, encoding a key component of the PBAF chromatin remodeling complex. We generated CRISPR-Cas9 transgenic mice harboring the human mutation in the mouse SMARCA4 orthologue. Mutant Smarca4 + /E1548K mice demonstrated marked hearing impairment demonstrated through acoustic startle response and auditory brainstem response tests. Isolated ossicles of the auditory bullae of mutant mice exhibited a highly irregular structure of the incus bone. Their in situ micro-CT studies demonstrated anomalous structure of the incus bone, causing disruption in the ossicular chain.

Conclusion: Otosclerosis can be caused by a variant in SMARCA4, with a similar phenotype of hearing impairment and abnormal bone formation in the auditory bullae in transgenic mice carrying the human mutation in the mouse SMARCA4 orthologue.

Grants: The Morris Kahn Family Foundation.

Conflict of Interest: None declared

P04.047.D Optimization of Capture Area coverage of spatial transcriptomics slide with cryosections of small specimen/ corneas

Alicja Wysocka[1], *Katarzyna Jaskiewicz[1], Małgorzata Rydzanicz[2], Rafał Płoski[2], Jakub Wozniak[3;4], Monika Udziela[5], Jacek Szaflik[5], Marzena Gajecka[1;3]*

[1]Institute of Human Genetics, Polish Academy of Sciences, Department of Cancer Genetics, Poznan, Poland; [2]Medical University of Warsaw, Department of Medical Genetics, Warsaw, Poland; [3]Poznan University of Medical Sciences, Chair and Department of Genetics and Pharmaceutical Microbiology, Poznan, Poland; [4]Poznan University of Life Sciences, Department of Genetics and Animal Breeding, Poznan, Poland; [5]Medical University of Warsaw, Department of Ophthalmology, Warsaw, Poland

Background/Objectives: Keratoconus (KTCN) is a progressive disorder of cornea characterized by its gradual thinning in the cone apex and histopathological changes noted in all five corneal layers. Due to the complexity of this tissue and the multilevel dimension of the KTCN-associated changes, a method combining and assessing both histology aspects and spatially performed whole transcriptome gene expression profiling should be implemented. Substantial technical challenges of using such protocols are sectioning and placement of the tissue on the slide.

Methods: The O.C.T.-embedded corneas, derived from KTCN patients qualified for the penetrating keratoplasty procedure, and unaffected corneas derived from deceased individuals as controls were evaluated. The microscopic H&E imaging assessment and Tissue Optimization step (Visium, 10xGenomics) were performed. Three corneal sections from each individual were placed within a single Capture Area of the Spatial Gene Expression Slide and proceeded to spatial gene expression analysis (Visium, 10xGenomics). The libraries prepared according to the manufacturer's protocol were subjected to next-generation sequencing (NovaSeq6000,Illumina).

Results: Cryosection thickness and tissue permeabilization time were successfully optimized. We managed to place three cryosections derived from one individual within a single Capture Area, measuring 6.5 × 6.5mm, from both affected and unaffected corneas. Thus, we increased the percentage of coverage and total sequencing depth threefold compared to one section per capture area, significantly improving the quality of results for low-input samples.

Conclusion: Preparation of experimental material – corneal samples according to our protocol, substantially increases the size of input data and the sequencing depth in spatial transcriptomics.

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Conflict of Interest: None declared

P04.048.A Diagnostic genome sequencing improves diagnostic yield in a single-center study of 100 patients with non-syndromic and syndromic hearing impairment

Nanna Dahl Rendtorff[1], *Lisbeth Tranebjærg[1;2], Mette Bertelsen[1]*

[1]The Kennedy Centre, Department of Clinical Genetics, Rigshospitalet, Copenhagen, Denmark; [2]Institute of Clinical Medicine, The Panum Institute, University of Copenhagen, Copenhagen, Denmark

Background: Hearing impairment (HI) is a common sensory disorder, which is genetically heterogeneous. Identification of causative variants underlying HI is challenging, since >100 genes for non-syndromic HI and >450 genes for syndromic HI have been reported.

Methods: 100 index patients with HI and variable additional clinical features underwent Whole Genome Sequencing (WGS) in clinical settings. The samples were analyzed using virtual gene-panels of 174 (76% of patients) or 500 genes, respectively, for patients suspected of having non-syndromic and syndromic HI. Nine patients had prior to WGS been prescreened for DFNB1, *SLC26A4*- and/or *STRC*-related HI and six using gene-panels for HI.

Results: A definite genetic diagnosis was made in 42/100 patients, distributed in 25 different genes. In total, 45 different likely pathogenic/pathogenic variants were detected, and 14 variants were novel. In addition, six patients had variants of uncertain significance (VUS) identified, where further work-up was recommended, which might change the classification to likely pathogenic. Finally, in an additional ten patients only one pathogenic variant was identified, so far. Variants in rare/recently identified genes causative of HI included *SLC12A2* and *ATOH1*.

Conclusion: WGS allowed detection of a definite or possible genetic diagnosis in ~48% of 100 cases (~53% excluding patients with unilateral HI and patients prescreened with previous NGS HI panel). Causative variants were found in >25 different genes, including both common and rare/recently identified HI genes, emphasizing the genetic heterogeneity of the condition. In non-solved selected families, the data will be re-analyzed with improved methods for detecting deep structural and intronic variants and with broader gene panels.

Conflict of Interest: None declared

P04.049.B Risk-adapted prevention and treatment in ophthalmogenetics based on a retrospective evaluation of a cohort of 455 patients

*Anica Leubner[1], Mareike Mertens[2;3], Saskia Biskup[4], Katrin Hoffmann[2], **Claudia Grünauer-Kloevekorn**[1;5], Pablo Villavicencio Lorini[2]*

[1]PraxisKlinik Augenärzte am Markt, Halle; [2]Martin-Luther-Universität Halle-Wittenberg, Institut für Humangenetik, Halle, Germany; [3]MVZ Universitätsklinikum Halle, Facharztzentrum für Pädiatrie & Humangenetik, Halle, Germany; [4]Zentrum für Humangenetik, Tübingen, Germany; [5]Martin-Luther-Universität Halle-Wittenberg, Medizinische Fakultät, Halle, Germany

Background/Objectives: We report on the ophthalmogenetic consultation and the results of molecular genetic testing and therapeutic options in 455 consecutive patients with suspected hereditary eye disease from our ophthalmogenetic consultation "rare eye diseases", which takes place since 2016.

Methods: An NGS-based in silico gene panel analysis based on exome enrichment was performed on a cohort of 455 patients during the period from 01/2019 to 01/2024 with a suspected hereditary ophthalmologic disease. Retrospectively, the diagnosis rate was determined depending on the clinical entity and the therapeutic options based on the molecular genetic results.

Results: The underlying molecular genetic cause was diagnosed in 266 patients (mean 58.5%, range 28.6% to 66.7% depending on the entity).

The highest diagnosis rate was found for hereditary retinal dystrophies (66.7 %) and diseases of the anterior segment (corneal dystrophies, 60.11 %). A lower rate was found for complex malformations (50 %) and hereditary optic atrophies (28.6 %). Therapeutic benefits were mainly seen in patients with corneal dystrophies (DMEK versus penetrating keratoplasty / limbokeratoplasty). Patients with retinal dystrophies benefited from rehabilitative measures and were assigned to study centers with gene therapy approaches.

Conclusion: Ophthalmogenetic consultation and diagnostics for suspected hereditary eye diseases are essential for prompt, personalized therapy and optical and social rehabilitation in affected families. Detailed medical history and diagnostics can optimize the detection rate and patient care can be provided quickly and adequately. Close interdisciplinary collaboration between human genetics and ophthalmology is crucial for the best possible treatment.

Grants: None.

Conflict of Interest: None declared

P04.050.C Insights into the FOXE3 Transcriptional Network from Exploration of the impact of a regulatory Variant in Complex Microphthalmia.

Lucas Fares Taie[1], *Julie Plaisancié[2], Clementine Angée[1], Elisa Erjavec[1], Isabelle Raymond Letron[3], Jean-Yves Douet[4], Mathilde Goetz[4], Catherine Vincent-Delorme[5], Ino Karemaker[6], Marijke Baltissen[6], Leonardo Valdivia[7], Yanad Abou Monsef[3], Patrick Calvas[8], Nicolas Chassaing[8], Jean-Michel Rozet[1]*

[1]Laboratoire de Génétique Ophtalmologique, INSERM U1163, Institut Imagine, Paris, France; [2]Laboratoire de Référence (LBMR) des anomalies malformatives de l'œil, Institut Fédératif de Biologie (IFB), CHU de Toulouse, Toulouse, France; [3]Ecole Nationale Vétérinaire de Toulouse (ENVT), Toulouse, France; [4]CHUVAC, Université de Toulouse, ENVT, Toulouse, France; [5]Service de Génétique Médicale, CHU de Lille, Lille, France; [6]Department of Molecular Biology, Faculty of Science, Radboud Institute for Molecular Life Sciences, Oncode Institute, Radboud University Nijmegen, Nijmegen, The Netherlands.; [7]Center for Integrative Biology, Facultad de Ciencias, Universidad Mayor, Santiago, Chile; [8]Centre de Référence des Affections Rares en Génétique Ophtalmologique (CARGO), site constitutif, CHU de Toulouse, Toulouse, France

Background/Objectives: *FOXE3* is a crucial transcription factor (TF) for lens and eye development, mutations of which cause microphthalmia associated with anterior segment dysgenesis (M-ASD). Previously, we reported a M-ASD case carrying p.Cys240* and an ultra-rare non coding regulatory variant (NCRV) in compound heterozygosity. In mice, homozygosity for the NCRV mildly affected eye development, whereas combined with a nonsense mutation it caused ASD. We aimed to understand the *FOXE3* transcriptional network in eye development by investigating how the NCRV influences *Foxe3* expression.

Methods: We conducted DNA pull-down assays and mass spectrometry, utilizing protein extracts from mouse ES, Rax-expressing ES or immortalized human epithelial lens cells, and wildtype (WT) or mutant (NCRV) oligonucleotides. TFs with differential binding were analyzed using RNAscope and siRNA-mediated silencing.

Results: 34 TFs displayed differential binding, with three being present in all three cell lines and co-localizing with *Foxe3* during ocular development. The binding of two of these three TFs was impeded by the NCRV, while there was a gain in binding for the remaining TF. Silencing these TFs in cultured lens cells from WT and homozygous NCRV mice demonstrated an impact on *Foxe3* expression.

Conclusion: We found three TFs binding to the WT or NCRV region of *FOXE3*. Two are linked to ocular malformations in mice, and one, a novel TF, binds to the WT sequence, suggesting a new role in lens development. This highlights the importance of studying unresolved cases in order to understand gene regulatory landscapes and to discover potential candidate genes for ocular malformations.

Grants: Retina France 2019

Conflict of Interest: None declared

P04.051.D Processing eye samples from patients with high myopia towards DNA methylation and gene expression analyses

Joanna Swierkowska[1], *Anna Mikolajczyk-Lorkiewicz[2], Marcin Stopa[2], Malgorzata Mrugacz[3], Marzena Gajecka[1;4]*

[1]Institute of Human Genetics, Polish Academy of Sciences, Poznan, Poland; [2]Department of Ophthalmology, Chair of Ophthalmology and Optometry, Poznan University of Medical Sciences, Poznan University Hospital, Poznan, Poland; [3]Department of Ophthalmology and Eye Rehabilitation, Medical University of Bialystok, Bialystok, Poland; [4]Chair and Department of Genetics and Pharmaceutical Microbiology, Poznan University of Medical Sciences, Poznan, Poland

Consortium: n/a

Background/Objectives: High myopia (HM), an eye disorder with a refractive error of ≤−6.0 diopters (D), has a multifactorial etiology with genetic and environmental factors involved. The relationship between changes in DNA methylation/gene expression and HM remains to be evaluated. The aim was to optimize protocols of processing biological material from eyes of patients with HM towards the investigation of DNA methylation and gene expression.

Methods: We have recruited 25 children with HM and 25 children without HM (<15 years old). Also, five adults with HM and five control individuals undergoing vitrectomy (a vitreo-retinal surgery) were ascertained. Patients' eyes were without inflammation, other diseases, and previous surgeries. Peripheral blood, serum, tear fluid, and conjunctival swabs as well as undiluted vitreous body and retinal samples from vitrectomy were collected.

Results: We optimized protocols for handling and storage of samples and chose commercial kits for nucleic acids isolations. We chose to place retinal samples in RNAlater and freeze vitreous body on dry ice for transport. Samples were processed for genome-wide DNA methylation analyses and RNA sequencing. Blood samples are designated for DNA/RNA isolations, serum and tear fluid for proteins and metabolites analyses, and conjunctival swabs for human and microbial DNA/RNA extractions. The influence of the type of biological material on the quality and quantity of extracted nucleic acids and obtained results will be presented.

Conclusion: Vitrectomy procedure is a good source of biological material from the eye that is normally impossible to obtain from living patients.

Grants: National Science Centre in Poland, 2019/35/N/NZ5/03150 (JS).

Conflict of Interest: None declared

P05

INTERNAL ORGANS & ENDOCRINOLOGY (LUNG, KIDNEY, LIVER, GASTROINTESTINAL)

P05.001.A Phenotypic spectrum of individuals with SLC16A2 variants in MCT8 deficiency

Kirsty McWalter[1], Houda Elloumi[1], Richard Sidlow[2], Ben Willis[3], Andrew Bauer[4], **Marianne Berrens**[3]

[1]GeneDx, Gaithersburg, United States; [2]Valley Children's Hospital, Madera, United States; [3]Egetis Therapeutics; [4]Children's Hospital of Philadelphia, United States

Background/Objectives: Variants in SLC16A2 cause MCT8 deficiency, an X-linked condition associated with hypotonia, failure to thrive, neurodevelopmental impairment, and thyrotoxicosis. Pathogenic SLC16A2 variants are known in ~320 individuals, with genotype-phenotype correlations suggested. Here, we contribute a cohort with SLC16A2 variants to the phenotypic spectrum of MCT8 deficiency.

Methods: By approved IRB protocol, we queried genetic testing laboratory deidentified data for SLC16A2 variants, classified by ACMG criteria. Clinical abstraction of medical records was performed, including Human Phenotype Ontology (HPO) terms reflecting clinical features and symptoms. Descriptive statistics were generated to define cohort characteristics.

Results: We identified 57 cases with likely pathogenic/pathogenic variants in SLC16A2 and 47 individuals with variants of uncertain significance (VUS). At time of testing, 49 individuals were ≤3yrs old; 10 individuals were adults (≥20yrs). Most cases (101/104) were identified by exome sequencing (ES), the remaining by panels or single gene sequencing. In total, 506 different HPO terms were associated with 99 individuals (332/506 terms seen only once). Common HPO terms included: 'global developmental delay' (75/99); 'generalized hypotonia' (37/99); 'seizure' (30/99); 'delayed speech and language development' (30/99); 'failure to thrive' (28/99); 'intellectual disability' (27/99); 'gastroesophageal reflux' (25/99); 'feeding difficulties' (24/99); 'constipation' (23/99); and 'delayed gross motor development' (20/99). HPO terms related to thyroid function and/or hormone levels were seen in 9/99 individuals.

Conclusion: This study indicates ES can be beneficial in diagnosing MCT8 deficiency, characterizing genotype-phenotype correlations in individuals with SLC16A2 variants, and targeting education on the benefits of confirmatory thyroid function testing including TSH, free T4, and T3.

Grants: none

Conflict of Interest: Kirsty McWalter GeneDx stock, GeneDx employee, Houda Elloumi HZE is an employee of GeneDx., Richard Sidlow RS is on the Advisory Board for Egetis/Rare Therapeutics and Guide Genetics, Ben Willis Egetis Therapeutics, BW is a paid consultant for Egetis Therapeutics, Andrew Bauer Research consultant with IBSA pharmaceuticals., Consultant with Egetis Therapeutics. Consultant with IBSA pharmaceuticals., Marianne Berrens MB owns stock options in Egetis Therapeutics., MB is an employee of Egetis Therapeutics.

P05.002.B Deep phenotyping of patients with congenital anomalies of the kidney and urinary tract (CAKUT) and *PAX2* loss-of-function (LOF) variants

Leonie Greipel[1;2], *Helge Martens[1], Lina Werfel[1;2], Robert Geffers[3], Bernd Auber[1], Anna Bjerre[4], Nele Kanzelmeyer[2], Dieter Haffner[2], Ruthild G. Weber[1]*

[1]Hannover Medical School, Department of Human Genetics, Hannover, Germany; [2]Hannover Medical School, Department of Pediatric Kidney, Liver and Metabolic Diseases, Hannover, Germany; [3]Helmholtz Centre for Infection Research, Genome Analytics Research Group, Braunschweig; [4]Oslo University Hospital, Division of Pediatric and Adolescent Medicine, Oslo

Background/Objectives: Patients with renal coloboma syndrome, caused by *PAX2* variants, may develop end-stage kidney disease (ESKD). This study aimed to identify and characterize patients with *PAX2* LOF variants in a CAKUT cohort, and to compile kidney function data from previously reported CAKUT patients with *PAX2* LOF variants.

Methods: Whole-exome sequencing was performed on 258 pediatric CAKUT patients. Patients carrying *PAX2* LOF variants underwent deep phenotyping. Twelve pertinent studies from the literature were reviewed.

Results: Six of 258 (2.3%) CAKUT patients carried heterozygous pathogenic *PAX2* LOF variants. Mean age of onset of ESKD was 9.6 years in 56 patients with *PAX2* LOF variants and ESKD reported here and previously. In our cohort, albuminuria was significantly higher, on average in the nephrotic range, in three patients with *PAX2* LOF variants and ESKD versus 41 patients with wildtype *PAX2* and ESKD. Phenotypic variability was highlighted by (i) three patients carrying the same *PAX2* variant presenting either with early-onset ESKD and marked albuminuria or CKD stage 3 and no severe albuminuria, and (ii) two parents carrying *PAX2* variants presenting either with adult-onset ESKD due to focal segmental glomerulosclerosis or sonographically normal kidneys.

Conclusion: As CAKUT patients with *PAX2* LOF variants are prone to marked albuminuria and early-onset ESKD, close monitoring and, if necessary, antiproteinuric measures are recommended. Then again, kidneys of *PAX2* LOF variant carriers may appear normal in early adulthood. Therefore, evaluation of

related living donors of CAKUT patients with *PAX2* LOF variants should include screening for *PAX2* variants.

Grants: PRACTIS-Clinician Scientist Program, Else Kröner-Fresenius-Stiftung (2018_Kolleg.12, TITUS-Clinician Scientist Program)

Conflict of Interest: None declared

P05.003.C Screening for MUC1 mutations based on a customized HTS library offers a new, complete approach to diagnosing patients with ADTKD.

Jose María García-Aznar Navajas[1], *Lara Besada[1], Ricardo Pan[1], Emilia Maneiro[1]*

[1]Healthincode, Immunology & Nephrology, La Coruña, Spain

Background/Objectives: *MUC1* has traditionally been a challenge to screen for genetic mutations because of its VNTR region. This region consists of repeated modules of 60 repeats, showing high diversity between individuals from 20 up to 125 times. Due to the complex nature of the analysis, conventional WES uncovers this region, so other techniques like SNapShot have been used as standard methodology targeted to the most frequent cause of MUC1-ADTKD (c.428dupC). We propose an alternative approach to analyze the MUC1-VNTR.

Methods: We designed an enriched library by using *SureSelect* HS protocol (*Agilent Technologies*), to be sequenced, including 25 probes that covered the MUC1-VNTR region. An especial computational filtering was applied to examine the VNTR region. We used this procedure in 1005 patients with unaffiliated CKD.

Results: We found four relevant variants in 16 patients (1.6%). We identified 7 patients with the variant c.428dupC. One patient harbored a similar change in a different module (c.488dupC). Moreover, this approach allowed us to detect other previously undescribed variants, potentially deemed disease-causing mutations *(table 1*).

MUC1 variant	No. patients	Domain	ACMG classification
c.428dupC; p.Ala144Serfs*86	7	VNTR	P
c.488dupC; p.Ala164Serfs*106	1	VNTR	P
c.54_55insT; p.Thr19Tyrfs*7	1	Extracellular	LP
c.888G>A; p.Trp296*	1	Transmembrane	LP
c.548G>A; p.Val260Ile	6	SEA	VUS-hot

Conclusion: We highlight the importance to completely analyze the whole sequence of *MUC1* in the context of ADTKD and unaffiliated CKD. Other prevalent mutations in *MUC1* could have been underestimated. The use of this HTS-customized library with an accurate pipeline adapted to MUC1-VNTR synthetizes the genetic analysis, improving the cost-effectiveness of techniques and diagnosis.

Conflict of Interest: Jose María García-Aznar Navajas Healthincode, Lara Besada Healthincode, Ricardo Pan Healthincode, Emilia Maneiro Healthincode

P05.004.D Skewed X-chromosome inactivation drives the proportion of *DNAAF6*-defective airway motile cilia and variable expressivity in primary ciliary dyskinesia

Lucie THOMAS[1], Laurence Cuisset[2;3], Jean-François Papon[4;5], Aline Tamalet[6], Isabelle Pin[7], Rola Abou Taam[8], Catherine Faucon[9], Guy Montantin[10], Sylvie Tissier[10], Philippe Duquesnoy[1], Florence Dastot-Le Moal[10], Bruno Copin[10], Nathalie Carion[2], Bruno Louis[4], Sandra Chantot-Bastaraud[1;11], Jean-Pierre Siffroi[1;11], Rana Mitri[9], Andre Coste[4;12], Estelle Escudier[1;10;13], Guillaume Thouvenin[6], Serge AMSELEM[1;10;13], Marie Legendre[1;10;13]

[1]INSERM U933, Childhood Genetic Diseases, Hôpital Armand-Trousseau, Paris, France; [2]Assistance Publique–Hôpitaux de Paris (AP-HP), Hôpital Cochin, Service de Génétique et Biologie Moléculaires, Paris, France; [3]Université de Paris, Paris, France; [4]Université Paris-Est, Faculté de Médecine, INSERM U955, CNRS ERL7240, Hôpital Henri-Mondor, Créteil, France; [5]AP-HP, Service d'ORL et de Chirurgie Cervico-Faciale, Hôpital Bicêtre, Le Kremlin–Bicêtre, France; [6]AP-HP, Service de Physiologie et Explorations Fonctionnelles, Hôpital Armand-Trousseau, Paris, France; [7]CHU de Grenoble Alpes, Grenoble, France; [8]AP-HP, Service de Pneumologie et Allergologie Pédiatriques, Hôpital Necker-Enfants Malades, Paris, France; [9]Service d'Anatomopathologie, Laboratoire de Microscopie électronique, CHIC, Créteil, France; [10]AP-HP, UF de Génétique Moléculaire, Hôpital Armand-Trousseau, Paris, France; [11]AP-HP, UF de Génétique Chromosomique, Hôpital Armand-Trousseau, Paris, France; [12]AP-HP, Service d'ORL, Hôpital Henri-Mondor et CHIC, Créteil, France; [13]Sorbonne université, Paris, France

Background/Objectives: Primary ciliary dyskinesia (PCD) is a rare airway disorder caused by defective motile cilia. Only male patients have been reported with pathogenic mutations in X-linked *DNAAF6*, which result in the absence of ciliary dynein arms, whereas their heterozygous mothers are supposedly healthy. Our objective was to assess the possible clinical and ciliary consequences of X-chromosome inactivation (XCI) in these mothers.

Methods: XCI patterns of 6 mothers of male patients with *DNAAF6*-related PCD were determined by DNA-methylation studies and compared to their clinical phenotype (6/6 mothers), as well as their ciliary phenotype (4/6 mothers), as assessed by immunofluorescence and high-speed videomicroscopy analyses. The mutated X chromosome was tracked to assess the percentage of cells with a normal inactivated *DNAAF6* allele.

Results: The mothers' phenotypes ranged from absence of symptoms to mild/moderate or severe airway phenotypes, closely reflecting their XCI pattern. Analyses of the symptomatic mothers' airway ciliated cells revealed the coexistence of normal cells and cells with immotile cilia lacking dynein arms, whose ratio closely mirrored their XCI pattern.

Conclusion: This study highlights the importance of searching for heterozygous pathogenic *DNAAF6* mutations in all female relatives of male PCD patients with a *DNAAF6* defect, as well as in females consulting for mild chronic respiratory symptoms. Our results also demonstrate that about 30% normal ciliated airway cells sufficed to avoid severe PCD, a result paving the way for gene therapy.

Grants: Institut National de la Santé et de la Recherche Médicale, Fondation pour la Recherche Médicale, Legs Poix from the Chancellerie des Universités.

Conflict of Interest: None declared

P05.005.C Three novel patients with lethal lung developmental disorders caused by variants in genes from the SHH-FOXF1 and TBX4-FGF10 signaling pathways

Katarzyna Bzdęga[1], *Gail Deutsch[2], Małgorzata Rydzanicz[3], Elżbieta Rafińska-ważny[4], Witold Błaż[5;6], Anna Terpin[7], Rafał Płoski[3], Tomasz Szczapa[8], Justyna Karolak[1]*

[1]Chair and Department of Genetics and Pharmaceutical Microbiology, Poznan University of Medical Sciences, Poznań, Poland;

[2]Department of Pathology, University of Washington School of Medicine, Seattle, United States; [3]Department of Medical Genetics, Medical University of Warsaw, Warsaw, Poland; [4]Department of Neonatology, Medical Center "Ujastek", Kraków, Poland; [5]Department of Neonatal and Intensive Care, Rzeszów Provincial Hospital No. 2, Rzeszów, Poland; [6]Faculty of Medicine, University of Rzeszów, Rzeszów, Poland; [7]Pro-Familia Hospital, Rzeszów, Poland; [8]II Department of Neonatology, Neonatal Biophysical Monitoring and Cardiopulmonary Therapies Research Unit, Chair of Neonatology, Poznan University of Medical Sciences, Poznań, Poland

Background/Objectives: Lethal lung developmental disorders (LLDDs), including alveolar capillary dysplasia with misalignment of the pulmonary veins (ACDMPV), acinar dysplasia (AcDys), congenital alveolar dysplasia (CAD), and primary pulmonary hypoplasias (PH) are rarely diagnosed lung anomalies in neonates, characterized by severe pulmonary arterial hypertension (PAH), refractory to treatment. In addition to histopathological evaluation, genetic testing plays a key role in accurate diagnosis of LLDD. In most newborns, LLDD is caused by abnormalities in genes from the SHH-FOXF1 (ACDMPV) and TBX4-FGF10 (AcDys, CAD, and PHs) signaling pathways. Here, we present molecular and histopathological findings in three Polish patients with LLDD.

Methods: Using whole-genome sequencing and histopathological lung evaluation, we analyzed three newborns who died due to respiratory failure within the neonatal period.

Results: Molecular analyses in Patient 1 revealed a heterozygous c.121G>T (p.Gly41Ter) variant in *TBX4* that was previously identified in two patients with PAH. In Patient 2, a novel heterozygous c.445C>T (p.Gln149Ter) variant in *FOXF1* was identified. A heterozygous recurrent ~2.2 Mb CNV deletion at 17q23.1q23.2 encompassing *TBX4* was detected in Patient 3. Histopathological assessment indicated LLDD in all three newborns, including PH (Patient 1), ACDMPV (Patient 2), and AcDys (Patient 3).

Conclusion: In neonatal cases of severe respiratory distress with PAH of an unknown etiology LLDD should be considered and confirmed by both genetic testing and histopathological evaluation. Our results further indicate that abnormalities in genes from the SHH-FOXF1 and TBX4-FGF10 signaling pathways, involved in human lung development, contribute to LLDD.

Grants: National Science Centre in Poland, 2019/35/D/NZ5/02896

Conflict of Interest: None declared

P05.006.B Kidney-Genetics – a reproducible and curated database of kidney-disease associated genes

Constantin Wolff[1], *Nina Rank*[1], *Jan Halbritter*[2], *Bernt Popp*[3]

[1]*Charité – Universitätsmedizin Berlin, Max Rubner Center for Cardiovascular Metabolic Renal Research, Berlin, Germany;* [2]*Charité – Universitätsmedizin Berlin, Department of Nephrology and Medical Intensive Care, Berlin, Germany;* [3]*Berlin Institute of Health, Center of Functional Genomics, Berlin, Germany*

Consortium: None.

Background/Objectives: Genomic insights are changing kidney disease (KD) diagnostics and treatment. Hundreds of monogenic KD genes have been identified to aid diagnosis, management, and monitoring. Lack of a unified and standardized KD gene database causes diagnostic blind spots and comparability issues in kidney genetics studies. To fill this gap, we created "Kidney-Genetics.org", a regularly updated, automated, and publicly accessible KD gene database.

Methods: KD-associated genes were obtained from (1) PanelApp, (2) a comprehensive literature review, (3) clinical diagnostic panels, (4) HPO-based searches in rare disease databases, and (5) automated PubTator-based search. Genes found in over two resources had high-evidence. Based on ClinGen recommendations, we created "Gene-Curator.org" for manual curation into entities and assignment of diagnostic genes to KD groups (e.g., syndromic vs. isolated; adult vs. pediatric; cystic, nephrotic).

Results: Kidney-Genetics.org contains annotations on gene expression, kidney phenotype, inheritance mode, disease onset, possible syndromic disease mode, and genetic variation for 3025 kidney-associated genes. The five analyzed sources yielded 534, 822, 956, 789, and 2158 genes. Of the 3025 genes, 598 (19.8%) are in two or more of the analyzed information sources, indicating high confidence and diagnostic potential. Of these high-evidence genes, 526 (88.0%) are in at least one and 56 (9.4%) in all 10 comprehensive diagnostic laboratory panels analyzed. We show that phenotypic groups correlate with functional gene clustering.

Conclusion: Kidney-Genetics.org helps clinicians, geneticists, and researchers analyze genomic data and understand diverse KDs. Our database tracks kidney research and diagnostics using a standardized pipeline and automated system.

Grants: None.

Conflict of Interest: Constantin Wolff: None declared, Nina Rank Junior Digital Clinician Scientist Programm of the BIH (DFG), Department of Nephrology and Medical Intensive Care, Charité University Medicine, Berlin, Jan Halbritter DFG (German Research Foundation), DFG (German Research Foundation), Department of Nephrology and Medical Intensive Care, Charité University Medicine, Berlin, Bernt Popp DFG (German Research Foundation), Labor Berlin - Charité Vivantes GmbH

P05.007.C Interpretation of risk factors and incidental findings in a patient with acute kidney injury requiring kidney replacement therapy

Nicole Meier[1], *Ulla T. Schultheiss*[1;2;3], *Eva Wohlleber*[1], *Juergen Kohlhase*[1]

[1]*Synlab MVZ Humangenetik Freiburg, Freiburg, Germany;* [2]*Institute of Genetic Epidemiology, Faculty of Medicine and Medical Center-University of Freiburg, Freiburg, Freiburg, Germany;* [3]*Department of Medicine IV, Nephrology and Primary Care, Faculty of Medicine and Medical Center-University of Freiburg, Freiburg, Germany*

Background: We report a sudden-onset acute kidney injury (AKI) case with no prior symptoms, requiring kidney replacement therapy. The patient's kidney function rapidly declined, with a creatinine level of 7.3 mg/dL and a proteinuria of 0.8 g/g. Nephropathology revealed preglomerular vascular thrombotic microangiopathy, arteriosclerosis, glomerular ischemia, severe tubular atrophy, and interstitial fibrosis (40-50%). No evidence of immunologic or complement-related glomerulonephritis or amyloidosis was found. The nephrologist referred the patient for diagnostics to assess recurrence risk before kidney transplantation.

Methods: We performed whole-exome sequencing (WES). Enrichment of target regions by the Twist Enrichment Kit for the Twist-Core/RefSeq Exome (Twist Bioscience). DNA sequencing (Next-Generation Sequencing, NGS) on Illumina NovaSeq 6000. Reference genome: GRCh37 (hg19). Bioinformatic analysis and gene dosage analysis via VarVis (Limbus Medical Technologies GmbH).

Results: WES detected no monogenic etiology but several risk factors for atypic hemolytic uremic syndrome (aHUS): a heterozygous variant in *CFHR5:* c.1411G>C, p.(Gly471Arg), a heterozygous deletion of *CFHR1/3* (confirmed by MLPA) and the CFH-H3 risk-haplotype. Incidentally homozygous FV-Leiden was detected.

Results were discussed with an interdisciplinary team. In this gremium we elucidated, that none of the variants were causal for the patients phenotype, but F5 should be taken into account regarding the functionality of the transplant. Regarding the risk-factors the transplanting facility may keep a complement suppressing therapy at the ready for the transplant procedure.

Conclusions: Even if no monogenic cause for the patient's phenotype was detected we should take risk factors and incidental findings into account regarding further therapeutic steps.

Conflict of Interest: None declared

P05.008.D Odyssey of the first female pioneer with an intragenic *TCF12* deletion in hypogonadotropic hypogonadism 26

Dilsu Dicle Erkan[1], *ABDULLAH SEZER*[2;3], *Prof Dr Semra Cetinkaya*[4], *Naz Guleray*[1]

[1]*Hacettepe University Faculty of Medicine, Medical Genetics, Ankara, Türkyie;* [2]*Health Science University Dr. Sami Ulus Obstetrics and Gynecology, Children Health and Disease Training and Research Hospital, Medical Genetics, Ankara, Türkyie;* [3]*Health Science University Ankara Etlik City Hospital, Medical Genetics, Ankara, Türkyie;* [4]*Health Science University Dr. Sami Ulus Obstetrics and Gynecology, Children Health and Disease Training and Research Hospital, Pediatric Endocrinology, Ankara, Türkyie*

Background/Objectives: The *TCF12* gene, part of the basic helix-loop-helix transcription factor family, is linked to craniosynostosis type-3 (MIM:615314) and ultra-rare hypogonadotropic hypogonadism 26 (HH26) (MIM:619718). Previously, isolated HH26 cases without craniosynostosis were limited to nine individuals, with no clinical information on women. Here we present the first thoroughly described female case with HH26.

Methods: Whole exome sequencing (WES;TwistExome-2.0) was complemented by CNV analysis (Muskat) and SNP-microarray (Illumina) for structural variant confirmation.

Results: A 15-year-old female, referred for delayed puberty and anosmia. The Tanner-stage I breast development and prepubertal pelvic ultrasonography results with low gonadotropin levels suggested Kallmann Syndrome. A year later estradiol was commenced to regulate menstruation. WES did not reveal any pathogenic SNVs but identified a novel intragenic deletion in *TCF12* spanning exons 6-8 through CNV analysis, which was further confirmed by microarray. This marks the first association of a structural variant with HH26. Intriguingly the individual also had multifocal papillary thyroid cancer, potentially related to *TCF12* impairment as previously *TCF12* somatic mutations associated with several malignancies.

Conclusion: The comprehensive detailing of this individual not only enrich the phenotypic spectrum in females but also expand the mutational landscape of this pleiotrophic disorder. Our findings reiterate the importance of integrating CNV analysis into next-generation sequencing workflows when clinical symptoms strongly suggest a genetic origin. Lastly, given the lack of genotype-phenotype correlation of *TCF12*-associated entities where identical mutations present as either isolated craniosynostosis or HH26, conducting a thorough systemic assessment, including radiological examination, and scrutinizing nuanced phenotypic features particular to females, will aid tailored phenotype-specific counseling.

Grants:

Conflict of Interest: None declared

P05.009.A Molecular-genetic analysis of Bulgarian patients with nephrotic disorders

Mila Sleptsova[1], *Slavena Atemin*[1], *Tihomir Todorov*[1], *Aleš Maver*[2], *Borut Peterlin*[2], *Albena Todorova*[1;3]

[1]*Genetic Medico-Diagnostic Laboratory "Genica", Molecular Genetics, Sofia, Bulgaria;* [2]*UMC Ljubljana, Clinical Institute of Medical Genetics, Ljubljana, Slovenia;* [3]*Medical University Sofia, Department of Medical Chemistry and Biochemistry, Sofia, Bulgaria*

Background/Objectives: In 2018 630 per million people in Bulgaria suffered from end stage renal disease. It is considered that the fifth most common cause of kidney failure are genetic renal disorders. Inheritable diseases can be avoided through genetic analysis, medico-genetic consultation, and family planning. We present 11 cases of nephrotic disease, where WES was used to determine the genetic etiology of the disease.

Methods: WES was performed in all 11 cases and kidney disease-related genes were analysed based on the phenotype observed. Sanger sequencing was used to confirm and segregate the variants of interest. A total of 43 samples were tested throughout the analysis.

Results: In total 9 cases were successfully concluded (or 82%) and of the reported genetic variants (presented below) 54% are novel. As a result of the high percentage genetically clarified cases, 2 prenatal samples and 3 relatives were additionally tested.

Case	Gene	Genetic Variant	Zygosity	Type of variant
1	*PKD1*	c.8354G>A	heterozygous	missense
2	*PKD1*	c.11494_11505del	heterozygous	in-frame deletion
3	*PKD1*	c.7937T>C	heterozygous	missense
	TSC2	c.3132-3T>C	heterozygous	intronic
4	*PKD1*	c.5615T>G	heterozygous	missense
	DSTYK	c.1558-4dup	heterozygous	intronic
5	*PKHD1*	c.6038del	heterozygous	frameshift deletion
		c.6866-2A>T	heterozygous	splice site
6	*MEFV*	c.2084A>G	heterozygous	missense
7	*NPHP4*	c.3292G>A	assumed homozygous	missense
8	*LAMA5*	c.8395C>G	heterozygous	missense
		c.4761C>A	heterozygous	synonymous
9	*COL4A5*	c.1339+1G>A	heterozygous	splice site

Conclusion: The diverse genetic variants in the presented cases enrich the spectrum of molecular-genetic diagnostics of nephrotic diseases in Bulgarian patients. Furthermore, the genetic analyses have been crucial in future family planning.

Grants:

Conflict of Interest: None declared

P05.010.B Associations of genetic factors with diabetes complications: an umbrella review

Xuan Zhou[1], *Nan Yang*[2], *Wei Xu*[1], *Xue Li*[2], *Athina Spiliopoulou*[1], *Evropi Theodoratou*[1]

[1]*Usher institute, Edinburgh, United Kingdom;* [2]*Zhejiang University, Hang Zhou Shi, China*

Background/Objectives: To comprehensively assess evidence from published systematic review and meta-analyses (SRMAs) on the genetics of diabetes complications.

Methods: A systematic literature search conducted in Medline and Embase identified 63 non-overlapping SRMAs. Meta-analyses were re-conducted to compare diabetes with and without complications using multiple genetic models. Associations were evaluated using Venice criteria and Bayesian false-discovery probability (BFDP); graded as highly credible, credible, and not credible. Highly credible and credible associations were contrasted to recent large genome-wide association studies (GWASs) of diabetes complications.

Results: Highly credible evidence was discovered for *MCP-1* (rs1024611) and *VEGF* (rs3025039) and type 2 diabetic retinopathy (DR); *ACACB* (rs2268388), *ACE* (Ins/Del), *MTHFR* (rs1801133), and *TCF7L2* (rs7903146) and type 2 diabetic kidney disease (DKD); and *SOD2* (rs4880) and type 1 diabetic peripheral neuropathy (DPN). When combining type 1 and 2 diabetes highly credible evidence was discovered for *ACE* (Ins/Del), *AKR1B1* (rs759853) and *ENPP1* (rs1044498) and DKD, and *EPO* (rs1617640) for the combined endpoint of DR and DKD. None of these associations were directly replicated in the latest GWASs for DR and DKD, however, another single nucleotide polymorphism (SNP), rs55853916 at *TCF7L2* had been detected as a GWAS hit for DKD.

Conclusion: This umbrella review rigorously assessed evidence on the genetics of diabetes complications, highlighting ten candidate genes not detected in recent GWASs. Mechanistic or bioinformatic studies are warranted to further assess the role of these genes in the pathology of diabetes complications and their potential as drug targets.

Grants: Diabetes UK Springboard Award [SBF006\1109]; CRUK Career Development Fellowship [C31250/A22804].

Conflict of Interest: None declared

P05.011.C The assessment of CDX1, IHH, SHH, GATA4, FOXA2, FOXF1 in congenital intra-abdominal adhesions

Helēna Freijere Pope[1;2], *māra pilmane*[1], *anna junga*[1], *aigars pētersons*[1;3]

[1]*Riga Stradiņš University, Department of Morphology, Rīga, Latvia;* [2]*Children's Clinical University Hospital, Medical Genetics, Rīga, Latvia;* [3]*Children's Clinical University Hospital, Surgery, Rīga, Latvia*

Background/Objectives: Congenital intra-abdominal adhesions are a rare condition that can result in a small bowel obstruction more in pediatric populations. The cause remains unknown, and the importance of congenital bands is related to the difficulty of diagnosis and early death. This research examines the expression of Caudal Type Homeobox 1 (CDX1), Indian Hedgehog (IHH), Sonic Hedgehog (SHH), GATA Binding Protein 4 (GATA4), Forkhead Box A2 (FOXA2) and Forkhead Box F1 (FOXF1) genes in human abdominal congenital adhesion fibroblast and endothelium cells.

Methods: The study group consisted of 14 children diagnosed with congenital intra-abdominal adhesions detected in various locations. The control group consisted of 6 children, their samples of peritoneal tissues were obtained during inguinal hernia repair surgeries. To view candidate gene mRNA transcripts, chromogenic in situ hybridization (CISH) was chosen. Semi-quantitative analysis investigated putative genes' signals.

Results: Significant correlations were found between CDX1 ($p < .001$) and SHH ($p = 0.032$) gene expression in congenital intra-abdominal adhesion fibroblasts compared to control peritoneal tissue. Statistically significant very strong correlations were found between the CDX1 and IHH gene comparing endothelium and fibroblast cells in congenital abdominal adhesion bands. The expression patterns of the genes IHH, GATA4, FOXA2, FOXF2, and SHH did not differ significantly.

Conclusion: Increased CDX1 gene expression in control group fibroblasts and endothelium, compared to congenital intra-abdominal adhesion-afflicted tissue, suggests involvement in congenital band formation. The control group's fibroblasts had a statistically significant increase in SHH-containing cells compared to the affected tissue of people with congenital intra-abdominal adhesions, suggesting that SHH may be involved in their development.

Conflict of Interest: None declared

P05.012.D Identification of the molecular mechanism underlying the involvement of mutations in the *MYO5B* gene and cholestasis

Cecilia Del Vecchio[1], *Mariateresa Di Stazio*[2], *Anna Monica Rosaria Bianco*[2], *Francesco Giambuzzi*[2], *Benedetta Blarasin*[3;4], *Cristina Bellarosa*[3], *Adamo Pio D'Adamo*[1;2]

[1]*University of Trieste, Department of Medicine, Surgery and Health Sciences, Trieste, Italy;* [2]*Institute for Maternal and Child Health – IRCCS, Burlo Garofolo, Trieste, Italy;* [3]*Italian Liver Foundation, Trieste, Italy;* [4]*University of Trieste, Department of Life Sciences, Trieste*

Background/Objectives: Progressive Familiar Intrahepatic Cholestasis (PFIC) represents an heterogenous group of rare diseases occurring in the first year of life, characterized by severe itching, hepatosplenomegaly, steatorrhea, and liver failure. MYO5B (NM_001080467) gene was recently described in PFIC patients but the molecular mechanism is still unknown. Some authors described that mutated MYO5B protein causes a wrong localization of canalicular proteins, which induces an alteration in bile salt secretion causing cholestasis.

Methods: In the last years, a retrospective and prospective study was done on 32 patients with intrahepatic cholestasis of unknown origin to identify causative variants by WES. To understand the pathogenetic mechanism we will evaluate the main canalicular transporters localization in patients-induced hepatocytes obtained from urine-derived induced Pluripotent Stem Cells (iPSC). When the sample is not available, iPSCs could be edited with the genetic variant of interest by CRISPR/Cas9 methodology.

Results: In 6 patients we identified causative mutations in MYO5B gene. We verified the canalicular protein localization on liver biopsies of two patients and, in both patients, the protein was normally expressed and located at the canalicular membrane.

Conclusion: Since immunostaining on our patients samples didn't clarify the pathogenetic mechanism and it's in contrast with previously published data, in which the missense mutation in MYO5B induces a protein mislocalization, there is an urgent need to perform functional studies of these genetic variants. In vitro-induced hepatocytes represent a promising model to verify the trafficking of canalicular proteins and to try to explain the cholestatic phenotype occurring in the presence of MYO5B mutations.

Grants: RC05/22 IRCCS "Burlo Garofolo", Trieste.

Conflict of Interest: None declared

P05.013.A Novel pharmacological approaches for metabolic-associated fatty liver disease based on a single patient with a de novo variant in *FASN*

Melina Musfeldt[1], *Anna Worthmann*[2], *Julius Ridder*[1], *Sharlaine Y. L. Piel*[1], *Ioannis Evangelakos*[1], *Hannah Voß*[3], *Marie O'Farrell*[4], *Alexander Fischer*[2;5;6], *Sangeeta Adak*[7], *Monica Sundd*[8], *Hasibullah Siffeti*[1], *Friederike Haumann*[1], *Katja Kloth*[1], *Tatjana Bierhals*[1], *Markus*

Heine[2], Paul Pertzborn[2], Mira Pauly[2], Julia-Josefine Scholz[1], Suman Kundu[9], Marceline M. Fuh[2], Axel Neu[10], Klaus Tödter[2], Maja Hempel[1;11], Uwe Knippschild[12], Clay F. Semenkovich[13], Hartmut Schlüter[3], Joerg Heeren[2], Ludger Scheja[2], Christian Kubisch[2], Christian Schlein[1]

[1]Institute of Human Genetics, University Medical Center Hamburg-Eppendorf, Hamburg, Hamburg, Germany; [2]Department of Biochemistry and Molecular Cell Biology, University Medical Center Hamburg-Eppendorf, Hamburg, Hamburg, Germany; [3]Section / Core Facility Mass Spectrometry and Proteomics, University Medical Center Hamburg-Eppendorf, Hamburg, Hamburg, Germany; [4]Sagimet Biosciences, California, United States; [5]Department of Molecular Metabolism, Harvard T. H. Chan School of Public Health, Harvard University, Boston, United States; [6]Department of Cell Biology, Harvard Medical School, Boston, United States; [7]Division of Endocrinology, Department of Medicine, Missouri, United States; [8]National Institute of Immunology, New Dehli, India; [9]Department of Biochemistry, University of Delhi South Campus, New Dehli, India; [10]Department of Pediatrics, University Medical Center Hamburg-Eppendorf, Hamburg, Hamburg, Germany; [11]Institute of Human Genetics, University Hospital Heidelberg, Heidelberg, Germany; [12]Department of General and Visceral Surgery, University Hospital Ulm, ULM, Germany; [13]Division of Endocrinology, Metabolism & Lipid Research, Department of Medicine, Washington University, Missouri, United States

Background/Objectives: The development of metabolic-associated fatty liver disease (MAFLD) is closely linked to aberrant synthesis of endogenous fatty acids in the liver mediated by fatty acid synthase (FASN). FASN produces saturated fatty acids, which are linked to cell stress and inflammation. Fatty acid synthesis can be reduced by supplementation of polyunsaturated fatty acids (PUFAs). However, it is not well understood, which factors determine the amount of PUFAs used in the liver from the diet and thus, the variable clinical outcome of its supplementation.

Methods: We performed trio-exome sequencing of a 5-year-old patient with autism and macrocephaly and performed plasma lipidomics via liquid chromatography mass spectrometry to study functional consequences in vivo. Further, we examined the effect of FASN in a clinical trial of patients with biopsy-confirmed MAFLD with a human FASN inhibitor, and performed studies combining FASN inhibitor with PUFA supplementation in mice.

Results: We identified a putative pathogenic de novo missense variant in *FASN*, leading to reduced FASN activity and surprisingly, increased PUFAs. This phenotype could be resembled in patients with MAFLD receiving a FASN inhibitor and mice receiving a combined FASN inhibition and PUFA supplementation treatment.

Conclusion: In common metabolic disease, the effectiveness of PUFA supplementation seems to depend on the rate of endogenous fatty acid synthesis. The combination of PUFA supplementation and FASN inhibition could contribute to the success of MAFLD treatment.

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P05.014.B Carriership revisited: phenotypic spectrum associated with heterozygyous variants in the IFT140 gene

Susanne Koning[1], *Dorien Lugtenberg[1], Marja Van der Meer[1], Anneke Vulto[1;2], Albertien M. van Eerde[3], Wendy Van Zelst-Stams[1;2], Ronald Roepman[1], Ernie Bongers[1], Marijn Stokman[1]*

[1]Radboud University Medical Center, Human Genetics, Nijmegen, Netherlands; [2]Maastricht University Medical Center, Human Genetics, Maastricht, Netherlands; [3]University Medical Center Utrecht, Genetics, Utrecht, Netherlands

Consortium: TheRaCil Consortium

Background/Objectives: Biallelic pathogenic variants in the IFT140 gene cause Mainzer-Saldino syndrome and isolated retinitis pigmentosa. Recently, heterozygous variants in IFT140 were reported in patients with polycystic kidney and liver disease. We investigated clinical manifestations of patients with heterozygous variants in IFT140 including carrier parents of children with recessive IFT140-related disease.

Methods: patients with mono- and biallelic variants in IFT140 were included. Clinical information was retrieved from medical records and additional phenotyping was performed for carrier parents including laboratory measurements and kidney ultrasound.

Results: Of 16 index patients, 11 had monoallelic and 5 had biallelic variants. The recurrent c.2399+1G>T splice variant was identified in 8 families. This variant was also detected in a patient with multiple kidney cysts who presented at age 15, and in compound heterozygosity with a variant of unknown significance in a patient with prenatally detected kidney cysts. These cases represent the earliest reported cysts in patients with heterozygous IFT140 variants. A minority of patients had chronic kidney disease, and no one reached end-stage kidney disease.

Conclusion: We expand the phenotypic spectrum associated with variants in the IFT140 gene and show an attenuated phenotype in carrier parents of patients with recessive IFT140-related disease. The heterozygous IFT140 phenotype is at the mild end of the ADPKD spectrum and shows reduced penetrance. We advise genetic testing in families with an IFT140 variant and surveillance according to ADPKD guidelines.

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Conflict of Interest: None declared

P05.016.D Functional characterization of *ATP7B* missense variants detected in patients with Wilson's disease

Amelie Stalke[1;2], *Annika Behrendt[3], Finja Hennig[1], Holger Gohlke[3;4], Nicole Buhl[1;2], Thea Reinkens[1], Ulrich Baumann[2], Brigitte Schlegelberger[1], Thomas Illig[5], Eva-Doreen Pfister[2], Britta Skawran[1]*

[1]Hannover Medical School, Department of Human Genetics, Hannover, Germany; [2]Hannover Medical School, Department of Pediatric Gastroenterology and Hepatology, Division of Kidney, Liver, and Metabolic Diseases, Hannover, Germany; [3]Heinrich Heine University Düsseldorf, Institute for Pharmaceutical and Medicinal Chemistry, Düsseldorf, Germany; [4]Forschungszentrum Jülich GmbH, John-von-Neumann-Institute for Computing, Jülich Supercomputing Center, Institute of Biological Information Processing (IBI-7: Structural Biochemistry), and Institute of Bio- and Geosciences (IBG-4: Bioinformatics), Gülich, Germany; [5]Hannover Medical School, Hannover Unified Bank, Hannover, Germany

Background/Objectives: Wilson's disease (WD) is an autosomal recessive disorder resulting in copper excess caused by biallelic variants in the *ATP7B* gene encoding a copper transporting P-type ATPase. *ATP7B* variants of unknown significance (VUS) are detected frequently, sometimes impeding a clear diagnosis. Functional analyses can help to classify these variants as benign or pathogenic. Additionally, variants already classified as (likely) pathogenic benefit from functional analyses to understand their pathomechanism, thus contributing to the development of future personalized treatment approaches.

Methods: We describe the clinical features of six WD patients, clinically confirmed by Leipzig score, and functionally characterized five *ATP7B* missense variants (two VUS, three yet uncharacterized likely pathogenic variants), detected in these patients. We determined ATP7B protein level, copper export capacity by a luciferase-based assay, and cellular localization using immunofluorescence staining in an in vitro model. Finally, we also analyzed the effects on protein stability using a 3D ATP7B protein model based on AlphaFold2.

Results: Protein levels and copper export capacity were decreased for all tested variants. Four variants resulted, at least partially, in ATP7B protein endoplasmic reticulum (ER)-retention. For all tested variants, except one ER-retained variant, our ATP7B protein model predicted ATP7B destabilization.

Conclusion: Our analyses allowed a reclassification of two VUS to 'likely pathogenic' and for two of the three 'likely pathogenic' variants to 'pathogenic'. For the ER-retained variants, especially the one with potentially preserved protein stability, therapies targeting ER-associated degradation (e.g. by pharmacological folding chaperones) may be promising.

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Conflict of Interest: Amelie Stalke German Research Foundation (DFG), Annika Behrendt: None declared, Finja Hennig: None declared, Holger Gohlke German Federal Ministry for Education and Research (BMBF), Nicole Buhl: None declared, Thea Reinkens: None declared, Ulrich Baumann Alexion, with no role in study design, collection, analysis, interpretation of data, Alexion, with no role in study design, collection, analysis, interpretation of data, Brigitte Schlegelberger: None declared, Thomas Illig: None declared, Eva-Doreen Pfister Alexion, with no role in study design, collection, analysis, interpretation of data, Britta Skawran: None declared

P05.017.A Improving diagnosis of Primary ciliary dyskinesia by genomic testing combined with functional assays

Martina Wilke[1], *Marianne Hoogeveen-Westerveld*[1], *Evelien Kroon*[1], *Frans W Verheijen*[1], *Tjeerd van der Veer*[2], *Menno M. van der Eerden*[2], *Sanne Kloosterman*[3], *Hettie Janssens*[3], *Yvonne den Boer*[3], *Paul Rebers*[2], *Andries Nagtegaal*[4], *Tjakko Van Ham*[1], *Lies Hoefsloot*[1]

[1]*Erasmus MC, University Medical Center Rotterdam, Department of Clinical Genetics, Rotterdam, Netherlands;* [2]*Erasmus MC, University Medical Center Rotterdam, Department of Respiratory Medicine, Rotterdam, Netherlands;* [3]*Erasmus MC, University Medical Center Rotterdam, Department of Pediatrics, Division of Respiratory Medicine and Allergology, Erasmus MC Sophia Children's Hospital, Rotterdam, Netherlands;* [4]*Erasmus MC, University Medical Center Rotterdam, Department of Otorhinolaryngology and Head and Neck Surgery, Rotterdam, Netherlands*

Background/Objectives: Primary ciliary dyskinesia (PCD) is a genetically heterogeneous, autosomal recessive disorder defined by dysfunction of the motile cilia. Diagnostic tests of PCD include functional laboratory measurements and genetic testing.

Methods: Over four years exome sequencing (ES) using a gene panel consisting of 40 PCD genes, was performed for individuals (n = 209) with clinical symptoms of PCD.

To improve the diagnostic yield and as an extension for the exome sequencing, functional assays were established in 2022: a) determine the ciliary beat frequency (CBF) and b) immunofluorescence imaging (IF). Nasal brushes of so far 39 individuals were collected, and used for IF/ CBF.

Laboratory findings and clinical phenotypes are discussed in a multidisciplinary expert group consisting of relevant clinical and laboratory personnel to obtain a final diagnosis of PCD.

Results: In our cohort, 18 (8,6%) individuals received a direct molecular diagnosis, 106 (51%) individuals had at least one sequence variant (variant of unknown significance (VUS), likely pathogenic, pathogenic) in a PCD gene and in 84 (40%) individuals no relevant variant was identified.

Aberrant staining could be observed in 19% (7/36:) of individuals. The diagnosis PCD was supported in six individuals (two variants). One individual had no relevant variants (PCD unlikely). Abnormal CBF was measured in seven individuals (19%; 7/36: 3x two variants (PCD positive); 1 carrier (VUS) and 3 with no relevant variant: both PCD unlikely).

Conclusion: The combination of genome diagnostics and functional assays will enhance the diagnostic yield for PCD and support the assessment of the likelihood of PCD.

Conflict of Interest: None declared

P05.018.B ADPKD phenocopies and a novel frameshift variant in *COL4A1*: a case report

Fiorenza Mioli[1;2], *Francesca Arruga*[1], *Valeria Bracciamà*[1;2], *Diana Carli*[1;2], *Angelo Corso Faini*[1;2], *Giulia Margherita Brach del Prever*[1;2], *maria luca*[1;2], *carmelo maria romeo*[1;2], *Caterina Scolari*[1;2], *Claudia Saglia*[1;2], *Massimo Gai*[3], *Roberta Giraudi*[3], *Luigi Biancone*[2;3], *Tiziana Vaisitti*[1;2], *Silvia Deaglio*[1;2]

[1]*AOU Città della Salute e della Scienza of Turin, Immunogenetics and Transplant Biology, Turin, Italy;* [2]*University of Turin, Department of Medical Sciences, Turin, Italy;* [3]*AOU Città della Salute e della Scienza of Turin, Nephrology Dialysis and Transplantation, Turin, Italy*

Background/Objectives: Different inherited conditions can lead to kidney cysts, including the most common autosomal dominant polycystic kidney disease (ADPKD) caused by *PKD1* or *PKD2* pathogenetic variants. However, ADPKD has phenocopies, making molecular analysis crucial for accurate diagnosis. Here, we present the case of a 47-year-old man with end-stage renal disease and a clinical diagnosis of ADPKD referred to our center for molecular diagnosis. The patient was also being evaluated for kidney transplant.

Methods: Clinical Exome Sequencing (CES) was performed and analysis restricted to specific in-silico gene panels. Identified variants were segregated by Sanger sequencing in family members

Results: Upon analysis of PKD-associated genes, two variants of unknown significance in *PKD1* were identified, one reported as hypomorphic and the other present in one sister and in the father, both without evidence of cysts. Analysis was extended to a panel of renal cysts-associated genes with identification of the heterozygous likely pathogenic variant c.1944del p.(Gly649fs*153) (NM_001845.6) in *COL4A1* gene, not previously reported in literature. *COL4A1* gene is associated with several disorders, including HANAC syndrome, which may present with isolated kidney cysts. Segregation analyses excluded the presence of the variant in the proband's sisters and father, strongly suggesting that this variant is responsible for the phenotype and arguing in favor of maternal origin. Indeed, the deceased mother had history

of recurrent strokes and muscle cramps, potentially compatible with HANAC syndrome.

Conclusion: This case highlights the importance of considering all phenocopies associated with ADPKD for accurate diagnosis, treatment and family counselling.

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Conflict of Interest: None declared

P05.019.C Unveiling a novel *INPP5E* mutation: Bridging the gap between Joubert Syndrome and MORM Syndrome

Nesibe Bulut[1], *Seda Hanife Oğuz*[2], *Selcuk DAGDELEN*[2], *Naz Guleray*[1]

[1]*Hacettepe University Medical Faculty, Department of Medical Genetics, Ankara, Türkyie;* [2]*Hacettepe University Medical Faculty, Division of Endocrinology and Metabolism, Ankara, Türkyie*

Background/Objectives: INPP5E encodes an inositol polyphosphate 5-phosphatase critical for primary cilium development and stability. Pathogenic biallelic INPP5E variants lead to ultra-rare ciliopathy spectrum ranging from Joubert Syndrome (OMIM:213300) to MORM Syndrome (Mental retardation, truncal obesity, retinal dystrophy, micropenis; MORMS; OMIM:610156). To date, approximately 35 mutations were reported only 2 of them being associated with MORMS. Adult phenotypic expression of this spectrum remains unclear. Here, we report a novel INPP5E mutation in an affected individual presenting with overlapping clinical features of both Joubert Syndrome and MORMS.

Methods: Clinical exome sequencing was performed to unravel underlying etiology.

Results: The affected 51-year-old woman exhibited congenital nystagmus, progressive retinal dystrophy, cataracts, hepatic fibrosis, portal hypertension and cirrhosis. She also had early-onset truncal obesity, hypertension, dyslipidemia, and obstructive sleep apnea. No developmental delay or ataxia was observed. Brain MRI demonstrated molar tooth sign. Three relatives older than 35-years also presented with similar findings. A novel homozygous mutation (c.1204A>G; p.Lys402Glu) affecting phosphatase catalytic domain in INPP5E (NM_019892) was identified.

Conclusion: The clinical findings of this patient align more with MORMS even though missense mutations affecting the catalytic domain were predominantly reported with Joubert phenotype and only truncating mutations in C-terminal domain were associated with MORMS. Therefore, the term "INPP5E-associated ciliopathies" is more appropriate to encompass the phenotypic spectrum of these separate entities. This study, in which the clinical course of multiple affected members is known until advanced age, emphasizes the importance of long-term follow-up of INPP5E-associated ciliopathies in terms of the progressive retinal and hepatic findings, and metabolic syndrome.

Grants: None

Conflict of Interest: None declared

P05.020.D Diagnostic utility of multi-faceted genetic screening in kidney failure of undetermined aetiology

Jeff Granhøj[1;2], *Katja Venborg Pedersen*[1], *Jesper Aagaard Graakjær*[1], *Dorte Lildballe*[3;4], *Bjarne Ørskov*[5], *Birgitte Godskesen Tougaard*[6], *Mads Jørgensen*[1], *Per Svenningsen*[7], *Henrik Birn*[4;6;8], *Maria Rasmussen*[1;2]

[1]*Lillebaelt Hospital - University Hospital of Southern Denmark, Department of Clinical Genetics, Vejle, Denmark;* [2]*University of Southern Denmark, Department of Regional Health Research, Odense, Denmark;* [3]*Aarhus University Hospital, Department of Molecular Medicine, Aarhus, Denmark;* [4]*Aarhus University, Department of Clinical Medicine, Aarhus, Denmark;* [5]*Zealand University Hospital, Department of Medicine, Roskilde, Denmark;* [6]*Aarhus University Hospital, Department of Renal Medicine, Aarhus, Denmark;* [7]*Institute of Molecular Medicine, University of Southern Denmark, Cardiovascular and Renal Research, Odense, Denmark;* [8]*Aarhus University, Department of Biomedicine, Aarhus, Denmark*

Background/objectives: The underdiagnosis of genetic kidney diseases calls for optimized screening strategies. We investigated the diagnostic yields of a three-part genetic screening in patients with kidney failure of undetermined aetiology (uKF).

Methods: We recruited 172 unrelated patients with suspected uKF for eligibility assessment through the review of medical records, excluding patients with specific causes of kidney disease. Inclusion criteria were: i) uKF ≤50 years or ii) undetermined kidney disease and family history of uKF ≤50 years. A total of 124 patients underwent initial screening, which included single-nucleotide-polymorphism (SNP)-array and SNaPshot *MUC1* analysis. Negative results prompted follow-up with short-read whole-genome sequencing (WGS) restricted to 542 genes. Only pathogenic or likely pathogenic variants in relevant genes were considered diagnostic.

Results: The median age of uKF was 37 years, and 91% of the cohort had adult-onset uKF. Diagnostic variants were identified in 33/124 patients (27%), with yields of 3/123 by SNP-array, 5/123 by *MUC1* analysis, and 25/116 by WGS. Diagnostic variants were most frequently identified in *COL4A3*/A4/A5, *NPHP1*, and *MUC1*, accounting for 58% of the genetic kidney diagnoses. A genetic diagnosis was obtained in 60% of the patients with a positive family history (15/25), of which 5/25 were detected by *MUC1* analysis. No significant difference in diagnostic yields was observed between childhood-onset versus adult-onset uKF.

Conclusion: Genetic screening revealed a monogenic cause in one-fourth of this uKF cohort. Notably, 20% of patients with positive family history had a *MUC1* variant undetectable by short-read sequencing.

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Astra Zeneca, Vifor Pharma, Boehringer Ingelheim, GSK: Advisory Board. Galapagos: IDMC., Maria Rasmussen: None declared

P05.021.A Monogenic Renal Calcium-Wasting Hypercalciuria: Unraveling *TRPV5* as the Cause of a Novel Mendelian Disorder

Naz Guleray[1;2], *Mark van Goor*[3], *Prof Dr Semra Cetinkaya*[4], *Jenny van der Wijst*[3], *melisa acun*[5], *fatma kurt colak*[2], *arda çetinkaya*[1;5], *joost hoenderop*[3]

[1]*Hacettepe University, Medical Faculty, Department of Medical Genetics, Ankara, Türkyie;* [2]*Health Science University, Dr. Sami Ulus Obstetrics and Gynecology, Children Health and Disease Training and Research Hospital, Department of Medical Genetics, Ankara, Türkyie;* [3]*Department of Medical Biosciences, Radboud University*

Medical Center, Nijmegen, Netherlands; [4]Health Science University, Dr. Sami Ulus Obstetrics and Gynecology, Children Health and Disease Training and Research Hospital, Department of Pediatric Endocrinology, Ankara, Türkyie; [5]Hacettepe University, Institute of Health Sciences, Department of Bioinformatics, Ankara, Türkyie

Introduction: Hypercalciuria is the most common metabolic risk factor associated with nephrolithiasis. Although the majority of hypercalciuria is explained by complex genetic factors, a monogenic cause of isolated renal calcium loss has not previously been described. Here, we demonstrate the first autosomal recessive pedigree with pure renal-leak type hypercalciuria and provide evidence that a loss-of-function variant in *TRPV5*, encoding a renal calcium channel, underlies the disorder.

Methods: We employed SNP-microarray-based homozygosity mapping followed by whole exome sequencing to elucidate the molecular basis of hypercalciuria in three affected siblings. Furthermore, the mutant TRPV5 channel was functionally characterized in HEK293 cells.

Results: We identified a homozygous ultra-rare variant in *TRPV5* (NM_019841.7:c.1792G>A; p.(Val598Met)), which co-segregates with hypercalciuria. This variant disrupts glycosylation and enhances proteasomal degradation of the mutant channel, thereby reducing its cell surface expression and diminishing calcium uptake. Intriguingly, besides hypercalciuria, the 10-year-old proband also displayed severe skeletal deformities, bone fractures, and hyperparathyroidism that could not be attributed solely to the *TRPV5* variant. Further investigation showed that the skeletal involvement unique to the proband was the outcome of a blended phenotype caused by an additional well-known pathogenic somatic *GNAS* mutation (NM_000516.7:c.601C>T; p.(Arg201Cys)).

Conclusion: *TRPV5* has long been proposed as a candidate for hypercalciuria in humans. This study provides the first evidence that biallelic *TRPV5* variants cause a novel form of monogenic hypercalciuria, which we name "Renal Calcium-Wasting Hypercalciuria" (RCWH). Given its recessive nature, RCWH might be overlooked as a cause of hypercalciuria and should be screened, particularly in inbred populations.

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Conflict of Interest: None declared

P05.022.B Loss of phospholipase PLAAT3 causes a mixed lipodystrophic and neurological syndrome due to impaired PPARγ signaling

*Nika Schuermans[1;2], **Salima EL CHEHADEH**[3;4;5], Dimitri Hemelsoet[6], Jérémie Gautheron[7], Marie-Christine Vantyghem[8;9], Sonia Nouioua[10;11], Meriem Tazir[11;12], Corinne Vigouroux[7;13], Martine Auclair[7;13], Elke Bogaert[1;2], Sara Dufour[2;14;15], Fumiya Okawa[16], Pascale Hilbert[17], Ramdane Cherif Ferroudja[10;11], Nike Van Doninck[18], Marie-Caroline Taquet[19], Toon Rosseel[1], Griet De Clercq[1;2], Elke Debackere[1;2], Paul Coucke[1;2], Carole Van Haverbeke[20], Jo Van Dorpe[20], Jon Andoni Urtizberea[21], Jean-Baptiste Chanson[22], Benoit Funalot[23;24], François-Jérôme Authier[24;25], Sabine Kaya[26], Wim Terryn[27], Steven Callens[28], Bernard Depypere[29], Bruce Poppe[1;2], Francis Impens[14;15;30], Noboru Mizushima[16], Arnaud Vanlander[31], Patrick Verloo[31], Christel Depienne[5;26], Isabelle Jéru[7;32], Bart Dermaut[1;2]*

[1]Center for Medical Genetics, Ghent University Hospital, Ghent, Belgium; [2]Department of Biomolecular Medicine, Faculty of Medicine and Health Sciences, Ghent University, Ghent, Belgium; [3]Service de Génétique Médicale, Institut de Génétique Médicale d'Alsace (IGMA), Hôpitaux Universitaires de Strasbourg, Strasbourg, France; [4]Laboratoire de Génétique Médicale, UMRS_1112, Institut de Génétique Médicale d'Alsace (IGMA), Université de Strasbourg et INSERM, Strasbourg, France; [5]I.G.B.M.C. - Institut de génétique et de biologie moléculaire et cellulaire, INSERM U1258, CNRS-UMR7104, Université de Strasbourg, Illkirch-Graffenstaden, France; [6]Department of Neurology, Ghent University Hospital, GHENT, Belgium; [7]Sorbonne Université, INSERM UMRS_938, Centre de Recherche Saint-Antoine (CRSA), Paris, France; [8]Endocrinology, Diabetology, Metabolism Department, National Competence Centre for Rare Diseases of Insulin Secretion and Insulin Sensitivity (PRISIS), Lille University Hospital, Lille, France; [9]INSERM U1190, University of Lille, European Genomic Institute for Diabetes, Lille, France; [10]Department of Neurology of the EHS of Cherchell, University Centre of Blida, Tipaza, Algeria; [11]NeuroSciences Research Laboratory, University of Algiers Benyoucef Benkhedda, Algiers, Algeria; [12]Department of Neurology, CHU Algiers (Mustapha Pacha Hospital), Algiers, Algeria; [13]Assistance Publique - Hôpitaux de Paris, Saint-Antoine University Hospital, National Reference Center for Rare Diseases of Insulin Secretion and Insulin Sensitivity (PRISIS), Department of Endocrinology, Diabetology and Reproductive Endocrinology, and Department of Molecular Biology and Genetics, Paris, France; [14]VIB-UGent Center for Medical Biotechnology, VIB, Ghent, Belgium; [15]VIB Proteomics Core, VIB, Ghent, Belgium; [16]Department of Biochemistry and Molecular Biology, Graduate School and Faculty of Medicine, The University of Tokyo, Bunkyo, Japan; [17]Department of Molecular and Cellular Biology, Institute of Pathology and Genetics, Charleroi, Belgium; [18]Department of Endocrinology and Diabetology, General Hospital VITAZ, Sint-Niklaas, Belgium; [19]Department of Internal Medicine and Nutrition, Hôpitaux Universitaires de Strasbourg, Strasbourg, France; [20]Department of Pathology, Ghent University Hospital, Ghent, Belgium; [21]Institut de Myologie, Paris, France; [22]Service de Neurologie et Centre de Référence Neuromusculaire Nord/Est/Ile de France, Hôpital de Hautepierre, Strasbourg, France; [23]Department of Medical Genetics, Hôpital Henri Mondor, Université Paris-Est-Créteil, Créteil, France; [24]INSERM UMR955, Team Relaix, Faculty of Medicine, Créteil, France; [25]Centre Expert de Pathologie Neuromusculaire/Histologie, Département de Pathologie, Hôpital Henri Mondor, Université Paris-Est-Créteil, Créteil, France; [26]Institut für Humangenetik, Universitätsklinikum Essen, Essen, Germany; [27]Department of Nephrology, Jan Yperman Hospital, Leper, Belgium; [28]Department of General Internal Medicine, Ghent University Hospital, Ghent, Belgium; [29]Department of Plastic and Reconstructive Surgery, Ghent University Hospital, Ghent, Belgium; [2]Department of Biomolecular Medicine, Faculty of Medicine and Health Sciences, Ghent University, Ghent, Belgium; [31]Division of Pediatric Neurology and Metabolic Diseases, Department of Pediatrics, Ghent University Hospital, Ghent, Belgium; [32]Department of Medical Genetics, DMU BioGeM, Sorbonne Université, AP-HP, Pitié-Salpêtrière Hospital, Paris, France

Consortium: Program for Undiagnosed Diseases (UD-PrOZA)

Background/Objectives: Phospholipase A/acyltransferase 3 (PLAAT3) is a phospholipid-modifying enzyme predominantly expressed in neural and white adipose tissue (WAT). It is a potential drug target for metabolic syndrome, as Plaat3 deficiency in mice protects against diet-induced obesity. We identified seven patients from four unrelated consanguineous families, with homozygous loss-of-function variants in *PLAAT3*, who presented with a lipodystrophy syndrome with loss of fat varying from partial to generalized, muscular hypertrophy and chronic muscle pain, associated with metabolic complications, as well as variable neurological features including demyelinating neuropathy and intellectual disability. We aimed to clarify the pathogenic mechanism of PLAAT3-related lipodystrophy syndrome and to further address the role of PLAAT3 in human adipogenesis.

Methods: We performed multi-omics analyses of mouse Plaat3−/− and patient-derived WAT. We next performed CRISPR-Cas9-mediated PLAAT3 inactivation in human adipose

stem cells and assessed the adipocyte differentiation signaling using morphological and expression studies.

Results: Multi-omics analysis of mouse Plaat3−/− and patient-derived WAT showed enrichment of arachidonic acid-containing membrane phospholipids and a strong decrease in the signaling of peroxisome proliferator-activated receptor gamma (PPARγ), the master regulator of adipocyte differentiation. Accordingly, CRISPR-Cas9-mediated PLAAT3 inactivation in human adipose stem cells induced insulin resistance, altered adipocyte differentiation with decreased lipid droplet formation and reduced the expression of adipogenic and mature adipocyte markers, including PPARγ.

Conclusion: These findings establish PLAAT3 deficiency as a hereditary lipodystrophy syndrome with neurological manifestations, caused by a PPARγ-dependent defect in WAT differentiation and function. The role of PLAAT3 in patients' neurological phenotype remains to be established.

Conflict of Interest: None declared

P05.023.C Out-of-frame translation restores the reading frame and rescues a homozygous loss-of-function variant in a novel *TBCE* phenotype

Peter Sparber[1], *Alexandra Filatova[1], Eugene Tatarskiy[1], Evgeniia Ulas[2], Grigory Perelman[1], Makretskaya Nina[3], Elena Nagaeva[4], Maria Kareva[4], Elena Frolova[5], Natalia Kalinchenko[4], Sergey Golyshev[6], Anton Burakov[6], Elena Nadezhdina[2], Vyacheslav Tabakov[7], Victoria Voinova[8], Anatoliy Tiulpakov[3], Mikhail Skoblov[1]*

[1]Research Centre for Medical Genetics, Laboratory of Functional Genomics, Moscow, Russian Federation; [2]Institute of Protein Research of Russian Academy of Science, Moscow, Russian Federation; [3]Research Centre for Medical Genetics, Genetics of Endocrine Diseases, Moscow, Russian Federation; [4]Institute of Pediatric Endocrinology, Endocrinology Research Centre, Moscow, Russian Federation; [5]National Medical Research Center for Children's Health Federal state autonomous institution of the Russian Federation Ministry of Health, Consulting and diagnostic, Moscow, Russian Federation; [6]A.N. Belozersky Institute of Physico-Chemical Biology, Lomonosov Moscow State University, Moscow, Russian Federation; [7]Research Centre for Medical Genetics, Moscow, Russian Federation; [8]Veltischev Research and Clinical Institute for Pediatrics Pirogov RNRMU, Moscow, Russian Federation

Background/Objectives: Pathogenic variants in the TBCE gene, which codes for a tubulin-specific chaperone are associated with three severe neurodevelopmental phenotypes. As complete absence of TBCE is lethal in all cases, at least residual activity of the TBCE protein was detected. Here we report a novel milder TBCE phenotype with unique molecular mechanism.

Methods: Splicing analysis was performed using deep NGS sequencing of RT-PCR products. Western-blot was used for TBCE detection. Translation efficiency was mmeasured using luciferase reporter assay. Overexpression experiments were performed in Hela cells with further tubulin staining. Golgi complex compactness was analyzed by immunofluorescence analysis. Microtubule repolymerization dynamics was analyzed after nocodazole treatment. Electron microscopy was used for ultrastructural studies.

Results: We assessed seven patients with a novel, distinct and clinically recognizable TBCE-associated phenotype featuring hypergonadotropic hypogonadism, amyotrophy and short stature. All patients were homozygous or compound-heterozygous for the c.100+1>A variant. RT-PCR confirmed splicing alteration leading to an early frameshift - p.Thr33delInsAla62fsTer24. Western-blot and luciferase reporter assay demonstrated translation of mutated TBCE protein from an alternative open reading frame compensation the frameshift starting from exon 4. The reduced translation of the mutant TBCE protein was attributed by both decreased stability of the altered N-terminus and competition for ribosome binding with the reference start codon. Overexpression experiments showed partial activity of the mutant protein. Reduced Golgi complex compactness was observed alongside normal microtubule repolymerization dynamics. Muscle sample electron microscopy displayed varying levels of acto-myosin complex degradation

Conclusion: Our findings unveil a novel TBCE phenotype with a unique molecular mechanism

Conflict of Interest: None declared

P05.024.D Study of the utility of genetic analyses in congenital hypothyroidism neonatal TSH screening

Silje Hogner[1], *William Nicholas Tourniaire[1], Emma Sofia Lundman[1], Janne Maren Strand[1], Julie Hellem AAby[1], Linda Karlsen Sørgjerd[1], Mona Christine Berge[1], Mari Eknes Ytre-Arne[1], Siv Merete Løvoll[1], Ingjerd Sæves[1], Cassandra Nicole Trier[1], Alexander Dominic Rowe[1], Magnus Odin Dahlseng[1], Andreas Øberg[1], Trine Tangeraas[1], Olve Moldestad[1], Asbjørg Stray-Pedersen[1], Jens Veilemand Jørgensen[1]*

[1]Oslo University Hospital, Norwegian National Unit for Newborn Screening, Oslo, Norway

Congenital hypothyroidism (CH) was included in the Norwegian newborn screening (NBS) program in 1979. Thyroid Stimulating Hormone (TSH) immunoassay (GSP, Revvity) are performed on all NBS dried blood spot samples. The Norwegian NBS program is regulated by an exception from the Biotechnology Act allowing for mass genetic screening for specific disorders. Genetic analyses have since 2012 been used as confirmatory test for most screening disorders, but not yet for CH. As part of improving the laboratory service, 42 genes associated with inherited CH were tested in samples with elevated TSH using Illumina DNA prep and bioinformatically filtered whole-genome sequencing.

After ~220,000 analyzed samples (2018-2022), a total of 349 infants were reported with elevated TSH and were subject for genome sequencing. So far, 146 samples have been sequenced. Nine individuals have a confirmed molecular cause of CH and 24 are carriers of one pathogenic variant in a gene related to autosomal recessive CH, six in compound heterozygozity with a variant of uncertain significance (VUS). In additional six cases, two VUS in the same gene were found, while in 24 samples one VUS was detected. In the remaining 83 samples, no variants of interest were found.

The current status of the project will be presented with regard to identified variants in TSH positive samples and evaluation of whether NGS panel determining the molecular causes of congenital hypothyroidism is useful in a NBS setting.

The study was approved by the local IRB (2017/2879) and funded by the South-Eastern Norway Regional Health Authority (2018080).

Conflict of Interest: None declared

P05.025.A A Cystic fibrosis case with combination of L467F-F508 del and novel 2217 delA mutation

Nadezhda Yaneva[1], *Guergana Petrova[2], Irena Bradinova[1], Tihomir Todorov[3], Albena Todorova[3;4], Alexey Savov[5]*

[1]National Genetic Laboratory, University Hospital of Obstetrics and Gynecology "Maichin Dom", Obstetrics and Gynecology, Sofia, Bulgaria; [2]Pediatric cilinic, UMHAT "Alexandrovska", Medical University of Sofia, Pediatric, Sofia, Bulgaria; [3]Genetic Medico-Diagnostic Laboratory "Genica", Sofia, Bulgaria; [4]Medical University Sofia, Medical Chemistry and Biochemistry, Sofia, Bulgaria; [5]National

Genetic Laboratory, University Hospital of Obstetrics and Gynecology "Maichin Dom", Obstetrics and Gynecology, Sofia, Bulgaria

Background/Objectives: Cystic fibrosis is an autosomal recessive condition caused by loss-of-function variants in the CFTR gene. Some people with CF carry complex alleles, with more than one CFTR variant on the same allele. Genetic variants located in cis can affect disease severity or treatment response.

Methods: At 24-weeks of gestation a couple was referred for CF testing because of ultrasound finding of hyperechogenic bowel suggesting fetal meconium ileus. Carrier screening was performed of the parents for the most common mutations in Bulgarian population.

Results: The mother was found to be carrier of complex L467F-F508del allele. The newborn examination after delivery found the maternal double variant L467-F508del and a novel pathogenic frameshift variant 2217delA subsequently confirmed to be carried by the father. Initially the paternal variant 2217delA was not detected likely because of allele dropout.

Conclusion: The existence of a complex allele does not always affect the responce to CFTR modulators. L467F-CFTR is reported to be damaging to protein maturation. The L467F-F508del complex allele is not responsive to Kaftrio treatment, so it should be considered an indication for prescription of mutation-specific therapies. Risk of allele dropout artifact on screening and diagnostic tests could lead to a wrong diagnosis, so it is important to detect and avoid it in clinical molecular diagnostics. Identification of the new pathogenic CFTR variants provides useful information for further observing the genotype-phenotype correlations and could open the door to integration of precision medicine into routine clinical practice in the near future.

Conflict of Interest: None declared

P05.026.B Pulmonary hemorrhage in CM-AVM2 due to disease-causing variant in EPHB4

Efrat Sofrin-Drucker[1;2], *Meir Mei-Zahav*[2;3], *lina basel-salmon*[4;5;6], *Benjamin Rothschild*[3;7], *Rotem Tal*[7;8], *Liora Harel*[7;8], *Oded Scheuerman*[2;9], *Naama Orenstein*[1;2], *Lily Bazak*[4]

[1]*Schneider- Children's Medical Center, Pediatric Genetics Unit, Petah Tikva, Israel;* [2]*Tel Aviv University, Faculty of Medicine, Tel Aviv-Yafo, Israel;* [3]*Schneider- Children's Medical Center, Pulmonary Institute, Petah Tikva, Israel;* [4]*Rabin Medical Center, Raphael Recanati Genetics Institute, Petah Tikva, Israel;* [5]*Tel Aviv District, Faculty of Medicine, Tel Aviv-Yafo, Israel;* [6]*Rabin Medical Center, Felsenstein medical research center, Petah Tikva;* [7]*Tel Aviv University, Faculty of Medicine, Tel Aviv-Yafo;* [8]*Schneider- Children's Medical Center, Pediatric Rheumatology unit, Petah Tikva, Israel;* [9]*Schneider- Children's Medical Center, Departments of Pediatrics A, B, C and Department of Day Hospitalization, Petah Tikva, Israel*

Background/Objectives: Capillary malformation-arteriovenous malformation 2 (CM-AVM2), caused by EPHB4 pathogenic variants, is part of a spectrum of autosomal dominant inherited vascular malformation syndromes. This group includes RASA1-mutated CM-AVM1 and hereditary hemorrhagic telangiectasia (HHT). CM-AVM2 is distinguished by the presence of arteriovenous malformations (AVMs) both within and outside the cranium, alongside multiple capillary malformations (CMs) and telangiectasias. We describe a familial case with lung involvement, suggesting a possible phenotypic expansion of the disease.

Methods: A 2-year-old boy had several episodes of life-threatening diffuse alveolar hemorrhage with severe anemia – (Hb – 4g/dl), was referred to our pediatric genetic unit. Despite extensive investigations, including repeated bronchoscopies, nasal endoscopy, lung MRI, CT imaging, lung biopsy, several cardiac and pulmonary arteries catheterizations, bubble echo, and RBC scan, the source of bleeding from the lung remained unidentified. Familial investigations revealed maternal lip telangiectasias and a capillary malformation on her forearm. Whole-exome sequencing (WES) was conducted for the child and his parents.

Results: The analysis identified a maternally inherited heterozygous c.2420G>A p.Gly807Glu variant in the EPHB4 gene, classified as likely pathogenic. This variant is not present in the gnomAD database. Computational prediction tools suggest a deleterious impact on the gene. A different missense variant at the same position has been previously reported in a patient with an AVM in the ear.

Conclusion: This case represents the first reported case of massive pulmonary hemprrhage, potentially as the primary symptom of CM-AVM2.

Grants: none

Conflict of Interest: None declared

P05.027.C Diagnostic challenges in very early onset polycystic kidney disease

Elena Luppi[1;2], *Carlotta Cristalli*[1], *Enrico Ambrosini*[3], *Amalia Conti*[1], *Claudio Graziano*[4], *Francesca Montanari*[1]

[1]*Medical Genetics Unit, IRCCS Azienda Ospedaliero-Universitaria di Bologna, Bologna, Italy;* [2]*University of Bologna, Department of Medical and Surgical Sciences, Bologna, Italy;* [3]*Medical Genetics Unit, University Hospital of Parma, Parma, Italy;* [4]*Medical Genetics Unit, AUSL Romagna, Cesena, Italy*

Background/Objectives: Very early onset polycystic kidney disease (VEO-PKD) defines the antenatal finding up to 18 months of age of cystic/hyperechoic enlarged kidneys. Differential diagnosis is challenging and encompasses recessive inheritance in autosomal dominant PKD genes.

We aim to define the occurrence of *PKD1/PKD2* genes variants in a cohort of VEO-PKD and their diagnostic implications by illustrative pedigrees.

Methods: We retrospectively reviewed clinical and molecular data of probands referred to the Medical Genetics Unit of Bologna from 2018 to 2023 for the perinatal detection of cystic/hyperechoic kidneys. All samples were tested by targeted NGS panel of cystic genes and multiplex ligation-dependent probe amplification of *PKHD1, HNF1B, PKD1 and PKD2*.

Results: Out of 20 VEO-PKD unrelated probands, 12 harboured likely causative variants in *PKD1/PKD2*. In 7 of them a single *PKD1/PKD2* variant was identified, while 5 patients had biallelic *PKD1* variants, of which 2 were inherited from a parent with a positive ADPKD history and 8 likely hypomorphic variants from otherwise healthy parents. The disease spectrum, even in biallelic genotypes, show a high variability from a prenatal onset with perinatal death to pauci symptomatic renal cysts in childhood. Of note, in the antenatal case we recorded a new genotype combining "in trans" a novel truncating variant (c.24_27dup; p.Ala10Profs) with the hypomorphic variant c.5830G>A (p.Gly1944Arg) and defining a severe phenotype.

Conclusion: Our results widen the VEO-PKD phenotypic and genotypic spectrum and underline the need for a careful ultrasound follow-up in pregnancies of ADPKD families and carrier testing/ultrasound screening of their partners.

Grants: None

Conflict of Interest: None declared

P05.029.A Decoding gastrointestinal puzzle: Unmasking Congenital enterokinase deficiency beyond the Cystic fibrosis in Bulgarian patient

Gabriela Kovacheva[1;2], *Tihomir Todorov*[2], *Slavena Atemin*[2], *Mila Sleptsova*[2], *Mila Baycheva*[3], *Vanyo Mitev*[1], *Albena Todorova*[1;2]

[1]*Medical University of Sofia, Department of Medical Chemistry and Biochemistry, Sofia, Bulgaria;* [2]*Independent Medical Diagnostic Laboratory Genome Center Bulgaria, Sofia, Bulgaria;* [3]*University Children's Hospital, Department of Paediatric Gastroenterology, Hepatology and Nutrition, Sofia, Bulgaria*

Background/Objectives: Congenital enterokinase deficiency is a rare autosomal-recessive genetic disorder characterized by the absence/dysfunction of enterokinase - a crucial enzyme in the digestive system, converting trypsinogen into its active form trypsin. The genetic basis of this gastrointestinal disorder involves different loss-of-function variants in the TMPRSS15 gene, which encodes the enterokinase enzyme. The clinical features of Congenital enterokinase deficiency can mimic those of Cystic fibrosis, a more prevalent genetic disorder. Both conditions may present in infants with failure to thrive, malabsorption, and chronic diarrhea, thus creating diagnostic challenges.

Methods: We report a case of a 3-month-old boy with pancreatic steatorrhea, hypoproteinemia and anemia. Genomic DNA from the proband was extracted from their peripheral blood and analyzed through whole exome sequencing (WES).

Results: The initial results of WES analysis revealed a heterozygous variant CFTR:c.2756G>A/p.Tyr919Cys, classified as a variant of uncertain significance (VUS). Subsequently, a multiplex ligation-dependent probe amplification (MLPA) analysis was performed. A normal result was yielded and excluded the diagnosis Cystic fibrosis. Further investigation uncovered a homozygous variant c.3004G>A/p.Gly1002Arg in the TMPRSS15 gene, confirmed through Sanger sequencing. Both of the proband's parents were found to be heterozygous carriers of this variant.

Conclusion: The clinical mimicry of Cystic fibrosis in patients with Congenital enterokinase deficiency poses a significant challenge in diagnosis and management, potentially impacting the well-being of those with enterokinase deficiency. Patients with enterokinase deficiency may be mistreated for Cystic fibrosis, leading to delays in addressing the specific enzymatic deficit.

Grants: -

Conflict of Interest: None declared

P05.030.B Investigation of genetic etiology in familial CAKUT cases

Durmuş Durmaz[1], *Güven Toksoy*[1], *Zeynep Yürük Yıldırım*[2], *bağdagül aksu*[2], *Zuhal Bayramoğlu*[3], *Zehra Oya Uyguner*[1], *alev yılmaz*[2], *Bilge Ozsait Selcuk*[1], *Birsen Karaman*[1;4], *Ayça Dilruba aslanger*[1]

[1]*Istanbul Faculty of Medicine, Istanbul University, Department of Medical Genetics, Istanbul, Türkyie;* [2]*Istanbul Faculty of Medicine, Istanbul University, Department of Pediatric Nephrology, Istanbul, Türkyie;* [3]*Istanbul Faculty of Medicine, Istanbul University, Department of Radiology, Istanbul, Türkyie;* [4]*Istanbul University, Institute of Health Sciences, Istanbul, Türkyie*

Background/Objectives: Congenital anomalies of the kidneys and urinary tracts (CAKUT) comprise a group of disorders that involve various structural anomalies resulting from defects in the embryologic development of the urinary system. Our objective is to identify the molecular etiology in CAKUT families, aiming to comprehensively understand the associated clinical and genetic characteristics.

Methods: Exome sequencing with CNV analysis was conducted on two affected individuals from each of the eight families. Variant confirmation and family segregation were carried out through Sanger sequencing and array-CGH analysis.

Results: We identified variants associated with CAKUT in four of the eight families. Family-I had a 9q32 duplication, including cases with renal hypoplasia and multicystic dysplastic kidney (MCKD). Heterozygous/novel variants in *TFAP2A* and *TFAP2B* were identified in Family-II with MCKD and subtle dysmorphic findings. A heterozygous novel variant in *GREB1L* was found in Family-III with horseshoe kidneys and vesicoureteral reflux. A heterozygous splice-site variant in *NOTCH2* and a heterozygous/novel variant in *ROBO2* were both found in Family-IV with renal agenesis, ureteropelvic junction obstruction, mild dysmorphic/skeletal findings.

Conclusion: This study reports novel variants associated with CAKUT and supports the pathogenicity of 9q32 duplication, which was previously reported in only one case with CAKUT. Identification of novel variants in genes involved in renal development in patients with syndromic CAKUT suggests that multigenic inheritance may influence the phenotype. In cases of CAKUT where genetic diagnosis is challenging, a detailed clinical examination and family study contribute to the diagnosis.

Grants: This project is supported by the Scientific Research Projects Coordination Unit of Istanbul University (Project-ID: TTU-2023-39892).

Conflict of Interest: None declared

P05.031.C CFTR diagnostics: patient data from 2020 to 2023

Bettina Desery[1], **Dorit Borchert**[1], *Anne Christians*[1], *Anna Teubert*[1], *Sarah Knippenberg*[1], *Gudrun Göhring*[1;1]

[1]*amedes MVZ wagnerstibbe für Laboratoriumsmedizin, Hämostaseologie, Humangenetik und Mikrobiologie Hannover, Hannover, Germany*

Background/Objectives: Cystic fibrosis (CF) is one of the most common autosomal recessive diseases worldwide. In addition to classic cystic fibrosis with severe lung involvement, atypical milder forms are also found, such as pancreatitis, disseminated bronchiectasis and infertility in men (congenital bilateral aplasia of the vas deferens (CBAVD)).

Methods: A total of 929 patients were examined for the presence of the most common variants and 1600 patients for the presence of additional (pathogenic) variants in the *CFTR* gene by next generations sequencing or hybridization assays. Relevant variants include (probably) pathogenic variants, variants of unclear significance (VUS) and the 5T allele (a disease-associated polymorphism).

Results: For the indication of CFTR-associated infertility, two relevant variants (suspected to be compound-heterozygous) were detected in 2.16% of patients. In 13.35% of patients, only one relevant variant (including VUS and 5T allele) was found. A total of 4.15% were carriers of at least one (probably) pathogenic variant.

In cases of cystic fibrosis, the diagnosis was confirmed with high probability in 13.19% of patients by detection of two pathogenic variants. Here, the F508del variant accounted for 78.95% of all detected compound-heterozygous or homozygous pathogenic variants. In additional 4.86%, only one pathogenic variant lacking a second variant could be detected.

Conclusion: The different proportions of confirmed diagnoses (two detected variants) in the indications suspected CFTR-associated infertility (2.2%) and suspected CF (13.2%) shows the higher diagnostic yield at the molecular level for a clinically well-defined suspected diagnosis such as cystic fibrosis than for CFTR-associated infertility.

Conflict of Interest: None declared

P05.032.D Involvement of p38 gamma/delta kinases in the development of alcoholic steatohepatitis

Daniel Salete Granado[1;2], *Beatriz Cicuéndez*[3], *Cintia Folgueira*[3], *Alfonso Mora*[3], *Marta León*[3], *María de los Ángeles Pérez Nieto*[2;4], *Antonio Javier Chamorro*[1;2;5], *Maura Rojas-Pirela*[1;2], *Ana-Belén Herrero*[1;2], *Guadalupe Sabio*[3], *Miguel Marcos*[1;2;5]

[1]*Institute of Biomedical Research of Salamanca-IBSAL, Salamanca, Spain;* [2]*University of Salamanca, Department of Medicine, Salamanca, Spain;* [3]*Spanish National Cancer Research Center, Madrid, Spain;* [4]*Instituto de Ciencias de la Salud de Castilla y León, Soria, Spain;* [5]*University Hospital of Salamanca, Department of Internal Medicine, Salamanca, Spain*

Background/Objectives: Alcoholic steatohepatitis (ASH) is a severe manifestation of alcoholic liver disease (ALD) characterized by steatosis and inflammation. The pivotal role of MAP kinases p38 γ/δ in liver steatosis and inflammation influences the progression of high-fat diet-induced non-alcoholic liver disease.

Methods: Male mice, both wild-type (WT) and p38 γ/δ knockout (KO), underwent a chronic and binge ethanol (EtOH) diet following the NIAAA model for liver damage induction. Histopathological analysis through oil red staining, qPCR, and western blot were employed to examine ethanol's molecular effects on the liver.

Results: Histopathology and gene expression revealed that EtOH-KO mice had reduced hepatic steatosis and lower inflammatory cytokine expression compared to EtOH-WT mice. Both EtOH-WT and EtOH-KO mice on the NIAAA diet experienced oxidative stress and mTOR pathway activation in the liver. EtOH-WT mice exhibited phosphorylation of p62 at Thr269 and Ser272, which was diminished in EtOH-KO mice.

Conclusion: The deletion of p38 γ/δ proves beneficial in mitigating ALD in mice by reducing inflammation and steatosis. An alcoholic diet increased oxidative stress and inhibited autophagic pathways, which was partially restored by p38 γ/δ knockout.

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Conflict of Interest: None declared

P05.033.A Molecular heterogeneity in idiopathic chronic and recurrent acute pancreatitis among highly consanguineous south Indian Tamils

Aruna Mannil[1], *Reena Gulati*[1], *Raja Kalayarasan*[2]

[1]*Jawaharlal Institute of Postgraduate Medical Education and Research (JIPMER), Pediatrics, Pondicherry, India;* [2]*Jawaharlal Institute of Postgraduate Medical Education and Research (JIPMER), Surgical Gastroenterology, Pondicherry, India*

Background/objective: Idiopathic chronic pancreatitis (ICP) is a debilitating disease characterized by irreversible damage to pancreas. ICP and recurrent acute pancreatitis (RAP) with early age onset are commonly associated with variants in multiple genes. We performed NGS to identify genetic variants in the highly consanguineous south Indian Tamil population.

Methods: We analyzed DNA extracted from peripheral blood of 27 patients with a diagnosis of ICP/RAP by NGS (Illumina).

Results: Clinically significant variants were identified in 25 of 27 patients (Table1).

Among them,11 (44%) have consanguineous parents (9 third degree and 2 second degree). Significant variants were identified in 7 genes associated with pancreatitis.

Conclusion: Percentage of consanguinity was more among patients compared to general Tamil population. The most common variant was c.101A>G in *SPINK1* gene. Two novel, two pathogenic and ten variants of unknown significance (VUS) predicted to be deleterious were identified. Proportion of CFTR variants identified were higher than in other populations.

Grants: Nil

Table 1: Variants identified

Age in year/ Gender	Variant	ACMG Classification	Gene
12/F	c.3205G>A	Likely pathogenic	*CFTR*
	c.1210-11T>G	VUS	
10/F	c.489+733C>A		
14/F	c.3047T>C		
29/M	c.508C>T		
23/F	c.4061T>C		
32/F	N1	Novel	
19/M	N2		
09/F	c.86A>T	Pathogenic	*PRSS1*
	c.40C>G	VUS	
15/M	c.86A>T	Pathogenic	
13/F	c.40C>G	VUS	
24/F	c.217G>A	Pathogenic	*CTRC*
	c.86A>T		*PRSS1*
14/M	c.101A>G	VUS	*SPINK1*
	c.47C>T	Likely pathogenic	*PRSS1*
24/M	c.86A>T	Pathogenic	*PRSS1*
30/M	c.47C>T	Likely pathogenic	
18/M	c.101A>G	VUS	*SPINK1*
16/M	c.101A>G		
16/F	c.101A>G		
14/M	c.101A>G		
18/M	c.101A>G		
39/M	c.101A>G		
	c.195-69_195-66dup		
	c.5637T>C		
10/F	c.101A>G		
	c.5637T>C		

Table h. continued

Age in year/ Gender	Variant	ACMG Classification	Gene
	c.195-69_195-66dup		
17/F	c.101A>G		
08/F	c.590G>A	Likely pathogenic	*LPL*
26/F	c.769G>A		*CTRC*
20/M	c.212G>A		*SLURP1*
	c.1778C>T	VUS	*ABCB4*

Conflict of Interest: None declared

P05.034.B Investigation of leukocyte telomere length and hTERT gene (MNS16A-VNTR) variant in obese adolescents

Aylin Yetim Şahin[1;2], *Yasemin Oyacı*[1;3], *Shahri Khudiyeva*[2], *Mustafa Pehlivan*[4], *Melike Tuğrul Aksakal*[2], *Sacide Pehlivan*[3]

[1]*Institute of Graduate Studies in Health Sciences, Istanbul University, Istanbul, Türkyie;* [2]*Istanbul Faculty of Medicine, Istanbul University, Department of pediatrics, Istanbul, Türkyie;* [3]*Istanbul Faculty of Medicine, Istanbul University, Department of Medical Biology, Istanbul, Türkyie;* [4]*Basaksehir Cam and Sakura City Hospital, Department of Hematology, Istanbul, Türkyie*

Background/Objectives: This study aims to investigate whether leukocyte telomere length (LTL) and human telomerase reverse transcriptase (*hTERT*) gene MNS16A-VNTR variant in obese adolescents differs from healthy, age/gender matched peers and if there is any association with clinical parameters.

Methods: The study included 151 participants, comprising 96 obese and 55 healthy adolescents. DNA isolated from peripheral blood of all individuals was used for LTL and *hTERT* gene analyses. Relative LTL was quantified by real-time PCR. LTL analysis was calculated as a fold change between patients and controls using the relative T/S ratio. The PCR method was used for the detection of *hTERT* gene VNTR variant.

Results: In the comparison of hTERT-MNS16A-VNTR between patients and healthy controls, statistically significant decrease was observed in the LL genotype ($p = 0.029$). In genotype comparisons of clinical parameters (LL/LS + SS genotypes), it was found that insulin, HOMA-IR and ALT levels significantly increased in LL genotyped patients (respectively, $p = 0.027$; $p = 0.033$; $p = 0.012$). Regarding LTL analysis, the T/S ratios of patients were marginally significantly decreased compared to controls ($p = 0.055$). In comparison of fold changes, 61% of patients had shorter telomeres than controls. Evaluation of T/S ratio median values with clinical parameters revealed no relationship between physical exercise, sleep, screen time, smoking exposure, breastfeeding duration.

Conclusion: In conclusion, it appears that LTL in obese Turkish adolescents is marginally shorter compared to healthy peers. The LL genotype of MNS16A-VNTR, acting as a regulator of hTERT promoter activity, suggests a potential association both in protecting against obesity with insulin resistance and hepatosteatosis.

Grants: This research received no specific grant.

Conflict of Interest: None declared

P05.035.C Silver-Russell like phenotype caused by variants in IGF1R and the imprinted IGF2 investigated with long read sequencing

Trine Maxel Juul[1], *Katja Venborg Pedersen*[2], *Emilie Lester*[1], *Caroline Hey Bækgaard*[1], *Martin Larsen*[1], *Susanne E. Boonen*[1], *Maria Kibaek*[3], *Niels Illum*[3], *Malene Heidemann*[3], *Signe Koch*[4], *Christina Fagerberg*[1;2]

[1]*Odense University Hospital, Dept. of Clinical Genetics, Odense, Denmark;* [2]*Sygehus Lillebælt, Vejle Hospital, Dept. of Clinical Genetics, Vejle, Denmark;* [3]*Odense University Hospital, H. C. Andersen Children's Hospital, Odense, Denmark;* [4]*The Hospital of Southern Jutland, Aabenraa Hospital, Dept. of Pediatrics, Aabenraa, Denmark*

Background/Objectives: The insulin-like growth factor system involves the insulin-like growth factors IGF1 and IGF2 that binds to the IGF1 receptor. Disturbances in this system are related to classic growth disorders including Silver-Russell syndrome.

The aim was to characterize the phenotype of the Silver-Russell like phenotype associated with *IGF1R* and the imprinted *IGF2* variants and describe the use of long read sequencing regarding parental allele origin of variants in imprinted genes.

Methods: Description of two individuals with a de novo variant in *IGF2*, and one family with an inherited variant in *IGF1R*. Long read sequencing was used to determine the phasing of the de novo variants.

Results: The two individuals with de novo missense variants in *IGF2* both had intrauterine and postnatal growth restriction. One of these, an adult female, had dysmorphic facial features and neurodevelopmental delay. In both cases, the de novo variant was showed to be located on the paternal allele.

In the IGF1R family, a paternal inherited missense variant in *IGF1R* was detected in three sisters, age from 10 months to 15 years old. All three had intrauterine and postnatal growth restriction, microcephaly and poor appetite.

Conclusion: We describe a phenotype of intrauterine and postnatal growth restriction in individuals affected by variants in *IGF2* and *IGF1R*. Phasing of the de novo variants in the imprinted *IGF2* can clarify which parental allele the de novo variant is located on. This information is of great importance to determine the significance of de novo variants in imprinted genes.

Conflict of Interest: None declared

P05.036.D PNPLA3-rs738409 and TM6SF2-rs58542926 missense variants are associated with laboratory parameters in obese adolescents

Shahri Khudiyeva[1], *Yasemin Oyacı*[2;3], *Aylin Yetim Şahin*[1;3], *firdevs baş*[1], *Sevde Sayın*[2;3], *İbrahim Kandemir*[4], *Edanur Karapınar*[5], *Sacide Pehlivan*[2]

[1]*Istanbul Faculty of Medicine, Istanbul University, Department of pediatrics, Istanbul, Türkyie;* [2]*Istanbul Faculty of Medicine, Istanbul University, Department of Medical Biology, Istanbul, Türkyie;* [3]*Institute of Graduate Studies in Health Sciences, Istanbul University;* [4]*Biruni University, Department of pediatrics, Istanbul, Türkyie;* [5]*Istanbul Faculty of Medicine, Istanbul University, Deparment of radiology, Istanbul, Türkyie*

Background/Objectives: This study aimed to examine the relationship between PNPLA3-rs738409 and TM6SF2-rs58542926 missense gene variants and NAFLD in obese adolescents.

Methods: We included 71 NAFLD(+), 30 NAFLD(-) obese and 58 healthy adolescents (HA) in this study. Hepatosteatosis status was examined by ultrasonography in obese adolescents. Genotyping analyzes of PNPLA3-rs738409 and TM6SF2-rs58542926 gene variants were performed using the PCR-RFLP method. The relationship between genotype and allele frequencies of gene variants between HA, obese NAFLD(-) and obese NAFLD(+) groups was examined. Bayesian Kendall's tau test was used for

independence hypotheses in the evaluation of laboratory findings and clinical variables.

Results: No statistically significant relationship was determined between the genotype and allele frequencies of PNPLA3-rs738409 and TM6SF2-rs58542926 gene variants among the three groups. In the NAFLD(-) group, AST levels of PNPLA3-rs738409 GC or GG genotype carriers were found to be 3.3 IU/L ($p = 0.046$) higher on average than CC genotype carriers. In the same group, BUN levels of TM6SF2-rs58542926 TC genotype carriers were found to be 2.9 mg/dL higher on average than CC genotype carriers ($p = 0.005$). In the NAFLD(+) group, the serum creatinine level of PNPLA3-rs738409 GC or GG genotype carriers was on average 1.3 mg/L lower than that of CC genotype carriers ($p = 0.021$).

Conclusion: A significant relationship was found between the genotype frequencies of PNPLA3 and TM6SF2 missense gene variants and laboratory parameters in adolescent obesity in the Turkish population.

Grants: This study was supported by the Scientific Research Project Coordination Unit of Istanbul University under Grant [number:TTU-2023-40010].

Conflict of Interest: None declared

P05.037.A Rare autosomal-recessive missense variants in *C2CD3* as a novel cause of non-syndromic nephronophthisis

Zachary Sentell[1], *Lina Mougharbel*[2], *Zachary Nurcombe*[1], *Sima Babayeva*[3], *Marc Henein*[4], *Lee Lee Chu*[2], *Murielle Akpa*[2], *Jean-Baptiste Rivière*[2], *Paul Goodyer*[5], *Elena Torban*[3], *Thomas Kitzler*[6]

[1]*McGill University, Department of Human Genetics;* [2]*The Research Institute of the McGill University Health Centre;* [3]*McGill University Health Centre, Department of Medicine;* [4]*McGill University, School of Medicine;* [5]*McGill University Health Centre, Department of Pediatrics;* [6]*McGill University Health Centre, Division of Medical Genetics*

Background/Objectives: Ciliopathies are heterogeneous genetic disorders predominantly caused by autosomal-recessive genetic variants that disrupt primary cilium function. Clinically, they manifest as a syndromic phenotypic spectrum, often involving kidney. Biallelic pathogenic variants in *C2CD3* disrupt ciliogenesis and cause a syndromic ciliopathy (Orofaciodigital syndrome XIV, OMIM #615948). We present evidence for compound heterozygous missense variants in *C2CD3* that result in an isolated renal-specific phenotype in a patient with non-syndromic nephronophthisis.

Methods: Exome sequencing identified biallelic *C2CD3* missense variants (p.Pro168Leu; p.Thr2079Met). Patient-derived fibroblasts and urinary renal epithelial cells (URECs), as well as human RPE-1 *C2CD3* knockout cell-lines were used for in vitro studies.

Results: Patient fibroblasts produced shorter cilia compared to a healthy sibling (2.309 vs. 2.850 µm, $P < 0.0001$), while URECs showed significantly shortened cilia (2.068 vs. 2.807 µm, $P < 0.0001$) and significantly reduced ciliation when compared to a healthy control. The latter was not observed in fibroblasts, suggesting a kidney-specific effect. Sonic hedgehog signaling was impaired in patient cells as expression of GLI3 activator protein and *GLI1* mRNA was reduced. Protein colocalization analysis demonstrated reduced C2CD3 localization to the basal body in patient URECs. Finally, rescue experiments in RPE1-*C2CD3*-KO cells corroborated the findings in patient-derived cells by demonstrating significantly reduced ability to restore ciliogenesis for each variant compared to wild-type.

Conclusion: Biallelic hypomorphic missense variants in *C2CD3* result in a novel non-syndromic nephronophthisis phenotype, as demonstrated by impaired ciliogenesis.

Grants: Fonds de recherche du Québec en Santé (FRQS) Salary Grant and the SickKids New Investigator Research Grant.

Conflict of Interest: None declared

P05.038.B Clinical features and genetic analysis of a case series of Primary Ciliary Dyskinesia: the importance of trio analysis.

Federico Rondot[1], *Amedeo Primerano*[2], *Giulia Vinci*[1], *Patrizia Dentelli*[1], *Barbara Pasini*[1;2]

[1]*University of Turin, Department of Medical Sciences, Turin, Italy;* [2]*AOU Città della Salute e della Scienza di Torino, Italy*

Background/Objectives: Primary ciliary dyskinesia (PCD) is a rare genetic disorder characterized by impaired ciliary function, leading to recurrent respiratory infections and various multi-organ manifestations. We present a case series of 8 subjects with a clinical diagnosis of PCD with variable clinical manifestations including situs viscerum inversus, perinatal respiratory distress and recurrent airways flogistic episodes. Subjects underwent high-speed video microscopy identifying abnormal ciliary motion patterns, that strengthed the clinical suspicion of PCD. To establish a definitive genetic diagnosis, trio exome sequencing was employed.

Methods: SureSelect-Agilent Custom-Constitutional-Panel-17Mb encompassing 5219 genes. Virtual panel "PCD" (41 genes).

Results: Definitive molecular diagnosis was achieved in 4 of 8 patients (DNAH5, RSPH9, CCDC40). Molecular diagnosis was not conclusive in 3 patients due to the presence of uncertain significance variants or heterozygous pathogenic variant (DNAH11, DNAH5, ODAD2, DNAI1, DNAH1). Finally, we report for the first time, at the best of our knowledge, a likely pathogenic ALU insertion predicted to disrupt the canonical donor site of DNAH5 exon 26.

Conclusion: This study highlights the significance of a trio approach in enhancing the diagnostic yield for PCD, providing valuable insights into the inheritance pattern, and facilitating genetic counseling for affected families. The case series enhances our understanding of PCD, emphasizing the need for integrated clinical and genetic approaches in diagnosis and management. Furthermore, the identification of an ALU insertion expands the genetic mechanisms of disease, serving as a crucial warning to carefully consider such mutations in ambiguous cases or those lacking molecular diagnoses.

Grants:

Conflict of Interest: None declared

P05.039.C The role of nonsense variants of RET in the mechanisms of pathogenicity of Hirschsprung's disease: the first Italian family carrying the c.208C>T p.(Gln70Ter) variant

Ludovica Fortuna[1;2], *Eugenia Tulli*[1;2], *Claudio Ricciardi Tenore*[1;2], *Maria Elisabetta Onori*[1;2], *Alessia Perrucci*[1;2], *Elisa De Paolis*[1;2], *Maria De Bonis*[1;2], *Daniela Mazzà*[3], *Anna Saramin*[4], *Angelo Minucci*[1;2], *Martina Rinelli*[1;2]

[1]*Fondazione Policlinico Universitario A. Gemelli IRCCS, Departmental Unit of Molecular and Genomic Diagnostics, Roma;* [2]*Genomics Research Core Facility, Gemelli Science and Technology Park (GSTeP), IRCCS Fondazione Policlinico Universitario Agostino Gemelli, Roma;* [3]*Institute for Maternal and Child Health, IRCCS Materno Infantile "Burlo Garofolo", Trieste;* [4]*University of Trieste, Department of Medicine, Surgery and Health Sciences, Triese*

Background. Hirschsprung's disease (HSCR) is a dominant condition, occurring in approximately 2-2.8 in 10.000 newborns. It is a classical model of enteric neuropathy characterized by bowel obstruction and absence of enteric ganglia in distal colon.

Pathogenic variants in *RET*, *NRG1*, and *L1CAM* genes have been found in most cases of HSCR. *RET* proto-oncogene encodes a receptor tyrosine kinase for members of glial cell line-derived neurotrophic factor (GDNF) family. *RET* activating point mutations are responsible for multiple endocrine neoplasia type 2A whereas, *RET*-inactivating point mutations or deletions, lead to development of HSCR. We report a family of isolated HSCR associated with a nonsense variant in the *RET* gene.

Materials and methods. A 30 years-old female subject and her son were both affected by isolated HSCR, which required the surgical partial colon resection.

Results. Clinical Exome analysis and Sanger sequencing performed in our two subjects allowed to identify a nonsense *RET* variant, *c.208C>T* p.(Gln70Ter), in both. The truncating variant occurs in exon 2 and causes the loss of the entire RET protein.

Conclusions. *RET* mutations have been found in up to 5% of sporadic HSCR and up to 50% of familial cases. Mutations in *RET* extracellular domain impair its cell surface expression, most likely due to misfolding of protein. Nonsense changes result in deleterious form of truncated proteins, depending on position of variants.

Our findings suggest a pathogenic effect of this nonsense variant according to others loss of function changes in *RET*. This mutation was previously reported only in just one other study about 601 Chinese patients.

Conflict of Interest: None declared

P05.040.D Novel biallelic missense variants in LSR supporting cause of pediatric cholestasis

Carolin Meier[1], *Maria Eugenia Rocha*[1], *Javier Martini*[1], *Maryam Najafi*[1], *Omar Bawazir*[1], *Mariana Ferreira*[1], *Emir Zonic*[1], *Uros Hladnik*[1], *paskal cullufi*[2], *Rabab al-Qaysi*[3], *Huma Cheema*[4], *Omid Paknia*[1], *Jorge Pinto Basto*[1], *Aida Bertoli-Avella*[1], *Kornelia Tripolszki*[1], *Peter Bauer*[1]

[1]*CENTOGENE GmbH, Rostock, Germany;* [2]*University Hospital Center "Mother Teresa", Tirana, Albania;* [3]*Children Welfare Teaching Hospital, Baghdad, Iraq;* [4]*University of Child Health Sciences, Lahore, Pakistan*

Background/Objectives: The role of the Lipolysis-stimulated lipoprotein receptor (LSR) gene in human disease has not yet been fully established. However, it has been associated with pediatric cholestasis caused by biallelic missense variants based on two patients. Familial intrahepatic cholestasis is an autosomal recessive heterogenous disorder characterized by disruption of bile formation leading to hepatocellular cholestasis with infantile onset. The LSR protein is presumed to be involved in clearing of triglyceride-rich lipoprotein from blood. Here, we report six unrelated patients presenting with early-onset cholestasis. Four of them were known to have parental consanguinity.

Methods: To identify causative variants, exome/genome sequencing was performed in two female and four male patients clinically presenting with childhood-onset cholestasis, jaundice and/or conjugated hyperbilirubinemia. Analysis of the data and our Biodatabank was performed. Gene-disease relationship was evaluated according to ClinGen guidelines.

Results: Our analysis revealed homozygous missense variants in the *LSR* gene in all six individuals. No patient had a positive finding in an alternative diagnostic gene explaining the cholestasis, therefore the variants in *LSR* remain the only explanation. In two patients the variant NM_205834.3:c.658G>A p.Val220Met was identified, whereas the other patients presented with the variants NM_205834.3:c.715C>T p.Leu239Phe, NM_205834.3:c.889T>C p.Cys297Arg, NM_205834.3:c.832G>C p.Gly278Arg and NM_205834.3:c.511C>T p.Arg171Cys. To our knowledge, all variants were novel and have not been reported in the literature to date.

Conclusion: Although gene-disease relationship for this gene remains limited, our findings further contribute to the association of *LSR* with a cholestasis phenotype and support *LSR* as potential diagnostic gene. Furthermore, it emphasizes the genetic heterogeneity of familial intrahepatic cholestasis.

Grants:

Conflict of Interest: Carolin Meier Centogene GmbH, Maria Eugenia Rocha Centogene GmbH, Javier Martini Centogene GmbH, Maryam Najafi Centogene GmbH, Omar Bawazir Centogene GmbH, Mariana Ferreira Centogene GmbH, Emir Zonic Centogene GmbH, Uros Hladnik Centogene GmbH, paskal cullufi: None declared, Rabab al-Qaysi: None declared, Huma Cheema: None declared, Omid Paknia Centogene GmbH, Jorge Pinto Basto Centogene GmbH, Aida Bertoli-Avella Centogene GmbH, Kornelia Tripolszki Centogene GmbH, Peter Bauer Centogene GmbH, Centogene GmbH

P05.041.A The Clinical and Molecular Spectrum of Bardet-Biedl Syndrome: 18 Cases

Turkan Turkut Tan[1], *Yusuf Dogan*[1], *Enise AVCI DURMUŞALİOGLU*[1], *Durdugül Ayyıldız Emecen*[1], *Esra Isik*[1], *Ozgur Cogulu*[1], *Tahir Atik*[1]

[1]*Ege University Faculty of Medicine, Department of Pediatric Genetic Diseases, İzmir, Türkyie*

Background / Objective:

Bardet-Biedl syndrome (BBS) is a rare, multisystemic autosomal recessive disorder caused by primary cilia dysfunction. It is a clinically and genetically heterogeneous group of diseases and commonly seen in populations where consanguineous marriages are prevalent. This study aims to evaluate the clinical and molecular features of Bardet-Biedl syndrome.

Method:

Clinical and molecular findings of 18 Bardet-Biedl syndrome cases followed at the Department of Pediatric Genetic Diseases, Ege University, were retrospectively reviewed.

Results: Of the cases diagnosed with BBS, 8 are female and 10 are male. Obesity was observed in 17/18 cases, retinal dystrophy in 8/18, polydactyly/brachydactyly in 10/18, renal anomalies in 4/18, learning difficulties in 18/18, and hypogonadism in 6/18. In 15 patients, 10 different single nucleotide variants/small indels in 5 genes (*BBS1, BBS2, BBS4, BBS9, BBS10*) are detected using NGS. One of these variants (*BBS1*:c.508C>T) has not been previously reported in the literature. In remaining 3 patients, NGS-based CNV analysis revealed one has a homozygous deletion in exons 18-19 of the *BBS9* gene, one in exon 16 of the *BBS9* gene, and one in exons 3-4 of the *BBS4* gene. According to the ACMG 2015 criteria, all the variants are classified as pathogenic.

Conclusion: Clinical and genetic diversity of BBS significantly complicates the diagnosis process. The use of next-generation sequencing (NGS) methods, along with NGS-based CNV analysis, has increased the rate of diagnosis. Identifying the underlying genetic cause allows for effective genetic counseling and provides predictions about the prognosis of the disease.

Conflict of Interest: None declared

P05.044.D A novel missense variant in the imprinted *IGF2* locus causes monogenic short stature in two German families

Malin Dewenter[1], *Annemarie Jonasson*[1], *Stephanie Schwarz*[2], *Barbara Oekl-Jaschkowitz*[2], *Tilman Rohrer*[3], *Dorna Hogeabri*[3], *Gerhard Binder*[4], *Hanno Bolz*[1]

[1]*Bioscientia Institute for Medical Diagnostics GmbH, Human Genetics, Ingelheim, Germany;* [2]*Bioscientia, Practice of Human Genetics, Homburg, Saar, Germany;* [3]*Saarland University Medical*

Center, University Children's Hospital, Homburg, Germany; [4]University Children's Hospital, Pediatric Endocrinology and Hormone Laboratory, Tübingen, Germany

Background/Objectives: The chromosome region 11p15 contains numerous imprinted genes, including *IGF2*. Paternally inherited heterozygous pathogenic variants in *IGF2* (MIM *147470) are associated with familial Silver-Russell syndrome (MIM #616489). Here, we report on two presumably unrelated families with multiple cases of smallness for gestational age (SGA), short stature and feeding difficulties. The index patients from both families were 5 years and 1 year old, respectively.

Methods: We performed whole-exome sequencing (WES), segregation and biochemical analyses.

Results: WES revealed the previously unreported, very rare heterozygous *IGF2* variant c.172C>T p.(Arg58Cys) in both patients and their fathers. Results from extensive segregation analysis were in line with an imprinted inheritance mode in both families. In family 1, two paternal half-brothers showed a comparable phenotype. In family 2, the index patient's father was similarly affected. All affected individuals carried the heterozygous *IGF2* variant on their paternal allele. In all available samples from unaffected carriers, the variant was present on the maternal allele. In Family 2, further investigations in the index patient showed reduced IGF2 levels and an IGF2/IGF1 ratio consistent with IGF2 deficiency.

Conclusion: Our extensive segregation and cross-family analysis together with biochemical data allowed for classification of the variant as pathogenic. Further analyses are planned to verify the assumed founder nature of the variant and to investigate the methylation pattern in this imprinted region.

Conflict of Interest: None declared

P05.045.A The importance of screening treatable congenital diseases in the context of three congenital sodium diarrhea cases

Ezgi Çevik[1], *Taha Bahsi*[1], *Zeynep Özdemir*[1], *İzzet Özgürlük*[2], *Haktan Bağış Erdem*[1]

[1]*Dr. Abdurrahman Yurtaslan Ankara Onkoloji Eğitim ve Araştırma Hastanesi, Medical Genetics, Ankara, Türkyie; [2]Ankara Bilkent Şehir Hastanesi, Obstetrics and Gynecology, Ankara, Türkyie*

Background/Objectives: Congenital sodium diarrhea (CSD) is a rare, genetically and clinically assorted enteropathy that comprises two forms. Recessive *SPINT2* mutations lead to the syndromic form, however, the non-syndromic form may occur due to dominant mutations in *GUCY2C* or biallelic mutations in *SLC9A3* which is liable for normal functioning of the Na + /H+ antiporter 3 of intestinal brush border. Common clinical symptoms are intractable watery diarrhea of intrauterine onset with high fecal sodium loss, dilation of bowel loops, growth retardation, and a history of polyhydramnios.

Methods: We present two consanguineous families with three affected children. Two of the children were sisters. All three patients had a history of polyhydramnios, watery diarrhea, and growth retardation. Apart from these, one of the sisters had neuropsychomotor developmental delay. Additionally, the other sister and the male patient had a history of surgery due to ileus. All three had abnormal intestinal histopathological findings. The male patient's growth is normal without supplementation although both of the sisters were deceased. WES analysis was performed on the male patient and the parents of the siblings.

Results: A novel homozygous mutation of c.1446+1G>C in *SLC9A3*(NM_004174.4) gene was identified as the likely cause of the diarrhea.

Conclusion: We identified three patients with a novel variant in the *SLC9A3* gene. Considering the variable expressivity of the disease, diagnostic process is challenging. Nevertheless, CSD is a treatable congenital disease, since good evolution is possible with immediate and adequate fluid-electrolyte supplementation. Therefore, we emphasize the importance of newborn genetic screening for treatable congenital diseases.

Conflict of Interest: None declared

P05.046.B Shorter telomeres in individuals with COPD exposed to biomass-burning smoke.

Angélica Domínguez-de-Barros[1], *Malena Gajate-Arenas*[1], *Gloria Pérez-Rubio*[2], *Ingrid Fricke-Galindo*[2], *Alejandra Ramírez Venegas*[3], *Rafael Hernández-Zenteno*[3], *Salvador García-Carmona*[2], *Robinson Robles-Hernández*[3], *Ramces Falfán Valencia*[2], *Elizabeth Córdoba-Lanús*[4]

[1]*Instituto Universitario de Enfermedades Tropicales y Salud Pública de Canarias (IUETSPC). Universidad de La Laguna, La Laguna, Tenerife, Spain., San Cristóbal de La Laguna, Spain; [2]Instituto Nacional de Enfermedades Respiratorias, Ismael Cosío Villegas (INER) - Ciudad de México (Mexico)., HLA Laboratory, Ciudad de México, Mexico; [3]Departamento de Investigación en Tabaquismo y EPOC, Instituto Nacional de Enfermedades Respiratorias Ismael Cosio Villegas, - Ciudad de México (Mexico)., Ciudad de México, Mexico; [4]Consorcio Centro de Investigación Biomédica en Red (CIBER) de Enfermedades Infecciosas CIBERINFEC, Instituto de Salud Carlos III, Madrid, Spain., Madrid, Spain*

Background/Objectives: Tobacco smoke is the leading cause of chronic obstructive pulmonary disease (COPD), but biomass-burning smoke (BBS) exposure is a significant risk factor, especially in low-income countries. COPD has been described as a disease of accelerated aging, induced cellular oxidative stress and inflammation, and telomere length as an aging marker. The clinical consequences of telomere shortening and cellular senescence in COPD caused by biomass exposure (BE) have not been explored.

Methods: 93 COPD cases secondary to BBS (BE-COPD) and 96 individuals exposed to BBS daily as controls were selected for the study. A subset of 41 individuals from each group were matched by age and hours/day of BBS exposure. Leucocyte relative telomere length (rTL) was determined by qPCR. Pulmonary function variables were registered.

Results: TL inversely correlated with age ($r = -0.182$, $p = 0.016$) and biomass exposure ($r = -0.301$, $p = 0.003$). The matched individuals' subsample showed BE-COPD cases to had shorter telomeres than controls (mean rTL 0.73 ± 0.43 vs. 1.38 ± 0.47; $p < 0.001$). This was confirmed in the whole cohort (OR = 0.134, CI 95% = 0.053-0.339; $p < 0.001$), adjusting by age and hours/day biomass exposure. A borderline association was found between shorter telomeres and impaired pulmonary function, as noted for FEV_1 and FVC% ($p < 0.05$ respectively) in matched BE-COPD subjects.

Conclusion: BE-COPD cases present shorter telomeres than controls, independently of age and biomass-burning smoke exposure. The preliminary association found between lung impairment (decreased FEV_1 and FVC%) and a shorter rTL in BE-COPD needs to be assessed in a large cohort to confirm these findings.

Grants: CIBERINFEC-CB21/13/00100

Conflict of Interest: None declared

P05.047.C The importance of genetic testing in adult-onset kidney diseases – Genetic Nephrology clinic overview

Galit Lazer Derbeko[1], *Omri Weiss*[1], *gheona Altarescu*[1;2]

[1]*Shaare Zedek Medical Center, Jerusalem, Israel;* [2]*Faculty of Medicine, The Hebrew University of Jerusalem, Jerusalem, Israel*

Background: Kidney disease has reached near pandemic proportions with as many as 1:7 affected in certain populations. Individuals with kidney-related genetic disorders are at a high risk of developing end-stage renal failure, raising the need for genetic clinics specializing in nephrology.

Methods:Summarizing diagnostic yields and demographic data of our adult Genetic Nephrology Clinic.

Results: In 12 months (12.2022-01.2024), a total of 62 patients visited our Genetic Nephrology clinic due to suspected genetic kidney diseases. 40 individuals (64%), from 32 families, underwent genetic testing. A gene panel was done for all 32 probands, with an average age of 42 years during testing. 8 (25%) Pathogenic, 13 (40.6%) variants of uncertain significance (VUS), and 11 (34.4%) negative results were obtained. We report an overall diagnostic yield of 25%, found at the low end of the previously reported findings range (20%-78%). We are also reporting a high ratio of VUS results (40.6%), highlighting an issue with gene panel reporting criteria. In addition, we have observed a high disparity in testing of females (22:66%) vs. male (10:34%), even though the acceptance rate at the clinic was 29 (54%) females vs 25 (46%) males.

Conclusion: A genetic diagnosis of adult genetic kidney diseases often involves treatments, follow-up monitoring, genetic counseling regarding reproductive opportunities, family member testing and in some cases, kidney transplantation. Early diagnosis and intervention are crucial for effectively managing these conditions and improving the quality of life for affected individuals. Our results show the importance of integration of genetic clinics in adult nephrology diagnosis.

Conflict of Interest: None declared

P05.048.D Common endocrine disorders- does genetic work-up influence clinical decisions? A joint perspective of geneticists and endocrinologists

Noa Shefer Averbuch[1;2;3], *Michal Levy*[3;4], *lina basel-salmon*[1;3], *Naama Orenstein*[1;3]

[1]*Rabin Medical Center, Beilinson campus, Recanati Genetic Institute, Israel;* [2]*schneider children's medical center of israel, The Jesse Z and Sara Lea Shafer Institute for Endocrinology and Diabetes, National Center for Childhood Diabetes, Israel;* [3]*Tel Aviv University, The Faculty of Medicine, Israel;* [4]*Rabin Medical Center, Beilinson campus, Recanati Genetic Institute, Ramat Hasharon*

Objective: This study aims to evaluate the impact of genetic testing on clinical decision-making in prevalent endocrine disorders.

Methods: Impact of 500 genetic consultations for diverse endocrinopathies performed between January 2018 to January 2024 was assessed, with a focus on short stature and diabetes, the two most common disorders referred for evaluation. The impact of genetic results on clinical decisions was assessed through two criteria: alterations in pharmaceutical treatment and modifications to the follow-up protocol that would not have been initiated otherwise.

Results: Out of 94 diabetes patients, 65 (70%) underwent genetic work-up, resulting in a significant clinical impact for 34 patients (52%). Notably, 8 patients (24%) experienced changes in pharmaceutical treatment, and in 26 (76%) modifications were made in medical follow-up. In the short stature group (118 patients), 77 (65%) completed genetic work-up, leading to substantial clinical impact for 30 patients (40%). This included medical treatment changes for 21 patients (27%) and alterations in medical follow-up for 56 patients (76%).

Discussion: Attaining a definitive diagnosis holds significant importance, impacting numerous family members and family planning decisions. While it may not always directly influence treatment, particularly in monogenic disorders where genetic treatments are limited, comprehending the molecular basis of the disorder, especially in endocrine disorders, frequently plays a pivotal role in determining the optimal medical therapy, choice of medications, and follow-up protocols.

Conflict of Interest: None declared

P05.049.A Unraveling the Genetic Tapestry of Obesity: Insights from a Diverse Patient Cohort

Ehud Banne[1], *tal Ben Ari*[1], *Marina Michelson*[1], *Dikla Pivko Levy*[1]

[1]*Wolfson Medical Center, Affiliated with the Tel Aviv University*

Background/Objectives: Obesity is a pervasive global health issue, especially prevalent among younger demographics and with multifaceted etiology. This study aimed to investigate the clinical and genetic factors associated with obesity in a diverse cohort of pediatric and adult patients.

Methods: Data from 10 patients aged 8-32 years were analyzed, focusing on variables such as BMI, hyperphagia presence, genetic variants, birth weight, and parental obesity association. Genetic analysis assessed variants in genes linked to obesity and other biological functions.

Results: Among pediatric patients, the mean BMI was 41.6 kg/m^2, surpassing the 99th percentile threshold, indicative of severe obesity. Hyperphagia was noted in 80% of patients, hinting at genetic predisposition. Genetic analysis revealed 0-4 variants per patient, with 50% harboring at least one obesity-related variant. Birth weight varied, mostly within appropriate gestational age, while parental obesity correlated significantly with pediatric obesity.

Conclusion: Identified variants spanned genes involved in diverse functions, including Bardet-Biedl syndrome and neurodevelopmental pathways. Most variants were classified as variants of uncertain significance (VUS), highlighting challenges in interpretation. The study underscores the need for discerning between pathogenic and benign variants, emphasizing personalized management approaches guided by genetic profiles. Integrating genetic testing into clinical practice may enable targeted interventions, while further exploration of genotype-phenotype correlations is vital for advancing our understanding and refining precision medicine strategies in obesity management.

This Study was aided by the RHYTHM company sponsored genetic testing. Genetic testing was conducted in CGC Genetics.

Conflict of Interest: None declared

P05.050.B Identification of genetic etiology in 130 patients with congenital hypopituitarism

Zehra Yavaş Abalı[1;2;3], *Guven Toksoy*[3], *Firdevs Bas*[4], *Tulay Guran*[2], *Ayse Pinar Ozturk*[4], *Volkan Karaman*[3], *Ayça Dilruba Aslanger*[3], *Sukran Poyrazoglu*[4], *Serap Turan*[2], *Abdullah Bereket*[2], *Feyza Darendeliler*[4], *Zehra Oya Uyguner*[3]

[1]*Istanbul University Institute of Health Sciences, Department of Genetics, Istanbul, Türkyie;* [2]*Marmara University School of Medicine, Department of Pediatric Endocrinology and Diabetes, Istanbul, Türkyie;* [3]*Istanbul University Istanbul Faculty of Medicine, Department of Medical Genetics, Istanbul, Türkyie;* [4]*Istanbul University*

Istanbul Faculty of Medicine, Department of Pediatric Endocrinology, Istanbul, Türkyie

Background Congenital hypopituitarism (CH) results from gene variants causing a deficiency in pituitary hormones. Identifying the genetic etiology is essential in predicting the degree of pituitary dysfunction and extra-pituitary features. We aimed to uncover the genetic causes in a large CH group using diverse techniques and an algorithmic approach.

Methods A targeted gene panel was used in 130 individuals (47 female) with CH. Multiplex ligation-dependent probe amplification (MLPA) was used in 67 cases, and exome sequencing (ES) was conducted for 22 of them with 16 indexes (including 2 trios and 2 paired) evaluated. Segregation analyses were performed.

Results The median age during molecular studies was 19 years (2-35 years), with a 35.6% consanguinity rate. Pathogenic/likely pathogenic variants/deletions in CH-associated genes were found in 19.6% of the cohort [*PROP1* = 4, *WDR11* = 3, *GH1* = 3, *IGSF1* = 2, *HESX1* = 2, *LHX3* = 2, *POU1F1* = 2, *PROKR2* = 2, *GHRHR* = 3, *GHSR* = 1, and *HHIP* = 1)]. ES revealed pathogenic variants in the *GLI2* (n = 2), and *RNPC3* genes (n = 2). Variants of uncertain significance (VUS) were found in *SMO* (n = 1), *ROBO1*(n = 1), *MFHAS1*(n = 1), and *SMCHD1*(n = 1) genes associated with CH. The synonymous variant in the *MFHAS1* gene is predicted to impact splicing with ongoing functional analyses. Further investigations on candidate genes are underway in two cases.

Conclusion The definitive molecular etiology could be determined in 24% of cases through various molecular techniques in a large cohort of CH. This implies that unknown molecular pathways or factors such as epigenetic and environmental influences may contribute to the etiology of CH.

Grants: Scientific Research Projects Coordination Unit of Istanbul University (TDK-2022-38742).

Conflict of Interest: None declared

P05.051.C Syndromic subtypes of monogenic diabetes account for a third of all cases diagnosed in a UK diagnostic laboratory

Kevin Colclough[1], *Kashyap Patel*[2]

[1]*Royal Devon and Exeter NHS Foundation Trust, Exeter Genomics Laboratory, Exeter, United Kingdom;* [2]*University of Exeter Medical School, Institute of Biomedical and Clinical Science, Exeter, United Kingdom*

Background/Objectives: The Exeter Genomics Laboratory provides a diagnostic service for monogenic diabetes using a targeted next generation sequencing (tNGS) assay that sequences both non-syndromic (MODY) and syndromic monogenic diabetes genes. We assessed the diagnostic utility of this non-selective sequencing approach.

Methods: We retrospectively collated data on 1811 children and young adults with a clinical suspicion of monogenic diabetes. All probands were tested using a tNGS assay of 9 MODY and 18 syndromic diabetes genes from January 2020 to November 2023. We determined the diagnostic yield of this assay and the proportion of patients diagnosed with a syndromic form of monogenic diabetes.

Results: A diagnosis of monogenic diabetes was made in 326/1811 individuals (18%). 103 individuals had a pathogenic variant in a syndromic diabetes gene, giving a diagnostic yield of 6% and accounting for 32% of all monogenic diabetes cases. A total of 13 different syndromic subtypes were diagnosed. The pathogenic variant *MT-TL1* m.3243A>G and pathogenic variants in *HNF1B* and *WFS1* accounted for 78% of all syndromic diabetes cases, with *MT-TL1* m.3243A>G being the 3rd most common cause of monogenic diabetes after *GCK* and *HNF1A*. Only 14% of individuals with a variant in a syndromic gene had a clinical suspicion of the disorder. Variable clinical penetrance, diabetes as the first presenting feature or lack of supporting information from the clinician accounted for these unexpected diagnoses.

Conclusion: Sequencing of both MODY and syndromic monogenic diabetes genes maximises diagnostic yield and overcomes the limitation of selective gene testing based on clinical features.

Conflict of Interest: None declared

P05.052.D Gene expression dynamics of immune cells and its genetic determinants in primary sclerosing cholangitis

Chiara Caime[1], *Pietro Demela*[2], *Rosanna Asselta*[3], *Pietro Invernizzi*[1], *Alessio Gerussi*[1], *Blagoje Soskic*[2]

[1]*University of Milano-Bicocca, Medicine and Surgery Department, Monza, Italy;* [2]*Human Technopole, Population and Medical Genomics Department, Milan, Italy;* [3]*Humanitas University, Biomedical Sciences Department, Pieve Emanuele (Milan), Italy*

Background/Objectives: Primary Sclerosing Cholangitis (PSC) is a rare autoimmune cholangiopathy with polygenic influences and unknown etiology, and the role of T cells in its pathogenesis remains unclear. Elucidating relevant T cell states in which disease-associated variants alter gene expression is critical for testing new drug targets and identifying biomarkers. Here, we aimed to systematically identify T cell states that are enriched for PSC-related susceptibility variants.

Methods: Single-cell (sc)RNA-seq data on intrahepatic T cells from 11 patients with PSC (cirrhotic = 9, non-cirrhotic = 2) were downloaded from the EBI ArrayExpress database (E-MAT-10143). To identify possible causative cell types shared across diseases, summary statistics (GWAS Catalog) were downloaded and integrated with scRNA-seq data using CELLECT-MAGMA. Metabolic-associated Steatotic Liver Disease (MASLD), primary biliary cholangitis (PBC), systemic lupus erythematosus (SLE), celiac disease (CD), multiple sclerosis (MS), rheumatoid arthritis and heart attack were used as control diseases.

Results: We quantified the association between cell-type expression specificity and heritability in PSC, revealing that Th1 cells and Tem/Trm cytotoxic cells are prioritized among all cell types (p-value = 0.007 and p-value = 0.002). In contrast, none of the subpopulations studied were implicated in the pathogenesis of MASLD and heart attack. The Th1 subpopulation is shared with PBC, SLE and CD (p-value = 0.048, p-value = 0.006, p-value = 0.011). The Tem/Trm subpopulation is shared with SLE, CD and MS (p-value = 8.56*10-6, p-value = 4.67*10-6, p-value = 0.001).

Conclusion: By analyzing T cell gene expression and genetic data in PSC, our study identifies Th1 and Tem/Trm cytotoxic cells as key players in its pathogenesis, linking PSC with broader autoimmune disease mechanisms.

Conflict of Interest: Chiara Caime: None declared, Pietro Demela: None declared, Rosanna Asselta: None declared, Pietro Invernizzi IPSEN, IPSEN, CALLIDITAS, ZYDUS, ADVANZ, Alessio Gerussi PRIN PNRR 2022, ADVANZ, IPSEN, CAMP4 Therapeutics, Blagoje Soskic: None declared

P05.053.A Renal and extrarenal disease progress in individuals with Alport syndrome and the "hypomorphic" variant p.Gly624Asp in *COL4A5*

Anna Neuhaus[1], *Annika Jens*[2], *Bastian Malte Krüger*[3], *Korbinian M. Riedhammer*[1;4], *Jasmina Comic*[1], *Julia Hoefele*[1], *Jan Halbritter*[2]

[1]*Klinikum rechts der Isar, Technical University of Munich, TUM School of Medicine and Health, Institute of Human Genetics, Munich, Germany;* [2]*Charité Universitätsmedizin Berlin, Department of Nephrology and Medical Intensive Care, Berlin, Germany;* [3]*University of Leipzig Medical Center, Division of Nephrology, Leipzig, Germany;* [4]*Klinikum rechts der Isar, Technical University of Munich, TUM School of Medicine and Health, Department of Nephrology, Munich, Germany*

Background/Objectives: Alport syndrome (AS) is a hereditary nephropathy characterized by microscopic haematuria and proteinuria leading to kidney failure (KF). Around 1/3 of individuals exhibit extrarenal symptoms such as hearing impairment. AS is predominantly linked to X-linked inheritance (disease-causing variants in *COL4A5)*. The variant p.Gly624Asp in *COL4A5* is most frequent in Eastern and Central Europe and was shown to be associated with a milder renal phenotype. The aim of this study was to collect clinical data of individuals with this variant and investigate their disease progression and extrarenal manifestation.

Methods: The cohorts comprised individuals from university hospitals in Munich, Berlin, and Leipzig. Data were collected from medical reports, and through direct communication with the treating physician and/or the patients.

Results: The total cohort comprised 93 individuals, 53 women and 40 men (mean age: 39 years). The control cohort consisted of 88 age- and sex-matched individuals (51 women and 37 men) with non-p.Gly624Asp-glycine missense variants in *COL4A5* (mean age: 37 years). A significant difference concerning the age of onset resp. proteinuria and in the reduction of glomerular filtration rate throughout the lifespan was observed among patients. There were no significant differences in the initial appearance of haematuria, the age of onset and the development of extrarenal symptoms, and the average age at first manifestation.

Conclusion: Although *COL4A5*-p.Gly624Asp is known to be a "hypomorphic" variant, the present data shows that affected individuals still have a substantial risk for the development of KF and extrarenal manifestations.

Grants: not applicable

Conflict of Interest: None declared

P05.055.C Partial duplication of HNF4A related to MODY1: molecular characterization and genotype-phenotype correlation

Marina Viñas-Jornet[1], *Carmen Asensio Ortega*[2], *Josep Oriola*[3;4], *Laura Piñol*[5], *Alba Cebollero Agusti*[6]

[1]*CLILAB Diagnostics, Genetics Lab;* [2]*Complex Hospitalari Moisès Broggi - Consorci Sanitari Integral, Endocrinology and Nutrition Service;* [3]*CDB. Hospital Clínic. Barcelona, Biochemical and Molecular Genetics Service;* [4]*University of Barcelona, Department of Biomedicine. Faculty of Medicine;* [5]*CDB. Hospital Clínic. Barcelona, Biochemical and Molecular Genetics Service;* [6]*CLILAB Diagnostics*

Background/Objectives: Maturity-onset diabetes of the young (MODY) is a group of monogenic autosomal dominant forms of diabetes mellitus, characterized by early onset (usually <25 years of age) and no autoantibodies. At least, 14 genes have been described related to this condition. One of them is *HNF4A*, specifically its haploinsufficiency, which is associated with MODY1. We report the case of a 20-year-old patient and her mother, both with clinical suspicion of MODY.

Methods: The proband DNA sample was processed using a custom NGS panel, a commercial MLPA (MRC Holland P241) and PCR followed by Sanger sequencing. Parental samples were analysed with MLPA.

Results: No pathogenic nor likely pathogenic point variants were detected by NGS sequencing. ExomeDepth copy number variation (CNV) suggested the presence of a partial duplication in tandem of exons 6 and 7 in the *HNF4A* gene. MLPA confirmed this duplication. Sequencing of an amplicon obtained using a forward primer located at 3′ of intron 6 and a reverse primer at 5′ of intron 6 confirmed it was in tandem and predicted the creation of a premature stop codon p.(Asp276GlyfsTer32). The study of her mother by MLPA confirmed she was also a carrier of this duplication. Proband and her mother had been previously diagnosed with idiopathic diabetes mellitus at the ages of 13 and 15 respectively.

Conclusion: We have characterized a new partial in tandem duplication of the gene *HNF4A* predicting a frameshift change in the protein. This duplication correlates with the MODY1 phenotype in the proband and her mother.

Grants:

Conflict of Interest: None declared

P05.056.D Molecular Diagnosis of Intrahepatic Cholestasis of unknown origin in pediatric patients

Francesco Giambuzzi[1], *Anna Monica Rosaria Bianco*[1], *Ilaria Ziccardi*[2], *Mariateresa Di Stazio*[1], *Cecilia Del Vecchio*[2], *Adamo Pio D'Adamo*[1;2]

[1]*Laboratory of Medical Genetics, Institute for Maternal and Child Health - IRCCS "Burlo Garofolo", Trieste, Italy;* [2]*Department of Medical, Surgical and Health Sciences, University of Trieste, Trieste, Italy*

Background/Objectives: Progressive Familiar Intrahepatic Cholestasis (PFIC) represents an heterogenous group of recessive diseases caused by mutations in genes encoding for hepatocanalicular acid transporters and phospholipids. At least 45% of pediatric cholestasis disorders have a genetic cause and 20% of liver transplants are performed for monogenic forms in which genetic characterization has important implications. Most of the genes responsible for PFIC are still unknown. Genes identification in PFIC patients may contribute to define cholestatic phenotype.

Methods: 37 patients, with diagnosis of intrahepatic cholestasis of unknown origin, with the aim to identify causative variants by whole exome sequencing (WES), were enrolled. Based on the results obtained, identified variants have been studied by means of functional tests such as immunofluorescence in liver biopsy.

Results: We found mutations in PFIC genes as ATP8B1 and ABCB4 as well as in MYO5B, PLEC, CFTR, ATP7B, NEK8, PHKD1, ATP6AP1 never described before as PFIC genes. We found heterozygous mutations in genes encoding proteins of the hepatocellular transport system, as well as in the cytoskeleton and liver regulatory proteins such as PLEC, which can cause cholestatic diseases with great variability and usually a less severe phenotype.

In patient's liver with PLEC mutations the immunofluorescence assay showed an impaired BSEP targeting and aberrant plectin distribution.

Conclusion: We identified genotype-phenotype correlation among mutations in PFIC genes as well as in genes not still associated with PFIC, which underscore the prognostic value of early genetic diagnosis and disease course could be favorably with positive effects on treatment choice and genetic counseling.

Grants: RC 26/17 IRCCS "Burlo Garofolo"

Conflict of Interest: None declared

P05.057.A Two rare cases of Cholestasis with novel heterozygous pathogenic *PLEC* variants.

Ilaria Ziccardi[1], *Anna Monica Rosaria Bianco*[2], *Mariateresa Di Stazio*[2], *Saveria Lory Croce*[1], *Adamo Pio D'Adamo*[1;2]

[1]*University of Trieste, Department of Medical, Surgical and Health Sciences, Trieste, Italy;* [2]*Institute for Maternal and Child Health - IRCCS "Burlo Garofolo", Trieste, Italy*

Background/Objectives: Progressive Familial Intrahepatic Cholestasis (PFIC) is a group of early onset cholestatic liver diseases. Recently, cytoskeleton linker plectin (*PLEC*) gene was found to protect liver from cholestatic injuries, and a pair of siblings with severe cholestasis with compound heterozygous PLEC mutations has been reported.

Methods: We report two female patients with severe cholestasis. The second is a case of intrahepatic cholestasis in a 66-year-old woman with jaundice, initially interpreted as drug-induced liver injury but that, despite the suspension of medications, has not improved. Suspecting PFIC we analyzed by Whole Exome Sequencing and identified variants have been studied by functional tests in liver biopsy.

Results: In the first patient we found compound heterozygous missense mutations in the *PLEC* gene (R1906W and P1146L). Immunofluorescence on liver biopsy showed a reduced PLEC and K8 colocalization; BSEP staining showed an aberrant canalicular pattern suggesting that PLEC mutations may affect BSEP targeting, causing cholestasis.

A liver biopsy of the 66-year-old woman revealed a morphological pattern of severe cholestatic liver disease with modest ductopenia and without significant hepatic inflammation. We found two in cis heterozygous missense mutations (S413R and R3485W) with conflicting interpretation of pathogenicity.

Conclusion: *PLEC* mutations in the first patient are associated with PFIC phenotype, while in the other patient the phenotype has clinically and spontaneously improved. So, *PLEC* heterozygous defects could be associated also with milder forms of cholestatic disease. Sequencing cholestasis genes in patients with adult-onset disease should be considered, with interpretation in collaboration with histopathologists and geneticists.

Grants: RC 26/17, RC 09/23 "IRCCS" Burlo Garofolo, Trieste
Conflict of Interest: None declared

P06

SKELETAL, CONNETIVE TISSUE, ECTODERMAL AND SKIN DISORDERS

P06.001.B Review of 400 genome analysis for patients with osteochondrodysplasia in the national french SeqOIA laboratory

Sophie Rondeau[1;2], *Audrey Briand-Suleau*[2;3], *Corinne Collet*[1;2], *Séverine Bacrot*[2;4], *Fabienne Escande*[2;5], *Perrine Brunelle*[2;5], *Tania Attie-Bitach*[1;2], *Laurence PACOT*[2;3], *Céline Gaucher*[2;3], *sophie monnot*[1;2], *Lucile Boutaud*[1;2], *Virginie Saillour*[2], *Marine Rajaoba*[1], *Barbara Girerd*[1], *Pauline Marzin*[1;6], *Caroline Michot*[1;6], *Genevieve Baujat*[1;6], *Valérie CORMIER-DAIRE*[1;6]

[1]*Hôpital Necker Enfants malades, Service de médecine génomique des maladies rares, Paris, France;* [2]*Laboratoire SeqOIA, Paris, France;* [3]*Hôpital Cochin, Service de Médecine Génomique des Maladies de Système et d'Organe, Paris, France;* [4]*CH A Mignot, Unité de génétique constitutionnelle, Service de Biologie, Versailles, France;* [5]*CHU Lille, Institut de Biochimie et Biologie Moléculaire, Lille, France;* [6]*Hôpital Necker Enfants malades, Centre de référence des Maladies Osseuses Constitutionnelles, Paris, France*

Background/Objectives: In 2018, a government-led genomic program was introduced in France, resulting in the creation of two national laboratories, namely SeqOIA and Auragen.

Methods: Since then, SeqOIA laboratory has provided genome sequencing for 400 osteochondrodysplasia patients with either a negative targeted panel sequencing or in first line for atypical phenotype. We present the results from this cohort.

Results: A diagnosis was reached in 124 patients (31%), 42 cases (10%) carried a variant of unknown significance, while 234 genomes were negative (59%).

Regarding the 124 positive genomes, 92 genes were involved, with a maximum of 5 patients carrying variants in the same gene (*COL2A1, FGFR3, RPL13*). 8 patients carried structural variants : 3 inversions, 3 large deletions, 1 large duplication and 1 translocation involving *NSD1* gene. We also identified 5 deep intronic variants and 3 uniparental disomies. 4 double diagnoses were also made. Those findings highlight the benefit of the WGS approach.

Variants of uncertain significance, located in 38 different genes, encompass intronic variants, variants in genes not yet involved in human pathology, and variants of unknown significance currently studied at the functional level

Conclusion: We conclude that Whole Genome sequencing has revolutionized the molecular diagnosis of osteochondrodysplasias, improving significantly the diagnostic yield. This advancement is particularly noteworthy as it enables diagnoses that were not accessible through panel or exome sequencing coupled with an array CGH.

Grants: /
Conflict of Interest: None declared

P06.002.C Functional study of the *GLI1* gene in Gorlin Syndrome

María Zubieta-Laseca[1;2], *Carlos Gutiérrez-Cerrajero*[1;2], *Angel Santos-Briz Terrón*[1;3], *Diego Iglesias-Corral*[1;2], *Laura Molinero-Sicilia*[1;2], *Miguel Ángel Collado-Pérez*[1;2], *Ana-Belén Herrero*[1;2], *Rogelio González-Sarmiento*[1;2]

[1]*Biomedical Research Institute of Salamanca (IBSAL), Salamanca, Spain;* [2]*University of Salamanca, Faculty of Medicine, Molecular Medicine Unit, Salamanca, Spain;* [3]*University Hospital Virgen de de la Vega, Dermathology, Salamanca, Spain*

Background/Objectives: Gorlin Syndrome is a rare disease characterized by various developmental abnormalities and a high susceptibility to different types of cancer, especially basal cell carcinomas and medulloblastomas. The underlying cause of this syndrome is a constitutive gain of function in the Sonic Hedgehog pathway, commonly resulting from heterozygous loss-of-function mutations in the *PTCH1* and *SUFU* genes. However, about 5% of Gorlin Syndrome patients lack mutations in these genes, and the cause of their disease remains unknown. Previous work in our group identified a heterozygous missense *GLI1* mutation (c.1591C>T/p.R531C) in three different patients without mutations in *PTCH1* and *SUFU*. In this study, we aimed to determine the pathogenicity of this mutation.

Methods: We cloned the *GLI1* cDNA both WT and mutated into a lentiviral vector that was used to infect the keratinocyte cell line Ker-CT. This way, we created two novel cell lines: one that overexpressed *GLI1* WT and another one that overexpressed *GLI1* R531C. We studied the expression of the GLI1 target genes in these cell lines and generated epidermal equivalents from them.

Results: The keratinocyte cell line that carried the *GLI1* mutation showed a higher expression of GLI1 target genes, consistent with

the behavior of a gain-of-function mutation. The epidermal equivalents showed that the cell line carrying the *GLI1* mutation suffered an altered differentiation and displayed features characteristic of Gorlin Syndrome.

Conclusion: These findings may point to a novel relationship between *GLI1* and the development of Gorlin Syndrome.

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Conflict of Interest: None declared

P06.003.D Novel biallelic *PISD* missense variants cause spondyloepimetaphyseal dysplasia with disproportionate short stature and fragmented mitochondrial morphology

Tess Holling[1], *Line Nolting*[2], *Gen Nishimura*[3], *Jakob Ek*[4], *Mads Bak*[4], *Merete Ljungberg1*[2], *Kerstin Kutsche*[1], *Hanne Hove*[2]

[1]*University Medical Center Hamburg-Eppendorf, Institute of Human Genetics, Hamburg, Germany;* [2]*Copenhagen University Hospital, Rigshospitalet, Center for Rare Diseases, Department of Pediatrics, København, Denmark;* [3]*Saitama Medical University Hospital, Center for Intractable Diseases, Saitama, Japan;* [4]*Copenhagen University Hospital, Rigshospitalet, Department of Clinical Genetics, København, Denmark*

Background/Objectives: Biallelic *PISD* variants cause a phenotypic spectrum ranging from short stature with spondyloepimetaphyseal dysplasia to a multisystem disorder affecting eye, ear, bone, and brain. *PISD* encodes the mitochondrial-localized enzyme phosphatidylserine decarboxylase. The PISD precursor is self-cleaved to generate a heteromeric mature enzyme that converts phosphatidylserine to the phospholipid phosphatidylethanolamine. We report a patient with novel biallelic *PISD* missense variants.

Methods: Clinical, genomic, and functional studies, including immunoblotting and immunofluorescence analyses, using patient and control fibroblasts.

Results: The 17-year-old male patient born to unrelated healthy parents presented with disproportionate short stature and spondyloepimetaphyseal dysplasia, including platyspondyly, prominent epiphyses, and metaphyseal dysplasia. Trio genome sequencing revealed compound heterozygous *PISD* variants NM_001326411.1:c.569C>T; p.(Ser190Leu) and c.799C>T; p.(His267Tyr). By using patient, parent, and control fibroblasts, we showed similar levels of the PISD precursor in all cell lines. However, patient cells had a significantly higher proportion of fragmented mitochondria compared to control cells cultured under basal condition and after treatment with 2-desoxyglucose that represses glycolysis and stimulates respiration. Data from the published structure of the *E. coli* PISD orthologue suggest that Ser190Leu and His267Tyr likely impair PISD's autoprocessing activity and/or phosphatidylethanolamine biosynthesis.

Conclusion: Our data demonstrate that the novel *PISD* p.(Ser190Leu) and p.(His267Tyr) variants act as hypomorphs and underlie the pure skeletal phenotype in the patient. Recent data showing targeting of PISD to the endoplasmic reticulum and lipid droplets and PISD's role in lipid droplet biogenesis and neutral lipid storage suggest that different functional consequences underlie the different phenotypes associated with biallelic *PISD* pathogenic variants.

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Conflict of Interest: None declared

P06.004.A Short anagen hair syndrome: association with mono- and biallelic variants in *WNT10A* and a genetic overlap with male pattern hair loss

Nicole Cesarato[1], *Agnes Schwieger-Briel*[2;3], *Yasmina Gossmann*[1], *Sabrina K. Henne*[1], *Kathrin Hillmann*[4], *Leonie Frommherz*[5], *Maria Wehner*[1], *Xing Xiong*[1], *Holger Thiele*[6], *Vincenz Oji*[7], *Donatella Milani*[8], *Iliana Tantcheva-Poor*[9], *Kathrin Giehl*[5], *Regina Fölster-Holst*[10], *Anne Teichler*[11], *Delphine Braeckmans*[11], *Peter Höger*[11], *Gabriela Jones*[12], *Jorge Frank*[13], *Lisa Weibel*[2], *Ulrike Blume-Peytavi*[4], *Henning Hamm*[14], *Markus Nöthen*[1], *Matthias Geyer*[15], *Stefanie Heilmann-Heimbach*[1], *F. Buket Basmanav*[1], *Regina C. Betz*[1]

[1]*Institute of Human Genetics, University of Bonn, Medical Faculty and University Hospital Bonn, Bonn, Germany;* [2]*Department of Pediatric Dermatology, University Children's Hospital Zurich, Switzerland;* [3]*Department of Dermatology, Medical Center - University of Freiburg, Germany;* [4]*Department of Dermatology, Venereology and Allergology, Charité - Universitätsmedizin Berlin, Germany;* [5]*Department of Dermatology and Allergy, Ludwig-Maximilian-University of Munich, Germany;* [6]*Cologne Center for Genomics, University of Cologne, Germany;* [7]*Department of Dermatology, University Hospital of Muenster, Germany;* [8]*Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy;* [9]*Department of Dermatology, Faculty of Medicine and University Hospital Cologne, University of Cologne, Germany;* [10]*Department of Dermatology, Venereology and Allergology, University Hospital Schleswig-Holstein, Kiel, Germany;* [11]*Department of Paediatric Dermatology, Catholic Children's Hospital Wilhelmstift, Hamburg, Germany;* [12]*Clinical Genetics Department, Nottingham University Hospitals NHS Trust, Nottingham, United Kingdom;* [13]*Department of Dermatology, Venereology and Allergology, University Medical Center Göttingen, Germany;* [14]*Department of Dermatology, Venereology, and Allergology, University Hospital Würzburg, Germany;* [15]*Institute of Structural Biology, University of Bonn, Medical Faculty and University Hospital Bonn, Germany*

Background/Objectives: Short anagen hair (SAH) is a rare, paediatric hair disorder characterised by the inability of the scalp hair to obtain length due to a shortened anagen phase. SAH has a strong negative psychological impact on the affected individuals. The genetic basis of SAH was so far unknown.

Methods: Exome sequencing was performed in 27 patients with SAH or with a complaint of short, non-growing hair. Data was screened to identify genes whose variants were enriched in this cohort. Sanger sequencing was used for validation, screening of 21 additional affected individuals and of relatives. Genetic association testing of SAH-related variants with male pattern hair loss (MPHL) was performed using UK Biobank data.

Results: The analyses revealed that 20 affected individuals (~40% of the SAH cohort) carried bi- or mono-allelic pathogenic variants in *WNT10A*. Most of these patients had light-coloured hair, and a regression of the fronto-parietal hairline. The most frequent variant in the cohort, c.682T>A;p.(Phe228Ile), was in linkage disequilibrium with four common *WNT10A* variants with a known association with MPHL. c.682T>A;p.(Phe228Ile), together with another rare SAH-associated variant, partially explained the known associations between *WNT10A* and MPHL.

Conclusion: Rare *WNT10A* variants are associated with a phenotypic spectrum ranging from no clinical signs to severe ectodermal dysplasia. Our results suggest that *WNT10A* is also associated with SAH and SAH has a genetic overlap with the common phenotype MPHL. The presumed shared biological effect of *WNT10A* variants in SAH and MPHL is the shortening of the anagen phase.

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Conflict of Interest: None declared

P06.005.B Bone age in achondroplasia - comparative study using visual and AI-based technologies

*Minu Fardipour[1], **Kyra Skaf**[1], Sebastian Rassmann[2], Christoph Beger[3], Primoz Kotnik[4], Tinatin Tkemaladze[5], Dr. Katja Palm[1], Peter Krawitz[2], Klaus Mohnike[1], Behnam Javanmardi[2]*

[1]Otto-von-Guericke-University Magdeburg, Medical Faculty, Magdeburg, Germany; [2]University of Bonn, Institute for Genomic Statistics and Bioinformatics, Bonn, Germany; [3]Leipzig University, Growth Network CrescNet, Medical Faculty, Leipzig, Germany; [4]University Medical Center Ljubljana, Department of Endocrinology, Diabetes and Metabolism, Ljubljana, Slovenia; [5]Tbilisi State Medical University, Department of Molecular and Medical Genetics, T'bilisi, Georgia

Background/Objectives: In achondroplasia, the Gly380Arg mutation of the Fibroblast Growth Factor Receptor 3 (FGFR3) gene, results in constitutive activation of chondrocyte proliferation and differentiation during bone growth. Consequently, FGFR3 activation results in characteristic radiological features, e.g. shortening of the tubular bones and impaired epiphyseal growth. These features are used to determine bone age to evaluate growth and puberty as well as timing for limb lengthening and epiphysiodesis procedures. A significant bone age delay was already described in 2010 by Pannier et al. using the Greulich and Pyle (GP) method. However visual determination of skeletal maturation in bone dysplasia suffers from high interrater variability.

Methods: To address the limitations of visual determination, this study aimed to evaluate the effectiveness of an open-source artificial intelligence (AI) tool, *Deeplasia* (Rassmann et al., 2024). The mean absolute difference (MAD) between the AI and the average of four visual determinations performed by four different human raters is used as a measure of the performance of *Deeplasia*.

Results: The preliminary analysis using left-hand radiographs from 25 achondroplastic children within the age range of 5 to 16 years (from University Hospital Magdeburg) resulted in a MAD of 6.5 months between the AI and the average of human raters, compared to the inter-rater error of 13.3 months. We are expanding our current cohort to more centres.

Conclusion: The open-source bone age assessment AI, *Deeplasia*, has a significantly smaller uncertainty in assessing the bone age of individuals with achondroplasia compared to the inter-rater error.

Grants:

Conflict of Interest: None declared

P06.006.C Deletion of the intracellular region of LRP4 leads to sclerosis via dysregulation of the Wnt and Smad pathways

*Caroline Caetano Da Silva[1], Genevieve Baujat[2], Anaik Previdi[2], Kelvin Valmorin[3], Adrien Bloch[3], Valérie CORMIER-DAIRE[2], **Corinne Collet**[2]*

[1]Leonard and Madlyn Abramson Pediatric Research Center, Philadelphia, United States; [2]INSERM UMR 1163, Imagine Institute, Genomic department, Necker-Enfants Malades Hospital, Paris, France; [3]Inserm 1132, Bioscar, Paris, France

Background/Objectives: Sclerosteosis type 1 and 2 are rare bone diseases characterized by high bone mass resulting from excessive osteoblast formation. These diseases are due respectively to pathogenic variants in *SOST* and *LRP4* genes, both involved in the canonical Wnt pathway. *LRP4* facilitates the inhibitory effect of sclerostin on the Wnt pathway by creating a complex with LRP4/SOST/LRP5/6. Pathogenic variants in sclerosteosis type 2 are mainly located in the binding site of sclerostin but other pathological mechanisms seem possible. Therefore, our aim was to better understand the role of *LRP4* during osteoblastic differentiation.

Methods: CRISPR-Cas9-mediated deletions of the intracellular and extracellular domains of LRP4 were generated in U2OS cell lines. To compare mutated and wt cells, differentiation and mineralization assays were performed, along with an estimation of canonical wnt pathway activation and a transcriptome.

Results: The generation of heterozygous variants led to decreased LRP4 protein expression at the plasma membrane, with an abnormal distribution of LRP4 in cells with extracellular deletion. The intracellular variant in LRP4 has an enhanced capacity for differentiation and mineralization; conversely, cells with extracellular deletion resulted in decreased differentiation and mineralization compared to control cells. The intracellular deletion of LRP4 revealed constitutional activation of the canonical wnt pathway. Transcriptome analysis showed dysregulation of various osteoblast-related pathways, in particular Smads.

Conclusion: The intracellular region of LRP4 is important for bone regulation not only through wnt pathways but also by interacting with other pathways such as smads.

Conflict of Interest: None declared

P06.007.D Functional studies of mutations responsible for the hereditary human limb malformations.

Akshaya Ramanujam[1], *Ewa Hordyjewska-Kowalczyk[2], Tylzanowski Przemko[3]*

[1]Medical University of Lublin, Department of Biomedical Sciences,Laboratory of Molecular Genetics, Lublin, Poland; [2]KU Leuven, Department of Human Genetics,Laboratory for Skeletal Dysplasia Research, Leuven, Belgium; [3]KU Leuven, Department of Development and Regeneration, Skeletal Biology and Engineering Research Centre,Laboratory for Developmental and Stem Cell Biology, Leuven, Belgium

Background/objectives: Our research concentrates on studying the molecular basis of Clubfoot, a hereditary limb disorder. We have recently identified *TPM2* as a candidate gene associated with that condition and the objective of the study is to establish the genotype-phenotype correlation.

Methods: The *wt* or *mt* mRNA will be injected into a single cell stage zebrafish embryos and the larvae will be analyzed during the next 5 days of development. The morphological analysis will include morphometric analyses and *whole mount* in situ *hybridization*. Loss-of-function experiments will use site directed mutagenesis using CRISPR/*Cas9* strategy by introducing the human mutation into the zebrafish genome and carry out the phenotypic analysis as described above.

Results: We have identified an autosomal dominant mutation, linked to Clubfoot, in *TPM2* gene. The gain of function experiments indicate that the mutation has a severe effect on the zebrafish development including the skeletal structures. The specific phenotypes include a change in the morphology of the somites and ventral tail bending suggesting a disturbance along the dorsal ventral axis. Larvae had also anterior-posterior axis shortening, empty swim bladders, malformed mandible and a pericardial edema. These phenotypes suggest defective *BMP signaling*. For the loss of function experiments, we are in the process of producing relevant crispants.

Conclusion: Our project aims to elucidate the phenotypic-genotypic correlation between the mutation in *TPM2* and the clubfoot phenotype. The identification of distinct genetic markers associated with this malformation will enhance our understanding of the molecular mechanisms governing skeletal morphogenesis.

Grants: OPUS (2019/35/B/NZ5/02955)

Conflict of Interest: None declared

P06.008.A Functional studies of hereditary human mutations leading to craniofacial malformations

Paulina Krzesinska[1], *Anna Jaruga*[1], *Krystian Kuźniarz*[2], *Tylzanowski Przemko*[1;3]

[1]*Medical University of Lublin, Department of Biomedical Sciences, Laboratory of molecular Genetics, Lublin;* [2]*Medical University of Lublin, Chair and Clinic of Maxillofacial Surgery, Lublin, Poland;* [3]*KU Leuven, Laboratory for Developmental and Stem Cell Biology, Department of Development and Regeneration, Skeletal Biology and Engineering Research Centre, Leuven, Belgium*

Background/Objectives: We have identified family with autosomal dominant, Non-Syndromic Cleft Lip and Palate (NCL/P) and linked it to mutations in *EHHAD* and *MASP1* genes respectively. In order to explore that link we chose the zebrafish gain and loss of function studies. The main objective of the presented work is to link the mutation to the phenotypic changes in the skeleton and explain the mechanism of action of these genes.

Methods: The *wt* or *mt* mRNA will be injected into a single cell stage zebrafish embryos and the larvae will be analyzed during the next 5 days of development. The morphological analysis will include morphometric analyses and *whole mount* in situ *hybridization*. Loss-of-function experiments will use site directed mutagenesis using CRISPR/Cas9 strategy by introducing the human mutation into the zebrafish genome and carry out the phenotypic analysis as described above.

Results: Injections of mRNAs encoding wt or mutant variants of both genes resulted in dose dependent phenotypes. The most striking defects were shortening of mandible and dorsal bending of the embryo. Additionally, mt mRNA had cyclopic eyes due to lack of forebrain and severe pericardial edema.

Conclusion: Our data suggests that the zebrafish is a viable model organism to study human skeletal malformations. We are currently in the process of finalizing the CRSPR driven genomic site directed mutagenesis. The identification of specific genetic markers associated with these malformations can contribute to a better understanding of the underlying molecular mechanisms and pathways involved in craniofacial development.

Grants: OPUS 2019/35/B/NZ5/02955

Conflict of Interest: None declared

P06.009.B Fifth patient with Silver-Russell Syndrome type 4 caused by a deletion of the entire *PLAG1* gene

Bianca Greiten[1], *Andreas Dalski*[1], *Malte Spielmann*[1;2], *Irina Hüning*[1;3]

[1]*Institute of Human Genetics, University of Lübeck, Lübeck, Germany;* [2]*Institute of Human Genetics, University of Kiel, Kiel, Germany;* [3]*MVZ für Humangenetik und Molekularpathologie GmbH, Rostock, Germany*

Background/Objectives: Silver-Russell Syndrome (SRS) is a genetically heterogeneous disorder primarily characterized by pre- and postnatal growth restriction, relative macrocephaly and facial dysmorphism. Recently, sequence variants in and deletions of *PLAG1* have been identified as the cause of SRS type 4. Haploinsufficiency of *PLAG1* leads to a decrease in IGF2 expression.

Methods: We report on an 8-year-old girl with SRS type 4 caused by a *PLAG1* deletion. She had intrauterine growth restriction and was born with birth length, weight and head circumference below the first percentile. Postnatally, she had severe feeding difficulties and mild developmental delay in the first year of life. After that, she reached developmental milestones in time. At the age of 4 years, she developed a tic disorder with motor and vocal tics. Her cognitive abilities were normal. Her body measurements at the age of 8 years were consistently below the 3rd percentile. Most severely impacted was her head circumference (-5,0 SD). She had mild craniofacial dysmorphisms, very suggestive for SRS, including a prominent forehead and triangular face.

Results: Array CGH analysis revealed a 1,2 Mb de novo microdeletion in 8p12.1 including the *PLAG1* gene (arr[GRCh37] 8q12.1(56897469_58105507)x1). It contained the genes from *LYN* to *LINC00968*.

Conclusion: The deletion of the *PLAG1* gene was the most suggestive cause of the patient's phenotype. Her clinical features were consistent with SRS type 4 and overlap with the phenotype of the four patients in the literature thus marking the fifth case. Although unusual for SRS, microcephaly has been described in another patient with *PLAG1* deletion.

Grants:

Conflict of Interest: None declared

P06.010.C Microbiome alterations in icthyotic skin

Judit San Segundo-Lozano[1;2], *Carla Sánchez-Jiménez*[1;2], *Carlos Gutiérrez-Cerrajero*[1;2], *María Zubieta-Laseca*[1;2], *Ana-Belén Herrero*[1;2], *Rogelio González-Sarmiento*[1;2], *Ángela Hernández-Martín*[3]

[1]*Faculty of Medicine, University of Medicine, Department of Medicine, Salamanca, Spain;* [2]*Biomedical Research Institute of Salamanca (IBSAL), Salmanca, Spain;* [3]*Hospital Infantil Niño Jesús, Department of Dermatology, Madrid, Spain*

Background/Objectives: Ichthyoses are disorders of cornification characterized by dry, scaly skin and a disruption of skin barrier function. This disruption promotes bacterial colonization, leading to recurrent infections that severely impair quality of life, can cause auditory complications and, in some cases, can be life threatening. However, very few studies explore the differences in cutaneous bacterial population profiles in the context of this disease.

Methods: A cohort of 34 patients from Hospital Niño Jesús under 18 years of age was analyzed. Clinical data were recorded and healthy and affected skin swabs were taken from scapula and armpit. Bacterial DNA was extracted and 16S rRNA, 18S rRNA and ITS sequences were sequenced in an Illumina NovaSeq X plus. Relative abundances of 246 bacterial *genera* were profiled using QIIME2 and statistical analyses were performed using RStudio.

Results: We initially performed a correspondence analysis comparing the profiles for healthy and affected samples. *Staphylococcus* was most predominantly associated to affected armpit and scapula samples. Meanwhile *Corynebacterium* was most predominantly associated to healthy armpit samples and *Streptococcus* and *Finegoldia* to healthy scapula. We then divided samples according to disease type and performed descriptive statistics. The most common forms of ichthyosis were epidermolytic ichthyosis (EI) and autosomal recessive congenital ichthyosis (ARCI). Within these groups, the most common genus were *Corynebacterium* for EI and *Staphylococcus* for ARCI.

Conclusion: The present study highlights differences in the microbiome between ichthyotic and healthy skin as well as between ichthyosis subtypes.

Grants: This project was funded by Fundación Nemesio Díez

Conflict of Interest: None declared

P06.011.D Allogenic Bone Marrow Transplantation efficacy in Craniometaphyseal Dysplasia with *ANKH* mutation.

Pauline Marzin[1;2], *Caroline Michot*[1;2], *Corinne Collet*[1;2], *sophie monnot*[1;2], *Cyril Gitiaux*[3], *Robert Matthieu*[4], *Sylvain Breton*[5], *Klervie Loiselet*[5], *Romain Luscan*[6], *Graziella Pinto*[7], *Anne-Cecile Chaux*[8], *Guillaume Morelle*[9], *Charlotte Boussard*[9], *Martin Castelle*[9], *Valérie Cormier-Daire*[1;2], *Despina Moshous*[9]

[1]*AP-HP, Hôpital Necker-Enfants Malades, Service de médecine génomique des maladies rares, Centre de référence des maladies osseuses constitutionnelles, Paris, France;* [2]*Institut Imagine, Université Paris Cité, UMR1163, INSERM, Paris, France;* [3]*Hôpital Necker-Enfants Malades, APHP, Service de Neurophysiologie Clinique Pédiatrique, Centre de Référence des Pathologies Neuromusculaires, France, France;* [4]*Hôpital Universitaire Necker - Enfants Malades, Service d'ophtalmologie, paris, France;* [5]*AP-HP, Hôpital Necker-Enfants Malades, Département de radiologie pédiatrique, Paris, France;* [6]*AP-HP, Hôpital Necker-Enfants Malades, Département d'ORL pédiatrique, Paris, France;* [7]*, AP-HP, Hôpital Necker-Enfants Malades, Département d'endocrinologie, diabétologie, gynécologie, Paris, France;* [8]*CHU de Saint-Etienne, Service de neuropédiatrie, Saint-Priest-en-Jarez, France;* [9]*AP-HP, Hôpital Necker-Enfants Malades, Unité d'immuno-hématologie, et rhumatologie, pédiatrique, Paris, France*

Craniometaphyseal dysplasia (CMD) is a rare genetic disease marked by progressive thickening of craniofacial bones and abnormal modelling of long bones metaphysis, related to monoallelic pathogenic variations in the *ANKH* gene. Diffuse and progressive hyperostosis is responsible for distinctive dysmorphism with upper airway stenosis, foramen magnum stenosis and cranial nerve compression causing optic atrophy, cranial nerve palsy and deafness.

The *ANKH* gene encodes a transmembrane protein that controls pyrophosphate levels, supporting a role of Ank in regulation of bone mineralization. In a AnkKI/KI mouse model mimicking CMD phenotype, bone matrix hypomineraisation and decreased osteoclastogenesis have been demonstrated. Moreover, transplantation of hematopoietic stem cells (HSCT) from healthy mice allowed bone mass reduction.

We present here the results of HSCT in three patients, with a severe form of CMD. Diagnosis was made at 3(P1), 13(P2) and 22(P3) months. Complications were already present with optic atrophy (3/3), facial nerve palsy (2/3), deafness (2/3) and nasal obstruction (3/3) requiring surgery (2/3). Patients were transplanted at the age of 6(P1), 21(P2) and 36(P3) months. To date, i.e. 6 years (P3), 15 months (P1) and 12 months (P3) after HSCT, we observed a significant improvement of patient condition, with decreased hyperostosis, slowdown in cranial perimeter growth velocity, stabilization of neurosensory status, and even an improvement in facial paralysis for patient P1.

Treatment with GCSH partially restores bone remodeling homeostasis in CMD, and therefore leads to stabilization of the disease. This report stresses the importance of an early diagnosis to improve the prognosis of this severe disorder.

Conflict of Interest: None declared

P06.012.A Transcriptome analysis reveals genetic compensation in a murine model of Ehlers-Danlos syndrome, classic type

Fransiska Malfait[1;2], *Zoë Malfait*[1;2], *Rachel Miller*[3], *Anne-Marie Malfait*[3], *Delfien Syx*[2;4]

[1]*Ghent University Hospital, Center for Medical Genetics, Gent, Belgium;* [2]*Ghent University, Department of Biomolecular Medicine, Gent, Belgium;* [3]*Rush Medical College, Department of Internal medicine, Chicago, United States;* [4]*Ghent University Hospital, Center for Medical Genetics, Ghent, Belgium*

Background/Objectives: The heritable connective tissue disorder classic Ehlers-Danlos syndrome (cEDS) is caused by heterozygous defects in the genes encoding type V collagen (*COL5A1* and *COL5A2*). A well-studied mouse model of cEDS, the *Col5a1* haploinsufficient (*Col5a1*$^{+/-}$) model (gift dr. David Birk, USF), recapitulates the most common molecular defect seen in human cEDS patients (i.e., *COL5A1* haploinsufficiency) and mimic the skin hyperextensibility and ultrastructural collagen abnormalities characteristic of human cEDS.

Methods: RNA sequencing was performed on lumbar L3-L5 bilaterally pooled dorsal root ganglia (DRG) of male and female wild-type and *Col5a1*$^{+/-}$ mice.

Results: Unexpectedly, a 2-fold upregulation of *Col5a1* expression was found in DRG of *Col5a1*$^{+/-}$ mice compared to wild-type littermates, but decreased protein levels for type V collagen were detected on immunohistochemistry. To further investigate this intriguing finding, RT-qPCR for *Col5a1* was performed on nine different tissues (e.g., DRG, brain, kidney, liver, colon, heart, lung, tail, skin) of both wild-type and *Col5a1*$^{+/-}$ mice. The expected 50% decrease in *Col5a1* expression was only observed in tails from female mice. *Col5a1* expression was higher in *Col5a1*$^{+/-}$ male versus female intestine and consistently upregulated in *Col5a1*$^{+/-}$ brain. No significant difference in *Col5a1* expression levels were observed in liver, DRG, kidney, skin and male tails.

Conclusion: These results indicate that *Col5a1* expression is not uniformly reduced by half in *Col5a1*$^{+/-}$ mice but highly variable, suggesting a tissue- and sex-specific genetic compensation response. This novel finding has never been associated with (c) EDS but can possibly explain part of the phenotypic features observed in cEDS.

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Conflict of Interest: Fransiska Malfait FWO-G041519N, Ghent University Hospital, ACR 2023, EDS Society, Zoë Malfait Ghent University, Rachel Miller: None declared, Anne-Marie Malfait: None declared, Delfien Syx FWO-G041519N, Ghent University

P06.013.B *EFEMP1*-related connective tissue disorder: distinct subtype of Ehlers-Danlos syndrome?

Merel Mol[1], *John Vlot*[2], *Johanna Escher*[3], *Tjakko Van Ham*[1], *Hennie Brüggenwirth*[4], *Natalja Bannink*[5], *Jeroen Renkens*[6], *Robert Verdijk*[4], *Johan Max Kros*[4], *Virginie Verhoeven*[1;7], *S. Demirdas*[1]

[1]*Erasmus MC University Medical Center, Department of Clinical Genetics, Rotterdam, Netherlands;* [2]*Erasmus MC University Medical Center, Department of Pediatric Surgery, Rotterdam, Netherlands;* [3]*Erasmus MC University Medical Center, Department of Pediatric Gastroenterology, Rotterdam, Netherlands;* [4]*Erasmus MC University Medical Center, Department of Pathology, Rotterdam, Netherlands;* [5]*Franciscus Gasthuis and Vlietland, Department of Pediatrics, Rotterdam, Netherlands;* [6]*Erasmus MC University Medical Center, Department of Orthopedics and Sportsmedicine, Rotterdam, Netherlands;* [7]*Erasmus MC University Medical Center, Department of Ophthalmology, Rotterdam, Netherlands*

Background/Objectives: Heritable connective tissue disorders (CTD) can be difficult to diagnose due to wide clinical and genetic heterogeneity. Biallelic variants in *EFEMP1*, encoding Fibulin-3, were reported as rare cause of CTD with recurrent herniations and joint hypermobility.

Methods: Three new CTD patients were clinically evaluated, followed by whole exome sequencing, targeted *EFEMP1* reanalysis including copy number variant analysis, and RNA-sequencing to investigate aberrant splicing and gene expression.

Results: Two male siblings (10/13yrs) presented with marfanoid habitus, recurrent inguinal/umbilical herniations, joint hypermobility, and scoliosis. Parents and halfsiblings reported joint

hypermobility and umbilical herniations. The eldest boy died (16yrs) from diaphragmatic herniation complicated by gastric perforation. Autopsy revealed widespread intestinal diverticula and pulmonary bullae. Both siblings harbored compound heterozygous pathogenic *EFEMP1*-variants (c.1320+2T>A,p.? and c.698G>A,p.Gly233Asp).

A non-related male patient (58yrs) had marfanoid features, myopia, recurrent diaphragmatic/inguinal herniations, and chronic gastrointestinal dilatation with severe malabsorption (brown-bowel-syndrome). Both his dizygotic twin-brother and mother had diaphragmatic/inguinal herniations and high myopia. The patient died (59yrs) of unclear cause; autopsy showed extensive diaphragmatic herniation, diverticula and pulmonary emphysema. A heterozygous *EFEMP1* splice-variant (c.81+1G>A,p.?) was identified, present in his mother and affected brother, absent in a healthy brother. The variant caused exon skipping leading to a start-loss. Targeted genome reanalysis nor RNA-sequencing revealed a second variant.

Conclusion: Biallelic pathogenic *EFEMP1*-variants cause severe CTD with recurrent herniations and diffuse gastrointestinal dilatation/diverticulosis, with increased risk for life-threatening rupture. Cardiovascular manifestations were not reported so far. Heterozygous carriers may present with a milder phenotype. We propose *EFEMP1*-related CTD as potential novel entity within the Ehlers-Danlos syndrome spectrum.

Conflict of Interest: None declared

P06.014.C Towards creation and characterization of *COL2A1*-SEDC and *BGN*-SEMD iPSC-derived chondrocyte models

Pauline De Kinderen[1], *Josephina (Jeannette) Meester*[1], *Silke Peeters*[1], *Geert Mortier*[2], *Bart Loeys*[1;3], *Aline Verstraeten*[1]

[1]*University of Antwerp and Antwerp University Hospital, Center of Medical Genetics, Antwerp, Belgium;* [2]*University of Leuven and Leuven University Hospital, Center of Human Genetics, Leuven, Belgium;* [3]*Radboud University Medical Center, Human Genetics, Nijmegen, Netherlands*

Background/Objectives: Endoplasmic reticulum (ER) stress attributed to aberrant protein folding is increasingly emerging as a converging chondrodysplasia mechanism. We aim to develop a human disease model to investigate whether ER stress also contributes to *COL2A1*-SEDC and *BGN*-SEMD. Induced pluripotent stem cell (iPSC)-derived chondrocytes exhibit known chondrodysplasia mechanisms and are thus highly suitable.

Methods and results: Somatic cells of two *COL2A1*-SEDC (p.Gly408Asp; p.Gly1107Arg) and two *BGN*-SEMD (p.Gly259Val) patients were reprogrammed into iPSCs using Sendai vectors delivering the four key reprogramming factors. Pluripotency was confirmed using immunocytochemistry for OCT4, SOX2, NANOG, TRA-1-60 and TRA-1-81 and by demonstrating the iPSCs' ability to differentiate into the three germ layers by means of directed differentiation and RT-qPCR. Genomic integrity was verified using the HumanCytoSNP-12 BeadChip. For each patient iPSC line, an isogenic control was made with CRISPR/Cas9. To assess performance of the iPSC-chondrocyte differentiation protocol, we next created control pellets with mesenchymal cell (MC)-like cells as an intermediate. >95% of the resulting MC-like cells were positive for the mesenchymal stem cell markers CD105, CD90, CD73 and CD44, while <2% expressed hematopoietic markers. The MC-like cells' ability to differentiate into osteoblasts and chondroblasts was demonstrated using Alizarin Red and Alcian Blue staining, respectively. Validation of growth plate cartilage-specific gene expression and collagen type II deposition in the iPSC-chondrocyte pellets was obtained by RT-qPCR and immunostaining.

Conclusion: We have established *COL2A1*-SEDC and *BGN*-SEMD iPSC lines and successfully implemented the iPSC-chondrocyte differentiation protocol in our lab as a first step in establishing chondrodysplasia disease models to study ER stress.

Grants: FWO PhD fellowship (G030524N)

Conflict of Interest: None declared

P06.015.D Expansion of the complex genotypic and phenotypic spectrum of *FGFR2*-associated neurocutaneous syndromes

Julia Schmidt[1], *Silke Kaulfuß*[1], *Hagen Ott*[2], *Marianne Gaubert*[1], *Nadine Reintjes*[3], *Felix Bremmer*[4], *Steffi Dreha-Kulaczewski*[5], *Philipp Ströbel*[4], *Gökhan Yigit*[1;6], *Bernd Wollnik*[1;6;7]

[1]*Institute of Human Genetics, University Medical Center Göttingen, Göttingen, Germany;* [2]*Children's Hospital Auf der Bult, Department of Pediatric Dermatology, Hannover;* [3]*Institute of Human Genetics, University Hospital Cologne, Cologne, Germany;* [4]*Institute of Pathology, University Medical Center Göttingen, Göttingen, Germany;* [5]*Department of Pediatrics and Adolescent Medicine, University Medical Center Göttingen, Göttingen, Germany;* [6]*DZHK (German Center for Cardiovascular Research), partner site Göttingen, Göttingen, Germany;* [7]*University of Göttingen, Cluster of Excellence "Multiscale Bioimaging: from Molecular Machines to Networks of Excitable Cells" (MBExC), Göttingen, Germany*

Background: The fibroblast growth factor receptors comprise a family of related but individually distinct tyrosine kinase receptors. Within this family, FGFR2 is a key regulator in many biological processes, e.g., cell proliferation, tumorigenesis, metastasis, and angiogenesis. Heterozygous activating non-mosaic germline variants in *FGFR2* have been linked to numerous autosomal dominantly inherited disorders including several craniosynostoses and skeletal dysplasia syndromes. So far, postzygotic somatic activating FGFR2 alterations were found in various cancer types.

Objectives: We aimed to identify the causal variant in an individual with cutaneous nevi, ocular malformations, macrocephaly, mild developmental delay, and the initial clinical diagnosis of Schimmelpenning-Feuerstein-Mims syndrome, a very rare mosaic RASopathies.

Methods: We performed exome sequencing as well as high-coverage disease-specific panel sequencing of different tissues, clinical phenotyping, structural mapping, and further functional characterization.

Results: We identified the mosaic variant c.1647=/T>G p.(Asn549=/Lys) in *FGFR2*. The variant is located in the tyrosine kinase domain of FGFR2 in a region that regulates the activity of the receptor. Further analysis to explore the functional consequences of the identified postzygotic *FGFR2* variant upstream of the RAS signaling pathway indicated a constitutive tyrosine kinase receptor activation.

Conclusion: Mosaic activating variants in *FGFR2* cause a new neurocutaneous condition characterized by cutaneous nevi, ocular malformations, macrocephaly, and mild developmental delay. Interestingly, there is a striking genotypic and phenotypic overlap with other mosaic RASopathies and *FGFR*-associated syndromes.

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Conflict of Interest: None declared

P06.016.A Genotypic and phenotypic features of tooth agenesis in Japanese population

Junichiro Machida[1;2], *Junya Adachi*[3], *Yoshihiko Aoki*[4], *Yasuto Sano*[2], *Michiyo Ando*[2], *Shunsuke Hyodo*[1], *Kosuke Saida*[1], *Mitsuo Goto*[2], *Yoshihito Tokita*[5]

[1]*Toyota Memorial Hospital, Oral and Maxillofacial Surgery, Toyota, Japan;* [2]*Aichi Gakuin University, School of Dentistry, Maxillofacial Surgery, Nagoya, Japan;* [3]*Toyohashi Municipal Hospital, Toyohashi, Japan;* [4]*Okazaki Municipal Hospital, Okazaki, Japan;* [5]*Aichi Devlopmental Disability Center Central Hospital, Kasugai, Japan*

Background/Objectives: Tooth agenesis is one of the most common congenital anomalies in humans, classified by the number of missing teeth: hypodontia is defined as five or fewer missing teeth, excluding the wisdom teeth, while oligodontia is defined as six or more missing teeth. The etiology of tooth agenesis remains unclear. The aim of this study is to analyze the phenotypes of nonsyndromic tooth agenesis linked to gene mutations in Japanese.

Methods: Fifty-nine unrelated subjects with apparent isolated tooth agenesis visited the Aichi-Gakuin University hospital from 2015 to 2023. The Classification, site, family history, and genetic factors (MSX1, PAX9, LRP6, and WNT10A) by mutation and/or exome analysis, were examined.

Results: Among the 32 Oligodontia, 9 had the WNT10A mutation, and 9 had the PAX9 mutation. There were 3 with LRP6 mutation, 2 with MSX1 mutation, and 9 were unknown. Among the 27 cases of hypodontia, 6 had WNT10A mutations, 2 had LRP6 mutations, and 19 were unknown. The incidence of oligodontia was statistically significantly higher in patients with genetic mutations in any of WNT10A, PAX9, LRP6, and MSX1 ($p = 0.0017$). In cases with MSX1 and PAX9 mutations, the first incisor teeth to molars are missing, but in cases with WNT10A and LRP mutations, missing sites are concentrated in teeth second incisor to premolar.

Conclusion: This suggests that hypodontia can lead to the development of multifactorial genetic diseases. In individuals with posterior tooth agenesis, molecular genetic testing of *MSX1* and/or *PAX9* may be considered first.

Grants: This work was supported in part by AMED under grant numbers JP17nk0101334 and JP20ek0109397 (to YT).

Conflict of Interest: None declared

P06.017.B Retrospective analysis of virtual gene panel analysis for genodermatoses reveals a high diagnostic yield in daily practice

Aude Beyens[1], *Jozefien Weytens*[1], *Lore Pottie*[1], *Sofie De Meulemeester*[2], *Karolien Aelbrecht*[1], *Silke De Feyter*[2], *Sofie De Schepper*[2], *Eline Van Holm*[1], *Sofie Symoens*[1], *Bert Callewaert*[1]

[1]*Ghent University Hospital, Center for Medical Genetics Ghent, Ghent, Belgium;* [2]*Ghent University Hospital, Department of Dermatology, Ghent, Belgium*

Background/Objectives: The complexity and heterogeneity of heritable skin disorders evidently cause a diagnostic delay impeding efficient clinical decision making. Virtual gene panel analysis (VPA) has proven to be a cost-effective, first-tier, genetic test in different settings, but results from dermatological practice are lacking. This study assesses the diagnostic yield of VPA for inherited skin disorders in an actual clinical setting without limitations on inclusion of patients.

Methods: A monocentric retrospective observational study to investigate the clinical characteristics, diagnostic yield, and management implications in 207 subjects referred for genetic testing.

Results: Molecular analysis yielded a definitive diagnosis in 38% and reached a probable diagnosis in an additional 3% of individuals. Extending the analysis to other panels in selected patients further increased the diagnostic rate with 2.4%. In 10% of the patients with a molecularly confirmed diagnosis, the findings changed the clinical diagnosis. Molecular diagnostics delineated previously unreported sub-entities in SAM and CHIME syndrome. The presence of mosaicism, absence of recognizable dermatological phenotypes, and panel settings are the main reasons of (false) negative results.

Conclusion: VPA for heritable skin disorders has a high diagnostic yield with implications to patient management, therapy, and genetic counseling. Our results highlight the feasibility and importance of implementing molecular testing into daily dermatological practice.

Grants: B.C. is a senior clinical investigator of the Research Foundation Flanders.

Conflict of Interest: None declared

P06.018.C Report of two new *KRT10* mutations associated to ichthyosis with confetti

Carlos Gutiérrez-Cerrajero[1;2], *Nerea Gestoso-Uzal*[1;2], *María Zubieta-Laseca*[1;2], *Nelmar Valentina Ortiz Cabrera*[3], *Ana-Belén Herrero*[1;2], *Irene Lara-Saez*[4], *Ángela Hernández-Martín*[3], *Rogelio González-Sarmiento*[1;2]

[1]*University of Salamanca, Faculty of Medicine, Department of Medicine, Salamanca, Spain;* [2]*Biomedical Research Institute of Salamanca (IBSAL), Salamanca, Spain;* [3]*Hospital Infantil Universitario Niño Jesús, Department of Dermatology, Madrid, Spain;* [4]*University College Dublin, Charles Institute of Dermatology, Dublin, Ireland*

Background/Objectives: Ichthyoses are disorders of cornification characterized by dry, scaly skin and a disruption of skin barrier function (1). A rare form is ichthyosis with confetti (IWC, ORPHA: 281190), characterized by generalized ichthyosiform erythroderma at birth, with the appearance of healthy focal spots later in life (2). IWC is caused by dominant mutations in the tail domain of *KRT10* and, rarely, *KRT1*, which alter its polarity from apolar to polar (2).

Methods: Two patients were referred to Hospital Infantil Niño Jesús in Madrid with generalized ichthyosiform erythroderma, mild ectropion, and palmoplantar, scalp, and nail involvement. Genomic DNA was extracted from peripheral blood by standard phenol/chloroform purification and mutations were found using whole exome sequencing (WES). The patients developed revertant spots after molecular diagnosis.

Results: Patient 1 was heterozygous for *KRT10* (NM_000421.3) c.1367_1368delAG and patient 2 was heterozygous for *KRT10* (NM_000421.3) c.1598_1603delinsC. Both mutations had not been previously reported and cause a frameshift in the last two exons of *KRT10*, which translate its tail domain. PepCalc was used to analyze the polarity of the aberrant tails and both were found to be positively charged, which is consistent with other IWC-causing mutations.

Conclusion: The present study reports two IWC patients with new *KRT10* frameshift mutations that alter tail polarity.

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Conflict of Interest: None declared

P06.019.D Type II collagenopathies: Unveiling a broader spectrum of phenotypic manifestations

Merve Soğukpınar[1;1], *Tuğba Daşar*[1], *Hatice Mutlu*[2], *Elçin Yıldız*[3], *gizem ürel-demir*[1], *Gulen Eda Utine*[1], *Pelin Simsek-Kiper*[1]

[1]*Hacettepe University, Department of Pediatrics, Division of Pediatric Genetics, Ankara, Türkyie;* [2]*Ankara University, Department of Pediatrics, Division of Pediatric Genetics, Ankara, Türkyie;* [3]*Hacettepe University, Division of Musculoskeletal Radiology, Department of Radiology, Ankara, Türkyie*

Background/Objectives: Type II collagen disorders include a distinct group of clinical phenotypes characterized by skeletal findings, orofacial features, ocular abnormalities, and hearing impairment. The spectrum ranges from severe perinatal lethal disorders to milder conditions presenting in adulthood. Herein, we aim to describe the clinical, radiological, and molecular findings of a group of type II collagenopathy patients.

Methods: The study included ten unrelated patients from Turkey with COL2A1 pathogenic variants.Medical history, anthropometric measurements, three-generation pedigree, phenotypic features, radiological images, and molecular features were evaluated for each patient retrospectively.

Results: Out of the ten patients, seven had severe short stature, two had short stature, and one had normal stature when they applied us.Additionally, 4/10 patients (40%) have midface retrusion, 4/10 (40%) patients have myopia, 3/10 (30%) patients have hearing impairment, 2/10 patients (20%) have cleft palate, and one patient have lower extremity length difference.The evaluation of the radiographic findings revealed a wide range of abnormalities, such as metaphyseal irregularity (6/10 patients), platyspondyly (6/10 patients), epiphyseal irregularity (4/10 patients), osteoporosis/osteopenia (4/10 patients), scoliosis (3/10 patients), and odontoid hypoplasia (2/10 patients).All patients had likely pathogenic or pathogenic variants in *COL2A1*.Four of the patients were diagnosed with spondyloepimetaphyseal dysplasia Strudwick type, three with spondyloepiphyseal dysplasia congenita, and one with Kniest dysplasia; two of the cases could not be classified due to their atypical presentation.

Conclusion: Type II collagenopathies constitute a heterogeneous group of diseases, forming a spectrum in terms of clinical features with significant phenotypic overlap.Therefore, any finding suggestive of type II collagenopathies should be evaluated from this perspective.

Grants: None

Conflict of Interest: None declared

P06.020.A Molecular aspects of hypohidrotic ectodermal dysplasias in Russia

Valeriia Kovalskaia[1], *Tatiana Cherevatova*[1], *Olga Shchagina*[1], *Aleksander Polyakov*[1], *Oksana Ryzhkova*[1]

[1]*Research Centre for Medical Genetics, Moscow, Russian Federation*

Background/Objectives: Hypohidrotic Ectodermal Dysplasia (HED) comprises a group of rare genetic disorders affecting two or more ectodermal derivatives (hair, teeth, nails, certain glands). X-linked ectodermal dysplasia is considered the most common form of HED; however, the precise contribution of individual genes (*EDA, EDAR, EDARADD, WNT10A*) to the development of HED has never been evaluated in Russia. The objective is to determine the mutational spectrum in a cohort of 233 Russian patients affected by HED to establish the prevalence of genetic variants in the four most frequently associated genes.

Methods: Targeted gene panel (NGS), Sanger sequencing, Multiplex Ligation-dependent Probe Amplification (MLPA), as well as segregation analysis to refine the pathogenicity of variants of uncertain significance, were performed.

Results: A total of 148 *EDA* mutations (including 50 novel ones) accounted for 63.5% of cases in our series, with gross deletions/duplications affecting one or more exons of the *EDA* found in 18 patients (7.7%). It is worth mentioning that about 1/6 of all variants in the *EDA* gene are missense variants affecting amino acids Arg153, Arg155, and Arg156. Moreover, almost 11% of the detected *EDA* variants constitute deletions of more than 10 b.p. in the exon 4. Recurrent variants in the *WNT10A* gene were detected in 12 Russian patients (5.1%), and 4.7% of all detected mutations account for the *EDAR* changes.

Conclusion: Molecular studies of HED have not been conducted in Russia until now; thus, investigating the mutational spectrum of different HED genes is crucial for the elaboration of an effective diagnostic approach.

Grants: No

Conflict of Interest: Valeriia Kovalskaia full-time, Tatiana Cherevatova full-part, Olga Shchagina full-part, Aleksander Polyakov full-part, Oksana Ryzhkova full-part

P06.021.B A serie of NPR2-related idiopathic short stature

Sena Cetin[1;2], *Hamdi Cihan Emeksiz*[3], *Mustafa Gunes*[1;2], *Filiz Ozen*[2], *Elif Yilmaz Gulec*[1;2]

[1]*Istanbul Medeniyet University Faculty of Medicine, Medical Genetics, İstanbul, Türkyie;* [2]*Istanbul Goztepe Prof. Dr. Suleyman Yalcin City Hospital, Medical Genetics, İstanbul, Türkyie;* [3]*Istanbul Medeniyet University, Faculty of Medicine, Pediatric Endocrinology, İstanbul, Türkyie*

Background/Objectives: The *NPR2* (MIM*108961) gene encodes the B-type natriuretic peptide receptor (NPR-B), functioning as a guanylyl cyclase in chondrocytes. Consequently, the *NPR2* signaling cascade plays a vital role in promoting bone matrix synthesis and regulating the growth plate. Biallelic variants lead to severe skeletal dysplasia, termed 'Acromesomelic dysplasia type Maroteaux (MIM#602875)'. Conversely, heterozygous variants cause a milder form known as 'Short stature with nonspecific skeletal abnormalities (MIM#616255)', mainly classified as idiopathic short stature (ISS).

Methods: Seven patients from different families, comprising four females and three males, were referred to us with a diagnosis of short stature. The mean age for females was 12, and for males, it was 13.6. The mean height SDSs were -2.96 and -2.68, respectively. They exhibited no prominent features other than mild facial dysmorphism. A NGS-based multigene panel test was conducted for evaluation.

Results: We identified seven monoallelic *NPR2* variants, including five missense, one nonsense, and one frameshift variant. Missense variants were classified as variants of uncertain significance (VUS), while the remaining two were considered likely pathogenic according to ACMG criteria. Two missense variants segregated with short stature in related families, specifically of maternal origin. Additionally, four missense variants and one frameshift variant were identified as novel.

Conclusion: Heterozygous variants in the *NPR2* gene were reported in 2-6% of cases of ISS. Affected patients displayed mild and variable degrees of short stature without a distinct phenotype. Variants in this gene could be a significant cause of nonsyndromic short stature and should be considered for testing in affected individuals.

Grants: None.

Conflict of Interest: None declared

P06.022.C comprehensive examination of the genotypic and phenotypic characteristics of turkish marfan syndrome patients.

Ceren Alavanda[1], *Bilgen Bilge Geçkinli*[2], *Serdar Epçaçan*[3], *figen akalın*[4], *Ahmet Arman*[2]

[1]*Van Research and Training Hospital, Department of Medical Genetics, Van;* [2]*Marmara University, School of Medicine, Department of Medical Genetics, Istanbul;* [3]*Van Research and Training Hospital, Department of Pediatric Cardiology, Van;* [4]*Marmara University, School of Medicine, Department of Pediatric Cardiology, Istanbul*

Background/Objectives: Marfan syndrome (MFS) is an autosomal dominant pleiotropic disease. The phenotype is associated with ocular, cardiovascular, and skeletal anomalies. Pathogenic variants in the *FBN1* gene that encodes fibrillin-1 cause MFS. Although a diagnostic criteria (Ghent criteria: GC) has been developed for MFS, it is difficult to diagnose every patient based solely on these criteria due to variable phenotype. Therefore, molecular diagnosis is important.

Methods: A total of 47 patients (23 female, 24 male) from 41 families were included in the study. The patients were evaluated according to GC. Single nucleotide variants and copy number variations in the *FBN1* gene were analyzed using next-generation sequencing, and segregation studies were performed via Sanger sequencing.

Results: The GC scores of the patients ranged from 2 to 11. Eight patients had ectopia lentis, 7 patients had aortic root dilatation. Mitral valve prolapsus was the most common cardiac anomaly. In the *FBN1* gene, 36 different variants (15 novel, 41.6%; 21 recurrent, 58.3%) were detected. Twenty-five (69.4%) of the variants were non-truncating (missense), 11 (30.5%) were truncating (6 were nonsense, 4 were splice-site and one was frameshift). Fifteen variants (41.6%) were causing substititon of cysteine residues. Twenty-three (65.7%) variants were localized in the EGF-like domains.

Conclusion: This study indicates that not all MFS patients fully met the GC. The identified 15 novel variants contribute to the variant spectrum of the *FBN1* gene. This study also showed that the variants were unique for patients and the majority of the variants were affecting cysteine residues.

Grants: None.

Conflict of Interest: None declared

P06.023.D Cleidocranial dysplasia: A comprehensive case series from a single center

Ipek Gorusen[1], *Nursel Elcioglu*[1], *Funda kokali*[1], *Ali Mentes*[2]

[1]*Marmara University Faculty of Medicine, Department of Pediatric Genetics, İstanbul, Türkyie;* [2]*Marmara University Faculty of Dentistry, Department of Pediatric Dentistry, İstanbul, Türkyie*

Background/Objectives: Cleidocranial dysplasia(CCD) is a rare autosomal dominant skeletal dysplasia characterized by delayed fontanelle closure, clavicle aplasia/hypoplasia and dental anomalies.Heterozygous loss of function in the RUNX2 gene located on chromosome 6p21.1 causes the clinical phenotype.We aimed to present our cases to improve the knowledge of this disorder.Cleidocranial dysplasia should be considered in patients with wide fontanel, frontal and parietal bossing, dental anomalies, clavicle hypoplasia/aplasia, bone deformities and short stature.

Methods: In the case series, eleven patients who were followed up clinically with cleidocranial dysplasia will be presented.Genetic results were provided by DNA exome sequencing.

Results:A total of 11 patients (male, $n = 2$ and female, $n = 9$) were enrolled in this study.The most common clinical findings were delayed closure of the anterior fontanel (72%, 8/11), frontal bossing (81%, 9/11), clavicle hypoplasia/aplasia (90%, 10/11) and short stature (90%, 10/11).Skeletal deformities were noted in all of the patients.Hypermobile shoulders, metopic groove and dental anomalies as supernumerary teeth were frequent findings.When the family history of the patients were evaluated, it was evident that (81%, 9/11) most of them had family members with similar clinical features.Various heterozygous mutation in the RUNX2 gene was detected in four patients from three families and the genetic analysis results of remaining patients are awaited.

Conclusion: Although cleidocranial dysplasia is rare, it is important to raise awareness for early diagnosis, provide symptomatic pediatric and dental care with multidisciplinary follow-up and provide accurent genetic counselling for affected patients and their parents.

Grants:I.Gorusen: None. **N.Elcioglu:**None. **F.Kokali:**None. **A.Mentes:**None.

Conflict of Interest: None declared

P06.024.A Clinical utility of diagnostic NGS for detection CNV variants in genes associated with skeletal dysplasia

Marija Mijovic[1], *Goran Cuturilo*[1;2], *Jelena Ruml Stojanovic*[1], *Aleksandra Miletic*[1], *Brankica Bosankic*[1], *Hristina Petrovic*[1]

[1]*University Children's Hospital, Department of Clinical Genetics, Belgrade, Serbia;* [2]*Faculty of Medicine, University of Belgrade, Belgrade, Serbia*

Introduction: Copy Number Variants (CNVs) may be a mechanism of gene dysfunction. It is traditionally considered that routine diagnostic Next Generation Sequencing (NGS) technology is not suitable for detecting CNVs. The goal of the present study was to determine NGS-based CNV calling yielded in genes for skeletal dysplasia.

Materials and Methods: CNVs were extracted from results reports of clinical or whole exome sequencing of 168 consecutive patients with different skeletal dysplasia. Genome sequencing was applied in the second step, only for highly suspicious patients.

Results: Molecular diagnosis by determining the causative gene variant(s) was established for 102 out of 168 patients (60.71%). CNVs were detected in the following genes: *IFT140*: heterozygous tandem duplication of exons 27-30 confirmed by long range PCR combined with another point variant in the same gene; *DYM*: homozygous exon 14 deletion confirmed by long range PCR; *TCF12*: heterozygous deletion of exons 9-19 confirmed by MLPA. Additionally, in two patients with skeletal dysplasia large chromosomal aberration was detected (14q32.33 deletion ~2.08 Mb; 15q26.2-26.3 deletion ~5.6 Mb) and confirmed by arrayCGH. In one negative patient a small deletion of 4.2 Kb encompassing the 3'UTR region of the *RUNX2* gene was identified using whole genome sequencing.

Conclusion: Among all diagnosed patients, CNVs were found in five patients (4,90%). Our results confirm that NGS should be the test of first choice in patients with skeletal dysplasia due to the high detection rate, including the possibility of detecting CNVs.

Conflict of Interest: None declared

P06.025.B Palmoplantar keratoderma related to FAM83G gene. Clinical and molecular characterization of a new case

Amaia Lasa-Aranzasti[1], *Lorraine Glennie*[2], *Marta Codina*[1], *Mar Xunclà*[1], *Gloria Aparicio Español*[3], *Elena García-Arumí*[1], *Eduardo Tizzano*[1], *Gopal Sapkota*[2]

[1]*Vall d'Hebron, Medicine Genetics Group, Vall d'Hebron Institut de Recerca (VHIR). Department of Clinical and Molecular Genetics, Barcelona, Spain;* [2]*School of Life Sciences, University of Dundee, MRC Protein Phosphorylation and Ubiquitylation Unit, School of Life Sciences, University of Dundee, Dundee, United Kingdom;* [3]*La Vall*

d'Hebron, Dermatology Service, Hospital Universitari Vall d'Hebron, Barcelona, Spain, Barcelona, Spain

Background/Objectives: Homozygous pathogenic variants in the *FAM83G* gene have been associated with palmoplantar keratoderma in dogs and humans. FAM83G activates WNT signalling through association with protein kinase CK1α. Here we report a novel case of palmoplantar keratoderma in humans due to a pathogenic variant in the *FAM83G* gene.

Methods: We report a woman with palmoplantar keratodermia and a homozygous variant in *FAM83G* identified by analysis of candidate variants in by whole-exome-sequencing (WES). A skin biopsy was obtained to perform functional studies in cultured fibroblasts. The ability of the pathogenic FAM83G mutant to bind to CK1α and activate WNT signalling was tested in various human cell lines, including cultured fibroblasts derived from the patient.

Results: A 60-year-old female with uneventful pregnancy and normal development first exhibited symptoms such as alterations in the hair and skin of both hands, and dystrophic nails at the age 3, and was diagnosed with palmoplantar keratoderma. Physical examination revealed features including thin, curly, and sparse hair, long upper eyelid eyelashes, poor dental enamel, and palmoplantar keratoderma. Family history included parental consanguinity and two unaffected siblings. WES identified a homozygous *FAM83G* gene variant (c.794G>C, p.Arg265Pro), which were heterozygous in unaffected siblings. Overexpression assays and skin fibroblasts analysis showed that theFAM83G^{R265P} showed less stability, inability to bind CK1α and attenuated WNT signalling response

Conclusions:

We report the second case of palmoplantar keratoderma in humans resulting from *FAM83G* mutations. Functionally, this novel variant implies impaired CK1α binding and the loss of WNT signalling leading to the pathogenesis of palmoplantar keratoderma.

Conflict of Interest: None declared

P06.026.C Urine-derived stem cells: A cutting-edge, non-invasive approach for investigating osteogenesis and chondrogenesis

Schulz Alexander[1], *Steffen Uebe*[1], *Arif B Ekici*[1], *Cornelia Kraus*[1], *Mandy Krumbiegel*[1], *André Reis*[1;2], *Christian Thiel*[1]

[1]*Universitätsklinikum Erlangen, Institute of Human Genetics, Erlangen;* [2]*Universitätsklinikum Erlangen, Center for Rare Diseases Erlangen (ZSEER), Erlangen*

Background/Objectives: Obtaining samples from relevant tissues for genetic analysis in affected individuals is often challenging, especially for tissues where biopsies cannot be readily obtained such as bone and cartilage. Therefore, it is necessary to establish cell culture models that can replicate pathophysiological processes in vitro. An easily accessible source of material is urine, containing epithelial cells, but also cells with stem cell properties, known as urine-derived stem cells (USC).

Methods: We successfully isolated cells with stem cell-like properties from urine of four healthy donors and differentiated two of them into osteogenic and chondrogenic lineages.

Results: Osteogenically induced cells exhibited positive calcific depositions and a significant upregulation of expression of the bone-specific *SPP1* gene. Conversely, chondrogenically induced cells, positive for glycosaminoglycans, showed overexpression of the specific chondrocyte marker *COL2A1*. To better mimic the in vivo environment, we established a 3D self-aggregating chondrogenic spheroid model of USC, confirmed by Immunofluorescence analysis demonstrating expression of the late chondrocyte marker aggrecan in the spheroid.

Conclusion: We confirmed the abundance of stem cells in human urine and highlights their potential to study developmental processes of the bone. Notably, we demonstrated the remarkable capability of USCs to form chondrogenic spheroids as a novel in vitro model for the study of chondrogenesis. Future research is dedicated to exploring protein alterations and their effect on chondrogenesis in individuals harbouring pathogenic variants linked to growth-related disorders. Our approach centers on the utilization of CRISPR Cas9 technology, strategically applied to three candidate genes *LAMA5, UBR4*, and *SMARCA5*.

Grants: TH896 7-1

Conflict of Interest: None declared

P06.027.D A study of Polish family with scoliosis and limb contractures expands the *MYH3* disease spectrum

Agnieszka Pollak[1], *Justyna Frasunska*[2], *Pawel Turczyn*[3], *Anna Kutkowska-Kaźmierczak*[4], *Jakub Peplowski*[5], *Rafał Płoski*[1], *Beata Tarnacka*[2]

[1]*Warsaw Medical University, Department of Medical Genetics, Warsaw, Poland;* [2]*Warsaw Medical University, Department of Rehabilitation, Warsaw, Poland;* [3]*National Institute of Geriatrics, Rheumatology and Rehabilitation, Clinic of Early Arthritis, Warsaw, Poland;* [4]*Institute of Mother and Child, Department of Medical Genetics, Warsaw, Poland;* [5]*Medical University of Warsaw, The Rare Diseases Laboratory, Laboratory of Genetics, University Center for Laboratory Medicine, Warsaw, Poland*

Background/Objectives: The causes underlying the congenital spine defects are heterogeneous and comprises variety of environmental and genetic factors. Furthermore, spine defects may be component of various diseases such as: hemivertebra, Klippel-Feil syndrome, Chiari malformation, tethered cord, spina bifida, diastematomyelia and many others. Some of these deformities are caused by genetic syndromes related to pathogenic variants in many genes i.e. fibrilin -1 gene (*FBN1*), fibriline – 2 gene (*FBN2*) or myosin heavy chain (*MYH3)* and share many similar defects such as scoliosis, joint limpness, joint contractures, arachnodactyly, muscle hypoplasia, often making the accurate diagnosis difficult. A disease associated with malfunction of the *MYH3* gene is characterized by scoliosis, contractures of the V fingers, knees and elbows, dysplasia of the calf muscles, feet deformity and limb lengths asymmetry. The aim of this study was to identify a cause of musculoskeletal deformities in a three-generation Polish family by exome sequencing.

Methods: Exome sequencing followed by family replication study and comprehensive phenotype analysis.

Results: Segregation of the newly described c.866A>C variant of the MYH3 gene in the family indicates an autosomal dominant model of inheritance. The detected MYH3 variant segregates with the disease within the family. Comprehensive phenotype analysis reveals features that have never been reported to date in MYH3-related disease.

Conclusion: The presented results expands the *MYH3* disease spectrum and emphasize the clinical diagnostic challenge in syndromes harbouring congenital spine defects and joint contractures.

Grants:

Conflict of Interest: None declared

P06.028.A Short stature in Klinefelter Syndrome: new cases and a review of literature

Ivo van Bostelen[1], *Jeroen Knijnenburg*[1], *Yvonne Hendriks*[1], *Sjoerd Joustra*[1;2], *Monique Losekoot*[1]

[1]*Leiden University Medical Center (LUMC), Leiden, Netherlands;* [2]*Erasmus MC, Rotterdam, Netherlands*

Klinefelter syndrome (KS; 47,XXY) occurs in ~150:100.000 males and is characterized by tall stature, gynecomastia, small testes, and azoospermia. KS patients have a distinct growth pattern, with normal birth length and early growth, acceleration of growth velocity between 5 and 8 years old, and high-normal adult height. In rare cases of KS short stature (SS) has been reported, a substantial number of which has growth hormone deficiency (GHD). GH-therapy has been an effective treatment to reach normal adult height in these patients. Other KS patients with normal-to-short stature might have a second genetic syndrome such as achondroplasia or Prader-Willi syndrome, or carry a rearrangement on one of the X-chromosomes that can explain the phenotype. In a third subgroup no clear cause of the SS has been defined.

GHD in KS patients may result from an underdeveloped pituitary, although normal brain MRIs are also reported in this group. Hence, the causes of SS in KS remain poorly understood. Here we present three cases of KS with SS. A complete genetic work-up of SNV and CNV analysis of a SS-associated gene panel revealed no additional abnormalities in two patients. The third patient carried the previously described 47.5 kb deletion in the 3'-regulatory region of the SHOX gene on one X-chromosome. A summary of clinical characteristics of these three cases is presented and compared with the published cases to provide an informative clinical overview of the rare association of SS in Klinefelter syndrome.

Conflict of Interest: None declared

P06.029.B Discovering the Laminins: A Groundbreaking Gene in Familial Hidradenitis Suppurativa

Elena Maria Nardacchione[1], *Ronald Rodrigues Moura*[2], *Gudrun Ratzinger*[3], *Barbara Böckle*[3], *Nina Frischhut*[3], *Wolfram Jaschke*[3], *Cecilia Del Vecchio*[2], *Cecilia Giacalone*[4], *Cecile Nait-Meddour*[5], *Adamo Pio D'Adamo*[2], *Niloofar Ghaffar*[6], *Esther von Stebut-Borschitz*[6], *Lucas André Cavalcanti Brandao*[7], *Michele Boniotto*[5;5], *Matthias Schmuth*[3], *Sergio Crovella*[8], *Paola Maura Tricarico*[2]

[1]*University of Trieste, Department of Medical Surgical and Health Sciences, Trieste, Italy;* [2]*Institute for Maternal and Child Health - IRCCS Burlo Garofolo, Department of Advanced Diagnostics, Trieste, Italy;* [3]*Medical University of Innsbruck, Department of Dermatology, Venereology and Allergy, Innsbruck, Austria;* [4]*University of Trieste, Department of Life Science, Trieste, Italy;* [5]*University Paris Est Créteil, INSERM, IMRB, Translational Neuropsychiatry, Créteil, France;* [6]*University of Cologne, Department of Dermatology, Köln, Germany;* [7]*Federal University of Pernambuco, Department of Pathology, Recife, Brazil;* [8]*Qatar University, Laboratory of Animal Research Center (LARC), Doha, Qatar*

Background and Objectives: Familial hidradenitis suppurativa (fHS) is a chronic autoinflammatory condition targeting the pilosebaceous unit, inherited in an autosomal dominant pattern. Despite its genetic component, only 10% of cases are linked to mutations in γ-secretase genes. Aiming to bridge this knowledge gap, our research, conducted under the auspices of the ERAPerMed European consortium, seeks to expand understanding of the genetic predisposition to fHS.

Methods: Our study included four families, encompassing 9 individuals with fHS and 3 without. We extracted DNA from saliva samples for Whole Exome Analysis (WES), utilizing the InterOMICsPro bioinformatics pipeline for analysis. We specifically looked for new or rare variants (GnomAD allele frequency<1%) with a significant predicted impact on protein function (CADD_Phred score>15), that were heterozygous and shared among affected family members but not present in unaffected.

Results: Our analysis revealed three distinct damaging non-synonymous single nucleotide variants (SNVs) in laminin genes: one shared SNV in the *LAMA3* gene across families 1-2, a different SNV in *LAMA3* in family-3, and a SNV in *LAMA5* in family-4.

Conclusion: Considering that laminins play a critical role in the structural and functional integrity of basement membranes in the skin; this investigation highlights the laminin gene family as a newly identified factor in the pathogenesis of fHS, enhancing our understanding of fHS. It opens avenues for targeted therapeutic strategies focusing on regulating laminin gene expression, offering new hope for managing this challenging condition.

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Conflict of Interest: None declared

P06.030.C Is severe epilepsy part of the ANO5 phenotype?

Mathilde Vester[1], *Janni Majgaard Jensen*[1], *Lone Sunde*[2]

[1]*Aalborg University Hospital, Department of Clinical Genetics, Aalborg, Denmark;* [2]*Aalborg University Hospital, Department of Clinical Genetics, Aalborg*

Background: Gnathodiaphyseal dysplasia (GDD) is an autosomal dominant, skeletal disorder characterized by cemento-osseous lesions of jawbones, bone fragility, and diaphyseal cortical thickening and bowing of tubular bones.

Methods: Trio whole-exome sequencing (WES) was performed using DNA obtained after amniocentesis. Re-analysis of trio WES was performed according to a childhood epileptic encephalopathy (CHE) panel consisting of 110 genes. Whole-genome sequencing (WGS) was performed as a trio, including the patient's grandmother. Also, analysis of mitochondrial DNA (mtDNA) was performed.

Results: We present a girl who, at prenatal ultrasound at $30+4$ weeks showed multiple fractures. Trio WES revealed heterozygosity for a de novo pathogenic variant (C5) of *ANO5* (c.15.33_1535dupGAT) consistent with the fractures. However, recurrent episodes of treatment-refractory epilepsy started at day 10. Scrutinizing genes related to CHE did not reveal any pathogenic variants. Magnetic resonance imaging revealed bilateral high signal changes in the basal ganglia, brain stem and thalamus. Interestingly, the patient's grandmother and other relatives had similar changes of the brain. However, neither WGS including the patient's grandmother, nor analysis of mtDNA, provided an explanation. The girl died at age two.

Conclusion: Variants in *ANO5* have been related to GDD and myopathy with autosomal dominant and recessive inheritance, respectively. Consistently we found heterozygotity for variant in *ANO5*, in a patient with GDD. However, the patient also had severe epilepsy and characteristic cerebral findings, of which the latter were also seen in relatives, suggesting a digenic cause of the epilepsy. Alternatively, the phenotypic spectrum of *ANO5* variants may be broader than thought.

Conflict of Interest: None declared

P06.031.D Gaining further insights in the molecular bases of spontaneous cervical artery dissection

VALENTINA TREVISAN[1;2], *Irene Scala*[3;4], *Pier Andrea Rizzo*[3], *Germana Viscogliosi*[1], *Lucrezia Perri*[1], *Maria De Bonis*[5;6], *Angelo*

Minucci[6], Paola Concolino[5], Giuseppe Zampino[1;7], Marco Castori[8], Aldobrando Broccolini[3;4], Chiara Leoni[1], Giovanni Frisullo[4]

[1]Fondazione Policlinico Universitario Agostino Gemelli IRCCS, Center for Rare Diseases and Birth Defects, Department of Woman and Child Health and Public Health, Roma, Italy; [2]Catholic University of the Sacred Heart, Section of Genomic Medicine, Department of Life Sciences and Public Health, Roma, Italy; [3]Catholic University of the Sacred Heart, Department of Neuroscience, Rome, Italy; [4]Fondazione Policlinico Universitario Agostino Gemelli IRCCS, Department of Neuroscience, Sensory Organs and Chest, Roma, Italy; [5]Fondazione Policlinico Universitario Agostino Gemelli IRCCS, Clinical Chemistry, Biochemistry and Molecular Biology Operations (UOC), Roma; [6]Fondazione Policlinico Universitario Agostino Gemelli IRCCS, Departmental Unit of Molecular and Genomic Diagnostics, Roma; [7]Catholic University of the Sacred Heart, Faculty of Medicine and Surgery, Roma, Italy; [8]Fondazione IRCCS Casa Sollievo della Sofferenza, Division of Medical Genetics, San Giovanni Rotondo (FG), Italy

Background/Objectives: Spontaneous dissection of cervical arteries (sCeAD) constitutes one of main cause of stroke in young age. Currently, a hereditary component has been identified approximately in 1-5% of sCeAD patients, particularly associated with monogenic diseases such as connective tissue diseases. However, in most cases the molecular bases still need to be elucidated. This study aims to define the prevalence of clinically actionable variants in sCeAD patients.

Methods: Adults with sCeAD were consecutively enrolled if meeting at least one of the following criteria: 1) multiple vascular wall abnormalities; 2) family history of arterial dissections/cerebrovascular disease; 3) clinical/radiological/dysmorphic findings suggestive of genetic diseases. Exclusion criteria were: 1) recent trauma; 2) cardiovascular/rheumatological disease. All patients performed a total-body CT and genetic evaluation. For eligible patients, a Next Generation Sequencing (NGS) using the Clinical Exome Solution® kit was performed, followed by the design of a virtual panel of 41 genes associated with vascular fragility. Bioinformatics analysis was performed using the SOPHiA DDM platform.

Results: Among 24 patients referred, molecular analysis was performed in 12 (5 males, median age 41 years). Five variants were identified: 1 incidental finding, 2 classified as variant of unknown significance (VUS), 2 evaluated as clinically actionable in connective-tissue genes and *MYLK* related disorder.

Conclusion: Clinically actionable variants have been identified in 16% of patients. In accordance with previous literature our results suggest that heritability in sCeAD plays a relevant role, and that genetic analyses should be performed in patients with at least one suggestive criterion.

Grants: none.

Conflict of Interest: None declared

P06.032.A Structural heart disease in patients with osteogenesis imperfecta

Eva Gonzalez-Roca[1;2], *Anastasia Mocritcaia[3], Chafik Alejandro Chacur[3], Marc Ramos Jovani[4], Ana Garcia[4], Helena Florez[3], Ana Monegal[3], Nuria Guañabens[3], Pilar Peris[3]*

[1]Hospital Clínic de Barcelona, Molecular Biology CORE Laboratory, Barcelona, Spain; [2]Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Barcelona, Spain; [3]Hospital Clínic de Barcelona, Rheumatology, Barcelona, Spain; [4]Hospital Clínic de Barcelona, Cardiology, Barcelona, Spain

Background/Objectives: Osteogenesis imperfecta (OI) is a genetic disorder characterized by connective tissue abnormalities of type I collagen (COL1) that leads to bone fragility. Few is known on the effects of OI in other COL1 containing tissues, such as the heart. Thus, the presence of heart disease should be evaluated in OI Subjects. Aim of the study: to evaluate the prevalence of structural heart disease in a cohort of adult subjects with OI and its relationship with the severity of the disease and the COL1 genotype.

Methods: We conducted a retrospective study of 28 adult patients diagnosed with OI. Clinical data, including type and genetic variant, fracture history, presence of hearing loss, blue sclerae and/or dentinogenesis was obtained from medical records. Laboratory, densitometric, and echocardiographic findings were collected.

Results: 26 out of 28 patients had OI type I. Thirteen exhibited echocardiographic alterations and 10 valvulopathies (4 moderate/severe). The remaining presented asymptomatic mild valve involvement. All four subjects with moderate/severe valvulopathies had OI type I, 3 were males with several previous fractures (<12), all had blue sclerae, and none presented hearing loss nor dentinogenesis. Moreover, 2 subjects with severe valvulopathies presented a marked low BMD (T-score <-4) and > 10 previous fractures. The *COL1A1* gene analysis identified pathogenic variants affecting glycine aminoacids (p.(Gly200Asp), p.(Gly221Arg)).

Conclusions: Patients with OI may present cardiac involvement, especially heart valvular disease. Periodic screening with echocardiography seems essential to identify and manage potential severe heart complications in this disorder. Whether some mutations favor valvular disease needs further studies.

Conflict of Interest: None declared

P06.034.D BMPER pathogenic variant heterogeneity in clinical presentation and prognosis

Sarah Chamieh[1], *Bashair Magadmi[1], YLINE CAPRI[2], sophie monnot[1], Céline Dupont[2], Jonathan Levy[2], Genevieve Baujat[1], Valérie CORMIER-DAIRE[1]*

[1]Hôpital Necker, Service de Médecine Génomique, France; [2]Hôpital Robert Debré, Service de Médecine Génomique, France

Background/Objectives: BMPER is a crucial gene for bone formation. Pathogenic variants in BMPER are largely associated with diaphanospondylodysostosis (DSD), a rare and often lethal phenotype characterised by skeletal and renal abnormalities. Lesser-known BMPER variant phenotypes include ischio-vertebral dysplasia (IVD), which often overlaps with spondylocostal dysostoses (SCDO) thereby challenging both descriptions in literature. This study aims to describe the clinical presentation and prognosis of 4 cases of bi-allelic BMPER-variant IVD phenotypes from 3 families.

Methods: Molecular analysis of confirmed BMPER mutations in 3 patients and 1 foetus revealed 3 cases of homozygous inherited mutations and 1 case of compound heterozygous de novo mutation.

Results: At the prenatal stage, IVD clinically presented with 4th and 5th finger hypoplasia, impaired vertebral body ossification, sacral agenesis, thoracic kyphosis, and thoracic cage anomalies. Postnatal skeletal features included kyphoscoliosis, wide iliac wings, and impaired ossification. One patient presented 4th and 5th finger hypoplasia, a novel finding for IVD. Two patients presented spinal cord compression, one of which complicated with post-surgical paraplegia. Imaging showed ischio-spinal dysplasia with impaired cervicothoracic segmentation, wide iliac wings, and impaired ossification. Dysmorphic features included wide anterior fontanel, downslanting and eversion of palpebral fissures, and long philtrum in all 3 live cases.

Conclusion: This study deepens our clinical understanding of IVD in the context of BMPER variants. Clinical consequences are orthopaedic and respiratory but can include impaired renal function and pituitary ectopy. There is no cognitive impairment. Challenges for prognosis in IVD are determined by surgical intervention and long-term respiratory function and re-education follow-up.

Grants: No

Conflict of Interest: None declared

P06.035.A Further evidence that biallelic variants in CRIPT cause a clinically recognizable poikilodermia syndrome

marianne perriere[1], Ali Dadban[2], Celine Klein[3], clemence mordacq[4], Anais Simmonot[5], Ba Ibrahima[6], Ange-Line Bruel[7], Jean Soulier[8], Pierre Vabres[9], Laurence Faivre[10], **Bénédicte DEMEER**[11]

[1]*CHU Amiens-Picardie, Genetics department, CLAD Nord de France, Amiens, France;* [2]*CHU Amiens-Picardie, dermatology department, Amiens, France;* [3]*CHU Amiens-Picardie, Pediatric orthopedy, Amiens, France;* [4]*CHU Lille, pneumology department, lille, France;* [5]*CHU Amiens-Picardie, neuropediatry department, Amiens, France;* [6]*Bichat claude Bernard hospital/LBMMS Seqoia, Genetics department /France Genomic Medicine Plan 2025, paris, France;* [7]*Bourgogne Franche-Comté University / CHU Dijon-Bourgogne, UMR1231-INSERM/ UF2294, dijon, France;* [8]*Paris Cité University/ Saint-Louis Hospital, AP-HP, Inserm/CNRS U944/7212 /Hematology Laboratory, paris, France;* [9]*UMR1231-Inserm, Bourgogne Franche-Comté University / CHU Dijon, France, UMR1231-Inserm, / MAGEC FHU Translad, dijon, France;* [10]*Bourgogne Franche-Comté University / CHU Dijon, UMR1231-Inserm/ Genetics department, CLAD, dijon, France;* [11]*CHU Amiens-Picardie/UPJV, Genetics department, CLAD Nord de France/ CHIMERE EA7516, Amiens, France*

Poïkiloderma is characterized by cutaneous atrophy, telangiectasia and macular pigmentation changes resulting in a patchy skin appearance. This cutaneous disorder may have an acquired or genetic basis. Few poïkiloderma syndromes have been described including Bloom and Rothmund-Thomson syndrome.

CRIPT is a short highly conserved 101 amino acid cysteine rich PDZ-binding protein, expressed in a wide range of tissues. In brain, it regulates interaction between the post synaptic density-95 protein through its PDZ binding domain, critical for CRIPT-induced dendrite growth. Outside the brain, a functional role for CRIPT is unknown.

Biallelic variants in *CRIPT* were initially reported in two individuals with primordial dwarfism and characteristic facies in 2014. A formal dermatological examination was not performed. Following a further publication of an individual with a similar clinical phenotype, an OMIM disease entity was recognized, defined as "short stature, microcephaly and distinctive facies (SSMCF", MIM #615789). Here, we report the comprehensive clinical phenotype and longitudinal follow-up of two additional individuals, each of whom were homozygous for the same frameshift mutation in the *CRIPT* gene. Both individuals manifested poikilodermia, microcephaly, growth restriction, skeletal anomalies, epilepsy and intellectual disability. In addition to these previously described features, we expand the phenotypic spectrum to include severe myopia and extremity anomalies. Our additional cases provide further evidence that bilallelic variants in CRIPT result in a recognizable clinical entity of syndromic poikiloderma. The two unrelated families are of Moroccan origin, suggestive of a founder effect in this geographic area.

We summarize the genotypic and phenotypic spectrum of CRIPT-related SSMFC, including two new individuals.

Conflict of Interest: None declared

P06.036.B Unveiling Cardiovascular Dimensions in Weyers Acrofacial Dysostosis: A Unique Case and Genetic Parallels with Ellis-van Creveld Syndrome

Gorjan Milanovski[1], *Meri Kirijas[1], Teodora Brnjarchevska Blazevska[1], Tamara Savevska[1], Aleksandar Petlichkovski[1]*

[1]*Ss. Cyril and Methodius University in Skopje, Faculty of Medicine-Skopje, Institute of Immunobiology and Human Genetics, Republic of North Macedonia*

Background/Objectives: *EVC* and *EVC2* encode transmembrane proteins of the single-pass type 1, forming a constitutive association near the ciliary transition zone. Pathogenic variants in these genes result in rare autosomal recessive and dominant skeletal dysplasias, exemplified by Ellis-van Creveld syndrome (EvCS) and Weyers acrofacial dysostosis (WAD), respectively. These conditions share clinical similarities, with WAD representing the milder form and notably lacking reported cardiovascular malformations thus far.

Methods and Results: We introduce a 33-year-old female patient displaying skeletal abnormalities, short stature, dysplastic nails and teeth, postaxial polydactyly, and congenital heart disease. Successful pediatric heart surgery addressed the single atrium defect. Gene panel sequencing via the Illumina NGS platform identified a heterozygous pathogenic frameshift *EVC* variant (c.1694del; p.Ala565Valfs*23), expected to yield an absent or disrupted protein product. This premature translational stop signal aligns with observations in EvCS cases, where *EVC* loss-of-function variants are recognized as pathogenic. The variant is exceptionally rare in the gnomAD database, with a frequency of 0.00005329. Comprehensive bioinformatic analysis did not uncover any additional trans pathogenic variants in the *EVC* gene.

Conclusion: This case potentially marks the first instance of WAD accompanied by cardiovascular defects, reinforcing the genetic and phenotypic parallels between WAD and EvCS. The observation prompts inquiry into whether heart defects are exclusive to EvCS or may extend to other related disorders. Future investigations should delve into larger datasets to validate and extend our comprehension of the cardiovascular spectrum in these rare skeletal dysplasias, advancing diagnostic precision and enhancing clinical management strategies.

Conflict of Interest: None declared

P06.037.C A novel form of autosomal dominant lamellar ichthyosis due to a missense mutation in the *NKPD1* gene

Katalin Komlosi[1], *Cristina Glocker[1], Hao-Hsiang Hsu-Rehder[1], Svenja Alter[1], Julia Kopp[1], Alrun Hotz[1], Andreas Zimmer[1], Ingrid Hausser[2], Roger Sandhoff[3], Vinzenz Oji[4], Judith Fischer[1]*

[1]*Institute of Human Genetics, Medical Center - University of Freiburg, Faculty of Medicine, University of Freiburg, Freiburg;* [2]*Institute of Pathology, Heidelberg University Hospital, Heidelberg;* [3]*Lipid Pathobiochemistry Group, German Cancer Research Center, Heidelberg;* [4]*Department of Dermatology, University Hospital Münster, Münster*

Background: The identification of monogenic causes for cornification disorders has enhanced our understanding of epidermal differentiation. While a dozen genes have been identified for non-syndromic autosomal recessive congenital ichthyoses, only few are known to cause autosomal dominant forms. Autosomal dominant lamellar ichthyosis (ADLI) is a rare condition, and *ASPRV1* was the only gene linked to ADLI to date.

Patients: Seven patients from a large German pedigree presented from birth with generalized lamellar ichthyosis with

very dry skin, whitish scaling of the trunk and scalp and brownish scales on the neck, in the axillary and inguinal areas.

Methods and Results: By WES of three affected members we identified a heterozygous variant (ENST00000686631.1:c.1372G>T, p.(Val458Phe)) in the *NKPD1* gene. Co-segregation was confirmed in all affected individuals tested. *NKPD1* is a member of the KAP family of P-loop NTPases and predicted to be involved in e.g. sphingolipid metabolism. Its role in human physiology and in the cornification cycle is not yet known. Skin histology showed acanthosis and compact orthohyperkeratosis, and the ultrastructure differed clearly from that seen in *ASPRV1*-ADLI. No changes in the expression of differentiation markers in 3D skin models of patient keratinocytes were observed, however, *NKPD1* mRNA expression increased during differentiation. While stratum corneum ceramides exhibited no significant alterations, patients showed an elevated ratio of protein-bound ceramides to omega-esterified ceramides.

Conclusion: We expand the clinicogenetic spectrum of ADLI, highlighting *NKPD1*'s role in impacting ceramide metabolism and skin lipid barrier formation, thereby raising the possibility of a novel pathway leading to cornification disorders.

Conflict of Interest: None declared

P06.038.D Genotype-phenotype correlations for missense variants in the *NF1* gene: the French experience for previously described associations and identification of a new correlation

Laurence PACOT[1;2], *Dominique Vidaud*[1;2], *Audrey Sabbagh*[3], *Ingrid Laurendeau*[2], *Audrey Briand-Suleau*[4], *Audrey Coustier*[1], *Theodora Maillard*[1], *Cecile Barbance*[1], *Salah Ferkal*[5;6], *Béatrice Parfait*[1;2], *Pierre Wolkenstein*[5;6], *Eric Pasmant*[1;2]

[1]*Fédération de Génétique et Médecine Génomique, DMU BioPhyGen, AP-HP.Centre-Université Paris Cité, Hôpital Cochin, PARIS, France;* [2]*Institut Cochin, Inserm U1016, CNRS UMR8104, Université Paris Cité, CARPEM, PARIS, France;* [3]*Université Paris Cité, IRD, UMR261, MERIT, PARIS, France;* [4]*Laboratoire de biologie médicale multisites SeqOIA, PARIS, France;* [5]*Hôpital Henri Mondor, Assistance Publique-Hôpitaux de Paris (AP-HP), Département de Dermatologie, CRETEIL, France;* [6]*INSERM, Centre d'Investigation Clinique 1430; Hôpital Henri-Mondor, Assistance Publique-Hôpitaux de Paris (AP-HP), Centre de référence des neurofibromatoses, CRETEIL, France*

Background/Objectives: Neurofibromatosis type 1 (NF1) is a clinically heterogeneous genetic disorder with an autosomal dominant inheritance resulting from loss-of-function variants in the *NF1* gene. Only a few genotype-phenotype correlations have been published for some missense variants in *NF1* in international cohorts. We describe our experience of these variants in the French cohort.

Methods: We compared the main clinical features of patients with those of a reference NF1 cohort.

Results: Clinical data were recorded from patients with missense variants modifying neurofibromin at the following codons: Met1149, Arg1809, Arg1276, Lys1423 and codons 844 to 848 (2, 21, 23, 31, and 25 patients respectively). None of the patients with missense variants at Met1149 or Arg1809 had neurofibromas. Cutaneous neurofibromas were less frequent in patients with missense variants at Arg1276 compared to the reference cohort, whereas spinal neurofibromas were more frequent. Patients with mutations at codons Arg1276, Lys1423 or 844 to 848 developed plexiform neurofibromas more frequently. Skeletal abnormalities were more frequently associated with mutations at Lys1423 and at codons 844-848. Cutaneous neurofibromas appeared less frequent and optic pathway gliomas more frequent in patients with missense variants at codons 844-848. We describe for the first time the association between a moderate form of NF1 and missense variants affecting neurofibromin at position Arg1204.

Conclusion: The description of the French cohort is in line with previously reported associations, except for missense variants affecting codons 844-848 of neurofibromin. We describe a new genotype-phenotype correlation, paving the way for future studies of NF1 pathogenic variants.

Conflict of Interest: None declared

P06.039.A Spondyloperipheral dysplasia caused by a non-coding 3-prime-UTR variant in *COL2A1* molecularly characterized using prime editing

Roberto Mendoza-Londono[1;2], *Ryan Marks*[2;3], *Lucie Dupuis*[1], *Gregory Costain*[1;2;3], *Megan Dickson*[1], *Shah Ahmed*[3], *Joseph Kozman*[3], *Evgueni Ivakine*[2;3;4]

[1]*Division of Clinical and Metabolic Genetics, Department of Paediatrics, The Hospital for Sick Children and University of Toronto, Toronto, Canada;* [2]*Department of Molecular Genetics, University of Toronto, Toronto, Canada;* [3]*Program in Genetics & Genome Biology, The Hospital for Sick Children, Toronto, Canada;* [4]*Department of Physiology, University of Toronto, Toronto, Canada*

Consortium: Care4Rare Canada Consortium

Background: Spondyloperipheral dysplasia is characterized by short stature, short extremities and brachydactyly caused by pathogenic variants in *COL2A1*. Structural perturbations that interrupt the assembly of the protein lead to COL2A1-related dominant negative effects. We describe an adult male who presented at birth with disproportionate short stature, narrow thorax, platyspondyly, short hands and feet, and scoliosis. Genome sequencing identified a de novo single variant in the 3' untranslated region (UTR) of *COL2A1* (NM_001844.5:c.*161A>G).

Methods: We hypothesized that the variant generates a cryptic splice site, leading to the generation of pathogenic alternative C-terminal isoforms. We utilized prime editing to model the variant in the HAP1MLH-/- human cell line and characterize splicing outcomes. Homozygous and heterozygous mutant cell lines were established, and RT-PCR was used to identify alternative COL2A1 isoforms involving exon 52 to the 3'UTR proximal to the variant described.

Results: Isolation and sequencing of alternative RT-PCR COL2A1 isoforms indicated the formation of novel isoforms with altered exon 52-54 splicing at moderate frequency. The predominant isoform generates a stop-loss variant, producing 40 de-novo amino acids at the conserved c-terminus and likely acts in a dominant negative manner to cause disease.

Conclusion: To date, only a limited number of 3' UTR changes associated with disease have been reported. We present a patient that remained molecularly undiagnosed until detailed analysis of the non-coding regions of the gene was completed. Moreover, we demonstrate how prime editing can be a rapid and efficient tool to model and characterize variants of unknown significance for meaningful interpretation.

Conflict of Interest: None declared

P06.040.B Partial deletion of TTN gene in two brothers affected by arthrogryposis multiplex congenita

Johanna Naab[1], *Steffi Döhnert*[1], *Birgit Eichhorn*[1], *Susanne Anders*[1], *Alexander Schulz*[2], *Mirjam Klaus*[1]

[1]*Mitteldeutscher Praxisverbund Humangenetik, Dresden, Germany;* [2]*Mitteldeutscher Praxisverbund Humangenetik, Erfurt, Germany*

Consortium: MVZ Mitteldeutscher Praxisverbund Humangenetik

Background: Titin, encoded by the gene *TTN* (OMIM *188840), is an important structural protein of the sarcomer and expressed in the cardiac and skeletal muscles. Pathogenic variants of *TTN* are associated with a variety of skeletal muscle diseases and cardiomyopathies with autosomal dominant or autosomal recessive inheritance. *TTN* is also discussed as candidate gene for distal arthrogryposis. We report on two brothers with arthrogryposis multiplex congenita and normal cognitive development.

Methods: A clinical exome diagnostic via sequencing by synthesis and an array-cgh analysis were conducted for both brothers on DNA from peripheral blood.

Results: We identified a heterozygous deletion in 2q31.2 for both patients. The deletion interval affects the *TTN* gene partially and includes exon 1 to 173 of 312 exons (NM_133378.4), thus likely resulting in haploinsufficiency. No other likely pathogenic or pathogenic variant was detected by the performed analyses, especially not in *TTN*. The deletion interval also partially involves the neighbouring gene *CCDC141* (OMIM *616031), for which no association to a disease is known so far.

Conclusion: Partial deletions affecting *TTN* are rarely described in literature. None of the already reported deletions encompasses the first coding exon of *TTN*. We propose the deletion as possibly causative for the phenotype of both patients, although an assured genotype-phenotype-correlation is hardly possible. Other affected and unaffected family members should be examined to further investigate the pathogenicity of the identified deletion.

Conflict of Interest: None declared

P06.041.C A homozygous synonymous *FAM111A* variant leading to FAM111A deficiency is associated with craniectodermal dysplasia and postnatal growth retardation

Loisa Dana Bonde[1], *Ibrahim Abdelrazek*[2], *Lara Seif*[1], *Malik Alawi*[3], *Khaled Matrawy*[4], *Karim Nabil*[5], *Ebtesam Abdalla*[2], *Kerstin Kutsche*[1], *Frederike L. Harms*[1]

[1]*University Medical Center Hamburg-Eppendorf, Institute of Human Genetics, Hamburg, Germany;* [2]*Alexandria University, Medical Research Institute, Department of Human Genetics, Alexandria, Egypt;* [3]*University Medical Center Hamburg-Eppendorf, Bioinformatics Core, Hamburg, Germany;* [4]*Alexandria University, Medical Research Institute, Diagnostic Radiology and Medical Imaging Department, Alexandria, Egypt;* [5]*Alexandria University, Faculty of Medicine, Department of Ophtamology, Alexandria, Egypt*

Background/Objectives: FAM111A (family with sequence similarity 111 member A) is a member of the family of trypsin-like serine proteases and removes covalent DNA-protein cross-links during DNA replication, which helps to prevent replication stress. De novo heterozygous *FAM111A* variants cause skeletal dysplasias, including the milder Kenny-Caffey syndrome (KCS) and the lethal osteocraniostenosis (OCS).

Methods: Clinical, genomic, and functional studies, including RNA analysis, immunoblotting, cell proliferation and viability assay, and cell cycle analysis by FACS, using subject- and control-derived fibroblasts were performed.

Results: We report two siblings born to consanguineous parents with craniofacial features, postnatal growth retardation, and ectodermal dysplasia. Whole-exome sequencing revealed a homozygous synonymous *FAM111A* variant NM_001312909.2: c.81G>A; p.Pro27= in both siblings that affects the last base of exon 4. We observed aberrantly spliced *FAM111A* transcripts and nearly absent FAM111A protein in patient-derived fibroblasts. Following the induction of covalent DNA-protein cross-links by the topoisomerase I inhibitor camptothecin, we identified a tendency towards a reduced proportion of metabolically active cells in patient compared to control fibroblasts. However, we could not detect a significant difference in cell cycle progression between patient and control cells.

Conclusion: Our data suggest that loss of FAM111A is associated with a novel autosomal recessive form of craniectodermal dysplasia with proportionate short stature that shows clinical overlap with autosomal dominant KCS. Similar to the proposed pathomechanism for KCS and OCS, a reduced ability to deal with replication stress induced by DNA-protein cross-links may underlie the autosomal recessive *FAM111A*-related disorder.

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Conflict of Interest: None declared

P06.042.D A familial UBA2 variant presenting with isolated skeletal involvement

ABDULLAH SEZER[1;2], **Zeynep Özdemir**[2], *Erdem Özkan*[3], *Prof Dr Semra Cetinkaya*[4]

[1]*Dr. Sami Ulus Kadın Doğum, Çocuk Sağlığı ve Hastalıkları Eğitim ve Araştırma Hastanesi, Medical Genetics, Ankara, Türkyie;* [2]*Ankara Etlik Şehir Hastanesi, Medical Genetics, Ankara, Türkyie;* [3]*Ankara Etlik Şehir Hastanesi, Radiology, Ankara, Türkyie;* [4]*Dr. Sami Ulus Kadın Doğum, Çocuk Sağlığı ve Hastalıkları Eğitim ve Araştırma Hastanesi, Pediatric Endocrinology, Ankara, Türkyie*

Background/Objectives: When considered from a syndromic perspective, Developmental Dysplasia of the Hip (DDH) could be attributed to some syndromes, such as Aplasia Cutis Congenita with Ectrodactyly Skeletal Syndrome (ACCES, OMIM #619959), caused by haploinsufficiency of the *UBA2* gene. This syndrome is extremely rare and presents with growth retardation, dysmorphic facial features, neurodevelopmental delay, skeletal problems such as ectrodactyly and scoliosis, skin findings such as aplasia cutis, and some internal organ problems. We present here a unique presentation of ACCES syndrome exhibiting isolated spondyloepiphyseal involvement.

Methods: A 13-year-old female patient presenting with disproportionate short stature and bilateral DDH was neurodevelopmentally normal and had mild dysmorphic facial features, and her father had osteoarthritis in addition to these findings. The dominant inheritance pattern in the pedigree suggested the prediagnosis of spondyloepiphyseal dysplasia tarda. WES analysis was conducted for the daughter using genomic DNA extracted from peripheral blood lymphocytes. The familial segregation analysis of the variants was evaluated using Sanger sequencing.

Results: The WES analysis has identified a novel heterozygous variant, NM_005499.2:c.460-2A>G, in the *UBA2* gene. It is shown that the father carried the same variant of the *UBA2* gene.

Conclusion: The *UBA2* gene in the SUMOylation pathway, which is known to cause degenerative skeletal problems, may cause spondyloepiphyseal dysplasia tarda. This case report investigates the potential phenotypic spectrum of conditions associated with the *UBA2* gene by emphasizing the distinctive clinical features of our case and highlighting the importance of comprehensive differential diagnosis in the evaluation of patients with bilateral DDH, short stature, and spondyloepiphyseal problems.

Conflict of Interest: None declared

P06.045.C Cancer incidence in neurofibromatosis 1: searching for subgroups associated with increased risk

Roope Kallionpää[1], *Jussi Leppävirta*[2], *Elina Uusitalo*[1], *Heli Ylä-Outinen*[1;3], *Minna Pöyhönen*[4;5], *Sirkku Peltonen*[2;6;7;8;9;10], *Juha Peltonen*[1]

[1]*University of Turku, Institute of Biomedicine, Turku, Finland;* [2]*Turku University Hospital, Department of Dermatology, Turku, Finland;* [3]*Turku University Hospital, Department of Pulmonary Diseases, Turku, Finland;* [4]*University of Helsinki, Department of Medical and Clinical Genetics, Helsingin yliopisto, Finland;* [5]*Helsinki University Hospital Diagnostic Center, Department of Genetics, HUSLAB, HUS, Finland;* [6]*University of Helsinki, Department of Dermatology and Allergology, Helsingin yliopisto, Finland;* [7]*Helsinki University Hospital, Skin and Allergy Hospital, HUS, Finland;* [8]*University of Gothenburg, Department of Dermatology and Venereology, Institute of Clinical Sciences, Sahlgrenska Academy, Gothenburg, Sweden;* [9]*Sahlgrenska University Hospital, Department of Dermatology and Venereology, Region Västra Götaland, Gothenburg, Sweden;* [10]*University of Turku, Department of Dermatology and Venereology, Turku, Finland*

Background/Objectives: Neurofibromatosis 1 (NF1) is an autosomal dominant disease that is associated with a high risk for cancer, yet there are few means to predict the personal cancer risk of an individual with NF1. The present study aims to search for indicators of cancer incidence among individuals with NF1.

Methods: The Finnish NF1 cohort consists of 1811 individuals, whose NF1 diagnoses have been verified using medical records. The cancers of individuals with NF1 were obtained from the Finnish Cancer Registry and summarized using standardized incidence ratio (SIR).

Results: Individuals with NF1 and a history of a prior cancer displayed a SIR of 3.69 (95% CI 2.79-4.76) for second primary cancers, which did not differ from the SIR of 4.15 (95% CI 3.68-4.66) for the first cancers. In addition, there were no significant differences between those with a history of any specific prior cancer compared with those without history of cancer. Two cancers were identified during pregnancy or the year following the delivery among women with NF1 (SIR 6.44, 95% CI 1.07-19.89), and 15 cancers were detected within 1-10 years after pregnancy (SIR 7.54, 95% CI 4.15-12.41). These SIRs did not differ from that of women with NF1 aged 20-49 years, and without a known history of deliveries (SIR 8.63, 95% CI 6.08-11.81).

Conclusion: The history of a prior cancer or pregnancy are not predictors of cancer incidence in NF1.

Grants: Children's Tumor Foundation Young Investigator Award (Award ID: 2023-01-006; https://doi.org/10.48105/CTF.CTF-2023-01-006.pc.gr.172004), Cancer Foundation Finland, Turku University Hospital.

Conflict of Interest: None declared

P06.046.D Deciphering multifaceted connections between NOTCH1 and SERCA2 proteins in the Darier's disease: a paradigmatic molecular network in the pathogenesis of a Mendelian genodermatosis

Valeria Barili[1], *Erika De Sensi*[1], *Ilenia Cannizzaro*[1], *Antonietta Taiani*[1], *Anita Luberto*[1], *Enrico Ambrosini*[2], *Davide Martorana*[2], *Vera Uliana*[2], *Patrizia Caggiati*[2], *Andrea Gherli*[1;3], *Anna Montanaro*[1;3], *bruno Lorusso*[1], *Giovanni Roti*[1;3], **Antonio Percesepe**[1;2]

[1]*University of Parma, Department of Medicine and Surgery, Parma, Italy;* [2]*University Hospital of Parma, Medical Genetics, Parma, Italy;* [3]*University Hospital of Parma, Hematology and BMT Unit, Parma, Italy*

Background/Objectives: Darier's Disease(DD) is a rare, dominantly inherited genodermatosis with the loss of desmosomal adhesion and abnormal keratinization, caused by variants in *ATP2A2*. *ATP2A2* encodes for a SERCA2 protein, a ubiquitously expressed cellular pump responsible for calcium translocation from cytosol to endoplasmic reticulum. The molecular mechanism underneath *ATP2A2* alterations is not known. Intriguingly, in other disease models SERCA2 is a master regulator of NOTCH1 signaling and SERCA2 inhibition causes NOTCH1 pathway inactivation. The project aims to define a DD transcriptional profile and the genetic, biochemical and physiological impact of DD *ATP2A2* alterations on NOTCH1 signaling and/or on other new pathways.

Methods: We collected patients' skin biopsies: i)to perform transcriptomic RNA-sequencing analysis on affected and unaffected subjects; ii)to generate keratinocytes and fibroblasts primary cells for expression analysis and immunofluorescence assays of the NOTCH1-downstream effector targets (*HES1*, *HEY1*, *c-MYC*); iii)to investigate NOTCH1 signaling deregulations through immunohistochemistry on FFPE bioptic samples. Furthermore, we generated plasmids containing patients' specific *ATP2A2* variants to evaluate their impact on the NOTCH1 signaling and the proteins interaction by performing in vitro functional and biochemical assays.

Results: We define a DD transcriptomic gene signature, focused on metabolic, ribosomal and immunological deregulations. We pointed out a molecular dependency of NOTCH1 pathways and *ATP2A2* defects through the effects evaluation of patient-specific variant overexpression and by ex vivo assays based on patient-derived primary cell lines and tissue IHC.

Conclusion: By understanding the molecular network between SERCA2 and NOTCH1 in DD, novel and effective therapeutic approaches may be developed and tested in DD

Conflict of Interest: Valeria Barili PRIN 2022, Erika De Sensi: None declared, Ilenia Cannizzaro: None declared, Antonietta Taiani: None declared, Anita Luberto: None declared, Enrico Ambrosini: None declared, Davide Martorana Biologist, Vera Uliana MD, Patrizia Caggiati Biologist, Andrea Gherli: None declared, Anna Montanaro: None declared, bruno Lorusso: None declared, Giovanni Roti Professor, Antonio Percesepe Equipment and supplies, PRIN 2022, Professor

P06.048.B Clinical and genomic profile of genodermatoses in an Indian cohort

Priyanka Patra[1], *Dhanya Lakshmi Narayanan*[1], *Gandham Sri Lakshmi Bhavani*[1], *Raghavendra Rao*[2], *Katta Girisha*[1;3], *Anju Shukla*[1], *Vivekananda Bhat*[1]

[1]*Kasturba Medical College, Manipal, Manipal Academy of Higher Education, Manipal, Medical Genetics, Manipal, India;* [2]*Kasturba Medical College, Manipal, Manipal Academy of Higher Education, Manipal, Department of Dermatology, Venereology and Leprosy, Manipal, India;* [3]*Sultan Qaboos University, Muscat, Oman*

Background: Genodermatoses are inherited disorders of skin with extensive genetic heterogeneity. Broad spectrum tests like exome sequencing have accelerated the rate of molecular diagnosis of genodermatoses. We describe clinical characteristics and genomic spectrum of genodermatoses from a tertiary care centre in India.

Methods: After clinical evaluation, affected individuals & their families were divided into broader subgroups and offered targeted gene testing or whole exome sequencing based on their clinical diagnosis.

Results: Over a period of 10 years, 155 individuals were clinically diagnosed as genodermatoses and offered genetic testing. Fifty-four individuals from 50 families opted for genetic testing. Consanguinity was recorded in 48% (24/50) families. Molecular diagnosis was achieved in a total of 45 out of 50 families (90%): five (11%) by targeted testing and in 40 (89%) by exome sequencing. Thirty-three monogenic disorders were identified in

50 families with predominance of autosomal recessive inheritance (25/33; 76%). The commonest group was disorders of cornification (9/33; 27%). Eighteen (36%) of 50 identified variants were novel. Forty-eight (48/50; 96%) variants were classified as disease causing according to ACMG/AMP guidelines. Copy number variant analysis of exome sequencing data was able to identify diagnosis in five families. Reproductive counselling was offered to 44% (22/50) of the families and prenatal testing was opted by 41% (9/22) of them.

Conclusion: Our study expands the genetic spectrum of genodermatoses in Indian population.

Grants

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Conflict of Interest: None declared

P06.049.C Geroderma osteodysplasticum: new data regarding the clinical evolution of a mild phenotype.

Isabelle Bacchi[1;2], *Stefano Giuseppe Caraffi*[2], *Roberta Zuntini*[2], *Allessandra Terracciano*[3], *Simona Viglio*[4], *Andrea Superti-Furga*[5;6], *Antonio Novelli*[3], *Livia Garavelli*[2]

[1]*Alma Mater Studiorum University of Bologna, Department of Medical and Surgical Science, Bologna, Italy;* [2]*Azienda USL-IRCCS di Reggio Emilia, Medical Genetics Unit, Reggio Emilia, Italy;* [3]*Bambino Gesù Children's Hospital, IRCCS, Laboratory of Medical Genetics, Translational Cytogenomics Research Unit, Rome, Italy;* [4]*University of Pavia, Biochemistry Unit, Department of Molecular Medicine, Pavia, Italy;* [5]*Lausanne University Hospital (CHUV) and University of Lausanne, Division of Genetic Medicine, Lausanne, Switzerland;* [6]*Genetica AG, Zurich and Lausanne, Switzerland*

Background/Objectives: Geroderma osteodyplasticum (GO) is a *GORAB*-related autosomal recessive disease: bone fragility, loose skin, hyperextensible joints and progeroid features are the main characteristics. This is a rare underdiagnosed condition with variable expressivity; there is limited amount of data regarding its natural history, especially concerning those who present mild phenotypes. Here we provide information on the good clinical evolution of a 20-year-old woman with GO.

Methods: Collagen production was evaluated on fibroblasts from a skin biopsy. Direct sequencing of selected genes and then Clinical Exome Sequencing (CES) were performed.

Results: At 8 months the proband was hospitalized for multiple spontaneous vertebral fractures. Persistent open bregmatic fontanelle, wormian bones, soft skin, joint hyperextensibility, malar hypoplasia were her main clinical features. She was treated with biphosphonate therapy for a year with success. The patient has consistently maintained a height at the upper limits of the normal range, in line with the family's genetic target. At 4 years, spine X-Ray findings showed great improvement. Beighton score is currently 0, bone density is normal. She has undergone dental interventions for dental overcrowding and prognathism. CES analysis identified two rare variants: c.14G>A and c.256delC in compound heterozygosity in the *GORAB* gene (NM_152281), respectively inherited from the mother and the father. They are predicted to lead to premature stop codon formation and likely transcript degradation, and truncating/loss-of-function variants are a known pathogenetic mechanism for *GORAB*.

Conclusions: This case provides new information about GO phenotype highlighting the presence of a novel variant not previously described in the literature.

Conflict of Interest: None declared

P06.050.D Enhancing molecular diagnosis of neurofibromatosis type 1: cultured melanocytes unveil somatic mosaicism in patients screened negative in blood samples

Annelies De Jaegher[1;2], *Suzanne Vanhauwaert*[1;2;3], *nathalie goderis*[1;2], *Hélène Verhaeghe*[1;2], *Aude Beyens*[1;2], *Kathleen Claes*[1;2;3]

[1]*Department of Biomolecular Medicine, Ghent University, Ghent, Belgium;* [2]*Center for Medical Genetics Ghent, Ghent University Hospital, Ghent, Belgium;* [3]*Cancer Research Institute Ghent (CRIG), Ghent University and Ghent University Hospital, Ghent, Belgium*

Objectives: Neurofibromatosis type 1, characterized by multiple cafe au lait macules (CALMs) and neurofibromas, affects 1 in 3500 individuals worldwide. While most diagnosed patients exhibit identifiable NF1 variants, detection rates are lower in sporadic cases, possibly due to mosaicism. This study aimed to uncover the underlying pathogenic NF1 variant within melanocytes from patients who previously tested negative in blood samples.

Methods: Melanocytes, harvested from skin biopsies taken from CALM were cultured from patients with (segmental) neurofibromatosis type 1, previously yielding negative blood screening results. We employed gDNA and cDNA sequencing of NF1, alongside MLPA analysis.

Results: Melanocytes from >1 CALM were available from 14 patients, identifying a common first hit in NF1 in 86% of the samples. Targeted deep sequencing failed to detect the variant in lymphocyte DNA (if available). The majority of identified first hits were nonsense variants (67%) and large deletions (25%). Second hits were not detected in 3/14 patients, but MLPA failed for 2 of them. Additionally, for 9 patients only melanocytes from 1 CALM were available, and in 78% of the samples, at least one pathogenic NF1 variant was found. For 5 additional patients, the analysis is ongoing.

Conclusion: The proposed workflow, addressing challenges such as correct biopsy collection and melanocyte growth, enhances the molecular diagnosis for mosaic NF1 cases and is implemented in clinical setting. Most patients are young adults seeking reproductive advice. Further investigations are warranted to assess transmission risks to offspring. Establishing an international database for somatic cases could serve as an invaluable resource for evaluating such risks.

Conflict of Interest: None declared

P06.051.A Discovering Common and Distinct Pathogenic Mechanisms between Alopecia Areata, Vitiligo and Hashimoto's Thyroiditis: an Integrated Gene Expression Analysis

Hadis Abdolahzadeh[1], *Yasmina Gossmann*[1], *Regina C. Betz*[1], *F. Buket Basmanav*[1]

[1]*Institut für Humangenetik, Universitätsklinikum Bonn, Bonn, Germany*

Background/Objectives: Alopecia areata (AA) is a common hair loss disorder with an estimated lifetime risk of 2%. AA is a T-cell mediated autoimmune disease directed against the hair follicle and affected individuals are at increased risk of developing Hashimoto's thyroiditis (HT) and vitiligo. The aim of the current study is to identify common and distinctive gene expression signatures between AA, HT and vitiligo to gain pathobiological insights into their comorbid and non-comorbid presentations, respectively.

Methods: Microarray-based transcriptomic datasets from AA, HT and vitiligo patients and their respective controls were retrieved from public resources and analyzed to identify common and distinct gene signatures (i.e differentially expressed genes; DEGs) using GEO2R and the Venny 2.1.0 tool. Enrichment analyses were performed by the Enrichr tool.

Results: We identified 1,659, 3,390 and 257 DEGs in AA, HT and vitiligo, respectively. AA shared 288 common DEGs with HT and 33

with vitiligo. The DEGs common to AA and HT were significantly enriched for pathways related to immune response, such as interferon gamma signaling, TCR signaling, and the adaptive immune system. The DEGs common to AA and vitiligo were significantly enriched for biological processes including cellular response to epinephrine stimulus, SNARE complex assembly and negative regulation of exocytosis.

Conclusion: Our results suggest that AA and HT share a common pathobiology over several components of the immune response, while the dysregulation or disruption of the exocytosis-mediated melanosome-transfer from melanocytes to keratinocytes could be a possible mechanistic link between AA and vitiligo.

Grants: Deutsche Forschungsgemeinschaft (German Research Foundation)

Conflict of Interest: None declared

P06.052.B *CLDN1* Arg81His founder variant causes Ichthyosis, leukocyte vacuoles, alopecia and sclerosing cholangitis (ILVASC) syndrome in Moroccan Jews

ilana aminov[1], *Vadim Dolgin*[1], *Elena Didkovsky*[2], *Anna Pikovsky*[3], *Noam Hadar*[1], *Eyal Kristal*[4], *Galina Ling*[4], *Idan Cohen*[5], *Uri Zilberman*[6], *Ohad Shmuel Birk*[1;5;7], *Marina Eskin-Shwartz*[1;7;8]

[1]*The Morris Kahn Laboratory of Human Genetics and National Center for Rare Diseases, Faculty of Health Sciences, Ben-Gurion University, Microbiology, immunology and genetics, Be'er Sheva, Israel;* [2]*Institute of Pathology, Rabin Medical Center, Petah Tikva, Israel;* [3]*Oral Medicine Unit, Department of Oral and Maxillofacial Surgery, Soroka University Medical Center, Be'er Sheva, Israel;* [4]*Outpatient Care and Pediatric Gastroenterology Units, Saban Pediatric Medical Center, Be'er Sheva, Israel;* [5]*The Shraga Segal Department of Microbiology, Immunology and Genetics, Faculty of Health Sciences, Ben-Gurion University, Be'er Sheva, Israel;* [6]*Pediatric Dental Unit, Barzilai Medical Center, Ashkelon, Israel;* [7]*Genetics Institute, Soroka University Medical Center, Be'er Sheva, Israel;* [8]*Faculty of Health Sciences, Ben-Gurion University, Be'er Sheva, Israel*

Background/Objectives: Ichthyosis, leukocyte vacuoles, alopecia, and sclerosing cholangitis (ILVASC), is a rare disease of autosomal recessive inheritance, resulting from loss of function of the tight junction protein claudin 1. Its clinical presentation is variable, and is characterized by liver and ectodermal involvement. Most ILVASC cases are attributed to homozygous truncating variants in *CLDN1*, but recently single missense variant CLDN1 p.Arg81His has been associated with isolated ichthyosis phenotype in a family of Moroccan Jewish descent. We aimed to delineate clinical features of ILVASC patients with p.Arg81His variant, to identify and determine carrier rate, and to examine for possible founder origin of this variant in Moroccan Jews.

Methods: For genotyping, whole exome sequencing, Sanger sequencing and restriction fragment length polymorphism analysis were used. Haplotyping was done using SNP arrays. Skin biopsies were assessed by light and electron microscopy.

Results: Seven patients with ILVASC from four families of North African Jewish ancestry, all harboring CLDN1 p.Arg81His variant, were found to have teeth, hair and liver/bile duct involvement, characteristic of ILVASC. Transmission electron microscopy of the affected skin revealed distorted tight junction architecture. Haplotype analysis in the vicinity of the *CLDN1* gene showed this variant to represent a founder variant in Moroccan Jews with an estimated carrier frequency of 1:220.

Conclusion: We emphasize the existence of diverse extra-cutaneous findings in individuals with ILVASC, bearing the CLDN1 p.Arg81His variant. Our data further support the pathogenicity of this variant and show it to represent a founder variant in Jews of Moroccan descent.

Conflict of Interest: None declared

P06.053.C Molecular diagnosis of hereditary ichthyosis in paediatric patients

Diana Monclús Arroyo[1], *Iván Monge Lobo*[1], *Barbara Fernández Garoz*[1], *Natalia Blanco Calvo*[1], *Elvira Cañedo Villarroya*[1], *Salvadora Aleza Esteras*[1], *Anna Duat Rodríguez*[1], *Alejandra Docampo Cancela*[1], *Saturnino Santos*[1], *Lucero Noguera Morel*[1], *Antonio Torrelo*[1], *Ángela Hernández-Martín*[1], **Nelmar Valentina Ortiz Cabrera**[1]

[1]*Hospital Infantil Universitario Niño Jesús, Madrid, Spain*

Molecular diagnosis of hereditary ichthyosis in pediatric patients

Background/Objectives: Congenital ichthyosis are a group of hereditary disorders of keratinization. Clinical findings are thickening of skin and desquamation, growth restriction, hearing and eye complications, infections, and thermodysregulation. A precise genetic diagnosis is crucial for proper genetic counselling and surveillance.

The aim of the study is to describe the phenotype and genotype of our sample

Methods: We show the data of cases studied at our department with clinical suspicion of from June 2018 to December 2023. Different techniques were used based on clinical suspicion. For suspected *STS* related ichthyosis, we used techniques oriented to copy number variation (CNV) detection. For suspected autosomal recessive congenital ichthyosis (ARCI) whole exome sequencing filtering by a virtual panel of 124 genes related to ichthyosis was used.

Clinical phenotype was described as mild, moderate, or severe.

Results: 25 patients were studied. A positive result was found in 88% of patients, non-conclusive results in 4% and negative results in 8%. The most common result was CNV involving *STS* in 30% (n = 7) of positive results, followed by ARCI related to *ABCA12* gene 13% (n = 3), *PNPLA1* and *TGM1* 9% (n = 2) each, and the rest of genes only were detected in one patient each.

Most of patients (55%) had a mild presentation, just 5% had a severe presentation.

Conclusion: We observed a high positivity rate among cases with clinical suspicion of ichthyosis referred for study in our laboratory. Clinical phenotype accurately oriented the genotyped encountered.

Conflict of Interest: None declared

P06.055.A Genetic aspects of IP locus in Incontinentia Pigmenti patients with silent autoimmunity

Ezia Spinosa[1], *Stefania Picascia*[1], *Mattia Ritorti*[1], *Carmela Casale*[1], *Alete Colella*[1], *Alessandra Pescatore*[1], *Maria Brigida Lioi*[2], *Matilde Valeria Ursini*[1], *Francesca Fusco*[1]

[1]*Institute of Genetics and Biophysics "A. Buzzati-Traverso" CNR, Naples, Italy;* [2]*Università degli Studi della Basilicata, Dipartimento di Scienze, Potenza, Italy*

Background/Objective: Incontinentia Pigmenti (IP; OMIM#308300) is a rare genetic disorder due to IKBKG/NEMO mutations. Recently, we detected neutralizing interferon (IFN)-α2 and IFN-ω Auto-Abs in 25% of IP patients, pre-existing to SARS-CoV-2 infection [1]. Here, we molecularly characterize the cohort of IP patients, analyzed by neutralizing assays in collaboration with IMAGINE Institut.

Methods: Clinical data and biological samples (DNA and plasma/serum) from 21 italian IP family trios, were collected in the Incontinentia Pigmenti Genetic Biobank (IPGB (http://www.igb.cnr.it/ipgb) and stored at -80°C. Additionally, PBMC from 4 patients were isolated by Ficoll-Plaque gradient centrifugation. A long-range PCR, Sanger sequencing were performed to identify the IP locus alteration.

Results: All patients (mean age 30 yrs; range 1-60 yrs) exhibited conventional IP skin phenotype. The alteration in IP locus was identified in 81% (17/21). Particularly, NEMOΔ4-10 recurrent deletion was found in 14 patients and a point mutation in 3 patients (c.628_651delinsCG, c.646delG; c.976_978del).

Conclusion: These results suggested that hidden autoimmunity is a new clinical aspect of IP, and further genetic aspects should be investigated.

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[1]Bastard P, et al. Science. 2020.

Conflict of Interest: None declared

P06.056.B Inherited Epidermolysis Bullosa - lessons learned from the highly inbred Bedouin community.

Ahmad Bleydi[1], *Olga Shor*[2], *Danielle Landau*[2;3], *Ohad Shmuel Birk*[1;3;4], *Marina Eskin-Shwartz*[1;3;4]

[1]*The Morris Kahn Laboratory of Human Genetics, National Center for Rare Diseases, at the Faculty of Health Sciences, Ben-Gurion University, Beer-Sheva, Israel;* [2]*Saban Pediatric Medical Center for Israel, Soroka Medical Center, Beer Sheva, Israel;* [3]*Faculty of Health Sciences, Ben-Gurion University, Beer-Sheva, Israel.;* [4]*Genetics Institute at Soroka Medical Center, Beer-Sheva, Israel*

Background/Objectives: Hereditary Epidermolysis Bullosa (EB) is a group of monogenic blistering skin diseases, resulting from genetic defects leading to increased skin fragility. Depending on the EB subtype, dominant and/or recessive modes of inheritance have been reported. In its severe forms, EB is a debilitating disease, with shortened lifespan, necessitating meticulous daily skin care and multidisciplinary treatment.

The Negev Bedouin community exceeds 250,000 residents and is subdivided into more than 100 tribal units. The estimated prevalence of consanguineous marriages is 44%, with strict tribal endogamy. Extremely high carrier rates for a wide range of recessively inherited disorders have been reported for this community.

Methods: Retrospective analysis of clinical and genetic data on the EB cases of Bedouin descent referred to Soroka Medical Center during 1998-2019.

Results: We analyzed 40 EB cases from 21 families (including simplex, junctional and dystrophic subtypes). All cases were recessively inherited. The relative prevalence of EB Simplex, Junctional EB and Dystrophic EB was 62%, 19% and 19% respectively. Six different mutations in EB -related genes were found to underlie this disorder in this cohort, most probably representing founder variants in this population.

Conclusion: We show that recessively inherited EB is extremely prevalent in the Negev Bedouin population and reveal its genetic heterogeneity to be limited to a small number of genetic variants.

These data underscore the importance of deciphering the mutational landscape of EB and possibly other recessively inherited disorders in isolated inbred communities to achieve rapid genetic diagnosis and effective carrier screening and disease prevention.

Conflict of Interest: None declared

P06.058.D A novel case of split hand/foot malformation associated with 4q25 deletion encompassing *LEF1*

Katrin Bahlke[1], *Yvonne Stratis*[1], *Albrecht Röpke*[1], *Cornelie Müller-Hofstede*[1], *Judit Horvath*[1], *Frank Tüttelmann*[1], *Eva Klopocki*[2], *Andreas Busche*[1]

[1]*Department of Medical Genetics, University Hospital Münster, Münster, Germany;* [2]*Institute of Human Genetics, Julius Maximilians University Würzburg, Würzburg, Germany*

Background/Objectives: Split hand/foot malformation (SHFM) is a clinically and genetically heterogeneous congenital limb defect. To date, several genes as well as critical chromosomal loci have been identified in association with SHFM. Amongst others, mono- and biallelic sequence variants in *LEF1* have been shown to be associated with SHFM as well as mild ectodermal dysplasia (ED). In contrast, heterozygous deletions encompassing *LEF1* have been reported to cause severe ED, yet no limb defects. We report on a 5-year old boy with congenital SHFM and mild developmental delay but without any symptoms of ED. Family history was unremarkable with respect to limb malformations or ED.

Methods: Quantitative PCR, SNP-array, and exome analysis were performed. Segregation analysis was conducted in the father only since no maternal blood sample was available.

Results: Quantitative PCR did not reveal SHFM-typical micro-duplications in critical regions 10q24 and 17p13. Exome analysis remained without identifying the cause for SHFM. SNP-array revealed a heterozygous 212.6 kb-sized deletion in 4q25 encompassing exons 1-11 out of 12 *LEF1* exons. The deletion was not paternally inherited.

Conclusion: We describe the first patient carrying a deletion encompassing *LEF1* without showing any symptoms of ED but SHFM instead. The phenotype is more in line with the clinical presentation of sequence variants in *LEF1*. Our findings support the concept of *LEF1*-associated phenotypes as a syndromic spectrum with variable expressivity comprising symptoms of SHFM and/or ED. However, further studies are required to understand the exact molecular mechanisms underlying the inconsistent genotype-phenotype-correlation.

Grants: None.

Conflict of Interest: None declared

P06.059.A Ante- and peri-natal presentation of classical Ehlers-Danlos syndrome: experience of the French national reference center.

Théa Lanfranchini[1], *Malika Foy*[2], *Fabrice Gillas*[2], *Baptiste Vierne*[2], *Karelle Benistan*[2], **Caroline Michot**[3]

[1]*Versailles-Saint-Quentin-en-Yvelines University, Versailles, France;* [2]*Raymond Poincaré Hospital, GHU Paris Saclay, Reference Center for non-vascular Ehlers-Danlos syndromes, Garches, France;* [3]*Necker Enfants malades Hospital, Department of Genomic Medicine for Rare Diseases, Reference Center for non-vascular Ehlers-Danlos syndromes (pediatric site), Paris, France*

Background: classical Ehlers-Danlos syndrome (cEDS) is an autosomal dominant condition associating joint hyperlaxity, skin hyperelasticity, atrophic scars and tissue fragility. Premature rupture of membranes, breech presentation and hip/shoulder neonatal dislocation have been reported, but literature on obstetric and neonatal complications of cEDS is poor. Data are also often confusing, mixing complications observed in different

types of EDS. Our study aims at identifying early signs of the syndrome to offer adequate management.

Methods: we performed a retrospective case-control study. The records of cEDS patients followed at the French national reference center for non-vascular EDS were reviewed and compared to those of their healthy siblings.

Results: we included 109 individuals, consisting in 77 cases and 32 controls. Severe IUGR was observed in 82% cases versus 40% controls. Intensive cares were required in 17% cases, including 5% of full-term newborns, versus in 6% controls. Congenital hip dislocation was found in 10% cases, equinovarus feet in 10% cases and abdominal wall hernia in 13% cases, compared to respectively 3%, 3% and 0% controls. Hypotonia was seen only in cases (5%), 75% of whom were full-term newborns. Hyperlaxity, major skin signs of cEDS and ecchymoses in axillary and contact areas were noted in only 5% of cEDS cases.

Conclusion: Severe IUGR, intensive care unit admissions at term, limb malposition and abdominal hernia are red-flags directing to the diagnosis of cEDS. Nevertheless, major signs of cEDS are rarely recognized at birth, preventing from specific management in sporadic cases. In familial cases, we advise a close ultrasound monitoring as in higher risk pregnancies.

Conflict of Interest: None declared

P06.060.B The effect of Tuberous sclerosis complex 1 (TSC1) deficiency in adipose progenitor cells - case report and in vitro studies

Julika Friedrich[1], *Antje Garten*[1], *Sandy Richter*[1], *Julia Hentschel*[2], *Maria Arelin*[1], *Konrad Platzer*[2], *Andreas Merkenschlager*[1], *Steffi Mayer*[3], *Wieland Kiess*[1], *Rami Abou Jamra*[2], *Diana le Duc*[2], *Henriette Kiep*[1]

[1]*Center for Pediatric Research, University Hospital for Children and Adolescents, Leipzig, Germany;* [2]*Institute of Human Genetics, Leipzig University, Leipzig, Germany;* [3]*University Hospital for Pediatric Surgery, Leipzig, Germany*

Background/Objectives: Activation of mechanistic target of rapamycin (mTOR) as a regulator of adipogenesis and lipid accumulation is controlled by upstream regulators tuberous sclerosis complex (TSC) 1 and TSC2. The impact of TSC1 deficiency is not clearly defined in human adipose tissue. We identified a TSC1 variant in a boy with developmental delay and a lipoma. We aim to assess the effect on adipogenesis and proliferation of adipose precursors and to establish a cell model to test potential therapeutic options.

Methods: The boy presented with a lipoma within the gluteal muscle which is not typical for tuberous sclerosis. In the lipoma tissue we identified a loss of heterozygosity on chromosome 9 including TSC1. To establish an in vitro model we used siRNA TSC1 knockdown (KD) in the human adipocyte models SGBS and LipPD1 and compared to control cells. We examined proliferation and adipocyte differentiation using Hoechst, Nile Red and Oil Red O stain. Expression of adipogenesis markers was measured by qPCR, activation of mTOR downstream effectors was assessed via Western blotting. We investigate the effects of in vitro incubation with different pathway inhibitors.

Results: Preliminary analyses suggested a higher proliferation of TSC1 KD cells compared to control cells. There was no signigicant effect on adipocyte differentiation. We detected an increased phosphorylation of mTORC1 downstream targets in KD cells and in SVF cells derived from the patient's lipoma.

Conclusion: We conclude that the TSC1 variant is probably causative for lipoma formation and a therapy with mTOR inhibitors may be beneficial.

Grants: DFG, SFB 1052.

Conflict of Interest: None declared

P06.061.C A second family with multiple epiphyseal dysplasia type 7 caused by the homozygous variant c.676G>A p.(Val226Met) in CANT1

André Travessa[1;2], *Silvia Modamio-Hoybjor*[3;4], *Luísa Brites*[5], *Miguel Beltrán*[6], *Karen E. Heath*[3;4], *Ana Berta Sousa*[1;2]

[1]*Department of Medical Genetics and ERN-BOND, ULS Santa Maria, Lisbon, Portugal, Portugal;* [2]*Faculty of Medicine, University of Lisbon, Lisbon, Portugal, Portugal;* [3]*Institute of Medical & Molecular Genetics (INGEMM), IdiPAZ, Hospital Universitario La Paz, Universidad Autonóma de Madrid (UAM), and CIBERER, ISCIII, Madrid, Spain, Spain;* [4]*Skeletal Dysplasia Multidisciplinary Unit (UMDE) and ERN-BOND, Hospital Universitario La Paz, UAM, Madrid, Spain, Spain;* [5]*Department of Rheumatology, ULS da Região de Leiria, Leiria, Portugal, Portugal;* [6]*Department of Orthopaedic Surgery, ULS Santa Maria, Lisbon, Portugal, Portugal*

Background: Variants in *CANT1* are responsible for Desbuquois dysplasia (DD) type 1, DD Kim type, and multiple epiphyseal dysplasia (MED) type 7, all with autosomal recessive inheritance. In 2017, *CANT1* variant c.676G>A p.(Val226Met), previously found in patients with DD Kim type, was shown to cause MED type 7 in one family. Here, we report an additional patient with the same homozygous variant and MED type 7.

Case report: 45-year-old woman, referred to our skeletal dysplasia multidisciplinary unit for clinical evaluation of severe osteoporosis. Her parents are distant cousins, and she has a brother with a similar phenotype. She has short stature (140cm, -7,11 SD), short trunk, kyphoscoliosis, left genu varum, right genu valgum, lumbar and knee pain, and astigmatism. She underwent multiple surgeries due to bilateral metatarsus varus, bilateral recurrent dislocation of the patella, disc herniation, left wrist fracture, and for evacuation arthrodesis of the right knee. Skeletal X-rays showed Swedish key appearance of the proximal femur, platyspondyly, and generalized epiphyseal dysplasia. Spine MRI revealed cervical-dorso-lumbar spondylosis, signs of L4 fracture, and multiple joint osteoarthritis. A NGS skeletal dysplasia panel revealed the presence of a homozygous pathogenic variant in *CANT1:* NM_001159773.2:c.676G>A p.(Val226Met).

Conclusions: In summary, *CANT1*-related MED is very rare, with only two families described so far. DD type 1, DD Kim variant, and MED type 7 are a continuum of severity, and their unification as *CANT1*-related dysplasias could be more appropriate. Description of further patients with p.(Val226Met) variant would contribute to understand their natural history.

Conflict of Interest: None declared

P06.062.D Enrichment of pathogenic copy number variants on 17q chromosome in patients with skeletal findings

Dijana Perovic[1], *Nela Maksimovic*[1], *Tatjana Damnjanovic*[1], *Biljana Jekic*[1], *Marija Dusanovic Pjevic*[1], *Milka Grk*[1], *Ana Djuranovic*[1], *Natasa Stojanovski*[1]

[1]*University of Belgrade - Faculty of Medicine, Institute of Human Genetics, Beograd, Serbia*

Background/Objectives: Human chromosome 17 (chr17) has the second highest gene density and is rich in segmental duplications. It is known for multiple microdeletion/microduplication syndromes, many of which include skeletal abnormalities. We aim to analyze the overall detection rate of pathogenic and likely

pathogenic copy number variations (pCNVs) in patients with complex phenotypes that include skeletal disorders, as well as the proportion and significance of CNVs of chr17 origin.

Methods: From the original sample of 1230 patients who underwent chromosomal microarray analysis (CMA) in our laboratory (2018-2023) by Agilent oligonucleotide $8 \times 60K$ or $4 \times 180K+SNP$ microarrays, those with skeletal abnormalities were selected. Detection rates of pCNVs were calculated for all chromosomes.

Results: 172/1230 (14,0%) patients had skeletal findings; 43 had pCNVs with a diagnostic yield of 25,0%, significantly higher than in the rest of the cohort (14,6%, $p < 0.001$). Chr17 was the most commonly affected in patients with skeletal findings (9/43). All but one pCNVs were on q arm, in region 17q12-q25.3. Of all detected pCNVs in our cohort (198/1230), those originating from chr17 were more frequently expressed in skeletal phenotype (9/17 for chr17 versus 34/198 for all other chromosomes; $p = 0.002$). One patient had a partial deletion of *the COL1A1* gene corresponding to osteogenesis imperfecta clinical diagnosis; all others had complex phenotypes and multiple genes involved, of which we distinguish *NOG* and *TBX4*.

Conclusion: Skeletal findings in phenotype could increase diagnostic yield of CMA. A high prevalence of CNVs in 17q12-q25.3 emphasizes the importance of genes in this region for skeletal development.

Grants:

Conflict of Interest: None declared

P07

CARDIOVASCULAR DISORDERS

P07.001.A Genetic causes of different types of cardiomyopathies in Bulgarian patients

Valentina Peycheva[1], *Kunka Kamenarova*[1], *Kalina Mihova*[1], *Darina Kachakova-Yordanova*[1], *Martin Georgiev*[1], *Maria Sredkova-Ruskova*[2], *Irena Bradinova*[3], *Emil Simeonov*[4], *Ivanka Dimova*[1], *Radka Kaneva*[1]

[1]*Medical University of Sofia, Laboratory of Genomic Diagnostics, Molecular Medicine Center, Department of Medical Chemistry and Biochemistry, Sofia, Bulgaria;* [2]*Medical University of Sofia, University Pediatrics Hospital, Department of Clinical Genetics, Sofia, Bulgaria;* [3]*Medical University of Sofia, Department of Obstetrics and Gynecology, Sofia, Bulgaria; 2UHOG "Maichin dom", National Genetic Laboratory, Sofia, Bulgaria;* [4]*Medical University of Sofia, UMBAL "Alexandrovska" hospital, Sofia, Bulgaria*

Background/Objectives: Congenital cardiac abnormalities have strong genetic component and can be either isolated heart defects or to be a part of more complex syndromic phenotype like the RASopathies. The aim of the present study was to find the genetic diagnosis in a group of Bulgarian patients with cardiomyopathies.

Methods: Nine patients with either isolated congenital heart defect or cardiomyopathy in the context of syndromic condition were referred to the genome diagnostics lab. Both targeted next-generation sequencing (NGS) or whole exome sequencing (WES) were performed using MiSeq and Novaseq6000 platforms (Illumina) respectively. Direct sequencing was used for confirmation of the discovered variants.

Results: In 6 patients NGS detected an overall of 9 variants in 7 genes. Three pathogenic and one likely pathogenic novel variant were found in *MYH7*, *PTPN11*, *BRAF* and *ACTB* genes. Five variants of unknown significance but with potential role in the disease phenotype were found in *TCAP*, *ACTN2* and *TTN* genes. Thus, the suspected diagnosis was confirmed in two patients from our cohort. In other two patients with suspicion for Noonan-Costello syndrome the diagnosis was refined based on the genetic findings. *PTPN11* gene, which is involved in the pathogenesis of Noonan and LEOPARD syndromes, was found to cause isolated cardiomyopathy without additional syndromic features.

Conclusion: Identifying the molecular basis of cardiac abnormalities could help define the pathophysiology of the disease, which will help to improve the individual therapy and prophylactics in order to reduce the risk of sudden cardiac death and will elucidate the recurrent family risk.

Grants: D01-302/17.12.2021, D01-165/28.07.2022

Conflict of Interest: None declared

P07.002.B A missense variant in SCN5A associated with specific ECG changes protects from multiple cardiac arrhythmia phenotypes

Julian Wanner[1;2], *Maren Krafft*[1], *Teemu Niiranen*[3;4], *Patrick T Ellinor*[5;6;7], *Sean Jurgens*[5;6], *Joel Rämö*[2;6;8], *Henrike Heyne*[1;2;9]

[1]*Hasso Plattner Institute (HPI), Potsdam, Germany;* [2]*Institute for Molecular Medicine Finland, Helsinki, Finland;* [3]*Finnish Institute for Health and Welfare, Helsinki, Finland;* [4]*University of Turku, Turku, Finland;* [5]*Broad Institute of MIT and Harvard, Cambridge, United States;* [6]*Massachusetts General Hospital, Cardiovascular Research Center, Boston, United States;* [7]*Harvard Medical School, Boston, United States;* [8]*Broad Institute of MIT and Harvard, Cardiovascular Disease Initiative, Cambridge, United States;* [9]*Hasso Plattner Institute for Digital Health, New York, United States*

Consortium: FinnGen

Background/Objectives: Cardiac arrhythmias pose a major public health burden, but diagnosis and prevention remain difficult. We recently described an *SCN5A* missense variant (rs45620037 [T220I], MAF = 5e-3 in Finland, MAF = 1.1e-3 in UK) with a protective effect from common cardiac arrhythmias in a heterozygous state.

Methods: Here, we investigate the variant's effects on ECG intervals, disease incidence and mortality and evaluate its interaction with polygenic background across lifetime in 3 cohorts totalling >1M individuals.

Results: We find significantly shorter QT intervals for heterozygous T220I carriers in the H2000 cohort (-7.84 ± 2.55 ms, $p = 2.1e-3$, $n = 6{,}048$), which we validate in the UK Biobank ($n = 81{,}195$), as hypothesized from the variant's experimentally determined small loss-of-function effect. Further, we find protective effects on multiple common arrhythmias in FinnGen ($n = 500{,}348$), including atrial fibrillation (AFib) (HR 0.60 [0.55, 0.66], $p = 3.13e-06$) which we replicate in the UK Biobank. The variant, however, increases the risk of sick sinus syndrome, especially in homozygotes (HR 12.65 [5.24, 30.53], $p = 1.68e-08$). Overall, the variant has a protective effect against mortality due to cardiac arrhythmia or heart failure (HR 0.74 (0.58, 0.95), $p = 1.93e-02$), while we do not observe significant differences in overall cardiovascular or all-cause mortality. In addition, we find a significant interaction ($p = 0.026$) between T220I carrier status and a polygenic score for AFib on lifetime risk of AFib.

Conclusion: We thus share results on a variant with effects on common arrhythmic diseases and associated endophenotypes with implications for disease prediction and drug development.

Grants: German Research Foundation (516649954)

Conflict of Interest: None declared

P07.004.D Characterization of a novel transgenic mouse model for arrhythmogenic cardiomyopathy: phenotype and heart transcriptome profile

Sara Vencato[1], Stefano Cagnin[1], Chiara Romanato[1], Karthik Murali[1], Sabina Ferron[1], Tommaso Becchi[1], Angelo Velle[1], Gabriele Sales[1], Chiara Romualdi[1], Agata Rakszewska[2], Christian Conrad[2], Paola Braghetta[3], Libero Vitiello[1], Alessandra Rampazzo[1], **Martina Calore**[1;4]

[1]Padova University, Biology, Padova, Italy; [2]Berlin Institute of Health, Center for Digital Health, Berlin; [3]Padova University, Department of Molecular Medicine, Padova; [4]Maastricht University, Cardiology-CARIM, Maastricht, Netherlands

Background:/Objectives Arrhythmogenic cardiomyopathy (ACM) is one of the most commonly inherited cardiomyopathies, characterized by the progressive substitution of the myocardium with fibrofatty tissue. Clinically, ACM is characterized by ventricular arrhythmias, syncope, and sudden cardiac death and shows wide phenotypic heterogeneity (1). Of the known disease genes, those encoding for plakophilin-2 (*PKP2*), desmoplakin (*DSP*), and desmoglein-2 (*DSG2*) are most commonly mutated. To study the pathogenic mechanisms of ACM, we generated a novel mouse model for the disease.

Methods: We generated transgenic mice overexpressing in cardiomyocytes human desmoglein-2 carrying the p.G100R mutation (TgG) found in an affected patient. To establish the transcriptomic ACM signature, we performed bulk and single nucleus-RNA seq on cardiac samples from 6 month-old TgG and control mice.

Results: TgG mice present several clinical features of ACM, such as fibrous replacement, increased inflammatory markers, and cardiac dysfunction. Accordingly, enrichment analysis and network construction performed on bulk RNA seq data identified upregulation of immune- and extracellular matrix-related processes. Ongoing data analysis from single nucleus-RNA seq revealed several (sub)clusters and differentially expressed genes in TgG hearts vs controls.

Conclusion: Collectively, these findings identified numerous processes and cell populations potentially altered in TgG mice that could pave the way for further studies focused on ACM pathogenic mechanisms.

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Conflict of Interest: None declared

P07.005.A Spectrum of *MYBPC3* variants in a cohort of hypertrophic cardiomyopathy patients

Giulia Margherita Brach del Prever[1;2], *Diana Carli[1;2], maria luca[1;2], Angelo Corso Faini[1;2], carmelo maria romeo[1;2], Fiorenza Mioli[1;2], Valeria Bracciamà[1;2], Francesca Arruga[1], claudia saglia[1;2], caterina scolari[1;2], tullia carradori[1;2], edoardo spagnolo[1;2], davide caldo[1;2], stefano pidello[3], claudia raineri[3], Gaetano Maria De Ferrari[2;3], marco cingolani[4], Walter Grosso Marra[4], matteo bianco[5], barbara mabritto[6], Tiziana Vaisitti[1;2], Silvia Deaglio[1;2]*

[1]Immunogenetics and Transplant Biology Service, Città della Salute e della Scienza University Hospital, Turin, Italy; [2]Department of Medical Sciences, University of Turin, Italy; [3]Division of Cardiology, Cardiovascular and Thoracic Department, Città della Salute e della Scienza University Hospital, Turin, Italy; [4]Division of Cardiology, Hospital of Ivrea, Italy; [5]Division of Cardiology, Coronary Intensive Care Unit, Clinical cardiology and cardiovascular imaging, A.O.U San Luigi Gonzaga, Orbassano, Italy; [6]Division of Cardiology, A.O. Ordine Mauriziano, Turin, Italy

Background/Objectives: a genetic etiology is identifiable in ~30% of cases of hypertrophic cardiomyopathy (HCM), being *MYBPC3* the most frequently involved gene, mutated in ~50% of cases with a confirmed genetic cause. Various mechanisms have been proposed as the *MYBPC3*-related HCM-pathogenesis, including haploinsufficiency. In this study, we aim to describe a cohort of molecularly characterized HCM patients, examine the *MYBPC3*-mutational spectrum and identify methods to assess variants pathogenicity.

Method: 396 HCM patients, recruited from 2018 to 2023, underwent next-generation sequencing analysis (NGS) of a panel of known HCM genes.

Results: 96 patients (24%) carried causative variants (pathogenic/likely pathogenic), 121 (31%) variants of uncertain significance (VUS) and 177 (45%) tested negative. Two patients, presenting both cardiac dilatation and hypertrophy, carried causative variants in DCM-associated genes (*LMNA* and *ABCC9*) suggesting a phenotypic overlap between HCM and DCM. 64 patients (16%) carried 71 *MYBPC3* variants, including 28 VUS (39%). Considering *MYBPC3* variants, 14 (20%) were truncating (frameshift, nonsense), 19 (27%) intronic splicing and 38 (53%) missense. 13 exonic variants (18%) were located near the exon-intron junction with potential impact on splicing.

Conclusion: NGS is a powerful tool to define molecular diagnosis of HCM. However, VUS represent a main clinical issue, limiting NGS usefulness in improving HCM clinical care and familial preventive measures. In our cohort, 32/71 (45%) *MYBPC3* variants, including many VUS, had a potential impact on splicing, thus complementary RNA studies may be of help for variants classification.

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Conflict of Interest: None declared

P07.006.B Two models, one mutation: functional characterisation of a Brugada syndrome associated CACNA1C variant in stem-cell-derived cardiomyocytes and zebrafish

Bert Vandendriessche[1], *Ewa Sieliwonczyk[1], Dogan Akdeniz[1], Hanne Boen[1], Merlijn Nemegeer[1], Charlotte Claes[1], Jolien schippers[1], Laura Rabaut[1], Bart Loeys[1], Dorien Schepers[1], Maaike Alaerts[1]*

[1]University of Antwerp and Antwerp University Hospital, Cardiogenomics, Center of Medical Genetics, Faculty of Medicine and Health Sciences, Edegem, Belgium

Background/Objectives: Brugada syndrome (BrS) is an inherited arrhythmic disorder which can lead to sudden cardiac death in the young (<40 years old). Only in circa 30% of BrS patients the underlying genetic cause can be identified with current cardiac arrhythmia gene panels. Moreover, numerous genetic "Variants of Uncertain Significance" (VUS) are being detected, but reliable functional models to prove their causality are lacking. Here I aim to model a VUS of the *CACNA1C* gene, detected in a BrS patient, both in human induced pluripotent stem-cell-derived cardiomyocytes (hiPSC-CM) and zebrafish to evaluate its pathogenicity. *CACNA1C* encodes the L-type calcium channel and the variant is hypothesised to induce a trafficking defect.

Methods and Results: First, the CRISPR gene-editing workflow was optimised to create the knock-in (KI) hiPSC and zebrafish lines. Using only Next Generation Sequencing (NGS) as an enrichment tool for the CRISPRed population, an average KI percentage of 2.99% (±5.09, N = 122) was achieved in zebrafish and even up to 64.2% (± 27.1%, N = 21) in hiPSCs. After downstream validation, we obtained homozygous and heterozygous lines of both study models. Fluorescence calcium imaging is a part of the functional

characterisation in both models and preliminary differences between calcium transient shapes could be noted in mutated versus wild type lines.

Conclusion: We showed that with our customised NGS workflow, we can successfully create KI lines irrespective of the study model. Preliminary characterisation of the models carrying the VUS hints at potential differences, but further in-depth analysis and more replicates are needed.

Grants: Research Foundation – Flanders

Conflict of Interest: None declared

P07.007.C Identification of a *NOTCH1*-specific methylation signature in non-syndromic CHD patients

Gregor Dombrowsky[1;2], *Liselot van der Laan*[3;4], *Ananilia Silva*[4], *Jeroen Breckpot*[5;6], *Enrique Audain Martinez*[1;2], *Anna Wilsdon*[7], *Michael Levy*[8], *Niels Vos*[3], *Sabine Klaassen*[9;10], *Felix Berger*[10], *Sven Dittrich*[11], *Brigitte Stiller*[12], *Hashim Abdul-Khaliq*[13], *Ingo Daehnert*[14], *Frances Bu'Lock*[15], *Thomas Pickardt*[16], *Ulrike Bauer*[16], *Hans-Heiner Kramer*[2], *Anselm Uebing*[2], *Siobhan Loughna*[7], *J David Brook*[7], *Peter Henneman*[3], *Bekim Sadikovic*[4], *Alex Postma*[3;17], *Marc-Phillip Hitz*[1;2]

[1]*Carl von Ossietzky University; Klinikum Oldenburg, Institute of Medical Genetics, Oldenburg, Germany;* [2]*University Hospital of Schleswig-Holstein, Department of Congenital Heart Disease and Pediatric Cardiology, Kiel, Germany;* [3]*Amsterdam Reproduction and Development Research Institute, Amsterdam University Medical Center, University of Amsterdam, Department of Human Genetics, Amsterdam, Netherlands;* [4]*Western University, Department of Pathology and Laboratory Medicine, London, Canada;* [5]*University Hospitals Leuven, Center for Human Genetics, Leuven, Belgium;* [6]*University of Leuven, Department of Human Genetics, Leuven, Belgium;* [7]*University of Nottingham, School of Life Sciences, Nottingham, United Kingdom;* [8]*London Health Sciences Centre, Verspeeten Clinical Genome Centre, London, Canada;* [9]*Charité - Universitätsmedizin Berlin and Max Delbrück Center, Experimental and Clinical Research Center (ECRC), Berlin, Germany;* [10]*Dept. of Congenital Heart Disease-Pediatric Cardiology, Deutsches Herzzentrum der Charité, Berlin, Germany;* [11]*University Hospital Erlangen; Friedrich-Alexander-University Erlangen-Nürnberg, Department of Pediatric Cardiology, Erlangen, Germany;* [12]*University Heart Center Freiburg-Bad Krozingen, Medical Center-University of Freiburg, Faculty of Medicine, Department of Congenital Heart Disease and Pediatric Cardiology, Freiburg, Germany;* [13]*University Hospital of Saarland, Department of Pediatrics and Pediatric Intensive Care Medicine, Homburg, Germany;* [14]*Heart Center Leipzig at University of Leipzig, Department of Pediatric Cardiology, Leipzig, Germany;* [15]*East Midlands Congenital Heart Centre and University of Leicester, Glenfield Hospital, Congenital and Paediatric Cardiology, Leicester, United Kingdom;* [16]*German Competence Network for Congenital Heart Defects, National Register for Congenital Heart Defects, Berlin, Germany;* [17]*Amsterdam University Medical Centers AUMC, Department of Medical Biology, Amsterdam, Netherlands*

Background/Objectives: Congenital heart defects (CHDs) are the most common malformation amongst newborns, with *NOTCH1*-haploinsufficiency as a major monogenetic cause. Here, we investigated whether a *NOTCH1*-specific DNA-methylation signature can be established. Methylation analysis can aid variant classification, especially regarding variants of uncertain significance (VUS), thus improving counselling of patients.

Methods: The initial analysis of an exome-based cohort (3907 CHD cases, 5157 controls) identified *NOTCH1*-variants in 34 cases, comprising different variant types and pathogenicity levels. These 34 cases were further extended by additional samples with similar *NOTCH1*-variants. DNA of assembled samples were subjected to DNA methylation profiling using the Illumina Infinium MethylationEPIC array. Raw data was Quality Control (QC) processed and analyzed using the EpiSign™ Discovery Software in R. DNA methylation profiles of controls (EpiSign Knowledge Database) were age and sex matched. A linear model (limma) was applied to obtain differential methylated positions between patients and controls. An unsupervised model was applied to evaluate the robustness of the classifying probes. Subsequently, a support vector machine was used to construct the final DNAm signature.

Results: All included samples passed QC-criteria. A selective DNA methylation signature was established using patients harbouring pathogenic *NOTCH1*-variants and control samples. Moreover, the signature showed robust specificity concerning >50 other episignatures related to neurodevelopmental disorders. Analysis of included VUSes demonstrated overlap with the *NOTCH1*-signature in 3 cases.

Conclusion: While previous DNA-methylation signatures have been mainly established for neurodevelopmental disorders, we demonstrate that a *NOTCH1*-specific signature can be identified in non-syndromic CHD patients. Our data also illustrates that DNA-methylation analysis can improve *NOTCH1* variant classification.

Grants:

Conflict of Interest: None declared

P07.008.D A novel approach in functional genomics: identification of genetic variants associated with congenital heart disease in Down syndrome

NAZIMAH NIK-MAHMOOD[1;2], *Farook Ahmad*[3], *Thiloka Ratnaike*[2;4], *Timothy Hearn*[1]

[1]*University of Cambridge, Department of Medical Genetics, United Kingdom;* [2]*Colchester Hospital, ESNEFT, Children's Department, Colchester, United Kingdom;* [3]*Royal Papworth, Critical Care Unit, Cambridge, United Kingdom;* [4]*University of Cambridge, Department of Paediatrics, Cambridge, United Kingdom*

Background/Objectives: Congenital heart disease (CHD) is a major complication associated with Trisomy-21 and affects 40-50% of them. This study explores a novel hypothesis: the enrichment of critical genetic variants present in a triple-state on all three copies of chromosome 21 may influence the penetrance of the CHD phenotype in individuals with Down Syndrome (DS).

Methods: We obtained genomics data of DS patients through the Genomics England Research Environment (GEL RE) from the 100,000 genomes project and NHS Genomic Medicine Service patients and performed a focussed genetic analysis within a region known as the Down syndrome critical region (DSCR) for CHD. We used a bespoke variant allele count approach incorporating haplotype phasing to interrogate the variants. We analysed data from forty-four participants and compared variant enrichment in the DS with CHD (Cases, $n = 13$) with the DS without CHD (Controls) group.

Results: We found six significantly (p-value < 0.05, odd ratio (OR) = 5.8-7.3) enriched triple-state variants in the Cases group compared to the Controls group. These include the most enriched intron variant, rs7280353 (p-value = 0.18, OR = 7.27), located within the *DSCAM* gene, a gene that is associated with DS and CHD, and one enhancer variant, rs2837922 (p-value = 0.02, OR = 5.8), located within a regulatory region, close to the *DSCAM* gene.

Conclusion: The study proposes the potential 'sensitising' effect of common but critical variants in their triple state in altering the expression of cardiac candidate genes such as the *DSCAM*, leading to increased risk for CHD. This study introduces a novel approach to functional genomics when exploring the 'trisomy' environment.

Conflict of Interest: NAZIMAH NIK-MAHMOOD Health Education England and Training and Education Charity Fund, Colchester Hospital., This was part of research dissertation submitted for masters degree., Full time consultant paediatrician Colchester Hospital, United Kingdom.

Part-time masters students University of Cambridge, Farook Ahmad: None declared, Thiloka Ratnaike: None declared, Timothy Hearn Employed- University of Cambridge

P07.009.A Genetic analysis in sudden unexpected death in infancy and children

Rika Kosaki[1], *Yoshiko Uchida*[2], *Kumiko Yanagi*[3], *Tadashi Kaname*[3]

[1]*National Center for Child Health and Development, Division of Medical Genetics, Japan;* [2]*National Center for Child Health and Development, Division of Pediatric Emergency and Transport Services, Tokyo, Japan;* [3]*National Research Institute for Child Health and Development, Department of Genome Medicine, Tokyo, Japan*

Background: Approximately 2,000 pediatric out-of-hospital cardiac arrests occur annually in Japan, with infants accounting for 40%, and the prognosis is poor.

Determining the cause of death is important for preventable deaths in children and for the bereaved families to accept the child's death. Especially in genetic cardiac diseases, which allows for diagnosis and treatment of survivors.

Methods: Comprehensive genetic test was performed on deaths of unknown cause at our hospital since 2017-2023. Pediatric Sudden Cardiac Death Gene Analysis Panel (450 genes, including disease genes known to affect the ECG and disease genes based on anatomical abnormalities) and WES were done.

Results: Genetic analysis was performed on 25 cases for which consent was obtained from the family.

In 6cases, we concluded that the variants of the *KCNH2, SNTA1, SMC1A, AKAP9,PKP2 and COL3A1* genes. Some were strongly associated with the primary disease and cause of death. In addition, three of the patients was identified with a family history of the same variants and symptoms considered to be arrhythmias, leading to future follow-up. Genetic counseling including disclosure of the results to the bereaved families of the patients was provided.

Conclusion: The results suggest that comprehensive genetic analysis is useful in determining the cause of death in pediatric out-of-hospital cardiac arrest. However, our study has several limitations, the genetic information can lead to health management of the bereaved family members.

Conflict of Interest: Rika Kosaki collaborator, full, Yoshiko Uchida principal investigator, full, Kumiko Yanagi collaborator, full, Tadashi Kaname collaborator, full

P07.010.B Biallelic NPR1 loss of function variants are responsible for neonatal systemic hypertension

YLINE CAPRI[1;2], *Theresa KWON*[3], *Olivia Boyer*[4], *Lucas Bourmance*[2], *Noe Testa*[2], *VERONIQUE Baudouin*[3], *Ronan BONNEFOY*[5], *Anne COUDERC*[4], *Laurence Heidet*[6], *Chakib Meziane*[7], *Elisabeth Tournier-Lasserve*[8], *Judith Melki*[2]

[1]*APHP Robert Debré, Genetics Department, Prais, France;* [2]*INSERM, UMR 1195, Le Kremlin Bicêtre, France;* [3]*APHP Robert Debré, Nephrology Department, Paris, France;* [4]*APHP Necker Enfants Malades, Nephrology, Paris, France;* [5]*APHP Robert Debré, Pediatric Cardiology Unit, Paris, France;* [6]*INSERM_Institut Imagine, UMR 1163, Paris, France;* [7]*Groupe Hospitalier Sud Ile de France, Neonatology Unit, Melun, France;* [8]*INSERM, UMR 1141, Paris, France*

Background: Early-onset isolated systemic hypertension is a rare condition of unknown genetic origin. Renovascular, renal parenchymal diseases or aortic coarctation are the most common causes of secondary systemic hypertension in younger children and neonates. We investigated the genetic bases of early- onset isolated systemic hypertension.

Methods: Whole- exome sequencing (WES) was followed by variant filtering and Sanger sequencing for validation and familial segregation of selected variants in a large consanguineous family. mRNA expression was performed to evaluate the impact of the predicted pathogenic variant on gene expression. WES or Sanger sequencing was performed in additional unrelated affected individuals.

Results: In one consanguineous family with four children presenting with isolated neonatal-onset systemic hypertension, we identified homozygous stop–gain variant in the NPR1 gene (NM_000906.4:c.1159C>T (p.Arg387Ter)) in the affected individuals. This variant leads to a dramatic reduction of NPR1 RNA levels. NPR1 gene analysis of additional families allowed the identification of another family with two affected children carrying homozygous frameshift variant in NPR1 (NM_000906.4:c.175del (p.Val59TrpfsTer8)).

Conclusion: We show for the first time that biallelic loss of function of NPR1 is responsible for isolated neonatal-onset systemic hypertension in humans, which represents a new autosomal recessive genetic cause of infantile systemic hypertension or cardiogenic shock. This is consistent with studies reporting early-onset systemic hypertension and sudden death in Npr1-deficient mice. NPR1 gene analysis should be therefore investigated in infants with early-onset systemic hypertension with or without cardiogenic shock of unknown origin.

Conflict of Interest: None declared

P07.011.C The role of haemoglobin levels and coagulation deficiency in the genetic risk of SCAD: a genetic correlation and MR study

Josephine Henry[1], *Takiy Berrandou*[1;2], *Margaux-Alison Fustier*[1], *Eric Camerer*[1], *Nabila Bouatia-Naji*[1]

[1]*INSERM - Universite Paris Cite, Paris cardiovascular research center, Paris, France;* [2]*Aarhus University, Quantitative genetics and genomics, Aarhus, Denmark*

Background/Objectives: Spontaneous coronary artery dissection (SCAD) is an under-studied form of myocardial infraction, prevalent among women <60yrs. The ischemic event is due to a hematoma, resulting in arterial dissection. Whether it originates from the arterial wall or the lumen is unclear. Our recent GWAS identified one locus near the tissue factor gene (*F3*), involved in coagulation cascade activation following tissue damage. Reduced *F3* expression in arteries correlates with increased SCAD risk. This motivates the investigation of the role of haematological traits and coagulation proteins in SCAD.

Methods: We assessed genetic correlations using LDSC and summary statistics for SCAD, 29 haematological traits and 12 circulating coagulation proteins from population-based GWAS. Inverse variance weighted (IVW) Mendelian randomization (MR) implemented in TwoSampleMR was applied to explore associations between correlated traits ($P_{Bonf} < 3.1 \times 10^{-3}$) and SCAD.

Results: We report positive genetic correlation between SCAD and haemoglobin levels (Hb) ($r_g = 0.11$, $P = 1.8 \times 10^{-5}$). MR indicated significant association of genetically higher Hb with increased SCAD risk ($N_{SNPs} = 296$, $P_{IVW} = 3.3 \times 10^{-5}$). Genetically decreased Factor VIII levels, a protein regulated by thrombin that activates Factor X, associated with increased SCAD risk ($N_{SNPs} =$

12, $P_{IVW} = 2.9 \times 10^{-3}$). Platelet levels did not seem to impact SCAD genetic risk.

Conclusion: Our study provides novel insights into the mechanisms behind SCAD hematoma formation. We genetically confirmed the role of coagulation cascade deficiency through the association of lower levels of Factor VIII. Higher Hb role may reflect higher erythropoietin production, reported to promote arterial dissection in mice. Overall, our findings exclude circulating platelets in SCAD, supporting the hypothesis of intramural hematoma formation.

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Conflict of Interest: None declared

P07.012.D Variations in hypoxia-inducible factor-1α in cardiac infarction

Ivana Škrlec[1], *Snježana Džijan[1;2], Jasminka Talapko[1], Vera Cesar[1;3]*

[1]Faculty of Dental Medicine and Health, Josip Juraj Strossmayer University of Osijek, Department of Biophysics, Biology and Chemistry, Osijek, Croatia; [2]Genos Ltd., DNA Laboratory, Zagreb, Croatia; [3]Department of Biology, Josip Juraj Strossmayer University of Osijek, Osijek, Osijek, Croatia

Background/Objectives: The *HIF1α* gene regulates oxygen homeostasis in mammalian cells and is activated under hypoxic conditions. It is involved in many hypoxia-related diseases, including cardiovascular diseases, and is crucial for adapting to changes in oxygen availability during cardiac infarction. As genetic variability of *HIF1α* has been associated with cardiovascular diseases, it should be linked with cardiac infarction, the leading cause of myocardial hypoxia. It occurs when the heart's metabolic demands exceed the oxygen delivered to the myocardium. HIF1α provides cardioprotective effects for the oxygen-deprived myocardium. The present study aims to investigate a possible association between *HIF1α* polymorphisms and susceptibility to cardiac infarction.

Methods: This case-control study involved 188 Croatian patients (107 males, 81 females) who had experienced non-fatal cardiac infarction and 94 participants in the control group who had no prior history of cardiovascular disease. The study analyzed two SNPs in the HIF1α gene (rs11549465 and rs11549467).

Results: The rs11549465 polymorphism was found to be associated with cardiovascular risk factors such as hypertension ($p = 0.014$) in patients with cardiac infarction, while rs11549467 was associated with excessive daytime sleepiness ($p = 0.043$). A statistically significant difference in allelic ($p = 0.008$) and genotypic distribution ($p = 0.05$) was found between the patients and the control group at gene locus rs11549465 HIF1α.

Conclusion: The study suggests that polymorphisms in the *HIF1α* gene may be related to cardiac infarction, particularly genetic variations within the *HIF1α* gene, specifically rs11549465, which may modify susceptibility to cardiac infarction in the Croatian population.

Grants: IP7-FDMZ-2023

Conflict of Interest: Ivana Škrlec: None declared, Snježana Džijan DNA Laboratory, Genos Ltd., Zagreb, Croatia, Jasminka Talapko: None declared, Vera Cesar: None declared

P07.013.A Prioritizing causal genes and cell types in calcific aortic valve disease: Insights from integrating single-cell expression with GWAS

Shilin Jin[1], Shishi Wu[1], Feng Shen[2], Di Yang[3], Mengyao Yu[4]

[1]Human Phenome Institute, Zhangjiang Fudan International Innovation Center, Fudan University, Shanghai, China; [2]Department of Cardiovascular Surgery, Shanghai General Hospital, Shanghai Jiao Tong University of Medicine, Shanghai, China; [3]Human Phenome Institute, Shanghai Pudong Hospital, Zhangjiang Fudan International Innovation Center, Shanghai Key Laboratory of Bioactive Small Molecules, Fudan University, Shanghai, China; [4]Human Phenome Institute, Shanghai Pudong Hospital, Zhangjiang Fudan International Innovation Center, Fudan University, Shanghai, China

Background/Objectives: Calcific aortic valve disease (CAVD) is the second leading cause of adult heart diseases. The functions of genetic risks exhibit tissue specificity, as well as the cellular heterogeneity in CAVD. In this study, we aimed to uncover potential cell populations and genes that contribute to the development of CAVD and to explore the intricate mechanisms of CAVD.

Methods: Three independent scRNA-seq datasets (embryonic, healthy, and CAVD groups, N = 75,442 cells) from the GEO database were integrated by Harmony. Subsequently, scPagwas was utilized to identify the CAVD-relevant cellular populations and genes by integrating pathway activation transformation of scRNA-seq data and GWAS summary statistics (10 cohorts involving 13,765 cases and 640,102 controls). Additionally, a cell-cell communication network analysis was performed by CellChat.

Results: Unbiased cluster analysis of the three scRNA-seq data identified 18 clusters, in which 9 clusters significantly enriched in genetic association signals related to CAVD, particularly monocytes and valve-derived stromal cells (VDSCs). Increased cell-cell interactions, notably involving monocytes, were observed in CAVD patients. Furthermore, 20 high-risk pathogenic genes were identified that exhibited intriguing patterns throughout the lifecycle of CAVD (PCC < 0.25). For instance, inflammation-related genes such as *CXCL8* and *IL6* are activated during the disease, while the gene *RPS20*, encoding ribosomal proteins, is suppressed from embryo to adult but reactivated during the disease.

Conclusion: We identified monocytes as the CAVD-related cellular population and unveiled a set of prioritized genes linked to CAVD. Our research has innovatively expanded the genetic landscape of CAVD from the embryonic, healthy, and disease perspectives.

Grants: Sponsored by Shanghai Sailing Program(21YF1452900)

Conflict of Interest: None declared

P07.014.B A pathogenic variant Gly148Arg of *ACTA2* gene causes left ventricular myocardial compaction: cardiac manifestations in a zebrafish model.

Wulan Apridita Sebastian[1], Masanori Inoue[1], Nobuyuki Shimizu[2], Ryosuke Sato[1], Saori Oguri[1], Tomoyo Itonaga[1], Shintaro Kishimoto[1], Hiroshi Shiraishi[2], Toshikatsu Hanada[2], Kenji Ihara[1]

[1]Oita University, Faculty of Medicine, Pediatrics, Yufu; [2]Oita University, Faculty of Medicine, Cell Biology, Yufu

Background/Objectives: The *ACTA2* gene encodes actin α2, a primary component of vascular smooth muscle cells. *ACTA2* missense variants are implicated in inherited thoracic aortic diseases, characterized by smooth muscle cell dysfunction in various organs including the lungs, brain vessels, intestines, pupils, bladder, and heart.

Methods: A heterozygous missense variant, Gly148Arg (G148R), was identified in a patient with thoracic aortic aneurysm,

dissection, and left ventricular non-compaction. To explore the potential functional and histopathological impacts of this variant, we employed zebrafish as a live model. We injected *ACTA2* mRNA (wild-type, G148R variant, and known pathogenic variant Arg179His [R179H]) into one-cell stage zebrafish embryos. These embryos were then observed up to 72 hours post-fertilization for cardiac analysis.

Results: We observed a significant reduction in heart shortening fractions in zebrafish with both pathogenic variants. Histopathologically, the myocardial walls in zebrafish with *ACTA2* pathogenic variants were thinner compared to the wild type, and a notable decrease in total myocardial cell count was evident. Additionally, the number of proliferating cells in endothelial and myocardial regions was significantly reduced in zebrafish with *ACTA2* variants, demonstrating the impact of G148R and R179H variants on left ventricular non-compaction and cardiac morphological abnormalities.

Conclusion: This study underscore the critical role of the *ACTA2* gene in various aspects of cardiovascular development, revealing previously unidentified implications of *ACTA2* variants in cardiac pathology.

Grants: This work was supported by JSPS Grant-in-Aid for Scientific Research (B) Grant Number JP22H03043.

Conflict of Interest: None declared

P07.015.C A novel mosaic *RASA1* variant in a child with Parkes Weber syndrome

Robin A. Pilz[1], *Dariush Skowronek*[1], *Tamara Ehresmann*[2], *Ute Felbor*[1], *Matthias Rath*[1;3]

[1]*Department of Human Genetics, University Medicine Greifswald, and Interfaculty Institute of Genetics and Functional Genomics, University of Greifswald, Greifswald, Germany;* [2]*Limbach Genetics GmbH, MVZ Humangenetik, Frankfurt, Germany;* [3]*Institute for Molecular Medicine, MSH Medical School Hamburg, Hamburg, Germany*

Background/Objectives: Capillary malformation-arteriovenous malformation (CM-AVM) syndrome, with or without Parkes Weber syndrome (PWS), is a rare autosomal dominant disease that is caused by pathogenic *RASA1* or *EPHB4* variants and can lead to life-threatening complications. Approximately 70 to 80 % of CM-AVM cases are inherited from an affected parent. However, postzygotic de novo variants must also be considered in genetic testing for CM-AVM.

Methods: We performed NGS gene panel sequencing for a 4-year-old female PWS patient presenting with multiple capillary malformations, two capillary stains on the left leg, and associated overgrowth of the second toe. We also conducted a literature review on cases with mosaic *RASA1* and *EPHB4* variants.

Results: A postzygotic *RASA1* loss-of-function variant was detected with a variant allele frequency (VAF) of 20 % in blood and oral epithelial cells of the index patient. The literature review revealed no correlation between the severity of the clinical phenotype and the VAF. We also identified a germline nonsense variant in the patient's *TEK* gene, which is not expected to cause a vascular phenotype but confers an increased risk for primary congenital glaucoma with variable expressivity.

Conclusion: Our case report and literature review strengthen the importance of testing for genetic mosaicism in CM-AVM and illustrate that a good knowledge of the sequenced genes and associated disease mechanisms is essential for adequate genetic counseling.

Grants: No specific grants were received.

Conflict of Interest: None declared

P07.016.D A novel splice-site *FHOD3* founder variant is a common cause of hypertrophic cardiomyopathy in the population of the Balkans – a cohort study

Nina Vodnjov[1;2], *Janez Toplišek*[3], *Aleš Maver*[1;4], *GORAN CUTURILO*[5;6], *Helena Jaklič*[1], *Nataša Teran*[1], *Tanja Višnjar*[1], *Maruša Škrjanec Pušenjak*[1], *Alenka Hodžić*[1], *Olivera Miljanovic*[7], *Borut Peterlin*[1], *Karin Writzl*[1;4]

[1]*University Medical Centre Ljubljana, Clinical Institute of Genomic Medicine, Ljubljana, Slovenia;* [2]*University of Ljubljana, Biotechnical Faculty, Ljubljana;* [3]*University Medical Centre Ljubljana, Department of Cardiology, Ljubljana;* [4]*University of Ljubljana, Faculty of Medicine, Ljubljana;* [5]*University Children's Hospital, Department of Medical Genetics, Belgrade, Serbia;* [6]*University of Belgrade, Faculty of Medicine, Belgrade, Serbia;* [7]*Clinical Centre of Montenegro, Podgorica, Montenegro*

Background/Objectives: Hypertrophic cardiomyopathy (HCM) is often caused by variants in genes encoding sarcomeric proteins. The contribution of HCM-causing variants in non-sarcomeric genes, such as *FHOD3*, has been less well investigated. We report a novel *FHOD3*:c.1646+2T>C variant found to be a common cause of HCM in Balkan population.

Methods: An in-house database of exome sequencing results, collected during routine diagnostics, was searched for recurrent variants in 134 probands with HCM. The heterozygous *FHOD3*(NM_001281740.3):c.1646+2T>C was observed in five probands. Segregation study and variant screening of the probands who had previously undergone panel or clinical exome sequencing was performed by Sanger sequencing. Whole genome sequencing was performed for haplotype analysis in 11 individuals. Medical history was obtained from institutional medical records.

Results: In total, 8/134 (16%) probands and seven relatives were found to have the *FHOD3*:c.1646+2T>C. The variant was classified as likely pathogenic (PS4_STR, PP1_SUP, PP3, PM2). *FHOD3*:c.1646+2T>C shows incomplete penetrance (13/15 affected individuals; unaffected individuals were 22 and 42 years old) and highly variable age of disease onset (mean 32 ± 18, range 4-63 years). Individuals with *FHOD3*:c.1646+2T>C had no history of sudden cardiac death (SCD), aborted SCD or atrial fibrillation. Haplotype and pedigree analysis suggested that the variant arose in an ancestor living in the Balkans.

Conclusion: *FHOD3*:c.1646+2T>C is the second most common HCM-causing variant in studied cohort. Consistent inclusion of non-sarcomeric HCM-causing genes in HCM genetic testing is necessary to better assess the number of disease-causing variants in these genes and their contribution to HCM development worldwide.

Grants: Co-financed by ARIS-RS (grant number P3-0326).

Conflict of Interest: None declared

P07.017.A Prenatal diagnosis of a PIK3R1- related overgrowth syndrome with cutaneous syndactyly and Peters anomaly

Eleonore Pairet[1;2], *Laurence Boon*[2;3], *Dana Dumitriu*[4], *Olivier Barbier*[5], *Pierre-Louis Docquier*[3;5], *Martina De Bortoli*[2], *Miikka Vikkula*[2], *Jean-Marc Biard*[6], *An Van Damme*[3], *Nicole Revencu*[1;3]

[1]*Cliniques universitaires Saint-Luc (UCLouvain), Human Genetics, Bruxelles, Belgium;* [2]*De Duve Institute UCLouvain, Human Genetics (GEHU), Woluwe-Saint-Lambert, Belgium;* [3]*Cliniques universitaires Saint-Luc (UCLouvain), Center for Vascular Anomalies, Bruxelles, Belgium;* [4]*Cliniques universitaires Saint-Luc (UCLouvain), Radiology, Bruxelles, Belgium;* [5]*Cliniques universitaires Saint-Luc (UCLouvain),*

Orthopedics, Bruxelles, Belgium; [6]Cliniques universitaires Saint-Luc (UCLouvain), Obstetrics, Bruxelles, Belgium

Background/Objectives: Somatic missense and indel variants in *PIK3R1* have recently been identified in a phenotypic spectrum that resembles *PIK3CA*-related overgrowth spectrum and in capillary malformation with dilated veins. The *PIK3R1*-associated phenotypic spectrum includes capillary malformations, venous ectasias/ engorgement, and mild soft tissue/bone overgrowth.

Results: We report a patient with a subcutaneous upper limb and thoracic mass identified at 25 weeks of gestation. An initial differential diagnosis included macrocystic lymphatic malformation and connective tissue tumour. Amniotic fluid analysis identified a *PIK3R1* variant (c.1735_1740del, p.(Gln579_Tyr580del)) with an allele fraction of 9.5%. The foetus developed anaemia at 31 weeks and had a transfusion. The child was born at term with an extensive capillary malformation of the 4 limbs and thorax, cutaneous syndactyly on 4 extremities, hypoplastic nails, and a left axillary mass with both lymphatic and venous components. A Peters anomaly was identified in his right eye. At day 9, a treatment by sirolimus was initiated because of disseminated intravascular coagulation. He is currently 2 years old and developing well and is under alpelisib treatment. Genetic testing performed postnatally showed the absence of the *PIK3R1* variant in the blood and its presence in a skin biopsy with an allele fraction of 16%.

Conclusion: This case represents the first prenatal diagnosis of the *PIK3R1*-associated phenotypic spectrum, confirming the risk of ocular phenotype and limb anomalies. Additionally, the evolution under sirolimus and subsequent alpelisib treatment will be discussed. This contribution enhances our understanding of the diverse manifestations and treatment strategies associated with this disease.

Conflict of Interest: None declared

P07.018.B Identification of *BCAR1* as a novel candidate gene for hydrops fetalis and lower limb lymphedema

Charlotte Kemper[1], *Rene Hägerling*[1;2]

[1]Charité - Universitätsmedizin Berlin, Institute of Medical Genetics and Human Genetics, Berlin, Germany; [2]Berlin Institute of Health (BIH), BIH Center for Regenerative Therapies, Berlin

Background/Objectives: Primary congenital lymphedema is a genetic disease caused by altered lymph vessel development during embryogenesis. There are over 20 known disease-causing genes, however, many cases remain to be unsolved. To close this gap, we performed trio whole exome sequencing in patients with primary lymphedema.

Methods: Following trio exome analysis, potential gene candidates were further investigated including state-of-the-art imaging techniques of human, murine and zebrafish samples as well as in vitro experiments on human dermal lymphatic endothelial cells (HDLECs).

Results: We identified the homozygous variant c.1238C>T p.(P413L) in *BCAR1*, a gene encoding for an adaptor protein in cell motility, in a two-year-old patient with lower limb lymphedema and prenatal hydrops fetalis. BCAR1 was previously described to act downstream of VEGF-receptors and be involved in cardiovascular development. The identified p.(P413L) variant is located between phosphorylation sites in its functional domain. To further investigate this variant as causative, we analyzed BCAR1 protein expression in murine and human tissue samples. We could show high expression in lymphatic vessels during mouse development, in adult mice and human skin samples. Further in vitro experiments including siRNA knock-down in HDLECs, highlighted an essential role of BCAR1 in focal adhesions and proliferation of LECs. These results were confirmed in zebrafish using *BCAR1*-morpholinos.

Conclusion: We present *BCAR1* as a novel candidate gene for primary congenital lymphedema underlying an autosomal recessive inheritance mode. We could show that BCAR1 is essential for cell proliferation and adhesion in LECs through in vitro and in vivo analysis in mice, zebrafish, and human tissue samples.

Grants: ERC-StG PREVENT

Conflict of Interest: Charlotte Kemper For the Project the ERC-StG PREVENT Grant of Dr. Dr. René Hägerling (Group leader) was used, Rene Hägerling: None declared

P07.019.C Genetic background of inherited arrhythmia syndromes in a cohort of Czech patients

Jana Zídková[1], *Markéta Bébarová*[2], *Martin Král*[2], *Olga Švecová*[2], *Štefan Zelenák*[3], *Jiří Pacherník3*[3], *Tomáš Bárta*[4], *Iva Synková*[1], *Tereza Kramářová*[1], *Tomáš Novotný*[5], *Samuel Lietava*[5], *Lenka Fajkusova*[1]

[1]University Hospital Brno, Centre of Molecular Biology and Genetics, Brno, Czech Republic; [2]Faculty of Medicine, Masaryk University, Department of Physiology, Brno, Czech Republic; [3]Faculty of Science, Masaryk University, Department of Experimental Biology, Brno, Czech Republic; [4]Faculty of Medicine, Masaryk University, Department of Histology and Embryology, Brno, Czech Republic; [5]University Hospital Brno, Department of Internal Medicine and Cardiology, Brno, Czech Republic

Background/Objectives: Inherited arrhythmia syndromes are rare genetic disorders that can range in severity from no symptoms to sudden cardiac death, even within affected families. The interpretation of genetic findings can be challenging, particularly when it comes to identifying variants of uncertain significance (VUS). Our study presents an overview of the molecular characteristics of a group of Czech patients with a causative variant in a gene associated with arrhythmia syndromes, as well as functional analysis of a selected VUS variant.

Methods: We use custom capture panel sequencing for variant detection and CNV analysis. Functional analysis of the *RYR2* p.(Y4734C) variant was performed using a multielectrode array on patient-specific hiPSC-derived cardiomyocytes of the proband and an unrelated healthy control.

Results: Causative variants were most frequently identified in the potassium channel genes *KCNQ1* and *KCNH2*, followed by *RYR2*, *SCN5A*, and other genes (*CACNA1C*, *CASQ2*, *HCN4*, *KCND3*, *KCNE1*, *KCNJ2*). Regarding functional analysis, the pilot data showed: (i.) a shorter field potential duration, (ii.) a reduced reactivity on β-adrenergic stimulation, and (iii.) a higher short-term variability of CL during pro-arrhythmic conditions.

Conclusion: Our findings provide valuable insights into the genetic background of inherited arrhythmia syndromes in Czech patients. Detailed functional analysis is necessary to reveal a possible relationship between the genotype and phenotype and can contribute to improved diagnosis and family counselling.

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Conflict of Interest: None declared

P07.020.D Epilepsy with faint capillary malformation or reticulated telangiectasia associated with mosaic AKT3 mutations

Nicole Revencu[1], *Martina De Bortoli*[2], *Marta Ivars*[3], *Marie-Cécile Nassogne*[4], *Cinzia Emilia Lavarino*[5], *Martin Lammens*[6], *Anne Renders*[7], *Dana Dumitriu*[8], *Raphaël Helaers*[2], *Laurence Boon*[9], *Eulalia Baselga*[3], *Miikka Vikkula*[2]

[1]Cliniques universitaires Saint-Luc (UCLouvain), Center for Human Genetics, Bruxelles, Belgium; [2]De Duve Institute UCLouvain, Human

Molecular Genetics, Woluwe-Saint-Lambert, Belgium; [3]Sant Joan de Déu Barcelona Hospital, Department of Dermatology, Esplugues de Llobregat, Spain; [4]Cliniques universitaires Saint-Luc (UCLouvain), Pediatric Neurology Unit, Bruxelles, Belgium; [5]Sant Joan de Déu Barcelona Hospital, Laboratory of Molecular Oncology, Pediatric Cancer Center Barcelona, Esplugues de Llobregat, Spain; [6]Cliniques universitaires Saint-Luc (UCLouvain), Service d'anatomopathologie, Bruxelles, Belgium; [7]Cliniques universitaires Saint-Luc (UCLouvain), Rehabilitation Department, Bruxelles, Belgium; [8]Cliniques universitaires Saint-Luc (UCLouvain), Pediatric Radiology Unit, Bruxelles, Belgium; [9]Cliniques universitaires Saint-Luc (UCLouvain), Center for Vascular Anomalies, Division of Plastic Surgery, Bruxelles, Belgium

Background/Objectives: Capillary Malformations (CM) are the most common type of vascular anomalies, affecting around 0.3% of newborns. They are usually caused by somatic pathogenic variants in *GNAQ* or *GNA11*. *PIK3CA* and *PIK3R1*, part of the PI3K-AKT-mTOR pathway, are mutated in fainter CMs such as megalencephaly-capillary malformation (M-CM) and diffuse capillary malformation with overgrowth (DCMO).

Results: In this study, we present two young patients with a CM-like phenotype, associated with cerebral anomalies and severe epilepsy. Pathogenic variants in *PIK3CA* and *PIK3R1*, as well as *GNAQ* and *GNA11* were absent in affected cutaneous tissue biopsies. Instead, we identified two somatic pathogenic variants in the *AKT3* gene. Subsequent analysis of the DNA obtained from surgically resected brain tissue of one of the two patients confirmed the presence of the *AKT3* variant. Focal cortical dysplasia was also detected in this patient. Genetic analysis thus facilitated work-up to reach a precise diagnosis for these patients, associating the vascular anomaly with the neurological symptoms.

Conclusion: This study underscores the importance to search for additional signs and symptoms to guide the diagnostic workup, especially in cases with atypical vascular malformations. Additionally, it strongly emphasizes the significance of genotype-phenotype correlation studies in guiding clinicians' informed decision-making regarding patient care.

Conflict of Interest: None declared

P07.021.A Whole exome sequencing identifies novel genetic variants associated with syndromic Tetralogy of Fallot

Fanni Tóth-Szumutku[1], *Ilona Kun[1], Anna Lengyel[1], Eva Pinti[1], Krisztina Nemeth[1], Tunde Abonyi[1], Seung Woo Ryu[2], Yongjun Song[2], Eva Kis[3], Laszlo Ablonczy[3], Vera Goda[4], Gergely Krivan[4], Iren Haltrich[1], Árpád Ferenc Kovács[1]*

[1]Semmelweis University, Pediatric Center, Tűzoltó Street Department, Budapest, Hungary; [2]3billion Inc., Seoul, Korea, Rep. of South; [3]Gottsegen National Cardiovascular Center, Budapest, Hungary; [4]Central Hospital of Southern Pest National Institute of Hematology and Infectious Diseases, Budapest, Hungary

Background/Objectives: Tetralogy of Fallot (TOF) is one of the most common cyanotic congenital heart diseases. The pathogenesis of this condition needs further clarification. The aim of our study was to ascertain genetic variants among patients with syndromic Tetralogy of Fallot.

Methods: Patients were retrospectively collected between 2005 and 2023 from two Hungarian pediatric centers. Karyotype analysis and fluorescent in situ hybridization (FISH) or multiplex ligation-dependent probe amplification (MLPA) specific for 22q11.2 microdeletion syndrome were performed for all patients. Whole exome sequencing (WES) was completed for patients lacking 22q11.2 deletion. Validation of identified variants was run via Sanger sequencing and FISH.

Results: 26 unrelated patients with syndromic TOF underwent WES. We identified 13 variants, including four pathogenic, six likely pathogenic variants (38% diagnostic yield) and three variants of uncertain significance (VUS). Two patients carried *JAG1* and three patients *CHD7* variants. Four novel likely pathogenic variants were identified: a nonsense variant in Patient 1 [*JAG1*:c.1080dup (p.Glu361Ter)], and a nonsense variant in Patient 2 [*PHF8*:c.718C>T (p.Arg240Ter)]. Patient 3 carried a nonsense *CHD7*:c.3310_3311del (p.Ile1104Ter) variant and Patient 4 carried a nonsense *CHD7*:c.4505del (p.Ser1502Ter) variant.

Conclusion: This is the first study conducted on a Hungarian patient cohort with syndromic TOF. The WES approach is an effective diagnostic tool in syndromic TOF. Importantly, we identified four novel variants, thus our results extend the genotypic spectrum of syndromic TOF associated *JAG1* and *CHD7* genes. To our knowledge, this is the first report of *PHF8* potentially associated with syndromic TOF.

Grants: 3billion's MEGA Grant Program

Conflict of Interest: Fanni Tóth-Szumutku: None declared, Ilona Kun: None declared, Anna Lengyel: None declared, Eva Pinti: None declared, Krisztina Nemeth: None declared, Tunde Abonyi: None declared, Seung Woo Ryu: None declared, Yongjun Song: None declared, Eva Kis: None declared, Laszlo Ablonczy: None declared, Vera Goda: None declared, Gergely Krivan: None declared, Iren Haltrich: None declared, Árpád Ferenc Kovács 3billion Megagrant 2022/20221123 to ÁFK

P07.022.B Clinical characteristics and cardiovascular disease outcomes of individuals with different familial hypercholesterolaemia gene-mutations in the UK Simon Broome Register

Nicole Omofoman[1], *Barbara Iyen[2], Steve Humphries[3], Nadeem Qureshi[2]*

[1]University of Leicester, Medical Genetics, School of Biological Sciences, Leicester, United Kingdom; [2]University of Nottingham, Centre for Academic Primary Care, Nottingham, United Kingdom; [3]University College London, Centre for Cardiovascular Genetics, London, United Kingdom

Background/Objectives: Familial hypercholesterolaemia (FH) is caused by a mutation in the *LDLR, APOB* or *PCSK9* genes. We investigated whether there are differences in cardiovascular disease (CVD). outcomes for the different FH genes, using the UK Simon Broome (SB) register linked with secondary care records in Hospital Episode Statistics (HES)

Methods: CVD incidence rates were determined (defined as a composite of coronary heart disease (CHD), myocardial infarction (MI), stable or unstable angina, stroke, TIA, peripheral vascular disease (PVD), heart failure or coronary revascularisation interventions). Conditional logistic regression determined hazards ratios for CVD associated with the different FH-genes.

Results: Of 2988 individuals in SB register with linked HES records, 399 (13.4%) had an identified FH-mutation (*LDLR* in 371(93%), *APOB* in 23(5.8%), and *PCSK9* in 5 (1.3%) individuals). At registration individuals with a *PCSK9* mutation had significantly higher mean pre-treatment total cholesterol (13.2mmol/l (SD1.6)), followed by individuals with *LDLR* and *APOB* gene mutations (10.3mmol/l (SD2.1) and 9.4mmol/l (SD1.3) respectively).

The composite CVD incidence rate (95%CI) (per 100,000 person-years) in individuals with an *LDLR* mutation was 23.8 (20.4-27.8), *APOB* was 17.2 (8.2-36.0), and *PCSK9* was 40.5 (13.5-125.5). Compared to clinical FH-individuals without an identified mutation, the highest CVD risk was observed in *PCSK9* mutation carriers (HR 1.9 [95%CI 0.6-6.1]), then *LDLR* (HR 1.06 [0.8-1.4]) and *APOB* (HR 0.5 [0.3-1.2]) carriers.

Conclusion: Individuals with *PCSK9* and *LDLR* gene mutations had more severe FH-phenotypes than those with *APOB* mutations, however there was no statistically significant difference in CVD risk between individuals with different FH gene mutations.

Grants: NIHR HTA award ID 15/134/02

Conflict of Interest: None declared

P07.023.C X-linked isolated dilated cardiomyopathy with myocardial emerin deficiency caused by missense variant in the EMD gene

Linda Bulmer[1;2], *Charlotta Ljungman*[2;3], *Johan Hallin*[1], *Pia Dahlberg*[2;3], *Carola Oldfors*[4], *Anders Oldfors*[4], *Anders Gummesson*[1;2]

[1]*Sahlgrenska University Hospital, Department of Clinical Genetics and Genomics, Gothenburg, Sweden;* [2]*Institute of Medicine, Sahlgrenska Academy, University of Gothenburg, Department of Molecular and Clinical Medicine, Gothenburg, Sweden;* [3]*Sahlgrenska University Hospital, Department of Cardiology, Gothenburg, Sweden;* [4]*Institute of Biomedicine, Sahlgrenska Academy, University of Gothenburg, Department of Laboratory Medicine, Gothenburg, Sweden*

Background/Objectives: The *EMD* gene, causative for Emery-Dreifuss muscular dystrophy type 1, has been suggested to associate with isolated dilated cardiomyopathy (DCM). However, evidence is currently insufficient to verify the gene-disease association. We investigated the causality for a missense variant, c.23C>G, p.(Ser8Trp), in *EMD* in a family with X-linked DCM without muscular dystrophy.

Methods: All family members confirmed to harbour the *EMD* variant underwent cardiac examination. Affected males were investigated for muscular dystrophy by physical examination, s-CK measurements and histopathological examination of skeletal muscle. Immunohistochemical analyses of emerin on myocardium and skeletal muscle were conducted. Segregation analysis was performed, and results quantified by estimation of LOD-score based on number of informative meioses and calculation of full-likelihood Bayes factor.

Results: Seven males and 19 females were included. Six hemizygous males were diagnosed with DCM at age 36-50 years. Conduction defects and ventricular arrythmias were prominent features, and three were heart transplanted due to heart failure. There was a family history of suspected sudden cardiac death in males. Muscular dystrophy was negated for all affected males and excluded by clinical examination in four. The *EMD* variant co-segregated with DCM (estimated LOD score 3.9, odds >2500:1 in favour of causality). Emerin immunostaining was profoundly reduced in myocardial specimens available from three affected males. Among the 17 heterozygous females, aged 20-87 years, one developed DCM at age 72.

Conclusion: We propose isolated DCM as a new *EMD*-phenotype, supported by the evidence implicating *EMD* in X-linked DCM without muscular dystrophy in this family.

Grants:

Conflict of Interest: None declared

P07.024.D Polygenic inheritance for common co-morbidities associated with congenital heart disease

Jamie-Lee Thompson[1], *Ingrid Tarr*[1], *Michael Troup*[1], *Gillian Blue*[2;3], *Emma Rath*[1;4], *David Winlaw*[1;2;3;4;5], *Sally Dunwoodie*[1;4], *Eleni Giannoulatou*[1;4]

[1]*Victor Chang Cardiac Research Institute, Computational Genomics, Darlinghurst, Australia;* [2]*Heart Centre for Children, Sydney, Australia;* [3]*The University of Sydney, Sydney Medical School, Sydney, Australia;* [4]*St Vincent's Clinical School, Faculty of Medicine, Sydney, Australia;* [5]*Ann & Robert H. Lurie Children's Hospital of Chicago, Chicago, United States*

Consortium: CHD Synergy Consortium

Introduction: Congenital Heart Disease (CHD) encompasses any structural abnormalities that are present in the cardiovascular system at birth. Individuals with CHD often have an added burden of co-morbidities, with approximately two thirds of patients affected by co-occurring medical conditions, which can significantly impact overall health and quality of life. The aim of this project was to assess the role of polygenic inheritance in the development of common co-morbidities.

Methods: Polygenic risk scores (PRS) for co-morbidities including hypertension, type 2 diabetes, obesity, and birth weight were calculated in two cohorts of CHD patients and their respective control groups. The first cohort consisted of 184 patients and 3,823 controls, while the validation cohort consisted of 486 patients and 503 controls.

Results: Several common CHD co-morbidities including hypertension, type 2 diabetes, and obesity had higher polygenic scores than comparable healthy controls, with significant associations for hypertension (9.8% higher mean) and type 2 diabetes (7.2% higher mean) in the first cohort. Results from the validation cohort supported this, with significant associations for hypertension (5.1% higher mean), type 2 diabetes (4.6% higher mean), and obesity (5.7% higher mean).

Conclusion: The data presented here suggests polygenic inheritance may contribute to the presence of some CHD co-morbidities, and therefore polygenic risk scores may provide a more comprehensive understanding of a patient's risk for development of co-morbidities.

Grants: NHMRC Synergy Grant (1181325)

Conflict of Interest: None declared

P07.025.A Re-evaluating the clinical validity of hypertrophic cardiomyopathy genes

Sophie Hespe[1], *Amber Waddell*[2], *Babken Asatryan*[3], *Emma Owens*[2], *Courtney Thaxton*[2], *Mhy-Lanie Adduru*[4], *Kailyn Anderson*[5], *Emily Brown*[6], *Lily Hoffman-Andrews*[7], *Elizabeth Jordan*[8], *Megan Mayers*[2], *Stacey Peters*[9], *Fergus Stafford*[1], *Richard Bagnall*[10], *Lucas Bronicki*[11], *Bert Callewaert*[12], *Anwar Chahal*[13], *Cynthia James*[6], *Olga Jarinova*[11], *Andrew Landstrom*[14], *Elizabeth McNally*[15], *Brittney Murray*[6], *Laura Muino Mosquera*[12], *VICTORIA PARIKH*[16], *Chloe Reuter*[16], *Roddy Walsh*[17], *Bess Wayburn*[18], *James Ware*[19], **Jodie Ingles**[1]

[1]*Garvan Institute of Medical Research, and University of New South Wales, Genomics and Inherited Disease Program, Sydney, Australia;* [2]*University of North Carolina at Chapel Hill, Department of Genetics, Chapel Hill, United States;* [3]*Johns Hopkins University School of Medicine, Division of Cardiology, Department of Medicine, Baltimore, United States;* [4]*Predictiv Care, San Francisco, United States;* [5]*University of Washington, Division of Genetic Medicine, Department of Pediatrics, Seattle, United States;* [6]*Johns Hopkins University, Division of Cardiology, Department of Medicine, Baltimore, United States;* [7]*Perelman School of Medicine at the University of Pennsylvania, Perelman Center for Advanced Medicine, Division of Cardiovascular Medicine, Department of Medicine, Center for Inherited Cardiovascular Disease, Philadelphia, United States;* [8]*Ohio State University, Division of Human Genetics, Department of Internal Medicine, Columbus, United States;* [9]*Royal Melbourne Hospital, Department of Cardiology and Genomic Medicine, Melbourne, Australia;* [10]*Centenary Institute, University of Sydney, Bioinformatics and Molecular Genetics, Sydney, Australia;* [11]*Children's Hospital of Eastern Ontario, Department of Genetics, Ottawa, Canada;* [12]*Ghent University, Department of Biomolecular Medicine, Ghent, Belgium;* [13]*WellSpan Health, Center for Inherited Cardiovascular Diseases,*

Lancaster, United States; [14]Duke University School of Medicine, Department of Pediatrics and Cell Biology, Durham, United States; [15]Northwestern University Feinberg School of Medicine, Center for Genetic Medicine, Dept of Medicine (Cardiology), Chicago, United States; [16]Stanford University School of Medicine, Stanford Center for Inherited Cardiovascular Disease, Division of Cardiovascular Medicine, Department of Medicine, Stanford, United States; [17]Amsterdam University Medical Centre, University of Amsterdam, Department of Experimental Cardiology, Amsterdam Cardiovascular Sciences, Amsterdam, Netherlands; [18]Ambry Genetics, Clinical Genomics, Aliso Viejo, United States; [19]Imperial College London, National Heart and Lung Institute & MRC Laboratory of Medical Sciences, London, United Kingdom

Consortium: ClinGen - Clinical Genome Resource

Background/Objectives: Hypertrophic cardiomyopathy (HCM) is an inherited cardiac condition affecting ~1 in 500 and exhibits extensive genetic heterogeneity. In 2018, The ClinGen Hereditary Cardiovascular Disease Gene Curation Expert Panel (HCVD-GCEP) curated 57 genes associated with HCM. This study, represents a reappraisal of the clinical validity of genes linked to HCM.

Methods: The ClinGen gene curation approach was employed to re-assess the validity of gene-disease relationships in HCM and related syndromic entities involving left ventricular hypertrophy.

Results: Thirty-five genes were re-curated based on their previous classification and time since last curation, and an additional 5 genes recently associated with HCM were included. Among the re-curated genes, 17 (50%) genes changed classification: 1 limited (previously no evidence), 4 disputed (previously no evidence), 9 disputed (from limited), and 3 definitive (previously moderate). Notably, 3 (9%) genes had a clinically relevant upgrade, including *TNNC1*, a 9th sarcomere gene with definitive HCM association. Nine (26%) genes were downgraded to disputed, further discouraging clinical reporting of variants in these genes. Two genes were curated for multiple inheritance patterns (*TRIM63*, disputed for autosomal dominant [AD] but moderate for autosomal recessive [AR]; *ALPK3*, strong for AD but definitive for AR). Five genes recently associated with HCM were curated: two limited (*RPS6KB1* and *RMB20*), 2 moderate (*KLHL24* and *MT-TI*), and 1 definitive (*FHOD3*).

Conclusion: Important reclassifications of previously curated and assessment of recently implicated HCM genes informs reporting of genetic variants for patients worldwide. Our work highlights the need for periodic evidence-based re-appraisal of gene curations.

Grants: NHGRI: Baylor/Stanford U24HG009649, Broad/Geisinger U24HG006834, UNC/Kaiser U24HG009650

Conflict of Interest: Sophie Hespe: None declared, Amber Waddell: None declared, Babken Asatryan: None declared, Emma Owens: None declared, Courtney Thaxton: None declared, Mhy-Lanie Adduru I have stock options with a company working on whole genome sequencing results integration with clinical data, Kailyn Anderson: None declared, Emily Brown I am a consultant for MyGene Counsel., Lily Hoffman-Andrews: None declared, Elizabeth Jordan: None declared, Megan Mayers: None declared, Stacey Peters: None declared, Fergus Stafford: None declared, Richard Bagnall: None declared, Lucas Bronicki Our laboratory has recently revamped our test menus for cardiomyopathies, including HCM. We may publish our experience with selecting genes to include in our CM panels., Bert Callewaert Work for a laboratory that offers fee-for-service testing related to the work of your ClinGen group, Anwar Chahal: None declared, Cynthia James Not directly related to variants or variant adjudication: Consultants for: Pfizer Inc, StrideBio Inc (uncompensated), Lexeo, Inc; Tenaya therapeutics (uncompensated). Research grant from: StrideBio Inc; Lexeo Inc, Olga Jarinova Our diagnostic laboratory offers testing for inherited cardiomyopathies. We are continuously optimizing the content of our panels and carrying out "mini-curations" of selected genes., Andrew Landstrom: None declared, Elizabeth McNally I intermittently consult for Invitae, a genetic testing company. I am part of the NIH sponsored EMERGE consortium (Electronic Health Records and Genomics). I am a consultant for AstraZeneca, Avidity, Amgen, Cytokinetics, PepGen, Pfizer, Tenaya Therapeutics, and I am the founder of Ikaika Therapeutics., Brittney Murray Consultant for MyGeneCounsel, Laura Muino Mosquera: None declared, VICTORIA PARIKH: None declared, Chloe Reuter: None declared, Roddy Walsh: None declared, Bess Wayburn work for a laboratory that offers fee-for-service testing related to the work of your ClinGen group, James Ware Past/present consultant or advisor to Pfizer; MyoKardia, Bristol-Myers Squibb; Foresite Labs; HealthLumen; Tenaya Past/present research support (to institution) from Pfizer; MyoKardia, Bristol-Myers Squibb (Not directly related to this work), Jodie Ingles: None declared

P07.026.B Genetic testing in a well-selected cohort of restrictive cardiomyopathy (RCM) detects variants in genes encoding sarcomeric proteins.

Markéta Adamová[1], *Alice Krebsová[1], Miloš Kubánek[1], Petra Peldová[2], Pavel Votýpka[2], Martin Kotrč[1], Lenka Piherová[3], Vojtěch Melenovský[1], josef Kautzner[1], Milan Macek[2]*

[1]Institute of Clinical and Experimental Medicine, Department of Cardiology, Prague, Czech Republic; [2]Charles University and Motol University Hospital, 2nd Faculty of Medicine, Department of Biology and Medical Genetics, Prague, Czech Republic; [3]Charles University in Prague and General University Hospital in Prague, First Faculty of Medicine, Department of Paediatric and Adolescent Medicine, Prague, Czech Republic

Background/Objectives: Restrictive cardiomyopathy (RCM) is the rarest form of cardiomyopathy. Its aetiology is rather complex ranging from infiltrative, inflammatory or hereditary causes, yet its genetic background is not entirely understood. Our goal was to analyse the genetic background in a selected cohort of Czech RCM cases.

Methods: RCM was defined according to the 2023 ESC Guidelines. A total of 12 probands (6 males, 6 females; mean age at diagnosis 43 ± 13 years) was included. Heart transplantation was necessary for terminal heart failure in 8/12 (67 %). All probands received clinical genetic consultation and molecular genetic analysis using next generation sequencing (targeted/exome sequencing; SOPHiA GENETICS, Switzerland). Family cascade screening was carried out in 38 relatives (3.2 per case).

Results: ACMG.net class 4 and 5 variants were disclosed in 10/12 (83 %) patients. Identified genes harbouring the pathogenic mutation encode sarcomeric proteins - *FLNC* (3/10), *DES* (2/10), *TNNT2* (2/10), *TNNI3* (2/10) and *MYBPC3* (1/10) genes. Among relatives, 10/38 persons were disclosed to be at risk of RCM or even already manifested cardiomyopathy (5/10), in its restrictive (2/10) or hypertrophic (3/10) forms.

Conclusion: The genetic yield in our selected cohort of familial RCM is high (83 %), mainly comprising variants in genes encoding sarcomeric and cytoskeletal proteins. The most frequently affected gene is *FLNC*. Even in a well-selected cohort with RCM, we found an apparent overlap with hypertrophic cardiomyopathy in relatives. Further studies are needed to elucidate the pathophysiological mechanisms leading to either restrictive or hypertrophic phenotype.

Grants: LX22NPO5104, ZD-ZDOVA2-001

Conflict of Interest: None declared

P07.027.C Genetic findings for cardiomyopathy and arrhythmia: a retrospective analysis of over 1,800 patients

Kirsi Alakurtti[1], *Julie Hathaway*[2], *Johanna Huusko*[1], *Marcos Cicerchia*[1], *Saija Ahonen*[1], *Johanna Tommiska*[1], *Kimberly Gall*[2], *Khalida Liaquat*[2], *Victoria Howell*[2], *Allison Sluyters*[2], *Janica Djupsjöbacka*[1], *Mikko Muona*[1], *Inka Saarinen*[1], *Eija H. Seppala*[1], *Tiia Kangas-Kontio*[1], *Lotta Koskinen*[1], *Pertteli Salmenperä*[1], *Samuel Myllykangas*[1], *Juha Koskenvuo*[1]

[1]*Blueprint Genetics, Espoo, Finland;* [2]*Blueprint Genetics Inc, Marlborough MA, United States*

Background/Objectives: Few cohort studies have examined the rate of informative test results in patients with inherited arrhythmias (IAs) and/or cardiomyopathies (CMs); but have not included the analysis of both the nuclear and mitochondrial (mtDNA) genes. We describe findings in an IA/CM cohort using a broad next-generation sequencing (NGS) panel that included both nuclear and mtDNA genes.

Methods: The NGS panel included 260 genes associated with IAs and/or CMs. 64.3% of patients had mtDNA genes included in their panel test. An informative test result was defined as the identification of pathogenic (P) or likely pathogenic (LP) variant(s) consistent with the patient's reported phenotype and known associated disease inheritance.

Results: 1,883 patients underwent testing, 81.1% were adults. 17.7% of patients received an informative test result. 1.2% had a LP/P deep-intronic variant and 4.5% had a LP/P copy number variant (CNV). 26.7% of these CNVs were single-exon deletions. 2.4% had a LP/P variant in a mtDNA gene. Children were more likely to receive an informative result (22.9%) than adults (16.6%). LP/P variants in *MYBPC3* were the most common finding in children (10%), followed by *RYR2* (8.8%). LP/P variants in *TTN* were the most common finding in adults (15.8%), followed by *MYBPC3* (7.9%).

Conclusion: 17.7% of patients received an informative result. Almost 5% were explained by a mtDNA variant, deep-intronic variant, or single-exon deletion. LP/P variants in *MYBPC3* were seen in 8% of patients. This study demonstrates that a NGS panel that includes the analysis of both nuclear and mtDNA genes has clinical utility for patients with IA and/or CM.

Conflict of Interest: Kirsi Alakurtti Blueprint Genetics, Julie Hathaway Blueprint Genetics, Johanna Huusko Blueprint Genetics, Marcos Cicerchia Blueprint Genetics, Saija Ahonen Blueprint Genetics, Johanna Tommiska Blueprint Genetics, Kimberly Gall Blueprint Genetics, Khalida Liaquat Blueprint Genetics, Victoria Howell Blueprint Genetics, Allison Sluyters Blueprint Genetics, Janica Djupsjöbacka Blueprint Genetics, Mikko Muona Blueprint Genetics, Inka Saarinen Blueprint Genetics, Eija H. Seppala Blueprint Genetics, Tiia Kangas-Kontio Blueprint Genetics, Lotta Koskinen Blueprint Genetics, Pertteli Salmenperä Blueprint Genetics, Samuel Myllykangas stock and stock options at Quest, Blueprint Genetics, Juha Koskenvuo stock and stock options at Quest, Blueprint Genetics

P07.028.D Unraveling Genetic Pathways: NOTCH1 Haploinsufficiency and Multi-Gene Involvement in Thoracic Aortic Aneurysms

Laura Torres-Juan[1], *Yolanda Rico*[2], *Elena Fortuny-Frau*[2], *Jaume Pons-Llinares*[2], *Susana Renee Avella-Klaassen*[1], *Tomás Ripoll-Vera*[3], *Jorge Alvarez-Rubio*[3], *Rafael Ramos*[4], *Fernando Santos-Simarro*[1], *Victor Asensio-Landa*[1], *Iciar Martínez*[1], **DAMIAN HEINE SUÑER**[1]

[1]*Hospital Universitari Son Espases/Health Research Institute of the Balearic Islands (IdISBa), Unit of Molecular Diagnostics and Clinical Genetics, Palma;* [2]*Hospital Universitari Son Espases/Health Research Institute of the Balearic Islands (IdISBa), Cardiology, Palma;* [3]*Hospital Universitario Son Llàtzer/Health Research Institute of the Balearic Islands (IdISBa), Cardiology, Palma, Spain;* [4]*Hospital Universitari Son Espases, Pathology, Palma*

Thoracic aortic aneurysms (TAA) represent a critical medical condition characterized by abnormal dilation of the ascending aorta, posing significant risks due to vessel wall weakness. The congenital bicuspid aortic valve (BAV) is recognized as a risk factor for TAA development, particularly attributed to the disruptive influence of asymmetric blood flow on the aortic wall. Although NOTCH1 mutations have been linked to non-syndromic TAAs resulting from BAV, limited understanding exists regarding NOTCH1 haploinsufficiency and its association with connective tissue abnormalities.

Our study involved the analysis of 127 cases through whole exome sequencing (WES) or whole genome sequencing (WGS), revealing suggestive variants in 44% (56 cases). Notably, two cases presented clear evidence that NOTCH1 gene alterations can be causative factors for TAA even in the absence of BAV. Specifically, a 117 Kb deletion encompassing a significant portion of the NOTCH1 gene, without affecting other coding genes, suggests haploinsufficiency as a potential pathogenic mechanism associated with TAA.

Furthermore, our findings shed light on the complexity of aortic pathology, exemplified by two brothers carrying variants in both the NOTCH1 and MIB1 genes. This observation underscores the involvement of different genes within the Notch pathway in the manifestation of aortic abnormalities, contributing to a comprehensive understanding of the genetic landscape underlying TAA development

Conflict of Interest: None declared

P07.029.A Identification of candidate genes for hypoplastic left heart syndrome using innovative exome sequencing data analysis and interpretation methods

Stella van Bergen[1], **Marrit M. Hitzert**[1], *Bart Charbon*[1], *Dennis Hendriksen*[1], *Jan Jongbloed*[1], *Cleo van Diemen*[1], *Wilhelmina S. Kerstjens-Frederikse*[1]

[1]*University Medical Center Groningen, Department of Genetics, Groningen, Netherlands*

Background/Objectives: Hypoplastic left heart syndrome (HLHS) is one of the most severe congenital heart diseases. Genetic causes are presumed to be heterogeneous. Although some causal genes have been identified, variants in these genes only explain a small subset of patients. We performed whole exome sequencing (WES) to identify novel candidate genes.

Methods: WES was performed on 42 index patients and their parents. Data was analysed with an in-house variant interpretation pipeline (VIP), using a deep learning-based trio variant caller and applying state of the art prediction algorithms. We filtered and interpreted candidate variants using dominant, recessive and complex inheritance models. Variant pathogenicity was determined using the ACMG guidelines and prioritisation of candidate genes was done by two clinical geneticists.

Results: In 26 of 42 patients, we identified at least one candidate variant. In total 37 variants were identified in 31 genes. In nineteen patients only one candidate gene was found while in seven patients multiple candidate genes (2-5) were identified.

Conclusion: This study revealed variants in various genes not previously associated with HLHS, results being in line with monogenic and multigenic aetiologies. Our findings need both replication in other patient cohorts as well as evaluation of gene function before a definitive causal role in HLHS pathogenesis can be established.

Grants: This research was funded by Dutch foundation for rare diseases (Stichting Zeldzame Ziekten Fonds)

Conflict of Interest: Stella van Bergen: None declared, Marrit M. Hitzert: None declared, Bart Charbon: None declared, Dennis Hendriksen: None declared, Jan Jongbloed: None declared, Cleo van Diemen: None declared, Wilhelmina S. Kerstjens-Frederikse Dutch foundation for rare diseases (Stichting Zeldzame Ziekten Fonds)

P07.030.B Unravelling Hypertension: A scientific exploration through integrative omics analysis

Aiman Farzeen[1;2;3], *Riccardo Berutti*[1;4], *Martin Pavlov*[1;4], *Harald Grallert*[2;3], *Annette Peters*[3], *Birgit Linkohr*[3], *Holger Prokisch*[1;4], *Christian Gieger*[2;5]

[1]*Technical University of Munich, Institute of Human Genetics, Munich, Germany;* [2]*Research Unit of Molecular Epidemiology, Neuherberg, Germany;* [3]*Institute of Epidemiology, Neuherberg, Germany;* [4]*Helmholtz Munich, Institute of Neurogenomics, Neuherberg, Germany;* [5]*Institute of Epidemiology, Molecular Epidemiology, Neuherberg, Germany*

Background/Objectives: Cardiovascular diseases (CVDs) are the most common non-communicable diseases, responsible for 17.9 million deaths each year. Hypertension a major risk factor for CVDs. GWAS studies have identified over 1,000 variants associated with hypertension. However, the functional impact of these variants is rarely known. Gene expression data generated by RNA-sequencing helps fill in the gaps by finding relevant genes affected by the identified variants.

Methods: RNA-sequencing was performed on 2,013 whole blood samples from a population cohort in Augsburg, Germany. This cohort is the first of its kind in the region. 10,685 genes passed QC and were used in dimensionality reduction analyses to find patterns in the data. Using these insights, differential gene expression analysis was done followed by pathway analysis. To investigate the connection between genetic variants and gene expression, genotyping array data was integrated to perform eQTL analysis.

Results: 132 genes in total were found significant after multiple testing correction, for systolic or Diastolic BP. The results of our analysis not only align with existing literature but also highlight new genes and pathways that could be further studied for hypertension. Some genes were reported for some cardiovascular phenotypes other than hypertension like LMNA and KCNJ2. Others, among many, were not reported for hypertension e.g TNFRSF10C, CEACAM3 and MIR23AHG. Our eQTL analysis also showed around 1,300 unique gene-snp pairs with some overlap with known hypertension GWAS loci, hence providing functional evidence.

Conclusion: RNA-sequencing data offers more information and unveils novel therapeutic avenues for managing hypertension.

Grants: DigiMed Bayern

Conflict of Interest: None declared

P07.031.C Genetic investigation of inherited cardiac diseases in Cypriot patients by targeted Next-Generation Sequencing analysis

Constantina Koutsofti[1], *Marios Ioannides*[2], *christiana polydorou*[1], *Gregory Papagregoriou*[1], *Apostolos Malatras*[1], *George Michael*[1], *Irene Hadjiioannou*[1], *Grigoris Constantinou*[1], *Katerina Stefanou*[1], *Stylianos Pieri*[1], *Eleni Loizidou*[1], *Christos Eftychiou*[2], *Elias Papasavvas*[3], *Theodoros Christophides*[2], *Anna Alkelai*[4], *Manav Kapoor*[4], *Alan R Shuldiner*[4], *PANAYIOTIS AVRAAMIDES*[2], *Constantinos Deltas*[1;5]

[1]*biobank.cy Center of Excellence in Biobanking and Biomedical Research, University of Cyprus, Nicosia, Cyprus;* [2]*Nicosia General Hospital, Department of Cardiology, Nicosia, Cyprus;* [3]*Platonas Medical Center, Nicosia, Cyprus;* [4]*Regeneron Genetics Center, Tarrytown, New York, United States;* [5]*School of Medicine, University of Cyprus, Nicosia, Cyprus*

Background/Objectives: Inherited cardiac diseases represent a highly heterogeneous group of conditions. Pathogenic variants in genes expressed in the cardiomyocytes cause a diverse spectrum of phenotypes. We focused on genetic testing of Cypriots with a clinical phenotype of an inherited heart condition.

Methods: We applied massive parallel DNA sequencing using a 72-gene-panel for studying inherited cardiac diseases. The detected variants were analysed with a bioinformatics approach. All filtered variants were validated using Sanger analysis.

Results: We analysed the data of probands in 40 and 10 families with inherited cardiomyopathies and arrhythmias respectively. In the 40 families with cardiomyopathies, we identified 72 variants, 48 novel. The 72 variants were classified: 47 (65%) as variants of uncertain significance, 18 (25%) as likely-pathogenic, and seven (10%) as pathogenic. In the 10 families with arrhythmias, we identified 16 variants, 10 novel. We classified the 16 variants: 10 (62%) as variants of uncertain significance, three (19%) as likely-pathogenic, and three (19%) as pathogenic.

Conclusion: We characterized genetically families with a cardiac phenotype. In some cases, the genetic heterogeneity and the multiplicity of variants make the definite molecular diagnosis challenging, especially when there is suspicion for incomplete penetrance. This is the first systematic study attempted in Cyprus for inherited cardiac conditions, enabling us to develop a genetic baseline and contribute to precision cardiology.

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Conflict of Interest: None declared

P07.033.A A young patient with an ajmaline induced type-I Brugada pattern in Cyprus and challenging genetic findings

Grigoris Constantinou[1], **Katerina Stefanou**[1], *Myria Zachariou*[1], *christiana polydorou*[1], *Gregory Papagregoriou*[1], *Pantelis Kourtellaris*[2], *Sofia Ourani*[3], *Elias Avraam*[4], *Emily Athanasiou*[3], *Constantinos Deltas*[1]

[1]*Molecular Medicine Research Center / Κέντρο Ερευνών Μοριακής Ιατρικής, biobank.cy, Center of Excellence in Biobanking and Biomedical Research, Nicosia, Cyprus;* [2]*American Medical Center, Cardiology, Strovolos, Cyprus;* [3]*Archbishop Makarios III Hospital, Clinical Genetics & Metabolic disorders clinic, Nicosia, Cyprus;* [4]*Archbishop Makarios III Hospital, Pediatric Nephrology Pediatric Rheumatology Pediatric Gastroenterology – Hepatology Pediatric Pulmonology Pediatrics Paediatric cardiology, Nicosia, Cyprus*

Background/Objectives: Brugada syndrome is associated with a high risk of cardiac arrhythmia and sudden cardiac death (SCD). Patients present with a structurally normal heart and a typical electrocardiographic (ECG) pattern, featuring right bundle branch block and persistent ST-segment elevation in right precordial

leads. The condition predominantly exhibits an autosomal dominant pattern of inheritance, while is mostly sporadic.

Methods: This study explores the case of an 11-year-old female admitted to the hospital in 2019 for an ajmaline provocation test, prompted by family history of Brugada Type-I as her father sustained an SCD at 37-yrs. Despite exhibiting normal heart structure and function, an ECG within normal limits and no arrhythmias, the patient displayed elevated blood pressure and a positive ajmaline test. Genetic testing was recommended and whole exome sequencing identified three variants of unknown significance as evaluated through the ACMG criteria.

Results: The patient bared a variant in KCND3:c.1348C>T (p.Leu450Phe), which was previously reported in patients with Brugada syndrome and independent electrophysiology studies in tissue culture systems further supported its pathogenicity. Interestingly, the same variant is carried by another 10 individuals out of a 1000 in the Cyprus general population Biobank (CYPROME).

Conclusion: Although not conclusive, data presented demonstrate that the KCND3 variant is pathogenic. However, the rather frequent carrier status general population (1:100) is now further addressed to investigate the true frequency of the disease in the Cypriot population.

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Conflict of Interest: None declared

P07.034.B The mutational spectrum of Jervell and Lange-Nielsen syndrome: insights from highly consanguineous families

Aya Hataba[1], *Mona Allouba*[1], *Omnia Kamel*[1], *Alaa Afify*[1], *Mariam Fathy*[1], *Mohamed ElMaghawry*[1], *Besra Samy*[1], *Aliaa Mahfouz*[1], *Eslam Ahmed*[1], *Amany Ellithy*[1], *Magdi H. Yacoub*[1;2;3], *Yasmine Aguib*[1;2]

[1]*Aswan Heart Centre, Magdi Yacoub Heart Foundation, Aswan, Egypt;* [2]*National Heart and Lung Institute, London, United Kingdom;* [3]*Harefield Heart Science Centre, Harefield, United Kingdom*

Background/Objectives: Jervell and Lange-Nielsen syndrome (JLNS) is an autosomal recessive disease caused by mutations in *KCNQ1* or *KCNE1*. It is characterized by prolonged QT interval on electrocardiogram, deafness and an increased risk of sudden cardiac death (SCD) (25%). Given the high consanguinity rate (35%) in the Middle East and North Africa region (MENA), an enrichment of JLNS is expected, providing a powerful opportunity to identify ancestry-specific genetic determinants. We report genetic data from the largest MENA JLNS cohort analyzed to date.

Methods: Prospective Egyptian JLNS families and ancestry-matched controls were recruited to Aswan Heart Centre for clinical phenotyping and genetic testing for genes associated with inherited cardiac conditions (Illumina). Variants were classified according to the ACMG/AMP guidelines.

Results: We followed up 39 unrelated probands (20 males and 19 females; mean age at enrollment: 5.8 years) for 4 years. Consanguinity was reported in 85% of probands with complete family data. SCD was reported in 2 probands. Homozygous *KCNQ1* mutations were observed in 82.4% of sequenced probands (28/34). Among these variants, 89% were truncating. Heterozygous parents were confirmed to be healthy. A novel, recurring *KCNQ1* variant (p.W304X) was identified in Egyptian probands (17%), suggesting that it could be a North African-specific founder.

Conclusion: Variants discovered in this underrepresented population adds to the mutational spectrum of JLNS and provides ancestry-specific genetic determinants of disease. Expanding the cohort will provide important genetic and mechanistic insights into the global understanding of inherited arrhythmia syndromes.

Grants:

Conflict of Interest: None declared

P07.036.D To Copy or not to Copy? Copy Number Variant (CNV) analysis for diagnosing inherited cardiomyopathies

Camilla Calandrini[1], *Judith Verhagen*[1], *Wouter te Rijdt*[1], *roza ali amin*[1], *Robert van der Helm*[1], *joan kromosoeto*[1], *Marjolein Weerts*[1], *Ingrid MBH van de Laar*[1], *Marja Wessels*[1], *michelle michels*[2], *Rogier Oldenburg*[1], *Marjon van Slegtenhorst*[1]

[1]*Erasmus MC, Clinical Genetics, Rotterdam, Netherlands;* [2]*Erasmus MC, Cardiology, Rotterdam, Netherlands*

Background/Objectives: In the past, genetic testing for cardiomyopathies (e.g. hypertrophic (HCM) and dilated (DCM) cardiomyopathies) mainly focused on the detection of sequence variants with Sanger sequencing and Next Generation Sequencing, with a high diagnostic yield of ~40% for HCM. In this study, we performed Copy Number Variant (CNV) analysis on Whole Exome Sequencing (WES) data to investigate its added value in diagnosing inherited cardiomyopathies.

Methods: We collected CNV data from 1710 cardiomyopathy patients tested between January 2020 and December 2023. CNV analysis was performed by read-depth method using dynamic bins (BAM Multiscale Reference Method by BioDiscovery) in a cardiomyopathy gene panel of ~55 genes. Detected copy number alterations were confirmed by an independent method.

Results: A CNV was reported in 7/1710 cases (0.41%), including two deletions and five gains encompassing cardiomyopathy genes. A previously described pathogenic deletion in the *MYBPC3* gene (exons 23-26) was detected in a young HCM patient, while a novel deletion of unknown significance in the *JPH2* gene (exon 3-4) was found in a DCM patient. The detected gains ranged from 44 kb to 25 Mb, and the affected cardiomyopathy-related genes included *RYR2*, *TNNC1*, *ALPK3*, *TXNRD2* and *PLN*. Of the 7 identified CNVs, 1 constituted a definitive diagnosis, 2 were highly suspicious findings and 4 were of unknown clinical significance.

Conclusion: The diagnostic yield of CNV analysis for cardiomyopathies is estimated to be <0.5%, which is low in comparison with other tested panels in our department. Nevertheless, CNV analysis may contribute to unraveling new disease mechanisms of inherited cardiomyopathies.

Grants: not applicable

Conflict of Interest: None declared

P07.037.A In isolated hypertrophic cardiomyopathy patients, importance of LZTR1 gene which is specific for the Noonan syndrome

selman yıldırım[1], *hülya tarım*[1], *betül turan*[1], *rahime laçin*[1]

[1]*Necmettin Erbakan University, Medical Faculty, Medical Genetics*

Objectives: Hypertrophic cardiomyopathy(HCM), characterized by unexplained left ventricular hypertrophy and genetic heterogeneity, is most frequently associated with the MYBPC3 and MYH7 genes. Noonan Syndrome is a syndrome characterized by characteristic facial features, short stature, congenital heart defects and often associated with pathogenic variants in PTPN11, SOS1, and LZTR1 genes. We present two cases from different families to emphasize the significance of the LZTR1 gene in non-syndromic HCM.

Methods: In the pediatric cardiology clinic clinical exome sequence analysis was performed two patients with HCM diseases. Variants were classified according to the ACMG standards.

Results: Proband-1(13 years,male) and Proband-2 (14 years,female) were both referred due to interventricular septal hypertrophy. Both patients had normal growth parameters; in addition to HCM, ptosis and curly hair were observed in proband-1, while proband-2 had no additional findings. In Proband-1, a novel splice donor variant in heterozygous form was identified in the LZTR1 gene (ENST00000215739:c.1353+2T>C) and classified as "Likely Pathogenic". In Proband-2, two heterozygous missense variants were detected in the LZTR1 gene. Among these variants, ENST00000215739:c.1735G>T/V579L was classified as a "Variant of Unknown Significance", and ENST00000215739:c.1397G>A/R466Q was classified as "Pathogenic".

Conclusion: The main objective of the study is to emphasize the significance of the LZTR1 gene, which is specific to Noonan Syndrome in isolated HCM patients. Furthermore with the obtain finding the study intends to contribute to the literature.

Conflict of Interest: None declared

P07.038.B The impact of genetic diagnosis in elderly cardiomyopathy patients

Micaela Oliveira[1], *Rafael Graça*[1], *Diana Antunes*[1;2;3], *Catarina Silveira*[1], *Yuri Chiodo*[1], *Maria Carmo-Fonseca*[4]

[1]*GenoMed - Diagnósticos de Medicina Molecular, S.A, Genetic diseases and pharmacogenetics, Lisboa, Portugal;* [2]*Santa Marta Hospital, Medical Genetics, Lisboa, Portugal;* [3]*Dona Estefânia Hospital, Medical Genetics, Lisboa, Portugal;* [4]*Instituto de Medicina Molecular João Lobo Antunes, Lisboa, Portugal*

Background/Objectives: Cardiomyopathies lead to serious structural and functional alterations of the heart. The underestimated prevalence of hypertrophic (HCM) and dilated cardiomyopathy (DCM) represent a major concern, specially due to their late onset, which can delay diagnosis and treatment. Therefore, it is crucial to consider a genetic aetiology of cardiomyopathies in the elderly, with genetic testing playing an important role in the screening and prevention of disease-related complications.

Methods: Here, we present a retrospective observational analysis of genetic testing in patients over 65 years old with suspected cardiomyopathies, between 2020 and 2023. The mean age of the studied cohort was 72 years old and the study focused on identifying the genetic diagnostic success rate of these patients.

Results: From the 255 index cases under study, 45 (17.65%) obtained a positive result, with the identification of a pathogenic or likely pathogenic genetic variant that could explain the phenotype. Among them, 26 (57.78%) presented a HCM phenotype and 12 (26.67%) showed a DCM phenotype. Concordantly, the majority of the variants (58.70%) were found in the sarcomeric genes *MYBPC3*, *MYH7*, *MYL2*, *TNNT2* and *TPM1*, being known that alterations in these genes are frequently associated with HCM. The remaining variants, mainly detected in DCM patients, were located in other genes, such as *FLNC*, *LMNA*, *PKP2*, *RYR2*, *TTN* and *TTR*.

Conclusion: The obtained results reinforce the critical role of genetic testing in the management of cardiomyopathies in the elderly population, not only to allow a more appropriate treatment, but also to provide adequate genetic counselling to family members at risk.

Conflict of Interest: None declared

P07.039.C Exploring genetic factors in infarct-like patients with myocarditis

Patricia Muñoz-Cabello[1], *Paula Fernandez-Alvarez*[1], *Amaia Lasa-Aranzasti*[1], *Mercedes Tomeo-Fresno*[1], *Roger Esmel-Vilomara*[2], *paola dolader*[2], *ferran gran*[2], *Elena García-Arumí*[1], *Eduardo Tizzano*[1]

[1]*hospital Vall d'Hebron, Department of Clinical and Molecular Genetics., Barcelona, Spain;* [2]*hospital Vall d'Hebron, Pediatric Cardiology, Barcelona, Spain*

Background: Acute myocarditis (AM) exhibits a diverse clinical presentation, ranging from subclinical to fulminant presentations. Teenagers often present with an infarct-like syndrome, characterized by subepicardial involvement and edema on cardiac magnetic resonance imaging. While virus infections are suggested to be its primary cause, it is unclear why a small proportion of paediatric patients develop AM, suggesting a genetic predisposition. Previous series including complicated and uncomplicated myocarditis have shown a wide range of prevalence between 4-60% of pathogenic variants.

Objectives: To assess the prevalence of pathogenic/likely pathogenic variants in cardiomyopathy genes among children with AM presenting an infarct-like pattern.

Methods: Retrospective cohort study involving paediatric myocarditis with an infarct-like pattern at Vall d'Hebron University Hospital and other Catalonia hospitals between 2007-2024. A customized NGS panel targeting 62 cardiomyopathy genes was employed.

Results: 12 patients with an infarct-like pattern were included. 4 out of 12 (33%) had pathogenic or likely pathogenic variants in dilated (*TTN*, *ALPK3*), hypertrophic (*MYBPC3*) and arrhythmogenic right ventricular cardiomyopathy (*DSP*) genes.

Conclusion: Our findings reveal a 33% prevalence of pathogenic or likely pathogenic variants in cardiomyopathy genes within our cohort, indicating a potentially bad prognosis and familial implications. It remains unknown if environmental factors trigger the activation of an inflammatory/immune response resulting in myocardial dysfunction in genetically predisposed patients. Another alternative explanation could be that inflammation in cardiomyopathies is caused by the heart failure itself in patients who were misdiagnosed as myocarditis. Our results support the value of genetic testing in AM patients with an infarct-like pattern

Grants:

Conflict of Interest: None declared

P07.040.D Genetic basis of end-stage cardiomyopathies that require heart transplant

Benjamin Rodríguez-Santiago[1], *Carles Moliner*[2], *Ivon Cuscó*[1], *Lourdes Vega*[1], *Marta de Antonio*[2], *Marta Campreciós*[2], *Vicenç Brossa*[2], *Jose Guerra*[2], *Manel Baena*[1], *Núria Cilvillé*[1], *Eudald Tejero*[3], *Clara Serra-Juhé*[1], *Sònia Mirabet*[2]

[1]*Hopital de la Santa Creu i Sant Pau, Genetics Department;* [2]*Hopital de la Santa Creu i Sant Pau, Cardiology Department;* [3]*Institut de Recerca - Hospital Santa Creu i Sant Pau, Barcelona, Spain*

Background/Objectives: The underlying etiologies of advanced heart failure requiring a heart transplant (HT) in Spain are mainly ischemic cardiomyopathy (ICM) and dilated cardiomyopathy (DCM). Other cardiomyopathies (CM) such as hypertrophic (HCM), restrictive (RCM), and arrhythmogenic right ventricular (ARVCM) account for 28-32% of HTs. The diagnostic yield of genetic testing in CM has been well established in recent genotype-phenotype correlation studies. However, specific studies addressing the genetic basis of HT patients are scarce and limited to familial cases. We have studied a cohort of heart transplanted patients diagnosed with CM regardless of whether they were

familial or sporadic cases by using NGS to assess diagnostic yield rate and genetic basis of HT.

Methods: Clinical variables (family history, habits, comorbidities), electrocardiogram and cardiac imaging were recorded for all participants. DNA from 114 alive HT patients with a CM (DCM, HCM, RCM or ARCV, ischemic or other obvious causes excluded) not genetically tested before. NGS (cardio panel or clinical exome using cardio virtual panel).

Results: A pathogenic or likely pathogenic variant was identified in 49% of heart transplanted patients in 18 different genes. Most represented affected genes were TTN (9), MYH7 (7), LMNA (5), FLNC (5), MYBPC3 (4), TNNI3 (4), followed by other genes with less cases (DSG2, DSP, RBM20, BAG3, TPM1, LAMP2, DES, FKTN, TNNT2, TMP1, PKP2, MT-TL1).

Conclusion: Gene testing should be incorporated in heart transplanted patient's management, regardless of CM type or family history. Patient's relatives at/not at risk could be identified and subsequently managed accordingly.

Grants: CM is supported by Rio Hortega grant (MCIN, Spain)

Conflict of Interest: None declared

P07.041.A Molecular basis of hereditary cardiovascular diseases (hCVDs) in a cohort of 486 Italian patients: a multi-step approach.

Giorgia Girotto[1;2], *Stefania Zampieri*[1], *Alessia Paldino*[3], *Beatrice Spedicati*[1;2], *Giovanni Turchetto*[1], *Daniela Mazzà*[1], *Matteo Dal Ferro*[3], *Gianfranco Sinagra*[2;3], *Stefania Lenarduzzi*[1]

[1]*IRCCS Burlo Garofolo, Trieste, Italy, SC Genetica Medica, Trieste;* [2]*University of Trieste, Trieste, Italy, Department of Medicine, Surgery and Health Sciences, Trieste, Italy;* [3]*Azienda Sanitaria Universitaria Giuliano-Isontina (ASUGI), University of Trieste, Center for Diagnosis and Treatment of Cardiomyopathies, Cardiovascular Department, trieste*

Background/Objectives: hCVDs are a leading cause of mortality globally, encompassing a broad range of heart conditions. This study provides a detailed genetic picture of hCVDs in a cohort of Italian patients.

Methods: 486 hCVD-patients recruited at the Medical Cardiological Unit of the Cattinara Hospital (Trieste, Italy) were analysed by Whole Exome Sequencing (WES). WES data allowed the identification of both single nucleotide and Copy number variants (CNVs), the latter further confirmed by MLPA or other high-throughput technology (i.e.,MinION).

Results: WES identified a molecular defect in 23% of patients, with a higher detection rate in familial cases than in sporadic ones (41% vs 16%). 108 causative variants in 22 genes were found. *TTN* was the most frequently mutated gene, followed by *MYH7, MYBPC3, FLNC*, *PKP2*, and *DSP*, characterizing 78% of resolved cases. Among them, two patients from the same geographic area carried the p.(Phe305Profs*27) *MYBPC3* variant, confirming a previously described founder effect. WES analysis also expanded the clinical spectrum of hCVDs with variants in *ACVRL1*, *GDF1*, *TMEM43*, *TRIM63*, and *TRPM4* genes, confirming the genetic heterogeneity of hCVDs.

Regarding CNVs analysis, the same deletion in *PKP2* was detected in three patients, while a novel large deletion, including *DSG2, DSC2*, and *TTR* genes, was identified in another proband. Both CNVs were confirmed by MLPA or MinION technology.

Conclusion: Our multi-step genetic diagnostic approach is a powerful tool to identify the molecular basis of hCVDs, highlighting their complex genetic background and providing crucial information for genetic counselling, clinical management and prognosis, and risk assessment.

Grants:.

Conflict of Interest: None declared

P07.043.C First cases of somatic mosaicism in non-syndromic dilated cardiomyopathy

Jan Jongbloed[1], *Lisette Leeuwen*[1], *Debby Hellebrekers*[2], *Bianca J.C. van den Bosch*[2], *Imke Christiaans*[1], *Yvonne Hoedemaekers*[3]

[1]*University of Groningen, University Medical Centre Groningen, Genetics, Groningen, Netherlands;* [2]*Maastricht University Medical Centre, Clinical Genetics, Maastricht, Netherlands;* [3]*Radboud University Medical Centre, Clinical Genetics, Nijmegen, Netherlands*

Background/Objectives: Somatic mosaicism in non-syndromic cardiomyopathies is rare and has not been previously described. This study presents the first cases in two dilated cardiomyopathy (DCM) families with somatic mosaicism in known cardiomyopathy genes.

Methods: Genetic analysis of cardiomyopathy genes was performed in the index patients and clinical characteristics and family history were collected. Sanger sequencing was used for cascade screening and mosaicism detection in different tissues.

Results: The first patient is a 64-year old male with DCM with a left ventricular ejection fraction (LVEF) of 16%. Genetic analysis revealed pathogenic variant c.614G>A, p.(Arg205Gln) in *TNNT2*, present in 20% in blood, urine, hair and buccal mucosa. Family history was positive for sudden death on both sides of the family. However, the variant was not found in the parents.

The second patient is a 34-year old male who was successfully resuscitated after ventricular fibrillation. Cardiac-MRI showed DCM with an LVEF of 19%. Family history was negative for sudden death and cardiac diseases. Genetic analysis revealed likely pathogenic variant c.73C>A, p.(Arg25Ser) in *LMNA*. Subsequently, the *LMNA* variant was detected in the father in ~5% in blood, urine, hair and buccal mucosa. He had a normal echocardiogram.

Conclusion: Although germline mosaicism in cardiomyopathy patients, as well as somatic mosaicism for *LMNA* variants in Hutchinson-Gilford progeria has been described previously, this is the first report on somatic mosaicism identified in non-syndromic DCM. Identifying somatic mosaicism for DCM is important for determining the need for genetic cascade testing, cardiac evaluation in relatives, and reproductive choices.

Grants: NA

Conflict of Interest: None declared

P07.044.D A novel homozygous c.401C>T variant in CAP2 detected in an infant with dilated cardiomyopathy

Grażyna Kostrzewa[1], *Karolina Rutkowska*[1], *Małgorzata Rydzanicz*[1], *Piotr Gasperowicz*[1], *Agnieszka Pollak*[1], *Tomasz Książczyk*[1], *Bożena Werner*[1], *Rafał Płoski*[1]

[1]*Medical University of Warsaw, Department of Medical Genetics, Warszawa*

Background/Objectives: Heart disorders are common causes of human morbidity and mortality. Sudden cardiac failure is the most prevalent cause of death, accounting for 20% of cases with cardiomyopathy. Dilated cardiomyopathy (DCM), along with hypertrophic cardiomyopathy (HCM), is among the most common cardiomyopathies.

Methods: We performed exome sequencing (ES) on a 10-month-old female infant with DCM. To confirm the ES results, amplicon deep sequencing was performed on the proband's sample, as well as on samples from her parents and sister.

Results: In the proband's sample, using exome sequencing (ES), a homozygous missense c.401C>T variant in the CAP2 gene (hg38: 6:017507269-C>T) was identified. The population frequency for the c.401C>T variant was 0.00004275 in the gnomAD database v.4, with no homozygotes reported. Homozygotes were also absent in the in-house datasets, which comprised over 10,000 ES of Polish individuals. The studied variant in the CAP2 gene was observed in a heterozygous state in unaffected parents and was absent in the proband's sister. The observed c.401C>T variant has not been previously described in the literature. So far, only three publications have described CAP2 gene variants in association with DCM in an autosomal recessive mode of inheritance.

Conclusion: In the current study, we present a novel homozygous mutation in the CAP2 gene in a Polish patient with DCM. Further examination of additional cases is required to understand the impact of the c.401C>T variant on phenotype and to provide prognostic and treatment-related recommendations.

Grants: N/A

Conflict of Interest: None declared

P07.045.A An in vitro system to study Desmoglein- 2 (*DSG2*) p.(N266S) related arrhythmogenic cardiomyopathy

Serena Pinci[1], *Rudy Celeghin*[1], *Maria Bueno Marinas*[1], *Marco Cason*[1], *Cristina Basso*[1], *Kalliopi Pilichou*[1]

[1]*University of Padua, Department of Cardiac, Thoracic, Vascular Sciences and Public Health, Padova, Italy*

Background: Desmoglein-2 (DSG2) p.(N266S) is a rare genetic variant previously identified in numerous families exhibiting arrhythmogenic cardiomyopathy (ACM) phenotypes (OMIM #107970; ORPHA247) while transgenic mice overexpressing the mouse equivalent variant recapitulated the pathognomonic features of the human disease. However, the molecular effect of this alteration remains an open question.

The purpose of this study is to use HL-1 (immortalized atrial mouse cardiomyocyte) cell line to define a stable in vitro model for investigating the pathogenesis of ACM related to the DSG2-N266S variant.

Methods: A plasmid carrying DSG2–N266S variant was synthesized through site directed mutagenesis and introduced into the HL-1 cells by using the 4-D Nucleofector System (Lonza). Since there was no specific protocol available for HL-1 transfection, a nucleofection protocol was optimized.

We investigated specific electrical and molecular properties in comparison to non-transfected HL-1 cells by performing Sanger sequencing, Real-time PCR, confocal microscopy, transmission electron microscopy (TEM), and Fluo-8 AM calcium imaging.

Results: Antibiotic selection, Real-Time PCR, and Sanger sequencing confirmed the ability of transfected HL-1 cells to express the plasmid carrying the DSG2–N266S variant. Confocal imaging confirmed the cardiac phenotype and revealed the insertion of the DSG2-N266S protein into desmosomes. TEM imaging showed the incapacity to form mature and well-defined desmosome in presence of DSG2-N266S protein compared to normal HL-1 cells. Finally, Fluo-8 AM calcium imaging revealed an alteration of calcium flux in transfected HL-1 cells.

Conclusion: Our study has provided a promising tool to investigate ACM pathogenic mechanisms, holding potential implication for future targeted therapeutic strategies.

Conflict of Interest: None declared

P07.046.B A cardiac emerinopathy without atrial standstill, expanding the phenotype of non-syndromic emerinopathies

Paula Fernandez-Alvarez[1;2], *Patricia Muñoz-Cabello*[1;2], *javier limeres-freire*[3], *Alejandro Moles-Fernandez*[1;2], *Miriam Masas*[1;2], *Elena García-Arumí*[1;2], *Eduardo Tizzano*[1;2]

[1]*Vall d'Hebron Hospital Universitari, Department of Clinical and Molecular Genetics, Barcelona, Spain;* [2]*Vall d'Hebron Institut de Recerca (VHIR), Medicine Genetics Group, Barcelona, Spain;* [3]*Vall d'Hebron Hospital Universitari, Department of Cardiology, Barcelona, Spain*

Background/Objectives: Pathogenic variants in EMD are associated with Emery-Dreifuss muscular dystrophy (EDMD) characterized by the triad of early joint contractures, progressive myopathy and cardiac conduction defects and arrhythmias. EDMD follows an X-linked inheritance pattern with usually asymptomatic heterozygous females. Most variants cause loss of function resulting in absence of emerin expression and tend to have a more severe phenotype than missense variants. Only recently, 4 cases of non-syndromic cardiac emerinopathy have been described as a progressive atrial standstill associated with left ventricular non compaction

Methods: We present a 31-year-old man with bradycardia and auricular flutter, diagnosed with left ventricular dilatation, normal ejection fraction and without non-compaction criteria. NGS cardiac panel identified a hemizygous 20 nucleotide deletion in EMD: c.266-15_368del affecting the acceptor site of intron 3. In vitro splice predictions predicted the loss of the canonical acceptor and the gain of a new one at -67pb. To assess the outcome of the variant we performed RNA studies.

Results: RNA studies showed that the variant did not produce the wild type RNA but 3 aberrant transcripts all of them causing frameshift and the appearance of a premature stop codon. The isophorms were (from most to less predominant): 1) skipping of exon 4; 2) deletion of 41 nucleotides of exon 4; 3) retention of 86 nucleotides from intron 3.

Conclusion: Our case expands the phenotype of cardiac emerinopathies with left ventricular dilatation and without the described characteristic atrial standstill.

Grants:

Conflict of Interest: None declared

P07.048.D Direct RNA sequencing detects base modifications caused by chemokine-mediated oxidative stress in vascular smooth muscle cells

Tobias Reinberger[1], *Inken Wohlers*[2]

[1]*University of Lübeck, Institute of Cardiogenetics, Lübeck, Germany;* [2]*Research Center Borstel, Biomolecular Data Science in Pneumology, Borstel, Germany*

Background/Objectives: Vascular smooth muscle cells (VSMCs) reside in the medial layer of the arterial wall and are involved in the regulation of vascular tone and vascular remodeling. However, VSMCs possess phenotypic plasticity which plays a role in subclinical atherosclerosis. We investigated the transition from contractile SMCs to pro-inflammatory SMC phenotypes and the impact of disease-relevant stimuli on gene regulation.

Methods: Primary human SMCs were cultured on a collagen I hydrogel and stimulated with TGF-β1 and a mixture of IL1-β and PDGF-B. These pro-inflammatory SMCs were further stimulated with PDGF-B to induce oxidative stress, which was confirmed by CellRox™ assays. SMCs were characterized using immunofluorescence and Illumina RNA sequencing (RNA-seq). RNA modifications

were detected using direct RNA-seq using Oxford Nanopore Technologies.

Results: Comparison of transcriptome profiles of SMC phenotypes with single cell RNA-seq data from carotid atherosclerotic plaques revealed that TGF-β1 stimulated SMCs best reflect canonical ACTA2+ SMCs (Kendall's rank correlation of ~0.4). Whereas SMCs treated with PDGF-B de-differentiate and adopt a pro-inflammatory state; indicated by enhanced expression of cytokine genes (*e.g.*, CXCL8). Further stimulation with PDGF-B resulted in higher levels of reactive oxygen species and in a shutdown of genes involved in DNA repair and cell cycle progression. Moreover, pro-inflammatory SMC phenotypes showed more RNA modifications as compared to TGF-β1 stimulated SMCs.

Conclusion: We have identified pro-inflammatory SMC phenotypes that are susceptible to oxidative stress and RNA modifications, which could be harmful at the late stages of human atherosclerotic plaque development.

Grants: Foundation Leducq (18CVD02, PlaqOmics); Junior Investigator Grant excellence cluster Precision Medicine in Chronic Inflammation

Conflict of Interest: None declared

P07.050.B A novel therapeutic strategy for Hypertrophic Cardiomyopathy caused by MYBPC3 variants using antisense oligonucleotides

Serena Evelina Margaretha Munteanu[1], *Elisa Nicolas Rocamora*[2], *Carolina Martinez Perez*[2], *Angel Bernabe Garcia*[1], *Jesús Wagih Gómez*[1], *Francisco Jose Nicolas Villaescusa*[3], *Juan Ramon Gimeno Blanes*[4], *Virginia Arechavala Gomeza*[5;6], *Maria Sabater Molina*[1;7]

[1]*Imib Instituto Murciano De Investigación Biosanitaria, Cardiogenetics, El Palmar, Spain;* [2]*Universidad de Murcia, Murcia, Spain;* [3]*Virgen of Arrixaca University Clinical Hospital, Regeneration, Molecular Oncology and TGFß, El Palmar, Spain;* [4]*Virgen of Arrixaca University Clinical Hospital, Unidad de Cardiopatías Familiares, El Palmar, Spain;* [5]*IIS Biobizkaia, Nucleic Acid Therapies for Rare Diseases, Barakaldo, Spain;* [6]*Fundación Ikerbasque, Bilbao, Spain;* [7]*Universidad de Murcia, Departamento de Medicina Legal, Murcia, Spain*

Background/Objectives: Non-sense or frameshift mutations in the MYBPC3 gene encoding cardiac myosin-binding protein C (cMyBP-C)are a major cause for inherited forms of Hypertrophic Cardiomyopathy(HCM).Antisese oligonucleotide mediated exon skipping attempts to reframe mutated MYBPC3 transcripts, resulting in shortened but functional protein. We aim to design and evaluate antisense oligonucleotides (AONs)for efficient exon-skipping in targeted MYBPC3 exons.

Methods: We've designed two specific AONs to mask the MYBPC3 exon 26 and confirmed successfully mediated exon skipping without disrupting the MYBPC3 reading frame.Primary Human Cardiac Myocytes(PHCM)(Promocell)were transfected with the splice-site targeting AONs.

Results: In this study we designed two chemically modified AONs (AON1 and AON2) through the addition of a 2′-O-methyl RNA phosphorothioate backbone, targeting exonic splicing enhancers(ESEs)or splice sites (SSs) to induce skipping of MYBPC3 exon 26.We evaluated that AON1(i26-3SS) and AON2(ESE)treated Primary Human Cardiac Myocytes(PHCM) show skipping of exons 26-27 in comparison to control samples, as we detected through RT-PCR and confirmed by Sanger sequencing.Preservation of both nucleotide sequences of exons 25 and 28 was observed. We've identified that 24h post-transfection both AONs affect cell lenght and number. We've observed that cell number and percentage of cellular area showed no difference 7 days post-transfeccion using AON1, indicating cellular death.

Conclusion: These results demonstrate that disruption of the MYBPC3 reading frame due to a truncating HCM mutation can be restored by exon skipping in cardiomyocytes in vitro,expanding the framework of future advancements in the therapeutic potential of antisense-mediated exon skipping MYBPC3 based HCM.

Grants: Proyecto de Investigación Básica en Cardiología de los Socios Estratégicos de la SEC2017.

Conflict of Interest: None declared

P07.051.C Rare genes in consanguineous families diagnosed as cardiomyopathy: A comprehensive review of autosomal recessive inheritance.

Farida Al Habib[1], *Reem M. Elshafie*[2], *Laila Bastaki*[2], *Ahmed Salem*[1], *Magdi Yacoub*[3]

[1]*Kuwait Foundation for Advancement of Science, Ministry of Health, Kuwait City, Kuwait;* [2]*Kuwait Medical Genetics Center, Ministry of Health, Kuwait City, Kuwait;* [3]*National Heart and Lung Institute, Imperial College London, United Kingdom*

Background/Objectives: Hereditary cardiomyopathy is a common cause of heart failure and sudden death. Molecular diagnosis is crucial in establishing the course of treatment, prognosis, and family screening. In contrast to more prevalent autosomal dominant forms, rare biallelic disease-causing variants linked to cardiomyopathies have been identified. These variants are frequently linked to an earlier clinical presentation and an increased risk of sudden cardiac death.

Methods: From a cohort of 145 patients in Kuwait diagnosed with cardiomyopathy, probands from consanguineous Middle Eastern families were selected. Whole exome sequencing was performed, and variants were classified according to the American College of Medical Genetics and Genomics guidelines.

Results: We identified homozygous missense variants in *ALPK3*, *MYL2*, *MYBPC3*, *OBSCN*, *FKTN*, and *TNNT2* linked to hypertrophic cardiomyopathy phenotype and *DSG2* linked to dilated cardiomyopathy. Segregation analysis in family members confirmed autosomal recessive inheritance presenting in childhood/early adulthood. Our results expand on the *OBSCN* gene's role as a culprit gene in recessive cardiomyopathies. We also provide evidence for novel recessive modes of inheritance of a well-established autosomal dominant *NEXN* gene, which might explain the clinical heterogeneity of cardiomyopathies.

Conclusion: This is the first comprehensive report of autosomal recessive cardiomyopathy cases in Kuwait. Gaining more knowledge about the genetic origins of recessive cardiomyopathies will have a significant impact on screening and diagnosis, especially in understudied Middle Eastern populations.

Grants: This work was supported by a research grant from the Kuwait Foundation for Advancement of Science (PII553MM 01) and the Ministry of Health, Kuwait.

Conflict of Interest: None declared

P07.052.D Next-generation sequencing reveals novel mutations in Kazakhstani patients with dilated cardiomyopathy

Ainur Akilzhanova[1], *Zhannur Abilova*[1], *Madina Zhalbinova*[1], *Ayaulym Chamoieva*[1], *Gulbanu Akilzhanova*[2], *Saule Rakhimova*[1], *Ulykbek Kairov*[1], *Ulan Kozhamkulov*[1], *Kenes Akilzhanov*[2;3], *Ayan Abdrakhmanov*[4], *Makhabbat Bekbossynova*[5], *Dos Sarbassov*[1;6]

[1]*National Laboratory Astana, Nazarbayev University, Center for Life Sciences, Astana, Kazakhstan;* [2]*Medical University Semey, Pavlodar*

Branch, Pavlodar, Kazakhstan; [3]Pavlodar City Clinic #1, Pavlodar, Kazakhstan; [4]Medical University Astana, Cardiology, Astana, Kazakhstan; [5]National Research Cardiac Surgery Center, Cardiology, Astana, Kazakhstan; [6]Nazarbayev University, School of Science and Humanities, Astana, Kazakhstan

Background/Objectives: Mutations of variety of genes contribute to dilated cardiomyopathy (DCM) development by altering cellular homeostasis of cardiomyocytes.

Methods: To identify the mutational spectrum in Kazakhstani cohort of DCM patients we performed targeted sequencing of 96 candidate genes for cardiomyopathies in 92 Kazakhstani patients with DCM ($n = 32$), coronary heart disease (CHD, $n = 23$), idiopathic VT (iVT, $n = 37$). 8 (25%) cases of DCM were familial and 24 (75%) were sporadic. ACMG/AMP guidelines were applied for variant interpretation of clinical significance.

Results: 307 unique variants in 74 genes totaling up in 456 variants for the overall study group were identified. Six (2.9% among all patient cohort) pathogenic genetic variants were observed in patients with DCM. Possible causative non-synonymous mutations (pathogenic and likely pathogenic) were identified in ~18.7% (6/32), VUS in 59.4% (19/32) patients, in 11% (4/32) no mutations were found in studied genes. We identified novel mutations in patients with DCM, not previously presented in HGMD. VUS in RBM20 gene G541A (G181R) was observed in patient with severe DCM (50 y.o. female) and missense variants - likely pathogenic variant C1898T (P633L) in 33 y.o. female with severe DCM. We identified 4 mutations in GAA gene, two of them were novel VUS C420A (N140K), C257G (P86R) in patients with DCM.

Conclusion: Novel mutations in DCM patients were revealed, and planned to be further assessed functionally using iPSC-derived cardiomyocites modeling.

Grants: Study supported by grants of Ministry of Science and Higher Education of the Republic of Kazakhstan (AP14869903, BR21881970) and Nazarbayev University CRP project *211123CRP1608*.

Conflict of Interest: None declared

P07.053.C Could telomere length serve as an early biomarker in hypertrophic cardiomyopathy?

Ángel Júdez Serrano[1], *Samira Louchachha Medhi*[1], *Serena Evelina Margaretha Munteanu*[1], *Elisa Nicolas Rocamora*[1], *Maria Sabater Molina*[1], *Juan Ramón Gimeno Blanes*[2]

[1]*Biomedical Research Institute of Murcia (IMIB-Arrixaca), Murcia, Spain;* [2]*Virgen de la Arrixaca University Clinical Hospital, Murcia, Spain*

Background/Objectives: Hypertrophic cardiomyopathy (HCM) is the leading cause of sudden death in people under 35 years of age. Its phenotype variability and incomplete penetrance have been an obstacle to a complete understanding of the disease. In recent years, there has been increasing interest in telomeric shortening and several authors have linked it to age-related diseases and HCM. Our aim was to study whether telomere length could affect the age at presentation and the severity of the HCM phenotype in genetic carriers.

Methods: Measurement of telomere length in circulating leukocytes (LTLc) was performed in 96 patients carrying a mutation in MYBPC3 causing HCM [58 affected (24 severely affected), 38 unaffected]. The variants included were c.2308+1G>A, p.Pro108Alafs*9, p.Arg891Alafs*160 and p.Glu258Lys, all considered founder mutations in our region.

Results: Our results show a lower LTLc in those affected patients with a more severe HCM phenotype compared to the affected and unaffected group ($p = 0.023$) independently of age and sex, which are two variables that affect LTLc. Similarly, a shorter telomere length was associated with greater severity of the phenotype, with patients presenting a larger left atrial size ($p = 0.032$), septal size ($p = 0.023$) and greater obstruction ($p = 0.044$). The presence of atrial fibrillation ($p = 0.039$) and sudden death ($p = 0.024$) was also higher in patients with shorter telomeres.

Conclusion: Telomere length is a variable that influences the expression of the phenotype in HCM patients carrying a MYBPC3 variant and it could have a prognostic role in the disease.

Grants: Research Project of the Spanish Society of Cardiology (SEC/FEC-INV-BAS21/021). Project Bristol Myers Squibb (CV027-063).

Conflict of Interest: None declared

P07.054.A Structural gene mutations in Brugada syndrome support its reclassification as cardiomyopathy

Emanuele Micaglio[1;2], **Sara Benedetti**[1;2], *Giuseppe Ciconte*[1], *Marco Villa*[2], *Roberto Manuello*[1], *Flavio Mastrocinque*[1], *Gabriele Negro*[1], *Emanuela Locati*[1], *Sara D'Imperio*[1], *Paola Carrera*[3], *Carlo Pappone*[1;2]

[1]*IRCCS Policlinico San Donato, Arrhythmology Department, San Donato (MI), Italy;* [2]*IRCCS Policlinico San Donato, Institute for Molecular and Translational Cardiology, San Donato (MI), Italy;* [3]*IRCCS San Raffaele, Unit of Genomics for Human Disease Diagnosis, Milano, Italy*

Background/Objectives: Brugada syndrome (BrS) is an inherited cardiac disorder characterized by specific electrocardiographic alteration and predisposition to ventricular arrhythmias. BrS has been traditionally considered a purely electrical disorder, mainly associated with mutations in the *SCN5A* gene, encoding the cardiac sodium channel α subunit, however alteration of structural genes has been recently suggested. Our aim was to evaluate whether defects in genes encoding structural proteins contribute to BrS pathogenesis.

Methods: A panel of genes encoding cardiac ion channels and structural proteins was analysed in 325 BrS patients by next generation sequencing. Molecular results were correlated with clinical and electrophysiological data.

Results: We identified likely pathogenic and pathogenic (class 4-5) variants in nine genes encoding sarcomeric, cytoskeletal and desmosomal proteins (*DSC2, DSG2, FLNC, LMNA, MYBPC3, MYH7, TNNI3, TNNT2, TPM1*) in 24 BrS patients and 13 relatives. By comparing this cohort to 154 BrS patients (90 probands and 64 relatives) carrying class 4-5 mutations in *SCN5A*, we observed that *SCN5A* patients were more susceptible to induction of ventricular arrhythmias during electrophysiological study (68% vs 47%, $p = 0.023$), while structural probands more frequently displayed right bundle branch block (24% vs 11%, $p = 0.034$). No difference was observed in proportion of patients displaying BrS electrocardiogram, size of arrhythmogenic substrate or sudden death family history.

Conclusion: Our data support a role for structural defects in BrS pathogenesis and suggest that it should be not be considered a simply electrical disorder. Further studies will be needed to elucidate how these defects result in electro-mechanical dysfuction in BrS patients.

Grant: 2016-CONT-0494/Ricerca Corrente

Conflict of Interest: None declared

P07.055.B Chromosomal Microarray Analysis in Congenital Heart Disease: Meta-Analysis of Diagnostic Yield

Ana Peterlin[1;2], *Tea Mladenić*[3;4], *Sanja Dević Pavlić*[3;4], *Borut Peterlin*[1]

[1]*Clinical Institute of Genomic Medicine, University Medical Centre Ljubljana, Ljubljana, Slovenia;* [2]*Institute of histology and embryology, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia;* [3]*Department of Medical Biology and Genetics, Faculty of Medicine, University of Rijeka, Rijeka, Croatia;* [4]*Center for genetic education, Faculty of Medicine, University of Rijeka, Rijeka, Croatia*

Background/Objectives: With a prevalence of approximately 1% in live births and even higher in foetuses, congenital heart disease (CHD) is the most common congenital abnormality. Pathogenic copy number variants are recognised as a prevalent and well-established genetic cause of CHD. Previous studies have highlighted the widespread adoption of chromosomal microarrays (CMA) as a first-tier genetic test for CHD. The primary outcome of this study was to determine the overall diagnostic yield of CMA, supplemented by subgroup analyses exploring patients with isolated CHD and those with both CHD and concomitant extra-cardiac congenital anomalies.

Methods: A systematic literature review was conducted in February 2024, using relevant keywords, within the PubMed database. Of the 531 identified studies, following the application of predefined inclusion and exclusion criteria, 36 were included in the proportional meta-analysis.

Results: In total, 9652 patients with CHD were included. The overall diagnostic yield of CMA was determined to be 10.6% (95% CI, 8.4%–13.2%), with a rate of 7.2% (95% CI, 5.1%–10.2%) observed among cases of isolated CHD and 17.4% (95% CI, 4.5%–48.4%) among patients presenting with both CHD and additional extra-cardiac congenital anomalies.

Conclusion: This meta-analysis provides evidence of the diagnostic efficacy of CMA in CHD and serves as a reference for expanding or replacing genetic testing with exome or genome sequencing.

Conflict of Interest: None declared

P07.057.D Losartan reduces aortic dilatation but does not affect aortic rupture force in MFS mice

Janine Meienberg[1], *Nicolo Dubacher*[1;2], *Mate Csonka*[1;3], *Mary Sheppard*[4], *Gabor Matyas*[1;5]

[1]*Swiss Foundation for People with Rare Diseases, Center for Cardiovascular Genetics and Gene Diagnostics, Schlieren-Zurich, Switzerland;* [2]*ETH Zurich, Department of Health Sciences, Zurich, Switzerland;* [3]*Semmelweis University, Heart and Vascular Center, Budapest, Hungary;* [4]*University of Kentucky, Department of Family and Community Medicine & Saha Aortic Center, Lexington, United States;* [5]*University of Zurich, Zurich Center for Integrative Human Physiology, Zurich, Switzerland*

Background/Objectives: Marfan syndrome (MFS) can lead to aortic aneurysms, dissections, and ruptures. Losartan, an angiotensin-II-receptor-type-1 inhibitor, has been shown to slow down aneurysm progression in mouse models and humans with MFS. However, it remains unclear whether losartan exerts its beneficial effect by the prevention of rises in blood pressure or by improving the biomechanical integrity of the aortic wall. We have recently established an objective approach to measure the rupture force of the murine aorta, enabling to assess the effect of drug treatments on the biomechanical integrity of the aorta at individual level, even in the absence of arterial events. Here, we applied our approach to losartan-treated mice modelling MFS.

Methods: 4-week-old homozygous (*Fbn1*$^{mgR/mgR}$) and wild-type mice were treated for 4 weeks with losartan and subsequently euthanized. Per mouse, three 1.5-mm rings of the thoracic aorta were prepared, mounted on a tissue puller, and uniaxially stretched until rupture.

Results: The mortality rate of losartan-treated *Fbn1*$^{mgR/mgR}$ mice was not significantly lower than that of untreated *Fbn1*$^{mgR/mgR}$ mice during the study interval. Losartan treatment reduced aneurysm formation, but had no impact on the aortic rupture force of *Fbn1*$^{mgR/mgR}$ mice. Aortic rupture force measurement revealed the weakened thoracic aorta prior to micro- and macroscopic changes.

Conclusion: In mice modelling MFS, the thoracic aorta treated with losartan remains susceptible to rupture despite the reduction in aortic diameter. Thus, aortic rupture force beyond aortic diameter may be useful for the investigation of drug therapies in mouse models of hereditary aortic diseases.

Conflict of Interest: None declared

P07.058.A Reinterpretation of genetic testing for Cardiomyopathies: Less is more

MArtina Manzoni[1], *Elena Sommariva*[1], *Francesca Pizzamiglio*[1], *Saima Mushtaq*[1], *Stefania Farina*[1], *alessandra volpe*[1], *MAria Luisa Biondi*[1], **Valeria Novelli**[2]

[1]*Centro Cardiologico Monzino, IRCCS;* [2]*Centro Cardiologico Monzino, IRCCS, Milan, Italy*

Background/Objectives: In the last years, the concept of reinterpretation of genetic testing has been considered as part of the diagnostic workflow. However, this process requires time, expert curators and new evidences to evaluate.

Recently, the European Society of Cardiology (ESC) published the new guidelines for the management of cardiomyopathies including a precise list of genes associated with the different phenotypes, including their level of contribution, posing a real support in the reinterpretation of variants in genes not clearly correlated with the disease.

To evaluate the impact of the new ESC indications, we reconsidered all genetic testing results performed in our Clinic in patients with confirmed or suspicious Cardiomyopathy.

Methods: From 2017 to 2023 we analyzed 296 probands for Cardiomyopathy, in particular 105 patients tested for Arrhythmogenic Cardiomyopathy (ACM), 113 for Hypertrophic Cardiomyopathy (HCM) and 78 for Dilated Cardiomyopathy (DCM), using a custom sequencing panel including 44 genes (11 genes for ACM, 34 for DCM and 29 for HCM).

Results: Comparison analysis with the genes reported from the ESC guidelines, considering only the definitive, moderate and strong genes, showed that 24 genes are not more associated with the phenotype and then not clinically actionable. In this scenario, we reconsidered 12 variants (8%), previously classified as VUS, changing the referral status of 8 patients, from uncertain to negative.

Conclusions: These results highlight the importance of the variant reinterpretation as a fundamental step of the genetic testing, posing the importance in the selection of the genes to analyse in order to improve the management of the patients.

Conflict of Interest: None declared

P07.060.C De novo variants in paediatric cardiomyopathies in a national rare disease initiative

Katherine Josephs[1;2], *Pantazis Theotokis*[1], *Rachel Buchan*[1;2], *Jemilat Orlandy*[1;2], *Shezan Elahi*[1;2], *Ellen Thomas*[3], *Angharad Roberts*[1;2;4], *James Ware*[1;2;5]

[1]*National Heart and Lung Institute & MRC Laboratory Institute of Medical Sciences, Imperial College London, London, United Kingdom;* [2]*Royal Brompton & Harefield, Guy's and St Thomas' NHS Foundation Trust, London, United Kingdom;* [3]*Genomics England, United Kingdom;* [4]*Great Ormond Street Hospital NHS Foundation Trust, London, United Kingdom;* [5]*Imperial College Healthcare NHS Trust, London, United Kingdom*

Consortium: Genomics England Research Consortium

Background/Objectives: Paediatric cardiomyopathies (PCM) are rare, serious diseases of the heart muscle with some of the worst outcomes in paediatric cardiology. The majority of children remain genetically unexplained. Trio analysis (comparing genomic data between a child and parents) is a powerful tool to detect rare causative variants when modelling for de novo dominant inheritance.

Methods: We studied protein coding de novo variants in 118 PCM trios and 448 rare disease adult controls in the 100,000 Genome Project, a UK large-scale genomics study. We used a previously reported de novo expectation model to assess and compare variant rates by variant class.

Results: We identify 141 protein-coding de novo variants in 118 PCM trios. Notably, there is a significant enrichment of protein truncating variants exome wide. In cardiac expressed genes, there is an excess of missense variants as well, findings absent in controls. We find 4 genes with de novo variants in >1 individual, significantly more than would be expected by chance. Two participants with dilated cardiomyopathy (DCM) harbour missense variants in *MAP3K7*, a gene we have recently identified as a novel candidate gene for DCM in adults. As part of this analysis, we uncover four new potential diagnoses in *MAP3K7*, *LZTR1*, *RYR2*, and *JAK1*.

Conclusion: This study, one of the largest investigations of paediatric cardiomyopathy trios to date, showcases the effectiveness of this approach even in the face of clinical and genetic heterogeneity. We identify new candidate disease genes and potential diagnoses for participants.

Grants: MRC, Wellcome Trust, Sir Jules Thorne

Conflict of Interest: Katherine Josephs: None declared, Pantazis Theotokis: None declared, Rachel Buchan: None declared, Jemilat Orlandy: None declared, Shezan Elahi: None declared, Ellen Thomas Genomics England, Angharad Roberts: None declared, James Ware Myokardia, Bristol-Myers Squibb, Pfizer, Foresite Labs, Health Lumen and Tenaya

P07.061.D The risk of different cardiovascular disease outcomes in individuals with familial hypercholesterolaemia in the UK Simon Broome register

Barbara Iyen[1], *Joe Kai*[1], *Steve Humphries*[2], *Ralph Akyea*[1], *Nadeem Qureshi*[1]

[1]*University of Nottingham, Centre for Academic Primary Care, Nottingham, United Kingdom;* [2]*University College London, Centre for Cardiovascular Genetics, London*

Background/Objectives: Familial hypercholesterolaemia (FH) is a known cause of premature coronary heart disease but emerging evidence suggests that FH may increase the risk of other atherosclerotic cardiovascular disease outcomes such as stroke and peripheral vascular disease (PVD). We investigated cardiovascular disease (CVD) outcomes in individuals with FH in the UK Simon Broome (SB) register, linked with secondary care hospitalisation records. This was compared with CVD incidence observed in the general population.

Methods: Of a total of 3553 individuals in the SB register, 2,988 (84.1%) had linked secondary care admission records from 1997 to 2018. We determined incidence rates for composite CVD (defined as a composite of coronary heart disease (CHD), myocardial infarction (MI), stable or unstable angina, stroke, TIA, peripheral vascular disease (PVD), heart failure, coronary revascularisation interventions), as well as CVD subtypes.

Results: Individuals were aged 20-79 years at SB registration, 1,570 (52.5%) were women, and the median follow-up was 17.4 years. 399 patients (13.4%) carried an FH-causing genetic variant. Incidence rate of composite CVD (per 1000 person-years) was 25.5 (95%CI: 24.1-26.9). The incidence rate of CHD (21.9, 95%CI 20.6-23.2) was higher than observed incidence rates of stroke (4.7, 95% CI 4.2-5.3) and PVD (4.9, 95%CI 4.4-5.5).

Conclusion: Individuals with FH in the SB register are at higher risk of all major types of cardiovascular disease compared to the general population, with the greatest risk for CHD. This further emphasizes the cardiovascular disease burden of FH, and underscores the importance of early diagnosis and optimal management.

Grants: NIHR Health Technology Award, project number 15/134/02

Conflict of Interest: Barbara Iyen: None declared, Joe Kai: None declared, Steve Humphries Grants from British Heart Foundation during the conduct of the study, Ralph Akyea: None declared, Nadeem Qureshi This project was funded by the NIHR Health Technology Assessment, project number 15/134/02, NQ was a member of the National Institute for Health and Care Excellence (NICE) familial hypercholesterolaemia guideline development group (CG71)

P07.062.A X-linked dilated cardiomyopathy: identification of de novo mutation of dystrofin gene: clinical characterization, genetic test and in silico analysis.

Alessandra Ranaldi[1], *Sara Cannito*[1], *Maria Francesca D'Ambrosio*[1], *Ilaria Ragnatela*[2], *Alessandra Margaglione*[2], *Rosa Santacroce*[1], *Giorgia Cordisco*[1], *Angelica Leccese*[1], *Giovanna D'Andrea*[1], *Francesco Santoro*[2], *Maurizio Margaglione*[1], *Maria d'Apolito*[1]

[1]*Medical Genetics, Department of Clinical and Experimental Medicine, Foggia, Italy;* [2]*Cardiology Unit, Foggia, Italy, University Polyclinic Hospital of Foggia, Foggia, Italy*

Background/Objectives: Dystrophin (DMD) gene mutations are associated with skeletal muscle disease as Duchenne and Becker Muscular Dystrophy (BMD) and X-linked dilated cardiomyopathy (XL-DCM)

Methods: We report the case of a 37 years old woman diagnosed with DCM at age of 33 that underwent genetic testing with whole-exome sequencing (WES) using the Illumina platform.

Results: A germline heterozygous A>G transversion at nucleotide 10103 in the DMD gene, leading to aspartic acid-glicine substitution at amino acid 3368 of the DMD protein (c.10103A>G p.Asp3368Gly) was identified and confirmed by Sanger's sequencing of the exon 70. In silico prediction suggests that this variant could have a deleterious impact on protein structure and function (CADD = 30). The genetic screening was extended to the first-degree relatives of the proband (his mother, father her sister) and due to absence of the variant in both parents, p.Asp3368Gly is considered de novo. The mutation has been searched in her 8-years-old son, the direct sequencing confirmed the hemizygosity. The young patient did not present signs and symptoms attributable to DCM, but reported asthenia, presented at clinical examination bilateral calf hypertrophy and at laboratory analysis,

there were increased levels of creatinine kinase (maximum value 19000 IU/L).

Conclusion: We report an early presentation of dilated cardiomyopathy in a 33 years old woman due to a de novo mutation of DMD gene (p.Asp3368Gly). Genetic identification of this mutation allowed an early diagnosis of skeletal muscle disease in her son.

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Conflict of Interest: None declared

P07.063.B Role of ADAMTSL6 in progressive TAA formation

Julia HUGUET HERRERO[1], *Zakaria Mougin*[1], *Angelique Bibimbou*[1], *Marie-Nathalie Lebel*[1], *Pauline Arnaud*[1;2], *Nadine Hanna*[1;2], *Jondeau Guillaume*[1;3], *Catherine Boileau*[1;2], *Carine Le Goff*[1]

[1]*Université Paris Cité and Université Sorbonne Paris Nord, INSERM U1148, Paris, France;* [2]*Département de Génétique, AP-HP, Bichat Hospital, Paris, France;* [3]*National Reference Centre for Marfan Syndrome and related disorders, AP-HP, Bichat Hospital, Paris, France*

Background/Objectives: Thoracic aortic aneurysm (TAA) is a permanent focal dilatation in the thoracic aorta. 20% of cases are genetic (HTAA). We identified pathogenic variants in *THSD4* related to a predisposition to HTAA. *THSD4* encodes the ADAMTSL6 protein, important in ECM by promoting the FBN1 matrix assembly. It was the first ADAMTS(L) associated with HTAA.The *Thsd4*$^{+/-}$ mice presented an aortic dilatation at 8 months old. Nevertheless, barely information is known about the exact role of ADAMTSL6 in HTAA. To elucidate this, we are studying at different ages 3, 6, 8 months old to understand the early steps of aneurysm formation/progression in this context.

Methods: To evaluate aneurysm formation, we studied TAA histological hallmarks: (i) the elastic fibre breaks; (ii) the unbalance in collagen content; (iii) the proteoglycans accumulation; (iv) the TGFβ signalling dysregulation.

Results: Preliminarily, we observed a pronounced TAA phenotype in heterozygous mice compared to the WT, which would confirm the role of ADAMTSL6 in TAA. Furthermore, there is a tendency of aneurysm formation age-depending, presenting the 8-month-old heterozygous mice significant TAA hallmarks. Interestingly, 3-month-old heterozygous mice present glycosaminoglycans accumulation, despite the premature age of the mice. It is tempting to think GAG could be a key factor for initiation of TAA formation in *Thsd4*$^{+/-}$ mice.

Conclusion: This study confirms the role of ADAMTSL6 in progressive TAA formation and suggests that the GAG is an initiator of the TAA formation. This will allow us to develop preventive approaches to stop the disease progression and prevent life-threatening outcomes.

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Conflict of Interest: None declared

P07.064.C Genotype-phenotype analysis of hypertrophic and dilated cardiomyopathy due to troponin complex mutations in Newfoundland and Labrador families

Ashley Baker[1], *Fiona Curtis*[2], *Emily Bonnell*[2], *Stephen Duffett*[3], *Lesley Turner*[2], *Anne Williams*[3], *Jenna Manuel*[4], *Peter Sutherland*[1], *Terry-Lynn Young*[1], *Kathy Hodgkinson*[4]

[1]*Memorial University of Newfoundland and Labrador, Faculty of Medicine, Department of Biomedical Sciences, St.John's, Canada;* [2]*Newfoundland and Labrador Health Services, Provincial Medical Genetics Program, St.John's, Canada;* [3]*Newfoundland and Labrador Health Services, Division of Cardiology, St.John's, Canada;* [4]*Memorial University of Newfoundland and Labrador, Faculty of Medicine, St.John's, Canada*

Background/Objectives: *TNNI3* p. R162Q and *TNNT2* p. K210del are two autosomal dominant pathogenic variants in the cardiac troponin complex, causing hypertrophic and dilated cardiomyopathy respectively. The natural history, penetrance, and range of symptoms for each variant is uncertain. *TNNI3* variants can increase calcium sensitivity: *TNNT2* variants can decrease sensitivity.

Methods: Six Newfoundland and Labrador families have one or both troponin variants. Extensive (up to seven generation) pedigrees are completed. Individuals born at an *apriori* 50% risk were defined as variant +ve, obligate carrier, severe cardiac event (sudden cardiac death (SCD), heart transplant, heart failure ≤50 years), variant -ve, or unknown (deceased, declined testing or not yet seen).

Results:

Variant	Sex	Variant positive		Variant negative	Obligate Carrier	Severe cardiac event
		Heterozygous	Homozygous			
TNNI3 p.R162Q	M	18	1	8	14	7
	F	25	2	19	12	7
TNNT2 p. K210del	M	1	0	1	1	1
	F	1	0	3	0	1

Three individuals have both *TNNI3* and *TNNT2* variants (2 F: 45y, 66y, and 1M deceased 84y). The females describe mild palpitations. The male never presented. Two individuals with the *TNNT2* variant have severe disease (onset < 20 yrs.). The diagnostic range for *TNNI3* heterozygotes is 16-67y. Thirty-six (5-77yrs) are variant +ve disease -ve. Five individuals had an SCD (range 32-57y). Homozygote *TNNI3* do not present differently.

Conclusion: *TNNT2* p.K210del is highly pathogenic. When paired with *TNNI3* p.R162Q, the pathogenicity is absent. The variant effect on calcium sensitivity likely has a cancelling effect. The *TNNI3* cohort is highly variable and homozygotes appear similar to heterozygotes.

Grants:

Conflict of Interest: None declared

P07.065.D The burden of cardiac genetic disease in Newfoundland and Labrador

Peter Sutherland[1], *Emily Bonnell*[2], *Fiona Curtis*[2], *Stephen Duffett*[3], *Sean Connors*[3], *Lesley Turner*[4], *Ashley Baker*[1], *Sajid Khayer*[5], *Kathy Hodgkinson*[6], *Ann Dorward*[7]

[1]*Memorial University of Newfoundland, Biomedical Sciences, St. John's, Canada;* [2]*Newfoundland and Labrador Health Services, Provinical Medical Genetics Program, St. John's, Canada;* [3]*Newfoundland and Labrador Health Services, Cardiology, St. John's, Canada;* [4]*Newfoundland and Labrador Health Services, Provincial Medical Genetics Program, St. John's, Canada;* [5]*Memorial University of Newfoundland, MUN Medicine, St. John's, Canada;* [6]*Memorial University of Newfoundland, Clinical Epidemiology, St. John's, Canada;* [7]*Memorial University of Newfoundland, Biomedical Sciences, St John's, Canada*

Background/Objectives: Catecholaminergic Polymorphic Ventricular Tachycardia (CPVT) is an autosomal dominant cardiac condition characterized by serious arrhythmia during exercise or stress. A Newfoundland and Labrador (NL) CPVT cohort was ascertained with a Ryanodine Receptor-2 (*RyR2* p.Y4962N) variant post a child's death. Classified likely pathogenic, it was downgraded to a variant of unknown significance (VUS). This study will determine the spectrum of CPVT phenotypes to aid classification in a family-based approach.

Methods: The extended family (n = 653, seven generations) was used to identify individuals with 50% a priori risk of inheriting the *RyR2* p.Y4962N variant. Clinical data is extracted from variant positive and negative individuals and placed in an SPSS.v28 dataset.

Results: Ninety family members at a priori 50% risk of inheriting the *RyR2* p.Y4962N variant are ascertained. Demographic data is available for 90: cardiac clinical data is currently available for 25.

Variant	Sex	Variant positive n = 29 (32.2%)		Variant negative n = 18 (20%)		Variant status unknown n = 43 (47.8%)	
		n	Age range(y)	N	Age range(y)	n	Age range(y)
RyR2-p.Y4962N	M	15	7-73	10	32-56	24	30-81
	F	14	15-92	8	7-91	19	29-83

Three cases of sudden death have occurred (range 7-29 years). Preliminary analysis reveals 4/25 with signs and symptoms of CPVT from mild (palpitations) to severe (syncope whilst driving).

Conclusion: Variant classification relies upon multiple lines of evidence. Segregation in large related cohorts with robust ascertainment is a powerful tool. For families experiencing severe clinical events, a VUS is psychologically traumatic. This study we hope will help solidify classification for this variant.

Grants:

Conflict of Interest: None declared

P07.066.A Cardiovascular disease outcomes in individuals with probable or definite FH diagnosis in the Simon Broome register

Barbara Iyen[1], Ralph Akyea[1], Steve Humphries[2], Joe Kai[1], **Nadeem Qureshi**[1]

[1]University of Nottingham, Centre for Academic Primary Care, Nottingham, United Kingdom; [2]University College London, Institute Cardiovascular Science, London, United Kingdom

Background/Objectives: The Simon Broome (SB) diagnostic criteria for definite or probable FH take into account combinations of laboratory tests, clinical criteria and family history. We assessed cardiovascular disease (CVD) risk in people with probable or definite FH by linking patient records in the UK SB register with secondary care records in Hospital Episode Statistics (HES).

Methods: Baseline characteristics and type of FH diagnosis (probable or definite) were assessed at time of SB registration. Using linked HES data, we determined incidence rates for CVD (a composite of coronary heart disease (CHD), myocardial infarction (MI), stable or unstable angina, stroke, TIA, peripheral vascular disease (PVD), heart failure, coronary revascularisation interventions). Cox proportional hazards regression models determined adjusted hazard ratios (aHR) for CVD in individuals with probable compared to definite FH.

Results: Of 2,988 individuals with linked SB register and HES data (median follow-up: 17.4 years), 1548 (51.8%) had definite FH while the remainder had probable FH diagnosis. Incidence rates (95%CI) of CVD (per 1000 person-years) were 25.1 (23.2-27.2) and 25.8 (24.0-27.7) with probable and definite-FH respectively. Compared to definite-FH individuals, those with probable FH had a lower risk of CHD (aHR 0.79, 95%CI 0.70-0.88), but no statistically different risk of stroke (aHR 0.89, 95%CI 0.71-1.13) and PVD (0.89, 95%CI 0.70-1.12).

Conclusion: Individuals with probable FH had a lower risk of CHD but a similar risk of stroke and PVD, compared to those with a definite FH. Improved risk-factor management in all patients with FH is still required to reduce risk of CVD.

Grants: NIHR HTA award ID 15/134/02

Conflict of Interest: None declared

P08

METABOLIC AND MITOCHONDRIAL DISORDERS

P08.001.B Long-chain fatty acid oxidation disorders: a novel gene database for LC-FAOD genes *ACADVL, CPT1A, CPT2, HADHA, HADHB*, and *SLC25A20*

Heather Richbourg[1], Omid K. Japalaghi[1], Vanessa Rangel Miller[1], Sean Daugherty[1], Moeen Al-Sayed[2], Peter Baker II[3], Aneal Khan[4], Mark Kiel[5], Lawrence Korngut[6], Deborah Marsden[1], Stephanie Monteleone[5], Gerard Vockley[7], **Nicole Miller**[1]

[1]Ultragenyx Pharmaceutical Inc., Novato, United States; [2]Al Faisal University, College of Medicine, Riyadh, Saudi Arabia; [3]University of Colorado, Anschutz Medical Campus, Aurora, United States; [4]M.A.G.I.C. (Metabolics and Genetics in Canada) Clinic Ltd, Calgary, Canada; [5]Genomenon, Inc, Ann Arbor, United States; [6]University of Calgary, Department of Clinical Neurosciences, Calgary, Canada; [7]UPMC Children's Hospital of Pittsburgh, Division of Medical Genetics and The Center for Rare Disease Therapy, Pittsburgh

Background/Objectives: Long-chain fatty acid oxidation disorders (LC-FAOD) comprise six rare, life-threatening conditions detected through plasma acylcarnitine profile abnormalities, identified either through NBS or via clinical presentation. Genetic testing is critical as limited functional testing is available. Correlation of clinical and biochemical features with genotype is incomplete, and numerous variants of uncertain significance (VUS) have been reported.

Methods: This database of variants in LC-FAOD genes (*ACADVL*, *CPT1A*, *CPT2*, *HADHA*, *HADHB*, *SLC25A20*) integrates data from: a systematic literature review using Mastermind, a sponsored gene panel program dataset, NBS results, and patient phenotypes.

Results: As of APR 2023, the LC-FAOD database reported 5365 variants from 3217 individuals with ≥1 gene variants, representing 975 unique variants: 662 (68%) pathogenic (P) or likely pathogenic (LP) and 301 (31%) VUS. 1842/3217 (57%) patients had a positive molecular diagnosis/MDx (≥2 P/LP) and 230/3217 (7%) had a potential MDx (LP/VUS, P/VUS), combined by gene as follows: *ACADVL* 38%, *HADHA* 28%, *CPT2* 21%, *CPT1A* 5%, *SLC25A20* 4%, *HADHB* 4. Of 2,059 LC-FAOD genotypes, 932 (45%) were private variants.

Phenotypic data were reported for 939 positive/potential LC-FAOD patients. Common phenotypes reported: *ACADVL* – Elevated CK (27%); *CPT2* – myoglobinuria (33%); *HADHA* – liver abnormality (28%); *SLC25A20* – liver abnormality (26%); *HADHB* – peripheral neuropathy (22%); *CPT1A* – liver abnormality (39%). The most common phenotype for patients <1y vs >12y: hypoglycemia (151; 47%) and myoglobinuria (56; 52%), respectively.

Conclusion: Gene databases are important for rare diseases: clinical information associated with variants are scarce; VUS are frequent and insufficient to support MDx. Genotype-phenotype data are important diagnostic aids.

Grants: None

Conflict of Interest: Heather Richbourg Ultragenyx Pharmaceutical Inc., Ultragenyx Pharmaceutical Inc., Omid K. Japalaghi Ultragenyx Pharmaceutical Inc., Vanessa Rangel Miller Ultragenyx Pharmaceutical Inc., Ultragenyx Pharmaceutical Inc, Sean Daugherty Ultragenyx Pharmaceutical Inc, Ultragenyx Pharmaceutical Inc, Moeen Al-Sayed investigator on study sponsored by Ultragenyx, Peter Baker II Investigator on study sponsored by Ultragenyx, Received educational grant from Ultragenyx, Aneal Khan Received research funding from Ultragenyx, Consultant for Ultragenyx, Mark Kiel Genomenon, Lawrence Korngut Received research funding from Ultragenyx, Ultragenyx, Deborah Marsden Ultragenyx Pharmaceutical Inc, Ultragenyx Pharmaceutical Inc, Stephanie Monteleone Genomenon, Gerard Vockley Received research funding from Ultragenyx, Nicole Miller Ultragenyx Pharmaceutical Inc.; BioMarin Pharmaceutical Inc., Ultragenyx Pharmaceutical Inc.

P08.002.C Analysis of lncrna expression in patients with metabolic syndrome: an investigation based on RNA-sequencing data

Yasemin Oyacı[1], *Naci Şenkal*[2], *Alpay Medetalibeyoğlu*[2], *Fatima Ceren Tunçel*[1], *Murat Köse*[2], *Tufan Tükek*[2], *Sacide Pehlivan*[3]

[1]*Institute of Graduate Studies in Health Sciences, Istanbul University, Medical Biology, Istanbul, Türkyie;* [2]*Istanbul Faculty of Medicine, Istanbul University, Department of Internal Medicine, Istanbul, Türkyie;* [3]*Istanbul Faculty of Medicine, Istanbul University, Medical Biology, Istanbul, Türkyie*

Background/Objectives: Long non-coding RNAs (lncRNAs) have been known to play a role in the pathogenesis of metabolic diseases. However, what we know about the regulatory role of lncRNAs in metabolic syndrome is limited. In our study we aimed to investigate the lncRNAs that are potentially involved in Metabolic Syndrome (MetS).

Methods: lncRNA profiles were analyzed in 12 MetS samples and 10 healthy control samples using Illumina Novaseq 6000 platform. Differentially expressed transcripts between two groups were identified, and Gene Ontology and Kyoto Encyclopedia of Genes and Genomes enrichment analyses were performed to identify the biological functions of lncRNAs.

Results: Out of a total of 15,771 lncRNA transcripts, 293 transcripts were significantly differentially expressed of which 144 were upregulated, 149 were downregulated and 180 novel lncRNAs. MIAT, associated with myocardial infarction, and LINC00299, associated with atherosclerosis, were identified as two of the lncRNAs that were highly upregulated in MetS patients.

PTPRN2-AS1, antisense of the PTPRN2 gene, which is associated with cardiometabolic diseases and ZFPM1-AS1, antisense of the ZFPM1 gene, which has a role in heart development have been identified as two of the lncRNAs that were significantly downregulated in Mets patients.

Conclusion: The study suggests that the functions of lncRNAs are important in MetS development and progression. Especially the change in the expression of MIAT, LINC00299, PTPRN2-AS1 and ZFPM1-AS1 lncRNAs in patients is a promising finding in terms of the increased CVD risk in MetS patients.

Grants: This study was supported by the Scientific Research Project Coordination Unit of Istanbul University under Grant [number: TOA-2022-39395].

Conflict of Interest: None declared

P08.003.D Nuclear Mitochondrial DNA disruption in KLHL10 gene causes Spermatogenic Failure

Murtadha Ali[1], *Najim Ameziane*[1], *Aboulfazl Rad*[1], *Gabriela Oprea*[1], *Shivendra Kishore*[1]

[1]*Arcensus GmbH, Rostock*

Consortium: Arcensus GmbH, Rostock, Germany

Background/Objectives: Translocation of Mitochondrial DNA (mtDNA) fragments into the nuclear genome, results in Nuclear Mitochondrial DNA segments (NUMTs). Novel or de novo NUMTs may show detrimental effects, contributing to human diseases, carcinogenesis, and ageing. Here we report a rare NUMT occurrence which disrupts the kelch like family member 10 gene (KLHL10), that plays an important role in male infertility.

Methods: A 41-year-old male patient with oligospermia and no family history (isolated case) was analysed via whole genome sequencing (WGS) to yield an average coverage depth of 30x for the nuclear genome and at least 1000x for the mitochondrial genome. Previous analysis showed no Y chromosome microdeletion. Chromosomal analyses revealed the presence of Robertsonian translocation between the long arm of chromosome 13 at 13q10 and the long arm of chromosome 15 at 15q10. However, this event did not explain the patient's phenotype.

Results: The WGS analysis unveiled the presence of a heterozygous insertion of the mitochondrial genome into exon 3 of KLHL10 gene (NM_152467.5), with breakpoints at chr17:41845555 (17q21.2) -chrM:3595. Consequently, this insertion disrupted the KLHL10 gene. Mutations in this gene have been previously associated with autosomal dominant spermatogenic failure type 11, a rare genetic male infertility medical condition.

Conclusion: To the best of our knowledge, this is the first reported instance of NUMTs causing a rare form of male infertility. Since NUMTs may create artefacts resembling mixed populations of mtDNA (pseudo-heteroplasmy), WGS is a comprehensive approach to accurately detect potential disease causing NUMTs.

Conflict of Interest: None declared

P08.004.A Perrault syndrome-associated missense variants affect HARS2 protein homodimerization and functional activity

Huw Thomas[1;2], *Maria Obregon Comino*[2], *Rob Harkness*[1;2], *Thomas Smith*[1;2], *William Newman*[1;2], *Raymond O'Keefe*[1;2]

[1]*Manchester University NHS Foundation Trust, St Mary's Hospital, Manchester Centre for Genomic Medicine, Manchester, United Kingdom;* [2]*The University of Manchester, Division of Evolution, Infection and Genomics, School of Biological Sciences, Manchester, United Kingdom*

Background/Objectives: Perrault syndrome is a rare, genetically heterogenous, autosomal recessive disorder characterised by sensorineural hearing loss and primary ovarian insufficiency. Bialleleic variants in nine genes to date have been linked to Perrault syndrome, with all associated with altered peroxisomal or mitochondrial function. Of these genes, *HARS2* – a mitochondrial histidyl-tRNA synthetase – has had 14 Perrault syndrome-associated variants reported, located throughout its protein sequence. Of note, 5 missense variants lie within a dimer-interface region and are predicted to influence the correct assembly of HARS2 homodimers - a prerequisite of normal HARS2 function.

Methods: Here, we undertook a series of in vitro analyses to investigate the consequences of Perrault syndrome-associated

missense variants on HARS2 protein homodimerization and function. We complemented these analyses with in silico modelling to validate and further identify additional HARS2 protein regions and residues of importance.

Results: We demonstrated that 2 of 5 variants studied caused significant disruption to HARS2 dimerization and identified residues 138, 139 and 150 as key contact points between HARS2 monomers. We also showed that some variants can lead to a decrease in aminoacylation-activity and a change in protein thermal stability.

Conclusion: These findings further our understanding of how missense changes in *HARS2* contribute to mitochondrial dysfunction and help to reclassify variants of uncertain significance as likely pathogenic, providing clinical utility.

Grants: This study was supported by the Medical Research Council (MR/W019027/1); Royal National Institute for the Deaf and Masonic Charitable Foundation (S60_Newman); Action Medical Research (GN2494); NIHR Manchester Biomedical Research Centre (NIHR203308).

Conflict of Interest: None declared

P08.005.B GLA p.Asp313Tyr variant in a large German cohort - genetic, biochemical, and clinical characterization

*Tobias Böttcher[1;2], Christian Beetz[1], Daniel Schulze[3], Deepa Saravanakumar[1], Emir Zonic[1], Sabine Schröder[1], Anett Kaune[1], Omid Paknia[1], **Jorge Pinto Basto**[1], Peter Bauer[1;4]*

[1]Centogene GmbH, Rostock, Germany; [2]Universitätsmedizin Greifswald, Neurology, Greifswald, Germany; [3]Charité-Universitätsmedizin Berlin Campus Charité Mitte, Institute of Biometry and Clinical Epidemiology, Berlin, Germany; [4]Universitätsmedizin Rostock, Rostock, Germany

Fabry disease (FD; OMIM #301500) is a lysosomal disorder, caused by pathogenic variants in the GLA gene, resulting in enzymatic deficiency of α-galactosidase A (AGLA). The pathogenicity of certain variants is controversially disputed, particularly the c.937G>T (p.Asp313Tyr) variant which causes an enzymatic pseudodeficiency in plasma [PMID 14680977]. According to the ACMG/AMP guidelines [PMID 25741868] most genetic laboratories had reclassified this variant to likely benign/benign over time. Nevertheless, there is ongoing discussion claiming p.Asp313Tyr a variant with FD-causing properties.

Genetic, biochemical, and clinical characteristics of GLA p.Asp313Tyr individuals were evaluated in patients from Germany screened in our laboratory for FD between 1st January 2015 and 31st December 2020.

From 18,290 male and 18,198 female samples examined, p.Asp313Tyr was detected in 204 and 309, respectively. The allele frequency was increased (males: 0.011, $p < 0.0001$, odds ratio 2.39, 95% CI 1.94-2.93; females: 0.008, $p < 0.0001$, odds ratio 1.98, 95% CI 1.68-2.35) compared to gnomAD data for European (non-Finish) population (0.0047 and 0.0043, resp.). Enzyme activity was not reduced to values accepted for provoking clinical symptoms of FD [PMID 30559317] and globotriaosylsphingosine levels were even normal in all cases. Furthermore, no significant associations with clinical symptoms were found in a multivariable analysis.

Applying ACMG/AMP criteria, the GLA variant p.Asp313Tyr must be classified as a benign variant and therefore does not cause FD. However, the fact of increased allele frequency in our screening cohort compared to general population reference data, demands further clarification of the role of this variant as a potential risk factor or modifier for a yet to be defined disorder.

Conflict of Interest: Tobias Böttcher Centogene, Centogene, Takeda, Amicus, Chiesi, Takeda, Amicus, Christian Beetz Centogene, Daniel Schulze Charité University Medicine, Centogene, Deepa Saravanakumar Centogene, Emir Zonic Centogene, Sabine Schröder Centogene, Anett Kaune Centogene, Omid Paknia Centogene, Jorge Pinto Basto Centogene, Peter Bauer Centogene, Centogene

P08.006.C Novel association of *COQ7* with frontal lobe leukodystrophy and isolated progressive spastic diplegia

*Clair Habib[1], Liana Semcesen[2], Jun Xiong Lee[1], Tegan Stait[3], Cas Simons[2], Eloise Uebergang1[3], John Christodoulou[2;3], Richard Leventer[1;2;3], David Thorburn[2;3], David Stroud[2], **Chloe Cunningham**[2;3]*

[1]Royal Children's Hospital, Parkville, Australia; [2]University of Melbourne, Department of Paediatrics, Parkville, Australia; [3]Murdoch Children's Research Institute, Parkville, Australia

Consortium: MitoMDT

Background/Objectives: *COQ7*-related CoQ_{10} deficiency 8 is a rare disorder that causes a spectrum of disease including spasticity, ranging from mildly progressive encephaloneuronephro-cardiopathy to fatal multisystemic disorder [MIM# 616733]. The phenotypic spectrum was recently expanded to include distal neuropathy with upper motor neuron signs. We present an individual with a novel phenotype of spastic diplegia and frontal lobe leukodystrophy with compound heterozygous *COQ7* variants and multi-omics functional analyses supporting causality.

Methods: Trio genome sequencing was undertaken in a study of unclassified leukodystrophies. Variants of uncertain significance in *COQ7* were investigated with functional studies of patient fibroblasts. Levels of COQ7 and associated proteins were assessed by proteomics and immunoblotting. CoQ_{10} levels were assessed by untargeted lipidomic analysis and complex II + III assays +/- CoQ_1 supplementation.

Results: The compound heterozygous variants in *COQ7* (c.319C>T;p.(Arg107Trp) and c.161G>A;p.(Arg54Gln)) had each been reported in a compound heterozygous state with different *COQ7* variants in unrelated individuals with limited evidence of pathogenicity. Immunoblotting demonstrated a marked deficiency of COQ7 protein, and proteomic data were consistent with previous studies of *COQ7* knockouts. Lipidomic analysis and complex II + III activity showed a deficiency of total CoQ_{10} and the CoQ_{10} pool accessible to respiratory chain enzymes.

Conclusion: This study provides strong evidence supporting the pathogenicity of the p.(Arg107Trp) and p.(Arg54Gln) variants in *COQ7*. We propose that the phenotypic spectrum associated with biallelic *COQ7* variants should be expanded to include leukodystrophy with isolated spastic diplegia and normal cognition.

Grants: This work is supported by grants from the Medical Research Future Fund (EPCD000034 and MRF2007959).

Conflict of Interest: None declared

P08.007.D Genetic diagnostic yield of obesity

Robert Künzel[1], *Helene Faust[1], Antje Garten[2], Matthias Blüher[3;4], Eric Göpel[2], Rami Abou Jamra[1], Mariami Jasaszwili[2], Anna Kirstein[2], Albrecht Kobelt[5], Antje Koerner[2;4], Johannes Lemke[1], Robert Stein[2;4], Diana le Duc[1;5]*

[1]University of Leipzig Medical Center, Institute of Human Genetics, Leipzig, Germany; [2]University Hospital for Children and Adolescents, Pediatric Research Center, Leipzig, Germany; [3]University of Leipzig Medical Center, Medical Department III—Endocrinology, Nephrology, Rheumatology, Leipzig, Germany; [4]Helmholtz Institute for Metabolic, Obesity and Vascular Research (HI-MAG) of the Helmholtz Zentrum

München at the University of Leipzig and University Hospital Leipzig, Leipzig, Germany; [5]Chemnitz Clinic, Diagnostic Center GmbH MVZ, Practice for Human Genetics, Chemnitz, Germany

Background/Objectives: Obesity poses a major public health concern. Although studies have shown that the heritability of BMI in individuals with obesity lies around 60-80%, the underlying genetics are still poorly understood. This study reports the genetic findings of a large cohort of patients with obesity, who underwent NGS-based diagnostic of exomes and large clinical panels. We aim to characterize the cohort in order to improve future diagnostic yield.

Methods: 560 patients with reported obesity underwent genetic diagnostics at the Institute of Human Genetics at Leipzig University between 2016 and 2023. The cohort includes 421 children and 139 adults, with 256 female and 304 male patients. 84.1% of patients (n = 471) received exome analyses, the rest underwent diagnostic based on large clinical panels.

Results: Initial results show that 4.8% of patients (27 of 560) received a genetic diagnosis explicitly explaining their obesity. Eight of these patients had *MC4R* variants. Syndromic obesity was diagnosed in 17 patients, four of whom had a 16p11.2 microdeletion. We reported a possible obesogenic variant in a further 7.7% of patients. Affected genes include *KSR2*, *SRRM2*, *PLIN1* and *NCOA1*. Definitive diagnostic yield was higher in children (5.5%) compared to adults (2.9%). Additionally, we reported a possible obesogenic variant in 7.4% of children and 8.6% of adults.

Conclusions: Only a small fraction of patients with obesity received a monogenetic diagnosis. Several identified variants may increase susceptibility to obesity. Further research is needed to reduce what appears to be a large diagnostic gap.

Grants: German Research Foundation CRC 1052 B10

Conflict of Interest: None declared

P08.008.A Comprehensive analysis of gut virome diversity in hypertriglyceridemia individuals

Shahbaz Raza[1], **Hanna Kim**[1;2]

[1]Sungkyunkwan University, Department of Clinical Research Design and Evaluation, Samsung Advanced Institute for Health Sciences and Technology, Seoul, Korea, Rep. of South; [2]Samsung Medical Center, Biomedical Statistics Center, Research Institute for Future Medicine, Seoul, Korea, Rep. of South

Background/Objectives: Hypertriglyceridemia is characterized by elevated triglyceride (TG) levels in the bloodstream and is a key component of metabolic syndrome. The prevalence of hypertriglyceridemia in Korea has increased from 10.2% in 1998 to 16.4% in 2017, with higher rates observed in men (25.2%) compared to women (8.0%). Recent studies have shown an association between gut virome and metabolic diseases. In this study, we investigated the gut virome in individuals with elevated TG levels (n = 402) compared to healthy individuals (n = 402).

Methods: We utilized gut metagenomic data from 1710 Korean individuals, calculating the relative abundance of the gut virome using the Kraken2 tool.

Results: We identified 1341 viral sequences, of which 17 were differentially abundant between the groups ($q < 0.05$). Among the 10 differentially abundant viral sequences in hypertriglyceridemia, 7 were phages. Notably, three *Escherichia* phage were significantly enriched in the hypertriglyceridemia group. While no significant differences in alpha diversity were observed, there were significant dissimilarities based on beta-diversity indices, including non-phylogenetic Bray-Curtis, Jaccard, and Canberra distances ($p < 0.05$). Correlation analysis revealed that Escherichia coli was associated with the majority of the significantly different viral species.

Conclusion: Our findings underscore the significance of viruses and phages in individuals with hypertriglyceridemia compared to healthy individuals. Further investigations are warranted to elucidate the role of these viral species in hypertriglyceridemia individuals.

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Conflict of Interest: None declared

P08.009.B Evaluation of newborn screening for X-linked adrenoleukodystrophy in Southern Spain

Maria Isabel Cabrera Gonzalez[1;2], Yolanda de Diego Otero[1], Rocio Calvo Medina[1], José Miguel Ramos Fernández[1], Javier Blasco-Alonso[1], María del Carmen Benito López[1], Marta García de Burgos[1;2], Sara Franco Freire[1], **RAQUEL YAHYAOUI MACIAS**[1]

[1]Malaga Regional University Hospital. IBIMA-Plataforma BIONAND; [2]

Background:

X-linked adrenoleukodystrophy (XALD) is the most common leukodystrophy and peroxisomal disease, with an estimated prevalence 1:10,000 live births. Early detection is essential to treat adrenal insufficiency, and in cases with cerebral XALD, hematopoietic stem cell transplantation may be curative if performed in early stages of the disease.

Methods: From July 2022 to December 2023, C26:0-LPC levels were measured by an inhouse LC-MS/MS method in 50,000 newborn screening (NBS) dried blood spot (DBS) samples. In newborns with a positive result, biochemical and genetic analysis were performed.

Results: Eight newborns had a positive result. Two patients displayed severe neonatal symptoms due to pathogenic variants in the *HSD17B4* and *PEX6* genes. The rest of newborns are asymptomatic. Four presented biochemical alterations, confirming the presence of a genetic variant in the *ABCD1* gene.

CASES	DBS C26:0-LPC (NV < 0,14 micromol/L)	Plasma C26:0 (NV < 0,72 micromol/L)	Plasma C26:0/C22:0 (NV < 0,016)	Genetic analysis
1 (female)	0,306	NA	NA	NA
2 (male)	1,512	18,07	0,904	***HSD17B4*** gene: c.1369A>T,p.Asn457Tyr (pathogenic) and c.1681G>C,p.Ala561-Pro (likely pathogenic)
3 (male)	0,229	0,58	0,030	NA
4 (male)	0,225	0,60	0,020	***ABCD1*** gene: c.1900G>A,(p.Ala634Thr) (VUS)
5 (male)	0,418	1,54	0,025	***ABCD1*** gene: c.1747G>A,p.(Val583Met) (VUS)
6 (male)	1,068	NA	NA	***PEX6*** gene: c.531delA,p.Pro179fs (pathogenic) and c.402delC,p.Gly135fs (likely pathogenic)
7 (male)	0,145	0,88	0,034	***ABCD1*** gene: c.2111C>T,(p.Ala704Val) (VUS)
8 (female)	0,687	2,58	0,059	***ABCD1*** gene: c.1415_1416delAG,p.Gln472fs (pathogenic)

Conclusion: Our study is the first universal XALD NBS program performed in Europe. Neonatal screening for XALD is an effective

tool for early detection of the disorder which allows early treatment, genetic counselling and carrier detection.

Conflict of Interest: None declared

P08.010.C A noval congenital generalized lipodystrophy type 2 patient caused by *BSCL2* gene

Ayse Busra Akalin[1], *Fatih Demircioğlu*[2], *Ibrahim Akalin*[3]

[1]*Bahcesehir University Faculty of Medicine, Medicine, Istanbul, Türkyie;* [2]*Medistate Hospital, Pediatric Hematology, Istanbul, Türkyie;* [3]*Metagentech Center for Genetic Diseases, Istanbul, Türkyie*

Background/Objectives: Lipodystrophies are genetically heterogenous disorders. It causes a person to lose adipose tissue in some parts of the body while gaining it to others, such as liver. The muscle tissue can be seen clearly which can be mistaken for hypertrophy due to loss of hypodermic fat loss.

Methods: A 2,5-year-old female patient was born to third degree consanguinity referred with dysmorphic facial appearance and distinct subcutaneous adipose tissue loss and elevated triglyceride levels. Physical examination revealed hepatomegaly. Whole exome sequencing was performed for mutation analysis.

Results: Exome sequencing analysis revealed germ line c.650C>G (p.S217X) homozygous mutation in *BSCL2* gene responsible for "Congenital Generalized Lipodystrophy Type 2 Syndrome [MIM#269700]", suggesting autosomal recessive inheritance.

Conclusion: Clinical findings detected in the patient align with the symptoms of Congenital Generalized Lipodystrophy Type 2 Syndrome, thus supporting the molecular diagnosis of the disorder in the patient. Additionally, the frequency of the variant has not been found in gnomAD database but was reported in literature only once, up to date.

Grants:

Conflict of Interest: None declared

P08.011.D A novel quantification method using digital PCR for Preimplantation Genetic Testing of Mitochondrial DNA disorder.

Yuki Mizuguchi[1;2], *Kou Sueoka*[3], *Suguru Sato*[1;4], *Mamoru Tanaka*[1]

[1]*Keio University School of Medicine, Obstetrics and Gynecology, Japan;* [2]*Nasu Red Cross Hospital, Obstetrics and Gynecology, Ohtawara, Japan;* [3]*Shizuoka Graduate University of Public Health, Graduate School of Public Health, Shizuoka, Japan;* [4]*Ogikubo Hospital Niji Clinic, Tokyo, Japan*

Background/Objectives: PGT for mitochondrial DNA (mtDNA) disorder is identifying embryos with mtDNA mutation ratio (mutant load; ML) below the threshold level. We have developed and applied a method for analyzing ML using quantitative multiplex PCR. However, the need of calibration curves makes this method complicated and time consuming. We aimed to develop a new quantification method for analyzing ML using digital PCR.

Methods: Blood sample from carrier of the 8993T>G mutation of mtDNA, which has a ML of 45%, was used. Experiments were conducted to find out the optimal settings of digital PCR for the mtDNA ML analysis by checking three points: PCR cycle number, sample concentration, and comparison of ML by analysis methods.

Results: Forty cycles were enough to produce a sufficient yield. Digital PCR was not able to analyze high concentration samples (1ng/μl). Low concentration samples, on the other hand, could be analyzed as low as 1 pg/μl. 50 pg/μl seemed to be the optimal concentration, at which stable amplifications of wild-type and mutant mtDNA were obtained. ML analyzed with optimal setting was 36.1% which was almost the same as the ML analyzed by pyrosequencing assay (36.3%).

Conclusion: This is the first report of the use of digital PCR for PGT in mitochondrial disorder. The results indicate that analysis using digital PCR is possible from limited number of cells, making it an ideal method for PGT. On the other hand, the ML was lower than the known value of 45% at all methods, and further study is needed to determine the cause.

Grants: None

Conflict of Interest: None declared

P08.012.A Molecular genetic analysis of familial hypercholesterolemia in a cohort of Czech patients

Radovan Bakalar[1], *Petra Slavikova*[1], *Veronika Todorovova*[1], *Renata Svacinova*[1], *Martin Reboun*[1], *Karolina Peskova*[1], *Pavel Jesina*[1], *Lenka Dvorakova*[1]

[1]*General University Hospital in Prague and 1st Faculty of Medicine, Charles University, Department of Pediatrics and Inherited Metabolic Disorders, Prague, Czech Republic*

Background/Objectives: Familial hypercholesterolemia (FH) is one of the most significant risk factors for atherosclerotic cardiovascular diseases (ASCVD). FH is predominantly caused by heterozygous variants in low density lipoprotein receptor (*LDLR*), apolipoprotein B (*APOB*) or proprotein convertase subtilisin/kexin type 9 (*PCSK9*) genes. Our aim was to monitor the frequency of variants, including CNVs, in individual genes in the Czech population.

Methods: Next-generation sequencing using a custom panel of 10 genes related to FH and FH-like phenotype was performed on Miseq platform (Illumina). An in-house pipeline including the ANNOVAR annotation tool and CNVkit was used for variant calling and CNV detection. Presence of CNVs was verified by SYBR Green qPCR and/or sequencing of the overlapping PCR products.

Results: In 53 out of 175 tested Czech suspected FH patients (30.3%), we detected various heterozygous causal variants in *LDLR* or *APOB*:c.10580G>A in 41 and 12 patients, respectively. Heterozygous variants of uncertain significance in *APOB* were identified in 6 patients. No causal variants were found in *PCSK9*. In *LDLR*, we identified a novel splicing variant c.2312-2A>C and 2 CNVs – a novel exon 2-14 duplication and a previously described exon 9-14 deletion. In other 10 patients, hypercholesterolemia could possibly be explained by variants in *LIPA*, *ABCG5*, *APOE*, *LDLRAP1 or LIPC*.

Conclusion: Pathogenic *LDLR* variants as the most common cause in our cohort are consistent with globally published data. Diagnostic genetic testing improves prognosis and personalized therapy for FH patients and helps to reduce the risk of ASCVD.

Grants: MZ ČR–RVO-VFN00064165

Conflict of Interest: None declared

P08.013.B Urine organic acid and acylglycine testing as auxiliary diagnostic tools for diagnosing flavin adenine dinucleotide synthase deficiency: A retrospective investigation

Zinandre Stander[1], *Zineb Ammous*[2], *Joanne Werker*[2], *Kai Muru*[3;4], *Katrin Ounap*[3;4], *April Studinski*[1], *Matthew Schultz*[1], *Patricia Hall*[1], *Dietrich Matern*[1], *Devin Oglesbee*[1], *Silvia Tortorelli*[1], **Dimitar Gavrilov**[1]

[1]*Mayo Clinic, Department of Laboratory Medicine and Pathology, Rochester, United States;* [2]*The Community Health Clinic, Topeka, United States;* [3]*University of Tartu, Department of Clinical Genetics,*

Tartu, Estonia; [4]University of Tartu, Institute of Clinical Medicine, Tartu, Estonia

Background: Flavin adenine dinucleotide synthase deficiency (FADSD) is a rare autosomal recessive disorder caused by pathogenic variants in *FLAD1*. Since FAD is a redox modulating cofactor of various acyl-CoA dehydrogenase reactions, FADSD patients generally present with neuromuscular symptoms and biochemical abnormalities resembling multiple acyl-CoA dehydrogenase deficiency (MADD). While investigations have characterized FADSD using plasma acylcarnitine profiles, literature regarding urinary signatures of these patients is lacking. Hence, this investigation aimed to provide insights into the urinary organic acid and acylglycine profiles of FADSD patients as auxiliary evidence for identification of the disorder prior to molecular analysis.

Methods: Retrospectively collected urine organic acid and acylglycine profiles of FADSD deficiency patients ($n = 4$) were qualitatively and quantitatively evaluated to: (1) establish a urinary signature for FADSD; and (2) determine whether these urinary signatures could be differentiated from MADD patients ($n = 21$).

Results: Collaborative Laboratory Integrated Reports (CLIR) based analysis of FADSD profiles revealed that ethylmalonic acid, methylsuccinic acid, and 12-associated ratios deviated notably from reference ranges (≤20% overlap). Although useful for the identification of FADSD, 5 ratios mainly related to the more prominent hexanoylglycine elevations in MADD cases, appear to be better suited for differentiation (0% overlap) of the two disorders.

Conclusion: Retrospective analysis of urinary FADSD profiles provided insights to a metabolic signature, that could be useful in the identification and differentiation of these patients from MADD cases prior to molecular testing. Hence further aiding in more timely diagnosis and/or treatment of FADSD patients.

Grants: PRG471 funding related to selected FADSD cases

Conflict of Interest: Zinandre Stander: None declared, Zineb Ammous: None declared, Joanne Werker: None declared, Kai Muru PRG471, Katrin Ounap PRG471, April Studinski: None declared, Matthew Schultz: None declared, Patricia Hall: None declared, Dietrich Matern: None declared, Devin Oglesbee: None declared, Silvia Tortorelli: None declared, Dimitar Gavrilov: None declared

P08.014.C Ubiquitination regulates the activity of GDP-mannose pyrophosphorylase B, which is associated with a congential disorder of glycosylation

Patricia Franzka[1], *Sonnhild Mittag[2], Obinna Umeh[3], Henriette Henze[4], Marie Juliane Jung[4], Véronique Blanchard[3;5], Julia von Maltzahn[4;6], Otmar Huber[2], Christian Hübner[1]*

[1]University Hospital Jena, Institute of Human Genetics, Jena, Germany; [2]University Hospital Jena, Department of Biochemistry II, Jena, Germany; [3]Charité-Universitätsmedizin Berlin, Institute of Diagnostic Laboratory Medicine, Clinical Chemistry and Pathobiochemistry, Berlin, Germany; [4]Leibniz-Institute on Aging-Fritz-Lipmann-Institute, AG von Maltzahn, Jena, Germany; [5]Medical School Berlin, Department of Human Medicine, Berlin, Germany; [6]Technische Universität Cottbus-Senftenberg, Stem Cell Biology of Aging, Cottbus, Germany

Background/Objectives: Glycosylation is the most common post-translational protein modification. Abnormal glycosylation can induce deleterious effects as observed in congenital disorders of glycosylation (CDGs) and often results in serious malfunctions of different organ systems such as brain and muscle. A typical example are mutations in the gene encoding for GDP-mannose-pyrophosphorylase-B (GMPPB). GMPPB generates GDP-mannose, a mannose donor for glycosylation. Its catalytically inactive paralogue GDP-mannose-pyrophosphorylase-A (GMPPA) serves as its feedback inhibitor and is associated with a CDG as well.

Methods: Co-immunoprecipitation followed by immunoblot analyses in HEK cells transfected with WT and variant GMPPB constructs together with ubiquitin and/or GMPPA constructs. GMPPB turnover was assessed in the presence of MG132 to inhibit proteasomal degradation or bafilomycin-A1 to inhibit lysosomal degradation. GMPPB activity was measured for GMPPB WT as well as different variant proteins. Myoblasts and N2a cells were used to study consequences of siRNA-mediated GMPPB knockdown on cell differentiation.

Results: We show that GMPPB is ubiquitously expressed and that its knockdown impairs myoblast and neuron-like differentiation. GMPPB is ubiquitinated and interacts with the E3 ubiquitin ligase TRIM67. Patient mutations localizing to the C-terminal part of GMPPB are associated with a diminished ubiquitination of GMPPB and display reduced enzymatic activity. Importantly, inhibition of GMPPB ubiquitination decreases its catalytic activity, while its turnover or interaction with GMPPA remains unaffected.

Conclusion: We here show a novel level of regulation of GMPPB activity by ubiquitination, which may serve as a potential target for future therapies.

Grants: DFG, IZKF.

Conflict of Interest: None declared

P08.015.D A challenging case of trichothiodystrophy: the patient's journey from clinical to genetic diagnosis

Albena Todorova[1;2], *Slavena Atemin[1], Mila Sleptsova[1], Tihomir Todorov[1], Dimitar Stamatov[3], Mila Baycheva[4;5]*

[1]Genetic Medico-Diagnostic Laboratory Genica and Genome Center Bulgaria, Sofia, Bulgaria; [2]Medical University of Sofia, Department of Medical Chemistry and Biochemistry, Sofia, Bulgaria; [3]University Children's Hospital, Sofia, Bulgaria, Department of Paediatric Neurology, Sofia, Bulgaria; [4]University Children's Hospital, Sofia, Bulgaria, Department of Paediatric Gastroenterology, Hepatology and Nutrition, Sofia, Bulgaria; [5]Medical University of Sofia, Department of Paediatrics, Sofia, Bulgaria

Background/Objectives: A 6-years old boy was referred for genetic testing after exhaustive clinical consultations and negative report from clinical exome sequencing. Born at term after pathological pregnancy with intrauterine growth retardation. The patient manifested with hypoproteinemia, hypoglycemia, physiological developmental delay (failure to thrive, severe hypotrophy, poor appetite, nasogastric feeding up to third year of age), neuro-psychological delay, microcephaly, dysmorphic features, brittle hair, trichorrhexis, EEG changes, no seizures, hepatomegaly, liver hemangiomas, hypothyroidism in treatment.

Methods: Genetic testing proceeded with whole exome sequencing – negative result. For the period of 2 years WES data were reanalyzed 6 times. Finally, two heterozygous missense variants were detected in the *CARS1* gene: c.1022G>A, p.Arg341His with maternal origin, and c.1108C>T, p.Arg370Trp with paternal origin.

Results: *CARS1* gene encodes cysteinyl-tRNA synthetase, mutations in which have been recently published in association with recessive multisystem disease that includes microcephaly, developmental delay, brittle hair and nails. Very few patients have been reported so far.

Conclusion: Trichothiodystrophy is a group of syndromes which affect mainly organs with neuroectodermal origin. The abnormalities, manifested at birth, always include brittle hair/nails, growth retardation and intellectual disability. *CARS1* gene has been recently included in the group of trichothiodystrophy-

causing genes. Here we present the first Bulgarian case of *CARS1*-related disorder. Genetic confirmation of the diagnosis is very important, because after treatment (special diet, rehabilitation, psychological and language help, ursodeoxycholic acid, levothyroxine, vitamins and minerals supplementation) and follow-up by gastroenterologist, endocrinologist and neurologist, the child's development has stabilized.

Grants:

Conflict of Interest: None declared

P08.016.A Rare forms of (non)syndromic monogenic diabetes in the Czech registry of patients susceptible for monogenic diabetes

Petra Dusatkova[1], *Klara Vesela*[1], *Katerina Kolarova*[1], *Jan Lebl*[1], *Zdenek Sumnik*[1], *Stepanka Pruhova*[1]

[1]*2nd Faculty of Medicine, Charles University and University Hospital Motol, Department of Paediatrics, Prague, Czech Republic*

Background/Objectives: Monogenic diabetes accounts for approximately 3.5% of patients manifesting diabetes till 30 years. Majority of these patients had subtypes of Maturity-Onset Diabetes of the Young (GCK/HNF1A/HNF4A-MODY). We aimed to describe rare forms of monogenic (non)syndromic diabetes in a Czech registry of patients susceptible for monogenic diabetes.

Methods: Probands with pathogenic variants in all pancreas-related but major MODY genes were selected. Data were obtained from the clinical questionnaires. The genetic testing was performed by Sanger sequencing, MLPA, followed by tNGS of 52 (63) genes since 2018.

Results: Out of 1903 probands with suspected monogenic diabetes, rare forms were detected in 52 families (87 persons). Median age at diabetes diagnosis was 17 (13-30) years. Median HbA1c reached 58 (45-69) mmol/mol (7.5%; 6.3-8.5%). Insulin was prevailing type of treatment (59.7%).

Gene	Persons/ families	Comorbidities
HNF1B	27/21	Renal cysts (n = 7), hypomagnesemia (n = 8), chronic renal insufficiency (n = 3), kidney transplantation (n = 1), kidney anomaly (n = 3), pancreas atrophy (n = 2)
INS	14/7	
WFS1	8/7	Optic hypoplasia (n = 3)
MT-TL1	14/6	Hearing impairment (n = 8), cardiomyopathy (n = 2), stroke (n = 2), heart transplantation (n = 1)
PDX1	8/4	
ABCC8	8/2	
INSR	2/2	
CEL	3/1	Pancreas atrophy and cysts (n = 2)
KCNJ11	1/1	
RFX6	2/1	

Diabetes alone displayed 19 persons with *HNF1B*, *MT-TL1* or *WFS1* anomaly.

Conclusion: Rare forms of monogenic diabetes caused by variants in 10 genes accounted for 7.1% of all families with monogenic diabetes. Genetic diagnosis not only clarify the diabetes aetiology but importantly allow to specifically investigate and treat in time additional comorbidities in probands with syndromic monogenic diabetes mimicking as "diabetes only" phenotype.

Conflict of Interest: None declared

P08.017.B Manifestations of ultra rare homozygous c.612C>G mutation in C1QBP gene in three members of one family

Sofiia Levandivska[1], *Margarita Marchuk*[2], *Ivanna Shymanska*[1;3], *Volodymyr Kravets*[1], *Anastasiya Honchar*[4], *Halyna Makukh*[1;3]

[1]*Scientific medical genetic center LeoGENE, Lviv, Ukraine;* [2]*Bogomolets National Medical University, Kyiv, Ukraine;* [3]*Lviv Regional Clinical Perinatal Centre, Lviv, Ukraine;* [4]*Universal clinic "Oberig", Kyiv, Ukraine*

Background/Objectives: *C1QBP* gene encodes the complement component 1Q subcomponent-binding protein. It is crucial for preserving normal mitochondrial structure and preventing fragmentation. Patients with homozygous or compound heterozygous *C1QBP* mutations have a diverse spectrum of clinical manifestations with an onset at any age. We report the clinical manifestations of patients with homozygous c.612C>G mutation of *C1QBP*, considering its ultra rare occurrence.

Methods: The analysis of C1QBP gene was conducted using Next Generation Sequencing (NGS), followed by Sanger sequencing of the region of interest in the proband`s family members.

Results: A 28-year-old woman sought medical attention with complaints of drooping eyelids, accompanied by rapid fatigue, morning nausea, weakness in facial and skeletal muscles and rhinolalia. A subsequent medical examination identified bilateral ptosis and ophthalmoplegia. The electromyography results revealed signs of chronic diffuse changes of the myopathic type. Further genetic analysis using NGS uncovered a homozygous c.612C>G mutation in *C1QBP*, with conflicting classifications of pathogenicity (NM_001212.4). Both parents and two of the four siblings carried the heterozygous mutation and haven't presented any symptoms. The other two siblings were found to have the homozygous c.612C>G mutation. The 22-year-old male exhibited droopy eyelids, muscle weakness, and nausea/vomiting when fatigued. The 15-year-old female displayed morning nausea and generalized weakness, with no facial manifestations noted thus far.

Conclusion: It is suggested a milder and non-fatal course of the disease when the homozygous c.612C>G mutation of *C1QBP* gene is present. The allele frequency of this mutation is extremely low, which raises the possibility of a shared ancestry.

Grants:

Conflict of Interest: None declared

P08.018.C Plasma-Lipidomics of mitochondrial disorders reveal disease- and phenotype-specific lipid signatures

Anna Worthmann[1], *Julia-Josefine Scholz*[2], *Melina Musfeldt*[2], *Christiane Neuhofer*[3;4], *Boriana Büchner*[5], *Thomas Klopstock*[5], *Christian Kubisch*[2], *Holger Prokisch*[3;4], **Christian Schlein**[2]

[1]*University Medical Center Hamburg-Eppendorf, Department of Biochemistry and Molecular Biology, Hamburg, Germany;* [2]*University Medical Center Hamburg-Eppendorf, Institute of Human Genetics, Hamburg, Germany;* [3]*Technical University of Munich, Institute of Human Genetics, München, Germany;* [4]*Helmholtz Munich, Institute of Neurogenomics, Oberschleißheim, Germany;* [5]*Friedrich-Baur-Institut, LMU Klinikum, Ludwig-Maximilians-University, Department of Neurology, München, Germany*

Consortium: mitoNET consortium

Background/Objectives: Mitochondrial disorders are a class of complex, heterogenous diseases, which show highly variable clinical and phenotypic differences. Many genetic diseases cause direct or indirect changes in lipid metabolism, such as in storage lipids, membrane lipids, metabolites or circulating lipid signaling molecules. As mitochondrial disorders are able to affect synthesis and degradation of lipids, these disorders reflect an interesting target for lipidomic biomarker analysis to evaluate signatures for distinct mitochondrial disorders, for phenotypic affection and for the prediction or progression of the diseases.

Methods: We conducted lipidomics of 299 human plasma samples of patients with confirmed genetic mitochondrial diseases via LC/MS-based lipidomics and correlated them to clinical chemistry parameters measured via the Roche Cobas system to measure affection of different internal organs by parameters, such as lipoproteins, liver transaminases, skeletal and heart muscle parameters, inflammation parameters and others.

Results: By conducting lipidomic analyses of 299 plasma samples with confirmed mitochondrial disorders, we could identify over 800 different lipid species, which included several potential biomarkers for distinct mitochondrial disorders, such as *OPA1*- or *ETFDH*-associated diseases. Further, we identified specific lipidomic signatures for organ affections distinctly describing cardiac involvement of mitochondrial disorders.

Conclusion: In sum, we show that plasma lipidomics are a powerful tool for investigation of lipid signatures for distinct mitochondrial diseases and its related organ manifestations.

Grants: DFG SCHL2276-2, TRR333-1/P15, UKE Medical faculty, Werner Otto foundation

Conflict of Interest: None declared

P08.020.A The shared genetic predisposition between type 2 diabetes and high blood pressure highlights insulin resistance and central obesity

Vincent Pascat[1;2;3], *Liudmila Zudina*[2;3], *Anna Ulrich*[3], *Jared Maina*[1], *Ayse Demirkan*[2;3;4], *Zhanna Balkhiyarova*[2;3;4], *Amélie Bonnefond*[1;3], *Igor Pupko*[2], *Marika Kaakinen*[2;3;4], *Lucas Maurin*[1], *Amna Abdelgader-Khamis*[1;3], *Patricia Munroe*[5;6], *Philippe Froguel*[1;3], *Inga Prokopenko*[1;2;4]

[1]*European Genomic Institute for Diabetes, University of Lille, INSERM UMR1283, CNRS UMR 8199, Lille, France;* [2]*University Of Surrey, Department of Clinical and Experimental Medicine, United Kingdom;* [3]*Imperial College London, Department of Metabolism, Digestion and Reproduction, United Kingdom;* [4]*University Of Surrey, People-Centred Artificial Intelligence Institute, United Kingdom;* [5]*Queen Mary University of London, William Harvey Research Institute, Barts and the London Faculty of Medicine and Dentistry, United Kingdom;* [6]*Queen Mary University of London, National Institute of Health and Care Research, Barts Cardiovascular Biomedical Research Centre, United Kingdom*

Background/Objectives: Type 2 diabetes (T2D) and elevated blood pressure (BP, including systolic [*SBP*] and diastolic [*DBP*]) are complex frequently co-occurring conditions. Understanding shared genetic factors between them could bring insight into the underlying pathophysiological processes.

Methods: We dissected the effects of 563 T2D and 999 BP variants established using genome-wide association studies (GWAS), by constructing polygenic score (PGS) in a set of 457,386 European individuals from UK Biobank (UKB). We gathered Z-scores from GWAS on 49 endophenotypes and utilised them to cluster 1,542 genetic variants into distinct pathophysiological processes and investigated cluster-specific PGSs for comorbidity. We performed bi-directional two-sample Mendelian Randomization (MR) to explore causal relationships between T2D/BP. We used GTEx expression quantitative trait loci (eQTL) in 49 tissues to identify co-localisation of these variants.

Results: T2D overall PGS was associated with risk of elevated BP, and vice versa (P-values ≤ 0.0167). MR suggested that T2D has an heterogenous causal effect on BP. Partitioning genetic variants defined five clusters of different pathogenetic processes, whereby Cluster2 featured individuals with metabolic syndrome characteristics, *i.e.*, central obesity, insulin resistance and shorter stature. MR using Cluster2 variants revealed a circle of causality between DBP, T2D, and SBP, with each increasing risk of comorbidity ($OR_{IVW\ DBP\rightarrow PP\ through\ Cluster2}$ [95% CI] = 1.04[1.02–1.06]; $\beta_{IVW\ T2D\rightarrow SBP\ through\ Cluster2}$[SE]=1.987 [0.278], P-values ≤ 1.86×10^{-5}). Cluster2 co-localised genes had enriched co-expression in adipose subcutaneous, thyroid and artery tibial tissues (*PP.H4.abf* >0.8).

Conclusion: Clustering SNPs by pathogenetic process highlighted the importance of central obesity and insulin resistance into causal effect of T2D leading to altered BP.

Grants: PreciDIAB, ANR-18-IBHU-0001

Conflict of Interest: None declared

P08.021.B Clinical courses and mutational spectrum of Thai patients with primary mitochondrial disorders

Phawin Kor-anantakul[1;2], *Rungroj Thangpong*[1;2], *Wuttichart Kamolvisit*[1;2], *Chupong Ittiwut*[1;2], *Rungnapa Ittiwut*[1;2], *Chanatjit Cheawsamoot*[1;2], *Wanna Chetruengchai*[1;2], *Phichittra Od-Ek*[1;2], *Adjima Assawapitaksakul*[1;2], *Aayalida Buasong*[1;2], *Vorasuk Shotelersuk*[1;2], *Kanya Suphapeetiporn*[1;2]

[1]*Faculty of Medicine, Chulalongkorn University, Center of Excellence for Medical Genomics, Medical Genomics Cluster, Department of Pediatrics, Bangkok, Thailand;* [2]*King Chulalongkorn Memorial Hospital, the Thai Red Cross Society, Excellence Center for Genomics and Precision Medicine, Bangkok, Thailand*

Background/Objectives: Primary mitochondrial disorders (PMD) exhibit clinical and genotypical heterogeneity, posing challenges in diagnosis for patients. Utilizing a comprehensive genetic testing approach, such as exome or genome sequencing, has shown to enhance diagnostic yield.

Methods: We conducted a retrospective review of patients who were molecularly diagnosed and followed-up at King Chulalongkorn Memorial Hospital from January 2020 to December 2023. Data on clinical presentations, investigations, Mitochondrial Disease Criteria scores (MDC; Witters et al., 2017), and genotypes were systematically collected.

Results: We identified seven patients with PMD with five cases attributed to nuclear DNA (nDNA) mutations (*SURF1*, *SDHA*, *SERAC1*, *SLC19A2*, *PDHA1*) and two cases associated with mitochondrial DNA (mtDNA) mutations (*MT-ATP6* m.9176T>G; *MT-ND5* m.12706T>C). Five out of seven cases can be definitive diagnosed by comprehensive genetic tests only. Notably, both patients with mtDNA origin exhibited symptoms of ophthalmoplegia and symptom onset during childhood (age 125 and 14 months old) and MDC scores reaching 8 (definite). The *MT-ND5* patient initially tested positive for acetylcholine receptor antibodies and experienced a misdiagnosis one year before the progressing neurological symptoms (stroke-like episode). In the nDNA group, the majority, four out of five patients, manifested symptoms in the infantile period. MDC scores for the nDNA group ranged from probable (5-7) in three patients to definite in two patients.

Conclusion: PMD can mimic other disorders. Distinct features such as external ophthalmoplegia and the onset age may suggest the aetiologic genetic classes (nDNA versus mtDNA). Utilizing comprehensive genetic testing is crucial for achieving a definitive diagnosis.

Grants: Health Systems Research Institute (#66-122)
Conflict of Interest: None declared

P08.022.C Impaired nuclear glycogen metabolism affects liver homeostasis in Argininosuccinic aciduria

*Leandro Soria[1], **Alfonso Manuel D'Alessio**[1], Elena Polishchuk[1], Claudia Perna[1], Rossella De Cegli[1], Youngmok Lee[2], Julien Baruteau[3], Nicola Brunetti-Pierri[1;4]*

[1]Telethon Institute of Genetics and Medicine, Pozzuoli; [2]UCONN Health Center, Department of Pediatrics, Farmington, United States; [3]UCL Great Ormond Street Institute of Child Health, London, United Kingdom; [4]Federico II University of Naples, Department of Translational Medicine, Naples, Italy

Consortium: N/A

Background/Objectives: Glycogen is synthesized de novo in the nucleus, and nuclear glycogenolysis provides a carbon pool for histone acetylation. Abnormal nuclear glycogen catabolism results in epigenetic changes. We found hepatic nuclear glycogen storage in $Asl^{Neo/Neo}$ mice, a model of Argininosuccinic Aciduria (ASA), an inherited urea cycle disorder due to argininosuccinate lyase deficiency. In $Asl^{Neo/Neo}$ mice we investigated mechanisms of aberrant nuclear glycogen storage and its consequences on liver homeostasis.

Methods: Nuclear glycogen storage in hepatocytes was investigated by electron microscopy. Enzymes and acetylated histones were determined by Western Blot on nuclear purified fractions. Hepatic gene expression was performed by QuantSeq. Survival of $Asl^{Neo/Neo}$ mice was investigated following intravenous administrations of an adeno-associated virus (AAV) vector encoding glycogen phosphorylase (PYGL), the rate-limiting enzyme in glycogenolysis or after intraperitoneal injections of vorinostat, a pan-Histone Deacetylase (HDAC) inhibitor.

Results: In nuclei of $Asl^{Neo/Neo}$ mouse livers, quantification of glycogen metabolizing enzymes suggested increased glycogen synthesis and reduced glycogenolysis. These nuclei also showed reduced acetyl-CoA content, and decreased lysine acetylation of histones. Neither nuclear glycogen accumulation nor reduced histone acetylation were observed in murine models of defective glycogen metabolism (PYGL deficiency) or ureagenesis. Hepatic transcriptomic analysis revealed H3K9-dependent changes. Increased histone acetylation, by hepatocyte-specific PYGL overexpression or HDAC inhibition, improved $Asl^{Neo/Neo}$ mouse survival.

Conclusion: Defective hepatic nuclear glycogen mobilization and histone hypoacetylation result in global transcriptomic changes and reduced survival in ASA mice. These findings may have implications for development of improved therapies for liver disease in ASA and potentially, other liver disorders with aberrant nuclear glycogen storage.

Grants: Fondazione Telethon.
Conflict of Interest: None declared

P08.023.D A single-center genotype-phenotype correlation cohort study of Hyperphenylalaninemia patients: Genetic analysis as a deterministic tool for treatment consistency

Alp Peker[1], *sezin yakut uzunuer[2], Erdoğan Soyuçen[3], Özden Altıok Clark[1], Gülay Dal Demirelli[4], Belgin Akcan Paksoy[3], banu nur[5], ercan mihci[5]*

[1]Akdeniz University Medicine Faculty, Medical Genetics, Antalya, Türkyie; [2]Akdeniz University Medicine Faculty, Medical Biology, Antalya, Türkyie; [3]Akdeniz University Medicine Faculty, Pediatric Metabolic Diseases, Antalya, Türkyie; [4]Akdeniz University Medicine Faculty, Pediatric Metabolic Diseases, Antalya; [5]Akdeniz University Medicine Faculty, Pediatric Genetics, Antalya, Türkyie

Background/Objectives: Hyperphenylalaninemia (HPA, increased blood phenylalanine levels) refers to a disorder spectrum, with Phenylketonuria (PKU) being a widespread autosomal recessive form caused by *PAH* gene mutations. Phenotypical outcomes and symptom severity are significantly influenced by genetic variations in HPA. This study investigates the genotypical-phenotypical correlation in a cohort of HPA patients, aiming to evaluate treatment consistency concerning specific genotypes and demonstrate that genotyping can provide guidance for optimal treatment and prognosis.

Methods: Genomic DNA was obtained from 31 PKU, 2 Tetrahydrobiopterin (BH4) deficiency, and 1 Dihydropteridin Reductase deficiency patients for Next-Generation Sequencing of HPA-associated genes. Variants were analyzed using Sophia DDM program, dbSNP, ClinVar, Ensembl services, and ACMG 2015 criteria. Allelic phenotypic values consistent with enzyme functionality regarding literature data were assessed with patient anamnesis'.

Results: Carriers of two null variants (homozygous/compound heterozygous) were unresponsive to BH4 therapy, reflecting the literature. Notably, BH4 therapy was also inadequate for two PKU patients who were compound heterozygous carriers with one null variant, albeit other variants' assumed protein function (*PAH* [NM_000277.3] c.1289T>C, c.143T>C). However, *PAH* variants c.1169A>G, c.533A>G, c.1114A>T, c.898G>T demonstrated positive BH4 responses when compounding a null variant. Homozygous c.782G>A variant, linked to classical PKU, responded to BH4 therapy along with diet.

Conclusion: This study implies genetic testing is plausible in predicting pre-treatment BH4 testing outcomes, aiding in decision-making before patient evaluation as it also provides valuable guidance to metabolism specialists during treatment. With more HPA genotypes analyzed and clinical data published, genotyping will hold better deterministic position towards patient prognosis and therapeutic management.

Grants: None
Conflict of Interest: None declared

P08.024.A A biallelic variant, c.1139T>G in *DAP3* is associated with encephalopathy, liver failure, lactic acidosis, and early infantile death

Mayuri Yeole[1], *Namanpreet Kaur[1], Vivekananda Bhat[1], Ramesh Bhat[2], Anju Shukla[1], Radhakrishnan Periyasamy[1]*

[1]Department of Medical Genetics, Kasturba Medical College, Manipal, Manipal Academy of Higher Education, Manipal, India; [2]Department of Pediatrics, Kasturba Medical College, Manipal, Manipal Academy of Higher Education, Manipal, India

Background/Objectives: Death-associated protein (DAP3), also known as mitochondrial ribosomal protein S29 (MRPS29), is a novel GTP binding protein, with dual function in apoptosis and mitochondrial protein translation.

Methods: We ascertained a six-month-old female, born to a consanguineously married couple. Singleton exome sequencing (ES) followed by Sanger validation and segregation was performed in the family. In silico protein modelling and analysis was done using HOPE and I-Mutant.

Results: Proband presented with recurrent episodes of vomiting and lethargy associated with fever and encephalopathy at five months. She developed hepatosplenomegaly, acute liver failure followed by multi-organ dysfunction, and later succumbed at six

months. Blood gas analysis indicated severe metabolic acidosis, mildly elevated liver enzymes, and deranged coagulation profile. Liquid chromatography with tandem mass spectrometry revealed malonyl-CoA decarboxylase and short-chain L-3-hydroxyacyl-CoA dehydrogenase deficiency. There is a history of deceased elder sibling with similar complaints at 11 months of age. On singleton ES, we identified a homozygous variant, c.1139T>G p.(Leu380Arg) in *DAP3* (NM_004632.4) in the proband. This variant is absent in population database gnomAD and in-house database of 3106 exomes. In silico prediction tools (REVEL, CADD, MutationTaster) are consistent in predicting the variant to be damaging to the DAP3 function. In silico mutagenesis predicted that the Arg380 mutant residue would affect the intramolecular interactions in the mutant protein, thus leading to conformational changes and affecting its interactions with other proteins.

Conclusion: With this report, we propose a novel disease-gene association in *DAP3* leading to multi-organ dysfunction with early infantile death.

Grants: IA/CRC/20/1/600002(DBT/Wellcome Trust Indian Alliance)

Conflict of Interest: None declared

P08.026.C Three Canavan disease patients with a single heterozygous pathogenic variant in ASPA gene – where is the missing part of the puzzle ?

Silvia Andonova[1;2], *Mariya Ivanova*[1], *Tihomir Todorov*[2], *Iliyana Pacheva*[3], *Dimitar Stamatov*[4], *Viktoriya Yordanova*[1], *Irena Bradinova*[1], *Alexey Savov*[1], *Albena Todorova*[2;5]

[1]*National Genetic Laboratory, UHOG "Maichin dom", Sofia, Bulgaria;* [2]*GMDL Genica, Sofia, Bulgaria;* [3]*Medical University-Plovdiv, Department of Pediatrics and Medical Genetics, Plovdiv, Bulgaria;* [4]*University Pediatrics Hospital, Department of Pediatric Neurology, Sofia, Bulgaria;* [5]*Medical University-Sofia, Department of Biochemistry, Sofia, Bulgaria*

Background/Objectives: Canavan disease is a rare condition due to an aspartoacylase deficiency that leads to a lethal infantile neurogenerative disease from the Leukodystrophies group. This autosomal recessive disorder is caused by pathogenic variants in *ASPA* gene, encoding the enzyme aspartoacylase. The disease is manifested in the early infancy with elevated levels of the substrate N-acetylaspartic acid (NAA) and neurologic findings including hypotonia, blindness, megalocephaly, etc. due to demyelination and spongy degeneration of the brain. Here we describe three infant patients from non-consanguineous families with clinical presentation and specific metabolic profile of Canavan disease.

Methods: Sanger sequencing of the *ASPA* gene and MLPA analysis were performed; the NAA levels were analyzed by GC/MS (gas-chromatography/mass-spectrometry).

Results: *ASPA* gene sequencing detected a different single heterozygous pathogenic variant in each patient - c.740T>C/p.Leu247Pro, c.863A>G/p.Tyr288Cys, and c.876_879del/p.Glu293-LeufsTer8, respectively. Segregation analysis revealed that all variants were inherited from a parent. Subsequently, MLPA analysis did not detect deletions or duplications in the targeted *ASPA* gene region.

Conclusion: Performing genetic analysis with advanced technologies (WES/WGS) would be beneficial for genetic profiling and revealing the precise molecular profile of Canavan disease in the presented Bulgarian patients. This could allow inclusion of these patients in clinical trials for a possible gene therapy with potential benefits.

Grants: The present study was financially supported by the Medical University Sofia (Grant D-175/2023).

Conflict of Interest: None declared

P08.027.D Molecular genetic analysis of positive cases of neonatal screening for galactosemia type I in Ukraine

Nataliia Olhovich[1;2], *Nataliia Mytsyk*[1;2], *Oksana Barvinska*[1], *Svetlana Kormoz*[1], *Iryna Hrehul*[1], *Yuliia Zhyvytsia*[1], *Olena Kutsyk*[1], *Olexandr Buryak*[2], *Volodymyr Olkhovych*[2], *Nataliia Samonenko*[3], *Marina Patsora*[3], *Mariia Haidei*[3], *Nataliia Gorovenko*[2;4], *Tetiana Ivanova*[3]

[1]*National Children Hospital OHMATDYT, Laboratory of Medical Genetics, Kyiv;* [2]*Institute of Genetic and Regenerative Medicine, Department of Genetic Diagnostics, Kyiv;* [3]*National Children Hospital OHMATDYT, Center of Orphan Diseases and Gene Therapy, Kyiv;* [4]*National University of Health named P.L. Shupyka, Department of Medical and Laboratory Genetics, Kyiv*

Actuality: Galactosemia type I (GALAC1, OMIM#230400) is an autosomal recessive disorder caused by pathogenic variants of *GALT* with frequency of 1:16,000-100,000 in Europe. The neonatal screening contributes to early detection and dietary treatment, which could prevent the acute manifestation and reduce the lethality.

Aim: To assess the molecular features of positive cases of neonatal screening for GALAC1 in Ukraine during the first year of its implementation.

Methods: The GALT activity in DBS by fluorometric method, NGS and Sanger sequencing.

Results and discussion: Neonatal screening for GALAC1 started in Ukraine in October 2022. During the first year of expanded screening, 145,693 newborns were examined, in 19 patients (0.013%) decrease in the activity of the GALT was detected (cut off 1.7 U/dL). Molecular genetic analysis of the *GALT* in patients with positive neonatal screening revealed only one patient with GALAC1 (5% of all positive cases) (p.Q188R/p.H114P). The rest of the patients had galactosemia Duarte (n = 13, 68%) or were heterozygous carrier of one pathogenic variant in *GALT* (n = 5, 26%). In patients with Duarte galactosemia, the genotype p.Q188R/p.N314D was detected in 54% cases (7/13), and another patients had a combination of the p.N314D variant with such variants as p.M142K (n = 2), p.K285N (n = 1), p.Y209D (n = 1), p.N314D (n = 2).

Conclusion: The frequency of confirmed cases of GALAC1 is 1:145,693 newborns, which is significantly lower than the European. These are preliminary data, a larger number of examined newborns is needed. The high frequency of the Duarte variant among positive cases was revealed compared to Europe.

Conflict of Interest: None declared

P08.028.A Novel treatments for primary Coenzyme Q10 deficiency

Cristina Cerqua[1], *Giulia Toffanin*[2], *Valeria Balmaceda*[3], *Cristina Calderan*[1], *Raffaele Cerutti*[3], *Carlo Viscomi*[3;4], *Eva Trevisson*[1;2]

[1]*Istituto di Ricerca Pediatrica IRP Città della Speranza, Padova;* [2], *Department of Women's and Children's Health, University of Padova, Padova, Italy;* [3]*Venetian Institute of Molecular Medicine, Padova, Italy;* [4], *Department of Biomedical Sciences, University of Padova, Padova, Italy*

Background/Objectives: Coenzyme Q (CoQ) is a mitochondrial electron transporter and one of the main cellular antioxidants. Mutations in human *COQ* genes, involved in CoQ biosynthesis, cause primary CoQ deficiency, associated with variable clinical phenotypes. Since treatment with CoQ is not always efficient, we

aimed to test two novel therapeutic strategies using a model of CoQ deficiency, the *Pdss2*$^{kd/kd}$ mouse, which harbors a mutation in *Pdss2* and develops a renal phenotype similar to that of most *PDSS2* patients, recognizable by increased proteinuria, decreased survival and glomeruli destruction.

Methods: First, we aimed to test the effects of gene replacement, by injecting in *Pdss2*$^{kd/kd}$ mice an adeno-associated vector (AAV) containing murine *Pdss2*. Then we decided to supplement mice with CoQ ring analogues, that represented a valid alternative to CoQ treatment in some CoQ models.

Results: In our hands, the gene replacement was ineffective in the mouse strain CBA/H, in which the *Pdss2* mutation occurred for the first time. After injecting the same vector at the same dose in a different mouse strain, the C57BL/6, we could observe a significant transduction increase in all tissues tested, compared to the CBA/H strain, demonstrating that the mouse genetic background influences the viral transduction efficiency. For the supplementation protocol, we studied mice survival, proteinuria and kidney histology.

Conclusion: Treatment with 2,4-dihydroxybenzoic acid, a CoQ analog, significantly prolonged the mice survival, reduced the proteinuria and improved the glomerular appearance. These results will be fundamental to encourage the development of analogous therapies for other *COQ* gene defects.

Grants: Fondazione Cariparo – Bando Ricerca Pediatrica 2020-2024

Conflict of Interest: None declared

P08.029.B Fine mapping and functional investigation of the novel type 2 diabetes (T2D) susceptibility locus *ACAD11/NPHP3*.

Hatice Bahar Sahin[1], *Hannah Maude*[1], *Bethany Quinton*[2], *David Michael*[1], *winston lau*[2], *Inês Cebola*[1], *nikolas maniatis*[2], **Toby Andrew**[1]

[1]*Imperial College, Metabolism, Digestion and Reproduction, London, United Kingdom;* [2]*UCL, Genetics, Evolution & Environment, London, United Kingdom*

Background/Objectives: We previously reported a replicated association between the *ACAD11/NPHP3* locus and type 2 diabetes (T2D) using population-specific genetic maps in a genome-wide study. Precise genetic co-localisation with expression quantitative trait loci (eQTL) implicated several target genes including *ACAD11*, which encodes the mitochondrial acyl-CoA dehydrogenase enzyme participating in fatty acid beta-oxidation. Here, we test the hypothesis that T2D-risk variants confer risk of disease by dysregulating the expression levels of *ACAD11* and neighbouring *cis*-genes in T2D-relevant tissues using in silico and functional experiments.

Methods: Chromatin activity datasets were used to prioritise non-coding variants that might disrupt enhancer activity. These were further investigated by transcription factor motif disruption analysis. Targeted sequencing of independent T2D cases and controls was used to identify candidate functional variants. Evidence of enhancer disruption by a candidate variant (rs73000573) was validated using a dual-luciferase reporter assay. To assess potential target *cis*-genes, CRISPR-mediated activation of the enhancer hosting the rs73000573 variant was undertaken in Hep3B cells, followed by RT-qPCR analysis of predicted *cis*-genes.

Results: *ACAD11* shows evidence of rare coding-variant enrichment. rs73000573 (MAF> = 0.01) disrupts TF binding at a validated enhancer in *ACAD11* intron 1, which in turn regulates *ACAD11* expression in Hep3B cells. GTEx data indicates rs73000573 acts as an eQTL for *ACAD11* and *NPHP3*.

Conclusion: The *ACAD11/NPHP3* locus contains multiple T2D-associated variants of interest, with a rare haplotype containing at least one functional variant that alters *ACAD11* expression and splicing, implicating altered mitochondrial fatty acid oxidation in the aetiology of T2D.

Grants: Wellcome Trust (209106/Z/17/Z) and the Medical Research Council (MR/X011070/1).

Conflict of Interest: Hatice Bahar Sahin Full time, Hannah Maude Full time, Bethany Quinton: None declared, David Michael: None declared, winston lau Full time, Inês Cebola Full time, nikolas maniatis Wellcome Trust (209106/Z/17/Z), Full time, Toby Andrew Medical Research Council (MR/X011070/1), Full time

P08.030.C Rare mtDNA variants in an Italian cohort of patients with suspected mitochondrial diseases.

Alberto Pietro Pasti[1], *Claudio Fiorini*[1], *Danara Ormanbekova*[1], *Eleonora Pizzi*[1], *Chiara La Morgia*[1;2], *Maria Lucia Valentina*[1;2], *Valerio Carelli*[1;2], *Leonardo Caporali*[1;2]

[1]*IRCCS Istituto delle Scienze Neurologiche di Bologna, Bologna, Italy;* [2]*Alma Mater Studiorum - Università di Bologna, Dipartimento di Scienze Biomediche e Neuromotorie (DIBINEM), Bologna, Italy*

Background/Objectives: Mitochondrial disorders comprise a group of heterogeneous rare pathologies, with a cumulative minimum prevalence of greater than 1 in 5000 in adults. For suspected mitochondrial primary diseases' molecular diagnosis, the analysis of mitochondrial DNA (mtDNA) is always suggested to identify both pathogenic SNVs and deletions.

Methods: As a reference center for the diagnosis of mitochondrial diseases in Northern Italy, the Institute of Neurological Sciences of Bologna between 2014 and 2023 has collected a cohort of 532 patients with suspected mitochondrial pathology which underwent mtDNA sequencing. We performed sequencing through a targeted NGS approach on DNA from blood and skeletal muscle tissues. The bioinformatic analysis followed the Mitoverse pipeline, with manual curation of predicted damaging variants.

Results: This cohort included cases of suspected optic neuropathy (n = 166, 31.2%), mitochondrial encephalomyopathy (n = 152, 28.6%), mitochondrial myopathy (n = 212, 39.9%), cardiomyopathy (n = 2, 0.3%). Although, the sampled cohort is depleted from the most common pathogenic variants (m.3243A>G, m.3460G>A, m.11778G>A, m.14484T>C), that are usually screened before the request of mtDNA testing, we solved 13.2% of the cases (n = 70). The most resolved category are optic neuropathies, with 16.2% of yield, while encephalomyopathies and myopathies resulted in 13.8% and 9.4% of solved cases, respectively. Notably, most of the pathogenic variants in myopathies involve the mitochondrial tRNA genes (60% of cases), and most of the variants causing optic atrophy are found in respiratory complex I subunits (81% of cases).

Conclusion: We expand the list of candidate pathogenic variants, to be confirmed through independent case reports or in vitro validation.

Grant: Italian Ministry of Health, Ricerca Corrente.

Conflict of Interest: None declared

P08.031.D Diagnosis of mtDNA disorders by exome and genome sequencing

Ana beatriz Hinojosa Amaya[1], *Ingrid Bader*[1], *Hans A. Mayr*[2], *Peter Freisinger*[3], *Stefanie Beck-Wödl*[1], *Marc Sturm*[1], *Susana Peralta*[1], *Tobias Haack*[1]

[1]*University of Tübingen, Institute of Medical Genetics and Applied Genomics, Tübingen, Germany;* [2]*Paracelsus Medical University,*

University Children's Hospital, Salzburger Landeskliniken (SALK), Salzburg, Austria; [3]Klinikum am Steinenberg, Children's Hospital Reutlingen, Reutlingen, Germany

Background/Objectives: Mitochondriopathies are clinically and genetically heterogenic disorders. Thanks to Next-Generation-Sequencing their diagnosis have been improved and accelerated. Nevertheless, in cases of mtDNA variations the diagnosis is still a challenge due to variable heteroplasmy level in different tissues. Our objective is to describe the cases diagnosed with pathogenic mtDNA variants in our institute by exome (ES) and genome sequencing (GS) and compare mtDNA depth coverage in both techniques.

Methods: We selected from our internal database cases with class 4 and 5 mtDNA variants in blood samples referred to our institute for a diagnostic ES or GS between 2016 and 2023. We calculated mean read depths and standard deviations (±SD) of mtDNA in exome (ES), short read (srGS) and long read genome sequencing (lrGS).

Results: We identified a total of 52 patients. In 35 cases (67%) variants were detected by ES and 17 (33%) by GS. The most frequent affected gene was *MT-ATP6* (62%). In only 35% a mitochondrial disorder had been suspected by the clinician. Most patients presented with neurological/neurodevelopmental phenotypes (61%). Mean read depth coverage values in mtDNA was as follows: ES: 98.41 ± 131.98, srGS: 1719.7 ± 751.38 and lrGS: 1806,36 ± 1189.

Conclusion: Our findings show the importance of including mtDNA analysis in patients with neurological symptoms. GS, particularly lrGS, showed a deeper and more uniform coverage across the mtDNA in comparison to ES. These findings together with the advantage of detecting copy number variants and also structural variants (particularly by lrGS) makes GS the preferred first-line diagnostic test for inherited mitochondrial disorders.

Conflict of Interest: None declared

P08.032.A Study of the frequent hypomorphic variant c.3045G>A in the *ATP7B* gene

Mikhail Skoblov[1], *Igor Bychkov[2], Nikita Vorobyov[1], Daria Akimova[1], Vyacheslav Tabakov[3], Alexandra Filatova[1]*

[1]Research Centre for Medical Genetics, Laboratory of Functional Genomics, Russian Federation; [2]Research Centre for Medical Genetics, Laboratory of hereditary metabolic diseases, Russian Federation; [3]Research Centre for Medical Genetics, Head of the Center for Collective Use "Biobank", Russian Federation

Background: Wilson's disease is a hereditary disorder that involves anomalous copper metabolism. Gene panel sequencing is commonly used to diagnose Wilson's disease but has an efficacy of only around 70%. RNA analysis offers an alternative method, detecting changes in RNA structure and expression levels caused by intron variants influencing splicing and regulatory variants affecting expression levels.

Methods: Total RNA was extracted from activated lymphocytes and primary cultured fibroblasts of patients using Trizol. An RT-PCR system with 8 loci was developed to detect pathogenic variants in the ATP7B gene. PCR products were analyzed by NGS on an Ion Torrent S5. Minigene and MPRA analyses characterized the hylomorphic variant. Splicing correction was performed using U7 snRNA in HEK293T cells.

Results: We performed RNA analysis on Wilson's disease patients to find the second pathogenic variant. We observed the partial skipping of 13 exon caused by the hypomorphic variant c.3045G>A which was missed during the initial panel sequencing due to its high population frequency. To investigate the molecular basis of this hypomorphic effect, the minigene with 13 exon of ATP7B was created. Analysis of the c.3045G>A variant confirmed splicing abnormalities. MPRA analyses on enhancer splicing sites in the minigene revealed other variants also caused exon skipping. We designed several U7 snRNA to compensate effect of hypomorphic variant thereby promoting the correct splicing of exon 13.

Conclusion: The investigation of frequent hypomorphic variants remains underexplored. Understanding their mechanisms not only enhances our comprehension of their actions but also leads to development of future treatments.

Conflict of Interest: None declared

P08.033.B Microbiome analysis of total blood RNAseq: Insights from a prediabetic population

Claudia Solari[1], *Nelba Perez[2], Maria Soledad Rodriguez Varela[1;2], Razan Hijazi[1], Alan Möbbs[1], Florencia De Lillo[1], Estefania Mancini[1], Ignacio Berdiñas[1], Patricio Rickert[1], Maria Olivera[1], Silvina Waldman[2], Rosana Poggio[1], Alejandro La Greca[1], Carlos Luzzani[1], Santiago Miriuka[1;2]*

[1]MultiplAI Health, Cambridge, United Kingdom; [2]FLENI, Buenos Aires, Argentina

Background/Objectives: Human microbiome has emerged as a key player in health and disease. Prediabetes represents a critical juncture where early intervention may mitigate progression to overt diabetes and its associated cardiovascular complications.

Our objective is to characterize the microbiome at the phylum and genus levels and assess their association with clinical features.

Methods: Blood samples from 336 subjects were collected at FLENI foundation, Buenos Aires, Argentina. Total RNA was isolated, purified, and deep sequenced (~100m reads). The RNAseq data was aligned with STAR. Unmapped reads were classified with Kraken2 software. Prediabetes was defined as glycemia of 110 to 125 mg/dl and/or glycated HbA1 between 6 and 6.4% without any diabetes treatment.

Results: The RNAseq data showed an extensive amount of microbiome reads (0.07%). In our cohort, the most represented phyla were Proteobacteria, Actinomycetota, Bacillota, Bacteroidota, and Cyanobacteria, and at a genus level, we found Cutibacterium, Moraxella, Acinetobacter, Staphylococcus, Streptococcus, among others. While no statistical differences emerged at the phylum level in the preDBT versus normal populations, genus-level analysis revealed elevated Staphylococcus reads in the preDBT group (p-value = 0.04257). Prediabetic individuals exhibited an inverse correlation between HbA1C levels and Bacillota phylum reads (R^2: 0.87) and Streptococcus genus reads (R^2: 0.73). No relationship was found with c-Reactive protein levels.

Conclusion: We observed an association between blood microbiome and metabolic parameters. These findings hint at a potential role in contributing to cardiometabolic risk, providing valuable insights that can inform the development of targeted preventive strategies.

Grants:

Conflict of Interest: Claudia Solari MultiplAI Health, MultiplAI Health, Nelba Perez MultiplAI Health, Maria Soledad Rodriguez Varela MultiplAI Health, Razan Hijazi MultiplAI Health, MultiplAI Health, Alan Möbbs MultiplAI Health, MultiplAI Health, Florencia De Lillo MultiplAI Health, MultiplAI Health, Estefania Mancini MultiplAI Health, MultiplAI Health, Ignacio Berdiñas MultiplAI Health, MultiplAI Health, Patricio Rickert MultiplAI Health, MultiplAI Health, Maria Olivera MultiplAI Health, MultiplAI Health, Silvina Waldman: None declared, Rosana Poggio MultiplAI Health, MultiplAI Health, Alejandro La Greca MultiplAI Health, MultiplAI Health,

Carlos Luzzani MultiplAI Health, MultiplAI Health, Santiago Miriuka MultiplAI Health, MultiplAI Health

P08.034.C Deoxyguanosine kinase deficiency: natural history and liver transplant outcome.

Eleonora Manzoni[1], Sara Carli[1], Pauline Gaignard[2], Lea Dewi Schlieben[3], Michio Hirano[4], Dario Ronchi[5], Emmanuel Gonzalès[6], Masaru Shimura[7], Kei Murayama[7], Okazaki Yasushi[8], Ivo Baric[9], Danijela Petkoviv[9], Daniela Karall[10], Hans A. Mayr[11], Diego Martinelli[12], Chiara La Morgia[1], Guido Primiano[13], René Santer[14], Serenella Servidei[15], Celine Bris[16], Aline Cano[17], Francesca Furlan[18], serena gasperini[19], Nolwenn Laborde[20], Costanza Lamperti[21], Dominic Lenz[22], Michelangelo Mancuso[23], Vincenzo Montano[23], Francesca Menni[18], Olimpia Musumeci[24], Victoria nesbtt[25], Elena Procopio[26], Cécile Rouzier[27], Chiara Ticci[26], Duccio Maria Cordelli[1], Valerio Carelli[1], Vincent Procaccio[16], Holger Prokisch[3], **Caterina Garone**[1]

[1]*Alma Mater Studiorum - Università di Bologna, Bologna, Italy;* [2]*Bicetre Hospital, Le Kremlin-Bicêtre, France;* [3]*Technical University of Munich, München, Germany;* [4]*Columbia University, New York, United States;* [5]*University of Milan, Milano, Italy;* [6]*University of Paris-Saclay, Gif-sur-Yvette, France;* [7]*Chiba Children's Hospital, Chiba, Japan;* [8]*Juntendo University Hospital, Bunkyo City, Japan;* [9]*University Hospital Centre Zagreb, Zagreb, Croatia;* [10]*Medizinische Universität Innsbruck, Innsbruck, Austria;* [11]*Paracelsus Medical University, Salzburg, Austria;* [12]*Ospedale Bambin Gesù, Ospedale Bambin Gesù, Italy;* [13]*Università Cattolica del Sacro Cuore, Roma, Italy;* [14]*University Medical Center Hamburg-Eppendorf, Hamburg, Germany;* [15]*Agostino Gemelli University Policlinic, Rome, Italy;* [16]*University of Angers, Angers, France;* [17]*Marseille University Hospital Timone, Marseille, France;* [18]*Mangiagalli Clinic IRCCS Cà Granda Foundation Ospedale Maggiore Policlinico, Milano, Italy;* [19]*Fondazione IRCCS San Gerardo dei Tintori, Monza, Italy;* [20]*Hospital Center University De Toulouse, Toulouse, France;* [21]*Istituto Neurologico "Carlo Besta" | Fondazione IRCCS, Milano, Italy;* [22]*Heidelberg University, Heidelberg, Germany;* [23]*University of Pisa, Pisa, Italy;* [24]*university of messina, Scilla, Italy;* [25]*Oxford Children's Hospital, Oxford, United Kingdom;* [26]*University Hospital Meyer, Firenze, Italy;* [27]*University of Nice Sophia Antipolis - Campus Trotabas, Nice, France*

Background/Objectives: Deoxyguanosine kinase (dGK) deficiency is an autosomal recessive disorder presenting with early-onset of liver failure with/without neurological involvement and a clinical course rapidly progressive to death. The lack of enzyme activity causes mitochondrial deoxynucleotides pool unbalance leading to quantitative and qualitative impairment of mtDNA synthesis and multiple OXPHOS defect. Here we present an international, multicentre retrospective natural history study.

Methods: Clinical, biochemical, metabolic and molecular genetics data for genetically confirmed patients with dGk deficiency were collected. A systematic literature review from January 2001 to June 2023 was also conducted.

Results: A cohort of 202 patients, 36 unreported, and 166 from the systematic literature review, were analyzed. Patients had a neonatal onset (≤ 1 month) in 55.7% of cases, infantile (>1 month and ≤ 1 year) in 32.3%, pediatric (>1 year and ≤18 years) in 2.5%, and adult (>18 years) in 9.5%. Kaplan-Meier analysis showed a statistically significant difference in the survival rate among the age-at-onset subgroups ($p < 0.0001$) with the highest mortality for the neonatal group.

We defined four different clinical subtypes: hepatocerebral (58.8%), isolated hepatopathy (21.9%), hepatomyoencephalopathy (9.6%), and isolated myopathy (9.6%). Muscle involvement was predominant in adult-onset cases whereas liver dysfunction causes morbidity and mortality in early-onset patients with a median survival of less than 1 year. Liver transplant (LTx) significantly modified the survival rate in 26 treated patients when compared with untreated.

Conclusion: In conclusion, dGk deficiency is a disease spectrum with a prevalent liver and brain tissue-specificity in neonatal and infantile-onset patients and muscle tissue-specificity in adult-onset cases.

Conflict of Interest: None declared

P08.035.D Epimutation in the MMACHC promoter due to PRDX1 variant causing epi-cblC methylmalonyl aciduria and homocystinuria

Martina Skopkova[1], *Pavlina Kabelikova[2], Andrea Andresova[1], Silvia Dallemule[3], Robert Petrovic[3], Katarina Brennerova[4], Daniela Gasperikova[1]*

[1]*Biomedical Research Center SAS, Institute of Experimental Endocrinology, Bratislava, Slovakia;* [2]*Biomedical Research Center SAS, Institute of Experimental Oncology, Bratislava, Slovakia;* [3]*Medical Faculty University Comenius and University Hospital, Institute of Medical Biology, Genetics, and Clinical Genetics, Bratislava, Slovakia;* [4]*Medical Faculty of Comenius University and National Institute of Children's Diseases, Department of Paediatrics, Bratislava, Slovakia*

Background/Objectives: Methylmalonic aciduria (MMA) is a group of autosomal recessive disorders resulting from impaired intracellular metabolism of cobalamin. Depending on the point in the metabolic pathway that is impaired, MMA can be accompanied by homocystinuria. That is also the case of mutations in the *MMACHC* gene causing combined MMA and homocystinuria type cblC. Besides the biallelic mutations in the *MMACHC* gene, CblC type MMA can occur due to specific mutations in the *PRDX1* gene located downstream from *MMACHC* in the opposite direction. The *PRDX1* mutations giving rise to an aberrant transcript overlapping the promoter region of the *MMACHC* gene cause its methylation and subsequent downregulation of expression (so-called epi-CblC).

Methods: The DNA of a patient with biochemically diagnosed MMA and homocystinuria was analysed by WES, methylation of the *MMACHC* promoter was assessed using methylation-specific PCR and pyrosequencing of the bisulphite-treated DNA.

Results: We identified a heterozygous known pathogenic variant c.271dup in the *MMACHC* gene in combination with a novel stop-to-stop variant c.599G>A in the *PRDX1* gene. Pathogenicity of this variant was supported by detection of increased methylation of the *MMACHC* promoter in the patient's blood and fibroblast DNA. We sequenced the aberrant *PRDX1* transcript reaching the *MMACHC* promoter and identified usage of a cryptic splice site in the last exon of the *PRDX1* gene.

Conclusion: We confirmed pathogenicity of a novel otherwise unsuspicious stop-to-stop variant in the *PRDX1* causing methylation of *MMACHC* gene promoter. The patient was thus diagnosed with epiCblC type of MMA.

Grants: APVV 22-0257, VEGA 1/0659/22

Conflict of Interest: None declared

P08.036.A Exploring the genetic interplay: oxidative stress and body mass index in the Spanish population

Francisco Lara-Hernández[1], *Elena Quiroz[1], Rebeca Melero[1], Celeste Moya-Valera[1], Laisa Briongos-Figuero[2], Juan Carlos Martin-Escudero[2;3], Guillermo Ayala[4], Javier Chaves[1;5], Ana Barbara Garcia-Garcia[1;5]*

[1]*INCLIVA Biomedical Research Institute, Genomics and Diabetes Unit, Valencia, Spain;* [2]*Rio Hortega University Hospital, Internal Medicine*

Service, Valladolid, Spain; [3]University of Valladolid, Department of Medicine, Valladolid, Spain; [4]University of Valencia, Department of Statistics and Operation Research, Valencia, Spain; [5]CIBERDEM, ISCIII, Madrid, Spain

Background/Objectives: Oxidative stress (OS) and obesity are interconnected conditions that play pivotal roles in predisponding individuals to various diseases. Our objective was to investigate the relationship between metabolism-related genes and OS and how they might influence the onset of obesity in general Spanish population.

Methods: SNPLex methodology was used to analyse about 900 SNPs from 272 candidate genes related to OS in 1502 subjects from the Hortega study. GSSG/GSH ratio, MDA and 8-oxo-dG were used as OS biomarkers. Linear regression models were used to calculate the interaction between OS parameters and selected SNPs in relation to BMI.

Results: Significant interaction were found between 60 SNPs and OS levels in relation to BMI. Adjusted p-values between 10^{-122} and 10^{-4}) was present in rs2694847 (*SP1*), rs11574777 (*IL6ST*), rs12665231 (*NOX3*), rs12524299 (*NOX3*), rs6816673 (*SLC39A8*) and rs198358 (*NPPA*) for GSSG/GSH ratio, rs35411892 (*SLC39A8*), rs697221 (*DDIT3*) and rs1800629 (*LTA*) for 8-oxo-dG and rs697221 (*DDIT3*) for MDA. These genes are involved in biological processes such as inflammation, endoplasmic reticulum stress, blood pressure and OS levels.

Conclusion: We have found an important effect of OS levels on genetic backgroung resulting in modulation of BMI by specific alleles. These data support the relationship between OS levels and BMI.

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Conflict of Interest: None declared

P08.037.B Elucidating mechanisms of mitochondrial dysfunction associated with BTRR complex deficiency

Marianne Gaubert[1], *Ipek Ilgin Gönenc[1], Halima Alachram[1], Kimia Mirzakhani[1], Lukas Cyganek[2;3;4], Gökhan Yigit[1;3], Bernd Wollnik[1;2;3]*

[1]University Medical Center Göttingen, Institute for Human Genetics, Göttingen, Germany; [2]University of Göttingen, Cluster of Excellence "Multiscale Bioimaging: From Molecular Machines to Networks of Excitable Cells" (MBExC), Göttingen, Germany; [3]German Center for Cardiovascular Research (DZHK), Partner site Göttingen, Göttingen, Germany; [4]University Medical Center Göttingen, Stem Cell Unit, Clinic for Cardiology and Pneumology, Göttingen, Germany

Background/Objectives: Bloom syndrome (BS) is an autosomal recessive disorder clinically characterized by primary microcephaly, short stature, immunodeficiency, and cancer predisposition, caused by biallelic mutations in genes encoding the members of the BTRR complex, i. e. *BLM, TOP3A, RMI1, and RMI2*. The topoisomerase TOP3A is crucial for relieving DNA torsional strain, highlighting its importance in genome maintenance. A mitochondrial isoform of TOP3A also exists, involved in the decatenation of mitochondrial DNA (mtDNA) molecules. Intriguingly, a subset of *TOP3A*-associated patients presented unusual phenotypic traits including dilated cardiomyopathy and clinical features of mitochondrial dysfunction. In the current study, we explore the mechanisms of BTRR-complex-related mitochondrial dysfunction in cardiomyopathies.

Methods: We employed BTRR complex knockout (KO) induced pluripotent stem cells to generate cardiomyocytes (iPSC-CMs). We assessed mitochondrial function through live cell imaging and Seahorse-based bioenergetics analysis. Furthermore, we established a high-throughput mtDNA ultra-deep sequencing strategy (30.000 x coverage), to explore mtDNA mutational signatures and evaluate the role of BTRR complex members in mitochondrial genome maintenance and stability.

Results: Our results demonstrate decreased mitochondrial membrane potential and respiration rates in TOP3A-KO iPSC-CMs, but also in RMI1-KO iPSC-CMs, previously thought to be devoid of function in mitochondria. Our mtDNA sequencing approach provides exceptional depth of coverage, enabling precise detection of rare low-frequency mtDNA variants and accurate estimation of heteroplasmy levels.

Conclusion: This study unveils new gene candidates in mtDNA maintenance and mtDNA mutational signatures linked to inherited forms of cardiomyopathy and offers insights into the occurrence and distribution of mtDNA mutations

Grants: Cluster of Excellence MBExC, Göttingen, Germany

Conflict of Interest: None declared

P08.038.C Is there a relationship between global 5-methylcytosine and 5-hydroxymethylcytosine levels and metabolic syndrome and its clinical findings?

Sacide Pehlivan[1], *Naci Şenkal[2;3], Yasemin Oyacı[1;3], Ebru Teberik[2], Mustafa Pehlivan[4], Tufan Tükek[2], Alpay Medetalibeyoğlu[2]*

[1]Istanbul Faculty of Medicine, Istanbul University, Department of Medical Biology, Istanbul, Türkyie; [2]Istanbul Faculty of Medicine, Istanbul University, Department of Internal Medicine, Istanbul, Türkyie; [3]Institute of Graduate Studies in Health Sciences, Istanbul University, Istanbul, Türkyie; [4]Basaksehir Cam and Sakura City Hospital, Department of Hematology, Istanbul, Türkyie

Background/Objectives: Metabolic syndrome (MetS) encompasses a set of metabolic features associated with an increased risk of cardiovascular disease and type 2 diabetes. One of the primary epigenetic processes controlling gene expression in eukaryotes is DNA methylation. In this study, we aimed to evaluate the relationship of 5-methylcytosine (5-mC) and 5-hydroxymethylcytosine (5-hmC) levels with MetS and its clinical findings.

Methods: A total of 303 people, 200 MetS patients, and 103 healthy controls were included in the study. The 5-mC and 5-hmC levels were measured quantitatively by the ELISA method using 100 ng of input genomic DNA sample. The relationship of participant characteristics and clinical variables with both 5-mC and 5-hmC levels were evaluated by performing median comparisons and correlation analyses.

Results: The 5-mC levels and 5-mC/5-hmC ratios were significantly increased in the patient group compared to the control group ($p = 0.001$). The 5-mC levels showed a negative correlation with HbA1c, creatinine, and Hb in the patients. Also, a negative correlation was detected in 5-hmC levels with triglyceride, fasting blood sugar, HbA1c, creatinine and a positive correlation was detected with HDL in the patient group.

Conclusion: Our results suggest that 5-mC levels and 5-mC/5-hmC ratios may play a role in the development of MetS. The fact that 5-mC and 5-hmC levels are associated with clinical findings shows that the global methylation profile is effective in the course of the disease. This could be a promising development for the treatment of MetS.

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Conflict of Interest: None declared

P08.039.D A digenic mechanism for Leigh syndrome on the background of the primary m.11778G>A LHON variant

Beryll Blickhaeuser[1;2], Sarah Stenton[3;4], Lea Dewi Schlieben[1;5], Thomas Klopstock[2], **Holger Prokisch**[1;5]

[1]Institute of Neurogenomics, Computational Health Center, Helmholtz Zentrum München, Germany; [2]Friedrich-Baur-Institute, Department of Neurology, LMU University Hospital, Ludwig-Maximilians-Universität München, Germany; [3]Division of Genetics and Genomics, Boston Children's Hospital, Boston, MA, United States; [4]Program in Medical and Population Genetics, Broad Institute of MIT and Harvard, Cambridge, MA, United States; [5]School of Medicine, Institute of Human Genetics, Klinikum rechts der Isar, Technical University of Munich, Germany

Consortium: GENOMIT

Background/Objectives: Leigh syndrome spectrum (LSS) is the most frequent clinical manifestation of pediatric mitochondrial disorders characterized by neurodegeneration and bilateral symmetrical lesions in the basal ganglia and/or brainstem. Defects in more than 100 genes spanning the nuclear and mitochondrial genome have been associated with LSS, most commonly related to mitochondrial complex I (CI) dysfunction. Rarely, LSS has been described in association with primary Leber hereditary optic neuropathy (LHON) variants of the mtDNA encoding CI subunits (m.3460G>A in MT-ND1, m.11778G>A in MT-ND4, and m.14484T>C in MT-ND6). The underlying mechanism of how these variants manifest as LSS, a severe neurodegenerative disease, instead of the LHON phenotype of isolated optic neuropathy, is unknown.

Methods: We performed exome sequencing in six patients with LSS carrying primary mtDNA LHON variants. Rare pathogenic nuclear variants were analysed for potential digenic interactions.

Results: In comparison to healthy controls, we found a significant enrichment of heterozygous pathogenic variants in CI subunits in (all) five LSS patients with the m.11778G>A variant, while none of the mtDNA LHON only patients carried a pathogenic variant in CI subunits.

Conclusions: The genetic findings support our hypothesis that the unusually severe clinical manifestation of LSS in patients harbouring the primary m.11778G>A (MT-ND4) LHON variant is caused by a synergistic digenic impairment of CI. This is the second example of digenic inheritance in which the combination of mutations in CI genes changed the phenotype from LHON to LSS.

Conflict of Interest: None declared

P08.040.A *PAH* genotype database: exploring population structure and personalized treatment recommendations for phenylketonuria patients

Polina Gundorova[1], *Mathias Woidy[1], Ania Carolina Muntau[2], Soeren Gersting[1]*

[1]University Medical Center Hamburg-Eppendorf, University Chidren's Research, Hamburg, Germany; [2]University Medical Center Hamburg-Eppendorf, University Children's Hospital, Hamburg, Germany

Background/Objectives: In autosomal-recessive disorders like phenylketonuria (PKU), understanding the combination of alleles within a patient's genotype is crucial for determining disease severity and potential treatment responses. This is especially relevant in PKU, where the genotype influences the efficacy of pharmacological analogues of tetrahydrobiopterin (BH_4), the natural cofactor and chaperone of phenylalanine hydroxylase (PAH).

Methods: An online database was developed to catalog *PAH* genotypes and their respective sequence variants, showcasing their distribution across populations. Additionally, PAH activity landscapes were performed through enzymatic activity assay in a 96-well format, utilizing gradients of L-phenylalanine and BH4 concentrations, with Tyrosine detection via high-performance liquid chromatography.

Results: The online application comprises data on over 10,000 country-specific *PAH* genotypes, offering search and analysis tools to facilitate population genetic research on PKU. Furthermore, the database provides access to functional data, including PAH activity landscapes for over 100 prevalent *PAH* genotypes, enabling personalized BH_4 testing and treatment recommendations for PKU patients.

Conclusion: The systematic organization of scientific publications on sequence variants and genotypes not only aids in population genetic studies but also enhances routine clinical patient care. By providing readily available functional studies, this database drives the advancement of personalized medicine, integrating the latest scientific findings to benefit patients.

Conflict of Interest: Polina Gundorova Biomarin, Iniuva, Mathias Woidy Biomarin, Ania Carolina Muntau Iniuva, Biomarin, Biomarin, Soeren Gersting Iniuva, Biomarin

P08.041.B Investigating the relationship between biochemical profiles and genotype in inborn errors of metabolism

Clarissa Berardo[1;2], *Alessandra Vasco[2], Simona Lucchi[2], Laura Cappelletti[2], Laura Assunta Saielli[2], Elisa Pratiffi[2], Salvatore Fazzone[2], Erika Benedetti[2], Andrea Meta[2], Alessia Mauri[1;2], Stephana Carelli[1;2], Francesca Furlan[3], Valentina Rovelli[4], Francesca Menni[3], Viola Crescitelli[5], Serena Gasperini[5], Luisella Alberti[2], Cristina Cereda[2]*

[1]University of Milan, Department of Biomedical and Clinical Science, Mil, Italy; [2]Buzzi Children's Hospital, Functional Genomic and Rare Diseases Unit, Department of Pediatrics, Milan, Italy; [3]Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Pediatric Intensive Care Unit, Milan, Italy; [4]ASST Santi Paolo Carlo Hospital, Clinical Department of Pediatrics, Mi, Italy; [5]Fondazione IRCCS San Gerardo dei Tintori, Pediatric Rare Diseases Unit, Monza, Italy

Background: Newborn screening is a public health program aimed at the rapid detection of treatable inborn errors of metabolism (IEMs), with a considerable rate of false positives. To reduce it, we aim to correlate biochemical markers to the genotype.

Methods: DBS were collected from newborns' heels at 48-72 hours after birth, in Lombardy region (Italy). FIA-MS/MS was performed to evaluate specific biochemical markers on DBS. If required, biochemical confirmation was performed on plasma and/or urine. Genetic confirmation was achieved by Sanger or NGS on newborns referred to clinical center.

Results: In Lombardy region, from 2016 to 2020, 102/386,431 (0.03%) newborns were affected by IEMs: 50% by FAO disorders, 32% by OA disorders, 17% by amino acidemias. We analyzed our data to unravel specific genotype-biochemistry correlation. As expected, the same mutation in homozygosity or heterozygosity in *ASS1* gene for citrullinemia type 1 showed different biochemical profiles. Only one newborn exhibited persisted high citrulline plasmatic levels, being negative to the citrullinemia panel genes. Two tweens with altered long-chain acylcarnitines and 50% of enzymatic activity of the VLCAD protein, resulted to be genetically negative. A newborn with one VUS in *ACADVL* gene showed the corresponding enzymatic activity of only 25%.

Conclusions: These observations show that it is not always possible to establish a correlation between the genotype and the biochemistry in IEMs. Hence, to properly diagnose IEMs, the search for biomarkers should be accompanied not only by clinic and genetic analysis, but also by whole exome sequencing, transcriptomic and long reads analysis.

Conflict of Interest: None declared

P08.042.C Novel discontinuous homozygous deletion in FUT8 leading to Congenital disorder of glycosylation with defective fucosylation 1

Nesligul Hasibe Gonen[1], nurcan ucuncu ergun[2], abdulkadir tekin[3], **sumeyra oguz**[4]

[1]Sanlıurfa Training and Research Hospital, Department of Medical Genetics, Sanlıurfa, Türkyie; [2]Sanlıurfa Training and Research Hospital, Department of Pediatric Nutrition and Metabolism Clinics, Sanlıurfa, Türkyie; [3]Sanlıurfa Training and Research Hospital, Department of Pediatrics, Sanlıurfa, Türkyie; [4]Zeynep Kamil Women and Children's Diseases Training and Research Hospital, Department of Medical Genetics, Istanbul, Türkyie

Background/Objectives: FUT8-Congenital disorder of glycosylation (FUT8-CDG, MIM #618005) is due to biallelic loss-of-function mutations of *FUT8*, encoding alpha 1,6 fucosyltransferase crucial for posttranslational modification that takes place in mammalian cells. FUT8-CDG is limited to nine individuals presenting with intrauterine growth retardation (IUGR), severe postnatal growth-developmental delays, recurrent respiratory infections, feeding problems, short limbs and neurological impairments.

Methods and results: The 8-month-old male proband, born to consanguineous parents via caesarean section due to IUGR and polyhydramnios, exhibited dysmorphic features, especially shortened lower extremities and severe growth-developmental delays. His affected sibling had polyhydramnios, IUGR, feeding problems and epilepsy and died at 1,5 months without a definitive diagnosis. The proband passed away at 10 months due to recurrent respiratory tract infections, feeding problems and refractory seizures. Targeted epilepsy gene panel analysis showed no disease-causing mutation. However, chromosomal microarray analysis revealed two discontinuous homozygous deletions at 14q23.3. Whilst, proximal deletion (~89 kb) (chr14:65,821,471-65,910,544; hg19) encompassed complete non-coding first exon, partial 5'UTR and first intron of *FUT8*, there was no coding gene in distal deletion (~229 kb) (chr14:66,214,378-66,444,344; hg19). Segregation studies are ongoing for these deletions.

Conclusion: Here, we present the tenth affected individual with FUT8-CDG in the literature. Although single nucleotide variations have been described in FUT8-CDG so far, our study shows that copy number variations can also be included in its molecular etiology. *FUT8* deletion in this study may have produced a loss-of-function effect by causing inhibition of transcription initiation through the deletion of the promoter region or impairment of post-transcriptional modification due to deletion of untranslated regions.

Grants:

Conflict of Interest: None declared

P08.043.D Effects of TXNIP-variants to systemic and cellular lipid and glucose handling

Sharlaine Y. L. Piel[1], *Julia-Josefine Scholz[1], Ioannis Evangelakos[1], Saskia Wortmann[2;3], Maja Hempel[4], Daniela Karall[5], Birgit Reinhart-Steininger[6], Anna Wredenberg[7;8], Elias Arnér[7], Katja Steinbrücker[9], Thomas M Stulnig[6;10], Hans A. Mayr[2], Richard Lee[11], Benedikt Schoser[12], Holger Prokisch[13;14], Joerg Heeren[15], Christian Kubisch[1], Anna Worthmann[15], Christian Schlein[1]*

[1]University Medical Center Hamburg-Eppendorf, Institute of Human Genetics, Hamburg, Germany; [2]Paracelsus Medical University and Salzburger Landeskliniken, University Children's Hospital Salzburg, Salzburg, Austria; [3]Radboud University Medical Center, Amalia Children's Hospital, Nijmegen, Netherlands; [4]University Medical Center Heidelberg, Institute of Human Genetics, Heidelberg, Germany; [5]Medical University of Innsbruck, Clinic for Pediatrics, Inherited Metabolic Disorders, Innsbruck, Austria; [6]Medical University of Vienna, Department of Medicine III, Clinical Division of Endocrinology and Metabolism, Vienna, Austria; [7]Karolinska Institute, Department of Medical Biochemistry and Biophysics and Wredenberg Laboratory, Stockholm, Sweden; [8]Karolinska Institute, Centre for Inherited Metabolic Diseases, Stockholm, Sweden; [9]Paracelsus Medical University, University Children's Hospital, Salzburg, Austria; [10]Hietzing hospital, Department of Medicine III and Karl Landsteiner Institute for Metabolic Diseases and Nephrology, Wien, Austria; [11]Massachusetts General Hospital and Harvard Medical School, Department of Medicine, MGH Cancer Center, Boston, United States; [12]Ludwig Maximilian University of Munich, Department of Neurology, Friedrich-Baur-Institute, München, Germany; [13]Helmholtz Munich, Institute of Neurogenomics, Neuherberg, Germany; [14]Technical University of Munich, Institute of Human Genetics, Munich, Germany; [15]University Medical Center Hamburg-Eppendorf, Department of Biochemistry and Molecular Cell Biology, Hamburg, Germany

Background/Objectives: Thioredoxin interacting protein, encoded by *TXNIP*, has been described to regulate systemic glucose metabolism in mice, making it an attractive target for the treatment of type 2 diabetes mellitus. However, recent findings link biallelic variants in *TXNIP* to cause a mitochondrial disorder in humans. Our research therefore investigates the effects of *TXNIP* variants on human glucose and lipid metabolism and studies potential therapeutic interventions in preclinical models.

Methods: The clinical presentation of eight patients with biallelic *TXNIP* variants and available plasma, organ samples, and fibroblasts were investigated using gene and protein expression studies, lipidomics, proteomics, histology and electron microscopy. To explore potential therapies, we treated patient fibroblasts and TXNIP-KO mice with pharmacological and dietary interventions.

Results: The patients presented with a range of clinical symptoms with an overlap in lactic acidosis, whereas hypoglycemia, combined dyslipidemia and cardiac and skeletal myopathy were variably present. Patient organ biopsies revealed the presence of glycogen and lipid deposits. Gene expression analysis indicated increased de novo fatty acid synthesis, which was resembled in TXNIP-KO mice and reversible by feeding low-carb diet in mice.

Conclusion: We introduce TXNIP to influence glucose and lipid metabolism in humans. Biallelic variants in *TXNIP* are associated with hypoglycemia and dyslipidemia, as well as muscular and hepatic lipid accumulation resulting from increased de novo fatty acid synthesis. Using preclinical models, we present a low-carb diet as a possible dietary symptomatic treatment for pathologically elevated organ lipid synthesis.

Grants: DFG - SCHL2276/2-1

Conflict of Interest: None declared

P08.044.A Could a shared intronic variant hold the key? Unraveling retinopathy and motor dysfunction in two individuals with variants of unknown significance in the FDXR gene

Janni Majgaard Jensen[1], *Helle L. Johnsen*[2], *lars kjaersgaard hansen*[3], *Else Gade*[4], *Mie Larsen*[5], *Caroline Hey Bækgaard*[5], *Kristina Sørensen*[5], *Elsebet Østergaard*[6], *Christina Fagerberg*[5;7]

[1]*Aalborg University Hospital, Departement of Clinical Genetics, Aalborg;* [2]*Aalborg University Hospital, Departement of Pediatrics, Aalborg;* [3]*Odense University Hospital, H. C. Andersen Children's and Youth Hospital, Odense, Denmark;* [4]*Odense University Hospital, Departement of Ophthalmology, Odense;* [5]*Odense University Hospital, Departement of Clinical Genetics, Odense;* [6]*Rigshospitalet, Departement of Clinical Genetics, København Ø, Denmark;* [7]*Sygehus Lillebaelt, Departement of Clinical Genetics, Vejle, Denmark*

Background: Biallelic variants in the *FDXR* gene, encoding ferredoxin reductase, are associated with a rare autosomal recessive progressive neurologic disorder characterized by visual impairment neuropathy, and hearing loss. Ferredoxin reductase is vital for iron-sulfur cluster biogenesis, impacting essential cellular processes such as mitochondrial respiratory chain function and iron homeostasis. We present two unrelated children carrying biallelic *FDXR* variants, one of which includes a suspected splicing variant, detected in both children.

Methods/Results: Individual 1, a 6-year-old girl, exhibited progressive visual impairment due to retinopathy, developmental delay, an unsteady gait, and tactile discomfort. Trio WGS detected two compound heterozygous variants of uncertain significance (VUS) in the *FDXR* gene: c.1402C>T, p.(Arg468Trp), and c.271-14G>A.

Individual 2, a 2-year-old boy, developed progressive retinopathy, hypotonia, dystonia and ataxia following a viral infection at the age of 1. He also exhibited touch-related discomfort on his lower legs. Trio WGS detected two compound heterozygous VUS' in the *FDXR* gene: c.310C>T, p.(Arg104Cys), and interestingly he also carried the intronic variant similar to individual 1, c.271-14G>A.

The c.271-14G>A variant was predicted to introduce a new splice acceptor site 14bp downstream of exon 4, indicating af longer transcript. Ongoing RNA analysis aims to classify the variant.

Conclusion: We present two unrelated patients with progressive retinopathy, motor dysfunction, and signs of peripheral neuropathy. Trio WGS revealed compound heterozygosity for two variants of uncertain significance in *FDXR*. Interestingly, one variant, c.271-14A>G, was observed in both individuals, supporting the possibility that this variant might indeed be pathogenic, thus reinforcing the diagnosis of *FDXR*-associated disease.

Conflict of Interest: None declared

P08.045.B New case of MEDNIK syndrome: feeding related non-motor seizures could be a new feature?

Adelaide Peruzzi[1;2], *Francesca Peluso*[2], *anna cavalli*[3], *daniele frattini*[3], *giovanni malmusi*[4], *Roberta Zuntini*[2], *gianluca contrò*[2], *Antonio Novelli*[5], *giancarlo gargano*[4], *Carlo Fusco*[3], *Livia Garavelli*[2]

[1]*Alma Mater Studiorum - Università di Bologna, Department of Medical and Surgical Science, Bologna, Italy;* [2]*Azienda USL - IRCCS di Reggio Emilia, Medical Genetics Unit, Reggio Emilia, Italy;* [3]*Azienda USL - IRCCS di Reggio Emilia, Child Neurology and Psychiatry Unit, Reggio Emilia;* [4]*Azienda USL - IRCCS di Reggio Emilia, Neonatal Intensive Care Unit, Department of Mother and Child, Reggio Emilia, Italy;* [5]*Bambino Gesù Children's Hospital, IRCCS, Laboratory of Medical Genetics, Translational Cytogenomics Research Unit, Roma, Italy*

Background/Objectives: MEDNIK Syndrome (MS) is a neurocutaneous disease associated with biallelic variants of the *AP1S1* gene. The involvement is multisystemic and includes ichthyosis, intellectual disability, deafness, peripheral neuropathy and a severe enteropathy often resulting in neonatal mortality. To date, only 14 cases have been reported in literature. We present the clinical course of a newborn girl with MS and a review of the literature.

Methods: SNP Array analysis revealed multiple regions of homozygosity. Subsequent clinical exome analysis identified the homozygous c.256C>T p.(Arg86Ter) variant in the *AP1S1* gene, resulting in a premature stop codon confirming the diagnosis.

Results: A girl, third child of consanguineous parents, born at term, presented since birth congenital non-infectious diarrhea. At 15 days of age, breastfeeding was ceased and total parenteral nutrition (TPN) was introduced. At 23 days of age, re-feeding was attempted but failed due to recrudescence of enteropathy and simultaneous onset of focal epileptic non-motor seizures, which resolved upon reinstating TPN. At 4 months of age, she was weaned of TPN and from 6 months onwards, she remained seizure free with anti-epileptic therapy and zinc-acetate supplementation. Additionally, she presented with ichthyosis, mild hearing loss and hyporigenerative anaemia. At 2 years of age she showed axial hypotonia, psychomotor delay and growth retardation.

Conclusion: Given the rarity of the condition, the high neonatal mortality and the limited therapeutic options available, we present this case along with a review of the literature, outlining the main clinical and molecular aspects of the disease including electrophysiological features never reported before.

Conflict of Interest: None declared

P08.046.C Mucopolysaccharidosis plus syndrome within identical twins having *VPS33A* and *VPS11* mutations

Ibrahim Akalin[1], *Fatih Demircioğlu*[2], *Nursel Elcioglu*[3], *Ayse Busra Akalin*[4], *Betül Tavil*[2]

[1]*Metagentech Center for Genetic Diseases, Medical Genetics, Istanbul, Türkyie;* [2]*Medistate Hospital, Pediatric Hematology, Istanbul, Türkyie;* [3]*Marmara University Faculty of Medicine, Pediatric Genetics, Istanbul, Türkyie;* [4]*Bahçeşehir University Faculty of Medicine, Medicine, Istanbul, Türkyie*

Background/Objectives: Mucopolysaccharidosis-plus syndrome (MPSPS) is inherited autosomal recessively in VPS33A gene and is an inborn error of metabolism result from diminished class C core vacuole/endosome tethering (CORVET) and the homotypic fusion and protein sorting (HOPS) complexes. Both are heterohexamers and consist of 4 subunits consisting of VPS33A, VPS11, VPS16 and VPS18. It presents with typical symptoms of mucopolysaccharidosis, as well as respiratory difficulties, congenital heart defects, renal and hematopoietic system disorders and delayed psychomotor activity in infants and early childhood. Most of the patients die because of cardiorespiratory failure in the beginning of their lives.

Methods: Two identical twins aged 3 year-old referred for genetic testing for undiagnosed cases whom represent dismorphic face, immunodeficiency, hepatomegaly, lethargy, distended abdomen, difficulty for breathing. One of the patient passed away due to worsening general conditions. Whole exam sequencing was run.

Results: WES analyses revealed heterozygous VPS33A (c.599G>A (p.Arg200Gln)), homozygous VPS11(c.101_103delATCinsGTT) and homozygous PRKDC (c.496dupA (p.I166Nfs*11)) mutations including PRKDC exon 5 an 23 deletions.

Conclusion: Mutations found in our patient suggesting a defect in endo-lysosomal pathway bey diminishing CORVET/HOPS complex not only with VPS33A but also with VPS11 and resulting the severe MPSPS with neurological components. Moreover, PRKDC mutations might be accomplished to immunodeficiency symptoms.

Grants:

Conflict of Interest: None declared

P08.047.D Characterising MRPL44(L156R) transgenic mouse model: insights into mitochondrial disorder pathogenesis and sex-dependent responses

Hamin Lee[1], *Chris Carroll*[1], *Alan Pittman*[1], *Susanna Cooper*[2]

[1]*St. George's, University of London, London, United Kingdom;* [2]*Oxford University, Oxford, United Kingdom*

Background/Objectives: Phenotypic heterogeneity is a hallmark of primary mitochondrial diseases (PMD). However, little is known about why variants in the oxidative phosphorylation (OXPHOS) system can lead to such variable and often tissue-specific disorders. We investigated whether biological sex modulates disease manifestation in response to mitochondrial dysfunction in PMD. Therefore, we generated MRPL44(L156R) transgenic knockin mice as a model of combined OXPHOS deficiency 16 and evaluated sex-specific differences in the disease pathogenesis of affected tissues.

Methods: MRPL44^{L156R} mice were generated using CRISPR. Tissues were harvested from young and mature MRPL44^{L156R} homozygous and wildtype mice, processed for histopathology, and molecular analyses of mitochondrial structure, content, and function. Additionally, RNA sequencing investigated the effect of the mutation on gene expression.

Results: The L156R variant destabilized the MRPL44 protein, impacting mitoribosomal large subunit assembly, leading to mitochondrial translation defects presenting as Complex I + Complex IV OXPHOS deficiency. Despite the marked biochemical deficiency, only mild tissue pathology was observed, indicating compensatory pathways activation, in line with the evidence of increased mitochondrial biogenesis. Females exhibited more severe OXPHOS and mitoribosome deficiency, along with induction of cardiomyocyte hypertrophy and inflammation markers. Transcriptomics unveiled a diametrically opposite response in mitochondria-related genes, with induction in females and reduction in males.

Conclusion: Our findings establish the MRPL44^{L156R} mouse model as a promising tool for studying mitochondrial diseases, elucidating the significance of sex specificity in these disorders. Moreover, our study identified a novel sex-specific mitochondrial-nucleus retrograde signalling pathway critical in PMD manifestation and respsonses to therapies targeting mitochondrial biogenesis.

Grants: MRC LID Studentship

Conflict of Interest: None declared

P08.048.A The landscape of autosomal-recessive pathogenic variants, consanguinity and homozygozity detected on newborn screening

Janne Maren Strand[1], *Trine Tangeraas*[1], *Emma Sofia Lundman*[1], *Silje Hogner*[1], *William Nicholas Tourniaire*[1], *Mari Eknes Ytre-Arne*[1], *Magnus Odin Dahlseng*[1], *Julie Hellem AAby*[1], *Andreas Øberg*[1], **Asbjørg Stray-Pedersen**[1]

[1]*Oslo universitetssykehus HF, Rikshospitalet, Norwegian National Unit for Newborn Screening, Oslo, Norway*

Consortium: NEWBORN SCREENING NORWAY

Background: In silico calculations predicts overrepresentation of positive newborn screening (NBS) among children with consanguineous parents. We present our numbers focusing on the screening disorders with an autosomal recessive (AR) trait. The Norwegian NBS program is regulated by an exception from the Biotechnology Act allowing for mass genetic screening for specific disorders. Genetic testing of DNA extracted from the blood-spots is used as confirmatory test prior to reporting a positive screening and clinical follow-up of the child.

Methods: The sequencing results from positive screening during the years 2019-2023 were selected, and variants in AR NBS disorders sorted into two groups: the compound heterozygous and homozygous. Information about ethnicity and parental consanguinity are part of the clinical evaluation at the follow-up consultations.

Results: Out of 267 563 newborns tested during 2019-2023, 227 were reported with an AR NBS disorder, 81 compound heterozygous, 46 homozygous. Out of the 46 with homozygous disease causing variants, 27 had parents without history of consanguinity, while 13 had consanguineous parents, and 12 of these couples were first cousins. In 6 of the newborns with homozygous disease causing variants, parental consanguinity was expected, but not confirmed. The ethnic origin of the confirmed consanguineous couples: Iraq, Syria, Afghan, Pakistan, Kurdistan, and Somalia.

Conclusion: Based on our numbers of reported newborns with abnormal results from the NBS and an AR NBS disorder, the true risk for unrelated couples in the general population to have a baby with a NBS disorder and the risk for consanguineous couples can be calculated.

Conflict of Interest: None declared

P08.049.B The effects of a dietary intervention for weight loss on quality of life components in adults with increased genetic risk for elevated BMI levels.

Maria Kafyra[1], *Ioanna-Panagiota Kalafati*[1,2], *Panagiota Giardoglou*[1], *iraklis varlamis*[3], *andriana kaliora*[1], *panagiotis moulos*[4], *George Dedoussis*[1,5]

[1]*Harokopio University of Athens, Department of Nutrition and Dietetics, Athens, Greece;* [2]*University of Thessaly, Department of Nutrition and Dietetics, Trikala, Greece;* [3]*Harokopio University of Athens, Department of Informatics and Telematics, Athens, Greece;* [4]*Biomedical Sciences Research Center "Alexander Fleming", Institute for Fundamental Biomedical Research, Vari, Greece;* [5]*Genome Analysis, Athens, Greece*

Background/Objectives: Current research highlights nutrigenetic effects on quality of life. The present explores gene-diet interactions and quality of life in adult participants of the iMPROVE study presenting overweight or obesity, following a 3-month high-protein or high-carbohydrate hypocaloric diet.

Methods: We used data from 80 participants to measure associations between the observed changes in physical and mental health post-intervention and 10 BMI-associated, candidate variants. Quality of life was assessed using the 12-item Short Form Survey (SF-12) physical (SF-PCS-12) and mental (SF-MCS-12) components. The level of statistical significance was corrected based on the Bonferroni method. Associations were examined through linear regression models adjusted for age and sex, using the PLINK version 1.9.

Results: SF-PCS-12 and SF-MCS were not significantly improved post-Intervention. Increase in SF-PCS-12 post-intervention was significantly lower among carriers of the BMI-raising rs17817449-G allele ($\beta = -3.758$, $p = 0.009$) and nominally lower among rs9939609-A alllele carriers ($\beta = -3.202$, $p = 0.028$). Increased SF-MCS-12 levels post-intervention were positively, nominally associated with four examined variants (rs1421085-C, rs1121980-A, rs17817449-G and rs9939609-A, $p < 0.05$ for all).

Conclusion: Although increase in SF-PCS-12 levels was lower in carriers of BMI-raising alleles, specific variants were associated

with higher levels of SF-MCS-12 increase. This denotes a favorable effect of a weight loss intervention on mental characteristics, even in the presence of favorable genetic makeup for increased BMI.

Grants: The BETTER4U project has received funding from the European Union's Horizon Europe Research and Innovation programme under Grant Agreement n° 101080117.

Conflict of Interest: None declared

P08.050.C A pathogenic deep intronic variant in the PCCB gene masked by a common benign intronic deletion

Alina Kurolap[1], *dalit barel*[1], *Nava Shaul Lotan*[2], *Isaiah Wexler*[3;4], *Hofit Gadot*[1], *Adi Mory*[1], *Ortal Barel Barel*[5], *shlomo almashano*[6], *Hagit Baris Feldman*[1;7]

[1]*Tel Aviv Sourasky Medical Center, The Genetics Institute and Genomics Center, Israel;* [2]*Hadassah Medical Organization, Department of Genetics, Israel;* [3]*Hadassah Medical Organization, Department of Pediatrics, Israel;* [4]*Hebrew University of Jerusalem, Faculty of Medicine, Israel;* [5]*Sheba Medical Center, Genomics Unit, the Center for Cancer Research, Israel;* [6]*Public Health Services, Ministry of Health, National Newborn Screening Program, Israel;* [7]*Tel Aviv University, Faculty of Medicine, Israel*

Background/Objectives: Propionic acidemia (PA) is a metabolic disorder caused by bi-allelic variants in either *PCCA* or *PCCB*, encoding the propionyl-CoA carboxylase (PCC) enzyme sub-units. PCC is required for catabolism of certain amino acids and odd-chain fatty acids. In its absence, toxic metabolites accumulate, leading to a variable presentation, including metabolic acidosis, neurologic symptoms, multi-organ dysfunction and possible death, with typical neonatal or early infantile onset.

Methods: Exome sequencing (ES) followed by genome sequencing (GS) were used to identify the genetic cause of PA in two unrelated children initially diagnosed by newborn screening and enzymatic studies. RNA analyses were performed to study variant outcomes.

Results: ES failed to identify a pathogenic variant. We proceeded with GS, demonstrating homozygosity for a deep intronic *PCCB* variant. RNA studies established that this variant creates an out-of-frame pseudoexon, leading to loss of wild type *PCCB* expression. Surprisingly, three of the variant-carrier parents also appeared homozygous due to a common benign intronic deletion on the second allele.

Conclusion: Reaching a precise diagnosis, whether biochemical or genetic, can be crucial in directing lifesaving care, concluding the diagnostic odyssey, and allowing future family planning. This study demonstrates the power of integrative genetic studies in reaching a diagnosis, utilizing GS and RNA analyses to overcome ES limitations and define variant pathogenicity. Importantly, the presumed parental homozygosity merits special attention, as it initially masked the causative variant. Intronic deletions should be taken into consideration when analyzing genomic data to avoid misinterpretation of pseudo-homozygosity and mislabeling pathogenic variants as benign.

Grants: None.

Conflict of Interest: None declared

P08.051.D Estimation of prevalence of Leigh Syndrome caused by three major nuclear genes mutations (*SURF1*, *SCO2*, and *NDUFV1*) in the Russian Federation

Denis Chistol[1], *Polina Tsygankova*[1], *Andrey Nekrasov*[1], *Nikita Beskorovayny*[2], *Ekaterina Zakharova*[1]

[1]*Research Centre for Medical Genetics, Laboratory of Inherited Metabolic Diseases, Moscow, Russian Federation;* [2]*Research Centre for Medical Genetics, Moscow, Russian Federation*

Background/Objectives: Leigh syndrome (LS) is the most common mitochondrial disorder in children, characterized by progressive neurological manifestations. The prevalence of LS and Leigh-like syndrome is usually estimated at 1:40,000 newborns, but the exact prevalence of LS in the Russian Federation is unknown. Our study presents an estimate of the prevalence of LS caused by the mutations in three major nuclear genes (*SURF1*, *SCO2* and *NDUFV1*) in the Russian Federation. *SURF1*, *SCO2* assumed to have founder mutations in Slavonic population.

Methods: We used the Russian Massive Parallel Sequencing dataset to approximate the prevalence of LS (total allele number = 18,012). For variant classification, we used the Franklin resource, which also classifies variants based on ACMG-AMP recommendations. Additional manual classification was performed for some variants based on knowledge accumulated in the laboratory of inherited metabolic diseases.

Results: For each gene, only those variants from the RuExac and Ruseq databases that were predicted to be pathogenic or probably pathogenic were included. The disease rate for each gene was calculated using the gene carrier rate (GCR). For each GCR we calculated a 95% CI (Wilson method): for *SURF1* = 1:237,419 (95%CI 1:127,849-1:441,248), for *SCO2* = 1:213,531 (95% CI 1:110,872-1:411,636), and for *NDUFV1* = 1:4,135,588 (95%CI 1:1,147,133-1:14,921,927).

Conclusion: The cumulative incidence of nuclear genes-associated LS in the Russian Federation is ~ 1:45,000. Based on our LS registry mtDNA-associated LS accounts not less than 1/3 of all cases. So the final estimation will be not less than 1:30,000. Which is probably the highest prevalence in the world.

Conflict of Interest: None declared

P08.052.A UCHL1 missense and loss-of-function variants as an emerging cause of autosomal dominant optic atrophy (ADOA)

Claudio Fiorini[1], *Giada Capirossi*[1;2], **Eleonora Pizzi**[1], *Federico Sadun*[3], *Maria Lucia Cascavilla*[4], *Chiara La Morgia*[1;2], *Marco Battista*[4], *Piero Barboni*[4], *Danara Ormanbekova*[1], *Valentina Del Dotto*[2], *Flavia Palombo*[1], *Valerio Carelli*[1;2], *Alessandra Maresca*[1], *Leonardo Caporali*[1;2]

[1]*IRCCS Istituto delle Scienze Neurologiche di Bologna, Programma di Neurogenetica, Bologna, Italy;* [2]*University of Bologna, Department of Biomedical and NeuroMotor Sciences (DIBINEM), Bologna, Italy;* [3]*Ospedale Oftalmico, Roma, Italy;* [4]*University Vita-Salute, IRCCS Ospedale San Raffaele, Department of Ophthalmology, Milano, Italy*

Background/Objectives: The ubiquitin carboxyl-terminal hydrolase isozyme L1 (*UCH-L1*) is a deubiquitinating enzyme highly expressed in neurons, where it solely accounts for 1-2% of total cytosolic proteins. Recently, several pedigrees were described with autosomal dominant hereditary spastic paraplegia (HSP) and optic atrophy due to heterozygous *UCHL1* loss-of-function variants.

Methods: We performed WES for a cohort of 249 hereditary optic neuropathies patients, adding phenotypic characterization experiments on fibroblasts derived from probands carrying novel likely pathogenic variants through RNA sequencing, immunoblotting and cellular respiration analysis.

Results: We identified two pedigrees harboring heterozygous variants in the *UCHL1* gene, segregating with a phenotype of isolated dominant optic atrophy (DOA). Family 1 members carry a splice-site variant (c.586-2A>C), while Family 2 members carry a missense variant with a predicted deleterious effect (c.284T>G, p.Leu95Arg). On Family 1 proband fibroblasts, we confirmed a marked reduction in UCH-L1 protein and a decrease in OXPHOS complexes subunits, especially significant for complex I and IV. No cellular respiration phenotype was evident, with only a higher glycolytic acidification profile in mutant cells. In addition, we

observed a significant reduction of pyruvate dehydrogenase E2 and E3 subunits and of citrate synthase levels. Moreover, by incubation with galactose medium for 48 h, both control and mutant cell lines showed an induced mitochondrial biogenesis with recovery of all protein levels.

Conclusion: In conclusion, we present heterozygous variants in *UCHL1* as a new cause of DOA, widening the phenotypic spectrum associated with *UCHL1* defect, possibly accompanied by metabolic rewiring and secondary mitochondrial dysfunction.

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Conflict of Interest: None declared

P08.053.B Unraveling the enigma of undiagnosed diseases: integration of multi-omics layers empowers precision diagnosis through unveiling pathogenic mechanisms on maple syrup urine disease

Alejandro Soriano-Sexto[1], *Juan Ramon Tejedor*[2;3;4], *Natalia Castejón-Fernández*[1], *Patricia Correcher*[5], *Lidia Sainz-Ledo*[2;3], *Juan José Alba-Linares*[2;3], *Rocío G Urdinguio*[2;3;4], *Magdalena Ugarte*[1], *Agustín F Fernández*[2;3;4], *Pilar Rodríguez-Pombo*[1], *Mario F Fraga*[2;3;4], *Belén Pérez*[1]

[1]*Centro de Diagnóstico de Enfermedades Moleculares (CEDEM), Centro de Biología Molecular Severo Ochoa (CBMSO), CIBERER, idiPAZ, Universidad Autónoma de Madrid (UAM), Madrid, Spain;* [2]*Centro de Investigación en Nanomateriales y Nanotecnología (CINN-CSIC), Centro Superior de Investigaciones Científicas (CSIC), Asturias, Spain;* [3]*Instituto de Investigación Sanitaria del Principado de Asturias (ISPA), Fundación para la Investigación y la Innovación Biosanitaria del Principado de Asturias (FINBA), CIBERER, Asturias, Spain;* [4]*Instituto Universitario de Oncología de Asturias (IUOPA), Asturias, Spain;* [5]*Unidad de Nutrición y Metabolopatías, Hospital Universitario la Fe, Valencia, Spain*

Background/Objectives: Maple syrup urine disease (MSUD) is a rare inherited metabolic disorder characterized by deficient activity of the branched-chain alpha-ketoacid dehydrogenase (BCKD) complex, required to metabolize the amino acids leucine, isoleucine and valine. Despite its profound metabolic implications, the molecular alterations underlying this metabolic impairment had not yet been elucidated.

Methods: We performed a comprehensive multi-omics integration analysis, including genomic, epigenomic and transcriptomic data from fibroblasts derived from a cohort of MSUD patients and unaffected controls to genetically characterize a MSUD case and to unravel the MSUD pathophysiology.

Results: The integration of multi-omics layers facilitated the identification of a strong epigenetic repression in the *DBT* promoter in a patient wherein no BCKD pathogenic variants had been detected, thereby unveiling alternative modes of disease inheritance and confirming its diagnosis potential. Furthermore, MSUD patients exhibit a defined episignature that reshapes the global DNA methylation landscape, resulting in the stimulation of HOX cluster genes and the restriction of cell cycle gene-related signatures. Subsequent data integration revealed the impact of AP1-related and CEBPB transcription factors on the observed molecular reorganization, with MEIS1 emerging as a potential downstream candidate affected by robust epigenetic repression in MSUD patients.

Conclusion: Integration of multi-omics data unveiled underlying molecular networks rewired in MSUD patients and represents a powerful approach with diagnostic potential for rare genetic disorders with unknown genetic bases.

Grants: PI22/00699 and PI21/01067

Conflict of Interest: None declared

P09

IMMUNOLOGY AND HEMATOPOIETIC SYSTEM

P09.001.C Score-Based Candidate Gene Identification in Inborn Errors of Immunity

Claire Dudler[1], *Amica Müller-Nedebock*[1], *Maria Fasshauer*[2;3], *Stephan Borte*[2;3], *Marie Gerisch*[2;3], *Rami Abou Jamra*[1], *Isabell Schumann*[1]

[1]*Institute of Human Genetics, University of Leipzig Medical Center, Leipzig, Germany;* [2]*St. Georg Hospital, IDCL (ImmunDefectCentrum Leipzig), Leipzig, Germany;* [3]*St. Georg Hospital, Center for Pediatric and Adolescent Medicine, Leipzig, Germany*

Background/Objectives: Inborn Errors of Immunity (IEI) exhibit considerable heterogeneity in clinical presentation, age of onset, impact of exogenous factors, penetrance as well as expressivity. Our in-house exome sequencing (ES) diagnostic yield for IEI individuals is currently only 18%. Thus, in this study, we aimed to reanalyse the ES data from unsolved IEI cases to identify new candidate genes. Furthermore, we set out to create a scoring tool incorporating gene- and variant-specific attributes to aid the identification of such IEI candidate genes.

Methods: The cohort consisted of 178 individuals with disorders of the immune system, who had undergone ES diagnostics but remained without genetic diagnosis. We searched for single nucleotide variants (SNVs) and applied filtering steps with regard to quality, in-silico predictions, impact and frequency. Subsequently we reviewed the selected variants and outlined a scoring tool incorporating these steps.

Results: We were able to identify several candidate genes (e.g. *PRKCB, PTK2B, GIT2, PDE7A, LGALS1, GCNT1, PPT2, GRWD1, FLOT1, CETP* and *MYO7B*) that are promising regarding the deleteriousness of the variant, tissue-specific expression, phenotypes of animal models, and predicted protein-protein interactions. We subsequently uploaded our findings to GeneMatcher to compare the potential phenotypic spectrum of the identified candidate genes.

Conclusion: With a focused approach to identifying candidate genes and efficiently estimating their relevance, we aim to increase the likelihood of detecting novel genes associated with IEI and improving future diagnostic yield.

Grants: None.

Conflict of Interest: None declared

P09.002.D Dissecting genetic susceptibility to Zika virus

Marie Bourdon[1;2;3], *Caroline Manet*[3], *Laurine Conquet*[3], *Corentin Ramaugé Parra*[3], *Etienne Kornobis*[4], *Eliette Bonnefoy*[5], *Xavier Montagutelli*[3]

[1]*LMU Klinikum, Comprehensive Childhood Research Center, München, Germany;* [2]*Helmholtz Munich, Neuherberg, Germany;* [3]*Institut Pasteur, Laboratoire de génétique de la souris, Paris, France;* [4]*Institut Pasteur, Biomics, Paris, France;* [5]*Institut Cochin, Inserm, Paris, France*

Background/Objectives: Zika virus (ZIKV) is a flavivirus transmitted by mosquitoes and responsible, for infections which consequences range from an absence of symptoms to severe complications. Using the Collaborative Cross, a collection of mouse inbred strains with large genetic diversity, we previously showed that host genetics affects the severity of symptoms and plasma viral load. CC071 was identified as the most susceptible

strain, characterized by high virus replication, severe clinical signs, and mortality. In vitro, CC071 mouse embryonic fibroblasts (MEFs) showed an increased viral titer and a delayed production of type I interferon.

Methods: The genetic factors associated with the susceptibility of CC071 was assessed using a backcross between CC071 and the resistant strain CC001. 51 unique MEF lines were derived and phenotyped after ZIKV infection. Embryos were genotyped using the MiniMUGA chip array.

Results: Phenotypic analysis of infected backcross MEFs suggested a monogenic inheritance of ZIKV susceptibility and QTL mapping analysis identified *Irf3* (one of the interferon transcription factors) as a strong candidate gene. RNA sequencing showed that the CC071 strain has a de novo mutation in *Irf3* which leads to abnormal splicing. However, after in vivo ZIKV infection, CC071 mice exhibited higher susceptibility than C57BL/6J *Irf3* KO mice, demonstrating the involvement of other susceptibility genes in the disease of CC071 mice.

Conclusion: Our results provide new insight into the in vitro and in vivo roles of *Irf3*, and into the genetic complexity of host responses to flaviviruses.

Grants: MB and CM were supported by doctoral fellowships from grant ANR-10-LABX-62-IBEID.

Conflict of Interest: None declared

P09.003.A Comprehensive and Integrative Single Cell Multi-omics Analysis of the Immune System of Private Mission Astronauts

JANGKEUN KIM[1], *Christopher E. Mason*[1], *Eliah Overbey*[1], *Braden Tierney*[1], *Deena Najjar*[1], *Krista Ryon*[1], *Cem Meydan*[1], *Jaime Mateus*[2], *Marissa Rosenberg*[2]

[1]*Weill cornell medicine, New York, United States;* [2]*SpaceX, Hawthorne, United States*

Background/objectives: Changes in immune function related to spaceflight are a common phenotype in astronauts, yet it remains poorly understood. Recent civilian astronaut missions, Inspiration4, Polaris Dawn, and Axiom-2 missions enabled single-cell multi-omics analysis, which enabled a state-of-the-art, multi-omic, single-cell profiling to address the gaps in our knowledge of the cellular response to space.

Methods: To better understand the immune response in human spaceflight, we generated single-cell, multi-ome, cell-free RNA (cfRNA), biochemical, and hematology data for the civilian astronauts from Inspiration4, Polaris Dawn, and Axiom-2 across pre-, in- and post-flight timepoints. We also integrated single-cell PBMC data with the NASA Twins Study, a set of 817 NASA GeneLab mouse transcriptomics, and other omics data from Inspiration4 to compare the changes in different samples.

Results: We found cell proportion, gene expression, chromatin accessibility, and overrepresented DNA motifs in differentially accessible regions after spaceflight. Also, we found that those biological changes mostly revert back to normal within a few months after spaceflight. We then identified a spaceflight gene signature of astronauts, representatively OXPHOS, translation, and UV response. This spaceflight signature was also observed in the other astronaut and spaceflight data.

Conclusion: In conclusion, these convergent signatures of spaceflight begin to detail the core, consistent set of human cellular and molecular responses to spaceflight and can help narrow the targets for countermeasures and monitoring in future studies. Moreover, any potential long-term changes need to be monitored longitudinally to scan for any reversion or exacerbation of the phenotypes before pharmacological or biomedical interventions can be considered.

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P09.005.C Meta-analysis of determinants of trans-acting regulatory variation in primary human monocytes across immune contexts

Isar Nassiri[1;2], *James Gilchrist*[3], *Julian C Knight*[4], *Benjamin Fairfax*[3]

[1]*University of Oxford, Department of Psychiatry, Oxford, United Kingdom;* [2]*Oxford-GSK Institute of Molecular and Computational Medicine (IMCM), Nuffield Department of Medicine, Oxford, United Kingdom;* [3]*University of Oxford, Department of Oncology, Oxford, United Kingdom;* [4]*University of Oxford, Centre for Human Genetics, Nuffield Department of Medicine, Oxford, United Kingdom*

Background: Characterizing the downstream regulatory impact of genetic variants on global gene expression changes remains a major challenge. Trans-acting expression quantitative trait locus (eQTL) provide agnostic insights into transcriptional pathways, and we have previously demonstrated that trans-eQTL in monocytes frequently involve master regulatory regions. To increase the statistical power, we carried out an eQTL meta-analysis in primary human monocyte samples and replicated the results.

Methods: We systematically explored the trans eQTL meta-analysis in post-exposure to lipopolysaccharide (LPS) or IFNγ monocytes. We performed an eQTL meta-analysis on 509 primary human monocyte samples and replicated the analysis using 100bp paired-end RNA-seq from a new cohort of 185 healthy Europeans.

Results: A total of 1635 trans-acting eQTL (FDR < 1^{-10}) were identified and replicated across treated conditions, with 19 of them being new master regulatory loci (those with > 5 trans associated genes). By using RNA-seq, we replicated 94% (14/15) of previously described master regulatory loci and confirmed their biological importance. To improve our understanding of trans-acting genetic variation, we incorporated associations with GWAS summary statistics for 84 complex traits. The enrichment of eSNPs in multiple sclerosis, celiac disease, and cancer traits was observed in treated monocyte cells. Finally, our allele-specific trans analysis revealed sparse gene networks where all genes exhibited similar expression patterns and were influenced by genetic variants.

Conclusions: The results provide insight into the downstream effects of genetic variants in induced monocytes linked to etiology of autoimmunity and cancer diseases.

Conflict of Interest: None declared

P09.006.D Bone Marrow Grafts with a Telomere Biology Defect

Nergis Güzel[1;2], *Yannic Schumacher*[2;3], *Kim Kricheldorf*[2;3], *Margherita Vieri*[2;3], *Martin Kirschner*[2;3], *Jens Panse*[2;3], *Andrea Gehrig*[4], *Erdmute Kunstmann*[4], *Laura Holthöfer*[5], *Susann Schweiger*[5], *Daniel*

Wolff[6], Florian Kraft[1;2], Miriam Elbracht[1;2], Ingo Kurth[1;2], Tim Henrik Brümmendorf[2;3], Fabian Beier[2;3], Robert Meyer[1;2]

[1]Institute for Human Genetics and Genomic Medicine, Medical Faculty, RWTH Aachen University, Aachen, Germany; [2]Center for Integrated Oncology Aachen Bonn Cologne Düsseldorf (CIO ABCD), Germany; [3]Department of Hematology, Oncology, Hemostaseology and Stem Cell Transplantation, Medical Faculty, RWTH Aachen University, Aachen, Germany; [4]Institute for Human Genetics, University of Würzburg, Würzburg, Germany; [5]Institute for Human Genetics, University Medical Center of the Johannes Gutenberg University Mainz, Mainz, Germany; [6]Department of Internal Medicine III, University Hospital Regensburg, Regensburg, Germany

Background/Objectives: Telomere Biology Disorders (TBD) are characterized by accelerated loss of telomeres. Bone marrow transplantation (BMT) can be a treatment option for hematological complications of TBD e.g. bone marrow failure (BMF). Here we report on three patients with TBD, who received a BMT from an asymptomatic matched family donor (MFD) also carrying an until then unrecognized germline variant for TBD.

Methods: All patients were included in the Aachen Telomere Registry. After obtaining informed consent, genetic analyses were performed, and clinical data were collected and analyzed.

Results: Patient 1 (NM_198253.3(*TERT*):c.2266C>T,-p.(Arg756Cys), homozygous) received a BMT from his brother (*TERT*:c.2266C>T,p.(Arg756Cys), heterozygous) at the age of 7, Patient 2 (NR_001566.1*(TERC):n.50C>A*, heterozygous) from her father (NM_032957.5(*RTEL1*):c.3604C>T,p.(Gln1202*), heterozygous) at the age of 5 and patient 3 (NM_198253.3(*TERT*):c.2851C>T,p.(Arg951Trp), heterozygous) from his identical twin brother at the age of 40. Surprisingly, two patients had had sufficient hematopoiesis for over 20 years without signs of BMF. In patient 3, BMF occurred nine years after BMT concurrently with a BMF in his twin brother. Telomere length testing showed premature telomere shortening in all three patients.

Conclusion: Here we provide evidence from three families that genetic testing of index patients with an unexplained BMF followed by their putative family donors prior to BMT could minimize the risk of transplanted TBD. If alternative donors are not available, BMT from a MFD with a pathogenic germline variant may represent a therapeutic option for BMF resulting in an improved clinical situation.

Grants: none

Conflict of Interest: None declared

P09.007.A Pilot study: Use of GestaltMatcher as a tool for the diagnosis of monogenetic Inborn Errors of Immunity

Annabelle Arlt[1], *Hannah Klinkhammer[1;2], Sandra von Hardenberg[3], Peter Krawitz[1], Tim Niehues[4]*

[1]Institute for Genomic Statistics and Bioinformatics, University Hospital Bonn, Bonn, Germany; [2]Institute for Medical Biometry, University Hospital Bonn, Bonn; [3]Department of Human Genetics, Hannover Medical School, Hannover; [4]HELIOS Childrens Hospital Krefeld, Krefeld

Background: GestaltMatcher is an artificial intelligence that has been developed to analyze phenotypic features in medical imaging data of patients with rare diseases and enables clinicians to match patients with facial similarity.

Inborn errors of immunity (IEI) encompass nearly 500 monogenic disorders characterized by susceptibility to infections, immunodysregulation and bone marrow failure. Due to their phenotypic variability, IEI are difficult to recognize. At the same time, a delay of diagnosis is associated with a significantly poorer outcome. We hypothesized that GestaltMatcher can be used to accelerate the clinical diagnosis of IEI patients associated with syndromic features.

Methods: An expert committee classified IEI into categories based upon the segment of the immune system that is affected. In the category of IEI with facial dysmorphisms, we applied GestaltMatcher for individuals diagnosed to have IEI. Here, we analyzed 19 images from patients with Nijmegen breakage syndrome, 100 images from patients with Kabuki syndrome and 16 images from patients with DiGeorge syndrome. These cohorts are compared to cohorts with different genetic disorders that were randomly sampled from the GestaltMatcher database (GMDB).

Results: Our statistical analysis showed that the Nijmegen breakage and Kabuki syndrome exhibit a recognizable facial gestalt, whereas the DiGeorge syndrome has less distinct features.

Conclusion: Our results indicate that facial dysmorphism in IEIs can be detected with GestaltMatcher and suggest that its application could accelerate the diagnosis of such disorders.

Conflict of Interest: None declared

P09.008.B Genome-wide DNA methylation profiling in Fanconi anemia identifies a robust signature for the clinical classification of VUS in *FANC* genes and reverted patients

Andrea Ciolfi[1], *Marco Ferilli[1], Daria Pagliara[2], Camilla Cappelletti[1], Lucia Pedace[2], Sadegheh Haghshenas[3], Massimo Bogliolo[4;5], María José Ramírez[4], María Roser Pujol[4;6], Michael Levy[3], Evelina Miele[2], Claudia Nardini[2], Raissa Relator[3], Angela Pitisci[2], Rita De Vito[7], Simone Pizzi[1], Jennifer Kerkhof[3], Haley McConkey[3;8], Francesca Nazio[2], Sarina Kant[9], Maddalena Di Donato[10], Emanuele Agolini[10], Marta Matraxia[10], Barbara Pasini[11], Alessandra Pelle[11], Tiziana Galluccio[12], Antonio Novelli[10], Stefan Barakat[9;13], Marco Andreani[12], Francesca Rossi[14], Cristina Mecucci[15], Anna Savoia[16], Bekim Sadikovic[3;8], Jordi Surrallés[4;17], Franco Locatelli[2;18], Marco Tartaglia[1]*

[1]Bambino Gesù Children's Hospital, Molecular Genetics and Functional Genomics, Roma, Italy; [2]Bambino Gesù Children's Hospital, Department of Hematology/Oncology and Cell and Gene Therapy, Roma, Italy; [3]London Health Sciences Centre, Verspeeten Clinical Genome Centre, London, Canada; [4]Hospital de la Santa Creu i Sant Pau, Institut d'Investigació Biomèdica Sant Pau, Barcelona, Spain; [5]Universitat Autònoma de Barcelona, Serra Hunter Fellow, Department of Genetics and Microbiology, Bellaterra, Spain; [6]Carlos III Health Institute, Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER), Madrid, Spain; [7]Bambino Gesù Children's Hospital, Department of Laboratories, Roma, Italy; [8]Western University, Department of Pathology and Laboratory Medicine, London, Canada; [9]Erasmus University Medical Center, Department of Clinical Genetics, Rotterdam, Netherlands; [10]Bambino Gesù Children's Hospital, Laboratory of Medical Genetics, Translational Cytogenomics Research Unit, Roma, Italy; [11]Ospedale Molinette, AOU Città della salute e della scienza di Torino, Torino, Italy; [12]Bambino Gesù Children's Hospital, Laboratory of Transplant Immunogenetics, Department of Hematology/Oncology, Cell and Gene Therapy, Roma, Italy; [13]Erasmus MC, ENCORE Expertise Center for Neurodevelopmental Disorders, Rotterdam, Netherlands; [14]University of Campania Studies "Luigi Vanvitelli", Department of Woman, Child and of General and Specialist Surgery, Napoli, Italy; [15]University and Hospital of Perugia, Institute of Hematology and Center for Hemato-Oncology Research, Perugia, Italy; [16]University of Verona, Department of Neurosciences, Biomedicine and Movement Sciences, Verona, Italy; [17]Universitat Autònoma Barcelona, Institut de Recerca IR SANT PAU, Hospital Santa Creu i Sant Pau, Barcelona, Spain; [18]Catholic University of the Sacred Heart, Department of Pediatrics, Roma, Italy

Background/Objectives: Fanconi anemia (FA) is a clinically variable and genetically heterogeneous cancer-predisposing disorder representing the most common bone marrow failure syndrome. It is caused by inactivating predominantly biallelic mutations in >20 genes with roles in the FA/BRCA DNA repair pathway. Molecular diagnosis of FA is challenging due to the wide mutational spectrum, requiring the assessment of chromosomal fragility with DNA cross-linking agents to definitively confirm diagnosis.

Methods: We assessed peripheral blood genome-wide DNA methylation (DNAm) profiles in 25 subjects with molecularly confirmed clinical diagnosis of FA (FANCA complementation group) by means of EPIC v.1 array to identify differentially methylated CpGs able to distinguish subjects with FA from healthy individuals and subjects with other genetic disorders. Then, a machine-learning model able to classify DNAm profiles in unsolved cases was applied.

Results: The DNAm signature was clinically validated using 49 samples belonging to 12 complementation groups and carrying biallelic pathogenic variants, which were collected from peripheral blood and/or bone marrow aspirates. The episignature properly classified FA individuals belonging to 10 complementation groups (A-C, D2, E, G, J, L, P, T), including 8 reverted cases. The signature was used to classify 22 molecularly and/or clinically unclassified patients.

Conclusion: The use of DNAm profiling for functional classification of VUS in *FANC* genes or classification of molecularly uncharacterized patients is an informative tool to validate or rule out a clinical suspicion for a wide range of complementation groups, even in presence of gene conversion events.

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P09.009.C Mutational load of somatic variants in autoinflammatory diseases-associated genes

Jaume Reig[1], *Juan Ignacio Aróstegui*[2], *Ferran Casals*[1;3]

[1]*Facultat de Biologia, Genètica, Microbiologia i Estadística, Barcelona, Spain;* [2]*Hospital Clinic, Servei d'immunologia, Barcelona, Spain;* [3]*Institut de Biomedicina de la Universitat de Barcelona (IBUB), Barcelona, Spain*

Background/Objectives: Mosaicism results from post-zygotic DNA variants, which may occur during embryonic development or later in life, often restricted to specific cells or tissues. Increasing evidence support that somatic variants contribute to non-cancer diseases, mostly monogenic but also complex disorders. Notably, gene mosaicism plays a relevant role in the pathogenesis of inborn errors of immunity, especially in the group of autoinflammatory diseases (AIDs). Clinical manifestations in certain AIDs appear later in life, suggesting the expansion of preexisting mutant clones through clonal hematopoiesis. Herein we aim to characterize the mutational load of somatic variants at subclinical frequencies in two genes related to AIDs.

Methods: We have analyzed whole-genome sequences from >400,000 individuals aged 40-60 years in the UK Biobank repository. A bioinformatic pipeline to detect low-frequency variants was applied, combined with a set of filters based on sequencing and mapping quality, minimum reads supporting the alternative allele, strand bias and proximity to read ends and indels was applied to reduce the number of false positives.

Results: We have identified carriers of pathogenic variants in *UBA1* and *NLRP3*. This preliminary evidence may establish the characterization of the load of somatic variants with low allele frequencies in these genes, enabling to perform a comparative analysis about the spatial distribution between germline and somatic variants.

Conclusions: The genetic load of somatic variants in unaffected individuals is informative on the number of individuals at risk of developing an AID of postzygotic origin, which can be of relevance for physicians visiting patients with late-onset inflammatory disorders.

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Conflict of Interest: None declared

P09.010.D Systematic analyses of exome sequences of individuals and families with suspected primary immunodeficiencies indicates expanded phenotypes associated with known immunodeficiency genes and reveals several new candidate variants

Ulrike Hüffmeier[1], *Heinrich Sticht*[2], *Cornelia Kraus*[1], *Mandy Krumbiegel*[1], *Benjamin Frey*[3], *Nora Naumann-Bartsch*[4], *Jakob Zierk*[4], *Gisela Fecker*[4], *Katalin Blum*[1], *Melissa Pauly*[1], *Sarah Schuhmann*[1], *Mona Walther*[1], *Annika Willms*[1], *Axel Hueber*[5], *Norbert Blank*[6], *Andre Hörning*[7], *Matthias Galiano*[8], *Tobias Krickau*[8], *Jürgen Rech*[9;10], *André Reis*[1;11]

[1]*Institute of Human Genetics, Universitätsklinikum Erlangen, FAU Erlangen-Nürnberg, Erlangen, Germany;* [2]*Institute of Biochemistry, Friedrich-Alexander-Universität Erlangen-Nürnberg, Erlangen, Germany;* [3]*Department of Radiation Oncology, Friedrich-Alexander-Universität Erlangen-Nürnberg (FAU) and Universitätsklinikum Erlangen, Erlangen, Germany;* [4]*Pediatric Hematology and Oncology, Department of Pediatrics and Adolescent Medicine, Comprehensive Cancer Center Erlangen-EMN (CCC ER-EMN), University Hospital Erlangen, Friedrich-Alexander-Universität Erlangen-Nürnberg, Erlangen;* [5]*Division of Rheumatology, Klinikum Nürnberg, Paracelsus Medical University, Nürnberg, Germany;* [6]*Department of Medicine V, Amyloidosis Center and Division of Haematology, Oncology and Rheumatology, University of Heidelberg, Heidelberg, Germany;* [7]*Pediatric Gastroenterology, Department of Pediatrics and Adolescent Medicine, Friedrich-Alexander-Universität Erlangen-Nürnberg, Erlangen, Germany;* [8]*Pediatric Rheumatology, Department of Pediatrics and Adolescent Medicine, University Hospital Erlangen, Friedrich-Alexander-University Erlangen-Nuremberg, Erlangen, Germany;* [9]*Department of Internal Medicine 3 - Rheumatology and Immunology, Friedrich-Alexander-Universität Erlangen-Nürnberg and Universitätsklinikum Erlangen, Erlangen, Germany;* [10]*Deutsches Zentrum Immuntherapie, Friedrich-Alexander-Universität Erlangen-Nürnberg and Universitätsklinikum Erlangen, Erlangen, Germany;*

[11]Center for Rare Diseases Erlangen (ZSEER), University Hospital Erlangen, Erlangen, Germany

Background/Objectives: In immunologic disorders with suspected Mendelian inheritance, the yield of exome sequencing is around 20%, lower than for many other disorders. To increase the diagnostic yield, we extended analysis of exomes to VUS and candidate genes, when results were normal.

Methods: We investigated 112 cases, 46% singletons and 47% trios, mainly for autoinflammatory disorders (82%). In the first tier, a panel of up to 449 genes (Tangye et al. *J Clin Immunol* 2022) was analysed. Rare copy number variants (CNVs) were determined using ExomeDepth. In 90 patients with research consent, the analysis was extended to all genes.

Results: In 20% of patients, disease-associated variants (class IV-V) in seven established genes were detected; additional two individuals carried a VUS in 9-11% of reads. Among seven "hot VUS" in genes previously associated with immunodeficiencies, two were classified as likely pathogenic by either RT-PCR or flow-cytometry, one further as benign. Three others are under investigation. To identify further candidates, we assessed de novo and recessive variants and identified 36 candidate variants in 30 genes, two in the same gene. The encoded proteins of 26 genes were predicted to be deleterious by structural protein-modelling, and these genes were enriched for GO-term 'leukocyte activation'. In one gene, we found rare CNVs in two patients. CNVs affecting ≥1 exon were enriched for molecular function 'complement binding'.

Conclusion: Systematic analysis of exome variants in unsolved cases increases the diagnostic yield to currently 21% and identifies additional candidate genes requiring further investigation.

Grants: None.

Conflict of Interest: None declared

P09.011.A Clinical exome sequencing has a diagnostic yield of 36% in patients with suspected monogenic hematological and immunological defects

carmelo maria romeo[1;2], *Angelo Corso Faini*[1;2], *Giulia Margherita Brach del Prever*[1;2], *maria luca*[1;2], *edoardo spagnolo*[1;2], *davide caldo*[1;2], *Fiorenza Mioli*[1;2], *Claudia Saglia*[1;2], *Caterina Scolari*[1;2], *tullia carradori*[1;2], *Emilia Parodi*[3], *Stefania Nicola*[4], *Iuliana Badiu*[4], *Luca Lo Sardo*[4], *Federica Corradi*[4], *Luisa Brussino*[4], *Carmen Fava*[5], *Valentina Bonuomo*[5], *Daniela Cilloni*[5], *Chiara dellacasa*[6], *Valentina Giai*[6], *Alessandro Busca*[6], *Eloise Beggiato*[7], *Federica Di Biase*[7], *Matteo Olivi*[7], *Ilaria Bersano*[7], *Irene Dogliotti*[7], *Giuseppe Lanzarone*[7], *Luisa Giaccone*[7], *Benedetto Bruno*[7], *Francesco Licciardi*[8], *Davide Montin*[8], *Loredana Farinasso*[8], *Ugo Ramenghi*[8], *Giovanni Del Borrello*[9], *Veronica Barat*[9], *paola quarello*[9], *franca fagioli*[9], *Valeria Bracciamà*[1;2], *Francesca Arruga*[1;2], *Tiziana Vaisitti*[1;2], *Silvia Deaglio*[1;2], *Diana Carli*[1;2]

[1]Immunogenetics and Transplant Biology Service, Città della Salute e della Scienza University Hospital, Turin, Italy; [2]University of Turin, Department of Medical Sciences, Turin, Italy; [3]Pediatric and Neonatology Unit, Ordine Mauriziano Hospital, Turin, Italy; [4]Department of Medical Sciences, Allergy and Clinical Immunology Unit, University of Torino and Mauriziano Hospital, Turin, Italy; [5]Department of Clinical and Biological Sciences, Università di Torino, Turin, Italy; [6]Division of Haematology, Città della Salute e della Scienza, Turin, Italy; [7]Department of Molecular Biotechnology and Health Sciences, Division of Hematology, University of Torino, Turin, Italy; [8]Department of Public Health and Pediatrics, University of Turin, Pediatria Specialistica U, "Regina Margherita" Children Hospital, Turin, Italy; [9]Pediatric Oncology Department, Regina Margherita Children's Hospital, AOU Città della Salute e della Scienza di Torino, Turin, Italy

Background: Numerous blood and immune disorders exhibit monogenic etiology. We describe a cohort of molecularly characterized pediatric and adult patients with suspected monogenic immuno-hematologic conditions, reporting diagnostic rates based on specific diseases.

Methods: We recruited 70 patients that underwent clinical exome sequencing (CES) in 2023.

Results: mean age was 16.8 ± 19.3 years (range 0-83 years), 51/70 patients (73%) had <18 years and 40/70 (57%) were males. 44 patients (63%) had bone marrow failure (BMF) (including hyporegenerative anemia, congenital neutropenia, non-immune thrombocytopenia); 18 (26%) had immune dysregulation (including combined immunodeficiency, autoimmune lymphoproliferative syndrome, complement deficiency and autoinflammatory disease) and 8 (11%) had congenital hemolytic anemia (CHA). The overall detection rate (patients with pathogenic and likely pathogenic variants consistent with the phenotype) was 36%, varying according to the macrocategory: 4/8 (50%) for CHA, 16/44 (36%) for BMF and 5/18 (28%) for immune dysregulation. In 13/70 of cases (19%) variants of unknown significance (VUS) were identified. *SPTB* was the most frequently involved gene in CHA while in BMF 11 different genes were involved, being ribosomal genes *GATA2* and *ELANE* the most represented. One case presented a double diagnosis (*ALAS2* and *G6PD*). No significant difference in diagnostic rates was observed between pediatric and adult patients (18/51, 35% vs 6/19, 32%, $p = 0.8$); however, the adult population was enriched in young adults with a heightened suspicion of monogenic disease.

Conclusion: our data confirm that CES analysis has a high diagnostic rate in immuno-hematological disease and is useful to define diagnosis, guide treatment and determine recurrence risk.

Conflict of Interest: None declared

P09.012.B A novel mutation in *MECOM* affects *MPL* regulation in vitro and results in thrombocytopenia and bone marrow failure

Daniele Ammeti[1], *Antonio Marzollo*[2], *Maria Gabelli*[2], *Melania Eva Zanchetta*[1], *Caterina Tretti-Parenzan*[2], *Roberta Bottega*[1], *Valeria Capaci*[1], *Alessandra Biffi*[2], *Anna Savoia*[3], *Silvia Bresolin*[2], *Michela Faleschini*[1]

[1]1Institute for Maternal and Child Health – IRCCS Burlo Garofolo, Trieste, Italy; [2]Padua University and Hospital, Paediatric Haematology, Oncology and Stem Cell Transplant Division, Women and Child Health Department, Padua, Italy; [3]University of Verona, Verona, Italy

Background/Objectives: MECOM-associated syndrome (MECOM-AS) is a rare genetic disease characterized by a continuous spectrum of clinical phenotypes ranging from isolated radioulnar synostosis to severe thrombocytopenia and bone marrow failure.

Of note, the haematological phenotype of MECOM-AS partially overlaps with other congenital amegakaryocytic thrombocytopenia (CAMT), caused by mutations in *MPL* or *THPO* genes.

MECOM-AS is caused by mutations in *MECOM* gene, which encodes the transcription factor EVI1. Although EVI1 plays an important role in haematopoiesis, the correlation between EVI1 dysfunction and thrombocytopenia remains largely unexplained.

Here we report the functional characterization of a novel variant of *MECOM* gene and suggest a possible role of *MPL* gene in the pathogenesis of MECOM-AS.

Methods: Mutational screening was performed through targeted NGS analysis. Luciferase reporter assays on the promoters of several target genes were conducted to determine the pathogenic role of the variant and to investigate the role of EVI1 on the transcriptional regulation of *MPL*.

Results: In a paediatric patient affected by a severe thrombocytopenia, we identified a novel de novo variant in *MECOM* gene (p.P634L). Functional studies showed that p.P634L impairs repressive activity of the transcription factor on several promoters. Moreover, we demonstrated that EVI1 plays a role in the transcriptional regulation of the *MPL* gene.

Conclusion: We demonstrated the pathogenicity of the variant, providing a certain molecular diagnosis to the patient. Furthermore, we suggest an involvement of *MPL* in the pathogenic mechanism underlying MECOM-AS, potentially explaining the overlap of the haematological defects observed in patients with *MECOM* or *MPL* mutations.

Conflict of Interest: None declared

P09.013.C Functional correction of DNA Ligase 1 deficiency in a homozygous patient-derived cell line

*Di Liu[1], **Yu-zhen Xiao**[1], Longlong Liu[2], Kristine Bousset[1], Britta Wieland[1], Michael Eckrich[3], Philip Roehrs[4], Virginia Thurston[5], Laurie Demmer[6], Niraj Patel[7], Jeffrey Huo[4], Thilo Dörk[1]*

[1]Hannover Medical School, Gynaecology Research Unit, Hannover; [2]University Hospital Münster, Department of Medicine A, Hematology, Oncology and Pneumology, Münster; [3]Texas Transplant Institute, Pediatric Blood & Marrow Transplant, Charlotte, United States; [4]Levine Children's Hospital, Pediatric Blood & Marrow Transplant, Charlotte, United States; [5]Carolinas Pathology Group, Charlotte, United States; [6]Levine Children's Hospital, Pediatric Genetics, Charlotte, United States; [7]Levine Children's Hospital, Pediatric Infectious Diseases, Charlotte, United States

Background: Human DNA ligase 1 (LIG1) is known to play a role in joining Okazaki fragments and participating in some DNA repair processes. LIG1 deficiency is a very rare autosomal recessive disorder with only seven patients described so far.

Patient/Methods: We used whole exome sequencing and Sanger validation to identify the genetic basis of disease in a seven-year-old girl with immunodeficiency, MDS, dysmorphic features, and growth retardation. We investigated a skin fibroblast line derived from the patient using immunoblotting, flow cytometry and proliferation assays, and we performed ABE single-base editing to correct the mutational defect.

Results: We found homozygosity for a missense variant, p.R305Q (*LIG1*c.914G>A), in the *LIG1* gene of the index patient. The variant was associated with a strong reduction of LIG1 protein in fibroblasts derived from the patient. Reversion of the p.R305Q homozygosity to the wildtype genotype using an ABE single-base editing approach fully restored LIG1 protein levels in the patient fibroblast line and corrected its aberrant cell cycle profile and proliferation in flow cytometry, EdU and xCELLigence measurements.

Conclusion: Homozygosity for the pathogenic missense variant p.R305Q gives rise to human LIG1 deficiency. Our isogenic pair of LIG1-deficient and LIG1-proficient fibroblast lines may become a useful tool to further study the functions of DNA ligase 1.

Conflict of Interest: None declared

P09.015.A Polygenic risk emerges from exome sequencing of inherited thrombocytopenia

Federica Isidori[1], *Federica Melazzini[2;3], Giulia Erini[4], Valeria Bozzi[2], Tania Giangregorio[1], Alessandro Mattiaccio[4], Carlo Sidore[5], Marco seri[1], Alessandro Pecci[2;3], Caterina Marconi[1], Tommaso Pippucci[1]*

[1]IRCCS Azienda Ospedaliero-Universitaria di Bologna, Bologna, Italy; [2]IRCCS Policlinico San Matteo Foundation, Medical Department, Pavia, Italy; [3]University of Pavia, Department of Internal Medicine, Pavia, Italy; [4]Alma Mater Studiorum-University of Bologna, Department of Medical and Surgical Sciences (DIMEC), Bologna, Italy; [5]Istituto di Ricerca Genetica e Biomedica-CNR, Cagliari, Italy

Background/Objectives: Inherited thrombocytopenia (IT) is a heterogeneous group of bleeding disorders characterized by low platelet count (PC). Exome Sequencing (ES) so far has focused on the identification of rare variants with large effect, discovering >50 genes and diagnosing <50% of patients. However, GWAS reported the presence of common variants with low or moderate effect that are overlooked in clinical analysis although their combined effect may have a large effect on PC. We sought to assess their contribution to IT risk.

Methods: ES was performed on a large cohort of IT patients (N = 219 from 152 families). Rare variants were searched across 52 IT-associated genes and 56 common SNPs among the 577 reported in a published PLT-GWAS. Polygenic risk scores (PRS) were calculated in the 152 IT family probands and in 1350 individuals ascertained for non-IT disorders (COVID-19, N = 603 and Epilepsy, N = 747).

Results: Identification and segregation of rare variants divided the probands in diagnosed (N = 45) and undiagnosed (N = 107). Median PRS distribution was significantly lower for IT patients versus non-IT individuals, and even lower in undiagnosed probands (Wilcoxon *p.adjust* = 0.001 and 0.00018, in the two non-IT cohorts, respectively). Interestingly, in 6 probands (4% of probands), a key contributor to this effect was identified in a common coding variant with large effect in an established Mendelian IT gene.

Conclusion: The genetic basis of IT is more complex than commonly thought, and PRS can be useful in clinical evaluation of IT patients.

Grants: Italian Ministry of Health Young Investigators Grant GR-2018-12367235

Conflict of Interest: None declared

P09.016.B VEXAS syndrome – expanding the phenotypic spectrum of this underdiagnosed disorder in rheumatology and hematology

Melissa Pauly[1], *Jürgen Rech[2;3;4], Norbert Blank[5;6;7], Alina Ramming[2;3], Melanie Hagen[2;3], Jochen Wacker[2;3], Bernhard Manger[2;3], Dorit Rech[4;8], Axel Hueber[9], Dorothee Kaudewitz[5;6], André Reis[1;4], Ulrike Hüffmeier[1]*

[1]Institute of Human Genetics, Universitätsklinikum Erlangen, Friedrich-Alexander-Universität Erlangen-Nürnberg, Erlangen, Germany; [2]Department of Internal Medicine 3 - Rheumatology and Immunology, Friedrich-Alexander-Universität Erlangen-Nürnberg and Universitätsklinikum Erlangen, Erlangen, Germany; [3]Deutsches Zentrum Immuntherapie, Friedrich-Alexander-Universität Erlangen-Nürnberg and Universitätsklinikum Erlangen, Erlangen, Germany; [4]Center for Rare Diseases Erlangen (ZSEER), University Hospital Erlangen, Erlangen, Germany; [5]Department of Internal Medicine V, Division of Rheumatology and Amyloidosis Center, University of Heidelberg, Heidelberg, Germany; [6]Zentrum Für Seltene Erkrankungen Heidelberg (ZSE HD), Heidelberg, Germany; [7]Internal Medicine 5, Amyloidosis Center and Division of Hematology, Oncology and Rheumatology, University Hospital Heidelberg, Heidelberg, Germany; [8]Department of Internal Medicine 5 - Hematology and Oncology, Friedrich-Alexander-Universität Erlangen-Nürnberg and Universitätsklinikum Erlangen, Erlangen, Germany; [9]Abteilung für Rheumatologie, Klinikum Nürnberg, Paracelsus Medizinische Privatuniversität, Nürnberg, Germany

Background/Objectives: VEXAS syndrome (vacuoles, E1 enzyme, X-linked, autoinflammatory, somatic; MIM#301054) is a recently

described rare, adult-onset autoinflammatory disorder in males caused by somatic *UBA1* variants. It is characterized by wide range of inflammatory and hematological manifestations. We now investigated a VEXAS cohort from Germany.

Methods: We obtained whole blood samples of eight individuals with suspicion of VEXAS syndrome – mainly based on clinical presentation, gender, and age – and performed exome sequencing to assess variants in a gene panel of periodic fever syndromes or in a targeted analysis of *UBA1*.

Results: In 7/8 males, we identified a pathogenic *UBA1* variant affecting amino-acid position 41 (3x p.(Met41Leu), 3x p.(Met41Thr), 1x p.(Met41Val)), somatic mosaicism ranged between 35-92%. A coding *UBA1* variant could not be identified in the eighth affected individual.

Mean age at genetic diagnosis was 70.3 years, average disease duration 2.5 years. Main manifestations were relapsing polychondritis, skin lesions, various autoinflammatory symptoms, and infections. Most individuals had macrocytic anemia and elevated inflammation parameters. We could not observe any correlation between degree of somatic mosaicism in PBMCs and disease severity.

Conclusion: This high detection rate indicates that VEXAS syndrome is more frequent than initially thought and highlights that molecular testing is indicated in all cases with suspected VEXAS syndrome. Testing should be expanded to individuals with less typical manifestations, especially since effective specific treatments were reported in individual patients, e.g. hypomethylating therapeutics leading to remission of clones with *UBA1* variant.

Conflict of Interest: None declared

P09.017.C Pathogenic Roles of a Human Telomerase RNA (hTR) template mutation in Dyskeratosis Congentia

Nathaniel Deimler[1], *Laura Holthöfer*[2], *Alex Orioli*[1], *Nadine Bobon*[1], *Matthias Linke*[2], *Verena Engelhardt*[2], *Mareike Selig*[2], *Fabian Beier*[3], *Susann Schweiger*[2;4], *Peter Baumann*[1;4]

[1]*Johannes Gutenberg University Mainz, Department of Biology, Mainz, Germany;* [2]*University Medical Centre of the Johannes Gutenberg University Mainz, Institute of Human Genetics, Mainz, Germany;* [3]*Medical Faculty, RWTH Aachen University, Department of Hematology, Oncology, Hemostaseology and Stem Cell Transplantation, Aachen, Germany;* [4]*Institute for Molecular Biology, Mainz, Germany*

Introduction: Dyskeratosis congenita (DKC), a telomere biology disorder resulting from mutations in telomere maintenance mechanisms, is characterized by a triad of dysplastic nails, reticular skin hyperpigmentation, and oral leukoplakia. DKC commonly leads to bone marrow failure, carcinomas, and pulmonary fibrosis. Telomerase, a ribonucleoprotein responsible for telomere elongation, is composed of a catalytic protein subunit (TERT), which reverse transcribes the non-coding RNA subunit (*TERC*). While many *TERC* mutations have been identified that affect the stability or folding of telomerase RNA, mutations in the template region are rare.

Methods: Clinical characterizations were coupled with RNA-Seq, WGS, and telomere length measurements by Flow-FISH and nanopore sequencing.

Results: The patient presented with a classic case of DKC carrying a heterozygous *TERC*-variant r.50C>A, inherited from his deceased mother and present in his deceased twin sister who died due to DKC-related complications at the ages of 58 and 21, respectively. r.50C>A hTR telomerase exhibits a decreased processivity compared to wild-type telomerase resulting in shortened telomeres, <10th percentile by Flow-FISH in all individuals carrying the mutation within this family. The maternal aunt, also carrying the *TERC*-mutation, showed no signs of DKC until age 58 with telomere lengths <10th percentile. The healthy aunt's daughter does not carry the *TERC* mutation and shows normal telomere lengths, despite inheriting mutant telomeric repeats from her mother.

Conclusion: While the effect of mutant repeat load in the absence of mutant telomerase is unknown, it is clear the mutant, non-processive telomerase results in severely shortened telomeres and the DKC phenotype observed in the index patient and his twin sister.

Conflict of Interest: None declared

P09.018.D Autoinflammatory diseases and germline vs. somatic mosaic variations

Eman Assrawi[1], *Camille Louvrier*[1;2], *William Piterboth*[2], *Florence Dastot-Le Moal*[2], *Farah Diab*[1], *aphrodite daskalopoulou*[1], *Rahma mani*[1], *romain Levergeois*[2], *quentin brandao*[1], *Bruno Copin*[2], *Angela Arenas-Garcia*[1], *Marie Legendre*[2], *Sonia Karabina*[1], *Serge AMSELEM*[1;2], **Irina Giurgea**[1;2]

[1]*Sorbonne Université, Inserm, Childhood Genetic Diseases Laboratory, Hôpital Armand-Trousseau, 75012 Paris, France., Paris, France;* [2]*Département de Génétique Médicale, APHP Hôpital Armand-Trousseau, 75012 Paris, France., Paris, France*

Background/Objectives: The study primarily investigates somatic mosaic variations within systemic autoinflammatory diseases (SAID) genes and their correlation with disease phenotypes, specifically focusing on variations in *NLRP3* and *TNFRSF1A* leading to NLRP3-AIDs and TRAPS (Tumor necrosis factor-Receptor-Associated Periodic Syndrome) respectively.

Methods: From 2018 to 2023, deep-targeted NGS was used to analyze the leukocyte DNAs of 800 patients using a SAID gene panel. All mosaic variations in SAID genes from PubMed and Infevers databases were reviewed.

Results: In *NLRP3*, 9 mosaic variations were identified in 12 independent patients, along with 10 germline variations in 10 patients. The literature reports 34 mosaic and 88 germline *NLRP3* variations.

Phenotype: genotype correlations suggest that *NLRP3* variations found only somatically may be incompatible with life if present germinally, while variations found only in germinal state could be asymptomatic in mosaic state.

In *TNFRSF1A*, two mosaic variations were found in three patients. The literature reports only three other mosaic variations. No obvious difference in disease severity between germline and mosaic variations has been observed.

Very few mosaic variations have been reported in the literature in other SAID genes: *NLRC4* (n = 4), *STING1* (n = 2), *NOD2* (n = 2), and *JAK1* (n = 1), precluding any genotype: phenotype correlation studies.

Most of mosaic variations identified in SAID genes were found to be widely distributed across different tissues.

Conclusion: The study underscores the high frequency of mosaic variations in NLRP3-AIDs and emphasizes the symptomatic nature of mosaic variations, even at very low allele frequencies (below 5%), potentially leading to false-negative diagnostic tests without deep sequencing.

Grants: H2020_European Union_779295.

Conflict of Interest: None declared

P09.019.C Bone marrow failure and immunodeficiency associated with human RAD50 variants

*Akihiro Hoshino[1;2], Masatoshi Takagi[1;3], **Kristine Bousset**[4], Jule Röddecke[4], Hanna Luisa Redeker[4], Iulia Folcut[4], Dan Tomomasa[1], Junya Kobayashi[5], Xi Yang[2;6], Naoki Sakata[7], Kenichi Yoshida[8], Satoru Miyano[9;10], Seishi Ogawa[8], Seiji Kojima[11], Tomohiro Morio[1], Thilo Dörk[4], Hirokazu Kanegane[12]*

[1]Tokyo Medical and Dental University, Department of Pediatrics and Developmental Biology, Graduate School of Medical and Dental Sciences, Tokyo, Japan; [2]Tokyo Medical and Dental University, Department of Pediatrics, Graduate School of Medicine and Pharmaceutical Sciences, Tokyo, Japan; [3]Tokyo Medical and Dental University, Department of Community Pediatrics, Perinatal and Maternal Medicine, Tokyo, Japan; [4]Hannover Medical School, Gynaecology Research Unit, Hannover, Germany; [5]Kyoto University, Department of Genome Repair Dynamics, Radiation Biology Center, Kyoto, Japan; [6]Children's Hospital of Chongqing Medical University, Division of Immunology, Chongqing, China; [7]Kindai University – Osakasayama Campus, Department of Pediatrics, Osakasayama, Japan; [8]Kyoto University, Department of Pathology and Tumor Biology, Graduate School of Medicine, Kyoto, Japan; [9]University of Tokyo, Laboratory of DNA Information Analysis, Human Genome Center, Institute of Medical Science, Tokyo, Japan; [10]University of Tokyo, Laboratory of Sequence Analysis, Human Genome Center, Institute of Medical Science, Tokyo, Japan; [11]Nagoya University, Department of Pediatrics, Nagoya University Graduate School of Medicine, Nagoya, Japan; [12]Tokyo Medical and Dental University, Department of Child Health and Development, Graduate School of Medical and Dental Sciences, Tokyo, Japan

Background/Objectives: The MRE11-RAD50-NBN (MRN) complex plays a key role in cellular DNA double-strand break response. Pathogenic variants in NBN and MRE11 give rise to the autosomal-recessive Nijmegen breakage syndrome (NBS) and ataxia telangiectasia-like disorder (ATLD), respectively. The clinical consequences of pathogenic variants in RAD50 are less well defined. RAD50 deficiency resembles NBS, but the first two patients described showed no immunodeficiency.

Methods: We report on a girl with microcephaly, mental retardation, bird-like face, short stature, bone marrow failure and B-cell immunodeficiency. We searched for candidate genes by whole-exome sequencing and analyzed the cellular phenotype of patient-derived fibroblasts using immunoblotting and functional complementation experiments.

Results: Compound heterozygosity for two variants in the *RAD50* gene (p.Arg83His and p.Glu485Ter) was identified in this patient. Expression of RAD50 protein and the MRN complex were maintained in patient derived fibroblasts. However, DNA damage-induced ATM kinase activity was markedly decreased, which was restored by the expression of wild-type RAD50. The RAD50*p.R83H missense substitution did not rescue the mitotic defect of RAD50-deficient fibroblasts, whereas wild-type RAD50 did. The RAD50*-p.E485X nonsense variant was associated with in-frame skipping of exon 10 (p.Glu485_545del).

Conclusion: These findings provide evidence for an important role of RAD50 in human bone marrow and immune cells. RAD50 deficiency should be considered in patients with an inborn bone marrow failure and B-cell immunodeficiency, in addition to its known features of microcephaly and short stature.

Grants: Research on Measures for Intractable Disease Project, Claudia von Schilling Foundation for Breast Cancer Research, MEXT/JSPS KAKENHI (# 22K07887).

Conflict of Interest: None declared

P09.020.A Genome-wide association study of COVID-19 vaccine side effects in Eastern Europe

Alexander Rakitko[1], *Elena Kovalenko[1], Alexey Kamelin[1], Anna Kim[1], Nicolay Plotnikov[1]*

[1]Genotek, Moskva, Russian Federation

Background/Objectives: COVID-19 vaccination has proven to be an effective method to reduce the incidence of coronavirus infection and the severity of its course. Post-vaccination adverse events varied among individuals, indicating a potential genetic basis for the different responses to the vaccine.

Methods: We studied the COVID-19 vaccine adverse reaction in a multi-ethnic group of 24381 vaccine (Sputnik-V) recipients (15545 controls, 8836 cases). The surveys encompassed inquiries regarding a spectrum of 11 symptoms, in addition to assessing the overall intensity of the post-vaccination reaction and its subsequent influence on daily activities. All samples were genotyped using Illumina GSA v3 microarray.

Results: Our study has detected several genome-wide significant loci on chromosome 6. Specifically, some alleles which showed the most significant association with severe COVID-19 vaccine reaction were HLA-DRB1*03:01 (OR = 0.807, p-value = 2.57×10^{-13}), HLA-DQA1*05:01 (OR = 0.806, p-value = 4.50×10^{-14}), HLA-DQB1*02:01 (OR = 0.809, p-value = 1.02×10^{-13}). This combination of alleles, known as DR3-DQ2, is associated with conditions such as celiac disease and diabetes mellitus. Our analysis has also revealed HLA-DRB1*07:01 (OR = 1.199, p-value = 1.92×10^{-14}), HLA-DQA1*02:01 (OR = 1.185, p-value = 1.28×10^{-13}).

Conclusion: We conducted the largest genome-wide association study to date on adverse reactions to the COVID-19 vaccine in a cohort of Eastern European descent. Our study underscores the significant associations between HLA genes and vaccine-related side effects. These insights have the potential to be integrated into clinical practice to guide personalized vaccine selection.

Conflict of Interest: None declared

P09.021.B Late onset of an autoinflammatory disease: identification and functional characterization of a mosaic variation of *NLRC4*

*Farah Diab[1], Camille Louvrier[1;2], Marc Fabre[3], aphrodite daskalopoulou[1], Eman Assrawi[1], **Rahma mani**[1], quentin brandao[1], romain Levergeois[2], Angela Arenas-Garcia[1], Florence Dastot-Le Moal[2], William Piterboth[2], frederic lezot[1], Sonia Karabina[1], Serge AMSELEM[1;2], Irina Giurgea[1;2]*

[1]Sorbonne Université, Inserm, Childhood Genetic Diseases Laboratory, Hôpital Armand-Trousseau, Paris, France; [2]Département de Génétique Médicale, Assistance Publique–Hôpitaux de Paris (APHP), Hôpital Armand-Trousseau, Paris, France; [3]Service de Médecine interne, Maladies infectieuses et Hématologie, Centre Hospitalier Pierre Oudot, Bourgoin-Jallieu, France

Background/Objectives: *NLRC4* gene encodes an essential component of the NLRC4 inflammasome, a multiprotein complex involved in activating inflammatory pathways. Germline *NLRC4* variations are associated with severe forms of autoinflammatory diseases (AID) in children, often involving enterocolitis and potentially leading to macrophage activation syndrome. Somatic mosaic variations in *NLRC4* have been reported in only two adult patients. This study aimed to identify the molecular basis of adult-onset AID without macrophage activation syndrome.

Methods: The study involved deep sequencing of the patient's leukocyte genomic DNA through a panel of AID genes and functional evaluation of the identified *NLRC4* variation in

HEK293T cells expressing different protein partners (ASC and Caspase1) enabling the detection of specks, a hallmark of NLRC4 inflammasome activation. NF-κB pathway activation was also studied by a reporter gene system.

Results: The patient, a 28-year-old man, had a history of unlabeled psychosis since the age of 19, and severe inflammatory episodes from the age of 21. Sequencing revealed the presence of a mosaic variation in *NLRC4*, c.398C>T p.(Thr133Ile), with the mutant allele represented at a ratio of 5%. Functional studies demonstrated that this variation has a gain-of-function pathogenic effect on inflammation, as evidenced by a significant increase in the percentage of specks in cells expressing mutant NLRC4 and the activation of the NF-κB pathway.

Conclusion: This study identified a novel mosaic variation of *NLRC4* associated with adult-onset autoinflammatory disease (AID) without macrophage activation syndrome, and demonstrated its gain-of-function pathogenic effect on inflammation.

Grants: H2020_European Union_779295.

Conflict of Interest: None declared

P09.022.C MR-PheWAS of circulating neutrophil characteristics and 20,740 disease outcomes, proteins and metabolites identifies multiple novel causal associations

Katy Fleming[1], *Emma Vincent*[2], *Borko Amulic*[1], *Andrew Mumford*[1], *Kate Burley*[1]

[1]*University of Bristol, School of Cellular and Molecular Medicine;* [2]*University of Bristol, Bristol Medical School*

Background/Objectives: Neutrophils mediate innate immunity and also inflammatory pathologies such as atherosclerosis and autoimmunity. Neutrophil granules are the likely effectors of these activities, yet evidence for causal associations between granularity and common human disease is sparse. Here we apply Mendelian randomisation (MR)-PheWAS to explore associations with circulating neutrophil count (NC) and side scatter (NSSC), a surrogate for granularity also measured in the blood count test.

Methods: Genetic variants independently associated with NC ($n = 154$) and NSSC ($n = 72$) in a prior European-ancestry GWAS were used to proxy exposures. Phenome-wide associations against 20,740 traits were identified by MR.

Results: NC and NSSC had weak phenotypic ($r = 0.03$) and genetic correlation ($rg = -0.02$), indicating that they are distinct cellular traits. NC had a causal effect (FDR $p < 0.05$) on 121 outcomes, including abundance of gut microbiota (e.g. Lactobacillus, -0.12 SD/SD increase in NC; FDR $p = 4.6 \times 10^{-2}$), products of anaerobic metabolism (e.g. lactate, 0.15 SD; FDR $p = 1.6 \times 10^{-18}$) and multiple measures of adiposity (e.g. BMI, -0.07 SD; FDR $p = 4.5 \times 10^{-2}$). NC-associated variants were predictive of Alzheimer's disease (OR 5.1 per SD increase in NC; FDR $p = 5.1 \times 10^{-4}$), consistent with previous association studies. NSSC had a causal effect on 26 outcomes, >80% of which were not associated with NC. These included dental problems (OR 0.99; FDR $p = 3.9 \times 10^{-2}$), a penetrant phenotype in rare Mendelian neutrophil granulopathies.

Conclusion: We report the first large-scale analysis of phenotypes causally associated with neutrophil characteristics. The approach identifies associations with infection and inflammation phenotypes already associated with neutrophils, and multiple hitherto unknown associations which warrant further functional investigation.

Grants:

Conflict of Interest: None declared

P09.023.D Bi-allelic MCM10 variants cause NK-cell deficiency manifesting a severe skin disorder and childhood malignancy

Yael Hoffman[1;2], *Alina Kurolap*[1;2], *Adi Mory*[1;2], *Hagit Baris Feldman*[1;2], *Daphna Marom*[1;2], *David Hagin*[2;3]

[1]*Tel Aviv Sourasky Medical Center, The Genetics Institute and Genomics Center, tel-aviv, Israel;* [2]*Tel Aviv University, Faculty of Medicine, tel-aviv, Israel;* [3]*Tel Aviv Sourasky Medical Center, Allergy and Clinical Immunology Unit, Department of Medicine, tel-aviv, Israel*

Background/Objectives: Minichromosome maintenance (MCM) proteins are essential for DNA replication and genome stability. MCM2-7 function as the core of replicative helicase, and together with the GINS complex and CDC45, form the CDC45:MCM2-7:GINS (CMG) helicase complex, which is stabilized by MCM10. Defects in CMG proteins, including MCM4, GINS1 and GINS4, were shown to be associated with natural killer (NK) cell deficiency in a limited number of individuals. Similarly, bi-allelic variants in *MCM10* were recently described as causing lethal NK cell deficiency in one subject, and prenatal cardiomyopathy with hypoplastic thymus and spleen in three embryos with progressive fetal hydrops. We report the first case of a rare lethal malignancy in a child homozygous for a novel *MCM10* variant.

Methods: Clinical case report, exome sequencing, RNA studies and flow cytometry-based immune-phenotyping.

Results: A six-year-old male presented with extensive poikyloderma, photosensitivity, and suspected chronic oral candidiasis. Urgent exome sequencing detected a homozygous pathogenic splice-site variant in *MCM10* (NM_018518.5): c.930+2T>A. The patient later developed and succumbed to an aggressive, EBV-positive, squamous cell carcinoma of the tongue. RNA studies confirmed that the identified variant leads to deletion of 51 bp from the 3'-end of exon 7, resulting in an in-frame deletion of 17 amino acids within the protein's oligonucleotide/oligosaccharide-binding (OB)-fold domain, critical for *MCM10* function. Immunophenotyping showed near absent NK cells with significant increase in NK-T cells and in terminally differentiated CD8 T-cells.

Conclusion: *MCM10* is a new autosomal recessive pediatric cancer predisposing gene, linking NK-cell deficiency and cancer risk. MCM10-deficiency belongs to an emerging group of syndromes we term "minichromosomopathies".

Conflict of Interest: None declared

P09.024.A Identification of a large novel inversion in the *F8* gene by long-read sequencing in two siblings with severe Haemophilia A

Gregor Gilfillan[1], *Marit Sletten*[1], *June Åsheim*[1], *Heidi Knudsen*[2], *Knut Erik Berge*[1], *Nina Iversen*[1]

[1]*Oslo University hospital Ullevål, Dept. Medical Genetics, Oslo, Norway;* [2]*Oslo University hospital, Dept. Rare Disorders, Oslo, Norway*

Background/Objectives: Two brothers diagnosed with severe Haemophilia A had FVIII levels <1% at birth, and were therefore suspected to harbour a mutation in the *F8* gene. The patients were first subjected to standard genetic testing: *F8* Intron 22 Inversion, Sanger sequencing (all 26 *F8* exons and exon–intron junctions), MLPA, whole genome sequencing (WGS) and mRNA analysis to detect splicing defects. No genomic defects were found, but *F8* mRNA could not be detected. We therefore tested for the presence of structural genomic variants using long-read sequencing with Pacific Biosciences technology.

Methods: High molecular weight DNA was extracted from blood and sequenced on a Pacific Biosystems Revio system. The genomic rearrangement detected by sequencing was confirmed using break-point PCR and Sanger sequencing.

Results: Long-read sequencing revealed a 143 kb inversion disrupting the *F8* gene. Interestingly, the inversion was not detected by the PacBio SV caller PBSV, but was obvious upon manual inspection of aligned reads. The inversion was confirmed by PCR spanning the breakpoints, and subsequent Sanger sequencing of the amplified products. Inversion breakpoints were in intronic segmental duplication sequences of ca. 1 kb, precluding diagnosis with standard-of-care methods. The rearrangement inverted the promoter and first exon of the *F8* gene, suggesting a full disruption of the gene. This conclusion is supported by the lack of detectable *F8* mRNA.

Conclusion: Long-read sequencing succeeded in diagnosing a case of FVIII insufficiency caused by a genomic inversion that was not possible to detect with standard diagnostic tools or RNA analysis.

Grants: None

Conflict of Interest: None declared

P09.025.B Molecular challenges in the diagnosis of X-linked chronic granulomatous disease: CNVs, intronic variants, skewed X-chromosome inactivation, and gonosomal mosaicism

Laura Batlle-Masó[1;2;3], *Jacques Riviere*[1;2], *Clara Franco-Jarava*[4;5], *Andrea Martin-Nalda*[1;2], *Marina Garcia-Prat*[1;2], *Alba Parra-Martínez*[1;2], *Aina Aguilo-Cucurull*[4;5], *Neus Castells*[6;7], *Monica Martinez-Gallo*[4;5], *Pere Soler-Palacin*[1;2], *Roger Colobran*[4;5;7]

[1]*Vall d'Hebron Research Institute (VHIR), Infection and Immunity in pediatric patients, Barcelona, Spain;* [2]*Vall d'Hebron University Hospital (HUVH), Pediatric Infectious Diseases and Immunodeficiencies Unit, Barcelona, Spain;* [3]*Universitat Pompeu Fabra, Barcelona, Spain;* [4]*Vall d'Hebron Research Institute (VHIR), Translational Immunology, Barcelona, Spain;* [5]*Vall d'Hebron University Hospital (HUVH), Immunology Division, Barcelona, Spain;* [6]*Vall d'Hebron Research Institute (VHIR), Medicine genetics research group, Barcelona, Spain;* [7]*Vall d'Hebron University Hospital (HUVH), Clinical and Molecular genetics department, Barcelona, Spain*

Chronic granulomatous disease (CGD) is a prototypical inborn error of immunity affecting phagocytes, in which these cells are unable to produce reactive oxygen species. CGD is caused by defects in genes encoding subunits of the NADPH oxidase enzyme complex (CYBA, CYBB, CYBC1, NCF1, NCF2, NCF4); inflammatory responses are dysregulated, and patients are highly susceptible to recurrent severe bacterial and fungal infections. X-linked CGD (XL-CGD), caused by mutations in the CYBB gene, is the most common and severe form of CGD.

In this study, we describe the analytical processes undertaken in 3 families affected with XL-CGD to illustrate several molecular challenges in the genetic diagnosis of this condition: in family 1, a girl with a heterozygous deletion of CYBB exon 13 and skewed X-chromosome inactivation (XCI); in family 2, a boy with a hemizygous deletion of CYBB exon 7, defining its consequences at the mRNA level; and in family 3, 2 boys with the same novel intronic variant in CYBB (c.1151 + 6 T > A). The variant affected the splicing process, although a small fraction of wild-type mRNA was produced. Their mother was a heterozygous carrier, while their maternal grandmother was a carrier in form of gonosomal mosaicism.

In summary, using a variety of techniques, including an NGS-based targeted gene panel and deep amplicon sequencing, copy number variation calling strategies, microarray-based comparative genomic hybridization, and cDNA analysis to define splicing defects and skewed XCI, we show how to face and solve some uncommon genetic mechanisms in the diagnosis of XL-CGD.

Conflict of Interest: None declared

P09.026.C A cohort of patients with cryopyrin-associated periodic syndromes caused by postzygotic NLRP3 pathogenic variants

Nuria Bonet[1], *Juan Ignacio Aróstegui*[2], *Ferran Casals*[3]

[1]*Universitat Pompeu Fabra, Genomics Core Facility, Barcelona, Spain;* [2]*Hospital Clínic, Immunology Department, Barcelona, Spain;* [3]*Universitat de Barcelona, Facultat de Biologia, Barcelona, Spain*

Background/Objectives: Cryopyrin-associated periodic syndrome (CAPS) is a rare autoinflammatory disorder (AID) caused by *gain-of-function NLRP3* variants. Although most CAPS patients are carriers of germline *NLRP3* pathogenic variants, several cases of post-zygotic variants have been reported.

We hypothesize that the clinical phenotype in those patients with mosaicism may be a direct consequence of its cellular distribution and/or the variant allele frequency (VAF). To test this hypothesis, we aim to deeply characterize the frequency and distribution of the somatic *NLRP3* variant in CAPS patients with mosaicism, and its evolution along time.

Methods: Amplicon-based deep sequencing (ADS) was used to assess the VAF at different time points, if possible, in peripheral blood, isolated leukocyte subpopulations and nail clippings.

Results: We identified 17 individuals carrying somatic (likely) pathogenic variants at *NLRP3*. At time of diagnosis, the mean VAF was 11.9%. All *NLRP3* variants were located at exon 4, with 5 of them being novel variants. VAF at different time points and mosaicism distribution were evaluated in 7 individuals, revealing either mosaicism stability along the time, and myeloid restriction in only one patient, respectively.

Conclusions: We herein genetically evaluated one of the largest cohort of patients with CAPS and *NLRP3* mosaicism. Collected evidences support for an early occurrence of the mutational event, as well as VAF stability along the time. Additional experiments are currently ongoing to evaluate the potential relationship of VAF with the patients' phenotype.

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Conflict of Interest: None declared

P10

INTELLECTUAL DISABILITY

P10.001.D

P10.001.D Variant-specific pathophysiological mechanisms of *AFF3* differently influence transcriptome profiles

Sissy Bassani[1;2], *Jacqueline Chrast*[1], *Ambrosini Giovanna*[3;4], *Norine Voisin*[1;5], *Frédéric Schütz*[6], *Alfredo Brusco*[7;8], *Fabio Sirchia*[9], *Lydia Turban*[10], *Susanna Schubert*[10], *Rami Abou Jamra*[10], *Jan-Ulrich Schlump*[11], *Desiree Demille*[12], *Pinar Bayrak-Toydemir*[13], *Gary Rex Nelson*[13], *Kristen Nicole Wong*[13], *Laura Duncan*[14;15], *Mackenzie Mosera*[14], *Christian Gilissen*[16], *Lisenka Vissers*[16], *Rolph Pfundt*[16], *Rogier Kersseboom*[17], *Hilde Yttervik*[18], *Geir Åsmund Myge Hansen*[18], *Marie Falkenberg Smeland*[19], *Kameryn M. Butler*[20], *Michael J. Lyons*[20], *Claudia Carvalho*[21;22], *Chaofan Zhang*[22], *James Lupski*[22;23;24;25], *Lorraine Potocki*[22;25], *Leticia Flores-Gallegos*[26], *Rodrigo Morales-Toquero*[26], *Florence Petit*[27], *Binnaz YALCIN*[28], *Annabelle Tuttle*[29], *Houda Elloumi*[29], *Lane Mccormick*[30], *Mary Kukolich*[30], *Oliver Klaas*[31], *Judit Horvath*[31], *Marcello Scala*[32;33], *Michele Iacomino*[33], *FRANCESCA FELICIA OPERTO*[34], *Federico Zara*[32;33], *Karin Writzl*[35;36], *Aleš Maver*[35], *Maria K. Haanpää*[37], *Pia*

Pohjola[37], Harri Arikka[38], Christian Iseli[3;4], Nicolas Guex[3;4], Alexandre Reymond[1]

[1]Center for Integrative Genomics, University of Lausanne, Lausanne, Switzerland; [2]Institute of Medical Genetics, University of Zurich, Zurich, Switzerland; [3]Bioinformatics Competence Center, Ecole Polytechnique Fédérale de Lausanne, Lausanne, Lausanne, Switzerland; [4]Bioinformatics Competence Center, University of Lausanne, Lausanne, Switzerland; [5]Sophia Genetics, St Sulpice, Lausanne, Switzerland; [6]Biostatistics platform, University of Lausanne, Lausanne, Switzerland; [7]Department of Neurosciences Rita Levi-Montalcini, University of Turin, Turin, Italy; [8]Medical Genetics Unit, Città della Salute e della Scienza University Hospital, Turin, Italy; [9]Department of Molecular Medicine, University of Pavia, Pavia, Italy; [10]Institute of Human Genetics, University of Leipzig Medical Center, Leipzig, Germany; [11]Department of Pediatrics, Centre for Neuromedicine, Gemeinschaftskrankenhaus Herdecke Gerhard Kienle Weg, Herdecke, Germany; [12]Genomics Analysis 396, ARUP Laboratories, Salt Lake City, United States; [13]Pediatric Neurology, University of Utah School of Medicine, Salt Lake City, United States; [14]Department of Pediatrics, Medical Center North, Vanderbilt University Medical Center, Nashville, United States; [15]Mayo Clinic, Rochester, Minnesota, United States; [16]Department of Human Genetics, Research Institute for Medical Innovation, Radboud University Medical Center, Nijmegen, Netherlands; [17]Center for genetic developmental disorders southwest, Zuidwester, Middelharnis, Netherlands; [18]Department of Medical Genetics, University Hospital of North Norway, Tromsø, Norway; [19]Department of Pediatric Rehabilitation, University Hospital of North Norway, Tromsø, Norway; [20]Greenwood Genetic Center, Greenwood, South Carolina, United States; [21]Pacific Northwest Research Institute (PNRI), Broadway, Seattle, United States; [22]Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, United States; [23]Human Genome Sequencing Center, Baylor College of Medicine, Houston, United States; [24]Department of Pediatrics, Baylor College of Medicine, Houston, United States; [25]Texas Children's Hospital, Houston, United States; [26]Hospital Ángeles Puebla, Puebla, Mexico; [27]CHU Lille, Clinique de génétique, Lille, France; [28]Inserm UMR1231, University of Burgundy, Dijon, France; [29]GeneDx, Gaithersburg, United States; [30]Department of Genetics, Cook Children's Medical Center, Cook Children's Health Care System, Fort Worth, United States; [31]Institute for Human Genetics, University Hospital Muenster, Muenster, Germany; [32]Department of Neurosciences, Rehabilitation, Ophthalmology, Genetics, Maternal and Child Health, University of Genoa, Genoa, Italy; [33]Medical Genetics Unit, IRCCS Istituto Giannina Gaslini, Genoa; [34]Child and Adolescent Neuropsychiatry Unit, Department of Medicine, Surgery and Dentistry, University of Salerno, Salerno, Italy; [35]Clinical Institute of Genomic Medicine, University Medical Centre Ljubljana, Ljubljana, Slovenia; [36]Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia; [37]Department of Genomics, Turku University Hospital, University of Turku, Turku, Finland; [38]Department of Pediatric Neurology, Turku University Hospital, University of Turku, Turku, Finland

Background: We described the KINSSHIP syndrome, a disorder associated with intellectual disability (ID), caused by de novo variants in the AFF3 degron. Mouse knock-ins/zebrafish overexpression provided evidence for a dominant-negative (DN) mode-of-action, wherein an increased level of AFF3 resulted in pathological effects.

Methods: Evolutionary constraints suggest other modes-of-inheritance. We challenged this hypothesis by screening ID cohorts and assessing the identified variants' deleteriousness.

Results: We detected twenty-one individuals displaying a milder syndrome with either hetero-/homozygous loss-of-function (LoF) or biallelic missense variants in *AFF3*. Matching zebrafish knockdowns exhibit neurological defects that could be rescued by expressing human *AFF3* mRNA, confirming their association with *aff3* ablation. Conversely, some of the human *AFF3* mRNAs carrying missense variants identified did not complement. Overexpression of mutated *AFF3* mRNAs in zebrafish embryos produced a significant increase of abnormal larvae compared to wild-type overexpression further demonstrating deleteriousness.

To further assess the effect of *AFF3* variation, we profiled the transcriptome of fibroblasts from affected individuals and engineered isogenic cells harboring +/+, DN/DN, LoF/+, LoF/LoF or DN/LoF *AFF3* genotypes. The expression of more than a third of the AFF3 bound loci is modified in either the DN/DN or the LoF/LoF lines. While the same pathways are affected, only about one-third of the differentially expressed genes are common to these homozygote datasets, indicating that *AFF3* LoF and DN variants largely modulate transcriptomes differently, e.g. the DNA repair pathway displayed opposite modulation.

Conclusions: Our results and the high pleiotropy shown by variation at this locus suggest that minute changes in *AFF3* function are deleterious.

Conflict of Interest: Sissy Bassani: None declared, Jacqueline Chrast: None declared, Ambrosini Giovanna: None declared, Norine Voisin Sophia Genetics, Frédéric Schütz: None declared, Alfredo Brusco: None declared, Fabio Sirchia: None declared, Lydia Turban: None declared, Susanna Schubert: None declared, Rami Abou Jamra: None declared, Jan-Ulrich Schlump: None declared, Desiree Demille ARUP Laboratories, Pinar Bayrak-Toydemir: None declared, Gary Rex Nelson: None declared, Kristen Nicole Wong: None declared, Laura Duncan: None declared, Mackenzie Mosera: None declared, Christian Gilissen: None declared, Lisenka Vissers: None declared, Rolph Pfundt: None declared, Rogier Kersseboom: None declared, Hilde Yttervik: None declared, Geir Åsmund Myge Hansen: None declared, Marie Falkenberg Smeland: None declared, Kameryn M. Butler: None declared, Michael J. Lyons: None declared, Claudia Carvalho Ionis Pharmaceuticals, Chaofan Zhang GeneDx, James Lupski 23andMe, Genome International, Lorraine Potocki: None declared, Leticia Flores-Gallegos: None declared, Rodrigo Morales-Toquero: None declared, Florence Petit: None declared, Binnaz YALCIN: None declared, Annabelle Tuttle GeneDx, Houda Elloumi GeneDx, Lane Mccormick: None declared, Mary Kukolich: None declared, Oliver Klaas: None declared, Judit Horvath: None declared, Marcello Scala: None declared, Michele Iacomino: None declared, FRANCESCA FELICIA OPERTO: None declared, Federico Zara: None declared, Karin Writzl: None declared, Aleš Maver: None declared, Maria K. Haanpää: None declared, Pia Pohjola: None declared, Harri Arikka: None declared, Christian Iseli: None declared, Nicolas Guex: None declared, Alexandre Reymond: None declared

P10.002.A Further evidence that the neurodevelopmental gene *FBXW7* predisposes to Wilms tumor

*Fabienne Meier-Abt[1], **Dennis Kraemer**[1;1], Nils Christian Braun[1], Michael Reinehr[2], Eveline Stutz-Grunder[3], Katharina Steindl[1], Anita Rauch[1]*

[1]University of Zurich, Institute of Medical Genetics, Schlieren, Switzerland; [2]University Hospital Zurich, Department of Pathology and Molecular Pathology, Zurich, Switzerland; [3]Children's Hospital Zurich, Department of Oncology, Zurich, Switzerland

Background/Objectives: Somatic variants in the NOTCH pathway regulator *FBXW7* are frequently seen in a variety of malignancies. Heterozygous loss-of-function germline variants in *FBXW7* have recently been described as causative for a neurodevelopmental syndrome. Independently, *FBXW7* was also considered as a susceptibility gene for Wilms tumor due to a few observations of heterozygous germline variants in patients with Wilms tumor.

Whether the same *FBXW7* variants are implicated in both, neurodevelopmental delay and Wilms tumor formation, remained unclear.

Methods: Trio-exome sequencing was performed in the propositus initially focussing on genes related to intellectual disability. Subsequently, DNA from the patient's Wilms tumor was evaluated for *FBXW7* second hit variants using exome sequencing. To test for diminished protein expression, immunohistochemical staining for FBXW7 protein was performed in tumor tissue.

Results: By clinical testing, we now observed a patient with neurodevelopmental delay due to a de novo constitutional mosaic *FBXW7* splice site pathogenic variant who developed Wilms tumor. In the tumor, we identified a second hit frameshift variant in *FBXW7*. Immunohistochemical staining was consistent with mosaic loss of FBXW7 protein expression in the tumor. Our data support the role of constitutional *FBXW7* pathogenic variants in both, neurodevelopmental disorder and the etiology of Wilms tumor.

Conclusion: Therefore, Wilms tumor screening should be considered in individuals with constitutional or germline pathogenic variants in *FBXW7* and associated neurodevelopmental syndrome.

Grants: University Research Priority Program of the University of Zurich ITINERARE–Innovative Therapies in Rare Disease

Conflict of Interest: None declared

P10.003.B "DYRK1A-associated neurodevelopmental disorder" - two new individuals further delineating the phenotype

Regina Rita Roth[1], *Rami Abou Jamra*[2], *Dagmar Wieczorek*[1], *Nuria Bramswig*[1]

[1]*Institute of Human Genetics, University Hospital Düsseldorf, Düsseldorf;* [2]*Institute of Human Genetics, University Hospital Leipzig, Leipzig*

Background/Objectives: *DYRK1A*-syndrome (OMIM #614104) is characterized by typical dysmorphic features, intellectual disability including impaired language development, autism spectrum disorder, seizures and microcephaly. About 200 cases have been reportet previously.

Methods: We present two further individuals with *DYRK1A*-syndrome from our cohort of patients with neurodevelopmental disorder. Both were examined with regard to their facial gestalt, dysmorphism and clinical abnormalities. The family anamnesis was taken and a pedigree was drawn up. Molecular genetic diagnostics was carried out in both cases.

Results: The first individual is a three-year-old boy with dysmorphic features and microcephaly. Clinically, he shows neurodevelopmental delay, particularly in the field of language, autistic traits, although an autism spectrum disorder has been excluded by neuropaediatric examination. He is prone to infections and febrile convulsions. Exome analysis identified a heterozygous pathogenic de novo nonsense variant in the *DYRK1A* gene.

The second patient is a four-year-old girl who also exhibits typical dysmorphia, but no microcephaly with a head circumference of 49 cm (-0.93 SD). She presents a speech-accentuated neurodevelopmental delay, an austism spectrum disorder, epilepsy and severe myopia. Exome based multigenepanel analysis identified a heterozygous likely pathogenic de novo nonsense variant in the *DYRK1A* gene.

Conclusion: We describe two individuals with *DYRK1A*-syndrome. Both patients have similar abnormalities that are described as being associated with de novo likely pathogenic and pathogenic variants in the *DYRK1A* gene. At the same time, both children differ in specific features like head circumference and autism spectrum disorder and thus broaden the phenotypic spectrum of *DYRK1A*-syndrome.

Grants:

Conflict of Interest: None declared

P10.004.C An IPSC-Model for the SRCAP-related neurodevelopmental disorder "non-Floating-Harbor Syndrome"

Johannes Rhode[1], *Lisa Hagenau*[1], *Stephanie Edwards*[1], *Falk Büttner*[2], *Ana Tzvetkova*[1;3], *Lars Jensen*[1], *Andreas Kuß*[1]

[1]*University Medicine Greifswald, Department of Functional Genomics, Greifswald, Germany;* [2]*Hannover Medical School, Institute of Clinical Biochemistry, Hannover, Germany;* [3]*University Medicine Greifswald, Institute of Bioinformatics, Greifswald, Germany*

Background/Objectives: The recently described Non-Floating-Harbor Syndrome (Non-FLHS or "Developmental Delay, Hypotonia, Musculoskeletal Defects and Behavioral Abnormalities", DEHMBA, OMIM # 619595) is a poorly understood neurodevelopmental disease caused by mutations in SRCAP. The aim of this study was to generate a disease model by introducing a non-FHS-causing mutation in the 3' end of SRCAP.

Methods: The iPSC line MHHi001-A (RRID:CVCL_QX51) was modified by introducing a frameshift mutation in a region known for non-FLHS mutations with an RNP-based CRISPR/Cas9 System. Resulting cells were screened for mutations and off-target effects. Expression of pluripotency markers was tested with qPCR and immunofluorescence microscopy. iPSC were differentiated as embryoid bodies, tested for differentiation markers with qPCR and immunofluorescence microscopy. Low coverage whole genome sequencing was performed on a MinION Mk1B to exclude chromosomal abnormalities.

Results: We generated an iPSC line (MHHi001-A-14) containing a heterozygous mutation (ENSG00000080603:c.[9307del]) in a locus known for non-FLHS associated mutations. The mutation was verified by Sanger sequencing of the target region and was classified as "deleterious" by MutationTaster (https://doi.org/10.1038/nmeth.2890). MHHi001-A-14 expresses the pluripotency markers OCT4, SOX2, NANOG and TRA-1-60. Embryoid bodies from this cell line tested positive for markers specific for all three germ layers. The cell line showed no off-target effects and no chromosomal abnormalities.

Conclusion: MHHi001-A-14 is a fully characterised iPSC, capable of differentiation in all three germ layers, with a newly introduced heterozygous frameshift mutation that resembles known non-FLHS mutations.

Conflict of Interest: None declared

P10.005.D Two hits in RAS/MAPK: a case report of dual molecular diagnoses of Neurofibromatosis type 1 and Noonan syndrome.

Sarah Josephi-Taylor[1;2], *Kristi Jones*[1;2]

[1]*The Children's Hospital at Westmead, Department of Clinical Genetics, Westmead, Australia;* [2]*University of Sydney, Discipline of Genomic Medicine, Sydney, Australia*

Background/Objectives: Neurofibromatosis type 1 (NF1) and Noonan Syndrome (NS), two distinct genetic conditions, are both caused by disruptions to proteins within the RAS/MAPK pathway. The RAS/MAPK cellular pathway regulates cell growth and differentiation. To the best of our knowledge, we present the first reported case of an individual with a confirmed dual molecular diagnosis of both NF1 and NS, surviving beyond infancy.

Methods: Clinical case report.

Results: Our patient, 9 years old, has a heterozygous nonsense pathogenic variant in exon 29 of the *NF1* gene (p.(Gln1841Ter)) and a recurrent pathogenic missense variant in the *PTPN11* gene (p.(Asn308Asp)). She presents with an amplified NF1 and NS phenotype. She has marked short stature (<1st centile, -4.7 SD), relative macrocephaly (50th-75th centile), bilateral ptosis, high myopia, hearing impairment, a complex asymmetric pectus deformity, thoracic scoliosis, bilaterally restricted elbow extension, mild pulmonary stenosis, a plexiform neurofibroma on her wrist, multiple café au lait macules, and mild to moderate intellectual disability. MRI brain shows mildly thickened optic nerves, a middle cerebellar peduncle lesion consistent with a low grade glioma and a borderline Chiari 1 malformation. Resolved issues include severe feeding difficulties necessitating gastrostomy feeds until age 6, and an atrial septal defect that closed spontaneously.

Conclusion: We hereby challenge previous conclusions in the medical literature that dual diagnoses within the RAS/MAPK pathway are neonatally lethal. The oncogenic potential of dual disruptions within this pathway remains a significant concern for our patient.

Grants:

Conflict of Interest: None declared

P10.006.A *MBD6*: A promising candidate gene for neurodevelopmental disorders

Elis Tiivoja[1;2], *Helen Raudik*[2], *Laura Mihkla*[1;2], *Riina Žordania*[1], *Sander Pajusalu*[1;2], *Charlotte Brasch-Andersen*[3], *Trine Maxel Juul*[3], *Lone Laulund*[3], *Matias Wagner*[4;5;6], *Juliane Winkelmann*[4], *A Micheil Innes*[7], *Julia Tagoe*[7], *amelle shillington*[8], *Susan Hiatt*[9], *Sandra von Hardenberg*[10], *Bernd Auber*[10], *Curtis C Rogers*[11], *Raymond Louie*[11], *Brooke Smith*[11], *Robert Meyer*[12], *Eva Lausberg*[12], *Cordula Knopp*[12], *Suneeta Madan-Khetarpal*[13], *Jessica Sebastian*[13], *Krzysztof Szczałuba*[14], *Bertrand Isidor*[15], *Benjamin Cogne*[15], *Sebastien Küry*[15], *Stefan Barakat*[16], *Katrin Ounap*[1;2]

[1]*Tartu University Hospital, Tartu, Estonia;* [2]*University of Tartu, Tartu, Estonia;* [3]*Odense University Hospital, Odense, Denmark;* [4]*Technical University of Munich, München, Germany;* [5]*Helmholtz Zentrum München, München, Germany;* [6]*Dr. von Hauner Children's Hospital, München, Germany;* [7]*University of Calgary, Calgary, Canada;* [8]*Cincinnati Children's Hospital Medical Center, Cincinnati, United States;* [9]*HudsonAlpha Institute for Biotechnology, Huntsville, United States;* [10]*Hannover Medical School, Hannover, Germany;* [11]*Greenwood Genetic Center, Greenwood, United States;* [12]*RWTH Aachen University, Aachen, Germany;* [13]*UPMC Children's Hospital of Pittsburgh, Pittsburgh, United States;* [14]*Medical University of Warsaw, Warsaw, Poland;* [15]*University Hospital of Nantes, Nantes, France;* [16]*Erasmus University Medical Center, Rotterdam, Netherlands*

Background: The *MBD6* gene encodes the MBD6 protein (methyl-CpG-binding domain protein 6, OMIM #619458), which is an important regulator of the BAP1 complex. Operating as a multiprotein complex, BAP1 functions as a general transcriptional activator by deubiquitinating epigenetic and transcription factors. Depletion of the MBD6 protein results in a reduction of BAP1-dependent gene expression. We hypothesize that germline variants in the *MBD6* gene may lead to a neurodevelopmental phenotype similar to the clinical syndrome caused by germline *BAP1* gene variants.

Methods: We collected information on twelve cases with a neurodevelopmental phenotype and suspected deleterious changes in the *MBD6* gene through GeneMatcher. The variants were detected by exome and genome sequencing.

Results: We gathered clinical data on twelve patients with potentially deleterious heterozygous changes in *MBD6* gene and a neurodevelopmental phenotype. Ten truncating, one missense and one intronic variant were identified. Variants occurred de novo in six patients and were inherited in two (not determined in four). Within our cohort the predominant clinical characteristics included global developmental delay (6/12), abnormal behaviour (7/12), epilepsy (4/12), and dysmorphic features (9/12). Among the observed behavioural abnormalities, 6/12 patients were reported to exhibit features consistent with autism spectrum disorder. Dysmorphic facial features were variable and nonspecific.

Conclusion: The data collected support the hypothesis that the *MBD6* gene is a candidate gene for neurodevelopmental disorders. We are planning to include functional studies in order to determine the molecular effect of the *MBD6* gene variants in our cohort.

Grants: Estonian Research Council grants PRG471, PRG2040, PSG774.

Conflict of Interest: None declared

P10.007.B Truncating *ADNP* variants as a recurrent monogenic cause of neurodevelopmental disorders

Mirjana Gusic[1], *Steffen Lott*[1], *Oliver Wachter*[1], *Julia Philippou-Massier*[1], *Konstanze Hörtnagel*[1]

[1]*Medicover Genetics, MVZ Martinsried GmbH, Martinsried, Germany*

Background/Objectives: Neurodevelopmental disorders (NDDs), impacting up to 17% of the population, currently include intellectual disabilities, communication disorders, autism spectrum disorder (ASD), ADHD, specific learning disorders (SLD), and movement disorders (Cainelli et al. 2022). Increased implementation of large-scale sequencing methods (especially the whole exome sequencing) in the last decade has revealed a significant enrichment of de novo variants in individuals with NDD and is becoming a standard tool in genetic diagnostics (Brunet et al. 2021).

Methods: Four individuals age 2–6 were referred to our diagnostic center for whole exome sequencing upon suspicion of a NDD. They shared a common phenotype of global developmental delay, delayed speech and language development and intellectual disability, as well as different types of facial dysmorphisms.

Results: Whole exome sequencing analysis revealed pathogenic variants in the *ADNP* gene, encoding for a transcription factor involved in the SWI/SNF remodeling complex, which were confirmed as de novo in three individuals, where trio-analysis was performed. All variants (three novel) were truncating, located in the fifth (last) exon of the gene and thus predicted to escape nonsense-mediated decay. Review of the literature revealed the *ADNP* gene as one of the most frequent causes of NDD (van Dijck et al. 2019; Gillentine et al. 2022).

Conclusion: This work expands the genetic spectrum and reviews the knowledge of *ADNP*-related disorder, highlighting its prevalence among patients with NDD as well as the clinical value of (trio) sequencing for the rapid discovery of pathogenic variants.

Conflict of Interest: None declared

P10.008.C Clinical and molecular characterisation of BAF complex-related syndromes in the Portuguese population

Daniela Oliveira[1;2;3], *Sara Pinho*[4], *Joana Catanho*[5], *Ana Rita Ferreira Pacheco Quental*[6], *Inês V. Carvalho*[7], *Inês Nunes Vicente*[8], *Carla Marques*[8], *Cristina Pereira*[9], *Margarida Venancio*[5], *Ana Berta Sousa*[4], *Jorge Saraiva*[1;3;10], *Sérgio B. Sousa*[1;2;3]

[1]*Medical Genetics Unit, Paediatric Hospital, Coimbra Hospital and University Centre, Coimbra, Portugal;* [2]*University Clinic of Genetics,*

Faculty of Medicine, University of Coimbra, Coimbra, Portugal; [3]Clinical Academic Center of Coimbra, Coimbra, Portugal; [4]Medical Genetics Unit, North Lisbon University Hospital Centre, Lisboa, Portugal; [5]Medical Genetics Unit, Central Lisbon University Hospital Centre, Lisboa, Portugal; [6]Medical Genetics Unit, São João University Hospital Centre, Porto, Portugal; [7]Neurology Unit, Coimbra Hospital and University Centre, Coimbra, Portugal; [8]Child Development Centre, Paediatric Hospital, Coimbra Hospital and University Centre, Coimbra, Portugal; [9]Paediatric Neurology Unit, Paediatric Hospital, Coimbra Hospital and University Centre, Coimbra, Portugal; [10]University Clinic of Paediatrics, Faculty of Medicine, University of Coimbra, Coimbra, Portugal

Background: BAF complex-related syndromes are a growing group of conditions resulting from mutations in over 29 genes that disrupt its function. Coffin-Siris and Nicolaides-Baraitser syndromes were the first to be recognised but several other intellectual disability (ID) phenotypes are being delineated.

Methods: Retrospective clinical and molecular characterisation of cases with BAF complex-related syndromes from four Portuguese hospital centres.

Results: We describe 38 patients with BAF complex-related syndromes due to variants in *ARID1B* (n:19), *ADNP* (n:3), *ARID2* (n:3), *SMARCA2* (n:3), *SOX11* (n:3), *SMARCB1* (n:2), *SMARCC2* (n:2), *ARID1A* (n:1), *BCL11A* (n:1) and *SMARCA4* (n:1). In two cases, the variant was inherited. We report 18 females and 20 males aged 3-34-year-old. All assessed patients presented developmental delay/ID: 31% mild, 42% moderate, 23% severe and 4% profound. Many patients had hypotonia (72%), CNS abnormalities (67%) and seizures (26%). Other frequent medical issues were visual impairment (69%), cryptorquidism (65%) and feeding problems (56%). Most common dysmorphisms included wide mouth (65%), broad nasal tip (56%), thick lower lip vermillion (55%), coarse facial features (54%), wide nasal base (53%), prominent eyelashes (49%), hypertrichosis (47%). and hypoplastic 5th finger/toenail (41%).

Conclusion: This multicentre study is the first on BAF complex-related syndromes on a large Portuguese cohort. Classical associated features, such as ectodermal anomalies, coarse face and 5th digit hypoplasia, were also commonly found in our cohort. *ARID1B* was confirmed as the most frequent gene involved. Thorough description contributes to increase knowledge of the natural history, genotype-phenotype correlations, and to accurate counselling and personalised management of these patients.

Conflict of Interest: None declared

P10.009.D Mutations in LRRC7, which encodes Densin-180, are linked to intellectual disability, autism, hyperphagia, and aggressive behaviour in human patients.

Jana Willim[1], *Daniel Woike*[2], *Christian Schlein*[3], *Prof. Dr. rer. nat. Hans-Jürgen Kreienkamp*[3]

[1]Human Genetics, University Hospital Salzburg, Salzburg, Austria; [2], Germany; [3]Human Genetics, University Medical Center Hamburg-Eppendorf, Hamburg, Germany

Background/Objectives: Densin-180/LRRC7 is a postsynaptic protein that acts as a scaffold of the postsynaptic density of glutamatergic synapses by connecting actinin and catenin proteins with Shank proteins and αCaMKII.

Methods: Exome-sequencing was performed to identify putative disease-causing variants. Functional analyses in HEK293T-cells were performed to confirm their pathogenicity.

Results: We identified eight individuals with heterozygous missense variants in *LRRC7*, affected by a neurodevelopmental disorder comprising intellectual disability, often accompanied by autism, ADHD and aggression. Variants affect conserved residues in the LRR region (six variants) and the PDZ domain (one variant) of the encoded Densin-180 protein. We analyzed the influence of these variants on the molecular and cellular functions of Densin-180. First, it was tested whether these LRR-specific variants have an influence on known interactions of Densin-180. The binding to αCaMKII, Shank3, and δ-catenin remained unchanged by the LRR domain variants. In contrast, the PDZ-specific missense variant strongly interfered with the interaction with δ-catenin, which is a known partner of the PDZ domain of Densin-180. This results in impaired synaptic targeting of Densin-180 in primary cultured neurons. For the LRR domain, we identified serine/threonine protein phosphatase 1, Erbin and MACF1 as new interaction partners. Four of the LRR-specific variants interfere with binding to all tested partners, while one variant showed a weakened, but non-significant effect. Again, several variants impaired synaptic targeting of Densin-180 in primary cultured neurons.

Conclusion: Taken together, our data suggest that the missense variants disrupt the scaffolding function Densin-180 at postsynaptic sites.

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Conflict of Interest: None declared

P10.010.A Expanding the Phenotype of PPP1R21-Related Neurodevelopmental Disorder

Mohammed Almannai[1;2], *Dana Marafi*[3;4], *Maha Zaki*[5;6], *Reza Maroofian*[7], *Stephanie Efthymiou*[7], *nebal waill saadi*[8], *Bilal Filmban*[9], *Hormos Dasfari*[10;11;12], *fatima rahman*[13], *shazia maqbool*[13], *Eissa Ali Faqeih*[9], *Fuad Al Mutairi*[1;2], *Hind Alsharhan*[3;14;15], *Omar Abdelaty*[15], *saadoun binhasan*[15], *Ruizhi Duan*[4], *Mahmoud Noureldeen*[16], *Alaa Alqattan*[15], *Henry Houlden*[7], *Jill Hunter*[17;18], *Jennifer Posey*[4], *James Lupski*[4;18;19], *Ayman El-Hattab*[20;21]

[1]King Abdullah Specialized Children's Hospital (KASCH), King Abdulaziz Medical City, Ministry of National Guard Health Affairs (MNG-HA), Genetics and Precision Medicine department (GPM), Riyadh, Saudi Arabia; [2]Medical Genomics Research Department, King Abdullah International Medical Research Center, Ministry of National Guard Health Affairs, King Saud Bin Abdulaziz University for Health Sciences, Ministry of National Guard Health Affairs, Riyadh, Saudi Arabia; [3]College of Medicine, Kuwait University, Department of Pediatrics, Kuwait; [4]Baylor College of Medicine, Department of Molecular and Human Genetics, United States; [5]Human Genetics and Genome Research Institute National Research Centre, Clinical Genomics department, Cairo, Egypt; [6]Armed Forces College of Medicine (AFCM), Department of Genetics, Cairo, Egypt; [7]UCL Queen Square Institute of Neurology, University College London, Department of neuromuscular disorders, London, United Kingdom; [8]College of Medicine, University of Baghdad, Pediatric Neurology, Children Welfare Teaching Hospital, Baghdad, Iraq; [9]King Fahad Medical City, Section of genetics, Riyadh, Saudi Arabia; [10]Faculty of Medicine and University Hospital Cologne, University of Cologne, Max Planck Institute for Biology of Ageing, Department of Pediatrics, Colonge, Germany; [11]Randall Centre for Cell and Molecular Biophysics, Faculty of Life Sciences and Medicine (FoLSM), King's College London, London, United Kingdom; [12]Max-Planck-Institute for Biology of Ageing, Colonge, Germany; [13]University of Child Health Sciences & The Children's Hospital, Lahore, Department of Developmental - Behavioral Pediatrics, Lahore, Pakistan; [14]Kuwait Medical Genetics Center, Ministry of Health, Sulaibikhat, Kuwait; [15]Farwaniya Hospital, Ministry of Health, Department of Pediatrics, Kuwait; [16]Faculty of Medicine, Beni-Suef University, Beni-Suef, Department of Pediatrics, Egypt; [17]Baylor College of Medicine, Department of Radiology, United States; [18]Texas Children Hospital, Houston, United States; [19]Human Genome Sequencing Center, Baylor College of Medicine, Houston, United States; [20]University of Sharjah, Sharjah, United Arab Emirates,

College of Medicine; [21]Genetics Clinics, University Hospital Sharjah, Sahrjah, United Arab Emirates

Background/Objectives: *PPP1R21* encodes for a conserved protein that is involved in endosomal maturation. Biallelic pathogenic variants in *PPP1R21* have been recently linked with a syndromic neurodevelopmental disorder. Here we present 12 additional individuals from ten unrelated families.

Methods: We recruited 12 individuals from 10 unrelated families from diverse ethnic backgrounds. The molecular diagnosis of *PPP1R21*-related NDD was made from clinical or research exome sequencing

Results: We identified nine different variants in *PPP1R21*, of which seven were novel. The main feature of the phenotype is neurodevelopmental delay and all subjects exhibited moderate to severe GDD and ID. Almost all of our subjects had hypotonia (11/12; 92%) while seizures were seen in only four subjects. Three subjects had optic atrophy, one had pale disc with poor tracking and one had nystagmus. Several key features overlapped with lysosomal storage disorders with coarse facial features observed in most individuals along with hypertrichosis in several of them. Two subjects have hepato-(splenomegaly) and one has left ventricular hypertrophy. Respiratory problems, in the form of apnea, recurrent respiratory infections and bronchitis, reported in five subjects. Common findings in brain imaging include delays in myelination, wavy outline of the bodies of the lateral ventricles, and slight prominence of the bodies of the lateral ventricles.

Conclusion:*PPP1R21*-related neurodevelopmental disorder is associated with a consistent phenotype and should be considered in highly consanguineous individuals presenting with developmental delay/intellectual disability along with coarse facial features. While we report the first three missense variants in *PPP1R21*, we did not observe any clear genotype-phenotype correlation.

Conflict of Interest: None declared

P10.011.B Neuronal phenotypes associated with *FBXO11*-deficiency can be alleviated by activation of the proteasome with small molecules

Anne Gregor[1;2], *Laila Distel*[3], *Arif B Ekici*[3], *Philipp Kirchner*[3;4], *Steffen Uebe*[3], *Mandy Krumbiegel*[3], *Soeren Turan*[5], *Beate Winner*[5;6], *Christiane Zweier*[1;2]

[1]*University of Bern, Department of Human Genetics, Inselspital Bern, Bern, Switzerland;* [2]*University of Bern, Department for Biomedical Research (DBMR), Bern, Switzerland;* [3]*Friedrich-Alexander-Universität Erlangen-Nürnberg, Institute of Human Genetics, Erlangen, Germany;* [4]*University of Bern, Institute of Tissue Medicine and Pathology, Bern, Switzerland;* [5]*Friedrich-Alexander-Universität Erlangen-Nürnberg, Department of Stem Cell Biology, Erlangen, Germany;* [6]*Friedrich-Alexander-Universität Erlangen-Nürnberg, Centre for Rare Diseases Erlangen (ZSEER), University Hospital Erlangen, Erlangen, Germany*

Background/Objectives: Haploinsufficiency of *FBXO11* is associated with a variable neurodevelopmental disorder. So far, neither the underlying pathomechanisms nor the function of FBXO11 in the nervous system are well understood. This study aims to shed light on pathways disturbed upon *FBXO11*-deficiency in neurons and to investigate potential therapeutic options of *FBXO11*-associated NDDs.

Methods: Using a combined approach, we induced *FBXO11*-deficiency in a human stem cell based neuronal model and a *Drosophila* model. We performed transcriptomic analyses on neuronal material as well as molecular phenotyping in both models. Additionally, we performed rescue experiments with two proteasome activating substances.

Results: We found that loss of FBXO11 is associated with a range of neuronal phenotypes, including defects in neuronal migration, dendritic arborization and abnormal proliferation/differentiation balance in *Drosophila* and human neurons. We could furthermore identify the stemness factor NANOG as a potential novel substrate of the ubiquitin ligase complex protein FBXO11 and therefore suggest a molecular link between disturbed neuronal function and proteasomal dysfunction in *FBXO11*-deficiency. Activating the proteasome with small molecules PD169316 and R-Verapamil could partially alleviate *FBXO11*-deficiency-associated phenotypes in the fly model.

Conclusion: Our study shows the importance of FBXO11 for neurodevelopment and highlights the reversibility of related phenotypes, which opens an avenue for potential development of therapeutic approaches.

Grants: MSCA Individual Fellowship (837547)

Conflict of Interest: None declared

P10.012.C MicroRNAs as potential biomarkers for childhood epilepsy

Sofia Domenech[1], *Amparo Andres-Borderia*[1], *Edna Ripolles*[1], *Nerea Gorria-Redondo*[2], *Francisco García-García*[3], *Sergio Aguilera Albesa*[2], **Carmen Espinós**[1]

[1]*Valencia Biomedical Research Foundation - Centro de Investigacion Principe Felipe (CIPF), Rare Neurodegenerative Diseases, Valencia, Spain;* [2]*Hospital Universitario de Navarra, Navarrabiomed, Paediatric Neurology Unit, Department of Paediatrics, Pamplona, Spain;* [3]*Valencia Biomedical Research Foundation, Centro de Investigacion Principe Felipe (CIPF), Computational Biomedicine Laboratory, Valencia, Spain*

Background/Objectives: Epilepsy is a prevalent chronic disease in childhood. It is estimated that one in 20 children may experience a seizure before the age of 5, and one in 150 will develop epilepsy. Additionally, one in three cases may be associated with behavioral problems or attention deficit hyperactivity disorder, and one in four may develop an intelligence quotient of less than 80, despite good seizure control. The objective of this study is to characterise biomarkers that help in predicting the disease progression and are useful in monitoring treatment.

Methods: We conducted a comparative study between patient cohorts and a sex- and age-matched control cohort. The patient cohort consisted of 200 patients (<14 years old) classified according to their epilepsy: focal idiopathic, generalized, structural and epileptic encephalopathy. We analysed the plasma presentation of 20 microRNAs previously associated with epilepsy. The analysis strategy included data processing and normalisation, differential expression analysis and assessment of the predictive performance of significant miRNAs from logistic regression models.

Results: A significant differential pattern was detected in the presentation of six microRNAs, namely miR-15a-5p, miR-128-3p, miR-139-5p, miR-155-5p, miR-181c-5p and miR-323a-5p, both intra- and inter-cohorts.

Conclusion: The analysis of the miRNA profile mentioned above allows differentiation between the four study groups, and combinations of them are specific signatures for each of the patient groups (focal idiopathic, generalized, structural and epileptic encephalopathy).

Grants: This study was supported by the Alicia Koplowitz Foundation and Generalitat Valenciana (CIAICO/2022/24).

Conflict of Interest: None declared

P10.013.D Insight into inter-individual phenotypic differences in Korean patients with 16p11.2 deletion

Ji Yoon Han[1]

[1]Deajeon St. Mary's Hospital, Pediatric Neurology, Daejeon, Korea, Rep. of South

Background/Objectives: The 16p11.2 copy number variations (CNVs) is increasingly recognized as one of the most frequent genomic disorders and the 16p11.2 deletion exhibits broad phenotypic variability and a diverse clinical phenotype. We describe on the neurodevelopmental course and discordant clinical phenotypes observed within and between individuals with the identical 16p11.2 deletion

Methods: The analysis of the CytoScan Dx Assay was conducted on the GeneChip System 3000Dx and the signal for the sample was then compared to a reference set using the Chromosome Analysis Suite (ChAs Dx) software.

Results: Ten patients from six separate families were identified with 16p11.2 deletion. Nine breakpoint (BP) 4-5 and one BP 2-5 of the 16p11.2 deletion were identified. All patients with 16p11.2 deletion exhibited developmental delay and/or intellectual disability. 60% of the patients presented with neonatal hypotonia, but muscle weakness improved with age. Benign infantile epilepsy manifested between the ages of 7-10 months (median 8 months) in six patients (60%). Vertebral dysplasia was observed in two patients (20%), and mild scoliosis was noted in three patients. 60% of patients were overweight.

Conclusion: We present six unrelated Korean families wherein identical 16p11.2 deletions resulted in diverse developmental trajectories and discordant phenotypes. The clinical variability and incomplete penetrance observed in individuals with 16p11.2 deletions remain unclear, posing challenges in accurate clinical interpretation and diagnosis.

Grants: none.

Conflict of Interest: None declared

P10.014.A Comprehensive in silico functional analysis of CDKL5 involved in a genetic Intellectual Disability

Tetsuya Inazu[1;2], Atsushi Morii[2], Yuuki Fujino[1], Kyoka Takeuchi[1]

[1]Ritsumeikan University, Department of Pharmacy, College of Pharmaceutical Sciences, Kusatsu, Shiga, Japan; [2]Ritsumeikan University, Graduate School of Pharmaceutical Sciences, Kusatsu, Shiga, Japan

Background/Objectives: Cyclin-dependent kinase-like 5 (CDKL5) deficiency disorder (CDD) is involved in X-linked epilepsy and intellectual disability. CDKL5 is a serine/threonine protein kinase whose pathological mutations cause CDD. Most missense mutations are concentrated in the catalytic domain.

As the mutations for the disease are increasing revealed by next-generation sequencing, variants of uncertain significance are occasionally determined.

Therefore, anticipating whether mutations in this region affect CDKL5 function is informative for clinical diagnosis.

We aimed to determine the comprehensively predicted the pathogenicity of all missense substitutions in the catalytic domain of CDKL5 using in silico analysis.

Methods: The analysis range is from amino acids 1 to 300 in the catalytic domain of CDKL5.

Each authentic human amino acid was virtually changed to 19 different amino acids for a total of 5700 missense substitution.

We used in silico prediction tools such as PolyPhen-2 HumDiv mode/HumVar mode, PROVEAN, and SIFT were selected individually or in combination with one another to analyze them. We also evaluated their accuracy using previously reported mutations as a reference. Each missense substitution was finally evaluated as "pathogenic" or "benign".

Results: Substitutions predicted as pathogenic were over 82.6% using each of the three tools.

A comprehensive in silico functional analysis of CDKL5 showed that the combination of PolyPhen-2 HumDiv, PolyPhen-2 HumVar, and PROVEAN tools gave the best performance for predicting the severity of mutations.

Conclusion: These results provide information that could accurately predict the pathogenicity of the disease.

Grants: JSPS KAKENHI 23K07235

Conflict of Interest: None declared

P10.015.B GenIDA, an international participatory database to better characterize comorbidities of genetic forms of intellectual disability: novel findings on KBG, SETD5 and DDX3X syndromes

Pauline Burger[1], Chloé soyer[2], Nicolas Chatron[2;3], Charlotte Ockeloen[4], Tjitske Kleefstra[4;5], Valentin Ruault[6], David Geneviève[6], Elana Forbes[7], Lottie Morison[7], Angela Morgan[7;8], Jean Louis Mandel[1;9]

[1]I.G.B.M.C. - Institut de génétique et de biologie moléculaire et cellulaire, Université de Strasbourg, Department of Neurogenetics and Translational Medicine, Illkirch-Graffenstaden, France; [2]Institut NeuroMyoGène - Université de Lyon, Laboratoire Physiopathologie et Génétique du Neurone et du Muscle, Lyon, France; [3]Hospices Civils de Lyon, Service de Génétique, Lyon, France; [4]Radboud University Medical Center, Dept. of Human Genetics, Nijmegen, Netherlands; [5]Erasmus MC, Dept. of Human Genetics, Rotterdam, Netherlands; [6]Université de Montpellier, Centre Hospitalier Universitaire Montpellier, Montpellier, France; [7]Murdoch Children's Research Institute, Speech & Language, Melbourne, Australia; [8]University of Melbourne, Department of Audiology and Speech Pathology, Parkville, Australia; [9]University of Strasbourg Institute for Advanced Studies, Strasbourg, France

Background/Objectives: GenIDA is an international participatory registry designed to better characterize the clinical manifestations and natural history of genetic forms of intellectual disability (ID).

Methods: Clinical information reported and updated by the patient's family using a structured questionnaire exploring physical parameters, cognitive and behavioural aspects, etc. is analysed to identify new medically relevant information for families and professionals concerned with a given condition. The questionnaire currently available in 8 languages has been completed for over 1900 patients.

Results: We have evaluated the benefit of caregivers' self-reported data on clinical characterization of 2 conditions that can hardly be distinguished: ANKRD11 variants, responsible for KBG syndrome, and SETD5 variants, responsible for a KBG-like phenotype. This approach enabled us to highlight quality of life issues, such as the consequences of epilepsy for KBG individuals ($n = 57$), or gastrointestinal problems for SETD5 individuals ($n = 37$).

The phenotypic description of DDX3X-related condition has been extended: our results, consistent with the literature regarding sleep disorders, and delays in developmental milestones in these individuals ($n = 62$), further indicate that behavioural problems seem more important than previously reported: besides ADHD, caregivers experience significant daily impulsivity, stereotypies and anxiety issues.

Conclusion: These results validate the interest of our participatory approach: through their direct involvement, families can

reveal previously underestimated aspects of the disorder and highlight their major concerns in their daily lives. More than a complement to medical reports, GenIDA can be used by specialists for "prospective phenotyping" to consolidate knowledge of ID syndromes and improve prognosis and patient management.

Grants:

Conflict of Interest: None declared

P10.016.C Missense variant in PDZ-domain of DLG3 causes syndromic X-linked intellectual developmental disorder.

Shiomi Otsuji[1], *Seiji Mizuno*[1], *Natsuki Nakamura*[1], *Tomoko Uehara*[1], *Mamiko Yamada*[2], *Hisato Suzuki*[2], *Toshiki Takenouchi*[3], *Mie Inaba*[1], *Kenjiro Kosaki*[2]

[1]*Aichi Developmental Disability Center Central Hospital, Department of Pediatric Internal Medicine and Clinical genetics, Kasugai, Japan;* [2]*Keio University School of Medicine, Center for Medical Genetics, Tokyo, Japan;* [3]*Keio University School of Medicine, Department of Pediatrics, Tokyo, Japan*

Background/Objectives: Truncating variants in *DLG3* were found to cause X-linked intellectual disability (XLID), however, clinical significance of the missense variants has been unknown. DLG3 gene is located at Xq13.1 and encodes synapse-associated protein 102 (SAP102), a member of the membrane-associated guanylate kinase protein family. Neuronal SAP102 is expressed during early brain development and is localized to the postsynaptic density of excitatory synapses. We identified a novel missense variant in *DLG3* in a male with severe intellectual disability and dysmorphic features.

Methods: We compared our patient with reported patients regarding clinical features and discussed pathogenicity of the variant.

Results: The patient was 22-year-old Japanese male, who was referred to our department for developmental delay when he was 9 months old. He had no family history of neurological disorders. He was born to unrelated, healthy Japanese parents. He showed overgrowth of +2SD in his childhood, while his final height was 173 cm (1.0SD). He showed some dysmorphic physical features similar to reported patients with truncating variants in the *DLG3*. Whole exome sequencing identified a hemizygous missense variant in the PDZ2-domain of *DLG3* (NM_021120.3:c.848A>C;p.Asn283Thr), which was evaluated as of uncertain significance according to the ACMG guideline. Multiple lines of computational evidence support a deleterious effect on the gene product.

Conclusion: The PDZ domains have been shown to interact with the NR2 subunits of the NMDA glutamate receptor. We speculate the N283T variant causes syndromic XLID via impaired interaction with the NMDA receptor. Functional studies are necessary to provide more conclusive evidence.

Grants: None.

Conflict of Interest: None declared

P10.017.D Determining the optimal quality and depth of phenotypic information to maximize diagnostic yield in neurodevelopmental disorders

Alexander Dingemans[1], *David Koolen*[1], *Nicole de Leeuw*[1], *Rolph Pfundt*[1], *Janneke Schuurs-Hoeijmakers*[1], *Bregje van Bon*[1], *Charlotte Ockeloen*[1], *Carlo Marcelis*[1], *Marjolein Willemsen*[1], *Anneke Vulto-van Silfhout*[1], *Bert de Vries*[1], *Lisenka Vissers*[1]

[1]*Radboud University Medical Center, Human Genetics, Nijmegen, Netherlands*

Background/Objectives: Although phenotypic data is crucial in diagnostics, it remains unclear at what 'quality and depth' such data is needed. We investigated the minimal level of phenotypic detail and depth needed to maximally impact diagnostic yield.

Methods: We deep-phenotyped 285 individuals with neurodevelopmental disorders (median 17 Human Phenotype Ontology (HPO) terms per individual) for whom a diagnosis was established using trio-exome sequencing. Using Exomiser as benchmark, we assessed the impact on diagnostic yields in different scenarios by comparative analyses of permuted input data using complete phenotypic profiles, different branches of the HPO tree, and varying information content of HPO terms.

Results: Using all phenotypic data achieved a top-1 sensitivity of 78%. The use of specific HPO branches like the nervous system maintained 75% sensitivity using only 1553 of 5173 terms (27%), while several others (such as the immune system) lowered accuracy to 67%, with no phenotypic data resulting in a sensitivity of 69%. A reduced set of HPO terms from strategically selected branches maintained a high sensitivity (77%), despite comprising only 40% of the HPO terms. Surprisingly, there was no correlation between the information content (Pearson's $r = 0.04$, $p = 0.45$) or number of HPO terms ($r = 0.02$, $p = 0.79$) and diagnostic yield. Phenotypic data was especially useful when interpreting missense variants (72% vs 46% without phenotypic data) and inframe deletions (57% vs 43%).

Conclusion: Our study establishes guidelines for phenotypic data collection, demonstrating that carefully selecting key HPO terms can maintain high diagnostic accuracy while reducing data volume by 60%, saving time and resources, without sacrificing diagnostic yield.

Grants: None

Conflict of Interest: None declared

P10.018.A Two novel distinguishable entities within the Coffin-Siris syndrome spectrum caused by specific missense variants in ARID1A and ARID1B

Eline van der Sluijs[1], *Alfredo M. Valencia*[2;3;4], *Ajinka Patil*[2;3], *Alexander Dingemans*[5], *Bert de Vries*[5], *Bekim Sadikovic*[6], *Marielle Alders*[7], *Cigall Kadoch*[2;3;8], *Gijs Santen*[1]

[1]*Leiden University Medical Center, Clinical Genetics, Leiden, Netherlands;* [2]*Dana-Farber Cancer Institute, Department of Pediatric Oncology, Boston, United States;* [3]*Broad Institute of MIT and Harvard, Cambridge, United States;* [4]*Stanford University, Department of Psychiatry and Behavioral Sciences, Stanford, United States;* [5]*Radboud University Medical Center, Department of Human Genetics, Nijmegen, Netherlands;* [6]*London Health Sciences Centre, Verspeeten Clinical Genome Centre, London, Canada;* [7]*Amsterdam UMC, locatie AMC, Department of Clinical Genetics, Amsterdam, Netherlands;* [8]*Howard Hughes Medical Institute, Chevy Chase, United States*

Consortium: ARID1 consortium

Haploinsufficiency of *ARID1A* and *ARID1B* causes Coffin-Siris syndrome (CSS) or non-specific intellectual disability (ID). Non-truncating variants in *ARID1A* and *ARID1B* are increasingly being identified owing to routine sequencing, however variant classification remains challenging due to the variable phenotype. We used two complementary approaches to investigate non-truncating variants: Phenoscore analysis, an artificial intelligence-based phenomics framework, using detailed phenotype data, photos, and DNA methylation analysis.

We identified 139 patients (*ARID1A* $n = 81$, *ARID1B* $n = 58$) with 124 unique non-truncating variants in *ARID1A* ($n = 70$) or *ARID1B* ($n = 54$). Sixteen variants were recurrent and 3 patients had

multiple non-truncating variants. Clinical data was available of 97 patients. DNA methylation analysis was performed for 64 variants.

Seventy-six variants were considered pathogenic because of a CSS-consistent phenotype, variant recurrence and/or de novo inheritance ($n = 46$), a BAFopathy episignature ($n = 8$), or both ($n = 22$). Pathogenic variants were predominantly located in either the ARID- DNA-binding($n = 26$) or C-terminal Core binding region (CBR)/Arm repeat-domains ($n = 33$) of both genes. In 5 *ARID1A* and 1 *ARID1B* patients with 4 unique variants in the CBR-domain and a CSS-compatible phenotype who lacked this episignature, a novel alternative episignature was identified. All patients had ID, other frequent features were short stature (*ARID1A* 85%, *ARID1B* 77%), brain anomalies (*ARID1A* 83%, *ARID1B* 75%) and cardiovascular anomalies (*ARID1A* 51%, *ARID1B* 15%, $p = 0.05$). PhenoScore analyses identified differences between *ARID1A* patients with variants in the ARID-domain compared to CBR-domain patients($p < 0.01$). ARID-domain patients have less severe ID ($p = 0.02$) and less myopia (100% versus 33%, $p = 0.03$) compared to CBR-domain patients.

Here, we report two novel clinical and (epi)genetic entities within the CSS spectrum.

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P10.019.B Effects of *WDFY3* loss-of-function in a human neuroblastoma model

Moritz Paha[1;2], *Sandy Richter*[2], *Max Holzer*[3], *Rami Abou Jamra*[1], *Johannes Lemke*[1], *Wieland Kiess*[2], *Antje Garten*[2], *Diana le Duc*[1;4]

[1]*Institute of Human Genetics, University of Leipzig Medical Center, Leipzig, Germany;* [2]*Center for Pediatric Research Leipzig, University Hospital for Children and Adolescents, Leipzig, Germany;* [3]*Paul Flechsig Institute for Brain Research, Department for Molecular and Cellular Mechanisms of Neurodegeneration, Leipzig, Germany;* [4]*Department for Clinical Genetics, Zentrum für Diagnostik GmbH am Klinikum Chemnitz, Chemnitz, Germany*

Neurodevelopmental disorders (NDD) are poorly understood and late diagnosis and interventions result in a high burden for the society and health system.

WD repeat and FYVE domain-containing protein 3 (WDFY3) acts as an adaptor and scaffolding protein in selective autophagy. Variants in *WDFY3* have been identified as a monogenic cause for NDD and are also associated with altered brain size. We (re-) analysed a large cohort of patients ($n = 26$) and present a comprehensive description of *WDFY3*-associated phenotypes. We found that brain size phenotypes are not related to specific protein domains.

To dissect effects of *WDFY3* loss-of-function we used the human neuroblastoma cell line SH-SY5Y to establish a *WDFY3* knockdown. Proliferation was increased as determined by cell counting, staining for Ki-67 and *PCNA* expression. Differentiation was decreased as shown by quantification of neurite tracing. We suggest that *WDFY3* plays a role in neuronal differentiation as *WDFY3* expression was increased in differentiated cells. These effects might be caused by downregulated wnt/β-catenin signalling since we observed lower levels of endogenous β-catenin protein. We also observed impaired clearance of aggregated Huntingtin protein and decreased protein levels of the ubiquitin-binding protein p62. We believe that this impairment of selective autophagy could be caused by incorrect interaction of WDFY3 and p62. Lastly, we found that treatment with the GSK-3β inhibitor lithium chloride was able to increase the expression of *WDFY3* and are currently investigating therapeutic implications.

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Conflict of Interest: None declared

P10.020.C Exome capture RNA-Seq from short-term lymphocyte cultures: A promising alternative to fibroblasts for diagnosing neurodevelopmental disorders

Thomas Besnard[1;2], *Laura Do Souto Ferreira*[1], *Virginie Vignard*[2], *Fabrice Airaud*[1], *Patricia Talarmain*[1], *Gaelle Landeau-Trottier*[1], *Eva Trochu*[1], *Delphine Quinquis*[1], *Jerome Buscail*[1], *Wallid Deb*[1;2], *Frédéric Ebstein*[2], *Sebastien Küry*[1;2], *Annastasia Voisine*[1], *Solène Conrad*[1], *Sandra Mercier*[1;2], *Mathilde Nizon*[1;2], *Marie Vincent*[1;2], *Bertrand Isidor*[1;2], *stéphane bezieau*[1;2], **Benjamin Cogné**[1;2]

[1]*CHU de Nantes, Service de Génétique médicale, Nantes, France;* [2]*Nantes Université, CHU de Nantes, CNRS, INSERM, l'institut du thorax, Nantes, France*

Background/Objectives: In France, the national genomic plan (PFMG 2025) has significantly advanced genome analysis for diagnosing neurodevelopmental disorders (NDD). However, with a current diagnostic rate of approximately 35%, many families still face diagnostic odyssey. RNA-Seq is being increasingly considered as the best complementary technique. However, whole blood yielded poor results and fibroblasts are currently the best clinically accessible tissue. Our objective was to find a clinical alternative to fibroblasts suitable for RNA-Seq analysis.

Methods: We collected 60 blood samples from probands with NDD or relatives. PBMCs were isolated and lymphocytes were cultured for 2 to 4 days on karyotyping medium with or without a 4-hour puromycin treatment. Automated exome capture RNA-Seq libraries were generated with SureSelect XTHS1 on the MAGNIS platform (Agilent) and sequenced on the NextSeq550 (2x75bp). Fastq were aligned with STAR on GRCh38, then splicing and expression outliers were identified using FRASER and OUTRIDER, respectively.

Results: Principal component analysis showed that short-term lymphocyte cultures exhibited homogenous gene expression, comparable to fibroblasts. We achieved at least 20X coverage for 66% of NDD genes, surpassing poly-A RNA-Seq on fibroblasts (58%) at equivalent sequencing depth (30 million paired-reads). We confirmed the pathogenicity of splicing-altering variants identified by genomics, alongside the detection of aberrant splicing and expression events in undiagnosed patients. We will show the first results of the study on undiagnosed patients confirming the interest of this strategy even after a negative genome.

Conclusion: Exome capture RNA-Seq from short-term lymphocyte cultures holds the potential for widespread application in NDD diagnosis.

Conflict of Interest: None declared

P10.021.D Optical genome mapping finally unveils the genetic cause of aniridia and intellectual disability in a 17-year-old after seven years of diagnostic odyssey

Wilena Telman[1], *Uwe Heinrich*[1], *Aysegül Klapperich*[2], *Jenny Schiller*[1], *Konstanze Hörtnagel*[1]

[1]*MVZ Martinsried GmbH, Planegg, Germany;* [2]*MVZ Humangenetik Köln GmbH, Köln, Germany*

Background/Objectives: We report on a 17-year-old female with aniridia, optic nerve hypoplasia, intellectual disability, and current overweight, raising suspicion of WAGR syndrome (Wilms tumor, aniridia, genitourinary anomalies, and impaired intellectual development syndrome). Microdeletions involving the *WT1* gene (*WT1* transcription factor) and the *PAX6* gene (paired box gene 6) cause WAGR syndrome. Previous chromosome analysis revealed a de novo reciprocal translocation t(11;22)(p13;q11.2).

Methods: Despite inconspicuous pre-diagnostics in 2015 (MLPA of *WT1* and *PAX6*, *PAX6* sequencing and microarray analysis), we were able to characterize the translocation breakpoints by using optical genome mapping (OGM, Bionano Genomics), uncovering the genetic cause for the patient's symptoms.

Results: The translocation was defined as seq(GRCh38) t(11;22)(p13;q12.1), pinpointing the breakpoints to a 5.5 kb region in intron 9 of the *ELP4* (elongation acetyltransferase complex, subunit 4) gene. This breakpoint lies approximately 103 kb downstream of the *PAX6* gene and causes the loss of the highly conserved regulatory element "SIMO" (ultra-conserved *PAX6* cis-regulatory element) within the *ELP4* gene, which is essential for *PAX6* gene expression. Causative variants in *PAX6* or its regulatory cis-elements can lead to aniridia, optic nerve hypoplasia and, rarely, autosomal dominant mental retardation.

Conclusion: This case underscores the diagnostic value of OGM, identifying the genetic basis for symptoms persisting over seven years of negative molecular pre-diagnostics.

Grants: -

Conflict of Interest: None declared

P10.022.A Rubinstein-Taybi syndrome: Characterization of epigenetic profiles to study the molecular mechanisms of the disease

Julien Van Gils[1], *Slim Karkar*[2], *Aurélien Barré*[2], *Sophie Nothof*[3], *Stéphane Claverol*[4], *Caroline Tokarski*[4], *Jean-Philippe Trani*[3], *Natacha Broucqsault*[3], *Claire El Yazidi*[3], *Didier LACOMBE*[1], *Patricia Fergelot*[1], *Frédérique Magdinier*[3]

[1]*Inserm U1211, Bordeaux, France;* [2]*Bordeaux Bioinformatic Center, Bordeaux, France;* [3]*Inserm U1251, Marseille, France;* [4]*Bordeaux Proteomic Platform, Bordeaux, France*

Rubinstein-Taybi syndrome (RTS) is a rare and severe genetic developmental disorder characterized by multiple congenital anomalies and intellectual disability. *CREBBP* and *EP300*, the two genes that are causative of RTS, encode transcriptional coactivators that possess a catalytic lysine acetyl transferase domain. RTS is considered as a model of epigenetic neurodevelopmental disorders. In RTS patients, nothing is known on the functional link between histone acetylation and transcriptomic profiles during neuronal differentiation.

We derived induced pluripotent stem cells from RTS patients carrying a recurrent *CREBBP* mutation. We differentiated these cells into cortical and pyramidal neurons. We compared their acetylome by LS-MS/MS and transcriptome by RNA-seq to cells derived from healthy donors at three different timepoints during neuronal differentiation.

We identified 25 specific acetylated histone marks that are specifically altered in RTS. All four core histones are regulated by CBP but H2B and H3 acetylation levels are the most impacted. By transcriptomic analyses, we identified the transition between neural progenitors and immature neurons as a critical step of the differentiation process, with an impact on genes involved in neuronal differentiation and brain development. Secondly, comparison of transcriptome analysis on fibroblasts with those obtained on neurons enabled us to identify 8 potential biomarkers that could define a transcriptomic signature of RTS in fibroblasts.

Our data highlight specific histone acetylation changes and a crucial phase of differentiation as a hallmark of RTS neuronal development. Analysis on fibroblasts reflects the alterations observed at neuronal level, making it a tissue of choice for the identification and testing of biomarkers in routine care diagnostics.

Conflict of Interest: None declared

P10.023.B Identification of relevant clinical and molecular data to reclassify variants of uncertain significance in the X-linked *HCFC1* gene

Sarah Cluzel[1], *Stefania Bigoni*[2], *Valentina Govoni*[2], *Alessandra Ferlini*[2], *Marianna Farnè*[2], *Rita Selvatici*[2], *Giorgia Girotto*[3], *Alice Goldenberg*[4], *Juliette Coursimault*[4], *François Lecoquierre*[4], *Aurore Garde*[5], *Laurence Faivre*[5;6;7], *Christel Thauvin-Robinet*[5;6;7], *Ange-Line Bruel*[6;7], *Christophe Philippe*[6;7], *Frederic Tran Mau Them*[6;7], *Julien Thevenon*[6;7], *Paul Kuentz*[6;7], *Antonio Vitobello*[6;7], *Arthur Sorlin*[6;7], *Gwenaël Le Guyader*[8], *Frederic Bilan*[8;9], *Renaud Touraine*[10], *Sarah Grotto*[11], *Boris Keren*[12;13], *Didier Devys*[14], *Jean Louis Mandel*[15], *Amelie Piton*[14;15], *Salima EL CHEHADEH*[1;16]

[1]*Service de Génétique Médicale, Institut de Génétique Médicale d'Alsace (IGMA), Hôpitaux Universitaires de Strasbourg, Strasbourg, France;* [2]*Medical Genetics Unit, Department of Medical Science, Ferrara University, Ferrara, Italy;* [3]*Institute for Maternal and Child Health-IRCCS Burlo Garofolo, Trieste, Italy;* [4]*Department of Genetics and Reference Center for Developmental Disorders, Rouen University Hospital, Rouen, France;* [5]*CRMRs "Anomalies du Développement et syndromes malformatifs" et "Déficiences Intellectuelles de causes rares", Centre de Génétique, CHU Dijon, Dijon, France;* [6]*Equipe INSERM UMR1231, Génétique des Anomalies du Développement, FHU TRANSLAD, Université Bourgogne Franche-Comté, Dijon, France;* [7]*UF "Innovation diagnostique dans les maladies rares", CHU Dijon, Dijon, France;* [8]*Service de Génétique Médicale, CHU de Poitiers, Poitiers, France;* [9]*INSERM U1084, LNEC, Université de Poitiers, Poitiers, France;* [10]*Service de Génétique Clinique, Chromosomique et Moléculaire, CHU-Hôpital Nord, Saint-Étienne, France;* [11]*UF de génétique clinique, Hôpital Armand Trousseau, APHP, Sorbonne Université, Paris, France;* [12]*Department of Genetics, Center for Rare causes of Intellectual Disabilities and UPMC Research Group "Intellectual Disabilities and Autism", Paris, France;* [13]*Department of Genetics, La Pitié-Salpêtrière Hospital, Assistance Publique-Hôpitaux de Paris, Paris, France;* [14]*Laboratoire de Diagnostic Génétique, Institut de Génétique Médicale d'Alsace (IGMA), Hôpitaux Universitaires de Strasbourg, Strasbourg, France;* [15]*INSERM U1258, CNRS-UMR7104, Institut de Génétique et de Biologie Moléculaire et Cellulaire (IGBMC), Université de Strasbourg, Illkirch Graffenstaden, France;* [16]*Laboratoire de Génétique Médicale, UMRS_1112, Institut de Génétique Médicale d'Alsace (IGMA), Université de Strasbourg et INSERM, Strasbourg, France*

Background: The *HCFC1* gene encodes a transcriptional co-regulator. Pathogenic missense variants have been reported in males with a X-linked neurodevelopmental disorder ranging from severe intellectual disability (ID) with drug-resistant epilepsy and cobalamin metabolism disorder (CblX) to non-specific ID without CblX, and even isolated epilepsy. A genotype-phenotype correlation has been evoked, with CblX associated variants being localized into the K1 and K2 repeats. However, as for other X-linked genes, the interpretation of inherited missense variants in *HCFC1* is a huge challenge, especially in case of non-CblX ID, as illustrated by the numerous variants of uncertain significance (VUS) reported (n = 205 VUS versus 7 pathogenic/likely pathogenic variants in ClinVar).

Objectives: The identification of relevant clinical and molecular data (including carrier mothers' X chromosome inactivation (XCI)

status) is therefore necessary to improve variants interpretation and increase the diagnostic of the non-CblX form of ID associated to *HCFC1* variants.

Methods and results: We identified one missense variant p.(Arg320Cys), located out of the K2 repeats, in three patients with moderate ID without CblX, from two unrelated families. Asymptomatic mothers presented with extremely skewed XCI, as in the three previously reported families. We collected clinical and molecular data for a dozen additional missense VUS through a French collaboration call.

Conclusion: These first results help to better describe the phenotypical spectrum of *HCFC1* related disorders, in particular the non-CblX ID form, confirm the correlation between variants' location and the CblX versus non-CblX phenotypes, and suggest that studying XCI in carrier females is useful to interpret *HCFC1* VUS.

Conflict of Interest: None declared

P10.024.C *EXOC1*: A novel candidate gene for syndromic intellectual disability with autosomal recessive inheritance

Lauren Badalato[1]

[1]*Queen's University, Pediatrics, Kingston, Canada*

Background/Objectives: *EXOC1* is a component of a multimeric complex involved in ciliogenesis, cell migration, and fusion of secretory vessels. While *EXOC1* has not yet been associated with a particular genetic condition in humans, other genes in the same exocyst complex, including *EXOC7* and *EXOC8*, have been associated with autosomal recessive neurodevelopmental disorders. One study reported that knockout of *Exoc1* was perinatal lethal in mice, suggesting a crucial biological importance of this gene.

Methods: Whole exome sequencing (WES) was performed in a clinical laboratory; the patient's records were reviewed and a clinical genetics exam was performed.

Results: The patient is currently 19 years of age and has a history of microcephaly, dysmorphism, and moderate intellectual disability.

WES identified biallelic frameshift variants in *EXOC1*: c.113dupT, p.Leu38PhefsX8 (pat), / c.31_32delGA, p.Asp11HisfsX6 (mat). Familial variant testing was also completed in the proband's unaffected sister, who was found to be heterozygous for the paternal variant.

Conclusion: We present *EXOC1* as a candidate gene for an autosomal recessive intellectual disability syndrome. While we are unable to draw definitive conclusions from single patient, the association of other exocyst subunit proteins with autosomal recessive neurodevelopmental disorders and microcephaly raises suspicion that complete loss of function of *EXOC1* may have similar effects. Studies are ongoing to better understand the functional impact of these variants.

Conflict of Interest: None declared

P10.025.D Novel UPF3B nonsense variant in two brothers with X-linked intellectual developmental disorder MRXS14

Maria Yusenko[1], *Silke Loeper*[2], *Kristina Doering*[1], *Krzysztof P Lubieniecki*[1], *Joanna Lubieniecka*[1], *Huu Phuc Nguyen*[1], *Sabine Hoffjan*[1]

[1]*Faculty of Medicine, Ruhr University Bochum, Department of Human Genetics, Bochum, Germany;* [2]*University Children's Hospital, Ruhr-University Bochum, Department of Neuropediatrics, Bochum, Germany*

Background/Objectives: MRXS14 is a rare X-linked intellectual developmental disorder (OMIM: 300676) with variable severity and type of clinical presentations. It is caused by *loss-of-function* variants in the *UPF3B* gene located on chromosome Xq24. As a member of the nonsense-mediated mRNA decay pathway, UPF3B is known to be important for normal development und function of the brain. So far only a limited number of patients and variants for this rare disorder have been reported.

Methods: In a family with two affected boys, we performed whole-exome-sequencing (WES) which revealed the novel nonsense variant c.240del; p.(Phe80Leufs*51) in *UPF3B* in both children in hemizygous state. The healthy mother is heterozygous carrier of this variant. We compare the clinical phenotype to that of other patients described in the literature.

Results: Both boys share some clinical features, including global developmental delay, hypertelorism, facial dysmorphism and muscle hypotonia. However, the overall clinical picture differs between the brothers, with the older boy additionally only showing macrocephaly, whereas his younger brother was born with severe congenital heart disease and frontal brain atrophy with hydrocephaly.

Conclusion: WES allowed to make the diagnosis in our patients. Our findings will help to further delineate the genotypic and phenotypic spectrum of MRXS14 disorder.

Conflict of Interest: None declared

P10.026.A YAF2: a novel candidate gene implicated in autosomal recessive non-syndromic intellectual disability

Ghazala Zafar[1], *Lubaba Bintee Khalid*[1], *Zafar Ali*[2], *Sohana Nadeem Hashami*[1], *Mathias Toft*[3;4], *Zafar Iqbal*[4], **Ambrin Fatima**[1]

[1]*Aga Khan University, Biological and Biomedical Sciences, Karachi, Pakistan;* [2]*University of Swat, Centre for Biotechnology and Microbiology, Swat, Pakistan;* [3]*University of Oslo, Institute of Clinical Medicine, Oslo, Norway;* [4]*Oslo University Hospital, Department of Neurology, Oslo, Norway*

Background/Objectives: Non-syndromic intellectual disability is a rare neurodevelopmental condition with significant limitation of cognitive functioning and adaptive behaviour as the primary disability. Extreme clinical and genetic heterogeneity has been witnessed for this condition.

Methods: We recruited a consanguineous Pakistani family manifesting severe non-syndromic intellectual disability. Two affected brothers were subjected to whole exome sequencing (WES) and shared variants were further prioritized based on population frequency, predicted pathogenicity and functional relevance.

Results: The WES data revealed a homozygous frameshift variant, c.470dup (NM_005748.6); (p.Asn157Lysfs*62) in the gene *YAF2*, which co-segregated with the disease. *YAF2* encodes a zinc finger containing protein that functions in transcription regulation and an interacting partner of a transcriptional repressor protein YY1, which has been implicated in developmental delay/intellectual disability (DD/ID). *YAF2* deletion in zebra fish and mouse embryonic stem cells (mESCs) causes developmental arrest and leads to impaired proliferation and abnormal differentiation. It primarily acts as a transcriptional repressor, notably affecting genes related to ectoderm cell fate.

Conclusion: Our findings indicate that *YAF2* is a novel candidate gene responsible for non-syndromic intellectual disability. Further, genetic recurrence and functional studies are warranted to consolidate the role of *YAF2* variants in disease manifestation.

Conflict of Interest: None declared

P10.027.B Phenotypic variability of patients with an identical *SHOC2* variant

Reena Buurman[1], *Karsten Thiel*[2], *Ulrich Baumann*[3], *Nataliya Di Donato*[1], *Sandra von Hardenberg*[1], *Bernd Auber*[1]

[1]*Institute of Human Genetics, Hannover Medical School, Hannover, Germany;* [2]*Clinic for Children and Adolescent Care, St. Clemens Hospital Geldern, Geldern, Germany;* [3]*Pediatric Pneumology, Allergology and Neonatology, Hannover Medical School, Hannover, Germany*

Background/Objectives: The SHOC2 protein is involved in the MAPK-signaling pathway. A specific variant in *SHOC2*:c.4A>C is known to cause Noonan-like syndrome with loose anagen hair (NS/LAH) (MIM 607721). NS/LAH is a RASopathy characterized by symptoms such as macrocephaly, growth retardation, mental retardation and cardiac defects. Here we present two NS/LAH patients with additional distinct symptoms which might add to the phenotypic spectrum of NS/LAH.

Methods: Whole genome sequencing was performed using the Illumina NovaSeq 6000 platform. Variant analysis was conducted using the Alamut (Interactive Biosoftware, Rouen, France) and GSvar Tübingen, Germany) programs. Variant classification followed the current ACMG Standards. *SHOC2* reference transcript: NM_001324336.2

Results: We identified a well-known pathogenic variant c.4A>C p.(Ser2Gly), that is typically associated with a consistent clinical phenotype of NS/LAH, in two individuals referred for genetic analysis because of additional symptoms, which were previously not described in the SHOC2 phenotype. Patient 1: A one-year-old boy, presented with unexplained hepatopathy and mental retardation. Initial testing for hepatopathy-associated genes was requested and showed no significant findings. However, an extended WGS analysis identified the recurrent *SHOC2* variant. Patient 2: An 18-year-old male with cardiac issues, sparse hair, short stature and therapy-refractory autoimmune diseases was initially tested for immunodeficiency-associated genes with inconclusive results. Subsequent WGS analysis revealed the *SHOC2* variant. Trio genome analysis revealed no additional variants that might be causative for immune phenotype.

Conclusion: We report two novel phenotypes associated with the recurrent pathogenic missense variant in *SHOC2*. This variant appears to have a broader phenotypic spectrum than previously recognized.

Grants: none

Conflict of Interest: None declared

P10.028.C Confirming *NUSAP1* as a disease gene for a syndromic neurodevelopmental disorder: insights from the third case

Maureen Jacob[1], *Susann Badmann*[1], *Theresa Brunet*[1;2], *Juliane Winkelmann*[1;3;4], *Matias Wagner*[1;2;4]

[1]*Institute of Human Genetics, School of Medicine, Technical University of Munich, Munich, Germany;* [2]*Dr. v. Hauner Children's Hospital, Department of Pediatric Neurology and Developmental Medicine, LMU - University of Munich, Munich, Germany;* [3]*Munich Cluster for Systems Neurology (SyNergy), Munich, Germany;* [4]*Institute of Neurogenomics, Helmholtz Zentrum München, Munich, Germany*

Background: By maintaining spindle function during metaphase, *NUSAP1* plays an important role in mitosis. Mo et al. recently described an association between a heterozygous de novo nonsense variant in *NUSAP1* in the penultimate exon and a neurodevelopmental symptom complex including congenital microcephaly, seizures, and severe developmental delay based on two unrelated individuals. Here, we present a third case with an overlapping phenotype and a heterozygous de novo nonsense variant in the last exon of *NUSAP1*.

Methods: DNA, extracted from blood samples of the patient and parents, was used for trio exome sequencing (ES). The patient's phenotype was systematically analyzed using HPO-terms (i.e. severe microcephaly, global developmental delay, seizures).

Results: ES failed to identify a pathogenic variant in established disease genes listed in OMIM. Subsequent analysis of rare de novo variants identified a heterozygous nonsense variant in the candidate gene *NUSAP1* (NM_016359.5:c.1264C>T, p.Arg422Ter), located in the last exon and not previously reported in the literature.

Conclusion: To date, we present the third case of *NUSAP1*-associated microcephaly and a neurodevelopmental phenotype, thereby strengthening its role as a disease gene. Notably, with a pLI of 0, *NUSAP1* appears to be tolerant to Loss-of-function variants. Our findings not only contribute to the growing evidence that variants located towards the C-terminus of *NUSAP1* escape nonsense-mediated decay but also raise the hypothesis that these variants, particularly in the charged helical domain, may have dominant-negative or gain-of-function effects. We propose that such variants could induce an over-stabilization of the spindle assembly and thereby disrupt cell division processes.

Grants:

Conflict of Interest: None declared

P10.029.D Deleterious ZNRF3 germline variants as a novel cause of neurodevelopmental disorders with mirror brain phenotypes due to distinct domain-specific effects on Wnt/β-catenin signaling

Paranchai Boonsawat[1], *Reza Asadolahi*[2], *Dunja Niedrist*[1], *Katharina Steindl*[1], *Anaïs Begemann*[1], *Pascal Joset*[3], *Elizabeth Bhoj*[4], *Dong Li*[4], *Elaine Zackai*[5], *Annalisa Vetro*[6], *Renzo Guerrini*[6], *Sandra Whalen*[7], *Boris Keren*[8], *Amjad Khan*[9], *Duan Jing*[10], *María Palomares-Bralo*[11], *Qin Hao*[12], *Britta Schlott Kristiansen*[12], *Bixia Zheng*[13], *Deirdre Donnelly*[14], *Markus Zweier*[1], *Michael Papik*[1], *Gabriele Siegel*[1], *Anselm Horn*[1;15], *Heinrich Sticht*[15], *Anita Rauch*[1;16]

[1]*Institute of Medical Genetics, University of Zurich, Zurich, Switzerland;* [2]*Faculty of Engineering and Science, University of Greenwich London, London, United Kingdom;* [3]*Medical Genetics, University Hospital Basel, Basel, Switzerland;* [4]*Center for Applied Genomics, Children's Hospital of Philadelphia, Philadelphia, United States;* [5]*Division of Human Genetics, Children's Hospital of Philadelphia, Philadelphia, United States;* [6]*Pediatric Neurology, Neurogenetics and Neurobiology Unit and Laboratories, Meyer Children's Hospital, University of Florence, Florence, Italy;* [7]*Unité Fonctionnelle de Génétique clinique, Hôpital Armand Trousseau, Paris, France;* [8]*Département de génétique, Hôpital de la Pitié-Salpêtrière, Paris, France;* [9]*Faculty of Science, Department of Biological Science (Zoology), University of Lakki Marwat, Khyber Pakhtunkhwa, Pakistan;* [10]*Shenzhen Children's Hospital, Guangdong, China;* [11]*Instituto de Genética Médica y Molecular (INGEMM), Unidad de trastornos del neurodesarrollo, Hospital Universitario La Paz, Madrid, Spain;* [12]*Department of Clinical Genetics, Odense University Hospital, Odense, Denmark;* [13]*Nanjing Key Laboratory of Pediatrics, Children's Hospital of Nanjing Medical University, Nanjing, China;* [14]*Belfast Health and Social Care Trust, Belfast, Ireland;* [15]*Institute of Biochemistry, Friedrich-Alexander-Universität Erlangen-Nürnberg (FAU), Erlangen, Germany;* [16]*Pediatric University Hospital Zurich, Zurich, Switzerland*

Background/Objectives: Zinc RING finger 3 (ZNRF3) is a negative-feedback regulator of Wnt/β-catenin signaling, which plays an

important role in human brain development. Although somatic mutations are frequently observed in cancer, germline variants in ZNRF3 have not been established as causative for neurodevelopmental disorders (NDDs).

Methods: We identified 12 patients with ZNRF3 variants and various phenotypes via GeneMatcher and evaluated genotype-phenotype correlation. After structural modelling, representative deleterious and control variants were assessed using in vitro transcriptional reporter assays with and without Wnt-ligand Wnt3a and Wnt-potentiator R-spondin (RSPO).

Results: We collected eight patients harboring de novo missense variants, who all presented with NDD. In addition, we obtained four patients with truncating variants, who showed non-NDD features, including heart, adrenal, or nephrotic problems. We found missense variants associated with macrocephalic NDD to cluster in the RING ligase domain. By structural modeling we predicted disruption of the ubiquitin ligase function likely compromising the Wnt receptor turnover. Accordingly, the transcriptional reporter assays showed enhanced Wnt/β-catenin signaling for these variants in a dominant negative manner. Contrarily, a patient with microcephalic NDD harbored a missense variant in the RSPO binding domain predicted to disrupt binding affinity to RSPO and showed attenuated Wnt/β-catenin signaling in the transcriptional reporter assays. In contrast to NDD-associated missense variants, the truncating variant behaved as the empty vector, and benign variants as the wildtype.

Conclusion: We provide evidence for mirror brain size phenotypes caused by distinct pathomechanisms in Wnt/β-catenin signaling through protein domain-specific deleterious ZNRF3 germline missense variants.

Grants: URPPs PRAECLARE and ITINERARE

Conflict of Interest: None declared

P10.030.A The cell division cycle protein 20 (*CDC20*) gene as a candidate gene for autosomal recessive syndromic microcephaly

Daniel L. Polla[1], *Mukunth Sadagopan*[1], *Javier Martini*[1], *Inês C Fernandes*[1], *Adnan Javed*[1], *Emir Zonic*[1], *Lia Abbasi Moheb*[1], *Tawfig Bin Omran*[2], *Peter Bauer*[1], *Aida Bertoli-Avella*[1]

[1]*CENTOGENE GmbH, Rostock, Germany;* [2]*Sidra and Hamad Medical Corporation, Genetic and Genomic Medicine, Doha, Qatar*

Background/Objectives: Microcephaly, characterized by a small head circumference, is usually caused by abnormal brain growth and development due to genetic and environmental factors. *CDC20* encodes a protein that regulates cell division and chromosome segregation, which is essential for the proper functioning of the anaphase-promoting complex (APC/C). It is also involved in apoptosis, tumorigenesis, immune responses, and stemness processes. *CDC20* has been previously associated with autosomal recessive oocyte/zygote/embryo maturation arrest type 14 due to its role in mitosis and meiosis, with *Cdc20* hypomorphic mice experiments indicating that female infertility results from dosage dependent CDC20 reduction, while *Cdc20* knockout mice were embryonic lethal. But its involvement in microcephaly is still unclear.

Methods NGS data was analyzed by CENTOGENE's bioinformatics pipeline and comprehensive variant filtering. Family history and clinical information provided were used to evaluate identified variants for pathogenicity and disease causality.

Results: We report four patients with microcephaly, neurodevelopmental delay, and behavioral abnormalities, who are homozygotes for a *CDC20* variant (NM_001255.2:c.556+1G>T, spliceAI score = 0.99) predicted to affect gene splicing. This variant is not reported in literature or population databases.

Conclusion: Previous studies showed that *CDC20* and APC/C play important roles in brain development, influencing the proliferation and differentiation of neural cells and the formation and function of synapses in rodents and *C. elegans*, and interaction with STIL, a centriole duplication factor associated with autosomal recessive primary microcephaly (OMIM 612703). While the applied ClinGen gene-disease validity guidelines showed limited relationship with the microcephaly phenotype, further research is needed to identify the underlying molecular mechanisms of this condition.

Conflict of Interest: Daniel L. Polla Employee at Centogene GmbH, Mukunth Sadagopan Employee at Centogene GmbH, Javier Martini Employee at Centogene GmbH, Inês C Fernandes Employee at Centogene GmbH, Adnan Javed Employee at Centogene GmbH, Emir Zonic Employee at Centogene GmbH, Lia Abbasi Moheb Employee at Centogene GmbH, Tawfig Bin Omran: None declared, Peter Bauer Managing Director at Centogene GmbH, Aida Bertoli-Avella Employee at Centogene GmbH

P10.031.B The Brain Gene Registry: Pairing Clinical Variants with Phenotype to Advance Intellectual and Developmental Disability Research

Maya Chopra[1;2], *Juliann Savatt*[3], *Taylor Bingaman*[3], *Molly Good*[3], *Alexis Morgan*[3], *Caitlin Cooney*[3], *Allison Rossel*[3], *Bryanna VanHoute*[3], *Ineke Cordova*[3], *Sonal Mahida*[1], *Abigail Sveden*[1], *Virginia Lanzotti*[4], *Dustin Baldridge*[4], *Christina Gurnett*[4], *Joseph Piven*[5], *Heather Hazlett*[5], *Scott Pomeroy*[1;2], *Mustafa Sahin*[1;2], *John Constantino*[6], *Erin Rooney Riggs*[3], *Philip Payne*[7]

[1]*Boston Children's Hospital, Rosamund Stone Zander Translational Neuroscience Center ; Intellectual and Developmental Disability Research Center, Boston, MA, United States;* [2]*Harvard University, Harvard Medical School, Boston, MA, United States;* [3]*Geisinger, Autism and Developmental Medicine Institute, Danville, PA, United States;* [4]*Washington University School of Medicine, Intellectual and Developmental Disability Research Center, St Louis, MO, United States;* [5]*University of North Carolina, Intellectual and Developmental Disability Research Center, Chapel Hill, NC, United States;* [6]*Children's Healthcare of Atlanta; Emory University, Departments of Psychiatry and Pediatrics, Atlanta, GA, United States;* [7]*Institute for Informatics, Washington University School of Medicine, St Louis, MO, United States*

Consortium: Brain Gene Registry Consortium

Background/Objectives: Clinically ascertained variants are under-utilized in intellectual and developmental disability (IDD) research; published reports are relied upon for the establishment of gene disease validity and understanding of disorder spectrum. We established the Brain Gene Registry (BGR) to co-register clinical variants with participant phenotypes. Here, we analyze the first 179 variants in the first 179 BGR registrants for novelty to ClinVar at the time of entry and presence in 6 other disease databases.

Methods: Participants with clinical variants (variant of uncertain significance [VUS] or above) in putative brain genes were enrolled from 12 IDD Research Centers, with co-registration of variant with standardized neurodevelopmental assessment and electronic health record. Individuals also enrolled into Genome Connect, the ClinGen patient registry. The first 179 variants were cross-referenced for previous presence in ClinVar and for presence in 6 other genetic databases.

Results: At present, there are 574 BGR registrants, representing 183 genes and 225 VUS classifications. Analysis of the first 179 variants showed that 76 (42.5%) were novel to ClinVar, and 62 (34.6%) were absent from all databases analyzed. Of the 103 variants present in ClinVar, 37 (35.9%) were uncertain (ClinVar aggregate classification of VUS or conflicting classifications).

Conclusion: A significant proportion of clinical variants that are novel or uncertain are siloed in healthcare systems, limiting the evidence base for new gene-disease relationships and full understanding of disorder spectrum. By co-registering variant and phenotype, the BGR serves as a unique resource for translational advances relating to rare IDDs.

Grants: IDDRC-CTSA BGR: 1U01T002764-01A. GenomeConnect: U24HG006834.

Conflict of Interest: None declared

P10.032.C Heterozygous variants in PACS1 are associated with a distinctive DNA methylation episignature

Sadegheh Haghshenas[1], *Raissa Relator*[1], *Michael Levy*[1], *Jennifer Kerkhof*[1], *Haley McConkey*[1;2], *Carolyn Lauzon-Young*[1;2], *Quentin Sabbagh*[3;4], *Camille Cenni*[5], *Alfredo Brusco*[6], *Giovanni Battista Ferrero*[7], *Victoria Siu*[8;9], *Irene Valenzuela Palafoll*[10], *David Geneviève*[3;4], *Bekim Sadikovic*[1;2]

[1]*Verspeeten Clinical Genome Centre, London Health Sciences Centre, London, Canada;* [2]*Western University, Department of Pathology and Laboratory Medicine, London, Canada;* [3]*Montpelier University, Inserm U1183, Montpellier, France;* [4]*Reference Center for Rare Disease and Malformative Syndrome, Clinical Genetic Department, Montpellier University Hospital, Montpellier, France;* [5]*Montpelier University, Department of Molecular Genetics and Cytogenomics, Rare and Autoinflammatory Diseases Unit, Centre Hospitalier Universitaire de Montpellier, Montpellier, France;* [6]*University of Torino, Department of Medical Sciences, Turin, Italy;* [7]*University of Torino, Department of Public Health and Pediatrics, Turin, Italy;* [8]*Western University, Department of Paediatrics, Division of Medical Genetics and Metabolism, London, Canada;* [9]*Children's Health Research Institute, London, Canada;* [10]*University Hospital Vall d'Hebron, Department of Clinical and Molecular Genetics, Medicine Genetics Group, Valle Hebron Research Institute, Barcelona, Spain*

Background/Objectives: Schuurs-Hoeijmakers syndrome (SHMS, OMIM # 615009) is a Mendelian disorder caused by pathogenic heterozygous variants in *PACS1*. It primarily manifests with global developmental delay, facial dysmorphisms, and various other congenital anomalies, including hypotonia, heart and eye abnormalities, and digestion problems. Given the clinical phenotypic overlap between SHMS and other neurodevelopmental disorders, definitively diagnosing the syndrome poses significant challenges.

The objective of this study is to identify a robust episignature for SHMS that can be used as a reliable biomarker for diagnosis in a clinical setting.

Methods: The EpiSign™ discovery pipeline was utilized to conduct genome-wide DNA methylation analysis on DNA samples extracted from peripheral blood of individuals with SHMS, comparing them with samples from controls and other known episignature disorders within the EpiSign Knowledge Database (EKD). Methylation levels were assessed using Illumina Infinium MethylationEPIC microarrays, and machine learning and statistical models were applied to identify the SHMS episignature.

Results: A robust episignature was detected for SHMS. Notably, this identified episignature successfully classified a previously unresolved subject within the EKD. Subsequent investigation revealed that the individual harbored one of only two known pathogenic *PACS1* variants associated with the syndrome, providing additional evidence for the episignature's robustness and sensitivity.

Conclusion: This study establishes a highly sensitive and specific episignature for SHMS, highlighting the expanding utility of episignature analysis in patients with suspected rare diseases.

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Conflict of Interest: Sadegheh Haghshenas: None declared, Raissa Relator: None declared, Michael Levy: None declared, Jennifer Kerkhof: None declared, Haley McConkey: None declared, Carolyn Lauzon-Young: None declared, Quentin Sabbagh: None declared, Camille Cenni: None declared, Alfredo Brusco: None declared, Giovanni Battista Ferrero: None declared, Victoria Siu: None declared, Irene Valenzuela Palafoll: None declared, David Geneviève: None declared, Bekim Sadikovic B.S. is a shareholder in EpiSign Inc, a biotech company involved in commercialization of EpiSign™ technology.

P10.033.D Causative single nucleotide variants in patients with mendelian disorders of the epigenetic machinery detected through whole exome sequencing in routine diagnostics

Judith Gottfreund[1], *Anna Munzig-Schmidt*[1], **Christian Albig**[1], *Julia Philippou-Massier*[1], *Konstanze Hörtnagel*[2]

[1]*MVZ Martinsried GmbH, Scientific Services, Planegg, Germany;* [2]*MVZ Martinsried GmbH, Medical Services, Planegg, Germany*

Background/Objectives: Conditions within the spectrum of mendelian disorders of the epigenetic machinery (MDEMs) comprise a group of multiple congenital disorders resulting from causative variants in genes encoding members of the epigenetic machinery. These mainly include epigenetic players responsible for defining DNA methylation, histone modifications and chromatin structure. Together with the transcription machinery, these components dynamically orchestrate gene expression and play an important role in mammalian development, environmental response and disease. MDEMs are phenotypically associated with neurodevelopmental disorder (ND), intellectual disability (ID) and abnormal growth. The recent improvement of NGS technologies and their use in genetic routine diagnostics has substantially refined the identification of MDEMs and provided enhanced opportunities for their diagnosis.

Methods: As part of our routine diagnostic procedures, most patients with sporadic ND undergo initial genetic testing by chromosomal analyses, array-CGH, and FMR1 repeat expansion analysis. In case of negative preliminary genetic tests, we perform WES as extended diagnostics, preferably as a trio analysis.

Results/Conclusion: Since 2019, we tested 1427 patients with unresolved ND, ID, and/or abnormal growth by WES analysis as part of our routine diagnostics. In 605 cases (42.4 %), we found at least one (likely) pathogenic variant or a variant of uncertain significance in genes associated with a ND. In 116 cases (19.2 % of positive cases), the reported variant was associated with MDEMs. Of these, 14 variants (12.0 %) can be assigned to the epigenetic regulation mechanism affecting DNA methylation, 53 variants (45.7 %) affecting histone modifications and 44 variants (37.9 %) affecting chromatin remodeling.

Conflict of Interest: None declared

P10.034.A Studies on X-linked ZC4H2 to better understand the molecular mechanisms and pathophysiology of ZC4H2-Associated Disorders

Jessica Gibbons[1], *Vanessa Suckow*[1], *Vera Kalscheuer*[1]

[1]*Max-Planck-Institute for Molecular Genetics, Development and Disease, Berlin*

Background/Objectives: Our group has long been involved in research into the genetic causes of rare neurodevelopmental disorders, with a focus on X-linked intellectual disability. One of the X-linked disease genes identified through our studies is *ZC4H2*, which encodes a zinc finger domain protein. We and others have associated ZC4H2 with variable clinical phenotypes of male and female patients presenting with a syndromic form of arthrogryposis multiplex congenital with central and peripheral nervous system involvement. Our understanding of the molecular functions and pathophysiology of ZC4H2-Associated Rare Disorders (ZARD) is currently limited.

Methods: CRISPR/Cas9, expression of ZC4H2 protein in bacterial cells and purification of stable protein, co-localization studies and biochemical assays.

Results: Since a complete knockout of *Zc4h2* in male mice is lethal, we have generated knockin mouse embryonic stem cells for two patient-specific missense variants. Furthermore, we have successfully purified ZC4H2 protein for antibody production, and validated that the microtubule tethering protein HOOK3 is a complex partner of ZC4H2. We are currently investigating the impact of mutant ZC4H2 protein on binding.

Conclusion: Establishing and characterizing knockin mice with patient-specific missense variants potentially serve as a valuable model for studying ZARD. Furthermore, successful purification of ZC4H2 protein is a crucial step for antibody establishment. Changes in protein interactions could disrupt normal cellular functions, contributing to the observed clinical phenotypes.

Grants: This work was supported by a research grant from the University of Pennsylvania Orphan Disease Center in collaboration with the ZCH42 Deficiency Research Foundation and the Max Planck Foundation.

Conflict of Interest: None declared

P10.035.B The phenotype and genotype spectrum of individuals with mono- or biallelic *ANK3* variants

*Francesca Furia[1;2], **Amanda Levy**[3], Stefan Barakat[4;5;6], Meghan Bartos[7], Emilia K.Bijlsma[8], Francesco Brancati[9;10], Jessica X. Chong[11], Wendy Chung[12], Joy Dean[7], Himanshu Goel[13;14], Alena Egense[15], Julie Fleischer[16], Paulino Gomez-Puertas[17], Ulrike Hüffmeier[18], Angela Kaindl[19], Eric Legius[20], Iñigo Marcos-Alcalde[17;21], Rikke Steensbjerre Moeller[1;2], Mary O'Connor[16], Marc Planes[22], Cornelia Potratz[19], Sylvia Quemener[23], David Ros-Pardo[17], Karen Rouault[24;25], Bitten Schoenewolf-greulich[3], Rachel Schot[4;5], Joseph Shen[15], Niclass Tanguy[26], Miel Theunis[27], Isabelle Thiffault[28;29], Kevin Uguen[24;30], Christiane Zweier[18;31], Elena Gardella[1;2], Zeynep Tümer[3;32]*

[1]Danish Epilepsy Centre, Dianalund, Denmark; [2]University of Southern Denmark, Odense, Denmark; [3]Copenhagen University Hospital, Rigshospitalet, Kennedy Center, Department of Clinical Genetics, Glostrup; [4]Department of Clinical Genetics, Erasmus MC University Medical Center, Rotterdam, Netherlands; [5]Discovery Unit, Department of Clinical Genetics, Erasmus MC University Medical Center, Rotterdam, Netherlands; [6]ENCORE Expertise Center for Neurodevelopmental Disorders, Erasmus MC University Medical Center, Rotterdam, Netherlands; [7]Children's Hospital Birmingham, University of Alabama Hospital, Birmingham, United States; [8]Department of Clinical Genetics, Leiden University Medical Centre, Leiden, Netherlands; [9]Human Genetics Institute, University of L'Aquila, L'Aquila, Italy; [10]Istituto Dermopatico dell'Immacolata, Rome, Italy; [11]University of Washington, Seattle, United States; [12]Columbia University, New York, United States; [13]Genetics Service, All India Institute of Medical Science, New Delhi, New Delhi, India; [14]University of Newcastle, Australia; [15]Division of Genomic Medicine, Department of Pediatrics MIND Institute University of California, Sacramento, United States; [16]SIU School of Medicine, Springfield, United States; [17]Molecular Modeling Group, Centro de Biología Molecular Severo Ochoa, Madrid, Spain; [18]Institute of Human Genetics, University of Erlangen-Nuremberg, Erlangen, Germany; [19]Sozialpädiatrisches Zentrum Neuropädiatrie, Charité Universitätsmedizin, Berlin, Germany; [20]Center for Human Genetics, University Hospital Leuven, Leuven, Belgium; [21]Bioscience Research Institute, School of Experimental Sciences, Universidad Francisco de Vitoria, UFV, Pozuelo de Alarcón, Spain; [22]University Hospital Center Pointe-à-Pitre Pointe-à-Pitre, Guadeloupe; [23]Institut National de la Santé et de la Recherche Médicale (INSERM), Brest, France; [24]University Brest, Inserm, EFS, Brest, France; [25]Hôpital Morvan, Laboratoire de Génétique Moléculaire et d'Histocompatibilité, Brest, France; [26]Service de Génétique, CHU Poitiers, Poitiers, France; [27]Clinical Genetics Centrum Menselijke Erfelijkheid Leuven, Leuven, Belgium; [28]Translational Genetics - Clinical lab. Children's Mercy Research Institute, Genomic Medicine Center, Kansas City, United States; [29]The University of Missouri-Kansas City (UMKC), School of Medicine, Kansas City, United States; [30]Service de Génétique Médicale, CHU de Brest, Brest, France; [31]Inselspital, Bern University Hospital, University of Bern, Bern, Switzerland; [32]Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark

Background/Objectives: *ANK3* encodes ankyrin-G, a protein involved in neuronal development and signaling, and is associated with a neurodevelopmental disorder (NDD) characterized with non-specific syndromic intellectual disability. To date, only 14 individuals with mono- or biallelic *ANK3* variants are reported in separate studies. The primary objective of this study was therefore to investigate and define the phenotype and genotype spectrum of *ANK3*-related NDD and explore a potential genotype-phenotype correlation.

Methods: Through GeneMatcher collaboration, we have collected the clinical and genetic information of 17 new individuals with *ANK3* variants and have reviewed the data from the 14 previously described individuals.

Results: In total, we describe 31 mono- or biallelic individuals with 35 different *ANK3* variants. The observed clinical spectrum spans language delay (93%), behavioral and/or psychiatric disturbances (88%), intellectual disability (73%), autism spectrum disorder (65%), motor delay (62%), hypotonia (63%), sleep disturbances (45%), and epilepsy (37%). Seventeen individuals have monoallelic *ANK3* variants and the remaining 14 have biallelic variants. While majority of the monoallelic variants are predicted to result in a truncated protein, the individuals with biallelic variants, which almost exclusively are missense, present with a more severe phenotype. Moreover, mono- and biallelic variants appears to be distributed differently across the different ankyrin-G isoforms.

Conclusion: Our data give a better understanding of the clinical phenotype of *ANK3*-related NDD and suggest that biallelic variants in *ANK3* are associated with increased severity of symptoms as well as an ankyrin-G isoform-specific mutational constraint.

Grants: None

Conflict of Interest: None declared

P10.036.C Mutation in mitochondrial chaperone TRAP1 results in male-specific autism

Malgorzata Rydzanicz[1], *Bożena Kuzniewska[2], Marta Magnowska[2], Tomasz Wójtowicz[3], Izabela Chojnicka[4], Ewelina Knapska[5], Andrzej Dziembowski[6;7], Rafał Płoski[8], Magdalena Dziembowska[2]*

[1]Medical University of Warsaw, Department of Medical Genetics, Warsaw, Poland; [2]University of Warsaw, Laboratory of Molecular Basis of Synaptic Plasticity, Centre of New Technologies, Poland; [3]Nencki Institute of Experimental Biology, Laboratory of Cell Biophysics, Poland; [4]University of Warsaw, Department of Health and Rehabilitation Psychology, Faculty of Psychology, Poland;

[5]Nencki Institute of Experimental Biology, Laboratory of Emotions Neurobiology, Poland; [6]International Institute of Molecular and Cell Biology in Warsaw, Laboratory of RNA Biology, Poland; [7]University of Warsaw, Faculty of Biology; [8]Medical University of Warsaw, Department of Medical Genetics

Background: There is increasing evidence of mitochondrial dysfunction in autism spectrum disorders (ASD), but the causal relationships are unclear.

Methods: ASD-discordant monozygotic twins were analyzed using exome sequencing. Knock-in mice with *Trap1* p.Q641* mutation (equivalent of human p.Q641*) were generated using CRISPR/Cas9 and characterized by social interaction and neuroanatomical analysis.

Results: In ASD-affected twin, but not in his healthy brother, we identified a postzygotic mosaic p.Q639* mutation in the *TRAP1* gene encoding a mitochondrial chaperone of the HSP90 family. Additional screening of 176 unrelated ASD probands revealed an identical heterozygous *TRAP1* variant in a male patient who had inherited it from a healthy mother. Notably, newly generated knock-in *Trap1* p.Q641* mice display ASD-related behavioral abnormalities exclusively in males. Accordingly, *Trap1* p.Q641* mutation also resulted in sex-specific changes in synaptic plasticity and the number of presynaptic mitochondria.

Conclusion: The *TRAP1* p.Q639* mutation is the first example of a monogenic ASD caused by impaired mitochondrial protein homeostasis.

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Conflict of Interest: None declared

P10.037.D Making sense of non-sense: Identification of an intronic deletion in ARID1A in Coffin-Siris syndrome

Sarah Schuhmann[1], *Georgia Vasileiou*[1;2], *Steffen Uebe*[1], *Andreas Fink*[1], *Antje Wiesener*[1], *André Reis*[1;2]

[1]Friedrich-Alexander-Universität Erlangen-Nürnberg, Institute of Human Genetics, Erlangen, Germany; [2]Friedrich-Alexander-Universität Erlangen-Nürnberg, Centre for Rare Diseases Erlangen, Erlangen, Germany

Consortium: Bavarian Genomes Network

Background: Disruption of chromatin regulatory genes belonging to the BAF complex represents one of the most frequent genetic causes for neurodevelopmental disorders (NDD). Coffin-Siris syndrome (CSS) is a multiple congenital anomalies NDD caused by pathogenic variants in 11 different genes encoding BAF complex subunits. However, in a subset of cases with a suspected CSS diagnosis the genetic basis remains unsolved.

Methods: Within the research project *Bavarian Genomes Network* we identified a four year old female individual from healthy non-consanguineous parents with global neurodevelopmental delay, muscular hypotonia, hypoplasia of the corpus callosum, coarse facial features, sparse scalp hair, hypoplastic toe nails, laryngo-tracheomalazia and dysplastic aortic valve suggestive of CSS. As trio exome sequencing and CNV analysis did not identify a causative variant, we applied short-read genome sequencing (GS) and other genomic assays for further characterization.

Results: Trio GS revealed a de novo 24 kb deletion, not detectable in exome sequencing, located in intron 4 of the *ARID1A* gene, which is associated with CSS type 2. RNA sequencing showed a 165 bp intron retention in 21% of the reads, predicted to result in a stop codon after 9 amino acids. This represents a loss-of-function allele compatible with the clinical diagnosis of CSS. Methylome analyses are pending.

Conclusion: To our knowledge this case represents the first intronic deletion identified in a patient with CSS. Our case highlights the benefits of genome sequencing in the diagnosis of unsolved cases as well as the necessity of additional genomic techniques to interpret variants in non-coding regions.

Conflict of Interest: None declared

P10.038.A *ARHGEF9*-associated developmental and epileptic encephalopathy: just an X-linked and not an X-linked recessive disease

Susann Badmann[1], *Maureen Jacob*[1], *Kristina Huß*[2], *Ingo Borggräfe*[2], *Saskia Biskup*[3], *Jerome Jüngling*[3], *Kathrine Bjørgo*[4], *Juliane Winkelmann*[1;5], *Matias Wagner*[1;5]

[1]Institute of Human Genetics, School of Medicine, Technical University of Munich, Munich, Germany; [2]Dr. v. Hauner Children's Hospital, Department of Pediatric Neurology and Developmental Medicine, LMU - University of Munich, Munich, Germany; [3]Center for Genomics and Transcriptomics (CeGaT), Tübingen, Germany; [4]Department of Medical Genetics, Oslo University Hospital, Oslo, Norway; [5]Institute of Neurogenomics, Helmholtz Zentrum München, Munich, Germany

Background: Variants in the X-chromosomal gene *ARHGEF9* cause a developmental and epileptic encephalopathy. Initially, the condition was considered as an X-linked recessive disorder, affecting males and females with a skewed X-inactivation pattern. A recent report of two affected females with loss-of-function (LoF) variants and random X-inactivation suggested an X-linked dominant inheritance for LoF and an X-linked recessive inheritance for missense variants (Ghesh et al., 2021).

Methods: Trio-exome sequencing was performed, and the X-inactivation pattern was assessed based on methylation analysis of *RP2* and *AR* from leukocyte DNA or RNA sequencing from leukocyte RNA.

Results: We report three affected females with heterozygous de novo variants in *ARHGEF9* (NM_001173479.2) and random X-inactivation pattern. In patient 1 a novel splice-donor variant c.222+1G>C was identified. Patient 2 has a novel microdeletion affecting exons 5-6 of *ARHGEF9*. Patient 3 carries a missense variant c.151C>T, p.(Arg51Trp). While patient 1 and 2 initially presented with developmental delay, learning difficulties/mild intellectual disability (ID) and developed epilepsy/hyperekplexia at 13 and 9 years respectively, patient 3 shows a developmental and epileptic encephalopathy with severe ID.

Conclusion: By presenting three affected females (in addition to two previously published) we provide further evidence that heterozygous LoF and missense variants in *ARHGEF9* can be disease causing also in females with random X-inactivation in blood DNA. Therefore, *ARHGEF9*-associated developmental and epileptic encephalopathy should be considered just as X-linked and not as X-linked recessive disorder. Based on this example we support the revision of the dichotomous model of X-chromosomal recessive and dominant inheritance.

Conflict of Interest: None declared

P10.039.B Recurrent cases of somatic mosaicism in KANSL1 identified in standard diagnostics

Vincent Arriens[1], *Kirstin Hoff*[1], *Anna-Sophie Liegmann*[2], *Christina Post*[2], *Irina Hüning*[2], *Nadine Hornig*[1], *Britta Hanker*[2], *Inga Vater*[1], *Malte Spielmann*[1], *Inga Nagel*[1]

[1]University Hospital Schleswig-Holstein, Campus Kiel, Institute of Human Genetics, Kiel, Germany; [2]University Hospital Schleswig-

Holstein, Campus Lübeck, Institute of Human Genetics, Lübeck, Germany

Background/Objectives: *KANSL1* variants are linked to Koolen-de Vries Syndrome, characterized by variable degrees of intellectual disability, speech and motor delay, and neonatal/childhood hypotonia. Additionally, epilepsy, behavioral abnormalities and dysmorphic features commonly accompany the disorder. Two cases with somatic mosaicism in *KANSL1* were identified during our standard diagnostics.

Patients/Methods: Exome, trio genome and Sanger sequencing were performed on two male patients aged 5 and 18 years. Both presented with global developmental delay (especially speech delay), hypotonia, epilepsy, brain abnormalities and macrocephaly.

Results: Two mosaic variants in *KANSL1* were detected by exome and genome sequencing and subsequently validated by Sanger sequencing. One patient carried a pathogenic de novo variant, c.611dup p.(Met205Tyrfs*9), in *KANSL1*. All three techniques showed somatic mosaicism, with an allelic frequency of 35% based on genome sequencing (exome: 21%). Similarly, in the second case, a likely pathogenic de novo variant (c.1420C>G p.(Arg474Gly)) in *KANSL1* showed somatic mosaicism across all three techniques, with an allelic frequency of 30% according to genome sequencing (exome: 28%).

Conclusion: The repeated occurrence of mosaicism in *KANSL1* raises the question of whether it is an incidental observation or due to a biological mechanism. Mosaic occurrences have been documented in genes linked to neurodevelopmental disorders and epilepsy and *KANSL1* might be another candidate. However, regarding a specific underlying mechanism causing mosaics in *KANSL1*, we can only speculate and further analyses are necessary.

Conflict of Interest: Vincent Arriens provision of free of charge sequencing reagents and software by Illumina within the framework of a collaborative research study, Kirstin Hoff provision of free of charge sequencing reagents and software by Illumina within the framework of a collaborative research study, Anna-Sophie Liegmann provision of free of charge sequencing reagents and software by Illumina within the framework of a collaborative research study, Christina Post provision of free of charge sequencing reagents and software by Illumina within the framework of a collaborative research study, Irina Hüning provision of free of charge sequencing reagents and software by Illumina within the framework of a collaborative research study, Nadine Hornig provision of free of charge sequencing reagents and software by Illumina within the framework of a collaborative research study, Britta Hanker provision of free of charge sequencing reagents and software by Illumina within the framework of a collaborative research study, Inga Vater provision of free of charge sequencing reagents and software by Illumina within the framework of a collaborative research study, Malte Spielmann provision of free of charge sequencing reagents and software by Illumina within the framework of a collaborative research study, Inga Nagel provision of free of charge sequencing reagents and software by Illumina within the framework of a collaborative research study

P10.040.C The additional clinical benefit of exome sequencing: the return of ACMG/AMP recommended incidental findings and data re-analysis

*Markéta Wayhelová[1], **Jan Smetana**[1], Vladimira Vallova[1], Petr Brož[1], Renata Gaillyova[2], Petr Kuglik[1]*

[1]Institute of Experimental Biology, Dept. of Molecular Genetics, Brno; [2]Institute of Medical Genetics and Genomics, Brno

Background/Objectives: Despite of the unquestionable clinical benefit of exome sequencing (ES) for the paediatric population with neurodevelopmental disorders (NDDs) and their families, the medical and clinical specialists have to face the increasing number of clinically actionable incidental findings and the need to reanalyze and reinterpret ES data, respectively.

Methods: We present our experience and yields for these incidental findings and data reanalysis based on the outlined ACMG/AMP general recommendation.

Results: In the paediatric population of 100 individuals with NDDs and their parents we returned the incidental findings. (*BRCA1, BRCA2, TGFBR1, RYR1, HFE, SDHB* genes) for 11 individuals from 7 families. Additionally, we revised the ES interpretation for two families, resulting in the elucidation of molecular diagnosis (the *CRAT* gene, 1 case) and the possible prevention of future medical complication due to the reclassification and report of the incidental finding (the *RYR1* gene, 1 case)

Conclusion: Our results declare and support the clinical benefit of returns of incidental findings and periodical data reanalysis. Future debates on the reporting of clinically actionable non-ACMG secondary findings for the carriers of causative variants for prevalent recessive congenital diseases and reinterpretation of the increasing number of novel variants may be required due to the widespread routine implementation of trio exome analyses in the clinical practice.

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Conflict of Interest: None declared

P10.041.D A homozygous stop variant in *DDIAS* is a plausible cause of a novel neurodevelopmental disorder

__Zafar Iqbal__[1], Bilal Ahmad Mian[2], lubaba khalid[2], Asmat Ali[2], Mohammad Raza[3], Mathias Toft[1;4], Ambrin Fatima[2], Grant S Stewart[5]

[1]Oslo University Hospital, Department of Neurology, Oslo, Norway; [2]The Aga Khan University, Department of Biological and Biomedical Sciences, Karachi, Pakistan; [3]The Aga Khan University, Department of Paediatrics and Child Health, Karachi, Pakistan; [4]University of Oslo, Institute of Clinical Medicine, Oslo, Norway; [5]University of Birmingham, Institute of Cancer and Genomic Sciences, Birmingham, United Kingdom

DNA damage-induced apoptosis suppressor protein is encoded by the gene *DDIAS* and has been implicated in activating the apoptotic signaling pathway in response to DNA damage. Unlike other DNA damage response genes, variants in *DDIAS* have never been implicated in human disease. However, recently we identified a five-year-old child from a consanguineous marriage, presenting with global developmental delay, intellectual disability, microcephaly, generalized clonic seizures, generalized spasticity, horizontal nystagmus, high arched palate, bilateral clinodactyly, and some hypopigmented macules on the neck. The patient is not able to sit, stand or hold his neck. Vision and hearing abilities of the patient are unaffected. Whole-exome sequencing of the affected child identified a homozygous stop variant in *DDIAS*: c.1631C>A (NM_145018.4); p.(Ser544*), which segregated with the phenotype. In keeping with a role for DDIAS in responding to DNA damage, skin fibroblasts from the affected child exhibited signs of increased genome instability and an inability to repair damaged DNA induced by mitomycin C but not aphidicolin or camptothecin. This suggests that DDIAS may work together with the Fanconi Anemia pathway to repair inter-strand DNA cross-links and/or DNA-protein cross-links. Further analysis is underway to identify

how defects in the DDIAS-dependent DNA damage response is responsible for causing disease.

Conflict of Interest: None declared

P10.042.A Homozygous truncating variant in *OGFOD1* in a patient with neurodevelopmental disorder: is it a novel ribosomopathy?

Amita Moirangthem[1], *Rajesh Kumar Maurya*[1], *Ankit Dhakad*[1], *Ashwin Dalal*[2]

[1]*Sanjay Gandhi Postgraduate Institute of Medical Sciences, Medical Genetics, Lucknow, India;* [2]*Center for DNA Fingerprinting and Diagnostics, Diagnostics division, Hyderabad, India*

Background/Objectives: 2-oxoglutarate Fe(II)dependent oxygenase domain containing protein (OGFOD1) plays a role in translational regulation by hydroxylation of the Pro62 residue of the small ribosomal protein subunit RPS23. De novo pathogenic variations in RPS23 have been reported to cause a developmental disorder with dysmorphism and variable cognitive dysfunction. OGFOD1 knockdown in human cell lines and in drosophila have also shown accumulation of stress granules, translational arrest and impaired cell growth. OGFOD1 has not been reported to be causative of any monogenic disorder yet.

Methods: We ascertained a 9 years old boy, born of healthy non-consanguineous parents, with global developmental delay, behavioral issues and epilepsy. He had moderate intellectual disability, microcephaly, low anterior hairline, downslanted eyes, flat occiput and overhanging columella. He was evaluated for possible genetic causes using exome sequencing (ES) and 750K SNP array.

Results: ES prioritized a homozygous variant c.1180_1181insA-TAG in *OGFOD1* (NM_018233). Segregation analysis of the variant in the family conformed to Mendelian pattern. In silico analyses predict this variant to lead to shifting of the reading frame and premature truncation of protein.Copy number variation analysis from ES data and SNP array was negative. A 5.5MB region of homozygosity was detected which included *OGFOD1*.

Conclusion: Existing literature suggests a close functional interaction between OGFOD1 and RPS23 and our patient shares overlapping phenotypic features with the RPS23 deficient patients. Therefore, OGFOD1 deficiency may also cause a ribosomopathy and we plan to study the functional effects in the patient derived cell lines.

Grants: Department of Biotechnology, India (BT/PR45460/MED/12/952/2022)

Conflict of Interest: None declared

P10.044.C Constitutional *PIK3CA* haploinsufficiency results in microcephaly and developmental delay

Michaela Rendek[1], *Camille Engel*[1], *Paul Kuentz*[2;3], *Peter Bauer*[4], *Boris Keren*[5], *Alexia Bourgois*[6], *Carla Lintas*[7;8], *Fiorella Gurrieri*[7;8], *Paola Grammatico*[9;10], *Irene Bottillo*[11], *Matthew Warman*[12], *Juliette Piard*[1;3]

[1]*Centre de Génétique Humaine, CHU Besançon, Besançon, France;* [2]*Oncobiologie Génétique Bioinformatique, PC Bio, CHU Besançon, Besançon, France;* [3]*UMR 1231 GAD, Inserm, Université de Bourgogne Franche Comté, Dijon, France;* [4]*CENTOGENE GmbH, Rostock, Germany;* [5]*Centre de Génétique Moléculaire et Chromosomique, AP-HP.Sorbonne Université - Hôpital de la Pitié-Salpêtrière, Paris, France;* [6]*Département de Génétique, CHU Caen, Caen, France;* [7]*Medical Genetics, University Campus Biomedico of Rome, Rome, Italy;* [8]*Fondazione Policlinico Universitario Campus biomedico, Rome, Italy;* [9]*Division of Medical Genetics, Department of Molecular Medicine, San Camillo-Forlanini Hospital, Rome, Italy;* [10]*Division of Medical Genetics, Department of Experimental Medicine, San Camillo-Forlanini Hospital, Rome, Italy;* [11]*Division of Medical Genetics, Depatment of Molecular Medicine, San Camillo-Forlanini Hospital, Rome, Italy;* [12]*Department of Genetics at Harvard Medical School and Pediatric Orthopedic Surgery, Boston, United States*

Background/Objectives: Post-zygotic, somatic mosaic, gain-of-function mutations in *PIK3CA* cause *PIK3CA*-related overgrowth syndrome (PROS). While germline gain-of-function variants affecting *PIK3CA* activity have also been described, we are not aware of published descriptions of patients with germline loss-of-function variants.

Methods: We performed whole exome sequencing and identified a putative germline loss-of-function *PIK3CA* variant in a proband with intellectual disability. We then used matchmaker exchange to identify and collect clinical and molecular data from additional patients for whom clinical sequencing identified a heterozygous putative loss-of-function variant.

Results: Three probands have predicted loss-of-function variants. Two mutations arose de novo and one was inherited from a mother who presents with learning difficulties. The 4 individuals exhibited a range of intellectual disability that tended to mild, two were microcephalic (<-2SD), and a third had a head circumference of -1.8 SD.

Conclusion: Our data suggest heterozygous loss-of-function *PIK3CA* variants are associated with neurodevelopmental disorders. The reduced head circumference observed in several of our patients is consistent with the observation that *PIK3CA* haploinsufficiency in mice also causes microcephaly. Also notable are that *PIK3CA* has a pLI of 1 in the gnomAD database and that putative loss-of-function alleles are associated with reduced brain grey matter volume in the Genebass database. A larger cohort of individuals with loss-of-function alleles is needed to better determine the spectrum of the haploinsufficiency phenotype.

Grants: None

Conflict of Interest: None declared

P10.045.D Further delineation of SCAF4 associated neurodevelopmental disorder

Cosima Meret Schmid[1;2], *Anna Ruiz*[3], *Carmen Manso*[3], *Isabella Herman*[4;5], *Farah Ammouri*[4], *Urania Kotzaeridou*[6], *Vanda McNiven*[7], *Lucie Dupuis*[7], *Katharina Steindl*[8], *Anais Begemann*[8], *Anita Rauch*[8], *Bertrand Isidor*[9], *Tobias Haack*[10], *Tobias Linden*[11], *Chad Haldeman-Englert*[12], *Charlotte Ockeloen*[13], *Elodie Lacaze*[14], *Jennifer Bassetti*[15], *Julia Hoefele*[16], *Theresa Brunet*[16], *Korbinian M. Riedhammer*[16], *Kirsten Cremer*[17], *Marie-Ange Delrue*[18], *Mathilde Nizon*[19], *pedro almeida*[20], *Fabiana Ramos*[20], *Siddarth Srivastava*[21], *Stephen Robertson*[22], *Sandra Mercier*[19], *Ruth Falb*[10], *Amelie Müller*[10], *Malgorzata Nowaczyk*[23], *Reem M. Elshafie*[24], *Paul Hillman*[25], *Leslie Dunnington*[26], *Hilde Braakman*[27], *Shane McKee*[28], *Francesca Mattioli*[29], *Angelica Moresco*[30], *Heinrich Sticht*[31], *Christiane Zweier*[1;2]

[1]*Inselspital Bern, Department of Human Genetics, Bern, Switzerland;* [2]*Department for BioMedical Research of University of Bern, Bern, Switzerland;* [3]*Genetics Laboratory, UDIAT-Centre Diagnòstic, Parc Taulí Hospital Universitari, Institut d'Investigació i Innovació Parc Taulí I3PT, Universitat Autònoma de Barcelona, Barcelona, Spain;* [4]*Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, United States;* [5]*Section of Pediatric Neurology and Developmental Neuroscience, Department of Pediatrics, Baylor College of Medicine, Houston, United States;* [6]*Division of Child Neurology and Inherited Metabolic Diseases, Centre for Paediatrics and Adolescent Medicine, University Hospital Heidelberg, Heidelberg, Germany;* [7]*Division of Clinical and Metabolic Genetics, The Hospital for Sick Children, University of Toronto, Toronto, Canada;* [8]*Institute of Medical Genetics, University of Zurich, Zürich, Switzerland;* [9]*Department of Medical Genetics, CHU Nantes, Nantes, France;* [10]*Institute of*

Medical Genetics and Applied Genomics, University of Tübingen, Tübingen, Germany; [11]University Children's Hospital, Klinikum Oldenburg, Department of Neuropediatrics, Oldenburg, Germany; [12]Mission Fullerton Genetics Center, Asheville, United States; [13]Department of Human Genetics, Radboud University Medical Center, Nijmegen, Netherlands; [14]Department of Medical Genetics, Le Havre Hospital, Le Havre, France; [15]Division of Medical Genetics, Department of Pediatrics, Weill Cornell Medicine, New York, United States; [16]Technical University of Munich, Institute of Human Genetics, München, Germany; [17]University Hospital Bonn, Institute of Human Genetics, Bonn, Germany; [18]CHU Sainte-Justine, Department of Genetics, Montréal, Canada; [19]Centre hospitalier universitaire de Nantes, Service de Génétique Médicale, Nantes, France; [20]Centro Hospitalar e Universitário de Coimbra, Medical Genetics Unit, Coimbra, Portugal; [21]Boston Children's Hospital, Department of Neurology, Boston, United States; [22]Dunedin School of Medicine, Department of Paediatrics and Child Health, Dunedin, New Zealand; [23]McMaster University, Department of Pathology and Molecular Medicine, Hamilton, Canada; [24]Kuwait Medical Genetic Center, Sulaibikhat, Kuwait; [25]Texas A&M University, Department of Molecular and Cellular Medicine, College Station, United States; [26]Memorial Hermann-Texas Medical Center, Houston, United States; [27]Amalia child hospital Nijmegen, Department of Pediatric Neurology, Nijmegen, Netherlands; [28]Belfast Health & Social Care Trust, Northern Ireland Regional Genetics Service, Belfast, United Kingdom; [29]UNIL - Université de Lausanne, Center for Integrative Genomics, Lausanne, Switzerland; [30]Division of Clinical Genetics, Children's Hospital, London Health Sciences Centre, Western University, London, Canada; [31]University of Erlangen-Nuremberg, Institut für Biochemie, Erlangen, Germany

Background/Objectives: Only recently, mostly de novo, truncating variants in *SCAF4* were identified in 18 individuals with variable neurodevelopmental phenotypes. However, knowledge on the molecular and clinical spectrum is still limited.

Methods: We assembled data on 37 novel cases with *SCAF4* variants and neurodevelopmental phenotypes, ascertained via GeneMatcher and personal communication. With detailed evaluation of clinical data, in silico predictions and structural modeling, we further characterized the molecular and clinical spectrum of the *SCAF4*-associated Fliedner-Zweier syndrome.

Results: The molecular spectrum in this cohort comprised 33 truncating (incl. splice-site) and four missense variants. While truncating variants were classified as (likely) pathogenic, significance of missense variants remained unclear. Two missense variants in the CID-domain of SCAF4 were predicted by structural modeling to destabilize the domain. Frequent clinical findings in affected individuals were mild developmental delay with prominent speech delay, seizures (37%), and variable skeletal abnormalities such as clubfoot, scoliosis or hip dysplasia. Cognitive abilities ranged from normal IQ to severe intellectual disability, with borderline to mild ID in the majority of individuals. Notably, there were more inherited *SCAF4* variants from similarly affected parents than de novo occurrences (15 vs 10).

Conclusion: Our study confirms the role of *SCAF4* variants in neurodevelopmental disorders and further delineates the associated clinical phenotype.

Conflict of Interest: None declared

P10.046.A *CTNNB1* variants: clinical and genetic observations from the UK's cohort

Taisiia Sazonova[1;2], *Sarah Richardson*[3], *Dianne Newbury*[4], *Usha Kini*[1;3]

[1]*University of Oxford, Nuffield Department of Clinical Laboratory Sciences, Oxford, United Kingdom;* [2]*University of Oxford, Radcliffe Department of Medicine, Oxford, United Kingdom;* [3]*Oxford University Hospitals NHS Foundation Trust, Oxford Centre for Genomic Medicine, Oxford, United Kingdom;* [4]*Oxford Brookes University, Department of Biological and Medical Sciences, Oxford, United Kingdom*

Background/Objectives: Catenin-beta 1 (*CTNNB1*) encodes a cadherin-associated protein and is crucial in embryonic development. It is linked to neurodevelopmental disorders and familial exudative vitreoretinopathy (FEVR). This study investigates genotype-phenotype correlations in the largest UK cohort of patients with reported *CTNNB1* variants, focusing on developmental, neurological, and ocular outcomes.

Methods: We studied clinical details and genotypes of 81 unreported patients: 45 from the Deciphering Developmental Disorders study, 23 from the 100,000 Genomes Project, and 13 via NHS. We ensured no double-counting of patients tested through multiple routes. Pathogenic variants were identified by exome or genome sequencing and classified via ACMG guidelines.

Results: All variants identified were loss-of-function variants, except for 1 missense variant. Recurrent variants (p.Arg535Ter, p.Arg474Ter, p.Tyr333Ter) did not show consistent phenotypes. Global developmental delay (87%), microcephaly (66%), and muscle tone abnormality (41%) were the most common findings. Spasticity was observed in 25% of patients, unrelated to any specific genotype. Motor milestones delay was observed: mean ages for sitting of 19 months and 34 months for walking independently. Progressive motor regression in 1 patient included transitioning from using a baby walker to knee walking and loss of independent standing ability. 41% had ocular symptoms, but FEVR was observed in 1 patient harbouring a p.Arg474Ter variant.

Conclusions: Our study is the largest UK cohort report on *CTNNB1* variants. The observed lack of genotype-phenotype correlations confirms that the genotype in this disorder cannot be used as a prognostic indicator. FEVR is rare complication but needs monitoring.

Grants: Merton College DPhil Scholarship partnered with RDM funding

Conflict of Interest: None declared

P10.047.B Experience using ExpansionHunter to identify diseases caused by STRs from exome sequencing data

Marta Codina[1;2], *Irene Valenzuela Palafoll*[1;2], *Jordi Leno*[1;2], *Héctor San Nicolás*[1;2], *Mar Costa-Roger*[1;2], *Paula Fernandez-Alvarez*[1;2], *Eulàlia Rovira-Moreno*[1;2], *Amaia Lasa-Aranzasti*[1;2], *Laura Trujillano*[1;2], *Barbara Masotto*[1;2], *Anna Maria Cueto Gonzalez*[1;2], *Desirée Martínez-Cruz*[1;2], *Anna Abuli*[1;2], *Elena García-Arumí*[1;2], *Eduardo Tizzano*[1;2]

[1]*Vall d'Hebron Institut de Recerca (VHIR), Medicine Genetics Group, Barcelona, Spain;* [2]*Vall d'Hebron Hospital Universitari, Department of Clinical and Molecular Genetics, Barcelona, Spain*

Background/Objectives: Expansion of short tandem repeats (STRs) cause more than 40 diseases, most affecting primarily the nervous system. Recently, bioinformatics tools such as ExpansionHunter (EH) have been developed to identify STRs from exome sequencing data (ES).

Methods: We applied EH to 1610 exomes from a cohort of rare disease patients to study 30 known loci. Expanded STRs in loci not related to the patient's clinical indication were discarded. The remaining were manually curated using REViewer to discard false positive results and validated with TP-PCR (Tripled repeat Primed-PCR).

Results: We identified 51 possible aberrant STR alleles in 49 patients (3% of the cohort). Correlation with the clinical indication

discarded 45/51 of the positive findings, resulting in a final number of six expanded STRs in six different patients (Myotonic dystrophy type I/DMPK: 4, Dentatorubral-pallidoluysian atrophy/ATN1: 1, SCA8/ATXN8OS; 1). Three expansions were already known, although unsuspected at the time of ES. All detected expanded STRs but one (ongoing) have been validated by TP-PCR. Of the four cases with an expansion in DMPK, three were full expansions in three patients diagnosed with Myotonic Dystrophy, whereas one was in the premutated range and considered an incidental finding.

Conclusion: Our work shows the utility of implementing STR analysis in clinical ES. Although ES is not the gold standard technic for STR analysis and has a high false positive rate, application of EH or other methods can pinpoint potential diagnosis. Our results show that STR evaluation could potentially increase diagnostic ES yield by approximately 0,4%.

Grants:

Conflict of Interest: None declared

P10.048.C Etiologies of megalencephaly, a series of 232 patients

Maxime MAZOWIECKI[1;2], *Daphné Lehalle*[1;3], *Perrine Charles*[1;4], *Anna Gerasimenko*[1;5], *Alexandra Afenjar*[1], *Thomas Courtin*[1], *Solveig Heide*[1;4], *Sarah Grotto*[1;4], *Sandra Whalen*[1;4], *Iydie BURGLEN*[1], *Jean-Madeleine De Saint Agathe*[1], *Caroline Nava*[1], *Sandra Chantot-Bastaraud*[1], *Florence Coulet*[1], *Mélanie Eyries*[1], *Julien Buratti*[1], *Boris Keren*[1], *BOURRAT Emmanuelle*[6], *Diane Doummar*[7], *Benedicte Heron*[7], *Madeleine Harion*[7], *Stéphanie Valence*[7], *Arnaud Isapof*[7], *Claudia Ravelli*[7], *Marie-Christine Nouges*[7], *Florence Renaldo*[7], *Diana Rodriguez*[7], *Pierre Vabres*[8], *Paul Kuentz*[9], *Delphine Heron*[1;4], *Cyril Mignot*[1;3;5]

[1]*APHP Sorbonne Université, GH Pitié Salpêtriére et Trousseau, Genetic department, Paris, France;* [2]*APHP Assistance publique Hôpitaux de Paris, Pediatric neurology department, MIRCEM, Le Kremlin-Bicêtre, France;* [3]*APHP- Assistance publique Hôpitaux de Paris, Center of Reference for Developmental Anomalies and Malformative Syndromes, Paris, France;* [4]*APHP - Assistance Publique Hôpitaux de Paris, Center of Reference for Intellectual Disabilities of Rare Causes, Paris, France;* [5]*APHP - Hôpital de la Pitié Salpêtrière, ICM- Paris Brain Institute - ICM, Inserm, CNRS, Paris, France;* [6]*APHP, Hôpital-Saint Louis, Service de Dermatologie, CRMR MAGEC Nord, Paris, France;* [7]*APHP- Trousseau Hospital, Pediatric Neurology Department, Paris, France;* [8]*University Hospital F.mitterand Dijon-Bourgogne, Dermatology Department, Dijon, France;* [9]*Hospital Jean Minjoz, Genetic Department, Besançon, France*

Background/Objectives: Megalencephaly (MEG), i.e. excessive brain growth, is diagnosed in individuals with macrocephaly and a brain imaging ruling out other causes of exaggerated head growth. When MEG is assessed, the choice of etiological investigations depends on a series of criteria, including associated cerebral / extracerebral malformations, neurodevelopment, clinical signs. During the last years, genome-wide studies allowed the identification of numerous genes of MEG with a large range of clinical presentations. However, there is no series of patients taking all these etiologies into account.

Methods: To provide the landscape of the genetic etiologies of MEG, we performed a comprehensive review of its genetic etiologies in PubMed and OMIM. We then collected data from the medical files of patients with OFC > + 3 SD and a MEG confirmed by brain imaging examined in our genetics unit since 20 years

Results: More than 70 genes of MEG are known to date. We retrieved 232 patients with MEG, including 52% with intellectual disability (ID). Pangenomic sequencing (ES/GS) were conducted in 147 with a diagnostic yield of 50%, including in patients without ID, and involved 61 genes. One third of the diagnoses were made in only 5 genes (PTEN first). 26% of the variants were associated with a tumor risk, they were inherited in 38% of cases.

Conclusion: Etiological investigations of MEG have a high diagnostic yield, even in the absence of ID. The great heterogeneity of the genes involved argues in favor of genome-wide testing. Diagnosis has a major impact on patient medical follow-up.

Conflict of Interest: None declared

P10.049.D WDR47-related neurodevelopmental disorders: a circular approach from mouse to human and vice versa

Binnaz YALCIN[1], *Efil Bayam*[2], *Stephan Collins*[3], *Peggy Tilly*[2], *Jose Rivera*[2], *Siwar Ben-Ayache*[4], *Lucile Tonneau*[4], *Sateesh Maddirevula*[5], *Fabiola Monteiro*[6], *Joao Kitajima*[6], *Fernando Kok*[6], *Mitsuhiro Kato*[7], *Ahlam Hamed*[8], *Mustafa A. Salih*[9], *Saeed Al Tala*[10], *Mais Omar Hashem*[5], *Hiroko Tada*[11], *Hirotomo Saitsu*[12], *Zafer Yüksel*[13], *Mitsuko Nakashima*[14], *Fowzan Alkuraya*[5], *Alexander Stegmann*[15], *Billie Au*[16], *Alina Christine Hilger*[17], *Fleur Vansenne*[18], *Linda Zuurbier*[19], *Boris Keren*[20], *Solveig Heide*[21], *Juliette D Godin*[2]

[1]*Inserm, CTM, Dijon, France;* [2]*IGBMC, TMN, Strasbourg;* [3]*University of Bourgogne, Dijon, France;* [4]*Inserm, CTM, Dijon, France;* [5]*King Faisal Specialist Hospital and Research Center, Riyadh, Saudi Arabia;* [6]*Mendelics Análise Genomica SA, CEP 04013-000, Sao Paulo, Brazil;* [7]*Showa University School of Medicine, Tokyo, Japan;* [8]*Faculty of medicine University of Khartoum, Khartoum, Sudan;* [9]*Health Sector, King Abdulaziz City for Science and Technology, Riyadh, Saudi Arabia;* [10]*Armed Forces Hospital, Khamis Mushayt, Saudi Arabia;* [11]*Tokyo Metropolitan Institute of Medical Science, Tokyo, Japan;* [12]*Department of Biochemistry, Hamamatsu University School of Medicine, 1-20-1 Handayama, Higashi-ku, Hamamatsu, Japan;* [13]*Human Genetics, Bioscientia GmbH, Ingelheim, Germany;* [14]*Hamamatsu University School of Medicine, 1-20-1 Handayama, Higashi-ku, Hamamatsu, Japan;* [15]*Maastricht University Medical Center, Maastricht, Netherlands;* [16]*University of Calgary, Alberta Children's Hosptial, Calgary, Canada;* [17]*University Hospital Erlangen, Erlangen, Germany;* [18]*University Medical Center Groningen, University of Groningen, Groningen, Netherlands;* [19]*Department of Human Genetics, University of Amsterdam, Amsterdam, Netherlands;* [20]*Department of Genetics, Unit of Developmental Genomics, AP-HP.Sorbonne Université, Pitié-Salpêtrière Hospital, Paris, France;* [21]*Department of Genetics and Referral Center for Intellectual disabilities of rare causes, AP-HP.Sorbonne Université, Assistance Publique-Hopitaux de Paris, Pitié-Salpêtrière Hospital, Paris, France*

Background: The WDR gene family, with 286 members including *WDR47*, plays an important role in physiology and development. WDR47's role in mouse brain development has been recently found, yet its link to human disorders remains unclear.

Methods: We generated mouse knock-out models for *Wdr47* and conducted detailed neuroanatomy studies across different developmental stages. These were then compared with human cases with WDR47 variants, collected through international data sharing. For human variants not indicative of loss of function, we examined knock-in models.

Results: Our study identified 11 unrelated families worldwide, with five showing bi-allelic and six mono-allelic missense variants in *WDR47*. All bi-allelic cases consistently displayed neuroanatomical phenotypes, including corpus callosum dysgenesis and microcephaly, along with intellectual disability and epilepsy. Bi-allelic cases with a clear loss-of-function mechanism were attributable to neuronal cell death due to disrupted cellular processes. By contrast, mono-allelic cases presented a broader range of symptoms, with some showing similar neuroanatomical

and neurological concerns. Our detailed study of the de novo missense variant p.Val660Ala in a mouse model revealed significant defects, confirming its pathogenicity and aiding in molecular diagnosis. The pathogenicity of the other monoallelic variants remains to be confirmed, and more patients are needed to fully understand the autosomal dominant phenotypes associated to WDR47-related neurodevelopmental disorders.

Conclusion: Our murine findings recapitulate the clinical features of the human diseases. Importantly, our study underscores the power of mouse models as starting point to find correspondence in humans especially for ultra-rare neurodevelopmental conditions like those related to *WDR47*.

Conflict of Interest: None declared

P10.050.A Secondary rare variants in Rett syndrome: toward a morbid gene card for personalized care and cure

Kristina Zguro[1;2], Susanna Croci[1;2], Giulia Brunelli[1;3], Roberto Canitano[4], Sabrina Buoni[4], Valeria Scandurra[4], simone furini[5], Caterina Lo Rizzo[6], **Alessandra Renieri**[1;2;6]

[1]Med Biotech Hub and Competence Center, University of Siena, Department of Medical Biotechnologies, Siena, Italy; [2]Medical Genetics, University of Siena, Siena, Italy; [3]Institute of Informatics and Telematics, CNR, Pisa, Italy; [4]Neuropsychiatric Unit Azienda Ospedaliera Universitaria Senese, Siena, Italy; [5]Department of Electrical, Electronic and Information Engineering "Guglielmo Marconi", University of Bologna, Cesena, Italy; [6]Genetica Medica, Azienda Ospedaliero-Universitaria Senese, Siena, Italy

Background and Methods: To understand the clinical variability of Rett syndrome (RTT), ranging from an autistic-like disorder to a very severe disease without speech, we performed whole-exome sequencing (WES) and genotyping in 142 MECP2-mutated girls. Clinical severity score was used for both global and domain-specific clinical assessment.

Results: Our analysis showed that the MECP2 variant type influences both global and specific severity scores, while polygenic risk score does not contribute to explaining epilepsy or cognitive level severity. Notably, 30% (43 patients) harbored co-occurring pathogenic variants in other genes relevant to RTT management and treatment. The mutated genes are associated with intellectual disability and epilepsy (*CHD2, GRIN2B, NHLRC1*), central (*TARDBP*), and peripheral (*TTR*) neurodegenerative disorders or scoliosis (GDF3). These results highlight the importance of performing WES in all RTT patients who undergo clinical trials, to avoid biases in drug efficacy assessment. Secondary rare variants hold aldo significant potential for personalized patient care in various areas, including dietary (*G6PD, MHCMAC, PHA, LDLR*), lifestyle (*CFTR, SERPIN1, AIRE*), post-surgery (*VWF*), and even oncological surveillance (*BRCA1, MUTYH*).

Conclusion: this study addresses unmet clinical needs in managing patients with complex and pleiomorphic diseases such as RTT. Furthermore, our findings open an innovative view on the pathogenesis of the so-called, monogenic diseases, showing the benefits of an accurate analysis of rare variants in every gene. This comprehensive approach is particularly relevant for an improved patient care and a reduced risk of false negatives/false positives in clinical trial outcomes.

Grants: INTERVENE, HORIZON Project 2020 n.101016775

Conflict of Interest: None declared

P10.051.B *IMMP2L* gene deletions detected by array-CGH – variants of uncertain clinicalsignificance or benign deletions?

Isabel Marques Carreira[1;2;3], *Susana Isabel Ferreira[3], Mariana Val[1], Alexandra Mascarenhas[1], Marta Pinto[1], Nuno Lavoura[1], Luís Miguel Pires[1], Ana Jardim[1], Joana Barbosa de Melo[1;2;3]*

[1]Laboratory of Cytogenetics and Genomics - Faculty of Medicine, University of Coimbra, Coimbra, Portugal; [2]Center for Innovative Biomedicine and Biotechnology (CIBB), University of Coimbra, Coimbra, Portugal; [3]Institute for Clinical and Biomedical Research (iCBR) and Center of Investigation on Environment Genetics and Oncobiology (CIMAGO), Faculty of Medicine, University of Coimbra, Coimbra, Portugal

Background/Objectives: *IMMP2L* gene has been reported as a candidate gene for Tourette syndrome, autism spectrum disorder (ASD) and additional neurodevelopmental disorders such as attention-deficit hyperactivity, speech-language delay and various developmental delays. There is some controversy in the literature concerning this association.

Methods: We have performed Agilent 180K oligonucleotide array-CGH in more than 4000 blood samples from patients with ASD, intellectual disability, language delay or global developmental delay.

Results: We detected 34 patients, 24 males and 10 females, with *IMMP2L* gene partial deletions, most of them involving the first exons of the gene. Inheritance determination was only possible in 7 patients, being maternally inherited in 5 cases and paternal in 2.

Conclusion: *IMMP2L* gene encodes a protein involved in processing the signal peptide sequences used to direct mitochondrial proteins to the mitochondria. In mice heterozygous for an inactive *Immp2l* gene, increase of ischemic brain damage and apoptosis of cerebellar neurons have been detected, and this observation lead to the notion that *IMMP2L* alterations may predispose to cognitive/behavioral disturbances in humans. However, some studies found no evidence of association.

We struggle with the question if we should continue reporting aCGH detected *IMMP2L* gene deletions, since most of them are inherited from normal progenitors, and considering the controversial literature reports. We also question the value of this diagnosis for the clinical management and genetics counseling of the diagnosed patients, and how to deal with genetics counseling in subsequent pregnancies of a couple with an affected child and a carrier normal progenitor.

Conflict of Interest: None declared

P10.052.C Coffin-Lowry syndrome: phenotypic spectrum in affected females

Anna Gerasimenko[1], Marie-Pierre Luton[1], Solveig Heide[1], Cyril Mignot[1], Fanny Phelippeau[1], Perrine Charles[1], Valentin Ruault[2], Mylene Tharreau[2], Gijs Santen[3], Mariette Hoffer[3], Bertrand Isidor[4;4], Mathilde Nizon[4], Isabelle Maray[1], Benedicte Gerard[5], Aurore Garde[6], Anne-Sophie Denommé-Pichon[6], Frédéric Tran Mau-Them[6], Cedric Le Caignec[4], Francesca Peluso[7], Melanie FRADIN[8], Daphné Lehalle[1], **Delphine Heron**[1]

[1]Pitié Salpêtrière, Clinical Genetics, Paris, France; [2]CHU Montpellier, Clinical Genetics, Montpellier, France; [3]Clinical Genetics, Leiden, Netherlands; [4]CHU Nantes, Cl; [5]CHU Strasbourg, Clinical Genetics; [6]CHU Dijon, Medical Genetics; [7]Azienda USL-IRCCS di Reggio Emilia, Reggio Emilia, Medi; [8]CHU Rennes, Clinical Genetics

Background/Objectives: The Coffin-Lowry Syndrome (CLS) is a rare condition associated with the loss of function of the *RPS6KA3* gene (OMIM 300075), located on the X chromosome. Diagnosis is frequently clinically considered in boys, presenting with severe

intellectual disability (ID) and craniofacial peculiarities. In females, the presentation is of a different spectrum, often leading to delayed diagnosis in adulthood, typically after identification of the condition in a male family member. Lack of knowledge of female phenotype impedes management for syndrome-specific issues, as well as genetic counseling and information to families.

Methods: This study reports the clinical, morphological, and neurodevelopmental phenotype of 9 females, diagnosed molecularly with CLS, who sought consultation at the Clinical Genetics Department of La Pitié-Salpêtrière Hospital between 2016 and 2023, as well as 43 patients via collaboration calls (ERN-ITHACA, DéfiScience, AnDDI-Rares).

Results: The majority of the patients in the series experienced learning difficulties, and did benefit from adaptions or specialized schooling. All evaluated patients exhibited neurodevelopmental disorders (ID or complex neurodevelopmental disorder). Additionally, almost all adult patients were referred for psychiatric follow-up, and presented with overweight or obesity.

Conclusion: To date, few reported cohorts have presented CLS in females. Literature data suggest inconsistent ID, learning difficulties, and rare psychiatric disorders. This study presents the largest phenotypic series of females with CLS, to our knowledge. Our findings suggest that female CLS encompasses a distinct clinical entity, with a neurodevelopmental phenotype that is more prevalent, and a majority of females exhibiting severe learning difficulties or ID, psychiatric comorbidities and obesity in adulthood.

Conflict of Interest: None declared

P10.053.D Exome reanalysis in patients candidates to participate in the national IMPaCT-GENOMICA program for Rare Diseases.

Emma Soengas Gonda[1], Marta Pacio Miguez[2], Berta Almoguera Castillo[2;3], Carmen Ayuso[2;3], Aránzazu Díaz de Bustamante[4], Mayte Darnaude[4], María Fenollar-Cortés[5], Belén Gil-Fournier[6], Soraya Ramiro León[6], Cristina González[7], Miguel A. Martin[8], Miguel Ángel Moreno-Pelayo[9], Matías Morín[9], Nelmar Valentina Ortiz Cabrera[10], Verónica Adriana Seidel[11], Pablo LAPUNZINA[1;12;13], **María Palomares-Bralo**[1;12;13]

[1]*Centro de Investigación Biomédica en Red en Enfermedades Raras (CIBERER), Madrid, Spain;* [2]*Centro de Investigación Biomédica en Red en Enfermedades Raras (CIBERER), Madrid, Spain;* [3]*Servicio de Genética, Fundación Jiménez Díaz, IIS-FJD, Madrid, Spain;* [4]*Unidad de Genética, Hospital Universitario de Móstoles, Madrid, Spain;* [5]*Genética Clínica, Servicio de Análisis Clínicos. Instituto de Medicina del Laboratorio. IdISSC. Hospital Clínico San Carlos, Madrid, Spain;* [6]*Servicio de Genética, Hospital Universitario de Getafe, Madrid, Spain;* [7]*Servicio de Genética, Hospital Universitario Infanta Sofía, Madrid, Spain;* [8]*Servicio de Genética, Hospital Universitario 12 de Octubre, IIS i + 12, Madrid, Spain;* [9]*Servicio de Genética, Hospital Ramón y Cajal-IRYCIS, Madrid, Spain;* [10]*Servicio de Análisis Clínicos. Consulta de Genética Clínica. Hospital Universitario Niño Jesús, Madrid, Spain;* [11]*Genética Clínica, Servicio de Pediatría. Hospital Gregorio Marañón, Madrid, Spain;* [12]*Instituto de genética médica y molecular, INGEMM, Idipaz. Hospital Universitario la Paz, Madrid, Spain;* [13]*European Research Network (ERN)-Ithaca*

Background/Objectives: A significant proportion of people with rare diseases (RD) don't have a genetic diagnosis after genomic studies. Reanalysis of genomic data has proven to be a useful approach to increase diagnostic yield. We present the results of reanalysis of genomic data from patients selected in the region of Madrid to participate in the national IMPaCT-GENóMICA RD program whose network is configured by 10 hospitals.

Methods: Researchers from this network selected a total of 285 patients with presumptive diagnosis of rare, monogenic disease without genetic diagnosis to participate. All of them had a previous complete exome study with inconclusive result. Those with an exome study performed in 2020 or earlier were considered for reanalysis. Exome data from 215 patients were reanalyzed.

Results: A conclusive diagnosis was obtained in 12% (n = 27/215). The causes that did not allow a diagnosis to be reached in the initial study were the absence of gene-disease association at the time of the first study, or the application of virtual panels that did not include the causal gene (66.7%; n = 18/27), the loss of the causal variant in the primary or secondary analysis (18.5%; n = 5/27) and differences between the phenotype of the patient and that of the published cases (14.8%; n = 4/27). In 23% of cases, the variant was classified as a pathogenic or probably pathogenic variant in Clinvar/HGMD

Conclusion: The diagnostic process is dynamic, reanalysis of genomic data and continuous clinical evaluation allow for improved diagnostic performance.

Grants:

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Conflict of Interest: None declared

P10.055.B The clinical phenotype of Bosch-Boonstra-Schaaf optic atrophy syndrome

Ilia Valentin[1], *Pilar Caro[1], Christine Fischer[1], Christian Schaaf[1]*

[1]*Institute of Human Genetics, University Heidelberg, Heidelberg, Germany*

Background/Objectives: (Likely) pathogenic variants in *NR2F1* are associated with Bosch-Boonstra-Schaaf optic atrophy syndrome (BBSOAS, OMIM 132890), a rare neurodevelopmental disorder. Patients present with a variety of symptoms including intellectual disability, developmental delay, visual impairment, hypotonia, seizures and/or autistic features. Since its initial description in 2014, the phenotype steadily expanded. However, there is limited information regarding the natural course of the disorder. Here we present data of 47 individuals who answered our questionnaire.

Methods: A questionnaire was created to assess the phenotype more comprehensively and to better understand the course of the symptoms. In addition, families sent medical documents and photos. To investigate genotype-phenotype correlation in our cohort, we compared the clinical features of two genotypic groups (variants in the DNA-binding domain (DBD, n = 17) vs. variants elsewhere in the gene (n = 30)).

Results: We observed a range of common symptoms, such as developmental delay, hypotonia, optic atrophy, nystagmus, strabismus, autistic features and thin corpus callosum on brain MRI. Overall, more improvements than worsenings were reported. Individuals with variants in the DBD showed higher prevalences of severe clinical features, such as infantile spasms (46.7% vs. 3.8%, $p = 0.002$) or being nonverbal (41.2% vs. 3.4%, $p = 0.002$).

Conclusion: Our study confirms characteristic clinical features of BBSOAS. Variants in the DBD are associated with a more severe clinical phenotype. We found no evidence that the disease is progressive; rather several symptoms are reported to improve over time. However, longitudinal studies are necessary to further investigate the course of the disease.

Grants: None

Conflict of Interest: None declared

P10.056.C Novel biallelic ABCC9 loss-of-function variants expand ABCC9-related intellectual disability and myopathy syndrome

*Stephanie Efthymiou[1], Marcello Scala[1;2;3], Vini Nagaraj[4], Katarzyna Ochenkowska[5], Fenne Komdeur[6], Robin Liang[7], Mohamad Abdelhamid[8], Tipu Sultan[9], Tuva Barøy[10], Marijke van Ghelue[7], Barbara Vona[11], Reza Maroofian[1], Faisal Zafar[12], Fowzan Alkuraya[13], Maha Zaki[14], MariaSavina Severino[15], Robert Tryon[16], Lin Brauteset[17], Kingsley Duru[4], Morad Ansari[18], Mark Hamilton[19], Mieke van Haelst[6], Gijs van Haaften[20], Federico Zara[3], Henry Houlden[1], Eric Samarut[5], Colin Nichols[16], **Marie Falkenberg Smeland**[21;22], Conor McClenaghan[4]*

[1]University College London, Dpt of neuromuscular disorders, UCL Queen Square Institute of Neurology, London, United Kingdom; [2]University of Genoa, Dpt of neurosciences, Rehab, Ophtalmology, Genetics, Maternal and Child Health, Genoa, Italy; [3]IRCCS Instituto Giannina Gaslini, U.O.C. Genetica Medica, Genoa, Italy; [4]Rutgers The State University of New Jersey, Center for Advanced Biotechnology and Medicine, and Dpts of Pharmacology and Medicine, Robert wood Johnson Medical School, New Jersey, United States; [5]Universite de Montreal, Centre de Recherche du Centre Hospitalier de l'Universite de Montreal and Dpt of Neuroscience, Montreal, Canada; [6]Amsterdam University Medical Centers, Section Clinical Genetics, Dpt Human Genetics and Amsterdam Reproduction and Development, Amsterdam, Netherlands; [7]University Hospital of North Norway, Dpt of Medical Genetics, Tromsø, Norway; [8]National Research Centre, Medical Molecular Genetics dpt, Human Genetics and Genome Research Institute, Cairo, Egypt; [9]Children Hospital, University of Child Health Sciences, Dpt of pediatric Neurology, Lahore, Pakistan; [10]Oslo University Hospital, Dpt of medical Genetics, Oslo, Norway; [11]University Medical Center Gôttingen, Institute for Human Genetics and Institute for Auditory Neuroscience and InnerEar lab, Gôttingen, Germany; [12]Childrens Hospital and Institute of Child Health, Dpt of pediatric neurology, Punjab, Pakistan; [13]King Faisal Specialist Hospital and Research Center, Dpt of Translational Genomics, Center for genomic medicine, Riyadh, Saudi Arabia; [14]National Research Centre, Clinical genetics dpt, Human Genetics and genome research institute, Cairo, Egypt; [15]IRCCS Instituto Giannina Gaslini, Neuroradiology Unit, Genoa, Italy; [16]Washington University St.Louis, MO, Dpt of cell biology and physiology and center for investigation of membrane excitability diseases, St. Louis, United States; [17]Innlandet Hospital Sanderud, Division of habilitation for Children, Hamar, Norway; [18]Western General Hospital, South East Scotland Genetic Service, Edinburgh, United Kingdom; [19]Queen Elizabeth University Hospital, West of Scotland Genetics Service, Glasgow, United Kingdom; [20]University Medical Center Utrecht, Dpt of Genetics, Utrecht, Netherlands; [21]University Hospital of North Norway, Dpt of pediatric rehabilitation, Tromsø, Norway; [22]UiT The Arctic University of Norway, Tromsø, Norway

Background/objectives: (NM_005691:c.1320+1G>A) loss-of-function (LoF) mutation of *ABCC9* was recently associated with autosomal recessive *ABCC9*-related intellectual disability and myopathy syndrome (AIMS)[1] in six patients with mild intellectual disability, white matter hyperintensities, myopathy and cardiac dysfunction. *ABCC9* encodes the SUR2 subunit of ATP sensitive-potassium (K_{ATP}) channels widely expressed throughout the body.

Methods: Phenotypic description including neuroradiologic evaluation of nine additional subjects and genotypic description of their different homozygous ABCC9 variants by heterologous expression studies. Three additional patients were recently identified, and will be included. In vivo studies in zebrafish and treatment trials in mice.

Results: All individuals show psychomotor delay and intellectual disability of variable severity associated with microcephaly, seizures, spasticity, short stature, muscle fatigability, and weakness. Cerebral MRIs demonstrate white matter abnormalities resembling periventricular leukomalacia, and corpus callosum hypoplasia. The AIMS phenotype expands to severe intellectual impairment, cerebral palsy, epileptic seizures in more patients, additionally, rhabdomyolysis in one patient.

K_{ATP} channels for AIMS variants are shown to be non-functional, implying LoF by severe truncations or in-frame deletions within SUR2.

abcc9 LoF zebrafish react to pentylenetetrazole (pro-convulsive) with exacerbated motor response, consistent with increased seizure-susceptibility. In skeletal muscle dominant-negative mice, verapamil (calcium-blocker) was ineffective in improving excercise tolerance, and unexpectedly lethal in SUR2-STOP mice[2].

Conclusion: Our findings further define a distinct *ABCC9*-related recessive phenotype, expand the genotypic and phenotypic spectrum of AIMS, revealing novel human pathologies arising from K_{ATP} channel dysfunction.

Grants: Wellcome trust. UCL-team funding. NIH award.

References:

1)Smeland et al (2019) PMID: 31575858
2)McClenaghan et al (2023) PMID: 37154692

Conflict of Interest: None declared

P10.057.D CTNND2 variants are associated with developmental delay, mild intellectual disability, and behavioral problems

*Denisa Weis[1], Andrej Ficek[2], **Ricarda Reiter**[1], Katarína Kušíková[3], Andrea Soltysova[2;4]*

[1]Institute of Medical Genetics, Kepler University Hospital, Dpt. Medical Genetics, Linz; [2]Faculty of Natural Sciences, Comenius University, Bratislava, Slovakia, Department of Molecular Biology, Bratislava 4, Slovakia; [3]Faculty of Medicine, Comenius University Bratislava and National Institute of Children's Diseases, Bratislava, Slovakia, Department of Pediatric Neurology, Bratislava, Slovakia; [4]Institute for Clinical and Translational Research, Biomedical Research Center, Slovak Academy of Sciences, Bratislava, Slovakia, Research Center, Bratislava, Slovakia

Consortium: CTNND2 Consortium

Background/Objectives: Heterozygous loss of CTNND2 has been linked to a broad spectrum of neurodevelopmental disorders such as autism, schizophrenia, mild intellectual disability, and neurocognitive abnormalities with developmental delay phenotype. Some authors report the association of CTNND2 variants with dysmorphic features, attention deficit, or myopia. Next-generation sequencing revealed many novel variants with low or no frequency in the European control database. While large deletions and translocation of the CTNND2 gene are reported as pathogenic, all missense variants are of uncertain significance in the ClinVar database.

Methods: In the present study, we evaluated 54 patients' clinical manifestations with whole exome sequencing, SNP array, and karyotyping methods. Patients with available facial photos were analyzed using Face2gene. Pathogenicity prediction, nonsense-mediated RNA decay, and sequence conservation regarding patients' phenotypes were tested in silico.

Results: The 54 patients carried 41 different CTNND2 variants. The main clinical presentation was intellectual disability (64.81%), developmental delay (61.11%), speech problems (50%), and apparent facial dysmorphism (50%). More than half of affected individuals presented abnormal behavior (51.85%), including impulse control difficulties (31.48%), emotional and social immaturity (24.07%), and aggressiveness (12.96%). Out of 27 cases with available family data, 40.7% of identified mutations were de novo, and disease phenotype was present in 25.9% of

parents with transmitting mutation. A broad spectrum of CTNND2 variants were identified; further studies of predominantly missense variants are needed to elucidate their role in the pathogenesis of the disease.

Conclusion: Our comprehensive monitored clinical data contribute to the knowledge of the CTNND2 variant's role in neurodevelopmental disorders.

Grants:

Conflict of Interest: None declared

P10.058.A MED13L is critical for OTX2 brain-specific regulatory element activation and neural cell fate commitment

jamal ghoumid[1], *Jerome Sige*[1], *Thomas Smol*[1], *Jade Fauqueux*[1], *Fiona Leduc*[1], *Caroline Thuillier*[2], *Marie Balerdi*[1], *Ryan Ziffra*[3;4], *Dianne Laboy Cintron*[3;4], *Florence Petit*[1], *Nadav Ahituv*[3;4]

[1]*Université de Lille - Pôle Recherche de la Faculté de Médecine, Lille, France;* [2]*Hospital Center University De Lille, Lille, France;* [3]*University of California San Francisco, San Francisco, Department of Bioengineering and Therapeutic Sciences, San Francisco, United States;* [4]*UCSF Institute for Human Genetics, San Francisco, United States*

Background: The Mediator Complex is a coordinator of cell lineage determination, forming and stabilizing enhancer-promoter loops. Mutations in MED13L, a subunit of the complex, lead to intellectual disability, suggesting MED13L has a neural developmental role. Despite this, its precise function remains unknown.

Methods: We performed MED13L ChIP-seq in human iPSC to identify MED13L-bound cis-regulatory elements (CREs). We generated homozygous *MED13L*-knockout iPSC and following 48 hours post neural induction carried out 4C-seq, to assess for chromatin interactions, using the *OTX2* promoter as the viewpoint, as this gene regulates cell fate decisions during neural patterning. We also generated cerebral organoids (CO) from these iPSCs and characterized them via single-cell (sc) RNA-seq and scATAC-seq.

Results: Single-cell transcriptome analysis showed that the *MED13L*-knockout COs had a neuroretinal-like phenotype compared to wild-type. scATAC-seq showed a loss of activation of *OTX2*-brain CREs and ectopic *OTX2*-retinal CRE activation in these COs. Analysis of chromatin interactions (4C-seq) at the *OTX2* locus showed loss of interaction between the *OTX2* promoter and brain-associated CREs that was replaced with *OTX2* retinal-associated CRE interactions.

Conclusion: In wild-type cells, brain CREs closely interact with the *OTX2* promoter, leading to a developmental program recapturing early brain development, as observed in our COs. In contrast, in the *MED13L*-knockout, the *OTX2* brain CREs show significant interactions with retinal-associated CREs, leading to a neuroretinal-like development in CO. These results suggest that MED13L is a major determinant of neural cell fate decisions and that MED13L-related intellectual disability could result from altered gene expression during this process.

Conflict of Interest: None declared

P10.059.B Implementation of episignatures to resolve undiagnosed neurodevelopmental diseases with irregular Mendelian patterns of inheritance

Slavica Trajkova[1;2;3], *Jennifer Kerkhof*[4;5], *Serena Rizzo*[2;3], *Chiara Giovenino*[6], *Lisa Pavinato*[1;7], *Simona Cardaropoli*[3;8], *Verdiana Pullano*[3;7], *Enza Ferrero*[3;7], *Tommaso Pippucci*[9], *Paola Dimartino*[10], *Jessica Rzasa*[5], *Haley McConkey*[4;5], *Elena Sukarova-Angelovska*[11], *Andrea Gazzin*[8], *Alessandro Mussa*[8], *Giovanni Battista Ferrero*[12], *Bekim Sadikovic*[4;5], *Alfredo Brusco*[1;2;3]

[1]*University of Turin, Department of Neurosciences Rita Levi-Montalcini, Turin, Italy;* [2]*Città della Salute e della Scienza University Hospital, Medical Genetics Unit, Turin, Italy;* [3]*University of Turin, Molecular Biotechnology Center "Guido Tarone", Turin, Italy;* [4]*Western University, Department of Pathology and Laboratory Medicine, London, Canada;* [5]*London Health Sciences Centre, Verspeeten Clinical Genome Centre, London, Italy;* [6]*Istituto di Candiolo SP142, Turin, Italy;* [7]*University of Turin, Department of Medical Sciences, Turin, Italy;* [8]*University of Turin, Department of Public Health and Pediatric Sciences, Turin, Italy;* [9]*Sant'Orsola-Malpighi University Hospital, Medical Genetics Unit, Bologna, Italy;* [10]*University of Pavia, Department of Molecular Medicine, Pavia, Italy;* [11]*Faculty of Medicine, University "Sv. Kiril I Metodij", Department of Endocrinology and Genetics, Skopje, Macedonia;* [12]*San Luigi University Hospital, Medical Genetics Unit and Thalassemia Center, Turin, Italy*

Background/Objectives: DNA methylation (DNAm) patterns, or episignatures, are poweful diagnostic biomarkers for single-gene neurodevelopmental disorders (NDDs). This report illustrates the power of episignatures to resolve familial NDDs with irregular inheritance patterns.

Methods: Our cohort is a small subset of NDD cases derived from a larger study involving almost 600 families examined by EpiSign™ DNAm. We focused here on (i) three pairs of siblings with similar phenotypes but different genetic findings and (ii) three cases with suspected chromatinopathy but negative exome

Results: Family #1: one boy had a de novo TRIP12 (L1044Ffs*3) variant, confirmed by the TRIP12 episignature. His brother had a de novo variant in FBN (A1728V), as yet without a diagnostic episignature. Family #2: two brothers with Kleefstra-like syndrome, one had a de novo EHMT1:K462Vfs*7 variant, confirmed by the EHMT1 episignature. Family #3: a boy with a ZMYM3:R441Q variant was confirmed by the ZMYM3 episignature; his sister had an isodicentric 15q11.2-q13.1 chromosome. The second group consisted in NDD cases with variants inherited from unaffected parents. Case #1: we discovered a maternally inherited variant (SMARCA2:G1129D) from an unaffected mosaic mother. Case #2: in a Sotos-like case, we found a paternally inherited variant (NSD1:K1002Efs*8). Case #3: the proband was diagnosed clinically with Cornelia de Lange but instead showed the ANKRD11-associated episignature, revealing an ANKRD11:N725Kfs*23 variant inherited from the mildly affected father

Conclusion: Episignature analysis is a powerful tool for identifying the molecular culprit in cases that show irregular Mendelian segregation patterns.

Conflict of Interest: None declared

P10.060.C Phenotypic and genotypic study of a large cohort of patients with *CNOT3*-related neurodevelopmental disorder

Camille Engel[1], *Michaela Rendek*[1], *Jessica Assoumani*[1], *Emanuela Argilli*[2], *Emilia K.Bijlsma*[3], *Lucia Bruno*[4], *Bert Callewaert*[5], *Sandra Coppens*[6], *Cynthia curry*[7], *Breanne Dale*[8], *Mette Handrup*[9], *Irina Hüning*[10], *Michele Iacomino*[11], *Bertrand Isidor*[12], *Stanislav Kmoch*[13], *David Koolen*[14], *Jana Lastuvkova*[15], *Carolyn Le*[2], *Maitz Silvia*[16], *Ghaydda Mirzaa*[17], *Inga Nagel*[18], *Sebastian Neuens*[19], *Lenka Nosková*[20], *Emily Pao*[21], *Anna Pecková*[15], *Julie Plaisancié*[22], *André Reis*[23], *Marlene Rio*[24], *Marcello Scala*[25], *Jolanda Schieving*[26], *Elliott H. Sherr*[2], *Andrew Shuen*[27], *Richard Sidlow*[28], *Julie Soblet*[19], *Pasquale Striano*[25], *Mohnish Suri*[29], *Georgia Vasileiou*[23], *Jolijn Verseput*[30], *Catheline Vilain*[19], *Emma Wakeling*[31], *Pia Zacher*[32], *Federico Zara*[25],

1458

Delphine Heron[33], Cyril Mignot[33], Jonathan Levy[34], Catherine Vincent-Delorme[35], David Geneviève[36], Boris Keren[37], Julien Van Gils[38], Anne-Laude Avice Denizet[1], Emílie Vyhnálková[39], Lukáš Ryba[40], Paul Kuentz[41], Juliette Piard[1]

[1]CHU Besançon, Centre de Génétique Humaine, Besançon, France; [2]University of California, Department of Neurology, San Francisco, United States; [3]Leiden University Medical Centre, Department of Clinical Genetics, Leiden, Netherlands; [4]Telethon Institute of Genetics and Medicine (TIGEM), Naples, Italy; [5]UZ Gent, Ghent University Hospital, Reference centre for Mendelian connective tissue disorders, Gent, Belgium; [6]Hôpital Erasme, Hôpital Universitaire de Bruxelles, Université Libre de Bruxelles, Center for Medical Genetics, Brussels, Belgium; [7]University of California, Genetic Medicine Central California, San Francisco, United Kingdom; [8]McMaster Children's Hospital, Genetics and Metabolics Clinic, Hamilton, Canada; [9]Aarhus University Hospital, Center for Rare Diseases, Pediatric and Adolescent Medicine, Aarhus, Denmark; [10]University of Lübeck, Institute of Human Genetics, Lübeck, Germany; [11]IRCCS Istituto Giannina Gaslini, Medical Genetics Unit, Genoa, Italy; [12]CHU Hôtel Dieu, Service de génétique médicale, Unité de génétique clinique, Nantes, France; [13]Faculty of Medicine, Charles University, Research Unit for Rare Diseases, Department of Pediatrics and Inherited Metabolic Disorders, Prague, Czech Republic; [14]Donders Institute for Brain, Cognition and Behaviour, Radboud university medical center, Department of Human Genetics, Nijmegen, Netherlands; [15]Masaryk Hospital in Ústí nad Labem, Regional Health Corporation, Department of Medical Genetics, Czech Republic; [16]MBBM Foundation, S. Gerardo Hospital, Clinical Pediatric Genetics Unit, Pediatrics Clinics, Monza, Italy; [17]University of Washington, Department of Pediatrics, Seattle, United States; [18]University Hospital Schleswig-Holstein, Institute of Human Genetics, Kiel, Germany; [19]Hôpital Universitaire des Enfants Reine Fabiola, Hôpital Universitaire de Bruxelles, Université Libre de Bruxelles, Department of Genetics, Brussels, Belgium; [20]Faculty of Medicine, Charles University in Prague, Research Unit for Rare Diseases, Department of Pediatrics and Inherited Metabolic Disorders, Prague, Czech Republic; [21]Division of Nephrology and Center for Clinical and Translational Research, Department of Pediatrics and Seattle Children's Hospital, Seattle, United States; [22]Hôpital Purpan, CHU de Toulouse, Service de Génétique Médicale, Toulouse, France; [23]Universitätsklinikum Erlangen, Friedrich-Alexander-Universität Erlangen-Nürnberg, Institute of Human Genetics, Erlangen, Germany; [24]Necker Hospital, APHP, Institut Imagine, University of Paris, Reference Center for Intellectual Disability, Genetics Department, Paris, France; [25]University of Genoa, Department of Neurosciences, Rehabilitation, Ophthalmology, Genetics, Maternal and Child Health, Genoa, Italy; [26]Radboud University Medical Center, Department of Neurology, Nijmegen, Netherlands; [27]The Hospital for Sick Children, Department of Pediatrics, Division of Clinical and Metabolic Genetics, Toronto, Canada; [28]Valley Children's Hospital, Department of Medical Genetics and Metabolism, Madera, United States; [29]Nottingham University Hospitals NHS Trust, City Hospital Campus, Nottingham Clinical Genetics Service, Nottingham, United Kingdom; [30]Radboud university medical center, Department of Human Genetics, Nijmegen, Netherlands; [31]Great Ormond Street Hospital for Children NHS Foundation Trust, North East Thames Regional Genetic Service, London, United Kingdom; [32]Epilepsy Center Kleinwachau, Radeberg, Germany; [33]APHP.Sorbonne Université, Hôpital Trousseau & Groupe Hospitalier Pitié-Salpêtrière, Département de Génétique, Paris, France; [34]AP-HP, Robert-Debré University Hospital, Genetics Department, Paris, France; [35]CHU Lille, Clinique de Génétique, Lille, France; [36]CHU Montpellier, Centre de référence anomalies du développement et syndromes malformatifs, Génétique Clinique, Montpellier, France; [37]Hôpital Pitié-Salpêtrière, AP-HP.Sorbonne Université, Département de génétique médicale, Paris, France; [38]University Hospital of Bordeaux, Department of Medical Genetics, Bordeaux, France; [39]Charles University 2nd Faculty of Medicine and University Hospital Motol, Department of Biology and Medical Genetics, Prague, Czech Republic; [40]Charles University - 2nd Faculty of Medicine and University Hospital Motol, Department of Biology and Medical Genetics, Prague, Czech Republic; [41]Centre Hospitalier Régional Universitaire, Université de Franche-Comté, Oncobiologie Génétique Bioinformatique, Besançon, France

Background/Objectives: Heterozygous de novo variants in *CNOT3* were first reported by Martin et al in 2018 in 16 patients with hypotonia, relative short stature, developmental delay, intellectual disability and behavioral disorders. Two years later, Meyer et al reported for the first time two families in which a variation of *CNOT3* segregated. A total of 31 affected individuals from 25 families have been reported in the literature to date.

Methods: Thanks to an international collaboration, we collected clinical and molecular data of 37 additional patients from 34 families. Together, with the addition of the data from litterature, this allowed us to build up a large cohort of 68 patients from 59 families.

Results: The study of this large cohort enabled us to highlight a homogenous clinical presentation between patients, including developmental delay, mild to moderate intellectual disability, autism spectrum disorder, behavioral disorders and morphological features. Some patients presented with epilepsy or deafness.

Conclusion: We have not identified any genotype-phenotype correlation for *CNOT3* variants, which could be explained by a loss-of-function effect of all of these variants. Our findings suggest that missense variants located in binding sites equally present with a loss-of-function effect.

The study of large cohorts is necessary to better characterize the phenotype of rare diseases, as well as to better understand their natural history and provide families with appropriate genetic counseling.

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P10.061.D Biallelic missense variants in *CSMD2* are associated with a neurodevelopmental disease and epilepsy

Clara Pailler-Pradeau[1], *Francesca Mattioli*[1], *Marianne Lemée*[2], *Hossein Darvish*[3], *Lorena Travaglini*[4], *Giovanna Stefania Colafati*[4], *Francesco Nicita*[4], *Shivarajan, M Amudhavalli*[5], *Caitlin Lawson*[5], *Luciana Musante*[6], *Giorgia Girotto*[6], *Flavio Faletra*[6], *Ginevra Zanni*[4], *Stylianos Antonarakis*[7], *Muhammad Ansar*[8], *Nicolas Guex*[1], *Christelle Golzio*[2], *Alexandre Reymond*[1]

[1]*UNIL - Génopode, CIG, Lausanne, Switzerland;* [2]*I.G.B.M.C. - Institut de génétique et de biologie moléculaire et cellulaire, Illkirch-Graffenstaden, France;* [3]*Golestan University of Medical Sciences, Neuroscience Research Center, Gorgan, Iran;* [4]*Bambino Gesù Children's Hospital, Roma, Italy;* [5]*Children's Mercy Hospital Kansas, Overland Park, United States;* [6]*Children's hospital "Burlo Garofolo", Trieste, Italy;* [7]*University of Geneva, Medical Faculty, Genetic Medicine, Geneva, Switzerland;* [8]*Hospital Ophthalmic Jules-Gonin Foundation Asylum Des Aveugles, Lausanne, Switzerland*

Background/Objectives: Common variants in the *CSMD1-3* paralogs were shown to be GWAS-associated with neurodevelopmental and psychiatric disorders. These CUB and Sushi domains-containing synaptic proteins play a role in neuron development.

Methods: We screened diseased cohorts for *CSMD2* variants and assessed the function of its zebrafish ortholog.

Results: GnomAD suggests that *CSMD2* is under constraint and that both haploinsufficiency and missense variation could be deleterious (pLI = 1; missense Z-score = 5.45). Consistent with this hypothesis, we identified sixteen individuals carrying biallelic missense mutations in *CSMD2* in developmental delay/intellectual disability cohorts. Patients present also with autism, attention deficit hyperactivity disorder, brain anomalies and epilepsy. While the identified mutations are spread along the gene, 3D in silico modelling of the encoded proteins shows that the mutated residues are structurally close. Their side chains cannot be incorporated without clashing into the backbone of the surrounding amino acids regardless of the rotamers.

Zebrafish ablated for the orthologous *csmd2* using crispr-cas presented with microcephaly (reduced size of optic tecta and increased distance between tecta), smaller cerebellum due to smaller Purkinje's cells, a decrease in the number of neural connections between optic tecta, a higher number of abnormal peripheral neuronal branches and a decreased swimming velocity further substantiating a possible role of this gene in neurogenesis and synapse formation. Both the 3D modelling and functional analyses in zebrafish of the variants identified in patients suggested that the encoded proteins were functionally impaired.

Conclusion: Our experiments demonstrate a link between missense mutations in *CSMD2* and an abnormal development of the central and peripheral nervous system.

Grants:

Conflict of Interest: None declared

P10.062.A Genetic architecture of neurodevelopmental disorders based on a series of 402 patients diagnosed with next generation sequencing.

Lucia Lopez-Lopez[1], *Laura Lapeña-Gil*[1], *María José Trujillo Tiebas*[1;2], *Almudena Avila Fernández*[1;2], *Inmaculada Martin Merida*[1;2], *Marta Corton*[1;2], *Pablo Mínguez*[1;2;3], *Fermina Lopez-Grondona*[1], *Ana Isabel Sánchez Barbero*[1], *Saoud Tahsin-Swafiri*[1;2], *Marta Rodriguez de Alba*[1;2], *Carmen Ayuso*[1;2], *Isabel Lorda*[1;2], *Fiona Blanco-Kelly*[1;2], **Berta Almoguera Castillo**[1;2]

[1]*Fundacion Jimenez Diaz University Hospital. IIS-FJD, Genetics and Genomics, Madrid, Spain;* [2]*CIBERER, Madrid, Spain;* [3]*Fundacion Jimenez Diaz University Hospital. IIS-FJD, Bioinformatics Unit, Spain*

Background/Objectives: The genetic architecture of neurodevelopmental disorders (NDDs) is complex with a great genetic heterogeneity and over 2,500 genes estimated to be involved. The aim of this work is to catalogue the main genetic causes of monogenic NDDs in a series of 402 patients diagnosed by next generation sequencing (NGS).

Methods: All patients with a definitive genetic diagnosis followed by NGS were included. Both Clinical Exome Sequencing using the Clinical Exome Solution v1-3 and the DDM platform (SOPHiA Genetics) and Whole Exome Sequencing were used. Variants were classified according to the ACMG guidelines. Segregation studies were performed using Sanger sequencing or array CGH.

Results: We identified 402 patients with a definitive genetic diagnosis, 16.7% of whom carried a pathogenic copy number (CNV) or structural variant detected with NGS. There were 175 different causative genes/*loci* with the most frequent diagnoses being a Bafopathy in 5.2% (N = 21), KBG syndrome in 4.2% (N = 17), Rett syndrome in 3.5% (N = 14), a Rasopathy in 2.2% (N = 9), and *CHD2* and *SCN1A*-related disorders in 2% of the patients each (N = 8). The rest of the genes/*loci* explained less than 2% of the diagnoses and included very rare forms of monogenic NDDs and three potential new gene-disease associations.

Conclusion: The spectrum of genetic causes seen in our sample of NDD patients reflects the high genetic heterogeneity associated with this condition and support the use of NGS as the first line approach.

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Conflict of Interest: None declared

P10.063.B At the frontiers of intellectual disability: a phenotypic study of the monoallelic effect of variants in the *KDM5B* gene

Jean-Sérène Laloy[1], *Euphrasie Servant*[2], *sacha schutz*[3;4], *Boris Keren*[2;3], *Juliette Coursimault*[5], *Cindy Colson*[6], *Jean-Baptiste Noury*[4], *Marlene Rio*[7], *Cyril Mignot*[2], *Claudia Ravelli*[8], *Alice Goldenberg*[5], *Anne-Marie Guerrot*[5], *Diane Doummar*[8;9], *Melanie FRADIN*[10], *Stéphanie Arpin*[11], *Blandine Dozieres*[12], *Perrine Charles*[2], *Aline Vincent*[1], *Sacha Weber*[1], *Heidi Schulz*[13], *François Lecoquierre*[3;5], *marion lesieur-sebellin*[3;7;9], *lydie BURGLEN*[3;8;9], *Paul Gueguen*[3;11], *Thomas Smol*[3;6], *Sophie Rondeau*[3;7], *Christele Dubourg*[3;10], *Jean-Madeleine de Sainte Agathe*[2;3;9]

[1]*University Hospital Center of Caen, Caen, France;* [2]*University Hospitals Pitié Salpêtrière - Charles Foix, Paris, France;* [3]*LBMMS SeqOIA, Paris, France;* [4]*Hospital Center Regional And University Chru, Brest, France;* [5]*Hospital Center University De Rouen, Rouen, France;* [6]*Hospital Center University De Lille, Lille, France;* [7]*Necker Hospital, Paris, France;* [8]*Hospital Armand Trousseau Ap-Hp, Paris, France;* [9]*Sorbonne Université, Paris, France;* [10]*CHU Rennes - Pontchaillou Hospital, Rennes, France;* [11]*Chru Hospitals Of Tours, Tours, France;* [12]*Hospital Robert Debré Ap-Hp, Paris, France;* [13]*Zentrum für Humangenetik Tübingen, Tübingen, Germany*

Background/Objectives: Diagnosing intellectual disability (ID) requires defining a frontier of normal intelligence that may

become permeable to neurodevelopmental disorders of varying severity. The KDM5B gene codes for an H3K4 demethylase that regulates the expression of numerous genes, and whose cerebral activity peaks during fetal development. The first deleterious monoallelic mutations in this gene were linked to ID of varying severity. However, the frequency of these mutations in the general population has called into question their diagnostic significance, and the disorder associated with KDM5B is now firmly established only in recessive forms (OMIM 618109).

Methods: We describe here a cohort of 44 carriers of a truncating or de novo monoallelic KDM5B variant from 23021 genome-wide sequenced individuals and 5 individuals added from exome-wide sequencing.

Results: Eighteen of them have a neurodevelopmental disorder and were phenotyped in detail. Comparison with 17 monoallelic and 5 biallelic cases in the literature reaffirms the existence of a dominant form of KDM5B-associated syndrome, distinct from the recessive form. A search for a modifier allele was carried out on the genomic data to explain the incomplete penetrance of the disease, but was inconclusive.

Conclusion: The diagnostic value of these variants remains difficult to establish. Recent genotype-phenotype studies in the general population suggest the involvement of KDM5B loss-of-function variants in cognition, and the existence of a continuum going beyond ID. Further studies are needed to understand what modulates the expression of these variants.

Grants: University of Caen Normandie

Conflict of Interest: None declared

P10.064.C Recurrent homozygous variant in the *NDST1* gene. A clinically recognizable syndromic intellectual disability.

Vanesa López González[1], *Ana Teresa Serrano Antón*[1], *María José Sánchez Soler*[1], *Marta Domínguez Jiménez*[1], *María Juliana Ballesta Martínez*[1], *Carmen María Dolores*[1], *LLuís Armengol*[2], *Encarna Guillén-Navarro*[1]

[1]*Hospital Clínico Universitario Virgen de la Arrixaca, Sección de Genética Médica. Servicio de Pediatría, El Palmar, Murcia, Spain;* [2]*Quantitative Genomic Medicine Laboratories, S.L "qGenomics", Barcelona, Spain*

Background/Objectives: Variants in *NDST1* are causative for autosomal recessive intellectual disability (ID) type 46 (MRT46, OMIM#616116), characterized by ID with language impairment, ataxia, epilepsy, behavioral disorder and growth retardation. Our objective is the clinical characterization of a group of patients with a recurrent homozygous variant.

Methods: Descriptive, retrospective study, through review of medical records of patients diagnosed with MRT46.

Results: 9 cases (5 males) of Roma ethnicity. Average age: 10,4 years. Consanguinity 5/9. All with moderate-severe ID. 4/9 hypotonia and 3/9 epilepsy. 4/9 stereotypies (hand flapping). None with brain structural abnormalities. 2/9 hearing loss and myopia respectively. Physical exam with relative macrocephaly (7/8), sparse eyebrows (5/8), wide nasal bridge (8/8), macrostomia (6/8), thick lips (5/8), dental diastema (3/7), prominent and low-set ears (8/9). All with skeletal anomalies (short and wide hands and feet, brachydactyly, clinodactyly, deep palmar and plantar creases, and elbow contracture). The homozygous pathogenic variant c.1831G>A, p.G611S in *NDST1* was identified in all cases.

Conclusion: Since its identification as a causative gene in 2011, just a few patients have been reported, these new 9 constituting an important contribution to the better clinical characterization of this entity. The existence of a recurrent variant in patients of Roma origin points to a founder effect, making it important to screen carriers, and to become familiar with its phenotype, in order to diagnose it early and in a targeted manner. A recognizable phenotype stands out, not described as specific in previous publications, with a behavioral phenotype in the Angelman-like spectrum.

Grants: none.

Conflict of Interest: None declared

P10.065.D *DDX3X*-related neurodevelopmental disorder in males

Milou Kennis[1], *Dmitrijs Rots*[1;2], *Arjan Bouman*[2], *Charlotte Ockeloen*[1], *Caroline Boelen*[3], *Carlo Marcelis*[1], *Bert de Vries*[1], *Mariet Elting*[4], *Quinten Waisfisz*[4], *Mieke van Haelst*[4], *Lukas Kruidenier*[5], *Mohnish Suri*[6], *Esperanza Font-Montgomery*[7], *Dawn Peck*[8], *Deirdre Donnelly*[9], *Curtis C Rogers*[10], *Ruth Richardson*[11], *Roseline Caumes*[12], *Boris Chaumette*[13], *Cécile Louveau*[13], *Tjitske Kleefstra*[1;2;14], *Lot Snijders Blok*[1]

[1]*Radboud University Medical Center, Clinical Genetics, Nijmegen, Netherlands;* [2]*Erasmus MC, Clinical Genetics, Rotterdam, Netherlands;* [3]*ADRZ, Pediatrics, Goes, Netherlands;* [4]*Amsterdam UMC, Human Genetics, Amsterdam, Netherlands;* [5]*Seattle Children's Hospital, Genetics Medicine, Seattle, United States;* [6]*Nottingham University Hospitals NHS, epartment of Clinical Genetics, Nottingham, United Kingdom;* [7]*Children's Hospital of Michigan, Department of Pediatrics/Genetics/Metabolism Disorders, Detroit, United States;* [8]*University of Missouri School of Medicine, Department of Child Health, Columbia, United States;* [9]*Patrick G Johnston Centre for Cancer Research Cell Biology, Queen's University, Department of Genetics Medicine, Belfast, United Kingdom;* [10]*Greenwood Genetic Center, Research Division, Greenwood, United States;* [11]*Newcastle upon Tyne hospitals NHS Foundation Trust, Northern Genetics Service, Newcastle, United Kingdom;* [12]*CHU Lille, Clinique de génétique Guy Fontaine, Lille, France;* [13]*GHU Paris Psychiatrie et Neurosciences, Paris, France;* [14]*Vincent van Gogh Institute for Psychiatry, Centre of Excellence for Neuropsychiatry, Venray, Netherlands*

Background/Objectives: *DDX3X*-related neurodevelopmental disorder is among the most common monogenetic causes of intellectual disability in females, with currently >1000 females diagnosed worldwide. However, reports on affected males with *DDX3X* variants are scarce. The limited knowledge on this X-linked disorder in males hinders the interpretation of hemizygous *DDX3X* variants in clinical practice.

Methods: In this study, we present a new cohort of 19 affected males with possibly disease-causing *DDX3X* variants, for whom we collected clinical and molecular data. Additionally, we reviewed the existing literature on hemizygous *DDX3X* variants.

Results: The vast majority of males have missense variants, mainly located in the helicase domains of the encoded protein. Two specific missense variants are recurrently present. No truncating variants have been reported, consistent with the presumed lethality of complete loss-of-function of *DDX3X* in males. In our cohort, four variants are de novo in the affected male and three variants are de novo in the carrier mother. The phenotype in males is diverse, including intellectual disability (mild-severe), speech delay, behavioral challenges and structural brain abnormalities. Interestingly, about half of the carrier mothers in our cohort have mild behavioral or learning difficulties.

Conclusion: This study presents the largest cohort to date of males with *DDX3X* variants, providing a comprehensive overview of the genetic and phenotypic spectrum. Our data show that specific missense variants in *DDX3X* cause an X-linked neurodevelopmental disorder in males, with unaffected or mildly affected carrier females. These insights will aid the interpretation of

hemizygous missense variants in *DDX3X* and can guide clinical management.

Grants: -

Conflict of Interest: None declared

P10.066.A Syndromic Intellectual Disability in Cypriot patients: variant discovery through diagnostic exome sequencing

Athina Theodosiou[1], *LUDMILA KOUSOULIDOU*[1], *angelos alexandrou*[1], *ioannis papaevripidou*[1], *Constantia Aristidou*[2], *paola evangelidou*[1], *Sofia Ourani*[3], *Emily Athanasiou*[3], *George A. Tanteles*[2;4], *carolina sismani*[1]

[1]*The Cyprus Institute of Neurology and Genetics, Cytogenetics and Genomics, Nicosia, Cyprus;* [2]*The Cyprus Institute of Neurology and Genetics, Clinical Genetics and Genomics, Nicosia, Cyprus;* [3]*Hospital Archbishop Makarios III, Department of Paediatrics, Clinical Genetics clinic, Nicosia, Cyprus;* [4]*Acropoleos Medical Centre, Nicosia, Cyprus*

Background/Objectives: Intellectual disability (ID) is characterized by significant limitations in both intellectual functioning and adaptive behaviour that originates before the age of 22. It can be isolated (non-syndromic ID) or present with one or multiple clinical features or co-morbidities in addition to ID (syndromic ID, SID). This study presents the results obtained during years 2021-2023 of Whole exome sequencing (WES) or Clinical Exome Sequencing (CES) in patients with SID and no diagnosis after extensive genetic workup including chromosomal analysis, array-CGH and other targeted tests.

Methods: WES/CES was performed on Illumina NextSeq500 or 2000 platforms using Illumina exome sequencing kits. Analysis was performed with VarSome Clinical platform (hg19 genome reference) to detect single-nucleotide variants and small insertions/deletions. Findings were confirmed by Sanger sequencing.

Results: A total of 235 samples from 88 families with SID were sequenced and variants were reported in 24 patients, giving a 27% detection rate for this specific group of patients. Findings include 20 dominant variants (16 de novo), two X-linked and one complex compound heterozygous in genes: *CDC42BPB*, *PHIP*, *ZBTB18*, *CHD8*, *SATB2*, *ANKRD11*, *CREBBP*, *MECP2*, *KCND2*, *SCN2A*, *CSNK2B*, *BICRA*, *TLK2*, *PCDH19*, *PRKAR1B, PPP3CA*, *PTEN*. Ten variants (~43%) were novel.

Conclusion: We present known and novel disease-causing variants within genes related to SID expanding the pathogenic variant spectrum of these genes. We emphasize the importance of trio analyses for novel variant discovery and highlight that exome sequencing, despite existing challenges, remains a cost-effective diagnostic tool for the benefit of the patients and their families.

Conflict of Interest: None declared

P10.067.B Deregulation of ion channels contributes to *RHOBTB2*-associated developmental and epileptic encephalopathy

Franziska Langhammer[1;2], *Anne Gregor*[1;2], *Niels R. Ntamati*[3], *Arif B Ekici*[4], *Beate Winner*[5;6], *Thomas Nevian*[3], *Christiane Zweier*[1;2]

[1]*Inselspital, Department of Human Genetics, Bern, Switzerland;* [2]*University of Bern, Department of Biomedical Research (DBMR), Bern, Switzerland;* [3]*University of Bern, Department of Physiology, Bern, Switzerland;* [4]*Friedrich-Alexander-University Erlangen-Nürnberg, Institute of Human Genetics, Erlangen, Germany;* [5]*Friedrich-Alexander-University Erlangen-Nürnberg, Department of Stem Cell Biology, Erlangen, Germany;* [6]*Friedrich-Alexander-University Erlangen-Nürnberg, Center of Rare Diseases Erlangen (ZSEER), Erlangen, Germany*

Background/Objectives: While de novo missense variants in the BTB domains of *RHOBTB2* cause a severe developmental and epileptic encephalopathy (DEE), de novo missense variants in the GTPase domain or bi-allelic truncating variants are associated with variable neurodevelopmental phenotypes, frequently with seizures. Apart from the observation of increased RHOBTB2 abundance resulting from BTB-domain variants and increased seizure susceptibility in *Drosophila* overexpressing *RhoBTB*, our knowledge on the *RHOBTB2*-related pathomechanisms is still limited.

Methods: Aiming to close this gap, we first performed RNA-Seq on fly heads overexpressing *RhoBTB*. Genetic interaction experiments were performed in flies with simultaneous overexpression of *RhoBTB* and dosage manipulation of either *paralytic, slowpoke* or *ir76a* and subsequent assessment of phenotypic modifications. Using CRISPR/Cas9, we established hiPSCs with either homozygous frameshifts or patient-specific heterozygous missense variants in the GTPase or the BTB domains. After differentiation into mature neurons, whole patch clamp recordings were performed.

Results: We found enrichment of genes encoding ion channels among the differentially expressed genes in transcriptomes from flies overexpressing *RhoBTB*. Subsequently, we confirmed a functional link between *RhoBTB* and *paralytic*, the orthologue of human sodium channels, including *SCN1A*, in vivo. In hiPSC-derived, mature neurons we observed altered neuronal activity and increased excitability resulting from heterozygous missense variants in the BTB domain region but not from variants in the GTPase domain or upon complete loss of RHOBTB2.

Conclusion: Our study indicates a role of deregulated ion channels in the pathogenesis of *RHOBTB2*-related DEE and points to different pathomechanisms underlying the observed genotype-phenotype correlations regarding variant zygosity, location and nature.

Conflict of Interest: None declared

P10.068.C Transcriptional study of early neural development in iPSCs-derived from Williams-Beuren Syndrome patients

Esmee ten Berk de Boer[1;2;3], *Mansoureh Shahsavani*[1], *Jesper Eisfeldt*[1;2;3], *Ann Nordgren*[1;2], *Anna Lindstrand*[1;2]

[1]*Karolinska Institutet, Molecular Medicine and Surgery, Sweden;* [2]*Karolinska University Hospital, Department of Clinical Genetics, Sweden;* [3]*Science for Life Laboratory, Sweden*

Background/Objectives: Williams-Beuren syndrome (WBS) is caused by a recurrent deletion of 26-28 genes on 7q11.23. It is characterized by cardiac abnormalities, characteristic facial features, and cognitive and behavioural impairments. Despite the clear genomic cause of WBS, the genotype-phenotype link remains unknown. This study aims to address this gap through a transcriptomics study using an in vitro neural model.

Methods: We generated induced pluripotent stem cells (iPSCs) from 4 WBS patients and 4 controls, differentiating them into neuroepithelial stem cells (NESCs) and further maturing them into neurons. Transcriptomics was done at 3 timepoints; the NESC stage, after 4 weeks, and 8 weeks of neural maturation. Our study is unique in looking at 3 timepoints to study the transcriptomic landscape in WBS patients during early neural development.

Results: At all timepoints, the genes in the deleted region show reduced expression. Furthermore, we observed global gene expression changes. Specifically, we identified 596, 374, and 598 differentially expressed genes (DEGs) in NESCs, 4 weeks, and 8 weeks neurons. Surprisingly, there is little overlap among these DEGs, with only 37 DEGs shared across all timepoints.

Conclusion: We see that there is little overlap in the transcriptomic landscape of WBS patients at different stages of neural development. Many timepoints have unique DEGs, and unique disturbed pathways. Thus, we conclude that to understand the pathology of WBS, it is essential to study various timepoints during development, as different parts of the phenotype may arise at different times.

Grants: Brain Foundation (Hjärnfonden, FO2022-0256), the Swedish research council (2019-02078)

Conflict of Interest: None declared

P10.069.D Determination of clinical significance and genotype-phenotype correlations in patients with copy number variations in the short arm of chromosome 16

Michal Pietrusinski[1], *Hanna Moczulska*[1], *Marcin Serafin*[2], *Edyta Budzyńska*[2], *Agnieszka Zmysłowska*[1]

[1]*Medical University of Lodz, Department of Clinical Genetics, Lodz, Poland;* [2]*Central Clinical Hospital, Outpatient Clinic for Clinical Genetics, Lodz, Poland*

Background/Objectives: Microdeletions or microduplications of chromosome 16p, including microdeletion syndromes 16p11.2, 16p12.2, 16p13.11 and microduplications have been found in patients with intellectual disabilities (ID), developmental delay (DD), autism spectrum disorders (ASD) and dysmorphic features, but the overall clinical significance of these chromosomal rearrangements remains controversial. There are several candidate genes associated with the neurological phenotype, but these loci have variable expression and incomplete penetrance. Our aim was to determine the genotype-phenotype correlation of these microaberrations in patients in order to improve genetic counseling and overall patient care.

Methods: The study included a total of 20 cases; 6 cases with 16p11.2 (3 microdeletions - 1 prenatal; 3 microduplications), 2 cases with 16p12.2 microdeletions (1 prenatal) and 12 cases with 16p13.11 (10 microduplications - 5 prenatal; 2 microdeletions - both prenatal) referred to the Outpatient Clinic for Clinical Genetics for ID, DD, ASD in postnatal diagnosis and high-risk microaberration syndrome in prenatal diagnosis. All cases underwent chromosome microarray analysis, in some cases confirmed by MLPA.

Results: Most of the patients were found to have ID, DD or ASD, and some had dysmorphic features, heart defects, diabetes in postnatal diagnosis, and fetal hypotrophy and heart defects in prenatal diagnosis.

Conclusion: It can be concluded that 16p microdeletions and microduplications are pathogenic/likely pathogenic when detected in the context of ID/DD/ASD and dysmorphic features at birth, as well as fetal hypotrophy, heart defects and abnormal ultrasound findings before birth. These results support the hypothesis that this region is dosage-sensitive, highlighting its clinical significance.

Conflict of Interest: None declared

P10.072.C Homozygous variants in the UNC5C gene lead to a neurodevelopmental disease phenotype

ABDULLAH SEZER[1], *Mohamad Abdelhamid*[2], *Lama Al-Abdi*[3], *Fowzan Alkuraya*[3], *Hasan Baş*[4;5], *AYŞE BAYRAM*[6], *Sebiha Cevik*[7], *Fatma Güzel*[7], *Mahmoud Issa*[8], *Öznur Kaya Güneş*[1], *Ludger Schöls*[9], *Elifcan Tasdelen*[1], *Edgard Verdura*[10], *Fatma N. Yetgin*[7], *Oktay I. Kaplan*[7]

[1]*Ankara Etlik City Hospital, Department of Medical Genetics, Ankara, Türkyie;* [2]*Human Genetics and Genome Research Institute, National Research Centre, Medical Molecular Genetics Department, Cairo, Egypt;* [3]*King Faisal Specialist Hospital and Research Center, Department of Genetics, Riyadh, Saudi Arabia;* [4]*Diyarbakir Gazi Yasargil Training and Research Hospital, Department of Medical Genetics, Diyarbakır, Türkyie;* [5]*Intergen Genetics and Rare Diseases Diagnosis Center, Ankara, Türkyie;* [6]*Ankara Etlik City Hospital, Department of Pediatric Neurology, Ankara, Türkyie;* [7]*School of Life and Natural Sciences, Abdullah Gul University, Rare Disease Laboratory, Kayseri, Türkyie;* [8]*Human Genetics and Genome Research Institute, National Research Centre, Clinical Genetics Department, Cairo, Egypt;* [9]*Hertie-Institute for Clinical Brain Research, University of Tübingen, Department of Neurodegenerative Diseases, Tübingen, Germany;* [10]*Bellvitge Biomedical Research Institute (IDIBELL), L'Hospitalet de Llobregat, Neurometabolic Diseases Laboratory, Barcelona, Spain*

Background/Objectives: *UNC5C* encodes a netrin receptor involved in axon guidance during brain development. Axon guidance is a fundamental process ensuring that neurons send out axonal projections to their designated targets, a pivotal step in nervous system formation. While some polymorphisms in *UNC5C* gene are linked to Alzheimer's disease susceptibility, its possible relation to neurodevelopmental disorders remain unexplored.

Materials/Methods: Exome sequencing analysis was used to identify variants in *UNC5C*. The GeneMatcher platform was utilized to identify additional families. The CRISPR/Cas9 system was employed to create equivalent variants of patients in *Caenorhabditis elegans*, and subsequent morphological and behavioral analyses were conducted on these mutants.

Results: In the patient group, consisting of eight individuals from six unrelated families, each patient harbored one of six different homozygous variants, three of which were loss-of-function variants, two missense variants, and one splice region variant. All patients exhibited an early-onset neurodevelopmental disease phenotype, characterized by global developmental delay and walking problems. Additionally, more than half of the patients shared clinical features such as short stature, feeding difficulties, retrognathia, short neck, finger contractures, and foot deformities. We generated a corresponding variant of p.Cys362* in *unc-5* in *C. elegans*, leading to null-like phenotypes such as locomotion difficulties and a shortened body. The generation of other mutants and ongoing morphological and behavioral analyses are currently underway.

Conclusion: Herein, we report the first human disease caused by homozygous variants in *UNC5C* gene presenting a novel neurodevelopmental disorder.

Conflict of Interest: None declared

P10.073.D tRNA 2'-O-methylation detection using mass spectrometry and nanopore sequencing in Ftsj1 deficient and WT mouse liver.

Lars Jensen[1], *Lisa Hagenau*[1], *Robert Hieronymus*[2], *Corinna Jensen*[1], *Johann Wurz*[2], *Sabine Müller*[2], *Andreas Kuß*[1]

[1]*University Medicine Greifswald, Department of Functional Genomics, Greifswald, Germany;* [2]*University of Greifswald, Bioorganic Chemistry, Greifswald*

Background/Objectives: Inherited forms of intellectual disability (ID) affect approximately 0.5% of the general population. A part of the mutations causing ID is found in genes involved in protein synthesis. These include certain ribosomal proteins, tRNA synthetases and tRNA modifying enzymes. Mutations in *FTSJ1* cause severe, non-syndromic ID. This gene encodes a 2'-O- methyltransferase involved in the modification of certain tRNAs and so far tRNA-methylation is the only known function of its gene

product. To show that mutations in *FTSJ1* reduce catalytic activity we established an assay for FTSJ1 deficiency in a murine model.

Methods: We used hybridization-based methods to purify tRNA phenylalanine ($tRNA^{Phe}$) from WT and $Ftsj1^{-/y}$ mouse liver. The purified tRNA was sequenced using nanopore sequencing or digested with RNase T1 and analyzed by mass spectrometry.

Results: The results show that both methods are applicable for detecting either absence or presence of methylation of $tRNA^{Phe}$ and thereby allows to determine whether Ftsj1 is functional or not in cells from which the tRNA was obtained.

Conclusion: Both methods are still time consuming as they rely on tRNA purification and relatively large amounts of purified $tRNA^{Phe}$ (>100 pmol) but analysis of unpurified tRNA may be feasible in the near future as mass spectrometry and nanopore sequencing data analysis improves.

Grants: DFG ID: 458950132

Conflict of Interest: None declared

P11
NEUROGENETIC AND PSYCHIATRIC DISORDERS

P11.001.A Investigating differences in genetic susceptibility to autism in preterm and term births

Yali Zhang[1;2], *Ashraf Yahia*[1;2], *Sven Sandin*[3;4], *Ulrika Ådén*[5], *Kristiina Tammimies*[1;2]

[1]*Karolinska Institute, Center of Neurodevelopmental Disorders (KIND), Centre for Psychiatry Research, Department of Women's and Children's Health, Stockholm, Sweden;* [2]*Karolinska University Hospital, Astrid Lindgren Children's Hospital, Solna, Sweden;* [3]*Karolinska Institute, Department of Medical Epidemiology and Biostatistics, Stockholm, Sweden;* [4]*Icahn School of Medicine at Mount Sinai, Department of Psychiatry, New York, United States;* [5]*Karolinska Institute, Department of Women's and Children's Health, Stockholm, Sweden*

Background/Objectives: Autism Spectrum Disorder (ASD) is an early-onset neurodevelopmental condition with heterogeneous presentation and strong genetic liability. However, some environmental stressors are implicated in increased liability for ASD, such as prematurity. We hypothesized that ASD-associated genetic susceptibility would be different in individuals born term and preterm.

Methods: We used phenotype and genetic data from two large ASD cohorts, Simons Foundation Powering Autism Research for Knowledge (SPARK) and Simons Simplex Collection (SSC), to investigate the differences across prematurity and ASD. Cohorts included 9,364 preterm and 66,549 term for phenotype and 465 and 4,472 for genomics, respectively. We analyzed differences in rare de novo and inherited variants rates and polygenic risk score (PRS) for ASD using generalized estimating equation models.

Results: ASD-preterm have a significantly higher frequency of additional diagnoses and more severe symptoms than ASD-term. Within ASD, no significant differences between the preterm and term for de novo variant event rate and PRS distribution were observed. Within preterm, those with ASD had more exonic de novo variants than non-ASD, but the difference was inconsistent. We observed a significant interaction between PRS and preterm birth for ASD diagnosis. Ongoing analyses are being added for the inherited variants and full genetic model.

Conclusion: We demonstrate that preterm ASD is associated with a higher burden of additional diagnosis but similar genetic liability to ASD. Further studies should investigate additional genetic variants contributing to preterm birth and the complex phenotype.

Grants: The Swedish Foundation for Strategic Research, KI Foundations, Sällskapet Barnavård, China Scholarship Council.

Conflict of Interest: Yali Zhang Sällskapet Barnavård, China Scholarship Council, Ashraf Yahia: None declared, Sven Sandin: None declared, Ulrika Ådén: None declared, Kristiina Tammimies The Swedish Foundation for Strategic Research, KI Foundations, Full

P11.002.B Disentangling the Genetic Architecture of Alzheimer's Disease from Aging and Dementia

César Cunha[1;2], *Tuomas Kilpeläinen*[1;2], *Ruth Loos*[1]

[1]*Novo Nordisk Foundation Center for Basic Metabolic Research, København, Denmark;* [2]*Novo Nordisk Foundation Center for Genomic Mechanisms of Disease at the Broad Institute of MIT and Harvard, Cambridge, Massachusetts, United States*

Consortium: EADB Consortium

Late-onset Alzheimer's Disease (LOAD) has a complex genetic architecture that has been challenging to differentiate from the general effects of aging and dementia. We aimed to identify genetic effects specific to LOAD by utilizing EADB GWAS results with and without LOAD-by-proxy cases (*i.e.* parental disease history) or non-age-matched control individuals. SNP-based heritability for LOAD increases from 1.3% to 5.6% after excluding proxies and non-age-matched controls, suggesting a more accurate estimate of the true SNP-based heritability. Out of 235 genome-wide significant loci in total, 85 are exclusive to LOAD, while 78 are linked to a broader dementia phenotype. LOAD-specific loci show higher effect sizes whereas dementia-specific loci show lower effect sizes in clinically-relevant against standard proxy-prevalent cohorts. We identify 21 age-specific loci of which we highlight rs58859091 as LOAD-exclusive and rs28577986 as dementia-exclusive. Among the 235 loci, gene prioritization by cS2G highlighted 155 genes, 71 of which have not been previously linked to LOAD. Gene expression enrichment analysis across brain cell types revealed two gene clusters: one specific to LOAD and another related to dementia. Pathway enrichment for LOAD-specific loci highlighted cholesterol, lipid, immune, and viral pathways, and revealed a connection to body weight regulation. Druggability analyses spotlighted compounds in cancer, cardiovascular, cardiometabolic, and calcium flux pathways. In conclusion, our LOAD-specific analyses elucidate some of missing heritability and unveil 18 variants specific to LOAD, distinct from variants associated with broad aging or dementia phenotypes. These findings uncover unprecedented biology, inform analysis strategies for LOAD, and may give clues for drug discovery.

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Conflict of Interest: None declared

P11.003.C Utility of Exome sequencing in the diagnosis of Hereditary spastic paraplegia: An Indian perspective.

Dr. Varun venkat Raghavan Mandikal Srinivasan[1], *Vykuntaraju K Gowda*[2], *himani pandey*[3]

[1]*SGPGIMS, Medical genetics, Lucknow, India;* [2]*Indira Gandhi institute of child health, Pediatric Neurology, Bangalore, India;* [3]*Redcliffe labs, Lab Head - Clinical Genomics, Noida, India*

Background: Hereditary spastic paraplegias (HSPs) are genetically heterogenous group of neurogenetic disorders affecting corticospinal tracts predominantly. The molecular diagnosis remains unknown in >30% cases.

Methods: To study the spectrum of gene mutations and the yield of whole exome sequencing in an Indian cohort of patients with HSP over a period of 2 years.

Results: A total of 44 families were included in the study with 22 cases being males. Consanguinity was seen in 19 families (43.2%) with similarly affected siblings in 5 families (11.2%). Thirty-three (75%) were categorized to have complicated HSP. Exome sequencing identified disease causing variants in 27/44 cases with a yield of 61.4%. 19 (43.2%) had recessive and 8 (18.2%) had dominant mutations. Novel variation was seen in 19 cases (43.2%). Most common disease-causing genes were *FA2H* in 5 (11.36%), *AP4M1 in* 3 (6.81%), *SPG7* in 2 (4.54%) cases. Recurrent variation in *FA2H* and *AP4M1* were observed in 2 unrelated families suggesting a probable founder effect. Three cases had presented with features of spastic paraparesis with no mutations in known HSP genes and had mutations In *ATAD3A, TNR* and *NDUFA11* genes. Dual diagnosis was seen in 2 cases with one case having pathogenic variants in *KIF1A, TANC2* and the other case having pathogenic variants in *SPG7, HEXA*. Only one case had an actionable finding in the *DSP* gene.

Conclusion: This study expands clinical spectrum, etiology and disease variability among Indian patients with HSP. The yield of WES in current study was on par with published literature.

Grants: None

Conflict of Interest: None declared

P11.004.D SCA4: low-cost detection of normal and expanded GCC repeats in *ZFHX3* by conventional PCR and fragment analysis

Andreas Dalski[1], *Yorck Hellenbroich*[1], *Christine Zühlke*[1], *Jassemien Strohschehn*[1], *Malte Spielmann*[1]

[1]*Institute of Human Genetics, University Hospital Schleswig-Holstein, University of Lübeck, Lübeck, Germany*

Background/Objectives: The introduction of long read sequencing is shedding light into the inaccessible regions of the human genome from telomer to telomer. We recently identified an exonic GCC-repeat expansion in *ZFHX3* causing spinocerebellar ataxia type 4 (SCA4), a dominant form of ataxia with sensory neuropathy has been published (Figueroa et al. 2024; Wallenius et al. 2024). However, long read sequencing is still expensive and technically challenging making it impractical for the screening of lager cohorts. Here we set out to develop a low-cost GCC-repeat test for clinical SCA4 screening.

Methods: Adapted PCR with *ZFHX3* specific primers was performed on the basis of a commercial FMR1-Kit (Bio-Techne AmplideX®). Fragment lengths were determined by capillary and classic agarose gel electrophoresis followed by Sanger sequencing.

Results: We present the results of 50 related (two independent kindreds) and unrelated patients and controls. Within the families clear anticipation is observed. Expanded alleles with 44 to 68 repeats show a striking effect on the age of onset ranging from 12 to 55 years.

Conclusion: Our protocol enables geneticists to perform analysis for the causative SCA4 repeat expansion on genomic DNA with a robust turn-around time at low costs. Furthermore, the exact repeat length prediction via this method and the proven clear anticipation allows an estimation of a possible disease onset for allele carriers at least in a research context.

Grants:

Conflict of Interest: None declared

P11.005.A De novo and Inherited Monoallelic Variants in Tubulin Alpha-4A (*TUBA4A*) cause Ataxia and Spasticity

Heba Morsy[1], *Mehdi Benkirane*[2;3], *Marion Bonhomme*[4], *Stephanie Safgren*[5], *Cecilia Marelli*[6], *Annabelle Chaussenot*[7], *Damian Smedley*[8], *Valentina Cipriani*[8;9], *Jean-Madeleine de Sainte Agathe*[10], *Can Ding*[11], *Lise Larrieu*[2], *Letizia Vestito*[8], *Henri Margot*[12], *Gaetan Lesca*[13], *Francis Ramond*[14], *Anna Castrioto*[15], *David BAUX*[2;16;17], *Jan Verheijen*[18], *Emna Sansa*[19], *Paola Giunti*[1;19], *Anne Bergougnoux*[2;3], *Morgane Pointaux*[2], *Olivier Ardouin*[2;17], *Charles VAN GOETHEM*[2;17], *Marie-Claire Vincent*[2], *Marios Hadjivassiliou*[20], *Mireille Cossee*[2;3], *Tiphaine Rouaud*[21], *Oliver Bartsch*[11], *William D. Freeman*[22], *Klaas J. Wierenga*[23], *Eric W. Klee*[18;24;25], *Henry Houlden*[1], *Anne Debant*[4], *Michel Koenig*[2;3]

[1]*UCL Queen Square Institute of Neurology, United Kingdom;* [2]*University Institute De Recherche Clinical, Montpellier, France;* [3]*PhyMedExp Univ Montpellier, France;* [4]*Centre de Recherche en Biologie cellulaire de Montpellier, Montpellier, France;* [5]*Mayo Clinic, Rochester, United States;* [6]*Hospital Center University De Montpellier, Montpellier, France;* [7]*Hospital Center Universitaire De Nice, Nice, France;* [8]*William Harvey Research Ltd, United Kingdom;* [9]*UCL Genetics Institute, London;* [10]*APHP, Sorbonne University, France;* [11]*University of Medicine, Mainz, Germany;* [12]*CHU of Bordeaux, France;* [13]*HCL, Lyon, France;* [14]*CHU of Saint-Etienne, France;* [15]*CHU of Grenoble, Grenoble, France;* [16]*Institut des neurosciences Montpellier, INSERM, France;* [17]*Montpellier BioInformatics for Clinical Diagnosis (MOBIDIC), Molecular Medicine and Genomics Platform (PMMG), France;* [18]*Mayo Clinic, Department of Oncology Research, Rochester, United States;* [19]*National Hospital for Neurology and Neurosurgery, United Kingdom;* [20]*Sheffield Teaching Hospitals NHS Foundation Trust, United Kingdom;* [21]*CHU of Nantes, France;* [22]*Mayo Clinic Jacksonville, United States;* [23]*Mayo Clinic, Jacksonville, United States;* [24]*Department of Clinical Genomics, Mayo Clinic, Rochester, United States;* [25]*Mayo Clinic, Department of Quantitative Health Sciences, Rochester, United States*

Consortium: Genomics England Research Consortium

Background/Objectives: Alpha-tubulin 4A encoding gene (*TUBA4A*) has been associated with familial amyotrophic lateral sclerosis (fALS) and fronto-temporal dementia (FTD), based on identification of likely pathogenic variants in patients from distinct ALS and FTD cohorts. Very recently, the p.Glu415Lys *TUBA4A* variant has been identified in a large Italian family with spastic cerebellar ataxia and in an unrelated sporadic patient with de novo occurrence.

Methods: We describe 12 patients from 11 unrelated families harbouring ultra-rare missense variants in *TUBA4A*. We carefully characterized the patients' phenotype by providing detailed clinical data and imaging, when available. A rare-variant gene-based burden case-control analysis was performed within the rare disease component of the UK 100,000 Genomes Project (100KGP). Functional studies on microtubules properties were performed on cultured fibroblasts from 3 patients.

Results: We report 12 patients presenting with spasticity and/or cerebellar ataxia and harboring a predicted pathogenic *TUBA4A* missense mutation, including 5 confirmed de novo cases and a mutation previously reported in a large family presenting with spastic ataxia. In addition, gene burden analyses in the UK 100K GP showed enrichment of *TUBA4A* rare variants in the inherited ataxia group compared to controls (OR: 14.3 [5.0 – 41.2]; $P < 10\text{-}4$). Cultured fibroblasts from 3 patients harboring distinct *TUBA4A* missense mutations showed significant alterations in microtubule organisation and dynamics, providing insights for *TUBA4A* variants pathogenicity.

Conclusion: Our data confirm the identification of a hereditary spastic ataxia disease gene with variable age of onset, expanding the clinical spectrum of *TUBA4A* associated phenotypes.

Conflict of Interest: None declared

P11.006.B The broad phenotypic spectrum of *AIMP1*-related disorders: unveiling a new case of hereditary spastic paraplegia

*Sara Morais[1;2;3;4], José Leal Loureiro[1;2;5], Eva Brandão[5], Jorge Sequeiros[1;2;3;4], Giovanni STEVANIN[6], **Mariana Santos**[1;2;3]*

[1]IBMC - Institute for Molecular and Cell Biology, Universidade do Porto, Porto, Portugal; [2]i3S - Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Porto, Portugal; [3]ICBAS School of Medicine and Biomedical Sciences, Universidade do Porto, Porto, Portugal; [4]CGPP - Center for Predictive and Preventive Genetics, IBMC - Institute for Molecular and Cell Biology, Universidade do Porto, Porto, Portugal; [5]Serviço de Neurologia, Centro Hospitalar de Entre o Douro e Vouga, Santa Maria da Feira, Portugal; [6]Université de Bordeaux, CNRS - Centre National de la Recherche Scientifique, INCIA - Institut de Neurosciences Cognitives et Intégratives d'Aquitaine, Bordeaux, France

Background/Objectives: Hereditary spastic paraplegias (HSP) are a heterogeneous group of neurodegenerative disorders, characterized by progressive lower limb spasticity and weakness. The clinical and genetic basis of HSP is very complex, with more than 80 genes associated with the disease. The genetic cause, however, remains elusive in nearly one half of all HSP cases. To improve diagnostic yield, and to identify novel HSP genes, a large Portuguese family with complex HSP was selected for this study.

Methods: Linkage analysis and whole-exome sequencing (WES) were performed to identify the causal gene in this HSP family with autosomal recessive inheritance. A minigene construct was created to confirm a predicted splicing effect.

Results: WES analysis focused on the defined linkage regions (maximum LOD score of 4.3 in chromosome 4), and led to the identification of an *AIMP1* variant (c.295G>A) in homozygosity. The minigene assay showed that this apparent *AIMP1* missense variant causes the loss of exon 3 donor splice site, resulting in the introduction of a premature stop codon, through the usage of an alternative splice site within the intron.

Conclusions: *AIMP1* was identified as a causal gene for complex HSP with early age-at-onset, contributing to the growing list of HSP genes. *AIMP1* variants have been previously associated with a spectrum of disorders, ranging from intellectual disability to more complex neurodegenerative diseases. Additional studies are required to definitively establish genotype-phenotype correlations.

Grants: FTC/Campus France, PESSOA 2023-2024 (2022.15027.CBM). MS: FCT, DL57/2016-Norma Transitória.

Conflict of Interest: None declared

P11.007.C Genetic study of SCA27B, CANVAS and other tandem repeat expansion disorders in Greek patients with late-onset cerebellar ataxia.

CHRISOULA KARTANOU[1], *Zoi Kontogeorgiou[1], Alexandros Mitrousias[1], Chrysoula Koniari[1], david pellerin[2;3], Marie-Josée Dicaire[2], Pablo Iruzubieta[3;4;5], Matt Danzi[6], Konstantinos Athanassopoulos[1], Maria Stamelou[7;8], Michail Rentzos[9], Evangelos Anagnostou[9], Stephan Zuchner[6], Bernard Brais[2;10], Henry Houlden[3], Marios Panas[1], Leonidas Stefanis[9], Georgia Karadima[1], Georgios Koutsis[1]*

[1]Neurogenetics Unit, 1st Department of Neurology, National and Kapodistrian University of Athens, Eginitio Hospital, Athens, Greece; [2]Department of Neurology and Neurosurgery, Montreal Neurological Hospital and Institute, McGill University, Montreal, Québec, Canada; [3]Department of Neuromuscular Diseases, UCL Queen Square Institute of Neurology London and The National Hospital for Neurology and Neurosurgery, University; [4]Department of Neurology, Donostia University Hospital, Biogipuzkoa Health Research Institute, Donostia-San Sebastián, Spain; [5]CIBERNED Centro de Investigacion Biomédica en Red en Enfermedades Neurodegenerativas-Instituto de Salud Carlos III (CIBER-CIBERNED-ISCIII), Madrid, Spain; [6]Dr. John T. Macdonald Foundation Department of Human Genetics and John P. Hussman Institute for Human Genomics, University of Miami Miller School of Medicine, Miami, Florida, USA; [7]Parkinson's disease and Movement Disorders Department, HYGEIA Hospital, Athens, Greece; [8]School of Medicine, European University of Cyprus, Nicosia, Cyprus; [9]1st Department of Neurology, National and Kapodistrian University of Athens, Eginitio Hospital, Athens, Greece; [10]Department of Human Genetics, McGill University, Montreal, Québec, Canada

Background/Objectives: Late-onset cerebellar ataxia (LOCA) encompasses heterogeneous neurodegenerative disorders with symptom onset after 30 years of age. A genetic diagnosis is established in fewer than 30% of LOCA patients. Intronic tandem repeat expansions (TREs) in *RFC1* and *FGF14* have recently emerged as common causes of LOCA. This study screened Greek LOCA patients for the commonest causative TREs.

Methods: Over a 28-year period, 206 consecutive LOCA patients were referred for genetic testing. Based on clinical data and inheritance pattern, patients were screened for FRDA, SCA1,2,3,6,7 and FXTAS, followed by testing for CANVAS and SCA27B. PCR, RP-PCR, agarose gel electrophoresis and fragment analysis were performed, as appropriate, to detect the pathogenic TREs.

Results: A genetic diagnosis was reached in 60 of 206 cases (29.1%). Mean age was 60.1 ± 11.2 (35-87) years and mean age at onset 52.5 ± 11.4 (30-80) years. SCA27B accounted for 9.7% of LOCA cases (n = 20), CANVAS for 6.8% (n = 14) and FRDA for 4.4% (n = 9). The overall frequency of SCA1 (n = 5), SCA2 (n = 6) and SCA7 (n = 3) was estimated at 6.8%. No cases of SCA3 and SCA6 were identified. FXTAS (n = 3) contributed another 1.5% of cases.

Conclusion: Our study provides comprehensive data on the genetic basis of LOCA in the Greek population. SCA27B, followed by CANVAS, represent the commonest known genetic causes of LOCA. FRDA is also a relatively common cause of LOCA. SCA1,2,7 and FXTAS are rare, whereas SCA3 and SCA6 are virtually absent. We recommend prioritizing testing for *FGF14* and *RFC1* expansions in the diagnostic algorithm of LOCA.

Grants: No

Conflict of Interest: None declared

P11.008.D Mutational screening of Greek patients with Charcot-Marie-Tooth disease using whole exome sequencing: Expanding the clinical and molecular spectrum

Zoi Kontogeorgiou[1], *Charalampos Tzebetzis[1], Chrisoula Kartanou[1], Chrysoula Koniari[1], Michail Rentzos[2], Panagiotis Kokotis[3], Evaggelos Anagnostou[3], Elisabeth Chroni[4], Vasiliki Zouvelou[2], Marios Panas[1], Georgios Koutsis[1], Georgia Karadima[1]*

[1]National and Kapodistrian University of Athens, Neurogenetics Unit, 1st Department of Neurology, Eginition Hospital; [2]National and Kapodistrian University of Athens, 1st Department of Neurology, Eginition Hospital; [3]National and Kapodistrian University of Athens, Clinical Neurophysiology Unit, 1st Department of Neurology, Eginitio Hospital,; [4]University of Patras, Department of Neurology, School of Medicine

Background: Charcot-Marie-Tooth disease (CMT) is the most common cause of hereditary neuropathy, exhibiting extensive genetic heterogeneity, which can be addressed using whole exome sequencing (WES). This study screened previously undiagnosed Greek patients with CMT, using WES.

Methods: Over a 6-year period, 441 consecutive patients were referred for CMT molecular diagnosis. Following targeted testing for CMT1A and in selected cases for CMTX, 173 index-cases remained undiagnosed, of which 65 with a strong suspicion of hereditary neuropathy. To date, 32 of these, including 13 cases with demyelinating and 19 cases with axonal neuropathy, have been screened by WES using Ion Torrent technology and confirmed by Sanger sequencing.

Results: Causative variants were detected in 19 of 32 patients (59.4%) across 14 genes known to cause CMT, of which 3 were novel (in *MPZ*, *SH3TC2* and *KIF1A*). In demyelinating forms the diagnostic yield was 76.9 % and in axonal forms 47.4%. Most causative variants were found in *SH3TC2* (5 cases) and *GJB1* (2 cases), all in demyelinating CMT. In patients with demyelinating CMT, single causative variants were found in *EGR2*, *MPZ* and *NDGR1*. In patients with axonal CMT, single causative variants were identified in *HINT1*, *NEFH*, *BSCL2*, *NEFL*, *MORC2*, *GDAP1*, *KIF1A*, *SORD1* and *MPV17*.

Conclusion: The overall diagnostic yield of WES was comparable to other CMT populations. Our results contribute to the further mapping of gene variants associated with hereditary neuropathy and highlight the importance of WES in the diagnostic algorithm of disorders with high genetic heterogeneity, in the context of precision medicine.

Conflict of Interest: None declared

P11.009.A The genetic landscape of sporadic adult-onset degenerative ataxia: a systematic multi-modal genetic study of 377 consecutive patients from the longitudinal multi-center SPORTAX cohort

Danique Beijer[1], *David Mengel*[1], *Demet Önder*[2], *Carlo Wilke*[1], *Andreas Traschütz*[1], *Jennifer Faber*[2], *Florian Harmuth*[3], *Claudia Dufke*[3], *Bernard Brais*[4], *Olaf Riess*[3], *Ludger Schoels*[5], *Tobias Haack*[3], *Stephan Züchner*[6], *david pellerin*[4], *Thomas Klockgether*[2], *Matthis Synofzik*[1]

[1]*Hertie-Institute for Clinical Brain Research, Division Translational Genomics of Neurodegenerative Diseases, Tuebingen, Germany;* [2]*University Clinic Bonn, Department of Neurology, Bonn, Germany;* [3]*University of Tuebingen, Institute of Medical Genetics and Applied Genomics, Tuebingen, Germany;* [4]*McGill University, Montreal Neurological Hospital and Institute, Montréal, Canada;* [5]*Hertie-Institute for Clinical Brain Research, Tuebingen, Germany;* [6]*University of Miami - Miller School of Medicine, Miami, United States*

Consortium: SPORTAX consortium

Background/Objectives: While most sporadic adult-onset neurodegenerative diseases have a relatively minor monogenic component, sporadic adult-onset ataxias might have a substantial monogenic component, due to several recently identified late-onset ataxia genes. Here, we systematically map the genetic landscape of sporadic adult-onset ataxia in a well-characterized and longitudinal cohort, combining multi-modal genetic screening techniques.

Methods: Systematic clinic-genetic analysis of a prospective longitudinal multi-center cohort of 377 sporadic adult-onset ataxia patients (SPORTAX cohort) - including clinically defined sporadic adult-onset ataxia of unknown etiology (SAOA) (n = 285) and multiple system atrophy of cerebellar type (MSA-C) (n = 92)-, combining GAA-*FGF14* and *RFC1* repeat expansion screening and next-generation sequencing (NGS).

Results: 86 out of 377 (22.8%) all patients carried a pathogenic or likely pathogenic variant, thereof 73/286 patients (= 25.5%) with clinical SAOA and 13/91 patients (= 14.3%) with clinical probable MSA-C. This included: 45 (11.9%) patients with GAA-*FGF14*>250 repeat expansions (5 with a clinically probable MSA), 17 (4.5%) patients with *RFC1* repeat expansions (3 with a clinically probable MSA); and 27 (7.2%) patients with SNVs identified by NGS (5 with a clinically probable MSA).

Conclusion: Leveraging a strictly consecutive cohort, we demonstrate that sporadic adult-onset ataxias – de-riched for genetic causes - have a substantial burden of monogenic variants, particularly GAA-*FGF14* and *RFC1* repeat expansions, and including a substantial share of patients with clinical probable MSA-C. These findings have important implications for genetic work-up and counselling of sporadic adult-onset ataxia patients both SAOA and MSA-C; and - with genetic targeted treatments now in reach -also for providing these patients with access to targeted gene-specific treatments.

Conflict of Interest: Danique Beijer Humboldt Postdoctoral Fellowship, Hertie Network of Excellence, Universitätsklinikum Tuebingen, David Mengel: None declared, Demet Önder: None declared, Carlo Wilke: None declared, Andreas Traschütz: None declared, Jennifer Faber: None declared, Florian Harmuth: None declared, Claudia Dufke: None declared, Bernard Brais: None declared, Olaf Riess: None declared, Ludger Schoels: None declared, Tobias Haack: None declared, Stephan Züchner: None declared, david pellerin: None declared, Thomas Klockgether: None declared, Matthis Synofzik: None declared

P11.010.B Human iPSCs-derived neuronal models to study the pathogenic mechanisms behind TANGO2-disease

Silvia Carestiato[1], *sofia galli*[2], *Paolo Scudieri*[2;3], *Lisa Pavinato*[1], *simona Baldassari*[2;3], *Federico Zara*[2;3], *Giovanni Battista Ferrero*[4], *Aldamaria Puliti*[2;3], *Alfredo Brusco*[5;6]

[1]*University of Turin, Medical Sciences, Turin, Italy;* [2]*University of Genoa, Department of Neurosciences, Rehabilitation, Ophthalmology, Genetics, Maternal and Child Health (DiNOGMI), Genoa, Italy;* [3]*IRCCS Istituto Giannina Gaslini, U.O.C. Medical Genetics, Genova, Italy;* [4]*University of Turin, Department of Clinical and Biological Sciences, Orbassano, Italy;* [5]*University of Turin, Department of Neurosciences "Rita Levi Montalcini", Turin, Italy;* [6]*Città della Salute e della Scienza University Hospital, Medical Genetics Unit, Turin, Italy*

Background/Objectives: The TANGO2 protein plays a significant role in vesicular trafficking, mitochondrial oxidation, and maintaining cellular homeostasis. Biallelic loss-of-function variants are associated to an autosomal recessive disorder characterized by cardiac arrhythmia, metabolic crises, rhabdomyolysis, and kidney damage. Additionally, a neurological component is observed, including neurodegeneration, developmental delay, and cerebral atrophy. Here, we investigated the previously unexplored role of TANGO2 loss in neurons by using neural stem cells and cortical neurons, and provided new insights into the disease pathogenesis.

Methods: iPSCs were generated from two siblings, who exibited different clinical presentation - one with a classical form and the other with milder features. iPSCs were used to derive neural rosettes (NRs), neural progenitor cells (NPCs) and mature neurons.

Results: In the patient with severe TANGO2 disease, analysis of NRs showed a reduction in area (~45%) and impaired morphological organization. The wound healing assay demonstrated altered migration in NPCs, which supports the previously reported role of TANGO2 in migration. Moreover, an increase cell death was observed during corticogenesis. The Reactive Oxygen Species (ROS) assay showed an oxidative stress condition, further supporting the involvement of TANGO2 in mitochondrial functions and cellular homeostasis.

In contrast, no significant differences were found in NRs, NPCs, and mature neurons in the milder sibling vs. controls.

Conclusion: We provide evidence for a crucial role of TANGO2 in early-stage neurodevelopment, particularly in neural tube formation, cell migration, and neuron maturation. Moreover, we suggest protective gene modifiers influencing patient phenotypes, highlighting the need for further transcriptomic and proteomic analyses.

Grants:

Conflict of Interest: None declared

P11.011.C Investigating the role of rare missense variants in RAB11B in Autism Spectrum Disorder

Marta Viggiano[1], *Laura Sandoni*[1], *Fabiola Ceroni*[1;2], *Paola Visconti*[3], *Annio Posar*[3;4], *Maria Cristina Scaduto*[3], *Alessandro Vaisfeld*[5], *Elena Bacchelli*[1], *Elena Maestrini*[1]

[1]*University of Bologna, Department of Pharmacy and Biotechnology, Bologna, Italy;* [2]*Oxford Brookes University, Faculty of Health and Life Sciences, Oxford, United Kingdom;* [3]*IRCCS Istituto delle Scienze Neurologiche di Bologna, UOSI Disturbi dello Spettro Autistico, Bologna, Italy;* [4]*University of Bologna, Department of Biomedical and Neuromotor Sciences, Bologna, Italy;* [5]*University of Bologna, Department of Medical and Surgical Sciences, Bologna, Italy*

Background/Objectives: Autism spectrum disorder (ASD) is a highly heritable neurodevelopmental disorder with a complex genetic architecture. The identification of rare de novo variants in ASD individuals have highlighted hundreds of high-confidence susceptibility genes, many of which are also associated with Intellectual Disability (ID) and/or other Neurodevelopmental Disorders (NDD). Recently, recurrent de novo missense variants in *RAB11B* have been associated with a NDD including ID and white matter anomalies. *RAB11B* encodes a small Rab GTPase involved in intracellular transport and endosomal trafficking, essential for numerous neurodevelopmental processes.

Methods: We performed whole exome and/or genome sequencing (WES/WGS) in a cohort of 116 ASD families, and a review of public databases and literature to identify deleterious variants in *RAB11B* in individuals with ASD/NDD. Then, immunofluorescence analysis was carried out to functionally characterize these variants.

Results: We identified a novel de novo missense variant in *RAB11B* (p.R33H) in our cohort, and two additional *RAB11B* uncharacterized missense variants in individuals with ASD/NDD from other studies. Co-localization experiments using ARPE-19 showed that after induction of the primary cilium assembly there was enriched localisation of the R33H mutant to the Golgi network, similar to inactive RAB11B.

Conclusion: Our study provides evidence that de novo missense variants close to *RAB11B* functional domains affect the protein sub-cellular localization during primary cilium assembly, suggesting a role for *RAB11B* in ASD aetiology.

Grants: #NEXTGENERATIONEU, MUR, NRRP: MNESYS (PE0000006) and National Center for Gene Therapy and Drug-based on RNA Technology (CN00000041).

Conflict of Interest: None declared

P11.012.D Genetic study of Portuguese Cerebral Cavernous Malformation patients using targeted gene panel sequencing

Maria Rosário Almeida[1], *João Durães*[1;2;3], *Ricardo Pereira*[4], *Ana Santos*[1], *Isabel Santana*[1;2;3]

[1]*Center for Innovative Biomedicine and Biotechnology, University of Coimbra;* [2]*Faculty of Medicine, University of Coimbra;* [3]*Neurology Department, Centro Hospitalar e Universitário de Coimbra;* [4]*Neurosurgery Department, Centro Hospitalar e Universitário de Coimbra*

Background/Objectives: Cerebral cavernous malformations (CCMs) are common vascular malformations in the central nervous system, known for causing headaches, hemorrhage, seizures, and neurologic deficits. CCMs can occur sporadically or in a familial autosomal dominant pattern, primarily linked to mutations in *CCM1* (*KRIT1*), *CCM2* (*MGC4607*), and *CCM3* (*PDCD10*) genes. This study aimed to unveil the mutation spectrum in patients diagnosed with CCMs in Portugal's central region and explore the related phenotypes.

Methods: A total of 24 subjects (20 patients and 4 family members) were investigated for the presence of pathogenic variants in the three CCM-related genes using targeted NGS panel or Sanger sequencing.

Results: The genetic analysis revealed five different pathogenic/likely pathogenic variants in 13 subjects (54%), including 9 patients and 4 family members (3 asymptomatic and 1 symptomatic) in the *CCM1* and *CCM2* genes, while no mutations were found in the *CCM3* gene. Of these pathogenic variants, three were novel, one in *CCM1* (p.N496Rfs*12) and two in *CCM2* (p.Q72*; p.G78Dfs*3). Furthermore, three variants were classified as VUS, two in *CCM1* (p.A30G; p.P114Q) and one in *CCM2* (p.A141T).

Conclusion: With the identification of three novel variants, this study expands the mutation spectrum of CCM-related genes and the associated clinical and imaging phenotypes. All identified pathogenic/likely pathogenic variants lead to a premature stop codon, while the three remaining missense variants were VUS. Thus, identification of underlying pathogenic variants in patients with CCMs has been essential to provide accurate genetic counselling to both patients and family members, and also to improve their clinical management.

Conflict of Interest: None declared

P11.013.A *ATXN1* intermediate repeat expansion among the Hungarian *C9orf72* positive cases

Zsófia Nagy[1;2;3;3], *Lilla Buzai-Kiss*[1], *Barbara Trombitas*[1], *Margit Pál*[2;3], *Adrienn Géresi*[1;4], *András Salamon*[5], *Péter Klivényi*[5], *Marta Szell*[2;3], *Maria Judit Molnar*[1;4]

[1]*Insitute of Genomic Medicine and Rare Disorders, Semmelweis University, Budapest, Hungary;* [2]*Department of Medical Genetics, University of Szeged, Szeged, Hungary;* [3]*ELKH-SZTE Functional Clinical Genetics Research Group, Szeged, Hungary;* [4]*ELKH-SE Multiomics Neurodegeneration Research Group, Budapest, Hungary;* [5]*Department of Neurology, University of Szeged, Szeged, Hungary*

Background/Objectives: The hexanucleotide repeat expansion in the *C9orf72* gene is the most common genetic cause of frontotemporal dementia (FTD) and amyotrophic lateral sclerosis (ALS). Recently, the mutation has been detected in other neurodegenerative diseases, such as Parkinson's disease and Huntington's like phenotype. Literature has shown that intermediate length *ATXN1* repeat expansion are more frequent among *C9orf72* positive ALS patients. However, no data is known about the involvement of *ATXN1* repeats in other *C9orf72* positive neurodegenerative conditions.

Methods: Patients positive for the *C9orf72* repeat expansion (N = 86) and 178 control patients diagnosed with oncohematological malignancies were screened for the *ATXN1* gene repeat length by fragment analysis. Intermediate repeat expansion was defined as 32-39 CAG units.

Results: Altogether 18 C9orf72 positive (C9 +) patients (20,93%) and 2 control patients (1,12%) carried an intermediate length repeat expansion of the *ATXN1* gene. Ten of the C9+ cases had ALS phenotype, one – one FTD and Alzheimer dementia respectively and four patients had Parkinsonian phenotype. The age at onset of the C9 + ALS patients carrying an intermediate

ATXN1 allele was lower and their survival was longer in irrespective of clinical presentation compared to the *C9orf72* positive cohort without an *ATXN1* intermediate allele.

Conclusion: Our results indicate the role of *ATXN1* as a phenotype modifier among *C9orf72* positive patients not only diagnosed with ALS but with neurodegenerative disorders. More research is being carried out to delineate the interplay of these genes and their role in disease mechanism.

Conflict of Interest: None declared

P11.014.B Heterozygous variants in *ESRRG* cause a neurodevelopmental disorder with ataxia

Brandon Bresack[1], *Alexandra Afenjar*[2], *Lydie Burglen*[2;3], *Rami Abou Jamra*[1]

[1]*Institute of Human Genetics, University of Leipzig Medical Center, Leipzig, Germany;* [2]*Centre de référence des malformations et maladies congénitales du cervelet Et Laboratoire de Neurogénétique Moléculaire Pédiatrique, Département de Génétique, AP-HP.Sorbonne Université - Hôpital d'Enfants Armand-Trousseau, Paris, France;* [3]*Developmental Brain Disorders Laboratory, Imagine Institute, Paris, France*

Background/Objectives: *ESRRG* is involved in regulation of mitochondrial genes and is expressed in the brain. Among its ESRR paralogues, *ESRRG* exhibits the highest mutational constraint but remains not associated with a disease trait. This study aims at proving if *ESRRG* is a novel neurodevelopmental disorder gene.

Methods: Exome or genome sequencing with standard filtering of variant in individuals with neurodevelopmental disorders. Systematic and standardized clinical documentation of symptoms. Molecular modelling of the identified variants. Literature and database search and bioinformatic tools regarding functional aspects of the variants and the gene.

Results: We report on 4 patients from 3 unrelated families carrying heterozygous missense variants in *ESRRG*; p.(Lys149Arg), p.(Arg184Cys), and p.(Leu449Arg). The latter variant was identified in an affected father and his affected child. All variants are absent in gnomAD (v4), are in silico pathogenic and are located in intolerant segments of the protein. All affected persons have developmental delay, ataxia and muscular hypotonia. Three individuals additionally feature eye movement disorders or ptosis. Other symptoms include dysarthria, myopia, glaucoma and optic nerve coloboma. Further patients with variants in *ESRRG* are still in clarification.

Conclusion: Taken the clinical overlap of neurodevelopmental delay, ataxia and muscular hypotonia, the function, size and constraints of the gene, the segregation and in silico predictions of the variants, we propose *ESRRG* as a novel gene for neurodevelopmental delay and ataxia.

Grants: none

Conflict of Interest: None declared

P11.015.C Human models for White Sutton syndrome: *POGZ* mutations change the transcriptome and induce defects in neural progenitor cell biology

Azza Soliman[1], *Jessica Leismann*[2], *Dewi Hartwich*[3], *Susanne Gerber*[3], *Susann Schweiger*[1], *Joan Barau*[2], *Hristo Todorov*[3], *Jennifer Winter*[1]

[1]*Institute of Human Genetics, Mainz, Germany;* [2]*Institute of Molecular Biology gGmbH (IMB), Mainz, Germany;* [3]*Institute of Human Genetics, Clinical Genomics and Bioinformatics, Mainz, Germany*

Background/Objectives: Pogo transposable element with ZNF domain (*POGZ*) has been identified as a frequently mutated gene in patients with neurodevelopmental disorders (NDDs), including autism spectrum disorder (ASD), intellectual disability and White-Sutton syndrome. However, the underlying disease-causing cellular and molecular mechanisms are still unclear.

Methods: We generated induced pluripotent stem cell lines (iPSCs) with heterozygous *POGZ* mutations, either derived from patient fibroblasts or through CRISPR/Cas9 editing, differentiated them into neural progenitor cells (NPCs) in 2D and 3D culture.

Results: We demonstrate that frameshift mutations, either in the N-terminus or in the HP1-binding zinc finger-like (HPZ) domain, decrease POGZ protein expression without affecting its nuclear localization. By using 3D neurospheres, we show that *POGZ* deficiency impairs self-renewal activity and enhances NPC differentiation and neuronal migration.

POGZ binds to chromatin and functions as a transcriptional regulator. RNA sequencing of the NPCs with *POGZ* mutations revealed widespread transcriptome changes. The differentially expressed genes were significantly enriched for GO terms including mitotic chromatid segregation, DNA repair, nonsense mediated decay and alternative splicing, among others. Interestingly, *POGZ* mutations were associated with a transcriptomic signature that was characterized by higher expression of neuron-specific genes. This finding might indicate "accelerated differentiation" in mutant NPCs similar to the phenotype observed in the 3D neurospheres. To identify direct POGZ targets, we are carrying out CUT&Tag sequencing. These experiments, which will be presented at the ESHG, will shed additional light into the regulatory role of POGZ during neural differentiation.

Conclusion: NPCs with *POGZ* mutations show alterations in POGZ expression, proliferation, differentiation and transcriptome, that suggest accelerated differentiation.

Conflict of Interest: None declared

P11.016.D NR2F1 in neural crest cells: a novel approach to model Bosch-Boonstra-Schaaf Optic Atrophy Syndrome (BBSOAS)

Ayat Ahmed[1], *Susanne Theiss*[1], *feven berhanne*[1], *tatyana kan*[1], *Michael Eibl*[1], *Malte Spielmann*[2], *veronica patricia yumiceba corral*[2], *Wilfred F. van Ijcken*[3], *Kerstin Ludwig*[4], *Ronja Hollstein*[4], *Christian Schaaf*[1], *Magdalena Laugsch*[1]

[1]*Institute of Human Genetics, Heidelberg University, Heidelberg, Germany;* [2]*Institute of Human Genetics, University Hospital Schleswig-Holstein, Lübeck, Germany;* [3]*Erasmus University Medical Center, University of Rotterdam, Rotterdam, Netherlands;* [4]*Institute of Human Genetics, University of Bonn, Bonn, Germany*

Background/Objectives: Bosch-Boonstra-Schaaf Optic Atrophy Syndrome (BBSOAS) is a rare autosomal dominant neurodevelopmental disease caused by aberrant NR2F1, which is crucial for brain and craniofacial development. Hence, we are investigating tissue-specific cis-regulatory landscape of *NR2F1* and unraveling the regulatory network controlled by NR2F1 during brain and craniofacial development.

Methods: Utilizing CRISPR/Cas9, we engineered induced pluripotent stem cells (hiPSCs) with *NR2F1* deletion and its putative regulatory elements (enhancers and neighboring lncRNA). We differentiated hiPSC into cranial neural crest cells (hNCC) to mimic craniofacial development and into neural progenitor cells (hNPC) and cortical neurons (CN) to mimic brain development. We then comprehensively examine the interaction of *NR2F1* and its cis-regulatory elements by Hi-C, 4C-seq. To explore the regulatory network controlled by NR2F1, we will combine RNA-seq and ChIP-seq to search for novel genetic

variants linked not only to BBSOAS but also to other neurocristopathies.

Results: We revealed that *NR2F1* lies within a topologically associated domain (TAD) divided into several sub-TADs harboring potential *NR2F1* enhancers and the neighboring lncRNA (*lncNR2F1*). Our findings indicate that *lncNR2F1* regulates *NR2F1* expression only in hNPC suggesting its temporal and tissue-specific action.

Conclusion: Investigating the regulatory landscape of *NR2F1* in its locus will allow us a better understanding of the BBSOAS phenotype and expand the molecular diagnostic of the disease. Our data on the regulatory NR2F1 network will provide new possible therapeutic targets as well as an excellent foundation for further studies of the relationship between brain and craniofacial development.

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Conflict of Interest: None declared

P11.017.A Comparative analysis of patient-specific *NR2F1* variants in mouse

Johann Maaß[1], *Susanne Theiss*[1], *Dominik Kamionek*[1], *Claudia Pitzer*[2], *Henning Fröhlich*[1], *Christian Schaaf*[1]

[1]*Institute of Human Genetics, Heidelberg, Germany;* [2]*Heidelberg University, Heidelberg, Germany*

Background/Objectives: Pathogenic *NR2F1* variants cause a rare neurodevelopmental disorder (BBSOAS, OMIM#615722), characterized by neurological symptoms such as autism, motor dysfunction, and intellectual disability, as well as morphological brain anomalies. Patient data suggest a genotype-phenotype correlation depending on the type and locus of the variant. As the most severe phenotypes are observed in patients with genomic alterations in the highly conserved DNA- and ligand-binding domain of NR2F1, a dominant-negative effect is suspected.

We compare three mouse lines, two with heterozygous patient-specific point variants in the DNA- or ligand-binding domain, as well as a heterozygous deletion, to improve our understanding of the etiology behind mild and severe BBSOAS cases.

Methods: We conducted behavioral experiments assessing autism-like behavior, social interaction, exploratory behavior, anxiety, sleep, motor skills, learning, and memory. Brain morphology was assessed using histological sections and MRI.

Results: All three mouse lines show signs of autism-like behavior, motor dysfunction, and intellectual disability. Mice harboring a variant in the DNA-binding domain were most severely affected, followed by mice with a variant in the ligand-binding domain, and mice with an *Nr2f1* deletion, respectively.

The two mouse lines with variants in the DNA- and ligand-binding domain displayed enlarged lateral ventricles, septal abnormalities, and striatal aplasia. Mice with an *Nr2f1* deletion showed cortical thinning.

Conclusion: Behavioral and brain morphology analysis revealed a clear genotype-phenotype correlation, supporting the dominant-negative effect associated with variants in the DNA- and ligand-binding domain. Our study provides valuable insights into the spectrum of BBSOAS severity and contributes to understanding the pathology associated with different *NR2F1* variants.

Grants:

Conflict of Interest: None declared

P11.018.B A novel homozygous in-frame deletion variant in *TPRKB* causing Galloway-Mowat syndrome 5

Namanpreet Kaur[1], *Khushbu Shirsat*[1], *Mayuri Yeole*[1], *Sheeba Farooqui*[1], *Vivekananda Bhat*[1], *Shahyan Siddiqui*[2], *Dhanya Lakshmi Narayanan*[1], *Anju Shukla*[1]

[1]*Department of Medical Genetics, Kasturba Medical College, Manipal, Manipal Academy of Higher Education, Manipal, India;* [2]*Department of Neurovascular Interventional Radiology, Yashoda Hospitals, Hitech city, Hyderabad, India*

Background/Objectives: Biallelic variants in *TPRKB* (TP53RK-binding protein) are known to cause Galloway–Mowat syndrome 5 (MIM#617731), characterized by early-onset nephrotic syndrome, facial dysmorphism, developmental delay and brain imaging abnormalities. TPRKB is part of a highly conserved kinase, endopeptidase and other proteins of small size (KEOPS) complex which plays important role in efficient translation and DNA damage response. To date, two families with missense pathogenic variants in *TPRKB* have been reported.

Methods: Singleton exome sequencing (ES) of proband followed by Sanger validation and segregation in the family was carried out. In silico mutagenesis of the protein was performed using Chimera v1.16.

Results: An 11-month-old male presented with global developmental delay, neuroregression, mild dysmorphism, atonic seizures, microcephaly (-4.09SD), hypertonia and repetitive hand movements with no signs of nephropathy at present. Brain magnetic resonance imaging showed mild cerebral atrophy, delayed myelination and hyperintensities along white matter tracts and globus pallidus in T2-weighted images. ES revealed a novel homozygous in-frame deletion, c.92_94del p.(Arg31del) in exon 2 of *TPRKB* gene (NM_016058.5). Segregation analysis revealed his parents to be carriers for this variant.

In silico analysis of TPRKB mutant protein showed alteration in the interactions with the neighbouring residues due to Arg31 deletion, thus leading to altered protein conformation as compared to wild-type protein. This could potentially disrupt the assembly of the KEOPS complex, leading to impaired complex functionality.

Conclusion: We report a novel in-frame deletion in *TPRKB* expanding the genotypic spectrum of Galloway-Mowat syndrome 5.

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Conflict of Interest: None declared

P11.019.C Somatic variations of brain tissue and their role in Multiple system atrophy.

Michal Izydorczyk[1], *Fritz Sedlazeck*[2;3], *Christos Proukakis*[3;4], *Ester Kalef-Ezra*[3;4], *Dominic Horner*[3;4], *Caoimhe Morley*[4], *Marco Toffoli*[3;4], *Nadine Holmes*[5]

[1]*Baylor College of Medicine, Human Genome Sequencing Center, Houston, United States;* [2]*Baylor College of Medicine, Houston, United States;* [3]*Aligning Science Across Parkinson's (ASAP) Collaborative Research Network, Maryland, United States;* [4]*UCL Queen Square Institute of Neurology, United Kingdom;* [5]*University of Nottingham, DeepSeq, United Kingdom*

Background: Multiple system atrophy (MSA) is a neurodegenerative disease, likely triggered by environmental and genetic factors, with similarities to Parkinson's disease, as they both involve aggregation of alpha-synuclein. We had previously shown somatic CNVs (gains) of the alpha-synuclein gene in MSA brains and selected two for WGS on a single-cell level. Other somatic

mosaicism (SNV, STR and SV) is difficult to assess, due to high diversity within tissues on single-cell level.

Methods: To assess these other types of mutations we utilized droplet multiple displacement amplification (dMDA) to generate single-cell amplificons >10kbp across samples of 2 MSA brains and control.

We used Nanopore sequencing with two library protocols, one digesting MDA side-chains with T7 endonuclease, and a PCR-based one, on 6 cells of each of the three brains. After filtering chimeric and low-quality signals, up to >50% of the human genome was analyzed using Sniffles2.

Results: Single cells showed ~80% accuracy for insertions when compared to bulk sequencing of the same brain regions. Furthermore, we identified hundreds of mosaic mutations private to single cell highlighting involvement of transposons. In addition, we found mutations within cellular signaling and metabolic genes, associated with MSA and other neurological diseases including GBAP1 and NOTCH2.

Conclusion: This all together demonstrates the power of single cell long read based DNA sequencing, uncovering previously unknown transposon mediated mutations in addition to potential diseases impacting mutations.

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Conflict of Interest: None declared

P11.020.D Genomic analysis of 116 families with Autism Spectrum Disorder: rare de novo and inherited variants further delineate the role of risk genes and highlight new candidates

Elena Bacchelli[1], *Marta Viggiano*[1], *Fabiola Ceroni*[1;2], *Paola Visconti*[3], *Annio Posar*[3;4], *Maria Cristina Scaduto*[3], *Laura Sandoni*[1], *Magali J. Rochat*[5], *Alessandra Maresca*[6], *Alessandro Vaisfeld*[7], *Davide Gentilini*[8;9], *Luciano Calzari*[9], *Valerio Carelli*[4;6], *Michael C Zody*[10], *Elena Maestrini*[1]

[1]*University of Bologna, Department of Pharmacy and Biotechnology, Bologna, Italy;* [2]*Faculty of Health and Life Sciences - Oxford Brookes University, United Kingdom;* [3]*IRCCS Istituto delle Scienze Neurologiche di Bologna, UOSI Disturbi dello Spettro Autistico, Bologna, Italy;* [4]*University of Bologna, Department Of Biomedical And Neuromotor Sciences, Bologna, Italy;* [5]*IRCCS Istituto delle Scienze Neurologiche di Bologna, Functional and Molecular Neuroimaging Unit, Bologna, Italy;* [6]*IRCCS Istituto delle Scienze Neurologiche di Bologna, Programma di Neurogenetica, Bologna, Italy;* [7]*University of Bologna, Department of Medical and Surgical Sciences, Bologna, Italy;* [8]*The University of Pavia, Department of Brain and Behavioral Sciences, Pavia, Italy;* [9]*Istituto Auxologico Italiano IRCCS, Bioinformatics and Statistical Genomic Unit, Milano, Italy;* [10]*New York Genome Center, New York, United States*

Background/Objectives: Autism spectrum disorder (ASD) is a complex neurodevelopmental condition with a strong genetic component in which rare variants contribute significantly to risk.

Methods: We performed whole genome and/or exome sequencing (WGS and WES) and SNP-array analysis to identify both rare sequence and copy number variants (SNVs and CNVs) in 435 individuals from 116 ASD families.

Results: We identified 37 rare potentially damaging de novo SNVs (pdSNVs) from 144 ASD individuals. Interestingly, two of them (one stop-gain and one missense variant) occurred in the same gene, *BRSK2*. Moreover, the identification of 8 de novo pdSNVs in genes not previously implicated in ASD (*AGPAT3, IRX5, MGAT5B, RAB8B, RAP1A, RASAL2, SLC9A1, YME1L1*) highlighted novel candidates. Potentially damaging CNVs (pdCNVs) provided support to the involvement of inherited variants in *PHF3*, *NEGR1*, *TIAM1* and *HOMER1* in neurodevelopmental disorders (NDD), although mostly acting as susceptibility factors with incomplete penetrance. Interpretation of identified pdSNVs/pdCNVs according to the ACMG guidelines led to a molecular diagnosis in 19/144 cases, although this figure represents a lower limit and is expected to increase thanks to further clarification of the role of likely pathogenic variants in new ASD/NDD candidates.

Conclusion: Our study highlights novel ASD candidate genes and contributes to characterize the allelic diversity, mode of inheritance and phenotypic impact of de novo and inherited risk variants in ASD/NDD genes.

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P11.021.A Molecular correlation in pediatric patients with drug-resistant focal epilepsy and structural lesion related to mTORpathies

Roger Prats[1], *Silvia Constancia Liendo Vallejos*[2], *Guerau Fernandez*[3;4;5], *Núria Brandi*[6], *Carlota Ros*[3], *Marta Tejedor*[3], *Clara Xiol*[3;4], *Cristina Jou*[7], *Judith Armstrong*[3;4;5], *Javier Aparicio*[2]

[1]*Fundació per la recerca Sant Joan de Déu, Esplugues de Llobregat, Spain;* [2]*Hospital Sant Joan de Déu, Department of neurology, Esplugues de Llobregat, Spain;* [3]*Hospital Sant Joan de Déu, Molecular Medicine and Genetics, Esplugues de Llobregat, Spain;* [4]*Institut de recerca Sant Joan de Déu, Esplugues de Llobregat, Spain;* [5]*CIBER-ER (Biomedical Network Research Center for Rare Diseases), Instituto de Salud Carlos III (ISCIII), Madrid, Spain;* [6]*Universitat de Barcelona, Barcelona, Spain;* [7]*Hospital Sant Joan de Déu, Department of neuropathology, Esplugues de Llobregat, Spain*

Background/Objectives: Focal cortical dysplasia (FCD) is a brain malformation due to a genetic disorder of neuronal migration (mTORpathy). It is characterised by cortical malformations in the cerebral cortex that can manifest with drug-refractory epilepsy. Here, a genetic analysis of 16 paediatric patients histologically diagnosed with FCD II after epilepsy surgery has been performed to try to find the molecular cause.

Methods: NGS sequencing using a gene panel specific for mTORpathies has been used to try to detect mosaic variants using paired peripheral blood and lesioned brain samples (applying a new bioinformatics programme capable of annotating variants with frequencies <5%). In parallel, a re-analysis of CES data was carried out to detect pathogenic variants in the germline using a larger panel of genes.

Results: The genetic cause has been elucidated in 4/16 patients (25% of diagnostic rate): three low frequency (<8%) mosaic variants have been identified in brain tissue, affecting the *MTOR* gene and not present in the germline. They have been previously described and explain the clinical manifestation of the patients. The fourth variant has been detected in the *DEPDC5* gene, in the germline in de novo heterozygosis, not reported and classified as probably pathogenic and disease-causing.

Conclusion: In view of the high diagnostic rate obtained, a workflow diagram has been designed for the molecular diagnosis of patients clinically diagnosed with FCD II. We hope this will improve diagnostic accuracy and contribute to build more robust and sustainable healthcare infrastructures that will have a positive impact on patients' lives.

Grants: PFNR00171 – PI: J. Aparicio

Conflict of Interest: None declared

P11.022.B Molecular yield of whole-genome sequencing for syndromic schizophrenia in clinical practice in France

Camille Verebi[1;2], *Cécile Louveau*[3], *Mylène Moyal*[2;3], *Adrien Legrand*[3], *Anton Iftimovici*[2;3], *Alinoe Lavillaureix*[4], *Giulia Barcia*[5], *Solène Conrad*[6], *Caroline Sarrosquy*[3], *Maud Lozé*[3], *Alice Poisson*[7], *Caroline Demily*[8], *Gaetan Lesca*[9], *Romain Rey*[8], *Alban Lermine*[10], *Pierre Blanc*[10], *Boris Chaumette*[2;3], *Thierry Bienvenu*[1;2]

[1]*Service de Médecine Génomique des Maladies de Système et d'Organe, Fédération de Génétique et de Médecine Génomique, Hôpital Cochin, APHP.Centre-Université Paris Cité, Paris, France;* [2]*Université Paris Cité, INSERM U1266, Institut de Psychiatrie et de Neurosciences de Paris, Paris, France;* [3]*Center for Rare Psychiatric Disorders, GHU Paris Psychiatrie et Neurosciences, Paris, France;* [4]*CHU Rennes, Service de Génétique Clinique, Centre de Référence Anomalies du développement, FHU GenOMedS, Univ Rennes, CNRS, INSERM, IGDR, UMR 6290, ERL U1305, Rennes, France;* [5]*Service de Médecine Génomique des Maladies Rares, Fédération de Génétique et de Médecine Génomique, Hôpital Necker - Enfants Malades, Assistance Publique-Hôpitaux de Paris, Paris, France;* [6]*Nantes Université, CHU de Nantes, Service de Génétique médicale, Nantes, France;* [7]*Hôpital Neurologique Pierre Wertheimer, Service de Neurologie C, Centre Expert Parkinson, Hospices Civils de Lyon, Lyon, France;* [8]*Centre de Référence Maladies Rares Troubles du Comportement d'Origine Génétique (GénoPsy Lyon), Centre d'excellence autisme iMIND, Centre Hospitalier Le Vinatier, UMR 5229, CNRS & Université Lyon 1, Bron, France;* [9]*Service de Génétique, Hospices Civils de Lyon, Bron, France;* [10]*Multi-site Medical Biology Laboratory SeqOIA, Paris, France*

Introduction: The French Medicine Genomics Plan (PFMG) 2025 has enabled access to genome sequencing in clinical settings throughout France.

Methods: Access to whole genome sequencing has been granted for patients with schizophrenia when additional criteria are met. These criteria include a normal CGH array as well as psychiatric and non-psychiatric clinical red flag features defining a "syndromic form of schizophrenia". To date, over 32 prescriptions have been made in the North of France. Some had previous genetic investigations such as karyotype, Fragile X, phenotypically oriented gene panels and even exome sequencing.

Results: We report here the yield of whole-genome sequencing for "syndromic schizophrenia" in a clinical setting: 8 genomes were conclusive (26%), 12 reported a variant of uncertain significance (39%) requiring further investigation, and 11 were inconclusive (35%). The diagnostic rate is similar to that previously published for similar clinical presentations. The positive genomes involved 8 genes: *AHDC1*, *ATP9A*, *CHD8*, *DLL1*, *FAAH2*, *KANSL1*, *MACF1 and TRIP12*. Variants of uncertain significance involved 15 different genes; eight of them were carried in a compound heterozygous, homozygous or hemizygous status. Of the remaining heterozygous variants, 4 arose de novo and the rest were inherited from an a priori asymptomatic parent. Most of the genes were not previously associated with schizophrenia, but with neurodevelopmental disorders.

Conclusion: Our data suggest that whole-genome sequencing may be useful and highly successful in the context of genetic work-up of patients with schizophrenia, and should be considered under appropriate conditions.

Conflict of Interest: None declared

P11.024.D Motor neuron disease, clinically similar to amyotrophic lateral sclerosis, as another extreme phenotype of *RFC1* spectrum disorder

Annalisa Schaub[1;2], *Hannes Erdmann*[1;2], *Veronika Scholz*[1], *Manuela Timmer*[3], *Peter Reilich*[2], *Angela Abicht*[1;2], *Florian Schöberl*[2]

[1]*Medical Genetics Center (MGZ), Munich, Germany;* [2]*University Hospital, Ludwig-Maximilians-University (LMU), Department of Neurology with Friedrich-Baur-Institute, Munich;* [3]*Gemeinschaftspraxis für Humangenetik, Dresden, Germany*

Background/Objectives: Biallelic pathogenic repeat expansions in *RFC1* have been recently identified as molecular origin of CANVAS and as one of the most common causes of adult-onset ataxia. The phenotypic spectrum has expanded massively and now is indicated as *RFC1* spectrum disease. Since clinical and subclinical motor neuron affection was described in patients with biallelic pathogenic *RFC1* repeat expansions, we systematically studied a cohort of altogether 107 ALS patients to analyze whether pathogenic *RFC1* repeat expansions can be found more frequently in sporadic ALS patients.

Methods: Genomic DNA was obtained from 107 patients with a clinical diagnosis of ALS (Gold Coast criteria). Known ALS-related genes were analyzed by NGS via gene targeted enrichment or exome sequencing. To identify pathogenic repeat expansions in *C9orf72* and *RFC1*, long-read sequencing by Oxford Nanopore Technology was used.

Results: In a cohort of 107 sporadic ALS patients, ACMG class 4/5 variants in genes associated with ALS were identified altogether in 12 % of the patients (*C9orf72*: 7%, *SOD1*: 3%, *TARDP*: 1%, *NEK1*: 1%). Including the patient previously described as case report by us, three patients in our cohort exhibited a biallelic pathogenic *RFC1* repeat expansion (i.e. ~3%).

Conclusion: *RFC1* spectrum disease should be considered in patients with ALS/MND, especially when additional atypical symptoms are present. Therefore, this highlights the relevance of genetic testing in addition to a clinical diagnosis. The usage of long-read sequencing in combination with deciphering the pathomechanism of *RFC1* spectrum disorder will be key for a more detailed understanding of the pathology and phenotypical variance.

Grants: No external funding

Conflict of Interest: None declared

P11.025.A Trajectory of neuroligin/neurexin dysregulation associates with the establishment of an ASD-like phenotype in a mouse model of tuberous sclerosis

Jennifer Krummeich[1;2], **Verena Engelhardt**[1], *Cosima Caliendo*[1], *Annabelle Arlt*[1;3], *Jannik Maier*[4], *Kamil Rolski*[5], *Kevin Vincze*[5], *Rainer Schneider*[5], *Michael Schmeisser*[4], *Susanne Gerber*[1], *Susann Schweiger*[1]

[1]*Institute of Human Genetics, University Medical Center of the Johannes Gutenberg-University, Mainz, Germany;* [2]*Institute of Pathology, University Medical Center of the Johannes Gutenberg-University, Mainz, Germany;* [3]*Institute for Genomic Statistics and Bioinformatics, University Hospital, Bonn, Germany;* [4]*Institute for Microscopic Anatomy and Neurobiology, University Medical Center of the Johannes Gutenberg-University, Mainz, Germany;* [5]*Insitute of Biochemistry, University of Innsbruck and Center for Molecular Biosciences, Innsbruck, Germany*

Background/Objectives: Tuberous sclerosis (TS) is an autosomal dominant disorder caused by mutations in *TSC1* or *TSC2* that negatively regulate the mechanistic Target of Rapamycin (mTOR), resulting in mis-regulation of cell growth and proliferation. TS is characterized by tumors in several organs, brain dysfunction that comprises epilepsy, and TSC-associated neuropsychiatric disorders (TAND) including autism spectrum disorders (ASD). The sequential appearance of these symptoms suggests a dynamic process of disease development. Since most studies have been conducted at single time points, the trajectory of symptom formation and underlying molecular changes have largely been neglected.

Methods: To model these, we used a conventional heterozygous *Tsc2* knockout mouse model. We longitudinally analyzed behavior at different time points and matched this with molecular changes throughout brain development by performing comparative proteome analysis of cortical homogenates and synaptosomes in early to late postnatal stages.

Results: We found that like in patients, TAND symptoms developed stepwise in a time-dependent manner, starting with aberrations in autism-like behavior at two months, followed by impairments in social behavior at three to four months. In addition, Tsc2 was reduced only at very early stages and fully compensated at later time points suggesting that the behavior phenotype develops independent of the primary defect. Furthermore, we found that the formation of behavioral aberrations correlates with a window of neuroligin and neurexin misexpression in cortical but not hippocampal tissue.

Conclusion: Taken together, our data suggests substantial homeostatic dynamics of gene expression underlying the TS phenotype and a correlation of ASD-like symptoms with cortical dysregulation of neuroligins and neurexin.

Grants:

Conflict of Interest: None declared

P11.026.B Spectrum of ultrarare neurogenetic disorders and gene variants in Bulgarian paediatric patients

Slavyana Yaneva Staykova[1], *Maya Atanasoska*[1;2], *Lubomir Balabanski*[1], *Spasimir Shishinyov*[1], *Veneta Bozhinova*[3], *Silvia Cherninkova*[4], *Ivaylo Tournev*[4], *Alexander Oscar*[4], *Elitsa Becheva-Kraichir*[5], *Rosen Kalpachki*[6], *Maria Gaydarova*[7], *Daniela Avdjieva-Tzavella*[7], *Irena Bradinova*[1;8], *Radoslava Vazharova*[1;9]

[1]*GMDL CellGenetics, Sofia, Bulgaria;* [2]*Sofia University "St. Kliment Ohridski", Faculty of Biology, Department of Genetics, Sofia, Bulgaria;* [3]*MHATNP St. Naum, Sofia, Bulgaria;* [4]*Aleksandrovska University Hospital, Sofia, Bulgaria;* [5]*Heart and Brain Center of Clinical Excellence, Pleven, Bulgaria;* [6]*St. Anna University Hospital, Sofia, Bulgaria;* [7]*SBAL Children's Hospital Prof. Dr. Ivan Mitev, Sofia, Bulgaria;* [8]*National Genetic Laboratory, Maichin Dom Hospital, Sofia, Bulgaria;* [9]*Sofia University "St. Kliment Ohridski", Faculty of Medicine, Department of Biology, Medical Genetics and Microbiology, Sofia, Bulgaria*

Background/Objectives: Rare diseases affect thousands of individuals in Bulgaria and contribute to morbidity and mortality. We present twelve cases of ultrarare neurogenetic disorders in the Bulgarian paediatric population.

Methods: Twenty-one patients aged 2 months to 17 years with suspected neurogenetic disorders were referred for genetic testing. Whole-genome sequencing with targeted bioinformatic analysis of genes associated with neurological conditions was performed.

Results: Fifteen patients received genetic diagnosis. In five individuals, potentially causative variants were identified. Twelve probands were carriers of homozygous, heterozygous or compound heterozygous pathogenic/likely pathogenic variants, associated with ultrarare genetic disorders:

Patient#	Gene variant/ variants	Genetic diagnosis	Prevalence (Orphanet)
1	*GABRB1*:c.898A>T (heterozygous)	Developmental and epileptic encephalopathy 45	-
2	*TAF6*:c.212T>C (homozygous)	Alazami-Yuan syndrome	-
3	*KIF1A*:c.760C>T (heterozygous)	NESCAV syndrome	-
4	*LARP7*:c.691_694del (homozygous)	Alazami syndrome	<1/1000000
5	*COL6A3*:c.6220G>T (heterozygous)	Bethlem myopathy	<1/1000000
6	*ALS2*:c.3703-2A>G (homozygous)	Infantile-onset ascending hereditary spastic paralysis	<1/1000000
7	*CSF1R*:c.2051del (heterozygous)	Hereditary diffuse leukoencephalopathy with spheroids 1	<1/1000000
8	*DDX3X*:c.865-2A>G (heterozygous)	Intellectual developmental disorder, X-linked syndromic, Snijders Blok type	<1/1000000
9	*VARS2*:c.502C>T, *VARS2*:c.1587G>T (compound heterozygous)	Combined oxidative phosphorylation deficiency 20	<1/1000000
10	*ASXL3*:c.3349C>T (heterozygous)	Bainbridge-Ropers syndrome	<1/1000000
11	*MTOR*:c.7141A>G (heterozygous)	Smith-Kingsmore syndrome	<1/1000000
12	*HINT1*:c.334C>A, *HINT1*:c.110G>C (compound heterozygous)	Neuromyotonia and axonal neuropathy, autosomal recessive	<1/1000000

Conclusion: The forementioned clinical cases contribute to the relatively narrow spectrum of documented ultrarare genetic disorders in the Bulgarian population. In addition, identifying the precise causative change in the genome in complex cases with neurological symptoms and overlapping phenotypic presentation can lead the way to novel treatments or therapies and improve outcomes.

Conflict of Interest: None declared

P11.027.C Increased Burden of Rare Protein-Truncating Variants in Constrained, Brain-specific and Synaptic Genes In Extremely Impulsively Violent Males with Antisocial Personality Disorder

Kateřina Hodaňová[1], *Dita Musalkova*[1], *Anna Přistoupilová*[1], *Hana Hartmannova*[1], *Helena Treslova*[1], *Lenka Nosková*[1], *Ivana Jedličková*[1], *Vaclav Jiricka*[2], *Michaela Langmajerová*[2], *Marc Woodbury-Smith*[3;4], *Mehdi Zarrei*[3], *Brett Trost*[3], *Stephen Scherer*[3;5], *Anthony J. Bleyer*[6], *Jan Vevera*[2], *Stanislav Kmoch*[1]

[1]*First Faculty of Medicine, Charles University, Prague, Department of Pediatrics and Inherited Metabolic Disorders, Praha 2, Czech Republic;* [2]*Faculty of Medicine in Pilsen, Charles University, Department of Psychiatry, Pilsen, Czech Republic;* [3]*, The Centre for Applied Genomics and Program in Genetics and Genome Biology, Toronto, Canada;* [4]*Newcastle University, Biosciences Institute, Faculty of*

Medical Sciences, Newcastle upon Tyne, United Kingdom; [5]University of Toronto, Department of Molecular Genetics and McLaughlin Centre, Toronto, Canada; [6]Wake Forest School of Medicine, Section on Nephrology, Winston- Salem, United States

Background/Objectives: The genetic correlates of extreme impulsive violence are poorly understood, and there have been few studies that have characterized a large group of affected individuals both clinically and genetically.

Methods: We performed WES in 290 males with the life-course-persistent, extremely impulsively violent form of antisocial personality disorder (APD) and analyzed the spectrum of rare protein-truncating variants (rPTVs). Comparisons were made with 314 male controls and publicly available genotype data.

Results: Participants were significantly more likely to harbor rPTVs in genes that are intolerant to loss-of-function variants (odds ratio (OR) 2.06; $p < 0.001$), specifically expressed in brain (OR 2.80; $p = 0.036$) and enriched for those involved in neurotransmitter transport and synaptic processes. In 60 individuals (20%), we identified rPTVs that we classified as clinically relevant these, 37 individuals harbored rPTVs in 23 genes that are associated with a monogenic neurological disorder, and 23 individuals harbored rPTVs in 20 genes reportedly intolerant to loss-of-function variants. Functional annotation tools were used for biological interpretation.

Conclusion: The analysis presents evidence in support of a model where presence of either one or several private, functionally relevant mutations contribute significantly to individual risk of life-course-persistent APD and reveals multiple individuals who could be affected by clinically unrecognized neuropsychiatric Mendelian disease. Thus, Mendelian diseases and increased rPTV burden may represent important factors for the development of extremely impulsive violent life-course-persistent forms of APD irrespective of their clinical presentation.

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Conflict of Interest: None declared

P11.028.D Strategies beyond WES in SpainUDP: Improvement of diagnostic rate by WES reanalysis, SR-WGS and RNASeq

Estrella Lopez-Martín[1], *Beatriz Martinez-Delgado[1;2], María J. Barrero[1], Lidia López[1], Lidia Mielu[1], Beatriz Baladron[1], Gema Gomez-Mariano[1], Sergio Casas-Tintó[1], Eva Bermejo-Sanchez[1]*

[1]Instituto de Investigación de Enfermedades Raras (IIER), Instituto de Salud Carlos III (ISCIII), Madrid, Spain; [2]Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER), CIBERER group 758, Madrid, Spain

Consortium: Spanish Undiagnosed Rare Diseases Program (SpainUDP)

Background/Objectives: Currently, the underlying etiologies for many rare diseases remain undiscovered and knowledge gaps continue to exist regarding genotype-phenotype correlation. To address these issues, the Spanish Undiagnosed Rare Diseases Program (SpainUDP) was established in Spain at the end of 2015 by the IIER-ISCIII.

Methods: The first phase of the SpainUDP protocol consists of deep phenotyping and trio-based WES in blood samples. However, more than 50% of patients continued undiagnosed after WES, so additional diagnostic strategies are being implemented to improve diagnostic rates of unsolved cases. Here, improvement of diagnostic rate after reanalysis of WES, SR-WGS and RNASeq was evaluated.

Results: Since the official launch of SpainUDP, 89 patients (42.4%) with ultrarare diseases have been diagnosed. Reanalysis of previous WES results led to the diagnosis of 8 patients (9 % of solved cases) and the use of technologies beyond the exome, such as SR-WGS and RNASeq, allowed the resolution of 3 additional cases (3% of solved cases). On the other hand, 121 undiagnosed patients (57.6%) are under study by using patient-derived cultured cells, organoids and animal models for functional assays, which are expected to allow the characterization of molecular mechanisms of unknown rare diseases. In addition, structural genomic variants are being investigated through LR-WGS and OGM with promising preliminary results.

Conclusions: Our experience highlights the need for establishing an integrated approach consisting of the use of combined omics methods, periodic reanalyses of results, as well as functional assays to support the phenotype-genotype relationship of a putative variant.

Conflict of Interest: None declared

P11.029.A Genetic Research and Testing for Epilepsy in a Resource-Constrained Setting – the South African experience.

Alina Esterhuizen[1;2;3], *Gemma Louise Carvill[4], Jo Wilmshurst[2;5]*

[1]University of Cape Town, Pathology, Division of Human Genetics, Cape Town, South Africa; [2]Neuroscience Institute, University of Cape Town, Cape Town, South Africa; [3]Groote Schuur Hospital, Human Genetics, Cape Town, South Africa; [4]Northwestern University, Ken and Ruth Davee Department of Neurology, Evanston, United States; [5]Red Cross War Memorial Children's Hospital, Paediatric Neurology, Cape Town, South Africa

Background: Sub-Saharan Africa bears the highest burden of epilepsy worldwide. A presumed proportion is genetic, but this aetiology is buried under the burden of infections and perinatal insults, in a setting of limited awareness and few options for testing. Children with developmental and epileptic encephalopathies (DEEs), are most affected by this diagnostic gap, as the rate of actionable findings is highest in DEE-associated genes. Here, we report findings of genetic analyses in local children with DEE, its relevance to precision medicine in South Africa. We emphasise the imperatives careful clinical assessment and multidisciplinary consultation, and describe the experience of translating this research into the South African, resource-constrained healthcare setting.

Methods: We recruited 234 genetically naïve infants clinically diagnosed with DEE. Genetic testing included gene panels, chromosomal microarray and exome sequencing of patient/parent trios. Statistical comparison of electroclinical features in children with and without candidate variants was performed to identify characteristics most likely predictive of a positive genetic finding.

Results: Of 41/234 children with likely/pathogenic variants, 26/234 had variants supporting precision therapy. Multivariate regression modelling highlighted neonatal or infantile-onset seizures and movement abnormalities as predictive of a positive genetic finding. We used this, coupled with an emphasis on precision medicine outcomes, to propose the pragmatic "Think-Genetics" decision tree for recognition of a possible genetic aetiology, pragmatic testing and multidisciplinary consultation.

Conclusion: Our findings emphasise the importance of access to early genetic testing in DEEs. The "Think-Genetics" strategy may facilitate early recognition, appropriate interim management and genetic testing in resource-constrained settings.

Grants: SA NHLS Trust, SA MRC, NIH.

Conflict of Interest: None declared

P11.030.B Investigating the extent and impact of splicing noise in Alzheimer's disease using genome-wide short-read RNA-sequencing

Sonia García-Ruiz[1], *Emil Gustavsson*[1], *Melissa Grant-Peters*[1], *David Zhang*[1], *Aine Fairbrother-Browne*[1], *Zhongbo Chen*[2], *Leonardo Collado-Torres*[3], *Mina Ryten*[1;2]

[1]*UCL Great Ormond Street Institute of Child Health, United Kingdom;* [2]*UCL Queen Square Institute of Neurology, United Kingdom;* [3]*Lieber Institute for Brain Development, Baltimore, United States*

Background/Objectives: Alternative splicing (AS) is a feature of most multi-exonic human genes and its dysregulation contributes to ageing and neurodegeneration, including Alzheimer's disease (AD). However, the genome-wide accuracy of AS in AD samples has received little attention despite the fact that high-depth RNA-sequencing data of diseased samples commonly contains partially unannotated reads within individuals implying the existence of splicing inaccuracies.

Methods: In this project, we investigated the characteristics of mis-splicing as detected using split reads partially mapping to known transcripts in the reference annotation. Using short-read RNA-sequencing data derived from 48 unique human postmortem brain samples (control and AD), we built an intronic database cataloguing all splicing events detected across the samples. We evaluated the structural differences and abundance of inaccurately spliced introns in AD samples as compared to controls.

Results: This analysis revealed significant increases in the levels of mis-splicing activity across the transcriptome in brain samples from AD patients as demonstrated by the higher rates of sequencing reads indicating the use of novel donor or acceptor splice sites. We observed that mis-splicing events in AD samples occurred more frequently at positions not divisible by three, equating to the size of a codon, and indicating a higher frequency of frame-shifting splicing events in AD samples as compared to controls. Finally, we detected that the genes containing higher frequencies of splicing inaccuracies in AD samples were enriched for synaptic functions.

Conclusion: Overall, these results suggest the key importance of splicing integrity at synapses, and that its failure could be a driving process in AD.

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P11.031.C *HTT*, *ATXN1* and *ATXN2* CAG repeat size and risk for cancer and Parkinson disease.

Sergio Pérez Oliveira[1;2], *Ignacio Álvarez Fernandez*[3], *Manuel Menendez-Gonzalez*[2;4;5], *Israel David Duarte-Herrera*[2], *Marta Blázquez-Estrada*[2;4], *Esther Súarez*[2;4], *Ciara García-Fernández*[2;4], *Pablo Siso-García*[2;6], *Pablo García-González*[7], *Maitee Rosende-Roca*[7;8], *Mercè Boada*[7;8], *Agustín Ruiz*[7;8], *Jon Infante*[8;9], *Beatriz De la Casa Fages*[10;11], *Isabel González-Aramburu*[8;9], *Maria Victoria Alvarez Martinez*[1;2], *Pau Pastor*[12;13]

[1]*Central University Hospital of Asturias, Laboratorio de Genética, Oviedo, Spain;* [2]*Instituto de Investigación Sanitaria del Principado de Asturias (ISPA), Oviedo, Spain;* [3]*Hospital Universitari MútuaTerrassa, Neurologia (Movement Disorders Unit), Terrassa, Spain;* [4]*Central University Hospital of Asturias, Neurología, Oviedo, Spain;* [5]*University of Oviedo, Medicine, Oviedo, Spain;* [6]*Hospital of Cabueñes, Neurologoia, Gijón, Spain;* [7]*Ace Alzheimer Centre Barcelona (Fundació ACE), Universitat Internacional de Catalunya, Barcelona, Spain;* [8]*ISCIII, Networking Research Center on Neurodegenerative Diseases (CIBERNED), Majadahonda, Spain;* [9]*Marqués de Valdecilla University Hospital, Neurology, Santander, Spain;* [10]*Gregorio Marañón General University Hospital, Neurologia (Movement Disorders Unit), Madrid, Spain;* [11]*Instituto de Investigación Sanitaria Gregorio Marañón, Madrid, Spain;* [12]*Hospital Germans Trias i Pujol, Unit of Neurodegenerative diseases, Department of Neurology, Badalona, Spain;* [13]*Germans Trias i Pujol Research Institute, Barcelona, Spain*

Background/Objectives: Parkinson's disease (PD) genetic risk embraces genetic and non-genetic factors. It has been suggested a link between CAG repeat number in the HTT, ATXN1 and ATXN2 genes and neurodegenerative diseases. In addition, some PD genetic factors are indeed associated with cancer pathways. Several studies found a low prevalence of cancer in neurodegenerative diseases that can be associated with a low? CAG repeats' size in several genes. This study aimed to investigate the influence of CAG repeat sizes in ATXN1, ATXN2, and HTT genes on the risk for developing the disease and cancer in a large cohort of PD patients and healthy controls.

Methods: The study included 1156 PD patients and 1070 controls of European ancestry. CAG repeat sizes in HTT, ATXN1, and ATXN2 genes were analyzed. Dunn's Multiple Comparison Test for quantitative variables and logistic and linear regression were used.

Results: The long ATXN1 allele and CAG size, and both, the ATXN2 short and long alleles were predictors for the PD risk. The long CAG ATXN1 allele gene was associated with the risk of cancer.

Conclusion: These results reinforce the hypothesis that ***α***-synucleinopathies and polyQ-linked neurodegenerative diseases can share common pathways. HTT, ATXN1, and ATXN2 CAG alleles' size can modify the risk of cancer among PD patients.

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Conflict of Interest: None declared

P11.032.D A framework for performing de novo variant analyses of complex psychiatric disorders in genetically isolated populations

Kate Bornais[1;2], *Jay Ross*[1;2], *Zoe Schmilovich*[1;2], *Miranda Medeiros*[1;2], *Dan Spiegelman*[2;3], *Patrick Dion*[2;3], *Guy Rouleau*[1;2;3]

[1]*McGill University, Human Genetics, Montréal, Canada;* [2]*McGill University, Montreal Neurological Institute-Hospital, Montréal, Canada;* [3]*McGill University, Neurology and Neurosurgery, Montréal, Canada*

Background/Objectives: Obsessive-compulsive disorder (OCD) is a common psychiatric disorder characterized by recurrent obsessions and compulsions. Childhood-onset OCD has a genetic component, with heritability estimates being ~45-65%. However, not all genetic factors are inherited. De novo variants (DNVs) arise spontaneously in zygotes and contribute significantly to childhood-onset OCD risk. To date, no DNV analyses of childhood-onset OCD have been performed in a genetically isolated population. Here, we identified DNVs in French-Canadian childhood-onset OCD trios.

Methods: Whole-exome sequencing was performed on 50 trios ($n = 150$). Variants were filtered for heterozygous genotype in the proband and wildtype in both parents. Single-nucleotide protein-coding DNVs with population minor allele frequency <1% were retained and confirmed in IGV. DenovolyzeR assessed the genome-wide burden of DNVs for different variant classes. Genes harbouring DNVs were examined for overlap with genes previously associated to OCD and other psychiatric disorders. Our candidate gene list was combined with those in previous OCD DNV publications for functional enrichment meta-analyses.

Results: 33 DNVs in 33 genes were observed across 36 probands (0.92 DNVs/proband, average CADD score = 16.1). An enrichment of splice-site DNVs was detected ($p = 0.0173$). 4 genes were identified in previous OCD studies (Halvorsen 2021; IOCDF-GC 2018). All 33 genes overlapped with genes associated to related psychiatric disorders. Genes involved in clathrin-dependent endocytosis (GO:0072583) and phosphatidylinositol binding (GO:0035091) were overrepresented in the combined gene list ($p\text{-}adj < 0.05$).

Conclusion: This study presents a framework for conducting DNV analyses in isolated populations. Using this framework, we have provided the first comprehensive list of candidate childhood-onset OCD genes specific to the French-Canadian population.

Grants:

Conflict of Interest: None declared

P11.033.A Bi-allelic *ATG12* variants are associated with a congenital disorder of autophagy

James Lambton[1], Fumi Suomi[2;3], Magali Prigent[4;5], Aliza Imam[6], Thomas McCorvie[7], Emma Hobson[6], Helen McCullagh[8], Eirik Frengen[9], Doriana Misceo[9], Anna Bjerre[10], Marie Falkenberg Smeland[11;12], Claes Klingenberg[12;13], Robert McFarland[1;14], Jack Collier[1;15], Wyatt Yue[7], Renaud Legouis[4;5], Thomas McWilliams[2;3], Charlotte Alston[1;14], Monika Olahova[1;16], **Robert Taylor**[1;14]

[1]*Newcastle University, United Kingdom;* [2]*University of Helsinki, Translational Stem Cell Biology & Metabolism Program, Finland;* [3]*University of Helsinki, Department of Anatomy, Finland;* [4]*Université Paris-Saclay, Institute for Integrative Biology of the Cell (I2BC), France;* [5]*INSERM U1280, France;* [6]*Leeds Teaching Hospitals NHS Trust, Yorkshire Regional Genetics Service, United Kingdom;* [7]*Newcastle University, Biosciences Institute, United Kingdom;* [8]*Leeds Teaching Hospitals NHS Trust, Department of Paediatric Neurology, United Kingdom;* [9]*Oslo University Hospital, Department of Medical Genetics, Norway;* [10]*Oslo University Hospital, Department for Specialised Paediatrics, Norway;* [11]*University Hospital of North Norway, Department of Paediatric Rehabilitation, Norway;* [12]*UiT - The Arctic University of Norway, Faculty of Health Sciences, Norway;* [13]*University Hospital of North Norway, Department of Paediatrics and Adolescence Medicine, Norway;* [14]*Newcastle upon Tyne Hospitals NHS Foundation Trust, NHS Highly Specialised Rare Mitochondrial Disorders Service, United Kingdom;* [15]*McGill University, Montreal Neurological Institute, Canada;* [16]*Northumbria University, Department of Applied Sciences, United Kingdom*

Background/Objectives: Autophagy is an essential homeostatic process, driving the endolysosomal degradation of protein aggregates, organelles and pathogens. Dysfunctional autophagy is implicated in complex human disease yet reports of congenital autophagy disorders were considered exceedingly rare until the recent report of unrelated families with bi-allelic *ATG7* variants encoding a core autophagy effector. In contrast to *Atg7* deletion in mice, causing perinatal lethality, affected patients survive into adulthood despite loss of ATG7 and diminished autophagic flux. We aimed to delineate the phenotypic spectrum of pathogenic *ATG12* variants and characterise associated molecular defects.

Methods: Bi-allelic *ATG12* variants were identified in three individuals from two families with complex neurological phenotypes. We performed structural modelling and functional studies (using patient-derived fibroblasts and yeast modelling) to determine pathogenicity.

Results: Affected individuals share a consistent phenotype comprising developmental delay and seizures, with cerebellar vermis hypoplasia evident on cranial MRI; one affected child had a nephritic syndrome, leading to chronic kidney failure; dysmorphic features included a broad nasal bridge, long philtrum, and low set ears. Fibroblasts from affected individuals demonstrated decreased steady-state levels of ATG12 protein and/or altered flux following starvation; Transmission Electron Microscopy revealed an accumulation of autophagic structures. Structural studies implicated disruption of ATG12-ATG5-ATG16N-ATG3 complex formation, supported by modelling of missense variants in yeast.

Conclusion: Our data emphasise an important, emerging group of genetic disorders characterised by dysfunctional autophagy, expanding the understanding of adaptive homeostasis in human health and disease.

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P11.034.B A previously unreported splice variant in *FLNA* causing periventricular nodular heterotopia 1 in a Danish family; explored by functional studies

Ellen Steffensen[1;2], *Jenny Blechingberg[1], Thomas van Overeem Hansen[3], Anneli Bolund[1], Stephen Robertson[4], Pernille Axél Gregersen[1], Ida Charlotte Bay Lund[1]*

[1]*Aarhus University Hospital, Department of Clinical Genetics, Aarhus, Denmark;* [2]*Aalborg University Hospital, Department of Clinical Genetics, Aalborg, Denmark;* [3]*Copenhagen University Hospital – Rigshospitalet, Department of Genetics, Copenhagen, Denmark;* [4]*Dunedin School of Medicine, University of Otago, Department of Women's and Children's Health, Dunedin, New Zealand*

Background/Objectives: Filamin A (FLNA)-related periventricular nodular heterotopia 1 (PVNH1) is an X-linked dominant disorder.

We report our experience with functional studies of a previously unreported splice variant in *FLNA*.

Methods: Whole genome sequencing targeted on a non-syndromic epilepsy gene panel. Additional functional studies and segregation analysis to assess the pathogenicity of a detected *FLNA* variant. The effect on mRNA splicing was studied though RT-PCR on RNA isolated from cultured fibroblasts and transformed lymphoblasts, respectively, under nonsense-mediated decay inhibition and subsequent semi-quantitative capillary electrophoresis and nanopore sequencing.

Results: The female proband presented with epilepsy and PVNH at age 17 years. Genetic testing revealed heterozygosity for a splice site variant, c.6503-1del (NM_001110556.2), in *FLNA*. Her sister suffered from migraines and was found to have PVNH. Co-segregation analysis revealed the variant in the sister, the sisters' mother, having asymptomatic PVNH and aortic valve insufficiency, but not in their unaffected maternal uncle.

Analysis of RNA from fibroblasts showed that in approx. 5% of transcripts, a cryptic splice acceptor site was used, resulting in a frameshift mutation and premature stop codon p.(Glu2160Alafs*5). In contrast, in transformed lymphoblasts, this alternative transcript constituted approx. 90% of all transcripts.

Based on the functional studies and co-segregation analysis, the variant in *FLNA* was reclassified from a "variant of uncertain significance" to a "pathogenic" variant.

Conclusion: This report highlights the value of functional studies in different cell types in females affected by *FLNA*-related PVNH1. Our findings may reflect skewed X-chromosome inactivation.

Grants: N/A

Conflict of Interest: None declared

P11.035.C A study of the natural history of *RARB*-related disorders

Valerie Chu[1], *Andre Tremblay*[1], *Jacques Michaud*[1]

[1]*CHU Sainte-Justine Azrieli Research Centre, Montréal, Canada*

Background/Objectives: Dominant variants in the Retinoic Acid Receptor Beta (*RARB*) gene underlie a syndrome known as MCOPS12 that is characterized by developmental eye defects, other congenital anomalies, and global developmental delay with dystonia. Although most affected individuals display severe developmental delay and motor impairment, some show minimal neurodevelopmental involvement. We initiated a natural history study of MCOPS12 to better characterize its clinical course.

Methods and Results: Individuals worldwide carrying pathogenic or likely pathogenic variants in *RARB* are eligible to participate. In addition to the baseline phenotype of the participants, developmental milestones, behavioural profiles, and dystonia severity are documented annually using standardized tools and video protocols. Clinical brain MRI images are also collected. The study framework includes a physician-driven arm and a participant-powered arm. For both arms, data are collected via an online REDCap platform.

Our group has characterized the functional impact of 26 pathogenic variants using in vitro transcriptional assays. We have found that these variants induce either a gain-of-function or a dominant-negative effect. We will use these assays to functionally classify novel variants identified in the participants. The natural history study will be piloted by 10 MCOPS12 families before its launch to all affected individuals.

Conclusion: As therapeutic options for MCOPS12 are being actively investigated, it is imperative to document the natural history of individuals with this condition. Our study will lead to the establishment of a framework for the development of clinical trials against MCOPS12.

Grants: Chaire Jonathan Bouchard, Cure MCOPS12, A Cure for Sienna

Conflict of Interest: None declared

P11.036.D Genome sequencing reanalysis increases the diagnostic yield in dystonia

Avi Fellner[1;2], *Gurusidheshwar Wali*[3], *Neil Mahant*[4], *Bianca R. Grosz*[5], *Melina Ellis*[5], *Ramesh K. Narayanan*[5], *Karl Ng*[6;7], *Ryan L. Davis*[2;6;8], *Michel Tchan*[6;9], *Katya Kotschet*[10], *Dennis Yeow*[2;6;11;12;13], *Laura I. Rudaks*[2;6;11;12;14], *Sue-Faye Siow*[6;14], *Gautam Wali*[6;8;13], *Con Yiannikas*[7;11], *Matthew Hobbs*[2], *Joseph Copty*[2], *Michael Geaghan*[2], *Paul Darveniza*[15], *Christina Liang*[6;7;13], *Laura Williams*[4], *Florence C.F. Chang*[4;6], *Hugo Morales-Briceño*[4;6], *Stephen Tisch*[15;16], *Michael Hayes*[11], *Scott Whyte*[17], *Sarah Kummerfeld*[2], *Marina L. Kennerson*[5;6;12], *Mark Cowley*[16;18], *Victor Fung*[4;6], *Carolyn M. Sue*[7;8;13;16], *Kishore R. Kumar*[2;6;11;12;16]

[1]*Rabin Medical Center, Beilinson Hospital, The Neurogenetics Clinic, Raphael Recanati Genetics Institute, Petah Tikva, Israel;* [2]*Garvan Institute of Medical Research, Darlinghurst, Australia;* [3]*Neurospecialities Centre, Belgaum, India;* [4]*Westmead Hospital, Movement Disorders Unit, Neurology Department, Westmead, Australia;* [5]*ANZAC Research Institute SLHD, Northcott Neuroscience Laboratory, Concord, Australia;* [6]*University of Sydney, Faculty of Medicine and Health, Sydney, Australia;* [7]*Royal North Shore Hospital, Department of Neurology, Sydney, Australia;* [8]*Kolling Institute, Faculty of Medicine and Health, University of Sydney and Northern Sydney Local Health District, Department of Neurogenetics, St. Leonards, Australia;* [9]*Westmead Hospital, Department of Genetic Medicine, Westmead, Australia;* [10]*St. Vincent's Hospital, Clinical Neurosciences, Melbourne, Australia;* [11]*Concord Repatriation General Hospital, Department of Neurology, Sydney, Australia;* [12]*Concord Repatriation General Hospital, Molecular Medicine Laboratory, Concord, Australia;* [13]*Neuroscience Research Australia, Sydney, Australia;* [14]*Royal North Shore Hospital, Department of Clinical Genetics, St. Leonards, Australia;* [15]*St. Vincent's Hospital, Department of Neurology, Darlinghurst, Australia;* [16]*UNSW Sydney, School of Clinical Medicine, UNSW Medicine & Health, Sydney, Australia;* [17]*Gosford Hospital, Department of Neurology, Gosford, Australia;* [18]*University of New South Wales, Children's Cancer Institute, Sydney, Australia*

Objective: We investigated the contribution of genomic data reanalysis to the diagnostic yield of dystonia patients who remained undiagnosed after prior genome sequencing.

Methods: Probands with heterogeneous dystonia phenotypes who underwent initial genome sequencing (GS) analysis in 2019 were included in the reanalysis, which was performed through gene-specific discovery collaborations and systematic genomic data reanalysis.

Results: Initial GS analysis in 2019 ($n = 111$) identified a molecular diagnosis in 11.7% (13/111) of cases. Reanalysis between 2020 and 2023 increased the diagnostic yield by 7.2% (8/111); 3.6% (4/111) through focused gene-specific clinical correlation collaborative efforts [*VPS16* (two probands), *AOPEP* and *POLG*], and 3.6% (4/111) by systematic reanalysis completed in 2023 [*NUS1* (two probands) and *DDX3X* variants, and a microdeletion encompassing *VPS16*]. Seven of these patients had a high phenotype-based dystonia score ≥3. Notable unverified findings in four additional cases included suspicious variants of uncertain significance in *FBXL4* and *EIF2AK2*, and potential phenotypic expansion associated with *SLC2A1* and *TREX1* variants.

Conclusion: GS data reanalysis increased the diagnostic yield from 11.7% to 18.9%, with potential extension up to 22.5%. While

optimal timing for diagnostic reanalysis remains to be determined, this study demonstrates that periodic re-interrogation of dystonia GS datasets can provide additional genetic diagnoses, which may have significant implications for patients and their families.

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P11.037.A Detection of repeat expansions using exome sequencing in spinocerebellar degeneration

Jean-Loup Méreaux[1], *Claire-Sophie Davoine*[1], *Marie Coutelier*[1], *Léna Guillot-Noël*[1], *Anna Castrioto*[2], *Perrine Charles*[3], *Giulia Coarelli*[1;3], *Claire Ewenczyk*[3], *Stephan Klebe*[4], *Anna Heinzmann*[1], *Aurélie Méneret*[5], *Jean-Madeleine de Sainte Agathe*[6], *Alexis Brice*[1], *Alexandra DURR*[1;3]

[1]*Sorbonne Université, Paris Brain Institute - ICM, Inserm, CNRS, AP-HP, PARIS 13, France;* [2]*University Hospital Centre Grenoble Alpes, Department of Neurology, Grenoble, France;* [3]*Pitié-Salpêtrière Hospital, Assistance Publique-Hôpitaux de Paris (AP-HP), Genetics Department, Paris, France;* [4]*University Hospital Essen, Department of Neurology, Essen, Germany;* [5]*Pitié-Salpêtrière Hospital, Assistance Publique-Hôpitaux de Paris (AP-HP), Neurology Department, Paris, France;* [6]*Pitié-Salpêtrière Hospital, Assistance Publique-Hôpitaux de Paris (AP-HP), Molecular and Cellular Neurogenetics Department, Paris, France*

Consortium: SPATAX network

Background/Objectives: Repeat expansions in 20 genes represent two thirds of the known variants causing inherited spinocerebellar degeneration. A targeted sizing of one locus after the other is generally used for diagnosis. However, high-throughput sequencing can also detect expansions with a specific calling. We report this approach to promote exome as a global screening test.

Methods: 813 individuals with from 663 families with spinocerebellar degeneration were included after exclusion of frequent repeat expansions in 50% of the cohort (SCA1,2,3,6,7,17). *Expansionhunter* was used to call repeat sizes from exomes (TWIST capture and Illumina short read sequencing paired-end at 150 cycles). A targeted fragment analysis was done to confirm the presence of the expansions.

Results: We identified 34 individuals from 27 families with pathogenic expansions (4.2%) in *ATXN2*, *ATXN3*, *HTT*, *NOP56*, *ATXN1*, *ATXN7*, *CACNA1A* and *TBP*. All coding repeats were highly covered as expected. These exomes covered also non-coding repeats (*NOP56*, *ZFHX3* and *FMR1*) but not all (*ATXN10*, *BEAN1*, *DAB1*, *FGF14*, *FXN* and *RFC1*). Interestingly, five out of seven *HTT* expansions were incidental findings unrelated to the causal gene.

Conclusion: Exome can be used as a first-tier test in spinocerebellar degenerations to detect both, single nucleotide variants and expansions. Adding the capture of the uncovered non-coding repeats could improve the diagnosis yield. Analysis of multiple genes must be interpreted closely with the clinical data to consider frequent incidental findings such as the *HTT* expansion.

Grants: Fondation pour la Recherche Médicale, thesis grant 13338 to JLM.

Conflict of Interest: Jean-Loup Méreaux Fondation pour la Recherche Médicale, grant 13338 for PhD funding, Claire-Sophie Davoine: None declared, Marie Coutelier: None declared, Léna Guillot-Noël: None declared, Anna Castrioto: None declared, Perrine Charles: None declared, Giulia Coarelli: None declared, Claire Ewenczyk: None declared, Stephan Klebe: None declared, Anna Heinzmann: None declared, Aurélie Méneret: None declared, Jean-Madeleine de Sainte Agathe: None declared, Alexis Brice: None declared, Alexandra DURR: None declared

P11.038.B Diagnostic findings and yield of investigations requested for children with developmental regression

Kirsten Furley[1;2], *Katrina Williams*[2;3], *Michael C. Fahey*[4], **Matthew Hunter**[5]

[1]*Monash University, Paediatrics, Melbourne;* [2]*Monash Children's Hospital, Developmental Paediatrics, Melbourne, Australia;* [3]*Monash University, Head of Paediatrics, Melbourne, Australia;* [4]*Monash Health, Head of Neurology, Melbourne, Australia;* [5]*Monash Health, Head of Genetics, Melbourne, Australia*

Background: Childhood conditions that feature developmental regression are diverse and poorly understood. Phenotype-genotype characterisation and diagnostic yield data are needed to advance understanding of causal processes and inform clinical decision making. The objective of this study is to report childhood conditions featuring developmental regression and assess diagnostic yields of investigations.

Methods: A retrospective chart review of ninety-nine children presenting with developmental regression to an Australian tertiary paediatric genetic clinic.

Results: Of 99 children, 31% of (N = 31) children had intellectual disability (ID), 19% (N = 19) were autistic, 39% (N = 39) were autistic with ID, and 9% (N = 9) children did not have an ID or autism diagnosis. Thirty-two percent (N = 32) of children received a new informative diagnosis, including potential phenotypic expansion of genes for developmental regression. Genomic testing provided the highest diagnostic yield (51.1%, N = 24) and was highest, 63.6% (N = 14) for children with an ID. Fifty percent (N = 3) of children without autism or ID, and fifty percent of autistic children with an ID (N = 6) received an informative genomic result.

Conclusion: We highlight the heterogeneity of conditions featuring developmental regression and report phenotypic expansion not previously reported. The high diagnostic yield of genomic testing offers opportunities to identify causes and associations with diagnoses not previously reported to include regression.

Grants: First author is a PhD student and receives a stipend scholarship from Monash University

Conflict of Interest: None declared

P11.039.C Diagnostic yield of exome sequencing in treatment-resistant schizophrenia

Sam Bradbrook[1], *Tatiana Maroilley*[2;3], *Maja Tarailo-Graovac*[2;3], *Chad Bousman*[1;2;4;5], *PY Billie Au*[1;2], *Mark Colijn*[5]

[1]*Cumming School of Medicine, University of Calgary, Medical Genetics, Calgary, Canada;* [2]*Alberta Children's Hospital Research Institute, University of Calgary, Calgary, Canada;* [3]*University of Calgary, Biochemistry and Molecular Biology, Cumming School of Medicine, Calgary, Canada;* [4]*University of Calgary, Physiology and Pharmacology, and Community Health Sciences, Calgary, Canada;* [5]*Hotchkiss Brain Institute, Mathison Centre for Mental Health Research and Education, Psychiatry, Calgary, Canada*

Background/Objectives: Schizophrenia is highly heritable, but the majority of affected individuals do not have an identifiable monogenic cause. Treatment-resistant schizophrenia (TRS) is characterized by positive symptoms despite ≥2 antipsychotic trials, requiring treatment with clozapine. We hypothesize that exome sequencing (ES) may be diagnostically useful in these individuals.

Methods: We completed singleton research ES and clinical SNP microarray on 34 probands who were previously diagnosed with TRS in Southern Alberta. The ES data analysis prioritized: (1) variants with a variant allele frequency <1% in gNOMAD v.2 and TopMED BRAVO databases; (2) variants reported by ClinVar; and (3) variants predicted as damaging by in silico predictors. A curated panel of genes with a strong association with schizophrenia or psychosis and their interacting genes was created through a literature review. Rare variants in these genes that met filtration criteria were identified. Finally, targeted pharmacogenomic testing for recognized variants related to drug metabolism was completed.

Results: 1/34 probands had a diagnostic result on ES, while 26/34 carried rare variants in panel-related genes. Two unrelated probands had an identical variant detected in *TYRO3*. To our knowledge, this candidate gene has not been associated with a psychiatric phenotype in humans. Pharmacogenomic analysis for rapid/ultrarapid metabolizer status did not entirely account for any individual's treatment resistance. Lastly, there were four variants identified on microarray, which are not conclusively associated with psychotic symptoms.

Conclusion: Our exploratory study's findings suggest that ES may be indicated in TRS. Larger studies will need to be completed to clarify its diagnostic yield.

Conflict of Interest: Sam Bradbrook: None declared, Tatiana Maroilley: None declared, Maja Tarailo-Graovac: None declared, Chad Bousman Founder, PY Billie Au: None declared, Mark Colijn: None declared

P11.040.D Phenotypic characterization of individuals with schizophrenia with expression and splicing outliers in whole transcriptomes

Laura Kilarski[1], *Isabelle Claus*[2], *Vicente Yépez*[3], *Franziska Degenhardt*[4], *Markus Wolfien*[5], *Kristina Adorjan*[6;7], *Monika Budde*[6], *Janos Kalman*[6;8], *Mojtaba Oraki Kohshour*[6], *Sergi Papiol*[6;8], *Fanny Senner*[6;8], *Andreas Forstner*[2], *Urs Heilbronner*[6], *Markus M. Nöthen*[2], *Peter Falkai*[8], *Thomas G. Schulze*[6;9], *Julien Gagneur*[10;11], *Eva C. Schulte*[1;2;6]

[1]*University of Bonn, Department of Psychiatry and Psychotherapy, Bonn, Germany;* [2]*University of Bonn, Institute of Human Genetics, Bonn, Germany;* [3]*Technical University Munich, Computational Molecular Medicine, Munich, Germany;* [4]*University of Essen, Department of Child and Adolescent Psychiatry, Essen, Germany;* [5]*University of Rostock, Department of Systems Biology & Bioinformatics, Rostock, Germany;* [6]*University of Munich, Institute of Psychiatric Phenomics and Genomics (IPPG), Munich, Germany;* [7]*University of Bern, University Hospital of Psychiatry and Psychotherapy, Bern, Switzerland;* [8]*University of Munich, Department of Psychiatry & Psychotherapy, Munich, Germany;* [9]*SUNY Upstate Medical University, Department of Psychiatry, Syracuse, NY, United States;* [10]*Technical University Munich, Computational Molecular Medicine, School of CIT, Munich, Germany;* [11]*Technical University Munich, Intitute of Human Genetics, School of Medicine and Health, Munich, Germany*

Background: Rare genetic variants with high effect sizes for schizophrenia (SCZ) have been identified, yet functional consequences and impact on individual phenotypes as well as their clinical relevance in unselected, naturalistic cohorts remain unclear.

Methods: Expression and splicing outliers were identified in whole blood transcriptomes of 538 individuals with DSM-IV SCZ-spectrum diagnoses from the PsyCourse Study[1] using OUTRIDER/FRASER[2] and filtered for known high-confidence candidate genes ($n = 417$) implicated in GWAS, rare variant association studies, and SCZ-linked copy number variants (CNVs). Comparisons were made between cases with and without outliers across >20 biometric and clinical characteristics.

Results: We found 41 expression or splicing outliers in candidate genes ($|z\text{-score}| > 5.0$, $FDR < 0.1$) across 5.2% of 538 individuals, half of which affected known CNVs. Splicing outliers in at least one gene were present in 10 individuals (1.9%), with three genes (*SRRM2, TMEM219, PDE4B*) showing the same pattern of aberrant splicing in more than one individual. We observed significantly lower clinical severity and higher verbal intelligence in cases carrying expression outliers. Outliers in genes overlapping a known CNV at 16p11.2 correlated with a younger age at onset.

Conclusion: Outlier analysis can identify individuals with severe changes in expression/splicing of genes implicated in SCZ, highlighting the role of rare, strong effect genetic factors in this common, genetically complex phenotype. Specific phenotypic profiles in individuals with outliers could be related to unrecognized subphenotypic groups. The identification of the genetic variants underlying the outliers is currently underway.

Grants: None.

References:

1.Budde, M *et al*. 2019

2. Yépez, VA *et al*. 2021

Conflict of Interest: None declared

P11.041.B Analysis of targeted epilepsy gene panel sequencing suggests that variants previously classified as uncertain significance in QARS1 and SCN9A genes may play an important role in epilepsy

Yara Hussein[1], **Hila Weisblum Neuman**[2], *Bruria Ben-Zeev*[2;3], *Shani Stern*[1]

[1]*University of Haifa, Sagol Department of Neurobiology, Faculty of Natural Sciences, Haifa, Israel;* [2]*Sheba, Pediatric Neurology Unit, The Edmond and Lily Safra Children's Hospital, Ramat Gan, Israel;* [3]*Tel Aviv University, Faculty of Medicine, Tel Aviv-Yafo, Israel*

Background/Objectives: Epilepsy, a chronic neurological disorder marked by seizures or epileptic syndrome, encompasses various etiologies, and carries implications for neurological, cognitive, psychological, and social well-being. There are over 500 genes associated with epilepsy occurrence. Therefore, Next-Generation Sequencing (NGS) epilepsy gene panels are commonly employed for patients with epilepsy. However, a substantial proportion of results remains uncertain and is not considered directly causative.

Methods: Between 2018-2022, a commercial epilepsy gene panel testing was conducted on pediatric patients at a single tertiary health center. Utilizing NGS technology, the panel

analyzed 187 genes associated with epilepsy, categorizing variants according to ACMG guidelines. A retrospective analysis of 156 probands and 74 parents delved into prevalence, population comparisons, and protein network path analysis, enhancing genetic comprehension. Advanced tools were employed for identifying notable variant combinations and calculating genetic correlations.

Results: Targeted gene panel sequencing revealed 18% negative, 22% positive and 60% uncertain outcomes among probands. Frequent pathogenic gene detections included SCN1A, MECP2, and CDKL5. Notably, two distinct variants, c.2133G>C in the SCN9A gene and c.316G>A in the QARS1 gene, were identified as likely pathogenic in contrast to their formal interpretation as variants of uncertain significance.

Conclusion: Epilepsy gene panel enables targeted gene analysis, emphasizing its clinical significance. The study elucidates the intricate genetic landscape of infantile and childhood epilepsy, with approximately 60% uncertainty in test results. We suggest an interpretation of two clinically significant variants in the QARS1 and SCN9A genes.

Grants: The Zuckerman STEM Leadership Program and Israel Science Foundation grants 1994/21 and 352/21.

Conflict of Interest: None declared

P11.042.A Whole genome sequencing reveals *CSTB* repeat expansions in two affected siblings with progressive myoclonus, ataxia, and dystonia

Ivana Dzinovic[1;2], *Matej Škorvánek*[3;4], *Robert Jech*[5], *Erik Tilch*[1;2], *Denisa Harvanova*[6], *Janette Baloghova*[7], *Barbara Schormair*[1;2], *Robert Kopajtich*[1;2], *Holger Prokisch*[1;2], *Juliane Winkelmann*[1;2;8], *Michael Zech*[1;2;9]

[1]Institute of Human Genetics, School of Medicine, Technical University of Munich, Munich, Germany; [2]Institute of Neurogenomics, Helmholtz Zentrum München, Munich, Germany; [3]P.J. Safarik University, Department of Neurology, Kosice, Slovakia; [4]University Hospital of L. Pasteur, Department of Neurology, Kosice, Slovakia; [5]Charles University in Prague, 1st Faculty of Medicine and General University Hospital in Prague, Department of Neurology, Prague, Czech Republic; [6]Faculty of Medicine, P. J. Safarik University, Associated Tissue Bank, Kosice, Slovakia; [7]Faculty of Medicine, P. J. Safarik University, Kosice, Slovakia, Department of Dermatovenerology, Kosice, Slovakia; [8]Munich Cluster for Systems Neurology (SyNergy), Munich, Germany; [9]Institute for Advanced Study, Technical University of Munich, Garching, Germany

Background/Objectives: Repeat expansions (REs) represent one of the major understudied types of genetic variation in cohorts of patients with movement disorders. Furthermore, variants residing outside of the coding regions usually escape detection via standard diagnostic methods still focused on exonic sequences. Therefore, within a cohort of patients with predominantly dystonic movement abnormalities and previously unyielding whole exome sequencing analysis we performed whole genome sequencing (WGS) studies.

Methods: ExpansionHunter algorithm (1) was implemented on WGS data to examine REs at thirty well-characterized disease-associated loci. Simultaneously, bulk proteomic and transcriptomic data were processed according to described protocols (2) from the patient-driven fibroblasts to check for expression changes associated with candidate genomic alterations.

Results: Pathogenic biallelic dodecamer REs (repeat count 38 and 32) within the 5`UTR of the gene *CSTB* (cystatin B) were identified in a pedigree with two similarly affected siblings presenting with adolescence-onset progressive myoclonus syndrome, epilepsy, ataxia, and upper-limb dystonia. Unaffected parents had one expanded allele each. Confirming the genomic findings, affected siblings had significantly reduced *CSTB* gene expression on both mRNA (p-value: $1.3e^{-22}$ and $5.2e^{-20}$) and protein level (p-value: $1.4e^{-10}$ and $3.2e^{-7}$) compared to control participants who did not carry *CSTB* REs.

Conclusion: Comprehensive genome analysis enables the detection of the pathogenic genetic variation beyond small coding variants and can significantly shorten the diagnostic odyssey for individual families with rare movement disorders.

References:

(1) Dolzhenko et al. *Bioinformatics* 2019; (2) Kopajtich et al. *medRxiv* 2021

Grants: Deutsche Forschungsgemeinschaft, Slovak Grant and Development Agency

Conflict of Interest: None declared

P11.043.B Pathophysiology of a neurodevelopmental disorder caused by biallelic SLC4A10 loss-of-function

Antje K. Huebner[1], Lutz Liebmann[1], James Fasham[2], Reham Khalaf-Nazzal[3], Reza Maroofian[4], Kai Kaila[5], Andrew Crosby[2], Emma Baple[2], **Christian Hübner**[1]

[1]Jena University Hospital, Institute of Human Genetics, Jena; [2]University of Exeter Medical School, RILD Wellcome Wolfson Centre, Exeter, United Kingdom; [3]Arab American University of Palestine, Jenin, Palestine; [4]Molecular and Clinical Sciences Institute, London, United Kingdom; [5]Helsinki Institute of Life Science, Helsinki, Finland

Background/Objectives: *SLC4A10* encodes a plasma-membrane HCO_3^- transporter, which utilizes the Na^+ gradient to mediate acid extrusion. In the brain, *SLC4A10* is broadly expressed in neurons and choroid plexus epithelial cells. We recently reported that biallelic *SLC4A10* loss-of-function variants cause a neurodevelopmental disorder with hypotonia, intellectual disability and behavioral abnormalities. Neuroradiological findings include microcephaly, abnormalities of the corpus callosum and collapsed brain ventricles.

Methods: We used constitutive *Slc4a10* deficient mice to study Slc4a10 loss of function in vitro and in vivo. Apart from behavioral experiments, we assessed the consequences for synaptic transmission and network excitability.

Results: *Slc4a10* deficient mice show mild behavioural abnormalities. As in some patients, the brain of *Slc4a10* deficient mice is smaller and the weight reduced compared to controls. While the analysis of the cortical structure does not show gross alterations, we note collapsed brain ventricles in Slc4a10 deficient mice resembling findings in patients. We further show that SLC4A10 is abundantly expressed at inhibitory presynapses. Suggesting that Slc4a10 is important for the regulation of the presynaptic pH, GABA release is compromised at inhibitory synapses. Importantly, modulating brain pH partially rescues GABA release.

Conclusion: As GABAergic inhibitory interneurons gate signal flow and contribute to the timing and synchronization of cortical oscillations, we propose that compromised synaptic inhibition due to SLC4A10 loss-of-function likely contributes to the pathophysiology of this disorder. We hypothesize that modulation of GABAergic transmission could be a possible therapeutic approach for ameliorating some of the neurological symptoms in patients.

Grants: DFG Hu 800/16-1 to CAH

Conflict of Interest: None declared

P11.044.C Association between polygenic risk and imaging derived phenotypes for the elucidation of essential tremor neuroanatomical vulnerabilities

Miranda Medeiros[1], *Alexandre Pastor-Bernier*[2], *Houman Azizi*[2;3;4], *Zoe Schmilovich*[1], *Patrick Dion*[2;5], *Alain Dagher*[2;5], *Guy Rouleau*[1;2;5]

[1]*McGill University, Human Genetics;* [2]*Montreal Neurological Institute;* [3]*McGill University;* [4]*Douglas Mental Health Institute;* [5]*McGill University, Neurology and Neurosurgery*

Background/Objectives: Essential tremor (ET) is a complex neurological disorder characterized by involuntary upper limb shaking. ET associated common variants can be leveraged to generate polygenic risk scores (PRS) to capture individuals' ET specific genetic risk. Here, we aim to identify regions of the brain made vulnerable due to heightened genetic risk of ET in healthy subjects by investigating the association of white matter diffusion-weighted magnetic resonance imaging (DTI), grey matter DTI and morphometry with ET PRS in the UK Biobank (UKB) cohort.

Methods: We paired genetic and imaging data from the UKB to model the associations of ET PRS on brain structure changes. Polygenic risk scores from healthy UKB participants were calculated using ET GWAS summary statistics (Liao et al., 2022) through PRS-CS. DTI mean diffusion measures (DM) fractional anisotropy (FA), mean diffusivity (MD), and free-water (FW), were obtained across 73 anatomically curated tracts in the ORG fiber clustering white matter atlas (Zhang et al., 2018). We performed multiple regression analysis with glmnet (R) for variable selection and cross-validation and conducted a white matter to grey matter tract crossing and volume analysis.

Results: Significant associations between ET PRS and DTI cerebellar microstructure (e.g., cerebellar peduncles and intracerebellar Purkinje tracts) and associations between ET PRS and grey mater DTI microstructure, in particular the red nucleus, hypothalamus, caudate, putamen, and several regions of a functional thalamus were identified.

Conclusion: We have found that most regions associated with ET PRS are part of a known motor network involved in ET pathogenesis.

Grants: N/A

Conflict of Interest: None declared

P11.045.D *RORA*-related neurodevelopmental disorder: A rare condition linking intellectual disability, cerebellar hypoplasia, and myoclonic seizure types.

Mariagrazia Talarico[1;2;3], *Julitta De Bellescize*[4], *Matthias De Wachter*[5], *Bertrand Isidor*[6;7], *Benjamin Cogne*[6;7], *Paul Rollier*[8], *Melanie FRADIN*[8], *Laurent Pasquier*[8], *Matthieu Egloff*[9;10], *Xavier Le Guillou Horn*[9;10], *Ilaria Guella*[11], *Scott Hickey*[12], *Paul Benke*[13], *amelle shillington*[14], *Candy Kumps*[15], *Olivier Vanakker*[15], *Erica Gerkes*[16], *Shenela Lakhani*[17], *Irina Romanova*[18], *Ilya Kanivets*[19], *Melanie Brugger*[20], *Katharina Vill*[20;21], *Raymond Caylor*[22], *Cindy Skinner*[22], *Rory Tinker*[23], *tommy stodberg*[24], *Astrid Nümann*[25], *Tobias Haack*[26], *Natalie Deininger*[26], *Holger Hengel*[27], *jeanne jury*[28], *Solène Conrad*[28], *grace yoon*[29], *Melissa Tsuboyama*[30], *Sonal Mahida*[30], *Giulia Barcia*[31], *Cyril Gitiaux*[31], *Marlene Rio*[31], *Andrea Bevot*[32], *Sylvia Quemener*[33;34;35], *Kevin Uguen*[33;34;35], *Antje Wonneberger*[36], *Dagmar Timmann-Braun*[37], *danielle hays Karlowicz*[38], *Katrin Ounap*[39;40], *Nicolas Chatron*[2;3], *Susanne Kjaergaard*[41], *Sebastien Küry*[28], *Sara Cabet*[3;42], *Gaetan Lesca*[2;3]

[1]*University Magna Graecia of Catanzaro, Department of Medical and Surgical Sciences, Catanzaro, Italy;* [2]*Hospices Civils de Lyon, Service de Génétique, Lyon, France;* [3]*Université de Lyon - Université Claude Bernard Lyon 1, INSTITUT NEUROMYOGÈNE, Pathology and Genetics of neuron and muscle, CNRS UMR, Lyon, France;* [4]*Hospices Civils de Lyon, Epilepsy, Sleep and Pediatric Neurophysiology Department, Lyon, France;* [5]*University of Antwerp, Department of Pediatric Neurology, Antwerp, Belgium;* [6]*CHU Nantes, Service de Génétique Médicale, Nantes, France;* [7]*University of Nantes, Institut du thorax, INSERM, CNRS, Nantes, France;* [8]*CHU Rennés, Service de Génétique Clinique, Centre Référence "Déficiences Intellectuelles de causes rares" (CRDI), Centre de référence anomalies du développement CLAD-Ouest, Rennés, France;* [9]*CHU de Poitiers, Service de Génétique Médicale, Poitiers, France;* [10]*Université de Poitiers, Poitiers, France;* [11]*University of British Columbia, Centre for Applied Neurogenetics, Djavad Mowafaghian Centre for Brain Health, Vancouver, Canada;* [12]*Ohio State University College of Medicine, Department of Pediatrics, Ohio, United States;* [13]*Joe DiMaggio Children's Hospital, Hollywood, United States;* [14]*Cincinnati Children's Hospital Medical Center, Division of Human Genetics, Cincinnatti, United States;* [15]*Ghent University Hospital, Center for Medical Genetics, Ghent, Belgium;* [16]*University Medical Center Groningen, Clinical Genetics, Groningen, Netherlands;* [17]*Brain and Mind Research Institute, Center for Neurogenetics, Weill Cornell Medicine, New York, United States;* [18]*Kazan State University, Department of Medical Genetics, Moscow, Russian Federation;* [19]*Svt. Luka's Institute of Child Neurology and Epilepsy, Department of Neurology, Moscow, Russian Federation;* [20]*Technical University of Munich, Institute of Human Genetics, School of Medicine, Munich, Germany;* [21]*Ludwig-Maximilians-University, Department of Pediatric Neurology and Developmental Medicine and LMU Center for Children with Medical Complexity, Munich, Germany;* [22]*Greenwood Genetic Center, Greenwood, United States;* [23]*Vanderbilt University Medical Center, Department of pediatrics, Medical Center North, Nashville, United States;* [24]*Karolinska University Hospital, Pediatric Neurology, Stockholm, Sweden;* [25]*Charité University Medcicine Berlin, Department of Neurology, Berlin, Germany;* [26]*University of Tübingen, Institute of Medical Genetics and Applied Genomics, Tübingen, Germany;* [27]*University of Tübingen, Center for Neurology and Hertie-Institute for Clinical Brain Research, Tübingen, Germany;* [28]*CHU de Nantes, Genetique medicale, Nantes, France;* [29]*The Hospital for Sick Children, Clinical and Metabolic Genetics, Toronto, Canada;* [30]*Boston Children's Hospital, department of neurology, Boston, United States;* [31]*Hôpital Necker-Enfants Malades, Fédération de Génétique, Service de Génétique Moléculaire, Paris, France;* [32]*University Children's Hospital Tübingen, Neuropediatrics, Tübingen, Germany;* [33]*CHU de Brest, Service de Génétique Médicale, Brest, France;* [34]*CHU de Brest, Centre de Référence Déficiences Intellectuelles, Service de Pédiatrie, Brest, France;* [35]*University of Brest, Brest, France;* [36]*Jena University Hospital, Department of Neuropediatrics, Jena, Germany;* [37]*Essen University Hospital, University of Duisburg-Essen, Department of Neurology and Center for Translational Neuro- and Behavioral Sciences (C-TNBS), Essen, Germany;* [38]*Duke University Medical Center, Division of Medical Genetics, Department of Pediatrics, Durham, United States;* [39]*Tartu University Hospital, Department of Clinical Genetics, Genetics and Personalized Medicine Clinic, Tartu, Estonia;* [40]*University of Tartu, Institute of Clinical Medicine, Tartu, Estonia;* [41]*Rigshospitalet, Clinical genetic department, Copenhagen, Denmark;* [42]*Hospices Civils de Lyon, Claude Bernard Lyon 1 University, 1)Pediatric and Fetal Imaging Department, Femme-Mère-Enfant Hospital, Lyon, France*

Background/Objectives: *RORA* encodes the retinoic acid receptor-related orphan receptor α (RORα), which plays a pivotal role in the cerebellar maturation and function. A recent study reported 16 individuals affected with *RORA*-associated neurodevelopmental disorder (*RORA*-NDD). Here, we report a larger series of patients with a special focus on the cerebellar pathology and the epilepsy spectrum.

Methods: 40 individuals carrying *RORA* pathogenic and likely pathogenic variants were recruited through an international collaboration. Clinical data were collected by a standardized survey. Brain MRI files were re-analysed when available.

Results: 33 *RORA* variants were identified, including 14 frameshift deletions, 4 frameshift insertions, 9 missense, and 6 nonsense variants. They occurred de novo in 18 individuals, were inherited

in 9, and not determined in 13. Three cardinal features identified in *RORA*-NDD were cognitive impairment, cerebellar hypoplasia or atrophy or both, and epilepsy. Four individuals presented progressive cerebellar symptoms. Three epilepsy phenotypic subgroups were identified: i) genetic generalized epilepsy (n = 10), falling within the eyelid myoclonia seizure spectrum in five cases and characterized by myoclonic absence epilepsy in two cases; ii) developmental and epileptic encephalopathy (n = 5, including one myoclonic); iii) unclassified myoclonic epilepsy (n = 3). Autism was diagnosed in 21% of individuals. Missense variants in the DNA-binding domain tended to induce a more severe phenotype than those in the ligand-binding domain.

Conclusion: This study provides a more comprehensive clinical and radiological analysis of *RORA*-NDD with deeper phenotyping of cerebellar manifestations and epilepsy (with a striking range of myoclonic seizures), each present in more than 50% of cases.

Grants: No grants to declare

Conflict of Interest: None declared

P11.046.A Genomics research of lifetime depression in the Netherlands

Floris Huider[1], *Yuri Milaneschi*[2], *Jouke-Jan Hottenga*[1], *Mariska Bot*[2], *Liset Rietman*[3], *Almar Kok*[4], *Tessel Galesloot*[5], *Leen 't Hart*[6], *Femke Rutters*[7], *Marieke Blom*[7], *Didi Rhebergen*[8], *Marjolein Visser*[9], *Ingeborg Brouwer*[9], *Edith Feskens*[10], *Catharina Hartman*[11], *Albertine Oldehinkel*[11], *Eco de Geus*[1], *Bart Kiemeney*[5], *Martijn Huisman*[4], *Susan Picavet*[3], *Monique Verschuren*[3], *Hanna van Loo*[11], *Brenda Penninx*[2], *Dorret Boomsma*[1]

[1]*Vrije Universiteit Amsterdam, Biological Psychology, Amsterdam, Netherlands;* [2]*Amsterdam UMC location Vrije Universiteit Amsterdam, Department of Psychiatry, Amsterdam, Netherlands;* [3]*Dutch National Institute for Public Health and the Environment, Center for Prevention, Lifestyle and Health, Bilthoven, Netherlands;* [4]*Amsterdam UMC location Vrije Universiteit Amsterdam, Department of Epidemiology and Data Science, Amsterdam, Netherlands;* [5]*Radboud University Medical Center, Nijmegen, Netherlands;* [6]*Leiden Medical Center, Leiden, Netherlands;* [7]*Amsterdam UMC location Vrije Universiteit Amsterdam, Amsterdam, Netherlands;* [8]*GGz Centraal, locatie Zon & Schild, Amersfoort, Netherlands;* [9]*Vrije Universiteit Amsterdam, Department of Health Sciences, Amsterdam, Netherlands;* [10]*Wageningen University & Research, Division of Human Nutrition and Health, Wageningen, Netherlands;* [11]*University of Groningen, University Medical Center Groningen, Department of Psychiatry, Groningen, Netherlands*

Consortium: BIONIC

Background/Objectives: Although broad phenotyping strategies have led to significant genetic discoveries for major depressive disorder (MDD), there is a call for uniform in-depth phenotyping that adheres to clinical criteria; both to replicate the findings thus far and to fine-tune the genetic signal in the search for clinically relevant findings.

Methods: In the Netherlands we have established the Biobanks Netherlands Internet Collaboration (BIONIC) among biobanks with genotype data assessed in their participants, with the aim of facilitating Genome-wide Association Studies (GWAS) in a relatively homogeneous population. We collected data on DSM-5 lifetime MDD diagnosis, sociodemographic traits, depressive symptom and episode characteristics and psychiatric history in 132,850 Dutch individuals with a standardized instrument that can be administered online; 66,684 individuals also have genome-wide SNP data available. To test for genetic associations between case-control status and imputed SNP data, the combined datasets were analyzed with fastGWA.

Results: Data from 16 cohorts were combined to create a dataset of N = 29,650 lifetime MDD cases (22%) meeting DSM-5 criteria and 94,300 screened controls. In the GWAS dataset of N = 66,684 Dutch individuals the prevalence of MDD was 25.3%. The heritability of the MDD phenotype - based on pedigree analyses - is 34%. The first GWAS of lifetime MDD in the Netherlands will be presented, with results obtained through mega- and meta-analysis approaches.

Conclusion: We initiated this project because the complex genetic basis of MDD requires large population-wide studies with uniform in-depth phenotyping, and show the feasibility of such an approach.

Conflict of Interest: None declared

P11.047.B Unraveling the impact of copy number variants in individuals in early phases of psychosis

Nadezhda Zhukova[1;2], *Guillaume Huguet*[3], *Qin He*[1;2], *Anton Iftimovici*[1;2;4], *Chuan JIAO*[1;2], *Ridha Joober*[5], *Marie-Odile Krebs*[1;2;4], *Sebastien Jacquemont*[3], *Guy Rouleau*[6], *Boris Chaumette*[2;4;5]

[1]*Université Paris Cité, Paris, France;* [2]*Institute of Psychiatry and Neuroscience of Paris (IPNP), INSERM U1266, Paris, France;* [3]*Centre de Recherche du Centre Hospitalier Universitaire Sainte-Justine, Montréal, Canada;* [4]*GHU-Paris Psychiatrie et Neurosciences, Paris, France;* [5]*McGill University, Department of Psychiatry, Montréal, Canada;* [6]*Montreal Neurological Institute and Hospital, McGill University, Department of Neurology and Neurosurgery, Montréal, Canada*

Background/Objectives: Psychotic disorders develop progressively during adolescence though multiple stages: ultra-high-risk (UHR) state, first episode of psychosis (FEP) and, ultimately, chronic presentation. Approximately 20% of UHR individuals transition to psychosis within a 2-year period (converters), while others maintain attenuated psychotic symptoms or recovered (non-converters). Additionally, FEP can be the first manifestation of schizophrenia or bipolar disorder. Accurate prediction of the outcome is crucial for targeted interventions. We hypothesized that genetic can help to develop prognostic biomarkers. Leveraging Next-Generation Sequencing (NGS) advancements, this study delves into the association between rare copy number variants (CNVs) and psychosis in UHR and FEP individuals, aiming to develop a predictive model.

Methods: A cohort of 396 individuals was sequenced using IlluminaSeq. Whole-genome sequencing data was analyzed with the Illumina Dragen pipeline. After calling, the CNVs were annotated with ClassifyCNV. Differences between FEP, converters, non-converters were tested for various CNV parameters (numbers, lengths, encompassed genes, etc.).

Results: This study found 5 known pathogenic and 3 likely pathogenic CNVs in the population. However, no significant differences were found between the clinical groups regarding the number of CNVs, their size, the presence of brain-expressed genes.

Conclusion: While confirming the presence of schizophrenia associated CNVs in this specific population of early phases of psychosis, the study highlights the current limitations in using CNV data for discrimination of individuals based on their clinical outcome. Consequently, future developments may consider incorporating other types of omics data besides clinical information to enhance the effectiveness of prediction strategies.

Grants: Brain Canada, MESSIDORE SchizOme

Conflict of Interest: None declared

P11.048.C Personalised penetrance estimation in *C9orf72*-related amyotrophic lateral sclerosis and frontotemporal dementia

Andrew Douglas[1;2], *Alexander Thompson*[2], *Martin Turner*[2], *Kevin Talbot*[2]

[1]*Oxford University Hospitals NHS Foundation Trust, Oxford Centre for Genomic Medicine, Oxford, United Kingdom;* [2]*University of Oxford, Nuffield Department of Clinical Neurosciences, Oxford, United Kingdom*

Background/Objectives: *C9orf72* hexanucleotide repeat expansions are the most common genetic cause of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) in European populations. Variation in disease penetrance between families presents a challenge for genetic counselling of at-risk relatives and reduces predictive utility of testing asymptomatic relatives. We have developed a model for estimating disease penetrance in individual families affected by *C9orf72* using available family history information, allowing calculation of personalised risk estimations for at-risk relatives.

Methods: Published aggregated age-of-onset data for *C9orf72*-related ALS/FTD were used to generate a cumulative relative risk curve for unaffected at-risk individuals within example pedigrees based on current ages. Age-related relative risks are combined with a priori chance of an individual carrying an expansion based on known pedigree information. Penetrance is calculated as number of affected individuals divided by sum of cumulative age-related risks of relatives being affected by 80 years.

Results: This model behaves as expected, with family history influencing penetrance estimation. Complete absence of family history information in an affected individual yields an assumed penetrance of 100%, while penetrance reduces as number and age of at-risk unaffected relatives increases. A sporadic case with a typical three-generation pedigree has an estimated penetrance similar to or even below that for *C9orf72* carriers in control populations.

Conclusion: Family history is currently the best indicator of penetrance in *C9orf72* carriers. Calculating family-specific penetrance would aid genetic counselling by allowing at-risk relatives a more accurate understanding of their individual risk.

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Conflict of Interest: Andrew Douglas: None declared, Alexander Thompson: None declared, Martin Turner Scientific Advisory role to Biogen and Novartis., Kevin Talbot: None declared

P11.050.A Rare variants in *PUM2* may cause a neurodevelopmental phenotype

Amica Müller-Nedebock[1], *Sabrina K. Phanor*[2], *Serena Raimo*[2], *Alice Ibrahima*[3], *Alison Male*[4], *annick REIN ROTHSCHILD*[5], *Anne-Marie Guerrot*[6], *Aurélia Jacquette*[7], *Boris Keren*[8], *Damian Smedley*[9], *Diana Carrasco*[10], *François Lecoquierre*[11], *Joel Krier*[12], *Kristen Park*[13], *Lauren C. Briere*[14], *Ludmila Jornea*[3], *Martine Doco-Fenzy*[15], *Nathalie Couque*[16], *Nino Spataro*[17], *Noor Smal*[18], *Pia Zacher*[19], *Pilar Cacheiro*[9], *sarah funtowiez*[5], *Sarah Weckhuysen*[20], *Sylvie Forlani*[21], *Thomas Husson*[11], *Wendy Jones*[4], *YLINE CAPRI*[22], *Vincenzo Alessandro Gennarino*[2], *Rami Abou Jamra*[1]

[1]*Institute of Human Genetics, University of Leipzig Medical Center, Leipzig, Germany;* [2]*Department of Genetics and Development, Columbia University Irving Medical Center, New York, NY, USA;* [3]*Paris Brain Institute-Institut du Cerveau-ICM, Sorbonne University, Inserm U1127, CNRS UMR 7225, AP-HP-Hôpital Pitié-Salpêtrière, 75013 Paris, France;* [4]*Department of Clinical Genetics, Great Ormond Street Hospital, London, UK;* [5]*Institute for Rare Diseases, Sheba Medical Center, Tel Hashomer, Israel;* [6]*Department of Genetics, Rouen University Hospital, Normandy Centre for Genomic and Personalized Medicine, 76821 Rouen, France.;* [7]*Genetic consultation, Alençon Hospital, Normandy France;* [8]*UF de Génomique du Développement, Centre de Génétique Moléculaire et Chromosomique, AP-HP.Sorbonne Université - Hôpital de La Pitié-Salpêtrière, Paris, France.;* [9]*William Harvey Research Institute, Charterhouse Square, Barts and the London School of Medicine and Dentistry Queen, Queen Mary University of London, London, UK;* [10]*Department of Clinical Genetics, Cook Children's Hospital, Fort Worth, Texas, USA;* [11]*Department of Genetics and Reference Center for Developmental Disorders, Normandie Université, UNIROUEN, Inserm U1245 and Rouen University Hospital, Rouen, France;* [12]*12 Division of Genetics, Department of Medicine, Brigham and Women's Hospital, Boston, MA 02115, USA;* [13]*Anschutz Medical Campus Department of Pediatrics and Neurology, University of Colorado School of Medicine, Aurora, CO, USA;* [14]*Center for Genomic Medicine & Department of Medical Genetics and Metabolism, Massachusetts General Hospital, Boston, MA 02114, USA;* [15]*Service de Génétique, CHU Reims, Reims and Service de Génétique, CHU Nantes, Nantes, France;* [16]*Department of Genetics, APHP-Robert Debré University Hospital, 75019 Paris, France.;* [17]*Center for Genomic Medicine, Parc Taulí Hospital Universitari, Institut d'Investigació i Innovació Parc Taulí (I3PT-Cerca), Universitat Autònoma de Barcelona, Sabadell, Spain;* [18]*VIB Center for Molecular Neurology, Edegem, Belgium;* [19]*Epilepsy Center Kleinwachau, Radeberg, Germany;* [20]*Division of Neurology, University Hospital Antwerp, Antwerp, Belgium;* [21]*ICM DNA and Cell Bank CNRS UMR7225, INSERM U1127, Sorbonne University, Hôpital de la Pitié-Salpêtrière, 47 Bd de l'Hôpital, 75013 Paris, France.;* [22]*Département de Génétique, Assistance Publique des Hôpitaux de Paris (AP-HP) Hôpital Robert Debré, 75019 Paris, France*

Background/Objectives: Many genetic variants have been associated with neurological disorders, but far less is known about the influence of changes at the level of post-transcriptional regulation. We have previously shown that the RNA-binding protein Pumilio1 (PUM1) is important for neurological function; variants in *PUM1* can cause either early-onset or late-onset disease (SCA47). However, there have not yet been reports of patients with variants in PUM1's homolog, PUM2, which recognizes the same RNA binding motif. We therefore sought to identify patients and characterize the effects of *PUM2* variants.

Methods: We identified 12 individuals exhibiting a neurodevelopmental phenotype with *PUM2* variants, and additional cases are currently under investigation. Comprehensive clinical data from consulting clinicians for all individuals was collected using standardized forms.

Results: Among the 12 cases, eight had variants predicted to lead to loss of function (comprising three canonical splice site, two nonsense, and three frameshift truncation variants). The remaining four cases had missense variants in regions intolerant to variation (Metadome). De novo variants were observed in ten individuals; for one elderly individual the parents were unavailable for testing and for another individual the father was unavailable for testing. The predominant clinical features encompassed developmental delay (11/12), seizures (6/12), and behavioral abnormalities (5/12).

Conclusion: Considering PUM2's established roles in neural development and synaptic function, we propose that *PUM2* variants may cause developmental delay with or without seizures and behavioral problems. Functional studies are currently in progress to gain a better understanding of the underlying disease mechanism.

Grants: Lauren C. Briere is supported by an NIH grant, U01NS134357

Conflict of Interest: None declared

P11.051.B SCA27B is a common cause of late-onset chronic ataxia in the Czech Republic

Emílie Vyhnálková[1], *Martin Vyhnálek*[2], *Jaroslava Paulasová Schwabová*[2], *Alena Zumrová*[3], *Jaroslav Jeřábek*[3], *Simona Karamazovová*[2], *Kuzmiak Michaela*[2], *Zuzana Blichová*[2], *Lucie Šťovíčková*[3], *Markéta Havlovicová*[4], *Zuzana Musova*[4]

[1]University Hospital at Motol and Charles University, Second Faculty of Medicine, Center of Hereditary ataxias, Department of Biology and Medical Genetics, Praha 5; [2]University Hospital at Motol and Charles University, Second Faculty of Medicine, Center of Hereditary ataxias, Department of Neurology, Praha 5; [3]University Hospital at Motol and Charles University, Second Faculty of Medicine, Center of Hereditary ataxias, Department of Paediatric Neurology, Praha 5; [4]University Hospital at Motol and Charles University, Second Faculty of Medicine, Department of Biology and Medical Genetics, Praha 5

Consortium: ERN-RND

Background/Objectives: SCA27B is a newly discovered autosomal dominant late-onset ataxia caused by the expansion of GAA repeats in the first intron of the fibroblast growth factor-14 gene (*FGF14*). Excellent response to treatment with 4-aminopyridine has been reported in patients.

Methods: Twenty-eight patients with chronic adult-onset degenerative ataxia of unknown origin, followed at the Center of Hereditary Ataxia, University Hospital at Motol, were tested for the *FGF14* GAA repeat expansion.

Results: The expansion in the *FGF14* gene within the pathological range ($GAA_{\geq 300}$) was found in 11 (39%) patients, and three patients (11%) had a borderline repeat length ($GAA_{250-299}$) with presumed incomplete penetrance. Eight patients had a positive family history of ataxia in a parent or sibling, and 6 cases were sporadic. The mean age of onset was 52 years. All patients had progressive cerebellar syndrome. Four patients (37%) presented with a parkinsonian syndrome. Significant fluctuations of symptoms during the day were present in 9 patients. Treatment with 4-aminopyridine was initiated in 5 patients, with an excellent effect in 4.

Conclusion: SCA27B is a newly described frequent cause of hereditary late-onset ataxia in the Czech Republic. Based on the estimated high frequency, we have included targeted molecular genetic testing for SCA27B in the standard protocol in patients with adult-onset chronic ataxia. This step is essential for accurate diagnosis and the significant therapeutic benefit observed with 4-aminopyridine treatment in SCA27B cases.

Grants: Supported by project No. LX22NPO5107 (Ministry of Education and Science of the Czech Republic).

Conflict of Interest: None declared

P11.052.C Novel bialleleic missense SVBP variant causes neurodevelopmental disorder with microcephaly.

Aboulfazl Rad[1], *Raman Kumar*[2], *Hafiz Muhammad Mustafa*[3], *Shivendra Kishore*[1], **Gabriela Oprea**[1]

[1]Arcensus GmbH, Rostock, Germany; [2]International Medical Center, National Stadium Road, Karachi, Pakistan; [3]Department of Biotechnology, University of Karachi, Karachi, Pakistan

Background/Objectives: Small vasohibin-binding protein (SVBP) functions as a chaperone and a co-factor for vasohibin 1 and 2 (VASH1 and 2). VASH1-SVBP and VASH2-SVBP complexes exhibit specific Tyr/Phe carboxypeptidase activity on microtubules. Recent literature has associated biallelic truncating variants in SVBP gene with neurodevelopmental disorders in five unrelated families. Here we report four patients from two unrelated families with similar neurodevelopmental disorders.

Methods: The SVBP variant was detected either by whole genome (WGS) or whole exome sequencing (WES). The family members were screened by Sanger sequencing.

Results: Both consanguineous Pakistani families had two affected siblings. The first family had two sibs aged 10 and 12 years with gait disturbance, global developmental delay, microcephaly, and motor delay. The second family had two sibs aged 1 and 4 years with delayed speech and language development, and with motor delay. Additionally, brain abnormalities were observed in the MRI scans of all analysed patients. Our analysis identified a novel homozygous missense variant (c.125T>C, p.Leu42Pro) located in exon 3 of SVBP gene (NM_199342.4) in all four patients. This rare variant segregated with disease in both families (parents and unaffected siblings).

Conclusion: Our findings expand the SVBP gene mutation spectrum, identified in patients with autosomal recessive neurodevelopmental disorder with ataxia, hypotonia, and microcephaly (NEDAHM). Further functional studies are necessary to elucidate the pathogenic mechanisms associated with missense variants in this gene.

Conflict of Interest: None declared

P11.053.D Spastic paraparesis as a relatively frequent manifestation of X-linked severe lethal adrenoleukodystrophy

Anna Uhrova Meszarosova[1], *Radim Mazanec*[2], *Jana Lastuvkova*[3], *Alena Santava*[4], *Lucie Osickova*[4], *Eva Vlckova*[5], *Petra Lassuthova*[1], *Dana Safka Brozkova*[1]

[1]Second Faculty of Medicine Charles University and University Hospital Motol, Prague, Neurogenetic Laboratory, Department of Paediatric Neurology, Czech Republic; [2]Second Faculty of Medicine Charles University and University Hospital Motol, Prague, Department of Neurology, Czech Republic; [3]Masaryk Hospital, Usti nad Labem, Department of Medical Genetics, Czech Republic; [4]Faculty Hospital Olomouc, Department of Medical Genetics, Czech Republic; [5]Faculty of Medicine Masaryk University and Faculty Hospital, Brno, Department of Neurology, Czech Republic

Background/objectives: Pathogenic variants in the *ABCD1* gene are cause of severe lethal X-linked adrenoleukodystrophy(X-ALD). X-ALD can manifest as rapidly progressive lethal adrenoleukodystrophy (ALD), milder adrenomyeloneuropathy (AMN), adrenal insufficiency (Addison's disease), or mild spastic paraparesis. Some female carriers can even remain asymptomatic. It makes the diagnosis confusing.

Methods: During nine years of routine diagnosis of hereditary spastic paraplegias (HSP) we performed targeted panel sequencing (the *ABCD1* gene was included) in approx. 350 patients suspected of having HSP.

Results: We detected six pathogenic variants in the *ABCD1* gene (two novel) in six index patients (four females, two men). The spastic paraparesis was in fact the clinical manifestation of the X-ALD with adult onset. The genetic result finally confirmed the proper diagnosis in the families even after many years which allowed retrospectively explain different clinical symptoms in families. Subsequently performed family segregation analysis revealed eight more heterozygous/hemizygous individuals.

Conclusion: The knowledge of the pathogenic variant in *ABCD1* gene is extremely important in families for preventive care particularly in male hemizygous still asymptomatic carriers. These men are in a high risk of fatal course of the disease due to X-linked inheritance.

The number of X-ALD families can be underdiagnosed, because the *ABCD1* pathogenic variant can be transferred via females in generations. X-linked inheritance, presence of spastic paraplegia, together with ALD, AMN or adrenal insufficiency (Addison's

disease/ crisis) in the family should be the good clue leading to *ABCD1* pathogenic variant.

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Conflict of Interest: None declared

P11.054.A Study of *RELN*-associated neurodevelopmental disorders using cerebral and cerebellar organoids

Philine Dinkel[1], *Ilka Reichardt*[2], *Mihail Sarov*[2], *Julia Sigl*[2], *Christina Eugster Oegema*[2], *Jula Peters*[2], *Evelin Schröck*[1;2], *William B. Dobyns*[3], *Michael Heide*[2;4], *Nataliya Di Donato*[1;5]

[1]*Institute for Clinical Genetics, University Hospital Carl Gustav Carus at TUD Dresden University of Technology and Faculty of Medicine of TUD Dresden University of Technology, Dresden, Germany;* [2]*Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany;* [3]*Department of Pediatrics, Division of Genetics and Metabolism, University of Minnesota, Minneapolis, United States;* [4]*German Primate Center, Leibniz Institute for Primate Research, Göttingen, Germany;* [5]*Department of Human Genetics, Hannover Medical School, Hannover, Germany*

Background/Objectives: Reelin is essential for correct lamination of the cortex and cerebellum. Its absence results in complete disorganization of neuronal layering in the mouse mutant reeler and patients with *RELN*-associated recessive lissencephaly. Reelin functions as a key signal for migrating neurons when secreted into the extracellular matrix. For several pathogenic *RELN* variants associated with autism or epilepsy, reduced Reelin secretion was demonstrated with transfected HEK293 cells, thereby suggesting a possible disease mechanism.

Methods: We use whole-brain and cerebellar organoids, which both harbor Reelin-expressing cells and allow to assess variant-specific effects on Reelin secretion in a 3D neuronal system for the first time. We first generated gene-edited induced pluripotent stem cells (iPSCs) with biallelic out-of-frame deletion and/or inversion of exon 8, representing a *RELN* knockout model. Secondly, we introduced a pathogenic splice site variant [NM_005045.3:c.4747+2T>G] seen in patients with *RELN*-associated lissencephaly.

Results: We realized that all potential knockout organoids express an unexpected alternative *RELN* transcript with in-frame skipping of exons 8-10 (130 amino acids) to rescue the reading frame. However, the alternative Reelin isoform is not secreted into the extracellular space, indicating a functional knockout in neuronal organoids. Currently, we characterize the impact of the alternative *RELN* transcript on morphology and cytoarchitecture of these organoids at different developmental stages.

Conclusion: Different *RELN* variants disrupt the secretion of Reelin, thereby presumably disabling its extracellular function on target neurons. Further investigations on how the secretion mechanism is arrested, and which intracellular function the alternative *RELN* transcript may pursue, will give new insights into pathomechanism of Reelinopathies.

Grants:

Conflict of Interest: None declared

P11.055.B TRAPPC6B biallelic variants cause a neurodevelopmental disorder with TRAPP II and trafficking disruptions

Hashem Almousa[1], *Sara A Lewis*[2], *Somayeh Bakhtiari*[2], *Sandra Hinz Nordlie*[2], *Alex Pagnozzi*[3], *Helen Magee*[2], *Stephanie Efthymiou*[4], *Jennifer Heim*[2], *Patricia Cornejo*[5], *Maha Zaki*[6], *Derek E. Neilson*[7], *Anusha Vemuri*[8], *Sheng Chih Jin*[9], *Xiao-Ru Yang*[10], *Abolfazl Heidari*[11], *Koen L. I. van Gassen*[12], *Aurélien Trimouille*[13], *Christel Thauvin-Robinet*[14], *James Liu*[2], *Ange-Line Bruel*[15], *Mais Omar Hashem*[16], *Ehsan Ghayoor Karimiani*[17], *Gozde Yesil Sayin*[18], *Lokesh Lingappa*[19], *Debangana Baruah*[19], *Julien Van Gils*[20], *Laurence Faivre*[21], *Mina Zamani*[22], *Hamid Galehdari*[22], **Rahema Mohammad**[4], *jasper van der Smagt*[12], *Alya Qari*[23], *John B. Vincent*[24], *A. Micheil Innes*[10], *Ali Dursun*[25], *R. Köksal Özgül*[25], *Halil Tuna Akar*[25], *Kaya Bilguvar*[26], *Cyril Mignot*[27], *Boris Keren*[27], *Claudia Ravelli*[28], *lydie BURGLEN*[29], *Alexandra Afenjar*[29], *Laura Donker Kaat*[30], *Fowzan Alkuraya*[31], *Henry Houlden*[4], *Segio Padilla-Lopez*[2], *Reza Maroofian*[4], *Michael Sacher*[1], *Michael C Kruer*[2]

[1]*Concordia University, Department of Biology, Montréal, Canada;* [2]*Barrow Neurological Institute, Phoenix, Arizona, United States;* [3]*The Australian e-Health Research Centre, Brisbane, Australia;* [4]*UCL Institute of Neurology, London, United Kingdom;* [5]*University of Arizona College of Medicine, Phoenix, Arizona, United States;* [6]*National Research Centre, Clinical Genetics Department, Cairo, Egypt;* [7]*Phoenix Children's Hospital, Phoenix, Arizona, United States;* [8]*University of Chicago, Chicago, IL, United States;* [9]*Washington University, United States;* [10]*Alberta Children's Hospital, S.W. Calgary, Canada;* [11]*Qazvin Medical University, Qazvin, Iran;* [12]*University Medical Center Utrecht, Utrecht, Netherlands;* [13]*CHU Bordeaux - Hôpital Pellegrin, Bordeaux, France;* [14]*Hôpital d'Enfants, Centre de Génétique, Dijon, France;* [15]*Université de Bourgogne, Laboratoire Génétique des Anomalies du Développement, Dijon, France;* [16]*Saudi Human Genome Program, Riyadh, Saudi Arabia;* [17]*St. George's, University of London, London;* [18]*Istanbul University, Istanbul Medical Faculty Department of Medical Genetics, Türkyie;* [19]*Rainbow Children Hospital, Hyderabad, India;* [20]*CHU Bordeaux - Hôpital Pellegrin, Laboratoire de Génétique Moléculaire, Service de Génétique Médicale, Bordeaux, France;* [21]*Centre de Génétique, Hôpital d'Enfants, Dijon, France;* [22]*Shahid Chamran University of Ahvaz, Department of Biology, Faculty of Science, Ahvaz, Iran;* [23]*Center for Genomic Medicine, King Faisal Specialist Hospital and Research Center, Saudi Arabia;* [24]*Campbell Family Mental Health Research Institute, Centre for Addiction and Mental Health, Toronto, Canada;* [25]*Hacettepe University, Türkyie;* [26]*Acibadem Mehmet Ali Aydinlar University, Istanbul, Türkyie;* [27]*APHP Sorbonne Université, Département de Génétique, Paris, France;* [28]*APHP Sorbonne Université, Paris, France;* [29]*Département de Génétique, APHP, Sorbonne Université, Paris, France;* [30]*Erasmus Medical Center, Rotterdam, Netherlands;* [31]*King Faisal Specialist Hospital and Research Centre, Saudi Arabia*

Background: Highly conserved TRAPP complexes are crucial for subcellular trafficking, essential for nervous system development. Variants in TRAPP complex subunits lead to neurodevelopmental disorders with overlapping phenotypes.

Methods: We examined TRAPPC6B, detailing clinical, neuroradiologic assessments, and disease mechanisms in 29 patients from 18 families with biallelic TRAPPC6B variants.

Results: We identified seven homozygous nonsense (n = 12 patients) and eight canonical splice-site variants (n = 17 patients). In addition, we identified one patient with compound heterozygous splice-site/missense variants with a milder phenotype and one patient with homozygous missense variants. These patients showed non-progressive microcephaly, developmental delays, intellectual disability, epilepsy, absent expressive language, and movement disorders. Brain imaging revealed reductions in cortex, cerebellum, and corpus callosum size with frequent white matter hyperintensity. Volumetric measurements indicated globally diminished volume. Molecular studies showed weakened interaction between mutant TRAPPC6B and TRAPPC3, reduced TRAPPC6B levels in patient-derived fibroblasts, and elevated TRAPPC6A levels. Co-immunoprecipitation suggested TRAPPC6B primarily affects TRAPP II complex functions. Drosophila models supported TRAPPC6B's role in neuromotor function.

Conclusion: Our findings confirm the association of damaging biallelic TRAPPC6B variants with microcephaly, intellectual

disability, language impairments, and epilepsy. These features overlap with disorders arising from pathogenic variants in other TRAPP subunits, particularly components of the TRAPP II complex. These findings suggest that TRAPPC6B is essential for brain development and function, and TRAPP II complex activity may be particularly relevant for mediating this function.

Grants: Supported by the Cerebral Palsy Alliance Research Foundation and the US National Institute of Neurological Disorders and Stroke.

Conflict of Interest: None declared

P11.056.C Novel compound heterozygous variants in *ALDH18A1* gene: expanding knowledge on autosomal recessive spastic paraplegia 9B

Silvia Cali[1;2], *Valeria Capra*[2], *Pasquale Striano*[3], *Maria Stella Vari*[4], *Francesca Madia*[5], *Edoardo Henzen*[6], *Francesca Faravelli*[2]

[1]*University of Catania, Biomedical and biotechnological sciences, Medical Genetics, Catania, Italy;* [2]*IRCCS Giannina Gaslini, Genomics and Clinical Genetics, Genova, Italy;* [3]*University of Genoa, Department of Neurosciences, Rehabilitation, Ophthalmology, Genetics, Maternal and Child Health, Genova;* [4]*IRCCS Giannina Gaslini, Pediatric Neurology and Muscular Diseases Unit, Genova;* [5]*IRCCS Giannina Gaslini, Medical Genetics Unit, Genova, Italy;* [6]*Italian Institute of Technology (IIT), Genomics Facility, Genova, Italy*

Background/Objectives: The ALDH18A1 gene, encoding pyrroline-5-carboxylate synthase (P5CS), is involved in the biosynthesis of proline, ornithine, and arginine. Mutations in ALDH18A1 cause hereditary spastic paraplegia (HSP), a neurodegenerative disorder characterized by progressive lower limb spasticity and weakness, known for its clinical and genetic heterogeneity. In particular, biallelic variants in ALDH18A1 lead to autosomal recessive spastic paraplegia-9B (SPG9B), an ultra-rare and complex neurological phenotype characterized by developmental delay, psychomotor regression, and facial dysmorphisms, which highlight its critical role in maintaining neurological integrity.

Methods: Here we present a 17-year-old patient exhibiting intellectual disability, paraplegia, and distinctive dysmorphic features. Whole exome sequencing (WES) revealed compound heterozygosity in ALDH18A1.

Results: Born to non-consanguineous parents, the patient experienced late intrauterine growth restriction. Recurrent regurgitation, vomiting, and psychomotor regression from 12 months onward were observed. Dysmorphic features included an elongated face, deep-set ears, long philtrum and pointed chin. Postnatal growth retardation, microcephaly and severe intellectual disability were also noted. Brain MRI disclosed abnormalities consistent with ALDH18A1-related HSP, showing altered morphology and signal intensity in supratentorial and subtentorial regions, along with a partially empty sella. Molecular analysis through WES identified compound heterozygosity for two novel *ALDH18A1* gene variants: c.1892_1902delinsC and c.1112G>A. Variants were classified likely pathogenic according to ACMG criteria.

Conclusion: SPG9B is an extremely rare condition, with only nine families described worldwide. To the best of our knowledge, this is the second familial instance reported in Italy. This case contributes to expand the knowledge about an ultra rare condition and confirm the heterogenous genetic landscape of ALDH18A1-related HSP.

Conflict of Interest: None declared

P11.057.D Impact of *MAGEL2* on development of human induced pluripotent stem cell (hiPSC)-derived neurons

Jannis Bücking[1], *Tobias Walczuch*[1], *Tobias Beschauner*[1], *Michael Eibl*[1], *Freya Hermann-Sim*[1], *Susanne Theiss*[1], *Melanie Spanjaard*[1], *Katrin Hinderhofer*[1], *Derek Tai*[2], *Celine De Esch*[2], *Michaela Bartusel*[3], *Byron Lee*[3], *Nima Jaberi-Lashkari*[3], *Eliezer Calo*[3], *Syed Azmal Ali*[4], *Michael Talkowski*[2], *Jeroen Krijgsveld*[4], *Christian Schaaf*[1], *Magdalena Laugsch*[1]

[1]*Heidelberg University, Institute of Human Genetics, Heidelberg, Germany;* [2]*Massachusetts General Hospital, Center for Genomic Medicine, Boston, MA, United States;* [3]*Massachusetts Institute of Technology, Department of Biology, Cambridge MA, United States;* [4]*German Cancer Research Center (DKFZ), Division Proteomics of Stem Cells and Cancer, Heidelberg, Germany*

Background/Objectives: Large chromosomal deletions or imprinting defects spanning 15q11-q13 cause Prader-Willi Syndrome (PWS), marked by the loss of expression of maternally imprinted genes, including the gene *MAGEL2*. The most prominent symptoms are neonatal hypotonia, neurodevelopmental delay, and hormonal abnormalities. Remarkably, mutations in *MAGEL2* alone result in a similar but more severe phenotype known as Schaaf-Yang Syndrome (SYS). Despite the shared features between these syndromes, SYS patients show a higher prevalence of intellectual disability, autism spectrum disorders, and neonatal death compared to those with PWS. However, the specific contribution of *MAGEL2* to SYS and PWS remains to be elucidated.

Methods: To determine cellular pathways affected by aberrant MAGEL2 in neurons, we first generated CRISPR/Cas9-engineered heterozygous and homozygous *MAGEL2* deletions in human induced pluripotent stem cells (hiPSCs). Following the differentiation along with isogenic PWS type I/II deletion and two SYS-associated *MAGEL2* mutation cell lines into cortical neurons, we conducted RNA-sequencing, whole proteome, and ubiquitome analyses. To determine the specific location of MAGEL2 in neurons and its interaction partners, we generated two additional hiPSC lines carrying either WT or SYS-mutated endogenously tagged MAGEL2 enabling the utilization of co-immunoprecipitation and immunofluorescence applications.

Results: Our preliminary multi-omics studies indicate various differentially affected pathways associated with synaptic transmission, transmembrane transport, and cytoskeleton organization which may provide new insights into the pathophysiology of MAGEL2 that are currently further investigated. Co-IP and localization studies are ongoing.

Conclusion: Our results will help us deepen our knowledge about the role of MAGEL2 in neurodevelopment and its contribution to PWS and SYS.

Grants: Start-Up Funds Christian Schaaf

Conflict of Interest: None declared

P11.058.A Exonic tandem repeats in patients with early phases of psychosis are over-represented among genes from lipid metabolism

rachele rubiu[1;2], *Brett Trost*[3], *Qin He*[1;2], *Chuan JIAO*[1;2], *Cecilia Carmignoto*[2], *Ridha Joober*[4], *Marie-Odile Krebs*[1;2;5], *Oussama Kebir*[2;5], *Guillaume Huguet*[6], *Sebastien Jacquemont*[6], *Guy Rouleau*[7], *Boris Chaumette*[1;2;4;5]

[1]*Université Paris Cité, Paris, France;* [2]*Institute of Psychiatry and Neuroscience of Paris (IPNP), INSERM U1266, Paris, France;* [3]*The Centre for Applied Genomics, The Hospital for Sick Children, Toronto, ON M5G 0A4, Toronto, Canada;* [4]*McGill Université, Department of Psychiatry, Montreal, Canada;* [5]*GHU-Paris Psychiatrie et Neurosciences, Paris, France;* [6]*Centre de Recherche du Centre Hospitalier Universitaire Sainte-Justine, Montreal, Canada;* [7]*Montreal*

Neurological Institute and Hospital, McGill University, Department of Neurology and Neurosurgery, Montreal, Canada

Background/Objectives: Clinical identification of individuals in early stages of psychosis, like ultra-high risk (UHR) or first episode of psychosis (FEP) has opened the way to investigate prognostic biomarkers. While genetic factors, including polygenic risk scores, show promise in predicting outcomes, rare variants such as tandem repeats (TRs) have been overlooked. We aimed to investigate their characteristics and functional implications in early psychosis.

Methods: A cohort of 396 individuals (318 FEP and UHR who developed FEP vs. 78 UHR who did not develop FEP) was sequenced using IlluminaSeq and genome-wide TRs and outlier identification was done by ExpansionHunter Denovo. Only TRs with a z-score >3.29 were characterized. Potential functional implications were assessed through gene-set confrontation, pathway, and functional enrichment analyses.

Results: Of the 2785 identified TRs, a subset of 156 TRs located in exonic regions was chosen for subsequent analysis resulting in 120 genes. While all these genes have already been associated with neuropsychiatric disorders, the gene-set confrontation analysis found that 63 of them were previously linked to schizophrenia. These genes were significantly expressed in brain tissue and were found to be enriched via functional and pathway analysis in the metabolic processes of unsaturated fatty acid, arachidonic acid, and eicosanoids.

Conclusion: This study highlights a potential link between TRs, lipid metabolism, and early psychosis. As lipid metabolism has been suggested to play an important role in psychosis vulnerability and as Omega-3 supplementation has been proposed as a preventive strategy for psychosis, these findings support the role of TRs in the pathogenesis of psychosis.

Grants: Brain Canada, SchizoREmics

Conflict of Interest: None declared

P11.059.B Disrupted oxytocin system development in rats with truncating *Magel2* mutation

Tim Schubert[1], *Yongxi Lu*[1], *Annabel Kleinwächter*[1], *Moritz Wimmer*[1], *Yuval Hazor*[1], *Colleen Bocke*[2], *Beril Demiryontar*[1], *Quirin Krabichler*[3], *Gina Yosten*[2], *Valery Grinevich*[3], *Christian Schaaf*[1], *Ferdinand Althammer*[1]

[1]*Institute of Human Genetics, Heidelberg University Hospital, Heidelberg, Germany;* [2]*St. Louis University, St. Louis, United States;* [3]*Central Institute of Mental Health, Department of Neuropeptide Research in Psychiatry, Mannheim, Germany*

Background/Objectives: The maternally imprinted *MAGEL2* gene gives rise to the MAGEL2 protein, which is linked to secretory granule formation and neuropeptide processing. *MAGEL2* is part of the Prader-Willi syndrome (PWS) deletion and truncating variants lead to Schaaf-Yang syndrome (SYS). Both are neurodevelopmental disorders with gross hypothalamic dysfunction and, in the case of SYS, a 75-85% prevalence of Autism Spectrum Disorder (ASD). Inconsistent improvements in patients receiving oxytocin (OT) in clinical trials suggest a critical period during brain development where treatment is most effective. This study compares OT system development in health and disease.

Methods: We studied rats harbouring a truncating *Magel2* mutation and performed semi-automated quantification of OT neurons throughout the entire rat hypothalamus in newborns, adolescents, and adults. Additionally, we mapped projections to socially relevant brain regions modulated by OT and analyzed oxytocin receptor mRNA levels via RNAScope hybridization.

Results: Our analysis reveals sex- and age-specific alterations in OT systems between wildtype and mutant rats. We show that *Magel2* deficient males display a transient increase in oxytocinergic neurons that disappears in adults. In females, these changes persist into adulthood, accompanied by reduced projections to the nucleus accumbens - a region crucial for social motivation and reward.

Conclusion: This work offers unprecedented insights into OT system development in health and disease, laying the foundation for further investigations into the development of oxytocinergic circuitry, its association with ASD and optimal windows for effective OT treatment in PWS and SYS.

Grants: FPWR Grant (Christian P. Schaaf), DFG AL2466/2-1 (Ferdinand Althammer)

Conflict of Interest: None declared

P11.060.C Confirming *KCNQ3* as a disease gene for autosomal-recessive developmental and epileptic encephalopathy

Ava Oberlack[1], *Maureen Jacob*[1], *Claudia Nussbaum*[2], *Juliane Winkelmann*[1;3], *Ingo Borggräfe*[4], *Matias Wagner*[1;3;4]

[1]*Klinikum rechts der Isar, Technical University of Munich, Institute of Human Genetics, Munich, Germany;* [2]*Dr. v. Hauner Children's Hospital, LMU - University of Munich, Department of Neonatology, Munich, Germany;* [3]*Helmholtz Zentrum München Deutsches Forschungszentrum für Umwelt und Gesundheit, Institute of Neurogenomics, Neuherberg, Germany;* [4]*Dr. v. Hauner Children's Hospital, LMU - University of Munich, Department of Pediatric Neurology, Munich, Germany*

Background: Heterozygous variants in *KCNQ3*, encoding a subunit of a voltage-gated potassium channel, are a well-known cause of autosomal-dominant self-limited (familial) neonatal epilepsy, self-limited (familial) infantile epilepsy and, rarely, neurodevelopmental disorder with or without autism and/or epilepsy. Recently, three families with *KCNQ3*-associated autosomal-recessive developmental and epileptic encephalopathy (DEE) showing neonatal-onset epilepsy and mild to severe intellectual disability have been described. Heterozygous parents and siblings had no history of seizures or developmental retardation.

Methods: Patient-parent trio exome sequencing was performed and analyzed using a systematic filtering approach.

Results: We present an additional case of a term-born child of consanguineous, asymptomatic parents, who showed migrating focal seizures from the second day of life. Trio exome sequencing identified a homozygous frameshift variant in exon 1 of *KCNQ3* (NM_004519.4:c.328_329del). The estimated prevalence of homozygous *KCNQ3*-associated disease based on the allele frequency of loss-of-function variants in gnomAD is around 1/6 million (> 20 new cases/year worldwide).

Conclusion: Our patient corroborates the significance of *KCNQ3* as a disease gene linked not only to autosomal-dominant, mainly self-limiting epilepsy syndromes, but also to autosomal-recessive DEE phenotypes. As the number of known patients is well below the estimated prevalence, the homozygous condition is probably underdiagnosed. Given the loss-of-function character of variants identified in recessive families, these should also be disease causing in a dominant context. However, heterozygous, healthy family members indicate a reduced penetrance below previous estimates. Retigabine, a modulator of potassium channels, could be a treatment option, but is currently not available in the EU.

Conflict of Interest: None declared

P11.061.D Biallelic variation in the choline and ethanolamine transporter *FLVCR1* underlies a pleiotropic disease spectrum

from adult neurodegeneration to severe developmental disorders

Daniel Calame[1;2;3], *Jovi Huixin Wong*[4], *Dat Tuan Nguyen*[4], *Puravi Panda*[4], *Riccardo Sangermano*[5], *Lama AlAbdi*[6;7], *Nancy C.P. Leong*[4], *Zain Dardas*[3], *Jawid Fatih*[3], *Stephanie Efthymiou*[8], *Mohamad Abdelhamid*[9], *Haowei Du*[3], *Shalini Jhangiani*[10], *Zeynep Coban Akdemir*[3;11], *Dana Marafi*[3;12], *Sedat Isikay*[13], *Subha Ramanathan*[14], *Kerby Oberg*[15], *Robin Clark*[14], *Catharina Wenman*[16], *Sam Loughlin*[16], *Tazeen Ashraf*[17], *Alison Male*[17], *Shereen Tadros*[17], *Ramy Saad*[17], *Davut Pehlivan*[1;2;3], *Jennifer Posey*[3], *Richard Gibbs*[3;10], *Henry Houlden*[8], *Fowzan Alkuraya*[7;18], *Reza Maroofian*[8], *Long Nam Nguyen*[4;19;20;21;22], *James Lupski*[2;3;10;23]

[1]*Baylor College of Medicine, Section of Pediatric Neurology and Developmental Neuroscience, Department of Pediatrics, Houston, United States;* [2]*Texas Children's Hospital, Houston, United States;* [3]*Baylor College of Medicine, Department of Molecular and Human Genetics, Houston, United States;* [4]*Yong Loo Lin School of Medicine, National University of Singapore, Department of Biochemistry, Singapore;* [5]*Massachusetts Eye and Ear, Harvard Medical School, Ocular Genomics Institute, Department of Ophthalmology, Boston, United States;* [6]*King Saud University, Department of Zoology, College of Science, Riyadh, Saudi Arabia;* [7]*King Faisal Specialist Hospital and Research Center, Department of Translational Genomics, Center for Genomic Medicine, Riyadh, Saudi Arabia;* [8]*UCL Institute of Neurology, Department of Neuromuscular diseases, London, United Kingdom;* [9]*Human Genetics and Genome Research Institute, National Research Centre, Medical Molecular Genetics Department, Cairo, Egypt;* [10]*Baylor College of Medicine, Human Genome Sequencing Center, Houston, United States;* [11]*School of Public Health, The University of Texas Health Science Center at Houston, Human Genetics Center, Department of Epidemiology, Human Genetics, and Environmental Sciences, Houston, United States;* [12]*Kuwait University, Department of Pediatrics, Faculty of Medicine, Kuwait;* [13]*Gaziantep Islam Science and Technology University, Department of Pediatric Neurology, Gaziantep, Türkyie;* [14]*Loma Linda University School of Medicine, Division of Genetics, Department of Pediatrics, Loma Linda, United States;* [15]*Loma Linda University School of Medicine, Department of Pathology and Human Anatomy, Loma Linda, United States;* [16]*Great Ormond Street Hospital for Children NHS Foundation Trust, Rare & Inherited Disease Laboratory, NHS North Thames Genomic Laboratory Hub, London, United Kingdom;* [17]*Great Ormond Street Hospital for Children NHS Foundation Trust, North East Thames Regional Genetic Service, London, United Kingdom;* [18]*Prince Sultan Military Medical City, Department of Pediatrics, Riyadh, Saudi Arabia;* [19]*National University of Singapore, Immunology Program, Life Sciences Institute, Singapore;* [20]*National University of Singapore, Singapore Lipidomics Incubator (SLING), Life Sciences Institute, Singapore;* [21]*Yong Loo Lin School of Medicine, National University of Singapore, Cardiovascular Disease Research (CVD) Programme, Singapore;* [22]*Yong Loo Lin School of Medicine, National University of Singapore, Immunology Translational Research Program, Singapore;* [23]*Baylor College of Medicine, Department of Pediatrics, Houston, United States*

Consortium: FLVCR1 International Collaborative Group

Background/Objectives: *FLVCR1* encodes a widely expressed transmembrane protein implicated in heme, choline, and ethanolamine transport. While *Flvcr1* knockout mice die in utero with skeletal malformations and defective erythropoiesis reminiscent of Diamond-Blackfan anemia, rare pathogenic *FLVCR1* variants are linked to autosomal recessive childhood or adult-onset neurodegeneration of the retina, spinal cord, and peripheral nervous system. Here we sought to better characterize the phenotypic spectrum of *FLVCR1*-related disease and the molecular consequences of pathogenic FLVCR1 variants.

Methods: We analyzed exome and genome sequencing data from >29,000 rare disease individuals to identify cases with biallelic *FLVCR1* variants. Additional cases were identified through GeneMatcher and international collaborations. The functional impact of *FLVCR1* variants were examined in an in vitro choline and ethanolamine transport assay as well as a mini-gene splicing assay.

Results: We identified 27 individuals from 21 unrelated families with biallelic ultra-rare missense and loss-of-function (LoF) *FLVCR1* variants. We characterize an expansive *FLVCR1*-related disease spectrum ranging from adult-onset retinitis pigmentosa to severe developmental disorders with profound developmental delay, microcephaly, reduced brain volume, epilepsy, spasticity, and premature death. The most severely affected individuals, including six individuals wih homozygous LoF variants, share traits with *Flvcr1* knockout mice and Diamond-Blackfan anemia including macrocytic anemia, skeletal malformations, and hydranencephaly. *FLVCR1* missense variants primarily fall within transmembrane domains and significantly reduce choline and ethanolamine transport; a subset also disrupt splicing.

Conclusion: This study expands our understanding of Mendelian disorders of choline and ethanolamine transporters and demonstrates the importance of choline and ethanolamine in hematopoietic, skeletal, and nervous system development and homeostasis.

Conflict of Interest: Daniel Calame: None declared, Jovi Huixin Wong: None declared, Dat Tuan Nguyen: None declared, Puravi Panda: None declared, Riccardo Sangermano: None declared, Lama AlAbdi: None declared, Nancy C.P. Leong: None declared, Zain Dardas: None declared, Jawid Fatih: None declared, Stephanie Efthymiou: None declared, Mohamad Abdelhamid: None declared, Haowei Du: None declared, Shalini Jhangiani: None declared, Zeynep Coban Akdemir: None declared, Dana Marafi: None declared, Sedat Isikay: None declared, Subha Ramanathan: None declared, Kerby Oberg: None declared, Robin Clark: None declared, Catharina Wenman: None declared, Sam Loughlin: None declared, Tazeen Ashraf: None declared, Alison Male: None declared, Shereen Tadros: None declared, Ramy Saad: None declared, Davut Pehlivan: None declared, Jennifer Posey: None declared, Richard Gibbs: None declared, Henry Houlden: None declared, Fowzan Alkuraya: None declared, Reza Maroofian: None declared, Long Nam Nguyen: None declared, James Lupski Stock ownership in 23andMe, is a co-inventor on multiple United States and European patents related to molecular diagnostics for inherited neuropathies, eye diseases, genomic disorders, and bacterial genomic fingerprinting, paid consultant for Genome International

P11.062.A A homozygous missense variant in *DMRTA2* causes colpocephaly with associated agenesis of corpus callosum and primary microcephaly

Ambrin Fatima[1], *Bilal Ahmad Mian*[1], *Lubaba Bintee Khalid*[1], *Farhan Bahadar Ali*[2], *Hijab Zahra*[1], *Asmat Ali*[1], *Mathias Toft*[3;4], *Zafar Iqbal*[4]

[1]*Aga Khan University, Biological and Biomedical Sciences, Karachi, Pakistan;* [2]*Aga Khan University, Department of Paediatrics and Child Health, Karachi, Pakistan;* [3]*University of Oslo, Institute of Clinical Medicine, Oslo, Norway;* [4]*Oslo University Hospital, Department of Neurology, Oslo University Hospital, Oslo, Norway*

Doublesex- and mab-3–related transcription factor a2 (*DMRTA2*), intricately balances cell cycle progression and neuronal differentiation by directly regulating the neurogenesis repressor Hes1. Here, we have studied a five-generation Pakistani consanguineous family having three affected individuals presenting with primary microcephaly with developmental disorder including intellectual

disability, seizures and developmental delay. The plausible candidate variant for the phenotype was identified by whole exome sequencing. We identified a rare homozygous missense variant, c.347G>C; p.Arg116Pro (NM_032110.3) in *DMRTA2*, which segregated with the phenotype. The variant p.Arg116Pro is predicted as "deleterious" by multiple insilico-prediction programs and classified as variant of uncertain significance, according to the ACMG classification criteria.The patient's MRI displayed a simplified gyral pattern along with agenesis of the corpus callosum and colpocephaly. The abnormal Electroencephalogram indicated mild encephalopathy. DMRTA2 safeguards neural progenitor cells (NPC) maintenance and its loss promotes neuronal differentiation and depletion of the NPC pool, resulting in microcephaly. So far a single variant c.1197delT in DMRTA2 has been reported in three siblings affected by a severe prenatal neurodevelopmental disorder characterised by fronto-parietal pachygyria, agenesis of the corpus callosum and progressive severe microcephaly. Moreover, we have initiated a set of functional experiments to unravel the pathogenicity of the novel variant identified in this study. Our findings add important details to the clinical and genetic spectrum of DMRTA2, and highlight p.Arg116Pro as a cause of colpocephaly with associated agenesis of corpus callosum and primary microcephaly.

Conflict of Interest: None declared

P11.063.B *GRIN2A* null variants confer a high risk for early-onset schizophrenia and other mental disorders and potentially enable precision therapy

Johannes Lemke[1], *Andrea Eoli*[2], *Ilona Krey*[1], *Bernt Popp*[1], *Vincent Strehlow*[1], *Dirk Alexander Wittekind*[1], *Anna-Leena Vuorinen*[3], *Stephen F Traynelis*[4], *Tim Benke*[5], *Henrike Heyne*[2], *Steffen Syrbe*[6]

[1]*Universtity of Leipzig Medical Center, Leipzig, Germany;* [2]*Hasso Plattner Institute (HPI), Potsdam, Germany;* [3]*University of Helsinki, Helsinki, Finland;* [4]*Emory University, Atlanta, United States;* [5]*University of Colorado Hospital, Aurora, United States;* [6]*University Hospital Heidelberg, Heidelberg, Germany*

Consortium: GRIN2A study group

Background: Mental disorders are considered highly polygenic. This is despite the observation of rare protein-truncating null variants in multiple genes that are believed to contribute to its heritability, particularly with respect to schizophrenia. *GRIN2A*, encoding the GluN2A subunit of the N-methyl-D-aspartate receptor (NMDAR) is one of very few such genes that had been identified to confer a substantial risk for schizophrenia.

Methods: We prospectively enquired the presence of psychiatric diagnoses in 235 individuals with pathogenic variants in *GRIN2A* who had previously enrolled in our global GRIN registry.

Results: We identified null variants in *GRIN2A* (*GRIN2A*null) to be significantly associated with mental disorders in general, including schizophrenia compared to missense variants (*GRIN2A*missense). *GRIN2A*null-related phenotypes can manifest as isolated mental disorder, i.e. as schizophrenia or mood disorder without further *GRIN2A*-specific symptoms, such as intellectual disability and/or epilepsy. *GRIN2A*null-related mental disorders manifest in early childhood or adolescence, which is significantly earlier than the adult onset in the general population.

Conclusion: *GRIN2A*null appears to be the first monogenic cause of isolated and early-onset mental disorders, including early-onset schizophrenia, and genetic testing should be considered in the diagnostic work-up of affected individuals. As L-serine is known to mediate co-agonistic effects on the NMDAR, it had been applied to four individuals with *GRIN2A*null-related mental disorders, all of whom experienced improvements of the neuropsychiatric phenotype. Thus, increasing brain concentrations of NMDAR co-agonists appears to be a promising precision treatment approach successfully targeting deficient glutamatergic signaling in individuals with mental disorders, i.e. due to *GRIN2A*null.

Conflict of Interest: Johannes Lemke Angelini Pharma, Jazz Pharmacyticals, UCB Pharma, GRIN Therapeutics, Andrea Eoli: None declared, Ilona Krey: None declared, Bernt Popp: None declared, Vincent Strehlow: None declared, Dirk Alexander Wittekind: None declared, Anna-Leena Vuorinen: None declared, Stephen F Traynelis: None declared, Tim Benke: None declared, Henrike Heyne: None declared, Steffen Syrbe: None declared

P11.064.C Combined effect of common and rare variants in genetic generalized epilepsy and subphenotypes

Emadeldin Hassanin[1], *Maryam Erfanian Omidvar*[2], *Dheeraj Bobbili*[1], *Holger Lerche*[2], *Patrick May*[1]

[1]*Luxembourg Centre for Systems Biomedicine, University of Luxembourg;* [2]*Hertie Institute for Clinical Brain Research*

Consortium: ILAE Consortium on Complex Epilepsies, EpiPGX Consortium, EuroEPINOMICS CoGIE Consortium, Epi25 Collaborative

Background/Objectives: In this study, we aimed to investigate the combined contribution of both common and ultra-rare genetic variants (URV) on the development of genetic generalized epilepsy (GGE) and subphenotypes.

Methods: We analyzed a large cohort of 669 GGE cases and 41,700 ethnically matched controls to identify individuals with URVs in specific gene-sets related to GGE, including GABA-A receptors, GGE-related genes, and focal epilepsy-related genes. We generated a novel polygenic risk score (PRS) for GGE and stratified individuals according to their PRS deciles and URV carrier status. Logistic regression models were applied while adjusting for the ethnicity, age and gender.

Results: Individuals with GGE had a significantly higher PRS than controls (P-value $= 2.22 \times 10^{-16}$). For URV carriers in the GABA gene-set, depending on PRS decile the ORs for non-carriers ranged between 0.33 (CI = 0.30-0.37) to 3.48 (CI = 3.43-3.53), whereas for the carriers it ranged from 0.75 (CI = 0.44-1.29) to 8.08 (CI = 5-12.63); Similar trend was observed for URV GGE-related gene carriers. However, there was no substantial difference in the ORs between URV carriers and non-carriers in the focal epilepsy-related genes. In the GGE-subphenotypes analysis, only juvenile myoclonic epilepsy (JME) cases showed differences in the ORs in the carriers vs non-carriers.

Conclusion: We suggest that considering the polygenic background alongside ultra rare variants is likely to increase the accuracy of risk assessment for individuals who inherit ultra rare variants associated with epilepsy.

Conflict of Interest: None declared

P11.065.D Identification of novel driver risk genes in CNV loci associated with neurodevelopmental disorders.

Sara Azidane[1], *Mario Caceres*[2;3], *laura perez-cano*[1], *xavier gallego*[1], *lynn durham*[4], *emre guney*[1]

[1]*STALICLA DDS, Barcelona, Spain;* [2]*Institute of Biotechnology and Biomedicine (IBB-UAB), Bellaterra (Barcelona), Spain;* [3]*Institució Catalana de Recerca i Estudis Avançats (ICREA), Barcelona, Spain;* [4]*STALICLA DDU, Genève, Switzerland*

Background/Objectives: Large and rare copy number vairants (CNVs) are known to entail risk for various neurodevelopmental disorders (NDDs). Nevertheless, given that NDD-risk CNVs cover

broad regions of the genome, it is particularly challenging to pinpoint the critical gene(s) responsible for the manifestation of the phenotype. Our aim was to identify NDD-risk regions, as well as genes within these genomic regions responsible for triggering the phenotype.

Methods: To identify NDD-risk regions we performed a meta-analysis of CNV data from the SFARI database. Dosage-sensitive genes within these risk loci were evaluated for enrichment of known NDD-risk pathways. Additional analyses of these genes included construction of protein-protein interaction networks, assessment of its expression in brain across development, and evaluation of transmitted deletions within multiplex ASD families. We also performed a burden analysis using data from Decipher, 1000 Genomes and iHART cohorts to validate the association of dose-sensitive genes with NDD-risk.

Results: Our findings revealed enrichment of dosage-sensitve genes within NDD-risk CNV loci, convergence in protein-protein interaction networks, high expression in brain across development, and overtransmission of deletions in ASD offspring. Burden analysis validated association for 154 dose-sensitive genes with NDD-risk, including 22 novel NDD-risk genes.

Conclusions:

This study highlights the complexity of NDDs, emphasizing a polygenic model in which multiple genes within NDD-risk CNV loci contribute to these heterogenous phenotypes. The identification of new risk genes enriches our understanding of the genetic basis of NDDs and opens routes for future research and therapeutic interventions.

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Conflict of Interest: Sara Azidane SA is a full time employee of STALICLA, Mario Caceres MC is cotutor of the PhD project reflected on the abstract., laura perez-cano LPC is a principal investigator on this project., LPC is a full time employee of STALICLA., xavier gallego XG is full time employee of STALICLA., lynn durham CEO and founder of STALICLA, emre guney EG is a full time employee at STALICLA

P11.066.A De novo variants in *SRSF3*, encoding an RNA binding protein involved in pre-mRNA splicing and nucleocytoplasmic shuttling, are associated with variable phenotypes

Jasmin Lisfeld[1], Ivan Chinn[2], Bo Yuan[3;4], Christian Casar[5], Christian Müller[5], Eyal Elron[6;7], gal zaks hoffer[6;7], Naama Orenstein[6;7], Kristian Tveten[8], Julian Delanne[9;10], Hana Safraou[10;11], Anne-Sophie Denommé-Pichon[10;11], Erfan Aref-Eshghi[12], Nathaniel H. Robin[13], Elizabeth D. Rothrock[13], James Lupski[3;4], Kerstin Kutsche[1], **Frederike L. Harms**[1]

[1]*University Medical Center Hamburg-Eppendorf, Institute of Human Genetics, Hamburg, Germany;* [2]*Baylor College of Medicine, Department of Pediatrics, Division of Immunology/Allergy/Retrovirology, Houston, United States;* [3]*Baylor College of Medicine, Human Genome Sequencing Center, Houston, United States;* [4]*Baylor College of Medicine, Department of Molecular and Human Genetics, Houston, United States;* [5]*University Medical Center Hamburg-Eppendorf, Bioinformatics Core, Hamburg, Germany;* [6]*Schneider- Children's Medical Center, Pediatric Genetics Unit, Petah Tikva, Israel;* [7]*Tel Aviv University, Faculty of Medicine, Tel Aviv-Yafo, Israel;* [8]*Telemark Hospital Trust, Department of Medical Genetics, Skien, Norway;* [9]*CHU Dijon, Hôpital d'Enfants, FHU TRANSLAD, Centre de Génétique et Centre de référence "Déficiences intellectuelles de causes rares", Dijon, France;* [10]*University of Burgundy, FHU TRANSLAD, INSERM UMR1231 GAD "Génétique des Anomalies du Développement", Dijon, France;* [11]*Dijon Bourgogne University Hospital, FHU TRANSLAD, Functional Unit for Diagnostic Innovation in Rare Diseases, Dijon, France;* [12]*GeneDx, Gaithersburg, United States;* [13]*University of Alabama at Birmingham, Heersink School of Medicine, Department of Genetics, Birmingham, United States*

Background/Objectives: This study investigates the impact of de novo heterozygous variants in *SRSF3*, which is involved in constitutive and alternative splicing and mRNA export.

Methods: Clinical, genomic, and functional studies using subject-derived fibroblasts and heterologous cellular systems were performed.

Results: We studied six individuals with variable phenotypes who had heterozygous de novo *SRSF3* (NM_003017.5) variants, including one missense variant [c.181G>C; p.(Asp61His)] affecting a highly conserved residue in the RNA recognition motif, three frameshift variants [c.330_331del; p.(Pro111Serfs*20), c.402_403del; p.(Arg137Ilefs*16), and c.477dup; p.(Ser160Valfs*3)] and one nonsense variant [c.445C>T; p.(Arg149*)] identified in two unrelated patients. The c.330_331del variant was predicted to cause nonsense-mediated mRNA decay, which was confirmed by RT-PCR in patient-derived fibroblasts. However, *SRSF3* mRNA and protein levels were similar in patient and control cells. As we lacked patient cells, we overexpressed the SRSF3-Asp61His, -Arg137Ilefs*16, -Arg149*, and -Ser160Valfs*3 mutants in HEK293T and HeLa cells. Our data revealed that the C-terminally truncated mutants had lower protein stability compared to wild-type SRSF3, correlating with reduced C-terminal phosphorylation of these mutants. All SRSF3 mutants showed decreased binding to polyadenylated RNA and reduced nucleocytoplasmic shuttling. Complex formation with YTHDC1, which recognizes N6-methyladenosine-modified mRNAs, and nuclear export factor 1 (NXF1) was reduced for SRSF3-Arg137Ilefs*16 and -Arg149*.

Conclusion: Our data suggest that impaired mRNA binding and reduced mRNA export from the nucleus contribute to the pathophysiology underlying variable phenotypes associated with de novo missense and truncating *SRSF3* variants. Notably, compensatory mechanisms were observed in fibroblasts with a heterozygous *SRSF3* loss-of-function variant to maintain normal SRSF3 levels.

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P11.067.B Genetic determinants of mitochondrial impairment in primary progressive multiple sclerosis

Melissa Sorosina[1], **Elisabetta Mascia**[1]*, Michela Crotti[2], Ferdinando Clarelli[1], Antonino Giordano[1;3], Laura Ferrè[1;3], Daniele De Ritis[2], Francesca Maltecca[2], Massimo Filippi[3;4;5;6], FEDERICA ESPOSITO[1;3]*

[1]*IRCCS San Raffaele Scientific Institute, Laboratory of human genetics of neurological disorders, Institute of Experimental Neurology, Division of Neuroscience, Milan, Italy;* [2]*IRCCS San Raffaele Scientific Institute, Mitochondrial Dysfunctions in Neurodegeneration Unit, Division of Neuroscience, Milan, Italy;* [3]*IRCCS San Raffaele Scientific Institute, Neurology and Neurorehabilitation Unit, Milan, Italy;* [4]*Vita-Salute San Raffaele University, Milan, Italy;* [5]*IRCCS San Raffaele Scientific Institute, Neurophysiology Service, Milan, Italy;* [6]*IRCCS San Raffaele Scientific Institute, Neuroimaging Research Unit, Division of Neuroscience, Milan, Italy*

Background/Objectives: Multiple sclerosis (MS) is a chronic, progressive neurological disorder. About 15% of patients experiences a progressive course from the onset, the primary progressive (PP) MS. Several lines of evidence demonstrate that mitochondrial dysfunction could be one of the driving factors of disease progression in MS. The purpose of this project is to explore the genetic determinants of mitochondrial impairment occurring in PPMS.

Methods: We performed a candidate gene-set enrichment analyses with magma tool, comparing 532 PPMS and 436 benign-MS (patients with EDSS < 4 after ≥20 years), starting from imputed whole-genome genotyping data (8.5M SNPs). A total of 166 gene-sets and 1704 nuclear genes covering the main mitochondrial functions were selected. Further functional evaluations to confirm the involvement of associated pathways were performed on 20 PPMS and 20 RRMS patient collected PBMCs, differentiated and expanded in $CD4^+$ T lymphocytes. We performed cytofluometric analysis of Tetramethylrhodamine Methyl Ester to measure mitochondrial membrane potential (MMP) and of MitoTracker staining to assess mitochondrial mass.

Results: Analyses highlighted gene-sets related to fatty acid beta oxidation ($p = 0.002$), mitophagy ($p = 0.004$) and mitochondrial uncoupling ($p = 0.03$) as associated with PPMS. At baseline, no differences were observed in terms of MMP comparing CD4+ enriched T lymphocytes collected from PP and Relapsing-Remitting patients, while a slight increase in mitochondrial mass was observed in PPMS (Kolmogorov-Smirnov test $p < 0.0001$), coherent with the presumed impairment of mitophagy-related processes.

Conclusion: Results suggest the presence of genetic-driven mitochondrial impairment in specific processes in PPMS. Additional functional assessments are ongoing.

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Conflict of Interest: Melissa Sorosina: None declared, Elisabetta Mascia: None declared, Michela Crotti: None declared, Ferdinando Clarelli: None declared, Antonino Giordano: None declared, Laura Ferrè: None declared, Daniele De Ritis: None declared, Francesca Maltecca: None declared, Massimo Filippi Editor-in-Chief of the Journal of Neurology, Associate Editor of Human Brain Mapping, Associate Editor of Radiology, and Associate Editor of Neurological Sciences, received compensation for consulting services from Alexion, Almirall, Biogen, Merck, Novartis, Roche, Sanofi, speaking activities from Bayer, Biogen, Celgene, Chiesi Italia SpA, Eli Lilly, Genzyme, Janssen, Merck-Serono, Neopharmed Gentili, Novartis, Novo Nordisk, Roche, Sanofi, Takeda, and TEVA, participation in Advisory Boards for Alexion, Biogen, Bristol-Myers Squibb, Merck, Novartis, Roche, Sanofi, Sanofi- Aventis, Sanofi-Genzyme, Takeda, scientific direction of educational events for Biogen, Merck, Roche, Celgene, Bristol-Myers Squibb, Lilly, Novartis, Sanofi-Genzyme, he re-ceives research support from Biogen Idec, Merck-Serono, Novartis, Roche, Italian Ministry of Health, Fondazione Italiana Sclerosi Multipla, and ARiSLA (Fondazione Italiana di Ricerca per la SLA), FEDERICA ESPOSITO received consulting and speaking fees from Novartis, Sanofi Genzyme

P11.068.C *FOXP2* alterations: A comprehensive look beyond speech and language

Caroline Utermann-Thuesing[1], *Almuth Caliebe*[1], *Yorck Hellenbroich*[2], *Malte Spielmann*[1;2], *Inga Nagel*[1]

[1]*University Hospital Schleswig-Holstein, Campus Kiel, Institute of Human Genetics, Kiel, Germany;* [2]*University Hospital Schleswig-Holstein, Campus Lübeck, Institute of Human Genetics, Lübeck, Germany*

Background/Objectives: Heterozygous disruptions in the *FOXP2* gene cause severe speech and language disorder (SLD). In addition to SLD, fine and gross motor dysfunction and facial dysmorphology can occur. Whether *FOXP2* variants cause autism spectrum disorder (ASD) is still on debate and other clinical findings such as global developmental delay and neurological movement disorders are currently not considered as part of *FOXP2*-associated disorders.

Methods: Exome and Sanger sequencing were performed in two unrelated families with speech and language developmental delay and additional clinical manifestations. In family A, the mother presented with a tremor-ataxia-dystonia syndrome. Her children (1 son, 1 daughter) displayed a similar phenotype (ataxia, tremor) along with hypotonia (both), suspected ASD (son) and global developmental delay (son). In family B, the only son of unaffected parents presented in addition to SLD with intellectual disability, hypotonia, behavioral abnormalities and dysmorphic facies such as low-set ears.

Results: Exome sequencing identified a missense variant of unclear significance in *FOXP2* (NM_014491.4:c.1661A>G; p.(His554Arg)) in all affected members of family A. In family B, the son carried a pathogenic *FOXP2*-loss of function variant (NM_014491.4:c.1540del; p.(Arg514Glyfs*12)). Notably, both *FOXP2* variants have not been described so far.

Conclusion: While previous case reports and the OMIM database emphasize SLD-related phenotypes, our findings, literature review and database research reveal potentially additional features for *FOXP2*-related diseases, including ASD and neurological movement disorders like ataxia and tremor. Furthermore, family A's clinical findings indicate phenotypic variability within the same family. This broader spectrum of manifestations suggests considering more variable clinical phenotypes in genetic counseling of individuals with *FOXP2* variants.

Conflict of Interest: None declared

P11.069.D Identification of rare structural risk variants in families with bipolar disorder

Priyadarshini Thirunavukkarasu[1], *Bettina Burger*[1], *sascha fischer*[1], *Jose guzman Parra*[2], *Lea Sirignano*[3], *Fabio rivas*[2], *Fermin Mayoral*[2], *Asensio gonzalez*[1], *Stefan Herms*[1;4;5], *Marcella Rietschel*[3], *Markus Nöthen*[4;5], *Andreas Forstner*[4;5;6], *Sven Cichon*[1;6;7]

[1]*University Hospital Basel and University of Basel, Department of Biomedicine, Switzerland;* [2]*University Regional Hospital, Biomedicine Institute (IBIMA), Mental Health Department, Spain;* [3]*Central Institute of Mental Health, Medical Faculty Mannheim, University of Heidelberg, Department of Genetic Epidemiology in Psychiatry, Germany;* [4]*University Hospital Bonn, Institute of Human Genetics, Germany;* [5]*University of Bonn, Platform Genomics, Life&Brain Center, Germany;* [6]*Research Center Juelich, Institute of Neuroscience and Medicine (INM-1), Germany;* [7]*University Hospital Basel, Medical Genetics, Institute of Medical Genetics and Pathology, Switzerland*

Background: Bipolar disorder (BD) is a genetically complex neuropsychiatric disorder with a lifetime prevalence of 1-2% and a twin-based heritability of 60-80%. Common SNPs account for ≈20% of the observed phenotypic variance. It is assumed that at least part of the unexplained phenotypic variance is explained by rare variants. Studies aiming at the identification of rare variants have mainly focused on short-read sequencing and single-nucleotide variants. In the present study, we aimed at exploring the contribution of rare structural variants (SVs) to BD by using long-read whole-genome sequencing (lrWGS).

Methods: We performed PacBio lrWGS in nine Spanish multi-generational families multiply affected with BD. From each family, we sequenced three affected individuals and filtered for rare SVs shared by all three individuals.

Results: We present preliminary results from two families, for which we had an initial callset of 68,217 and 31,956 SVs, respectively. After a rigid filtering process, we retained 14 SVs (family1) and 7 SVs (family2). Notably, 6/14 and 4/7 of these SVs segregate in genes previously implicated in BD: *AGAP1, CACNA1C, CAMTA1, FAM135B, NXN, TENM4* (family 1), and *DLGAP2, NOL4, PRKG1, PTPRD* (family 2).

Conclusions: Our study tentatively identified rare SV in novel and previously implicated BD genes segregating with BD. We are currently following-up these findings in additional affected individuals from the two families. To better understand pathophysiological aspects of the most promising variants, we are planning functional work in iPSCs.

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Conflict of Interest: None declared

P11.070.A Genetic contributions to epilepsy and febrile seizure: A study of narrow-sense and single-nucleotide polymorphism (SNP) heritability

Janne Auning Hansen[1;2], *Bjarni Jóhann Vilhjálmsson*[1;3;4], *Julie Werenberg Dreier*[1;5], *Betina Trabjerg*[1], *Bjarke Feenstra*[6], *Jakob Christensen*[2;7]

[1]*National Centre for Register-based Research, Aarhus University, Aarhus, Denmark;* [2]*Department of Neurology, Aarhus University Hospital, Aarhus, Denmark;* [3]*Bioinformatics Research Centre, Aarhus University, Aarhus, Denmark;* [4]*Novo Nordisk Foundation Center for Genomics Mechanisms of Disease, Broad Institute of MIT and Harvard, Cambridge, United States;* [5]*Centre for Integrated Register-based Research, Aarhus University, Aarhus, Denmark;* [6]*Statens Serum Institut, Copenhagen, Denmark;* [7]*Department of Clinical Medicine, Aarhus University, Aarhus, Denmark*

Background/Objectives: This study aims to elucidate the heritable aspect of epilepsy and febrile seizures by estimating the narrow-sense heritability of these disorders using data from the Danish population-based health registers and compare with the SNP-heritability estimated using genotype data from the Lundbeck Foundation Initiative for Integrative Psychiatric Research (iPSYCH) sample.

Methods: We utilized the Danish nationwide registers to create a twin cohort with which we estimated the narrow-sense heritability utilising a modified Falconer's formula [1]. Additionally, we conducted genome-wide association studies (GWAS) in the iPSYCH cohort, estimating the SNP-heritability using LDpred2-auto.

Results: We identified 40,205 twin pairs, born in Denmark from 1977 to 2018 including 1140 persons with epilepsy and 2940 persons with febrile seizures. The narrow-sense heritability was 57% (95% CI: 17-91%) for epilepsy and 42% (95% CI: 23-62%) for febrile seizures, while the SNP-heritability for epilepsy was 7.8% (95% CI: 7.7-7.9%) and 28.3% (95% CI: 27.9-28.6%) for febrile seizures.

Conclusion: We demonstrated significant narrow-sense heritabilities underscoring the substantial genetic component in these disorders. The SNP-heritability estimates indicate that common genetic variants play a notable role. However, they account for less than a quarter of the heritable variation in epilepsy, pointing towards the involvement of e.g. structural and rare variants in its pathogenesis.

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P11.071.B Does genetic risk for depression influence microglial function? Analyses in human induced pluripotent stem cell-derived microglia

Eva Beins[1], *Thomas Bajaj*[2], *Ronja Hollstein*[1], *Friederike David*[1], *Frederike Stein*[3], *Susanne Meinert*[4], *Fabian Streit*[5], *Petra Ina Pfefferle*[6], *Kerstin Ludwig*[1], *Jonathan Schmid-Burgk*[7], *Nils Gassen*[2], *Per Hoffmann*[1], *Udo Dannlowski*[4], *Stephanie Witt*[5], *Tilo Kircher*[3], *Markus Nöthen*[1], *Andreas Forstner*[1]

[1]*Institute of Human Genetics, University of Bonn and University Hospital Bonn, Bonn, Germany;* [2]*Department of Psychiatry and Psychotherapy, University Hospital Bonn, Bonn, Germany;* [3]*Department of Psychiatry and Psychotherapy, University of Marburg, Marburg, Germany;* [4]*Institute for Translational Psychiatry, University of Münster, Münster, Germany;* [5]*Department of Genetic Epidemiology in Psychiatry, Central Institute of Mental Health, Medical Faculty Mannheim, University of Heidelberg, Mannheim, Germany;* [6]*Medical Faculty, Comprehensive Biobank Marburg (CBBMR), Member of the German Biobank Alliance (GBA) and the German Center for Lung Research (DZL), Philipps University of Marburg, Marburg, Germany;* [7]*Institute of Clinical Chemistry and Clinical Pharmacology, University of Bonn and University Hospital Bonn, Bonn, Germany*

Consortium: FOR2107

Background/Objectives: Depression is a common psychiatric disorder caused by both genetic and environmental risk factors. Dysregulation of microglia - the brain-resident immune cells - has been implicated, but human data in this context is scarce. Here, we use induced pluripotent stem cell (iPSC) derived microglia (iMG) from patients with major depressive disorder (MDD) and healthy controls (HC) selected from the extreme ends of the genetic risk spectrum.

Methods: iPSC-lines were generated for donors selected from the German FOR2107 cohort based on depression polygenic risk scores and family history for psychiatric disorders ($n = 3$ MDD high risk, $n = 3$ HC low risk). Cells were differentiated into iMG, stimulated with lipopolysaccharide (LPS) and NLRP3 inflammasome activators and analysed using immunocytochemistry, ELISA, immunoblotting and 3'mRNA-sequencing.

Results: In first experiments, all iPSC-lines were successfully differentiated into iMG without major differences in cellular development or transcriptome profiles between MDD and HC. There was no difference in cytokine release after stimulation with LPS alone, however, MDD-iMG showed increased interleukin-β processing and release after activation of NLRP3 by LPS+nigericin, but not LPS + ATP. We further identified several differentially expressed genes between stimulated MDD- vs. HC-iMG, some of which have previously been associated with depression or related traits.

Conclusion: If replicated in upcoming experiments, these findings would suggest that high genetic risk for depression leads to dysregulated microglial NLRP3 inflammasome activation. Overall, our new iPSC-lines provide a valuable tool to study genes and mechanisms involved in depression pathogenesis, analyse gene-environment interactions (e.g. "stress") in a defined cellular model and potentially identify pathways relevant for therapy.

Grants: O-149.0137

Conflict of Interest: None declared

P11.072.C Diagnostic yield of whole-exome sequencing in 400 patients with neurodevelopmental disorders

Asuman Koparir[1], *Eva Metzger*[2], *Konstantinos Kolokotronis*[3], *Paulina Bahena Carbajal*[1], *Erkan Koparir*[1], *Yvonne Jelting*[1], *Michaela AH Hofrichter*[1], *Thomas König*[4], *Eva Runkel*[5], *Juliane Spiegler*[4], *Nicole Stachelscheid*[5], *Delia Lorenz*[6], *Neda Dragicevic Babic*[6], *Helge Hebestreit*[6], *Erdmute Kunstmann*[1], *Thomas Haaf*[1], *Eva Klopocki*[1]

[1]*Institute of Human Genetics, Julius Maximilians University Würzburg, Würzburg, Germany;* [2]*MVZ genetikum GmbH, Neu-Ulm, Germany;* [3]*Institute of Medical Genetics, University of Zurich, Switzerland;* [4]*Department of Pediatrics, University Hospital Würzburg, Würzburg, Germany;* [5]*Department of Pediatrics and Neuropediatrics, Klinikum Aschaffenburg-Alzenau, Aschaffenburg, Germany;* [6]*Center for rare diseases, University Hospital Würzburg, Würzburg, Germany*

Background: Neurodevelopmental disorders (NDDs) are a genetically and clinically heterogeneous group of conditions, affecting 1%–3% of children. NDDs comprise several disorders including intellectual disability, autism spectrum disorder, attention deficit hyperactivity disorder or developmental delay, among others.

Methods: In this study, we evaluated 400 individuals with neurodevelopmental delay and additional clinical features between 2018 and 2023. Most of our patients were previously tested and negative for molecular karyotyping and *FMR1* CGG expansion. We performed whole-exome sequencing to identify the underlying molecular pathology.

Results: 205 of 400 (51%) affected individuals carry 208 different variants in 156 NDD genes. According to the ACMG/AMP criteria we identified 61 variants as pathogenic, 81 as likely pathogenic and 66 as variants of unknown significance. 151 (73.7%) individuals display heterozygous variants in autosomal dominant genes, 37 (18%) carry biallelic variants in autosomal recessive genes, and 17 (8.3%) patients have hemizygous variants in X-chromosomal genes. Furthermore, we detected 8 CNVs, including single-exon or whole-gene deletions.

Conclusion: We performed a retrospective study to estimate the diagnostic yield of whole-exome sequencing in 400 patients with neurodevelopmental disorders. In 36% of our patients pathogenic or likely pathogenic variants were identified and thus, the patients received a genetic diagnosis. In the present study, we identified 145 novel variants in NDD genes and expand the genotypic spectrum of NDDs.

Conflict of Interest: None declared

P11.073.D Copy number variant analysis identifies diagnostic events across diverse epilepsies

Tania Giangregorio[1], *Lorenzo Serra*[2], *Paola Dimartino*[3], *Federica Isidori*[1], *Pamela Magini*[1], *Raffaella Minardi*[4], *Laura Licchetta*[4;5], *Francesca BISULLI*[4;5], *Tommaso Pippucci*[1]

[1]*IRCCS Azienda Ospedaliero-Universitaria di Bologna, Bologna, Italy;* [2]*University of Bologna, Department of Pharmacy and Biotechnology (FaBiT), Bologna, Italy;* [3]*University of Bologna, Department of Medical and Surgical Sciences (DIMEC), Bologna, Italy;* [4]*IRCCS Istituto delle Scienze Neurologiche di Bologna, Reference Center for Rare and Complex Epilepsies-EpiCARE, Bologna, Italy;* [5]*University of Bologna, Department of Biomedical and NeuroMotor Sciences (DIBINEM), Bologna, Italy*

Background/Objectives: Different genetic architectures underlie the main types of epilepsy including genetic generalized epilepsies (GGE), focal epilepsies and developmental and epileptic encephalopathies (DEE). Copy number variants (CNVs) can cause or confer genetic risk for epilepsy. Recent studies show an increased burden of CNVs in epilepsy patients compared to controls and the strong association of CNV hotspots with specific epilepsy types. Our study investigate the landscape of CNVs and their role in the aetiology of epilepsy.

Methods: We extracted DNA from blood samples and collected detailed phenotyping data of 781 individuals with epilepsy. Genotyping data were generated on the Illumina Infinium Global Screening Array. To infer CNVs calls we designed a portable workflow using PennCNV algorithm. We filtered out all samples with a standard deviation of log Rratio > 0.3, Ballele frequency drift > 0.01, waviness factor > 0.05 and a CNV load > 100. CNVs calls were annotated using AnnotSV software.

Results: We identified rare CNV in seizure-associated loci in three patients. An individual with GGE carried a 1.3 MB deletions at 16p13.11 locus previously associated with generalized tonic-clonic seizures, while two patients with DEE carried a 1.6 Mb deletion at 3q29 and a 4.8 Mb deletion at 15q13.1-q11.2 respectively. Almost 1% of patients carry rare pathogenic CNVs spanning known epilepsy genes.

Conclusion: Our findings reflect the complexities of phenotype-genotype relationships in epilepsy and highlight the emerging significance of CNVs that raise risk for specific epilepsy type.

Grants: Epi25 collaborative

Conflict of Interest: None declared

P11.075.B Looking beyond labels: genetics of psychiatric traits cluster with insulin-related measures

Barbara Šakić[1;2], *Izel Erdogan*[2;3], *Giuseppe Fanelli*[2;3;4], *Martina Arenella*[2;3;5], *Nina Roth Mota*[2;3], *Janita Bralten*[2;3]

[1]*Radboud University Medical Center, Human Genetics, Cognitive Neuroscience, Nijmegen, Netherlands;* [2]*Donders Institute for Brain, Cognition and Behaviour, Nijmegen, Netherlands;* [3]*Radboud University Medical Center, Human Genetics, Nijmegen, Netherlands;* [4]*University of Bologna, Department of Biomedical and Neuromotor Sciences, Bologna, Italy;* [5]*King's College London, Institute of Psychiatry, Psychology and Neuroscience, London, United Kingdom*

Background/Objectives: Psychiatric disorders, like attention-deficit/hyperactivity disorder (ADHD), obsessive-compulsive disorder (OCD) and autism spectrum disorder (ASD) and insulin-related diseases co-occur and are genetically correlated, but an understanding of the molecular genetic underpinnings of this overlap remains sparse. Here we aim to explore the existence of dimensional genetic clusters based on ADHD, OCD and ASD symptomatology as well as insulin-related continuous measures observed in the general population.

Methods: We applied multivariate genomic structural equation modeling (genomicSEM) to investigate latent genetic factors underlying publicly available summary statistics of the largest genome-wide association studies (GWASs) of ADHD symptoms (n = 17,666), OCD symptoms and cases (n = 17,992) and sociability linked to ASD (n = 342,461) with GWASs of insulin-related traits (n = 98,210-697,734). Subsequently, we performed multivariate genome-wide association analyses on the observed genetic clusters including both psychiatric and insulin-related traits and follow-up annotation with Functional Mapping and Annotation of Genome-wide association studies (FUMA).

Results: A three-factor model fitted our data the best (AIC = 57, CFI = 0.99, SRMR = 0.068), highlighting a latent genetic factor including ADHD symptom scores together with body mass index, fasting glucose and glycated hemoglobin and a latent genetic factor including OCD symptoms together with glycated hemoglobin. Significant genes (n = 58) and gene-sets (n = 17) associated to these factors indicate a potential role for glycemic regulation

and insulin-related processes as well as neurodevelopmental biological pathways.

Conclusion: Our findings show that latent genetic factors exist reflecting shared liabilities underlying psychiatric traits with insulin-related measures introducing the concept of potential biologically informed subgroups in psychiatry.

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Conflict of Interest: None declared

P11.076.C DNA damage repair genes as modifiers of clinical outcomes in Cuban patients with Spinocerebellar Ataxia type 2

Luis Almaguer-Mederos[1;2], *Dany Cuello-Almarales*[3], *Raúl Aguilera-Rodríguez*[1], *Dennis Almaguer-Gotay*[1], *Yanetza González-Zaldivar*[1], *Suzana Gispert-Sánchez*[2], *Georg W. J. Auburger*[2]

[1]*Center for the Investigation and Rehabilitation of Hereditary Ataxias, Molecular Neurobiology, Holguín, Cuba;* [2]*Goethe University Frankfurt, University Hospital, Clinic of Neurology, Exp. Neurology, Frankfurt am Main, Germany;* [3]*Center for the Investigation and Rehabilitation of Hereditary Ataxias, Clinical Medicine, Holguín, Cuba*

Background/Objectives: DNA damage repair mechanisms have been associated with outcomes of disease severity in Huntington's disease and spinocerebellar ataxias, and are involved in the pathogenesis of these polyglutamine disorders. Here, we assess the role of 10 single nucleotide variants (SNVs) as candidate modifiers of clinical severity in a large cohort of patients with Spinocerebellar ataxia type 2 (SCA2).

Methods: A study involving 307 Cuban SCA2 patients was conducted. The *ATXN2* CAG repeat length was determined by PCR followed by polyacrylamide gel electrophoresis. Most SNVs (*MLH1* rs1800734, *MSH3* rs26279, *ERCC2* rs13181, *ERCC3* rs4150407, *ERCC6* rs2228528, *LIG1* Exon 6 A→C, *XRCC1* rs25487, *XRCC3* rs861539, and *OGG1* rs1052133), were assessed by PCR/RFLP; *MLH3* rs175080 was assessed by 4P-ARMS-PCR. The age at disease onset, SARA, and INAS scores were used as clinical outcome variables.

Results: All examined genotypes were in Hardy-Weinberg equilibrium ($p<0.05$). Age at onset was significantly associated with *MLH1* rs1800734, *ERCC3* rs4150407, *LIG1* Exon 6 A→C, and *OGG1* rs1052133. Nominally significant associations were also obtained between the SARA score and *XRCC1* rs25487, *XRCC3* rs861539, and *OGG1* rs1052133. Besides, the INAS score showed nominal significant associations with *MSH3* rs26279 and *MLH3* rs175080. Contributions of SNVs to clinical outcomes varied between 1.1 and 3.3 percent.

Conclusion: Evidence for modifier effects of SNVs on clinical outcomes of patients with Spinocerebellar ataxia type 2 is provided, suggesting that DNA damage repair pathways might be relevant to SCA2 physiopathology. However, further studies in additional SCA2 cohorts are needed to account for potential effects of population structure.

Conflict of Interest: None declared

P11.077.D 25 years of genetic studies in movement disorders: in-depth insights from a cohort of >9,000 patients

Ana Filipa Brandão[1;2], *Alexandra Lopes*[1;2], *Miguel Alves-Ferreira*[1;2;3], *Ana Lopes*[1;2], *Sara Morais*[1;2], *Patrícia Isabel Marques*[1;2], *Fátima Lopes*[1;2], *Joana Sá*[1;2], *Rita Bastos Ferreira*[1;2], *Diana Pinto*[1;2], *Liliana Rocha*[1;2], *Paulo Silva*[1;2], *Maria João Sá*[1;2], *Jorge Sequeiros*[1;2;3], *João Parente Freixo*[1;2], **Jorge Oliveira**[1;2]

[1]*CGPP – Centre for Predictive and Preventive Genetics, IBMC – Institute for Molecular and Cell Biology, Univ. Porto, Porto, Portugal;* [2]*i3S – Instituto de Investigação e Inovação em Saúde, Univ. Porto, Porto, Portugal;* [3]*ICBAS – School of Medicine and Biomedical Sciences, Univ. Porto, Porto, Portugal*

Background/Objectives: Movement disorders (MD) encompass neurological diseases with abnormal voluntary or involuntary movements. The genetic etiology of MD has advanced with next-generation sequencing (NGS) and gene discovery in recent years. Our aim is to delineate the mutational spectrum in a substantial cohort of MD patients from 1998 to 2023.

Methods: We reviewed our database for MD genetic studies (NGS multigene panel or fragment analysis), targeting disease-causing variants or those of unknown clinical significance (VUS).

Results: For hereditary ataxias due to repeats' expansions ($n = 2,836$) the diagnostic yield was 26.9%($n = 764$) [522 SCAs patients (mostly Machado-Joseph Disease/SCA3), 143 with FRDA and 99 CANVAS] and for non-repeat expansions by NGS ($n = 593$) 9.8%($n = 58$) had a molecular diagnosis, 49.1%($n = 291$) had VUS, and 41.2%($n = 244$) were negative.

Regarding Huntington's disease ($n = 2,203$), 44.0%($n = 973$) had a molecular diagnosis, 54.0%($n = 1189$) were negative, and ~2% reduced-penetrance disease-causing alleles.

For Parkinson's disease ($n = 318$), 10.7%($n = 34$) had a molecular diagnosis, 41.8%($n = 133$) had VUS, and no reported variants in 47.5%($n = 151$).

Concerning spastic paraplegia ($n = 495$), 21.0%($n = 105$) had a molecular diagnosis, 38.0%($n = 186$) had VUS and in 41.0% ($n = 204$) no variants were reported.

For dystonia ($n = 292$) 9.0%($n = 25$) had a molecular diagnosis, 51.0%($n = 150$) had VUS, while in 40.0%($n = 117$) no variants were reported.

Reassessment is ongoing to include >4,400 cases studied by single-gene analysis (Sanger seq. and/or MLPA), which will expand our results.

Conclusion: The diagnostic yield of MD patients was ~34%, mostly attributable to repeats' expansions. Further 14% have uncertain outcomes requiring clinical revaluation and/or additional studies for a definitive diagnosis. Globally, this data is crucial for developing a comprehensive genetic test, leveraged by ongoing NGS improvements.

Conflict of Interest: None declared

P11.078.A Variation profiling of protein-coding genes in Bulgarian paediatric patients with specific language impairment

Iglika Sotkova-Ivanova[1;2], *Hristo Ivanov*[1;3], *Aleksandar Linev*[1;3], *Ivan Ivanov*[2;3], *Iliyana Pacheva*[1;2], *Nikolay Popov*[4], *Tihomir Vachev*[5], *Radoslava Vazharova*[6], *Vili Stoyanova*[1;3]

[1]*Medical University Plovdiv, Department of Pediatrics and Medical Genetics, Plovdiv, Bulgaria;* [2]*University Hospital "St. George" Plovdiv, Department of Pediatrics, Plovdiv, Bulgaria;* [3]*University Hospital "St. George" Plovdiv, Department of Medical Genetics, Plovdiv, Bulgaria;* [4]*Child Center Spectar, Povdiv, Bulgaria;* [5]*Plovdiv University "Paisii Hilendarski", Department of Plant Phisyology and Molecular Biology, Plovdiv, Bulgaria;* [6]*SU "St. Kliment Ohridski", Faculty of Medicine, Sofia, Bulgaria*

Background/Objectives: Specific Language Impairment (SLI) affects 7-10% of children, causing delayed speech development. The language deficit in SLI children is not due to cognitive, motor, sensory, or socio-emotional factors. Our study aims to characterize the variation profile of protein coding genes in SLI patients.

Methods: We conducted whole exome sequencing on 40 children with proven SLI and performed enrichment analysis of biological pathways using KEGG and Reactome.

Results: Sequencing and enrichment analysis revealed diverse genetic variants and interactions between genes associated with sound sensory processing, neural network development, axon guidance, and synaptic formation. We identified variants in new candidate genes: MSTO1, BAP1, PMABP1, and PRODH. The CACNA gene family, regulating calcium channels, and the CDH gene family, involved in cell adhesion and neuronal connectivity, emerged as compelling candidates.

Conclusion: Many variants linked to language deficits in our patients are implicated in pathways related to sound sensory processing. These genetic variants may coexist with other benign variants, leading to minor alterations in sound perception that result in SLI. Variants in genes associated with neural network development, axon guidance, and synaptic formation may subtly interfere with normal neural network development and functioning, resulting in impaired language abilities. The specific nature of the mutation and its impact on the function of their products may also contribute to the severity of the resulting phenotype. Further functional studies are warranted to fully comprehend the relationship between these mutations and SLI. This hypothesis offers a valuable framework for exploring the underlying mechanisms of this complex disorder.

Conflict of Interest: None declared

P11.079.B A novel pathogenic *PTRH2* variant indicates seizure association with protein-truncating variants in Infantile-Onset Multisystem Neurologic, Endocrine and Pancreatic Disease (IMNEPD)

Ömer Çağrı Akçin[1], *Okan Kurtçu*[2], *Neşe Dericioğlu*[3], *Sevim Erdem Özdamar*[3], *Can Ebru Bekircan-Kurt*[3], *Şule Altıner*[2], *arda çetinkaya*[1;4]

[1]*Hacettepe University, Faculty of Medicine, Department of Medical Genetics, Ankara, Türkyie;* [2]*Ankara University, Faculty of Medicine, Department of Medical Genetics, Ankara, Türkyie;* [3]*Hacettepe University, Faculty of Medicine, Department of Neurology, Ankara, Türkyie;* [4]*Hacettepe University, Center For Genomics and Rare Diseases, Ankara, Türkyie*

Background: Infantile-Onset Multisystem Neurologic, Endocrine and Pancreatic Disease (IMNEPD) is an ultra-rare disorder with variable expressivity, resulting from biallelic loss-of-function variants in *PTRH2*. *PTRH2* encodes for peptidyl-tRNA hydrolase, possibly involved in release of peptides from stalled mitochondrial ribosomes, which is also involved in apoptosis.

Methods: Whole exome sequencing (WES) and detection of homozygosity regions were utilized to explore the disease-causing variant.

Results: Here, we report a novel IMNEPD-associated pathogenic *PTRH2* variant in a 24-year-old male born to consanguineous parents. The proband exhibited mild intellectual disability, sensorineural hearing impairment, absence seizures, demyelinating peripheral neuropathy, distal muscle weakness, pes equinovarus, ataxic gait and exocrine pancreatic insufficiency. He lacked significant motor delay in childhood. Cerebellar atrophy, endocrine abnormalities and hepatomegaly were absent. WES revealed a single homozygous nonsense variant within a homozygosity region, which results in a truncated PTRH2 protein (NM_016077.5:c.100C>T, p.Arg34Ter) lacking the hydrolase domain and part of mitochondrial localization sequence.

Conclusion: In contrast to previous studies associating severe disease phenotype with loss-of-function variants (PMID: 37239392), the proband had a mildly progressing clinical phenotype with previously unreported absence seizures. Non-absence seizures and EEG anomalies have only been associated with 4 of the 6 reported nonsense/frameshift variants and none of the 3 missense variants. For other phenotypic features, phenotypic variability is extensive even within the same families. In conclusion, we suggest that seizures in IMNEPD are associated with *PTRH2* genotypes leading to a truncated protein. However, the variability in other phenotypic features still need to be addressed, which are possibly explainable by *PTRH2*-independent causes.

Grants: None.

Conflict of Interest: None declared

P11.080.C World-second report of a family with familial narcolepsy, cataplexy and novel *MOG* variant

Jakub Trizuljak[1;2;3], *Jakub Duben*[1], *Tomáš Gyönyör*[2], *Zuzana Vrzalová*[1;3], *Renáta Michalovská*[4], *Věra Hořínová*[2;5]

[1]*University Hospital Brno, Department of Internal Medicine - Hematology and Oncology, Brno-Bohunice, Czech Republic;* [2]*University Hospital Brno - Children's Hospital, Department of Medical Genetics and Genomics, Czech Republic;* [3]*CEITEC MU, Czech Republic;* [4]*GHC GENETICS, s.r.o., Czech Republic;* [5]*Reprofit International Brno, Czech Republic*

Background/Objectives: Narcolepsy is a neurological disorder that is marked by the recurrent, sudden, uncontrollable compulsion to sleep. Narcolepsy is often associated with cataplexy, sleep paralysis, hypnagogic hallucinations (pre-sleep dreams), and automatic behaviours. Familial narcolepsy accounts for less than 10% of all narcolepsy cases. However, documented families are very rare and causative mutation has been identified only in one case. We present a 4-generational pedigree with autosomal dominant cataplexy and sleep disorder, caused by a novel mutation of myelin-oligodendrocyte glycoprotein (*MOG*) gene.

Methods: We performed whole exome sequencing (WES) and segregation analysis of both affected and healthy family members.

Results: WES identified a novel, likely pathogenic variant c.284G>C, (p.Arg95Pro, rs752019554) in *MOG* gene, segregating with sleep disorder and cataplexy in the family, but absent in unaffected family members. The affected individuals also show elevated prolactin serum levels. To date, only one Spanish family with *MOG* causal variant has been reported (Hor et al., 2011).

Conclusion: We present a second report of a family with autosomal dominant narcolepsy and cataplexy, segregating with a *MOG* likely pathogenic variant. These findings support a role for myelin and oligodendrocytes in disease susceptibility to this neurobehavioral disorder.

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Conflict of Interest: None declared

P11.081.D Application of HiFi long-read genome sequencing reveals large 125 CAG repeat expansion in *HTT* in a patient with infantile Huntington's disease

Juliane Martinez Sena[1], *Christina Pontes*[2], *Roland Achmann*[3], *Rainer Koenig*[1], *Ulrich Seidel*[4], *Zafer Yüksel*[1], *Christian Betz*[1], *Hanno Bolz*[1]

[1]*Bioscientia Institute for Medical Diagnostics GmbH, Bioscientia Human Genetics, Ingelheim, Germany;* [2]*Heidelberg University Hospital, Pediatric Neurology, Heidelberg, Germany;* [3]*Labor Lademannbogen MVZ GmbH, Human Genetics, Hamburg, Germany;* [4]*Klinikum Worms gGmbH, Pediatric Neurology, Worms, Germany*

Background/Objectives: Huntington's disease (HD) is an autosomal dominant disorder caused by a CAG trinucleotide repeat expansion in the huntingtin gene (*HTT*). Repeat expansion correlates inversely with age of onset. HD is characterized by progressive decline of motor and cognitive function, and psychiatric changes, with onset mostly at 35-45 years. Infantile HD is rare and often accompanied by non-specific symptoms delaying the diagnosis. We describe a male with infantile HD and a large *HTT* repeat expansion.

Methods: The patient had previously been tested for HD in a reference laboratory using an established approach, triplet repeat-primed PCR (TP-PCR). After PacBio HiFi long-read whole-genome sequencing (LR-WGS) based on DNA from peripheral blood, data were processed using the tandem repeat genotyper (TRGT, PacBio). Further, samples from patients with confirmed repeat diseases (n = 8, FraX/*FMR1*, SCA1/*ATXN1*, SCA17/*TBP*, HD/*HTT*, BSMA/*AR*) were analysed by LR-WGS, and results compared with the respective repeat ranges identified by conventional tests.

Results: While TP-PCR displayed ">100, approximately 118 CAG repeats", LR-WGS identified 125 CAG repeats and repeat interruptions on the mutant *HTT* allele. LR-WGS results for the 8 additional samples from other repeat disorders closely or exactly corresponded to values identified by conventional methods.

Conclusion: HiFi LR-WGS represents a comprehensive approach for diagnosing pediatric patients with a differential diagnosis of HD. Although more comparative analyses are needed for *HTT* and other repeat disorder-associated loci, LR-WGS appears to be suitable for quantifying the respective expansions and additionally provides information on repeat interruptions as potential modifiers.

Conflict of Interest: None declared

P11.082.A Detection of low VAF somatic variants in focal cortical dysplasias and brain tumors using Single-molecule Molecular Inversion Probes

Ester Cifaldi[1;2], *Paola Dimartino*[3], *Lorenzo Ferri*[4;5], *Raffaella Minardi*[5], *alessia perciavalle*[3], *laura licchetta*[5], *Marco seri*[1;3], *Marco de Curtis*[6], *Rita Garbelli*[6], *Laura Rossini*[6], *Laura Tassi*[7], *Francesca Gozzo*[7], *michele rizzi*[8], *matteo martinoni*[9], *Elena Pasinis*[5], *Roberto Michelucci*[5], *gianluca marucci*[10], *sofia asioli*[4;5], *Francesca BISULLI*[4;5], *Leonardo Caporali*[5], *Tommaso Pippucci*[11]

[1]*Polyclinic Sant'Orsola-Malpighi University Hospital, Medical Genetics Unit, Bologna, Italy;* [2]*University of Siena, Department of Molecular and Developmental Medicine, Siena, Italy;* [3]*University of Bologna, Department of Medical and Surgical Sciences (DIMEC), Bologna, Italy;* [4]*University of Bologna, Department of Biomedical and NeuroMotor Sciences (DIBINEM), Bologna, Italy;* [5]*IRCCS Institute of Neurological Sciences of Bologna (full member of the European Reference Network EpiCARE) Reference Center for Rare and Complex Epilepsies-EpiCARE), Bologna, Italy;* [6]*IRCCS Foundation, Neurological Institute "C.Besta", Epilepsy Unit, Milano, Italy;* [7]*Niguarda Hospital, "Claudio Munari" Epilepsy Surgery Center, Milano, Italy;* [8]*IRCCS Foundation, Neurological Institute "C.Besta", Department of Neurosurgery, Milano, Italy;* [9]*IRCCS Institute of Neurological Sciences of Bologna, Division of Neurosurgery, Bologna, Italy;* [10]*IRCCS Foundation, Neurological Institute "C.Besta", Neuropathology Unit, Milano, Italy;* [11]*IRCCS Azienda Ospedaliero–Universitaria di Bologna, Computational Genomics Unit, Bologna, Italy*

Background: Drug resistant focal epilepsy (DRFE) accounts for a significant proportion of epilepsy burden worldwide. DRFE-associated mutations can be brain-only somatic events often limited to the epileptogenic zone. Given their relatively low variant allelic fraction (VAF), their detection is challenging.

Methods: DNA was extracted from matched Fresh Frozen (FF) or Formalin-Fixed, Paraffin-Embedded (FFPE) brain and blood samples of 94 DRFE patients. We designed separate panels for Focal Cortical Dysplasia (FCD, 14 genes) and brain tumors (BT, 10 genes) using single molecule Molecular Inversion Probes (smMIPs).

Results: smMIPs sequencing obtained an adequate read depth required for the detection of low VAF variants. We obtained 3000X mean coverage for uncollapsed reads and target regions were covered with >200 unique reads on average. In the 175 tested samples, we identified a pathogenic variant in SLC35A2 with VAF 2.49% in FF lesional tissue of a FCD type I patient and not present in the blood, somatic mutations in MTOR with VAFs ranging from 1.07% to 5.89%, germline variants in DEPDC5, TSC1 and NPRL3 genes in brain-blood matched samples from FCD type II patients. We also detected BRAF somatic mutations (VAFs from 2% to 35%) and TP53 and IDH1 mutations in BT patients. For FFPE FCD type II samples, we discovered somatic mutations in RHEB, in MTOR and TSC2 (from 1.10% to 3.86%) and a germline pathogenic mutation in TSC1 gene.

Conclusion: smMIPs showed accurate detection of low-VAF mosaicisms in both FF and FFPE brain samples.

Grant: Italian Ministry of Health RF-2019-12370564

Conflict of Interest: None declared

P11.083.B Diagnostic yield of chromosomal microarray analysis in 1.398 patients with autism spectrum disorder: A single Hospital experience over 9 years.

Anna Brunet-Vega[1], *Neus Baena*[1], *Laura Capel*[1], *Eduardo Perez-Romero*[1], *Núria Capdevila*[1], *Juan Pablo Trujillo Quintero*[1], *Carmen Manso*[1], *Victor Martinez-Glez*[1]

[1]*Parc Taulí Hospital Universitari. Institut d'Investigació i Innovació Parc Taulí (I3PT-CERCA). Universitat Autònoma de Barcelona., Center for Genomic Medicine, Sabadell, Spain*

Background/Objectives: Autism spectrum disorder (ASD) comprises a group of neurodevelopmental disorders characterized by impairment in social interaction, communication and behavioural challenges. Genetic factors play a role, and chromosomal microarray analysis (CMA) is a rapid and effective method for detecting copy number variants (CNV).

This work aims to evaluate the efficiency of using CMA as a first-line test in patients with ASD and to determine relevant CNV.

Methods: We enrolled 1.398 patients with ASD who underwent CMA between 2014-2023. Cases were evaluated by a clinical geneticist or neuropediatrician and had a clinical diagnosis of ASD (HP:0000717, HP:0000729, HP:0000753). An 8 × 60K array-CGH was performed on 714 patients and 4X180K exon-level on 684 patients.

Results: We identified pathogenic and likely pathogenic CNVs in 83 patients (59 males and 24 females), reaching an overall diagnostic yield of 6%. Most frequent CNVs were recurrent: 1q21.1 duplication (4 patients), 15q11.2 deletion (7 patients), 15q13.3 duplication (7 patients), 16q11.2 deletion (5 patients), 16p13.11 duplication (4 patients), and 22q11.21 duplication (4 patients). Nine patients carried unique large pathogenic CNVs (4-18Mb), 5 gains (2q31q33, 3q25q26, 16q21 and Xp11) and 4 losses (1p34, 4q34q35, 10q11 and 22q13). We highlight that 17% of pathogenic CNVs were small deletions (7.4 to 222Kb) of only one or few exons on *IMMP2L* (3 patients), *DMD* (2 patients), *TMLHE* (2 patients), SLC9A9, *PRKN*, *CNTNAP2*, *RBFOX1*, *GRIN2A* and *SHANK3* genes with an important role in neurodevelopment.

Conclusion: This series reaffirms the diagnostic yield of CMA in ASD and highlights the utility of exon-focus array-CGH for diagnosis.

Conflict of Interest: None declared

P11.084.C Investigating the early phases of psychosis in the light of human evolutionary genetics

Veronica Sandroni[1], *Qin He*[1], *Ridha Joober*[2], *Marie-Odile Krebs*[1;3], *Sebastien Jacquemont*[4], *Guy Rouleau*[5], *Boris Chaumette*[1;2;3]

[1]*Université Paris Cité, Institute of Psychiatry and Neuroscience of Paris (IPNP) - INSERM U1266, Paris, France;* [2]*McGill Université, Department of Psychiatry, Montreal, Canada;* [3]*GHU-Paris Psychiatrie et Neurosciences, Paris, France;* [4]*Centre de Recherche du Centre Hospitalier Universitaire Sainte-Justine, Montreal, Canada;* [5]*Montreal Neurological Institute and Hospital - McGill University, Department of Neurology and Neurosurgery, Montreal, Canada*

Background/Objectives: Schizophrenia appears gradually during adolescence after a period called ultra-high risk (UHR). The reasons why some UHR individuals convert (UHR-C) to a first-episode of psychosis (FEP) while other do not (UHR-NC) is poorly understood. We aim at testing the Olduvai domain, to explain this differential outcome. This domain is the highest amplified coding sequence in the human genome and six subtypes have been described: conserved (CON1-3), and human-lineage specific (HLS1-3). The number of copies has been associated with increased neuronal proliferation, brain volume, better cognitive performance, and symptoms severity in schizophrenia.

Methods: From a cohort of 396 individuals with Whole-Genome sequencing data (IlluminaSeq), we tested the association between the CON1 and HLS1 copy numbers and the evolution toward the disease (UHR-C, UHR-NC, FEP).

Results: CON1 mean copy number was 80.91 (± 4), 80.85 (± 3.76), and 81.29 (± 4.49) for UHR-NC, UHR-C and FEP respectively and no statistical difference was found. HLS1 mean copy number was 132.73 (± 22.44), 131.41 (± 15.41), and 148.24 (± 34.33) for UHR-NC, UHR-C and FEP respectively. Statistical difference in HLS1 copy number was found for UHR-NC vs UHR-C + FEP (p-value = 0.0002), UHR-C vs FEP (p-value = 7.10^{-7}), UHR-C vs UHR-NC vs FEP (p-value = 5.10^{-5}). No difference was found between UHR-NC and UHR-C.

Conclusion: CON1 copy number did not differ between UHR-NC, UHR-C and FEP. HLS1 copy number differed between the three groups with higher values in UHR-C and FEP.

Grants : année recherche, bourse CFP, Brain Canada, SchizoREmics

Conflict of Interest: None declared

P11.085.D Voltage-gated ion channels and their role in epilepsy

Magdalena Soukup Vodickova[1;2], *Barbora Jeřábková*[2], *Kamila Reblová*[2;3], *Lenka Fajkusova*[1;2]

[1]*Faculty of Science Masaryk University, National Centre for Biomolecular Research, Brno, Czech Republic;* [2]*University Hospital Brno - Children's Hospital, Centre of Molecular Biology and Genetics, Brno, Czech Republic;* [3]*Faculty of Medicine Masaryk University, Institute of Medical Genetics and Genomic, Brno, Czech Republic*

Background:

Epilepsy and epileptic syndromes are a group of heterogeneous neurological diseases characterized by spontaneous recurrent seizures caused by an imbalance of neuron excitability. Next-generation sequencing (NGS) brought to light multiple genes responsible for monogenic epilepsies. Some of the most commonly diagnosed genes are those coding voltage-gated ion channels. However, epilepsy management remains challenging, and patients often suffer from seizures even after receiving a diagnosis.

Methods: Patients with epilepsy underwent NGS-based targeted panel sequencing followed by bioinformatics analysis for variant detection.

Results: We have identified 53 pathogenic and probably pathogenic variants in our cohort of patients in eight ion channel genes. The most commonly diagnosed gene was *SCN1A*, which encodes alpha-1 subunit of the voltage-gated sodium channel Nav1.1. We have found a variant in *SCN1A* in 22 affected patients. Variants in this gene can cause a wide spectrum of phenotypes, and the management of epilepsy can defer depending on whether the variant is gain or loss of function. The second most common gene in our cohort, with 10 diagnosed patients, was *KCNQ2*, a gene encoding potassium channel subunit Kv7.2. *KCNQ2* is associated with neonatal epilepsies that can result either in benign familial neonatal epilepsy or in severe developmental epileptic encephalopathy. The localization of the variant within a protein may help predict the development of epilepsy.

Conclusion: This highlights the importance of finding genetic and molecular evidence supporting the pathogenic role of variants in ion channel genes. Moreover, these findings might play a crucial role in therapeutic approaches of precision medicine.

Conflict of Interest: None declared

P11.086.A Novel autism risk genes inferred by comparative sociogenomics and molecular network analysis

Alice Chiodi[1], *Francesca Anna Cupaioli*[1], *Ettore Mosca*[1], **Alessandra Mezzelani**[1]

[1]*Institute for Biomedical Technologies (CNR-ITB), National Research Council, Segrate (MI), Italy*

Background/Objectives: Autism spectrum disorders (ASD) are a group of complex neurodevelopmental conditions characterized by difficulty with social interaction and communication. Although ASD etiology is still uncertain, genetics play a key role: heritability is estimated at around 80% and thousands of autism risk genes have been reported in the Simons Foundation Autism Research Initiative (SFARI) database. Since some of these genes are involved in social behavior of different animals, including humans, we hypothesized that the genetic underpinnings of sociability should be in common and stable across multiple species and may be implicated in autism.

Methods: Network diffusion analysis was applied to 659 genes linked to sociability in several animals, including humans. The resulting top network was further studied in terms of communities, pathways and enrichment of SFARI genes.

Results: The top network, composed by 240 genes, is significantly associated with SFARI (46 genes, p < 10^-15), with 194 possible novel autism risk genes. Among the latter, the analyses highlighted cadherin and catenin family genes (*CDH-2/6/8, CTNN-A2/D1 and ARVCF*), acting in cell adhesion and cell-cell communication and involved in neurodevelopment. Impairment of *CDH2* and *CTNNA2* is related to blood-brain barrier immune cell transmigration, while of *CDH6/8* to a syndromic deafness.

Conclusion: Comparative sociogenomics, coupled with advanced bioinformatic methods, allows the identification of conserved gene networks involved in sociability and ASD. This approach is also powerful in predicting novel autism risk genes that, if validated, could represent novel biomarkers of the condition, shedding light on its complex etiology.

Grants: EU "GEMMA" (825033); EU/MUR "BBMRI.it" (IR0000031).

Conflict of Interest: None declared

P11.087.B Additional Evidence of *ERBB4* as a Haploinsufficient Gene Associated with Neurodevelopmental Disorders

Irene Mademont-Soler[1;2], *Maria Camós-Carreras*[2;3], *Aida Palacín*[2;4], *Dolors Casellas-Vidal*[2;3], *Gemma Hernández-Gallego*[2;3], *Carles Garrido*[5], *Xavier Queralt*[1], *Susanna Esteba-Castillo*[2;4], *María Obón*[1;2]

[1]*Hospital Universitari de Girona Dr. Josep Trueta, Àrea de Genètica Clínica i Consell Genètic, Salt;* [2]*Institut d'Investigació Biomèdica de Girona (IDIBGI-CERCA), Salt;* [3]*Hospital Universitari de Girona Dr. Josep Trueta, Servei de Neuropediatria, Girona;* [4]*Institut Assistència Sanitària, Salt;* [5]*Reference Laboratory, Barcelona, Spain*

Background/Objectives: The gene *ERBB4* plays a crucial role in numerous neurobiological processes in both the developing and adult brain. It has been suggested that its haploinsufficiency may be related to intellectual disability (ID) or epilepsy, but evidence is still limited.

Methods: Array CGH was performed on a 6 year-old boy diagnosed with autistic spectrum disorder (ASD), who did not exhibit intellectual disability (ID) or language difficulties. Genetic studies were also conducted on five family members. All of them underwent a comprehensive evaluation covering cognition, mental health and behaviour.

Results: The proband presented a 310 Kb deletion of 2q34, involving exons 3-16 of the *ERBB4* gene. Both his father and paternal grandmother also carried the deletion and were diagnosed with ASD (without ID), although their phenotypes were milder. Mental health examination of non-carriers did not reveal any psychiatric disorder.

Conclusion: The identified intragenic deletion of *ERBB4* adds further evidence regarding the involvement of this gene in neurodevelopmental disorders and provides the first evidence of its association with ASD. Notably, expressivity appears to be variable among carriers, which is an important consideration when providing genetic counselling.

Grants: No grants were secured for this project.

Conflict of Interest: None declared

P11.088.C Novel *GNB1* pathogenic variants as a cause of developmental delay

Emilija Shukarova Stefanovska[1], *Predrag Noveski*[1], *Ivana Maleva Kostovska*[1], *Gabriela Novotni*[2], *Learta Alili-Ademi*[3], *Dijana Plaseska-Karanfilska*[1]

[1]*Macedonian Academy of Sciences and Arts, Research Center for Genetic Engineering and Biotechnology "Georgi D. Efremov", Skopje, Macedonia;* [2]*University Neurology Clinic, Medical Faculty, Skopje, Macedonia;* [3]*University Pediatric Clinic, Medical Faculty, Skopje, Macedonia*

Background/Objectives: Neurodevelopmental disorders (NDD) represent the most prevalent chronic disorders encountered in pediatric setting. Comprehensive care for individuals with NDD includes a search for an underlying etiologic diagnosis, primarily through a genetic evaluation. Recently, pathogenic variants in guanine nucleotide-binding protein, beta 1 (*GNB1)* gene have been identified as a cause of developmental delay. *GNB1*-encephalopathy is inherited in an autosomal dominant manner and is typically caused by de novo pathogenic variants, most of them missense variants in exons 6 and 7. So far, only around 60 cases with *GNB1*-encephalopathy have been reported.

Methods: Genetic testing was performed by whole exome sequencing.

Results: Here we present three cases with *GNB1* pathogenic variants, detected by WES analyses. Two novel pathogenic variants: c.1009A>G; p.Lys337Glu in exon 11 and c.343G>A; p.Gly115Ser in exon 7, occurring as de novo events were identified in a 33-years moderately affected male and 28-years mildly affected female patients, respectively, both with developmental delay and dystonia. A well-known pathogenic variant c.239T>A, p.Ile80Asn in exon 6 was identified in a male newborn with severe hypotonia and dysmorphic features and was inherited from a mosaic unaffected father.

Conclusion: In conclusion, our study describes the first cases of *GNB1*-encepholopathy in N. Macedonia with various phenotypes and contributes to the spectrum and associated clinical presentations of *GNB1* pathogenic variants.

Grants: N.A.

Conflict of Interest: None declared

P11.089.D Detection of a de novo loss-of-function variant in *KDM5A*: expanding the mutational and phenotypic spectrum of *KDM5A*-associated autism spectrum disorder

Kirstin Hoff[1], *Monika Kautza-Lucht*[1], *Daniel Kaschta*[1], *Christina Post*[2], *Katharina Schau-Römer*[1], *Inga Vater*[1], *Malte Spielmann*[1;2], *Inga Nagel*[1]

[1]*University Hospital Schleswig-Holstein Campus Kiel, Institute of Human Genetics, Kiel, Germany;* [2]*University Hospital Schleswig-Holstein Campus Lübeck, Institute of Human Genetics, Lübeck, Germany*

Background/Objectives: Despite the wide genetic and clinical variability of neurodevelopmental disorders (NDDs), proteins involved in NDD belong to only a few common frequently affected processes, including chromatin remodeling. In an individual with NDD and autism spectrum disorder (ASD), we report a de novo truncating variant in *KDM5A*, a lysine-specific histone H3 demethylase contributing to chromatin architecture modification.

Methods: Genome sequencing was performed on a four-year-old male patient and his parents. The patient exhibited NDD, ASD, macrocephaly, tall stature, urethral malformation and dysmorphic facial features.

Results: We could identify a de novo likely pathogenic variant c.1726C>T p.(Gln576*) in *KDM5A*. This variant, absent in population databases, introduces a premature termination codon, presumably resulting in protein truncation or nonsense mediated decay. It has been reported as an inherited variant of unknown significance in one individual with *KDM5A*-associated NDD (ClinVar-ID 1701668).

Conclusion: Little is known about KDM5A-associated NDDs, with potentially disease-relevant variants reported in both monoallelic and biallelic contexts. In a study by El Hayek et al. (2020, eLife), reduced levels of KDM5A were observed in a patient with a de novo loss-of-function variant. Recent functional studies indicate that KDM5A is essential for establishing hippocampal cell identity during early development. Given the hippocampus's involvement in NDDs and ASD, truncating variants in *KDM5A* may play a disease-relevant role.

Grants:

Conflict of Interest: Kirstin Hoff provision of free of charge sequencing reagents and software by Illumina within the framework of a collaborative research study, Monika Kautza-Lucht provision of free of charge sequencing reagents and software by Illumina within the framework of a collaborative research study, Daniel Kaschta provision of free of charge sequencing reagents and software by Illumina within the framework of a collaborative research study, Christina Post provision of free of charge sequencing reagents and software by Illumina within the framework of a collaborative research study, Katharina Schau-Römer provision of free of charge sequencing reagents and software by Illumina within the framework of a collaborative research study,

Inga Vater provision of free of charge sequencing reagents and software by Illumina within the framework of a collaborative research study, Malte Spielmann provision of free of charge sequencing reagents and software by Illumina within the framework of a collaborative research study, Inga Nagel provision of free of charge sequencing reagents and software by Illumina within the framework of a collaborative research study

P11.090.A Impact of de novo variants on epilepsy in neurodevelopmental disorders

Andrea Eoli[1;2], *Henrike Heyne*[1;2]

[1]*Hasso Plattner Institute for Digital Engineering gGmbH, University of Potsdam, Potsdam, Germany;* [2]*Icahn School of Medicine at Mount Sinai, Mount Sinai School of Medicine, New York, United States*

Background/Objectives: Epilepsy is often a comorbidity in neurodevelopmental disorders (NDDs), a significant proportion of which can be attributed to rare de novo variants (DNVs). Although previous studies identified more than 360 disease-associated genes, many more remain undiscovered at increasing sample sizes, which prompts the exploration of larger datasets.

Methods: To increase statistical power, we recruited and meta-analyzed eight different cohorts of parent-offspring trios of individuals with NDDs. After extracting DNVs from coding regions and harmonizing them across the cohorts, we applied a mutational model (DeNovoWEST) to compare each gene's observed *versus* expected DNV rate and discover novel genetic associations contributing to these complex phenotypes. Eventually, we compared DNVs in NDDs with versus without epilepsy, performing gene set enrichment analyses and phenotype enrichment analyses (using HPO terms).

Results: We meta-analyzed DNVs across 16,538 individuals diagnosed with different NDDs, of which 6,964 (42%) also had epilepsy, to identify disease genes associated with and without epilepsy. We compared DNVs in NDDs with versus without epilepsy.

Conclusion: With our findings, we gain insights into the pathobiology of epilepsy.

Grants: German Research Foundation (516649954).

Conflict of Interest: None declared

P11.091.B The pathophysiology underlying congenital hydrocephalus

Marlene Dallmayer[1], *Karina Violou*[1], *Heymut Omran*[1], **Julia Wallmeier**[1]

[1]*University clinic Münster, Department of General Pediatrics, Münster*

Consortium: Hydrocephalus-Study Group

Background/Objectives: One in 1.000 newborns suffers from accumulation of cerebrospinal fluid, referred to as hydrocephalus, which is frequently caused by intracranial hemorrhage, infections or tumors. In about 10% of cases there is no external cause of hydrocephalus. In this group genetic defects have been identified underlying hydrocephalus development. Whereas some genetic defects are associated with cortical malformations, or multi-systemic disorders such as ciliopathies, RASopathies or craniosynostosis others only cause enlarged ventricles.

Methods: We performed whole exome sequencing / whole genome sequencing analyses in 62 affected (27 hydrocephalus only / 35 hydrocephalus + brain malformation). We first applied a virtual panel including 190 genes previously associated with hydrocephalus. Next, we performed virtual panels (~500 genes) for ciliopathies, brain malformations, craniosynostosis, RASopathies as well as congenital disorders of glycosylation.

Results: Utilizing the „Hydrocephalus"-panel we identified variants in eight families in seven different genes (*CCDC40, KIAA1109, L1CAM, MPDZ, POMGNT1, POMGNT2*), which we considered likely disease causing. These were very rare (<0.001 (aut.-rez.), <0.0001 (dom.)) nonesense-mutations or considered likely disease causing in Varsome. Applying the combined virtual panel, we identified five additional variants in *SMARCA4, TUBB4B*, *SLC12A6, KRAS* and *CDH2*, which were very rare and either homozygous or inherited de novo.

Conclusion: Consistent with previous reports we were able to identify the underlying genetic defect in ~ 20% of patientents with idiopathic hydrocephalus. Our data point to specific pathophysiological entities causing congenital hydrocephalus in the heterogenous group of disorders with idiopathic hydrocephalus. However, there is still a large group of genetically and pathophysiolgically unsolved cases.

Grants: DFG WA 4283/1-1

Conflict of Interest: Marlene Dallmayer: None declared, Karina Violou: None declared, Heymut Omran DFG OM6/10, Ethris, Recode, Julia Wallmeier DFG WA 4283/1-1

P11.092.C Novel bi-allelic *DMXL2* gene variants in patients with Ohtahara syndrome

Myriam Essid[1;2], *Maxime Colmard*[3], *Maryline Carneiro*[4], *Pauline MONIN*[1], *Valentin Ruault*[5], *Nicole Chemaly*[6], *Blandine Dozieres*[7], *Laurence Perrin*[7], *Reza Maroofian*[8], *Farzad Hashemi-Gorji*[9], *Julie Piarroux*[3], *Nicolas Chatron*[1;2], *Laurent Villard*[10], *Rima Nabbout*[6], *Giulia Barcia*[11], *Gaetan Lesca*[1;2]

[1]*Groupement Hospitalier Est, Hôpital Femme-Mère-Enfant, Hospices Civils de Lyon, Department of Medical Genetics, Lyon, France;* [2]*Institut Neuromyogène, CNRS UMR 5310 - INSERM U1217, Université de Lyon, Université Claude Bernard Lyon 1, Lyon, France;* [3]*Guy de Chauliac Hospital, CHU of Montpellier, Neuropaediatrics Department, Montpellier, France;* [4]*Groupement Hospitalier Est, Hôpital Femme-Mère-Enfant, Hospices Civils de Lyon, Neuropaediatrics Department, Lyon, France;* [5]*CHU de Montpellier, Department of Medical Genetics, Montpellier, France;* [6]*Necker Enfants Malades University Hospital, APHP, Pediatric Neurology Department, Paris, France;* [7]*Robert Debré University Hospital, APHP, Department of Pediatric Neurology and Metabolic Diseases, Paris, France;* [8]*Queen Square Institute of Neurology, University College London, Department of Neuromuscular Disorders, London, United Kingdom;* [9]*Faculty of Medicine, Shahid Beheshti University of Medical Sciences, Department of Medical Genetics, Tehran, Iran;* [10]*Hopital de la Timone-Enfants, Marseille Medical Genetics, Marseille, France;* [11]*Necker-Enfants Malades Hospital, Université de Paris, Department of Medical Genetics, Paris, France*

Background/Objectives: Bi-allelic pathogenic variants in the *DMXL2* gene(MIM# 612186) have recently been recognized as a cause of Ohtahara syndrome, a very severe form of early-infantile developmental and epileptic encephalopathy(EIDEE). *DMXL2* encodes a vesicular protein highly expressed in brain and involved in autophagy. To date, six patients with this condition have been reported.

Herein, we describe new cases of DMXL2-related EIDEE.

Methods: A series of six unrelated patients with EIDEE and harboring novel homozygous variants of *DMXL2* was assembled through an international collaboration using GeneMatcher and the European Reference Networks(ITHACA and EPiCARE).

Results: All affected individuals were born to consanguineous parents. Family history was notable for decreased affected siblings and for deafness in two distinct cases.

Clinical findings were consistent with neonatal onset and drug-resistant seizures, suppression-burst pattern on EEG recordings, profound developmental delay with severe hypertonia, quadriparesis, peripheral polyneuropathy and sensorineural hearing impairment.

Repeated brain MRI scans showed progressive grey and white matter abnormalities including hypomyelinisation and leukodystrophy.

Genetic testing was based on exome(n = 3) or genome sequencing(n = 3). Six novel biallelic *DMXL2* pathogenic variants were identified including two splice site variants, a missense substitution, a nonsense variant, a deletion and a duplication. These variants were predicted to be damaging by in silico tools. Family segregation analysis showed parents to be heterozygous carriers for the causative mutation

Conclusion: Our results support the presence of core features and contribute to widen the genotypic spectrum of *DMXL2*-retated EIDEE. We consider performing functional studies to broaden our knowledge of the pathomechanism of *DMXL2* variants.

Grants: None declared

Conflict of Interest: None declared

P11.093.D Evaluation of the hTERT-MNS16A variant and leukocyte telomere length in patients with cannabinoid or synthetic cannabinoid use disorder

*Sacide Pehlivan[1], Ayşe Feyda Nursal[2], **Hasan Mervan Aytac**[3], Yasemin Oyacı[3], Eren Aytaç[4], Mustafa Pehlivan[5]*

[1]Istanbul Faculty of Medicine, Istanbul University, Department of Medical Biology, Istanbul, Türkyie; [2]Faculty of Medicine, Hitit University, Department of Medical Genetics, Çorum, Türkyie; [3]Institute of Graduate Studies in Health Sciences, Istanbul University, Istanbul, Turkey, Istanbul, Türkyie; [4]Konya Beyhekim Training and Research Hospital, Department of Psychiatry, Konya, Türkyie; [5]Basaksehir Cam and Sakura City Hospital, Department of Hematology, Istanbul, Türkyie

Background/Objectives: Recent studies have reported that cannabis use predicts epigenetic changes associated with accelerated aging. The variable number of tandem repeats (VNTR) MNS16A in the human telomerase reverse transcriptase (*hTERT*) gene organizes the extension of telomeres. Therefore, we aimed to evaluate the relationship of the *hTERT* MNS16A VNTR variant and leukocyte telomere length (LTL) with the risk of cannabinoid or synthetic cannabinoid use disorder (CUD/SCUD).

Methods: A total of 228 subjects, including 147 CUD/SCUD patients and 81 healthy controls participated in the study. The MNS16A VNTR was genotyped using polymerase chain reaction (PCR). Relative LTL was quantified by real-time PCR. For a single-copy gene (S), the T/S ratio (telomere, T) was determined; LTL was expressed as the relative T/S ratio, and the relative quantification of telomeric DNA, rather than its absolute length, was assessed.

Results: There was no significant difference between patients and controls in terms of MNS16A VNTR genotype distribution. There was no relationship found between MNS16A VNTR genotype distribution and clinical symptoms. The log-transformed T/S ratio showed that LTL shortening was greater in the patient group than in the controls ($p = 0.001$). Psychotic symptoms and violent behavior were more common in patients with LTL fold changes L˃1 ($p = 0.022$ and $p = 0.011$, respectively). Also, substance usage time was higher in patients with LTL fold changes L˃1 ($p = 0.033$).

Conclusion: Although our results showed that the MNS16A VNTR variant was not a risk factor for CUD/SCUD, we found that LTL shortening was present in patients.

Grants: This research received no specific grant.

Conflict of Interest: None declared

P11.094.A Lessons learnt from a genotype-first analysis of voltage-gated ion channels

Nathan Routledge[1], *Reza Maroofian[1], Stephanie Efthymiou[1], Rauan Kaiyrzhanov[1], Henry Houlden[1]*

[1]UCL Queen Square Institute of Neurology, London, United Kingdom

Background/Objectives: Voltage-gated ion channels (VGCs), comprising over a hundred genes, regulate neuronal action potential and trigger multiple ion-dependent processes. While some VGCs are linked to channelopathies like epilepsy and developmental epileptic encephalopathy, many remain unassociated. The study of ion channels is a promising avenue for successful treatment development, as understanding their functional and malfunctioning processes can lead to novel corrective treatments. The highly conserved and analogous structure of ion channel alpha units across channels enhances the value of variant observations in one channel for understanding others.

Methods: Using a genotype-first strategy, we screened the Queen's Square Institute of Neurology genomic database, housing 30,000 exomes from diverse backgrounds, focusing on Sodium VGC genes. This approach aimed to assess the genetic epidemiology and prevalence of channelopathies, exploring the clinical and genetic spectrum within a substantial dataset.

Results: Analyzing 3,947 variants indicated a prevalence of higher-than-variant-of-uncertain-significance (VUS) classifications in voltage-gated and pore-forming domains across nine channels. *SCN8A* exhibited a unique pattern, with a concentration of higher-than-VUS variants in the C-terminal. Surprisingly, likely pathogenic variants were found in unexpected contexts, including healthy individuals. While novel homozygous cases were identified in *SCN1A* and *SCN3B*.

Conclusion: The study's outcomes highlight the voltage-sensor and pore-forming domains as hotspots in Sodium VGCs, validating existing knowledge while proposing a distinct hotspot in *SCN8A*. Adopting a genotype-first diagnostic approach may uncover new causal genes and inheritance patterns. However, the robustness of variant classifications warrants scrutiny. This analysis underscores the dynamic nature of channelopathies and emphasizes the need for ongoing evaluation in variant classification methodologies.

Grants: N/A

Conflict of Interest: None declared

P11.095.B Biallelic variants in *SLC4A10* lead to a neurodevelopmental disorder

Paria Najarzadeh Torbati[1], *Ehsan Ghayoor Karimiani[2;3], Mina Zamani[4], Rauan Kaiyrzhanov[5], Lutz Liebmann[6;6], Barbara Vona[7;8], Antje K. Huebner[9], Daniel Calame[10;11], Vinod K. Misra[12], Saeid Sadeghian[13], Reza Azizimalamiri[14], Mohammad Hasan Mohammadi[13], Jawaher Zeighami[15], sogand heydaran[16], Mehran Beiraghi Toosi[17;18], Javad Akhondian[17], Meisam Babaei[19;19], Narges Hashemi[20], Rhonda Schnur[21], Mohnish Suri[22], Jonas Setzke[8;23], Matias Wagner[24;25], Theresa Brunet[24;25], Christopher M. Grochowski[11], Lisa Emrick[10;11], Wendy K. Chung[26], Ute Hellmich[27;28], Miriam Schmidts[29;30], James Lupski[11;31;32], Hamid Galehdari[16], MariaSavina Severino[33], Henry Houlden[34], Christian Hübner[6;35], Reza Maroofian[34]*

[1]Department of Medical Genetics, Next Generation Genetic polyclinic, Mashhad, Iran; [2]Next Generation Genetic Polyclinic, Mashhad, Iran, Department of Medical Genetics, Iran; [3]Molecular and Clinical Sciences Institute, St. George's, University of London; [4]Narges Medical

Genetics and Prenatal Diagnosis Laboratory, Ahvaz, Iran, Narges Medical Genetics and Prenatal Diagnosis Laboratory; [5]UCL Queen Square Institute of Neurology, London, UK, Department of Neuromuscular Diseases; [6]Institute of Human Genetics, Jena University Hospital, Friedrich Schiller Universität, Am Klinikum 1, 07747, Jena, Germany; [7]Institute of Human Genetics, University Medical Center Göttingen, Göttingen, Germany, University Medical Center Göttingen, Göttingen, Germany; [8]Institute for Auditory Neuroscience and InnerEarLab, University Medical Center Göttingen, Göttingen, Germany; [9]4. Institute of Human Genetics, Jena University Hospital, Friedrich Schiller Universität, Am Klinikum 1, 07747, Jena, Germany; [10]Division of Neurology and Developmental Neuroscience, Department of Pediatrics, Baylor College of Medicine, Houston, TX, USA; [11]Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX, USA; [12]Division of Genetic, Genomic & Metabolic Disorders, Discipline of Pediatrics College of Medicine, Central Michigan University, USA; [13]Department of Pediatric Neurology, Golestan Medical, Educational, and Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran; [14]12. Department of Pediatric Neurology, Golestan Medical, Educational, and Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran; [15]Narges Medical Genetics and Prenatal Diagnosis Laboratory, Kianpars, Ahvaz, Iran; [16]Department of Biology, Faculty of Science, Shahid Chamran University of Ahvaz, Ahvaz, Iran; [17]Pediatric Neurology Department, Ghaem Hospital, Mashhad University of Medical Sciences, Mashhad, Iran; [18]Neuroscience Research Center, Mashhad University of Medical Science, Mashhad, Iran; [19]Department of Pediatrics, North Khorasan University of Medical Sciences, Bojnurd, Iran; [20]Department of Pediatrics, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran; [21]Clinical Genomics Program GeneDx | GeneDx.com; [22]Clinical Genetics Service, Nottingham University Hospitals NHS Trust, Nottingham, UK; [23]Institute of Human Genetics, University Medical Center Göttingen, Göttingen, Germany; [24]Institute of Human Genetics, Klinikum rechts der Isar, School of Medicine, Technical University of Munich, 81675 Munich, Germany; [25]Institute of Neurogenomics, Helmholtz Zentrum München, Neuherberg 85764, Germany; [26]Departments of Pediatrics and Medicine Columbia University Irving Medical Center New York NY. USA; [27]Friedrich Schiller University Jena, Faculty of Chemistry and Earth Sciences, Institute of Organic Chemistry and Macromolecular Chemistry, Humboldtstrasse 10, 07743 Jena, Germany; [28]Cluster of Excellence Balance of the Microverse, Friedrich Schiller University Jena, Jena, Germany; [29]Pediatrics Genetics Division, Center for Pediatrics and Adolescent Medicine, Faculty of Medicine, Freiburg University, Mathildenstrasse 1, 79106 Freiburg, Germany; [30]CIBSS-Centre for Integrative Biological Signalling Studies, University of Freiburg, Freiburg, Germany; [31]Texas Children's Hospital, Houston, TX, USA; [32]Human Genome Sequencing Center, Baylor College of Medicine, Houston, TX, USA; [33]Neuroradiology Unit IRCCS, Istituto Giannina Gaslini, Genoa, Italy; [34]Department of Neuromuscular Diseases, UCL Queen Square Institute of Neurology, London, UK; [35]Center for Rare Diseases, Jena University Hospital, 07747 Jena, Germany

Background/Objectives: SLC4A10 encodes a plasma membrane-bound ion transport protein, which mediates Na + -dependent HCO3− import thus mediating net acid extrusion. Recently neurodevelopmental disorders have shown in Slc4a10 knockout (KO) mice including collapsed brain ventricles, an increased seizure threshold, also mild behavioral abnormalities, impaired vision, and deafness. It is not reported before that pathogenic variants in SLC4A10 have been associated with monogenic human disease traits.

Methods: In an extensive international data sharing, we performed exome/genome sequencing and identified 11 patients from 6 independent families with biallelic predicted-damaging variants in SLC4A10. A minigene assay, localization studies, intracellular pH recordings, and protein modelling were performed to study the possible functional consequences of the variant alleles. Clinical features, neuroimaging data, and facial images were reviewed by a group of clinical geneticists, neurologists.

Results: The cohort contains 8 rare/ultra-rare biallelic SLC4A10 variants, which segregate with the disease within the families. Patients' conditions are characterised by global developmental delay/intellectual disability and central hypotonia, microcephaly, cerebellar ataxia, epilepsy, and facial dysmorphism. Neuroimaging features range from some non-specific to distinct neuroradiological findings. 6/7 missense variants affect evolutionarily highly conserved residues and are predicted to be damaging according to various in silico analyses. Functional analyses supported the pathogenic effect for 4/7 missense variants.

Conclusion: We provide evidence that pathogenic biallelic SLC4A10 variants can lead to neurodevelopmental disorder. We also demonstrate the challenges of assessing sequence variants identified in patients and distinguishing pathogenic variants from other potentially functional variants.

Conflict of Interest: None declared

P11.096.C Biallelic loss-of-function variants in *IST1* are associated with a complex neurologic disorder

Annette Lischka[1], Katja Eggermann[1], Andrea Maier[2], Dimah Hasan[3], Lena Franken[1], Emilia Michalewicz[4], Rebekka Götzl[5], Tibor Karl Lohmann[4], Martin Häusler[6], Miriam Elbracht[1], Ingo Kurth[1], Florian Kraft[1], **Cordula Knopp**[1]

[1]Institute of Human Genetics and Genomic Medicine, RWTH Aachen University, Aachen, Germany; [2]Medical Treatment Center for Adults with Intellectual Disabilities and/or Severe Multiple Disabilities, RWTH Aachen University, Aachen, Germany; [3]Department of Diagnostic and Interventional Neuroradiology, RWTH Aachen University, Aachen, Germany; [4]Department of Ophthalmology, RWTH Aachen University, Aachen, Germany; [5]Department of Plastic Surgery, Hand and Burn Surgery, RWTH Aachen University, Aachen, Germany; [6]Division of Neuropediatrics and Social Pediatrics, Department of Pediatrics, RWTH Aachen University, Aachen, Germany

Background:

IST1 (increased sodium tolerance 1) is an atypical member of the endosomal sorting complexes required for transport-III (ESCRT-III). For several ESCRT-III proteins (CHMP1A, CHMP2A, CHMP3, VSP4) as well as other direct interaction partners of IST1 like SPART or SPAST, complex neurologic or neurodegenerative disorders have been reported. However, *IST1* has not been associated with a human phenotype.

Methods: Trio WES was performed in an index patient and his parents. *IST1* protein expression was studied in patient-derived fibroblasts using western blot (WB) analysis. We are currently conducting functional studies on fibroblasts to show possible effects on cytokinesis, autophagy and endosomal morphology.

Results: Compound heterozygous loss-of-function (LOF) variants in *IST1* (NM_001270975.2:c.151C>T, p.(Arg51*); c.792T>A, p.(Tyr264*)) were identified in the patient. Complete loss of IST1 protein in fibroblasts was demonstrated by WB. The 20-year-old patient showed intellectual disability, tetra spasticity, hypotension of the trunk, optic nerve hypoplasia, nystagmus, microcephaly, short stature, and underweight. cMRI at the age of 14 years showed bilateral diffuse hypomyelination of the supratentorial white matter with slight reduction in brain volume of cerebrum and brainstem.

Conclusion: The clinical features of our patient resemble neurological diseases that are caused by genes that are direct

interaction partners of *IST1*. This implicates that biallelic LOF variants in *IST1* are associated with a neurological disorder that is clinically characterized by features of complex hereditary spastic paraplegia and hypomyelinating leukodystrophy. Additional patients are needed to confirm the link between *IST1* and neurological disease.

Conflict of Interest: None declared

P11.097.D Clinico-genetic findings in a cohort of 2750 frontotemporal dementia patients

Guillaume Cogan[1;2], Isabelle Leber[2], Dario Saracino[1], Foudil Lamari[1], Khrouf Walid[1], Julie Bogoin[1], Isabelle David[1], Patricia Moreau[1], Anne-Laure Fauret[1], Lionel Arnaud[1], Marion Houot[2], Eric Le Guern[1;2], **Fabienne Clot**[1]

[1]Assitance Publique - Hopitaux de Paris, La Pitié-Salpêtrière, Medical genetics; [2]Paris Brain Institute

Background/Objectives: Frontotemporal dementia (FTD) is the third most frequent neurodegenerative dementia after Alzheimer and Lewy body disease. It encompasses different clinical sub-types including behavioral variant of FTD (bvFTD), non-fluent variant Primary Progressive Aphasia (nfvPPA) and semantic variant PPA (svPPA). It can be associated with Parkinsonism, apraxia and motor neuron disease (MND). Genetics play an important role since approximatively 40% of cases have a positive family history of neurologic disorders, predominantly with a dominant pattern. Global genetic diagnostic yield in FTD vary greatly from 10 to 38% depending on cohorts and studies design. However, the relative small size of cohort (less than 1000) limit the ability to draw a comprehensive view of the phenotype-genotype correlation in FTD.

Methods: Using a three-steps genetic screening strategy, we report the genetic architecture of a French diagnostic cohort including 2750 index cases with sporadic or familial FTD.

Results: Overall diagnostic yield is 11.5% (n = 316). *C9orf72, GRN* and *MAPT* altogether represent 91% of diagnoses. We further performed multiple statistical analyses to decipher the association between all possible clinical variables (gender / clinical sub-type / type of FTD-associated symptoms / age at onset / positivity and type of family history etc.) and the probability to identify a causal variant.

Conclusion: Those results enable to suggest new guidelines for the genetic diagnosis strategy of FTD. We hope this cohort – the largest to date - will help European genetics and neurology departments to provide accurate genetic tests for FTD patients.

Grants: No

Conflict of Interest: None declared

P11.098.A Epilepsies of presumed genetic etiology show enrichment of rare variants that occur in the general population

Linnaeus Bundalian[1], Yin-Yuan Su[2], Siwei Chen[3], Akhil Velluva[1], Anna Kirstein[4], Antje Garten[4], Saskia Biskup[5], Florian Battke[5], dennis lal[3], Henrike Heyne[6;7], Konrad Platzer[1], Chen-Ching Lin[2], Johannes Lemke[1], **Diana le Duc**[1;8]

[1]Institute of Human Genetics, University Hospitals Leipzig; [2]Institute of Biomedical Informatics, National Yang Ming Chiao Tung University; [3]Analytic and Translational Genetics Unit, Broad Institute of MIT and Harvard; [4]Pediatric Research Center, University Hospitals Leipzig; [5]CeGaT GmbH; [6]Program in Medical and Population Genetics, Broad Institute of MIT and Harvard; [7]Hasso-Plattner-Institut for Digital Engineering, University of Postdam; [8]Clinical Genetics, Center for Diagnostics GmbH at Chemnitz Clinics

Consortium: Epi25 Collaborative

Background/Objectives: Previous studies suggested that severe epilepsies, e.g., developmental and epileptic encephalopathies (DEEs), are mainly caused by ultra-rare de novo genetic variants. For milder disease, rare genetic variants could contribute to the phenotype.

Methods: To determine the importance of rare variants for different epilepsy types, we analyzed a whole-exome sequencing cohort of 9,170 epilepsy-affected individuals and 8,436 control individuals. Here, we separately analyzed three different groups of epilepsies: severe DEEs, genetic generalized epilepsy (GGE), and non-acquired focal epilepsy (NAFE). We required qualifying rare variants (QRVs) to occur in control individuals with an allele count ≥ 1 and a minor allele frequency ≤ 1:1,000, to be predicted as deleterious (CADD ≥ 20), and to have an odds ratio in individuals with epilepsy ≥ 2.

Results: We identified genes enriched with QRVs primarily in NAFE (n = 72), followed by GGE (n = 32) and DEE (n = 21). This suggests that rare variants may play a more important role for causality of NAFE than for DEE. Moreover, we found that genes harboring QRVs, e.g., HSGP2, FLNA, or TNC, encode proteins that are involved in structuring the brain extracellular matrix.

Conclusion: The present study confirms an involvement of rare variants for NAFE that occur also in the general population, while in DEE and GGE, the contribution of such variants appears more limited.

Grants: EKFS 2020_EKEA.42 to D.L.D.

Conflict of Interest: None declared

P11.099.B Preliminary investigation on the contribution of risk alleles and copy number variants in Indian schizophrenia patients.

K Naga Mohan[1], *Minali Singh[1]*

[1]Birla Institute of Technology & Science Pilani, Hyderabad Campus, Biological Sciences, Secunderabad, India

Background: Schizophrenia is a complex disorder with multiple etiologies of which genome-scale studies focusing on genetic risk identified multiple risk alleles and CNVs. However, most data are on Caucasian patients and negligible from Indians. We conducted a pilot study to test the presence of risk-conferring SNPs and CNVs in patients and controls from South India.

Methods: DNA samples from 168 controls and 168 patients were analyzed using PsychArrays, followed by GenomeStudio, PLINK1.9 and PennCNV. Further analysis involved publicly available data from SZDB2.0.

Results: Medium-sized deletions (100 kb – 1Mb) occurred at a significantly higher frequency in patients ($p = 4.5E-5$; FDR = 2.7E-5). Of the total 30 CNVs identified of interest, 13 were previously reported; 17 had candidate genes with either de novo mutations or methylation or expression abnormalities. 4q35.1-35.2 duplication containing *TLR3* with a reported intronic variant, expression and methylation abnormality in schizophrenia occurred at a significantly higher frequency in controls ($p = 0.02$).

GWAS yielded five variants in *NTM* (FDR adjusted $p = 1E04$) and an overall, 17 variants that showed significant differences between controls and patients ($p = 1E03$-$1E04$). Of these, 12 were reported by the Psychiatric Genomics Consortium (PGC).

Conclusions: This pilot indicated potential of replication of PGC-identified risk-conferring SNPs, reported as well as unreported CNVs containing candidate genes. This lays the foundation for replication studies on larger samples from India.

Grants: Department of Science and Technology and Indian Council for Medical Research, BITS Pilani Hyderabad Campus, India.

Conflict of Interest: None declared

P11.100.C The analysis of NGS data revealed a high incidence of pathogenic tandem repeat expansions in Czech patients with rare neurological diseases

Alena Musilova[1], *Petra Lassuthova*[1], *Lucie Sedlackova*[1], *Zuzana Musova*[2], *Anna Uhrova Meszarosova*[1], *Barbora Straka*[1], *Dana Safka Brozkova*[1]

[1]*Second Faculty of Medicine, Charles University and University Hospital Motol, NGL Laboratory, Department of Pediatric Neurology, Prague, Czech Republic;* [2]*Second Faculty of Medicine, Charles University and University Hospital Motol, Department of Biology and Medical Genetics, Prague, Czech Republic*

Background/Objectives: Tandem repeats (TR) are DNA regions of tandemly repeated nucleotide motifs. They are highly polymorphic, with very high mutation rates. Pathogenic TR expansions represent causal genetic events for various diseases (mainly neurological).

Methods: TR expansions were analyzed in patients who underwent whole-exome (WES) or whole-genome sequencing (WGS) of blood-derived DNA for the diagnosis of a rare neurological disease. The analysis was performed by the bioinformatic tool ExpansionHunter on the known set of 65 TR sites associated with disease onset in published literature.

Results: In our cohort of 1123 patient samples (1070 WES, 53 WGS) we discovered 99 TR loci within the pathogenic range in 95 (8.46 %) patients (84 WES, 11 WGS). Causal pathogenic TR expansions for the patient primary neurological disease were found in 5 patients in the genes *AR*, *CNBP*, and *DMPK*. Twelve patients were disease carriers with a pathogenic TR expansion in the genes *DMD*, *FXN*, *PABPN1*, *PRDM12*, *VWA1*, and *ZIC3*. As secondary findings, we also detected pathogenic TR expansions in the genes *HOXD13*, *HTT*, *NIPA1*, *PHOX2B, RUNX2, TCF4*, and *ZIC3*. The majority (71) of these pathogenic TR expansions were located in the *TCF4* gene. *TCF4* expansions are known to cause Fuchs endothelial corneal dystrophy, which has a high incidence in the Caucasian population.

Conclusion: TR analysis of WES and WGS data is profitable in the diagnostics of patients with rare neurological diseases and for discovering disease carriers, which is important for the prevention of disease reoccurrence in the family.

Grants: Supported by the MHCR AZV NU22-04-00097

Conflict of Interest: None declared

P11.101.D A case of an Angelman syndrome caused by intragenic duplication of *UBE3A* uncovered by adaptive nanopore sequencing

Laura Holthöfer[1], *Stefan Diederich*[1], *Lioba Lehmann*[1], *Charlotte Hewel*[1], *Susann Schweiger*[1], *Susanne Gerber*[1], *Matthias Linke*[1]

[1]*Institute for Human Genetics, University Medical Center of the Johannes Gutenberg University Mainz, Mainz, Germany*

Background/Objectives: Angelman syndrome patients are characterized by severe intellectual disability, speech impairment, ataxia, epilepsy and distinct behavioural profiles. It is caused by a loss of function of the maternal copy of *UBE3A* and other genes on chromosome 15q11–13. AS is a genomic imprinting disorder underlying four possible molecular mechanisms: (1) deletions of the imprinted region on 15q11.2q13 on the maternal allele, (2) maternally inherited *UBE3A* genomic point mutations, (3) imprinting defects causing aberrant DNA methylation within chromosome 15q11–q13 and (4) uniparental disomy (UPD) of the paternal chromosome 15q11–q13.

Methods: Barcoded Nanopore libraries were subject to adaptive sampling of medically relevant regions with parent-of-origin methylation or Epi-variants.

Results: Adaptive Nanopore sequencing as a diagnostic method for imprinting disorders and episignature analysis revealed an intragenic duplication of Exon 6 and 7 in *UBE3A* (NM_000462.5) in a patient with relatively mild Angelman-like syndrome. In an all-in-one nanopore sequencing analysis DNA hypomethylation of the *SNURF*:TSS-DMR, known contributing deletions on the maternal allele and point mutations in *UBE3A* were excluded. In contrast, breakpoints and orientation of the tandem duplication could clearly be defined. Segregation analysis in the family showed that the duplication derived de novo in the maternal grandfather.

Conclusion: To our knowledge, this is the first report on a family with an intragenic

tandem duplication of Exon 6 and 7 in *UBE3A* causing Angelman syndrome. Our study shows the benefits of an all-in-one nanopore sequencing approach for the diagnostics of Angelman syndrome and other imprinting disorders.

Grants: -

Conflict of Interest: None declared

P11.102.A Reduced cerebrospinal fluid levels of the PCK2 protein in patients with Alzheimer's disease

Nazlıcan İlhan[1], *Sumeyra Ildız*[2], *Erdi Sahin*[3], *Pelin Sordu*[2], *Bedia Samanci*[3], *Merve Alaylioglu*[2], *Hasmet Ayhan Hanagasi*[3], *Ibrahim Hakan Gurvit*[3], *Başar Bilgiç*[3], *Erdinç Dursun*[2], *Duygu Gezen-Ak*[2]

[1]*Istanbul University-Cerrahpasa, Department of Medical Biology, Cerrahpasa Faculty of Medicine, Istanbul, Türkyie;* [2]*Istanbul University-Cerrahpasa, Institute of Neurological Sciences, Department of Neuroscience, Istanbul, Türkyie;* [3]*Istanbul University, Istanbul Faculty of Medicine, Department of Neurology, Behavioral Neurology and Movement Disorders Unit, Istanbul, Türkyie*

Background/Objectives: Alzheimer's disease (AD) is the most common form of dementia. Neurofibrillary tangles, β-amyloid plaques, and neuronal death in specific brain regions are the characteristics of the disease. Amyloid beta has ability to translocate to nucleus and induce gene transcription, thus it may function as a transcription factor. We identified Jun, Fos, and RELA transcription factors have direct interactions with Aβ by using FpClass prediction tool. These transcription factors regulate the expression of mitochondrial Phosphoenolpyruvate Carboxykinase 2 (PCK2) protein. PCK2 is known to be a key enzyme in the gluconeogenic pathway. In the brain, mitochondria are the main sources of ATP to supply the high energy demands of neurons so that PCK2 protein could have a significant effect on both neuronal activity and neurodegeneration. We hypothesized that PCK2 levels in cerebrospinal fluid (CSF) of AD patients may be linked to the disease's progress and could be used as a biomarker.

Methods: CSF levels of PCK2 were determined by ELISA in 48 Alzheimer's patients, 12 mild cognitive impairment (MCI) patients, and 14 age-matched cognitively healthy individuals. Graphpad Prism was used for the statistical analysis. $p < 0.05$ was considered statistically significant.

Results: The results indicated that PCK2 levels were lower in AD patients compared to control group. PCK2 levels were also lower in MCI patients but this was not statistically significant.

Conclusion: This is the first study investigating the possibility of PCK2 as a biomarker that may be monitored in CSF of Alzheimer's patients.

Grants: This project was supported by IUCBAP (project no:37292).

Conflict of Interest: None declared

P11.103.B Unveiling the genetic diversity of cerebral palsy mimics using trio-WES in the Turkish cohort

Ayca Yigit[1;2;3], *OZLEM AKGUN DOGAN*[1;3;4;5], *Gunseli Bayram Akcapinar*[6], *zeynep ozkeserli*[6], *semih ayta*[7], *Ozden Hatırnaz Ng*[1;3;8], *Özkan Özdemir*[1;3;8], *kaya bilguvar*[1;3;4;9], *ugur ozbek*[1;2;3]

[1]*Acıbadem University, Genome Studies, Institute of Health Sciences, Istanbul, Türkyie;* [2]*IBG - Izmir Biomedicine and Genome Center, Rare and Undiagnosed Disease Platform, Istanbul, Türkyie;* [3]*Acıbadem University, Rare Diseases and Orphan Drugs Application and Research Center (ACURARE), Istanbul, Türkyie;* [4]*Acıbadem University, Department of Medical Genetics, Faculty of Medicine, Istanbul, Türkyie;* [5]*Acıbadem University, Pediatric Genetics Unit, Department of Pediatrics, Faculty of Medicine, Istanbul, Türkyie;* [6]*Acıbadem University, Department of Medical Biotechnology, Institute of Health Sciences, Istanbul, Türkyie;* [7]*Spastic Children's Foundation of Turkey, Department of Pediatric Neurology, Istanbul, Türkyie;* [8]*Acıbadem University, Department of Medical Biology, Faculty of Medicine, Istanbul, Türkyie;* [9]*Yale School of Medicine, Departments of Neurosurgery and Genetics, Yale Center for Genome Analysis, New Haven CT, United States*

Background/Objectives: Cerebral palsy (CP) is a group of disorders that involves permanent, non-degenerative motor function deficits. Regardless of the presence of prenatal and perinatal risk factors, CP is linked to an underlying genetic cause, despite its unclear origins. This genetic landscape overlaps with neurodevelopmental disorders, including brain malformations, epilepsy, and autism.

Parental sequencing, or 'trio-WES,' is used to assess the many variations in the index patient using whole exome sequencing (WES). Our approach aims to enhance diagnostic precision by using trio-WES data and reanalyzing clinical and WES data from unresolved cases in the Turkish CP cohort.

Methods: In the Turkish cohort, unsolved cases with CP and CP-like symptoms were selected according to essential criteria for CP clinical diagnosis. Biological materials and omics data from consenting patients were integrated into the ACU-Biobank. Genome analysis was conducted with our custom-built genome analysis pipeline. When necessary, candidate variants were confirmed by Sanger sequencing following variant filtering and prioritizing.

Results: Exome analysis revealed that 41% (26/63) of patients carried pathogenic or likely pathogenic variants, including 8 novel variants, causing or increasing risk of CP. *SPAST, KIF1A, and COL6A1* each harbor two or more candidate variants. Also, VUS were detected in 33% (21/63) of cases within genes correlating to the observed phenotypes. Notably, Trio-WES revealed de novo mutations in *KIF1A* and *SYNGAP1* genes.

Conclusions: These preliminary findings highlight the intricate genetic landscape of CP. Further research will significantly contribute to identifying the genetic etiology of CP and improving precision in differential diagnosis.

Grant: This project was granted by TUBITAK #S221889.

Conflict of Interest: None declared

P11.104.C Oligogenic inheritance in sporadic Parkinson disease: a retrospective study

Chiara Fallerini[1;2], *Elena Pasquinelli*[1;2], *Samantha Minetto*[1;2], **Giulia Casamassima**[1;2], *Anna Maria Pinto*[3], *Lucia Monti*[4], *Alessandra Renieri*[1;2;3]

[1]*Med Biotech Hub and Competence Center, University of Siena, Department of Medical Biotechnologies, Siena, Italy;* [2]*Medical Genetics, University of Siena, Siena, Italy;* [3]*Genetica Medica, Azienda Ospedaliero-Universitaria Senese, Siena, Italy;* [4]*Unit of Neuroimaging and Neurointervention, "Santa Maria alle Scotte" Medical Center, Siena, Italy*

Background/Objectives: About 15% of Parkinson disease (PD) cases are considered monogenic rare disorders due to one (dominant) or 2 (recessive) mutations in one gene, transmitted following Mendelian rules. However, some hints indicate that in the remaining cases, genetics is likewise relevant, but the number and the nature of genes involved is still unclear. Here we explore the hypothesis that combination of rare variants in a handful of genes is the major etiology and pathophysiology in sporadic PD.

Methods: We performed exome-sequencing in a cohort of 14 sporadic PD patients and 26 controls analysing a panel of 68 genes involved in either PD or Parkinsonism (PanelApp).

Results: Gene combination analysis reveals that each patient has at least one rare variant in one of the three areas of PD pathophysiology: endolysosomal/lysosomal function (*LRRK2, VPS35, ATP13A2, GBA, LYST, ATP1A3, ATP6AP2, VPS13A*), synaptic vesicle trafficking (*SYNJ1, RAB39B, SNCAIP, SLC6A3, TH*) and mitochondrial impairments (*PINK1, HTRA2, PANK2, PLA2G6, c12orf12, COASY*), often in addition with one gene related to calcium (*PDGFB*) or magnesium (*SLC30A10*) deposits, dopaminergic neuron transcription factor (*NR4A2*),neuroinflammation (*GNR*) or axonal transport (*TUBB4A, MAPT, or DICTN1*).

Conclusion: Rare variants in PD/Parkinsonism genes are the main genetic determinant in sporadic PD with a sensitivity of 0.75 and specificity of 0.86 for PD recognition. This is relevant for clinical policy (identification of patients at risk in the population), clinical practice (identification of specific gene card for personalized treatment, including gene editing) and future research (for better understanding pathophysiological mechanisms).

Grants: INTERVENE, HORIZON Project 2020 n.101016775.

Conflict of Interest: None declared

P11.105.D RNA-seq performed in an in vitro cellular model of Congenital Central Hypoventilation Syndrome confirmed the involvement of ROS production and cell cycle deregulation in the pathogenesis of the disease

Chiara Africano[1;2], *Tiziana Bachetti*[3], *Genny Del Zotto*[4], *Ignazia Prigione*[5], *Giada Recchi*[5], *Paolo Uva*[6], *roberto cusano*[7], *Eleonora Di Zanni*[2;8], *Marta Rusmini*[2;6], *alice grossi*[2], *Francesca Rosamilia*[6], *Isabella Ceccherini*[2]

[1]*Università di Genova, Dipartimento di Neuroscienze, Riabilitazione, Oftalmologia, Genetica e Scienze Materno-Infantili, Genova, Italy;* [2]*Giannina Gaslini Institute, UOSD Laboratory of Genetics and Genomics of Rare Diseases, Genova, Italy;* [3]*IRCCS AOU San Martino, Proteomics and Mass Spectrometry Unit, Genova, Italy;* [4]*Giannina Gaslini Institute, Core Facilities, Dept Research and Diagnostics, Genova, Italy;* [5]*Giannina Gaslini Institute, UOC Rheumatology and Autoinflammatory Diseases, Genova, Italy;* [6]*Giannina Gaslini Institute, Clinical Bioinformatics Unit - Scientific Direction, Genova, Italy;* [7]*CRS4, Science and Technology Park Polaris, Pula, Italy;* [8]*Consiglio Nazionale delle Ricerche (CNR), Institute of Biophysics, Genova, Italy*

Background/Objectives: Congenital Central Hypoventilation Syndrome (CCHS) is a rare autosomal dominant genetic disorder, characterised by abnormal ventilatory response to hypoxia and

hypercapnia, associated with heterozygous PHOX2B mutations. More than 80% of the variants are expansions from +4Ala to +13Ala of the polyalanine stretch, thus leading to cytoplasmic protein retention and aggregation. Little is known about the pathogenesis of CCHS. We applied transcriptomics to identify the pathways deregulated by the severe +13Ala mutation and to evaluate how a pharmacological treatment can recover a correct cellular phenotype.

Methods: RNA-sequencing was performed in cells expressing PHOX2B-WT and PHOX2B+13Ala, treated or not with 17AAG, an analogue of the antibiotic Geldanamycin, followed by GSEA analysis. Among the pathways resulted differentially involved in PHOX2B+13Ala pathogenesis, the production of reactive oxygen species (ROS) and cell cycle progression were validated in vitro.

Results: Consistent with the in-silico analysis, a relevant oxidative status, i.e. increased ROS production and stress-induced hypoxic cells, was confirmed in the presence of expanded PHOX2B protein compared to WT. A delayed cell cycle was also revealed by RNA-sequencing analysis in PHOX2B+13Ala cells, along with reduced percentage of G2 cells in PHOX2B+13Ala compared to PHOX2B-WT, implying a cell block at the S-G2 checkpoint.

Conclusion: RNA-sequencing is a promising approach to study the pathogenesis of CCHS in vitro. A transcriptome study of mesenchymal cells from CCHS patients carrying different PHOX2B mutations is also underway.

Grants: This work was supported by both the 2016 CCHS-Pilot Grant Awarded by the CCHS Family Network and "Associazione Italiana per la Sindrome da Ipoventilazione centrale Congenita" (AISICC)

Conflict of Interest: None declared

P11.106.A TUBB2A, what if it wasn't so malformative? A new series of patients and an expanded spectrum of severity in brain malformations.

Louis Januel[1], *Nicolas Chatron*[1;2], *Pauline MONIN*[1], *Massimiliano Rossi*[1], *Audrey Putoux*[1], *Henri Margot*[3], *Sophie Naudion*[3], *Francoise Durand Dubief*[4], *Quentin Thomas*[5], *Francis Ramond*[6], *Marine Lebrun*[6], *Frédéric Tran Mau-Them*[7], *Julian Delanne*[7], *Hana Safraou*[7], *Laurence Faivre*[7], *Ange-Line Bruel*[7], *Estelle Colin*[8], *Julie Chavany*[9], *Florence Riccardi*[10], *Olivier Monestier*[11], *Isabelle Maystadt*[11], *Anne de Saint Martin*[12], *Amelie PITON*[12;13], *Elise Schaefer*[13], *Charlotte Dubucs*[14], *Judith Melki*[15], *Alain Verloes*[16], *Xenia Latypova*[16], *Christele Dubourg*[17], *Laurent Pasquier*[18], *Alinoe Lavillaureix*[18], *Melanie FRADIN*[18], *Audrey Labalme*[1], *Claire Bardel*[1], *Sara Cabet*[2;19], *Gaetan Lesca*[1;2]

[1]*Hospices Civils de Lyon, Service de Génétique, Bron, France;* [2]*Institut NeuroMyoGène, Univ Lyon 1, CNRS, INSERM, Physiopathologie et Génétique du Neurone et du Muscle, UMR5261, U1315, Lyon, France;* [3]*GH Pellegrin, CHU de Bordeaux, Service de génétique médicale, Bordeaux, France;* [4]*Hospices Civils de Lyon, Service de neurologie - sclérose en plaques, pathologies de la myéline et neuro-inflammation, Bron, France;* [5]*CHU de Dijon, Services de Neurologie et de Génétique clinique, Dijon, France;* [6]*CHU Saint Etienne, Génétique Clinique Chromosomique et Moléculaire, Saint-Etienne, France;* [7]*CHU de Dijon, Service de Génétique, Dijon, France;* [8]*CHU d'Angers, Service de Génétique médicale, Angers, France;* [9]*CHITS Toulon, Service de pédiatrie, Toulon, France;* [10]*CHITS, Toulon, Service de génétique médicale, Toulon, France;* [11]*Institut de Pathologie et de Génétique, Centre de Génétique Humaine, Gosselies, Belgium;* [12]*CHU de Strasbourg, Service de Neuropédiatrie, Strasbourg, France;* [13]*CHU de Strasbourg, Service de génétique médicale, Strasbourg, France;* [14]*CHU de Toulouse, Service de Génétique médicale, Toulouse, France;* [15]*Hôpital Bicêtre, Unité de Génétique Médicale, Paris, France;* [16]*Hôpital Robert Debré, Service de Génétique clinique, Paris, France;* [17]*CHU de Rennes, Laboratoire de génétique moléculaire et génomique médicale, Rennes, France;* [18]*CHU de Rennes, Service de génétique clinique, Rennes, France;* [19]*Hospices Civils de Lyon, Service d'imagerie médicale, Bron, France*

Background/Objectives: Tubulinopathies encompass a spectrum of rare central nervous system malformations, classically featuring variable combinations of microlissencephaly, lissencephaly, pachygyria, polymicrogyria, cortical dysplasia, and characteristic abnormalities in the basal ganglia, thalami, corpus callosum, cerebellum, and brainstem. Typically, patients exhibit varying degrees of neurodevelopmental disorders, along with epilepsy, hypotonia, and features associated with autism spectrum disorders (ASD).

Several genes encoding different alpha or beta tubulins are implicated in these pathologies. *TUBB2A* pathogenic variants were first described in 2014 in a patient with neurodevelopmental disorders and typical brain anomalies associated with tubulinopathies. Since then, twelve patients have been reported, some of them having more subtle abnormalities, or even normal brain MRI (one patient).

Methods and Results: Here, we report on a new series of patients carrying de novo missense variants in *TUBB2A*, to better understand the phenotype associated with this disorder. Some patients, notably, exhibit mild to severe neurodevelopmental disorders with epilepsy and autistic traits, along with mild and inconspicuous brain anomalies. As suggested by recent literature on this gene, we hypothesize that the phenotypic spectrum associated with variations in the *TUBB2A* gene may extend to patients with neurodevelopmental disorders but without the brain anomalies typically described in tubulinopathies.

We also present challenges in interpreting variations within the *TUBB2A* gene due to sequence homology with several homologous genes and pseudogenes. We propose a technological variant confirmation pipeline to overcome these challenges.

A collaboration is about to commence to study the impact on neuronal migration in a murine model with several identified variations in the cohort.

Conflict of Interest: None declared

P11.107.B Recurrent *PACS2* variant in two children with developmental and epileptic encephalopathy 66: expanding the phenotype with relative macrocephaly and migrating focal seizures in one of them

Beáta Bessenyei[1], *Katalin Koczok*[1], *Judit Cservenyák*[2], *Tímea Margit Szabó*[3], *Mónika Bessenyei*[3], *Eszter Kovács*[1], *Orsolya Nagy*[1], *László Madar*[1], *Aniko Ujfalusi*[1], *Istvan Balogh*[1;4], **Katalin Szakszon**[3]

[1]*University of Debrecen, Faculty of Medicine, Department of Laboratory Medicine, Division of Clinical Genetics, Debrecen, Hungary;* [2]*Borsod-Abaúj-Zemplén County Hospital and University Hospital, László Velkey Child Health Centre, Miskolc, Hungary;* [3]*University of Debrecen, Faculty of Medicine, Institute of Paediatrics, Debrecen, Hungary;* [4]*University of Debrecen, Faculty of Medicine, Department of Human Genetics, Debrecen, Hungary*

Background: The *PACS2*-related early developmental and epileptic encephalopathy (MIM 618067) is a rare condition with only 53 patients reported so far. The causality of a recurrent variant c.625G>A, p.(Glu209Lys) was first recognized in 2018.

Methods: We report on two unrelated children with early-infantile focal seizures, one with clonic clusters, the other with migrating focal seizures manifesting in asymmetrical tonic semiology. Both children showed developmental delay, hypotonia, speech delay and recognizable facial gestalt. They are able to walk independently and became seizure free under antiepileptic

treatment. The first child developed good verbal communication, the second was able to speak some simple words at 4 years of age and had relative macrocephaly. WES analyses using Illumina sequencing technology were performed in both individuals. Pathogenic variant was confirmed by Sanger sequencing.

Results: WES identified the identical pathogenic heterozygous variant c.625G>A, p.(Glu209Lys) in the *PACS2* gene in both individuals.

Conclusion: Pathogenic *PACS2* variants are associated with developmental epileptic encephalopathy type 66. The recurrent c.625G>A, p.(Glu209Lys) missense variant has been reported in all but one individual to date with nearly consistent phenotype including neonatal- or infantile-onset epilepsy, developmental delay, facial dysmorphism. Relative macrocephaly is infrequently reported in the medical literature while migrating focal seizures are first described here.

Conflict of Interest: None declared

P11.108.C Identification of mitochondrial DNA variants enriched in primary progressive multiple sclerosis patients

Kaalindi Misra[1], *Melissa Sorosina*[1], *Antonino Giordano*[1;2], *Elisabetta Mascia*[1], *Sara Scirè*[1], *Laura Ferrè*[1;2], *Ferdinando Clarelli*[1], *Chiara Zanetta*[2], *Massimo Filippi*[2;3;4;5], *FEDERICA ESPOSITO*[1;2]

[1]*IRCCS San Raffaele Scientific Institute, Laboratory of Human Genetics of Neurological disorders, Institute of Experimental Neurology, Milano, Italy;* [2]*IRCCS San Raffaele Scientific Institute, Neurology and Neurorehabilitation Unit, Milano, Italy;* [3]*IRCCS San Raffaele Scientific Institute, Neurophysiology Service, Milano, Italy;* [4]*Vita-Salute San Raffaele University, Milano, Italy;* [5]*IRCCS San Raffaele Scientific Institute, Neuroimaging Research Unit, Division of Neuroscience, Milano, Italy*

Background/Objectives: Multiple sclerosis (MS) is a disorder where the immune system attacks the central nervous system, leading to gradual loss of neurological function. Mitochondria (mt) are important organelles within cells that produce energy. Researchers have found evidence of damage to mitochondria in people with primary progressive multiple sclerosis (PPMS), which is a type of MS that progresses from the beginning. The project aims to identify alterations in mtDNA genes that are more common in PPMS patients.

Methods: A cohort of 279 MS patients, comprising 113 with benign relapsing-remitting multiple sclerosis and 166 with PPMS was investigated. DNA from each patient was extracted, and mtDNA was amplified through two PCR reactions, generating two amplicons per sample. Amplification, library preparation and sequencing were performed with the TaKaRa LA PCR Kit, Nextera XT Library preparation kit and Illumina MiSeq (paired-end, 250bp reads). Variant calling and annotation were performed with MutServe (v2.0) by implementing the in-house pipeline. Variants were tested for association using an allelic chi-squared test and gene burden analysis.

Results: mtDNA analysis found six shared variants between PPMS and benign MS, with different frequencies ($p < 0.05$) among groups, and ten unique variants in the PPMS group. Six of them were classified as pathogenic, affecting genes encoding complex I proteins ND3, ND4, ND5, ND6, and CO1.

Conclusions: Our results suggest the existence of potentially pathogenic mtDNA mutations enriched in PPMS. Analyses of a larger cohort of patients are underway to replicate these findings.

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Conflict of Interest: Kaalindi Misra: None declared, Melissa Sorosina: None declared, Antonino Giordano: None declared, Elisabetta Mascia: None declared, Sara Scirè: None declared, Laura Ferrè: None declared, Ferdinando Clarelli: None declared, Chiara Zanetta: None declared, Massimo Filippi He receives research support from Biogen Idec, Merck-Serono, Novartis, Roche, Italian Ministry of Health, Fondazione Italiana Sclerosi Multipla, and ARiSLA (Fondazione Italiana di Ricerca per la SLA)., Massimo Filippi is Editor-in-Chief of the Journal of Neurology, Associate Editor of Human Brain Mapping, Associate Editor of Radiology, and Associate Editor of Neurological Sciences Scientific direction of educational events for Biogen, Merck, Roche, Celgene, Bristol-Myers Squibb, Lilly, Novartis, Sanofi-Genzyme, Bayer, Biogen, Celgene, Chiesi Italia SpA, Eli Lilly, Genzyme, Janssen, Merck-Serono, Neopharmed Gentili, Novartis, Novo Nordisk, Roche, Sanofi, Takeda, and TEVA, Alexion, Almirall, Biogen, Merck, Novartis, Roche, Sanofi, Bristol-Myers Squibb, Merck, Novartis, Roche, Sanofi-Aventis, Sanofi-Genzyme, and Takeda, FEDERICA ESPOSITO Novartis and Sanofi Genzyme, Novartis and Sanofi Genzyme

P11.109.D Rare variant enrichment analysis in pediatric European Moyamoya Angiopathy patients unveils novel candidate susceptibility genes.

Elena Cabello[1], *Katharina Steindl*[1], *Ivan Ivanovski*[1], *Paranchai Boonsawat*[1], *Paolo Zanoni*[1], *Michael Papik*[1], *Angela Bahr*[1], *Nadia Khan*[2], *Anita Rauch*[1;2]

[1]*Institute of Medical Genetics - UZH, Schlieren, Switzerland;* [2]*Universität Kinderspital Zürich, Moyamoya Center, Zürich, Switzerland*

Background/Objectives: Moyamoya angiopathy (MMA) is a rare condition characterized by stenosis of the intracranial internal carotid artery and its branches resulting in transient ischemic attacks and strokes in children. Previous studies, mainly focusing on adult patients and/or Asians, have suggested few susceptibility genes, with *RNF213* being the most prevalent gene with a recurrent susceptibility variant p.R4810K in Asian populations. In adult European MMA patients, diverse *RNF213* rare variants have been positively associated, but not p.R4810K.

Methods: Exome sequencing was performed from blood samples in a pediatric cohort of 105 MMA patients and 234 healthy control individuals. Rare-variant enrichment test was performed using the R package SKAT. To verify the independence of our results, we repeated the test against a different control cohort of 307 individuals. A third test was done including 78 cases and 204 controls predicted as 100% of European origin.

Results: In the three tests there is significant enrichment of rare variants in two genes previously associated with MMA (*RNF213*, *NF1*), which underlines the validity of our approach and, among others, *COL18A1* (endostatin), which is known for its association with brain anomalies. As RNF213, endostatin is involved in angiogenesis and endothelial cell proliferation, and was previously considered to predispose to MMA in Trisomy 21 patients.

Conclusion: We present a MMA case-control study focusing exclusively on pediatric patients, mostly of European origin, tested with two independent control cohorts. The results show novel candidate genes that could improve the understanding of MMA.

Conflict of Interest: None declared

P11.110.A Analysis of the leptin gene in patients with anorexia nervosa

Luisa Rajcsanyi[1;2;3], *Sieglinde Düerkop*[1;2;3], *Yiran Zheng*[2;3], *Beate Herpertz-Dahlmann*[4], *Jochen Seitz*[3;4], *Martina de Zwaan*[5], *Wolfgang Herzog*[6], *Stefan Ehrlich*[7;8], *Stephan Zipfel*[9;10], *Katrin Giel*[9;10], *Karin Egberts*[11], *Roland Burghardt*[12], *Manuel Föcker*[13;14], *Jochen Antel*[2;3],

Martin Wabitsch[15], Pamela Fischer-Posovszky[15], Johannes Hebebrand[2;3], Anke Hinney[1;2;3]

[1]Section for Molecular Genetics of Mental Disorders, University Hospital Essen, Essen, Germany; [2]Center for Translational Neuro- and Behavioural Sciences, University Hospital Essen, Essen, Germany; [3]Department of Child and Adolescent Psychiatry, Psychosomatics and Psychotherapy, University Hospital Essen, Essen, Germany; [4]Department of Child and Adolescent Psychiatry and Psychotherapy, University Hospital of the RWTH Aachen, Aachen, Germany; [5]Department of Psychosomatic Medicine and Psychotherapy, Hannover Medical School, Hannover, Germany; [6]Department of Internal Medicine II, General Internal and Psychosomatic Medicine, University of Heidelberg, Heidelberg, Germany; [7]Eating Disorders Research and Treatment Center, Department of Child and Adolescent Psychiatry, Faculty of Medicine, TU Dresden, Dresden, Germany; [8]Translational Developmental Neuroscience Section, Division of Psychological and Social Medicine and Developmental Neurosciences, Faculty of Medicine, TU Dresden, Dresden, Germany; [9]Department of Psychosomatic Medicine and Psychotherapy, Medical University Hospital Tübingen, Tübingen, Germany; [10]Center of Excellence in Eating Disorders KOMET, Tübingen, Germany; [11]Department of Child and Adolescent Psychiatry, Psychosomatics and Psychotherapy, University Hospital Würzburg, Würzburg, Germany; [12]Oberberg Clinic for Child and Adolescent Psychiatry, Psychosomatics and Psychotherapy, Fasanenkiez, Berlin, Germany; [13]Department of Child and Adolescent Psychiatry, University Hospital Münster, Münster, Germany; [14]LWL-University Hospital Hamm for Child and Adolescent Psychiatry, Ruhr-University Bochum, Bochum, Germany; [15]Department of Pediatrics and Adolescent Medicine, University of Ulm, Ulm, Germany

Background/Objectives: Body weight is predominantly regulated by the leptin-melanocortin system. Mutations in leptin (*LEP*) are known to cause a dysregulated energy homeostasis and are associated with monogenic forms of obesity. Leptin is also relevant for anorexia nervosa (AN).

Methods: Thus, we have screened the coding region of *LEP* in 462 female patients with AN, and 445 healthy lean controls. Functional implications of detected variants were analysed utilizing in silico tools. Additionally, we investigated the prevalence of pathogenic *LEP* variants in the general population.

Results: We detected one non-synonymous leptin mutation (p.Val94Met, rs17151919) in a patient with AN (15.94 kg/m^2). In silico analyses revealed a protein destabilizing effect. We further identified two rare heterozygous synonymous variants in patients with AN (rs201523305 and novel SNP g.128154532C/T) and an additional synonymous variant in the lean group (rs13306517). Further, we analysed 75 non-synonymous and loss-of-functions variants in *LEP* extracted from gnomAD and predicted their pathogenicity with in silico tools. The prevalence for pathogenic variants was estimated based on the Hardy-Weinberg-Equilibrium. Across all populations, the prevalence of heterozygosity of a pathogenic variant was 1:2,100, while the prevalence for homozygosity was 1:17,830,000.

Conclusion: Consequently, given the unexpected identification of a *LEP* variant in patients with AN that was previously associated with obesity and the fact that *LEP* variants are typically rare in the general population, we can assume that *LEP* variants might have a relevance for AN.

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Conflict of Interest: None declared

P11.111.B LSM7 variants involving key amino-acids for LSM complex assembly cause a neurodevelopmental disorder with leukodystrophy and cerebellar atrophy.

Matis CRESPIN[1], *Pauline Marzin[1], Thomas Courtin[1;2], Karine Siquier-Pernet[2], christine bole[3], VALÉRIE MALAN[1;2], Patrick Nitschke[4], Charles-Joris Roux[5], Michel Lemoine[6], Marlene Rio[1], Nathalie Boddaert[5], Vincent Cantagrel[2]*

[1]Necker Hospital, Service de médecine génomique des maladies rares, Paris, France; [2]Institute Imagine, Developmental brain disorders, Paris, France; [3]Institute Imagine, Genomics Platform, Paris, France; [4]Institute Imagine, Bioninformatics Core Facility, Paris, France; [5]Necker Hospital, Département de radiologie pédiatrique, Paris, France; [6]Necker Hospital, Service de médecine physique et de réadaptation, Paris, France

Background/Objectives: Cerebellar atrophy and hypoplasia are usually identified on MRI performed on children presenting signs of cerebellar ataxias, including imbalance, poor coordination, developmental delay and intellectual disability. These signs can be observed in the context of metabolic defects associated with hypomyelinating or demyelinating leukodystrophies. Known genetic defects include mutations in genes encoding subunits of the RNA polymerase III complex and enzymes involved in aminoacyl-tRNA synthetase pathway. However, the molecular diagnosis for many patients remains unsolved. A recent study reported two cases, a first one diagnosed with leukodystrophy and cerebellar atrophy and a second one who died in utero, with each of them having a different homozygous variant in the LSM7 gene. This gene has never been described in human pathology before, it encodes for a subunit of the LSM complex that is involved in pre-RNA maturation and mRNA degradation and consequently it has been suggested as a candidate disease gene. Presently, we report a patient with neurodevelopmental defects, leukodystrophy and cerebellar atrophy.

Methods: We performed a cerebral MRI and whole genome sequencing.

Results : The patient harbours compound heterozygous variants in the LSM7 gene. One of these variants is the same as the one carried by the first case previously reported and the other one is at the same position as the variant of the second case.

Conclusion :

Based on comparable neuroimaging, clinical features and the involvement of the same amino-acids previously demonstrated as key for LSM complex assembly, we propose that LSM7 disruption causes a neurodevelopmental disorder with leukodystrophy and cerebellar atrophy.

Grants :

Conflict of Interest: None declared

P11.112.C NKX6-2- related leukodystrophy: natural history study and novel genetic therapeutic approaches

Kristina Zhelcheska[1], *Viorica Chelban[1], Henry Houlden[1]*

[1]UCL Queen Square Institute of Neurology, Neuromuscular Disorders, London, United Kingdom

Background/ Objectives: Hypomyelinating leukodystrophies are a heterogeneous group of genetic disorders characterized by impaired formation of the myelin sheath in the CNS, leading to a wide spectrum of phenotypes. Bi-allelic variants in the *NKX6-2* gene were identified to cause spastic ataxia 8 (SPAX8), autosomal recessive, with hypomyelinating leukodystrophy. There are currently no disease modifying treatments nor biomarkers available for *NKX6-2* patients, where only supportive and palliative care is available.

Methods: We conducted a natural history study involving affected individuals with *NKX6-2*-related leukodystrophy from unrelated families to assess the progression of the disease.

Immunocytochemical staining of NKX6-2 protein in *NKX6-2* patient-derived fibroblasts was conducted to evaluate the consequence of pathogenic variants on NKX6-2 expression compared to unaffected healthy controls.

Results: We have identified affected individuals from families worldwide harbouring pathogenic variants in *NKX6-2*. Our findings reveal there is variation in phenotypic severity, both according to differences in the onset of disease and the specific disease-causing variant.

Conclusion: *NKX6-2*- related leukodystrophy is an untreatable neurological disorder and there is clinical need to develop new treatment strategies for this disorder. Our natural history study will be also used to identify serial biomarkers in patients with *NKX6-2*-related leukodystrophy, helping to provide outcome measures for potential future trials. Furthermore, we aim to use in vitro models to develop novel genetic therapeutic approaches to replace and rescue NKX6-2 protein loss.

Grants: Ataxia UK

Conflict of Interest: None declared

P11.113.D Kaya-Barakat-Masson syndrome: Delineation of the clinical phenotype of an autosomal recessive YIF1B-related neurodevelopmental disorder and examination of the underlying mechanisms using in vivo disease modelling with zebrafish

Eva Medico Salsench[1], **Leslie Sanderson**[1], *Stefan Barakat*[1]

[1]*Erasmus MC University Medical Center, Department of Clinical Genetics, Rotterdam, Netherlands*

Consortium: International YIF1B study group

Background/Objectives: Intracellular vesicular trafficking is crucial to human brain development and its dysfunction is implicated in many neurodevelopmental disorders. YIF1B is an essential protein involved in anterograde trafficking from the endoplasmic reticulum to the cell membrane and in the architecture of the Golgi apparatus. We recently described bi-allelic variants in *YIF1B* as the cause of a severe neurodevelopmental disorder (Kaya-Barakat-Masson syndrome, KABAMAS, OMIM #619125) in 25 affected individuals from 20 independent families to date. KABAMAS is characterized by progressive encephalopathy with varying degrees of movement disorders, severe global developmental delay, microcephaly, and epilepsy. Both pathogenic truncating and missense variants have been identified. Here, we compare the clinical phenotype between all currently known YIF1B cases and expound on the disease mechanism through in vivo modeling using zebrafish.

Methods: Using CRISPR/Cas9 we generated *yif1b* knock-out zebrafish and extensively phenotyped their development and cellular alterations using transgenic lines, in vivo imaging, and multi-omics technologies including proteomics.

Results: *Yif1b* depletion causes delayed development and altered touch-evoked response. Neuropathological analysis and in vivo imaging shows increased apoptotic signaling and a swollen microglial phenotype. Further, the *yif1b* genotype impacts microglial morphology and ER stress response following tunicamycin treatment. The capacity of human missense variants to rescue these phenotypes has been assessed.

Conclusion: Our findings provide insight into the role of YIF1B during neurodevelopment in zebrafish and human. In addition, we provide a model system to study this disorder in vivo and further examine the essentiality of intracellular trafficking during brain development.

Grants: ZonMw Vidi 09150172110002

Conflict of Interest: None declared

P11.114.A Uniparental IsoDisomy: a new mechanism in Friedreich ataxia

Brian Sperelakis Beedham[1], *Maryse Magem*[1], *Jean-Madeleine de Sainte Agathe*[2], *Zahra Assouline*[2], *Cyril Gitiaux*[3], *Isabelle Desguerre*[3], *Julie Steffann*[1], *Giulia Barcia*[1]

[1]*Necker Enfants Malades Hospital, Médecine Génomique des Maladies Rare, Paris, France;* [2]*Pitié Salpétrière Hospital, Génétique, Paris, France;* [3]*Necker Enfants Malades Hospital, Neurologie Pédiatrique, Paris, France*

Background/Objectives: Friedreich's Ataxia (FRDA) is the most common hereditary ataxia. Most patients are homozygous for a (GAA)n expansion in the first intron of the *FXN* gene. The remaining patients carry *FXN* point mutations or intragenic deletions.

We describe the first FRDA patient with a chromosome 9 segmental Uniparental isoDisomy (UPiD) unmasking a homozygous expansion of the *FXN* gene that initially went unnoticed by TP-PCR due to a deletion in the target region of one primer.

Methods: Molecular screening for *FXN* typically centers on the analysis of (GAA)n expansions, commonly performed by triplet-repeat primed PCR (TP-PCR). This method was initially employed before undergoing further investigation through genome sequencing (GS) and modified TP-PCR.

Results: The child (5 year-old) presented with a progressive proprioceptive ataxia since the age of 4, associated with peripheral neuropathy and severe scoliosis. Cardiac ultrasound and ECG were normal.

At first, TP-PCR failed to amplify the *FXN* locus for the patient. However, GS subsequently identified an expansion of *FXN*, coupled with a maternal segmental Uniparental Isodisomy (UPiD) encompassing the *FXN* gene. This diagnosis was ultimately validated through modified TP-PCR.

Conclusion: To the best of our knowledge, this is the first case of FRDA associated to segmental UPiD. This case highlights the complexity of the molecular diagnosis of FRDA, and emphasises the importance of integrating results from various approaches before dismissing FRDA as a potential diagnosis, particularly in cases with compelling clinical evidence.

Grants: No grants

Conflict of Interest: None declared

P11.115.B Chromosomal microarray analysis in a cohort of 200 pediatric patients with epilepsy

Maria Chiara Baroni[1;2], *Luca Bergonzini*[2;3], *Giulia Olivucci*[1;4], *Giulia Severi*[1], *Francesca Montanari*[1], *Valentina Marchiani*[3], *Valentina Gentile*[3], *Luca Soliani*[3], *Antonella Boni*[3], *Tullio Messana*[3], *Angelo Russo*[3], *Duccio Maria Cordelli*[2;3], *Pamela Magini*[1], *Caterina Garone*[2;3]

[1]*IRCCS Azienda Ospedaliero-Universitaria di Bologna, Medical Genetics Unit, Bologna, Italy;* [2]*Alma Mater Studiorum, University of Bologna, Department of Medical and Surgical Sciences, Bologna, Italy;* [3]*IRCCS Istituto delle Scienze Neurologiche di Bologna, UOC Neuropsichiatria dell'Età Pediatrica, Member of the ERN EpiCare, Bologna, Italy;* [4]*Università di Palermo, Dipartimento di Discipline Chirurgiche, Oncologiche e Stomatologiche, Palermo, Italy*

Background/Objectives: Copy number variants (CNVs) are established risk factors for neurodevelopmental disorders. Our aim was to evaluate the role of CNVs detectable with chromosomal microarray in a cohort of pediatric patients with epilepsy.

Methods: We reviewed 200 pediatric patients referred to our Genetic and Neuropsychiatric Units for epilepsy diagnosed according to ILAE Classification (2017), who underwent array-CGH between 2017 and 2023. Clinical (age at onset, seizures, neurodevelopment, dysmorphisms, comorbidities) and neuroradiological data were collected and analyzed. CNVs were classified according to the ACMG guidelines.

Results: 34/200 probands had (likely) pathogenic CNVs, resulting in a diagnostic yield of 17%; 19/34 had focal epilepsy, 15/34 generalized epilepsy; 25/34 (73,5%) had additional signs of developmental disorder. 23 patients were diagnosed with deletion/duplication syndromes and reciprocal CNVs at 22q11.21, 1p36.33, and 16p13.11 were described in the cohort. Six patients had CNVs including genes previously associated with epilepsy/neurodevelopmental disorder (*NPRL3, MBD5, CDKL5, MECP2, EHMT1, ANKRD11*). Four probands had a 2p16.3 deletion, involving *NRXN1*, one had an intragenic deletion of *ANKS1B*, recently associated with a neurodevelopmental phenotype. Parental testing revealed de novo CNVs in 42,8% (9/21) and inherited CNVs in 57,1% (12/21). 37 patients (18,5%) had CNVs of unknown significance, including a de novo deletion within the neuronal splicing regulator *RBFOX1*, which is undergoing further evaluation.

Conclusion: We confirmed that CNVs contribute significantly to epilepsy, particularly when associated with neurodevelopmental delay and dysmorphism. We also highlighted incomplete penetrance and provided detailed phenotypic descriptions of patients with positive results, which can guide genetic counseling and clinical management.

Conflict of Interest: None declared

P11.116.C Somatic alterations in drug-resistant focal epilepsy

Joana Ribeiro[1;2;3], Luís Miguel Pires[4], Ilda Patrícia Ribeiro[1;2;3;5], Olinda Rebelo[6], Francisco José Sales Almeida Inácio[6], Antonio Freire[4], **Joana Barbosa de Melo**[1;2;3;5]

[1]Lab of Cytogenetics and Genomics, Faculty of Medicine, University of Coimbra, Coimbra, Portugal; [2]CIBB- Centro de Inovação em Biomedicina e Biotecnologia, University of Coimbra, Coimbra, Portugal; [3]iCBR CIMAGO – Centro Investigação em Meio Ambiente Genética e Oncobiologia, Faculty of Medicine, University of Coimbra, Coimbra, Portugal; [4]Faculty of Medicine, University of Coimbra, Coimbra, Portugal; [5]Clinical Academic Center of Coimbra, CACC, Coimbra, Portugal; [6]Serviço de Neurologia, CHUC, Unidade de Saude Local Coimbra, Coimbra, Portugal

Background/Objectives: Epilepsy stands as one of the most severe neurological disorders, impacting nearly 50 million individuals globally. The main objective of this study was to perform a comprehensive molecular analysis of pathological brain samples from epilepsy surgery in order to identify the most prevalent (cyto)genomic alterations and aberrant DNA methylation patterns in key genes for disease development.

Methods: Patients with drug-resistant focal epilepsy, both children and adults, associated to histologically proven focal cortical dysplasia and long-term epilepsy associated tumor who underwent surgical treatment were included. Different high-throughput genomic and epigenetic techniques were applied.

Results: Eight patients with long-term epilepsy associated tumors (4 dysembryoplastic neuroepithelial tumors, 3 gangliogliomas, 1 pilocytic astrocytoma) and 2 patients with focal cortical dysplasia (types Ia and IIa) were included. Somatic variants (*WDR24* p.Trp259Gly; *MICAL1* p.Lys1036Arg; *KATNB1* p.Leu566Ile; *MATN4* p.Phe91Val; *ANKRD6* p.His386Gln) were identified in patients with focal cortical dysplasia. Chromosomal abnormalities were observed in long-term epilepsy associated tumors, namely monosomy 19, deletions of 1p, 4p, 12p, 22q and gains of 20p. The promotor region of the genes *MGMT* and *CD44* exhibited methylation in one ganglioglioma.

Conclusion: Relevant molecular alterations related to the main histopathological diagnoses of epilepsy surgery were detected, which may contribute to the identification of new diagnostic biomarkers and targeted treatments and to decipher the molecular and pathological landscape of focal epilepsy.

Conflict of Interest: None declared

P11.117.D *SCN1A* related channelopathies, one gene - multiple phenotypes

Danai Veltra[1;2], *VIRGINIA THEODOROU[3], marina katsalouli[3], Pelagia Vorgia[4], George Niotakis[5], FILIO TSAPROUNI[6], Roser Pons[7], Konstantina Kosma[1], Aphrodite Kampouraki[1], Eirini Tsoutsou[1], Periklis Makrythanasis[1;8;9], Kyriaki Kekou[1], Jan Traeger-Synodinos[1], Christalena Sofocleous[1]*

[1]Medical School, National and Kapodistrian University of Athens, Laboratory of Medical Genetics, Athens, Greece; [2]National and Kapodistrian University of Athens, Research University Institute for the Study and Prevention of Genetic and Malignant Disease of Childhood, Athens, Greece; [3]St. Sophia's Children's Hospital, Pediatric Neurology Department, Athens, Greece; [4]Hellenic Mediterranean University, Agrifood and Life Sciences Institute, Heraklion, Greece; [5]Venizelion Hospital, Pediatric Neurology Department, Heraklion, Greece; [6]Tzaneio Hospital, Pediatric Neurology Department, Piraeus, Greece; [7]Medical School, National and Kapodistrian University of Athens, First Department of Pediatrics, Athens, Greece; [8]Medical School, University of Geneva, Department of Genetic Medicine and Development, Geneva, Switzerland; [9]Biomedical Research Foundation of the Academy of Athens, Athens, Greece

Background/Objectives: Epileptogenesis is related to various mechanisms including channelopathies, synaptopathies, and interneuronopathies caused by alterations in many genes. Of those *SCN1A* is considered a key epilepsy gene, especially in infants experiencing febrile seizures (FS) within the first year of life. The study reports and comments on findings and genotype-phenotype correlations assessed following testing for *SCN1A* in hospitalized patients.

Methods: Sanger sequencing and/or MLPA was performed on DNA from 338 patients for whom medical and family histories, as well as informed consent, were available. Five additional patients, underwent Whole Exome Sequencing (WES) with xGen Exome Research v2 kit on Illumina NextSeq-500 system. Bioinformatic analysis used Franklin and VarSome Clinical platforms. All variants were classified according to ACMG and ClinGen recommendations.

Results: Amongst 23 patients (18 with Sanger and 5 after WES) a causative *SCN1A* variant (14 known, 9 novel) was detected, associated with a wide variety of seizures and accompanying phenotypes. Segregation analysis performed in 16/23 cases revealed the variant in 8 additional family members, while 10 cases were considered de novo. In four patients, the first seizure occurred following an infection (e.g Covid-19).

Conclusion: Although in three cases genetic diagnosis was achieved immediately after the first seizure, many families, despite family history, were referred for *SCN1A* testing only after hospitalization, whence single gene tests are compensated by the National Health System. The variant type and the correlated range of clinical presentations (FS to Developmental Epileptic Encephalopathy), as well as impact of infections (specifically Covid-19) will be discussed.

Grants: None.

Conflict of Interest: None declared

P11.118.A Whole Exome Sequencing in Microcephaly: Assessing Clinical and Genetic Heterogeneity

Saadet Nur Bozgeyik[1], Enise AVCI DURMUŞALİOGLU[2], Turkan Turkut Tan[2], Tahir Atik[2], Ozgur Cogulu[2], Ferda Ozkinay[2], **Esra Isik**[2]

[1]Ege University, Medical Faculty, Türkyie; [2]Ege University, Türkyie

Background/Objectives: Microcephaly is a clinical and genetic heterogeneous condition that typically manifests as a neurodevelopmental disorder. It may occur isolated or as an accompanying feature of a syndrome. The aim of this study to evaluate the usefulness of whole exome sequencing (WES) in a heterogeneous group of patients presented with microcephaly.

Methods: Thirty patients from 27 unrelated families presenting with microcephaly and analyzed using WES during their diagnostic work-up were included. Patients were classified into two groups (non-syndromic/syndromic) based on the presence of accompanying additional anomalies.

Results: Twelve patients were classified as non-syndromic while 18 as syndromic. In 16 of 27 families (59.2%) a pathogenic or likely pathogenic variant in genes already known to be responsible for microcephaly were identified. In one family including two affected cases, a homozygous variant (c.511C>T, p.Arg171Cys) was detected in *XAB2* gene (NM_020196.3) which could be a candidate novel gene related with microcephaly and neurodevelopmental disorder.

Conclusion: In this study, a high diagnostic rate has been achieved in syndromic and non-syndromic microcephaly cases via WES. WES has revealed a potential novel gene XAB2 encodes a multi-functional protein that plays critical role in processes including transcription-coupled DNA repair and mRNA splicing. WES might be considered as the first-tier test in diagnostic approach of microcephaly.

Grants: None.

Conflict of Interest: None declared

P11.120.C Rapid genome sequencing for infantile-onset epilepsy within a national healthcare setting

Hannah Robinson[1], *Karen Stals[1], Lucy Mallin[1], Hayley Lees[1], Nicholas Head[1], Lewis Pang[1], Ewa Goljan[1], Natasha Grumbt[1], Suzanne Hocking[1], Jessica Adams[1], Verity Woodhall[1], Matthew Wakeling[2], Sue Hill[3], Caroline Wright[2], Sian Ellard[3], Andrew Parrish[1], Emma Baple[1;2;4]*

[1]Exeter Genomics Laboratory, Exeter, United Kingdom; [2]University of Exeter, Exeter, United Kingdom; [3]NHS England, London, United Kingdom; [4]Peninsula Clinical Genetics Service, Exeter, United Kingdom

Background/Objectives: The Exeter NHS Genomics Laboratory provides the rapid genome (previously exome) sequencing service in England for acutely unwell children with a likely monogenic disorder. Children <1 year with unexplained probable monogenic epilepsy are eligible for testing, irrespective of previous admission to intensive care (ICU), due to the high actionability of a precise molecular diagnosis in this group.

Methods: We conducted a review of all children sequenced through the service since 2019 to investigate diagnostic yield and actionability in epilepsy. The exome/genome analysis strategy employed was family-trio gene-agnostic (95%) when possible, or otherwise virtual gene-panel analysis on duos (4%) or singletons (1%).

Results: 2365 children were tested (1745 exomes, 620 genomes), 28% displayed signs of epilepsy/seizures (n = 654). Excluding those whose seizures had a probable environmental cause or were accompanied by multiple congenital abnormalities, 53% were referred for rapid testing by age 1 year (n = 346), with 62% from ICU settings (n = 214) and 38% from non-ICU settings (n = 132). Diagnostic yield was 55% (n = 189), with a maximum yield of 61%, including possible variants of uncertain significance and partial diagnoses (n = 210). Diagnostic yield from non-ICU settings (55%, n = 72) was similar to ICU settings (55%, n = 117). Results were communicated to referring clinicians within a median of 9 days (range 4–27 days). *KCNQ2* and *SCN2A* were the most frequent diagnoses in both ICU and non-ICU settings.

Conclusion: We demonstrate the feasibility and utility of implementing diagnostic rapid genome sequencing as a much needed first-line test for infantile-onset epilepsy, at scale, within a national healthcare setting.

Conflict of Interest: None declared

P11.121.D The CHGB rs742710 variant and its association with early ALS onset: a cross-cohort analysis

Ivan Tourtourikov[1;2], *Kristiyan Dabchev[2], Tihomir Todorov[2], Teodor Angelov[3], Teodora Chamova[3], Ivaylo Tarnev[4;5], Vanyo Mitev[1], Albena Todorova[1;2]*

[1]Medical University of Sofia, Department of Medical Chemistry and Biochemistry, Sofia, Bulgaria; [2]Genetic Medico-Diagnostic Laboratory Genica and Genome Center Bulgaria, Sofia, Bulgaria, Sofia, Bulgaria; [3]Medical University of Sofia, Department of Neurology, Faculty of Medicine, Sofia, Bulgaria; [4]Medical University of Sofia, UMBAL Aleksandrovska, Department of Neurology, Clinic of Nervous Diseases, Sofia, Bulgaria; [5]New Bulgarian University, Department of Cognitive Science and Psychology, Sofia, Bulgaria

Background/Objectives: Amyotrophic lateral sclerosis (ALS) is a multifactorial neurodegenerative disease, with a wide spectrum of behavioral and motor symptoms. ALS pathogenic models based on chromogranin (CHGB)-mediated secretion of toxic mutant SOD1 molecules have been studied, leading to reports of a specific variant, P413L, being associated with an earlier age of onset in some, but not all populations. Our aim was to investigate the effect of this variant, and the replicability of the findings across patients from Bulgaria and the Answer ALS (AALS) program.

Methods: Genotyping was performed on 150 sporadic ALS patients from Bulgaria via Sanger sequencing for the CHGB variant rs742710. Clinical and genotyping data for 700 individuals with sporadic ALS were obtained from the Answer ALS Foundation Program, 'Answer ALS' (https://www.answerals.org). Statistical analysis was performed via IBM SPSS Statistics v26.

Results: Carriers of the G allele of rs742710 demonstrated a 2-year earlier onset for both patient cohorts. Sex was found to cause an earlier onset in both cohorts, with males having a 3-year ($p < 0.001$, for AALS) and 4-year ($p = 0.016$, for the Bulgarian cohort) age of onset. The G allele was strongly associated with ALS for both cohorts, with $p < 0.0001$ for AALS and $p < 0.00062$ for the Bulgarian population.

Conclusion: Our findings, consistent across both Bulgarian and AALS cohorts, highlight the importance of genetic factors in ALS heterogeneity and underscore the need for further research to explore the mechanisms through which CHGB influences ALS onset and progression.

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Conflict of Interest: None declared

P11.122.A RBL2/Rbf loss-of-function disrupts neurodevelopment in humans and flies and reveals a key role for an Rb proteins in post-mitotic neurons

Gabriel Aughey[1], *Elisa Cali*[1], *Henry Houlden*[1], *Reza Maroofian*[1], *James Jepson*[1]

[1]*University College London*

Background/Objectives: RBL2 is a key regulator of cell division and propagates senescence by repressing repair genes and controlling DNA methylation and telomere lenght. It has been found to be an important regulator of neuronal differentiation and survival in post-mitotic neurons, and its dysfunction is associated to BRUWAG syndrome, a neurodevelopmental disorder characterized by severe developmental delay, dysrmorphic features, microcephaly and behavioural abnormalities. To date, clinical features have been described in only six individuals carrying biallelic loss of function (LOF) variants in *RBL2*. Futhermore, the cell-types in which RBL2 expression is required to promote robust neural development remains unclear.

Methods: The affected individuals were identified through extensive data sharing and collaboration. Rbf knockdown was induced in all cells and in specific cell types. Behavioural and neuronal phenotypes were assessed using the *Drosophila* Activity Monitor (DAM) system, larval locomotion and climbing assays.

Results: We identified 30 affected indviduals with a neurodevelopmental disorder characterized by severe GDD/ID, behavioural abnormalities, dysmorphic features, severe microcephaly, and variable presence of seizures. *Rbf* fly mutants displayed morphological phenotypes and presented behavioural and locomotor defects that were reminescent of the human phenotype. Pan-neuronal expression of *Rbf* in post-mitotic neurons fully rescued the phenotype in the *Rbf* hypomorph mutants.

Conclusion: Taken together, our study expands the phenotypic and genotypic specturm of *RBL2*-related disorder, illustrates parallels between human and fly RB protein function in the nervous system and suggests RBL2/Rbf is important in neural precursors and post-mitotic neurons to promote normal brain morphology and motor development.

Grants:

Conflict of Interest: None declared

P11.123.B Proposed Practice Guidelines for the clinical and genetic diagnosis of ASD/ID: Insights from a Cohort of 500 Consecutive Subjects

Federica Francesca L'Erario[1], *Elena Sonnini*[1], *Domizia Pasquetti*[1], *Clarissa Modafferi*[1], *Annalisa Gazzellone*[1], *Pino D'Ambrosio*[1], *Ilaria Contaldo*[2], *Domenico Marco Romeo*[3], *Claudia Brogna*[3], *Vincenzo Nigro*[4;5], *Giuseppe Marangi*[1], *Daniela Orteschi*[1;6], *Maria Grazia Pomponi*[7], *Anna Gloria Renzi*[1], *Marina Carapelle*[1], *Paolo Niccolò Doronzio*[1], *Daniela Pia Rosaria Chieffo*[8;9], *Domenica Immacolata Battaglia*[10;11], *Eugenio Maria Mercuri*[12], *Chiara Veredice*[13], **Marcella Zollino**[1;14]

[1]*Section of Genomic Medicine, Department of Life Sciences and Public Health, Università Cattolica del Sacro Cuore, Roma;* [2]*Child Neurology and Psychiatric Unit, Fondazione Policlinico Universitario Agostino Gemelli, IRCCS, Roma;* [3]*Paediatric Neurology Unit, Fondazione Policlinico Universitario A. Gemelli IRCCS, Roma;* [4]*Telethon Institute of Genetics and Medicine, Pozzuoli, Naples, Italy;* [5]*Department of Precision Medicine, Università degli Studi della Campania "Luigi Vanvitelli," Naples, Italy.;* [6]*Medical Genetics Unit, Fondazione Policlinico Universitario "A. Gemelli" IRCCS, 00168 Rome, Italy.;* [7]*UOC Genetica Medica, Fondazione Policlinico Universitario "A. Gemelli" IRCCS, 00168 Roma, Italy.;* [8]*Clinical Psychology Unit, Fondazione Policlinico Universitario Agostino Gemelli IRCCS, 00168 Rome, Italy;* [9]*Department of Woman, Children and Public Health, Catholic University of Sacred Heart, 00168 Rome, Italy;* [10]*Department of Life Sciences and Public Health, Università Cattolica del Sacro Cuore, Rome, Italy.;* [11]*Child Neurology and Psychiatric Unit, Fondazione Policlinico Universitario Agostino Gemelli, IRCCS, Rome, Italy.;* [12]*Catholic University of the Sacred Heart, Rome, Italy; Complex Operational Unit of Child Neuropsychiatry, Catholic University of the Sacred Heart, Rome, Rome, Italy.;* [13]*Pediatric Neuropsychiatry Unit, Fondazione Policlinico Universitario Agostino Gemelli IRCCS, Rome, Italy.;* [14]*Department of Life Sciences and Public Health, Catholic University, Rome, Italy*

Consortium: TUDP Study Group

Background: The nosological definition of Autism Spectrum Disorders (ASD)/intellectual disability (ID) continues to be confusing. Various monogenic conditions are categorized within ASD/ID spectrum along with primary ASD, which is often multigenic in nature. Similarly, several risk alleles are mentioned as single-gene mutation in ASD/ID.

Aim: To determine whether distinct diagnostic flowcharts can be established for syndromic/monofactorial and non-syndromic/oligo-multigenic forms of ASD/ID.

Methods: Analysis of 500 consecutive patients referred to us for pre-test clinical evaluation due to ID and/or ASD. Pregnancy factors, including in vitro fertilization, hypotonia, peculiar facial characteristics, motor delay, micro/macrocephaly, brain anomalies, epilepsy and non-Mendelian family history for behavioural and psychiatric disorders were also recorded.

Results: ASD occurred in comorbidity with ID in of the half cases. One-third of them exhibited a monofactorial condition, including both complex diseases (i.e. *TCF4, CHAMP1, ASXL3*) and apparently non-syndromic presentation. Monofactorial conditions mimicking primary ASD were primarily *FMR1, RAI1, MECP2, PTEN*, del22q13, *ANRKD11* in children, *PPP2R5D* and (UPD)(15)pat associated disorders. However, additional key features were recorded in each.

Conclusions: Novel distinctive guidelines for primary or syndromic ASD/ID are proposed for application at a diagnostic level. A scoring system for the most sensitive clinical manifestations leading to the hypothesis of non-syndromic or syndromic ASD/ID is discussed. Our goal is to achieve a precise diagnosis, proper recurrence risk assessment, and an adequate pipeline for genetic investigations. The importance of pre-test and post-test clinical evaluations by the same clinical geneticist is highlighted.

Conflict of Interest: None declared

P11.124.C ELFN1 deficiency is associated with an autosomal recessive neurodevelopmental disorder with epilepsy

Rhys Dore[1], *Alden Huang*[2], *Mojtaba Movahedinia*[3], *Nadirah S. Damseh*[4], *Ijaz Anwar*[5], *Mohammad Yahya Vahidi Mehrjardi*[6], *Mehdi Khorrami*[7], *Fatemeh Eghbal*[8], *Mohammad Reza Mirjalili*[9], *Mohammadreza Dehghani*[10], *Ehsan Ghayoor Karimiani*[8], *Sergey Oreshkov*[5], *Muhammad Ansar*[5;11], *Stylianos Antonarakis*[12], *Julian Martinez-Agosto*[13], *stanley f. nelson*[2], *Undiagnosed Disease Network*[14], *Kirill Martemyanov*[15], *Henry Houlden*[16], *Reza Maroofian*[16]

[1]*Department of Clinical Genetics, Guy's Hospital, Guy's and St Thomas' NHS Foundation Trust, London, United Kingdom;* [2]*Institute for Precision Health, David Geffen School of Medicine, University of California Los Angeles, Los Angeles, United States;* [3]*Children Growth Disorder Research Center, Shahid Sadoughi University of Medical Sciences, Yazd, Iran;* [4]*Faculty of Medicine, Al-Quds University, Palestinian National Authority, Abu Deis, Palestine;* [5]*Department of Ophthalmology, University of Lausanne, Jules Gonin Eye Hospital, Fondation Asile Des Aveugles, Lausanne, Switzerland;* [6]*Diabetes Research Center, Shahid Sadoughi University of Medical Sciences, Yazd, Iran;* [7]*Department of Genetics and Molecular Biology, Faculty of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran;* [8]*Next Generation Genetic Polyclinic, Mashhad, Iran;* [9]*Internal*

Medicine Department, Medical College, Shahid Sadoughi University of Medical Sciences, Yazd, Iran; [10]Abortion Research Centre, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran; [11]Advanced Molecular Genetics and Genomics Disease Research and Treatment Centre, Dow University of Health Sciences, Karachi, Pakistan; [12]Department of Genetic Medicine and Development, University of Geneva Medical Faculty, Geneva, Switzerland; [13]Department of Human Genetics, David Geffen School of Medicine, University of California Los Angeles, Los Angeles, United States; [14]Undiagnosed Diseases Network, Common Fund, Office of the Director, NIH, Bethesda, United States; [15]Department of Neuroscience, UF Scripps Biomedical Research, Jupiter, Florida, United States; [16]Department of Neuromuscular Diseases, Queen Square Institute of Neurology, University College London, London, United Kingdom

Background/Objectives: *ELFN1* encodes a postsynaptic protein found throughout the nervous system. *Elfn1*$^{-/-}$ mice demonstrate an increased propensity for seizures and locomotion, with reduced thigmotaxis and limb strength. Biallelic loss-of-function (LOF) variants in *ELFN1* were initially identified in a single family affected by developmental and epileptic encephalopathy.

Methods: Eight individuals from five independent families, aged between 10 months to 21 years, including 3/5 families with consanguinity, underwent comprehensive clinical and genetic evaluations.

Results: All individuals carried homozygous LOF deletions in *ELFN1*. These included three families with frameshift deletions (c.1767del, p.(Val591CysfsTer93); c.128_137del, p.(Ile43ArgfsTer120); c.1522del, p.(Gln508SerfsTer176)), of which the third was associated with uniparental disomy of chromosome 7. One family had an in-frame deletion (c.475_477del, p.(Val159del)), resulting in loss of Elfn1's localization and binding function. One family had a 5'UTR deletion (g.1697729_1779918del) confirmed by RNA-seq as exhibiting loss of expression. All cases exhibited neurodevelopmental delay/intellectual disability, with varying severity, and experienced epilepsy. Seizure onset varied, ranging from the neonatal period to four years, encompassing generalized tonic-clonic seizures, tonic seizures, and intractable infantile spasms. Neuroimaging revealed corpus callosum thinning and white matter loss in one case, while others were reported as normal or with non-specific findings. Autism and ADHD were diagnosed in three and two cases, respectively. Clinical examinations showed various neurological abnormalities and dysmorphism. Hypotonia was the commonest feature (6/8), with less frequent features including muscle weakness, muscle atrophy, ataxia and nystagmus.

Conclusion: This study expands the phenotypic spectrum and reinforces the association between *ELFN1* deficiency and a neurodevelopmental disorder with epilepsy.

Grants:

Conflict of Interest: None declared

P11.125.D Postzygotic Point Mutations in Genetic Diagnostics and Counselling in Neurodevelopmental Disorders

Anna Walczak[1], *Małgorzata Rydzanicz*[1], *Agnieszka Pollak*[1], *Rafał Płoski*[1]

[1]*Medical University of Warsaw, Department of Medical Genetics, Warszawa, Poland*

Background: De novo mutations are spontaneous, non-inherited changes in the genome sequence. Postzygotic variants, arising spontaneously in an organism, lead to mosaicism – the presence of genetically different cell lines. Mosaicism types include somatic, gonosomal, and germinal, with implications ranging from asymptomatic states to severe diseases.

Methods: This study aimed to estimate the clinical significance threshold of variant allele frequency (VAF), the frequency of germinal or gonosomal mosaicism among parents

of affected children, and the highest VAF of a pathogenic variant not causing symptoms.

The WES test and ADS based on next-generation sequencing were employed.

Results: Among 55 tested families (proband and parents), we identified 25 postzygotic variants in 17 genes linked to mostly neurodevelopmental human diseases, manifest mainly by ASD, ID and DD. Among probands, 9 variants in 5 gene were detected, while asymptomatic parents showed 16 different variants in 14 genes (4 in mothers, 12 in fathers). Eight of the 12 paternal variants led to gonosomal mosaicism. Paternal germinal mosaicism had an estimated frequency of 13% (4/30 tested semen samples). VAF ranged from 3% (proband) to 35% (asymptomatic mother).

Conclusion: This research underscores the significance of scrutinizing both probands and parents for postzygotic variants within a VAF range of 1-35%. It is essential to recognize potential "de novo" variants by excluding their presence in additional tissues of parents during diagnostics and genetic counseling, crucial for pinpointing causative variants. The recurrence risk associated with a specific variant detected in a mosaic form in an asymptomatic parent cannot be overlooked.

Conflict of Interest: None declared

P11.126.A Multi-polygenic risk score approach to neurodevelopment-associated trait prediction in a high risk admixed cohort

Catarina S Gomes[1], *Veronica Euclydes*[1], *Caroline camilo*[1], *Gisele Gouveia*[1], *Helena Brentani*[1]

[1]*University of São Paulo, Institute of Psychiatry, São Paulo, Brazil*

Background/Objectives: The polygenic scores (PGS) aim to summarize the effect of SNPs on an individual's genetic susceptibility to a phenotype. The predictive performance of polygenic scores (PGS) is largely dependent on the target phenotype, the ancestry of the target and development population, and the number of samples available to train the PGS. In the context of psychiatry genetics, it is very well known the existence a high genetic correlation among psychiatry disorders and neurodevelopmental-associated phenotypes. Therefore, this study aims to utilize a multi-polygenic risk score approach to predict emotional and cognitive problems in a high-risk Brazilian cohort.

Methods: As target phenotypes, we seek to predict the Regional Project on Child Development Indicators (PRIDI) and the Strengths and Difficulties Questionnaire (SDQ) measured in 524 children in a situation of high social vulnerability in a large Brazilian metropolis. The children had their DNA collected from the saliva and were genotyped by using the Illumina Global Diversity Array. The genotyping data was processed using the *gtc2vcf* pipeline and the polygenic scoring models will be built using GWAS performed for ADHD, schizophrenia and ASD as a basis.

Results: So far, we have carried out the ancestry characterization of the sample and verified the expected miscegenation characteristic of the sample (average European ancestry below 65%). This proves to be a problem for the use of PRS constructed in European populations and to overcome this issue, we will use PRS calculation approaches based on genetic correlation of predictive characteristics.

Grants: Fundação de Amparo à Pesquisa do Estado de São Paulo (Grant #18/18560-6).

Conflict of Interest: None declared

P11.128.D Dissecting the impact of noncoding variation in ASD etiology

Julieta Bonacina[1], *julia scotellaro*[2], *Lili Kelley*[2], *Soleha Patel*[2], *taehwan shin*[2], *Ashish Jain*[2], *Paulina Gonzalez Tovar*[2], *Ryan Doan*[1]

[1]*Boston Children's Hospital and Harvard Medical School, Pediatrics, Boston, United States;* [2]*Boston Children's Hospital, Pediatrics, Boston, United States*

Background/Objectives: Despite heritability, only approximately 25% of individuals with Autism Spectrum Disorder (ASD) have an identifiable causal variant impacting a protein coding gene. Recently, many efforts, including ours, have suggested that noncoding regulatory variation may account for a substantial proportion of missed genetic diagnoses. In this study, we analyze the functional impact of de novo and inherited recessive noncoding promoter variants in individuals with ASD.

Methods: We examined available whole genome sequencing data from ASD cohorts (SPARK, HMCA and SSC) for predicted damaging rare de novo and inherited recessive promoter variation using our custom classification pipeline. Then, variants found only in cases were experimentally profiled using a massively parallel reporter assay. Thus, for each variant, we calculated a $\log_2$ score representing the comparative transcriptional regulatory activity of the alternative allele in relation to the reference allele, averaged across strands and replicates.

Results: Surprisingly, 12.4% (200/1617) of candidate noncoding variants significantly altered transcriptional activity and had a strong effect (absolute score > 0.2, FDR < 5%), with 76.5% of them leading to a decreased expression. Genes impacted by these 200 variants were enriched for neuron projection morphogenesis ($p = 0.04$) and histone modification ($p = 0.05$) processes, and included 30 known ASD genes (SFARI Gene database). Interestingly, 3 out of 4 of the variants identified in a regulatory element near HTATSF1, and 3 out of 7 associated with KDM5C, had strong functional impact.

Conclusion: Our results provide a systematic functional dissection of risk de novo and inherited promoter variants for ASD.

Grants: SFARI Bridge to Independence Award.

Conflict of Interest: None declared

P11.129.A Delineation of the *DLL1*-associated phenotype in 47 individuals

Leonard Koch[1], *Simona E Bianconi*[2], *Silvia Carestiato*[3], *Juliette Coursimault*[4], *Laurence Faivre*[5], *Jan Fischer*[6], *Himanshu Goel*[7], *Dorothy Grange*[8], *Christopher B Griffith*[9], *Anne-Marie Guerrot*[4], *Malavika Hebbar*[10], *Vera-Maria Herrmann*[11], *Callie L Hornbuckle*[12], *Stephen G Jones*[13], *Louisa Kalsner*[14], *Henriette Kiep*[15], *Cordula Knopp*[16], *Klaas Koop*[17], *Carmen Manso*[18], *Chaya N Murali*[19], *Lisa Pavinato*[3], *Valentin Ruault*[20], *Wolfgang M Schmidt*[21], *Shruti Shenbagam*[22], *Marwan Shinawi*[23], *Sarah Spinette*[24], *Anna Tietze*[25], *Alice Zalan*[26], *Björn Fischer-Zirnsak*[1], *Nadja Ehmke*[1]

[1]*Charité - Universitätsmedizin Berlin, Institute of Medical Genetics and Human Genetics, Berlin, Germany;* [2]*Southern California Permanente Medical Group, Kaiser Permanente, Department of Genetics, San Diego, California, United States;* [3]*University of Turin, Department of Medical Sciences, Torino, Italy;* [4]*Univ Rouen Normandie, Inserm U1245 and CHU Rouen, Department of Genetics and Reference Center for Developmental Disorders, Rouen, France;* [5]*UMR1231 GAD, Inserm - Université Bourgogne-Franche Comté, Dijon, France;* [6]*University Hospital Carl Gustav Carus at the Technische Universität Dresden, Institute for Clinical Genetics, Dresden, Germany;* [7]*Hunter New England Local Health District, Hunter Genetics, Waratah, Australia;* [8]*Washington University School of Medicine, St. Louis Children's Hospital, Division of Genetics and Genomic Medicine Department of Pediatrics, St. Louis, Missouri, United States;* [9]*University of South Florida, Tampa, Florida, United States;* [10]*BC Children's Hospital, University of British Columbia, Division of Neurology, Department of Paediatrics, Vancouver, Canada;* [11]*Leipzig University Medical Center, Institute of Human Genetics, Leipzig, Germany;* [12]*Children's Medical Center Dallas, Department of Genetics, Dallas, Texas, United States;* [13]*University of Arkansas for Medical Sciences, Little Rock, Arkansas, United States;* [14]*Connecticut Children's Medical Center and University of Connecticut School of Medicine, Divisions of Genetics and Neurology, Farmington, Connecticut, United States;* [15]*Hospital for Children and Adolescents, University Hospital Leipzig, Division of Neuropaediatrics, Leipzig, Germany;* [16]*Medical Faculty, RWTH Aachen University, Institute for Human Genetics and Genomic Medicine, Aachen, Germany;* [17]*University Medical Center Utrecht, Department of Pediatrics, Utrecht, Netherlands;* [18]*Parc Taulí Hospital Universitari, Institut d'Investigació i Innovació Parc Taulí I3PT, Universitat Autònoma de Barcelona, Center for Genomic Medicine, Sabadell, Spain;* [19]*Baylor College of Medicine, Department of Molecular and Human Genetics, Houston, Texas, United States;* [20]*Hôpital Arnaud de Villeneuve, Département de Génétique Médicale, Maladies Rares et Médecine Personnalisée, CHU Montpellier, France;* [21]*Medical University of Vienna, 1Neuromuscular Research Department, Center for Anatomy and Cell Biology, Vienna, Austria;* [22]*Al Jalila Children's Specialty Hospital, Al Jalila Genomics Center, Dubai, United Arab Emirates;* [23]*St. Louis Children's Hospital, Washington University School of Medicine, Division of Genetics and Genomic Medicine, Department of Pediatrics, St. Louis, Missouri, United States;* [24]*The University of Vermont Medical Center, Cancer Genetics, Burlington, Vermont, United States;* [25]*Charité - Universitätsmedizin Berlin, Institute of Neuroradiology, Berlin, Germany;* [26]*University of Illinois at Chicago, Department of Pediatrics, Section of Genetics, Chicago, Illinois, United States*

Background/Objectives: Delta-like 1 (DLL1) is a Notch receptor ligand, forming an important pathway for brain morphogenesis. Heterozygous pathogenic *DLL1* variants cause neurodevelopmental disorder with nonspecific brain abnormalities and with or without seizures (NEDBAS; MIM 618709). So far, detailed clinical and molecular data of 15 individuals from 12 unrelated families have been published.

Methods: The cohort was identified using GeneMatcher and ClinVar. We designed a questionnaire systematically evaluating clinical symptoms only described in single individuals before. It was also applied to the case reports of the previously published cohort for joint analysis.

Results: We report the clinical and molecular features of 32 unpublished individuals from 28 unrelated families with 25 different heterozygous de novo or inherited (likely) pathogenic *DLL1* variants (frameshift, splice site, nonsense, missense, multiexon deletions). Common clinical features in all 47 individuals were developmental delay and/or intellectual disability (88%), brain abnormalities (62%), attention deficit and hyperactivity disorder (50%), autism spectrum disorder (44%) and seizures (36%). Newly uncovered comorbidities in the current cohort include aggressive and/or self-injurious behavior (53%), sleeping problems (31%), anxiety (23%) and affective disorders (19%). 81% of the affected individuals receive help with developmental issues, physical therapy, and/or psycho/-pharmacological therapy. Seizures were almost exclusively associated with brain malformations and/or micro-/macrocephaly.

Conclusion: This work accentuates the complexity of the predominantly neurologic and psychiatric *DLL1*-associated phenotype, indicating the importance of specialized care in these domains. Functional in vivo and in vitro studies are needed to fully investigate the *DLL1*-related pathomechanism.

Grants: Not applicable.

Conflict of Interest: None declared

P11.130.B Parenti-Mignot neurodevelopmental syndrome (PMNDS): three novel patients with disease-causing variants in *CHD5*

Lara Hartmayer[1], *Antonia Paes*[2], *Sven Hethey*[2], *David Overberg*[3], *Jan-Ole Gehrmann*[3], *Corinna Doege*[3], *Gregor Dückers*[4], *Sandra von Hardenberg*[1], *Bernd Auber*[1], *Anke Katharina Bergmann*[1], *Tim Ripperger*[1]

[1]*Deparment of Human Genetics, Hannover Medical School, Hannover, Germany;* [2]*Department of Neuropediatric, Child and Youth Hospital AUF DER BULT, Hannover, Germany;* [3]*Eltern-Kind-Zentrum Prof. Hess, Department of Pediatric Intensive Care and Department of Pediatrics and Adolescent Medicine, Neuropediatrics, Klinikum Bremen Mitte, Bremen, Germany;* [4]*HELIOS Childrens Hospital, Krefeld, Germany*

Background/Objectives: CHD5 plays an important role in radial migration and terminal differentiation of neuronal cells. Disease-causing variants in *CHD5* are linked to PMNDS, a rare autosomal dominant neurodevelopmental disorder with less than twenty reported cases. Causal variants frequently occurred de novo, including mosaic patients. We present three novel patients with PMNDS expanding its clinical spectrum.

Methods: Whole genome sequencing (WGS).

Results: A 3-year-old boy with mild developmental delay, mild facial dysmorphism, and respiratory infection-associated onset of seizures at 2 years was referred with suspected GLUT1-deficiency due to abnormal liquor results. While GLUT1-deficiency was genetically not confirmed, the variant NM_015557.3(CHD5):c.2514del p.(Ile839Serfs*8) [heterozygous] was identified. Parents were not available for testing.

A critically-ill 11-month-old girl with pre-existing global developmental delay, hypotonia, and mild facial dysmorphism presented with a viral encephalitis-associated left hemispheric recurrent status epilepticus. By rapid trio-WGS, we identified the de novo variant NM_015557.3(CHD5):c.2662C>T p.(His888Tyr) [heterozygous].

A critically-ill 5-year-old Syrian boy with consanguine parents, recurrent severe infections, neutropenia, anemia, mild facial dysmorphism, global developmental delay, hypotonia, and dental anomalies was referred for rapid WGS. We identified two disease-causing variants: NM_006118.4(HAX1):c.130_131insA p.(Trp44*) [homozygous] and NM_015557.3(CHD5):c.3988C>T p.(Gln1330*) [heterozygous], confirming the co-occurrence of severe congenital neutropenia-3 and PMNDS. Parents have not been tested yet.

Conclusion: PMNDS is characterized by an unspecific and variable phenotype including developmental delay, hypotonia, behavioral disturbances, epilepsy, mild non-specific facial dysmorphism, and variable degrees of intellectual disability. Our report complements known clinical phenotypes and associates PMNDS with severe epilepsy adding it to the list of differential diagnosis in critically-ill children.

Conflict of Interest: None declared

P11.131.C Establishing the Molecular Foundation of Brain Anatomy in Living Individuals

Anina Lund[1], *Lora Liharska*[1], *Eric Vornholt*[1], *Ryan Thompson*[1], *Yuyang Luo*[2;3;4], *Esther Cheng*[1], *You Jeong Park*[1], *Brian Fennessy*[1], *Lillian Wilkins*[5], *Deepak Kaji*[1], *Hannah Silk*[1], *Kimia Ziafat*[6], *Alice Hashemi*[1], *Emily Moya*[7], *Claudia Feng*[8], *Lisa Linares*[1], *Marysia-Kolbe Rieder*[9], *Joseph. Scarpa*[10], *Prashant Kota*[11], *Vanessa Cohen*[1], *Brendan Sullivan*[1], *Punit Shah*[12], *Weiping Ma*[1], *Jessica Johnson*[13], *Greg Miller*[14], *Pei Wang*[1], *Nadkarni Girish*[1], *Michael Kiebish*[14], *Nischal Chand*[14], *Niven Narain*[14], *Eric Schadt*[1], *Brian Kopell*[1], *Alexander Charney*[1], *Noam Beckmann*[1]

[1]*Icahn School of Medicine at Mount Sinai, New York, United States;* [2]*Ocular Genomics Institute, Department of Ophthalmology, Massachusetts Eye and Ear, Boston, MA;* [3]*Department of Ophthalmology, Harvard Medical School, Boston, MA;* [4]*Broad Institute of MIT and Harvard, Cambridge, MA;* [5]*UCLA department of neurobiology;* [6]*Faculty of Medicine, University of British Columbia, Canada;* [7]*Stanford University School of Medicine;* [8]*Wellcome Sanger Trust Cambridge, UK;* [9]*Yale School of Medicine, DNA Diagnostic Laboratory;* [10]*Department of Anesthesiology, Weill Cornell Medicine;* [11]*Goldman Sachs;* [12]*Berg Health;* [13]*Department of Psychiatry, University of North Carolina at Chapel Hill,;* [14]*BPGbio, Inc*

Background/Objectives: The relationship between brain molecular traits (i.e., gene and protein expression) and brain anatomical features in living individuals, each independently shown to be associated with disease, has yet to be defined. Here, we use neuroimaging-linked molecular data, generated from 320 biopsies from the prefrontal cortex from 202 older individuals undergoing the deep brain stimulation electrode implantation procedure to characterize this relationship.

Methods: Gene expression (single-nuclei [snRNAseq], N = 31 and bulk RNA-seq, N = 289) as well as protein expression (liquid chromatography-mass spectrometry, N = 155) data generated as part of the Living Brain Project, were processed, and integrated with structural MRI-derived anatomical features (N = 197, cortical thickness, area, and volume) measured in the same individuals. Associations of molecular traits to anatomical features were performed for each imaging feature using linear mixed models. Associations were replicated between -omics and annotated to specific cell type effects using snRNA-seq data.

Results: Molecular signatures of brain anatomical features in living individuals were identified, with multiple features associated with bulk gene expression (19 features with ≥1gene at FDR ≤ 0.05). These associations replicated in protein expression (median absolute Spearman rho = 0.13). SnRNAseq replicated gene and protein expression signatures, and annotated them to specific cell types, providing further characterization of these signatures and their pathways (e.g., for medial orbitofrontal thickness, snRNAseq signature in excitatory neurons is correlated to gene [rho = 0.22] and protein [rho = 0.24] expression).

Conclusion: By associating molecular traits with imaging anatomical features generated from the same individuals, we provide an in-depth characterization of the molecular foundation of brain anatomy in living individuals.

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Abbott Neuromodulation, Consultant

Turing Medical, Consultant, Alexander Charney: None declared, Noam Beckmann: None declared

P11.132.D From hereditary spastic paraplegia to epileptic encephalopathy: the first series of patients with biallelic variants expands the phenotypic spectrum associated with the SPAST gene

Manon DEGOUTIN[1], *Chloé Angelini*[1], *Claire Bar*[1], *Traki Ben Hassine*[2], *Claire Ewenczyk*[3], *ziv gan-or*[4], *Didier LACOMBE*[1], *Claire Lefeuvre*[5], *Purvi Majethia*[6], *Mouna Messaoud*[2], *Guy Rouleau*[4], *Oksana Suchowersky*[7], *Anju Shukla*[6], *Marine Guillaud Bataille*[8], *Giovanni Stevanin*[9], *Cyril GOIZET*[1]

[1]*Hospital Pellegrin Bordeaux, Bordeaux, France;* [2]*University Of Science And Technology Houari Boumediene, Bab Ezzouar, Algeria;* [3]*Sorbonne Université, Paris, France;* [4]*Department of Human Genetics, Montréal, Canada;* [5]*Raymond Poincaré University Hospital - (AP-HP), Garches, France;* [6]*KASTURBA MEDICAL COLLEGE, Manipal, India;* [7]*University of Alberta, Medicine, Edmonton, Canada;* [8]*University Hospitals Pitié Salpêtrière - Charles Foix, UF Neurogénétique Moléculaire et Cellulaire, Paris, France;* [9]*Faculté de Médecine - Université de Bordeaux, Bordeaux, France*

Background/Objectives: Heterozygous pathogenic variants in *SPAST* are known to cause Hereditary Spastic Paraplegia 4 (SPG4), the most common form of HSP, characterized by progressive bilateral lower limb spasticity with frequent sphincter disorders. However, there are very few descriptions in the literature of patients carrying biallelic variants in *SPAST*.

Methods: Targeted Sanger sequencing, panel sequencing and exome sequencing were used to identify the genetic causes in 8 patients from 5 unrelated families with symptoms of HSP or developmental and epileptic encephalopathy.

Results:

We describe 8 patients with pure HSP with variable age of onset, mostly in infancy, and 3 patients with severe early-onset developmental and epileptic encephalopathy with a progressively worsening tetrapyramidal syndrome. The patients' parents, heterozygous carriers of pathogenic *SPAST* variants, included both asymptomatic carriers and patients with a classic form of HSP4.

These different phenotypes are consistent with the very rare descriptions available in the literature: a patient carrying a homozygous variant in *SPAST* presenting with developmental encephalopathy and epilepsy had been reported, as well as 3 patients with pure spastic paraplegia, of infantile or late onset.

Conclusion: Biallelic variants of SPAST may explain cases of hereditary spastic paraplegia with autosomal recessive inheritance. Furthermore, these biallelic variants may also cause epileptic encephalopathy with a tetrapyramidal syndrome, a new phenotype associated with the *SPAST* gene.

Grants: No grants

Conflict of Interest: None declared

P11.133.A Spatial transcriptomics reveals mechanisms of degeneration and regeneration in multiple sclerosis

Jia Hui Khoo[1], *A. M. R. Van Den Bosch*[2], *Zhigang Lu*[1], *Dennis Wever*[2], *Li Pu*[1], *Bart Eggen*[3], *Joost Smolders*[2;4], *Jörg Hamann*[2;5], *Zhouchun Shang*[1], *Jan Mulder*[6], *Inge Huitinga*[2;7]

[1]*BGI Research Foundation Latvia, Riga, Latvia;* [2]*Netherlands institute for Neuroscience, Neuroimmunology Research Group, Amsterdam, Netherlands;* [3]*University Medical Center Groningen, Department of Neuroscience, Section Medical Physiology, Groningen, Netherlands;* [4]*Erasmus Medical Center, MS Center ErasMS, Department of Neurology and Immunology, Rotterdam, Netherlands;* [5]*Amsterdam University Medical Center, Amsterdam Infection and Immunity Institute, Amsterdam, Netherlands;* [6]*Karolinska Institute, Department of Neuroscience, Stockholm, Sweden;* [7]*University of Amsterdam, Swammerdam Institute for Life Sciences, Amsterdam, Netherlands*

Background/Objectives: In multiple sclerosis (MS), various lesion types are found across the central nervous system, with varying potential for demyelination, remyelination and scar formation. Mixed active/inactive (mA/I) lesions with foamy microglia correlate with neuro-axonal damage while those with ramified microglia do not, suggesting biological differences. Investigating these differences may elucidate critical mechanisms driving MS lesion progression and repair.

Methods: Postmortem tissue of n = 8 MS donors and n = 3 control donors was provided by the Netherlands Brain Bank. We performed high-resolution spatial transcriptomics and immunohistochemistry analyses, focusing on the demyelinated center, demyelinating border and the peri-lesion border of mA/I lesions with ramified microglia to those with foamy microglia within the same donors.

Results: Stereo-seq and IHC results at the single-cell and bin50 level showed that lesion border and demyelinated center had significantly different cellular composition as compared to the peri-lesion region. Unsupervised clustering and cell-bin pseudo bulk analysis identified distinct gene expression patterns. Gene expression of mA/I lesions with foamy microglia indicated higher levels of ongoing tissue damage, demyelination, complement activation, iron metabolism, reactive astrocytes, and immunoglobulin production in the centre, border, and peri-lesion region. In contrast, mA/I with ramified microglia showed higher levels of neurite outgrowth, stability of myelin, and lipid export in the border and peri-lesion.

Conclusion: mA/I lesions with ramified microglia may be more prone to remyelination, while those with foamy microglia may be more prone to lesion expansion. This study sheds light on the molecular mechanisms driving MS lesion dynamics and identifies potential targets for therapeutic interventions.

Grants:

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P11.134.B Dynamic X-chromosomal reactivation enhances female brain resilience

Marco Bertin[1], *Hristo Todorov*[1], *Stephan Kaeseberg*[1], *Radhika Menon*[2;3;4], *Elisa Gabassi*[3;5], *Sarah Frank*[3], *Heiko Brennenstuhl*[6;7], *Benjamin Lohrer*[3], *Jennifer Winter*[1], *Jennifer Krummeich*[1], *Jürgen Winkler*[8], *Beate Winner*[9;10], *Eva Weis*[1], *Dewi Hartwich*[1], *Stefan Diederich*[1], *Katja Luck*[11], *Susanne Gerber*[1], *Peter Lunt*[12], *Nadine*

Bobon[13], Peter Baumann[11;13], Benedikt Berninger[4;14;15;16;17], Sven Falk[3], Marisa Karow[3], Susann Schweiger[1]

[1]University Medical Center of the Johannes Gutenberg University Mainz, Institute of Human Genetic, Mainz, Germany; [2]MIMETAS — Organ-on-a-Chip, Oegstgeest, Netherlands; [3]Friedrich-Alexander-Universität Erlangen-Nürnberg, Institute of Biochemistry, Erlangen, Germany; [4]University medicine at the Johannes Gutenberg University in Mainz, Institute of Physiological Chemistry, Mainz, Germany; [5]Institute of Molecular Biology & CMBI Leopold-Franzens-University Innsbruck, Department of Genomics, Stem Cell Biology and Regenerative Medicine, Innsbruck, Austria; [6]University Hospital Heidelberg, Division of Child Neurology and Metabolic Medicine, Center for Child and Adolescent Medicine, Heidelberg, Germany; [7]Heidelberg University, Institute of Human Genetics, Heidelberg, Germany; [8]University Hospital Erlangen, Department of Molecular Neurology, Erlangen, Germany; [9]University of Erlangen-Nuremberg, Department of Stem Cell Biology, Erlangen, Germany; [10]University of Erlangen-Nuremberg, Center of Rare Diseases Erlangen (ZSEER), Erlangen, Germany; [11]Institute of Molecular Biology, Mainz, Germany; [12]University of Bristol, Centre for Academic Child Health, United Kingdom; [13]Johannes Gutenberg University of Mainz, Faculty of Biology, Mainz, Germany; [14]King's College London, Institute of Psychology, Psychiatry &Neuroscience, Centre for Developmental Neurobiology, London, United Kingdom; [15]King's College London, MRC Centre for Neurodevelopmental Disorders, London, United Kingdom; [16]Francis Crick Institute, United Kingdom; [17]Johannes Gutenberg University of Mainz, Focus Program Translational Neuroscience, Mainz, Germany

Background/Objectives: While random X-chromosome inactivation in female cells of placental mammalians silences one allele of the majority of X-chromosomal genes, a considerable fraction is only incompletely and variably inactivated resulting in a tissue-specific pattern of mono- and biallelic expression.

Methods: Here we used clonal human female induced pluripotent stem cells (iPSCs) allowing us to trace the (in)activation status of the two X-chromosomes individually along neural differentiation trajectories.

Results: We discovered X-chromosome-wide locus- and lineage-specific dynamic usage of the two X-chromosomal alleles in female cells induced by differentiation. Leveraging iPSCs derived from patients with an X-linked neurodevelopmental disorder, we demonstrate that activation of alleles on the inactive X-chromosome can exert protective effects on the manifestation of disease phenotypes in female neural cells and tissue.

Conclusion: Taken together, our data demonstrate that alleles on the inactive X-chromosome can serve as a critical reservoir reactivated during differentiation, thereby enhancing the resilience of female neural tissue.

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Conflict of Interest: None declared

P11.135.C Terminal duplications of the 17p13.3 region: Further case evidence of a cause for polymicrogyria

Eleanor Ludington[1], *David Francis[1], Tiong Yang Tan[1;2;3;4], George McGillivray[1;5], Anand Vasudevan[5], Richard Leventer[2;3;6], Chloe Cunningham[1;2;3]*

[1]Victorian Clinical Genetics Services, Parkville, Australia; [2]Murdoch Children's Research Institute, Parkville, Australia; [3]The University of Melbourne, Department of Paediatrics, Parkville, Australia; [4]The Royal Children's Hospital Melbourne, Parkville, Australia; [5]The Royal Women's Hospital, Department of Clinical Genetics, Parkville, Australia; [6]The Royal Children's Hospital Melbourne, Department of Paediatric Neurology, Parkville, Australia

Background/Objectives: The chromosome 17p13.3 microduplication syndrome is recurrent, reported in only 40 individuals. This interstitial duplication involves the Miller-Dieker critical region and can be categorised into class I (*PAFAH1B1*-absent) and class II (*PAFAH1B1*-present). It produces a variable phenotype including developmental delay, behavioural disorders, facial dysmorphism, and structural brain abnormalities. Terminal duplications incorporating this region are very rare, usually complicated by other structural CNVs, and only one individual in the literature has been reported to have polymicrogyria. The aetiology of polymicrogyria is heterogeneous and poorly understood. Genetic causes include chromosomal or monogenic variants. We report 3 individuals with terminal 17p13.3 microduplications, two of whom have bilateral perisylvian polymicrogyria.

Methods: Three unrelated individuals were seen by clinical genetics at a tertiary paediatric hospital in Melbourne, Australia for assessment of polymicrogyria and/or developmental delay. Each had a standard SNP chromosomal microarray (Illumina Infinium GSA-24 v3.0).

Results: In all 3 individuals standard SNP chromosomal microarray identified overlapping 17p13.3 duplications ranging from 4Mb - 4.3Mb. One individual also had a 2.3Mb terminal deletion of 5q35.3. Each duplication included *PAFAH1B1*, *YWHAE* and *CRK* within the Miller-Dieker critical region. MRI brain of two of the individuals demonstrated polymicrogyria. The third individual had expressive language delay, hypotonia and oromotor dysfunction without indication for a brain MRI.

Conclusion: Our observation of two cases of 17p13.3 microduplication syndrome with polymicrogyria strengthens the association of duplication of the Miller-Dieker critical region with polymicrogyria and underscores the variable neurodevelopmental phenotype.

Grants: N/A

Conflict of Interest: None declared

P11.136.D Phenotypic and mutational spectrum in dystonia genes: Insights from gene panel screening

Mirja Thomsen[1], *Katrin Marth[1;2], Sebastian Loens[1;3], Judith Charlotte Everding[1;4], Johanna Junker[1;5], Friederike Borngräber[6], Fabian Ott[7], Silvia Jesús[8], Mathias Peter Gelderblom[9], Thorsten Odorfer[10], Gregor Kuhlenbäumer[4], Han-Joon Kim[11], Eva Schäffer[4], Jos Becktepe[4], Meike Kasten[1;12], Norbert Brüggemann[1;5], Robert Pfister[13], Katja Kollewe[14], Joachim K. Krauss[15], Ebba Lohmann[16;17], Frauke Hinrichs[1], Daniela Berg[4], Beomseok Jeon[11], Hauke Busch[7], Eckart Altenmüller[17], Pablo Mir Rivera[8;18], Christoph Kamm[2], Jens Volkmann[10], Simone Zittel-Dirks[9], Andreas Ferbert[19], Kirsten Zeuner[4], Arndt Rolfs[20;21], Peter Bauer[22], Andrea Kühn[6], Tobias Bäumer[3;5;23], Christine Klein[1], Katja Lohmann[1]*

[1]University of Lübeck, Institute of Neurogenetics, Lübeck, Germany; [2]University Hospital Rostock, Department of Neurology, Rostock, Germany; [3]University of Lübeck, Institute of Systems Motor Science, Lübeck, Germany; [4]University Hospital Schleswig-Holstein, Campus

Kiel, Department of Neurology, Kiel, Germany; [5]University Hospital Schleswig-Holstein, Campus Lübeck, Department of Neurology, Lübeck, Germany; [6]Charité – Universitätsmedizin Berlin, Department of Neurology, Berlin, Germany; [7]University of Lübeck, Medical Systems Biology Group, Lübeck Institute of Experimental Dermatology, Lübeck, Germany; [8]Hospital Universitario Virgen del Rocío/CSIC/Universidad de Sevilla, Unidad de Trastornos del Movimiento, Servicio de Neurología y Neurofisiología Clínica, Seville, Spain; [9]University Medical Center Hamburg-Eppendorf, Department of Neurology, Hamburg, Germany; [10]University Hospital Würzburg, Department of Neurology, Würzburg, Germany; [11]Seoul National University Hospital, Department of Neurology, Seoul, Korea, Rep. of South; [12]University Hospital Schleswig-Holstein, Campus Lübeck, Department of Psychiatry, Lübeck, Germany; [13]Neurological Practice, Neusäß, Germany; [14]Hannover Medical School, Department of Neurology, Hannover, Germany; [15]Hannover Medical School, Department of Neurosurgery, Hannover, Germany; [16]University of Tübingen, Hertie Institute for Clinical Brain Research, Tübingen, Germany; [17]Hanover University of Music, Drama and Media, Institute of Music Physiology and Musicians' Medicine, Hannover, Germany; [18]Centro de Investigación Biomédica en Red sobre Enfermedades Neurodegenerativas (CIBERNED), Spain; [19]Klinikum Kassel, Department of Neurology, Kassel, Germany; [20]University of Rostock, Medical Faculty, Rostock, Germany; [21]Agyany Pharmaceuticals, Jerusalem, Israel; [22]Centogene AG, Rostock, Germany; [23]University Hospital Schleswig-Holstein, Campus Lübeck, Center of Rare Diseases, Lübeck, Germany

Consortium: DysTract consortium

Background/Objectives: Dystonia is a movement disorder characterized by excessive muscle contractions leading to involuntary movements and abnormal postures. Pathogenic variants in several genes (*GCH1*, *GNAL*, *KMT2B*, *PRKRA*, *SGCE*, *THAP1*, and *TOR1A*) have been linked to monogenic forms of dystonia. To further elucidate the phenotypic and genetic spectrum, we screened a large sample of patients with dystonia or Parkinson's disease (PD), where dystonic signs are not infrequent.

Methods: We employed a next-generation sequencing-based gene panel to screen 1,207 dystonia and 1,036 PD patients for variants in dystonia-causing genes. Pathogenicity was assessed using the ACMG and MDSGene (www.mdsgene.org/methods) scoring systems. The impact on DNA methylation of *KMT2B* variants was evaluated by analyzing the gene's characteristic episignature.

Results: We identified 171 carriers (109 with dystonia [9.0%]; 62 with PD [6.0%]) of 131 rare variants (minor allele frequency <0.005). Thereof, 52 patients (48 dystonia [4.0%]; four PD [0.4%, all with *GCH1* variants]) carried 33 different (likely) pathogenic variants, including 17 previously unreported changes. Episignature analysis of 48 *KMT2B* variants suggested only two variants as pathogenic.

Conclusion: This study validates pathogenic variants in several genes as relevant contributors to dystonia and expands the mutational spectrum. Notably, likely pathogenic variants only in *GCH1* were also found in PD patients. For DYT-KMT2B, the recently described episignature served as a reliable readout to determine the functional effect of newly identified variants. Subsequent exome and genome sequencing in unresolved patients will identify additional genetic causes of dystonia.

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P11.138.B Genome sequencing results of a cohort of pediatric patients with autism and neurodevelopmental disorders

Mariia Parfenenko[1], *Ilya Dantsev*[1], *Olga Groznova*[2;3], *Victoria Voinova*[2]

[1]*Veltischev Research and Clinical Institute for Pediatrics and Pediatric Surgery of the Pirogov Russian National Research Medical University of the Ministry of Health of the Russian Federation, laboratory of clinical genomics and bioinformatics, Moscow, Russian Federation;* [2]*Veltischev Research and Clinical Institute for Pediatrics and Pediatric Surgery of the Pirogov Russian National Research Medical University of the Ministry of Health of the Russian Federation, clinical genetics, Moscow, Russian Federation;* [3]*2. Charity Fund for medical and social genetic aid projects «Life Genome», Moscow, Russian Federation*

Background/Objectives: Autism is a neurodevelopmental disorder (NDD) characterized by communication difficulties and stereotypic behavior, which frequently co-occurs with intellectual disability, developmental delay, epilepsy, and other conditions. SFARI (Simons Foundation Autism Research Initiative) Gene database categorizes autism-associated genes into four levels of evidence: 1 – high confidence, 2 – strong candidate, 3 – suggestive evidence, and an extra category "S" for genes linked to additional features.

Methods: 116 unrelated patients (table 1) (mean age: 7.4 y/o (3-20), female: 41 (35%), male: 75 (65%)) with NDDs underwent genome sequencing. We viewed pathogenic, likely pathogenic, and variants of unknown significance (VOUS) in autism-associated genes as likely causative.

Feature	Autism	Signs of autism	Developmental delay	Speech disorders	Intellectual disability	Epilepsy	Known normal karyotype	NDDs in relatives
Number of patients (N, %)	49, 42%	116, 100%	50, 43%	56, 48%	12, 10%	25, 22%	30, 26%	20, 17%

Results: 156 variants (table 2) in 113 genes included in SFARI Gene were likely causative, 66 genes – associated with syndromic forms, 47 – isolated autism; 63 belonged to category 1, 34 – category 2, 16 – to others. 88 patients had 1 involved gene, 23 had 2, the rest – 3 or 4. The most frequently involved genes: *CHD8* (6 cases) and *SCN2A* (4 cases).

Pathogenic	8 (5%)
Likely pathogenic	21 (13%)
VOUS	127 (82%)
Inherited from parent	12 (8%)

Conclusion: Genome sequencing of 116 patients with NDDs provided insight into likely genetic causes of autism and autism-like phenotypes in our population.

Grants:

Conflict of Interest: None declared

P11.139.C Single cell RNA sequencing-based phenotyping to detect early signatures of neurodegenerative diseases

Lioba Elvers[1], *Jana Henck*[2], *Saranya Balachandran*[1], *Kristian Händler*[1], *Nathalie Kruse*[1], *Josh Kim*[2], *Mai Phan*[2], *Lars Wittler*[3], *Elisa Giorgio*[4], *Varun Sreenivasan*[1], *Malte Spielmann*[1]

[1]*University Hospital Schleswig-Holstein, University of Lübeck and Kiel University, Institute of Humangenetics, Lübeck;* [2]*Max-Planck-Institut für molekulare Genetik, Research Group Development & Disease, Berlin, Germany;* [3]*Max-Planck-Institut für molekulare Genetik, Berlin, Germany;* [4]*Università di Pavia, Department of Molecular Medicine, Pavia, Italy*

Background/Objectives: For many neurodegenerative diseases, comprehensive genotype-phenotype correlations are challenging. But it has been proposed that disease-causing variants may lead to cellular and molecular perturbations long before the onset of clinical symptoms. Here we set out to explore whether the late-onset autosomal dominant leukodystrophy (ADLD), has early detectable signatures.

Methods: We sampled the brains of mouse mutants replicating known ADLD-associated variants. Based on the differences detected in the 3D genome between the two mutants, we subjected brains of two embryonic stages (E11.5 and E18.5) and in youth (P30) to single cell RNA sequencing (sc-RNAseq). We applied sc-RNAseq to provide an unbiased characterization of different SVs that result in the same clinical phenotype. In the analysis we compared changes in cell composition, differential gene expression, and intercellular signaling in an objective and comprehensive manner.

Results: Interestingly, we detected several significant changes in the cell composition and gene expression already at early embryonic stages in multiple cell types, including neuronal cell trajectories, indicating a gap between molecular and clinical onset of disease. Further, although categorized as the same disease, there are differences between the two SVs in early development, indicating alternate disease mechanisms.

Conclusion: Analysis of the single cell dataset revealed changes in brain development in the mouse models of ADLD, for example in the cellular composition, gene expression and specific gene pathways detectable. Further extensive and unbiased characterization is underway to uncover more subtle changes in the brain architecture of the developing embryo.

Conflict of Interest: None declared

P11.141.A Transcriptomic and epigenomic consequences of heterozygous loss of function mutations in AKAP11, the first large-effect shared risk gene for bipolar disorder and schizophrenia

Nargess Farhangdoost[1;2], *Yumin Liu*[2], *Patrick Dion*[2;3], *Boris Chaumette*[4], *Anouar Khayachi*[2], *Guy Rouleau*[2;3]

[1]*McGill University, Human Genetics, Montréal, Canada;* [2]*Montreal Neurological Institute-Hospital, Montréal, Canada;* [3]*McGill University, Montréal, Canada;* [4]*Institute National De La Santé Et De La Recherche Médicale, Paris, France*

Background/Objectives: Bipolar disorder (BD) is a complex psychiatric condition with psychotic symptoms similar to schizophrenia (SCZ). Despite extensive research, the roots of BD remain largely unknown. *AKAP11* is a loss-of-function (LoF) rare-variant shared risk gene between BD and SCZ with large effects. Here, the effect of heterozygous LoF mutations in *AKAP11* is being investigated at the transcriptomic and epigenetic level in induced pluripotent stem cell (iPSC)-derived neurons.

Methods: Using a human iPSC, we generated 3 *AKAP11*-Knockout (*AKAP11*-KO) isogenic neuronal clones, each carrying a different heterozygous frameshift mutation. Subsequently, we carried out RNA sequencing, whole-genome bisulfite sequencing, and chromatin immunoprecipitation followed by sequencing of histone H3 lysine 27 acetylation.

Results: We identified an overall downregulation in genes related to ribosome function and transcription factor activity, and an upregulation in signaling receptor interactions, cytokine activity, and cytoskeletal dynamics. We detected global DNA methylation changes, predominantly in intergenic and intronic regions. Intergenic enhancers with reduced activity were associated with downregulated genes also primarily involved in transcription factor activity. Conversely, intronic enhancers with increased activity were linked to upregulated genes involved in signaling receptor binding, cytokine receptor binding, and cytoskeleton-related functions.

Conclusion: Our exploration into the epigenomic and transcriptomic impacts of heterozygous LoF of AKAP11 has revealed insights into significant outcomes in the regulation of gene expression. This is particularly evident in genes that function as transcription factors and co-factors, regulators of actin and cytoskeleton, and cytokine receptors, primarily through the modulation of intergenic and intronic cis-regulatory elements.

Fellowship: Fonds de Recherche du Québec-Santé

Conflict of Interest: None declared

P11.142.B *UBE2I*: a promising candidate gene in syndromic neurodevelopmental disorders involved in sumoylation pathways

Verdiana Pullano[1], *Slavica Trajkova*[2], *Alessandra Aulino*[1], *Elena Sukarova-Angelovska*[3], *Henrike Sczakiel*[4;5], *Susan Hiatt*[6], *Anna C.E. Hurst*[7], *Benjamin Cogne*[8], *Karishma Bakshi*[9], *Andrea Pichler*[9;10], *Tommaso Pippucci*[11], *Paola Dimartino*[12], *Simona Cardaropoli*[13], *Alessandro Mussa*[13], *Silvia De Rubeis*[14], *Joseph Buxbaum*[14], *Giovanni Battista Ferrero*[15], *Alfredo Brusco*[2]

[1]*University of Turin, Department of Medical Sciences, Turin, Italy;* [2]*University of Turin, Department of Neurosciences Rita Levi-Montalcini, Turin, Italy;* [3]*University Clinic for Children's Diseases, Faculty of Medicine, University "Sv. Kiril I Metodij", Department of Endocrinology and Genetics, Skopje, Macedonia;* [4]*Institute of Medical Genetics and Human Genetics, Charité - Universitätsmedizin Berlin, Corporate Member of Freie Universität Berlin and Humboldt-Universität zu Berlin, Berlin, Germany;* [5]*BIH Biomedical Innovation Academy, Clinician Scientist Program, Berlin Institute of Health at Charité - Universitätsmedizin Berlin, Berlin, Germany;* [6]*HudsonAlpha Institute for Biotechnology, Huntsville, AL, United States;* [7]*University of Alabama, Department of Genetics, Birmingham, AL, United States;* [8]*Service de Génétique Médicale, Centre Hospitalier Universitaire de Nantes; Université de Nantes, Inserm UMR 1087 / CNRS UMR 6291, Institut du thorax, Nantes, France;* [9]*Institute of Biochemistry, ETH Zürich, Zürich, Switzerland;* [10]*Max Planck Institute of Immunobiology and Epigenetics, Department of Epigenetics, Freiburg, Germany;* [11]*IRCCS Azienda Ospedaliero-Universitaria di Bologna, Bologna, Italy;* [12]*University of Pavia, Department of Molecular Medicine, Pavia, Italy;* [13]*University of Turin, Department of Public Health and Pediatrics, Turin, Italy;* [14]*Seaver Autism Center for Research and Treatment, Icahn School of Medicine at Mount Sinai, New York, United States;* [15]*University of Turin, Department of Clinical and biological sciences, Turin*

Background/Objectives: Defects in SUMOylation, a regulatory post-translational modification process, are an emerging cause of neurodevelopmental disorders (NDDs). SUMOylation involves the conjugation of SUMO protein to specific Lys residues of target substrates by E1, E2, and E3 enzymes. We investigated *UBE2I*, encoding UBC9, the only E2 enzyme in the SUMO system, as a potential causative gene for NDD.

Methods: Using trio-ES on a comprehensive survey of more than 600 NDD families, we discovered a de novo missense change, NM_194260.3:p.(Asn124Ile), in the *UBE2I* in a pediatric patient displaying a syndromic phenotype. GeneMatcher platform allowed us to identify three additional *UBE2I* patients (p.Val25Met; p.Trp53Leu; p.Glu143Gly) and gather clinical data. We generated stable inducible cell lines using the Flp-In T-Rex system, to express the wild-type (WT) or the variant forms. Using western blot analysis, we examined the impact of these variants on UBC9 protein levels and sumoylation.

Results: We have identified four unrelated cases with deleterious missense variants in *UBE2I*, affecting highly evolutionarily conserved aminoacids. All patients displayed common features, including neurodevelopmental delay, thick eyebrows, hypertelorism/telecanthus, almond shaped eyes, nasal shape anomalies and hand anomalies. In our cellular model, the expression levels of the *UBE2I* variants were lower compared to the wildtype. However, we also noted an increase in sumoylation suggesting that these variants may disrupt the normal regulation of sumoylation processes.

Conclusion: We propose *UBE2I* as a potential candidate gene for a novel NDD. Confirming its involvement in NDDs will provide valuable insights into the significance of sumoylation in brain function and development.

Grants:

Conflict of Interest: None declared

P11.143.C Improving work up amongst patients with rare movement disorders according to diagnostic yield findings– Update from single center Neurogenetic Clinic

Dvir Penn[1], *Yam Amir*[1], *Gili Ben David*[1], *Tatyana Gurevich*[1], *Hagit Baris Feldman*[1], *roy alcalay*[1], *yuval yaron*[1], **Penina Ponger**[1]

[1]*Tel Aviv Sourasky Medical Center - Ichilov, Tel Aviv-Yafo, Israel*

Background/Objectives: The diagnostic yield of genetic testing for movement disorders is increasing as next-generation-sequencing (NGS) and advanced molecular testing are incorporated into routine work-up. We present the genetic testing diagnostic yield in a tertiary center Neurogenetic Clinic, focusing on rare movement disorders.

Methods: Retrospective analysis (2019-2023) of 190 patients aged 1-84 years diagnosed with cerebellar ataxia (CA, N = 92), spastic paraparesis (SPP, N = 50) and hyperkinetic movement disorders, including dystonia and dyskinesia (HMD,N = 48). Work-up included NGS and repeat expansion testing. Undiagnosed cases underwent exome-sequencing (ES) or genome-sequencing.

Results: Amongst 190 patients, 107 were newly referred undiagnosed patients. Amongst these a genetic diagnosis was reached in 46 cases (43%): 29 (63%) by NGS and 17 (37%) by repeat expansion analysis. Diagnostic rate reached 46% in CA, 31% in SPP and 32% in HMD. Diagnostic yield of NGS panels was 26% and of ES (singleton and trio) 33%. Diagnostic yield was higher among patients with younger onset of disease and isolated phenotypes. 29 patients had at least two brain MRI examinations

during their work-up and 24 patients completed non diagnostic CMA tests.

Conclusion: Our Neurogenetic clinic reports higher diagnostic yield compared with reported literature (20-30%), especially in patients with early-onset disease. ES increased diagnostic yield, compared with panel testing, especially amongst patients with complex phenotypes. Our findings indicate that performing a tailored genetic work-up fashion according to phenotype complexity and age of onset can reduce the need for auxiliary and molecular testing. Further cost-benefit analysis is required to decrease expenses and ensure timely diagnosis.

Grants:None

Conflict of Interest: None declared

P12

NEUROMUSCULAR DISORDERS

P12.001.D Whole-exome sequencing in the diagnostics of rare diseases - solving the mystery of a 12-year-old case

Natalie Friedova[1], *Jana Tajtlova*[1], *Renata Kejkulova*[1], *Robert Rusina*[2], *Martina Langova*[1]

[1]*Thomayer University Hospital, Department of Medical Genetics, Prague, Czech Republic;* [2]*Department of Neurology 3rd Faculty of Medicine Charles University and Thomayer University Hospital, Prague, Czech Republic*

Background/Objectives: The method of massively parallel sequencing at the level of whole-exome sequencing (WES) brings fundamental benefits in the diagnosis of the genetic cause of the disease in patients in whom previously used methods have failed and no clinically relevant result has been achieved.

We present a case of a 51-year-old man who was undergoing the diagnostic procedure since the age of 39 for ataxia and chorea with subsequent progression of cognitive impairment and movement disorder. MRI revealed progressive brain damage and cerebellar atrophy. Since 2011, our patient has been gradually investigated for suspected Huntington's disease, early-onset Alzheimer's disease, and related neurodegenerative disorders with negative test results.

Methods: Sequencing of whole-exome was performed as part of the SSELXT HS Human All Exon V8 + NCV (Agilent) sequencing on the NextSeq 550DX (Illumina) high-capacity sequencer. Bioinformatic analysis was performed by GENOVESA (Bioxys) by virtual panel (neurological diseases, 189 genes).

Results: By using a virtual panel of neurodegenerative diseases, a heterozygous pathogenic variant

the HGVS/HGVp: FIG4(NM_014845.6):c.447-2A>T p.?; dbSNP:rs866895236 (deep reads 104) was detected. Pathogenic variants in the *FIG4* gene (FIG4 Phosphoinositide 5-Phosphatase) are associated with amyotrophic lateral sclerosis 11 (OMIM 61257).

Conclusion: Whole-exome sequencing marked a breakthrough in the diagnostics of our patient. Whole exome sequencing has the potential to significantly shorten the diagnostic procedures, leading to the successful diagnosis of rare genetic conditions.

Grants: Supported by MH CZ – DRO (Thomayer University Hospital – TUH, 00064190)

Conflict of Interest: Natalie Friedova MH-CZ - DRO (Thomayer University Hospital - TUH, 00064190), Thomayer University Hospital First Faculty of Medicine, Charles University and the General Teaching Hospital, Charles University in Prague, Jana Tajtlova MH-CZ - DRO (Thomayer University Hospital - TUH, 00064190), Thomayer University Hospital, Renata Kejkulova MH-CZ - DRO (Thomayer University Hospital - TUH, 00064190), Thomayer University Hospital, Robert Rusina MH-CZ - DRO (Thomayer University Hospital - TUH, 00064190), Thomayer University Hospital Charles University in Prague General Faculty Hospital in Prague, ERN - neurological diseases Members Executive Board - Czech Neurological Society, Martina Langova MH-CZ - DRO (Thomayer University Hospital - TUH, 00064190), Thomayer University Hospital Charles University in Prague

P12.002.A Genome-wide meta-analysis provides novel insights into the polygenic architecture of myasthenia gravis

Alice Braun[1], *Stephan Ripke*[1], *Frauke Stascheit*[1], *Andreas Meisel*[1]

[1]*Charité – Universitätsmedizin Berlin, Berlin, Germany*

Consortium: 23andMe Research Team

Background/Objectives: Myasthenia gravis (MG) is a rare autoantibody-mediated disease of the neuromuscular junction that is characterized by muscle weakness and fatigue. We present a comprehensive genetic analysis of MG utilizing data from 12 cohorts and a replication sample provided by 23andMe, Inc.

Methods: We conducted discovery and replication case-control genome-wide association study, association analyses of imputed human leukocyte antigen (HLA), polygenic risk scoring (PRS), and genetic correlation analyses through linkage disequilibrium score regression. The discovery GWAS meta-analysis included 5,708 patients with clinically diagnosed MG and 432,028 control subjects. The results were replicated and meta-analyzed with a sample of 3,989 self-reported MG cases and 226,643 controls.

Results: The GWAS identified 12 genome-wide significant ($P < 5e^{-8}$) loci. Association analyses of imputed HLA alleles highlighted inverse effect sizes for late- (aged ≥50) and early-onset (aged <50), suggesting a potential modulatory influence on the time of disease manifestation. This study is the first to assess the performance of PRS for MG, which significantly predicted disease status in an independent unstratified target cohort, explaining 4.21% of the phenotypic variation ($P = 5.12e^{-9}$). We identified five significant genetic correlations with MG, with the strongest observed for type 1 diabetes ($r_g = 0.523$, $P = 1.42e^{-6}$, SE = 0.109) and rheumatoid arthritis ($r_g = 0.508$, $P = 1.11e^{-6}$, SE = 0.104).

Conclusion: The discovery and replication meta-analyses identified six novel associations and validated six known loci, enhancing our understanding of the genetic architecture of MG. Furthermore, polygenic risk scores effectively predicted MG in an independent cohort, presenting a promising addition to existing diagnostic and prognostic procedures.

Grants: German Research Foundation grant number 461427996

Conflict of Interest: None declared

P12.003.B Identification of deep intronic mutations in previously unsolved Duchenne and Becker muscular dystrophy cases through whole genome sequencing

Natalie Pluta[1], *Uri Hamiel*[2], *Bar Levy*[2], *Alina Kurolap*[2], *Hagit Baris Feldman*[2], *Heike Kölbel*[3], *Friedrich Stock*[4], *Elke Hobbiebrunken*[5], *Astrid Pechmann*[6], *Eberhard Gilberg*[7], *Juliane Lippert*[1], *Ann-Kathrin Zaum*[1], *Simone Rost*[1;8]

[1]*Institute of Humangenetics, University of Wuerzburg, Würzburg, Germany;* [2]*Genetics Institute and Genomics Center, Tel Aviv Sourasky Medical Center, affiliated with Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel;* [3]*Department of Paediatric Neurology, Center for Neuromuscular Disorders in Children and Adolescents, University Clinic Essen, University of Duisburg-Essen, Essen, Germany;* [4]*Institute of Human Genetics, University Clinic Essen, Essen, Germany;* [5]*Devision of Neuropaediatrics, Department of Paediatrics and Adolescent Medicine, University Medical Centre Goettingen,*

Göttingen, Germany; [6]Department of Neuropediatrics and Muscle Disorders, Medical Center-University of Freiburg, Faculty of Medicine, University of Freiburg, Freiburg, Germany; [7]Department of Human Genetics, University Medicine Greifswald, and Interfaculty Institute of Genetics and Functional Genomics, University of Greifswald, Greifswald, Germany; [8]MGZ - Medical Genetics Center, München, Germany

Background/Objectives: Duchenne and Becker Muscular Dystrophy (DMD/BMD) are genetic disorders characterized by progressive muscle weakness, primarily caused by mutations in the *DMD* gene. Despite advances in genetic diagnostics, approximately 2-7%, of DMD/BMD cases remain genetically unsolved. The *DMD* gene consists of 99% intronic sequences, which emphasizes the importance of exploring deep intronic regions for the identification of new variants.

Methods: In this study, four cases are presented in which the causative variant could not be identified by conventional genetic analysis. All four patients have muscle weakness, elevated CK values (>10,000 U/l) and altered dystrophin in the muscle biopsy.

Results: Using whole genome sequencing, we were able to identify the four different causative intronic variants in the mentioned cases: two known variants in intron 55 and 61, and two novel variants in intron 15 and 53, respectively. These deep intronic variants in the *DMD* gene were previously missed by conventional sequencing methods.

Conclusion: The identification of these variants not only resolves the genetic ambiguity in these cases, but also emphasizes the importance of going beyond exonic sequences to understand the genetic basis of DMD/BMD. Our findings help to close the gap in the genetic characterization of unsolved DMD/BMD cases, paving the way for more accurate diagnosis and potentially treatments. This study highlights the need for further exploration of deep intronic regions to improve diagnostic yield in genetically complex diseases such as DMD.

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Conflict of Interest: None declared

P12.004.C Diagnostic capabilities of phenotype-driven analysis of trio genome sequencing data from 58 unsolved children with neuromuscular diseases

Berta Estevez-Arias[1;2], *Leslie Matalonga[3], Delia Yubero[4;5], Kiran Polavarapu[6], anna codina[7;8], Carlos Ortez[1;4;7], Laura Carrera-García[1;7], Jessica Expósito-Escudero[1;7], Cristina Jou[4;7;8], Stefanie Meyer[6;9], Özge Aksel-Kiliçarslan[6], Alberto Aleman[6], Rachel Thompson[6], Rebeka Luknárová[10], Anna Esteve-Codina[3], Steven Laurie[3], german demidov[11], Vicente Yépez[10], Sergi Beltran[3], Julien Gagneur[10;12;13], Ana Töpf[14], Hanns Lochmuller[3;6;15], Andrés Nascimento[1;4;7], Janet Hoenicka[2;4], Francesc Palau[2;4;5], Daniel Natera-de Benito[1;4;7]*

[1]Hospital Sant Joan de Déu, Neuromuscular Unit, Department of Neurology, Barcelona, Spain; [2]Sant Joan de Déu Research Institut, Laboratory of Neurogenetics and Molecular Medicine – IPER, Barcelona, Spain; [3]Centro Nacional de Análisis Genómico (CNAG), Barcelona, Spain; [4]Center for Biomedical Research Network on Rare Diseases (CIBERER), ISCIII, Spain; [5]Sant Joan de Déu Hospital and Sant Joan de Déu Research Institut, Department of Genetic and Molecular Medicine – IPER, Barcelona, Spain; [6]Children's Hospital of Eastern Ontario Research Institute, Ottawa, Canada; [7]Sant Joan de Déu Research Institut, Applied Research in Neuromuscular Diseases, Barcelona, Spain; [8]Hospital Sant Joan de Déu, Department of Pathology, Barcelona, Spain; [9]University Medical Center Göttingen, Georg-August University, Department of Neurology, Göttingen, Germany; [10]School of Computation, Information and Technology, Technical University of Munich, Munich, Germany; [11]Institute of Medical Genetics and Applied Genomics, University of Tübingen, Tübingen, Germany; [12]Computational Health Center, Helmholtz Center Munich, Neuherberg, Germany; [13]Institute of Human Genetics, School of Medicine, Technical University of Munich, Munich, Germany; [14]The John Walton Muscular Dystrophy Research Centre, Translational and Clinical Research Intitute, Newcastle University and Newcastle Hospitals NHS Foundation Trust, Newcastle, United Kingdom; [15]The Ottawa Hospital, Division of Neurology, Department of Medicine, Ottawa, Canada

Background/Objectives: Neuromuscular diseases (NMD) constitute a diverse spectrum of disorders with a collective global prevalence of 100-200 cases per 100,000 individuals. The integration of next-generation sequencing into clinical practice has revolutionized their genetic diagnosis and has taken the lead over neurophysiological and histopathological tests. However, a large percentage of patients remain undiagnosed (50%).

Methods: We have assessed the usefulness of combining a standardized high-quality phenotyping with trio genome sequencing, and RNASeq if muscle biopsy was available (13 samples), to elevate the diagnostic rate for 58 individuals with unsolved childhood-onset NMDs that had previously undergone inconclusive exome studies.

Results: A genetic diagnosis was established in 23 out of 58 index patients (40%) and in two additional families we identified candidate variants whose pathogenicity is still awaiting confirmation. SNVs or smalls indels were identified as causative in 22 of the 23 solved cases, while a complex structural variant in *PLEKHG5* explained the condition in the remaining case.

Interestingly, the trio analysis uncovered a monoallelic *RYR1* variant in an asymptomatic mother and her offspring affected by congenital myopathy, proven to be in mosaic in the mother. Furthermore, in another patient with a congenital myopathy, the combination of RNASeq and genome sequencing let us identify a pseudoexon in the *NEB* gene and the causative deep-intronic variant.

Conclusion: With these examples, we illustrate how our genetic analysis approach proved to be effective to end the diagnostic odyssey of 23 patients.

Grants: The Solve-RD project has received funding from the European Union's Horizon 2020 research and innovation programme (No 779257).

Conflict of Interest: None declared

P12.005.D Large-scale Proteomics Profiling of Peripheral Blood of DM1 patients identifies biomarkers for disease severity and physical activity

Daniel Van As[1;2], Tine Claeys[3], Renee Salz[1], Ralf Gabriels[3], Vera Dobelmann[4], Francis Impens[3], Pieter-Jan Volders[3], Andreas Roos[5], Elise Duchesne[6], Cynthia Gagnon[7], Lennart Martens[3], Baziel van Engelen[2], **Peter-Bram t Hoen**[1]

[1]Radboud university medical center, Medical BioSciences, Nijmegen, Netherlands; [2]Radboud university medical center, Neurology, Nijmegen, Netherlands; [3]Ghent University, Ghent, Belgium; [4]University of Duesseldorf, Duesseldorf, Germany; [5]University of Essen, Essen, Germany; [6]University of Quebec, Chicoutimi, Canada; [7]University of Sherbrooke, Sherbrooke, Canada

Consortium: The OPTIMISTIC Consortium, the ReCognitION consortium

Background: Myotonic Dystrophy type 1 (DM1), caused by CTG repeat expansions in *DMPK*, is a progressive multisystem disease with large heterogeneity in disease onset, severity and

progression. This study aims to identify proteomic biomarkers that can serve as surrogate endpoints in clinical trials.

Methods: We profiled 450 peripheral blood samples from adult DM1 patients participating in the clinically well-characterised OPTIMISTIC trial cohort, using bottom-up mass spectrometry-based proteomics. Blood samples of an external cohort of 57 DM1 patients were used to validate the negative associations of ITIH3 expression with clinical measures of physical activity.

Results: We observed 165 protein groups significantly associated with CTG-repeat length and 73 with physical activity, as reflected by the 6-minutes-walk-test (6MWT) score. Severity biomarkers were associated with hypogammaglobulinemia, whereas reduced physical activity was mostly associated with increased levels of complement factors and the complement-associated factor ITIH3 (Rho -0.3, $p < 0.01$). The association between ITIH3 and physical activity has been confirmed in an external cohort, where similar negative associations were observed with the 6MWT score (Rho -0.2, $p = 0.19$) and grip strength (Rho -0.43, $p < 0.01$). Multivariate Elastic-Net analysis resulted in the selection of 10 proteins that explain up to 31% of the variance in CTG-repeat length and 27% in 6MWT scores.

Conclusion: DM1 induces a broad disease fingerprint in the peripheral blood proteome. We suggest a panel of carefully selected proteins for monitoring disease severity that deserves validation in longitudinal studies spanning larger time frames than the OPTIMISTIC study.

Grants: This study was funded by e-RARE JTC2018 grant N°643578.

Conflict of Interest: None declared

P12.006.A Genetic and epigenetic profiling for FSHD diagnosis by nanopore sequencing

Florentine Scharf[1], *Hannes Erdmann*[1;2], *Morghan Lucas*[1;3], *Stefanie Gehling*[1], *Ariane Hallermayr*[1;3], *Anna Benet-Pages*[1;4], *Sibylle Jakubiczka*[5], *Martin Zenker*[5], *Annika Saak*[2], *Jochen Schaefer*[6], *Udo Koehler*[1], *Teresa Neuhann*[1], *Elke Holinski-Feder*[1;3], *Maggie C. Walter*[2], *Benedikt Schoser*[2], *Angela Abicht*[1;2]

[1]*MGZ - Medical Genetics Center, München, Germany;* [2]*Friedrich Baur Institute at the Department of Neurology, LMU University Hospital, Ludwig-Maximilians-Universität, München, Germany;* [3]*Department of Medicine IV, LMU University Hospital, Ludwig-Maximilians-Universität, Munich, Germany;* [4]*Institute of Neurogenomics, Helmholtz Research Center, Neuherberg, Germany;* [5]*Institute of Human Genetics, University Hospital Magdeburg, Magdeburg, Germany;* [6]*Department of Neurology, University Hospital Carl Gustav Carus, Dresden, Germany*

Background/Objectives: The genetic diagnosis of facioscapulohumeral muscular dystrophy (FSHD) poses a significant clinical challenge. Conventional diagnostic strategies address the required presence of a permissive haplotype, a contraction of the D4Z4 repeat of chromosome 4q35 (FSHD1), or a pathogenic variant in an epigenetic suppressor gene (FSHD2). However, it was recently shown that methylation serves as a reliable marker of disease severity, given an exact assignment of haplotype, which to date remains challenging.

Methods: To circumvent the known limitations of conventional diagnostics we leverage Oxford Nanopore Technologies (ONT) targeted long-read sequencing to simultaneously characterize haplotypes, number of repeat units, methylation profiles, single nucleotide and structural variants, hybrid alleles and somatic mosaicism within a single sequencing run.

Results: As proof of concept, we were able to clarify the unexpected high average distal methylation in a paucisymptomatic patient homozyguous for 4qA, harboring a repeat contraction with two repeat units on one allele. The ONT data revealed the presence of the contraction as a mosaic, causing an increase in the average percent methylation. In another patient, resolving a complex hybrid allele revealed the presence of a hypermethylated rare 4qC166H haplotype, which makes FSHD unlikely as reason for the patient's symptoms.

Conclusion: ONTs ability to provide long reads and base modification data allows the characterization of genetic parameters involved in development of FSHD in its entirety. The methylation data can unambiguously be assigned to single alleles. This approach not only enables a more precise diagnostic but will help answer yet unresolved questions regarding the pathomechanism of this highly complex disease.

Grants: N/A

Conflict of Interest: None declared

P12.007.B Soft tissue sarcoma in a patient with limb-girdle muscular dystrophy

Carolina Maya Gonzalez[1], *Fulya Taylan*[1;2], *Bianca Tesi*[1;2;3], *Sandra Wessman*[4;5], *Teresita Diaz de Stahl*[4;5], *Kristina Lagerstedt-Robinson*[1;2], *Milena Dukic*[6;7], *Gustaf Ljungman*[8], *Giorgio Tettamanti*[1;9], *Anna Poluha*[6;10], *Ann Nordgren*[1;11;12]

[1]*Karolinska Institutet, Department of Molecular Medicine and Surgery, Centre for Molecular Medicine, Stockholm, Sweden;* [2]*Karolinska University Hospital, Department of Clinical Genetics and Genomics, Stockholm, Sweden;* [3]*Karolinska Institutet, Department of Medicine Huddinge, Stockholm, Sweden;* [4]*Karolinska Institutet, Department of Oncology-Pathology, Stockholm, Sweden;* [5]*Karolinska University Hospital, Department of Pathology and Cancer Diagnostics, Stockholm, Sweden;* [6]*Uppsala University, Department of Immunology, Genetics and Pathology, Uppsala, Sweden;* [7]*Uppsala University Hospital, Department of Clinical Pathology, Uppsala, Sweden;* [8]*Uppsala University, Pediatric Oncology, Department of Women's and Children's Health, Uppsala, Sweden;* [9]*Karolinska Institutet, Institute of Environmental Medicine, Unit of Epidemiology, Stockholm, Sweden;* [10]*Uppsala University Hospital, Clinical Genetics, Uppsala, Sweden;* [11]*Sahlgrenska University Hospital, Department of Clinical Genetics and Genomics, Gothenburg, Sweden;* [12]*University of Gothenburg, Institute of Biomedicine, Department of Laboratory Medicine, Gothenburg, Sweden*

Background/Objectives: Limb-girdle muscular dystrophy recessive 1 (LGMDR1) is a rare neuromuscular disorder caused by biallelic pathogenic *CAPN3* variants. The prevalence of cancer in patients with LGMDR1 is uncertain. Here, we present the clinical findings of a 17-year-old patient with LGMDR1 who developed a Soft Tissue Sarcoma (STS). Additionally, we conducted a literature review on muscular dystrophies (MD) and cancer.

Methods: As part of the nationwide Genomic Medicine Sweden Childhood Cancer project, the patient was offered paired germline-tumor whole genome DNA sequencing and tumor RNA-sequencing. A PubMed literature review on cancer in MD was performed.

Results: In the patient, identification of two germline biallelic pathogenic *CAPN3* variants (NP_000061.1:p.(Arg448Cys) and NP_000061.1:p.(Thr184ArgfsTer36)), confirmed the LGMDR1 diagnosis. STS analyses led to the diagnosis of a desmoplastic small round cell tumor with near-triploid karyotype and multiple numerical/segmental chromosomal aberrations. RNA-sequencing showed a EWSR1::WT1 fusion transcript. No germline variants in childhood cancer predisposition genes were detected.

MD mice are susceptible to STS development. In our literature review including 66 patients with MD and cancer, we observed an overrepresentation of STS in Duchenne muscular dystrophy (DMD,

10/24 patients) and LGMD (2/8 patients) and an early age at cancer onset amongst reported MD cases (average 31.3 years).

Conclusion: We describe the first patient with LGMDR1 who developed a STS. Our literature review revealed an early age at cancer onset in reported patients with MD, with an over-representation of STS in DMD and LGMD. Further studies involving population-based cohorts addressing the association between LGMDR1 and cancer are warranted.

Grants: The Swedish Childhood cancer fund.

Conflict of Interest: None declared

P12.008.C Autosomal dominant tibial muscular dystrophy in Estonia

Siiri Sarv[1], *Tiia Reimand*[1;2], *Eve Õiglane-Shlik*[1;2], *Sanna Puusepp*[2], *Lisanna Põlluaas*[1], *Laura Mihkla*[2], *Sander Pajusalu*[1;2], *Sandra Ütt*[3], *Katrin Gross-Paju*[3], *Tiina Kahre*[1;3], *Marco Savarese*[4;5], *Peter Hackman*[4;5], *Bjarne Udd*[4;5;6], *Katrin Ounap*[1;2]

[1]*University of Tartu, Estonia;* [2]*Tartu University Hospital, Estonia;* [3]*West Tallinn Central Hospital, Estonia;* [4]*Folkhälsan Research Center, Helsinki, Finland;* [5]*University of Helsinki, Helsinki, Finland;* [6]*Tampere University Hospital, Finland*

Background/Objectives: Tibial muscular dystrophy (TMD; MIM#600334, ORPHA:609) is an adult onset distal myopathy that was first described by Udd et al in Finnish patients. An 11-bp insertion/deletion in the last exon (exon 364) of the *TTN* gene is the Finnish founder variant (FINmaj). In heterozygous state, FINmaj causes autosomal dominant (AD) TMD with slow progression affecting preferentially the tibialis anterior muscle. To date, FINmaj variant is not described outside the Finnish population. After identification of a first family, this study aimed to retrospectively find more TMD patients and to estimate the FINmaj frequency in the Estonian population.

Methods: We retrospectively analyzed all large gene panel and whole exome sequencing data from 2011-2021 to find FINmaj carriers in Genetics and Personalized Medicine Clinic (GPMC) cohort (n = 4632) and in the cohort of unsolved myopathy/neuropathy patients in West Tallinn Central Hospital cohort (n = 52). Magnetic resonance imaging (MRI) was performed on seven FINmaj patients.

Results: We found four families with 16 patients who carry the heterozygous FINmaj variant: two patients with autosomal recessive limb-girdle muscular dystrophy type LGMD R10 and 14 patients with AD TMD. All the TMD patients in the cohort carried the FINmaj variant and had clinical symptoms by the age of 50 years. MRI analysis showed TMD-specific findings in >50yo patients. FINmaj variant incidence in the Estonian GPMC cohort is 1/1500. Haplotype analysis is at work.

Conclusion: AD TMD is the most common hereditary muscle disease in Estonia and is still underdiagnosed.

Grants: EJP-RD IDOLS-G, PRG471, PRG2040.

Conflict of Interest: None declared

P12.009.D Clinical characterization of a cohort of female carriers of X-linked myotubular myopathy (XL-MTM) in Germany – extending the phenotypic spectrum and knowledge about the impact of heterozygous variants in the *MTM1* gene

Frederik Braun[1;2], *Frank J. Kaiser*[2], *Ulrike Schara-Schmidt*[1]

[1]*Centre for Neuromuscular Disorders, Centre for Translational Neuro- and Behavioral Sciences, University Duisburg-Essen, Department of Pediatric Neurology, Essen, Germany;* [2]*Institute of Human Genetics, University Hospital Essen, University Duisburg-Essen, Essen, Germany*

Background/Objectives: The mode of inheritance of X-linked myotubular myopathy (XL-MTM) is still considered recessive, although female carriers are frequently symptomatic. We aimed to characterize the phenotypic spectrum and burden of disease in a carrier cohort.

Methods: We performed a cross-sectional study among XL-MTM carriers in Germany, recruiting predominantly through a patient association. We used a custom-made questionnaire on medical and family history, the Checklist Individual Strength (CIS20-R), Short Form 12 (SF-12) health survey, Pain Detect, Short-Form McGill Pain Questionnaire (SF-MPQ-D), Graded Chronic Pain Scale (GCPS), and Depression Anxiety Stress Scale (DASS21). All participants received a neurologic examination including manual muscle testing (MMT), a 6-minute walking test (6MWT), and timed up-and-go (TUG) test. Where possible, we performed ultrasound of the abdomen and vastus lateralis muscles, echocardiography, pulmonary function testing, blood count, and further laboratory testing. Carriers were classified as manifesting based on the degree of muscular weakness and ambulatory status.

Results: In our study population (n = 14), most carriers were classified as manifesting with a mild (n = 8; independent ambulation, with limb or axial weakness) or minimal phenotype (n = 3; only facial muscle weakness). We consider only the minority non-manifesting (n = 3). Frequent features include a high-arched palate, fatigue, muscle pain and stiffness, and pulmonary problems. Many carriers are caregivers of boys with XL-MTM (n = 11). Families are burdened with stillbirth and death in early childhood (n = 8).

Conclusion: The majority of female carriers of XL-MTM show neuromuscular and additional symptoms. This should be considered in diagnostics, clinical care, and future clinical trials.

Grants: DFG FU 356/12-2

Conflict of Interest: Frederik Braun This work was supported by the DFG in the framework of the DFG Clinician Scientist Programme UMEA, FU 356/12-2, Frank J. Kaiser: None declared, Ulrike Schara-Schmidt: None declared

P12.010.A Novel deep intronic variant in the CAPN3 gene in trans with pathogenic variant as a cause of calpainopathy

Tihomir Todorov[1], *Slavena Atemin*[1], *Mila Sleptsova*[1], *Stoyan Bichev*[2], *Alexey Savov*[2], *Albena Todorova*[1;3]

[1]*Genetic Medico-Diagnostic Laboratory "Genica", Sofia, Bulgaria;* [2]*University hospital of Obstetrics and Gynecology, National Genetics Laboratory, Sofia, Bulgaria;* [3]*Medical University of Sofia, Department of Medical Chemistry and Biochemistry, Sofia, Bulgaria*

Background/Objectives: Patient with muscular dystrophy was referred for molecular-genetic analysis in order to identify the genetic reason for the observed phenotype. Based on whole exome sequencing (WES) a heterozygous pathogenic variant c.598_612del in the *CAPN3* gene was detected. Segregation analysis in the family showed that the variant was inherited from the patient's healthy father. Therefore autosomal-dominant model of inheritance was excluded. Additional molecular-genetic analyses were performed to determine the genetic etiology.

Methods: WES was performed and a panel of genes related to muscular dystrophies was analyzed. Variants flagged through the analysis were confirmed and segregated in the patient's family via Sanger sequencing. MLPA analysis for deletions/duplications along the *CAPN3* gene was performed.

Results: MLPA analysis did not show aberrations along the *CAPN3* gene. The analysis of WES data did not result in finding a potentially pathogenic variant in any of the other analyzed genes. Further analysis of the WES data revealed a novel intronic variant c.1914+309C>T in the *CAPN3* gene. Segregation analysis

confirmed maternal inheritance of the detected variant and hence both *CAPN3* variants are localized in trans.

Conclusion: Based on the performed analysis two biallelic variants in the *CAPN3* gene were detected. This suggests autosomal-recessive mode of inheritance. Muscular biopsy revealed lack of 3 calpain isoforms which supports pathogenic nature of the detected *CAPN3* genetic variants and confirms the genetic diagnosis of calpainopathy in the patient.

Grants: No

Conflict of Interest: None declared

P12.011.B Unexpected cause of spastic ataxia

Martina Langova[1;2], *Natalie Friedova*[1;3], *Jana Tajtlova*[1], *Renata Kejkulova*[1], *Magdalena Vitkova*[1], *Antonin Sipek Jr*[1;2;3]

[1]*Thomayer University Hospital, Department of Medical Genetics, Prague, Czech Republic;* [2]*Third Faculty of Medicine, Charles University in Prague, Department of Medical Genetics, Prague, Czech Republic;* [3]*Institute of Biology and Medical Genetics, First Faculty of Medicine, Charles University and General University Hospital, Prague, Czech Republic*

Background/Objectives: Whole-exome sequencing (WES) has in recent years significantly expanded the range of diagnostic possibilities. WES helped to reveal the cause of difficulties in patients with so far unresolved diagnoses.

A 43-year-old woman was consulted for slowly progressive mobility impairment with associated spasticity and ataxia with the early onset of symptoms. The initial symptoms (walking and coordination disability) had occurred in early childhood. The latest MRI showed mild brain atrophy, and EMG revealed severe demyelination polyneuropathy.

Methods: Sequencing of whole-exome was performed as part of the SSELXT HS Human All Exon V8 + NCV (Agilent) sequencing on the NextSeq 550DX (Illumina) high-capacity sequencer. Bioinformatic analysis was performed by GENOVESA (Bioxys) by virtual panel (hereditary spastic paresis).

Results: Using WES were detected one pathogenic and two likely pathogenic heterozygous variants in gene *SACS*.

SACS(NM_014363.6):c.12150_12156del p.Glu4051PhefsTer5); dbSNP: - (deep reads 39)

SACS(NM_014363.6):c.12158_12159del p.(Leu4053ProfsTer7); dbSNP: - (deep reads 44)

SACS(NM_014363.6):c.4020T>A p.(Tyr1340Ter) dbSNP: - (deep reads 44)

All of them were confirmed by Sanger sequencing.

Conclusion: Conclusion: Pathogenic variants in the *SACS* (Sacsin Molecular Chaperone) gene are associated with spastic ataxia Charlevoix-Saguenay type (ARSACS; OMIM: 270550) with autosomal recessive inheritance. The symptoms are starting in the age of 12-18 months. Our patient was referred to the specialized center for hereditary ataxia for further treatment. Thanks to WES, it was possible to achieve a diagnostic result in a patient with a long diagnostic odyssey.

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Conflict of Interest: Martina Langova Supported by MH CZ - DRO (Thomayer University Hospital - TUH, 00064190), Thomayer University Hospital Charles University in Prague, Natalie Friedova Supported by MH CZ - DRO (Thomayer University Hospital - TUH, 00064190), Thomayer University Hospital General University Hospital Charles University in Prague, Jana Tajtlova Supported by MH CZ - DRO (Thomayer University Hospital - TUH, 00064190), Thomayer University Hospital, Renata Kejkulova Supported by MH CZ - DRO (Thomayer University Hospital - TUH, 00064190), Thomayer University Hospital, Magdalena Vitkova Supported by MH CZ - DRO (Thomayer University Hospital - TUH, 00064190), Thomayer University Prague, Antonin Sipek Jr Thomayer University Hospital General University Hospital in Prague Charles University

P12.012.C 5q spinal muscular atrophy epidemiology in Russia: insights from a large-scale pilot project involving 202,908 newborns.

Irina Efimova[1], *Andrey Marakhonov*[1], *Natalya Balinova*[1], *Kristina Grishina*[1], *Olga Schagina*[1], *Alexander Polyakov*[1], *Sergey Voronin*[1], *Rena Zinchenko*[1], *Sergey Kutsev*[1]

[1]*Research Centre for Medical Genetics, Moscow, Russia;, Moscow, Russian Federation*

Background/Objectives: Spinal muscular atrophy (SMA) is the one the most common neurodegenerative disease in childhood. We report here the results of a pilot project of newborn screening (NBS) for SMA5q in 8 regions across Russia for 2022 year. The objective was to assess the viability, consistency, and utility of (NBS) for SMA5q across diverse populations.

Methods: A real-time PCR kit, the Eonis SCID-SMA, was used for NBS. Verification of the homozygous *SMN1* deletion was performed using MLPA.

Results: 26 patients with SMA were identified. Birth prevalence appeared to be 1:7804 newborns (95%CI:5263-12500). 38.46% (95%CI:20.23-59.43%) had two SMN2 copies, 42.31% (95%CI:23.35-63.08%) had three copies, 15.38% (95%CI:4.36-34.87%) had four copies, and 3.85% (95%CI:0.10-19.64%) had five copies of SMN2.

All infants possessing 2-3 copies of SMN2 underwent immediate treatment. Newborns with 4 and 5 copies followed a "watchful waiting" strategy and received treatment upon the emergence of clinical symptoms, with two manifesting symptoms at 3 months of age. Among those treated, the significant majority (82%, 18/22) underwent gene therapy. Subsequent short-term monitoring post-gene therapy demonstrated improvements in motor function.

Conclusion: The study underscores the necessity for a standardized algorithm facilitating early diagnosis and management through NBS, thereby providing substantial benefits to affected families. Future research should focus on long-term outcomes and evolving medical needs of NBS-diagnosed children. Overall, our pilot project experience let to expansion of our SMA5q NBS to the whole of Russia and reduce the time interval between a screening result and prompt SMA-specific treatment.

Grants: Novartis Pharma LLC, Skopinfarm LLC.

Conflict of Interest: None declared

P12.013.D Clinical Laboratory Experience of Frataxin Quantification in Blood for the Diagnosis of Friedreich Ataxia

Iris Pantovich[1;2], *Amy White*[2], *April Studinski*[2], *Weiyi Mu*[3], *Bonnie Kaas*[4], *Matthew Bower*[5], *Gisele Bentz Pino*[2], *Dawn Peck*[2], *Kyle Salsbery*[2], *Emily Lauer*[2], *Angela Pickart*[2], *Kandelaria Rumilla*[2], *Wei Shen*[2], *Zhiyv Niu*[2], *Patricia Hall*[2], *Matthew Schultz*[2], *Dimitar Gavrilov*[2], *Silvia Tortorelli*[2], *Dietrich Matern*[2], *Ralitza Gavrilova*[6], **Devin Oglesbee**[2]

[1]*University of Minnesota, Genetic Counseling Program, Minneapolis, United States;* [2]*Mayo Clinic, Laboratory Medicine and Pathology, Rochester, United States;* [3]*Johns Hopkins University, Genetic Medicine, Baltimore, United States;* [4]*Ascension Medical Group, Neurology, Baltimore, United States;* [5]*M Health Fairview University of Minnesota Medical Center, Genetic Counseling, Minneapolis, United States;* [6]*Mayo Clinic, Neurology, Rochester, United States*

Background/Objectives: Friedreich ataxia (FA) is characterized by progressive spasticity, ataxia, dysarthria, and hypertrophic cardiomyopathy. FA typically presents in childhood but late-onset forms can complicate a diagnosis. Homozygous intron 1 GAA-repeat expansions in *FXN* occur in 98% of cases, reducing frataxin levels, and causing disease. A rapid diagnosis can expedite omaveloxolone treatment and slow disease progression. Our laboratory has offered frataxin levels for rapid FA screening and diagnosis.

Methods: From 2011 to 2023, 1,126 blood samples were sent for frataxin analysis. Quantitation used a published method (Oglesbee et al. Clin Chem. 2013). Final diagnostic outcomes were obtained from 2011-2023 by chart review or external clinician contact from 2018-2023.

Results: Frataxin levels (< 12 ng/mL) consistent with FA were observed in 15.6% patients, while 5.8% had results overlapping possible carrier status. The remaining cohort (78.6%) demonstrated normal frataxin levels (21-74 ng/mL). Among intermediate frataxin levels (12-21 ng/mL), molecular testing for 12/65 resulted in a diagnosis for seven patients, carrier status for three individuals, and two patients with negative molecular results. Five cases are highlighted including two siblings, 10 and 13-years-old, with low frataxin levels, but atypical GAA repeat results; a 30-year-old with late-onset FA and GAA repeats (418 and 418 repeats); a 44-year-old with a neurological presentation, but a single expansion allele (>900 repeats) and frataxin of 23 ng/mL; and a patient presenting in their mid-50's.

Conclusion: Early detection of FA is important to initiate treatment to slow disease progression and frataxin concentrations are useful for rapid FA screening.

Grants: None

Conflict of Interest: None declared

P12.015.B Whole genome sequencing reveals compound heterozygous *RYR1* variants in a patient with severe congenital myopathy: case report and comparison with additional cases of recessive *RYR1*-related myopathy

Sören Janßen[1], Leoni Erbe[2], Moritz Kneifel[3], Matthias Vorgerd[3], Kristina Döring[2], Krzysztof P Lubieniecki[2], Joanna Lubieniecka[2], Wanda Gerding[2], Nicolas Casadei[4], Thomas Luecke[1], Huu Phuc Nguyen[2], Cornelia Köhler[1], **Sabine Hoffjan**[2]

[1]University Children`s Hospital Bochum, Department of Neuropediatrics, Bochum; [2]Ruhr-University Bochum, Department of Human Genetics, Bochum; [3]University Hospital Bergmannsheil, Department of Neurology, Heimer Institute for Muscle Research, Bochum; [4]University of Tübingen, Institute of Medical Genetics and Applied Genomics, Tübingen, Germany

Background/Objectives: *RYR1*-related myopathies comprise a wide spectrum of myopathic phenotypes, including malignant hyperthermia, central core or multiminicore myopathy and severe congenital myopathy. Inheritance can be autosomal dominant, more frequently in milder phenotypes, or autosomal recessive, mostly in severe congenital forms. However, due to the large gene and heterogeneous clinical presentation, genotype-phenotype correlations are challenging. Further, there is limited knowledge about intronic or structural variation not covered by exome analysis.

Methods: We present a 6-year-old patient from a non-consanguineous German family with severe congenital myopathy requiring a wheelchair and constant ventilation, who was known to carry a heterozygous pathogenic, presumably recessive *RYR1* variant transmitted from the healthy mother. No other pathogenic variant in *RYR1* or another myopathy gene was evident from exome analysis. In this patient we performed optical genome mapping (OGM) and whole genome sequencing (WGS) to clarify the genetic cause of his myopathy.

Results: OGM did not reveal structural variants affecting myopathy-related genes. However, WGS identified an additional heterozygous intronic variant (c.10441-48G>A) that has recently been described in compound heterozygous state in another patient with severe congenital myopathy and shown to affect splicing. Segregation analysis confirmed the two *RYR1* variants to be compound heterozygous.

Conclusion: WGS confirmed the diagnosis of a *RYR1*-related myopathy in our patient. We compare his clinical symptoms to the patient with the same intronic variant from the literature as well as other patients with compound heterozygous *RYR1* variants from our muscle center.

Grants: This project was funded by the "Dr. Georg E. und Marianne Kosing-Stiftung"

Conflict of Interest: None declared

P12.016.C Molecular insights into Pompe Disease: a study of PPARgamma, COL13A1 and cellular glycogen metabolism

Sophie Uyttebroeck[1], *DT Laura Vo Ngoc[1], Randy Osei[1], Bart Dequeker[1], Sara Seneca[1], Frederik Hes[1], Alexander Gheldof[1]*

[1]Vrije Universiteit Brussel (VUB), Universitair Ziekenhuis Brussel (UZ Brussel), Clinical Sciences, Research group Genetics, Reproduction and Development, Centre for Medical Genetics, Jette, Belgium

Background/Objectives: Pompe disease, caused by bi-allelic pathogenic variants in the *GAA* gene leading to acid-alpha glucosidase enzyme deficiency and subsequent lysosomal glycogen accumulation, manifests predominantly with hypotonia and cardiomyopathy. The correlation between cellular glycogen accumulation and clinical phenotype in Pompe disease remains unclear.

Methods: Transcriptomic analysis of Pompe patients' fibroblasts versus healthy controls revealed downregulated COL13A1 mRNA levels and upregulated PPARG expression in patient cells. Promotor analysis identified two PPARG binding sites in the *COL13A1* gene promotor. Inhibiting PPARG by an siRNA-mediated knockdown led to COL13A1 upregulation. Experiments showed cytosolic glycogen depletion upregulating PPARG and COL13A1 in patient samples.

Results: COL13A1 encodes an atypical collagen crucial for neuromuscular junction (NMJ) function by stabilizing the acetycholine receptor clustering. PPARG is a transcription factor regulating energy metabolism. Our findings show an enhanced PPARG expression linked to cellular energy deficits. Additionally, PPARG influences the transcriptional regulation of COL13A1 expression to a certain extent, thereby linking glycogen accumulation in Pompe cells with the NMJ functionality. Upregulation of PPARG and COL13A1 post-glycogen depletion shifts focus from lysosomal to cytosolic glycogen in Pompe disease pathogenesis.

Conclusion: We show that COL13A1, an important mediator of the NMJ, is downregulated in Pompe patients' fibroblasts and is regulated by the transcription factor PPARG. Moreover, both COL13A1 and PPARG expression are regulated by cytosolic, rather than lysosomal glycogen. These findings uncover a potential novel disease mechanism in Pompe disease.

Conflict of Interest: None declared

P12.017.D A novel SGCE gene variant found in a Hungarian myoclonus-dystonia syndrome family

*Katalin Koczok[1], **Eszter Kovács**[1], László Madar[1], Gabriella Csorba[1], Judit Boczán[2], Istvan Balogh[2]*

[1]University of Debrecen, Faculty of Medicine, Division of Clinical Genetics, Debrecen, Hungary; [2]University of Debrecen, Faculty of Medicine, Department of Neurology, Debrecen, Hungary

Background/Objectives: Hereditary myoclonus was suspected in four siblings suffering from myoclonic jerks mainly affecting the neck and upper limbs. Pedigree analysis suggested autosomal dominant inheritance pattern with reduced penetrance on maternal transmission. After thorough neurologic examination they were referred to genetic counseling for further investigation.

Methods: Based on the clinical symptoms and inheritance pattern we performed single gene testing by Sanger sequencing of the coding region of the *SGCE* gene in one of the affected siblings (female proband). In the case of the three other affected (two females and one male) and seven unaffected family members (children of the three female patients) targeted mutational testing by Sanger sequencing was perfomed. Variant classification was based on the ACMG variant classification guideline.

Results: In the proband and all other affected siblings the c.874G>T, p.(Glu292*) nonsense variant was detected in heterozygous form in the *SGCE* gene. This novel genetic alteration was classified as pathogenic. The parents did not consent to testing, we assume paternal inheritance of the variant. Testing of the female patients' children revealed 4 mutation carriers and 3 non-carrier individuals. The male sibling has no offspring.

Conclusion: Genetic diagnosis of myoclonic dystonia-11 was established in the siblings by single gene testing, which still has a place in the era of next-generation sequencing if sound clinical examination and pedigree analysis suggests a specific condition. All of the carrier children (aged >7 years) were unaffected as expected since the maternally derived mutant allele generally (~95%) does not result in disease.

Conflict of Interest: None declared

P12.018.A Clarifying the etiology of cerebral palsy in pediatric patients by whole exome sequencing

Kristina Stefanova[1], *Nevyana Ivanova[1], Kalina Mihova[1], Genoveva Tacheva[2], Teodora Paneva[2], Ivan Litvinenko[2], Maya Koleva[3], Veneta Bojinova-Tchamova[3], Albena Jordanova[4], Ivanka Dimova[5], Radka Kaneva[1]*

[1]Medical University - Sofia, Molecular Medicine Center, Department of Medical Chemistry and Biochemistry, Sofia, Bulgaria; [2]Medical University - Sofia/SBAL "Prof.Ivan Mitev", Sofia, Bulgaria; [3]University Hospital for Neurology and Psychiatry "Sveti Naum", Sofia, Bulgaria; [4]VIB - UAntwerp, Center for Molecular Neurology, Antwerpen, Belgium; [5]Medical University - Sofia, Laboratory of Genome Diagnostics, Department of Medical Chemistry and Biochemistry, Sofia, Bulgaria

Background/Objectives: Cerebral palsy is a complex neurological condition that appears in infancy or early childhood and affects motor and developmental skills. It is generally accepted that it is not a genetic disease and occurs as a result of brain damage before, during birth or in early childhood. In many cases, it is difficult to distinguish cerebral palsy from the neurologic disorders with genetic origin; however, it has implications on the recurrence risk, therapeutic approach and prognosis. In this study we used whole exome sequencing (WES) to clarify the etiology of the disease in two patients with cerebral palsy.

Methods: WES was performed to look for potentially pathogenic variants in two pediatric patients with initial diagnosis of unspecified cerebral palsy. Sanger sequencing was used to confirm the variants and for segregation analysis in the families.

Results: WES-data analysis revealed disease-causing variants in 5 genes, including *CPLANE, COL1A1, RERE, AP1G1* which are associated with comparable symptoms. A known likely pathogenic variant in *KIDINS220* was found in one of the patients. This gene is associated with a complex form of spastic paraplegia, which is the most common differential diagnosis of cerebral palsy.

Conclusion: Our result showed that two pediatric patients have been misdiagnosed with cerebral palsy and that WES could be used as a robust method for differential diagnosis of the disease. Making a precise genetic diagnosis in these cases is crucial for prognosis, treatment, risk of recurrence and for informed reproductive decisions.

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Conflict of Interest: None declared

P12.019.B X-chromosome inactivation in women with dystrophinopathies: key to symptom manifestation or coincidental occurrence

Ekaterina Vorontsova[1], *Elena Zinina[1], Aleksander Polyakov[1], Sergei A. Kurbatov[2], Dariya Guseva[1], Aysylu Murtazina[1], Olga Schagina[1]*

[1]Research Centre for Medical Genetics, Moscow, Russian Federation; [2]Research Institute of Experimental Biology and Medicine Voronezh State Medical University named after N.N. Burdenko, Voronezh, Russian Federation

Background/Objectives: Dystrophinopathies are a spectrum of X-linked muscular disorders caused by pathogenic/likely pathogenic variants in the dystrophin gene (*DMD*). Symptoms of the disease have also been observed in women, which is often attributed to skewed X-inactivation.

Methods: The study group included 12 women with symptoms of muscular dystrophy and a confirmed pathogenic genetic variant in the *DMD* gene and control group of 16 healthy women. All DNA samples were extracted from blood. Quantitative methylation-sensitive fluorescent PCR (QF-PCR) was used to assess X-chromosome inactivation levels.

Results: The female patients included in our study had the following symptoms: decreased muscle tone, fatigue, muscle cramps and increased blood CK level (more than 500 U/L). All patients had pathogenic *DMD* variants: full exon and small deletions/insertion, nonsence mutations and splicing variants. A family history of the disease was traced in 4 (33%) patients, the other 8 patients were the only affected family. Unbalanced X-inactivation was detected in 17% of female with symptoms and *DMD* mutations. In the healthy control group, it was in 31% female.

Conclusion: According to the study, only a small number of women with *DMD* symptoms have unbalanced inactivation. The proportion of women with skewed X-chromosome inactivation does not differ from the proportion observed among controls. Thus, unbalanced inactivation may be only one of the many mechanisms of dystrophinopathies penetration in heterozygotes.

Conflict of Interest: None declared

P12.020.C Investigation of clinical and genetic heterogeneity in human fetuses with arthrogryposis multiplex congenita

Shravya M S[1], *Subrahmanya Vasishta[1], Akhila Vasudeva[2], Mary Mathew[3], Sujatha Jagadeesh[4], Beena Suresh[5], Jayarekha Raja[6], Apurv Barche[7], Prasanth Adiga[8], Vijay Kumar Shetty[9], Sheela Nampoothiri[10], Priya Ballal[11], Nanda Sundresh[12], Vivekananda Bhat[1],*

Shruti Bajaj[13], Malavika Hebbar[1], Radhakrishnan Periyasamy[1], Shambhavi Haspare[14], Sushma Sawkar[15], Shyamala Guruvare[16], Dhanya Lakshmi Narayanan[1], Anju Shukla[1], Katta Girisha[1], Shalini Nayak[1]

[1]Kasturba Medical College, Manipal, Medical Genetics, Manipal, India; [2]Kasturba Medical College, Manipal, Division of Fetal Medicine, Department of Obstetrics and Gynaecology, Manipal, India; [3]Kasturba Medical College, Manipal, Pathology, Manipal, India; [4]Mediscan systems, Department of Clinical Genetics and Genetic Counseling; [5]Mediscan Systems, Department of Clinical Genetics and Genetic Counseling, Chennai; [6]Mediscan Systems, 4Department of Clinical Genetics and Genetic Counseling, Chennai; [7]Kasturba Medical College, Manipal, Department of Pediatrics, Manipal, India; [8]Kasturba Medical College, Manipal, Department of Reproductive Medicine and Surgery, Manipal, India; [9]Nidhi Maternity Clinic, Lalith Hospital, Department of Obstetrics and Gynecology, Udupi; [10]Amrita Institute of Medical Sciences and Research Center, AIMS, Department of Pediatric genetics, Cochin; [11]Kasturba Medical College, Mangalore, Department of Obstetrics and Gynaecology, Mangalore; [12]Annapurna Hospital, Chikmangalur; [13]Sir H. N. Reliance Foundation Hospital and Research Centre, Department of Pediatrics, Mumbai; [14]Doctors Hospital, Kumbla; [15], Sri Sai Speciality Hospital, Haveri; [16]Kasturba Medical College, Manipal, Department of Obstetrics and Gynaecology, Manipal, India

Background/Objectives: Arthrogryposis is a condition with contractures in two or more joints associated with approximately 400 conditions. The study aims to elucidate the clinical and genomic profile of human fetuses with arthrogryposis

Methods: We performed perinatal evaluation of fetuses referred for autopsy following pregnancy loss and clinical evaluation in neonates after taking informed consent. Fetal tissues and blood samples were used to obtain genomic DNA. Chromosomal microarray (CMA), whole exome, and genome sequencing were performed as required. Spinal muscular atrophy (SMA) and common aneuploidies were ruled out whenever necessary.

Results: We have recruited 29 families (33 subjects; males -19 ; females- 14) with arthrogryposis in the study. Eleven consanguineous families (41%) were noted. Gestation ranged from 14-32 weeks and there were three neonatal losses (3-28 days). The study identified rare phenotypes associated with *ADGRG6*, *ANKS6*, *AGRN, CHRNA1*, *CEP55, GUSB*, *COASY, SYNE2, CEP290, PIGN and ASCC1*. Gene of uncertain significance, *KIF21A* and *UNC50* were identified. Biallelic loss of function variants in *KIF21A* caused severe arthrogryposis and brain anomalies in a family. A homozygous variant c.644-13_644-9delCTTTC in *UNC50 lead to* aberrant splicing with an insertion of 17 nucleotides following reverse trancriptase-polymerase chain reaction and identified as cause of recurrent fetal akinesia deformation sequence in a family.

Conclusion: We achieved a molecular diagnosis in fifteen families. Most of the families had autosomal recessive (10 families) inheritance and were lethal. Detailed perinatal evaluation with integration of genomic tests in fetuses with arthrogryposis improves diagnosis.

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Conflict of Interest: None declared

P12.021.D Genetic findings from multi-gene panel for inherited myopathies

Monica Segura Castell[1], *Kimberly Gall[2], Kirsi Alakurtti[1], Julie Hathaway[2], Åsa Hagström[1], Heli Kuisma[1], Janica Djupsjöbacka[1], Mikko Muona[1], Tuuli Pietilä[1], Pertteli Salmenperä[1], Inka Saarinen[1], Lotta Koskinen[1], Ray Veeraraghavan[2], Samuel Myllykangas[1], Juha Koskenvuo[1]*

[1]Blueprint Genetics, Espoo, Finland; [2]Blueprint Genetics Inc., Marlborough MA, United States

Background/Objectives: Positive genetic test in inherited myopathies (IMs) can provide information about prognosis, risk to family members, and enable participation in clinical trials or precision medicine therapies. Using next-generation sequencing (NGS), we investigated the yield of genetic testing in an IMs-suspected cohort determining the clinical utility of simultaneously testing nuclear and mitochondrial genes.

Methods: Patients included underwent Comprehensive Muscular Dystrophy/Myopathy Panel testing at Blueprint Genetics (CLIA-certified diagnostic laboratory). Testing included sequence and copy number variant (CNV) analyses of NGS data from a validated whole or clinical exome. Variant interpretation adhered to ACMG/AMP guidelines. A positive result was defined as a pathogenic (P) or likely pathogenic (LP) variant(s) consistent with the patient's reported phenotype and associated disease inheritance.

Results: We reviewed 489 patient reports, being 45.2% children (0-18 years) and 57.7% male. Positive results occurred in 24.3%, with 33.9% children and 16.4% adults. *DMD* was responsible for almost one-third of diagnoses (31.2%), while variants in ten genes accounted for over two-thirds (66.4%). CNVs contributed to 21% of the positive results, with 80% found in *DMD*. Three (12%) CNVs were smaller than 500 bps.

Conclusion: This study highlights the clinical utility of multi-gene panel testing, covering nuclear and mitochondrial genes, as almost 25% of patients with suspected IMs received a molecular diagnosis. *DMD* accounted for almost one-third of positive results, with notable implications given the recent FDA approval of gene therapy for IMs such as Duchenne muscular dystrophy. The importance of sensitive CNV detection capabilities is emphasized.

Conflict of Interest: Monica Segura Castell Blueprint Genetics, Kimberly Gall Blueprint Genetics, Kirsi Alakurtti Blueprint Genetics, Julie Hathaway Blueprint Genetics, Åsa Hagström Blueprint Genetics, Heli Kuisma Blueprint Genetics, Janica Djupsjöbacka Blueprint Genetics, Mikko Muona Blueprint Genetics, Tuuli Pietilä Blueprint Genetics, Pertteli Salmenperä Blueprint Genetics, Inka Saarinen Blueprint Genetics, Lotta Koskinen Blueprint Genetics, Ray Veeraraghavan Blueprint Genetics, Samuel Myllykangas stock and stock options at Quest, Blueprint Genetics, Juha Koskenvuo stock and stock options at Quest, Blueprint Genetics

P12.022.A Methylation epi-signature help to determine the pathogenicity of VUS in two cases of *KMT2B*-related dystonia

Gleyson Carvalho[1], Cláudio Gusmão[2], Matheus Castro[2], Mayara Sakuma[2], Beatriz Wolff[1], Lucas Vieira[1], Mariana Costa[1], Rafaela Mendes[1], Maria Fernanda Chagas[1], Yanca Gasparini[1], **Leslie Kulikowski**[1]

[1]Faculdade de Medicina da Universidade de São Paulo, Pathology; [2]Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo, Neurology

Background/Objectives: *KMT2B*-related dystonia (OMIM #617284), is an autosomal dominant progressive neurological condition characterized by early-onset of movement disorder. Variants of uncertain significance (VUS) accounts for a significant proportion of genomic variation in patients with dystonia. In this study, we describe the usage of a genome methylation epi-signature consisting of 113 CpG previously described by Mirza-

Schreiber et al. (2022) and how can it impact the diagnostic in patients with variants of uncertain significance.

Methods: Genome-wide methylation status was accessed using the EPIC methylation assay in peripheral blood samples from two subjects with dystonic posturing and gait imbalance with novel variants classified as VUS in *KMT2B* (p.Leu1720Phe and p.Tyr2515Cys). After QC and normalization steps, we compared the M-values for all 113 probes from two subjects and 6 controls using a support vector machine classification model.

Results: The subject with p.Tyr2515Cys variant shows a hypermethylation results comparable to the individuals with pathogenic and likely pathogenic variants in *KMT2B*. The observed hypermethylation in the subject with p.Tyr2515Cys variant allowed us to enhance the classification to probably pathogenic, while the individual with p.Leu1720Phe variant showed no significant difference in methylation status compared to controls without variants in *KMT2B*.

Conclusion: The study of methylation can be a powerful tool to accumulate evidence of the pathogenicity of variants and improve the diagnostic in patients with variants of uncertain significance.

Grants: National Council for Scientific and Technological Development (CNPQ- 402951/2021-2).

Conflict of Interest: None declared

P12.023.B *SORD* and *SORD2P* inversion: long read sequencing identifies a novel genetic mechanism underlying inherited neuropathy.

Arianna Manini[1;2], *Stefano Facchini*[2], *Ricardo Parolin Schneckenberg*[2], *Chiara Pisciotta*[3], *Adriana Rebelo*[4], *Jacquelyn Raposo*[4], *Elisa Vegezzi*[5], *Riccardo Currò*[2], *Tiffany Grider*[6], *Shawna Feely*[6], *Paola Saveri*[3], *Luca Crivellari*[3], *Stefania Magri*[3], *Franco Taroni*[3], *Matilde Laura*[2], *Mary M Reilly*[2], *Michael Shy*[6], *Stephan Züchner*[4], *Davide Pareyson*[3], *Andrea Cortese*[2]

[1]*University of Milan, Milan, Italy;* [2]*UCL Queen Square Institute of Neurology, Department of Neuromuscular Diseases, London, United Kingdom;* [3]*Fondazione IRCCS Istituto Neurologico Carlo Besta, Milan, Italy;* [4]*Dr. John T. Macdonald Foundation Department of Human Genetics, John P. Hussman Institute for Human Genomics, University of Miami Miller School of Medicine, Miami, United States;* [5]*University of Pavia, Department of Brain and Behavioral Sciences, Pavia, Italy;* [6]*Roy and Lucille Carver College of Medicine, University of Iowa, Iowa City, United States*

Background/Objectives: Despite the best efforts, over 50% of axonal CMT cases do not receive genetic confirmation. Notably, current short read technologies, including whole exome (WES) and whole genome sequencing (WGS), present major shortcomings in the study of structural variants and repeated regions, contributing to the missing heritability in CMT.

Methods: Here we leverage long read sequencing (LRS) and non-sequencing based optical genome mapping (OGM) to identify a large structural variant involving *SORD* and its pseudogene *SORD2P*, which disrupts *SORD* reading frame and causes SORD = CMT in multiple families.

Results: In three unrelated axonal CMT patients with high serum sorbitol and a heterozygous c.757delG *SORD* variant, LRS and OGM revealed a 200Kb inversion spanning two highly homologous *SORD/SORD2P* introns, undetectable by WES or WGS. Additionally, inverse PCR in 37 CMT cases with a heterozygous pathogenic *SORD* mutation identified this inversion in 4 (~10%) of them. This inversion occurred de novo in one family and was inherited from an asymptomatic parent in another. These cases resemble previously described SORD = CMT cases. Public data on 3D chromatin structure suggest *SORD* and *SORD2P*'s proximity in the nucleus could facilitate such recombination events.

Conclusion: In conclusion, *SORD/SORD2P* inversion is key in axonal CMT cases with a single *SORD* mutation, especially when serum sorbitol is high. This study demonstrates LRS and OGM's effectiveness in identifying genetic causes in unresolved CMT cases, revealing a new mechanism by which structural variants in gene/pseudogene pairs (~3000 in the human genome) can cause genetic disease, often undetected by short-read sequencing technologies.

Grants:

Conflict of Interest: None declared

P12.024.C Medaka fish *Oryzias latipes* a promising model to study titinopathies: the HMERF case study.

Viviana Cetrangolo[1], *Marco Linari*[2], *Elena Polishchuk*[1], *Marco Savarese*[3], *Jaakko Sarparanta*[3], *Ivan Conte*[4], *Bjarne Udd*[3], *Vincenzo Vincenzo Nigro*[1;5]

[1]*Telethon Institute of Medicine and Genetics-Tigem, Pozzuoli, Italy;* [2]*Università degli Studi di Firenze - Dipartimento di Biologia, Firenze, Italy;* [3]*Folkhälsan Research Centre, Biomedicum, Helsinki, Finland;* [4]*UNINA - Dipartimento di Biologia, Napoli, Italy;* [5]*Università degli Studi della Campania "Luigi Vanvitelli", Napoli, Italy*

Background/Objectives: Titinopathies are severe diseases, affecting skeletal and heart muscles. They are caused by variants in the titin gene, whose protein is crucial for sarcomere architecture. Titin A150/FN3-119 domain variants are associated with hereditary myopathy with early respiratory failure (HMERF), a life-threatening disorder manifesting respiratory failure and proximodistal muscle weakness. Mouse models for several titinopathies have been developed, but an HMERF model is missing. This work aims to generate and characterize the first HMERF fish model.

Methods: *Oryzias latipes* (medaka) is a teleost fish, used for genetic studies. Medaka has one copy of titin gene paralog, which protein sequence is evolutionarily conserved. This allows a straightforward genome-editing, through CRISPR/Cas9 strategy.

Results: We generated the first HMERF vertebrate model, harboring a missense variant found in patients (p.C31712R). The morphological characterization shows a perturbation of muscle fiber organization at the ultrastructure level and immunofluorescence analyses show a reduction of sarcomeric protein signals, especially in homozygous individuals. Moreover, locomotor analyses reveal a decrease in homozygous movement. These differences are supported by the force-velocity relationship analyses, which reveal that the shortening velocity (V), at any load (T), is lower in homozygotes. Consequently, in homozygotes, the power (VxT) at any load drops drastically.

Conclusions: The medaka larvae phenotype seems to recapitulate HMERF patients' pathological conditions. This work highlights the potential of medaka fish model for our understanding of the mechanism underlying HMERF disease.

Grant: This work was supported by the European Joint Program on Rare Diseases, Telethon Undiagnosed Disease Program (TUDP), the Research Council of Finland and AFM-Telethon.

Conflict of Interest: None declared

P12.025.D Long-read whole-genome sequencing in peripheral neurodegeneration: challenges, insights, and paths forward

Ayse Candayan[1;2], **Luiza Lorena Pires Ramos**[1;2], *Els De vriendt*[1;2], *Albena Jordanova*[1;2;3]

[1]*VIB-UAntwerp Center for Molecular Neurology, Antwerpen, Belgium;* [2]*University of Antwerp, Department of Biomedical Sciences,*

Antwerpen, Belgium; [3]Medical University of Sofia, Department of Medical Chemistry and Biochemistry, Sofia, Bulgaria

Background/Objectives: Inherited peripheral neuropathies (IPN) are genetically heterogeneous disorders that still present a significant diagnostic challenge, suggesting the presence of novel causative genes or 'non-conventional' mutational mechanisms yet to be identified. Using long-read sequencing (LRS) to bridge this heritability gap seems promising, as it allows identification of variants that were intractable by other technologies.

Methods: We selected 15 families with IPN that had undergone extensive analyses using short-read sequencers but remained genetically undiagnosed. Nanopore whole-genome sequencing was performed on 32 individuals on a PromethION device. Downstream data analyses were conducted using an in-house pipeline.

Results: Challenges surfaced across multiple layers of the pipeline, including issues related to the high-quality sample requirements for LRS from "historical" cases and the quality control process. Selecting parameters for accurate base-calling proved critical, along with combining structural variants (SVs) shared among different individuals due to ambiguous breakpoints. Our experience also highlighted the need for comprehensive SV databases for downsizing the list of candidate variants. Verification of candidate variants also proved challenging, requiring tailoring of different techniques for each variant of interest.

Conclusion: LRS is poised to become the next paradigm-shifting tool in human genetics and will help us bridge the many heritability gaps in genetic diseases. As technologies rapidly evolve, researchers embarking on such studies face numerous challenges, thus sharing experiences among early-stage LRS users will greatly facilitate advancement in the field.

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Conflict of Interest: None declared

P12.026.A A novel deep intronic MFN2 mutation in a patient with autosomal recessive CMT2.

Roxane van Heurck[1], *Thomas RIO FRIO[1], Caterina Marconi[1], Anne Vannier[1], Jean-Louis Blouin[1], Iman Boukrid[2], Joel Fluss[3], Marc Abramowicz[1]*

[1]Hôpitaux Universitaires de Genève (HUG), Genetic Medicine and Development, Genève, Switzerland; [2]Hôpital de La Tour, Neurology, Meyrin, Switzerland; [3]Hôpitaux Universitaires de Genève (HUG), Pediatric Neurology, Genève, Switzerland

Background/Objectives: Charcot Marie Tooth disease type 2A (CMT2A) is a rare axonal neuropathy caused by dominant (90%) or recessive (10%) pathogenic variants in the mitofusin gene *MFN2*. To date, anomalies have only been reported in *MFN2* coding sequence.

Methods: We collected clinical data in a patient presenting with mild intellectual deficiency and clinical CMT2. Molecular investigations were performed with primary analysis of 1694 genes with Whole Exome Sequencing (WES), followed by Whole Genome Sequencing (WGS) and mRNA analyses.

Results: A 23-Year-old female patient born of non-consanguineous parents of French and Swiss origins, was first seen at ten years with foot drop, *pes cavus*, absent lower limbs tendon reflexes and partial sphincter dysfunction. Initial ENMG showed signs of a predominant sensory axonal neuropathy. Subsequent motor involvement was noted and the patient became wheelchair dependent at age 16, displayed optic atrophy at age 21 and complained of neuropathic pain.

WES identified a founder recessive single allelic intragenic deletion in *MFN2*. WGS and RNA analyses revealed the presence of a deep intronic single nucleotide substitution in trans, that caused the inclusion of a cryptic exon in 50% of allele-derived mRNA. The combination of this hypomorphic allele that showed drastically reduced expression of MFN2 mRNA, with the allele carrying the deletion is compatible with a recessive CMT2A.

Conclusion: This is the first report of a deep intronic mutation in *MFN2* that causes a recessive CMT2A. This study highlights the benefit from WGS combined with the functional analysis at the RNA level for non-coding variants.

Grants: HUG QS07-38

Conflict of Interest: None declared

P12.027.B Accelerated Whole Genome Sequencing provides rapid diagnosis and treatment in a 4-months-old girl with Congenital Myasthenic Syndrome

Maria Isaksson[1], *Lone Laulund[2], Maria Grevit[3], Ulla Larsen[3], Bjørk Larsen[4], Pernille Torring[1]*

[1]Odense University Hospital, Department of Clinical Genetics, Odense C, Denmark; [2]Odense University Hospital, H. C. Andersens Children's Hospital, Odense C, Denmark; [3]Odense University Hospital, Department of Anaesthesiology and Intensive Care, Odense C, Denmark; [4]Odense University Hospital, Department og Clinical Genetics, Odense C, Denmark

Background/Objectives: Congenital Myasthenic Syndromes (CMS) are a heterogeneous group of genetic disorders, characterized by impaired neuromuscular transmission, resulting in weakness of the skeletal muscles. The course and severity of disease is variable, with onset typically in the first two years. Neonates may present with respiratory insufficiency, multiple joint contractures, and feeding difficulties and dysmorphic features. Some subtypes may respond well to treatment with acetylcholinesterase inhibitors. We present a case, where Whole Genome Sequencing (WGS) ensured rapid CMS-diagnosis in a four-months-old girl.

Methods: Trio-WGS was performed on DNA extracted from peripheral blood, with Next Generation Sequencing technology (Illumina NovaSeq 6000).

Results: A girl was admitted twice to the paediatric intensive care unit, at two and four months of age respectively. In both occasions, she presented with respiratory insufficiency, episodes of apnoea and required intubation. Clinical features included hypotonia with reduced head control, frog-legged posture, reduced coughing, excessive drooling, a high arched palate and feeding difficulties. Electroencephalogram, brain MRI and Creatinine Kinase level were normal. Accelerated trio-WGS detected that the patient was compound heterozygote of a pathogenic variant (c.264C>A) and a likely pathogenic variant (c.690G>T) in the RAPSN-gene, compatible with the diagnosis of CMS with autosomal recessive inheritance. She responded well to treatment with acetylcholinesterase inhibitor (pyridostigmine) and was extubated after 4 days. She is currently within age-appropriate development at nine-months-old.

Conclusion: This case illustrates that accelerated WGS can ensure a fast diagnosis, immediately providing initiation of relevant treatment, significantly altering the disease course and improving quality of life for the patient.

Conflict of Interest: None declared

P12.028.C Identification of a novel neurodevelopmental syndrome caused by variants in the gene *PRKAR2B*

Ella Whittle[1], *marc ruiz*[1], *Hamin Lee*[1], *hannah gulliford*[1], *ratna romy*[1], *Debby Hellebrekers*[2], *Melanie O'Leary*[3], *Lynn Pais*[3], *Grace E VanNoy*[3], *Jean-Madeleine de Sainte Agathe*[4], *Mohamad Abdelhamid*[5], *Paria Najarzadeh Torbati*[6], *erika ignatius*[7], *Yalda Jamshidi*[1], *Alan Pittman*[1], *Shamima Rahman*[8], *Henry Houlden*[9], *reza maroofian*[9], *Ehsan Ghayoor Karimiani*[1], *Chris Carroll*[1]

[1]*St George's University of London, United Kingdom;* [2]*Maastricht UMC+, Maastricht, Netherlands;* [3]*Broad Institute of MIT and Harvard, Cambridge, United States;* [4]*Faculty of Medicine Pierre et Marie Curie, Paris, France;* [5]*National Research Centre (NRC), Egypt;* [6]*Islamic Azad University of Mashhad, Mashhad, Iran;* [7]*University of Helsinki, Helsinki, Finland;* [8]*UCL Great Ormond Street Institute of Child Health, United Kingdom;* [9]*UCL Queen Square Institute of Neurology, United Kingdom*

Background/Objectives: Protein Kinase A (PKA) is a well-known cell signalling complex essential to cellular proliferation, homeostasis and growth. PKA consists of two catalytic subunits under the control of a regulatory subunit dimer encoded by the gene protein kinase cAMP-dependent type II regulatory subunit type II beta (*PRKAR2B*). Here, we report for the first time recessive pathogenic variants in *PRKAR2B* as a cause of a neurodevelopmental disorder.

Methods: Whole-exome sequencing was employed followed by a prioritisation strategy to identify rare variants (absent or MAF <0.001 in gnomAD) predicted to have a deleterious impact (CADD Phred scores >20) within individuals. Suspected variants were validated with Sanger sequencing and functionally investigated utilising in silico and in vitro techniques. We generated HEK293 *PRKAR2B* knockout cells to investigate the impact of PRKAR2B protein loss alongside patient fibroblasts collected.

Results: We present five *PRKAR2B* variants, three loss-of-function variants, one splice variant and one missense variant, in six unrelated families. Patients presented with an overlapping clinical phenotype characterised by dystonia, brain atrophy, spasticity, seizure, delayed speech and language development. Pathogenicity of variants has been supported through the measurement of endogenous PRKAR2B protein from patient cells and through a minigene exon-trapping assay. Results indicate that the loss of PRKAR2B protein reduces the phosphorylation activity of the PKA complex and causes aberrant DRP1 signalling, a checkpoint for mitochondrial fission, suggesting perturbed mitochondrial dynamics as a possible molecular mechanism of PRKAR2B-related disease.

Conclusion: We have identified variants in *PRKAR2B* as a cause of rare monogenic neurodevelopmental disease.

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Conflict of Interest: None declared

P12.029.D A rare muscle disease: 3 cases diagnosed with myotonia congenita

Ali DURU[1], *Mert Coşkun*[1], *Özden Altıok Clark*[1], *Gokcen Karamik*[2], *Nuray Ozturk*[2], *Öznur YILMAZ BAYER*[2], *nadide cemre randa*[3], *Aslı Toylu*[1], *banu nur*[2], *ercan mihci*[2]

[1]*Akdeniz Üniversitesi Hastanesi, Medical Genetic, Antalya, Türkyie;* [2]*Akdeniz Üniversitesi Hastanesi, Pediatric Genetics, Antalya, Türkyie;* [3]*Antalya Education and Research Hospital, Medical Genetic, Antalya, Türkyie*

Background/Objectives: Myotoni Congenita (MC) is a disease characterized primarily by myotonia during childhood, along with muscle hypertrophy and the presence of the warm-up phenomenon. Blood creatinine kinase (CK) increases in these patients. Spontaneous electrical activity can be seen in electromyography. This disease is caused by pathogenic variants in the *CLCN1* gene.

Methods: Genomic DNA from patients were examined for genes associated with Myotonic syndromes using Next-Generation Sequencing method. During the evaluation of variants, we applied the ACMG 2015 criteria.

Results: Patient 1 underwent genotyping following the observation of extensive contractions in the lower extremities, alongside the occurrence of warm-up phenomena and spontaneous electrical activity detected during electromyography. Analysis of the sequencing data revealed a homozygous variant of c.568_569delinsTC in *CLCN1* gene. This missense variant was classified as pathogenic in Clinvar.

Patient 2 and Patient 3, siblings of consanguineous parents, were applied to our clinic with involuntary contractions of arms. Serum CK elevation was detected in both cases. Electromyography was performed in one case and myotonic discharges were detected. In genetic analysis, c.2533G>A homozygous missense variant was detected in the *CLCN1* gene in both patients. Later, the parents were analyzed to be heterozygous carriers of the variant. This variant was reported as a variant of uncertain clinical significance in the Clinvar database.

Conclusion: Variant c.2533G>A is presumed a candidate variant associated with MC because of its predicted functonal effect and affirming segregation analysis. Functional analysis' are expected to better elucidate the clinical outcomes of the observed variant.

Grants: None

Conflict of Interest: None declared

P12.030.A Neuromuscular junction deficits in PURA syndrome

Magdalena Mroczek[1], *Magdalena Chrościńska-Krawczyk*[2], *Dimitrios Zafeiriou*[3], *Anna Małges*[2], *Volkan Adak*[4], *Adela Della Marina*[5], *Michał Bielak*[2], *Christoph Handschin*[4], *Andreas Roos*[5], *Stanley Iyadurai*[6]

[1]*University Hospital of Basel, Department of Neurology, Basel, Switzerland;* [2]*University Children Hospital, Department of Child Neurology, Lublin, Poland;* [3]*Aristotle University of Thessaloniki, "Hippokration" General Hospital, Thessaloniki, 1st Paediatric Department, Developmental Centre "A. Fokas", Thessaloniki, Greece;* [4]*University of Basel, Biozentrum, Basel, Switzerland;* [5]*University Duisburg-Essen, Department of Pediatric Neurology, Centre for Neuromuscular Disorders, Centre for Translational Neuro- and Behavioral Sciences, Essen, Germany;* [6]*Johns Hopkins All Childrens Hospital, Division of Neurology, St. Petersburg, United States*

Background/Objectives: PURA syndrome is a rare genetic central nervous system disorder caused by heterozygous variants in the *PURA* gene. Several phenotypic features recently suggested a neuromuscular junction (NMJ) source of weakness. We investigated if treatment response to the pyridostigmine, an NMJ agent, is generalizable over centers and questioned if PURA protein is localized at the NMJ on the molecular level.

Methods: Observational study with patients from Poland and Greece. Pyridostigmine was administered at 3 to 7 mg/kg in divided doses 4 to 6 times. Proteomics was performed on one human muscle biopsy and fibroblasts. Murine data were collected from the snRNAseq and the LCMseq (soleus/ extensor digitorum longus).

Results: 7 patients underwent treatment and one is to be included. There was an improvement after pyridostigmine, including neurodevelopmental improvements and muscle strength increase when assessed in a systematic way. Neurophysiological studies performed on 5 patients suggested postsynaptic deficits in 4 patients and pre- and post-synaptic deficits in 1 patient. Proteomics from the muscle biopsy and fibroblasts

showed interaction of PURA with synaptic proteins. In the single nuclei studies Pura expression was noted in subsynaptic NMJ nuclei at higher levels compared to the remaining myonuclei.

Conclusion: PURA syndrome although deemed a CNS disorder, localizes to the NMJ and manifests with NMJ deficits, which effectively responds to NMJ modulation. Some central nervous system disorders may have a peripheral component that can be treated effectively.

Grants: none

Conflict of Interest: None declared

P12.031.B Genetic characterization of *RYR1* variants: Insights into neuromuscular disorders and malignant hyperthermia in Czech patients

Tereza Kramářová[1;2], *Johana Kopčilová[1;3], Jana Zídková[1;2], Kamila Reblová[1;2], Lenka Fajkusova[1;2]*

[1]University Hospital Brno, Centre of Molecular Biology and Genetics, Brno-Bohunice, Czech Republic; [2]Faculty of Medicine Masaryk University, Institute of Medical Genetics and Genomics, Brno, Czech Republic; [3]Faculty of Science Masaryk University, National Centre for Biomolecular Research, Brno, Czech Republic

Background:

The *RYR1* gene encodes a ryanodine receptor, which works as a sarcoplasmic reticulum calcium release channel in the skeletal muscle. RYR1-related diseases represent a wide range of disorders that affect the neuromuscular system and can be inherited in a dominant or recessive manner or result from de novo variants. The European Malignant Hyperthermia Group reports a direct association between malignant hyperthermia (MH) and sixty-six diagnostic mutations in the *RYR1* gene and two diagnostic mutations in the *CACNA1S* gene.

Methods: Targeted sequencing of genes associated with neuromuscular disorders, bioinformatic analysis for detection of small-scale gene variants and copy number variants.

Results: We present *RYR1* genetic findings in Czech patients with myopathy and/or MH. Pathogenic/likely pathogenic variants were identified in 75 unrelated patients – 17 patients had dominantly inherited myopathy, 21 patients recessively inherited myopathy, and 37 patients had a diagnostic MH variant without a myopathy phenotype. Significant clinical heterogeneity was noted among patients with the same type of inheritance, which correlated to some extent with the localization of the variant in the structure of the RyR1 protein.

Conclusion: Analysis of *RYR1* variants is essential for understanding the pathogenesis of RYR1-related diseases. Characterisation of variants in patients with suspected or confirmed MH can contribute to the expansion of MH diagnostic mutations and risk prediction of this life-threatening disease.

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Conflict of Interest: None declared

P12.032.C The WWOX gene variants detection in patient with cerebellar ataxia using optical genome mapping

Karolina Rutkowska[1], *Agnieszka Pollak[1], Magdalena Krygier[2], Marta Zawadzka[2], Maria Mazurkiewicz-Bełdzińska[2], Rafał Płoski[1]*

[1]Medical University of Warsaw, Department of Medical Genetics, Warsaw, Poland; [2]Medical University of Gdańsk, Department of Developmental Neurology, Gdańsk, Poland

Background/Objectives: Cerebellar ataxias (CAs) are a heterogeneous group of neurological disorders characterized by degeneration of the cerebellum and other parts of nervous system. Clinical features of CAs include loss of coordination of limb and eye movements, dysarthria. Diagnosis of CAs remains a challenge due to large phenotypic and genetic heterogeneity.

Methods: We used optical genome mapping (OGM) to study ultra-high molecular weight DNA of the 16 year old female patient with cerebellar ataxia. The patient was previously examined using exome sequencing, however no pathogenic variants associated with proband's phenotype were found.

Results: In the patient's sample two heterozygous structural variants (SVs) in the *WWOX* gene were found: insertion (hg38 16:78,393,619-78,396,002; 3,688 bp) and deletion (hg38 16:78,275,761-78,281,462; 5,700 bp). We also performed OGM in proband's mother sample and found that she was a carrier of the *WWOX* insertion. OGM data suggest that both variants in our patient with high probability are an in trans configuration. Extensive study showed association of *WWOX* gene with developmental and epileptic encephalopathy and esophageal squamous cell carcinoma. The *WWOX* gene was also related to autosomal recessive spinocerebellar ataxia characterized by onset of generalized seizures in infancy, delayed psychomotor development with mental retardation, and cerebellar ataxia. The variants found in OGM study may be related to the proband's phenotype.

Conclusion: OGM is a valuable a high-resolution technology that enables detection all types of SVs in the genome. OGM can be a potential supplement to the available clinical genetics diagnostics methods.

Conflict of Interest: None declared

P12.033.D RNA sequencing as a complementary diagnostic tool in a cohort of undiagnosed patients with muscle disease

Alba Segarra-Casas[1], *maria josé rodriguez[2], cristina domínguez[3;4], aurelio hernández-laín[3], Solange Kapetanovic[5], Laura González-Mera[6], Velina Nedkova[6], Adolfo Lopez de Munain[7], Daniel Natera-de Benito[8], Carlos Ortez[8], Cecilia Jimenez-Mallebrera[4;8], Andrés Nascimento[4;8], Benjamin Rodríguez-Santiago[4;9], Eduard Gallardo[4;9], Montse Olivé[4;9], pia gallano[1;4], lidia gonzalez-quereda[1;4]*

[1]Institut de Recerca - Hospital Santa Creu i Sant Pau, Genetics Department, Barcelona, Spain; [2]Hospital de la Santa Creu i Sant Pau, Genetics Department, Barcelona, Spain; [3]imas12 Research Institute, Hospital Universitario 12 de Octubre, Neuropathology Unit, Madrid, Spain; [4]CIBERER, Instituto de Salud Carlos III, Madrid, Spain; [5]Hospital de Basurto, Neurology Department, Bilbo, Spain; [6]IDIBELL-Hospital Universitari de Bellvitge, Neuromuscular Diseases Unit, Neurology Department, L'Hospitalet de Llobregat, Spain; [7]Biodonostia-Osakidetza Basque Health Service, Biodonostia Neurosciences Area Group of Neuromuscular Diseases, San Sebastian, Spain; [8]Hospital Sant Joan de Déu, Neuromuscular Unit, Department of Neurology, Esplugues de Llobregat, Spain; [9]Institut de Recerca - Hospital Santa Creu i Sant Pau, Neuromuscular Diseases Unit, Neurology Department, Barcelona, Spain

Background/Objectives: It is estimated that 50% of patients with neuromuscular diseases remain undiagnosed after exome sequencing (ES) or gene panel sequencing. Here, we evaluate total RNA sequencing from muscle biopsy as a diagnostic tool in genetically unsolved patients following genomic DNA testing.

Methods: Total RNAseq was extracted from muscle biopsies of 29 undiagnosed patients after ES/gene panel sequencing, 3 positive controls with an identified RNA defect in the *DMD* gene and 5 healthy controls. We evaluated the presence of aberrant splicing (FRASER, LeafCutterMD, rMATS), outlier gene expression (OUTRIDER) and monoallelic expression in autosomal recessive genes. In addition, variant calling was performed from RNAseq data (GATK).

Results: In 7/29 patients (24%) we detected pathogenic alterations at the mRNA level including: two exon skipping events (*COL6A1*), one intronic retention (*TTN*), one alternative splicing event caused by a synonymous variant (*RYR1*) and two cases of monoallelic expression in autosomal recessive genes (*GNE*).

Conclusion: RNAseq analysis improves the diagnostic rate in patients with muscle diseases after inconclusive results from exome sequencing or gene panel sequencing by identifying molecular events that would not have been detected by other methods, and also by reclassifying VUS variants identified at the gDNA level. With this pilot study, we demonstrate the utility of RNAseq as a complementary diagnostic tool in muscle diseases, being particularly useful in patients with a clear clinical suspicion focused on a subset of genes.

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Conflict of Interest: None declared

P12.034.A Congenital myopathy in male twins with biallelic variants in *TTN*: a case report

lisa isabel olfe[1], *Alisa Förster*[1], *Uta Diebold*[2], *Tim Ripperger*[1], *Corinna Hendrich*[1], *Anke Katharina Bergmann*[1]

[1]*Hannover Medical School, Department of Human Genetics, Hannover, Germany;* [2]*Children- and Teenager Hospital Auf der Bult, Social-Pediatric Center, Hannover, Germany*

Background/Objectives: Autosomal recessive titin-related myopathies are a heterogeneous group of potentially severe congenital muscle disorders caused by disease-causing variants in titin (*TTN)*. With its 364 exons, *TTN* encodes the largest known human protein involved in sarcomere formation. Due to its size and manifold splice variants, classification of genetic variants in *TTN* is challenging.

Methods: Whole genome sequencing (WGS)

Results: We report on 14-year old male twins with congenital myopathy. The boys were born from non-consanguineous parents with a gestational age of 36 + 3 weeks. The more severly affected sibling presented as floppy infant. Their phenotype was characterized by muscle weakness, neonatal feeding difficulties, delay in motor development milestones, short stature, dystrophy and scoliosis. MRI showed fatty infiltration of pelvis and leg muscles. Muscle biopsy showed a myopathic tissue pattern. No cardiac involvement was observed. Their parents and one older brother are healthy.

Phenotype-based interpretation of quattro whole genome sequencing revealed compound heterozygous variants in *TTN* in both index patients: The likely pathogenic variant NM_001267550.1(TTN):c.19709_19710del p.(Val6570Glufs*18) transmitted from the mother, and the variant of uncertain significance (VUS) NM_001267550.1(TTN):c.106531+5G>A r.(spl?) p.? inherited from the father.

Conclusion: We suspect an autosomal recessive *TTN*-related myopathy in accordance with the symptoms of the two brothers and RNA analysis to confirm functional impact of the VUS is ongoing. This case highlights the need for further (functional) data to reclassify detected VUS in *TTN* in order to provide a diagnosis and adequate genetic counselling for the affected family.

Grants: none

Conflict of Interest: None declared

P12.035.B Multiexon *TRAPPC11* gene deletions as a cause of muscular dystrophy and their estimated genesis

Johana Kopčilová[1;2], *Tereza Kramářová*[2;3], *Jana Zídková*[2;3], *Kamila Reblová*[2;3], *Lenka Fajkusova*[1;2]

[1]*Faculty of Science Masaryk University, National Centre for Biomolecular Research, Brno, Czech Republic;* [2]*University Hospital Brno, Centre of Molecular Biology and Genetics, Brno-Bohunice, Czech Republic;* [3]*Faculty of Medicine Masaryk University, Institute of Medical Genetics and Genomics, Brno, Czech Republic*

Background: Transport protein particle (TRAPP) is a multiprotein complex involved in the localization of proteins to the Golgi compartment. To this day, only small-scale variants in the gene encoding TRAPPC11 subunit have been identified as a cause of muscular dystrophy. We present clinical and molecular genetic findings in three patients, all compound heterozygotes for a missense variant and a structural variant in the *TRAPPC11* gene. We assessed the possible mechanisms of origin of the structural variants by analysing the breakpoint junctions.

Methods: Custom capture panel sequencing and/or whole genome sequencing; bioinformatics analysis detecting small-scale gene variants and large gene deletions/duplications; long-range PCR for the sequencing of breakpoint junctions.

Results: In P1, a known missense variant and a deletion of exons 6-9 were found. In P2 and P3, a novel missense variant and a deletion of exons 24-25 and 26-30 were identified, respectively. The presumed mechanisms of origin are nonhomologous end joining in P1 and P2 and nonallelic homologous recombination in P3.

Conclusion: We describe patients in whom copy number variants along with single nucleotide variants in the *trans* position are associated with disease manifestation. Sequencing of breakpoint junctions of identified gene deletions allowed us to elucidate the upstream mechanisms leading to genomic instability. This knowledge enables us to improve strategy for identifying disease causing mutations.

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Conflict of Interest: None declared

P12.036.C Comparison of two different doses of vitamin D3 supplementation on bone health in ambulatory boys with DMD- a double blind randomized controlled trial

Reena Gulati[1], *pranay reddy sangala*[1]

[1]*Jawaharlal Institute of Postgraduate Medical Education and Research, Pediatrics, Puducherry, India*

Background/Objectives: Duchenne muscular dystrophy (DMD) is a progressive, disabling genetic condition with high risk of osteoporosis. Vitamin D deficiency, aggravated by glucocorticoid treatment leads to fractures in some cases. However, the optimal supplemental dose is still under investigation. We compared the effect of two different daily doses of cholecalciferol supplementation for 3 months on bone mineral density (BMD) and muscle functional parameters in boys with DMD.

Methods: Forty-four DMD patients aged 4-15 years were randomized into 2 blinded groups (22 patients each), who received either 2000 or 4000 IU/day of Cholecalciferol along with routine calcium supplementation for 3 months. BMD by Dual-energy X-ray absorptiometry (DXA) at lumbar spine and 'total body less head' assessments, serum 25-hydroxy vitamin D (25-OHD) and functional scales were evaluated at entry and after 3 months.

Results: Both groups exhibited significant improvement in BMD after 3-month cholecalciferol supplementation [p = 0.006 (4000 IU/day), and 0.008 (2000 IU/day) at lumbar spine and p = 0.006 (4000 IU/day), and 0.024 (2000 IU/day) at 'total body less head' respectively]. Serum 25-hydroxy vitamin D levels improved to 'sufficient' category in 80% patients compared to 27% before supplementation. Functionality tests did not show any significant change in either group. Improvement in BMD and serum 25-OH D levels was comparable between the two groups.

Conclusion: In 4-15-year-old boys with DMD, Vitamin D3 supplementation at 2000 or 4000 IU/day for 3 months significantly improved BMD and serum 25-hydroxy vitamin D levels to similar extent. Functional scores may need longer supplementation to show improvement.

Grants: Intramural-JIPMER, India

Conflict of Interest: None declared

P12.037.D Patient-derived iPSC lines as an essential resource for in vitro modelling studies on ALS pathogenesis and the development of new therapeutic approaches.

Paolo Niccolò Doronzio[1], Francesco Martello[1], Anna Gloria Renzi[1], Mariagrazia Longo[1], Claudia Mancini[1], Daniela Orteschi[2], Amelia Conte[3], Giulia Bisogni[3], Daniela Bernardo[3], Katia Patanella[3], Marcella Zollino[1;2], Mario Sabatelli[4;5], Marco Luigetti[4;5], Serena Lattante[1;6], **Giuseppe Marangi**[1]

[1]*Università Cattolica del Sacro Cuore, Section of Genomic Medicine, Department of Life Sciences and Public Health, Rome, Italy;* [2]*Fondazione Policlinico Universitario A. Gemelli IRCCS, Unit of Medical Genetics, Department of Laboratory and Infectious Disease Sciences, Rome, Italy;* [3]*Fondazione Policlinico Universitario A. Gemelli IRCCS, Adult NEMO Clinical Center, Unit of Neurology, Department of Aging, Neurological, Orthopedic and Head-Neck Sciences, Rome, Italy;* [4]*Università Cattolica del Sacro Cuore, Section of Neurology, Department of Neuroscience, Rome, Italy;* [5]*Fondazione Policlinico Universitario A. Gemelli IRCCS, Department of Neuroscience, Sensory Organs and Chest, Rome, Italy;* [6]*Università del Salento, Department of Experimental Medicine, Lecce, Italy*

Background/Objectives: Amyotrophic lateral sclerosis (ALS) is a lethal neurodegenerative disease, mainly due to the progressive loss of motor neurons. The great clinical and genetic heterogeneity of ALS mirrors the different underlying pathogenic mechanisms, yet to be fully understood. A major hurdle in identifying the causes of ALS is the inaccessibility of the disease target tissue, i.e. patient motor neurons. Human induced pluripotent stem cells (iPSCs) derived from ALS patients and differentiated into target cells has been taking hold as a precious resource for both molecular mechanism analysis and drug development in ALS.

Methods/Results: We established 40 iPSC cultures from ALS patient-derived fibroblasts by Sendai virus reprogramming: 17 from familial cases and 23 from sporadic. Causative genetic variants had been previously identified in 29 patients, including: 5 *C9orf72* repeat expansions, 6 variants in *SOD1*, 10 in *TARDBP*, 3 in *FUS*, 3 in *TBK1*, 1 in *VCP* and 1 in *OPTN*. Furthermore, we obtained iPSCs from 2 pairs of twins discordant for the phenotype (with a *TARDBP* variant in one pair), and two unaffected relatives carrying pathogenic variants (in *TARDBP* and *FUS*). For result comparison, we likewise generated iPSCs from healthy controls.

Conclusion: We provide a detailed characterization of both patients' phenotypes and iPSC lines genetic and molecular features. We discuss about iPSC differentiation into motor neurons we are carrying on, aiming to be able to study the consequences of specific genetic variants on those cells. In conclusion, we believe that our iPSC lines constitute a valuable tool for ALS research.

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Conflict of Interest: None declared

P12.038.A A systematic investigation of the role of the oligogenic/digenic inheritance in Amyotrophic lateral sclerosis with machine learning tools on WGS data

Lucia Corrado[1], **Fjorilda Caushi**[1]*, alessio bottrighi[2], Nicola Pomella[1], Fabiola De Marchi[3], Susanna Zucca[4], Federica De Paoli[4], Beatrice Piola[1], Benedetta Bartilomo[1], Fatima Masoomi Sarvestani[1], Adriano Chio[5], Letizia Mazzini[3], Maurizio Grassano[5], Andrea Calvo[5], sandra d'alfonso[1]*

[1]*Università degli Studi del Piemonte Orientale, Health Sciences, novara, Italy;* [2]*Università degli Studi del Piemonte Orientale, Dipartimento di Scienze e Innovazione Tecnologica, Alessandria, Italy;* [3]*Università degli Studi del Piemonte Orientale, Dipartimento di Medicina Traslazionale, Università del Piemonte Orientale, Clinica Neurologica AOU Maggiore della Carità, novara, Italy;* [4]*Engenome, Engenome s.r.l., Pavia, Italy;* [5]*università degli Studi di Torino, ALS Centre, "Rita Levi Montalcini" Dipartimento di Neuroscienze, Università degli Studi di Torino, SC Neurologia 1U, Azienda Ospedaliero-Universitaria Città della Salute e della Scienza di Torino, torino, Italy*

Background/Objectives: ALS is a neurodegenerative motor neuron disease. Its genetic cause is unknown in 85% of cases despite identifying over 40 ALS genes. Digenic Inheritance (DI) represents the simplest model for complex genetic diseases. Here, a Machine Learning (ML) approach was used to study the impact of DI on ALS using a pilot WGS dataset.

Methods: We used DIVAs, a ML tool, to analyze 60 WGS data. DIVAs identified and classified digenic variant combinations based on the patient's phenotype, variant pathogenicity, and gene-gene interaction. For each combination, DIVAs assigns a score (0-1) proportional to the evidence of causation. We selected combinations with a score higher than 0.7 and prioritized pairs with likely pathogenic, pathogenic, or uncertain significance (VUS) variants.

Results: We obtained 6 patients with digenic combinations that met our criteria. Among these, two presented interesting digenic combinations (all including VUS) suggesting a possible pathway interaction. Patient 22 showed a missense mutation in *DCTN1* ALS-associated susceptibility gene, combined with a missense in *SPG11* juvenile ALS-associated gene, both involved in cytoskeletal and axon-transport defect. Patient 60 showed a 5'UTR deletion in *ERBB4* ALS gene coupled with a missense in *TRPM7* ALS susceptibility gene, both involved in secondary ion channel defects as transmembrane receptors.

Conclusions: Our initial findings suggest that DIVAs ML tool can detect digenic couples related to patient's disease. We are comparing the results with other ML tools (*DE profile*, VarCoPP, *OligoPVP*, ORVAL, DiGePred) to complete a pipeline to test a larger ALS cohort.

Grants: DIG-ALS (FG_19/2023, AriSLA, Foundation, Italy)

Conflict of Interest: None declared

P12.039.B A global analysis of the CMT1A locus: implications for the origin and susceptibility to Charcot- Marie-Tooth disease type 1A across populations

Alexandra Alitsiou[1;2;2;2]*, Pedro M Rodriguez Cruz[1;2;3;4], Jon Andoni Urtizberea[5], Anne-Sophie Lia[6], Karima Ghorab[7], Sergi Beltran[1;8], Oscar Lao[9]*

[1]*Centro Nacional de Análisis Genómico - CNAG, Barcelona, Spain;* [2]*Universitat de Barcelona, Barcelona, Spain;* [3]*Université Cheikh Anta Diop De Dakar, Dakar, Senegal;* [4]*UCL Institute of Neurology,*

Department of Neuromuscular Diseases, London, United Kingdom; [5]Institut de Myologie, Paris, France; [6]University of Limoges, Department of Biochemistry and Molecular Genetics, Limoges, France; [7]University of Limoges, Department of Neurology, Limoges, France; [8]Universitat de Barcelona, Faculty of Biology, Department of Genetics, Microbiology and Statistics, Barcelona, Spain; [9]Institut de Biologia Evolutiva (CSIC - UPF), Barcelona, Spain

Background/Objectives: Charcot-Marie-Tooth type 1A (CMT1A) is an inherited neuropathy with an estimated prevalence of 1/5000 in the general population. CMT1A results from abnormal dosage of the *PMP22* gene, arising from non-allelic homologous recombination (NAHR) events between paralogous sequences called CMT1A-REPs, flanking the gene. While extensively reported in Caucasian individuals, CMT1A remains undocumented in those of African genetic ancestry. We hypothesise this is due to differences in CMT1A-REP regions between individuals of African and non-African genetic background. Our objective is to study the variability of CMT1A-REPs between populations using available genetic data and in silico analyses.

Methods: Leveraging data from previous whole genome sequencing projects, we built a combined dataset, with a focus on capturing African genetic variation. Using this dataset, we explore haplotypes based on both common and rare SNPs, structural variants in CMT1A-REP regions, recombination-related elements, and their potential interactions, as well as their distribution across different populations.

Results: Individuals of African genetic ancestry harbour distinct CMT1A-REP haplotypes with lower recombination rates and reduced sequence similarity compared to other ethnicities. This suggests potential genetic variation protective against the NAHR-mediated events causing CMT1A. Additionally, recombination regulators like PRDM9 might differ between African and non-African ethnicities, hinting at distinct mechanisms at play.

Conclusion: Our study suggests genetic differences in CMT1A-REPs between African and non-African ancestries, potentially contributing to the lack of CMT1A reports in individuals of African genetic origin. However, further research is needed to fully understand the epidemiology of CMT1A in Africa and how CMT1A-REP variations influence disease risk.

Funding: AFM-Telethon (25078), La Caixa Foundation (LCF/BQ/PI21/11830012).

Conflict of Interest: None declared

P12.040.C Prenatal description of three cases of 5q SMA type 0 detected by exome sequencing

Anna Abuli[1], *Mar Costa-Roger*[1], *Marta Codina*[1], *Carlota Rodó*[2], *Laura Blasco-Pérez*[1], *Desirée Martínez-Cruz*[1], *Jordi Leno*[1], *Eduardo Tizzano*[2]

[1]Vall d'Hebron Barcelona Hospital, Department of Clinical and Molecular Genetics, Barcelona, Spain; [2]Vall d'Hebron Barcelona Hospital, Maternal-Fetal Medicine Unit, Department of Obstetrics, Barcelona, Spain

Background/Objective: Spinal muscular atrophy (SMA) is a recessive neuromuscular disorder characterized by progressive muscle atrophy with high phenotypic variability. Cases of the most severe type, SMA 0, are uncommon and prenatal manifestations, beyond loss of fetal movements, are barely described. This work reports the presentation of three fetuses with SMA type 0 within one family.

Methods: A couple was referred to our Hospital due to ultrasound findings at 31 weeks of gestation. A similar presentation was observed in the couple's two following pregnancies. All pregnancies were terminated. After negative arrayCGH results, exome sequencing (ES) was performed in all fetuses and parents, using ExomeDepth to evaluate CNVs. Results were validated by MLPA.

Results: Ultrasound findings included short corpus callosum, ventricular septal defect and foot edema in fetuses 1 and 3; and increased nuchal translucency and hydrops fetalis in fetus 2. Results from ES indicated a possible deletion in the *SMN* locus, which was corroborated by manual IGV revision and quantification of the paralogous sequence variant c.840C>T. MLPA confirmed 0 *SMN1* and 1 *SMN2* copy in all fetuses, allowing the molecular diagnosis of SMA type 0.

Conclusions: This work highlights the challenges of recognizing the fetal presentation of known genetic entities due to lack of knowledge of their prenatal signs. When congenital heart defects together with increased NT and fetal edema are present, SMA should be suspected. Although ES is not the gold-standard technique for SMA, targeted analysis of the SMN locus may guide the diagnosis in prenatal cases or atypical presentations.

Conflict of Interest: None declared

P12.041.D Validation of a Machine Learning tool for rapidly identifying FSHD patients

Claudia Strafella[1], *Domenica Megalizzi*[1;2], **Giulia Trastulli**[1;2], *Luca Colantoni*[1], *Emma Proietti Piorgo*[1], *Carlo Caltagirone*[3], *Raffaella Cascella*[1;4], *Emiliano Giardina*[1;2]

[1]Santa Lucia Foundation IRCCS, Genomic Medicine Laboratory-UILDM, Rome, Italy; [2]Tor Vergata University, Department of Biomedicine and Prevention, Rome, Italy; [3]Santa Lucia Foundation IRCCS, Department of Clinical and Behavioral Neurology, Rome, Italy; [4]Catholic University Our Lady of Good Counsel, Department of Biomedical Sciences, Tirana, Albania

Background/Objectives: FacioScapuloHumeral Dystrophy (FSHD) is a myopathy characterized by the loss of repressive epigenetic features affecting the *D4Z4* locus (4q35). The study aimed at validating a Machine Learning (ML) pipeline for discriminating FSHD subjects according to the DNA methylation profile of *D4Z4*.

Methods: 251 subjects with clinical signs of FSHD sent to our center during 2022-2023 were analyzed. The recruited subjects were tested for the 4q subtelomeric variant by means of specific PCR. Subjects carrying at least a 4qA allele underwent methylation levels analysis of two *D4Z4* regions. The identification of methylation profiles compatible with FSHD was performed by running a ML algorithm based on methylation levels. The presence of a short *D4Z4* allele and/or of pathogenic variants in FSHD genes was simultaneously evaluated.

Results: Among the 251 subjects, the 4q variant type distribution was 41% 4qA/4qA, 51% 4qA/4qB and 8% 4qB/4qB. Of the 232 carriers of at least a 4qA allele, the ML model predicted 122 subjects as FSHD in line with the reduced methylation levels compatible with the disease. The remaining 110 subjects were predicted as non-FSHD and showed high methylation levels. By comparing the results of *D4Z4* allele sizing, for 122 hypomethylated subjects the FSHD prediction was fully concordant with the presence of FSHD genetic signatures. Among the 110 subjects classified as non-FSHD, 85 were concordant with the absence of FSHD genetic alterations and 25 subjects displayed borderline methylation levels.

Conclusion: The present method emerges as a powerful tool to be implemented into the FSHD diagnostic practice.

Conflict of Interest: None declared

P12.042.A The diagnostic role of Next generation sequencing on Facioscapulohumeral muscular dystrophy (FSHD)

Beyza Yavuzcan[1], *Manar Kaptan*[1], *Serpil Eraslan*[2;3], *Şahin Avcı*[2;3], *İlker Eren*[3;4], *Gulshan Yunisova*[3;5], *Mehmet Demirhan*[3;4], *Piraye Oflazer*[3;5], *Hülya Kayserili*[2;3;6]

[1]*Koc University, Graduate School of Health Sciences, Istanbul;* [2]*Koc University Hospital, Genetic Diseases Evaluation Center, Istanbul, Türkyie;* [3]*Koc University Hospital, Muscle Diseases Center;* [4]*Koc University School of Medicine, Ortopedics and Traumatology Department, Istanbul, Türkyie;* [5]*Koc University School of Medicine, Neurology Department, Istanbul, Türkyie;* [6]*Koc University School of Medicine, Medical Genetics Department*

Background/Objectives: FSHD is a genetic neuromuscular disorder. D4Z4 macrosatellite repeat contractions on chromosome 4q35 constitute 95% of all cases as FSHD type 1. The remaining cases, FSHD type 2, the underlying genetic defect is pathogenic variants in associated genes such as *SMCHD1*, *DNMT3B*. Variants in these genes could potentially modulate the phenotype of FSHD1 patients with borderline D4Z4 contractions[1]. The diagnostic algorithm of FSHD involves the analysis of D4Z4 Repeat Units (RU) and the analysis of the FSHD associated genes to confirm/exclude diagnosis and distinguish cases that require differential diagnosis[2].

Methods: Molecular combing (MC) technology was utilized for the detection of D4Z4 RUs in 86 Turkish index patients. Whole exome sequencing (WES) was performed in 12 potential FSHD2 patients with no contractions or borderline RUs with their consent.

Results: MC revealed 71/86 patients with contracted 1-10 RUs. 5/71 patients had borderline 8-10 RUs and 14/86 patients had no contractions. Pathogenic mutations were observed in *SMCHD1* in 3 patients, one in non-contracted group and two in borderline group. *CAPN3* variants were identified in two patients, shifting the diagnosis to LGMD. In the remaining patients did not reveal any results.

Conclusion: Our findings largely coincide with literature and underscore the significance of additional testing. It is crucial to explore the impact of NGS to reveal related phenotypes and overlapping genetic factors that may contribute to the clinical heterogeneity of FSHD and its differential diagnosis.

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Conflict of Interest: None declared

P12.044.C Unveiling the clinical and molecular characteristics of a mild phenotype in McArdle Disease: A Case Study

Rocio Garrido-Moraga[1], *Pablo Serrano Lorenzo*[1;2], *Adrian González-Quintana*[1;2], *Alberto Blazquez Encinar*[1;2], *Tomàs Pinós*[2;3], *Alejandro Lucia*[4], *Alfredo Santalla*[5], *Gisela Nogales Gadea*[6], *Joaquín Arenas*[1;2], *Cristina Domínguez-González*[2;7;8], **Miguel A. Martin**[1;2;9]

[1]*Instituto de Investigación Sanitaria Hospital 12 de Octubre (imas12), Mitochondria and Neuromuscular Disorders Group, Madrid, Spain;* [2]*CIBERER, Madrid;* [3]*Vall Hebron Institut de Recerca, Neuromuscular and Mitochondrial Pathology Group, Barcelona, Spain;* [4]*Universidad Europea de Madrid, Faculty of Sport Sciences, Madrid, Spain;* [5]*Universidad Pablo de Olavide, 3Department of Computer and Sport Sciences, Sevilla, Spain;* [6]*Institut d'Investigacio en Ciencies de la Salut Germans Trias i Pujol Can Rubi, 4Grup de Recerca en Malalties Neuromusculars i Neuropediàtriques, Barcelona, Spain;* [7]*Instituto de Investigación Sanitaria Hospital 12 de Octubre (imas12), Mitochondrial and Neuromuscular Disorders Group, Madrid, Spain;* [8]*"12 de Octubre" University Hospital, Neurology. Neuromuscular Disorders., Madrid, Spain;* [9]*"12 de Octubre" University Hospital, Clinical and Molecular Genetics, Madrid, Spain*

Background/Objectives: McArdle disease (GSDV) is an autosomal recessive disease resulting from mutations in the *PYGM* gene, which encodes the muscle glycogen phosphorylase. The spectrum of this disorder ranges from moderate exercise intolerance to recurrent rhabdomyolysis or muscle weakness. Most patients exhibit undetectable levels of PYGM protein and enzyme activity. This study aims comprehensively characterize the clinical, biochemical and molecular features of a patient expressing a mild phenotype, harboring a homozygous deep intronic variant in the *PYGM* gene.

Methods: Exercise testing was conducted using cycle-ergometry. Genetic testing was performed by whole exome sequencing. Enzyme histochemistry and activity (spectrophotometry), PYGM expression (cDNA analysis by real-time PCR), and protein levels [western blot-(WB)] were studied in muscle biopsy.

Results: We identified a homozygous *PYGM*:c.425-26A>G variant in a 66-year-old male presenting with acute hyperCKemia and mild myalgia. This variant, previously reported in compound heterozygosis in one patient, presented an atypical clinical profile. Exercise testing was not consistent with those typically observed in McArdle patients. Histochemistry displayed normal staining, yet enzyme activity was very low (nearly 3% of controls) but significantly higher than in other PYGM patients. cDNA studies revealed a splicing defect and lower *PYGM* expression compared to controls and other GSDV patients. WB demonstrated undetectable PYGM protein levels.

Conclusion: Our findings support previous data indicating that the c.425-26A>G variant retains residual PYGM enzyme activity, sufficient to modulate the clinical phenotype, despite causing a gene expression defect and undetectable protein levels; and emphasizes the need for detailed molecular characterization in atypical presentations of GSDV.

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Conflict of Interest: Rocio Garrido-Moraga: None declared, Pablo Serrano Lorenzo: None declared, Adrian González-Quintana: None declared, Alberto Blazquez Encinar: None declared, Tomàs Pinós Principal Investigator. Spain, Alejandro Lucia Principal Investigator. Spain, Alfredo Santalla: None declared, Gisela Nogales Gadea Principal Investigator. Spain, Joaquín Arenas: None declared, Cristina Domínguez-González Principal Investigator. Spain., UCB Pharma, Miguel A. Martin Principal Investigator.Spain, Consultant UCB Pharma

P12.045.D DMD indel variant in exon 75 causes intellectual disability and congenital cataract in a family of four affected male patients

Mathilde Faurholdt Lauridsen[1], *Martin Sokol*[1], *Maria Rasmussen*[1;2]

[1]*Lillebaelt Hospital, University Hospital of Southern Denmark, Clinical Genetics, Vejle, Denmark;* [2]*University of Southern Denmark, Institute of Regional Health Research, Odense, Denmark*

Background/Objectives: Pathogenic variants in the *DMD* gene are known to cause X-linked Dystrophinopathies. The muscular dystrophies are sometimes accompanied by intellectual disability especially if the pathogenic variant is located in the distal part of the gene affecting most isoforms. Some of these isoforms are expressed in neuronal cells and fiber cells of the crystalline lens like the Dp71 isoform. Only very few patients have been described with pathogenic *DMD* variants primarily causing intellectual disability without muscular symptoms and only very rarely *DMD* variants have been associated with congenital cataract.

Methods: Trio whole genome sequencing and segregation analysis were performed in a family with four male patients affected by intellectual disability and congenital cataract.

Results: Trio whole genome sequencing revealed the indel variant *DMD*[NM_004006.3]:c.10751_10764delAACAGCTGGAGTCA, p.(Lys3584Thrfs*54) in the proband and his mother. Subsequently, segregation analyses found that the affected brother and maternal uncle also had the variant, whereas it was not found in two unaffected male family members. The variant is located in exon 75 and affects most isoforms of the *DMD* gene including Dp71 expressed in the brain and fiber cells of the crystalline lens.

Conclusion: Pathogenic frame-shift variants affecting isoforms primarily expressed in the brain and crystalline lens of the *DMD* gene are rarely described in the literature and should be considered a rare cause of intellectual disability and congenital cataract.

Grants:

Conflict of Interest: None declared

P12.046.A Biallelic variants in the *PLPP7* gene (Net39) cause a new neuromuscular disease in humans.

Katarína Kušíková[1], *Rene G. Feichtinger*[2], *Hans A. Mayr*[2], *Miriam Kolníková*[1], *Ognian Kalev*[3], *Serge Weis*[3], *Denisa Weis*[4]

[1]*Department of Pediatric Neurology, Faculty of Medicine, Comenius University Bratislava and National Institute of Children's Diseases, Bratislava, Slovakia;* [2]*University Children's Hospital, Salzburger Landeskliniken (SALK), Paracelsus Medical University (PMU) Salzburg, Salzburg, Austria;* [3]*Division of Neuropathology, Department of Pathology and Molecular Pathology, Neuromed Campus, Kepler University Hospital, Johannes Kepler University, Linz, Austria;* [4]*Department of Medical Genetics, Kepler University Hospital Med Campus IV, Johannes Kepler University, Linz, Austria*

Background/Objectives: The *PLPP7* gene (OMIM: *618743) encodes the nuclear envelope transmembrane protein 39 (Net39), which is crucial in muscle chromatin organization, gene expression, and myoblast differentiation. Loss of Net39 explored in vivo in Net39 knockout mice has been shown to cause juvenile mice lethality and muscle abnormalities consistent with Emery-Dreifuss muscular dystrophy. However, the Net39 deficiency phenotype has never been described in humans.

Methods: We present the first documented case report of an eight-year-old boy of consanguineus parents originating from Turkey with delayed motor development due to muscle weakness, positive Gowers's sign, gait disturbance, progressive muscle atrophy, but normal creatine kinase in blood.

Results: Muscle biopsy from vastus lateralis muscle showed fibrosis, endo-, peri- and epimysial lipomatosis, and numerous atrophic and hypertrophic fibers, corresponding to clearly pronounced myopathic changes. Karyotyping, array CGH, and NGS panels focused on neuromuscular disorders (NMDs) revealed no causal variants. WES analysis revealed a homozygous variant c.199G>A (p.Glu67Lys) in the *PLPP7* gene. This variant affects a conserved amino acid in the interaction domain of the membrane protein. Functional studies on patient muscles are currently ongoing.

Conclusion: *PLPP7* deficiency, previously considered to be a potential cause of neuromuscular phenotype in humans, is now reality and could be the cause of NMDs phenotype in undiagnosed patients. Therefore, we recommend to include the *PLPP7* gene in routine NGS neuromuscular panels.

Grants: Austrian Science Fund (FWF; I6478-B, GENOMIT to JAM)

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Conflict of Interest: None declared

P12.047.B Role of the *GFAP* gene in Alexander disease: in-depth statistical analyses using a large database of clinical signs and genetic variants

Francesca Rosamilia[1], *alice grossi*[2], *Silvia Carestiato*[3], *Marta Rusmini*[1,2], *Chiara Africano*[4], *Giada Recchi*[5], *Ettore Salsano*[6], *Isabella Ceccherini*[2], *Tiziana Bachetti*[7]

[1]*IRCCS Istituto Giannina Gaslini, Clinical Bioinformatics Unit - Scientific Direction, Genova, Italy;* [2]*IRCCS Istituto Giannina Gaslini, UOSD Laboratory of Genetics and Genomics of Rare Diseases, Genova, Italy;* [3]*University of Turin, Department of Medical Sciences, Torino, Italy;* [4]*University of Genova, Department of Neuroscience, Rehabilitation, Ophthalmology, Genetics and Maternal Child Sciences (DINOGMI), Genova, Italy;* [5]*IRCCS Giannina Gaslini Institute, UOC Rheumatology and Autoinflammatory Diseases, Genova, Italy;* [6]*Fondazione IRCCS Istituto Neurologico C. Besta, SC Malattie Neurologiche Rare, Milano, Italy;* [7]*IRCCS Ospedale Policlinico San Martino, Proteomics and Mass Spectrometry Unit, Genova, Italy*

Background/Objectives: Heterozygous mutations of the gene encoding glial fibrillary acidic protein (*GFAP*) are responsible for Alexander disease (AxD), a rare autosomal dominant astrogliopathy classified, based on age of onset (AOO), in infantile, juvenile and adult. Although some mutational hotspots along the *GFAP* gene appear to be preferentially associated with specific AOO, the final effect of most variants and the clinical course of their carriers is still unpredictable.

Methods: Starting from an extensive literature review, we created a database containing 550 AxD-associated *GFAP* mutations, including observations from in-house AxD molecular diagnosis. The characterization of these variants in terms of affected protein regions, amino acid change, heritability, CADD score, distance from splice site boundaries, and associated disease, including AOO and neuroradiological symptoms, were analyzed to draw a genotype-phenotype correlation.

Results: Numerous suggestive associations were observed, in particular almost half (49.6%) of variants involve an arginine codon, of which 45.8% occur in infantile cases, 34.4% of these latter being de-novo. Consistently, infantile cases have mutations with higher CADD values than adults. Considering the most frequent mutations (p.R239C, p.R79H, p.R88C, p.R79C, p.R416W, p.R239H), the associated clinical signs were used to identify mutation-specific enrichments. To confirm our results, we conducted several meta-analyses on specific variants across distinct patient categories.

Conclusion: The creation of a large database of *GFAP* variants offers a tool to investigate still neglected aspects of the variants role in the onset and progression of AxD, such as the genotype-phenotype correlation, the incomplete penetrance and variable expressivity of some variants, opening new perspectives in diagnosis and genetic counseling.

Conflict of Interest: None declared

P13

MULTIPLE MALFORMATION/ANOMALIES SYNDROMES

P13.001.C Alkuraya-Kučinskas syndrome *BLTP1* brain-conditional knock-out leads to severe disruption in neocortical lamination

Jacqueline Chrast[1], *Siwar Ben-Ayache*[2], *Stephan Collins*[2], *Catherine Roger*[1], *Hector Gallart Ayala*[3], *Séverine Lorrain*[4],

Manfredo Quadroni[4], Julijana Ivanisevic[3], Binnaz YALCIN[2], Alexandre Reymond[1]

[1]Université de Lausanne, Center for Integrative Genomics, Lausanne, Switzerland; [2]University of Burgundy, INSERM U1231 Center for Translational and Molecular medicine, Dijon, France; [3]Metabolomics Unit, Faculté de Biologie et de Médecine, Lausanne, Switzerland; [4]Université de Lausanne, Protein Analysis Facility, Lausanne, Switzerland

Background/Objectives: We previously described the Alkuraya-Kučinskas syndrome, a disorder associated with biallelic variants in *BLTP1* (bridge-like lipid transfer protein), a.k.a *KIAA1109*. Most probands die in utero or soon after birth, and surviving patients present with corpus callosum agenesis, ventriculomegaly and arthrogryposis. Homozygous ablation of mouse *Bltp1* resulted in similar preweaning lethality.

Methods: To bypass this lethality, we engineered *Emx1*-Cre-mediated conditional knockouts (cKO) in which *Bltp1* expression is only removed in cortical and hippocampal neurons.

Results: This restricted ablation of *Bltp1* recapitulated the preweaning lethality observed in the constitutional knockouts, suggesting that lack of expression of *BLTP1* in neurons is sufficient to cause death. We also observe a significant decrease of heterozygote pups' survival suggesting that hemizygous carrier fitness is impacted. Consistent with this hypothesis, *BLTP1* pLOEUF is 0.58. A single homozygous cKO survived to adulthood out of 21 expected. It presented reduced thickness of the cortical plate, complete agenesis of the corpus callosum, 5-fold size decrease of the anterior commissure, and a malformed hippocampus. Brain histology of 18.5-day cKO embryos similarly showed a thinning of the cortex, as well as a lack of defined structural layers with total absence of both *Pax6*- and *Tbr2*-expressing apical and intermediate progenitors, respectively.

Consistent, with the recent description of BLTP1 as a tube-forming lipid transport protein that allows rapid lipid transport between the endoplasmic reticulum and the plasma membrane, we found that it interacted with membrane-bound and vesicle proteins and a disruption of the lipidome in our cKO models.

Conclusion: We engineered a clinically-relevant model of Alkuraya-Kučinskas syndrome.

Grants:

Conflict of Interest: None declared

P13.002.D First Patient With Rasmussen Syndrome Caused By A Small Deletion Encompassing The TSHZ1 Gene

Britta Hanker[1], *Vincent Arriens[2], Malte Spielmann[1;2], Inga Nagel[2]*

[1]Institute of Human Genetics, University of Lübeck, Lübeck, Germany; [2]Institute of Human Genetics, University of Kiel, Kiel, Germany

Background/Objectives: To date, the genetic cause of Rasmussen syndrome has not yet been described. In 1979, Rasmussen reported a family with congenital bilateral vertical talus, bilateral atresia of the external auditory canal and increased interocular distance. Until now pathogenic variants in *TSHZ1* are only associated with atresia or stenosis of external auditory canal.

Methods: We report on a three-year-old girl with facial features including epicanthus, hypertelorism as well as a broad nasal root. She had hearing impairment and stenosis of the external auditory canal as well as rocker bottom feet. The mother presented with the same foot deformity and hearing impairment due to bilateral atresia of the auditory canal.

Results: Exome sequencing in the child revealed a heterozygous deletion of 210 kb encompassing *TSHZ1*. We identified the same deletion in the mother.

Conclusion: Systematic review of the literature showed that patients with mutations in *TSHZ1* show atresia of external auditory canal but vertical talus (rocker bottom feet) has not been described. Feenstra *et al*. reported in 2011 on families with deletions or mutations in *TSHZ1* of more than 2,5 Mb in 18q22.3 including *TSHZ1*, which showed vertical talus, too. The patient's phenotype overlaps with the clinical signs of a family with a heterozygous frameshift variant we presented in 2019. Therefore, our findings support the hypothesis that deletions encompassing *TSHZ1* or mutations in this gene cause Rasmussen syndrome with incomplete penetrance and variable clinical expressivity. Atresia or stenosis of external auditory canal and foot malformation and dysmorphic feature are a consistent sign in Rasmussen syndrome.

Conflict of Interest: None declared

P13.003.A Novel mutation c.564C>A in SNAP25 gene, revealed in a baby with prenatal abnormalities and postnatal death

Ivanka Dimova[1], *petya chaveeva[2], Kunka Kamenarova[1], Kalina Mihova[1], Darina Kachakova[1], Gergana Stancheva[1], Radka Kaneva[1]*

[1]Medical University Sofia, Molecular Medicine Center, Sofia, Bulgaria; [2]SAGBAL "Dr Shterev", Sofia, Bulgaria

Background/objectives: Deviations from fetal screening for congenital anomalies always raise serious concerns about the future health of the child. Here we report a case of a pathologically progressed pregnancy with persistently increased nuchal fold, intrauterine growth retardation (IUGR), oligohydramnios and increased resistance index in the umbilical artery. A chorionic biopsy with array CGH analysis was performed, with no aberrations found. After birth, the baby was hospitalized for respiratory distress syndrome; dysmorphic facies, pterygium colli, oblique eye slits, micrognathia; decreased muscle tone, hand contractures. At 1 year - severe retardation in physical and neuropsychological development, hospitalized due to severe shock state and ended with exitus letalis.

Method: Whole exome sequencing (using NovaSeq 6000, Illumina) of the isolated child's DNA was performed searching for the possible genetic etiology of the condition.

Results: A novel genetic variant c.564C>A (p.Asn188Lys) in *SNAP25* gene was discovered and classified as likely pathogenic, based on the established association between the gene and the patient's phenotype, the absence of the variant in control populations, and in silico predicted pathogenicity. The parents were tested for the carriership and they didn't carry the variant. Mutations in *SNAP25* gene are associated with autosomal dominant congenital myasthenic syndrome 18 (CMS18).

Conclusion: CMS18 is a presynaptic neuromuscular disorder characterized by early-onset muscle weakness with delayed psychomotor development and ataxia. Here we report de novo occurrence of mutation for this syndrome with fatal outcome. Our case demonstrates non-specific, but markable prenatal presentation of the syndrome with IUGR, increased nuchal fold and oligohydramnios.

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Conflict of Interest: None declared

P13.004.B A Pfeiffer phenotype in association with the common Apert FGFR2 S252W mutation: A clinical report and likely molecular explanation

Ramy Saad[1], *Caitlin Barns-Jenkins*[1], *Claire Lawn*[1], *Gregory James*[2], *Noor ul Owase Jeelani*[2], *Andrew Wilkie*[3], *Louise Wilson*[1]

[1]*North East Thames Regional Genetics Service, Great Ormond Street Hospital for Children NHS Foundation Trust, London, UK;* [2]*UCL Great Ormond Street Institute of Child Health and Craniofacial Unit, Great Ormond Street Hospital for Children, London, UK;* [3]*MRC Weatherall Institute of Molecular Medicine, University of Oxford, Oxford, UK; Oxford Centre for Genomic Medicine, Oxford University Hospitals NHS Foundation Trust, Oxford, UK*

Background/Objectives: Apert Syndrome (AS) and Pfeiffer Syndrome (PS) result from different heterozygous FGFR2 missense variants. While the craniofacial features overlap, AS is characterised by symmetrical cutaneous and bony 'mitten' syndactyly of all 4 limbs, with complete syndactyly of the 3 central rays and variable involvement of the first and fifth rays. PS is characterised by broadened deviated thumbs and halluces with variable minor skin syndactyly.

There have been occasional reported cases of a PS phenotype in association with a common AS variant, for which no molecular mechanism has been elucidated.

Methods: We investigated an 18-year-old patient with brachyturricephaly, right coronal craniosynostosis, hypertelorism, exorbitism, mid-face hypoplasia, mildly broadened deviated halluces, wide sandal gaps and right 2/3 cutaneous toe syndactyly. Her forearm movements and hands were normal. Her phenotype is consistent with PS. Genetic testing was performed on lymphocyte DNA by Next Generation Sequencing of a gene panel comprising *FGFR1, FGFR2, FGFR3, EFNB1, TCF12, ERF* and *TWIST1*.

Results: We identified a heterozygous *FGFR2* c.755C>G p.(Ser252Trp) pathogenic missense variant, which is the commonest cause of AS. A second novel missense variant (Gly261Arg) was identified *in cis*.

AlphaMissense modelling suggests that G261R is likely pathogenic for loss of function (LOF) (Score: 0.993). Substitution of the equivalent residue in FGFR1 is associated with LOF and Kallmann syndrome.

Conclusion: We provide evidence that the second variant ameliorates the gain of FGFR2 function associated with the AS substitution resulting in the milder phenotype. Our findings provide further insight into the mechanism of the severe syndactyly in AS.

Grants:

Conflict of Interest: None declared

P13.005.C Phenotypic descriptions of individuals with pathogenic variations of *PPP1R12A* gene

Océane COUDRIEU[1;2], *Alice Goldenberg*[3], *Christine Francannet*[1], *Fanny Laffargue*[1], *Laïla El Khattabi*[4], *Céline Richard*[5], *Isabelle Creveaux*[6], *Cordula Knopp*[7], *Bert Callewaert*[8], *Florence Petit*[9], *Simon Boussion*[9], *Catherine Sarret*[10], *Aurelien JUVEN*[1;2]

[1]*CHU Estaing, Medical Genetics Department, Clermont-Ferrand, France;* [2]*Université Clermont Auvergne, Clermont-Ferrand, France;* [3]*CHU de Rouen, Medical Genetics Department, Rouen, France;* [4]*Sorbonne Université - Hôpitaux Armand Trousseau et Pitié-Salpêtrière, Département de Génétique Médicale - DMU BioGeM, Paris, France;* [5]*CHU Estaing, Cytogenetics department, Clermont-Ferrand, France;* [6]*CHU Gabriel Montpied, Molecular Biology department, Clermont-Ferrand, France;* [7]*Institute for Human Genetics and Genomic Medicine, Aachen, Germany;* [8]*Ghent University Hospital, Center for Medical Genetics, Gent, Belgium;* [9]*Hopital Jeanne de Flandre, CHU Lille, Genetics Department, LIlle, France;* [10]*CHU Estaing, Pediatric Department, Clermont-Ferrand, France*

Background/Objectives: The PPP1R12A gene, located at position 12q21.2-q21.31, encodes a protein also known as MYPT1 and serves as a subunit of myosin phosphatase. It regulates interactions between myosin and actin and plays a crucial role in controlling cell morphology and contractility of smooth muscle cells. Pathogenic variations in PPP1R12A have been associated with various congenital malformations of the urogenital system, the digestive system, brain malformations, and neurodevelopmental disorders. To our knowledge, only 18 patients have been described in the literature.

Methods: Following a European call for collaboration, we collected patients presenting pathogenic variation in PPP1R12A identified by CGH Array, WES and WGS.

Results: We have identified an additional 7 individuals, including sporadic and familial cases. The pathogenic variations found in our patients all resulted in loss of function, similar to those described in the literature. The previously mentioned digestive or urogenital malformations are also observed in most patients in our cohort. Among our 7 patients, only one exhibited brain malformations, consisting of mild cortical atrophy and nodular heterotopias, which did not lead to intellectual disability. Notably, in 4 of our patients, the variation was inherited from a seemingly pauci- or asymptomatic parent, who may only exhibit subtle facial morphological characteristics such as narrow palpebral fissures and a high forehead.

Conclusion: Our results highlight a significant variability in expression within this phenotypic spectrum. It would be important to be able to describe a larger number of individuals in order to refine our understanding of the phenotype and potentially clarify the genotype-phenotype relationship.

Grants:

Conflict of Interest: None declared

P13.006.D Biallelic variants in centrosome linker component LRRC45 cause a novel ciliopathy

Radhakrishnan Periyasamy[1], *Neha Quadri*[1], *Florian Erger*[2], *Iba Kordorkhyriem*[1], *Priyanka Upadhyai*[1], *Anju Shukla*[1]

[1]*Kasturba Medical College, Manipal, Department of Medical Genetics, Udupi, India;* [2]*Institute of Human Genetics and Center for Rare Diseases Cologne, University Hospital Cologne and University of Cologne, Germany*

Background: Recently, biallelic variants in *LRRC45* have been reported in individuals from two unrelated families. Developmental delay and intellectual disability occurred in one family, while rod-cone retinal dystrophy was described in the other.

Methods: A detailed clinical and radiological evaluation followed by genetic testing was performed in probands from families 1 (P1) and 2 (P2&P3). Functional characterization of the candidate variant in P1 was performed using patient-derived skin fibroblasts.

Results: P1 is a 3-year-old female who presented with developmental delay, seizures, visual impairment, and dysmorphism. Cranial MRI revealed polymicrogyria, malformed brainstem, periventricular heterotopia, thin corpus callosum, and cerebellar vermian hypoplasia. Exome sequencing identified the homozygous variant c.1402-2A>G in *LRRC45* (NM_144999.4). The variant c.1402-2A>G triggers aberrant splicing, the resultant LRRC45 transcript lacks exon 14. The mRNA and protein levels of LRRC45 were drastically reduced in fibroblast cells. The number of ciliated cells in P1 was significantly reduced.

P2 presented with respiratory insufficiency, hypotonia, swallowing difficulty, club feet, and ventricular septal defect. Cranial MRI

revealed pachygyria, ventriculomegaly, corpus callosum hypoplasia, cerebellar and vermian hypoplasia. A subsequent affected fetus (P3) from the same family had polyhydramnios and similar CNS malformations. Exome sequencing identified the homozygous variant c.1262G>C (p.Arg421Thr) in *LRRC45* in P2 and P3.

Conclusion: Leucine-Rich Repeat-Containing Protein 45 serves as a centrosome proteinaceous linker and plays a role in centrosome cohesion and ciliogenesis. We propose that pathogenic variants in *LRRC45* are associated with a novel spectrum of ciliopathy phenotypes.

Grants: Centre for Rare Disease Diagnosis, Research and Training-IA/CRC/20/1/600002:DBT/Wellcome Trust India Alliance.

Conflict of Interest: Radhakrishnan Periyasamy Full time, Neha Quadri: None declared, Florian Erger: None declared, Iba Kordorkhyriem: None declared, Priyanka Upadhyai PI, Full time, Anju Shukla PI, Full time

P13.007.A Genotype-phenotype correlations point to the emerging *KDM2B*-associated syndrome caused by variants in the CxxC domain

Amber van Oirsouw[1], *Michael Hadders*[2], *Edith Peters*[1], *Thirza Verhoeff*[1], *Stefan Barakat*[3], *Arjan Bouman*[3], *Marie-Noëlle Bonnet-Dupeyron*[4], *Mathilde Pujalte*[5], *Louis Januel*[5], *Phillip Boone*[6], *Casie Genetti*[6;7], *Leslie Dunnington*[8], *Laura Farach*[8], *Bertrand Isidor*[9], *Benjamin Cogne*[9], *Richard van Jaarsveld*[1], *Renske Oegema*[1]

[1]*University Medical Center Utrecht, Department of Genetics, Utrecht, Netherlands;* [2]*University Medical Center Utrecht, Utrecht University, Oncode Institute and Center for Molecular Medicine, Utrecht, Netherlands;* [3]*Erasmus MC University Medical Center, Department of Clinical Genetics, Rotterdam, Netherlands;* [4]*Centre Hospitalier de Valence, Consultations de Génétique, Valence, France;* [5]*Hospices Civils de Lyon, Department of Genetics, Lyon, France;* [6]*Boston Children's Hospital, Division of Genetics and Genomics, Boston, United States;* [7]*Boston Children's Hospital, Manton Center for Orphan Disease Research, Boston, United States;* [8]*Houston McGovern Medical School, UT Health Science Center, Houston, United States;* [9]*Centre Hospitalier Universitaire de Nantes, Nantes, France*

Background/Objectives: Heterozygous pathogenic *KDM2B* variants are associated with a novel neurodevelopmental disorder. *KDM2B* encodes the epigenetic regulator lysine-demethylase 2B (KDM2B), which has histone-modifying activity through its JmjC domain and DNA-binding ability through its CxxC domain. Both loss-of-function and missense variants have been identified in individuals with neurodevelopmental delay, autism/ADHD, congenital organ anomalies and facial dysmorphism. Missense variants appeared to cluster in the CxxC domain. Our goal is to further characterize this *KDM2B*-related disorder, including its molecular mechanisms and genotype-phenotype correlations.

Methods: We expanded our patient cohort of individuals with heterozygous *KDM2B* variants through GeneMatcher and personal communication. To assess DNA binding ability of 8 CxxC variants, we performed electrophoretic mobility shift assays (EMSAs).

Results: We collected data from 34 individuals with *KDM2B*-associated syndrome, including 14 individuals with 11 CxxC variants. Individuals with CxxC variants have a higher frequency of de novo occurrence and congenital organ anomalies compared to those with other variants. Cardiovascular anomalies are observed in 11/14, most commonly atrial septal defect. Ophthalmological and urogenital anomalies are both present in 6/14.

EMSA analysis shows 7/8 CxxC variants have a significantly decreased DNA-binding ability compared to wildtype.

Conclusion: Our study highlights the CxxC domain as a mutational hotspot and the excess of organ anomalies associated with CxxC variants. Our results indicate a distinct mechanism of CxxC variants through a dominant-negative mechanism as opposed to a loss-of-function effect in other patients. To further investigate the role of the CxxC domain during early development, we have created a patient-derived iPSC model.

Grants: Wilhelmina Kinderziekenhuis Research Fund

Conflict of Interest: None declared

P13.009.C Wiedemann-Steiner syndrome: clinical and molecular characterization of six Brazilian patients

Ana Mondadori dos Santos[1], *Caio Graco Bruzaca*[2], *Luciana Cardoso Bonadia*[3], *Carmen Silvia Bertuzzo*[3], *The Rare Genomes Project Consortium*[4], **Carlos Eduardo Steiner**[3]

[1]*São Leopoldo Mandic, Campinas Unit, Campinas, Brazil;* [2]*Hospital Municipal Infantil Menino Jesus, Sao Paulo, Brazil;* [3]*UNICAMP Universidade Estadual de Campinas, Translational Medicine, Campinas, Brazil;* [4]*Albert Einstein Israelite Hospital, Laboratory Medicine, Sao Paulo, Brazil*

Consortium: The Rare Genomes Project Consortium

Background/Objectives: Wiedemann-Steiner syndrome (WDSTS, MIM #605130) is neurodevelopmental disorder also characterized by hypertrichosis, short stature, and facial dysmorphisms. It follows autosomal dominant inheritance due to heterozygous variants in the *KMT2A* gene. This study describes six patients with WDSTS confirmed by molecular tests and compares clinical and molecular findings, characterizing the individuals in typical or atypical phenotypes.

Methods: Patients were seen in a Reference Center for Rare Diseases. Molecular investigation by target gene sequencing or next-generation sequencing had been previously carried out. All patients were seen by the same clinical geneticist and were classified as typical (classic) or atypical (expanded) phenotypes based on the descriptors OMIM, Genereviews, and two primary international studies.

Results: Of the six patients (3M:3F), four have the typical phenotype, including the patient from the original description by Steiner and Marques (2000), and two were considered atypical due to the absence of either growth deficiency and/or the characteristic facies. Short stature and hypertrichosis were found in four patients, and developmental delay in five. Most variants were novel and caused by frameshift mutations, and recurrence of the same variant was identified only in a pair of monozygotic twins. Five variants were novel, and only one variant was previously described, showing no concordance of clinical presentation between both individuals.

Conclusion: In addition to contributing to the expanded phenotypic description of WDSTS, the present study comprised a local sample with typical and atypical phenotypes that represented the findings in the Brazilian population.

Conflict of Interest: None declared

P13.010.D KDM2B-associated syndrome can present with macroglossia and other Beckwith- Wiedemann syndrome features

Renske Oegema[1], *Amber van Oirsouw*[2;2], *Nour Elkhateeb*[3], *Sarju Mehta*[3], *Leslie Dunnington*[4], *Laura Farach*[4], *Richard van Jaarsveld*[2], *Marielle Alders*[5]

[1]*University Medical Center Utrecht, Genetics, Utrecht, Netherlands;* [2]*University Medical Center Utrecht, Department of Genetics, Utrecht, Netherlands;* [3]*Cambridge University Hospitals NHS Foundation Trust, Department of Clinical Genetics, Cambrigde, United Kingdom;* [4]*McGovern Medical School at the University of Texas Health Science Center at Houston (UTHealth Houston) and Children's Memorial*

Hermann Hospital, Division of Medical Genetics, Department of Pediatrics, Houston, United States; [5]Amsterdam University Medical Center, University of Amsterdam, Amsterdam Reproduction and Development Research Institute, Department of Human Genetics, Amsterdam, Netherlands

Background: *KDM2B*, encoding the epigenetic regulator Lysine-demethylase 2B, is associated with a neurodevelopmental syndrome characterized by developmental delay and/or intellectual disability, behavioral challenges, and congenital organ anomalies. KDM2B acts as a histone demethylase through its JmjC domain as well as protects the DNA from hypermethylation through its DNA-binding CxxC domain. Recently, we have shown in a collaborative effort that affected individuals show a distinct episignature with a strong hypermethylated profile. Previously, an individual with a deletion of 12q24.31 including *KDM2B* was reported who had features of Beckwith – Wiedemann syndrome (BWS): high birth weight (p90-p95), macroglossia, and hyperinsulinism. We wanted to further explore an overlap between these syndromes and hypothesized that KDM2B influences methylation of the BWS 11p15 locus.

Methods: We searched our KDM2B cohort and the literature for additional cases. We tested 11p15 methylation in available patient samples of KDM2B patients with and without BWS features.

Results: We identified 34 individuals with KDM2B-associated syndrome of which 7 (21%) had BWS features, macroglossia was present in all seven individuals. Four of them fulfill the clinical criteria for classical BWS. Although both BWS and macroglossia are associated with an increased pediatric cancer risk, malignancies were not reported in this cohort. 11pq15 methylation was normal in all tested patient samples.

Conclusion: KDM2B-associated syndrome should be included in the differential diagnosis of individuals with BWS features, especially macroglossia, and normal 11p15 methylation. Further studies are needed to explore the underlying mechanisms, which possible involve somatic 11p15 methylation events or epigenetic disturbances of other loci.

Grants: WKZ Research Fund to RO

Conflict of Interest: None declared

P13.011.A MED13L syndrome: Contribution of the GenIDA database to patient phenotyping

Roseline caumes[1], *Thomas Smol*[2], *Pauline Burger*[3], *Jean Louis Mandel*[4], *Jamal Ghoumid*[1]

[1]CHU Lille, Clinique de Génétique, Lille, France; [2]CHU Lille, Institut de Génétique Médicale, Lille, France; [3]Université de Strasbourg, INSERM U1258, CNRS UMR7104, GenIDA, IGBMC, Illkirch, France; [4]Université de Strasbourg, Institute for Advanced Studies of the University of Strasbourg (USIAS), strasbourg, France

Background : MED13L pathogenic variations cause the so-called MED13L-syndrome. The condition is characterized by a developmental delay in both motor and language acquisition, leading to a mild to severe intellectual disability (ID). A majority of patients may also exhibit malformations, particularly in the cardiac or skeletal system. GenIDA international project aims to improve the knowledge and management of rare genetic diseases.

Methods : The present study uses standardized HPO terms to comprehensively phenotype individuals' descriptions extracted from the literature (100 patients) and to compare patient phenotypic data from the GenIDA database. Additionally, by comparing variant identified in patients reported in the literature, we aim to identify potential phenotype-genotype correlation.

Results : There was no significant difference in the phenotype description between individuals from the GenIDA cohort and individuals from the literature. Nevertheless, the results highlight that developmental delay, dysmorphic features, and cardiac defects are predominantly described in the cohort based on scientific reports, whereas sensory defects, walking difficulties, and limb malformations are more emphasized in reports from families.

Further analysis comparing patients with missense variants to those with loss-of-function variants revealed statistically significant differences in four criteria : absence of speech, lack of independent walking, epilepsy, and cardiopathy were more prevalent in individuals with missense variations.

Conclusion : GenIDA provided a reliable description of patients with MED13L syndrome, in

emphasizing items few described in the literature. Patients with missense variants presented statistically more pronounced neurological symptoms than those with loss-of-function variants.

Conflict of Interest: None declared

P13.012.B Myhre syndrome in adulthood: clinical variability and emerging genotype-phenotype correlations

Eva Vanbelleghem[1;2], *Tim Van Damme*[1], *Aude Beyens*[1;2], *Sofie Symoens*[1;2], *Kathleen Claes*[1;2], *Julie De Backer*[1;3], *Ilse Meerschaut*[4], *Floris Vanommeslaeghe*[5], *Sigurd Delanghe*[5], *Jenneke Van den Ende*[6], *Tessi Beyltjens*[6], *Eleanor Scimone*[7], *Mark Lindsay*[8;9], *Lisa Schimmenti*[10], *Alicia Hinze*[11], *Emily Dunn*[12], *Natalia Gomez-Ospina*[12], *Isabelle Vandernoot*[13], *Thomas Delguste*[13], *Sandra Coppens*[13], *Valérie Cormier-Daire*[14], *Marco Tartaglia*[15], *Livia Garavelli*[16], *Joseph Shieh*[17], *Şenol Demir*[18], *Esra Arslan Ateş*[19], *Martin Zenker*[20], *Mersedeh Rohanizadegan*[21], *Greysha Rivera-Cruz*[22], *Sofia Douzgou HOUGE*[23], *Angela Lin*[7], *Bert Callewaert*[1;2]

[1]Ghent University Hospital, Center for Medical Genetics, Gent, Belgium; [2]Ghent University, Department of Biomolecular Medicine, Gent, Belgium; [3]Ghent University Hospital, Department of Cardiology, Gent, Belgium; [4]University Hospital Brussels, Department of pediatric cardiology, Brussels, Belgium; [5]Ghent University Hospital, Department of Nephrology, Gent, Belgium; [6]Antwerp University Hospital and University of Antwerp, Department of Medical Genetics, Antwerp, Belgium; [7]MassGeneral Hospital for Children, Department of Pediatrics, Genetics Unit, Boston, United States; [8]Massachusetts General Hospital, Cardiovascular Genetics Program, Cardiology Division, Boston, United States; [9]Massachusetts General Hospital, Pediatric Cardiology Division, Boston, United States; [10]Mayo Clinic, Department of Clinical Genomics, Rochester, United States; [11]Mayo Clinic, Division of Rheumatology, Rochester, United States; [12]Stanford University, Department of Pediatrics, Division of Medical Genetic, Stanford, United States; [13]Hôpital Universitaire de Bruxelles, Université Libre de Bruxelles, ULB Center of Human Genetics, Brussels, Belgium; [14]Necker-Enfants Malades Hospital, Paris Cité University, Centre of Reference for Constitutional Bone Diseases (MOC), Department of Genetics, Paris, France; [15]Bambino Gesù Children's Hospital, Molecular Genetics and Functional Genomics, Roma, Italy; [16]Azienda USL - IRCCS di Reggio Emilia, Medical Genetics Unit, Reggio Emilia RE, Italy; [17]University of California, San Fransisco, Institute for Human Genetics and Department of Pediatrics, San Francisco, United States; [18]Marmara University, Department of Medical Genetics, School of Medicine, Istanbul; [19]Istanbul University, Department of Medical Genetics, Faculty of Medicine, Istanbul, Türkyie; [20]University Hospital Magdeburg, Institute of Human Genetics, Magdeburg, Germany; [21]University of Pennsylvania, Department of Medicine, Division of Translational Medicine & Human Genetics, Philadelphia, United States; [22]Stanford University School of Medicine, Division of Reproductive Endocrinology and Infertility, Stanford, United States; [23]Haukeland University hospital, Department of Medical Genetics, Bergen, Norway

Background/Objectives: Myhre syndrome (MS, MIM 139210) is a rare multisystemic disorder caused by recurrent pathogenic missense variants in *SMAD4*. The clinical features have been mainly documented in childhood and comprise variable neurocognitive development, recognizable craniofacial features, short stature, hearing loss, thickened skin, joint limitations, diverse cardiovascular and airway manifestations, and increased fibrosis often following trauma or surgery. Guidance for the medical management of adults with MS is currently lacking.

Methods: We describe 24 adults with MS and review the literature on adults with MS.

Results: Overall, our cohort shows a milder phenotype as well as lower mortality rates compared to what has been published in literature. Individuals with a codon 500 variant in *SMAD4* present with a more pronounced phenotype for both altered neurodevelopment as well as systemic manifestations. However, in contrast to the literature, we observe cardiovascular abnormalities in individuals with the p.(Arg496Cys) variant. In addition, we describe scoliosis as a new manifestation and we report fertility in two additional males with the p.(Arg496Cys).

Conclusion: Our study contributes novel insights into the clinical variability of MS and underscores the importance of variant-specific considerations. We provide recommendations for the management of MS in adulthood.

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Conflict of Interest: None declared

P13.013.C Biallelic variants in *BRF2* are associated with perinatal death and craniofacial anomalies

Francesca Mattioli[1], *Anne Hebert*[1], *Sissy Bassani*[1], *Rún Friðriksdóttir*[2], *Nazia Ibrahim*[1;3], *Shagufta Naz*[3], *Ásmundur Oddsson*[2], *Patrick Sulem*[2], *Gisli Halldórsson*[2;4], *Páll Melsted*[2;4], *Daniel Guðbjartsson*[2;4], *Flavia Palombo*[5], *Tommaso Pippucci*[6], *Nayereh Nouri*[7], *Marco seri*[6], *Emily Farrow*[8;9], *Carol J Sauders*[8;10], *Nicolas Guex*[11], *Muhammad Ansar*[12;13], *Kári Stefánsson*[2;14], *Alexandre Reymond*[1]

[1]*Center for Integrative Genomics - UNIL, Lausanne, Switzerland;* [2]*deCODE genetics/Amgen Inc, Reykjavik, Iceland;* [3]*Lahore College for Women University, Lahore, Pakistan;* [4]*University of Iceland, School of Engineering and Natural Sciences, Reykjavik, Iceland;* [5]*IRCCS Istituto delle Scienze Neurologiche, Bologna, Italy;* [6]*IRCCS Azienda Ospedaliero-Universitaria di Bologna, Bologna, Italy;* [7]*Isfahan University of Medical Sciences, Craniofacial and Cleft Research Center, Isfahan, Iran;* [8]*Children's Mercy Research Institute, Genomic Medicine Center, Kansas City, United States;* [9]*University of Missouri, School of Medicine, Kansas City, United States;* [10]*Children's Mercy, Department of Pathology and Laboratory Medicine, Kansas City, United States;* [11]*University of Lausanne, Bioinformatics Competence Center, Lausanne, Switzerland;* [12]*University of Lausanne, Department of Ophthalmology, Lausanne, Switzerland;* [13]*Jules-Gonin Eye Hospital, Lausanne, Switzerland;* [14]*University of Iceland, Faculty of Medicine, School of Health Sciences, Reykjavik, Iceland*

Background/Objectives: Large-scale sequencing among 1.52 million healthy individuals allows exploring genetic of recessive lethality, for example, a significant underrepresentation of double transmission of a protein-altering *BRF2* splicing variant. *BRF2* encodes a subunit of the RNA-polymerase III complex whose subunits were associated with autosomal recessive disorders, such as Treacher-Collins syndrome.

Methods: We combined NGS with functional analyses to investigate the effects of *BRF2* variants.

Results: We identified three pairs of Icelandic parents from the early 20th century heterozygous for this *BRF2* splicing variant. Five of their seventeen offsprings died before 3 months of age, while all surviving siblings are heterozygous. Using data aggregation, we identified seven more individuals worldwide from three unrelated families carrying biallelic variants in *BRF2*. Affected individuals present a variable phenotype ranging from severe craniofacial anomalies with early death to intellectual disability with motor and speech development, depending on the combination of the variants, *i.e.* homozygous stop-gain, truncation paired with missense in the DNA-binding domain, homozygous missense in the zinc-finger domain that recruits the polymerase III subunit, respectively. In silico 3D modelling and functional analyses suggested functional impairment of the identified variants. Zebrafish knocked-down for the orthologous *brf2* presented with abnormal escape response, reduced swimming velocity and head malformations, which could be complemented by the human wild-type but not mutated BRF2 mRNA further demonstrating their deleteriousness.

Conclusion: Our results show that *BRF2* biallelic alterations are associated with early death. We provide a new link between brain anomalies and RNA polymerase III subunits and its targets.

Grants:

Conflict of Interest: Francesca Mattioli: None declared, Anne Hebert: None declared, Sissy Bassani: None declared, Rún Friðriksdóttir deCODE genetics/Amgen Inc., Nazia Ibrahim: None declared, Shagufta Naz: None declared, Ásmundur Oddsson deCODE genetics/Amgen Inc., Patrick Sulem deCODE genetics/Amgen Inc., Gisli Halldórsson deCODE genetics/Amgen Inc., Páll Melsted deCODE genetics/Amgen Inc., Daniel Guðbjartsson deCODE genetics/Amgen Inc., Flavia Palombo: None declared, Tommaso Pippucci: None declared, Nayereh Nouri: None declared, Marco seri: None declared, Emily Farrow: None declared, Carol J Sauders: None declared, Nicolas Guex: None declared, Muhammad Ansar: None declared, Kári Stefánsson deCODE genetics/Amgen Inc., Alexandre Reymond: None declared

P13.014.D *ELMO2*-related Intraosseous Vascular Malformation: Report of four new cases with novel pathogenic variants, clinical follow-up and therapeutic approaches

Mert Karakaya[1], *iman ragab*[2], *Vera Riehmer*[1], *Florian Erger*[1], *Nihal Hussien Aly*[2], *Seung Woo Ryu*[3], *Go Hun Seo*[3], *Anne Schultheis*[4], *Marc Hoemberg*[5], *Boris Decarolis*[5], *Christian Netzer*[1]

[1]*Institute of Human Genetics, Faculty of Medicine and University Hospital Cologne, Cologne, Germany;* [2]*Ain Shams University Cairo, Pediatric Hospital, Hematology Oncology Unit, Cairo, Egypt;* [3]*Division of Medical Genetics, 3billion, Inc., Seoul, Korea, Rep. of South;* [4]*Institute of Pathology, Faculty of Medicine and University Children's Hospital of Cologne, Cologne, Germany;* [5]*Department of Pediatric Oncology, Faculty of Medicine and University Children's Hospital of Cologne, Cologne, Germany*

Background/Objectives: Primary intraosseous vascular malformation (VMPI) is an ultra-rare disorder characterized by severe intraosseous vascular malformations with autosomal-recessive inheritance. It is caused by biallelic germline loss-of-function variants in *ELMO2*. Therapeutic options are limited, often involving interventions such as sclerotherapy and radical surgical procedures, with significant risks and complications.

Methods: We present four unreported patients with VMPI. Genetic analysis was performed using whole exome sequencing, revealing pathogenic variants in *ELMO2*. Additionally, an extensive histopathological analysis was conducted in one patient. Clinical follow-up from prenatal diagnosis onwards, along with novel pharmacotherapeutic approaches, was evaluated.

Results: Clinical presentations of the affected individuals were consistent with VMPI. Genetic analysis identified homozygous

variants in *ELMO2*, including a novel homozygous 25bp-duplication (c.579_603dup). Therapeutic approaches included coil embolization, sclerotherapy, as well as pharmacotherapeutic agents, such as propranolol, sirolimus, and bisphosphonates. Notably, the pharmacotherapeutic strategies reported in our study represent novel approaches in the management of VMPI. Coil embolization addressed acute bleeding episodes, while pharmacotherapies showed variable responses and associated adverse effects. One patient, who was prenatally diagnosed, showed milder symptoms without any therapy to date.

Conclusion: VMPI remains a challenging and life-threatening condition with limited therapeutic options. Early genetic diagnosis enables preventive measures, yet the optimal time for intervention remains uncertain. This report contributes insights into the clinical, genetic and histopathological spectrum, expanding our understanding of VMPI. We underscore the importance of close monitoring, novel therapeutic strategies, and comprehensive care approaches. *ELMO2's* role in VMPI pathogenesis remains elusive, which is crucial to reveal for developing targeted therapies.

Grants:

Conflict of Interest: None declared

P13.015.A Acytokinetic mitosis results in impaired neurogenesis and erythropoiesis in *Mcph1* knockout mice

Yoann Vial[1;2;3], *Jeannette Nardelli*[2], *Adeline Bonnard*[1;3], *Justine Rousselot*[1], *Michele Souyri*[3], *Pierre Gressens*[2], *Hélène Cavé*[1;3], *severine drunat*[1;2]

[1]*Robert Debré Hospital - APHP, Genetics, Paris, France;* [2]*Neurodiderot, Paris, France;* [3]*Research Institute Saint Louis Université De Paris, Paris, France*

Background/Objectives: Microcephaly is a common feature of inherited bone marrow failure syndromes observed in 27% and 9% of patients with Fanconi anemia and dyskeratosis congenita, respectively. However, whether there are common fundamental pathways that are critical for both neuronal and hematopoietic development remains to be explored.

Methods: Using a well-characterized *Mcph1* knockout mouse model specifically developed for microcephaly, we examined erythropoietic abnormalities at birth and during fetal development by flow cytometry. Dysregulated pathways were identified by RNA-seq analysis. To validate our findings at the protein level, we performed Western blot and immunofluorescence analyses. The same methods were used to study *Mcph1* knockout fetal brains.

Results: Our research revealed that *Mcph1*-knockout mice exhibited congenital macrocytic anemia due to impaired terminal erythroid differentiation during fetal development. Anemia's cause is a failure to complete cell division, evident from tetraploid erythroid progenitors with DNA content exceeding 4n. Gene expression profiling demonstrated activation of the p53 pathway in *Mcph1*-deficient erythroid precursors, leading to overexpression of *Cdkn1a/p21*, a major mediator of p53-dependent cell cycle arrest. Surprisingly, fetal brain analysis revealed hypertrophied binucleated neuroprogenitors overexpressing p21 in *Mcph1*-knockout mice, indicating a shared pathophysiological mechanism underlying both erythroid and neurological defects. However, inactivating p53 in *Mcph1*$^{-/-}$ mice failed to reverse anemia and microcephaly, suggesting that p53 activation in *Mcph1*-deficient cells resulted from their proliferation defect rather than causing it.

Conclusion: These findings shed new light on Mcph1's function in fetal hematopoietic development, emphasizing the impact of disrupted cell division on neurogenesis and erythropoiesis - a common limiting pathway.

Conflict of Interest: None declared

P13.016.B Etiology of congenital thyroid dysfunction in Down syndrome

Peter Lauffer[1], *Nitash Zwaveling-Soonawala*[2], *Bernadette de Bakker*[3], *Maurice van den Hoff*[4], *Anita Boelen*[5], *Peter Henneman*[6], *Paul van Trotsenburg*[2]

[1]*Amsterdam UMC, Clinical Genetics, Amsterdam, Netherlands;* [2]*Amsterdam UMC, Pediatric Endocrinology, Amsterdam, Netherlands;* [3]*Amsterdam UMC, Obstetrics and Gynaecology, Amsterdam, Netherlands;* [4]*Amsterdam UMC, Medical Biology, Amsterdam, Netherlands;* [5]*Amsterdam UMC, Endocrine Laboratory, Amsterdam, Netherlands;* [6]*Amsterdam UMC, Human Genetics, Amsterdam, Netherlands*

Background/Objectives: Down syndrome (DS) is a chromosomal disorder characterized by trisomy of chromosome 21 and is associated with various health problems. Congenital thyroid dysfunction is a common endocrine abnormality in DS, but its exact cause remains unknown. This work aimed at studying the etiology of congenital thyroid dysfunction in DS.

Methods: Thyroid tissue of fetuses with DS ($n = 4$) and fetuses without a genetic/developmental abnormality ($n = 5$) were analyzed using histopathological evaluation, RNA sequencing (RNA-seq), and DNA methylation (DNAm) profiling.

Results: Histological examination showed abnormally developed DS thyroid tissue compared to non-DS/healthy tissue. RNA-seq analysis showed numerous differences in gene expression between DS and healthy thyroid tissue, including significant downregulation of thyroid genes *FOXE1*, *IYD* and *DIO2*. Gene set enrichment analysis showed disruption of vital cellular processes and functions in DS fetal thyroid tissue. Analysis of DNAm and expression quantitative trait methylation (eQTM) analysis identified functional and biologically relevant associations, that can be linked to plausible disrupted pathways in DS.

Conclusions: Based on these observations, we conclude that congenital non-autoimmune thyroid dysfunction in DS is probably a DS-specific form of thyroid dysfunction, characterized by abnormal thyroid development, altered expression of genes that regulate thyroid development and hormone production, and altered expression and DNAm of genes pertaining to numerous vital pathways leading to significant metabolic disturbances. The disturbances as investigated by multi-omics analyses (RNA-seq, DNAm and eQTM analyses) are widely spread over the genome, and presumably caused by the direct and downstream effects of overexpressed chromosome 21 genes (dosage effect), and (epi)genomic disturbances.

Conflict of Interest: None declared

P13.017.C Reclassification of a novel synonymous *PGAP1* variant disrupting splicing

Giada Moresco[1], *Martina Miceli*[2], *Carola Conca Dioguardi*[2], *Ornella Rondinone*[1], *Gianluca Tolva*[2], *Roberta Villa*[2], *Maria Saponaro*[2], *Laura Guarnaccia*[3], *Tiziano Palazzotti*[1], *Federica Alberti*[1], *Laura Fontana*[1;2], *Monica Miozzo*[1;2]

[1]*Università degli Studi di Milano, Medical Genetics, Department of Health Sciences, Milan, Italy;* [2]*ASST Santi Paolo e Carlo Hospital, Medical Genetics Unit, Milan, Italy;* [3]*Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Laboratory of Experimental Neurosurgery and Cell Therapy, Neurosurgery Unit, Milan, Italy*

Background/Objectives: Variants of uncertain significance (VUS) represent a major challenge in clinical genetic testing and their impact is often underestimated. In particular, synonymous variants may affect splicing mechanisms causative of multiple monogenic

disorders. In-silico tools are not always effective in predicting the impact of these variant and their characterization remains challenging.

Methods: We performed trio whole-exome sequencing (WES) and targeted mRNA sequencing in a 14-years-old girl born to healthy consanguineous parents and affected by global neurodevelopmental delay, absent speech, flaccid tetraparesis, and brain atrophy.

Results: We identified in the proband the novel homozygous synonymous variant c.1272G>A (p.Lys424Lys) in the *PGAP1* gene, inherited from her heterozygous parents. This variant is classified as a VUS and predicted to alter the donor splice site of the *PGAP1* exon 12. Targeted mRNA sequencing confirmed the expression of a mutated isoform with skipping of exon 12 in the proband and the presence of both the wildtype and exon 12-skipped isoforms in her parents. Exon 12-skipping leads to a frameshift and the generation of a premature stop codon, further resulting in the synthesis of a truncated non-functional protein or in nonsense mediated mRNA decay. Biallelic *PGAP1* mutations are responsible for the autosomal recessive neurodevelopmental disorder with dysmorphic features, spasticity, and brain abnormalities (NEDDSBA; OMIM #615802), a syndrome strongly correlated with the proband's phenotype.

Conclusion: The characterization of this *PGAP1* synonymous variant allowed its reclassification as likely pathogenic, further supporting the importance of synonymous variant evaluation in cases with a strong gene-disease correlation.

Grants: None.

Conflict of Interest: None declared

P13.018.D Biallelic mutation of CWF19L2, encoding a core spliceosome subunit, causes a combined neurodevelopmental and immunodeficiency disorder.

James Poulter[1], *Fatima Nadat*[2], *Eleanor O'Callaghan*[1], *Dylan Lawless*[1], *Alaa Alghamdi*[1], *Ailish McCafferty*[1], *Alisa Rose*[1], *Clive McKimmie*[1], *Daniel Peckham*[1], *Jacquelyn Bond*[1], *Asif Iqbal*[3], *Clive Carter*[2], *Christopher Watson*[1], *Helen McGettrick*[3], *Gina Doody*[1], *Sinisa Savic*[1;2]

[1]*University of Leeds, Leeds Institute of Medical Research, Leeds, United Kingdom;* [2]*Leeds Teaching Hospitals Trust, Clinical Immunology, Leeds, United Kingdom;* [3]*University of Birmingham, Birmingham, United Kingdom*

Background: CWF19L2 plays a key role in the post-mRNA release spliceosome complex during pre-mRNA splicing. Here we present biallelic CWF19L2 mutations in a family with microcephaly, autism and immunodeficiency.

Methods: Immunological assessment included immunophenotyping, functional T and B cell assessment and neutrophil migration assays. Quad exome sequencing was used to identify pathogenic variants. Long-read transcriptomes were performed on a PromethION-P48. Mitotic and cell proliferation defects were studied using siRNA of *CWF19L2* in HEK293s.

Results: An affected sib-pair presented with early onset bronchiectasis, recurrent sinopulmonary infections and microcephaly. Immunoglobulin profiling showed reduced IgM, but normal IgG and IgA levels. Lymphocyte populations and T cell proliferation were normal except for a reduction in naïve T-cells. B cells could differentiate in vitro and secrete antibodies (IgM, IgA and IgG) following T dependent stimulation. Whilst neutrophil burst test was normal, their migration towards IL-8 or across inflamed endothelium was severely impaired. Exome sequencing identified compound heterozygous *CWF19L2* variants (c.2200C>T, p.Gln734Ter; c.2251T>C, p.Cys751Arg), encoding a core component of the spliceosome, as the only variants segregating with the disease. Long-read nanopore RNA sequencing confirmed altered splicing in the affected sibling as well as differential expression of genes associated with immune and inflammatory response, chemokine-mediated signalling and actin cytoskeleton. *CWF19L2* knockdown in HEK293s resulted in reduced cellular proliferation and signficant mitotic defects.

Conclusion: We show biallelic loss-of-function variants in *CWF19L2* cause a novel neurodevelopmental and immunodeficiency disorder characterised by defects in splicing, mitosis and neutrophil migration.

Grants: UKRI Future Leaders Fellowship (JP) & Kennedy Trust (SS)

Conflict of Interest: None declared

P13.019.A A further example of the role of small non-coding RNAs (sncRNAs) in syndromic diseases that also highlights the diagnostic gap of Whole Exome Sequencing (WES)

Sylvia Hütter[1], *Marie Arlt*[1], *Marion Bermúdez*[2], *Karl Hackmann*[1], *Doreen William*[1], *Oliver Kutz*[1], *Tim Hutschenreiter*[1], *Franziska Steinz*[1], *Tanita Kretschmer*[3], *Andreas Dahl*[4], *Jens Schallner*[5], *Evelin Schröck*[1;6], *Joseph Porrmann*[1;3]

[1]*Institute for Clinical Genetics, University Hospital Carl Gustav Carus at TUD Dresden University of Technology and Faculty of Medicine of TUD Dresden University of Technology, Dresden, Germany;* [2]*Mitteldeutscher Praxisverbund Humangenetik, Erfurt, Germany;* [3]*UniversityCenter for Rare Diseases, University Hospital Carl Gustav Carus at TUD Dresden University of Technology, Dresden, Germany;* [4]*DRESDEN-concept Genome Center, Center for Molecular and Cellular Bioengineering, TUD Dresden University of Technology, Dresden, Germany;* [5]*Department of Pediatrics, Faculty of Medicine of TUD Dresden University of Technology and University Hospital Carl Gustav Carus at TUD Dresden University of Technology, Dresden, Germany;* [6]*Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany*

Background/Objectives: Non-coding RNAs (ncRNAs), a group of ribonucleic acids that do not encode for proteins, exert regulatory roles in crucial biological processes implicated in cancer and syndromic diseases.

Methods: We previously reported the case of a 6-year-old boy diagnosed with cartilage-hair-hypoplasia, harboring compound heterozygous variants in the RNA-coding *RMRP* gene. Here, we present another 6-year-old boy who presented with global developmental delay, microcephaly and short stature, accompanied by phenotypic abnormalities such as clinodactyly, hypoplasia of the penis and distinctive facial dysmorphic features.

Results: Whole Genome Sequencing (WGS) revealed two variants in the *RNU4ATAC* gene (NR_023343.1:n.13C>T and NR_023343.1:n.51G>A). Family-based segregation analysis confirmed compound heterozygosity. *RNU4ATAC* encodes the small nuclear RNA (snRNA) U4atac that does not result in a protein product. This snRNA is a constituent of the minor (U12-type) spliceosome, responsible for specific intron splicing in the human genome. Biallelic pathogenic variants in the *RNU4ATAC* gene have been intricately linked to a spectrum of diseases collectively known as RNU4atac-opathy, encompassing Taybi-Linder syndrome (TLS)/Microcephalic osteodysplastic primordial dwarfism, type I (MOPD1, OMIM #210710), Roifman syndrome (RFMN, OMIM #616651) and Lowry-Wood syndrome (LWS, OMIM #226960). Common clinical manifestations across these syndromes include growth retardation, skeletal dysplasia/short stature, microcephaly and developmental delay/intellectual disability. Both of the identified variants have already been described in trans with other pathogenic variants. Functional studies indicated reduced U12-dependent splicing activity compared to wildtype.

Conclusion: In this case, WGS bridges a diagnostic gap by revealing non-coding pathogenic variants associated with syndromic disease.

Grants:

Conflict of Interest: None declared

P13.020.B A novel autosomal recessive telomere biology disorder caused by biallelic variants in POLA2

Malin Kvarnung[1], *Maria Pettersson*[1], *Maryam Rafati*[2], *Lisa Mcreynolds*[2], *Anna Norberg*[3], *Ida Pesonen*[4], *Roza Chaireti*[5], *Boa Grönros*[6], *Julia Burlin*[6], *Jenny Ryden*[5], *Eva Hellström Lindberg*[5], *Giri Neelam*[2], *Sharon A. Savage*[2], *Suneet Agarwal*[7], *Ann Nordgren*[1], *Bianca Tesi*[1]

[1]Karolinska Institutet, Clinical genetics, Stockholm, Sweden; [2]National Cancer Institute, Clinical Genetics Branch, Division of Cancer Epidemiology and Genetics, Bethesda, MD, United States; [3]Umeå University, Department of Medical Biosciences, Medical and Clinical Genetics, Umeå, Sweden; [4]Karolinska Institutet, Respiratory Medicine Unit, Department of Medicine Solna, Stockholm, Sweden; [5]Karolinska University Hospital, Department of Hematology, Stockholm; [6]Danderyd University Hospital, Division of Nephrology, Stockholm, Sweden; [7]Harvard Medical School, Division of Hematology/Oncology, Pediatric Oncology, Dana-Farber Cancer Institute; Department of Pediatrics, Boston, MA, United States

Background/Objectives: *POLA2* encodes the accessory subunit of DNA polymerase α (polα)/primase, which is crucial for telomere C-strand fill-in. Incomplete fill-in of the C-rich telomeric strand after DNA replication has been proposed as a mechanism for Coats plus syndrome, a phenotype within the broader spectrum of telomere biology disorders (TBD), so far associated with pathogenic variants in *POT1*, *CTC1*, and *STN1*. The objective of the study was to describe the detailed phenotype and decipher the molecular etiology of TBD in two families negative for pathogenic variants in the known TBD disease genes.

Methods: Whole genome sequencing, segregation analysis and telomere length analyses.

Results: We report the findings of biallelic deleterious rare variants in the *POLA2* gene in all five affected individuals. All patients harbored the variant p.(Ile96Thr) in a heterozygous compound state with either p.(Pro424Leu) or an intragenic structural variant (deletion-inversion-deletion, 7.8 kb in total). All of the affected individuals displayed abnormally short telomeres and a clinical phenotype compatible with a TBD disorder / Coats plus, including retinal and gastrointestinal (GI) telangiectasias.

Conclusion: Our results suggest *POLA2* as a novel autosomal recessive gene for a TBD with Coats plus features.

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Conflict of Interest: None declared

P13.022.D The EXOSEX project: EXome sequencing in a cohort of fetuses with syndromic differences of SEX development.

Laura Mary[1], *Chloe Quelin*[2], *Anna Lokchine*[1;3], *Erika Launay*[1], *Bénédicte Nouyou*[1], *Laurent Pasquier*[2;4], *Marc-Antoine Belaud-Rotureau*[1;3], *Melanie FRADIN*[2], *Catherine Henry*[1], *Alinoe Lavillaureix*[2], *Florence Demurger*[2], *Philippe Loget*[5], *Sylvie Nivot Adamiak*[6], *Gwenaelle Le Bouar*[7], *Sylvie Odent*[2;4], *Sylvie Jaillard*[1;3]

[1]CHU de Rennes, Service de Cytogénétique et Biologie Cellulaire, Rennes; [2]CHU de Rennes, Service de Génétique Clinique, Centre de Référence Anomalies Du Développement, CLAD Ouest, Rennes, France; [3]Université de Rennes, Inserm, EHESP, Irset, UMR_S, 1085, Rennes; [4]Université de Rennes, IGDR, CNRS UMR 6290, INSERM ERL 1305, Rennes, France; [5]CHU de Rennes, Service D'Anatomie Pathologique, Rennes, France; [6]CHU de Rennes, Service D'endocrinologie Pédiatrique, Rennes; [7]CHU de Rennes, Unité de Médecine Foetale, Service de Gynécologie-Obstétrique, Rennes

Background/Objectives: Differences of sex development (DSDs) are a group of congenital conditions, either isolated or syndromic, characterized by a discrepancy between chromosomal, gonadal, and genital sex development of an individual. Despite significant advances, almost half of the patients remains undiagnosed to date. Previous reports of exome sequencing (WES) of large cohorts of patients presenting with various phenotypes that may include DSD (for example the DDD cohort) showed that this approach allowed the identification of new DSD-causing genes. Since most studies focus on postnatal patients, we thus aimed at studying a cohort of 10 fetuses presenting with syndromic DSD.

Methods: trio/quatuor WES on Illumina platform, after dismissing pathogenic chromosomal imbalances through karyotype and aCGH (with exception of 1 patient).

Results: We found pathogenic or likely pathogenic variants in 5 disease-causing genes and 1 candidate gene, leading to a diagnosis yield of 60%, in accordance with previously reported postnatal DSD cohorts. Of the 5 disease-causing genes, 3 were previously reported as causing DSD (*MYRF, PORCN, AMH*). For the last 2 genes (*MMP21* and *PPP2R1A*), DSD were not usually reported, linking DSD to primary cilia anomalies (*MMP21*) or abnormal chromatin remodeling (*PPP2R1A*). Finally, we found a homozygous variant in a candidate gene, *SLC25A26*, usually responsible for a combined mitochondrial oxidative phosphorylation deficiency without DSD reported to date.

Conclusion: Deciphering the mechanisms of sex development is challenging. Studying syndromic DSD patients through WES remains a fast and interesting tool to highlight new genes and pathways, when WGS is not routinely available.

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P13.023.A Investigating the DNA methylation profile of Mowat-Wilson syndrome: Call for patients with atypical presentation or uncertain genotypic correlation

Stefano Giuseppe Caraffi[1], *Liselot van der Laan*[2], *Kathleen Rooney*[3;4], *Slavica Trajkova*[5;6], *Roberta Zuntini*[1], *Raissa Relator*[3], *Sadegheh Haghshenas*[3], *Michael Levy*[3], *Chiara Baldo*[7], *Giorgia Mandrile*[8], *Carolyn Lauzon-Young*[3], *Duccio Maria Cordelli*[9;10], *Ivan Ivanovski*[11], *Anna Fetta*[9;10], *Elena Sukarova-Angelovska*[12], *Alfredo Brusco*[5;13], *Lisa Pavinato*[5;6], *Verdiana Pullano*[5;6], *Marcella Zollino*[14], *Haley McConkey*[3;4], *Giuseppe Merla*[15;16], *Marco Tartaglia*[17], *Giovanni Battista Ferrero*[18], *Bekim Sadikovic*[2;3;4], *Livia Garavelli*[1]

[1]Azienda USL-IRCCS di Reggio Emilia, Medical Genetics Unit, Reggio Emilia, Italy; [2]Amsterdam Reproduction & Development Research Institute, Amsterdam University Medical Centers, University of Amsterdam, Netherlands; [3]Verspeeten Clinical Genome Centre, London Health Science Centre, London, Canada; [4]Western University, Department of Pathology and Laboratory Medicine, London,

Canada; [5]University of Torino, Department of Medical Sciences, Torino, Italy; [6]Molecular Biotechnology Center "Guido Tarone", University of Torino, Torino, Italy; [7]IRCCS Istituto Giannina Gaslini, Laboratory of Human Genetics, Genova, Italy; [8]San Luigi University Hospital, University of Torino, Medical Genetics Unit and Thalassemia Center, Orbassano (TO), Italy; [9]IRCCS Istituto delle Scienze Neurologiche di Bologna, UOC Neuropsichiatria dell'Età Pediatrica, Bologna, Italy; [10]University of Bologna, Department of Medical and Surgical Sciences (DIMEC), Bologna, Italy; [11]Institute of Medical Genetics, University of Zürich, Zürich, Switzerland; [12]University Clinic for Pediatric Diseases, Ss. Cyril and Methodius University in Skopje, Department of Endocrinology and Genetics, Skopje, Macedonia; [13]Città della Salute e della Scienza University Hospital, Medical Genetics Unit, Torino, Italy; [14]Institute of Genomic Medicine, 'Sacro Cuore' Catholic University of Rome, Department of Life Sciences and Public Health, Roma, Italy; [15]University of Naples Federico II, Department of Molecular Medicine and Medical Biotechnology, Napoli, Italy; [16]Fondazione IRCCS Casa Sollievo della Sofferenza, Laboratory of Regulatory and Functional Genomics, San Giovanni Rotondo (FG), Italy; [17]Ospedale Pediatrico Bambino Gesù, IRCCS, Molecular Genetics and Functional Genomics Research Unit, Roma, Italy; [18]University of Torino, Department of Clinical and Biological Science, Torino, Italy

Background/Objectives: Mowat-Wilson syndrome (MOWS) is a rare congenital disease caused by haploinsufficiency of *ZEB2*, encoding a key developmental transcription factor. Individuals with MOWS show a variable phenotype including intellectual disability, epilepsy, typical facial features, short stature, Hirschsprung disease, brain and heart defects. By performing genome-wide DNA methylation (DNAm) analysis in a cohort of affected individuals, we defined a MOWS-specific DNAm profile or "episignature" as a novel diagnostic biomarker. We propose to apply this episignature to study uncertain and atypical MOWS cases.

Methods: By analyzing the DNAm profiles of 29 individuals with clinical and molecular diagnosis of MOWS using EpiSign™ software relative to the EpiSign Knowledge database reference, we defined and validated a specific MOWS episignature. Through international calls for patients, we are collecting individuals with: i) suspected MOWS-like disorder and a *ZEB2* variant of uncertain significance (eg. missense/noncoding, C-terminal truncation), or ii) typical MOWS presentation but no *ZEB2* gene/locus alteration detected. Their DNAm profiles are then compared to the MOWS episignature.

Results: We identified a reproducible MOWS episignature, with the potential to reclassify variants of uncertain significance. We highlighted its consistency with ZEB2 main role as a transcriptional repressor and with *ZEB2* previously suggested autoregulation ability.

Conclusion: Clinically or molecularly uncertain MOWS cases may be caused by variants affecting specific ZEB2 domains/functions, or by altered *ZEB2* auto- or eteroregulation. Investingating their DNAm profile relative to MOWS episignature will help define their diagnosis, and elucidate the epigenetic correlations between typical and atypical forms.

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P13.024.B Novel 14q32.2 microdeletion encompassing BCL11B: a case with complex phenotype including neurodevelopmental disorders, dysmorphisms, Parkinsonism/dystonia and T-cell Acute Lymphoblastic Leukemia

Daniele Guadagnolo[1], *Gioia Mastromoro[1], Nader Khaleghi Hashemian[1], Barbara Torres[2], Maria Cecilia D'Asdia[2], Alessandro De Luca[2], Laura Bernardini[2], Antonio Pizzuti[1]*

[1]Sapienza University of Rome, Department of Experimental Medicine, RM - ROMA; [2]Casa Sollievo della Sofferenza, Laboratory of Medical Genetics, San Giovanni Rotondo

Background/Objectives: In patients presenting multisystemic involvement, the attribution of all clinical characteristics to a single novel molecular finding is challenging. Integrating cytogenetics and sequencing genome-wide analyses can help define new clinical entities. We describe a novel 14q32.2 microdeletion in a patient with neurodevelopmental, neurological, and hematological features.

Methods: The proband was a 26-year-old male. He had plagiocephaly at 6 months. By age 3 he presented global developmental delay. At age 26, he developed T-cell Acute Lymphoblastic Leukemia (T-ALL). He presented wide eyes, beaked nose, receding chin as facial dysmorphisms. He had Parkinsonism/dystonia signs: amimic facies, dystonic head tremor, rigidity, abnormal posture. Brain imaging was normal. Chromosomal Microarray Analysis (CMA) and the Next Generation Sequencing (NGS) analysis of Parkinsonism/dystonia genes were performed on peripheral blood.

Results: CMA identified a 14q32.2 de novo Likely Pathogenic 3.2Mb microdeletion, [GRCh37]14q32.2(96961518_100129367). The germline origin was confirmed on buccal swab. The region included 3 OMIM Morbid genes: *VRK1* (*602168), *BCL11B* (*606558), *CCNK* (*603544). Of these, *BCL11B* and *CCNK* can be associated with dominant syndromic neurodevelopmental disorders. The deletion was not reported in the literature or in DECIPHER. NGS analysis did not identify Pathogenic/Likely Pathogenic variants.

Conclusion: The novel 14q32.2 microdeletion is the likely cause of the neurodevelopmental phenotype of the patient. The exclusion of known causes of Parkinsonism/dystonia suggests that movement disorders can be associated with the microdeletion. The occurrence of T-ALL in the proband, and the proven role of somatic *BCL11B* loss in sporadic T-ALL, support a possible association with hematological malignancy susceptibility.

Grants: None

Conflict of Interest: None declared

P13.025.C Microduplication encompassing *CCL2* and segregating in a family with anencephaly and pilonidal cyst: a possible link between chemokine and neural tube defects?

Gioia Mastromoro[1;2], *Daria Darelli[3], Daniele Guadagnolo[2], Nader Khaleghi Hashemian[2], Marta Tocchi[1], Antonella Mennella[1], Gianandrea Traversi[1], Stefania Mariani[1], Serena Bucossi[1], Laura*

Bernardini[4], Antonio Pizzuti[2], Andrea Zumpano[1], Mauro Ciro Antonio Rongioletti[1]

[1]Ospedale Isola Tiberina - Gemelli Isola, Roma, Italy; [2]Sapienza University of Rome, Roma, Italy; [3]San Pietro Hospital, Roma, Italy; [4]Casa Sollievo della Sofferenza, San Giovanni Rotondo, Italy

Background/Objectives: Anencephaly is a multifactorial condition, with a recurrence risk of 2-5% following a single case. Neural tube defects are usually induced by environmental factors, such as hyperthermia or vitamin deficiency.

Methods: A pregnant woman was referred for history of fetal anencephaly in a prior pregnancy. Since no genetic investigations had previously been performed, prenatal karyotype and Chromosomal Microarray Analysis (CMA) were offered in the ongoing pregnancy.

Results: CMA revealed a paternally inherited 640Kb microduplication encompassing *CCL2* and *CCL11*. At clinical re-evaluation, the father reported a personal history of pilonidal cyst and disclosed a previous pregnancy with another partner terminated following ultrasound findings of anencephaly in that fetus as well.

CCL2 (C-C Motif Chemokine Ligand 2; MIM + 158105) encodes a cytokine that displays chemotactic activity for monocytes and basophils and has a role in the MIF Mediated Glucocorticoid Regulation and TGF-Beta Pathway. The *CCL2* -2518A>G promoter polymorphism is associated with an increased risk factor for spina bifida in pregnant women with this variant in homozygosity. Exposure to heat in the first trimester is associated with an increased risk for neural tube defects. Previous studies demonstrated that chemokine receptors, including CCR2 (receptor of CCL2), are expressed in the brain by neural progenitor cells, playing a role in chemokine-directed migration in the context of normal neurogenesis.

Conclusion: The present family suggests that the *CCL2* overexpression might disrupt the early embryonic microenvironment by hindering the closure of the anterior and posterior neuropore, configuring itself as a susceptibility factor for neural tube defects.

Grants: None

Conflict of Interest: None declared

P13.026.D Antenatal presentation of Mitochondrial Inorganic Pyrophosphatase (PPA2) deficiency: a report on two cases.

Anne Guimier[1;2], *Paul Gueguen[3;4], Jeanne Médéric[3], Anna C.E. Hurst[5], Lucile Boutaud[1;2], Nicolas Derive[4], Stanislas Lyonnet[1;2], Hans A. Mayr[6], Kit Doudney[7], Chris Gordon[2], Jeanne Amiel[1;2]*

[1]Hôpital Necker-Enfants Malades, APHP, Service de Médecine Génomique des Maladies Rares, PARIS, France; [2]Institut Imagine, Université Paris Cité, Laboratory of Embryology and Genetics of Malformations, Inserm U1163, PARIS, France; [3]CHRU de Tours, Université de Tours, Service de Génétique et UMR1253, iBrain, Inserm, TOURS, France; [4]SEQOIA, Laboratoire de Biologie Médicale Multi-Sites, PARIS, France; [5]University of Alabama at Birminghan, Department of Genetics, Birmingham, United States; [6]Paracelsus Medical University Salzburg, Department of pediatrics, SALZBURG, Austria; [7]University of Otago, Centre for Postgraduate Nursing Studies and the Department of Pathology and Biomedical Science, CHRISTCHURCH, New Zealand

Background: PPA2 is a mitochondrial pyrophosphatase essential for cellular energy metabolism. PPA2 biallelic alterations were first described in families with recurrence of sudden cardiac death in early childhood or adolescence. Subsequent studies extended the clinical spectrum to include neurological signs in adults and, in one prenatal case, the association of cerebral malformations and cardiomyopathy that awaited confirmation with further cases.

Methods: Trio genome sequencing was performed in two patients with antenatal presentation.

Results: The previously described fetal case presented severe intrauterine growth retardation (IUGR), ventriculomegaly, agenesis of the corpus callosum (ACC), cerebellar hypoplasia and dilated cardiomyopathy. He was born prematurely and died at 2 days of life. Two variants were identified in trans in PPA2: a recurrent missense (c.514G>A p.Glu172Lys) and a truncating variant (c.938C>A p.Ser313*). Here we report a second prenatal case, unrelated to the first, yet harbouring the same two PPA2 variants, with the association of IUGR, microcephaly, ACC, cerebellar hypoplasia and abnormal gyration, leading to termination of pregnancy at 24 weeks of gestation. Cardiac examination was normal. These cases combine a truncating variant and a hypomorphic variant with low residual pyrophosphatase activity, leading to severe prenatal developmental anomalies.

Conclusion: We report the first two prenatal cases of PPA2 deficiency sharing the same genotype and severe IUGR and cerebral malformations. Similar prenatal presentations of ACC and cerebellar hypoplasia have been reported in other energy metabolism disorders such as pyruvate dehydrogenase deficiency. The consequences of complete deficiency of PPA2 on development remain to be described and may be embryonic lethal.

Conflict of Interest: None declared

P13.027.A 18p11 microdeletion involving PTPRM in monozygotic triplets concordant for increased STAT3 activation, Th17 cell preponderance, and juvenile onset autoimmunity

Morten Krogh Herlin[1], *Jens Magnus Bernth Jensen[2], Lotte Andreasen[1], Mikkel Steen Petersen[2], Jonas Lønskov[3], Mette Bendixen Thorup[4], Niels Holtum Birkebæk[5], Trine Hyrup Mogensen[3;6], Troels Herlin[5;7], Bent Deleuran[3;8]*

[1]Aarhus University Hospital, Department of Clinical Genetics, Aarhus N, Denmark; [2]Aarhus University Hospital, Department of Clinical Immunology, Aarhus N, Denmark; [3]Aarhus University, Department of Biomedicine, Aarhus, Denmark; [4]Aarhus University Hospital, Department of Radiology, Aarhus, Denmark; [5]Aarhus University Hospital, Department of Pediatrics and Adolescent Medicine, Aarhus N, Denmark; [6]Aarhus University Hospital, Department of Infectious Diseases, Aarhus, Denmark; [7]Aarhus University, Department of Clinical Medicine, Aarhus N, Denmark; [8]Aarhus University Hospital, Department of Rheumatology, Aarhus, Denmark

Background/Objectives: 18p deletion syndrome is a chromosomal disorder with an estimated prevalence of 1 in 50,000 characterized by intellectual disability, facial dysmorphology, and brain abnormalities. Some patients also present with autoimmune disease, including thyroiditis, although a cause for this association has not been established. Here, we investigated a unique case of monozygotic triplets concordant for developmental delay and juvenile-onset Graves' thyroiditis.

Methods: We performed chromosomal microarray analysis and subsequently whole-genome sequencing. We also performed various immunological assays investigating STAT3 phosphorylation, Th1/Th2/Th17 cell fractions, Treg cells, and overall immunophenotype.

Results: We identified a de novo interstitial 5.9 Mb deletion of chromosome 18p11.31p11.21 spanning 19 protein-coding genes, NC_000018.10: g.5420142_11286542del. Due to concordance for juvenile-onset autoimmunity in the triplets, we hypothesized the deletion as a possible cause hereof and therefore searched the literature on the genes involved. Based on recent experimental studies identifying protein tyrosine phosphatase receptor type mu (PTPRM) as a phosphatase of STAT3, we hypothesized PTPRM hemizygosity as a potential cause for autoimmune susceptibility in

the triplets. This was supported by increased cell fractions with phosphorylated STAT3 and higher Th17 cell fractions.

Conclusion: We identified monozygotic triplets concordant for developmental delay and juvenile-onset autoimmunity with an interstitial 18p deletion. Based on increased STAT3 phosphorylation and Th17 cell preponderance, we suggest PTPRM hemizygosity as a putative cause of autoimmune susceptibility in 18p deletion syndrome, which potentially could be targeted by IL-17 inhibition.

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Conflict of Interest: None declared

P13.028.B *DNAJC17* biallelic variants cause cerebellar hypoplasia and microcephaly - a novel gene-disease relationship

Aida Bertoli-Avella[1], *Mariana Ferreira*[1], *Nadine Jalkh*[1], *Indrani Manjunath*[1], *Rocio Garcia*[1], *Susan Zielske*[1], *Ruxandra Aanicai*[1], *Javier Martini*[1], *Claudia Krüger*[1], *Dragana Markovic*[1], *Fuad Al Mutairi*[2;3;4], *Hülya Kayserili*[5], *Jorge Pinto Basto*[1], *Peter Bauer*[1;6]

[1]*CENTOGENE GmbH, Rostock, Germany;* [2]*King Abdullah Specialized Children's Hospital (KASCH), Genetic and Precision Medicine Department, Riyadh, Saudi Arabia;* [3]*King Abdullah International Medical Research Center (KAIMRC), Riyadh, Saudi Arabia;* [4]*Ministry of National Guard - Health Affairs, Riyadh, Saudi Arabia;* [5]*Koç University School of Medicine (KUSoM), Medical Genetics Department, Istambul, Türkyie;* [6]*University of Rostock, Department of Internal Medicine III - Hematology, Oncology, and Palliative Medicine, Rostock, Germany*

Background/Objectives: Recently, *DNAJC17* was reported as a candidate gene for retinal dystrophy (RD). Given the limited gene-disease relationship (GDR), we the role of *DNAJC17* in human disease.

Methods: We queried our Biodatabank containing information from over 800,000 individuals for biallelic, rare variants in *DNAJC17* (NM_018163.2), and related phenotypes. Human phenotypes are recorded as Human Phenotype Ontology (HPO) terms. GDR was evaluated according to the ClinGen recommendations.

Results: Four unrelated patients were identified with ages ranging from childhood to adulthood with nyctalopia, reduced visual acuity, RD, and immunodeficiency. Additionally, one patient had global developmental delay, and another had seizures. All patients were homozygous for the same previously reported synonymous variant affecting splicing (c.681G>A p.Ala227Ala). Interestingly, we observed a different phenotype in patients with other biallelic variants including nonsense (c.898delG p.Glu300fs and c.560delG p.Gly187fs) and one missense predicted to affect splicing (c.792G>T p.Lys264Asn). A total of 11 patients (nine families) were identified with cerebellar hypoplasia (CH), intrauterine growth retardation/failure to thrive, global developmental delay, ataxia, renal malformations, and nystagmus. Furthermore, a knockout mice model (Dnajc17) has been reported to cause embryonic lethality prior to implantation.

Conclusion: The validity of the GDR for this new neurodevelopmental phenotype reached moderate evidence, supporting a causal role of biallelic *DNAJC17* variants for a new syndrome with cerebellar hypoplasia and microcephaly. The evidence for ophthalmological phenotype remains limited. The current result suggests *DNAJC17* pleiotropism and/or variable expressivity. Our results indicate the value of careful GDR assessment and the utility of biodatabanks in medical genetics.

Conflict of Interest: Aida Bertoli-Avella CENTOGENE, Mariana Ferreira CENTOGENE, Nadine Jalkh CENTOGENE, Indrani Manjunath: None declared, Rocio Garcia CENTOGENE, Susan Zielske CENTOGENE, Ruxandra Aanicai CENTOGENE, Javier Martini CENTOGENE, Claudia Krüger Centogene, Dragana Markovic Centogene, Fuad Al Mutairi: None declared, Hülya Kayserili: None declared, Jorge Pinto Basto: None declared, Peter Bauer Stocks, CENTOGENE

P13.029.C A unique missense mutation in the RING domain impairs MID1 E3 Ubiquitin ligase activity and localisation and is associated with uncommon Opitz Syndrome-like signs

Martina Mascaro[1], **Elisa Lazzari**[2], *Luigi D'Ambrosio*[3], *Berta Almoguera Castillo*[4], *Saoud Tahsin-Swafiri*[4], *Melania Eva Zanchetta*[2], *Germana Meroni*[3]

[1]*University of Udine, Udine, Italy;* [2]*IRCCS "Burlo Garofolo", Trieste, Italy;* [3]*University of Trieste, Trieste, Italy;* [4]*Fundación Jiménez Díaz University Hospital, Madrid, Spain*

Background/Objectives: Mutations in *MID1*, a RING-type E3 ubiquitin ligase, are associated with the rare genetic disease X-linked Opitz Syndrome (XLOS; OMIM: #300000), characterized by incorrect development of the embryonic midline.

Methods: Genetic studies including karyotyping, CGH array and clinical exome sequencing were performed on the proband. Furthermore, ubiquitin ligase activity and subcellular localization were analysed in cells overexpressing exogenous mutant MID1.

Results: We have identified a novel mutation in MID1 RING, a domain in which XLOS-associated mutations have never been reported, in a family with a history of midline developmental defects. The proband, hemizygous for the *MID1* mutation, presents with signs compatible with XLOS in the context of a broader spectrum of intellectual disability. Mechanistically, the novel mutation identified in MID1 impairs the E3 ubiquitin ligase activity consistent with potential disruption of the catalytic RING domain. Furthermore, altered subcellular localization of mutated MID1 was also observed together with its impact on the stability of the microtubular network, the latter observation in contrast to what previously reported for classical XLOS-mutants.

Conclusion: Our study demonstrates that a newly discovered aminoacidic substitution within the RING domain impairs MID1 E3 ubiquitin ligase activity and alters its microtubular localisation in a manner that is different from the XLOS-associated mutations reported to date, potentially explaining the additional neurological signs in the proband. Altogether these observations raise the question of the relationship between MID1 activity and its microtubular localisation, an issue worth further investigation to understand the function of MID1 and its role in the pathogenesis of midline developmental defects.

Conflict of Interest: None declared

P13.030.D Functional validation of a novel *RPS6KA3* splice-affecting variant causing Coffin-Lowry Syndrome: a case report.

Emanuele Coccia[1;2], *Maria Chiara Baroni*[1;2], *Gabriele Massaccesi*[1], *Chiara Diquigiovanni*[1], *Flavia Palombo*[3], *Alessandro Vaisfeld*[1;2], *Giovanni Innella*[1;2], *Daniela Turchetti*[1;2], *Elena Bonora*[1]

[1]*Department of Medical and Surgical Sciences (DIMEC), University of Bologna, Bologna, Italy;* [2]*Medical Genetics Unit, IRCCS Azienda Ospedaliero-Universitaria di Bologna, Bologna, Italy;* [3]*IRCCS Istituto di Scienze Neurologiche di Bologna, Programma di Neurogenetica, Bologna, Italy*

Background/Objectives: A two-year-old boy was evaluated for delayed neurodevelopment, restrictive cardiomyopathy, bilateral profound hearing loss, dysmorphic features. Whole-exome-sequencing analysis detected the hemizygous c.1354-12T>G

variant in *RPS6KA3*, inherited from the healthy mother, a novel variant classified as of uncertain significance but predicted to alter splicing. To clarify the clinical role of the variant and to provide the parents with proper recurrency risk for the new ongoing pregnancy, we concurrently performed transcript analysis and segregation study.

Methods: Total RNA was extracted from peripheral blood and then retrotranscripted into cDNA. RT–PCR was performed, and its products separated by electrophoresis. Bands were excised, purified, sequenced and electropherograms visualized. Amplification of exon 16 of *RPS6KA3* and intron-exon splicing sites was performed on DNA extracted from peripheral blood of the proband's maternal grandparents.

Results: RT-PCR and sequencing analysis revealed only a shorter amplified product in the proband, corresponding to the abnormally spliced form lacking exon 16, whereas the heterozygous mother presented the band corresponding to the wild-type allele and the same shorter isoform. This demonstrated that the variant causes the "skipping" of exon 16 of *RPS6KA3*, altering the downstream reading frame. The segregation study demonstrated the de novo origin of the variant in the mother.

Conclusion: These findings supported the pathogenicity of the variant, allowing to confirm the diagnosis of Coffin-Lowry Syndrome in the proband. Moreover, these insights made it possible to quickly provide the parents with accurate prenatal counseling for the ongoing and future pregnancies, also offering the possibility of carrying out a prenatal diagnosis.

Conflict of Interest: None declared

P13.031.A Deletion of RAI1 non-coding exons 1-2 causes Smith-Magenis syndrome

Uri Hamiel[1;2], *Alina Kurolap*[1], *Hofit Gadot*[1], *Daphna Marom*[1;2], *Adi Mory*[1], *Anat Bar Shira*[1], *Hagit Baris Feldman*[1;2]

[1]*Tel Aviv Sourasky Medical Center, Genetics Institute and Genomics Center, Tel Aviv, Israel;* [2]*Tel Aviv University, Faculty of Medicine, Tel Aviv, Israel*

Background:

Smith-Magenis syndrome (SMS) is a complex genetic disorder characterized by distinctive physical features, developmental delay, and a typical behavioral phenotype. Interstitial 17p11.2 deletions, which include the *RAI1* gene, are detected in >90% of patients, while exon 3 single nucleotide variants are detected in the rest. The primary transcript of the *RAI1* gene is transcribed from exons 3 onwards, while exons 1-2 are non-coding. It is unclear whether genetic variants preceding exon 3 cause SMS. We evaluated an 15-month-old infant for developmental delay and infantile spasms. An exome-based gene panel revealed a deletion encompassing exons 1-2 of *RAI*.

Methods: Chromosomal microarray (CMA) was done to confirm exome sequencing results and suspected de novo inheritance. Real time quantitative PCR (RT-qPCR) analysis was conducted to evaluate *RAI1* mRNA expression.

Results: CMA confirmed a de novo 738 Kbp deletion on 17p11.2, which includes exons 1-2 of *RAI1* but spares exons 3 onwards. RT-qPCR analysis on RNA extracted from the patient, father and three unrelated controls revealed that the patient has *RAI1* haploinsufficiency.

Conclusion: While deletions encompassing the *RAI1* coding region are well established in SMS pathogenesis, only one patient with an SMS phenotype and exons 1-2 deletion was previously reported. We show, for the first time, that a deletion of the *RAI* non-coding exons 1-2, which spares the gene's coding region, causes *RAI1* haploinsufficiency by eliminating regulatory elements, including the gene promoter, thus causing Smith-Magenis syndrome.

Conflict of Interest: None declared

P13.032.B Modeling a novel ciliopathy in *C.elegans*

Elena Tacchetto[1;2], *valeria morbidoni*[2], *luca pannone*[3], *Simone Martinelli*[3], *Eva Trevisson*[1;2]

[1]*Università degli Studi di Padova, Salute della Donna e del Bambino, Padova, Italy;* [2]*Istituto di Ricerca Pediatrica, Città della Speranza, Padova, Italy;* [3]*Istituto superiore di Sanità, Roma, Italy*

Background/ Objectives: Two novel heterozygous variants in *KIF3B* were identified in a patient with severe ciliopathy, and inheritance compatible with an autosomal recessive condition. KIF3B is a molecular motor that exploit ATP hydrolysis for intraflagellar transport along microtubules. *KIF3B* mutations have been previously identified as a cause for an autosomal-dominant ciliopathy [1], mainly characterized by retinitis pigmentosa. We propose to generate and characterize a *C.elegans* model to investigate the molecular consequences KIF3B defects.

Methods: The worm genome was edited with CRISPR/Cas9 reproducing patient's mutations and cilia-dependent behaviors were analyzed (chemosensation, osmotic avoidance, dye filling uptake). Cilia length and integrity was assessed by confocal microscopy to check on possible defects. Finally, movement and lifespan were evaluated to investigate possible different KIF3B function.

Results: Chemotaxis assays using different attractants resulted in significant differences between wild-type and *klp-11* mutant worms. In the dye-filling assay, to check for morphological intactness of cilia and sensory neurons, a significant decrease in dye uptake was observed. Moreover, *klp-11* knock-out worms showed a reduction in cilia length. Finally, *KIF3B* orthologue mutant worms showed a significant motor impairment and a significant reduction in lifespan compared to control worms.

Conclusion: These results suggest that KIF3B is not only important for cilia-dependent behaviours, as chemotaxis and chemosensation, but also for cilia structure and integrity as demonstrated by decreased cilia length and impaired dye uptake in mutants. Interestingly, *KIF3B* seems also to have roles in cilia-unrelated pathways, such as movement and ageing.

Grants: IRP 2024-2026

References: [1] Cogné B. AJHG 2020;106:893-904

Conflict of Interest: Elena Tacchetto IRP Research Grant 2024-2026, valeria morbidoni IRP Research Grant, 2024-2026, luca pannone: None declared, Simone Martinelli: None declared, Eva Trevisson IRP Research Grant 2024-2026

P13.033.C New insights into the molecular mechanism and pathogenesis of the *FBRSL1*-associated syndrome

Gina Kastens[1], *Roser Ufartes*[1], *Miriam Franken*[1], *Michaela Mischak*[1], *Christof Lenz*[2;3], *Silke Pauli*[1]

[1]*University Medical Center Göttingen, Institute of Human Genetics, Göttingen, Germany;* [2]*University Medical Center Göttingen, Department of Clinical Chemistry, Göttingen, Germany;* [3]*Max Planck Institute for Multidisciplinary Sciences, Bioanalytical Mass Spectrometry Group, Göttingen, Germany*

Background/Objectives: Pathogenic variants in *Fibrosin Like 1* (*FBRSL1*) cause a recently described congenital malformation syndrome. The affected patients presented with respiratory insufficiency, postnatal growth restriction, microcephaly, global developmental delay, heart defects, skin creases and other

malformations. Initial studies showed the existence of different wild-type FBRSL1 isoforms. All affected patients carry a truncating variant in the N-terminal region of the gene. To gain molecular insights into the pathogenesis of the *FBRSL1*-associated syndrome, we analyzed the nuclear function of wild-type FBRSL1 by performing protein interaction and gene expression analysis.

Methods and Results: Using affinity purification mass spectrometry and co-immunoprecipitation, we demonstrated an association of different FBRSL1 isoforms with chromatin remodeling complexes, which play a key role in embryonic development. In addition, we showed by ChIP-Seq that wild-type FBRSL1 is involved in the regulation of gene expression. Downstream enrichment analysis of FBRSL1 targets revealed an enrichment of genes associated with fundamental biological processes and developmental defects.

Conclusion: In summary, we were able to elucidate the nuclear function of FBRLS1 that seems to be particularly important in embryonic development. These findings and further investigations on the effect of pathogenic variants in FBRSL1 will help us to understand the pathogenesis of the FBRSL1-associated syndrome.

Grants: DFG (PA 2030/5-3)

Conflict of Interest: None declared

P13.034.D Expanding the phenotypic sprectrum of intellectual developmental disorder with dysmorphic facies and ptosis (IDDDFP)

Oğuz Çilingir[1], *Elif Saraç*[1], *Ezgi Susam*[1], *Derya Hazal Özbakır*[1], *Ebru Erzurumluoglu Gokalp*[1], *Sinem Kocagil*[1]

[1]*Eskisehir Osmangazi University, Meselik Campus, Eskisehir*

Background/Objectives: Intellectual developmental disorder with dysmorphic facies and ptosis (IDDDFP) (MIM#617333) is an autosomal dominant disorder identified by delayed psychomotor development, intellectual disability, and distinctive facial features, notably ptosis/blepharophimosis. EEG abnormalities and epilepsy also occur. IDDDFP is associated with pathogenic variations in the Bromodomain and PHD Finger Containing Protein (BRPF1) gene which encodes a multivalent chromatin reader that engages with and stimulates histone acetyltransferases, contributing to its involvement in epigenetic regulation.

Methods: 5 year-old female patient with history of hypotonia, psychomotor developmental delay, autistic features, sleep disturbances, hyperventilation, and seizures was referred to our outpatient clinic for evaluation. On examination her weight was 12 kg (-2,7 SD), length was 98 cm (-2,3 SD), occipitofrontal circumference was 48 cm (-1,8 SD). She had short and upslanted palpebral fissures, red hair and fair skin.

Results: A de novo pathogenic *BRPF1* variant (NM_001003694.2:c.3298C>T, p.(Arg1100Ter)) were identified at WES (Twist Human Core Exome capture kit, MGI DNBSEQ-G40).

Conclusion: Initially described in 2017, IDDDFP has been reported in 48 patients to date. Here we present an additional case diagnosed with this rare entity. Our patient had a neurocognitive phenotype and dysmorphic features similar to cases reported in the literature but sleep disturbances and abnormal breathing pattern has not been previously described. We believe that these novel findings will contribute to expanding the clinical spectrum of IDDDFP and our understanding of this rare phenotype.

Conflict of Interest: None declared

P13.035.A Molecular insights into a new syndrome caused by *KMT2D* missense variants in exon 38 and 39 distinct from classical Kabuki syndrome

Michaela Mischak[1], *Roser Ufartes*[1], *Gina Kastens*[2], *Christof Lenz*[2;3], *Silke Pauli*[1]

[1]*University Medical Center Göttingen, Institute of Human Genetics, Göttingen, Germany;* [2]*University Medical Center Göttingen, Department of Clinical Chemistry, Göttingen, Germany;* [3]*Max Planck Institute for Multidisciplinary Sciences, Bioanalytical Mass Spectrometry Group, Göttingen, Germany*

Background: Pathogenic variants in *KMT2D* cause Kabuki syndrome type 1, a rare autosomal dominant developmental disorder characterized by a typical facial gestalt, developmental delay, mental retardation, and a variable degree of congenital malformations. *KMT2D* encodes a histone methyl transferase, which acts as a significant epigenetic regulator. Kabuki syndrome can have a remarkable phenotypic overlap with CHARGE syndrome, another congenital malformation disorder. Recently, a syndrome caused by missense variants in exon 38 and 39 (Ex38/39) in *KMT2D* has been described, with a phenotype that overlaps with but is distinct from CHARGE and Kabuki syndromes. The most striking clinical features of the patients were choanal atresia, hypoplastic nipples, anomalies of the branchial apparatus, nuchal pits, anomalies of the lacrimal canal, hearing loss, external ear malformations, and thyroid abnormalities, while intellectual disability was largely absent.

Objectives: Identification of the pathogenic effect of Ex38/39 missense variants in *KMT2D* that leads to this particular phenotype of the newly identified syndrome.

Methods and Results: To determine whether the described missense variants cause a loss- or gain-of-function in KMT2D, we analyzed the interaction of a part of KMT2D containing the missense variants observed in patients with the recently described atypical Kabuki syndrome. Additionally, we searched for KMT2D interaction partners in this region and analyzed the effect of the variants on the interaction.

Conclusion: In summary, we elucidated the function of the Ex38/39 region of KMT2D, which will help us to understand this new syndrome and potentially provide further insights into the epigenetic role of KMT2D.

Grants: DFG (PA 2030/2-3)

Conflict of Interest: None declared

P13.036.B Three additional cases with biallelic *CRIPT* variants. Broadening the phenotype of this ultra-rare disorder.

Maria Garcia-de Paso[1], *Irene Lazaro-Rodriguez*[2], *Victor Asensio-Landa*[1], *Damian Heine*[1], *Laura Torres-Juan*[1], *Ana Martín-Santiago*[3], *María Angeles Muñoz Miguelsanz*[4], *Juan Francisco Quesada Espinosa*[5], *Lucía Garzón Lorenzo*[2], *Jaime Cruz*[2], *Iciar Martínez*[1], **Fernando Santos-Simarro**[1]

[1]*Hospital Universitari Son Espases, Molecular Diagnostics and Clinical Genetics Unit, Palma, Spain;* [2]*Hospital Universitario 12 de Octubre, Pediatric Endocrinology Department, Madrid, Spain;* [3]*Hospital Universitari Son Espases, Pediatric Dermatology, Palma;* [4]*Hospital Universitari Son Espases, Pediatric Surgery Department, Palma, Spain;* [5]*Hospital Universitario 12 de Octubre, Genetics, Madrid, Spain*

Background: Biallelic *CRIPT* variants (Cysteine-Rich PDZ Domain-BindingProtein) have only been described in six patients and the clinical phenotype has only recently been delineated to include growth retardation, neurodevelopmental delay and Rothmund-Thompson-like features.

Case presentation and methods: We present three additional cases with biallelic *CRIPT* variants. Two of the cases are sisters, aged two and five years, born to a healthy consanguineous couple of Moroccan origin. Their common manifestations include:

prenatal growth retardation, moderate psychomotor delay, microcephaly, dysmorphic features and ectodermal abnormalities. In addition, the eldest has a cervical aneurysmal vascular lesion and the younger has a congenital heart defect and unilateral microphthalmia. The third case, unrelated to the previous two, is a 12-year-old girl born to consanguineous Moroccan parents. Her clinical features include prenatal growth retardation, moderate psychomotor delay, microcephaly, dysmorphic features and ectodermal abnormalities in addition to epilepsy, myopia magna and skeletal abnormalities.

Results: Whole exome sequencing identified the homozygous *CRIPT* (NM_014171.6): c.132delA; (p.Ala45GlnfsTer86) variant in both sisters and the homozygous *CRIPT* (NM_014171.5): c.126delA (p.A45Qfs*85) variant in the third case. The parents were confirmed to be heterozygous carriers.

Conclusions: We present three additional cases with biallelic *CRIPT* variants and contribute to the delineation of the clinical phenotype of this ultra-rare disorder. The *CRIPT* (NM_014171.6): c.132delA seems to be a recurrent variant in the Moroccan population, described in all (four) identified cases from this population.

Conflict of Interest: None declared

P13.037.C Validation of a hypomorphic variant in CDK13 as the cause of CHDFIDD with autosomal recessive inheritance through determination of an episignature

Jan Fischer[1], *Karl Hackmann*[1], *Marielle Alders*[2], *Marcel Mannens*[2], *David Geneviève*[3], *Bekim Sadikovic*[4], *Evelin Schröck*[1;5], *Joseph Porrmann*[1]

[1]*Institute for Clinical Genetics, University Hospital Carl Gustav Carus at TUD Dresden University of Technology, and Faculty of Medicine of TUD Dresden University of Technology, Dresden, Germany;* [2]*Amsterdam Reproduction and Development Research Institute, Amsterdam UMC, University of Amsterdam, Department of Human Genetics, Amsterdam, Netherlands;* [3]*Reference Center for Rare Disease, Montpellier University, Medical Genetic Department for Rare Disease and Personalize Medicine, Montpellier, France;* [4]*Verspeeten Clinical Genome Centre, London Health Sciences Centre, Department of Pathology and Laboratory Medicine, Western University, London, Canada;* [5]*Max-Planck-Institut of Molecular Cell Biology and Genetics, Dresden, Germany*

Background: Heterozygous pathogenic variants in *CDK13* have been associated with an autosomal dominant disorder characterized by congenital heart defects, dysmorphic facial features and intellectual developmental disorder (CHDFIDD) (OMIM:617360). Besides haploinsufficiency, missense variants with a dominant-negative effect are also considered disease causing (PMID:30702837). All previously described pathogenic missense variants are located in the kinase domain of CDK13 with the phenotype likely being associated to the degree of functional domain alteration. CDK13 has been shown to influence DNA methylation and individuals with the CHDFIDD have recently been found to exhibit a characteristic episignature (PMID:35063350).

Methods/Results: We now report on a 2.5-year-old boy with an atrial septal defect, a mild pulmonary valve stenosis, a horseshoe kidney, corpus callosum hypoplasia, cerebellar hypoplasia, bilateral clubfoot with syndactyly (toes 2 and 3), feeding difficulties (requiring a PEG tube), psychomotor developmental delay and facial dysmorphism. Notably, the consanguineous parents both had mild intellectual disability without further signs of CHDFIDD.

Exome-wide sequencing of the boy revealed a previously undescribed homozygous missense variant of unknown significance in the kinase domain of *CDK13* (NM_003718.5:c.2330C>G, p.(Ala777Gly)). Due to the phenotypic overlap but discrepant mode of inheritance with CHDFIDD, a genome-wide methylation screen (EpiSignTM DNA methylation analysis) was conducted for improved variant assessment. This revealed the presence of a CHDFIDD-typical episignature in the index patient and milder methylation abnormalities in the heterozygous mother.

Conclusion: This suggests that p.Ala777Gly is a hypomorphic variant, which uniquely results in autosomal recessive inheritance. The general approach further highlights the utility of episignatures for the assessment of variants of unknown significance.

Conflict of Interest: None declared

P13.038.D Expanding molecular and clinical phenotype of Seckel syndrome: ATRIP deficient patient reveals novel insights in ATR signalling pathway

Kathleen Claes[1;2;3], *Lynn Backers*[1;2;3], *Elien Beyls*[4;5], *Evi Duthoo*[4;5], *Bram Parton*[3], *Veronique Debacker*[4], *Kim De Leeneer*[1;2;3], *Marieke De Bruyne*[3], *Levi Hoste*[4], *Ans Baeyens*[1;6], *Anne Vral*[1;6], *Peiquan Huang*[7], *Thorkell Gudjónsson*[7], *Tessa Kerre*[1;5;8], *Qiang Pan-Hammarstrom*[9], *Claus Storgaard Sørensen*[7], *Filomeen Haerynck*[4;5], *Simon J Tavernier*[3;4;10]

[1]*Cancer Research Institute Ghent (CRIG);* [2]*Ghent University, Department of Biomolecular Medicine;* [3]*Ghent University Hospital, Center for medical genetics, Gent, Belgium;* [4]*Ghent University Hospital, Primary immunodeficiency Research Lab (PIRL), Center for Primary Immunodeficiencies Ghent, Jeffrey Modell Foundation diagnosis and research center, Gent, Belgium;* [5]*Ghent University, Department of Internal Medicine and Pediatrics;* [6]*Ghent University, Radiobiology, Department of Human Structure and Repair, Gent, Belgium;* [7]*University of Copenhagen, Department of Biosciences and Nutrition, Copenhagen, Denmark;* [8]*Ghent University Hospital, Department of Hematology and, Jeffrey Modell Diagnosis and Research Center, Belgium;* [9]*Karolinska Institutet, Department of Medical Biochemistry and Biophysics, Division of Immunology, Stockholm, Sweden;* [10]*VIB-UGent Center for Inflammation Research, Unit of Molecular Signal Transduction in Inflammation*

Background/Objectives: This study presents a unique case of ATRIP-deficient Seckel syndrome (SS), wherein the patient also suffers from combined immunodeficiency (CID), a rarely observed condition in SS. The objective is to understand ATRIP's role in the DNA damage response and its connection to the immune system.

Methods: Expression of ATRIP and its partners was analysed at RNA and protein levels. Chromosomal sensitivity post DNA damage was confirmed using micronucleus assays. Flow cytometry (FCM) assessed cell cycle dynamics. Live-cell imaging and CRISPR-SELECT^{TIME} quantified fitness of ATRIP-deficient cells. Immunophenotyping utilized FCM, single-cell RNA sequencing (scRNAseq) and linear amplification-mediated high-throughput genome-wide sequencing (LAM-HTGS).

Results: The proband harboured a homozygous ATRIP splice variant (c.829+5G>T), inherited from healthy parents, leading to a complete loss-of-function. In contrast to current state-of-the-art, residual ATR kinase activity was observed, albeit impaired. Similar to the ATR-SS phenotype, patient's cells exhibit compromised DNA replication, cell cycle progression, and increased susceptibility to genotoxic agents. CRISPR-SELECT^{TIME} affirmed reduced cell fitness in presence of the ATRIP variant. Immunophenotyping unveiled diminished B, CD4 + T, and NK cells. Both B and T cell maturation and function were impaired upon analysis. Aberrations in immunoglobulin class switch frequency and distribution suggest a recombination deficiency. scRNAseq identified profound immune dysregulation.

Conclusion: This study broadens the clinical and molecular spectrum of SS, revealing a CID phenotype linked with ATRIP

deficiency. It delineates the molecular and functional impact of ATRIP deficiency on the ATR pathway and immune development.

Grants: FWO.TBM.2018.0001 - RAPID

Conflict of Interest: None declared

P13.039.A Assessment of ageing in Cornelia de Lange Syndrome

Feliciano Ramos[1], *Cristina Lucia Campos*[2], *Ana Latorre-Pellicer*[2], *Enrique Váquez*[3], *Marta Gil-Salvador*[2], *Beatriz Puisac*[2], *María Arnedo*[2], *Ariadna Ayerza Casas*[4], *Ana Dopazo*[3], *Julia del Rincón*[1], *Juan Pie*[2]

[1]*Hospital Clínico Universitario 'Lozano Blesa', Facultad de Medicina, Universidad de Zaragoza, Pediatría, Zaragoza, Spain;* [2]*Universidad de Zaragoza, Facultad de Medicina, Fisiología. Laboratorio Genética Clínica y Genómica Funcional, Zaragoza, Spain;* [3]*CNIC, Cardiology, Madrid, Spain;* [4]*Hospital Infantil Universitario 'Miguel Servet', Cardiología, Zaragoza, Spain*

Background/Objectives: Cornelia de Lange syndrome is a rare, congenital disorder characterized by a widely variable clinical presentation. In addition to distinctive facial features, affected individuals present physical abnormalities, growth retardation and cognitive disabilities. Its genetic cause is related to variants in genes related to the cohesin complex, with variants in the *NIPBL* gene being the most frequent.

There is a lack of understanding of the evolution and implications of pathogenic variants at the systemic and cellular level in adult individuals. Therefore, our aim is to characterize six adult individuals at the clinical, cellular and molecular level.

Methods: Application of the CdLS clinical score. Characterization of primary fibroblasts by evaluation of growth curves. Study of the transcriptome by RNA sequencing and set enrichment analysis.

Results: All patients presented a classic phenotype and pathogenic de novo variants in the *NIPBL* gene. Growth charts showed significant differences with controls. RNA sequencing showed a deregulation of genes mainly involved in neurodevelopment and mRNA processing, with a downward trend. In addition, gene set enrichment analysis showed a significant enrichment of processes related to mRNA regulation and maintenance. Finally, study of genes deregulated during the ageing process showed a trend of deregulation in all patients.

Conclusion: Differences at the cellular level seem to indicate an accumulation of damage that impairs proper cell proliferation. In addition, deregulation trend of genes involved in ageing could suggest an acceleration in this process.

Grants: M.G-S is supported by a Predoctoral Fellowship from the DGA and C.L-C by a Predoctoral Fellowship from the Spanish Ministry of Health-ISCIII.

Conflict of Interest: None declared

P13.040.B A novel NR2F2 gene variant in a child with atrioventricular septal defect, growth delay and additional syndromic features

Anna Lengyel[1], *Eva Pinti*[1], *Rita Bertalan*[1], *Gyorgy Fekete*[1], *Iren Haltrich*[1]

[1]*Semmelweis University, Pediatric Center, Hungary*

Background: Nuclear receptor subfamily 2 group F member 2 (*NR2F2*) encodes a member of the nuclear receptor superfamily of ligand-activated transcriptional factors. Heterozygous loss-of-function variants are associated with congenital heart defects and 46,XX disorder of sexual development.

Methods: A 5-year-old boy presented with short stature, decreased weight, atrioventricular septal defect and cryptorchidism. The patient's history was notable for single umbilical artery, increased nuchal fold (NIPT was performed and revealed a normal fetal 46,XY karyotype), meconium aspiration, feeding difficulties in infancy (requiring transient tube feeding), strabismus, hypermetropia, astigmatism and delayed motor development. Speech and language development were normal; currently the child has mild learning difficulties. Physical examination identified a long face, thin lip vermillions, low-set ears and abnormal pinna morphology. CNV analysis inclusive WES was performed from peripheral blood.

Results: A novel heterozygous frameshift variant was identified in *NR2F2* gene: c.423del, p.(Val142Trpfs*3). This truncating variant is absent in gnomAD. Associated disorders are inherited in an autosomal dominant manner. As mentioned above, variants in *NR2F2* are associated with congenital heart defects, and have recently been reported in relation to a more complex phenotype including developmental delays, intellectual disability, hypotonia, craniofacial minor anomalies, feeding difficulties, cryptorchidism, renal failure, hearing loss, strabismus, and, less frequently, asplenia and congenital diaphragmatic hernia.

Conclusions: We report a novel likely pathogenic *NR2F2* variant in a patient with syndromic features, expanding the genotypic spectrum linked to the recently expanded NR2F2-associated phenotype.

Grants: n/a

Conflict of Interest: None declared

P13.041.C Lissencephaly with dysgenesis of the corpus callosum and congenital cataract due to a novel de novo variant of the *DYNC1H1* gene

Mikulas Zich[1], *Pavel Seeman*[1;2], *Sarka Cerna*[1], *Petra Cibulkova*[3], *Valentyna Kaduchova*[3], *Jana Lastuvkova*[1]

[1]*Krajska zdravotni, a.s. - Masarykova nemocnice Usti nad Labem, o.z., Department of Medical Genetics, Usti nad Labem, Czech Republic;* [2]*2nd Faculty of Medicine, Charles University and Motol University Hospital, Department of Paediatric Neurology, Prague 5, Czech Republic;* [3]*University Hospital Ostrava, Department of Molecular and Clinical Pathology and Medical Genetics, Ostrava-Poruba, Czech Republic*

Background/Objectives: Lissencephaly is a set of congenital gyrification disorders resulting from abnormal neuronal migration that affects the development of the cerebral cortex. Pathogenic heterozygous variants of the *DYNC1H1* gene are the 4th most common cause of lissencephaly and account for approximately 3% of its genetic causes.

Methods: Next-generation sequencing (NGS), Magnetic resonance imaging (MRI), electroencephalography, neurological and ophthalmic examination

Results: We present a 27-month-old male patient born with right-sided talipes equinovarus. By 3 months of age, diagnosis of a severe central coordination disorder and delayed psychomotor development had been established. The patient was also diagnosed with bilateral perinuclear cataract. At 5 months, the patient suffered status epilepticus. Subsequent brain MRI revealed lissencephaly, dysgenesis of the corpus callosum and delayed myelination. Seizures became well-controlled with antiepileptic medication. NGS of a gene panel for lissencephaly revealed a novel, previously undescribed heterozygous missense variant of the *DYNC1H1* gene (NM_001376.5): c.10886T>C, p.Phe3629Ser. This variant has not been detected in either parent, so it has emerged de novo. A few other de novo variants of the *DYNC1H1* gene, e.g. c.10973G>A, have been described in patients with a

very similar phenotype, i.e. lissencephaly, cataract, bilateral talipes equinovarus, delayed psychomotor development and epilepsy.

Conclusion: Given the similarity of our patient's phenotypic features to those associated with other previously described de novo variants of the *DYNC1H1* gene, we consider the c.10886T>C variant of the *DYNC1H1* gene to be the cause of our patient's medical condition.

Conflict of Interest: None declared

P13.042.D Neurodevelopmental anomalies associated with the disruption of the plasminogen pathway

Kevin Uguen[1], Tanja Frey[2], Osama Muthaffar[3], Jean-Claude Décarie[4], Najim Ameziane[5], Sarah Boissel[1], Yalda Baradavan-Heravi[2], Anita Rauch[2], Gabriela Oprea[5], Aboulfazl Rad[5], Katharina Steindl[2], **Jacques Michaud**[1]

[1]CHU Sainte-Justine Azrieli Research Centre, Montréal, Canada; [2]Institute of Medical Genetics - UZH, Schlieren, Switzerland; [3]King Abdul Aziz University, Department of pediatrics, Jeddah, Saudi Arabia; [4]CHU Sainte-Justine, Department of medical imaging, Montréal, Canada; [5]Arcensus GmbH, Rostock, Germany

Background/Objectives: Congenital hydrocephalus (CH) is a condition characterized by an accumulation of cerebrospinal fluid within the brain, sometimes associated with other brain anomalies such as Dandy-Walker malformation (DWM). In order to gain insight into the molecular basis of these disorders, we performed genome-wide sequencing in consanguineous families with CH and DWM.

Methods: Exome or genome sequencing was performed in three families with CH and DWM.

Results: We identified homozygous truncating variants in the *PLAT* gene in the four affected family members from these families. All of them showed tetraventricular hydrocephalus. In two individuals, a membrane at the inferior aspect of the fourth ventricle was likely the cause of their hydrocephalus. Three cases exhibited DWM whereas the two oldest individuals displayed intellectual disability. *PLAT* encodes the tissue-type plasminogen activator (tPA), a serine protease whose main function is to cleave the proenzyme plasminogen to produce active plasmin, which is a potent serine protease with an extensive range of substrates, including fibrin, the chief constituent of thrombus. Interestingly, plasminogen deficiency has also been shown to cause CH and DWM, suggesting that loss of *PLAT* function causes these defects by disrupting plasmin production.

Conclusion: In summary, we describe a new recessive disorder characterized by obstructive CH, DWM and intellectual disability in individuals with loss-of-function variants in *PLAT*. This discovery further strengthens the involvement of the plasminogen pathway in the pathogenesis of these neuro-developmental disorders.

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Conflict of Interest: None declared

P13.043.A Expanding the nosology of CHAMP1-related disorders

Simona Amenta[1;2], *Giuseppe Marangi[3;4], Daniela Orteschi[4], Marcello Scala[5;6], Valeria Capra[7], Lucia Ulgheri[2], Francesca Peluso[8], Vincenzo Nigro[9;10], Marcella Zollino[3;4]*

[1]university of sassari, Department of Biomedical Sciences, Sassari, Italy; [2]University hospital of Sassari, Genetics and Developmental Biology, Sassari, Italy; [3]Catholic University, Rome, Italy, Department of Life Sciences and Public Health, Rome, Italy; [4]Policlinico Universitario "A. Gemelli" Foundation IRCCS, Genomic Medicine, Rome, Italy; [5]University of Genoa, Department of Neurosciences, Rehabilitation, Ophthalmology, Genetics, Maternal and Child Health, Genoa, Italy; [6]IRCCS Istituto Giannina Gaslini, Pediatric Neurology and Muscular Diseases Unit, Genoa, Italy; [7]IRCCS Istituto Giannina Gaslini, Medical Genetics Unit, genoa, Italy; [8]Azienda USL-IRCCS Reggio Emilia, 42123 Reggio Emilia, Italy, Medical Genetics Unit, Reggio Emilia, Italy; [9]University of Campania "Luigi Vanvitelli", Department of Precision Medicine, Naples, Italy; [10]Telethon Institute of Genetics and Medicine, Pozzuoli, Italy

Consortium: Telethon Undiagnosed Diseases Program (TUDP) Study Group

Background: *CHAMP1* disease is a rare condition, described in 40 individual so far, typically caused by truncating gene variants. The underlying pathomechanism in these cases is a dominant negative effect. Although a homogeneous phenotype is described, characterized by moderate to severe intellectual disability, autism and distinctive facial characteristics, the phenotypic spectrum of the disease is poorly understood. In addition, different pathomechanisms, namely haploinsufficiency as a consequence of gene deletion, and gain-of-function as a consequence of missense variants, cause different phenotypes, as we recently inferred. These preliminary suggestions need confirmation. Importantly, the role of missense variants remains conflicting.

Methods: We collaborated with the *CHAMP1* foundation to refine phenotype-genotype correlations through direct clinical evaluations of nine subjects during the July 2023 convention in Verona and online interviews with 18 families (21 with truncating variants, 4 with missense variants and 2 with unbalanced translocations).

Results: New insights were achieved by comparative evaluation of a larger patients series. **Truncating variants:** the clinical phenotype undergoes great variability in individual patients; a subset of patients who received early and intensive habilitation interventions presented with a very mild neurodevelopmental impairment, suggesting a high responsiveness to the treatments; whether it reflects the individual genomic background is under investigation. **Unbalanced translocations/13q deletions:** no homogeneous correlations were observed, reflecting the heterogeneity of the chromosome rearrangements; however small and isolated 13q deletions are associated with a milder phenotype. **Missense variants**: preliminary data point at different clinical outcome of certain variants, likely depending on the impairment of different protein domains.

Conflict of Interest: None declared

P13.044.B Delineating the phenotype associated with the 15q25.2-15q25.3 microdeletion

Maja Todorovic[1], *Daryl Scott[2], Himanshu Goel[3;4], Mohnish Suri[5], Levinus Bok[6], Rolph Pfundt[7], Ornella Galesi[8], Jill A. Rosenfeld[9], Antonio Novelli[10], Enrico Grosso[11], Vincenzo Antona[12], Marco Fichera[8;13], Han Brunner[14], Giovanni Battista Ferrero[15], Alfredo Brusco[1;11]*

[1]University of Torino, Città della Salute e della Scienza University Hospital, Department of Neurosciences "Rita Levi Montalcini", Torino, Italy; [2]Baylor College of Medicine, Department of Molecular and Human Genetics, Houston, United States; [3]Hunter Genetics, Waratah, Waratah, Australia; [4]School of Medicine and Public Health, University of Newcastle, Newcastle, Australia; [5]School of Medicine University of Nottingham, Clinical Genetics Service, University of Nottingham, UK, United Kingdom; [6]Maxima Medisch Centrum Veldhoven, Department of Pediatrics, Brabant, Netherlands; [7]Radboud University Nijmegen Medical Centre, Department of Human Genetics, Division of Genome Diagnostics (848), Nijmegen, Netherlands; [8]Oasi Research Institute-

IRCCS, Troina, Italy, Reseach Unit of Rare Diseases and Neurodevelopmental Disorders, Troina, Italy; [9]Baylor College of Medicine, Undiagnosed Diseases Network, Houston, United States; [10]Bambino Gesù Children's Hospital, IRCCS, Laboratory of Medical Genetics, Rome, Italy; [11]Città della Salute e della Scienza University Hospital, Medical Genetics Unit, Torino, Italy; [12]University Hospital Policlinico "Paolo Giaccone", University of Palermo, Department of Health Promotion, Mother and Child Care, Internal Medicine and Medical Specialities "G. D'Alessandro", Palermo, Italy; [13]Medical Genetics, University of Catania, Department of Biomedical and Biotechnological Sciences, Catania, Italy; [14]Radboud University Nijmegen Medical Centre, Department of Human Genetics and Donders Center for Neuroscience, Nijmegen, Netherlands; [15]Department of Public Health and Pediatric Sciences, University of Torino, Torino, Italy

Background/Objectives: The 15q25 region contains various copy number variants (CNV), some of which have already been linked to neurodevelopmental (NDD) and neuropsychiatric disorders. Our aim is to gather data to further characterize distal 15q25.2 deletion that is associated with an NDD.

Methods: We conducted a thorough search across multiple databases, including PubMed, Decipher, ClinVar, Database of Human CNVs IRCCS Troina, and DGV, to identify cases with 15q25.2-15q25.3 deletions (approximate coordinates hg19: chr15:85089979-85728844). Furthermore, we contacted clinicians to gather pertinent genetic and clinical information related to these deletions.

Results: We identified a total of 33 cases with a recurrent distal deletion 15q25.2-15q25.3. The core phenotypic features observed in most cases include autism spectrum disorder, mild to moderate developmental delay or intellectual disability, speech and language delay, behavioral abnormality. Morphological features were reported in a subset of cases and included esophageal atresia, scoliosis with lumbar hyperlordosis, bifid uvula, pulmonary atresia with intact ventricular septum, congenital cardiac anomaly, café-au-lait spots and mild facial dysmorphism such as synophrys, upslanted palpebral fissure, flat mid face on profile, a short philtrum, posteriorly rotated ears, macrocephaly. In 12% of the cases the deletion occurred de novo, while in 38% it was inherited, suggesting incomplete penetrance or variable expressivity; for half of the cases the parents' information was not available. The microdeletion encompasses 17 genes, with only ZNF592 being suggested as haploinsufficient (pLI = 1, LOEUF = 0.21).

Conclusion: Our findings provide additional evidence of a recurrent 15q25.2-15q25.3 deletion that contributes to neurodevelopmental disorders.

Grants: Ministry of Education and Merit; Torino University

Conflict of Interest: None declared

P13.045.C *Fetal anomalies of Potter sequence, IUGR, renal tubular dysgenesis, and osteopenia associated with placenta disorder of massive perivillous deposition with/without chronic histiocytic intervillositis: a report on four new cases with a focus on prenatal presentation*

Stanley Neufeld[1], *Mary Ann Thomas*[1;2], *Elaine Chan*[3;4;5], *seemab haider*[6;7], *weiming yu*[3;4;5], *Julie Lauzon*[1;2]

[1]*Alberta Children's Hospital, Department of Medical Genetics;* [2]*University of Calgary, Alberta Children's Hospital Research Institute;* [3]*University of Calgary, Cumming School of Medicine, Pathology and Laboratory Medicine;* [4]*Alberta Precision Laboratories;* [5]*Alberta Children's Hospital;* [6]*University of Calgary, Department of Radiology;* [7]*Alberta Children's Hospital, Department of Radiology*

Background/Objectives: Investigation of fetal anomalies in pregnancy often focuses on molecular testing of the fetus itself. However, placental disorders are increasingly being recognized for their pathogenic role. Here we present four new fetal cases involving growth restriction, shortened long bones, and osteopenia, where a fetal disorder such as skeletal dysplasia was suspected, but where the likely cause is in fact a certain placental disorder.

Methods: Four fetal cases with similar placental and fetal anomalies were reviewed including the prenatal imaging, fetal autopsy, and placental examination.

Results: Oligo-/anhydramnios and growth restriction developed between 19 and 28 gestational weeks, variably coinciding with the detection of anomalies including increased cardio-thoracic ratio and shortened long bones. Spontaneous fetal demise occurred between 28 and 30 weeks of gestation in three cases; in the fourth case, the pregnancy was interrupted at 21 weeks' gestation. Autopsy showed pulmonary hypoplasia, flattened facies, and talipes, all in keeping with decreased amniotic fluid/Potter sequence. Histologic examination revealed renal tubular dysgenesis in some cases, and placental massive perivillous fibrin deposition (MPFD) in all cases. Genetic testing for trisomies 13, 18, and 21 was normal.

Conclusion: The mechanism for the fetal anomalies appears to be MPFD leading to reduced fetal kidney perfusion, causing renal tubular dysgenesis and subsequently oligo/anhydramnios. The placental and renal abnormalities also lead to osteopenia, possibly via altered vitamin D metabolism. These case studies highlight the association of a placental disorder with fetal anomalies, suggesting that the placenta may have a broader role in fetal development than is currently appreciated.

Conflict of Interest: None declared

P13.047.A Ophthalmological evaluation in Brazilian patients with Cornelia de Lange Syndrome presenting variants in the *NIPBL* gene

Thainá Vilella[1;2], *Beatriz Carvalho Nunes*[1], *Giulia Del Valle*[2], *Chong Kim*[3], *Isabel Pinheiro*[3], *Juliana Sallum*[2], *Hiromi Aoi*[4], *Naomichi MATSUMOTO*[4], *Maria Isabel Melaragno*[1], *Priscila Cardoso Cristovam*[2]

[1]*Universidade Federal de São Paulo, Department of Morphology and Genetics, São Paulo, Brazil;* [2]*Universidade Federal de São Paulo, Department of Ophthalmology and Visual Sciences, São Paulo, Brazil;* [3]*Instituto da Criança da Universidade de São Paulo, Department of Genetics, São Paulo, Brazil;* [4]*Yokohama City University, Department of Human Genetics, Yokohama, Japan*

Background/Objectives: Cornelia de Lange Syndrome (CdLS) is a multisystemic genetic disease. Mutations in the NIPBL gene cause Cornelia de Lange syndrome 1 (CdLS1; OMIM #122470), which accounts for approximately 60% of CdLS cases. This study aims to evaluate the ophthalmological phenotype of CdLS1 and its correlation with NIPBL genetic variants.

Methods: Sixteen probands with confirmed *NIPBL* mutations by whole exome sequencing (WES) underwent a thorough ophthalmological examination. Assessments encompassed facial distance measurements, ectoscopy, visual acuity, pupillary reaction, ocular motility, slit lamp examination, tonometry, cycloplegic refraction, and dilated fundus examination, performed according to individual tolerance levels.

Results: Among the identified *NIPBL* gene mutations, 5 were splicing site mutations, 4 were missense mutations, and 7 were loss of function (LoF) mutations. Consistently observed ophthalmological features included synophrys (100%), arched and thick eyebrows (94%), long eyelashes (94%), and curled eyelashes (88%). Downslanted palpebral fissures and moderate ptosis were exclusively associated with LoF variants, whereas missense or splicing variants were associated with neutral or positively slanted

palpebral fissures and mild ptosis. Strabismus affected 37.5% of the patients, and myopia and high myopia were observed in 68.75% and 18.75% of subjects, respectively. Additional findings included thinning of the iris pupillary zone and optic disc peripapillary atrophy.

Conclusion: The study is the first ophthalmological study within the Brazilian population and contributed to a better understanding of ophthalmological changes in patients with CdLS1. Furthermore, it suggests a potential correlation between the type of genetic variant and distinct ophthalmological phenotypes.

Grants: #2019/21644-0, São Paulo Research Foundation (FAPESP).

Conflict of Interest: None declared

P13.048.B Single-chromosome methylation sequencing and gene expression data unravel spreading of X-chromosome inactivation into gene-rich autosomal regions in an unbalanced t(X;13) translocation

Bianca Pereira Favilla[1], *Ahmed Al-Rikabi*[2], *Danielle C. F. Bruno*[3;4], *Paula Asprino*[5], *Vera Ayres Meloni*[1], *Pedro A. F. Galante*[5], *Iscia Lopes-Cendes*[3;4], *Fernanda Teixeira Bellucco*[1], *Thomas Liehr*[2], *Maria Isabel Melaragno*[1]

[1]*Genetics Division, Department of Morphology and Genetics, Universidade Federal de São Paulo, São Paulo, Brazil;* [2]*Institute of Human Genetics, Jena University Hospital - Friedrich Schiller University, Jena, Germany;* [3]*Department of Translational Medicine, School of Medical Sciences, University of Campinas, Campinas, Brazil;* [4]*Brazilian Institute of Neuroscience and Neurotechnology, Campinas, Brazil;* [5]*Molecular Oncology Center, Instituto de Ensino e Pesquisa (IEP) - Hospital Sírio-Libanês, São Paulo, Brazil*

Background/Objectives: Patients with unbalanced X-autosome translocations present skewed X-chromosome inactivation (XCI) and autosomal silencing due to variable spreading of XCI into the translocated autosomal regions. Investigating chromosome-specific autosomal silencing in these cases not only offers an improved genotype-phenotype characterization of patients but also provides unbiased insights into intrinsic DNA and chromatin features that may mediate the XCI spreading.

Methods: Here, we combined chromosome-specific methylation sequencing analysis using an innovative chromosome-microdissection-based approach, bulk RNA-seq, and allele-specific expression analysis from peripheral blood cells to unveil silencing across the autosomal portion of the derivative chromosome from a patient with a t(X;13) translocation.

Results: Our analyses revealed differentially hypomethylated sites at gene bodies, exclusively on the derivative chromosome, which overlap with the downregulated genes (FDR < 0.05) across chromosome 13. Allele-specific expression analysis showed that differentially downregulated genes present inactivation of the allele from the derivative chromosome with different effect sizes, and are mostly clustered together in gene-rich topologically associating domains (TADs) across chromosome 13, while genes within gene-poor TADs tend to resist the spreading of XCI in the autosomal context. Moreover, downregulated genes are related to phenotypic characteristics that the patient presented, such as abnormalities of the eyes, neurodevelopmental delay, and microcephaly.

Conclusion: Our results suggest clinically relevant alterations in gene expression that can be attributed to the spreading of XCI into autosomal sequences and propose a variable XCI spreading in this context, with gene-rich autosomal regions being more prone to silencing.

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Conflict of Interest: None declared

P13.049.C A metabolic signature for *NADSYN1*-dependent congenital NAD deficiency disorder

Justin Szot[1], *Hartmut Cuny*[1], *Ella Martin*[1], *Delicia Sheng*[1], *Kavitha Iyer*[1], *Dimuthu Alankarage*[1], *Gavin Chapman*[1], *Kayleigh Bozon*[1], **Sally Dunwoodie**[1]

[1]*Victor Chang Cardiac Research Institute, Sydney, Australia*

Background/Objectives: Nicotinamide Adenine Dinucleotide (NAD) is essential for embryonic development. Biallelic loss-of-function variants in *KYNU*, *HAAO or NADSYN1*, enzymes of the NAD de novo synthesis pathway cause Congenital NAD Deficiency Disorder (CNDD). Congenital malformation can be prevented in *Kynu* or *Haao* mouse models with vitamin B3 (NAD precursor). To further understand CNDD causes and possible prevention, we assess 13 additional individuals with *NADSYN1* variants, a mouse model, vitamin B3 supplementation in mice, and quantified the NAD metabolome in patients and mice.

Methods: Patient variants in *NADSYN1* were assessed for pathogenicity using enymatic activity assays. A mouse model of *NADSYN1*-dependent CNDD was generated using CRISPR-Cas9, embryo phenotyping assesed using micro-CT, and various maternal NAD precursor supplements (vitamin B3 vitamers) were assessed to prevent adverse pregnancy outcomes. Some patients were orally supplemented with vitamin B3 vitamers. NAD and related metabolites were quantified in patients or mice using UHPLC-MS/MS.

Results: NADSYN1 variant proteins were functionally impaired in a domain-specific manner.

Nadsyn1 loss-of-function mouse embryos recapitulated the multiple malformations present in human cases. The efficacy of vitamin B3 supplementation depends on which gene (*KYNU*, *HAAO or NADSYN1)* caused the NAD deficiency and the type (amidated or deamidated) of vitamin B3 used. The circulatory NAD metabolome in humans and mice pre- and post-supplementation revealed a consistent metabolic signature with utility for patient identification and treatment.

Conclusion: Our data collectively improves clinical diagnostics of CNDD, provides guidance for the therapeutic prevention of CNDD, and suggests an ongoing need to maintain NAD levels via amidated vitamin B3 supplementation post-birth.

Conflict of Interest: None declared

P13.050.D Spectrum of 33 individuals with Sotos syndrome in a single facility

Tomoko Uehara[1], *Shiomi Otsuji*[1], *Natsuki Nakamura*[1], *Seiji Mizuno*[1], *Mie Inaba*[1]

[1], *Japan*

Background: Sotos syndrome is caused by microdeletion of chromosome 5q35 or loss-of-function valiants of NSD1 gene. It is known that there are differences in the clinical phenotypes between patients with microdeletions and valiant types.

Methods: We examined retrospectively the clinical characteristics of 33 patient with Sotos syndrome. All patients were genetically diagnosed by FISH analyses, microarray, NSD1 sequencing, or whole exome sequencing.

Results: 17 patients had 5q35 microdeletion, 15 patients had NSD1 valiants, and one patient had NSD1 intragenic (exon 22 and the last exon) deletion. 8 of 33 patients showed normal intelligence or mild intellectual disability (ID). 6 of the 8 patients had substitutional mutations in NSD1. Macrocephaly were shown

in 66.7% (14 of 21) of patients with moderate to severe ID and 100% (all 8 patients) of patients with mild ID or normal intelligence. Congenital heart diseases (CHD) or renal tract abnormalities were occurred in 10 patients (25%) with severe to moderate ID. Nine and eight of them had 5q35 microdeletions, respectively. Large extremities and accelerated tooth eruption were shown in 14 of 20 patients and 21 of 30 patients, respectively.

Conclusion: As having been previously reported, our results support that intellectual disability can be more severe in Sotos syndrome patients with chromosomal microdeletions than with genetic valiants. In addition, it is speculated that CHD or renal tract abnormalities may occur more often in Sotos syndrome due to NSD1 deletion. Macrocephaly, large extremities, and accelerated tooth eruption are distinctive phenotypes and critical for making diagnosis.

Conflict of Interest: None declared

P13.051.A Burden of Mendelian disorders in a large Middle Eastern biobank

Waleed Aamer[1], *Aljazi Al-Maraghi*[1], *Najeeb Syed*[1], *Elbay Aliyev*[1], *Geethanjali Devadoss Gandhi*[1], *Alya Al-Kurbi*[1], *Omayma Al-Saei*[1], *Muhammad Kohailan*[1], *Navaneethakrishnan Krishnamoorthy*[1], *Sasirekha Palaniswamy*[1], *Khulod Al-Malki*[1], *Saleha Abbasi*[2], *Nourhen Agrebi*[2], *Fatemeh Abbaszadeh*[3], *Ammira Akil*[2], *Ramin Badii*[3], *Tawfeg Bin Omran*[4], *Bernice Lo*[2], *Younes Mokrab*[2], *Khalid Fakhro*[1]

[1]*Sidra Medicine, Laboratory of Genomic Medicine, Research Branch, Ar Rayyan, Qatar;* [2]*Sidra Medicine, Human Genetics Section, Research Branch, Ar Rayyan, Qatar;* [3]*Hamad Medical Corporation, Section of Clinical and Metabolic Genetics, Department of pediatrics, Doha, Qatar;* [4]*Sidra Medicine, Division of Genetic & Genomics Medicine, Ar Rayyan, Qatar*

Consortium: Qatar Genome Program Consortium

Background/Objectives: Genome sequencing of large biobanks from under-represented ancestries provides a valuable resource for the interrogation of Mendelian disease burden at population level, complementing familial studies.

Methods: We examine a set of 2,648 Mendelian genes from 20 panels, annotating known and novel pathogenic variants, and assessing their penetrance/impact on the measured traits.

Results: We find 62.5% of participants are carriers of at least 1 known pathogenic variant relating to recessive conditions, with homozygosity observed in 1 in 150 subjects, for which Peninsular Arabs are enriched versus other ancestries. On average, 52.3 loss-of-function variants were found per genome, 6.5 of which affect a known Mendelian-disorder gene. Several variants annotated in Clinvar as pathogenic appeared at intermediate frequencies (1-3%), highlighting Arab founder effect, while others have high frequencies (>5%) prompting reconsideration as benign. Cumulative gene burden analysis revealed 56 genes having gene carrier frequency >1/50, including 5 ACMG Tier3 genes. Additionally, we systematically assess the impact of novel/rare variants on biobank phenotypes and discover 39 candidate large-effect variants associating with extreme quantitative traits. Further, through rare-variant burden testing we discover 5 genes with high mutational load and impact on traits relevant to disease conditions, including metabolic disorder and type 2 diabetes.

Conclusion: This study on the first-phase of the Qatar Genome Program cohort provides a comprehensive resource from a Middle Eastern population to understand the global mutational burden in Mendelian disease genes, and their impact on traits in seemingly healthy individuals in high consanguinity settings.

Grants: QNRF award (QF-QBB-RES-PUB-003 and NPRP10-1219-160035)

Conflict of Interest: None declared

P13.052.B Self-reported health status in adults with Bardet-Biedl syndrome in Norway

Cecilie F. Rustad[1;2], *Kristian Tveten*[3], *Ragnheidur Bragadottir*[2;4], *Jeanette Ullmann Miller*[1], *Charlotte von der Lippe*[3], *Solrun Sigurdardottir*[1]

[1]*Oslo University Hospital, Centre for Rare Disorders, Oslo, Norway;* [2]*University of Oslo, Faculty of Medicine, Oslo, Norway;* [3]*Telemark Hospital Trust, Department of Medical Genetics, Skien, Norway;* [4]*Oslo University Hospital, Department of Ophthalmology, Oslo, Norway*

Background/Objectives: Bardet-Biedl syndrome (BBS) is a rare condition belonging to the ciliopathies. Retinal dystrophy, central obesity, postaxial polydactyly and renal disease are some of the clinical features. We examined health-related quality of life (HRQoL) in adults with BBS.

Methods: This study was designed in the context of outpatient hospital clinics between January 2022 and February 2023. Data included demographics, clinical characteristics, genetic analysis and measures of health status. Self-reported HRQoL was measured using the EQ-5D-5L (mobility, self-care, usual activities, pain/discomfort, anxiety/depression). The EQ-5D-5L scores were compared with Norwegian norms and patient populations with chronic eye diseases.

Results: Thirty adults with BBS (50% female, median age: 36 years, range 20-69) were included. Pathogenic or likely pathogenic variants in *BBS1* and *BBS10* were found in 69% of the samples. Greater proportions of adults with BBS reported problems with mobility (53%), self-care (23%) and usual activities (53%) compared to the general population ($p < 0.001$). Problems with pain/discomfort (63%) and anxiety/depression (43%) had similar proportions to those in the general population. The mean EQ-index scale score was 0.79 (SD 0.18) and was similar to the index score of adults with chronic eye diseases ($p = 0.08$).

Conclusion: Adults with BBS were more likely to report problems with mobility, usual activities and self-care as compared with the general population. Impaired vision might be a contributing factor associated with these health problems, but due to the complexity of clinical features of BBS other factors cannot be ruled out.

Grants: Raagholtstiftelsen in Norway.

Conflict of Interest: Cecilie F. Rustad Raagholtstiftelsen in Norway, Kristian Tveten: None declared, Ragnheidur Bragadottir: None declared, Jeanette Ullmann Miller: None declared, Charlotte von der Lippe: None declared, Solrun Sigurdardottir Raagholtstiftelsen in Norway

P13.053.C A rare co-occurance of Mayer-Rokitansky-Küster-Hauser and Sotos syndromes in a patient with novel *NSD1* variant

guldeniz oner[1], *Gokcen Karamik*[1], *Nuray Ozturk*[1], *Öznur YILMAZ BAYER*[1], *banu nur*[1], *Mustafa Gökhan Ertosun*[2], *Mesut Parlak*[3], *ercan mihci*[1]

[1]*Akdeniz Üniversitesi Hastanesi, Pediatric Genetics, Antalya, Türkyie;* [2]*Akdeniz Üniversitesi Hastanesi, Medical Genetics, Antalya, Türkyie;* [3]*Akdeniz Üniversitesi Hastanesi, Pediatric Endocrinology, Antalya, Türkyie*

Background/Objectives: Sotos syndrome is an autosomal dominant neurodevelopmental disorder characterized by overgrowth and dysmorphic facial features. Pathogenic sequence and copy number variants in the *NSD1* gene cause this syndrome. Mayer-

Rokitansky-Küster-Hauser (MRKH) syndrome is characterized by agenesis or aplasia of the uterus and upper part of the vagina in females. Here, we present a patient with clinical manifestations of both syndromes. We aimed to confirm the patient's diagnosis by next generation sequencing analysis.

Methods: Genomic DNA was extracted from the patient's peripheral blood sample. Exons and exon-intron transition regions of the candidate genes were investigated by next generation sequencing using Sophia DDM program. Variant information servers dbSNP, ClinVar, Ensembl and ACMG 2015 criteria were used for evaluations.

Results: In the sixteenth exon of *NSD1* gene, c.5326_5336del (p.His1776Cysfs*6) frameshift variant was detected in heterozygous state. This variant was expected to cause functional effects due to premature ending of the protein translation. It was not reported in large population studies or disease-specific databases.

Conclusion: The detected novel frameshift variant in our study was evaluated as in relation with Sotos syndrome because of its predicted deleterious effects. Our patient is the third to be reported with both Sotos syndrome and MRKH syndrome. To better elucidate a possible association between these syndromes, variant segregation status in the patient's family and larger population data will be examined.

Grants: None

Conflict of Interest: None declared

P13.054.D A novel homozygous variant in *DCHS1* in a Brazilian boy with clinical features of Van Maldergem syndrome

Gabriela Correia-Costa[1], Bruna Fagundes Rodrigues[1], Carlos Eduardo Steiner[1], Vera Lucia Gil-da-Silva-Lopes[1], **Társis Paiva Vieira**[1]

[1]*School of Medical Sciences - Universidade Estadual de Campinas - Unicamp, Medical Genetics and Genomic Medicine, Department of Translacional Medicine, Campinas, Brazil*

Background/Objectives: Van Maldergem syndrome (VMLDS) is an extremely rare autosomal recessive disorder and is caused by biallelic variants in the *DCHS1* or *FAT4* gene. Less than 20 patients have been described, and few have variants in *DCHS1*. Here, we report a Brazilian patient with clinical features of VMLDS and a novel variant in *DCHS1*.

Methods: Exome sequencing (ES) was performed on genomic DNA. Analysis and classification of variants were performed following the American College of Medical Genetics and Genomics (ACMG) recommendations.

Results: The patient, first evaluated at four months of age, had hypotonia, chronic feeding difficulties, and was fed through a nasogastric tube. Dysmorphological evaluation showed large fontanelle, almond shaped eyes, epicanthus inversus, micrognathia, camptodactyly in fingers and toes, bilateral single transverse palmar crease, clinodactyly of the 5th toes, and tibial bowing, suggesting VMLDS. In his last evaluation, at one year of age, he was still being fed through a tube, with a swallowing disorder, had motor delay, hydrocephalus, joint laxity, and could not speak yet. ES detected a novel nonsense and homozygous variant in *DCHS1* - Chr11(GRCh38):g.6622021 T>A; c.9655A>T; p.Lys3219*; NM_003737.4. Since this variant is in the last exon, is not predicted to undergo nonsense-mediated decay, and removes <10% of protein, it was classified as a variant of uncertain significance. **Conclusion:** Since the patients' phenotype matches VMLDS, we believe this variant may be pathogenic, and further functional analysis may support this finding.

Grants: FAPESP #2022/05126-1.

Conflict of Interest: Gabriela Correia-Costa: None declared, Bruna Fagundes Rodrigues: None declared, Carlos Eduardo Steiner: None declared, Vera Lucia Gil-da-Silva-Lopes FAPESP #2022/05126-1, Társis Paiva Vieira FAPESP #2022/05126-1

P13.055.A Curious case of recurrence in Mowat Wilson Syndrome: report of two siblings

ahmet kablan[1], **Esma Ertürkmen Aru**[1]

[1]*Ankara Etlik Şehir Hastanesi, Medical Genetics, Ankara, Türkyie*

Background/Objectives: Mowat–Wilson syndrome (MWS) is a neurodevelopmental disorder marked by distinctive facial features and a range of congenital anomalies. It is characterized by de novo monoallelic mutations in *ZEB2* gene. To date, MWS has been reported in over 300 individuals, with only a rare familial recurrence in the literature. Specifically, the recurrence in siblings reported in four families, which nonsense and frameshift mutations were identified. Here, we present a sister and a brother who exhibit neurodevelopmental delay, EEG anomalies, characteristic dysmorphic facial features, constipation and self-mutilation behavior. Both are diagnosed with MWS, exhibiting a de novo *ZEB2* deletion spanning 6 exons. We aim to explore recurrence rates, investigate gonadal mosaicism likelihood, and report the first sibling recurrence case with a large exon deletion.

Methods: Due to strong phenotypic indications of MWS and normal karyotype results, one sibling underwent single nucleotide polymorphism (SNP) chromosomal microarray analysis for CNV, while the other had clinical exome sequencing.

Results: The CNV analysis conducted on the younger brother revealed a heterozygous deletion of approximately 35 kb in size, encompassing exons 3-9 of the *ZEB2* located in the 2q22.3 region. The CNV analysis of the sister's clinical exome sequencing data displayed an exon 3-9 deletion identical to brother. Karyotype and SNP microarray analyses of parents did not reveal any carrier status, although father has 46,XY,t(9;16)(q34;p11.2) rearrangement which evaluated as incidental.

Conclusion: Herein, we aim to present the first report of siblings recurrence in MWS with a large de novo deletion of 3-9 exons in *ZEB2*, to our knowledge.

Conflict of Interest: None declared

P13.056.B International Long COVID Host Genetics Initiative tackling genetics of Long COVID

Vilma Lammi[1], *Tomoko Nakanishi[2;3], Samuel Jones[1], Hugo Zeberg[4;5], Hanna Ollila[1;6;7]*

[1]*University of Helsinki, Institute for Molecular Medicine (FIMM), Helsinki, Finland;* [2]*McGill University, Department of Human Genetics, Québec, Canada;* [3]*Kyoto University, Kyoto-McGill International Collaborative Program in Genomic Medicine, Graduate School of Medicine, Kyoto, Japan;* [4]*Karolinska Institutet, Department of Physiology and Pharmacology, Stockholm, Sweden;* [5]*Max Planck Institute for Evolutionary Anthropology, Department of Evolutionary Genetics, Leipzig, Germany;* [6]*Broad Institute of MIT and Harvard, Cambridge, MA, United States;* [7]*Massachusetts General Hospital and Harvard Medical School, Center for Genomic Medicine. Anesthesia, Critical Care, and Pain Medicine., Boston, MA, United States*

Consortium: Long COVID Host Genetics Initiative

Background/Objectives: Long COVID is characterized by symptoms including fatigue, dyspnea, and cognitive dysfunction. Long COVID Host Genetics Initiative aims to elucidate genetic factors contributing to the risk of prolonged symptoms after SARS-CoV-2.

Methods: 33 studies from 19 countries conducted genome-wide association studies (GWAS) comparing Long COVID

(N = 15,950) to subjects recovered from COVID-19 or population controls (N = 1,892,830).

Results: A multiancestry meta-analysis of Long COVID after test-verified SARS-CoV-2 (N = 3,018) and 994,582 population controls identified a haplotype associated with increased risk for Long COVID (rs9367106-C, P = 1.8 × 10-10, odds ratio = 1.63(1.40-1.89), risk allele frequency = 4.2%). The association replicated in an independent sample of nine studies with 9,500 Long COVID cases and 798,835 controls.

Long COVID risk variant associated with increased *FOXP4* expression in lung (P = 5.3 × 10-9) and hypothalamus (P = 2.6 × 10-6). *FOXP4* expression in blood associated with Long COVID (P = 0.0063).

FOXP4 locus has earlier been associated with lung cancer and severity of acute COVID-19. Mendelian Randomization supported the role of COVID-19 severity as a causal risk factor for Long COVID (P = 2.4 × 10-3), but Bayesian modelling comparing the effects of *FOXP4* variants and other severity variants suggested an independent *FOXP4* association with Long COVID.

Conclusion: The global Long COVID Host Genetics Initiative has identified the *FOXP4* locus as a genetic risk factor for prolonged sequelae of SARS-CoV-2, suggesting the regulation of lung functions and immune system involved in the pathophysiology of Long COVID.

Grants: We acknowledge the work of healthcare professionals, technicians, patients, researchers, and funders working hard together to tackle SARS-CoV-2 and its long-term consequences. Full list of authors contributing to the Long COVID HGI: http://tinyurl.com/AuthorsLongCOVIDHGI

Conflict of Interest: None declared

P13.057.C The crucial role of exome sequencing in clinical care of an infant patient cohort

Vanessa Geiger[1], Manuel Lüdeke[2], Gabriel Miltenberger-Miltenyi[1], Birgit Zirn[3], **Cord-Christian Becker**[1]

[1]MVZ genetikum, München, Germany; [2]MVZ genetikum, Neu-Ulm, Germany; [3]MVZ genetikum, Stuttgart, Germany

Background/Objectives: Critically ill infants and infants with multisystem involvement profit from early and precise diagnosis. Gene panel testing as well as whole exome and genome sequencing have evolved as first-tier diagnostic options. These methods not only identify the diagnosis and support clinical decision making, but also avoid more invasive (and harmful) diagnostic techniques. Here, we studied clinical usability of next generation sequencing (NGS) in a pediatric patient cohort below the age of 1 year.

Methods: Retrospective analysis of all neonates and infants aged 0-12 months who had next generation sequencing in our laboratory between 2021 and 2023. Patients were stratified by age (group A ≤2 months, group B 2-6 months, and group C 6-12 months) and analyzed for outcome and diagnostic yield.

Results: 259 patients were included in the study. Pathogenic and likely pathogenic gene variants were identified in 45,3% (24/53) of patients in group A, 42,2% (35/83) in group B and 38,2% (47/123) in group C. Younger age was associated with a higher rate of genetic diagnoses (group A vs. group C; OR 1,34). Infants ≤12 months with complex syndromal phenotypes (defined as ≥2 organs involved) received a genetic diagnosis in 47,6% (39/82). The highest diagnostic yield was observed in group A patients with cardiovascular/respiratory disorders (75%, 3/4) and group B patients with eye/ear anomalies (66,7%, 6/9).

Conclusion: Our study confirms that NGS in critically ill infants is an effective tool and has a high diagnostic yield. Examples from our cohort elucidate that patients benefit from early implementation.

Grants: -

Conflict of Interest: None declared

P13.058.D Epigenomic and phenotypic characterization of DEGCAGS syndrome

Karim Karimi[1], **Denisa Weis**[2]*, Ingvild Aukrust[3;4], Tzung-Chien Hsieh[5], Marie Horackova[2], Julie Paulsen[6], Roberto Mendoza-Londono[7], Lucie Dupuis[7], Megan Dickson[7], Hellen Lesmann[5], Małgorzata Rydzanicz[8], Mateusz Biela[8], Mafalda Saraiva Santos[9], Abdulrahman Aldeeri[10], Khalid Hama Salih[11], Bassam Al-Musawi[12], Tracy Lau[13], David Murphy[13], Henrik Døllner[6;14], Franco Laccone[15], Andrea Soltysova[16], Raissa Relator[1], Michael Levy[1], Jennifer Kerkhof[1], Jessica Rzasa[1], Henry Houlden[13], Sérgio Sousa[9;17], Reza Maroofian[13], Timothy Yu[10], Peter Krawitz[5], Bekim Sadikovic[1;18], Sofia Douzgou HOUGE[3]*

[1]London Health Sciences Centre, London, Canada, Molecular Diagnostics Program, and Verspeeten Clinical Genome Centre, London, Canada; [2]Kepler University Hospital, Johannes Kepler University, Department of Medical Genetics, Med Campus IV, Linz, Austria; [3]Haukeland University Hospital, Department of Medical Genetics, Bergen, Norway; [4]University of Bergen, Department of Clinical Science, Bergen, Norway; [5]Institute for Genomic Statistics and Bioinformatics, University Hospital Bonn, Rheinische Friedrich-Wilhelms-Universität Bonn, Bonn, Germany; [6]St. Olav's hospital, Trondheim University Hospital, Department of Medical Genetics, Trondheim, Norway; [7]The Hospital for Sick Children, Division of Clinical and Metabolic Genetics, Toronto, Canada; [8]Medical University of Warsaw, Department of Medical Genetics, Warsaw, Poland; [9]Hospital Pediátrico, Centro Hospitalar e Universitário de Coimbra, Medical Genetics Unit, Coimbra, Portugal; [10]Boston Children's Hospital, Harvard Medical School, Boston, Division of Genetics and Genomics, Boston, United States; [11]College of Medicine, Sulaimani University, Department of Pediatrics, Sulaymaniyah, Iraq; [12]Department of Pathology and Forensics Medicine, College of Medicine, University of Baghdad, Baghdad, Iraq; [13]University College London, Institute of Neurology, London, United Kingdom; [14]Norwegian University of Science and Technology, Department of Clinical and Molecular Medicine, Trondheim, Norway; [15]Hospital of the Sister of Merci, Department of Pediatrics, Linz, Austria; [16]Faculty of Natural Sciences, Comenius University, Department of Molecular Biology, Bratislava, Slovakia; [17]Faculty of Medicine, Universidade de Coimbra, University Clinic of Genetics, Coimbra, Portugal; [18]Western University, Department of Pathology and Laboratory Medicine, London, Canada

Background/Objectives: Developmental Delay with Gastrointestinal, Cardiovascular, Genitourinary, and Skeletal Abnormalities syndrome (DEGCAGS, OMIM #619488) is caused by biallelic, loss-of-function (LoF) *ZNF699* variants, and is characterized by variable neurodevelopmental disability, discordant organ anomalies among siblings of consanguineous families and infant mortality. *ZNF699* encodes for a KRAB-Zinc Finger Protein of unknown function. We aimed to investigate the geno-phenotypic spectrum of DEGCAGS and the possibility of a diagnostic episignature.

Methods: We collected data on 28 affected individuals (11 new). We analysed 53 facial photographs from five individuals using GestaltMatcher. We applied methylation arrays on peripheral blood DNA samples in nine affected individuals. The methylation levels were fitted in a multivariate linear regression model to identify the differentially methylated probes. We constructed a binary Support Vector Machine classification model to differentiate DEGCAGS from controls.

Results: We expand the ZNF699-related molecular spectrum. We show that loss of ZNF699 causes novel, age-dependent clinical

findings and a similar facial gestalt. We identify a robust and distinct episignature for biallelic, LoF samples.

Conclusion: We further delineate the epi-geno-phenotypic spectrum of DEGCAGS. We discovered a sensitive and specific episignature for DEGCAGS, a robust biomarker for screening and diagnosis. Analysis of differentially methylated regions suggests an effect on genes potentially involved with disease manifestations.

Grants: Grant #43066, the Norwegian Advisory Unit on Rare Disorders, to Sofia Douzgou Houge, Project 2629394: The post-exome clinic: improving the impact of exome sequencing for developmental disorders in Norway.

Conflict of Interest: Karim Karimi: None declared, Denisa Weis: None declared, Ingvild Aukrust: None declared, Tzung-Chien Hsieh: None declared, Marie Horackova: None declared, Julie Paulsen: None declared, Roberto Mendoza-Londono: None declared, Lucie Dupuis: None declared, Megan Dickson: None declared, Hellen Lesmann: None declared, Małgorzata Rydzanicz: None declared, Mateusz Biela: None declared, Mafalda Saraiva Santos: None declared, Abdulrahman Aldeeri: None declared, Khalid Hama Salih: None declared, Bassam Al-Musawi: None declared, Tracy Lau: None declared, David Murphy: None declared, Henrik Døllner: None declared, Franco Laccone: None declared, Andrea Soltysova: None declared, Raissa Relator: None declared, Michael Levy: None declared, Jennifer Kerkhof: None declared, Jessica Rzasa: None declared, Henry Houlden: None declared, Sérgio Sousa: None declared, Reza Maroofian: None declared, Timothy Yu: None declared, Peter Krawitz: None declared, Bekim Sadikovic Professor Sadikovic is a shareholder in EpiSign Inc, company involved in commercialization of EpiSignTM technology., Sofia Douzgou HOUGE: None declared

P13.059.A An immortalised lymphatic cell line for modelling EPHB4 variants causative of lymphatic anomalies using CRISPR-Cas9 protocols

Ruby Moy[1], *Kazim Ogmen*[1], *Silvia Martin Almedina*[1], *Alan Pittman*[1], *Pia Østergaard*[1], *jeroen hagendoorn*[2], *lotte van den bent*[2]

[1]*St George's University of London, United Kingdom;* [2]*UMC Utrecht, Utrecht, Netherlands*

Background/Objectives: Primary Lymphatic Anomalies (PLA) have been associated with pathogenic variants in *EPHB4*, a receptor tyrosine kinase that binds to its ligand EphrinB2 regulating the development of blood and lymphatic vessels. Further work is required to characterise these variants and assess the mechanisms of disease. This will provide valuable information about targetable molecular pathways necessary to delineate novel drug treatment strategies.

Methods: This project aims to develop new in vitro tools using CRISPR-Cas9 protocols to create patient-modelled cells for in-depth investigation of the EPHB4 signalling pathway and the impact of PLA variants.

For this purpose, we have further characterised an immortalised lymphatic endothelial cell (imLEC) line, created by Frenkel and colleagues (PMID:34185991), in terms of lymphatic identity using immunofluorescence and western blot. RNA- sequencing and the label-free imaging microscopy system Livecyte have been used to study the response to Vascular Endothelial Growth Factor-C (VEGFC), the main growth factor promoting lymphangiogenesis.

Results: Results show that imLECs express lymphatic markers even in long-term culture, and after VEGFC treatment gene set enrichment suggests they regulate similar downstream pathways as primary lymphatic endothelial cells. VEGFC-driven proliferation was also validated by live imaging.

Using imLECs we can generate EPHB4 knock-in cell lines with CRISPR-Cas9 protocols using ribonucleoprotein complexes and fluorescence-labelled guide RNAs to induce single nucleotide changes using homology-directed repair.

Conclusion: In conclusion, imLECs retain a lymphatic identity and respond to VEGFC even when cultured for long time, making them a valuable new in vitro model to use CRISPR-Cas9 methodologies and investigate EPHB4 disease mechanisms in PLA.

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Conflict of Interest: None declared

P13.061.C 3q29 deletion syndrome in a patient with intellectual disability, facial dysmorphism, bilateral breast cancer, and secondary myelofibrosis

Jakub Duben[1], *Jakub Trizuljak*[1;2;3], *Tomáš Gyönyör*[2], *Ivona Blahakova*[1;3], *Zuzana Vrzalová*[1;3], *Karla Plevová*[1;2;3], *Paulína Likavcová*[3], *Katerina Stano Kozubik*[2;3], *Sarka Pospisilova*[1;2;3], *Michael Doubek*[1;2;3]

[1]*University Hospital Brno, Department of Internal Medicine - Hematology and Oncology, Brno-Bohunice, Czech Republic;* [2]*University Hospital Brno - Children's Hospital, Department of Medical Genetics and Genomics, Czech Republic;* [3]*CEITEC MU, Czech Republic*

Background/Objectives: Recurrent 3q29 deletions are characterized by neurodevelopmental and psychiatric manifestations, including mild-to-moderate intellectual disability, autism spectrum disorder, anxiety disorders, attention-deficit/hyperactivity disorder, executive function deficits, graphomotor weakness and schizophrenia. Other common findings are nutrition deficiency, gastrointestinal disorders, ocular issues, dental anomalies and congenital heart defects. We present an unusual occurrence of this disorder: a 22-year-old female with facial dysmorphism, myopia, bilateral breast cancer and significant chemo-and radiotoxicity.

Methods: We performed whole exome sequencing (WES) for testing of cancer susceptibility genes followed by chromosomal microarray analysis (CMA).Additionally, we carried out comprehensive bone marrow testing for myeloid malignancies.

Results: WES identified no causal variant for bilateral breast cancer. CMA revealed heterozygous

1,2 Mb-sized 3q29 deletion, subsequently confirmed by CNV analysis from WES. The extent of deletion corresponds to autosomal dominant recurrent 3q29 microdeletion syndrome. Bone marrow histology showed diffuse reticulin fibrosis, probably secondary to breast cancer treatment.

Conclusion: We present the first report of a patient with 3q29 deletion and early-onset breast cancer. While WES testing did not provide a definitive answer for the genetic cause of bilateral breast cancer, the 3q29 deletion explains the facial dysmorphy and intellectual disability. What is more, the deletion involves *RNF168* gene, a potential tumor suppressor gene, which has been suggested to play a role in the development of sporadic malignancies.

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Conflict of Interest: None declared

P13.062.D Longterm survival in a patient with triploidy

Maike Dittmar[1], *Eva Maria Murga Penas*[2], *Rebecca. Gembicki*[2], *Yorck Hellenbroich*[2], *Malte Spielmann*[1;2], *Ann Carolin Longardt*[3], *Martin Schrappe*[3], *Christel Eckmann-Scholz*[4], *Kristin Andresen*[4], *Almuth Caliebe*[1]

[1]*Institute of Human Genetics, Universitätsklinikum Schleswig-Holstein, University of Kiel, Kiel;* [2]*Institute of Human Genetics, Universitätsklinikum Schleswig-Holstein, University of Lübeck, Lübeck;* [3]*Department of Pediatrics and Adolescent medicine I, University Medical Center Schleswig-Holstein (UKSH), Campus Kiel, Kiel;* [4]*Department of Obstetrics and Gynecology, University Medical Center Schleswig-Holstein, Campus Kiel, Kiel*

Background/Objectives: Triploidy is diagnosed in approximately 10-20% of investigated miscarriages with intrauterine growth restriction. Affected children usually die perinatally or within a few days after birth. In the literature 13 patients with non-mosaic triploidy surviving for more than 30 days have been reported (PMID: 35131773).

Methods: Herein we report a new patient with triploidy. The patient was born at 34 weeks gestation with a weight of 900g (-3,2z). The pregnancy was complicated by growth retardation due to placental insufficiency. Non-invasive prenatal testing was unsuccessful due to insufficient free fetal DNA quantity.

Physical examination revealed dysmorphism (pointed nose, hypertelorism, low-set ears, a high arched palate, hands with clinodactyly (3rd-5th fingers) and short thumbs, single palmar crease). The feet were disproportionately long with significantly shortened and receding big toes and a prominent calcaneus.

Organ malformations included suspected partial agenesis of the corpus callosum, horseshoe kidney, cardiomegaly with persistent ductus arteriosus, respiratory insufficiency with apnea-bradycardia syndrome, pulmonary hypertension, and mild right pleural effusion. Additionally, there was suspected hearing impairment and various eye abnormalities.

Results: By chromosomal analysis on blood lymphocytes and fluorescent in situ-hybridization of lymphocytes and buccal mucosa cells non-mosaic triploidy was diagnosed.

Conclusion: If the additional chromosome set is of maternal origin (digynic triploidy), life expectancy can be several months. Prenatal signs of digynic triploidy include a small fibrotic placenta and severe intrauterine growth restriction. Given that our patient is now almost 8 months old, this also suggests a maternal origin.

Conflict of Interest: None declared

P13.063.A Identification and functional characterisation of ACTN1 variants in individuals with a distinct clinical subtype of frontonasal dysplasia without platelet disorder

Tiong Yang Tan[1;2;3], *Peter Houweling*[2;3], *Shengping Huang*[4], *Thanh-Binh Nguyen*[5;6], *Patrick Yap*[1;2], *Marieke van Dooren*[7], *Irene Mathijssen*[7], *Martin Delatycki*[1;2;3], *Luisa Clucas*[8], *Kathryn North*[1;2;3], *David Ascher*[5;6;9], *Katrina Bell*[2;3], *Timothy C. Cox*[4;10]

[1]*Victorian Clinical Genetics Services, Parkville, Australia;* [2]*Murdoch Children's Research Institute, Parkville, Australia;* [3]*The University of Melbourne, Department of Paediatrics, Parkville, Australia;* [4]*University of Missouri-Kansas City, Department of Oral & Craniofacial Sciences, Kansas City, United States;* [5]*The University of Queensland, School of chemistry and molecular biosciences, Saint Lucia, Australia;* [6]*Baker Heart and Diabetes Institute, Computational Biology and Clinical Informatics, Melbourne, Australia;* [7]*Erasmus MC Sophia Kinderziekenhuis, Department of Clinical Genetics, Rotterdam, Netherlands;* [8]*The Royal Children's Hospital Melbourne, Haematology, Parkville, Australia;* [9]*University of Cambridge, Department of Biochemistry, United Kingdom;* [10]*University of Missouri-Kansas City, Department of Pediatrics, Kansas City, United States*

Background/Objectives: Alpha-actinins are dimeric crosslinking proteins involved in bundling and anchoring of the actin cytoskeleton to the cell membrane, with at least six isoforms encoded by four genes (*ACTN1-4*) in mammals. Pathogenic variants in *ACTN1* are associated with autosomal dominant congenital macrothrombocytopenia [MIM: 615193].

Methods & Results: By exome sequencing and global matchmaking we identified four de novo and inherited monoallelic *ACTN1* variants in six individuals from four unrelated families (including a three-generation family) segregating a distinctive frontonasal dysplasia phenotype with craniosynostosis, hypertelorism, down-slanting palpebral fissures, low-set posteriorly rotated ears, wide anterior fontanelle, broad nasal tip, costovertebral and genitourinary anomalies. All probands are *EFNB1*-negative and none have macrothrombocytopenia. Three of the variants are missense substitutions and one is an in-frame deletion. Protein modelling suggests that craniofacial variants occur in residues less tolerant of variation and are predicted to be more damaging compared to macrothrombocytopenia-associated variants. Ectopic expression of GFP-tagged protein suggests that craniofacial variants perturb localisation in a manner distinct from macrothrombocytopenia-associated variants. Cultured fibroblasts have been obtained from two patients and an unaffected parental control to examine the effects of these variants in vitro. We generated a CRISPR-gene-edited mouse to investigate whether the cranioskeletal anomalies are recapitulated and thus validate a pathogenic role for these variants.

Conclusion: *ACTN1* variants may be overlooked in genomic analysis of craniofacial patients because of the prior association with a haematological disorder. Our clinical and functional characterisation provide evidence of an *ACTN1*-related craniofrontonasal phenotype allelic to congenital macrothrombocytopenia.

Grants: MCRI Theme grant; AFGN; UDP-Vic

Conflict of Interest: None declared

P13.064.B Functional variability in Wolf-Hirschhorn syndrome.

Julián Nevado[1;2;3], *Xana Da Silva-Mori*[1], *Raquel Blanco-Lago*[4], *Cristina Bel-Fenellós*[5], *Maria Angeles Mori-Alvarez*[1], *Elena Mansilla*[1;2], *Fe Amalia García Santiago*[1;2], *Isabel Vallcorba*[1;2], *Harry Pachajoa*[6], *Jair Tenorio*[1;2;3], *Pablo LAPUNZINA*[1;2;3]

[1]*La Paz University Hospital, INGEMM, Madrid, Spain;* [2]*CIBERER, Madrid, Spain;* [3]*ITHACA network, Madrid, Madrid, Spain;* [4]*Central University Hospital of Asturias, Neuropediatrics, Oviedo, Spain;* [5]*Universidad Complutense, Dpto. Investigación y Psicología en Educación, Madrid, Spain;* [6]*ICESI University, Cali, Colombia*

Background/Objectives: Wolf-Hirschhorn syndrome (WHS, OMIM #194190) is caused by the loss of genetic material at the distal region of the short arm of chromosome-4. It is a neurodevelopmental characterized by global developmental delay, microcephaly, delayed speech, dysmorphic features, and mostly epilepsy. Individuals affected by this syndrome may have shown a high heterogeneity and variability established by parents, caregivers, and different clinical specialists. The main aim of this work is to compare functionally individuals in a large series of patients with WHS.

Methods: 140 diagnosed WHS individuals (75 from Spain and 65 from Latino-America), who are deeply phenotyped at clinical and molecular level by SNP-arrays. We estimated and validated an individual functional severity score (Global Functional Assessment of the Patient; GFAP), using different features taken from two questionnaires and weighed them by HPO-term frequencies.

Results: We highlight a positive correlation-ship between GFAP and the size of deletion, and other variables associated with a worst prognosis. Thus, low values for GFAP correlated with a better prognosis. We found high GFAP values in males, and single 4p deletions, but no differences have been found between origin-subpopulations.

Conclusion: Using this score, we established that, the functional variability in WHS individuals is mainly associated with the gender, the size of the deletions, and eventually with additional genetic rearrangements. Altogether this data are supporting a role for GFAP mimicking the evolve-prognosis in WHS. Which is also associated directly with size of the deletions, according with the idea of the existence of genotype/phenotype correlationships.

Grants: PI-18/01433

Conflict of Interest: None declared

P13.066.D De novo pathogenic OTX2 variant in a paediatric patient with hypopituitarism and congenital ocular anomalies: a case report and review of literature

Nelly Miteva-Marcheva[1], *Hristo Ivanov*[1], *Tsvetelina Tsvetanova*[1], *Mariya Georgieva*[2], *Vili Stoyanova*[1]

[1]*Medical University of Plovdiv, Paediatrics and Medical Genetics, Plovdiv, Bulgaria;* [2]*Outpatient Clinic for Specialized Pediatric Endocrinology, Metabolic Disorders, and Pediatrics, Pediatric Endocrinology and Metabolism, Stara Zagora, Bulgaria*

Background/Objectives: OTX2, a member of the bicoid subfamily of homeodomain-containing transcription factors, governs brain, craniofacial, and sensory organ development. Heterozygous gene variants correlate with syndromic microphthalmia, combined pituitary hormone deficiency, and early-onset retinal dystrophy, with or without pituitary dysfunction.

Methods: We present a case of a 7-year-old boy exhibiting congenital hypopituitarism due to specific anomalies: markedly hypoplastic infundibulum, poorly developed adenohypophysis, bilateral cryptorchidism, and ocular abnormalities including congenital glaucoma and leucoria. The child's clinical diagnosis was initially set at 5 years of age. Subsequently, at the age of 7, the child underwent genetic consultation and diagnosis. No familial precedent of a similar condition is reported, though the father has poliosis.

Results: Blueprint Genetics Whole Exome Family Plus analysis revealed a de novo heterozygous frameshift variant, OTX2 c.106dup, p.(Arg36Profs*52), not present in the patient's parents.

Conclusion: The pathogenic classification of OTX2 c.106dup, p.(Arg36Profs*52) is supported by its association with the patient's phenotype, rarity in control populations, presence in two individuals with a related phenotype, frameshift nature, and de novo occurrence. Absence in the patient's parents lowers the recurrence risk for future siblings, yet a slight risk persists due to potential parental germline mosaicism. Genetic counseling is advised.

Grants: Diaczok D, Romero C, Zunich J, Marshall I, Radovick S. A novel dominant negative mutation of OTX2 associated with combined pituitary hormone deficiency. J Clin Endocrinol Metab. 2008 Nov;93(11):4351-9. https://doi.org/10.1210/jc.2008-1189. Epub 2008 Aug 26. PMID: 18728160; PMCID: PMC2582563.

Conflict of Interest: None declared

P13.067.A Step-by-Step Genetic Insight into a Case of Growth Retardation

Ayşe Tekin[1], *Güven Toksoy*[1], *büşra gizem kına*[1], *Ayça Dilruba Aslanger*[1], *Birsen Karaman*[1], *Bilge Ozsait Selcuk*[1], *Cagri Gulec*[1], *Zehra Oya Uyguner*[1], *Esma Nur Konur*[1], *Gozde Yesil Sayin*[1], *firdevs baş*[2], *tuğçe kandemir*[2], *Ayse Pinar Ozturk*[2]

[1]*Istanbul University, Medicinal genetic, İstanbul;* [2]*Istanbul University, Department of Pediatrics, İstanbul*

Background. Uniparental disomy (UPD) is an inheritance pattern where both copies of a chromosomal pair originate from a single parent, notably affecting chromosomes that carry genes subject to parental-origin-dependent imprinting. The frequency of UPD is reported as 1 in 2,000. Russell-Silver Syndrome (RSS) is characterized by hypomethylation of the imprinting control region on chromosome 11, maternal UPD7 or pathogenic variants in related gene regions with an estimated prevalence of 1 in 3.000.

Methods: A patient presenting with growth retardation underwent clinical assessment, pedigree analysis, and hormone profiling. Step-by analysis included Sanger sequencing for *CUL7* and *OBSL1*, exome sequencing (ES), arrayCGH, MLPA-ME030 and MLPA-ME032 testing.

Results: A 16-year-old male patient was primarily considered for RSS. The parents, without consanguineous marriage, were healthy, with no affected individuals in the family. Initial symptoms began at the age of two with growth retardation. He had distinctive facial features, mild intellectual disability, and prominent heels. Sanger sequencing, ES, and MLPA-ME030 yielded normal results. Array CGH detected loss of heterozygosity across chromosome 7. MLPA-ME032 demonstrated methylation at HHA1 cleavage sites targeting the *GRB10* and *MEST* genes.

Conclusion: While maternal UPD7 frequency in RSS ranges from 7-10%, genetic heterogeneity complicates the molecular and cytogenetic RSS diagnosis. Genetic testing to elucidate rare molecular pathologies can only be performed in the presence of a strong clinical diagnosis. Molecular diagnosis contributes to better clinical monitoring of the patient

Grants: I would like to convey special thanks to Scientific Research Projects Coordination Unit of İstanbul University(Project ID 40482)

Conflict of Interest: None declared

P13.068.B *BCL11B*-related disease: a single phenotypic entity?

Juliana Heather Vedovato-dos-Santos[1;2], *Rebecca Tooze*[1], *Emma Mccann*[3], *Sivagamy Sithambaram*[3], *Yasemin Alanay*[4;5], *David Johnson*[6], *Özlem Akgün Doğan*[4;7], *Meltem Kilercik*[8], *Aysen Bingol*[9], *Memet Ozek*[8], *Christoffer Nellaker*[10], *Stephen Twigg*[1], *Andrew Wilkie*[1]

[1]*MRC Weatherall Institute of Molecular Medicine (Oxford University & John Radcliffe Hospital), Clinical Genetics Group, Oxford, United Kingdom;* [2]*Jesus College, Oxford, United Kingdom;* [3]*Liverpool Women's NHS Foundation Trust, Department of Clinical Genetics, Liverpool, United Kingdom;* [4]*Acıbadem University School of Medicine, Division of Pediatric Genetics, Department of Pediatrics, Istanbul, Türkyie;* [5]*Acıbadem University School of Medicine, Rare Diseases and Orphan Drugs Application and Research Center-ACURARE, Istanbul, Türkyie;* [6]*Oxford University Hospitals NHS Foundation Trust, Craniofacial Unit, Oxford, United Kingdom;* [7]*Acıbadem University School of Medicine, Division of Medical Biochemistry, Department Of Basic Sciences, Istanbul, Türkyie;* [8]*Acıbadem University School of Medicine, Department of Neurosurgery, Istanbul, Türkyie;* [9]*Akdeniz Üniversitesi, Division of Pediatric Immunology and Allergy, Department of Pediatrics, Antalya, Türkyie;* [10]*Nuffield Department of Women's & Reproductive Health (NDWRH), University of Oxford, Oxford, United Kingdom*

Consortium: The Genomics England research consortium

Background/Objectives: Heterozygous pathogenic variants in BCL11B have been described as causative for two Mendelian phenotypes. Here we provide a systematic review of the literature, providing evidence to support the notion that BCL11B-related disease (BRD) should be regarded as a single phenotypic entity. Furthermore, we supply four new patient reports, three with sequence variants and one with a deletion encompassing BCL11B. All of them presented with craniosynostosis (CRS), thus refining the phenotype of BRD and highlighting CRS as an important diagnostic clue.

Methods: Two databases were searched (MEDLINE and Embase) and snowballing was conducted on all selected results (backward via reference lists). Newly reported patients were identified through data from the Genomics England 100,000 Genomes Project, international collaboration and the Craniofacial Unit in Oxford. Pathogenic variants in BCL11B were detected through next generation sequencing and validated by Sanger sequencing.

Results: We present an analysis of the largest cohort of patients with pathogenic sequence variants in BCL11B: fifty cases, including three newly reported patients. We have defined the phenotype of BRD as a neurodevelopmental disorder with three cardinal features: neurodevelopmental delay, facial dysmorphism and immunodeficiency.

Conclusion: Major points of interest are the delineation of the phenotype, the addition of CRS to the major features, and the discussion on the role of 14q32 microdeletions in the molecular pathogenesis of the syndrome. We hope that the facial photographs and reconstructions of the skull presented here will prove useful in further defining this phenotype.

Grants: VTCT Foundation Scholarship, Clarendon Fund Scholarship, Jesus College Graduate Scholarship

Conflict of Interest: None declared

P13.069.C Impact of deep phenotyping: High diagnostic yield in a diverse pediatric population of 172 patients through clinical whole-genome sequencing at a single center

*OZLEM AKGUN DOGAN[1;2;3], Ecenur Tuc Bengur[4;5], Beril Ay[5], **Gulsah Sebnem Ozkose**[2;3], Emre Kar[5], Fuat Baris Bengur[5], Aybike Bulut[2;3], Ayça Yiğit[2;3], Eylül Aydın[2;3], Fatma Nisa Esen[6], Özkan Özdemir[2;7], Ahmet Yeşilyurt[6], Yasemin Alanay[1;2;3]*

[1]Acibadem Mehmet Ali Aydinlar University, School of Medicine, Department of Pediatrics, Division of Genetics, Istanbul, Türkyie; [2]ACURARE-Rare and Undiagnosed Diseases Center; [3]Acibadem Mehmet Ali Aydinlar University, Health Sciences Institute, Department of Genome Studies, Istanbul, Türkyie; [4]Washington University in St. Louis, School of Medicine, Department of Pediatrics, Division of Genetics & Genomic Medicine, Missouri, United States; [5]Acibadem Mehmet Ali Aydinlar University, School of Medicine, Istanbul, Türkyie; [6]Acibadem Labgen Genetic Diagnosis Center, Istanbul, Türkyie; [7]Acibadem Mehmet Ali Aydinlar University, School of Medicine, Department of Basic Sciences, Division of Medical Biology, Istanbul, Türkyie

Background/Objectives: Pediatric patients with undiagnosed conditions, particularly those suspected of having Mendelian genetic disorders, pose a significant challenge in healthcare. This study investigates the diagnostic yield of whole genome sequencing (WGS) in a pediatric cohort with diverse phenotypes, particularly focusing on the role of clinical expertise in interpreting WGS results.

Methods: A retrospective cohort study was conducted at Acibadem University's Maslak Hospital in Istanbul, Turkey, involving pediatric patients (0-18 years) who underwent diagnostic WGS testing. Clinical assessments, family histories, and previous laboratory and imaging studies were analyzed. Variants were classified and interpreted in conjunction with clinical findings.

Results: The cohort comprised 172 pediatric patients, aged 0-5 years (62.8%). International patients (28.5%) were from 20 different countries. WGS was utilized as first-tier in 61.6% of patients. The diagnostic yield of WGS reached 61.0%, enhanced by the reclassification of variants of uncertain significance (VUS) through reverse phenotyping by an experienced clinical geneticist. Consanguinity was 18.6% of the overall cohort. Dual diagnoses were present in 8.5% of solved patients.

Discussion: Our study particularly advocates for the selection of WGS as a first-tier testing in infants and children with rare diseases under 5 years of age, thereby potentially shortening the duration of the diagnostic odyssey. The results also emphasize the critical role of a single clinical geneticist's expertise in deep phenotyping and reverse phenotyping, which contributed significantly to the high diagnostic yield.

Conflict of Interest: None declared

P13.071.A Heterozygous deletions of BCL11B broaden the spectrum of severe combined intellectual developmental disorder with dysmorphic facies, speech delay and T-cell abnormalities (IDDSFTA)

Corinna Hendrich[1], *Jana Lentes[1], Eva Tolosa[2], Hendrik Langen[3], Davor Lessel[4], Nataliya Di Donato[1], Tim Ripperger[1], Oscar de la Calle-Martín[5], Anke Katharina Bergmann[1]*

[1]Hannover Medical School, Human Genetics, Hannover, Germany; [2]University Medical Center Hamburg-Eppendorf, Institut für Immunologie, Hamburg, Germany; [3]Children- and Teenager Hospital Auf der Bult, Sozialpädiatrisches Zentrum Hannover, Hannover, Germany; [4]Paracelsus Medical University, Universitätsinstitut für Humangenetik der PMU, Salzburg, Austria; [5]Hospital de Sant Pau, Immunology Dept. Universitat Autonoma de Barcelona., Barcelona, Spain

Background Heterozygous missense, frameshift, splice site and truncating variants as well as chromosomal rearrangements of the *BCL11B* gene coding for a transcription factor are associated with a developmental disorder syndrome with language and cognitive impairment as well as facial abnormalities and a characteristic reduction in ILC2 T cells (innate lymphoid cells) (IDDSFTA). In addition, somatic alterations of *BCL11B* are known to be a contributory cause of lymphoid neoplasia. Heterozygous deletions of the *BCL11B* gene have been described in isolated cases in the literature and have not yet been classified as disease-causing.

Methods Whole exome sequencing, array cGH and karyotyping were performed in two unrelated patients with an IDDSFTA-typical phenotype. Due to the rarity of the variant and the lack of functional analyses regarding *BCL11B* deletions, this deletion is currently classified as a variant of undetermined significance (VUS).

Results Both patients carry a heterozygous deletion including the *BCL11B* gene. Additionally, significant reduction of ILC2 T-cells were shown in both patients.

Conclusion We postulate that heterozygous deletions of *BCL11B* contribute to the IDDSFTA genotypic spectrum with possible clinical consequences for the further surveillance of these patients with regard to immunological or hematological secondary diseases

Conflict of Interest: None declared

P13.072.B Next-generation phenotyping improves the diagnostic yield in a Cornelia de Lange Syndrome Brazilian cohort

Beatriz Carvalho Nunes[1;2], *Thainá Vilella[2], Isabel Pinheiro[3], Hiromi Aoi[4;5], Rie Seyama[4;5], Naomichi Matsumoto[4], Fernanda Teixeira Bellucco[2], Chong Kim[3], Maria Isabel Melaragno[2], Peter Krawitz[1], Tzung-Chien Hsieh[1]*

[1]Rheinische Friedrich-Wilhelms-Universität Bonn, Institute for Genomic Statistics and Bioinformatics, Bonn, Germany; [2]Universidade Federal de São Paulo, Department of Morphology and Genetics, Genetics Division, São Paulo, Brazil; [3]Universidade de São Paulo, Genetics Unit, Instituto da Criança, São Paulo, Brazil; [4]Yokohama City University, Department of Human Genetics, Yokohama, Japan;

[5]Juntendo University, Department of Obstetrics and Gynecology, Tokyo, Japan

Background/Objectives: Cornelia de Lange Syndrome (CdLS), a rare multisystemic developmental disorder, results mainly from germline mutations. However, around 15%-20% of cases present mosaicism, often undetectable in peripheral blood, and the overlapping features of CdLS with other syndromes complicate accurate diagnosis. This study employs next-generation phenotyping to prioritize CdLS patients for further investigation based on facial gestalt, offering a potentially more efficient diagnostic approach.

Methods: Out of the 62 patients, 38 were diagnosed with CdLS, eight received negative results in Whole Exome Sequencing (WES), and 16 were diagnosed with other conditions despite initial suspicion of CdLS. Facial analysis was conducted by GestaltMatcher, and we reported the top-10 detected syndromes. Buccal cells WES was performed with paired-end libraries in Illumina NOVASeq.

Results: Among diagnosed CdLS patients, 87.2% had CdLS ranked as their top-1 syndrome, with all presenting it within their top-10 syndromes. Among patients with negative WES results, 75% had CdLS as their top-1 syndrome and all within their top-10. For patients diagnosed with other conditions, 60% had their respective diagnosis within the top-10 syndromes, 66.6% had CdLS within the top-10, and one patient had it in their top-1. This patient was submitted to further analysis of buccal cells WES which revealed a likely pathogenic mosaic variant on the SMC1A gene (NM_006306.4:c.256G>T [p.Glu*]). Therefore, GestaltMatcher can identify potential syndromes even in cases where WES yielded inconclusive results.

Conclusion: This study highlights the effectiveness of GestaltMatcher in guiding the selection of appropriate tests in cases where WES results are inconclusive.

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Conflict of Interest: None declared

P13.073.C Kabuki Syndrome: prenatal and neonatal diagnosis and improved phenotyping

paolo infantino[1], *Elena Gennaro*[2], *Maria Teresa Divizia*[1], *Giulia Rosti*[1], *Pierangela De Biasio*[3], *Maria Gnazzo*[4], *Silvia Buratti*[5], *Francesca Faravelli*[1]

[1]*Giannina Gaslini Institute, Genomics and Clinical Genetics, Italy;* [2]*Giannina Gaslini Institute, Human Genetic Laboratory, Italy;* [3]*Ospedale San Martino, Diagnosi prenatale e Medicina perinatale, Italy;* [4]*Ospedale Pediatrico Bambino Gesù, UO di Malattie Rare, Italy;* [5]*Giannina Gaslini Institute, Intensive Care Unit, Italy*

Background: Kabuki Syndrome (KS) represents an heterogeneous entity, caused by pathogenic variants (PV) in the KMT2D or KDM6A gene. The most frequently associated prenatal findings are: cardiac anomalies, polyhydramnios, genitourinary anomalies, single umbilical artery and hydrops /pleural effusion/ascites. Prenatal and neonatal diagnosis of KS diagnosis remains uncommon, but the advance of the sequencing technology is covering the gap, revealing a broader clinical spectrum.

Methods: Two KS cases were ascertained through WES trio analysis. Case 1: a fetus with augmented nuchal translucency and augmented right atrium volume, non-visualization of the gallbladder, single umbilical artery and pleural effusion; case 2: a newborn with hyperbilirubinemia, cardiac malformation, mild immunodeficiency, diaphragmatic hernia, skeletal and cerebral abnormalities. In both cases, prenatal a-CGH and RASopathies NGS panel didn't reveal any causative variant.

Results: KMT2D c.10740+1G>A, p.(?) and c.16438_16441del, p.(Asn5480Valfs*) PVs were respectively identified and confirmed to be de novo; the first located at a canonical splicing site and the second predicted to cause a frameshift. Based on ACMG classification the two variants were classified as pathogenic, and considered causative of the clinical findings.

Conclusion: We describe two cases which confirm the heterogeneity of KS phenotype, and its prenatal and early onset features. In both cases the condition was ascertained through WES trio testing, confirming the value of the technique in prenatal/neonatal complex cases, shortening the diagnostic odissey and providing important information to clinicians and families.

Conflict of Interest: None declared

P13.074.D The presence of cardiological manifestations in SHANK3-mutated patients

Lucia Dougherty de Miguel[1], *Roger Esmel-Vilomara*[2], **Alícia Artigas Baleri**[3], *Asunción Díaz*[1], *Eulàlia Turón-Viñas*[1], *Lourdes Vega*[3], *Luisa Panades de Oliveira*[4;5;6], *Ivon Cuscó*[3;7], *Susana Boronat*[1]

[1]*Institut d'Investigació Biomèdica Sant Pau (IIB Sant Pau), Hospital de la Santa Creu i Sant Pau, Paediatric Neurology, Barcelona, Spain;* [2]*Institut d'Investigació Biomèdica Sant Pau (IIB Sant Pau), Hospital de la Santa Creu i Sant Pau, Paediatric Cardiology, Barcelona, Spain;* [3]*Institut d'Investigació Biomèdica Sant Pau (IIB Sant Pau), Hospital de la Santa Creu i Sant Pau, Genetics Department, Barcelona, Spain;* [4]*Hospital del Mar, Epilepsy Monitoring Unit, Department of Neurology, Barcelona, Spain;* [5]*Hospital del Mar Medical Research Institute (IMIM), Epilepsy Research Group, Barcelona, Spain;* [6]*Universitat Pompeu Fabra (UPF), Barcelona, Spain;* [7]*Instituto de Salud Carlos III, Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER), Barcelona, Spain*

Background/Objectives: *SHANK3* has been established as the causative gene of 22q13.3 microdeletion syndrome. Patients with *SHANK3* point mutations have also been identified with a wide range of clinical manifestations. No genotype-phenotype correlation has been described. Recent studies showed that *SHANK3* plays an important role in cardiac function and suggest that genetic alterations may be associated with cardiac abnormalities. To date, cardiac alterations have been poorly associated with the 22q13.3 deletion, but not with *SHANK3* point mutations. The aim of this study is to expand the clinical manifestations related to *SHANK3* mutations.

Methods: We carried out a retrospective study of patients with *SHANK3* (NM_001372044.2) mutations. Detailed clinical data were collected, including psychomotor development, neuropsychiatric manifestations and electroencephalographic patterns. An exhaustive cardiological examination was also performed.

Results: We included five patients with a *SHANK3* mutation: two frameshifts (c.3286del, c.3610_3611del), one nonsense (c.4090C>T), one splice mutation (c.2448+1G>A) and a complete deletion (secondary to unbalanced translocation). In accordance with previous studies, our 4 loss-of-function mutations were located between the PDZ and SAM domains. Cardiological examination was performed in 4 patients, all of them had cardiac abnormalities: postictal atrial fibrillation, mild-moderate left ventricular dysfunction, borderline left ventricular function and persistent ductus arteriosus.

Conclusion: In our series we have observed cardiac abnormalities in most patients. Although the relationship of these findings remains to be clarified, the results showed the importance of performing a complete cardiological screening in these patients, especially in those with refractory epilepsy, which could contribute to the increase in the risk of SUDEP.

Grants:

Conflict of Interest: None declared

P13.075.A Two novel IGF2 missense variants in four patients with Silver-Russel(-like) syndrome

Stephany Donze[1], *Hermine van Duyvenvoorde*[2], *Arie van Haeringen*[2], *yvonne hilhorst- hofstee*[2], *Carlo Marcelis*[3], *Petra van Setten*[4], *Monique Losekoot*[2], *Daniëlle van der Kaay*[5]

[1]*Erasmus University Medical Center, Clinical Genetics, Rotterdam, Netherlands;* [2]*Leiden University Medical Center (LUMC), Clinical Genetics, Leiden, Netherlands;* [3]*Radboud University Medical Center, Clinical Genetics, Nijmegen, Netherlands;* [4]*Radboud University Medical Center, Pediatrics, Nijmegen, Netherlands;* [5]*Erasmus University Medical Center, Pediatric Endocrinology, Rotterdam, Netherlands*

Background/Objectives: Silver-Russell syndrome (SRS) is a rare disorder characterized by being born small for gestational age, postnatal growth failure, relative macrocephaly, a prominent forehead, feeding difficulties, and body asymmetry. Most cases are due to loss of methylation of the imprinting control region 1 on chromosome 11p15. Recently, pathogenic paternal IGF2 variants have been associated with a SRS phenotype. The variants and clinical spectrum remain, however, limited.

Methods: Four patients with a SRS(-like) phenotype and a variant in IGF2, detected by whole exome sequencing (WES), were collected.

Results: Clinical characteristics are summarized in Table 1. In patients 1, 2 and 3, WES showed a missense variant in the IGF2 gene, NM_000612.4:c.100G>A p.(Gly34Ser). In patient 1 and 2 from family 1, the variant was paternally inherited. In patient 3 from family 2 the variant occurred de novo, and was located on the paternal allele. In patient 4 from family 3 WES showed a de novo missense variant in the IGF2 gene, NM_000612.6:c.148T>C; p.(Phe50Leu). Unfortunately, paternal origin could not be confirmed.

Conclusion: In this case series we report 2 novel IGF2 missense variants in 4 patients with a variable SRS-like phenotype. In contrast to previous reports, pre- and postnatal growth retardation and congenital anomalies were less pronounced and two patients with pre- and/or postnatal growth retardation showed spontaneous catch-up growth. Behavioural issues were prominent and could present a new feature of SRS patients with an IGF2 pathogenic variant. This case series expands the genotypic and phenotypic spectrum of SRS patients with an IGF2 pathogenic variant.

Grants: Not applicable.

Table 1. Characteristics of four patients with an IGF2 variant

Pregnancy and delivery	**Patient 1**	**Patient 2**	**Patient 3**	**Patient 4**
Gestational age (weeks + days)	38 + 4	39 + 6	37 + 3	34 + 5
Birth weight SDS	-1,1	-2.2	-2.0	0,5
Birth height SDS	-	-	-1.6	-1.2
Height at age 4 weeks	-1.9	-2.6	-	-
Growth at last assessment				
Height SDS	-0.5	-1.4	-1.5	-3.2
Weight for height SDS	2,4	-0.7	-0.1	1,7
Head circumference SDS	0,2	-0.1	0,3	3,2
Sitting height / height ratio SDS	-0.4	-1.6	1,6	2,9
NH-CSS score				
Birth length and/or weight ≤ -2 SDS	-	+	+	-
Postnatal height ≤ -2 SDS	-	-	-	+
Relative macrocephaly at birth	+	+	-	+
Prominent forehead	+	+	+	+
Body asymmetry	-	-	-	-
Feeding difficulties and/or low BMI	-	+	-	-
Total score	2 / 6	4 / 6	2 / 6	3 / 6
Other clinical features not in NH-CSS score				
Triangular face	+	+	+	-
Low-set / posteriorly rotated ears	-	+	-	-
Down turned mouth	-	-	+	-
Micrognathia	-	-	-	-
5th finger clinodactyly	-	-	+	+
Speech delay	+	+	+	+
Motor delay	-	+	+	+
Behavioural problems	+	+	+	?
Other	autism	autism, sensory processing disorder, intellectual disability	pointy chin, congenital hip dysplasia	shoulder dimples
Endocrine findings				
Serum IGF-I SDS	0,2	-0.5	-	-2.0
Serum IGFBP-3	-	-	-	-2,9
Serum IGF-II	349 ng/ml (220-620)	530 ng/ml (280-610)	-	63 ng/ml (190-580)

Conflict of Interest: None declared

P13.076.B Prenatal characterization of an additional chromosome 9 derivative

Juliane Maria Beuschlein[1], Niklas Hirschberger[1], Moneef Shoukier[1], Florian Vogel[1], Eva Kahles[1], Daniela Bayer[1], Markus Stumm[2], Rainer Wimmer[2], Julia Flunkert[2], Cornelia Daumer-Haas[1]

[1]Eurofins Humangenetik und Pränatal-Medizin MVZ GmbH, Munich, Germany; [2]Medicover Humangenetik Berlin-Lichtenberg MVZ, Berlin, Germany

Background/Objectives: Tetrasomy 9p is characterized by an additional isochromosome consisting of two short arms of chromosome 9. It is associated with a high variability of symptoms. About 25 cases have been described prenatally with the following findings: Intrauterine growth restriction (IUGR), heart defects, ventriculomegaly, Dandy-Walker malformation, cleft lip/palate and facial dysmorphism. Two third of these cases were non-mosaics.

Methods: A 37-year-old woman presented with the following abnormal fetal sonographic findings at 18 + 6 weeks of gestation: IUGR, brachycephalus, Dandy-Walker malformation, left choroid plexus cyst, absent nasal bone, micrognathia, persistent left superior vena cava and white spot. Amniocentesis was performed at 19 + 3 weeks. Array comparative genomic hybridization (CGH) analysis of uncultured amniocytes, chromosome and fluorescence in situ hybridization (FISH) analyses of cultured amniocytes and optical genome mapping of fetal lymphocytes were performed.

Results: Array CGH analysis detected a signal increase indicating a tetrasomy of the chromosome region 9pter→p22.3 (16 Mb) and a trisomy of the region p22.3→p13.1 (24 Mb). Considering the results of the array CGH analysis, chromosome and FISH analyses revealed the following non-mosaic karyotype: 47,XY,+i(9)(pter→q11.1::p22.3→pter). In contrast, optical genome mapping suggested that the region 9q11.1→q21.1 might be present in the additional chromosome 9 derivative. The patient opted for termination of pregnancy.

Conclusion: There are difficulties in interpreting the results regarding the pericentromeric region, as there were discrepancies between array CGH analysis and optical genome mapping. Advantages and disadvantages of the different methods, as well as their limitations, need to be taken into account when evaluating the findings.

Grants: None declared.

Conflict of Interest: Juliane Maria Beuschlein full-time, Niklas Hirschberger full-time, Moneef Shoukier full-time, Florian Vogel full-time, Eva Kahles full-time, Daniela Bayer part-time, Markus Stumm full-time, Rainer Wimmer full-time, Julia Flunkert full-time, Cornelia Daumer-Haas part-time

P13.077.C A *NEDD4* homozygous frameshift variant in a boy with multiple congenital anomalies overlapping the *Nedd4* knock-out murine model

Giulia Severi[1], Federica Isidori[2], Alessandra Vancini[3], Maria Chiara Baroni[1], Emanuela Scarano[4], Marco seri[5], Tommaso Pippucci[2]

[1]IRCCS Azienda Ospedaliero-Universitaria di Bologna, UOC Genetica Medica, Italy; [2]IRCCS Azienda Ospedaliero-Universitaria di Bologna, Computational Genomics, Italy; [3]Maggiore Hospital, Neonatal Intensive Care Unit, Bologna, Italy; [4]IRCCS Azienda Ospedaliero-Universitaria di Bologna, Rare Disease Unit, Department of Pediatrics, Italy; [5]IRCCS Azienda Ospedaliero-Universitaria di Bologna, Italy

Background/Objectives: *NEDD4* encodes a E3 ubiquitin ligase and to date it is not associated to disease in humans. In the murine model, functional studies demonstrate that *Nedd4* regulates the craniofacial development, promoting neural crest cells survival and maintaining their staminality. When *Nedd4* expression is abolished, neural crest cells migrating from the posterior forebrain, midbrain and rostral hindbrain may undergo aberrant apoptosis, resulting in an insufficient population of these cells for proper brain functioning. *Nedd4*$^{-/-}$ mice have severe cranio-facial defects with a prevalent maxillary and mandibular involvement, clef lip and palate, and deficiency of the trigeminal ganglia. They are also significantly smaller than the wild type littermates.

Methods: We performed whole exome sequencing (WES) in a boy, first child of consanguineous parents from Pakistan, who presented prenatally with left cleft lip and palate and right clubfoot. At birth he also showed post-axial right polydactyly, bilateral iris and optic nerve coloboma, patent foramen ovale and ductus arteriosus and dysmorphic features. He showed developmental delay: at 4 years he could walk only if assisted and he was still non-verbal. He had short stature (-3.3 SD) and microbrachycephaply (-4.1 SD; -3.3 SD related to his stature).

Results: WES revealed a homozygous c.1369_1375delATTTCATinsCG (p.Ile457AlafsTer15) mutation in *NEDD4*; his parents were both heterozygous. RNA-seq, transcriptome, ubiquitinome and other functional studies are ongoing on skin fibroblasts.

Conclusion: The phenotype of this patient shows consistency with phenotypic features in mice, but more patients are needed to prove that *NEDD4* is implicated in human disease.

Conflict of Interest: None declared

P13.078.D Results of rapid trio exome sequencing in routine care of 90 critically ill newborns and infants with an unclear symptom complex at the University Hospital Cologne, Germany

Clara Velmans[1], Mert Karakaya[1], Louisa Lepkes[1], Sarah Thull[1], Zempel Hans[1], Jutta Becker[1], Nico Fuhrmann[1], Sophie Kaspar[1], Nadine Reintjes[1], Vera Riehmer[1], Nora Winnerling[1], Bodo B. Beck[1], Florian Erger[1], Julia Schreml[1], Christian Netzer[1]

[1]Institute of Human Genetics, University of Cologne, Faculty of Medicine and University Hospital Cologne, Cologne, Germany

Background: The majority of congenital syndromic disorders are genetically determined. By means of trio exome analysis a genetic cause can currently be confirmed in up to half of those affected.

Methods: Since the beginning of 2021 rapid trio exome sequencing was implemented in routine care of hospitalized newborns and infants with an unclear symptom complex (abnormalities in at least two organ systems with unclear etiology) at the University Hospital of Cologne, Germany. The genetic diagnostics were embedded in extensive pre- and post-test genetic counselling and required close cooperation between pediatricians and clinical geneticists.

Results: The diagnostic result was available after an average of two weeks. Of the 90 patients examined so far a genetic cause could be confirmed in 46 patients (diagnostic rate: 51 %). In almost half of these patients the genetic diagnosis resulted in a direct clinical relevance or therapeutic benefit. The diagnoses were very heterogeneous ranging from more common rare diseases with atypical presentation to very rare syndromes with less than 50 described patients in the literature so far. 52 % of the confirmed diagnoses were caused by a de novo genetic variant, 28 % were autosomal recessive disorders and surprisingly in 9

patients (20 %) the dominant genetic disease was inherited from an unaffected or mildly affected parent.

Conclusion: Rapid trio exome sequencing in routine care of critically ill newborns and infants with an unclear symptom complex represents a good diagnostic option with a high diagnostic yield and considerable clinical relevance.

Conflict of Interest: None declared

P13.079.A Atypical Prader-Willi Syndrome: Insights into a rare mechanism and its implications

Sarah Graine[1], *Laurence Cuisset*[1], *jean-luc alessandri*[2], *Fatma Maazoun*[1], *Berenice Herve*[1], *Martine Montagnon*[1], *Thierry Bienvenu*[1], *Jean-Michel Dupont*[1], *Aziza Lebbar*[1]

[1]*Cochin Hospital, APHP Centre-Universite Paris Cité, Service de Médecine Génomique des Maladies de Système et d'Organe, Fédération de Génétique et de Médecine Génomique, Paris, France;* [2]*Hospital Center Félix Guyon, Department of Medical Genetics, Saint-Denis, France*

Background/Aims: 70% of Prader-Willi syndrome (PWS) result from a microdeletion mediated by NAHR between segmental duplications BP1 or BP2 and BP3.

This report describes a rare deletion mechanism affecting the imprinted PWS/AS locus within the 15q11-q13 region, resulting from a unique 3:1 malsegregation of a reciprocal translocation t(11;15)(p15;q12).

This 4.14 Mb deletion, is smaller than usual cases, expanding from BP1 to the *GABRB3* gene while leaving *GABRA5*, *GABRG3*, *OCA2*, and *HERC2* intact. This may have implications for the reduction of neuropsychiatric consequences. The clinical description and potential impact on genetic counseling will be discussed.

Methods: The patient underwent clinical assessment and genetic testing, including DNA methylation analysis (methylation-specific PCR), FISH (*SNRPN* probe), aCGH, and karyotyping to identify the specific rare mechanism involved.

Results: The 4-month-old patient presented with global hypotonia, and orality disorders at the time of examination.

The diagnosis of PWS was confirmed by methylation analysis, which indicated a loss of the paternal allele. Additionally, cytogenetic analysis identified a derivative chromosome 15 from a translocation t(11;15)(p15;q12), leading to an atypical 4.14 Mb deletion of the 15q11.2-q12 region. However, since no imbalance related to chromosome 11 has been detected by aCGH, the phenotype will be entirely linked to the 15q deletion. Parental karyotyping will be performed, to check for an inherited translocation.

Conclusion: This case emphasises the relevance of detailed chromosomal analysis in PWS with Karyotype for accurate genetic counselling.

Reduce size of deletion may suggest a milder PWS phenotype, which will be confirmed as the child ages.

Grants: None.

Conflict of Interest: None declared

P13.080.B Usefulness of new generation sequence analysis in rare diseases of childhood: dual molecular diagnosis

Esra Isik[1], *Enise AVCI DURMUŞALİOGLU*[1], **Mehmet Mert Topaloğlu**[1], *Turkan Turkut Tan*[1], *Fayize Maden Bedel*[2], *Tayfun Cinleti*[3], *Tahir Atik*[1], *Ozgur Cogulu*[1]

[1]*Ege University, Pediatric Genetics;* [2]*Erzurum City Hospital, Pediatric Genetics;* [3]*İzmir City Hospital, Pediatric Genetics*

Background/Objectives: The term "dual molecular diagnosis" denotes the identification of two distinct molecular causes contributing to a patient's clinical symptoms or disease manifestations. This can be especially important in rare diseases. Most rare diseases manifest during childhood, the age of onset of clinical symptoms, individual variability, modifying factors, and coexisting conditions introduce complexities to the diagnostic process. NGS enables the rapid, simultaneous analysis of a multitude of genes, facilitating the detection of multiple genetic changes underlying the patient's clinical manifestations. This study aims to showcase patients with dual molecular diagnoses within the pediatric patient group, utilizing clinical exome sequencing (CES) and whole exome sequencing (WES) results. By doing so, the study underscores the clinical importance of NGS in unraveling the intricate genetic landscape of rare diseases and enhancing diagnostic precision.

Methods: Between November 2021 and May 2023, we retrospectively reviewed 288 CES and 324 WES results from children aged 0-18 with various indications at the Ege University Department of Pediatric Genetic Diseases.

Results: Dual molecular diagnoses occurred in 17 (2.77%) cases, with 14 patients exhibiting parental consanguinity. Patient ages ranged from 1 to 16 years. Thirty-five different variants in 33 genes were detected, with 33 being single nucleotide changes/indels and two being intragenic copy number variations. Eighteen of those variants were novel.

Conclusion: Identifying dual molecular diagnoses in pediatric rare diseases is crucial for tailored patient management and effective genetic counseling. NGS methods enable early and precise diagnoses in complex phenotypes, providing insights beyond traditional diagnostic approaches.

Grants: None

Conflict of Interest: None declared

P13.081.C The era of candidate genes: biallelic frameshift variants in *FAT1* and a new malformation syndrome?

Sara Pinho[1], *Aida Bertoli-Avella*[2], *Ligia Almeida*[2], *Juliette Dupont*[1]

[1]*Unidade Local de Saúde Santa Maria, Serviço de Genética, Departamento de Pediatria, Lisbon, Portugal;* [2]*CENTOGENE GmbH, Rostock, Germany*

Background: The *FAT1* gene belongs to a cadherin family involved in epithelial cell adhesion, migration and polarity. In 2019, Lahrouchi et al. reported 10 cases with homozygous frameshift variants in *FAT1* and ocular abnormalities, nephropathy, syndactyly and facial dysmorphisms.

Methods: We present an 18-year-old female, first child of a healthy consanguineous couple with unremarkable family history, with bilateral congenital ptosis, toe syndactyly, learning difficulties and facial dysmorphisms, such as elongated face, high arched eyebrows, micrognathia, narrow palate and oligodontia. She underwent surgery for biliary atresia in the neonatal period. Due to secondary biliary cirrhosis, she is now waiting a liver transplant.

Results: No pathogenic/likely pathogenic copy number variant was found by arrayCGH. Subsequent exome sequencing identified the homozygous frameshift variant NM_005245.3: c.1068_1069del, p.(Pro358Thrfs*10), in *FAT1* gene, still not linked to an OMIM clinical entity. However, following ClinGen framework for clinical validity, this gene is rated with strong evidence for autosomal recessive colobomatous-microphthalmia, ptosis, nephropathy and syndactyly syndrome. Both parents are heterozygotes.

Conclusion: Our patient's phenotype is consistent with other cases described in the literature with biallellic frameshift variants

in *FAT1*. Most patients have ptosis, toe syndactyly and similar facial features. Other ocular abnormalities can occur. Intellectual disability and nephropathy only exist in some individuals. No other cases of biliary atresia have been reported.

Although loss of function appears to be a mechanism of disease in this gene, additional evidence is necessary. This case adds evidence to the role of *FAT1* in a new multi-system disorder and contributes to the characterization of its associated phenotype.

Conflict of Interest: None declared

P13.082.D The role of rapid whole exome sequencing in critically ill newborns with a preliminary diagnosis of genetic syndrome

Durdugul Ayyildiz Emecen[1], *esra isik*[1], *Enise AVCI DURMUŞALİOGLU*[1], *aysegul yildirim*[1], *demet terek*[1], *Ozge Altun Koroglu*[1], *mehmet yalaz*[1], *mete akisu*[1], *esra er*[2], *pelin teke kisa*[3], *ozlem sarac sandal*[2], *gulhan atakul*[2], *Ozgur Cogulu*[1], *Ferda Ozkinay*[1], *Tahir Atik*[1]

[1]*Ege University Faculty of Medicine, Türkyie;* [2]*S.B.Ü Dr. Behçet Uz Çocuk Hastalıkları Ve Cerrahisi Eğitim Ve Araştırma Hastanesi, Türkyie;* [3]*Dokuz Eylul University Faculty of Medicine, Türkyie*

Introduction: Whole-exome sequencing has become one of the first-line diagnostic tests for patients with multisystem abnormalities and congenital anomalies. Although it has started to be used quite frequently in clinics, it is not yet widely utilized in neonatal intensive care services. It is vital to make a quick and accurate diagnosis when approaching a critically ill patient. In our study, we aimed to evaluate the utility of rapid whole-exome sequencing in critically ill patients.

Method: Whole-exome sequencing was performed on 8 critically ill patients hospitalized in the intensive care unit who were younger than 3 months.

Results: Five of the patients were female. The mean age was 22 days. Five patients had a certain diagnosis with whole-exome sequencing (P-1, P-4, P-5, P-7, P-8). Two of the patients had heterozygous pathogenic variants in the PTPN11 gene (P-5, P-8). One patient (P-7) was diagnosed with Stickler syndrome with a novel homozygous variant c.1152del (p.Trp384*) in the FOXL3 gene. In one patient (P-4) with dilated cardiomyopathy, a de-novo heterozygous pathogenic variant was detected in the TPM1 gene. One patient (P-2) was diagnosed with Kabuki syndrome. Three patients (P-5, P-6, P-7) died before receiving whole-exome sequencing results.

Conclusion: With the help of rapid whole-exome sequencing, timely changes in treatment, initiation of palliative treatment, creation of a long-term treatment program, and quality genetic counseling can be provided for patients diagnosed in a short time.

Therefore, it is inevitable that rapid whole-exome sequencing will become the first-line test in diagnosing critically ill patients in the future.

Conflict of Interest: None declared

P13.083.A Hickam's dictum: Keeping up with the Diagnoses

Susana Lemos Ferreira[1], *Joana Catanho*[1], *Mafalda Melo*[1], *Sofia Nunes*[1], *Rui Gonçalves*[1], *Marta Amorim*[2], *Diana Antunes*[1], *Márcia Rodrigues*[3], *Teresa Kay*[1], *Inês Carvalho*[1], *Margarida Venancio*[1]

[1]*Hospital Dona Estefânia, Unidade Local de Saúde São José, Centro Clínico Académico de Lisboa, Serviço de Genética Médica, Lisboa, Portugal;* [2]*Hospital dos Lusíadas, Consulta de Genética Médica, Lisboa, Portugal;* [3]*Unidade Local de Saúde Santa Maria, Serviço de Genética Médica, Lisboa, Portugal*

Background/Objectives: Hickam's dictum sustains that a patient with multiple symptoms is more likely to have a combination of diseases rather than only one. Dual genetic diagnoses (DGD) consist of distinct or blend diagnoses of more than one locus that segregates independently. Whole exome sequencing (WES) is associated with increased DGD. Literature estimates that 7% of cases have DGD.

Methods: Retrospective review of paediatric patients with DGD referred to our Genetics Department (2001-2023).

Results: 25 patients were identified with a confirmed or potentially DGD. In 20/25, DGD were confirmed, clinical consistent with the phenotype and genetic results were classified as likely pathogenic/pathogenic. In 5/25, one variant was classified as uncertain significance but considered to be of potential clinical relevance. The main indication for referral was multisystemic disease (22/25). 3/25 patients were referred with a previous genetic diagnosis that did not fully explain the phenotype. Of the cases without previous diagnosis (22/25), 12 presented atypical features, 2 had a blended phenotype, and 8 had a second diagnosis after clinical validation of incidental/secondary findings. In 15/25, the first-tier test provided DGD. Primary diagnoses were obtained by WES (13/25) and array (7/25), and secondary diagnoses by WES (14/25) and array (8/25).

Conclusion: DGD should be considered in patients with atypical features and a multisystemic phenotype. Clinical suspicion was the main drive to continue investigation. WES improved our diagnostic capacity by identifying diagnoses that might be difficult to assert. Diagnostic investigation is not necessarily complete after achieving one diagnosis, and pursuing a DGD have implications to the patients and families.

Grants: yes

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P13.084.B Identification of novel *MN1* genotype-phenotype associations and in vitro studies of the pathogenic mechanisms involved

Elise Pisan[1;2], *Louis Januel*[3], *Massimiliano Rossi*[3;4], *Julie Jurgens*[5;6], *Brenda J. Barry*[6;7], *Joseph L. Demer*[8], *Caroline Pottinger*[9], *Kay Metcalfe*[10], *Rainer König*[11], *Michelle De Silva*[12;13], *Chloe Cunningham*[12;13;14], *Elizabeth Engle*[5;6], *Jeanne Amiel*[1;2], *Chris Gordon*[1]

[1]*Institute Imagine, Embryology and Genetics of Human Malformations, Paris, France;* [2]*Hôpital Necker-Enfants Malades, Service de Médecine Génomique des Maladies Rares, Paris, France;* [3]*Hospices Civils de Lyon - HCL, Service de Génétique, Lyon, France;* [4]*Claude Bernard University Lyon 1, INSERM U1028, CNRS UMR5292, CRNL, GENDEV Team, Bron, France;* [5]*Boston Children's Hospital, F.M. Kirby Neurobiology Center, Boston, United States;* [6]*Boston Children's Hospital and Harvard Medical School, Department of Neurology, Boston, United States;* [7]*Howard Hughes Medical Institute, Chevy Chase, United States;* [8]*University of California, Pediatric Ophthalmology and Strabismus Division, Departments of Ophthalmology and Neurology, Los Angeles, United States;* [9]*Wrexham Maelor Hospital, All Wales Medical Genetics Service, United Kingdom;* [10]*Manchester Centre for Genomic Medicine, United Kingdom;* [11]*Bioscientia Institute for Medical Diagnostics, Department of Human Genetics, Ingelheim, Germany;* [12]*Victorian Clinical Genetics Services, Parkville, Australia;* [13]*Murdoch Children's Research Institute, Parkville, Australia;*

[14]University of Melbourne, Royal Children's Hospital, Department of Paediatrics, Parkville, Australia

Background/Objectives: MN1 is a transcriptional cofactor of poorly understood function. Two phenotype groups associated with heterozygous *MN1* variants have been described: i) mild neurodevelopmental delay, deafness and minor dysmorphisms ascribed to loss-of-function by nonsense-mediated mRNA decay (NMD) or large deletions; and ii) severe intellectual disability (ID), craniofacial and brain malformations ascribed to *MN1* C-terminal truncating (MCTT) variants escaping NMD, leading to gain-of-function (GoF) via increased protein half-life. Here, we present probands with phenotypes and *MN1* variants not fitting either of these categories and explore their molecular mechanisms.

Methods: *MN1* variants were identified in probands by exome sequencing. Plasmids encoding wild-type or mutant MN1 were transfected into HEK-293T cells, with or without a translation inhibitor, followed by immunofluorescence assays and Western blots to assess subcellular localization and protein stability.

Results: Six novel *MN1* variants were identified in seven probands: i) p.(Ala1318Asp) in the C-terminal tail; ii) a cluster of four truncating variants in a region (Tyr878-Ala887) predicted to undergo NMD but whose phenotypic consequences are more severe than that expected for haploinsufficiency; and iii) p.(Ser829Phe) in two unrelated probands with frontonasal dysplasia and ID. As demonstrated previously for C-terminally truncated *MN1*, all variants induced nuclear aggregates upon overexpression. Relative to wild-type MN1, protein stabilization was increased by p.(Ala1318Asp) and by the truncations but was unchanged by p.(Ser829Phe).

Conclusion: Our results suggest that p.(Ala1318Asp) and the four truncating variants result in a GoF mechanism similar to the MCTT variants, while p.(Ser829Phe) has a unique qualitative effect on MN1 activity.

Conflict of Interest: None declared

P13.085.C Rahman syndrome - clinical and molecular analysis of 5 patients.

Anna Kutkowska-Kaźmierczak[1], *Katarzyna Wojciechowska*[2], *Castaneda Jennifer*[1], *Karolina Śledzińska*[3], *Paweł Gawliński*[1], *Mateusz Dawidziuk*[1], *Aleksandra Landowska*[1], *Olga Malinowska*[1], *Anna Abramowicz*[1], *Milena Poryszewska*[1], *Beata Twardowska*[1], *Larysa Pylyp*[1], *Beata Nowakowska*[1], *Jerzy Bal*[1], *Ewa Obersztyn*[1], *Monika Gos*[1]

[1]Institute of Mother and Child, Department of Medical Genetics, Warsaw, Poland; [2]Independent Laboratory of Genetic Diagnostics, Medical University of Lublin, Lublin, Poland; [3]Medical University of Gdańsk, Department of Paediatrics, Haematology and Onkology, Gdańsk, Poland

Background/Objectives: Rahman syndrome (RS), also called HIST1H1E syndrome, is a rare neurodevelopmental disorder characterized by distinctive phenotype with the following symptoms: **h**ypotonia, **i**ntellectual disability with behavioral problems, **s**keletal, **t**estes (cryptorchidism), **t**hyroid, **h**eart (ASD) and **e**ctodermal anomalies (sparse hair, thin nails and abnormal dentition), seizures, craniosynostosis and deafness. To date, all patients were reported to have a heterozygous pathogenic de novo variant in the *H1-4* gene. The objective of this work is thorough analysis of clinical picture of patients with Rahman syndrome.

Patients and methods: We present clinical evaluation of 5 patients with the pathogenic variant in *H1-4* gene identified in high-throughput sequencing analysis and clinical features of RS, aged from 10 to 19 years.

Results: All patients presented generalized hypotonia at infancy, macrocephaly and dysmorphic features with thin hair, all but one - intellectual disability from mild to moderate, delayed or absent speech, motor developmental delay, strabismus and/or astigmatism Congenital heart defects (ASD) were found in three individuals. Two patients presented short stature, hypothyroidism, ADHD and anxiety. One patient had deafness, one cryptorchidism, one scoliosis and one submucosal cleft palate with thin nails and severe dental caries. Molecular analysis revealed the presence of pathogenic or likely pathogenic variants: c.116dup (p.Val40GlyfsTer7), c.389dup (p.Pro131AlafsTer65), c.435dup (p.Thr146HisfsTer50), c.441dup (p.Lys148GlnfsTer48) and c.436_458del (p.Thr146AspfsTer42) in the *H1-4* gene.

Conclusions: Our patients present with typical clinical features of Rahman syndrome including macrocephaly with hypotonia, intellectual disability and dysmorphic features. We conclude that this disease should be considered in the differential diagnosis of macrocephaly.

Conflict of Interest: None declared

P13.087.A Extended panel next generation sequencing in patients suspected of RASopathy.

Monika Gos[1], *Olga Malinowska*[1], *Beata Twardowska*[1], *Mateusz Dawidziuk*[1], *Aleksandra Landowska*[1], *Anna Abramowicz*[1], *Milena Poryszewska*[1], *Anna Kutkowska-Kaźmierczak*[1], *Castaneda Jennifer*[1], *Jakub Klapecki*[1], *Natalia Braun-Walicka*[1], *Własienko Paweł*[1], *Maciej Geremek*[1], *Artur Barczyk*[1], *Karolina Śledzińska*[2], *Monika Cichoń-Kotek*[2], *Anna Kłosowska*[2], *Beata Lipska-Ziętkiewicz*[2], *Jolanta Wierzba*[2], *Damian Bieszczad*[2], *Aleksandra Gintowt-Chakour*[2], *Małgorzata Piotrowicz*[3], *Tatiana Chilarska*[3], *Nina Wieczorek-Cichecka*[3], *Katarzyna Końska*[4], *Magdalena Janeczko*[4], *Jacek Pilch*[5], *Katarzyna Wojciechowska*[6], *Ilona Jaszczuk*[6], *Renata Posmyk*[7], *Anna Jakubiuk-Tomaszuk*[8], *Magdalena Badura-Stronka*[9], *Maria Lassota*[10], *Krzysztof Szczałuba*[11], *Robert Smigiel*[12], *Ewa Obersztyn*[1], *Jerzy Bal*[1]

[1]Institute of Mother and Child, Deparment of Medical Genetics, Warsaw, Poland; [2]Medical University of Gdańsk, University Clinical Centre, Gdańsk, Poland; [3]Research Institute of Polish Mother's Memorial Hospital, Department of Genetics, Łódź, Poland; [4]Jagiellonian Medical University, Clinical Genetics Outpatient Clinic, Krakow, Poland; [5]Medical University of Silesia, Department of Pediatric Neurology, Katowice, Poland; [6]Lublin Medical University, Clinical Genetics Outpatient Clinic, Lublin; [7]Medical University in Bialystok, Department of Clinical Genetics, Białystok, Poland; [8]Medical University of Bialystok, Department of Pediatric Neurology and Rehabilitation, Białystok, Poland; [9]Poznan University of Medical Sciences, Department of Medical Genetics, Poznań, Poland; [10]Genetic Clinic at SPZOZ No. 1, Rzeszów, Poland; [11]Warsaw Medical University, Department of Medical Genetics, Warsaw, Poland; [12]Wroclaw Medical University, Clinical Department of Pediatrics, Endocrinology, Diabetology and Metabolic Diseases, Wrocław, Poland

Background/Objectives: Rasopathies are a group of systemic disorders that present with distinctive dysmorphic features, short stature, congenital heart defect, delayed psychomotor development and skin abnormalities. They are caused by the presence germline pathogenic variants in genes of the RAS/MAPK signalling pathway. The aim of our study was the assessment of the utility of NGS panel in case of RASopathy suspicion.

Methods: The gene panel was designed based on the literature review and included RASopathy genes, genes related to dysmorphic syndromes that clinical symptoms are similar to those observed in RASopathy patients. The study included 698 patients with RASopathy suspicion that were tested with targeted gene panel (KAPA SeqCap/HyperCap Workflow, Roche). The analysis has

been performed with in-house analysis pipeline based on bcbio and Variant Effect Predictor software.

Results: The analysis of variants in known RASopathy genes revealed the presence of pathogenic / likely pathogenic variant in 149 patients (21.3%) and variants of unknown significance in further 42 individuals (6.2%). The most commonly mutated genes were *PTPN11* (49 cases), *NF1* (28), *SOS1* (22) and *BRAF* (11). The analysis of other genes included in our panel revealed the presence of pathogenic / likely pathogenic variants in 59 (8.4%) individuals, including 10 patients with *ANKRD11* variant (supporting diagnosis of KBG syndrome).

Conclusion: Panel next generation sequencing is a powerful tool in the diagnosis of patients with RASopathy suspicion. The application of extended gene panel allowed to provide differential diagnosis in our patients.

Grants: The study was partially supported from EJP-RD/I/NSEuroNet/01/2021.

Conflict of Interest: None declared

P13.088.B Unveiling a pathogenic hotspot in PUM1 gene associated with PADDASS

Catarina Macedo[1], *Teresa Moreno*[2], *Mariana Soeiro e Sá*[1]

[1]*Hospital de Santa Maria, Serviço de Genética Médica, Departamento de Pediatria, Lisboa, Portugal;* [2]*Hospital de Santa Maria, Unidade de Neuropediatria, Departamento de Pediatria, Lisboa, Portugal*

Background/Objectives: *PUM1* gene encodes an RNA-binding protein linked to the Pumilio protein family, responsible for translation regulation during embryogenesis and cell differentiation. Diseases linked to *PUM1* encompass Spinocerebellar Ataxia 47 and *PUM1*-Associated Developmental Disability-Ataxia-Seizure Syndrome (PADDASS). PADDASS manifests with developmental delay (DD), intellectual disability (ID), ataxia, seizures, and short stature. Notably, four out of nine reported cases harbour the c.3439C>T, p.(Arg1147Trp) variant, with refractory seizures, hypotonia, and DD/ID being present in most patients. Dysmorphic features have been described in some of these patients.

Methods: We present a case of a girl born to nonconsanguineous parents, displaying fetal growth restriction and short bones in the second-trimester ultrasound. At the initial genetic assessment, in her first month of life, she exhibited refractory seizures, hypotonia, and distinctive dysmorphic features overlapping with Schinzel-Giedion syndrome (hirsutism, enlarged anterior fontanelle; low-set ears; sparse eyebrows; hypertelorism; short, narrow and up-slanted palpebral fissures; broad nasal bridge; bulbous nose; anteverted nares; long philtrum; thin upper lip; wide-spaced nipples; fifth finger clinodactyly; single transverse palmar crease). She evolved with severe DD, divergent strabismus, and recurrent respiratory infections. ArrayCGH yielded normal results.

Results: The first trio Whole Exome Sequencing (WES) in 2018 showed no diagnostic variants, but WES reanalysis in 2023 identified the de novo c.3439C>T, p.(Arg1147Trp) variant in *PUM1* gene.

Conclusion: Our findings contribute to the recognition of the c.3439C>T, p.(Arg1147Trp) variant as a disease-causing hotspot in PADDASS. This case emphasizes the distinctive facial gestalt as a cardinal feature, together with early-onset refractory epilepsy, hypotonia, and DD/ID.

Grants:

Conflict of Interest: None declared

P13.089.C Expanding the phenotypic spectrum of TRAF7 syndrome: report of eleven new cases and literature review

Aina Prat-Planas[1;2;3], *Carmen Palma Milla*[4;5;6], *Emma Soengas Gonda*[2;4], *Mónica Centeno-Pla*[1;2;7], *Jaime Sánchez-Pozo*[4;8], *Irene Lazaro-Rodriguez*[4;8], *Juan F. Quesada-Espinosa*[4;5], *Ana Rosa Arteche López*[4;5], *Jonathan Olival*[9], *Marta Pacio Miguez*[10], *María Palomares-Bralo*[2;10;11], *fernando santos-simarro*[12], *Ramón Cancho-Candela*[13], *María Vázquez-López*[14], *Verónica Adriana Seidel*[15], *Antonio Federico Martínez-Monseny*[3;16], *Didac Casas-Alba*[3;16], *Daniel Grinberg*[1;2;3], *Susanna Balcells*[1;2;3], *Mercedes Serrano*[2;3;17], *Raquel Rabionet*[1;2;3], *Miguel A. Martin*[2;4;18], *Roser Urreizti*[2;3;7]

[1]*Universitat de Barcelona, Department of Genetics, Microbiology and Statistics, Institute of Biomedicine (IBUB), Barcelona, Spain;* [2]*Instituto de Salud Carlos III (ISCIII), Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER), Madrid, Spain;* [3]*Institut de Recerca Sant Joan de Déu (IRSJD), Esplugues de Llobregat, Spain;* [4]*Hospital Universitario 12 de Octubre, Unidad de Dismorfología y Genética (UDisGen), Madrid, Spain;* [5]*Hospital Universitario 12 de Octubre, Department of Genetics, Madrid, Spain;* [6]*Member of the Genetics Commission of the Spanish Society of Clinical Chemistry (SEQC), Spain;* [7]*Hospital Sant Joan de Déu, Clinical Biochemistry and Genetics Department, Esplugues de Llobregat, Spain;* [8]*Hospital Universitario 12 de Octubre, Department of Pediatric Endocrinology, Madrid, Spain;* [9]*Hospital Sant Joan de Déu, Genomic Unit, Molecular and Genetic Medicine Section, Esplugues de Llobregat, Spain;* [10]*Hospital Universitario La Paz, INGEMM, Institute of Medical Genetics, Madrid, Spain;* [11]*ITHACA - European Reference Network, Spain;* [12]*Hospital Universitario Son Espases, IdISBa, Unidad de Diagnóstico Molecular y Genética Clínica, Palma de Mallorca, Spain;* [13]*Universidad de Valladolid, Pediatric Neurology, Hospital Universitario Río Hortega, Faculty of Medicine, Valladolid, Spain;* [14]*Hospital Gregorio Marañón, Neuropediatrics Section, Madrid, Spain;* [15]*Hospital Gregorio Marañón, Clinical Genetics, Pediatrics Department, Madrid, Spain;* [16]*Hospital Sant Joan de Déu, Department of Genetic Medicine, Pediatric Institute of Rare Diseases (IPER), Esplugues de Llobregat, Spain;* [17]*Hospital Sant Joan de Déu, Pediatric Neurology Department, Esplugues de Llobregat, Spain;* [18]*Instituto de Investigación Hospital 12 de Octubre (imas12), Grupo de Enfermedades Mitocondriales y Neurometabólicas, Madrid, Spain*

Background/Objectives: TRAF7 syndrome (MIM#618164) is an ultrarare multisystemic neurodevelopmental disorder with heterogeneous clinical presentations caused by germline heterozygous missense variants in the *TRAF7* gene.

Methods: We present a detailed description of eleven new TRAF7 syndrome cases, featuring eight distinct genetic variants, including a novel one. Phenotypic analysis and a comprehensive review of the 58 previously reported individuals outline consistent clinical findings, emphasizing dysmorphic features, developmental delay, adaptive and behavioural phenotype, endocrine manifestations, and congenital heart defects.

Results: In this large cohort, new findings include a wider range of cognitive dysfunction, with some individuals exhibiting normal intellectual abilities despite early psychomotor developmental delay. Communication difficulties, particularly in expressive language, are common and often require alternative communication methods. Autistic traits, notably lack of cognitive flexibility, are observed in our cohort. Additionally, hearing loss, sleep disturbances, and endocrine anomalies, including short stature are described. Cardiac defects, frequently severe, cause early-life complications. The novel missense variant p.(Arg653Leu) further underscores the complex relationship between germline *TRAF7* variants and somatic changes linked to meningiomas and sheds new light into the controversial oncologic issue.

Conclusion: Our comprehensive analysis and description expands the phenotypic spectrum, emphasizing the need for oncological evaluations and proposing an evidence-based

guideline for clinical management. This study contributes to a better understanding of TRAF7 syndrome, offering insights for improved diagnosis, intervention, and patient care.

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Conflict of Interest: None declared

P13.090.D Neurological and cognitive outcome in primary microcephaly and chorioretinopathy.

Mathilde Gras[1], *severine drunat*[2], *Anais Ernault*[2], *Alain Verloes*[2], *Sandrine Passemard*[1]

[1]*APHP, Hôpital Robert Debré, Child neurology department, PARIS, France;* [2]*APHP, Hôpital Robert Debré, Genetic Department, PARIS, France*

Background/Objectives: Primary Microcephalies (PMs) are characterized by a reduction in brain size, associated or not with short stature or sensorineural disorders, and mainly recessively inherited. Although more is known about the cognitive outcomes of the three most common forms of PM, with or without dwarfism (*ASPM, WDR62*, and *CDK5RAP2*), patients with PM and neurosensory disorders remained unexplored.

Methods: We studied i) frequency of each genotype, ii) growth parameters, iii) sensorineural features and iv) cognitive profile of patients with PM and retinopathy caused by mutations in *PLK4, TUBGCP6, TUBGCP4, KIF14, KIF11* and *RBBP8*. We compared our data with those of the literature.

Results: Among the 267 reported patients, 211 patients had mutations in *KIF11*. Developmental delay (DD)/ID was identified in 53/149 (35,5%), and learning disabilities were described in 38/149 (25%). However, only 1/149 patient was assessed (FSIQ 77)

Patients with mutations in *PLK4, TUBGCP6, TUBGCP4, KIF14, KIF11* and *RBBP8* were much less frequent. DD was described in 8/9 reported patients with mutations in *KIF14*, 14/14 in *PLK4*, 4/8 in *TUBGCP4*, 11/11 in *TUBGCP6* and 11/11 in *RBBP8* genes. None of these patients were cognitively assessed.

We provide molecular and cognitive data from 4 new patients [*KIF11* (1), *TUBGCP6* (2), *PLK4* (1)] with a FSIQ ranging from 43 to 82.

Conclusion: The cognitive profile of patients with PM and retinopathy encompass a wide phenotypic spectrum ranging from normal to severe ID, making genetic counselling difficult. Increasing our knowledge on the outcome of these affections through European networks are mandatory.

Grants: none

Conflict of Interest: None declared

P13.091.A Targeted re-sequencing of Neural Tube Defects patients and families identifies rare variants in genes candidate from animal models

Ferruccio Romano[1], *Michele Iacomino*[2], *Marzia Ognibene*[2], *Marcello Scala*[2], *Irene Schiavetti*[3], *Marco Pavanello*[4], *Gianluca Piatelli*[4], *Marco Di Duca*[2], *Yeraldin Chiquinquira Castillo De Spelorzi*[5], *Francesca Faravelli*[1], *Federico Zara*[2], *Patrizia De Marco*[2], *Valeria Capra*[1]

[1]*Istituto Giannina Gaslini, Unità di Genomica e Genetica Clinica, Genova, Italy;* [2]*Istituto Giannina Gaslini, Unità di Genetica Medica, Genova, Italy;* [3]*Università di Genova, Dipartimento di Scienze della Salute, Genova, Italy;* [4]*Istituto Giannina Gaslini, Unità di Neurochirurgia, Genova, Italy;* [5]*Istituto Italiano di Tecnologia, Genomics Facility, Genova, Italy*

Consortium: ERN-ITHACA

Background/Objectives: Neural tube defects (NTD) are congenital malformations arising when the neural tube, precursor of the brain and spine, fails to properly close during neurulation. Etiology is multifactorial, with environmental and genetic factors variably contributing, on a case by case basis. Molecular genetic studies of orthologues of the mouse NTD genes have been precious in the identification of predisposing NTD genes in humans, highlighting the peculiar role of the planar cell polarity (PCP) pathway in a fraction of human NTD patients.

Methods: Seventy-eight NTD patients were selected. Next-generation targeted re-sequencing of 29 genes encoding for components of the PCP pathway or for family members of proteins (including SHROOM and GRHL) underlying NTDs in well-known animal models was carried-out. A gene-burden analysis was also performed to assess for potential enrichment of rare damaging variants in the NTD cohort compared to the *in house* control exome dataset.

Results: 38 individuals (50%) presented with at least one putatively damaging rare variant, most of which were missense (64/76, 84%). *GRHL1* and *WNT5A* and, among gene families *GRHL* and *SHROOM*, were significantly enriched in the patients' cohort, compared to controls.

Conclusion: This study highlights the involvement of human orthologues of mouse genes in human NTD phenotype. Further re-sequencing, or whole exome sequencing of large groups of cases might give the clues for a better clinical management of patients and their families.

Grants: This research was supported by the "5xmille" founding to IRCCS Istituto Giannina Gaslini, and by Italian Health Ministry-Ricerca Corrente 2023.

Conflict of Interest: None declared

P13.092.B Simultaneous copy number and methylation analysis using methylation-specific digitalMLPA in congenital abnormalities and intellectual disability

Irene Zwarts[1], *Marrit Rinsma*[2], *Santiago Castanedo*[2], *Lisette Stolk*[2]

[1]*MRC Holland, IVD Development, Amsterdam, Netherlands;* [2]*MRC Holland, Cytogenetics, Amsterdam, Netherlands*

Background/Objectives: In the general population, the prevalence of intellectual disability (ID) is estimated at 1-3% and that of congenital abnormalities (CA) at 2-4%. CA and ID can be caused by many genetic defects, as well as imprinting changes. When no distinct syndromic features are displayed, finding the (epi)genetic cause often proves to be challenging. Testing a broad range of regions and imprinting-associated differentially methylated regions (iDMRs) would therefore be a useful contribution to elucidate the genetic causes of CA and ID.

Methods: SALSA® digitalMLPA™ Probemix DM025 Congenital Anomalies is being developed for simultaneous detection of copy number variation in more than 40 genes/regions across all chromosomes, and methylation status of 11 iDMRs. This assay was tested with 136 control samples and more than 15 positive samples, including 4 imprinting disorders, 5 microdeletion and 1 microduplication syndromes.

Results: In control samples, the expected copy number was found and imprinting regions showed a median methylation status of 44-67% per iDMR. The positive samples with previously identified deletions, duplications and methylation aberrations were analysed and were 100% in concordance with the known genotype and methylation status.

Conclusion: This data shows the ability of this methylation-specific digitalMLPA assay to correctly determine both copy

number and methylation status of targets that are frequently altered in individuals with CA and ID. This assay provides a quick and easy solution for the challenge of finding genetic defects causing CA and ID within one assay.

Grants: N/A

Conflict of Interest: Irene Zwarts MRC Holland, Marrit Rinsma MRC Holland, Santiago Castanedo MRC Holland, Lisette Stolk MRC Holland

P13.093.C Functional Insights of Truncated MAGEL2 in Schaaf-Yang Syndrome

Mónica Centeno-Pla[1;2;3], *Estefanía Alcaide-Consuegra*[1;2], *Sophie Gibson*[1], *Jonas Setzke*[4], *Aina Prat-Planas*[1;2], *Daniel Tornero*[4], *Isaac Canals*[5], *Daniel Grinberg*[1;2], *Roser Urreizti*[2;3], *Raquel Rabionet*[1;2], *Susanna Balcells*[1;2]

[1]*University of Barcelona, Department of Genetics, Microbiology and Statistics, IBUB, IRSJD, Barcelona, Spain;* [2]*Instituto de Salud Carlos III, Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER), Madrid, Spain;* [3]*Hospital Sant Joan de Déu, Clinical Biochemistry Department, CIBERER, Esplugues de Llobregat, Spain;* [4]*Institute of Neuroscience, University of Barcelona, Department of Biomedical Sciences, Barcelona, Spain;* [5]*University Children's Hospital Zurich, University of Zurich, Division of Metabolism and Children's Research Center, Zurich, Switzerland*

Background/Objectives: Schaaf-Yang syndrome (SYS) is an ultra-rare neurodevelopmental disorder caused by truncating mutations in *MAGEL2*, a gene that contributes to the regulation of retrograde transport, circadian rhythm, and mRNA metabolism. Previous heterologous expression assays in HeLa cells revealed a shift from a cytoplasmic to a more nuclear localization of a truncated MAGEL2 protein compared to the WT. We now extend the analysis to six other SYS mutations. Additionally, we generate neurons (iNs) and astrocytes (iAs) from SYS iPSC lines to study MAGEL2 in more relevant cellular contexts.

Methods: ICC assays were conducted in transfected HeLa cells to quantitatively assess the nuclear localization of FLAG-tagged MAGEL2 using Mander's colocalization coefficient. SYS and control iPSCs were differentiated into iNs and iAs, and co cultured.

Results: Truncated MAGEL2 proteins consistently displayed a predominantly nuclear localization, irrespective of the position and chemistry of the tag. Variants associated with arthrogryposis multiplex congenita showed a more pronounced nuclear retention phenotype. iNS and iAs were successfully generated from both SYS and control iPSCs. Calcium imaging and proteomic analyses revealed functional traits of SYS physiopathology.

Conclusion: Results suggest a correlation between clinical severity and the degree of nuclear mis-localization. Assays in different cell models will unveil deeper insights into MAGEL2 functional implications, now pointing to a pathogenic neomorphic effect of the truncated protein.

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Conflict of Interest: None declared

P13.094.D Delineation of the adult phenotype of Coffin-Siris syndrome in 35 individuals

Ariane Schmetz[1], *Hermann-Josef Lüdecke*[1], *Harald Surowy*[1], *Sugirthan Sivalingam*[1], *Ange-Line Bruel*[2], *Roseline Caumes*[3], *Perrine Charles*[4], *Nicolas Chatron*[5], *Krystyna Chrzanowska*[6], *Marta Codina*[7], *Cindy Colson*[3], *Ivon Cuscó*[7], *Anne-Sophie Denommé-Pichon*[2], *Charles-Patrick Edery*[5], *Laurence Faivre*[8], *Andrew Green*[9], *Solveig Heide*[4], *Tzung-Chien Hsieh*[10], *Alexander Hustinx*[10], *Lotte Kleinendorst*[11], *Cordula Knopp*[12], *Florian Kraft*[12], *Peter Krawitz*[10], *Amaia Lasa-Aranzasti*[7], *Gaetan Lesca*[5], *Vanesa López González*[13], *Julien Maraval*[14], *Cyril Mignot*[4], *Teresa Neuhann*[15], *Christian Netzer*[16], *Barbara Oekl-Jaschkowitz*[17], *Florence Petit*[3], *Christophe Philippe*[18], *Renata Posmyk*[19], *Audrey Putoux*[5], *André Reis*[20], *María José Sánchez Soler*[13], *Julia Suh*[12], *Tinatin Tkemaladze*[21], *Frédéric Tran Mau-Them*[2], *André Travessa*[22], *Laura Trujillano*[7], *Irene Valenzuela Palafoll*[7], *Mieke van Haelst*[11], *Georgia Vasileiou*[20], *Catherine Vincent-Delorme*[3], *Mona Walther*[20], *Pablo Emilio Verde*[23], *Nuria Bramswig*[1], *Dagmar Wieczorek*[1]

[1]*Institute of Human Genetics, Medical Faculty and University Hospital Düsseldorf, Heinrich Heine University Düsseldorf, Düsseldorf, Germany;* [2]*Inserm UMR1231 team GAD, University of Burgundy and Franche-Comté, Dijon, France;* [3]*CHU Lille, Clinique de Génétique, Lille, France;* [4]*Assistance Publique-Hôpitaux de Paris, Département de Génétique, Groupe Hospitalier Pitié-Salpêtrière, Paris, France;* [5]*Service de Génétique, Hospices Civils de Lyon, Bron, France;* [6]*Department of Medical Genetics, The Children's Memorial Health Institute, Warsaw, Poland;* [7]*Area of Clinical and Molecular Genetics, Vall d'Hebron University Hospital, Barcelona, Spain;* [8]*Centre de Génétique et Centre de Référence Anomalies du Développement et Syndromes Malformatifs, Fédération Hospitalo-Universitaire TRANSLAD et Institut GIMI, Dijon Bourgogne University Hospital, Dijon, France;* [9]*Department of Clinical Genetics, Children's Health Ireland at Crumlin, and University College Dublin School of Medicine and Medical Science, Dublin, Ireland;* [10]*Institute for Genomic Statistics and Bioinformatics, University Hospital Bonn, Rheinische Friedrich-Wilhelms-Universität Bonn, Bonn, Germany;* [11]*Department of Clinical Genetics, Amsterdam UMC, Amsterdam, Netherlands;* [12]*Institute for Human Genetics and Genomic Medicine, Medical Faculty, RWTH Aachen University, Aachen, Germany;* [13]*Sección Genética Médica, Servicio de Pediatría, Hospital Clínico Universitario Virgen de la Arrixaca (HCUVA), Murcia, Spain;* [14]*Centre de Référence Déficiences Intellectuelles de causes rares, Dijon Bourgogne University Hospital, Dijon, France;* [15]*Medizinisch Genetisches Zentrum, München, Germany;* [16]*Institute of Human Genetics, University of Cologne, Faculty of Medicine and University Hospital Cologne, Cologne, Germany;* [17]*BIOSCIENTIA, MVZ Labor Saar, Praxis für Humangenetik, Homburg Saar, Germany;* [18]*Functional Unit of Innovative Diagnosis for Rare Diseases, Dijon Bourgogne University Hospital, Dijon, France;* [19]*Department of Clinical Genetics, Medical University in Bialystok, Bialystok, Poland;* [20]*Institute of Human Genetics, Universitätsklinikum Erlangen, Friedrich-Alexander-Universität Erlangen-Nürnberg, Erlangen, Germany;* [21]*Department of Molecular and Medical Genetics, Tbilisi State Medical University, Tbilisi, Georgia;* [22]*Medical Genetics Department, Centro Hospitalar Universitário Lisboa Norte, Lisbon, Portugal;* [23]*Coordination Centre for Clinical Trials, Heinrich Heine University Düsseldorf, Düsseldorf, Germany*

Background/Objectives: Over the past decade, pathogenic variants in genes encoding subunits of the SWI-SNF complex were identified as causal for Coffin-Siris syndrome (*ARID1B, ARID1A, SMARCB1, SMARCA4, SMARCE1, ARID2, DPF2, SMARCC2, SOX11, SOX4, SMARCD1, BICRA*). However, a significant knowledge gap persists regarding the clinical manifestations of Coffin-Siris syndrome in adulthood. Long-term outcomes and associated risks remain unclear, contributing to considerable uncertainty among caregivers and clinicians.

Methods: Through collaborative efforts, including an ERN-ITHACA call for collaboration, social media engagement, partnerships with syndrome-specific foundations, and collaboration with colleagues, we successfully recruited a cohort of adult individuals with Coffin-Siris syndrome. A comprehensive questionnaire was utilized to assess their phenotypes.

Results: We included 35 adult individuals with Coffin-Siris syndrome, revealing a broad spectrum of phenotypes. This cohort particularly emphasizes the high percentage of overweight/obesity, visual impairment, and behavioral anomalies.

Conclusion: Initiating our study as a collaborative project, we aimed to investigate and document the adult phenotype of Coffin-Siris syndrome. We will present the phenotypic data from our adult cohort of affected individuals, delineating the evolving features of this syndrome. The collected data allows for a more accurate prediction of risks and is of great value to affected individuals and their caregivers in making preventive and therapeutic decisions.

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P13.095.A Loss-of-function variants in ERF are associated with a Noonan syndrome-like phenotype with or without craniosynostosis

Maria Lisa Dentici[1], *Marcello Niceta*[2], *Francesca Romana Lepri*[3], *Cecilia Mancini*[2], *Manuela Priolo*[4], *Camilla Cappelletti*[2;5], *Andrea Ciolfi*[2], *Chiara Leoni*[6;7], *Simone Pizzi*[2], *Viviana Cordeddu*[8], *Mafalda Mucciolo*[3], *Vito Luigi Colona*[1], *Lorenzo Sinibaldi*[1], *Silvana Briuglia*[9], *Andrea Gazzin*[10], *Diana Carli*[11], *Luigi Memo*[12], *Eva Trevisson*[13], *Concetta Schiavarello*[14], *maria luca*[11], *Antonio Novelli*[3], *Emanuela Scarano*[14], *Giuseppe Zampino*[6;7], *Alessandro Mussa*[11], *Bruno Dallapiccola*[2], *Maria Cristina Digilio*[1], *Marco Tartaglia*[2]

[1]*Ospedale Pediatrico Bambino Gesù, IRCCS, Rare Diseases and Medical Genetics, Rome;* [2]*Ospedale Pediatrico Bambino Gesù, IRCCS, Molecular Genetics and Functional Genomics, Rome;* [3]*Ospedale Pediatrico Bambino Gesù, IRCCS, Translational Cytogenomics, Rome;* [4]*Ospedale Cardarelli, Genetica Medica e di Laboratorio, Napoli;* [5]*Università di Roma "Tor Vergata", Department of Biomedicine and Prevention, Rome;* [6]*Fondazione Policlinico Universitario A. 19 Gemelli IRCCS, Department of Woman and Child Health and Public Health, Rome;* [7]*Università Cattolica del Sacro Cuore, Department of Life Sciences and Public Health, Rome;* [8]*Istituto Superiore di Sanità, 00161 Rome, Italy., Department of Oncology and Molecular Medicine, Rome;* [9]*Ospedale Universitario "Gaetano Martino", Genetics and Pharmacogenetics, Messina;* [10]*Ospedale Pediatrico "Regina Margherita", Pediatric 1 Clinical Genetics, Torino;* [11]*Università of Torino, Department of Medical Sciences, Torino;* [12]*IRCCS, Burlo Garofolo, Institute for Maternal and Child Health, Trieste;* [13]*Università di Padova, Department of Women's and Children's Health, Padova;* [14]*IRCCS Azienda Ospedaliero-Universitaria di Bologna, Department of Pediatrics, Bologna*

Background/Objectives: Heterozygous inactivating variants in the ETS2 repressor factor (*ERF*) gene, encoding a transcriptional regulator negatively controlling RAS-MAPK signaling, have been associated with syndromic craniosynostosis involving various cranial sutures and Chitayat syndrome, an ultrarare condition with respiratory distress, skeletal anomalies, and facial dysmorphism. Recently, a single patient with craniosynostosis and a phenotype resembling Noonan syndrome (NS), the most common disorder among the RASopathies, was reported to carry a de novo loss-of-function variant in *ERF*.

Methods: Here, we clinically profile 20 individuals from 10 unrelated families carrying different germline heterozygous mutations in *ERF* and showing a phenotype reminiscent of NS.

Results: The majority of subjects presented with a variable degree of global developmental and/or language delay. Their shared facial features included absolute/relative macrocephaly, high forehead, hypertelorism, palpebral ptosis, wide nasal bridge, and low-set/posteriorly rotated ears. Stature was below the 3rd centile in two-third of the individuals, while no subject showed typical NS cardiac involvement.

Notably, craniosynostosis was documented only in two unrelated individuals, while a dolichocephalic aspect of the skull in absence of any other evidence supporting a premature closing of sutures was observed in other 9 subjects. Unilateral Wilms tumor was diagnosed in one individual. Variants were nonsense and frameshift changes, supporting *ERF* haploinsufficiency.

Conclusion: These findings provide evidence that loss-of-function variants in *ERF* cause a disorder resembling NS with or without craniosynostosis, and allow a first dissection of the molecular circuits contributing to MAPK signaling pleiotropy.

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Conflict of Interest: None declared

P13.096.B Multilocus Imprinting Disturbances (MLID) analysis in a cohort of Beckwith-Wiedemann and Silver-Russell Syndromes: Comparison between specific MS-MLPA and methylation microarray

alejandro parra[1;2;3], *Jair Tenorio*[1;2;3], *mario cazalla*[1;2;3], *Patricia Pascual Vinagre*[1;2;3], *Lucía Miranda-Alcaraz*[1;2;3], *Natalia Gallego*[1;2;3], *Pedro Arias*[1;2;3], *cristina silvan*[1;2;3], *carlos rodriguez-antolin*[1;2;3], *the sogri consortium*[1;2;3], *julian nevado*[1;2;3], **Pablo LAPUNZINA**[1;2;3]

[1]*La Paz University Hospital, Institute of Medical and Molecular Genetics (INGEMM), Madrid, Spain;* [2]*CIBERER, Centro de Investigación Biomédica en Red de Enfermedades, Instituto de Salud Carlos III, Madrid, Spain;* [3]*ERN-ITHACA, European Reference Network on Rare Malformations Syndromes, Intellectual and other Neuro-developmental Disorders, Belgium, Belgium*

Consortium: The SOGRI Consortium

Background/Objectives: Imprinting disorders comprise a group of diseases which molecular basis is due to defects in the pattern of methylation in specific regions, which either inactivate or activate specific genes. To date, several syndromes associated

with defects in the methylation pattern have been identified, including Beckwith Wiedemann syndrome and Silver-Rusell syndrome. In some cases, multilocus imprinting disturbances (MLID) have been identified, in cases in which more than one methylation loci is altered.

Methods: A cohort of 54 patients with BWS (n = 99) and SRS (n = 13) who already have a methylation defect at the pathology-associated locus (11p15) was analyzed. Methylation analysis was performed by two techniques: (1)MS-MLPA using the commercial kit MRC-Holland ME034 (MRC Holland, Amsterdam, Netherlands, Netherlands). This kit allows the analysis of 11 different imprinting regions. (2) Methylation arrays. Also, available DNA from mothers of patients with MLID have been also analyzed.

Results: In, approximately, 20% of the total number of patient studied, a Multi-locus Imprinting Disturbance was found.

Conclusion: A significant percentage of patients with BWS and SRS syndromes have methylation alterations in more than one locus. These "additional" alterations may be associated with a much more heterogeneous clinical features of the patients, confirming the multilocus syndrome. There are maternal genes associated with multilocus syndromes in their offspring. A consensus should be established for the clinical and molecular testing and reporting of patients with multilocus imprinting disturbances.

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Conflict of Interest: None declared

P13.097.C Expanding the clinical and molecular spectrum of Kabuki syndrome cases

Yusuf Dogan[1], *Enise AVCI DURMUŞALİOGLU[1], Filiz Hazan[2], Turkan Turkut Tan[1], Tahir Atik[1], Esra Isik[1], Ozgur Cogulu[1]*

[1]Ege University, Department of Child Genetics; [2]Dr. Behçet Uz Children's Education and Research Hospital, Department of Medical Genetics

Background/Objectives: Kabuki syndrome is a rare disorder characterized by typical facial features, minor skeletal anomalies, fetal fingertip pads, intellectual disability, and growth retardation, caused by variants in the *KMT2D* (56–76%) or *KDM6A* (5–8%) genes. This study aims to expand the clinical and molecular spectrum of Kabuki syndrome.

Methods: This study included ten Kabuki syndrome cases followed at the Department of Pediatric Genetic Diseases. The clinical and molecular findings were evaluated retrospectively.

Results: Ten Kabuki syndrome cases, aged 9 months to 14 years, were referred to the Pediatric Genetic Diseases outpatient service due to developmental delay and dysmorphic features. Developmental delay, intellectual disability, and renal anomalies were present in the majority of the cases. Additionally, strabismus, coloboma, cleft palate, atrial and ventricular septal defects, horseshoe kidney, kidney atrophy, and anal stenosis were observed. Using next-generation sequencing (NGS), eight variants were identified in *KMT2D*; five of them were truncating, one was missense, and one was in-frame deletion. NGS-based copy number variation (CNV) analysis revealed a 1699 bp heterozygous deletion covering exons 5 and 9 of the *KMT2D* gene. All of the variants were classified as either likely pathogenic or pathogenic. Furthermore, the KDM6A gene also had two variants: the missense variant (c.871G>A), inherited from the unaffected mother, and the novel frameshift variant (c.1302del), which was likely pathogenic.

Conclusion: Clinical manifestations in Kabuki syndrome exhibit a wide spectrum. NGS aids in refining the diagnosis and providing appropriate genetic counseling. NGS-based CNV analysis serves as a helpful method for identifying deletions responsible for Kabuki syndrome.

Conflict of Interest: None declared

P13.098.D Branchio-oto-renal (BOR) syndrome caused by a deep intronic variant in EYA1 in a Danish family

Marie Lorans[1], *Pernille Axél Gregersen[1;2;3]*

[1]Department of Clinical Genetics, Aarhus University Hospital, Aarhus, Denmark, Denmark; [2]Centre for Rare Diseases, Pediatrics and Adolescent Medicine, Aarhus University Hospital, Aarhus, Denmark, Denmark; [3]Department of Clinical Medicine, Aarhus University, Aarhus, Denmark, Denmark

Background/Objectives: Branchio-oto-renal (BOR) syndrome is a rare autosomal dominant condition characterised by: 1) branchial arch defects; 2) structural defects of the ear; and 3) renal malformations. We describe a deep intronic variant in the *EYA1* gene that segregates with BOR-syndrome in a Danish family.

Methods: DNA from the index patient was processed and sequenced using an Illumina NovaSeq 6000 instrument. The whole genome sequencing (WGS) data was analysed with VarSeq software 2.4.0 (Golden Helix). SpliceAI prediction was used to evaluate splice variants. Sanger Sequencing was used to test relatives. In-vitro exon-trapping assay was applied to test the functional effect of splice-variants.

Results: A previously unreported heterozygous deep intronic variant in *EYA1*; NM_000503.6(EYA1):c.556+2123C>T, (p.(?)) was identified in the proband and 3 affected relatives. According to SpliceAI, the C-to-T transition creates a cryptic splice donor site in intron 7, 2 bp upstream of the variant and a cryptic acceptor site 118 bp upstream of the variant. The exon-trapping assay produced altered splicing products for the variant-specific sequencing libraries when compared to wild-type sequencing libraries. A transcript with inclusion of a 117 bp long cryptic exon was exclusively observed in the variant-specific sequencing libraries. This abnormal transcript is expected to undergo nonsense mediated decay resulting in *EYA1* haploinsufficiency.

Conclusion: We identified a deep intronic variant in *EYA1* that segregates with BOR syndrome. To the best of our knowledge, this is the first report of a causative deep intron variant in BOR-syndrome. Our results demonstrate the clinical utility of WGS in cases with highly specific phenotypes.

Conflict of Interest: None declared

P13.100.B Exploring *ASXL1* variants in Bohring-Opitz Syndrome: Analysis of four novel cases and literature review on clinical and molecular features

Juan Diego Gutiérrez Ávila[1;2], *Aina Prat-Planas[1;2], Mónica Centeno-Pla[1;2;3], Roser Urreizti[2;3], Susanna Balcells[1;2], Daniel Grinberg[1;2], Deepthi De Silva[4], Valeria Capra[5], Romesh Gunasekera[6], Silvia Cali[5], Maria Margherita Mancardi[7], Annalaura Torella[8;9], Marcello Scala[10;11], Raquel Rabionet[1;2]*

[1]University of Barcelona, Faculty of Biology, Department of Genetics, Microbiology and Statistics, Barcelona, Spain; [2]Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER)- Instituto de Salud Carlos III (ISCIII), Madrid, Spain; [3]Institut de Recerca Sant Joan de Déu (IRSJD), Esplugues de Llobregat, Spain; [4]Faculty of Medicine, University of Kelaniya, Department of Physiology, Ragama, Sri Lanka; [5]IRCCS Giannina Gaslini Institute, Genomic and Clinical Genetics, Genoa, Italy; [6]Lady Ridgway Hospital, Colombo; [7]IRCCS Giannina Gaslini Institute, Pediatric Neurology, Genoa, Italy; [8]University of Campania "Luigi Vanvitelli", Department of Precision Medicine, Naples, Italy; [9]Telethon Institute of Genetics and Medicine,

Pozzuoli, Italy; [10]University of Genoa, Department of Neurosciences, Rehabilitation, Ophthalmology, Genetics, Maternal and Child Health, Genoa, Italy; [11]IRCCS Giannina Gaslini Institute, Pediatric Neurology and Muscular Disease Unit, Genoa, Italy

Background/Objectives: Bohring-Opitz Syndrome (BOS) is a rare neurodevelopmental disorder caused by germline variants in the *ASXL1* gene. Its clinical presentation is heterogeneous but is mainly characterized by feeding difficulties in the neonatal period, hypotonia, seizures, the BOS posture, myopia, and distinct craniofacial features. Here, we report four novel cases, all harboring mutations in *ASXL1*.

Methods: *ASXL1* variants were identified by whole exome sequencing (WES) or direct sequencing, segregation analysis was performed to determine whether the variants were de novo. Additionally, we conducted a comprehensive review of the clinical and molecular characteristics documented in the existing literature on BOS.

Results: Three different truncating variants in *ASXL1* were identified: c.1544_1545del [p.(Val515Glyfs13)], located in exon 12, was found in two patients; c.3637_3640del [p.(Leu1213Ilefs3)] and c.3424C>T (p.Gln1142*), both in exon 13. The first variant is expected to undergo nonsense-mediated mRNA decay (NMD), while the mutations located in exon 13 are expected to escape this process. Including the variants in this report, a total of 36 pathogenic variants have been identified so far, with one of them located in exon 11 and the rest clustered in exons 12 and 13. No clear genotype-phenotype correlations have been established.

Conclusion: This study presents a comprehensive analysis of four previously unreported cases of BOS, contributing to the expansion of the molecular and clinical understanding of the syndrome. The novel patients exhibit distinctive clinical features that align with the diverse spectrum characterizing this syndrome, our clinical data review further enriches the knowledge of the presentation and underlying genetic variations.

Conflict of Interest: None declared

P14

CYTOGENETICS, GENOME VARIATION AND ARCHITECTURE

P14.001.C Blindspots in short-read genome sequencing for classic chromosomal rearrangements

Lucas GAUTHIER[1], *Claire Caillot[1], Mathilde Pujalte[1], Marianne Till[1], Damien Sanlaville[1;2], Nicolas Chatron[1;2]*

[1]Genetics Department, Lyon University Hospital, Lyon, France; [2]Univ Lyon, Univ Lyon 1, CNRS, INSERM, Physiopathologie et Génétique du Neurone et du Muscle, UMR5261, U1315, Institut NeuroMyoGène, Lyon, France

Background/Objectives: Short-read Genome Sequencing (srGS) has emerged as a powerful tool in medical genetics, providing comprehensive insights into the genetic landscape of individuals. However, detecting structural variants may pose significant challenges, particularly those involving repeated sequences as satellites repeats, telomeric and centromeric repeats, transposons or low complexity regions.

Methods: We previously described the first case of insertion of an alpha satellite DNA outside centromeric and regions (Giannuzzi et al., 2021) and herein present two other cases where default structural variant calling failed at the characterization of a karyotype-visible event. The first case is the recurrent t(11;22)(q23;q11.2) translocation, a clinically relevant variant obscured by the presence of low complexity regions in the chromosome 22 region but where we could fetch out spanning reads through manual inspection of alignments. The second case entails an addition of 57Mb of 8q telomeric region on top of the short arm of a chromosome 4. Manual inspection of read alignments identified reads spanning the junction between the chromosome 8 added region and the TTAGGG hexamere repeat. Only the combination of genome sequencing and karyotype could fully resolve this post-telomere addition.

Results: These case reports have allowed us to suggest new layers to our structural variant calling pipeline to detect such variants reputed invisible to srGS but still present in the data.

Conclusion: These three observations affect large repeats that can also be challenging for long-read sequencing technologies. We emphasize the need to describe these limitations and work on technological improvements to deliver the full power of these technologies to patients.

Grants: None

Conflict of Interest: None declared

P14.002.D Mind the reference gap

Kristine Bilgrav Saether[1], *Claudia Carvalho[2], Jakob Schuy[1], Adam Ameur[3], Ann Nordgren[1], Jesper Eisfeldt[3], Anna Lindstrand[1]*

[1]Karolinska Institutet, Molecular Medicine and Surgery; [2]Pacific Northwest Research Institute; [3]Science for Life Laboratory

Background/Objectives: Inversions are a class of structural variants that are especially challenging to detect due to their copy number neutral state as well as their association with repetitive regions. Reference genome builds such as GRCh37 and GRCh38 lack sequence across large regions of centromeric, telomeric and repetitive DNA. This makes detection of structural variants, such as inversions particularly challenging, due to their association with these regions. The recently published Telomere to Telomere (T2T) reference genome offers a continuous sequence spanning the previously missing regions, promising heightened structural variant detection and improved mapping.

Methods: We reanalyzed five cytogenetically visible inversions previously unresolved using GRCh37 or GRCh38, short read genome sequencing (srGS) and in certain cases also long read GS (lrGS). Next, by pairwise comparison between five human/primate reference genomes, GRCh37, GRCh38, T2T, the chimpanzee and bonobo, we characterize Differential Reference Regions (DRRs), genomic segments that were missing in at least one reference genome.

Results: Two inversions were resolved where the breakpoints were located in regions missing in reference genomes GRCh37 or GRCh38. We found 217 Mb of DRR sequence missing from GRCh38 and present in T2T. Finally, we characterize the presence of DRRs using srGS from 100 healthy Swedish individuals.

Conclusion: Our findings call attention to the importance of complete, high-quality reference genomes for accurate structural variant detection. This seems to be especially important for inversions that often elude detection in both clinical and research analysis of srGS data.

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P14.003.A New insights for the interpretation of *SMCHD1* gene variants effect: a T2T-based functional study on a heterogeneous cohort of patients

Sara Pini[1], *Maria Francesca Di Feo*[1;2;3], *Matteo Chiara*[4;5], *Marco Savarese*[6;7], *Paolo Uva*[8], *Michele Iacomino*[8], *Maria Iascone*[9], *Filippo Maria Santorelli*[10], *Marie Vandroux*[11], *Jocelyn Laporte*[11], *Aldamaria Puliti*[2;12], *Noemi Albano*[1], *Marco seri*[13;14], *Tommaso Pippucci*[14], *Federica Isidori*[14], *Anna Cereda*[15], *Bjarne Udd*[6;7;16], *Valentina Salsi*[1], *Rossella Tupler*[1;17;18]

[1]*University of Modena and Reggio Emilia, Department of Biomedical, Metabolic and Neural Sciences, Modena, Italy;* [2]*University of Genoa, Department of Neuroscience, Rehabilitation, Ophthalmology, Genetics, and Maternal and Child Health (DINOGMI), Genova, Italy;* [3]*IRCCS Policlinico San Martino, Genova, Italy;* [4]*Università degli Studi di Milano, Dipartimento di Bioscienze, Milano, Italy;* [5]*Consiglio Nazionale delle Ricerche, Istituto di Biomembrane, Bioenergetica e Biotecnologie Molecolari, Bari, Italy;* [6]*Folkhälsan Research Center, Helsinki, Finland;* [7]*University of Helsinki, Department of Medical Genetics, Medicum, Helsinki, Finland;* [8]*IRCCS Istituto Giannina Gaslini, Clinical Bioinformatics, Genova, Italy;* [9]*ASST Papa Giovanni XXIII, Laboratorio di Genetica Medica, Bergamo, Italy;* [10]*IRCCS Fondazione Stella Maris, Pisa, Italy;* [11]*Université de Strasbourg, IGBMC (Institut de Génétique et de Biologie Moléculaire et Cellulaire), Illkirch, France;* [12]*IRCCS Istituto Giannina Gaslini, Medical Genetics Unit, Genova, Italy;* [13]*University of Bologna, Department of Medical and Surgical Sciences, Bologna, Italy;* [14]*Sant'Orsola-Malpighi University Hospital, Medical Genetics Unit, Bologna, Italy;* [15]*ASST Papa Giovanni XXIII, Paediatric Unit, Bergamo, Italy;* [16]*Vaasa Central Hospital, Department of Neurology, Vaasa, Finland;* [17]*University of Massachusetts Medical School, Department of Molecular, Cell, and Cancer Biology, Worcester, United States;* [18]*University of Massachusetts Medical School, Li Weibo Institute for Rare Diseases Research, Worcester, United States*

Background/Objectives: Facioscapulohumeral muscular dystrophy (FSHD), an hereditary myopathy, has prevalence of 1:20.000. FSHD1 is associated with the reduction of the number of D4Z4 tandem repeats (DRA) located at 4q35. FSHD2 classifies 5% of FSHD clinical cases with no DRA. In 80% of FSHD2 cases heterozygous mutations have been reported in the chromatin remodeling factor SMCHD1 which is required for normal DNA methylation and transcriptional repression. Pathogenic variants in SMCHD1 have been also associated with Bosma Syndrome and hypopituitarism. Considering this clinical heterogeneity, we investigated if D4Z4 methylation status serves as a readout of the biological effects of *SMCHD1* variants in relation with different phenotypes.

Methods: We collected aggregated sequencing data from six centers in Italy, France and Finland. Three centers provide diagnosis for muscle genetic diseases and three investigate genetic syndromes and monogenic diseases.

Results: We evaluated sequencing data from 22.370 samples and identified 44 *SMCHD1* variants of interest. DNA samples from 22 subjects carrying 18 variants underwent T2T-based methyl-seq analysis using a customized molecular and bioinformatic approach to test D4Z4 methylation status. Reduced D4Z4 methylation was observed in 9 subjects presenting pathogenic variants: 8 from the myopathic and 1 from the non-myopathic cohorts. These findings indicate that our D4Z4 methylation assay can be applied to functional investigation of *SMCHD1* variant biological effect.

Conclusion: Our results point out the importance of reusing genomic data from ample cohorts to extend and deepen the correlation between gene variants and the observed individual phenotypic variability for preventing biased interpretations.

Grants: None

Conflict of Interest: None declared

P14.004.B A targeted amplification-free sequencing protocol for base-resolution profiling of short tandem repeat expansions

Guilherme De Sena Brandine[1], *Valeriya Gaysinskaya*[1], *Janet Aiyedun*[1], *Julian Rocha*[1], *Duncan Kilburn*[1], *Sarah Kingan*[1], *Egor Dolzhenko*[1], *Zoi Kontogeorgiou*[2], *Christina Zarouchlioti*[3], *Robert Thaenert*[4], *Pilar Alvarez Jerez*[5], *Kimberley Billingsley*[5], *Sonia Lameiras*[6], *Sylvain Baulande*[6], *Aidan Hennigan*[4], *Alesia Piselli*[4], *Chelsea Roselund*[4], *Alice Davidson*[3], *Georgios Koutsis*[2], *Georgia Karadima*[2], *Stéphanie Tomé*[7], *Michael Eberle*[1]

[1]*Pacific Biosciences (PacBio), Menlo Park, United States;* [2]*National and Kapodistrian University of Athens, Neurogenetics Unit, 1st Department of Neurology, Eginition Hospital, School of Medicine, Athens, Greece;* [3]*University College London, Institute of Ophthalmology, United Kingdom;* [4]*Quest Diagnostics, Marlborough, United States;* [5]*National Institutes of Health, Center for Alzheimer's and Related Dementias, National Institute on Aging, Bethesda, United States;* [6]*Institut Curie, PSL Research University, ICGex Next-Generation Sequencing Platform, Paris, France;* [7]*Sorbonne Université, Inserm, Institut de Myologie, Centre de Recherche en Myologie, F-75013, Paris, France*

Background/Objectives: Short tandem repeats (STRs) are DNA sequences composed of multiple copies of 1-6bp motifs. STRs are ubiquitous in the human genome, and some are prone to expansions that cause disease. Many pathogenic STR expansions have highly variable genomic sequences, both at the individual and population scale. Motif composition, sequence length and CpG methylation often dictate disease onset and severity. An ideal approach to identifying pathogenic STR expansions requires accurate assessments of all these properties in a single assay.

Methods: We describe a robust amplification-free protocol to generate long-read HiFi sequencing libraries containing a panel of loci associated with 20 pathogenic STR expansions. The protocol can be multiplexed to sequence up to 48 samples at 20 to 1000x coverage per locus in one PacBio sequencing run. We measured accuracy in 86 samples with validated pathogenic expansions at loci including *CNBP*, *DMPK*, *RFC1* and *C9orf72*. We tested 1720 sample-expansion combinations, including technical replicates, for expansions between 66bp and >10kb.

Results: Our assay correctly categorized all (86/86) expansions, including the detection of hypermethylation in the *FMR1* expansion and differentiating the pathogenic AAGGG motif in *RFC1*. We identified additional expansions in *FXN*, *RFC1* and *TCF4*, consistent with these loci having carrier frequencies between 1:50 and 1:20. Excluding these three genes, we found no unexpected expansions (0/1405) in any sample-loci combinations.

Conclusions: The protocol provides an accurate description of the tissue-level molecular landscape of various pathogenic STRs and is adaptable to other loci in the human genome.

Conflict of Interest: Guilherme De Sena Brandine Guilherme de Sena Brandine is an employee and shareholder of Pacific Biosciences., Guilherme de Sena Brandine is an employee and shareholder of Pacific Biosciences., Valeriya Gaysinskaya Valeriya Gaysinskaya is an employee and shareholder of Pacific Biosciences., Valeriya Gaysinskaya is an employee and shareholder of Pacific Biosciences., Janet Aiyedun Janet Aiyedun is an employee and shareholder of Pacific Biosciences., Janet Aiyedun is an employee and shareholder of Pacific Biosciences., Julian Rocha Julian Rocha is an employee and shareholder of Pacific Biosciences., Julian Rocha is an employee and shareholder of Pacific Biosciences., Duncan Kilburn Duncan Kilburn is an employee and shareholder of Pacific Biosciences., Duncan Kilburn is an employee and shareholder of Pacific Biosciences., Sarah Kingan Sarah B. Kingan is an employee and shareholder of Pacific Biosciences., Sarah B. Kingan is an employee and shareholder of Pacific Biosciences., Egor Dolzhenko Egor Dolzhenko is an employee and shareholder of Pacific Biosciences., Egor Dolzhenko is an employee and shareholder of Pacific Biosciences., Zoi Kontogeorgiou: None declared, Christina Zarouchlioti: None declared, Robert Thaenert Robert Thaenert is an employee at Quest Diagnostics, Pilar Alvarez Jerez: None declared, Kimberley Billingsley: None declared,

Sonia Lameiras: None declared, Sylvain Baulande: None declared, Aidan Hennigan Aidan Hennigan is an employee at Quest Diagnostics, Alesia Piselli Alesia Piselli is an employee at Quest Diagnostics, Chelsea Roselund Chelsea Roselund is an employee at Quest Diagnostics, Alice Davidson: None declared, Georgios Koutsis: None declared, Georgia Karadima: None declared, Stéphanie Tomé: None declared, Michael Eberle Michael A. Eberle is an employee and shareholder of Pacific Biosciences, Michael A. Eberle is an employee and shareholder of Pacific Biosciences

P14.005.C Phenotypic variability in rare NF2 patients carrying 22q12 microdeletions: exploring genetic and epigenetic mechanisms

Viviana Tritto[1], *Micol Pozzi*[1], *Rosina Paterra*[2], *Arianna Borghi*[2], *Serena Redaelli*[3], *Marica Eoli*[2], *Paola Riva*[1]

[1]*University of Milan, Department of Medical Biotechnology and Translational Medicine, Segrate (Milan), Italy;* [2]*IRCCS Foundation Carlo Besta Neurological Institute, Unit of Molecular Neuro-Oncology, Milan, Italy;* [3]*University of Milano-Bicocca, School of Medicine and Surgery, Monza (MB), Italy*

Background/Objectives: Neurofibromatosis type 2 (NF2) is an autosomal dominant tumor-prone disorder, mainly due to point mutations or intragenic deletions of the *NF2* gene, encoding Merlin protein, that inhibits proliferation pathways. Rare NF2 patients carrying 22q12 microdeletions, characterized by a severe and variable phenotype, have also been described. We characterized three NF2 microdeletion patients with a complex phenotype to evaluate the incidence of 22q12 microdeletions and the role of specific genetic/epigenetic mechanisms.

Methods: We carried out aCGH, in silico analysis, gDNA qPCRs, Long-Range PCRs, and Sanger sequencing to ascertain the deletions' extent, gene content, and breakpoints. To study a possible position effect generated by the chromatin alteration, we performed in silico analysis of 22q12 TADs and regulatory elements, and gene expression analysis by qPCR on 12 genes flanking patients' deletions. The possible phenotypic contribution of variants identified by Whole Exome Sequencing was evaluated.

Results: Patients 690, 568, and 647 show deletions spanning 2.35, 3.08, and 4.64 Mb, respectively, involving 59-65 genes and 5-9 TADs. The position effect on some flanking genes was detected by qPCR. Genotype-phenotype correlation studies reveal a possible association between *AP1B1* gene haploinsufficiency and cognitive impairment in patients 568 and 647. Furthermore, patient 647 showed *MN1* gene deletion possibly related to Toriello-Carey syndrome, and *SHANK3* gene duplication, which could be linked to neurobehavioral disorders.

Conclusion: Our results pinpoint the need to identify the incidence of 22q12 microdeletions in NF2 patients, suggesting a novel prognostic significance of these genetic lesions, contributing to provide genotype-phenotype correlations.

Grants: Academic fund

Conflict of Interest: None declared

P14.006.D Identifying the role of somatic copy number variations in Parkinson's disease using single-cell whole genome sequencing

zeliha gozde turan[1;2], *Ester Kalef-Ezra*[1;2], *Dominic Horner*[1;2], *Caoimhe Morley*[1], *Diego-Perez Rodriguez*[1], *Zane Jaunmuktane*[1;2;3], *Jonas Demeulemeester*[2;4;5;6], *Fritz Sedlazeck*[2;7], *Christos Proukakis*[1;2]

[1]*UCL Queen Square Institute of Neurology, London, United Kingdom;* [2]*Aligning Science Across Parkinson's (ASAP) Collaborative Research Network, United States;* [3]*UCL Queen Square Institute of Neurology, Queen Square Brain Bank for Neurological Disorders, London, United Kingdom;* [4]*The Francis Crick Institute, Cancer Genomics Laboratory, London, United Kingdom;* [5]*KU Leuven, Department of Oncology, Leuven, Belgium;* [6]*Center for Cancer Biology, Leuven, Belgium;* [7]*Baylor College of Medicine, Human Genome Sequencing Center, Houston, United States*

Background/Objectives: Parkinson's disease (PD) is the most common neurodegenerative movement disorder with partly unexplained etiology. The heritability of PD is ~30%, leading to further research into other potential contributors, such as somatic copy number variations (CNVs).

Methods: To determine the role of CNVs on PD pathology, we generated and analyzed low-coverage single-cell whole genome sequencing data. We focused on neuronal (NeuN-positive) and non-neuronal (NeuN-negative) cell types from the cortex of three PD patients, three age-matched controls, and one individual with incidental Lewy body disease (ILBD). Out of the 532 analyzed cells, 90% passed the quality control criteria. Additional brain samples, including those from the substantia nigra, are being processed.

Results: The percentage of cells with at least one CNV was highest in the ILBD case (43.8%), followed by controls (14.1%), and then PD patients (7.6%) ($p < 0.001$). Notably, in PD, the number of NeuN-negative cells with at least one CNV is 3.39 times greater than that of NeuN-positive cells ($p = 0.004$). In contrast, this ratio is 1.37 for the control group ($p = 0.37$). These findings suggest that neurons in PD might be more susceptible to the CNV load, potentially resulting in increased cell death. This could have significant effects on both the progression and severity of the disease.

Conclusion: This study contributes to a better understanding of complex PD pathology and opens new avenues for further research into the factors influencing PD.

Grants: This research was funded in whole by Aligning Science Across Parkinson's [564669] through Michael J. Fox Foundation for Parkinson's Research.

Conflict of Interest: None declared

P14.007.A The distribution of single nucleotide polymorphisms across consecutive exons and introns of the human genome

Magdalena Fraszczak[1], *Jakub Liu*[1;2], *Magda Mielczarek*[1;2], *Paula Dobosz*[2;3], **Joanna Szyda**[1;2]

[1]*Wroclaw University of Environmental and Life Sciences, Biostatistic Group, Department of Genetics, Wroclaw, Poland;* [2]*Poznan University of Medical Science, University Cancer Diagnostic Center, Poznan, Poland;* [3]*University of Warsaw, Institute of Genetics and Biotechnology, Warsaw, Poland*

Background/Objectives: Single nucleotide polymorphisms (SNPs) are the most common type of genomic variation. We explored the number of SNPs in the human genome, focusing on differences in SNP counts among consecutive introns and exons.

Methods: The analysed cohort consisted of 41836187 SNPs identified in the genomes of 1222 individuals of Polish origin. HSA01 and HSA22 were selected for downstream analysis representing of two markedly different DNA molecules. Statistical analysis consisted of testing the variability of SNP counts between genes, among exons/introns, and the pairwise differences in SNP counts between exons/introns.

Results: Considering exons, the number of SNPs differed significantly between genes and between exons. Pairwise comparisons showed a significant excess of SNPs in the outer exons (1st and last) in HSA01 (all genes) and in HSA22 (all genes except genes with 6, 7, 8 exons). For introns, the number of SNPs

differed significantly between genes, while the variability among introns was significant in all genes except for genes with 4, 6, 7 introns on HSA22. Pairwise comparisons demonstrated significantly higher SNP counts in the first intron in HSA01, with a less clear pattern in HSA22.

Conclusion: The special role of the first intron as well as the first and terminal exon has been discussed in the literature, where it was demonstrated that these functional units are characterised by sequence features distinct from the internal exons/introns. This phenomenon was also demonstrated by our study that utilised SNPs identified in a well-defined population representing individuals of Polish origin, albeit yielding contradictory results.

Conflict of Interest: None declared

P14.008.B Spatial exploration of human germline mutational signatures

Rahul Sharma[1], *Marcin Karpinski*[1], *Slavé Petrovski*[1], *Keren Carss*[1], *Dominik Glodzik*[2], *Dimitrios Vitsios*[1], *Xueqing Zou*[1]

[1]*AstraZeneca, Centre for Genomics Research, Discovery Sciences, R&D, Cambridge, United Kingdom;* [2]*Harvard Medical School, Department of Biomedical Informatics, Boston, United States*

Background/Objectives: Mutational signature analysis has been proven instrumental in gaining insights into underlying DNA damage and repair processes, particularly in cancer genomics. In contrast, germline mutational processes have received negligible attention. This study explores human germline single nucleotide variations (SNVs) to comprehensively understand germline mutagenesis and its dependence on genomic features and location.

Methods: We created spatial mutational profiles by binning filtered SNVs for 44,589 samples with EUR ancestry from Genomic England (GE) 100K genomes project into 10,000 base-pairs non-overlapping windows. For unique and robust mutational signatures, we employed an advanced volume-regularized non-negative matrix factorization approach.

Results: We identified eight SNV germline mutational processes, three strand-dependent and five strand-independent. Strand-dependent processes were associated with endogenous processes that create mutation strand bias, including transcription-coupled repair on transcribed strand and higher replication errors on lagging strand. Specific signatures correlate with replication timings, distribution of CpG islands, gene bodies, or specific chromatin regions. Several signatures align with previous TopMed dataset findings underlining their robustness. Notably, four GE germline mutational signatures have >0.85 cosine similarity with four COSMIC cancer signatures.

Conclusion: We obtained statistically robust germline mutational signatures from ~45K GE samples, demonstrated their variation spatially across the genome and revealed their associations with different genomic features. Future study will extend the analysis to double nucleotide substitutions, indels, and structural variants to identify all possible germline mutational processes. Such studies will advance our understanding of how germline mutations cause diseases, potentially revealing new avenues for diagnosis and treatment.

Grants: AstraZeneca

Conflict of Interest: Rahul Sharma AstraZeneca employee, AstraZeneca employee working on this project/grant, AstraZeneca employee working on this project/grant, AstraZeneca employee, Marcin Karpinski AstraZeneca employee, Data science support, AstraZeneca employee working on this project/grant, AstraZeneca employee, Slavé Petrovski AstraZeneca employee, VP Centre for Genomics Research, AstraZeneca employee, VP Centre for Genomics Research, AstraZeneca employee, Keren Carss Senior Director, Genomics Analytics, Senior Director, Genomics Analytics, Senior Director, Genomics Analytics, AstraZeneca Employee, Dominik Glodzik AstraZeneca Collaborator, AstraZeneca Collaborator, project supervision, AstraZeneca Collaborator, project supervision, AstraZeneca Collaborator, project supervision, Dimitrios Vitsios AstraZeneca Employee, principal investigator, principal investigator, AstraZeneca Employee, Xueqing Zou AstraZeneca Employee, principal investigator, principal investigator, AstraZeneca Employee

P14.009.C De novo structural variation in the Lithuanian population from short-read WGS data

Ingrida Domarkienė[1], *Giorgi Sokhadze*[1], *Alina Urnikyte*[1], *Laura Pranckeniene*[1], *Svetlana Dauengauer-Kirlienė*[1], *Aušra Matulevičienė*[1], *Ingrida Pylipienė*[2], *Vaidutis Kučinskas*[1]

[1]*Department of Human and Medical Genetics, Institute of Biomedical Sciences, Faculty of Medicine, Vilnius University;* [2]*Clinic of Children`s Diseases, Institute of Clinical Medicine, Faculty of Medicine, Vilnius University*

Background/Objectives: Structural variants (SVs) can have profound consequences in evolution and human disease. There is an increasing recognition in the scientific community of the need to explore SVs across different populations. Identifying de novo SVs is crucial for exploring variation diversity, the origins of disorders and assessing their occurrence rates, which is key to understanding evolutionary milestones in population history.

Methods: Illumina short-read whole genome sequencing (WGS) data of 25 families (trios) of Lithuanian origin from the general population was generated (on Illumina instrument). Data was of good quality (Q30 value: >90%), average coverage ~38x. SVs were called with *DELLY* and *LUMPY* software. De novo SVs were filtered by using at least 70% overlap. Called SVs (>49 bp) were annotated by using *AnnotSV*.

Results: The algorithms identified various types of de novo SVs. DELLY detected deletions (3%) and translocations (82%), while LUMPY identified duplications (11%) and inversions (4%). Two individuals excluded as outliers due to a significantly higher number of SVs. Average number of de novo SVs per person was approximately 6 with a range from 0 to 19 SVs. About 70% of de novo SVs were classified as singletons. Nearly half of these SVs were found to encompassed genes suggesting potential functional implications.

Conclusion: It is the first attempt to characterise de novo SVs from the short-read WGS data in the Lithuanian population. Further detailed SV characterization and integrative analysis will provide useful information on local human population genomic variation and will add data to the databases.

Grants: ANELGEMIA (No.S-MIP-20-34) funded by the LMTLT.

Conflict of Interest: None declared

P14.010.D Prevalence and inheritance pattern of recurrent copy number variations (RecCNVs) associated with genetic disorders in the Czech population.

Pavlína Čapková[1,2], *Šárka Trávníčková*[2], *Andrea Štefeková*[1], *Zuzana Fabíková*[1], *Václava Curtisová*[1], *Josef Srovnal*[3], *Enkhjargalan Mracká*[1], *Lucia Punová*[1], *Jana Petřková*[1], *Marketa Routilova*[1], *Radek Vrtěl*[1]

[1]*University Hospital Olomouc, Department of Medical Genetics, Olomouc, Czech Republic;* [2]*Faculty of Medicine and Dentistry, Palacky University Olomouc, Department of Medical Genetics, Olomouc, Czech Republic;* [3]*Faculty of Medicine and Dentistry,*

Palacky University Olomouc, Institute of Molecular and Translational Medicine, Olomouc, Czech Republic

Consortium: CzechArray

Background/Objectives: CNVs are a common cause of various genetic disorders and occur at defined genomic regions that are particularly prone to recurrent deletions and duplications and often exhibit variable expressivity and incomplete penetrance. Classification of the variants diverges. In our study we focused on selected RecCNVs: del/dup -1q21.1(*GJA5, RBM8A*), 2p16.3(*NRXN1*), 2q13(*NPHP1*), 3p26.3(*CNTN4*), 9p24.3(*DOCK8*), 15q11.2 (*NIPA1,2*), 15q13.3(*CHRNA7, OTUD7A*), 16p13.11(*MYH11*), 16p11.2 (*SH2B1, TBX6*), 17q12 (*HNF1B*), 22q11.2 (*TBX1*), Xp22.33-31(*STS, SHOX*) to determine their prevalence, impact and inheritance pattern. This group we compared to the group of the rare well-known pathogenic variants (RareCNVs): del/dup - 1q36.3, 4p16.3 (WHS), 5p15 (cri-du-chat), 7q11.23 (WBS), 15q11.2-q13 (PW/AS), 17q11.2 (PTLS, SMS), 17p13.3 (MDS), 22q11.2(DGS) detected in our cohort.

Methods: A cohort of 2342 patients was tested with microarray (Agilent/Affymetrix) and/or MLPA (MRC Holland) (SALSA MLPA microdeletions 1 and 2, Subtelomeres, Autism, DiGeorge, PWS/AS).

Results: We assessed 11 deletions and 12 duplications in the RecCNVs group (mostly associated with neurodevelopmental disorders) and 8 deletions and 4 duplications (associated with multi-systems disorders) in the RareCNVs group. The overall prevalence of the variants in RecCNVs and RareCNVs was 4.52 % and 1.67 % resp. Healthy carriers (parents or siblings) were detected in 1.28 % vs 0.09 %. Variants were inherited in 1,4% and 0.17 % in RecCNV and RareCNV group resp.

Conclusion: RecCNVs are widely disseminated and frequently inherited in the population contrary to the RareCNVs. This should be considered in genetic counselling especially in prenatal diagnosis.

Grants: Supported by MH CZ – DRO (FNOl, 0098892)

Conflict of Interest: None declared

P14.011.A A high resolution SNP array increases the diagnostic yield in genomic testing of undiagnosed neurocognitive disorders(UDD) and rare disease

David Francis[1], *Paul Kalitsis*[1], *Vivian Wei*[1], *Vida Petrovic*[1], *Simon Sadedin*[1;2], *Meghan Wall*[1]

[1]*MCRI, VCGS, Parkville, Australia;* [2]*The University of Melbourne, Pediatrics, Parkville, Australia*

Background/Objectives: SNP Microarrays have been utilised as the gold standard for detecting recurrent and novel pathogenic CNVs in UDD. High resolution arrays have tested these patients sporadically for detection of intragenic CNVs with varying detection rates. In this study we utilised a SNP microarray platform designed for intragenic CNV detection, the illumina GDA-Cyto.

Methods: Validation of the GDA-Cyto platform against whole genome (WG) data showed sensitivity down to single exon detection (dependant on probe coverage). The platform was implemented for confirmation of ES and WG CNVs, detection of 2nd hit CNVs in AR genes and more recently for UDD and rare disease cases post normal standard array or ES/panel (431 cases).

Results: The 431 cases showed a high pathogenic detection rate overall of 9.5% over standard genomic testing rates, and for specific clinical referrals, intellectual disability (ID) (17%), Epilepsy and ID (9.3%), capillary malformation disorders (25%) and stature/skeletal (25%). Data analysis showed that most pathogenic findings were partial or intragenic deletions (75%), non-mosaic (98%), involved autosomal dominant genes (70%), loss of function (100%), were 32kb in size(median) and showed 3 exon (median) involvement. Cases which involved untranslated regions, mosaicism, the first or last coding exons and enhancer regions were likely to be missed by standard testing.

Conclusion: : Clinical referrals showed a normal GSA standard MA or ES result, high resolution microarray being the next affordable genomic test. This study informs diagnostic laboratories and clinicians of high resolution microarray utility in their genomic workflows.

Conflict of Interest: None declared

P14.012.B Gene Expression Profiles Induced by RNA interference on NSD1 Δ5Δ7, and NSD1 Δ19–23 isoforms in Fibroblast Cells.

Giuseppina Conteduca[1], *Davide Cangelosi*[2], *Chiara Baldo*[3], *Alessia Arado*[3], *barbara testa*[3], *Michela Malacarne*[3], *gilberto filaci*[1], *Domenico Coviello*[3]

[1]*IRCCS San Martino, Biotherapy, Genova, Italy;* [2]*IRCCS Giannina Gaslini, Clinical bioinformatics unit, Genova, Italy;* [3]*IRCCS Giannina Gaslini, Human Genetics, Genova, Italy*

Background/Objectives: Germline variants in NSD1 gene are responsible of Sotos syndrome (SoS), while somatic variants promote neoplastic cell transformation. Molecular pathways affected by NSD1 variants are to date uncharacterized. Our previously studies, showed three alternative RNA isoforms of NSD1 present in fibroblast cells lines (FBs): the canonical full transcript and 2 alternative transcripts, indicated as AT2 (NSD1 Δ5Δ7) and AT3 (NSD1 Δ19–23 5'end).

Methods: In order to elucidate the role of NSD1 isoforms in different molecular pathways, NSD1 isoforms expression was suppressed by siRNA knockdown in FBs cultures. In this study, using microarray gene expression analysis, transcriptome data was explored.

Results: We demonstrate that all 3 isoforms are involved both in cell cycle regulation and in tumoral and neoplastic development. We showed that one NSD1 isoform AT2 target is ARP3 actin-related protein 3 homolog B (ACTR3B). It may act downstream of the stress fibers and bundle of actin filaments formation and it might mediate the cellular division mechanisms. In vitro findings showed that loss of both NSD1 canonical isoform and AT2 isoform, impaired the ability of fibroblasts to regulate the actin cytoskeleton and we observed that this caused selective loss of the stress fibers.

Conclusion: Our findings provide novel insights of NSD1 function and demonstrate an essential role of NSD1 in regulating actin cytoskeleton and stress fibers formation in fibroblasts.

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Conflict of Interest: None declared

P14.013.C Optical genome mapping identifies hidden structural variants in 58 undiagnosed rare disease patient-parent trios

Bart van der Sanden[1], *Sandra Vorimo*[2], *Theresa Brunet*[3;4], *Aïcha Boughalem*[5], *Maureen Jacob*[3], *Ronald van Beek*[1], *Eveline Kamping*[1], *Elisa Rahikkala*[6], *Outi Kuismin*[6], *Jukka Moilanen*[6], *Katri Pylkäs*[2;7], *Elisabeth Graf*[3], *Sandy Loesecke*[3], *Lisenka Vissers*[1], *Juliane Winkelmann*[3;8;9;10], *Kornelia Neveling*[1], *Thomas Meitinger*[3], *Tuomo Mantere*[2], *Detlef Trost*[5], **Alexander Hoischen**[1;11]

[1]*Department of Human Genetics, Research Institute for Medical Innovation, Radboud university medical center, Nijmegen, Netherlands;* [2]*Laboratory of Cancer Genetics and Tumor Biology, Cancer*

and Translational Medicine Research Unit and Biocenter Oulu, University of Oulu, Oulu, Finland; [3]Institute of Human Genetics, School of Medicine, Technical University Munich, Munich, Germany; [4]Dr. v. Hauner Children's Hospital, Department of Pediatric Neurology and Developmental Medicine, LMU - University of Munich, Munich, Germany; [5]Laboratoire CERBA, Saint-Ouen l'Aumône, France; [6]Department of Clinical Genetics, Medical Research Center Oulu and PEDEGO Research Unit, Oulu University Hospital and University of Oulu, Oulu, Finland; [7]Northern Finland Laboratory Centre Nordlab, Oulu, Finland; [8]Institute of Neurogenomics, Helmholtz Zentrum München, Munich, Germany; [9]Lehrstuhl für Neurogenetik, Technische Universität München, Munich, Germany; [10]Munich Cluster for Systems Neurology (SyNergy), Munich, Germany; [11]Department of Internal Medicine; Radboud Expertise Center for Immunodeficiency and Autoinflammation and Radboud Center for Infectious Disease (RCI), Radboud University Medical Center, Nijmegen, Netherlands

Background/Objectives: Next-generation sequencing has unraveled the genetic cause for many rare disease patients, but a significant number remain undiagnosed using standard-of-care tests. Here, optical genome mapping (OGM) identified structural variants (SVs) that have remained undetected by extensive prior genetic testing including exome and/or genome sequencing.

Methods: We performed OGM for 58 patient-parent trios and prioritized SVs using a two-step approach. First, we systematically identified all de novo SVs and subsequently we studied all rare inherited SVs. Potential pathogenic SVs were confirmed using orthogonal methods.

Results: On average, we identified 6,295 SVs >500bp per proband, primarily insertions (69.8%) and deletions (27.0%). In total, we identified 11 de novo SVs, including 3 insertions, 3 deletions, 3 duplications, 1 translocation, and 1 inversion. These SVs potentially impacted multiple candidate disease-associated genes, such as *ACSL6*, *CAP2*, *FAM8A1*, *FGF9*, *KIF13A*, *MSI2*, *NSF*, and *NUP153*. Additionally, on average per sample, we identified 11 inherited SVs overlapping with (the regulatory region of) an established OMIM disease gene, including a homozygous deletion affecting *SCN9A* causing congenital indifference to pain and a ~115kb inter-chromosomal insertion in the CMTX locus at Xq27.1 causing Charcot-Marie-Tooth disease, both leading to a novel diagnosis. In total, we identified (likely) pathogenic SVs in two individuals and candidate disease-causing SVs in five other individuals.

Conclusion: OGM enables the accurate detection of challenging de novo SVs. We detected a de novo SV mutation rate per genome of 0.19. Overall, our results suggest a potential additional yield of 12%, demonstrating that OGM can unravel previously hidden SVs in extensively tested patients.

Grants:

Conflict of Interest: None declared

P14.014.D Prenatal suspicion of monosomy X (NIPT) - postnatal complex aberrant X-chromosome

Susanna Schubert[1], *Senta Schönnagel[1], Jana Knorr[1], Robin-Tobias Jauss[1], Anne-Christin Teichmann[1]*

[1]Institute of Human Genetics, University of Leipzig Medical Center, Leipzig

Background/Objectives: We present a 1.5-year-old female patient without clinical manifestations. Prenatally, the fetus was notable for borderline nuchal translucency only, and there was suspicion of monosomy X in the non-invasive prenatal testing (NIPT). Prenatal invasive diagnostic was not performed.

Methods: For initial postnatal testing only EDTA-blood was available, so we conducted a short tandem repeat (STR) analysis to validate the NIPT result. Following this, a series of diagnostic procedures, including MLPA, Exome sequencing, and cytogenetic analysis were performed to elucidate the complexity of the X chromosome. Finally, the methylation pattern was determined to check for X-inactivation.

Results: STR analysis identified three homozygous X chromosome markers, one showing a distinct peak height ratio. MLPA confirmed the presence of *SHOX*-gene but suggested a duplication Xp and deletion Xq, indicative of an Iso-Xp chromosome. Subsequent CNV (copy number variation) and cytogenetic analysis revealed X chromosome complexities, including duplication of the p-arm and centromere-near region, triplication of the interstitial q-arm, and a large deletion up to Xqter. Methylation pattern analysis identified abnormal CAG repeats, indicating a pathologically skewed X-inactivation in the patient.

Conclusion: Based on these findings, the patient exhibits a complex structurally abnormal karyotype characterized by a dicentric X chromosome. The 100% X-inactivation (non-random X-inactivation) of the aberrant paternal X chromosome suggests that the patient is likely to undergo normal development. However, potential issues such as puberty and menstrual abnormalities, along with a higher risk of primary ovarian insufficiency (as the deletion includes the POF1 region), are anticipated.

Grants: None

Conflict of Interest: None declared

P14.015.A Deciphering a Complex Short Size RERE Rearrangement: Importance of Combined Approaches for Duplication Interpretation

Jade Fauqueux[1], *Jean-Pascal Meneboo[2;3;4;5;6], Emilie Ait Yahya[7], Caroline Thuillier[8], Elise Boudry[8], Celine Villenet[2;3;4;6;8], Roseline Caumes[9], Martin Figeac[2;3;4;5;6], Jamal Ghoumid[1;9], Thomas Smol[1;8]*

[1]Univ Lille, ULR7364 RADEME, Lille, France; [2]Univ Lille, Lille, France; [3]CNRS, Lille, France; [4]INSERM, Lille, France; [5]CHU Lille, Lille, France; [6]Institut Pasteur, Lille, France; [7]CHU Lille, Cellule Bioinformatique, Plateau Commun de Séquençage, Lille, France; [8]CHU Lille, Institut de Génétique Médicale, Lille, France; [9]CHU Lille, Clinique de Génétique, Lille, France

Background: *Arginine-glutamic acid dipeptide repeats*, encoded by the *RERE* gene at locus 1p36.23, is a nuclear receptor coregulator that positively regulates retinoic acid signaling. Monoallelic pathogenic variations of *RERE* have been associated with neurodevelopmental disorders with or without brain, eye, or heart abnormalities.

Methods: We performed a comprehensive structural analysis of an initially suspected de novo duplication of the *RERE* gene identified in a patient with non-syndromic intellectual disability, using Nanopore long-read sequencing, secondarily combined by optical mapping.

Results: Reanalysis of the profile of each CGH array probe revealed that the duplication partially involving the *RERE* gene could be consistent with a duplication-triplication-duplication rearrangement (DUP-TRP-DUP). Long-read sequencing confirmed the presence of the unbalanced DUP-TRP-DUP, but also provided two other major information. First, the rearrangement of the additional *RERE* locus was more complex than might appear, with an inversion of the last duplication of the *RERE* rearrangement: DUP-INV(TRP-DUP). Second, the complex structural variant, supported by optical mapping, was inserted into the locus 22q11.2, in a region with a pattern of repeated PATRR motifs at the breakpoint sites on chromosome 22, which appears to destabilize the region and increase the likelihood of insertions.

Conclusion: Long-read sequencing approaches have provided a comprehensive understanding of a highly complex structural variant involving the *RERE* gene while shedding light on its pathogenicity, which may act more as an environmental disorganization structural factor within the locus 22q11.2, rather than an abnormality of *RERE* itself.

Grants: BPI 2022 CHU Lille

Conflict of Interest: None declared

P14.016.B Structural Characterization of Recurrent Duplications Involving *FGF12* by Long-Read Nanopore Sequencing

Jade Fauqueux[1], Jean-Pascal Meneboo[2], Caroline Thuillier[3], Celine Villenet[2], Roseline Caumes[4], Marion Gerard[5], nicolas gruchy[5], Martin Figeac[2], Jamal Ghoumid[1;4], **Thomas Smol**[1;3]

[1]Univ Lille, ULR7364 RADEME, Lille, France; [2]Univ Lille, CNRS, Inserm, CHU Lille, Institut Pasteur de Lille, US 41 - UAR 2014 - PLBS, Lille, France; [3]CHU Lille, Institut de Génétique Médicale, Lille, France; [4]CHU Lille, Clinique de Génétique, Lille, France; [5]CHU Caen, Service de Génétique, Caen, France

Background: Fibroblast growth factor 12, encoded by the *FGF12* gene, is a member of the fibroblast growth factor homologous factor family. FGF12 interacts with voltage-gated sodium channels and enhances their excitability by delaying rapid inactivation. Pathogenic variations within *FGF12*, characterized by de novo heterozygous point variants, have been associated with developmental and epileptic encephalopathies. Here, we investigate structural variations encompassing *FGF12* by long read approaches.

Methods: A structural analysis of de novo duplications involving the *FGF12* gene, in patients with epileptic encephalopathies, was performed. We realized a targeted long-read sequencing approach on the *FGF12* locus using adaptive sampling from Nanopore. The data were then confirmed by PCR and nested PCR, followed by Sanger sequencing of the respective amplicons.

Results: Targeted long-read sequencing, covering 1% of the genome with a focus on *FGF12*, achieved coverage of the region to a depth of approximately 16X, allowing observation of the targeted duplications. Analysis of the reads covering the duplication revealed the mechanism as a tandem direct duplication, which was confirmed by PCR and Sanger sequencing. Furthermore, the observation of repeated intragenic LINE sequences at the duplication not only underscored the involvement of the non-allelic homologous recombination mechanism but also contributed to the understanding of their recurrent nature.

Conclusion: Long-read approaches, through Nanopore sequencing, provide a means for the detection of tandem duplications within a segment of the *FGF12* gene and offer a comprehensive understanding of their recurrent nature within *FGF12*.

Grants: BPI 2022 CHU Lille

Conflict of Interest: None declared

P14.017.C Complete characterization of human polymorphic inversions and other complex variants from long read data

Ricardo Moreira Pinhal[1;2], *Konstantinos Karakostis[1;2], Illya Yakymenko[1;2], maria diaz[1;2], andres santos[3], jaime urtaza[3], Marta Puig[1;3], Mario Caceres[1;2;4]*

[1]Institut de Biotecnologia i de Biomedicina, Universitat Autònoma de Barcelona, Bellaterra, Barcelona, Spain; [2]Research Programme on Biomedical Informatics (GRIB), Hospital del Mar Research Institute (IMIM), Barcelona, Spain; [3]Departament de Genètica i de Microbiologia, Universitat Autònoma de Barcelona, Bellaterra, Barcelona, Spain; [4]ICREA, Barcelona, Spain

Consortium: InvFEST

Background/Objectives: Inversions are a special type of structural variants whose study has lagged behind due to their balanced nature and the presence of large inverted repeats (IR) at their breakpoints. New techniques are finally making possible to identify the full spectrum of human inversions. However, in most cases, just a limited number of individuals has been studied, which precludes the analysis of the effects of the detected variants. Here, we take advantage of long reads to generate and characterize an exhaustive catalogue of IR-mediated inversions.

Methods: We have developed a computational pipeline that uses Oxford Nanopore Technologies (ONT) long read data to genotype inversions. Using this method, we have interrogated 636 candidate inversions from different studies, ranging from 265 bp to 7.9 Mb and flanked by up to 190 kb long IRs, in a diverse set of 90 individuals.

Results: We detected both orientations in 218 inversions, validating 140 novel inversions, with frequencies ranging between 1-50%. We also show that ONT genotypes are highly accurate, matching perfectly previous experimental genotypes of 53 inversions. Importantly, additional structural variants were identified in 136 predictions, revealing the complexity of repeat-rich regions. Finally, some of these regions show discrepancies with the new Pangenome reference, suggesting that they might not be well resolved.

Conclusion: Long reads therefore have a great potential for the characterization of currently missed inversions and other complex genomic regions in multiple individuals, opening the door to determine their real functional impact.

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Conflict of Interest: None declared

P14.018.D Search for monogenic causes of Hashimoto's thyroiditis (HT) in a family with a history of early-onset HT

Vesna Boraska Perica[1], *Dean Kaličanin[1], Robert Belužić[2], Ana Barić[3;4], Marko Vuletić[3], Sanda Gračan[3], Vedrana Čikeš Čulić[5], Ante Punda[3], Veselin Škrabić[6], Maja Barbalić[7]*

[1]School of Medicine University of Split, Department of Medical Biology, Split, Croatia; [2]Rudjer Boskovic Institute, Division of Molecular Medicine, Laboratory for Metabolism and Aging, Zagreb, Croatia; [3]University Hospital of Split, Department of Nuclear Medicine, Split, Croatia; [4]School of Medicine University of Split, Department of Nuclear Medicine, Split, Croatia; [5]School of Medicine University of Split, Department of Chemistry and Biochemistry, Split, Croatia; [6]University Hospital of Split, Department of Pediatrics, Split, Croatia; [7]Faculty of Science University of Split, Department of Biology, Split, Croatia

Background/Objectives: Hashimoto's thyroiditis (HT) is a complex autoimmune disorder of thyroid gland. To date, only one monogenic form of HT has been identified. Our aim was to investigate monogenic causes of HT in a family with a history of early-onset HT.

Methods: Whole exome sequencing (WES) was performed in six family members with HT (four with early-onset HT diagnosed between 5-12 years of age) and three healthy family members in order to find candidate variants segregating with the disease. Following WES findings, ELISA for the substrate (5-hydroxytryptofan, 5-HTP) of the candidate gene (*TPH2*) was performed in all family members and 19 control individuals to assess the functional impact of the detected variant.

Results: Under an autosomal dominance model, we identified a rare missense variant (rs17110563, MAF (CEU) = 0.0017) in the *TPH2* (tryptophan hydroxylase 2) gene as the likely causative

variant. The *TPH2* gene encodes an enzyme responsible for converting L-tryptophan to 5-HTP, which is further converted into serotonin. The levels of 5-HTP were shown to be slightly lower in family members carrying the variant, compared to controls, however this difference was not statistically significant.

Conclusion: By exploring a large pedigree burdened with early-onset HT, we identified a candidate variant within *TPH2*, a gene involved in serotonin metabolism. While largely unexplored, recent studies indicate a potential association between decreased serotonin levels and hypothyroidism. In addition, *TPH2* is located in close proximity of another promising candidate, *TRHDE* gene, involved in thyroid-axis regulation, which warrants further research of this region in relation to HT.

Conflict of Interest: None declared

P14.019.A Characterizing the genetic architecture of thousands of different outcomes using LDpred2-auto.

Ole Hansen[1], *Lucas Rasmussen*[1], *Clara Albiñana*[1], *Zhihong Zhu*[1], *Jakob Grove*[2;3;4], *Florian Privé*[1], *Bjarni Jóhann Vilhjálmsson*[1;2;5]

[1]*Aarhus University, NCRR, Aarhus, Denmark;* [2]*Bioinformatics Research Centre, Aarhus, Denmark;* [3]*Department of Biomedicine, Aarhus University, Aarhus, Denmark;* [4]*Aarhus University, Aarhus, Denmark;* [5]*Broad Institute of MIT and Harvard, Cambridge, United States*

Background: Understanding the genetic architecture of health outcomes is important when planning future genetic studies, as well as for developing personalized medicine strategies and public health interventions. Recently we presented an updated version of LDpred2-auto (Privé *et al*., AJHG 2023) that models the genetic architecture (e.g. polygenicity and selection coefficients) to improve the estimation of SNP-based heritability and the prediction of polygenic risk scores. This method only requires genome-wide association studies (GWAS) summary statistics.

Methods: Here we develop a LDpred2-auto pipeline and apply it to thousands of different human phenotypes using GWAS summary statistics that are made available in the GWAS Catalog (https://www.ebi.ac.uk/gwas/). This pipeline provides a comprehensive map of genetic architectures of those human traits. The output of this pipeline includes the estimates of polygenic score, heritability, polygenicity, and a selection coefficient. Additionally, we output a range of metrics and quality control measures making the user more informed.

Results: We estimate adjusted R2, polygenicity p, (SNP) h2, and selection coefficient α for many different disorders and phenotypes. We infer out-of-sample prediction accuracy of the scores, and when possible, observed out-of-sample prediction accuracy in the iPSYCH cohort.

Conclusions: Our research introduces a new systematic approach in large-scale genetic analysis, integrating biological and selection perspectives using LDpred2-auto. This will enhance our understanding of genetic architecture in general and for specific traits in particular.

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Conflict of Interest: Ole Hansen: None declared, Lucas Rasmussen: None declared, Clara Albiñana: None declared, Zhihong Zhu: None declared, Jakob Grove: None declared, Florian Privé: None declared, Bjarni Jóhann Vilhjálmsson BJV is a member of the scientific advisory board for Allelica

P14.020.B *AGAP* duplicons underlie genomic instability at chromosome 10q11.22

Vincenza Simona Delvecchio[1], *Stefania Fornezza*[1], *William Harvey*[2], *Philip Dishuck*[2], *Evan Eichler*[2;3], **Giuliana Giannuzzi**[1]

[1]*University of Milan, Department of Biosciences, Milan, Italy;* [2]*University of Washington School of Medicine, Department of Genome Sciences, Seattle, United States;* [3]*Howard Hughes Medical Institute, Seattle, United States*

Background/Objectives: Chromosome 10q11.22 is a duplication-rich interval of our genome and among the latest to be fully assembled. It carries copy-number variable genes that have been associated with brain diseases and obesity. Here, we sought to characterize structural diversity at this locus and gain insights into mechanisms of genomic instability.

Methods: We performed a detailed analysis of the region in 94 haploid assemblies that were produced from a panel of 47 diverse samples by the Human Pangenome Reference Consortium. We validated the structures by Illumina-based copy-number genotyping and by distance between unique k-mers in Nanopore reads. We narrowed down the breakpoints to a few kbp intervals.

Results: We identified eleven alternative haplotypes differing in the copy number of two main and large (200 and 450 kbp) genomic segments and by a 700 kbp inversion polymorphism. The size disparity from the most common haplotype ranged from 20 kbp to 1.7 Mbp, with 2.4 Mbp difference between the shortest and the longest haplotypes. The boundaries between structurally variant intervals correspond to duplicons containing functional or pseudogenized *AGAP* copies that emerged in the last 25 million years of human evolution. Longer duplicons mediated the rearrangements and they were generally broken at different positions.

Conclusion: We discovered extensive structural diversity at the 10q11.22 locus that includes large duplications, deletions, and inversion toggling. Such genomic instability derives from non-allelic homologous recombination between high-identity duplicons associated with a Catarrhini-specific subgroup of the *AGAP* gene family. Our breakpoint analysis suggests general rules of the molecular mechanism of repeat-mediated rearrangements.

Grants: PRIN 2022

Conflict of Interest: None declared

P14.021.C Exploring somatic structural variants in leukemia patients through chromatin interaction assessment

Jakub Porc[1;2], *Karolina Cernovska*[1;2], *Kristyna Zavacka*[1;2;3], *Kamila Stranska*[1;2;3], *Sabina Adamova*[2;3], *Natalie Kazdova*[3], *Eva Ondrouskova*[3], *Marie Jarošová*[2;3], *Sarka Pospisilova*[1;2;3], *Michael Doubek*[1;2;3], *Karla Plevová*[1;2;3]

[1]*CEITEC MU, Center for Molecular Medicine, Brno, Czech Republic;* [2]*Faculty of Medicine Masaryk University, Institute of Medical Genetics and Genomics, Brno, Czech Republic;* [3]*University Hospital Brno, Department of Internal Medicine – Hematology and Oncology, Brno-Bohunice, Czech Republic*

Background/Objectives: Chronic lymphocytic leukemia (CLL) is a disease with a very heterogeneous course and genetic background. Numerous somatic structural variants (SVs) have been linked to this malignancy, but underlying pathogenetic mechanisms are not yet fully understood. We employed Micro-C, a chromatin conformation capture method, on CLL patient samples with complex genomic rearrangements to investigate long-distance and SV-related interactions and their potential impact on disease behavior.

Methods: We selected 10 CLL cases with clonally expanded complex SVs as a suitable model to provide insights into disease biology. Chromatin crosslinking was carried out on B cells

separated from peripheral blood of the patients. Classical cytogenetics, chromosomal microarray and whole genome sequencing were available for them to evaluate the results comprehensively. EagleC and NeoLoopFinder tools were used to determine the CNV profile, perform CNV-aware contact matrix normalization, detect novel loops, topologically associating domains (TADs), and recreate a map of SVs and affected regions.

Results: Numerous SVs were detected, including insertions, deletions, and translocations, that were independently confirmed by other methods. We also identified novel loops and TADs caused by those aberrations, providing insights into the molecular consequences of detected SVs and mechanisms underlying the disease development.

Conclusion: We assume that chromatin conformation analysis has great potential for detecting genomic rearrangements and their molecular consequences, worth future studies on larger datasets.

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Conflict of Interest: None declared

P14.022.D Rearrangements of the *NUP98* gene in adult patients with de novo acute myeloid leukemia (AML)

Sarka Ransdorfova[1], *Jana Markova*[1], *Marie Valeriánová*[1], *Iveta Mendlikova*[1], *Kristina Rochlova*[1], *Zuzana Zemanova*[2], *Libuse Lizcova*[2], *Lenka Pavlistova*[2], *Anna Jonasova*[3], *jan valka*[1]

[1]*IHBT, Prague 2, Czech Republic;* [2]*Center of Oncocytogenomic, Institute of Medical Biochemistry and Laboratory Diagnostics, Prague 2, Czech Republic;* [3]*1st Medical Department, General University Hospital and First Faculty of Medicine, Prague 2, Czech Republic*

Background/Objectives: *NUP98* gene rearrangements (11p15.4) belong to cryptic recurrent genetic alterations in cytogenetically normal AML patients that are associated with poor prognosis. The most frequent *NUP98* fusion gene partner is *NSD1*. It occurs most frequently in children (16%) and young (2%) AML patients, and is rare in adults.

Methods: Since 2017, we examined the bone marrow cells of 470 newly diagnosed adult patients with AML using conventional karyotyping in combination with molecular techniques: FISH (Abbott, MetaSystems), mFISH/mBAND (MetaSystems). Fusion genes were detected by RT-PCR followed by direct sequencing.

Results: We identified *NUP98* gene rearrangements in nine cytogenetically normal patients (median age 53 years, three > 60 years). We confirmed t(5;11)(q35;p15.5) with *NUP98::NSD1* gene fusion in seven of these cases (7/470; 1.5%). Sequence analyses proved the NUP98-NSD1 transcript, arising from fusion of exon 12 of *NUP98* with exon 6 in the *NSD1* gene, in all of them. In two other patients, we proved t(11;12)(p15.4;p13.33) and t(7;11)(p15.4;p15.2) with a *NUP98::KDM5A* and *NUP98::HOXA9* gene fusion, respectively. *FLT3*/ITD was detected in all nine patients. Specific primers and probes have been designed to monitor minimal residual disease during therapeutic treatment of the patients. Four patients died (median OS 13 months). Five patients are alive, three after allogeneic bone marrow transplantation.

Conclusion: We demonstrated the occurrence of *NUP98* aberrations with *FLT3*/ITD in cytogenetically normal adult AML patients older than 60 years. Subsequent molecular analyzes of these rearrangements provided information important for precise monitoring of the course of therapy.

Grants: Supported by MH CZ-DRO IHBT00023736, MH CZ-DRO-0064165

Conflict of Interest: None declared

P14.023.A Evaluation of cytogenetic response in chronic myeloid leukemia with variant translocation

Marie Valeriánová[1], *Kristina Rochlova*[1], *Iveta Mendlikova*[1], *Hana Klamova*[2], *Adam Laznicka*[2], *Zuzana Zemanova*[3], *Sarka Ransdorfova*[1]

[1]*IHBT, Cytogenetics, Prague, Czech Republic;* [2]*IHBT, Prague, Czech Republic;* [3]*Institute of Medical Biochemistry and Laboratory Diagnostics, Center of Oncocytogenomics, Prague, Czech Republic*

Background/Objectives: Variant Philadelphia (Ph) translocations involving chromosomes 9 and 22 and one or more other chromosomes occur in 5-10% of newly diagnosed cases of chronic myeloid leukemia (CML). Some previous studies have suggested a suboptimal response to TKIs in variant translocations compared to classical CML. However, the prognostic impact of partner chromosome loci remains unclear due to insufficient data.

Methods: We analyzed variant Ph translocations using cytogenomic methods to determine their molecular mechanisms and evaluate the response to treatment.

Results: Of 307 CML patients examined between 2013 and 2023, we detected variant Ph translocation in 21 patients in chronic phase and two patients in blast crisis (i.e. 7.5%). FISH revealed a one-step mechanism of translocation in 13/23 cases and two-step or multi-step mechanism in 10/23 cases. Cytogenetic evaluation of treatment response was performed in 18 of 23 patients. In 14 patients, treatment resulted in complete cytogenetic remission. Three patients, who achieved partial cytogenetic remission, two of them had high-risk additional chromosomal abnormalities - ACAs, failed first-line TKIs treatment. One patient achieved no cytogenetic response, had high-risk ACAs and a mutation in the *BCR::ABL1* tyrosine kinase domain (TKD).

Conclusion: Partner chromosomes and translocation mechanisms are unlikely to influence the therapeutic response in CML with a variant Ph translocation. The failure of therapeutic response is due to other aberrations or TKD mutations rather than the type of variant Ph translocation. Reporting new cases of variant translocations is essential to further elucidate their prognostic significance.

Grants: Supported by MH-CZ-DRO IHBT00023736 and MH CZ-DRO-0064165.

Conflict of Interest: None declared

P14.024.B A new case with multiple de novo copy number variant (MdnCNV) phenomenon

Deborah Bartholdi[1], *Nijas Aliu*[1], *Dominique Braun*[1], *Mandy Krumbiegel*[2], *Christiane Zweier*[1]

[1]*Department of Human Genetics, Bern University Hospital, University of Bern, Bern, Switzerland;* [2]*Institute of Human Genetics, University Hospital Erlangen, Friedrich-Alexander University, Erlangen-Nürnberg, Germany*

Background/Objectives: We report on a 10 year-old individual with developmental delay, microcephaly and mild skeletal and neuromuscular features (kyphosis, camptodactyly of fingers, contractures of knees). The clinical features were not indicative of a specific genetic disorder.

Methods: We performed trio exome sequencing including screening for copy number variants and subsequently chromosomal microarray analysis (CMA), karyotyping, and FISH.

Results: Trio exome did not reveal pathogenic or likely pathogenic SNVs but showed six de novo copy number gains, each encompassing between 3-4 Mb on chromosomes 3

(3q26.2q26.31), 4 (4q13.3q21.1), 5 (5p15.32p15.31 and 5q34), 6 (6p21.2p21.1) and 13 (13q13.3q14.11), respectively. Chromosomal analysis did not show a complex chromosomal rearrangement (karyotype 46,XY). The CNVs were confirmed by CMA and FISH. The latter showed that the duplications were located at the original chromosome bands. All of the six duplications were classified as VUS.

Conclusion: This case fulfils the criteria for the MdnCNV (multiple de novo CNV) phenomenon, which is characterized by the carrying of four or more constitutional de novo CNVs arising independently throughout the genome within one generation in individuals with developmental disorders. MdnCNV seems to favor copy number gains and was first described in 2017 (PMC5407901). It is estimated to occur in 1 per 12,000 individuals referred for CMA. It is hypothesized that the CNVs in MdnCNV arise by a microhomology-mediated, break-induced replication mechanism during the peri-zygotic period.

This case adds to this very rare genome instability syndrome, the impact of which on human disease is not yet fully understood.

Conflict of Interest: None declared

P14.025.C Positive Predictive Value of Balanced Structural Variants reported through Short-Read Genome Sequencing: do we need orthogonal confirmation ?

Renaud Touraine[1;2], Damien Sanlaville[1;3;4], Anne-Françoise Roux[1;5], Florence Amblard[6], Virginie Bernard[1], Frederic Bilan[1;7], Tristan Celse[1;8], Nicolas Chassaing[1;9], Isabelle Creveaux[1;10], Anne-Sophie Denommé-Pichon[1;11], Matthieu Egloff[1;7], Vincent Gatinois[12], Claire Goursaud[1;13], Radu Harbuz[1;6], Gaelle Hardy[1;14], Louis Januel[1;3], Paul Kuentz[1;15], Marine Lebrun[1;2], Gaetan Lesca[1;3;4], Delphine Mallet[1;13], Luke Mansard[1;5], Laurence Michel-Calemard[1;13], Franck Pellestor[12], Fabienne Prieur[2], Mathilde Pujalte[3], Francis Ramond[1;2], John Rendu[1;16], Florence Riant[1;17], Véronique Satre[1;6;18], Anouck Schneider[1;12], Mylene Tharreau[1;12], Julien Thevenon[1;18;19], Marianne Till[3], Antonio Vitobello[1;11], Caroline Schluth-Bolard[1;20;21], Charles Coutton[1;12;18], **Nicolas Chatron**[1;3;4]

[1]GCS AURAGEN, Lyon, France; [2]CHU-Hôpital Nord, Service de Génétique Clinique, Chromosomique et Moléculaire, Saint-Etienne, France; [3]Hospices Civils de Lyon, Service de Génétique, Lyon, France; [4]Univ Lyon, Univ Lyon 1, CNRS, INSERM, Physiopathologie et Génétique du Neurone et du Muscle, UMR5261, U1315, Institut NeuroMyoGène, Lyon, France; [5]Univ Montpellier, CHU Montpellier, INSERM, Institute for Neurosciences of Montpellier (INM) and Molecular Genetics Laboratory, Montpellier, France; [6]University Hospital of Grenoble, Cytogenetics Laboratory, Grenoble, France; [7]Université de Poitiers and CHU de Poitiers, INSERM U1084, LNEC and Service de Génétique, Poitiers, France; [8]Centre Hospitalier Universitaire de La Réunion, Service de Génétique Médicale, La Reunion, France; [9]CHU Toulouse, Purpan Hospital, Department of Medical Genetics, Toulouse, France; [10]CHU Clermont-Ferrand, Service de Biochimie et Génétique Moléculaire, Clermont-Ferrand, France; [11]FHU TRANSLAD, University of Burgundy, Functional Unit for Diagnostic Innovation in Rare Diseases and INSERM UMR1231 GAD "Génétique des Anomalies du Développement", Dijon, France; [12]University Hospital of Montpellier, Department of Molecular Genetics and Cytogenomics, Montpellier, France; [13]Hospices Civils de Lyon, Service de Biochimie et Biologie Moléculaire, Lyon, France; [14]Centre Hospitalier Universitaire de Grenoble Alpes, Laboratory of Molecular Genetics, Grenoble, France; [15]Université de Franche-Comté, CHU Besançon, FHU TRANSLAD et Institut GIMI, Oncobiologie Génétique Bioinformatique, Besançon, France; [16]CHU Grenoble Alpes and Université Grenoble Alpes, Inserm U1216 and Grenoble Institut Neurosciences, Grenoble, France; [17]AP-HP, Saint-Louis Hospital and Lariboisiere Hospital, Department of Neurovascular Molecular Genetics and Reference Center for Rare Vascular Diseases of the Central Nervous System and the Retina (CERVCO), Paris, France; [18]University of Grenoble Alpes, INSERM U1209 Institute for Advanced Biosciences, Grenoble, France; [19]University Hospital of Grenoble, Medical Genetics service, Grenoble, France; [20]Hôpitaux Universitaires de Strasbourg, Laboratoire de Diagnostic Génétique, Institut de Génétique Médicale d'Alsace, Strasbourg, France; [21]Université de Strasbourg, INSERM UMRS_1112, Strasbourg, France

Background/Objectives: Genome Sequencing has transformed our practice of rare disease diagnosis. For cytogeneticists, who first applied GS to characterize balanced structural variants (BSV), GS is now upfront as a first-tier test where karyotypes and microarrays once stood with the benefit of maximizing diagnostic yield combining SNV, CNV and BSV detection. While short-read GS performances for SNV and CNV calling are known, we sought to evaluate the positive predictive value of BSV reported in a diagnostic setting.

Methods: We identified 47 rare disease patients carriers of a BSV identified through short-read GS.

Results: Nineteen of these were inversions, 17 reciprocal translocations, 11 more complex events including one 18-breakpoint chromoanagenesis. 28 were deemed (likely) pathogenic due to either gene disruption (n = 25) or position effect (n = 3). While an orthogonal confirmation was not performed in 20 cases, 26 out of 27 investigated variants were confirmed: 18 through conventional cytogenetics techniques, 7 with junction fragment PCR, 2 through optical mapping. The sole unconfirmed case was a false positive t(1;11)(p31.1;p13) from the bioinformatics pipeline during its development phase. In 3 cases, short-read GS analysis was deemed incomplete and led to the recommendation of a more thorough characterization.

Conclusion: The robustness of BSVs reported here (PPV = 96%) suggests that orthogonal techniques might be relevant for further familial or prenatal testing but optional for confirmation when a clear BSV signal could be observed. This performance is the result of validated pipelines and human expertise combined for optimal diagnostics. Such expertise and retrospective validations will be critical for future technologies' implementation (e.g.: long-read sequencing).

Grants:

Conflict of Interest: None declared

P14.026.D Direct evidence of deletion and gene conversion as origin of SMA alleles revealed by long-read sequencing

Maria Zwartkruis[1;2], *Martin Elferink[2], Demi Gommers[1;2], Laura Blasco-Pérez[3;4], Mar Costa-Roger[3;4], Ilaria Signoria[1], Ivo Renkens[2;5;6], Amber den Ouden[7], Ronny Derks[7], Jared Green[1], Joris Kortooms[1], Carlo Vermeulen[5;8], Roy Straver[5;8], Hanneke van Deutekom[2], Marjan Weiss[7], Fay-Lynn Asselman[1], Eduardo Tizzano[3;4], Renske Wadman[1], WL Van Der Pol[1], Gijs van Haaften[2], Ewout Groen[1]*

[1]University Medical Center Utrecht, Department of Neurology and Neurosurgery, UMC Utrecht Brain Center, Utrecht, Netherlands; [2]University Medical Center Utrecht, Department of Genetics, Utrecht, Netherlands; [3]Vall d'Hebron Research Institute (VHIR), Medicine Genetics Group, Barcelona, Spain; [4]Hospital Vall d'Hebron, Department of Clinical and Molecular Genetics, Barcelona, Spain; [5]University Medical Center Utrecht, Center for Molecular Medicine, Utrecht, Netherlands; [6]University Medical Center Utrecht, Utrecht Sequencing Facility, Center for Molecular Medicine, Utrecht, Netherlands; [7]Radboud University Medical Center, Department of Human Genetics and Radboudumc Research Institute for Medical Innovation, Nijmegen, Netherlands; [8]University Medical Center Utrecht, Oncode Institute, Center for Molecular Medicine, Utrecht, Netherlands

Background/Objectives: Spinal muscular atrophy (SMA) is a debilitating neuromuscular disease, caused by homozygous defects in the *survival motor neuron 1 (SMN1)* gene. *SMN1* and SMA-modifying paralog gene *SMN2* are located in the highly complex *SMN* locus. We aimed to elucidate the still unresolved structure of the *SMN* locus in SMA patients to determine the origin of SMA alleles as a possible disease modifier.

Methods: We performed long-read Nanopore sequencing for 32 SMA patients and additional PacBio HiFi sequencing for five patients. Combining both methods, we performed de novo assembly of the *SMN* locus. For Nanopore data, directed mapping was performed, followed by polyploid haplotype phasing.

Results: De novo assembly revealed the structure of the *SMN* locus in SMA patients. Firstly, we found alleles showing absence of *SMN1* in its expected location, but partial presence of surrounding genes, indicating *SMN1* deletion. Secondly, we found *SMN2* at the expected location of *SMN1*, indicating gene conversion of *SMN1* to *SMN2*. By polyploid phasing of Nanopore reads, we determined 200 kilobase-long haplotypes of *SMN2* and surrounding genes, which were linked to specific locations on the *SMN* locus. Of the resolved *SMN2* haplotypes, 61% were located in an *SMN2* environment and 39% in an *SMN1* environment.

Conclusion: We provide direct evidence for deletion and gene conversion as origin of SMA alleles lacking *SMN1*. Varying environments of the *SMN2* gene could have direct implications for gene expression through regulatory sequences or epigenetic signatures, providing a possible explanation for genotype-phenotype discordances in SMA.

References:

Grants: Prinses Beatrix Spierfonds; Spieren voor Spieren.

Conflict of Interest: None declared

P14.027.A A rare case of mosaic Turner Syndrome with Duchenne Muscular Dystrophy and Biotinidase Deficiency

Mustafa Gunes[1;2], *Sena Cetin*[1;2], *Filiz Ozen*[2], *Elif Yilmaz Gulec*[1;2]

[1]*Istanbul Medeniyet University Medical School, Medical Genetics, İstanbul;* [2]*Istanbul Goztepe Prof. Dr. Suleyman Yalcin City Hospital, Medical Genetics, İstanbul*

Background/Objectives: Turner Syndrome (TS) is a common sex chromosome aneuploidy characterized by short stature and ovarian insufficiency in females. Approximately, half of the patients with TS have mosaicism for 45,X with another karyotype. Duchenne muscular dystrophy (DMD, MIM #310200) is a severe X-linked recessive neuromuscular disease. Biotinidase Deficiency (BD, MIM #253260) is a rare autosomal recessive metabolic disorder with variable neurological and cutaneous findings. We present a little girl expressing the phenotypes of these three disorders.

Methods: A 2-year-old girl, who was diagnosed with mosaic TS and BD in early infancy, was referred to us due to high serum creatine kinase (CK) levels since infancy. Since her aunt's son died due to DMD, the patient underwent *DMD* gene deletion/duplication MLPA analysis and *DMD* gene next-generation sequencing (NGS) respectively.

Results: The girl had the feature of TS with short stature (-2.71 SDS). Her karyotype was 45,X [10]/47,XXX[20] revealing a TS mosaicism, she also had homozygous c.1336 C>G variant in *BTD gene*. While *DMD* MLPA analysis was normal, a known pathogenic nonsense variant c.1912C>T (p.Gln638*) in *DMD* gene was identified via NGS. The variant was confirmed by Sanger sequencing in the proband and her mother.

Conclusion: X-linked recessive disorders such as DMD rarely affect women. Although our case additionally had trisomy X karyotype, her high CK levels ranging between 7000-24.000 IU/L at this early age concluded the diagnosis with DMD. This irrelevance is probably due to the higher mosaicism percentage of 45,X karyotype on myocytes. This patient is unique with the coexistence of mosaic TS, DMD and BD.

Conflict of Interest: None declared

P14.028.B Utility of methylation episignature analysis for CNVs: A systematic review and case series

Sunwoo Lee[1], *France Docquier*[1;2], *Fay Rodger*[1;2], *Rhiannon Evans*[1;2], *Stephanie Allen*[3], *Dominic McMullan*[3], *Soo-mi Park*[4], *Richard Sandford*[4], *Marc Tischkowitz*[4], *Jose-Ezequiel Martin Rodriguez*[1;2], *Ana Toribio*[1;2], *Eamonn Maher*[1;2]

[1]*University of Cambridge, Department of Medical Genetics, Cambridge, United Kingdom;* [2]*Stratified Medicine Core Laboratory NGS Hub, Cambridge, United Kingdom;* [3]*West Midlands Regional Genomics Laboratory, Central and South Genomic Laboratory Hub, Birmingham, United Kingdom;* [4]*Cambridge University Hospitals NHS Trust, Cambridge, United Kingdom*

Background/Objectives: Copy number variants are an important cause of neurodevelopmental disorders but variability in size and gene content can make interpretation of the causation of the phenotype challenging. Pathogenic variants in chromatin modifying genes (CMGs) are also an important cause of neurodevelopmental disorders and many are associated with specific DNA methylation (DNAm) episignatures that can facilitate interpretation of variant pathogenicity. It can be postulated that if CMGs are within or close to CNVs, then episignature analysis could inform the potential role of the CMG in the associated phenotype unless other genes within the CNV modified or mitigated the phenotype.

Methods: To investigate genotype-epigenotype-phenotype relationships for CMG-associated CNVs we undertook a systematic literature review and undertook methylation profiling in a series of patients.

Results: We identified 93 CMG-CNV cases that satisfied eligibility criteria (82 published and 11 unpublished); 84 deletions, 9 duplications). DNAm profiling was performed by methylation array ((n = 82) or NGS-based methods (n = 11). Examples of deletion CNVs containing a CMG which were associated with the relevant CMG episignature were identified and the relationships between CNV type, size and gene content and presence/absence of episignature patterns will be presented with illustrative case reports.

Conclusion: Episignature analysis would appear to have potential for providing pathogenetic and diagnostic insights into a range of recurrent CNVs though more extensive analysis is required to determine the sensitivity and limitations of episignature analysis for specific CNVs.

Grants: NIHR Cambridge Biomedical Research Centre (BRC-1215-20014 and NIHR203312)

Conflict of Interest: None declared

P14.029.C Optical genome mapping of a patient with ring chromosome 3 shows the importance of choosing the proper reference genome for the analysis

Bruna Burssed[1], *André Rodrigueiro Clavisio Pereira de Oliveira*[2], *Marco Antonio Ramos*[1], *Mei Ling Chong*[3], *Peining Li*[3], *Fernanda Teixeira Bellucco*[1], *Maria Isabel Melaragno*[1]

[1]*Universidade Federal de São Paulo, Department of Morphology and Genetics, São Paulo, Brazil;* [2]*Uniscience do Brasil, Uniscience Molecular Laboratory, São Paulo;* [3]*Yale University, Department of Genetics, New Haven, United States*

Background/Objectives: Ring chromosomes are rare chromosomal alterations formed by circular DNA molecules. The proposal of a mechanism of formation relies on the investigation of the breakpoints' location, which is done by comparing the altered genome with a reference genome, regardless of the used technique.

Methods: We studied a male patient with intellectual disability, short stature, dysmorphisms, and clinodactyly. Karyotype and chromosome microarray revealed a ring chromosome 3 – r(3) – with a 5.7 Mb deletion in 3p. Short-read whole genome sequencing (SR-WGS) and optical genome mapping (OGM) were performed to find the precise location of the ring's breakpoints.

Results: OGM, first analyzed using GRCh37 and GRCh38 as references, detected an unexpected translocation between 3p and chromosome 19 with no indication of a ring chromosome. Using the T2T (telomere-to-telomere) as reference, OGM revealed the r(3), displaying a junction point between 3p (chr3:6,012,432) and 3q (chr3:201,097,227). SR-WGS using the T2T as reference located the breakpoint in 3p (chr3:6,010,296), which appears to have joined with a tandem repeat in 3q.

Conclusion: To locate the breakpoints in this r(3), the lack of a deletion in 3q creates a challenge when using the GRCh37 and GRCh38 as reference genomes since they contain unsequenced gaps that include the telomeres. The T2T presents the complete genome sequence, allowing for the precise characterization of the 3q subtelomeric region. The karyotype, which proved the presence of a r(3), was particularly important since it showed inaccuracy in the first alignments of the OGM analysis.

Grants: São Paulo Research Foundation (FAPESP), Brazil.

Conflict of Interest: None declared

P14.030.D Breaking The Boundaries: Deciphering Elusive Structural Variants Through Short Reads Mapping Interpretation

Tomer Poleg[1], *ilana aminov*[1], *Noam Hadar*[1], *Amit Safran*[1], *Vadim Dolgin*[1], *Matan M. Jean*[1], *ofek freund*[1], *Nadav Agam*[1], *Marina Eskin-Shwartz*[1;2], *Gali Heimer*[3], *Bruria Ben-Zeev*[3], *Ohad Shmuel Birk*[1;2]

[1]*The Morris Kahn Laboratory of Human Genetics, Faculty of Health Sciences, Beer sheva;* [2]*Genetics Institute, Soroka Medical Center, Beer Sheva;* [3]*Edmond and Lily Safra Children's Hospital, Sheba Medical Center, Ramat Gan*

Background/Objectives: Structural variants (SVs) pose challenges for detection using short-read sequencing (SRS) techniques. SRS encounters difficulties in spanning the exact SV breakpoints and accurately mapping them, hindering detection of SVs, especially those found in non-coding regions or those characterized by combinations of structural changes, known as complex structural variants (cSVs). Solving cases with SV detection software is a complex task, as it reveals thousands of SVs per genome, with a significant number of false positives and inaccurate variant predictions.

Methods: Whole Genome Sequencing (WGS) was conducted. HomozygosityMapper (HM) was employed for linkage analysis. MANTA software facilitated identification of potential SV breakpoints, focusing on linkage areas or genes known to be associated with the observed phenotype. Integrative Genomic Viewer (IGV) was utilized to visualize read mapping, enabling interpretation and hypothesizing the SV types, and accurately determining their respective boundaries. Borders of identified SVs were verified by PCR and Sanger sequencing.

Results: We present an effective approach that allowed us to uncover novel disease-causing SVs, deciphering unresolved cases. Examples include two distinct de-novo SVs in MECP2 causing Rett Syndrome: a cSV with two different deletion sites (~5Kbp and ~60Kbp) combined with a ~105Kbp inversion, and a~200Kbp translocation; A cSV involving a ~12Kbps deletion and 10bp duplication within FRA10AC1 causing a recessive neurodevelopmental disorder; And a novel ~20Kbp tandem duplication within NSMCE2 causing recessive Primordial Dwarfism.

Conclusion: Deciphering both SVs and cSVs with SRS is attainable through visualization of abnormal split reads and interpretation of the unusual distances and orientations between paired-end reads.

Conflict of Interest: None declared

P14.031.A HLA-DRB1 single nucleotide polymorphism variability in patients with inflammatory bowel disease

Patrik Krumpolec[1], *Klaudia Babišová*[1], *Andrej Gnip*[1], *Oliver Petrovic*[1], *Michaela Hyblova*[1]

[1]*Medirex Group Academy, Nitra, Slovakia*

Background/Objectives: Inflammatory bowel diseases (IBD), including Crohn's disease (CD) and ulcerative colitis (UC), are characterized by chronic, relapsing-remitting inflammation of the gastrointestinal tract. These conditions are recognized as having a multifactorial etiology, resulting from a dysregulated mucosal immune response to commensal gut flora in genetically predisposed individuals, compounded by environmental risk factors. A suggested link exists between specific HLA genes and IBD development, notably the HLA-DRB1 gene. However, the mechanisms by which HLA genes influence IBD remain to be fully elucidated.

Methods: Our study cohort comprised 35 IBD patients (12 CD and 23 UC). Whole-genome sequencing with 30X coverage was used for SNPs determination.

Results: A comparative analysis of HLA-DRB1 single nucleotide polymorphisms (SNPs) variability between CD and UC patients revealed a significantly higher frequency of SNPs for HLA-DRB108 ($p = 0.0006$), HLA-DRB109 ($p = 0.0167$), HLA-DRB110 ($p = 0.0002$), HLA-DRB112 ($p = 0.039$), and HLA-DRB114 ($p = 0.003$). Among UC patients, a higher frequency of SNPs for the HLA-DRB112 allele was observed in patients experiencing repeated relapses compared to those with long-term remission ($p = 0.0007$).

Conclusion: The observed differences in HLA-DRB1 SNP frequencies between CD and UC patients suggest a potential role in distinguishing between these IBD forms. Furthermore, the frequency of HLA-DRB1*12 SNPs may serve as a genetic predictor of relapse rate in UC patients.

Grants: This work was supported by the OPII programme as the project: Serious diseases of civilization and COVID19, co-financed by the European Regional Development Fund (ITMS code:313011AVH7).

Conflict of Interest: None declared

P14.032.B "Towards characterizing inversions in 1000 Individuals across the human population using single-cell pooled and long-read data"

Vasiliki Tsapalou[1], *Wolfram Hoeps*[2], *Tobias Rausch*[1], *Thomas Weber*[1], *Eva Benito*[1], *Siegfried Schloissnig*[3], *Tobias Marschall*[4], *Jan Korbel*[1]

[1]*EMBL Heidelberg, Genome Biology Research Unit, Heidelberg, Germany;* [2]*Radboud University Medical Center, Department of Human Genetics, Nijmegen, Netherlands;* [3]*Vienna Biocenter, Research Institute of Molecular Pathology (IMP), Vienna, Austria;* [4]*Institute for Medical Biometry and Bioinformatics, Medical Faculty, Heinrich Heine University Düsseldorf, Düsseldorf, Germany*

Consortium: Human Genome Structural Variation Consortium (HGSVC)

Background/Objectives: Structural variants (SVs) are key contributors to human genomic diversity. Despite technological advances in SV detection, knowledge gaps remain, notably for inversions. These copy-number neutral variations, often flanked by identical repeat sequences, are notoriously challenging to detect. Using data from 1000 individuals in the 1000 Genomes Project, we aim to comprehensively identify inversions, understand their formation mechanisms, and explore their phenotypic impacts.

Methods: Employing single-cell sequencing (Strand-Seq) and Oxford Nanopore Technologies (ONT) data from the 1000 Genomes IMP/MARVL study, we aim to identify a broad spectrum of previously undetected inversions. Strand-Seq's ability to reveal read directionality is crucial for detecting large inversions, particularly near complex regions. In this regard, we pursued an innovative single-cell data generation method, by coupling cell line pooling with strand-specific sequencing. This approach enables efficient sequencing of diverse human samples, optimizing cell numbers for precise inversion identification and genotyping. Concurrently, we leverage ONT data to capture smaller to mid-sized genomic rearrangements. A particular focus was to refine existing computational methods for detecting small inversions up to 1kb.

Results: Our research has identified several novel polymorphic inversions, establishing the most comprehensive inversion dataset to date. We will discuss these findings, emphasizing their biological significance and population distribution.

Conclusion: This endeavor is poised to advance large-scale population-genetics studies, providing a unique opportunity to decipher naturally occurring and disease-causing structural variations within various human population groups, and their role in adaptation and genome evolution.

Grants: Research funded by the Human Genome Structural Variation Consortium (HGSVC).

Conflict of Interest: None declared

P14.033.C SNP array in 19 original cases of paternal genome wide uniparental disomy: from diagnosis to mechanistic

Uriel Bensabath[1], *Laïla El Khattabi*[1;2], *Irène NETCHINE*[3;4], *Sandra Chantot-Bastaraud*[1], *Frederic Brioude*[3;4]

[1]*AP-HP Sorbonne Université - Hôpital Armand Trousseau, UF de Génomique Chromosomique, Département de Génétique médicale, Paris, France;* [2]*Institut du Cerveau, ICM - Pitié Salpêtrière, Equipe Développement du Cerveau, Paris, France;* [3]*INSERM, Sorbonne Université, Centre de Recherche Saint-Antoine, Paris, France;* [4]*Sorbonne Université - Hôpital Armand Trousseau, Endocrinologie moléculaire et pathologies d'empreinte, Paris, France*

Background/Objectives: Genome-wide paternal uniparental disomy (GW-pUPD) is a rare disorder with common and variable clinical features depending on the percentage and tissue distribution of the disomic lineage. Phenotypic features may include those of any pUPD syndrome, with the Beckwith-Wiedemann syndrome (BWS) being the most represented, which is characterized by lateralized overgrowth, macroglossia, abdominal defects, hyperinsulinism, and predisposition to embryonal tumors.

About 35 GW-pUPD live-borns have been reported, with multiple possible mechanisms.

Methods: We present 19 GW-pUPD cases (12 live-borns, 7 fetuses), including 17 females and 2 males. We collected clinical data and molecular analysis results using single nucleotide polymorphism (SNP) microarray (Illumina Human OmniExpress-24v1-3). We analyzed the B Allele frequency profile in terms of numbers of clusters and allelic frequency, in autosome as well as in sex chromosomes.

Results: SNP microarray analysis suggested:

Single spermatozoon fertilization, followed by irregular reduplication of the male pronucleus, in 16 cases, leading to XX biparental and XX isodisomic paternal cell populations.
Multi-fertilization by two spermatozoa, resulting in a biparental (XX or XY) cell population and XX isodisomic paternal cell population, in two cases.
The remaining case, exhibited a XY heterodisomic paternal cell population and a XY biparental cell population. This unique case, with heterodisomic cell population, offers valuable insights into fertilization mechanisms.

Conclusion: Our study highlights utility of SNP-array in GW-UPD to understand the underlying mechanisms. We also suggest an abacus and a systematic methodology for interpretation. Additionally, we delineate the phenotype of 19 new GW-pUPD cases.

Grants : none

Conflict of Interest: None declared

P14.034.D Intragenic Deletions in NRXN1: Insights from aCGH Analysis in Neurodevelopmental Disorders

Sofia Dória[1], *Joel Pinto*[1], *Carolina Almeida*[1], *Maria João Pinho*[1], *Maria Helena Santos*[2], *Ana Grangeia*[1;3]

[1]*Faculty of Medicine - Genetics, Pathology, Porto, Portugal;* [2]*Centro Hospitalar Vila Nova de Gaia/Espinho – CHVNG/E, Child and Adolescent Neuroscience Unit, Vila Nova de Gaia, Portugal;* [3]*Centro hospitalar Universitário de São João, Medical Genetics, Porto, Portugal*

Background/Objectives: Neurexins, pivotal pre-synaptic cell adhesion molecules, play a crucial role in facilitating and sustaining synaptic connections within diverse brain structures. The *NRXN1* gene encodes neurexin and is essential for efficient neurotransmission, contributing to the formation of synaptic contacts. Heterozygous intragenic deletions of *NRXN1* have been implicated in autism spectrum disorder, schizophrenia, attention deficit hyperactivity disorder, and intellectual disability. Our objective was to assess the prevalence of *NRXN1* intragenic deletions using array comparative genomic hybridization (aCGH) and establish genotype-phenotype correlations.

Methods: Patients with intragenic *NRXN1* deletions were selected from a cohort of 2608 individuals with neurodevelopmental disorders who underwent aCGH (Agilent 4 × 180K platform) at the Genetics Unit of the Faculty of Medicine, University of Porto.

Results: Nine patients (0.35%) with intragenic *NRXN1* deletions were identified, ranging in size from 17Kb to 314Kb. Three deletions involved exons, while the others six occurred within introns. The deletions were classified using the ACMG criteria and Franklin variant assessment platform. Two exonic deletions were classified as Likely Pathogenic, one as Pathogenic, and all intronic deletions as Variants of Uncertain Significance (VUS), with five involving intron 6. Phenotypic variability included epilepsy, autism, and cognitive impairment with severity ranging from mild to severe global developmental disorders.

Conclusion: Despite some intronic *NRXN1* deletions being considered polymorphisms, others were deemed pathogenic, emphasizing the need for a comprehensive understanding of their significance. Our findings revealed significant clinical heterogeneity in *NRXN1* deletions, indicating variable expressiveness and incomplete penetrance, complicating genotype-phenotype correlations and presenting challenges for genetic counselling.

Grants: None
Conflict of Interest: None declared

P14.036.B Timing of de novo mutations in neurodevelopmental disorders: exploring low-level parental mosaicism in blood and sperm via integrated genomic approaches

François Lecoquierre[1], Kevin Cassinari[1], Olivier Quenez[1], Steeve Fourneaux[1], Sophie Coutant[1], Myriam Vezain[1], anne rovelet-lecrux[1], marion rolain[1], Fanny Jumeau[2], Angèle May[1], nathalie drouot[1], Charbonnier Françoise[1], celine derambure[1], Anne Boland[3], Robert Olaso[3], Vincent Meyer[3], Jean-François Deleuze[3], Pascale Saugier-Veber[1], Alice Goldenberg[1], Anne-Marie Guerrot[1], Charbonnier Camille[1], **Gaël Nicolas**[1]

[1]*Univ Rouen Normandie, Inserm U1245, Normandie Univ, CHU Rouen, Department of genetics, Rouen, France;* [2]*Rouen University Hospital, Reproductive Biology, Rouen, France;* [3]*Cnrgh G2, Évry-Courcouronnes, France*

Background/Objectives: De novo variations (DNVs), typically assumed to occur in the zygote, actually happen mostly pre-zygotically, at various pre- or postnatal stages. Identifying the cell undergoing the mutation is crucial for genetic counseling. Our aim was to trace hundreds of DNVs by quantifying parental mosaicism.

Methods: We analyzed the proportions and rates of parental mosaicism in two distinct datasets. The first set consisted in 189 disease-associated DNVs from patients with neurodevelopmental disorders. Parental blood mosaicism was assessed through high-depth sequencing (smMIPs, median depth 7000x). The second dataset was based on 5 children in whom all DNVs (49 to 111 per individual) were identified and phased using trio-based short and long-read genome sequencing. Parental mosaicism was identified by deep sequencing in parental blood and paternal sperm samples.

Results: In both datasets, a similar proportion of 4% of variants were detectable in the blood of one of the parents, with allele fractions ranging from 0.02% to 31.5%, indicating parental embryonic mosaicism. Among the 5 trios deeply analyzed, 90% of variants could be phased on a parental haplotype, with paternal bias and paternal age effect consistent with prior studies. Variants on the maternal haplotype were 3x more likely to be mosaic in blood, and 3% of DNVs showed sperm mosaicism without being detectable in the father's blood.

Conclusion: We suggest that phasing DNVs and studying sperm offers accurate recurrence risk quantification of DNV on paternal haplotypes, suggesting that no further prenatal testing is required for future pregnancies in case of absent sperm mosaic.

Conflict of Interest: François Lecoquierre Grant received from Girci Nord Ouest for this study, Kevin Cassinari: None declared, Olivier Quenez: None declared, Steeve Fourneaux: None declared, Sophie Coutant: None declared, Myriam Vezain: None declared, anne rovelet-lecrux: None declared, marion rolain: None declared, Fanny Jumeau: None declared, Angèle May: None declared, nathalie drouot: None declared, Charbonnier Françoise: None declared, celine derambure: None declared, Anne Boland: None declared, Robert Olaso: None declared, Vincent Meyer: None declared, Jean-François Deleuze: None declared, Pascale Saugier-Veber: None declared, Alice Goldenberg: None declared, Anne-Marie Guerrot: None declared, Charbonnier Camille: None declared, Gaël Nicolas: None declared

P14.037.C DNA Extraction Methodologies for Ultra Long Nanopore Sequencing to Determine Chromosome Specific Rodent Telomere Lengths

Elliot Kidd[1], *Ravi Mooruth[1], Eirini Meimaridou[1], Andrew Walley[2], Una Fairbrother[1]*

[1]*London Metropolitan University, School of Human Science, London, United Kingdom;* [2]*St George's University of London, Centre for Biomedical Education, London, United Kingdom*

Background: Telomeres are involved in gene expression through Telomere Position Effect Over Long Distance (TPE-OLD) and indirectly through telomeric repeat containing RNA (TERRA). It is important to fully elucidate relationships between TL and disease, by observing TL, not only on average, per tissue or per cell, but of specific chromosome ends. Ultra long sequencing has been attempted with Human TL (5-15Kb) with some success. However, animal model TL, such as rat and mouse, are longer (20-100kb, and 30-150kb, respectively) and more problematic.

Method: Previous work in our lab shows that phenol/chloroform extraction damages DNA less than spin columns and magnetic beads and that extraction methods are crucial for accurate TL determination. We have generated long reads using low impact DNA extraction methods and nanopore sequencing with a Mk1C. Rat DNA was isolated using isotachophoresis (Purigen) and a mouse DNA sample isolated using phenol/chloroform. We designed a system of tagging telomere ends with a custom probe that hybridises to the 3' overhang present at the end of telomeric DNA, to ensure we are capturing the entire telomere.

Results: We generated sequence of similar ultra long reads of 120-170kb for both mouse and rat samples extracted with isotachophoresis and phenol/chloroform methodologies respectively. We have generated putative telomeric/subtelomeric reads of < 455Kb using an adaptive sampling approach, focussing on mouse chromosome 1.

Conclusion: Direct sequencing of highly repetitive stretches of DNA is difficult, using specific DNA extraction techniques and adaptive sampling and tagging approaches for targeted enrichment has generated chromosome specific ultralong reads in rodents.

Grants: Internal funding
Conflict of Interest: None declared

P14.038.D Delving into the variation of human meiotic recombination

Philip Zahariev[1], *winston lau[1], Sioban Sen Gupta[2], nikolas maniatis[1]*

[1]*University College London, GEE, London, United Kingdom;* [2]*University College London, Institute for Women's Health, London, United Kingdom*

Background/Objectives: Meiotic recombination is the most important mechanism behind genetic variation and a fundamental process to evolution and gene mapping for human diseases. The study of recombination utilises genetic maps based on different methods and data. The low coverage of informative meiosis is a major limitation when estimating current recombination events with family-based linkage maps in centiMorgans (cM). Sperm genotyping increase resolution, but can only be done in small regions. Maps from WGS data of unrelated individuals evade such limitations but rely on linkage disequilibrium (LD), which can be influenced by factors other than recombination.

Methods: We conduct an in-depth investigation of the differences between family-based and population-based genetic maps across different populations. We use the NF1 gene-region as

a case study to investigate their relationship with recombination-estimates from embryo data of families undergoing IVF and 32 previously-studied regions using sperm genotyping data.

Results: Analysis of the embryo data shows clear evidence of recombination within NF1, which is only captured by population-based genetic maps in LD units (LDU). This overlap co-locates with a cluster of the well-established sequence motif CCNCCNTNNCCNC which is associated with recombination hot-spots. Analysis of the 32 hotspots using sperm genotyping also demonstrates better correlation between sperm-specific cM and LDU distances compared to family-based sex-average cM and population-based cM.

Conclusion: There is clear variation between different genetic maps when compared to gamete data. We show LDU maps offer the highest accuracy and resolution, and alongside their population-specificity, make them valuable in trans-ancestry genome-wide association studies

Grants: MRC (MR/X011070/1)

Conflict of Interest: Philip Zahariev UCL, winston lau UCL, Sioban Sen Gupta UCL, nikolas maniatis Medical Research Council (MRC), UCL

P14.039.A Genotype-phenotype correlation of deletions and duplications on chromosome 15q in a cohort of 2370 clinical cases using an oligonucleotide array CGH platform

Vlasta Cejnova[1], *Lenka Vancová*[1], *Lucie Liskova*[1], *Vjaceslav Harmas*[1], *Dana Safka Brozkova*[2], *Jan Jencik*[2], *Renata Chladova*[3], *Lilly Dejová*[1], *Monika Stará*[1], *Anna Pecková*[1], *Jana Lastuvkova*[1]

[1]*Department of Medical Genetics, Masaryk Hospital in Usti nad Labem, Regional Health Corporation, Usti nad Labem, Czech Republic;* [2]*Neurogenetic laboratory, Department of Paediatric Neurology, 2nd Faculty of Medicine, Charles University and University Hospital Motol, Prague, Czech Republic;* [3]*GENvia Genetic Laboratory, Prague, Czech Republic*

Background/Objectives: The chromosome 15q locus is an enormously complex region of the human genome. Low-copy repeats in this region predispose genes to dosage changes mediated by non-allelic homologous recombination events, leading to chromosomal deletions and duplications.

Methods: Array CGH (Agilent SurePrint G3 8x60K), MLPA, karyotyping and FISH.

Results: We found genomic imbalances on chromosome 15q in 161 cases in a cohort of 2370 samples. We detected Prader-Willi syndrome in two newborns with hypotonia and failure to thrive. The 15q13.3 microdeletion syndrome was identified in eleven patients, in five cases the ~1.6 Mb 15q13.3 microdeletion was maternally inherited and in one case was de novo. Array CGH also revealed a 110 kb maternal heterozygous intragenic deletion of *IGF1R* gene at 15q26.3, Dup15q syndrome, a 15q26.3 terminal duplication as a part of an unbalanced translocation, a de novo terminal deletion of 15q26.2q26.3 leading to the formation of a ring chromosome, a 15q13.2q13.3 BP4-BP5 interstitial duplication, and a homozygous/heterozygous deletion of the *STRC* and *CATSPER2* genes at 15q13.3 as the cause or transmission of Deafness-infertility syndrome. The 15q11.2 BP1-BP2 ~500 kb microdeletion/microduplication, a susceptibility region for neurological dysfunction including developmental and speech delay, was present in thirty-six cases. Twenty individuals had deletion/duplication of 15q13.3 encompassing only the *CHRNA7* gene.

Conclusion: The chromosome 15q is associated with several devastating neurodevelopmental syndromes, autism spectrum disorders, and other genetic conditions. Array CGH is a suitable and reliable method for rapid and effective detection of deletions/duplications on chromosome 15q.

Grants:

Conflict of Interest: None declared

P14.040.B In depth genomic studies in a female Prader-Willi syndrome patient carrying a novel balanced translocation

Constantia Aristidou[1;2], *Ioanna Charalampous*[1;2], *Athina Theodosiou*[1], *ioannis papaevripidou*[1], *Marios Tomazou*[3], *Mads Bak*[4;5], *Mana M. Mehrjouy*[4], *Niels Tommerup*[4], *Sophia Kitsiou-Tzeli*[6], *carolina sismani*[1]

[1]*The Cyprus Institute of Neurology and Genetics, Department of Cytogenetics and Genomics, Nicosia, Cyprus;* [2]*The Cyprus Institute of Neurology and Genetics, Department of Clinical Genetics and Genomics, Nicosia, Cyprus;* [3]*The Cyprus Institute of Neurology and Genetics, Bioinformatics Department, Nicosia, Cyprus;* [4]*University of Copenhagen, Wilhelm Johannsen Centre for Functional Genome Research, Department of Cellular and Molecular Medicine, Copenhagen, Denmark;* [5]*Copenhagen University Hospital, Department of Clinical Genetics, Copenhagen, Denmark;* [6]*University of Athens, Department of Medical Genetics, Medical School, Athens, Greece*

Background/Objectives: Prader-Willi syndrome (PWS) is a neurodevelopmental disorder resulting from the disruption of paternally expressed genes (PEGs) in the 15q11-q13 imprinted region, commonly caused by a paternal deletion or maternal uniparental disomy. The remaining cases have imprinting defects, or rarely, balanced translocations involving 15q11-q13. This study aimed to explain the PWS phenotypes in a female carrying a novel apparently balanced translocation 46,XX,t(3;15)(q21;q11.2)dn.

Methods: Detailed breakpoint delineation and identification of the translocation's parental origin was performed using Whole-Genome Mate-Pair Sequencing and Transposase Enzyme Linked Long-read Sequencing. The methylation status of PEGs was assessed with MS-MLPA and bisulfite sequencing; gene expression profiles were obtained by RT-PCR using RNA from lymphoblastoid cell lines.

Results: Initial data indicated that the translocation is truly balanced and of paternal origin. The der(15) breakpoint mapped ~5kb upstream *NDN*, maintaining the integrity of the SNURF-SNRPN locus and separating PEGs located centromeric to the breakpoint from the PWS imprinting center (PWS-IC). Normal biparental methylation was observed for PEGs on either side of the breakpoint, while gene expression levels were indistinguishable from those in controls except from an apparent over-expression of *NDN* and *MAGEL2*, both mapping upstream the breakpoint.

Conclusion: This is the first PWS case with a unique translocation mapping upstream the PWS-IC that maintains gene-promoter integrity of all 15q11-q13 critical genes. Positioning of a chr3 enhancer upstream *NDN* and *MAGEL2* could explain the differential expression observed. Further studies are required to support genotype-phenotype correlations in our patient and potentially provide novel insights into the pathogenic mechanisms for PWS.

Conflict of Interest: None declared

P14.041.C Beyond the coding DNA: deep-intronic splicing variants are a frequent cause of missing heritability in Joubert syndrome.

Fulvio D'Abrusco[1], *Elisa Giorgio*[1], *Monica Boggioni*[1], *Luca Cattaneo*[1], *Chiara Di Fini*[1], *Valentina Serpieri*[1], *Roebrta Scalise*[2], *Roberta Battini*[2;3], *Enza Maria Valente*[1;4]

[1]*University of Pavia, Department of Molecular Medicine, Pavia, Italy;* [2]*IRCCS Stella Maris, Department of Developmental Neuroscience, Pisa, Italy;* [3]*University of Pisa, Department of Clinical and Exerimental Medicine, Pisa, Italy;* [4]*IRCCS Mondino Foundation, Neurogenetics Research Centre, Pavia, Italy*

Background/Objectives: Despite the NGS revolution, the diagnostic yield of many inherited disorders remains unsatisfactory. One example is Joubert syndrome (JS), a recessive ciliopathy. After WES, up to 30-35% patients fail to receive a definite diagnosis. By focusing on a cohort of JS patients in whom WES only disclosed single heterozygous coding pathogenic variants in a known JS gene, we already showed that a proportion of these cases are caused by CNVs or intronic variants detectable through WES reanalysis. However, some patients still remained negative, suggesting the existence of deep intronic variants not sequenced by WES.

Methods: Three JS patients carrying single heterozygous coding variants in a JS gene underwent WGS combined with RNAseq. We searched for deep intronic variants in the gene of interest, and the impact of candidate variants was validated on patients' cDNA.

Results: In each of the three patients tested, we identified a distinct heterozygous deep intronic variant, in trans with the exonic pathogenic variant. Notably, all three variants were shown to induce the insertion a pseudo-exon, resulting in the insertion of a premature stop codon.

Conclusion: The combined analysis of WGS and RNAseq allowed detecting deep-intronic "second hits" in all patients tested. Along with our previous data on cryptic intronic variants detectable on WES data, we provide proof of principle that cryptic variants are highly frequent mutational events in JS, and their search results in a significant increase of the diagnostic yield.

Grants: Telethon-GGP20070

Conflict of Interest: None declared

P15
DIAGNOSTIC IMPROVEMENTS AND QUALITY CONTROL

P15.001.A RNA-sequencing expression outlier detection in an unsolved cohort of neurodevelopmental disorders improves the diagnostic yield by up to 10%

Kaisa Teele Oja[1;2], *Piret Mertsina*[1;2], *Kai Muru*[1;2], *Sanna Puusepp*[3], *Tiia Reimand*[1;2], *Karit Reinson*[1;2], *Laura Roht*[1;2], *Mikk Tooming*[1;2], *Ikeoluwa A. Osei-Owusu*[4], *Vijay S. Ganesh*[4], *Anne O'Donnell-Luria*[4], *Ben Weisburd*[4], *Sander Pajusalu*[1;2], *Katrin Ounap*[1;2]

[1]*Tartu University Hospital, Genetics and Personalized Medicine Clinic, Tartu, Estonia;* [2]*University of Tartu, Institute of Clinical Medicine, Tartu, Estonia;* [3]*Tartu University Hospital, Pathology Department, Tartu, Estonia;* [4]*Broad Institute of MIT and Harvard, Program in Medical and Population Genetics, Cambridge, United States*

Background/Objectives: Diagnosing rare inherited disorders has improved significantly in recent years with the emergence of new technologies. Whole exome sequencing (WES) enables the detection of the molecular cause in ~50% of syndromic neurodevelopmental disorders (NDDs) and 25-30% of isolated NDDs. Different omics methods have been shown to improve the diagnostic yield. We aimed to implement RNA expression outlier detection in a cohort of unsolved cases at Tartu University Hospital.

Methods: Skin fibroblasts were collected from patients who had signed an informed consent form after a non-diagnostic WES. The patients belong to three general categories: strict NDD cohort (group 1), expanded NDD cohort (group 2), and patients with muscular or mitochondrial disorders (group 3). RNA was sequenced at GenomeScan or Broad Institute and analyzed using the OUTRIDER algorithm for detecting expression outliers.

Results: Overall, RNA sequencing was performed in 167 patients. Group 1 consisted of 54 patients (median age 8.6 years), group 2 of 85 patients (median age 9.6 years), and group 3 of 28 patients (median age 34.9 years). All cohorts included slightly more male participants (~60%). In group 1, RNA sequencing aided in finding the molecular cause of four patients (~7%) and revealed good candidate genes in two other cases. In groups 2 and 3, results are still pending.

Conclusion: RNA sequencing is a complementary analysis in undiagnosed patients and in patients with NDDs expression outlier detection can improve the diagnostic yield by up to 10%.

Grants: PRG471, PRG2040, PSG774, NIH UM1HG008900

Conflict of Interest: None declared

P15.002.B Benefits and risks of gnomAD v4 in a diagnostic setting

Stephan Drukewitz[1], *Amica Müller-Nedebock*[1], *Maximilian Radtke*[1], *Konrad Platzer*[1], *Rami Abou Jamra*[1]

[1]*Institute of Human Genetics, University of Leipzig Medical Center, Leipzig*

Background: The Genome Aggregation Database (gnomAD) is the largest database for human genetic variations and an essential reference for variant interpretation.

With the release of gnomAD version 4.0.0, the number of individuals included was raised to 807,162. However, collecting more samples from various sources adds additional challenges in quality control and increases the risk of including individuals with an undiagnosed genetic disorder.

Methods: We included all clearly pathogenic ClinVar variants and determined their respective allele frequencies in gnomAD v.2.1.1 versus v.4.0.0. In addition, we assessed the potential effect on our routine diagnostic work by focusing on ~1700 pathogenic and ~1600 likely pathogenic variants, reported in patients from our facility.

Results: Looking at developmental delay alone, we identified 252 pathogenic variants in 58 of 129 genes associated with this phenotype, 120 of those variants are exclusively present in gnomAD v.4.0.0. For 15 of those genes, no pathogenic ClinVar variants were identified in gnomAD v.2.1.1. Analyzing our in-house variant dataset, we identified 116 pathogenic or likely-pathogenic variants, present exclusively in v.4.0.0 and thus potentially impacting variant classification and necessitating an adaptation of our in-house filtering steps.

Discussion: The rapid growth of gnomAD will undoubtedly help to increase the quality of genetic diagnostics, especially for currently underrepresented ethnicities. However, the nearly five-fold increase in contributing individuals compared to previous releases requires adaptation in the way we work with this data to interpret clinically relevant variants. Here we present a first insight into the challenges and benefits of incorporating gnomAD v.4.0.0 in a contemporary NGS-based diagnostic setting.

Conflict of Interest: None declared

Variants in intron 6 of SMN1 causing misdiagnosis in genetic detecting of SMA

1588

Yujin Qu[1], Jinli Bai[1], hui jiao[2], hong qi[3], xiaoyin peng[2], yuwei jin[1], hong wang[1], Fang Song[1]

[1]Capital Institute of Pediatrics, Medical Genetics, BEIJING, China; [2]Children's Hospital Affiliated to Capital Institute of Pediatrics, Department of Neurology, BEIJING, China; [3]Prenatal Diagnosis Center, Beijing Haidian District Maternal and Child Health Care Hospital, BEIJING, China

Consortium: no

Abstract

Background : An updated consensus on the approach to the diagnosis and management of SMA was published in 2018. However, clinicians should remain alert to some pitfalls of genetic testing that can occur when following a routine diagnosis.

Methods: Three unrelated individuals, misdiagnosed as homozygous deletion of *SMN1*, were enrolled. MLPA (P060 and P021) and qPCR were used to detect copy number of *SMN* gene. *SMN1* variants were identified by *SMN1* Clone Sequencing and next-generation sequencing. Transcription of *SMN1* variants were detected using qRT-PCR and ex vivo splicing analysis.

Results: Three individuals were eventually identified as a SMA patient carrying a heterozygous deletion and pathogenic variant (c.835-17_835-14delCTTT) of *SMN1*, a carrier only carrying one *SMN1* gene with healthy phenotype, and a patient with nemaline myopathy 2 with heterozygous deletion of *SMN1* exon 7, respectively. We found that the misdiagnosis can be attributed to the presence of the c.835-17_835-14delCTTT or c.835-17C>G variants in intron 6 of the *SMN1* gene, which generate a false signal for the SMN1 exon 7 due to their influence on the amplification of *SMN1* exon 7 during the MLPA-P060 kit assay and the qPCR kit testing.

Conclusion: Accurate genetic diagnosis is the primary basis for guiding treatment of spinal muscular atrophy (SMA). We recommend that additional detecting methods are considered in cases where the *SMN1* copy number is ambiguous to minimize the misdiagnosis of SMA.

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Conflict of Interest: Yujin Qu Natural Science Foundation of Beijing Municipality (No. L212035 and 7212005), Jinli Bai: None declared, hui jiao: None declared, hong qi: None declared, xiaoyin peng: None declared, yuwei jin: None declared, hong wang: None declared, Fang Song National Key Research and Development Program of China (No. 2016YFC0901505)

P15.004.D Optimization and quality control of library preparation in bulk and single-cell ATAC-sequencing

Patrick Schindele[1], Woo-Jin Kim[2]

[1]Agilent Technologies, Waldbronn, Germany; [2]Seoul National University School of Dentistry, Seoul, Korea, Rep. of South

Background/Objectives: The assay for transposase-accessible chromatin with sequencing (ATAC-Seq) is currently the most widely used technique for determining chromatin accessibility and relies on the activity of the hyperactive DNA transposase Tn5. ATAC-Seq libraries aim to have a nucleosomal fragmentation pattern that is crucial to achieving reliable outcomes. Here, we investigated and optimized various conditions for both bulk ATAC-Seq and single-cell ATAC-Seq for improving ATAC-Seq library preparations.

Methods: Bulk ATAC-Seq library preparation was performed according to the original publication[1]. Single-cell ATAC-Seq library preparation was performed using the Chromium Next GEM Single Cell Multiome ATAC kit (10x Genomics). Library quality control (QC) was performed using the Agilent 4150 TapeStation System with the High Sensitivity D5000 ScreenTape.

Results: For bulk ATAC-Seq, a custom protocol was established by optimizing total cell number, lysis conditions and Tn5 concentration that shows equal to superior nucleosomal patterning of the library than comparable commercially available kits. For single-cell ATAC-Seq, methods were tested to assess optimal lysis conditions for efficient isolation of individual nuclei. Determining the amount of nuclei RNA turned out to be a reliable indicator to confirm optimal lysis conditions and high quality libraries.

Conclusion: The findings from this study offer insights into improving and how to assess the efficiency and quality of ATAC-Seq library preparations for both bulk ATAC-Seq and single-cell ATAC-Seq.

Grants: [1]Buenrostro, J., Giresi, P., Zaba, L. et al. Transposition of native chromatin for fast and sensitive epigenomic profiling of open chromatin, DNA-binding proteins and nucleosome position. Nat Methods 10, 1213–1218 (2013). https://doi.org/10.1038/nmeth.2688

Conflict of Interest: None declared

P15.005.A Targeted Oxford nanopore long-read sequencing of the *LDLR* gene with CRISPR/Cas9-mediated selection

Dariush Skowronek[1], Robin A. Pilz[1], Agnieszka Gach[2], Tadeusz Kałużewski[2], Loisa Bonde[1], Ute Felbor[1], Matthias Rath[1;3]

[1]Department of Human Genetics, University Medicine Greifswald, and Interfaculty Institute of Genetics and Functional Genomics, University of Greifswald, Greifswald, Germany; [2]Department of Genetics, Polish Mother's Memorial Hospital Research Institute, Lodz, Poland; [3]Institute for Molecular Medicine, MSH Medical School Hamburg, Hamburg, Germany

Background/Objectives: Familial hypercholesterolemia (FH) affects around 1 in 250 people. Most FH cases are caused by pathogenic variants in the *LDLR* gene, with copy number variations (CNVs) accounting for about 10 %. However, short-read sequencing is limited in the specification of CNV breakpoints and the identification of complex structural variants (SVs).

Methods: High-molecular weight DNA was extracted from blood of two FH patients with a known deletion of the first two exons of *LDLR* or a multi-exon *LDLR* duplication, respectively. Four crRNAs were used for CRISPR/Cas9-mediated target selection of the *LDLR* gene in a dual-cut excision approach. Long-read sequencing was performed on an Oxford Nanopore MinION device.

Results: Complete coverage of the *LDLR* gene was achieved with a mean read length of 22.9 kb and 21.05 kb, respectively. Long-read sequencing allowed us to specify CNV breakpoints and showed that the *LDLR* deletion is 19.2 kb in size and encompasses the first two exons as well as the 5'-untranslated and promotor regions of *LDLR*. Breakpoints were located in *Alu* repeat elements. Furthermore, long-read sequencing verified an *in tandem* localization of a large *LDLR* duplication covering exons 4-8. Both CNVs could be clearly classified as loss-of-function.

Conclusion: Accurate characterization of CNVs with CRISPR/Cas9-mediated target selection and Oxford Nanopore long-read sequencing facilitates variant interpretation and enables cost-effective PCR-based variant confirmation in subsequent familial analyses. Screening for more complex SVs with this amplification-free approach may also help to elucidate the missing heritability in mutation-negative FH patients.

Grants: No specific grants were received.

Conflict of Interest: None declared

P15.006.B Virtual panels have a superior diagnostic yield for inherited rare diseases relative to static panels

Massomeh Sheikh Hassani[1], *Ahmad Abou Tayoun*[1]

[1]*Al Jalila Childrens Hospital, Dubai, United Arab Emirates*

Background/Objectives: Exome-base panels – also known as slices or virtual panels – are now a popular, cost-effective and efficient method of analyzing a capture backbone using a phenotype guided set of genes, especially in heterogenous disorders, performed in clinical laboratories optimizing genetic testing diagnosis. Given the advantages of potential interrogation of all the known human genes in exome slices, this strategy allows analysis flexibility by enabling frequent gene updates based on disease association and specific order requests.

Methods: Here we perform exome slice testing on 949 mostly pediatric patients (50.7% females; age rage 3 months–82 years old) referred from multiple pediatric /clinics within a single Center in the Middle East specialties (82.8% Arabs; 17.2% non-Arabs).

Results: The overall diagnostic yield was 27% out of the total exome slices reported. Interestingly, 13.2% of the positive cases were due to pathogenic variants identified in genes outside the "panel" gene list at the time of testing, and could have been missed in "static" panels as result of novel gene-disease associations and phenotype spectrum broadening.

Conclusion: We highlight specific cases where this practice has resulted in better clinical management of patient upon the extracurricular findings; suggesting regular and guided disease-centric updates and modifications to gene lists composed and maintained by clinical laboratories for best practices and high-quality analysis of exome slices.

Conflict of Interest: None declared

P15.007.C Initial analysis of long-read whole genome sequencing data generated using an Oxford Nanopore Technologies PromethION.

Christopher Watson[1;2], *Laura Crinnion*[1;2], *Sarah Dixon*[2], *Florentina Sava*[3], *Harry Daisley*[1], *Anne-Cecile Hogg*[3], *Rodney Nyanhete*[3], *Verity Hartill*[4], *Emma Hobson*[4], *Rosalyn Jewell*[4], *James Poulter*[1]

[1]*University of Leeds, Leeds Institute of Medical Research, Leeds, United Kingdom;* [2]*North East and Yorkshire Genomic Laboratory Hub, Central Lab, Leeds Teaching Hospitals NHS Trust, Leeds, United Kingdom;* [3]*North East and Yorkshire Genomic Laboratory Hub, Sheffield Diagnostic Genetics Service, Sheffield, United Kingdom;* [4]*Yorkshire Regional Genetics Service, Leeds Teaching Hospitals NHS Trust, Leeds, United Kingdom*

Background/Objectives: The widespread availability of short-read next generation sequencing data has transformed the speed and cost of molecular diagnostic testing. Nevertheless, there are a proportion of patients whose diagnosis remains incomplete.

Methods: Here we present two exemplar scenarios where long-read "third generation" whole genome sequencing has been used as a follow-up to routine genetic testing.

Results: The first patient presented with congenital diarrhoea, trichorrhexis nodosa and cleft palate. Targeted mutation analysis of coding exons and immediate splice site sequences of a 47 gene panel identified a heterozygous pathogenic mutation c.2808G>A p.(Trp936*) in the autosomal recessive disease gene TTC37. To identify a second plausibly pathogenic variant long-read sequencing was performed. This revealed a single heterozygous candidate variant, c.2634+679A>G, in intron 25 which was observed to be in trans. In silico predictions supported the creation a novel splice donor site (SpliceAI score of 0.98). A globin-depleted whole transcriptome library, in combination with short-read sequencing, validated the predicted novel splice junction and confirmed a molecular diagnosis of trichohepatoenteric syndrome. In a further pair of unrelated families, we investigated the recurrent de novo duplication, c.136_138dup p.(Leu46dup), identified in GNAS exon 1. Analysis of bulk genomic DNA (which removes the need for PCR amplification) enabled characterisation of methylation status at this imprinted locus. By identifying single nucleotide variants that tag the parental haplotypes we could determine the parent-of-origin of the mutations and assess future disease severity.

Conclusion: Adoption of single molecule nanopore sequencing, by diagnostic laboratories, promises to aid the analysis and interpretation of incompletely resolved molecular investigations.

Conflict of Interest: Christopher Watson Dr Watson has received travel expenses from Oxford Nanopore Technologies., Laura Crinnion: None declared, Sarah Dixon: None declared, Florentina Sava: None declared, Harry Daisley: None declared, Anne-Cecile Hogg: None declared, Rodney Nyanhete: None declared, Verity Hartill: None declared, Emma Hobson: None declared, Rosalyn Jewell: None declared, James Poulter: None declared

P15.008.D Leveraging Nextflow for the Development of FAIR-Compliant Somatic Variant Calling Workflows

Kübra Narci[1;2;3], *Nagarajan Paramasivam*[3], *ivo Buchhalter*[1;2], *Daniel Huebschmann*[1;2;3;4;5;6;7], *Christian Mertes*[8;9;10]

[1]*Omics IT and Data Management Core Facility (ODCF), DKFZ, Heidelberg, Germany;* [2]*German Human Genome-Phenome Archive (GHGA, W620), DKFZ, Heidelberg, Germany;* [3]*Computational Oncology Group (CO), Molecular Precision Oncology Program, National Center for Tumor Diseases (NCT) Heidelberg and German Cancer Research Center (DKFZ), Heidelberg, Germany;* [4]*Innovation and Service Unit for Bioinformatics and Precision Medicine (BPM), DKFZ, Heidelberg, Germany;* [5]*Pattern Recognition and Digital Medicine Group (PRDM), Heidelberg Institute for Stem Cell Technology and Experimental Medicine (HI-STEM), Heidelberg, Germany;* [6]*Section for Translational Precision Medicine, Institute of Human Genetics, Heidelberg University, Heidelberg, Germany;* [7]*German Cancer Consortium (DKTK), Heidelberg, Germany;* [8]*School of Computation, Information and Technology, Technical University of Munich, Garching, Germany;* [9]*Institute of Human Genetics, School of Medicine, Technical University of Munich, Munich, Germany;* [10]*German Human Genome-Phenome Archive (GHGA), Technical University of Munich, Germany*

Consortium: German Human Genome-Phenome Archive consortium

Background: The National Center for Tumor Diseases (NCT) Heidelberg and the German Cancer Research Center (DKFZ) have developed, maintained, and used somatic variant calling pipelines for high-throughput data analysis. These pipelines have played a significant role in large consortia, including the International Cancer Genome Consortium (ICGC). The workflows encompass diverse software components for calling somatic single nucleotide variations (SNVs), short insertions and deletions (indels), structural variants (SVs), and allele-specific somatic copy number aberrations (sCNAs).

Methods: With the ultimate aim of providing adequate resources for data sharing and harmonizing the processing of data, as a part of the federated European Genome-Phenome Archive (EGA), The German Human Genome-Phenome Archive (GHGA) is committed to the utilization of FAIR-compliant bioinformatic workflows which requires efforts on secure portability of data and workflows, flexibility, scalability and automation

of the processes. Nextflow, a prevalent workflow management language, has emerged as a solution for such reproducible workflows aligning with the goals of GHGA. In a fruitful collaboration with nf-core, a community effort supporting nextflow, GHGA is ensuring standardized and non-repetitive work in this respect. The translation of DKFZ somatic variant calling workflows to nextflow enables us to create a framework for the standardization of workflow processes.

Results: The initial releases of standardized nf-platypusindelcalling, nf-snvcalling, and nf-aceseq workflows are readily available under the GHGA GitHub repository.

Conclusion: GHGA collaborates with the nf-core community to provide FAIR-optimized versions of the DKFZ/NCT Somatic Variant calling pipelines.

Grants: Deutsche Forschungsgemeinschaft (DFG) - project number 441914366 (NFDI 1/1); Molecular Precision Oncology Program (MPOP), NCT Heidelberg.

Conflict of Interest: None declared

P15.009.A Genome sequencing clearly outperforms exome in identifying small deletions: two case reports

Luise Kulosik[1], *Arne Herting*[2], *Rami Abou Jamra*[1]

[1]*Institute of Human Genetics, University of Leipzig Hospitals and Clinics, Leipzig;* [2]*Department of Epileptology, Mara Hospital, University of Bielefeld Hospitals and Clinics, Bielefeld*

Background/Objectives: Neurodevelopmental disorders and epilepsy often remain unsolved in exome sequencing (ES). We propose that genome sequencing (GS) offers distinct advantages and could increase the diagnostic yield in such cases. In this study, we highlight the impact on identifying copy number variants.

Methods: In the context of a medical thesis, we performed GS for 43 individuals with neurodevelopmental delay and/or epilepsy who remained negative after ES. We performed standard wet-lab methods. We used Emedgene for the bioinformatics analysis and variant filtering. Our filtering steps included single nucleotide variants as well as structural variants, repeat expansions and mitochondrial variants.

Results: In two cases, we identified deletions that could not be detected in WES. We identified and confirmed a pathogenic de novo 50% mosaic deletion in *CDKL5* (seg[GRCh38] Xp22.13p22.13(18493442-18556487)) in a male patient with epileptic encephalopathy, severe combined developmental delay, microcephaly, and short stature. Furthermore, we identified a de novo deletion of uncertain significance in *GRIN2B* (seg[GRCh38] 12p13.1p13.1(13959860-14015156)x1) in a female patient with moderate global developmental delay, absence epilepsy, autistic features and falls.

Conclusion: Due to consistent coverage, also of the GC rich 5` exons, and the precise mapping of the intronic breakpoints, GS offers enhanced capabilities for the CNV analysis, presenting a notable advantage over ES.

Grants: None

Conflict of Interest: None declared

P15.010.B Accelerating reanalysis in 100,000 Genomes Project participants with a cohort wide bioinformatics approach

Cassandra Smith[1], *Liam Abrahams*[1], *Nadezda Volkova*[1], *Ken Hanscombe*[1], *Alex Stuckey*[1], *Chris Odhams*[1], *Zandra Deans*[2], *Rachael Mein*[2], *Sue Hill*[2], *Ellen Thomas*[1], *Augusto Rendon*[1], *Susan Walker*[1]

[1]*Genomics England, London, United Kingdom;* [2]*Genomics Unit, NHS England & NHS Improvement, United Kingdom*

Background/Objectives: The overall diagnostic yield following primary analysis for the families in the 100,000 Genomes Project was 20%. Reanalysis of genomic data increases diagnostic yield, but is challenging to perform efficiently and at scale, especially because a lower number of high-confidence candidate variants and a larger contribution of diagnostic variants which are challenging to identify or interpret are anticipated, compared with the primary analysis.

Methods: We have developed a large-scale research-based bioinformatics workflow for cohort-wide reanalysis of whole genome sequencing data, and applied it to >70,000 genomes, including 39,400 affected individuals, in the 100,000 Genomes Project.

Results: The workflow identifies and annotates rare small variants, copy number variants, inversions, insertions and breakends in targeted genes or regions. Variants can be prioritised according to the mode of inheritance and associated mechanism for diagnostic variants in a gene of interest. Combinations of small variants and other variant types identified in the same participant are flagged, supporting identification of compound heterozygous variants. Parameter flexibility allows tailored analysis for complex genes or regions. To date, over 2,500 genes and regions known to be associated with rare disorders have been analysed for >39,000 affected individuals from the 100,000 Genomes Project.

Conclusion: Our cohort-wide approach for reanalysis improves efficiency by focusing analyst time on variants with a high potential to be relevant to patient phenotypes. It helps mitigate limitations of family-based analysis and supports interpretation through shared variants. The candidate diagnostic variants identified are being returned to NHS laboratories through the Diagnostic Discovery pathway.

Grants: N/A

Conflict of Interest: None declared

P15.011.C Utility of Mosdepth Coverage Analysis Tool to Identify Copy Number Variations in Exome Sequencing Data

Ashwin Dalal[1], *pragna lakshmi t*[1], *aparna roy*[1]

[1]*CENTRE FOR DNA FINGERPRINTING AND DIAGNOSTICS, DIAGNOSTICS DIVISION, HYDERABAD, India*

Background/Objectives: Copy number variations (CNVs) are important causes of genetic disorders. Existing CNV pipelines have limited capability for detection of homozygous and partial exonic CNVs. Mosdepth, a coverage analysis tool, can be adopted to identify these abnormalities.

Methods: Mosdepth calculates the sequencing coverage from BAM or CRAM files using a "start-stop" method to count coverage at each base position without double-counting overlaps. It offers 2x faster per base depth calculation than the bedtools. Here, we show two case studies of utilization of Mosdepth to identify the disease causing variant from exome data.

Results: Exome sequencing in two families with proband suspected to be a case of Bardet-Biedl syndrome and Microcephalic Osteodysplastic Primordial Dwarfism, type II (MOPD II) respectively, did not reveal any significant variant in any of the genes related to phenotype. Coverage analysis by Mosdepth for the respective genes associated with the clinical phenotype revealed absence of reads for four consecutive exons which is very likely to be due to homozygous deletion of exon 20 – 23 in *BBS9* gene (Likely Pathogenic, with the criteria 2A-2E, 3A) in case 1 and a homozygous 393bp deletion encompassing part of exon 19 and subsequent intron of *PCNT* gene (Pathogenic with PVS1 and PM2 criteria) in case 2.

Conclusion: The two cases exemplify the utility of MosDepth as a tool for detection of homozygous whole exon or partial exon deletions which may be missed by common SNV/CNV pipelines.

Grants: Department of Biotechnology, Government of India (Project Id BTI/AAQ/01/CDFD-Flagship/2019)

Conflict of Interest: None declared

P15.012.D Ancestry-related differences in clinical prioritisation of genetic variants in the 100,000 Genomes Project Cancer Programme

Thuy Nguyen[1], *Samuel Tallman*[1], *Yoonsu Cho*[1;2;3], *John Ambrose*[1], *Alona Sosinsky*[1], *Maxine Mackintosh*[1;4], *Loukas Moutsianas*[1], *Matt Silver*[1], *Karoline Kuchenbaecker*[1;5]

[1]*Genomics England, United Kingdom;* [2]*University of Bristol, Medical Research Council Integrative Epidemiology Unit, United Kingdom;* [3]*University of Bristol, Population Health Sciences, Bristol Medical School, United Kingdom;* [4]*Alan Turing Institute, London, United Kingdom;* [5]*University College London, Division of Psychiatry, London, United Kingdom*

Background/Objectives: The 100,000 Genomes Project Cancer Programme (100KGPC) served as a precursor for the NHS (National Health Service) Genomic Medicine Service in England, the first national healthcare programme to offer routine whole-genome sequencing. We examined how representative the 100KGPC was of demographics in England's cancer registry and assessed the effect of genetically-inferred ancestry on genetic variants prioritised for clinical review.

Methods: We analysed ancestry-related differences in detection rates for germline pathogenic variants (GPVs) and actionable somatic mutations by regression modelling using data from 14,775 cancer patients.

Results: Ratios of age-adjusted cancer incidence rates between self-reported ethnicities were not significantly different from those in England. Exceptions included breast, bladder, and prostate cancers where minority ethnicities were included in higher proportions (>1.4x, $p < 0.05$).

Non-European ancestries were more likely to have one or more high priority GPVs than European ancestry (>1.9x, $p < 0.05$). Non-European ancestries also had more lower priority GPVs (>1.5x, $p < 1e\text{-}3$). At least 20% of the lower priority GPVs in patients of African ancestry were putative benign variants that could have been excluded through application of ancestry-stratified allele frequency filtering.

In contrast, fewer actionable somatic mutations were identified in South Asian ancestry than in European ancestry (0.83x, $p < 0.05$). Ancestry-related differences diminished after accounting for age and tumour mutation burden.

Conclusion: Our analysis demonstrates the need for better variant classification across diverse ancestries and showcases opportunities for improvement, which will be crucial to ensure equitable implementation of genomics in cancer care.

Grants: Data supplied by the National Genomic Research Library.

Conflict of Interest: None declared

P15.013.A Incorporating Nanopore Sequencing into a Diverse Diagnostic Toolkit for Incontinentia Pigmenti

Simone Ahting[1], *Denny Popp*[1], *Henry Oppermann*[1], *Vincent Strehlow*[1], *Maria Fasshauer*[2;3], *Bernt Popp*[1;4], *Maike Karnstedt*[1], *Isabell Schumann*[1]

[1]*Institute of Human Genetics, University of Leipzig Medical Center, Leipzig;* [2]*Center for Pediatric and Adolescent Medicine, St. Georg Hospital Leipzig, Leipzig;* [3]*IDCL (ImmunDefectCentrum Leipzig), St. Georg Hospital Leipzig, Leipzig;* [4]*Berlin Institute of Health at Charité, Center of Functional Genomics, Berlin, Berlin, Germany*

Background/Objectives: Incontinentia pigmenti (IP) is a rare hereditary multisystemic disorder predominantly affecting females. Conventional short-read sequencing is complicated by a highly homologous pseudogene, which usually makes alternative approaches such as long-range PCR or analysis of skewed X-chromosome-inactivation (XCI) necessary. This study proposes incorporating Nanopore Sequencing into the diagnostic framework for IP. It enables determination of both, the precise localization of the variant in the gene or pseudogene, as well as its prevalence on the active or inactive X-chromosome.

Methods: We employed a comprehensive multi-method approach, incorporating whole-exome sequencing (WES), long-range PCR, RT-PCR and XCI analysis to identify a small heterozygous deletion in the *IKBKG* gene in a family affected with IP. Furthermore, we used Nanopore Sequencing to analyze haplotype-specific methylation at the *IKBKG* promoters.

Results: After variant detection through WES, XCI analysis and long-range PCR both indicated the variant to be located in *IKBKG*. It was undetectable at the RNA level, potentially due to nonsense-mediated mRNA decay or an insufficiently low allelic fraction. Nanopore Sequencing however, not only confirmed the variant to be located in *IKBKG*, but also allowed us to confirm the skewed XCI, with the variant-carrying allele found to be predominantly inactivated.

Conclusion: This study underlines the importance of thorough investigation, exceeding the mere variant detection, especially in genes with homologous pseudogenes. It also demonstrates for the first time, the potential of Nanopore Sequencing as a tool for precise variant mapping in the context of differentially methylated promoter regions due to skewed XCI.

Grants: None

Conflict of Interest: None declared

P15.014.B Implementation of routine diagnostic genome sequencing: understanding the diagnostic performance

Joohyun Park[1], *Marc Sturm*[1], *Olga Kelemen*[1], *Stephan Ossowski*[1;2], *Tobias Haack*[1;2]

[1]*Institute of Medical Genetics and Applied Genomics, Tübingen, Germany;* [2]*Center for Rare Diseases, Tübingen*

Background/Objectives: Short-read genome sequencing (GS) is now adopted as first-tier diagnostic testing method for rare genetic disorders in our center. Here, we share our experience we have gained while establishing GS as a reliable diagnostic tool, covering aspects from validation of sequencing pipelines to the practical implementation in clinical routine.

Methods: The small variant calling performance of our pipeline was evaluated by benchmarking against the GiaB reference NA12878. After thorough quality control, all datasets were analyzed based on our in-house standard filtering scheme. Diagnostic ES ($n = 1{,}016$) and GS ($n = 1{,}977$) datasets from 2022 were compared in terms of coverage, the number of detected rare variants and identified causal findings.

Results: Our standard benchmarks for ES yielded an F1 score of 0.9875 (SNVs) and 0.9427 (InDels). GS outperformed with F1 scores of 0.9955 (SNVs) and 0.9858 (InDels). Regarding coverage, ES had insufficient coverage (<20x) in 2.80% of OMIM regions whereas GS had low coverage in only 0.23%. In total, GS detected $24{,}809 \pm 5{,}655$ rare (MAF < 0.1%) and $5{,}533 \pm 1{,}746$ private ($MAF = 0$) small variants, and ES detected 302 ± 86 and 73 ± 28, respectively. Finally, almost 9% of solved diagnostic cases by GS

had either a non-coding variant, small deletions (<3 exons) or structural genomic variations as causal variant.

Conclusion: Our benchmarks and coverage statistics indicate that GS outperforms ES in both variant detection and achieving uniform coverage. Furthermore, GS can identify causal genomic variants that are undetectable by ES. Despite various challenges, the successful implementation of GS in our diagnostic center demonstrates its feasibility.

Conflict of Interest: None declared

P15.015.C The effect of cryopreservation on DNA methylation signals

*Ning Yuan Lee[1], Melissa Hum[1], Guek Peng Tan[2], Ai Choo Seah[3], Patricia Kin[3], Ngiap Chuan Tan[3;4], Hai Yang Law[2], **Ann Lee**[1;5;6]*

[1]National Cancer Centre Singapore, Division of Cellular and Molecular Research, Singapore, Singapore; [2]KK Women's and Children's Hospital, DNA Diagnostic and Research Laboratory, Singapore, Singapore; [3]SingHealth Polyclinics, Singapore, Singapore; [4]Duke-NUS Medical School, SingHealth Duke-NUS Family Medicine Academic Clinical Programme, Singapore, Singapore; [5]Duke-NUS Medical School, SingHealth Duke-NUS Oncology Academic Clinical Programme, Singapore, Singapore; [6]National University of Singapore, Department of Physiology, Singapore, Singapore

Background/Objectives: Genetic or epigenetic studies often use cryopreserved DNA samples. However, long-term cryopreservation may introduce biases and changes in DNA methylation. The objective of this study was to determine the effect of cryopreservation on DNA methylation profiles.

Methods: DNA methylation profiling was performed using the Infinium MethylationEPIC bead chip on DNA samples extracted from peripheral blood samples. Two cohorts were profiled: a discovery ($n = 126$) and a validation ($n = 136$) cohort.

Results: DNA methylation profiling revealed no significant change in mean global methylation across increasing storage durations of up to four years. For individual CpG methylation levels, both cohorts had a higher number of hypomethylated CpGs than hypermethylated CpGs at q-value < 0.05 (4,049 hypomethylated and only 50 hypermethylated CpGs in discovery; 63 hypomethylated and only 6 hypermethylated CpGs in validation). No difference was found when controlling for age, estimated cell type composition and storage duration as buffy coat prior to DNA extraction. In addition, it was observed that CpGs are more likely to be hypomethylated the closer they are to CpG islands.

Conclusion: This study has quantified the effects of cryopreservation on DNA methylation profiles and shown a detectable bias towards hypomethylation at the level of individual CpG methylation. Future studies using cryopreserved DNA samples should be mindful of this bias, and further efforts can be made to develop more stable methods for DNA storage.

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P15.016.D CF screening challenges in Georgia

*Nino Vardosanidze[1], Nana Kavlashvili[1;2], Lali Margvelashvili[3], **Saba Iordanishvili**[4], Oleg Kvlividze[5;6], Dodo Agladze[3;4]*

[1]Tbilisi State Medical University, Tbilisi, Georgia; [2]M. Iashvili Children's Central Hospital, Tbilisi, Georgia; [3]Medical Genetics and laboratory diagnostic center, Tbilisi, Georgia; [4]Petre Shotadze Tbilisi Medical Academy, Tbilisi, Georgia; [5]New Vision University, Internal Medicine, Tbilisi; [6]Georgian Foundation for Genetic and Rare Diseases (GeRaD)

Background/Objectives: Cystic fibrosis is an autosomal-recessive multisystem disorder caused by mutations of the *CFTR* gene. It is typically characterized by recurrent respiratory tract infections, pancreatic insufficiency leading to malnutrition and growth impairment. In Georgian national guideline for CF diagnostics, sweat chloride test is used as a gold standard

Methods: The results of 40,420 patients born in 2022 were statistically analyzed. Patients with elevated IRT were identified. Data was analyzed to determine the efficiency of the protocol for newborn screening in Georgia.

Results: 40,420 newborns were screened in 2022. 768 newborns with elevated IRT levels (1,9%) were detected. From 768, only 460(59,9%) were followed up. 308(40.1%) patients did not undergo further evaluation. 460 tested newborns, 62(13,5%) showed elevated chloride levels(>29mmol/l). When retested, only 6(9,7%) patients showed increased levels. In 2022, 8 new CF patients were registered, 6 of them via national screening program and 2 of them late diagnosis.

Conclusion: Data analyzes of 2022 screening in Georgia, emphasizes prevalence and diagnostic challenges of CF. Individuals retested with ST were diagnosed, but only 59,9% of cases were tested. Only 18,7% of patients with elevated IRT underwent the recommended second checkup. 1:5053 individuals born in 2022 was diagnosed with CF. 308 individuals with elevated IRT were not retested. Mandatory retest implementation can be a solution, involving collaboration with the Ministry of Health, the social agencies and the national screening center. It is also important to raise awareness among health care professionals.

Grants: Shota Rustaveli National Science Foundation (SRNSF) (PHD-23-638)

Conflict of Interest: None declared

P15.017.A Genome sequencing in clinical diagnostics in a heterogeneous patient cohort

Chen Du[1], *Sandra von Hardenberg[1], Reena Buurman[1], Alisa Förster[1], Anja Schöner-Heinisch[1], Brigitte Pabst[1], Michaela Losch[1], Bernd Haermeyer[1], Benedikt Schnur[1], Winfried Hofmann[1], Florian Kaisen[1], Nataliya Di Donato[1], Gunnar Schmidt[1], Bernd Auber[1]*

[1]Medical School Hannover, Hanover, Germany

Background/Objectives: We implemented short-read genome sequencing for genetic analysis of rare hereditary disorders because of the superior quality of genome sequencing over exome sequencing, including detection of non-coding variants and structural variations. We report the results of two years of genome sequencing in clinical diagnostics.

Methods: (1) library preparation with an IDT DNA Library Prep Kit, (2) 160 bp paired-end genome sequencing with an insert size of 350-400 bp on an Illumina NovaSeq 6000 or X Plus instrument, (3) inhouse data analysis using megSAP, (4) variant interpretation according to standard guidelines, (5) clinical reporting.

Results: We analyzed >1700 independent consecutive index cases (58% pediatric). 91% of samples were analyzed as singleton, 9% as trio or other. Median turnaround time was 35 days. The overall diagnostic rate was 18% with substantial variation across disease groups (40% for metabolic vs. <10% for immunodeficiencies and liver disorders). In 19% of cases a variant of unknown significance was reported. 2% of cases generated candidate variants in genes of unknown significance. Variants in positive reports were 48% truncating, 38% missense, 3% small in-frame deletions, 2% single exon deletions/inversion, 4% large deletions,

2% recurrent microdeletion/duplication syndromes and 3% intronic or regulatory. Trio genome sequencing following unremarkable singleton genome analysis was performed in 35 cases and unmasked the diagnosis in 20% of cases.

Conclusion: Genome sequencing is a feasible tool in diagnostic routine to detect various classes of variation and diagnostic yield is enhanced by trio analysis. A major limitation is the lack of annotation of non-coding variants.

Grants: -

Conflict of Interest: None declared

P15.018.C Interhospitalary Network of Genetic Variants.

Guerau Fernandez[1;2], *Joe Kane*[1], *Alejandro Moles-Fernández*[3], *Manuel Rueda*[4], *Marc Pybus*[5], *Cinthia Aguilera*[6], *Ania Alay*[7], *Celia Badenas*[8;9], *Nidia Barco-Armengol*[1], *Ignacio Blanco*[10;11], *Natalia Blay*[12], *ELISABETH CASTELLANOS*[10;11], *Rafael de Cid*[12], *David Cordero Romera*[7], *Eduardo Delgado De la Iglesia*[7], *Anna Esteve-García*[6], *Laura Gort*[2;8;9], *Jordi Leno*[3], *Oriol Lopez-Doriga*[13], *Irene Madrigal*[8;9], *Gemma Milla Martin*[13], *Aina Montalban-Casafont*[9;14], *Josep Oriola*[8;9], *Miriam Potrony*[8;9], *Jordi Rambla*[13], *Laia Rodriguez-Revenga*[8;9], *Arnau Soler Costa*[13], *Nino Spataro*[15], *Eudald Tejero*[16], *Xavier Solé*[9;14], *Babita Singh*[13], *Eva Gonzalez-Roca*[9;14], *Lauren Fromont*[13], *Ariadna Padró Miquel*[6], *José Villanueva-Cañas*[9;14], *Elena García-Arumí*[2;3], *Benjamin Rodríguez-Santiago*[2;16;17], *Delia Yubero*[1;2]

[1]*Institut de Recerca Sant Joan de Déu (IRSJD), Department of Genetic and Molecular Medicine-IPER, Barcelona, Spain;* [2]*Centro de Investigación Biomédica en Red de Enfermedades Raras, Madrid, Spain;* [3]*Vall d'Hebron University Hospital, Department of Clinical and Molecular Genetics, Barcelona, Spain;* [4]*Centro Nacional de Análisis Genómico - CNAG, Barcelona, Spain;* [5]*Fundació Puigvert, Molecular Biology Laboratory, Barcelona, Spain;* [6]*IDIBELL Institut d'Investigació Biomèdica de Bellvitge, L'Hospitalet de Llobregat, Spain;* [7]*Institut Català d'Oncologia (ICO), Unit of Bioinformatics for Precision Oncology, Barcelona, Spain;* [8]*Hospital Clínic de Barcelona, Biochemistry and Molecular Genetics, Barcelona, Spain;* [9]*Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Barcelona, Spain;* [10]*Germans Trias i Pujol Research Institute, Clinical Genomics Research Group, Barcelona, Spain;* [11]*Hospital Germans Trias i Pujol, Genetics Department, Badalona, Spain;* [12]*Germans Trias i Pujol Research Institute, Genomes for Life-GCAT lab Group, Barcelona, Spain;* [13]*Centre for Genomic Regulation (CRG), European Genome-Phenome Archive, Barcelona, Spain;* [14]*Hospital Clínic de Barcelona, Molecular Biology CORE Laboratory, Barcelona, Spain;* [15]*Park Taulí University Hospital, Genetics Unit, Sabadell, Spain;* [16]*Institut de Recerca - Hospital Santa Creu i Sant Pau, Barcelona, Spain;* [17]*Hospital de la Santa Creu i Sant Pau, Genetics Department, Barcelona, Spain*

Background/Objectives: DNA analysis using next-generation sequencing (NGS) techniques has become routine in the diagnosis of genetically-based diseases in most hospitals world wide due to reductions in sequencing costs and simplification of technique methods. The study of exomes and even whole genomes has increased the complexity of data analysis and interpretation. In order to improve genetic healthcare is increasingly recognized the need to eliminate data and knowledge "silos". Hence, a network of seven hospitals has been established to query genetic variants using standard and secure tools compliant with GDPR regulations.

Methods: Approximately 70 variables are to be shared encompassing both phenotypic and genotypic information. To ensure clarity and consistency, a common vocabulary has been established for each field using specific dictionaries or internationally accepted ontologies.

Results: Data has been stored in an aggregated manner and integrated by each institution into a local database following a common structure defined by the Beacon_v2 reference implementation from the Global Alliance for Genomics and Health (GA4GH). More than 15.000 genomic tests will be accessible within the network to be interrogated (increasing 4000/year approximately).

Conclusion: To our knowledge, this is the first federated hospital centered network to query genomic data. The exchange of genomic and associated data between centers can help interpret and classify variants, leading to multiple benefits: 1) consolidating information to interpret a variant as a cause of the disease or not, 2) identifying interpretation conflicts, reducing the possibilities of a less robust interpretation, 3) improving the quality and effectiveness of diagnosis.

Grants: MaratóTV3-202040

Conflict of Interest: None declared

P15.019.D Nine years study of Initiative on Rare and Undiagnosed Diseases (IRUD) study in Osaka Women's and Children's Hospital

Nobuhiko Okamoto[1], *Eriko Nishi*[1], *Yuiko Hasegawa*[2], *Kumiko Yanagi*[3], *Tadashi Kaname*[4], *Fuyuki Miya*[5], *Mamiko Yamada*[6], *Hisato Suzuki*[7], *Toshiki Takenouchi*[8], *Kenjiro Kosaki*[9]

[1]*Osaka Women's and Children's Hospital, Department of Medical Genetics, Izumi, Osaka, Japan;* [2]*Osaka Women's and Children's Hospital, Department of Medical Genetics, Izumi, Osaka, Japan;* [3]*National Research Institute for Child Health and Development, Department of Genome Medicine, Setagaya-ku,Tokyo, Japan;* [4]*National Research Institute for Child Health and Development, Center for Medical Genetics, Setagaya-ku,Tokyo, Japan;* [5]*Keio University School of Medicine, Center for Medical Genetics, Shinjuku-ku, Japan;* [6]*Keio University School of Medicine, Genome Medicine, Shinjuku-ku, Tokyo, Japan;* [7]*University of Tsukuba, Institute of Medicine, Tsukuba, Japan;* [8]*Keio University School of Medicine, Department of Pediatrics, Shinjuku-ku, Tokyo, Japan;* [9]*Keio University School of Medicine, Center for Medical Genetics, Shinjuku-ku, Tokyo, Japan*

Background/Objectives: Patients with rare and intractable diseases face difficulties finding diagnoses and treatments for their conditions, including diagnostic odysseys, multiple incorrect diagnoses, and unreached treatments. The Japan Agency for Medical Research and Development (AMED) launched a nationwide study called the Initiative on Rare and Undiagnosed Diseases (IRUD) in 2015. IRUD plays an important role in the national medical support network for rare and intractable diseases together with university hospitals as well as children's hospitals. Many patients with rare and intractable diseases in Osaka and neighboring prefectures visit Osaka Women's and Children's Hospital (OWCH). OWCH is consisted with a perinatal center, children's hospital, and research institute. The mission of the Department of Medical Genetics in OWCH is to pursue basic and clinical research for the diagnosis and treatment of genetic diseases.

Methods: Whole-exome sequencing and whole-genome sequencing have been used for the genetic diagnoses of patients who remain otherwise undiagnosed. Furthermore, IRUD constitutes an international data-sharing framework.

Results: We have registered nearly 800 pedigrees with undiagnosed diseases since 2015. Genetic analyses revealed their diagnoses with a high diagnostic rate of approximately 54%. We have identified novel causative genes and new disease entities, including Takenouchi-Kosaki syndrome, *CDK8*-associated disorder, *CDK19*-related disorder, and abnormalities in *DHX9* and *STARD9*. Model organism approaches play important roles in the resolution of these issues.

Conclusion: We are investigating a number of novel genes. The promotion of IRUD is essential for elucidating the causes of and, ultimately, providing cures for rare and undiagnosed diseases. We report our 9-year experience in OWCH.

Grants: AMED

Conflict of Interest: None declared

P15.020.A Evaluating the UTRannotator tool in a clinical diagnostic environment

Shilu Amin[1;2], *Sam Nalty*[1], *Michael Spiller*[1]

[1]*Sheffield Children's NHS Foundation Trust, Sheffield Diagnostic Genetics Service, Sheffield, United Kingdom;* [2]*The University of Manchester, Manchester, United Kingdom*

Background/Objectives: Over 98% of the human genome consists of non-protein-coding regions including a wide range of regulatory regions. Whole genome sequencing (WGS) within the NHS Genomic Medicine Service (GMS) can now explore these regions. However, software tools to annotate potentially pathogenic non-coding variants are still limited. To expand WGS analysis, focusing on untranslated regions (UTRs) of transcripts is highly relevant; variants within 5'UTRs have been shown to lead to rare/developmental disorders.

The recently developed UTRannotator tool identifies 5'UTR variants with functional consequences, and outputs detailed annotations for predicting the likely impact on protein translation. The aim of this study is to test UTRannotator in a clinical diagnostic environment for patients with developmental disorders.

Methods: Variant call format files from reported local GMS WGS cases on the R27 panel (congenital malformation and dysmorphism syndromes) were filtered and annotated using the UTRannotator Ensembl Variant Effect Predictor plug-in. After filtering for R27 green genes, 5'UTR consequence and absence in gnomAD, 5'UTR variants in autosomal dominant (AD)/X-linked (XL) genes were prioritised. The UTRannotator outputs were used for downstream analysis following non-coding variant interpretation guidelines.

Results: From a total of 254 local R27 cases, the UTRannotator provided annotations for 31 5'UTR variants (in 27 cases; 3 had >1 variant); 20 variants in AD/XL genes were prioritised for further analysis. Final results will be presented at the conference.

Conclusion: UTRannotator is a promising tool to aid in the interpretation of 5'UTR variants. However, classification and interpretation of these variants remain difficult.

Grants:

Conflict of Interest: None declared

P15.021.B Determining the impacts of a critical genotyping error identified by External Quality Assessment (EQA); are laboratories considering the implications for patients?

India Roderick[1], *Victoria Williams*[1], *Nicola Wolstenholme*[1]

[1]*EMQN CIC, Manchester, United Kingdom*

Background/Objectives: In line with our educational objectives, EMQN CIC provides a form to guide the root causes analyses (RCA) for critical errors in EQA (errors with potential to cause patient harm). Here, we present the data from a single year's submissions, identifying the most common causes of genotyping error, good practice in RCA and suggesting improvements to the investigation process.

Methods: 255 Participating laboratories made critical genotyping errors in the 2022 scheme year. RCA Forms received from 95 laboratories were analysed by RCA category, reflection on consequences and risks for similar errors in their usual practice, and corrective and preventative actions performed.

Results: The most common error was a false negative result, accounting for over 75% of all critical genotyping errors. The most common root causes were issues with the test method (30.5%), and transcription errors (21.1%). 95% of forms submitted had considered corrective and preventative actions, whereas only 76% had fully considered the consequences and risks for other patients tested.

Conclusion: Good practice was observed for corrective and preventative measures, but 24% submissions did not fully consider the extent and impact of the error, leaving residual risk in existing tests and potential consequences for patient care. It is vital to review if the cause of an error could have affected other patients. Participating in EQA can help laboratories improve the quality of testing for patients by identifying errors proactively so the root cause can be resolved, and services improved.

Conflict of Interest: None declared

P15.022.C Identification of a novel deep intronic *DDC* variant in patients with aromatic l-amino acid decarboxylase deficiency

Naomichi MATSUMOTO[1]

[1]*Yokohama City University Graduate School of Medicine, Department of Human Genetics, Yokohama, Japan*

Background: Aromatic l-amino acid decarboxylase (AADC) deficiency is an autosomal recessive neurotransmitter disorder prevalent in Southeast Asia. AADC deficiency caused by biallelic pathogenic variants in the *DDC* gene. We previously reported Japanese sibling cases with AADC deficiency, their diagnosis was confirmed by the lack of DDC activity but only with a heterozygous known variant in the *DDC* (c.329C>A, p.Ala110Glu) as one-hit.

Methods: To detect a missing pathological variant, we performed the targeted long-read sequencing with Nanopore sequencer for both siblings using adaptive sampling. Entire region of the *DDC* was successfully captured with a coverage depth of brother and sister were 40 and 30, respectively. After the haplotypes phasing and variant calling, we searched for candidate variants (as second-hit) in an allele harboring no c.329C>A.

Results: We identified a novel deep intronic variant (c.714+255C>A). This variant is common in siblings and was predicted by SpliceAI to potentially activate the noncanonical splicing acceptor site. We performed a minigene assay to determine the impact of the variant on splicing. Three transcripts, including the canonical transcript, were detected with the wild-type, but only the noncanonical cryptic exon was produced with the c.714+255C>A.

Conclusion: This noncanonical cryptic exon introduced a premature stop codon, which could lead to nonsense-mediated decay, supporting c.714+255C>A is pathogenic (second-hit).

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Conflict of Interest: None declared

P15.023.D Bioinformatics fieldnorm for Genetic medical laboratories.

Tobias Beers[1], Roxane Boyer[2], Robert Ernst[3], Geert Geeven[4], Bart de Koning[5], Thomas Leenders[6], Rick Medemblik[2], Pieter Neerincx[7], Ruben Vorderman[8], **Hanneke van Deutekom**[3]

[1]The Netherlands Cancer Institute (NKI), Department of Pathology, Amsterdam, Netherlands; [2]Amsterdam University Medical Center, Department of Human Genetics, Amsterdam, Netherlands; [3]UMC Utrecht, Department of Genetics, Utrecht, Netherlands; [4]Erasmus MC, Department of Clinical Genetics, Rotterdam, Netherlands; [5]Maastricht UMC+, Department of Clinical Genetics, Maastricht, Netherlands; [6]Radboud University Medical Center, Department of Human Genetics, Nijmegen, Netherlands; [7]University Medical Center Groningen, Department of Genetics, Groningen, Netherlands; [8]Leiden University Medical Center (LUMC), Department of Biomedical Data Sciences, Leiden, Netherlands

Background/Objectives: In the past years bioinformatics has become a crucial part in several medical laboratories, such as Genetics, Pathology and Medical Microbiology. Hence, the work of bioinformaticians within these laboratories needs to comply to both the ISO-15189 and the IVDR. Where the IVDR contains requirements for software development, "software shall be developed and manufactured in accordance with the state-of-the-art taking into account the principles of development life cycle, risk management, … ", the ISO-15189 does not include requirements for bioinformatics or software development processes. These processes are described in the IEC-62304, a harmonized standard for "Medical Device Software – Software life cycle processes". The use of this standard is voluntary and gives good guidance to demonstrate compliance with legal requirements.

Methods: Bioinformaticians from all the genetic medical laboratories in the Netherlands gathered to discuss the processes used to develop bioinformatic workflows, i.e. software development. The processes within the different centers and the IEC-62304 were used as a guidance to create a fieldnorm for bioinformatics.

Results: The first version of the fieldnorm is available within GitHub. The main goal of this document is to give guidance to what should be documented and registered to comply to the ISO-15189 and the IVDR. The document could also serve as a guidance for (internal) auditors.

Conflict of Interest: None declared

P15.024.A Comprehensive blood group genomic profiling: a leap forward with Adaptive Sampling

Morgan Gueuning[1], Gian Andri Thun[1], Maja P. Mattle-Greminger[1], **Stefan Meyer**[2]

[1]Blood Transfusion Service Zurich SRC, Research and Development, Schlieren, Switzerland; [2]Blood Transfusion Service Zurich SRC, Molecular Diagnostics and Cytometry, Schlieren, Switzerland

Background/Objectives: In transfusion medicine, genetic characterization of blood groups is of great importance, especially for patients regularly transfused like those with thalassemia or sickle cell disease. While SNV-based genotyping is generally effective for characterizing most of 45 known blood group systems, some systems involve paralogous genes prone to complex variation, which is challenging to resolve. Oxford Nanopore Technologies (ONT) has recently introduced a long-read sequencing enrichment method called 'adaptive sampling', which computationally eliminates off-target reads during real-time sequencing. As this approach does not pose restrictions on DNA fragment length, it is highly suitable to detect structural variants.

Methods: Our study aimed to evaluate adaptive sampling's effectiveness in the characterization of the entire blood group genome, particularly in cases where the genetic underpinnings for observed blood group phenotypes were unclear. We targeted ~8.6 Mb of the human genome, covering 51 blood group genes, along with 2 hematopoietic transcription factors and 7 human platelet antigen genes.

Results: Latest PromethION flow cells provided high mean read coverage across target regions (>45x), enabling accurate variant calling. Validation against genotype data for 17 blood group systems revealed 100% concordance with up to 73 pre-typed variants, including complex systems like RH and MNS. Notably, the sequencing data uncovered novel structural variants, such as a ~8.6-kb deletion in *RHCE*, providing explanations for previously unresolved blood group phenotypes.

Conclusion: In summary, adaptive sampling proved promising for complementing current tools in transfusion medicine, offering an accurate, user-friendly, and comprehensive approach to unravel the entire individual blood group genome.

Grants:

Conflict of Interest: None declared

P15.025.B Detection of BRCA1/2 ctDNA using a custom AmpliSeq™ panel in patients with metastatic castration-resistant prostate cancer (mCRPC)

Claudio Antonio Coppola[1], *Brunella Pilato[1], Giuseppina Matera[1], Rosanna Lacalamita[1], Antonia Lasorella[1], Graziana Gramegna[1], Simona De Summa[1], Debora Traversa[1], Vito Di Lorenzo[1], Alessandro Caniglia[1], Fulvia Colonna[1], Stefania Tommasi[1]*

[1]Giovanni Paolo II / I.R.C.C.S. Cancer Institute, Bari, Italy

Background/Objectives: To date, the Italian Association of Medical Oncology (AIOM) recommends the evaluation of *BRCA1* and *BRCA2* gene alterations in plasma of mCRPC patients when tissue is not available for therapeutic setting. However, only a limited number of tools are available to detect these alterations in depth and at an affordable cost.

The aim is to establish and validate a custom panel to identify *BRCA1/2* mutations in ctDNA of mCRPC patients suitable for therapeutic use.

Methods: Plasma from 10 mCRPC patients and 3 synthetic plasma from the EMQN EQA scheme were collected and ctDNA was automatically extracted (KingFisher Duo Prime). The custom panel was designed using Ion AmpliSeq Designer software. Libraries were prepared using the Ion AmpliSeq™ HD Library Kit and sequenced using the Ion GeneStudio™ S5 System (Thermo Fisher). Data were analysed using Torrent Suite and Ion Reporter software. The entire procedure was performed according to the manufacturer's protocols.

Results: The results of *BRCA1/2* analysis were completely concordant between tissue and plasma of the 10 patients. Five of them presented pathogenic alterations also at the germline level. *BRCA1/2* alterations found in synthetic plasma samples were confirmed by CQ assessment.

Conclusion: The data obtained demonstrate the utility of developing a customised panel for *BRCA1/2* alterations in ctDNA for use in the therapeutic setting. However, the study still has major limitations due to the sensitivity of the analysis, which needs to be optimised. This could be overcome with a larger cohort and the inclusion of patients carrying only somatic mutations.

Grants: Early Career Awards

Conflict of Interest: None declared

P15.026.C A novel DNA extraction column to improve the sensitivity of circulating cell free DNA (cfDNA) detection in liquid biopsies

Juliette Goulay[1], Corentin Germain[1], Ludivine Beaussire[2], Frederic DiFiore[2;3], Florian Clatot[2;3], Florine Hallez[1], Michel Arotçarena[1], Kaynoush Naraghi[1], Sami Bayoudh[1], **Nasrin Sarafan-Vasseur**[2]

[1]AFFINISEP SAS, Le Houlme, France; [2]Université de Rouen Normandie, Inserm U1245, IRON Group, Rouen, France; [3]Centre Henri Becquerel, Department of Medical Oncology, Rouen, France

Background/Objectives: Cell free DNA (cfDNA) has been implicated as an important biomarker in cancer management. The natural characteristics of cfDNA make it a challenging analyte to extract because in various liquid biopsies, it is poorly concentrated and highly fragmented (~180bp). Moreover, interfering genomic DNA can make it difficult to accurately detect rare cancer mutant targets. Thus, precision liquid biopsy requires precision cfDNA extraction.

The extraction of DNA from biological matrices is usually performed using an extraction column in the presence of a chaotropic agent. A new thin and uniform membrane was designed for DNA extraction. Thanks to its dense and uniform particles distribution, this membrane shows high capacity for extraction and purification of molecules. It can also be used for miniaturization in µspin columns or Spin Tips format, to reduce loading and elution volumes. Moreover, the miniaturized formats are perfectly compatible with automated liquid handlers and can be used for high throughput analysis (HTS).

Methods: For our proof of concept, we used control plasmas and urines spiked with fragmented DNA harboring HPV-18 sequences, to mimic samples from cervical cancer patients.

The new extraction support was compared, in the form of a complete kit with all the required reagents (new buffer solutions), to other kits.

Results: The results showed an efficient and a highly quantitative extraction, with recovery yields up to 100%.

Conclusion: This innovative kit improves the efficiency of short fragment isolation by removing genomic DNA compared other methods and could be used for both plasma and urine matrices.

Grants: France Relance

Conflict of Interest: None declared

P15.027.D The clinical utility of DNA methylation episignatures in neurodevelopmental disorders: a single center experience

Daphne Smits[1], *Alice Brooks[1], Arjan Bouman[1], Virginie Verhoeven[1], Laura Donker Kaat[1], Sarina Kant[1], Stefan Barakat[1]*

[1]Erasmus Medical Center Rotterdam, Department of clinical genetics, Netherlands

Background/Objectives: Disease causing variants in genes encoding chromatin regulators are the cause of numerous neurodevelopmental disorders. Episignatures, disorder-specific DNA-methylation patterns, have been developed for many disorders and have emerged as a diagnostic tool to provide insights into disease pathophysiology or classification of variants identified with other DNA diagnostics. Here, we evaluated the diagnostic utility of episignatures in a routine clinical setting.

Methods: We analyzed the reports of 293 cases seen at Erasmus MC that underwent commercial EpiSign™ testing between June 2019 and June 2023. The cohort included 70 targeted analyses, in subjects with a previously identified genetic finding, and 223 complete analyses when previous diagnostics testing has not resulted in a diagnosis.

Results: Of the cases that underwent targeted analyses, a positive episignature was observed in 22% (11/50) for variants of unknown significance (VUS), 81.8% (9/11) for likely pathogenic variants, and 100% (9/9) for pathogenic variants. For the 223 cases in which a complete episignature was requested, a positive episignature was observed in 9.4%. Among 61.8% of these, the suggested profile matched the phenotypic features and provided valuable insights into the diagnosis. In about half of them, a causative variant was found retrospectively. For the other 38.2% of positive episignatures, the observed profile did not explain the clinical features for which the test was requested.

Conclusion: This study supports the clinical utility of comprehensive episignature analysis in cases without previous genetic findings, even beyond extensive exome sequencing, as well as during the interpretation and classification of VUS.

Grants: ZonMw Vidi 09150172110002

Conflict of Interest: None declared

P15.028.A Unveiling the Diagnostic Potential: Comprehensive Analysis of 61 Repeat Expansion Loci in 20,000 Exomes

Helene Faust[1], *Theresa Sujatta[1], Stephan Drukewitz[1], Björn Schulte[2], Jerome Jüngling[2], Florian Battke[2], Martin Schulze[2], Christine Zühlke[3], Malte Spielmann[3], Ina Schanze[4], Bernt Popp[5], Rami Abou Jamra[1], Julia Hentschel[1], Denny Popp[1]*

[1]Institute of Human Genetics, University of Leipzig Medical Center, Leipzig, Germany; [2]Center for Genomics and Transcriptomics (CeGaT), Tübingen, Germany; [3]Institute of Human Genetics, University Hospital Schleswig-Holstein, Lübeck, Germany; [4]Institute of Human Genetics, University Hospital Magdeburg, Magdeburg, Germany; [5]Center of Functional Genomics, Berlin Institute of Health at Charité, Berlin, Germany

Background/Objectives: Although the bioinformatic tool ExpansionHunter was developed for short-read genomes, several studies showed the tool's good performance detecting repeat expansions in exome data. However, these studies analyzed only a small number of the disease-associated repeat expansion loci and focused on movement disorders. In the gnomAD data set v3.1.2 are currently 59 disease-associated repeat expansion loci available. We wondered whether these could be detected in exome data.

Methods: In this study, we applied ExpansionHunter v5.0 for 61 repeat expansion loci in 20,000 exomes from two institutes. We filtered for pathogenic repeat expansions in the loci with sufficient coverage to detect clinically relevant repeat expansions. In addition, we investigated different setups for repeat expansion detection with ExpansionHunter and compared the influence of the different wet lab conditions at the two institutes.

Results: Sufficient coverage was detected for 50 loci. Of these, we found repeat expansions in the pathogenic range in 38 loci. For 17 loci, repeat expansions were confirmed in patients with a disease-overlapping phenotype, including movement disorders, neurodegenerative and muscular diseases as well as malformation syndromes. We observed atypical disease manifestations in many patients. Furthermore, optimizing the pre- and post-processing of each locus enhances reliable repeat expansion detection.

Conclusion: We showed the benefit of repeat expansion detection in exome sequencing, also beyond movement disorders. With this study, we developed a guide for implementing repeat expansion detection with ExpansionHunter for exome data and, in perspective, for short-read genome data in a diagnostic setting.

Grants: None.

Conflict of Interest: None declared

P15.029.B Reclassification of variants of uncertain clinical significance in mendelian cardiomyopathies and arrythmias using targeted blood RNA analysis

Thomas RIO FRIO[1], *Anne Vannier*[1], *Caterina Marconi*[1], *Marc Abramowicz*[1;2], *Jean-Louis Blouin*[1;2], *Siv Fokstuen*[1;2]

[1]*University Hospitals of Geneva, Division of Genetic Medicine, Geneva, Switzerland;* [2]*University of Geneva-School of Medicine, Department of Genetic Medicine and Development, Geneva*

Background/Objectives: Molecular diagnosis of mendelian cardiomyopathies and arrythmias is performed through screening of associated genes by NGS. Some rare non-coding or missense variants may be classified as of uncertain clinical significance (VUS) although predicted altering RNA splicing in silico.

Methods: Using mRNA from blood, we investigated 4 patients with hypertrophic cardiomyopathy and one with long-QT syndrome who carried a VUS in *MYBPC3*, *TPM1* or *KCNH2* identified outside canonical ±1,2 splice-site positions and predicted to alter/create splice sites.

Results: An intronic deletion c.2737+4_2737+7delAGTG in *MYBPC3* was shown to lead to 3 transcripts with different abnormal splicing patterns. Deep intronic variant c.1927+337G>T in *MYBPC3* caused inclusion of a cryptic exon. Missense variant p.Gly148Arg in *MYBPC3* created a splice acceptor site partially used to produce a transcript with a partial exonic deletion. An intronic variant c.1945+3G>C in *KCNH2* was shown to abolish the canonical splice donor site. Finally, a missense variant in *TPM1* (p.Asp84Glu) was shown not to alter splicing.

Conclusion: Of the 5 VUS with supporting in silico predictions, we could reclassify the 3 intronic variants as pathogenic. Although an incomplete or absent effect on splicing was shown for the 2 missense variants, we could not downgrade them, as uncertainty remains regarding the effect of the amino acid substitution on the protein function. This targeted variant-specific RNA approach is a cost-effective functional test applicable in blood, which allowed to solve 60 % of VUS predicted to alter splicing. Such complementary approach is expected to increase significantly diagnostic sensitivity of NGS-based gene tests.

Grants: HUG QS07-38

Conflict of Interest: None declared

P15.030.C The *TANGO2* Lesson

Siren Berland[1], *Lene Hjertnes*[1], *Anna Owczarz*[2], *Espen Lien*[3], *Julie Paulsen*[4], *Henrikke Nilsen Hovland*[1], *Lars Utne Haukland*[5], *Marianne Jortveit*[6], *Cathrin Lytomt Salvador*[7], *Trine Tangeraas*[8]

[1]*Haukeland University Hospital, Department of Medical Genetics, Bergen, Norway;* [2]*Ski Barneklinikk, Ski, Norway;* [3]*Trondheim University Hospital, Department of Pediatrics, Trondheim, Norway;* [4]*Trondheim University Hospital, Department of Medical Genetics, Trondheim, Norway;* [5]*Nordland Hospital Trust, Department of Paediatrics, Bodø, Norway;* [6]*Sørlandet Hospital, Department of Paediatrics, Kristiandsand, Norway;* [7]*Oslo University Hospital, Department of Medical Biochemistry, Oslo, Norway;* [8]*Oslo University Hospital, Norwegian National Unit for Newborn Screening, Division of Paediatric and Adolescent Medicine, Oslo, Norway*

Background: TANGO2 deficiency, described in 2016, is a recessive disorder of cellular trafficking characterised by developmental delay, seizure, spasticity, and hypothyroidism. Sudden "TANGO2-spells" with hypotonia, dysarthria, ataxia, and head tilt can last for hours without symptoms in between. Acute metabolic decompensation can occur, with life-threatening cardiac arrhythmia, rhabdomyolysis, and hypoglycaemia. Treatment with B-vitamin complex might prevent decompensation and thus be lifesaving. The most common allele is a deletion of exons 3-9 (60%). We aimed to search for missed diagnoses among patients analysed with chromosomal microarray.

Methods: We reanalysed all 2013-2023 Cytoscan HD SNP arrays for intragenic *TANGO2* deletions. *TANGO2* is included in the 22q11.2 microdeletion (DiGeorge), but these were not reanalysed.

Results: Among 13000 arrays, we identified one homozygous and nine heterozygous deletions. One carrier had a suspicious phenotype on the 2014-referral form, and WES disclosed *TANGO2* VUS+ (NM_152906.7) c.473C>T p.(Ala158Val) in trans. Clinical follow-up showed a typical phenotype in both patients; one was wrongly assigned with alternating hemiplegia and treated with a calcium blocker. The deletion was ~ 34kb and included ~ 28 array probes, below our cut-off of 30. The homozygous patient already had a normal WES, but our WES pipeline does not call CNVs.

Conclusion: A manual search for *TANGO2* deletions resulted in two patients reaching a correct diagnosis and treatment that might prevent early death. The carrier frequency of the deletion in Western Norway is ~ 1/1000. We implemented interventions to detect homozygous *TANGO2* deletions on WES.

Conflict of Interest: None declared

P15.031.D Genes with limited proof of disease causality - to report or not to report

Adela Chirita-Emandi[1;2], *Maria Puiu*[1;2]

[1]*Clinical Emergency Hospital for Children Louis Trucanu, Regional Center for Medical Genetics Timis, Timisoara, Romania;* [2]*Univeristy of Medicine and Pharmacy Victor Babes, Genetics, Timisoara, Romania*

Background/Objectives: Significant efforts were made to establish whether particular DNA variants identified, are (likely) pathogenic or not. The interpretation criteria designed by ACMG, have become standard for variant interpretation in clinical practice. Yet, other guidelines are available, such as the ABC System that also take into account the disease. There is less consensus regarding which are genes are indeed associated with well-described phenotypic features in humans. For many, well-described conditions, gene–disease relationships(GDR) are usually clear, however much less for very rare or heterogeneous conditions(e.g. cardiomyopathies, intellectual disability). There are curated databases such as OMIM(omim.org) or ClinGen(clinicalgenome.org) that focus on establishing GDR. Yet, there is no guideline to advise on criteria regarding GDR. Consequently, the reporting of genes with limited proof of disease causality(GLPDC) varies between laboratories.

Methods:A retrospective analysis regarding GLPDC on NGS reports from the Regional Center of Medical Genetics Timis, Romania, evaluated panels(1494), exomes(105) and genomes(55) in people with various rare diseases.

Results:Depending on disease there could be 50% or more negative results for panel, exome and genome sequencing. Reporting variants in GLPDC depended on the NGS test, as the panel versus exome and genome sequencing had less chance to identify variants in GLPDC. Trio analysis increased the chance for GLPDC. Less than 5% of results had reported variants in GLPDC.

Conclusion: Reporting variants in GLPDC could be valuable as they could represent indeed monogenic causes of disease or could represent disease modifiers. However, reporting variants in GLPDC may also cause distress to the families. Guideline for GDR reporting are needed.

Grants:-

Conflict of Interest: None declared

P15.032.A Variant-Curator: An open platform for the development and use of variant interpretation guidelines

Max Xiaohang Zhao[1;2], *Felix Boschann*[1;2;3], *Bernt Popp*[4]

[1]*Charité - Universitätsmedizin Berlin, Institute of Medical Genetics and Human Genetics, Berlin, Germany;* [2]*Berlin Institute of Health at Charité, Universitätsmedizin Berlin, Berlin, Germany;* [3]*Berlin Institute of Health at Charité, Universitätsmedizin Berlin, BIH Biomedical Innovation Academy, Clinician Scientist Program, Berlin, Germany;* [4]*Berlin Institute of Health at Charité, Universitätsmedizin Berlin, Center of Functional Genomics, Berlin, Germany*

Background/Objectives: Since the ACMG's 2015 sequence variant interpretation recommendation, variant interpretation systems have formalized, diversified, and become more complicated. The guidelines lack accompanying tools that allow easy utilization. Also, no standardized formats exist for exchanging interpretation information. We propose an open solution for creating, validating, and utilizing diverse variant interpretation frameworks that is capable of fully implementing currently published best practices for variant interpretation.

Methods: Our interpretation framework solution is web-based and built using modern web-development methodologies, including TypeScript and vue.js for the front-end interface. Our tool does not require a central server and can be used in a decentralized manner. We also suggest an interchange format for variant interpretation schemas that includes a self-contained format with evidence and variant information.

Results: We present an early version of our framework, which includes a functional web service for assessing variants using flexible schemas and full support for ClinGen criteria specification (CSpec) interpretation guidelines. We provide a schema editor tool for editing and creating schemas. We provide a draft of the proposed schema data structure and tools for reproducible variant interpretation and structured inclusion of evidence data points. This will allow for automation of individual criteria. Our tools make variant assessments sharable and reproducible. The system is easy to install locally and integrate into existing pipelines.

Conclusion: Our variant interpretation platform enables reproducibility in variant interpretation while remaining open to future developments in the interpretation frameworks used. Our tools and code are open, free to use by anyone and can be tested at variant-curator.arsbrevis.de.

Grants: None.

Conflict of Interest: None declared

P15.033.B One-test-for-all in rare disease: Genome sequencing outperforms standard of care approaches

Daniel Kaschta[1], *Christina Post*[2], *Franziska Gaass*[2], *Bianca Greiten*[2], *Anna-Sophie Liegmann*[2], *Rebecca. Gembicki*[2], *Jelena Pozojevic*[2], *Katharina Schau-Römer*[1], *Maj-Britt Salewski*[1], *Kristin Schulz*[2], *Franka Rust*[1], *Christine Zühlke*[2], *Eva Maria Murga Penas*[2], *Nadine Hornig*[1], *Kirstin Hoff*[1], *Juliane Köhler*[1], *Vincent Arriens*[2], *Caroline Utermann-Thuesing*[1], *Varun Sreenivasan*[2], *Kristian Händler*[2], *Valerie Katharina Berge*[2], *Maike Dittmar*[1], *Britta Hanker*[2], *Yorck Hellenbroich*[2], *Irina Hüning*[2], *Monika Kautza-Lucht*[1], *Almuth Caliebe*[1], *Inga Vater*[1], *Inga Nagel*[1], *Malte Spielmann*[1;2]

[1]*Universitätsklinikum Schleswig-Holstein, Campus Kiel, Institute for Human Genetics, Kiel, Germany;* [2]*Universitätsklinikum Schleswig-Holstein, Campus Lübeck, Institute for Human Genetics, Lübeck, Germany*

Consortium: UKSH Genom Konsortium

Background/Objectives: Genome sequencing (GS) promises to be a one-test-for-all strategy, but prospective studies on the benefits of GS for patients in a real-world setting are still limited. We performed a prospective real-world study, by directly comparing single GS (SGS) and trio GS (TGS) in rare diseases patients with the established tiered standard of care (SC) approach. We analyzed 1148 genomes of individuals affected by developmental disorders, congenital anomalies, or monogenic neurological conditions. Our study aimed to assess diagnostic yield and turnaround time in a head-to-head comparison.

Methods: Between January 2022 and April 2023 345 trios and 85 singletons underwent the SC workflow (cytogenetics, Array-CGH, index exome sequencing) and in parallel short-read SGS and TGS. Three independent scientific teams performed: 1. SC workflow 2. SGS and 3. TGS analysis. Results were compared afterwards.

Results: A Molecular diagnosis with clinical assertion was reported via cytogenetics in 1,8%, Array-CGH in 4,3%, and exome sequencing in 43% reaching a total of 49,1% for SC compared to 50,7% for SGS and 51,3% for TGS. Discrepancies were caused by deep intronic variants and small copy number variants. Turnaround times averaged at 18 weeks for SC, 10 weeks for SGS, and 6 weeks for TGS. TGS significantly reduced time for segregation analysis and improved evaluation of variants.

Conclusion: We demonstrate that SGS and TGS outperform SC approaches, significantly reduce turnaround times, and can serve as a one-test-for-all strategy in rare disease. We anticipate that diagnostic yields for TGS will increase with deeper knowledge about the noncoding variants.

Conflict of Interest: Daniel Kaschta provision of free of charge sequencing reagents and software by Illumina within the framework of a collaborative research study, Christina Post provision of free of charge sequencing reagents and software by Illumina within the framework of a collaborative research study, Franziska Gaass provision of free of charge sequencing reagents and software by Illumina within the framework of a collaborative research study, Bianca Greiten provision of free of charge sequencing reagents and software by Illumina within the framework of a collaborative research study, Anna-Sophie Liegmann provision of free of charge sequencing reagents and software by Illumina within the framework of a collaborative research study, Rebecca. Gembicki provision of free of charge sequencing reagents and software by Illumina within the framework of a collaborative research study, Jelena Pozojevic provision of free of charge sequencing reagents and software by Illumina within the framework of a collaborative research study, Katharina Schau-Römer provision of free of charge sequencing reagents and software by Illumina within the framework of a collaborative research study, Maj-Britt Salewski provision of free of charge sequencing reagents and software by Illumina within the framework of a collaborative research study, Kristin Schulz provision of free of charge sequencing reagents and software by Illumina within the framework of a collaborative research study, Franka Rust provision of free of charge sequencing reagents and software by Illumina within the framework of a collaborative research study, Christine Zühlke provision of free of charge sequencing reagents and software by Illumina within the framework of a collaborative research study, Eva Maria Murga Penas provision of free of charge sequencing reagents and software by Illumina within the framework of a collaborative research study, Nadine Hornig provision of free of charge sequencing reagents and software by Illumina within the framework of a collaborative research study, Kirstin Hoff provision of free of charge sequencing reagents and software by Illumina within the framework of a collaborative research study, Juliane Köhler provision of free of charge sequencing reagents and software by Illumina within the framework of a collaborative

research study, Vincent Arriens provision of free of charge sequencing reagents and software by Illumina within the framework of a collaborative research study, Caroline Utermann-Thuesing provision of free of charge sequencing reagents and software by Illumina within the framework of a collaborative research study, Varun Sreenivasan provision of free of charge sequencing reagents and software by Illumina within the framework of a collaborative research study, Kristian Händler provision of free of charge sequencing reagents and software by Illumina within the framework of a collaborative research study, Valerie Katharina Berge provision of free of charge sequencing reagents and software by Illumina within the framework of a collaborative research study, Maike Dittmar provision of free of charge sequencing reagents and software by Illumina within the framework of a collaborative research study, Britta Hanker provision of free of charge sequencing reagents and software by Illumina within the framework of a collaborative research study, Yorck Hellenbroich provision of free of charge sequencing reagents and software by Illumina within the framework of a collaborative research study, Irina Hüning provision of free of charge sequencing reagents and software by Illumina within the framework of a collaborative research study, Monika Kautza-Lucht provision of free of charge sequencing reagents and software by Illumina within the framework of a collaborative research study, Almuth Caliebe provision of free of charge sequencing reagents and software by Illumina within the framework of a collaborative research study, Inga Vater provision of free of charge sequencing reagents and software by Illumina within the framework of a collaborative research study, Inga Nagel provision of free of charge sequencing reagents and software by Illumina within the framework of a collaborative research study, Malte Spielmann provision of free of charge sequencing reagents and software by Illumina within the framework of a collaborative research study

P15.034.C Somatic TP53 mutations (sTP53m) in cell-free circulating DNA (cfDNA) in high-grade serous ovarian cancer (HGSOC)

Catherine Ramsden[1], *John McDermott*[1;2;3], *Andrew Clamp*[4;5], *Jurjees Hasan*[4], *Claire Mitchell*[4], *Zena Salih*[4], *Emma Woodward*[1;2;3], *Gareth Evans*[1;2;3], *Stephen Taylor*[5], *Gordon Jayson*[4;5], *Helene Schlecht*[1;2], *George Burghel*[1;2;3], *Robert Morgan*[4;5]

[1]*Manchester Centre for Genomic Medicine, North West Genomic Laboratory Hub, Manchester, United Kingdom;* [2]*Department of Clinical Genetics, Manchester University NHS Foundation Trust, Manchester, United Kingdom;* [3]*Division of Evolution, Infection and Genomics, School of Biological Sciences, Faculty of Biology, Medicine and Health, University of Manchester, Manchester, United Kingdom;* [4]*Department of Medical Oncology, The Christie NHS Foundation Trust, Manchester, United Kingdom;* [5]*Division of Cancer Sciences, School of Medical Sciences, Faculty of Biology, Medicine and Health, University of Manchester, Manchester, United Kingdom*

Background/Objectives: The commonest type of ovarian cancer, HGSOC, is characterised by near ubiquitous s*TP53*m. Percutaneous diagnostic biopsies of abdominopelvic tumours are invasive and risk complications; therefore, we developed a non-invasive liquid biopsy to detect s*TP53*m in cfDNA (cf*TP53*m) for HGSOC.

Methods: Whole blood was centrifuged twice to extract plasma, and cfDNA was isolated using the cobas® cfDNA Sample Preparation Kit. gDNA was isolated from FFPE tissue using the cobas® DNA Sample Preparation Kit. Single-primed PCR enrichment used a QIAseq Targeted DNA Custom Panel, including UMIs, and next-generation sequencing was performed on an Illumina platform. An in-house bioinformatic pipeline reported variants with an allele frequency (VAF)≥5% and sequencing data was manually inspected for variants with lower VAFs.

Results: 33 women (median age = 53, range = 39-78) with HGSOC donated tumour/plasma. Plasma was donated at 61 timepoints (TPs; 14 women donated plasma at ≥2TPs) including pre-chemotherapy (n = 28), mid-chemotherapy (n = 4) and during surveillance (n = 29). s*TP53*m were detected in tumour DNA in 26/29 (90%; VAF 5-81%; n = 4 had no solid tumour available). cf*TP53*m were detected in 26/61 (43%; VAF 1-50%) plasma samples (21/33 women). cf*TP53*m were most often detected in pre-chemotherapy plasma (19/28, 68%). In 17 women with s*TP53*m detected in tumour and cfDNA, the concordance was 100%. 2/4 women with no solid tumour had a detectable cf*TP53*m. 2/3 women with no s*TP53*m in tumour DNA had a detectable cf*TP53*m.

Conclusion: We report a proof-of-concept liquid biopsy to detect cf*TP53*m in HGSOC. Further optimisation is required to enrich for cf*TP53*m in post-chemotherapy plasma.

Grants: Donation from Red Sea Pedestrians charity-1158475.

Conflict of Interest: None declared

P15.035.D Single-molecule real-time sequencing of an amplicon containing two highly identical homologous genes may lead to sequence errors.

Jeroen Knijnenburg[1], *Youp Zegers*[2], *Rebecca Van Esveldt*[1], *Leon Hailiang Mei*[2], *Susan Kloet*[3], *Sahila Balkassmi*[1], *Tamara Koopmann*[1], *Cornelis Harteveld*[1]

[1]*Leiden University Medical Center, Clinical genetics, Leiden, Netherlands;* [2]*Leiden University Medical Center, Sequence analysis support core, Leiden, Netherlands;* [3]*Leiden University Medical Center, Human genetics, Leiden, Netherlands*

Background/Objectives: The human alpha globin A (HBA) gene cluster region is involved in recessive alpha-thalassemia disease, which can be defined by reduced synthesis of alpha-globin chain and consequently a mild to severe (hemolytic) anemia. A series of founder mutations are well known, ranging from single nucleotide variants to larger deletions, duplications and gene conversions. Correct diagnostics of variants is hampered by the fact that the region is built up out of seven homologous genes and pseudogenes, of which the coding genes HBA1 and HBA2 are embedded in a duplicated homologous area. They differ slightly over the introns and the 5′ untranslated region, but more over the 3′ untranslated regions. Multiple techniques - usually deletion PCR, Sanger sequencing of several fragments and MLPA - are needed for a correct molecular diagnosis.

Methods: Single-molecule real-time (SMRT) sequencing of a 6,2 kb long-range PCR fragment including the HBA1 and HBA2 genes was set up to detect the most common variants in the HBA gene cluster. A pilot set with known variants was tested. Electrophoresis of the long-range PCR fragments showed the expected fragment length. SMRT sequencing was expected to generate reads of the same length.

Results: The majority of generated reads were shorter than expected, showing a hybrid 2,5 kb sequence fragment with an in vitro gene conversion, fusing HBA1 to HBA2 and splicing out the region in between during the sequencing process.

Conclusion: This technical artefact may hamper a correct identification of variants in amplicons covering homologous regions.

Conflict of Interest: None declared

P15.036.A Unveiling the potential: evaluating prenatal genetic diagnosis through exome trio sequencing

Victor Asensio-Landa[1], *Sara Sanchez*[1], *Rosa Martorell*[2], *DAMIAN HEINE SUÑER*[1], *Laura Torres-Juan*[1], *Fernando Santos-Simarro*[1], *Maria Garcia-de Paso*[1], *Susana Renee Avella-Klaassen*[1], *María Carmen Prado-Farnos*[1], *Maria Vila*[2], *Andrea Alegre*[2], *Rosa Ruiz de Gopegui*[2], *Albert Tubau*[3], *Iciar Martínez*[1]

[1]*Hospital Universitario Son Espases, Unidad de Diagnóstico Molecular y Genética Clínica, palma, Spain;* [2]*Hospital Universitario Son Espases, Servicio de Ginecología y Obstetricia, palma, Spain;* [3]*Hospital Universitario Son Llatzer, Servicio de Ginecología y Obstetricia, palma, Spain*

Background/Objectives: Congenital anomalies, affecting around 2-3% of births, predominantly stem from genetic disorders. The nascent implementation of trio-based exome sequencing for prenatal genetic diagnosis varies significantly among hospitals, yielding highly variable diagnostic rates.

This study aimed to assess the efficacy of prenatal genetic diagnosis through trio-based exome analysis.

Methods: Over the period 2019-2023, the Genetics Unit conducted 173 prenatal studies on pregnancies with ultrasound anomalies following genetic counselling and informed consent. Exome sequencing was performed on fetal DNA samples and parental DNA. Variants were classified according to ACMG criteria in genes directly linked to ultrasound abnormalities, categorized as pathogenic or non-informative.

Results: Of the 173 studies, 30% (n = 52) yielded pathogenic outcomes, while the remainder were non-informative. Pathogenic results comprised variants classified as pathogenic or probably pathogenic, with 60% being of de novo origin and 29% autosomal recessive.

Ultrasound abnormalities demonstrated varying detection rates, with skeletal anomalies leading at 40% (16/40). Central nervous system anomalies and intrauterine growth retardation/hydrops fetalis both exhibited a pathogenic report in 34% (10/29 and 14/41, respectively). Cardiovascular anomalies had the lowest detection rate at 21% (6/29).

Conclusion: Exome trio sequencing proves valuable for prenatal diagnosis, though its application necessitates specific guidelines and the establishment of comprehensive gene databases linked to prenatal ultrasound phenotypes. This study sheds light on the potential of this approach while emphasizing the need for standardized protocols and genetic databases to enhance its clinical utility.

Conflict of Interest: None declared

P15.037.B Implementation of an automated diagnostic WES workflow for multiple sample types

Tom Hofste[1], *Fallon Berkhout*[1], *Jordi Corominas Albany*[1], *Michiel Oorsprong*[1], *Hilde Swinkels*[1], *Manon Oud*[1], *Sanne Smeekens*[1], *Remco van Cruchten*[2], *Marjan Weiss*[1], *Christian Gilissen*[1], *Marcel Nelen*[1], *Helger Yntema*[1]

[1]*Radboudumc, Human Genetics, Nijmegen, Netherlands;* [2]*Radboudumc, Pathology, Nijmegen, Netherlands*

Background/Objectives: The Genome Diagnostics laboratory of the Human Genetics department at Radboud University Medical Center provides rapid whole exome sequencing since 2017, with a maximum turnaround time of 15 days (from sample to report). With the continuously increasing number of requests, the manual workflow was no longer sufficient. Automated workflows are needed more than ever to meet the increasing demand in sample throughput and sequencing capacity, whilst minimizing the risk of human errors. A major goal was to enlarge the sample volume in the clinical rapid WES service while retaining the turnaround time.

Methods:

Results: We implemented an automated WES workflow based on Twist Bioscience protocol on a Hamilton Microlab® STAR Liquid Handling platform. This workflow has enabled high-quality exome data with less hands-on time and a decrease in human error potential with end-to-end sample tracking. However, being a core facility, our laboratory receives patient DNA samples from a large number of national and international laboratories, often varying in concentration, volume, and buffer composition. We noticed that these variables have a significant impact on enzymatic fragmentation showing more vulnerability to failures. In order to eliminate these concerns around sample quality, we integrated mechanical shearing using the Covaris R230 Focused-ultrasonicator on-deck, enabling standardized and robust shearing, regardless of sample input.

Conclusion: Using this automated library preparation workflow, we have been able to expand our clinical rapid WES service, through increased sample throughput for multiple sample types, such as blood, saliva, amnion, chorion, cell line and FFPE, whilst maintaining the turnaround time to patient report.

Conflict of Interest: Tom Hofste Radboudumc, Fallon Berkhout RadboudUMC, Jordi Corominas Albany Radboudumc, Michiel Oorsprong Radboudumc, Hilde Swinkels Radboudumc, Manon Oud Radboudumc, Sanne Smeekens Radboudumc, Remco van Cruchten Radboudumc, Marjan Weiss Radboudumc, Christian Gilissen Radboudumc, Marcel Nelen Radboudumc, Helger Yntema Radboudumc

P15.038.C Closing diagnostic gaps by long-read sequencing: from phasing to repeat expansion detection

Helene Faust[1], *Patricia Duffek*[1], *Henry Oppermann*[1], **Denny Popp**[1]

[1]*University of Leipzig Medical Center, Insitute of Human Genetics, Leipzig, Germany*

Background/Objectives: In routine diagnostics, the analysis of short fragments by short-read NGS and state-of-art molecular genetic methods, e.g. Sanger sequencing and qPCR, is often a limiting factor for phasing variants, discriminating genes from the respective pseudogenes, analyzing structural variants, methylation and repeat expansions. In contrast, long-read sequencing can overcome these limitations due to technically unlimited read lengths and methylation data. Here, we provide examples for a variety of long-read sequencing applications.

Methods: Nanopore sequencing was applied for clinical cases when a pathogenic variant could not be found or pinpointed by state-of-art molecular genetic methods. Either genomic DNA or long-range PCR amplicons were subjected to Nanopore sequencing optionally using adaptive sampling. Data was analyzed mainly by Integrative Genomics Viewer (IGV).

Results: Long-range amplicons were used to phase a de novo and an inherited variant in *AFG3L2* proving them as compound heterozygous and to locate a rare splicing variant in the gene *NBEA* excluding it in its pseudogenes. A 3.4 Mb inversion affecting *SLC19A2* was validated and segregated via differential annealing-mediated racket PCR and long-read sequencing. In another case, allele-specific methylation information was exploited to locate a pathogenic variant in *IKBKG* on the preferentially inactivated X-chromosome. A repeat expansion of the pathogenic motif ATTTC was identified in *DAB1* establishing the diagnosis of spinocerebellar ataxia 37.

Conclusion: Long-read sequencing is a valuable addition to the toolbox of routine genetic diagnostic methods as long read and methylation information can be used to close current diagnostic gaps and address manifold diagnostic questions.

Grants: None

Conflict of Interest: None declared

P15.039.D Effects of rare protein-coding variants in reported monogenic obesity genes in a general population study

Raina Jia[1], *felix day*[1], *katherine kentistou*[1], *john perry*[1;2], *ken ong*[1]

[1]*University of Cambridge, MRC Epideimology Unit, Cambridge, United Kingdom;* [2]*University of Cambridge, MRC Metabolic Diseases Unit, Wellcome-MRC Institute of Metabolic Science, Cambridge, United Kingdom*

Background/Objectives: Previous clinical studies have identified variants in genes associated with monogenic obesity, but their penetrance in the general population remains largely unknown. This study uses exome sequencing data from the UK Biobank (UKBB) to evaluate the effect of heterozygous variants in these genes on adult BMI and recalled childhood body size.

Methods: This study assessed the impact of rare (MAF < 0.1%) deleterious protein-coding variants in nine monogenic obesity genes in 419,581 UKBB participants of white European ancestry. We examined loss-of-function variants predicted by in silico tools, and by literature extracted in vitro evidence. Moreover, we examined the interaction effects of rare variants with a polygenic risk score for adult BMI.

Results: Carriers of predicted loss-of-function heterozygous variants in *MC4R*, *PCSK1*, and *POMC* had a higher risk of increasing adult BMI and increasing childhood body size (all p <0.003, Bonferroni corrected). *KSR2* also had a significant effect on childhood body size. No significant interaction was found between rare variant carrier status in any gene and a common variant score ($p < 0.006$, Bonferroni corrected). Among heterozygous carriers of reported experimentally characterised loss-of-function variants, obesity penetrance ranged from 8% to 29% (median = 23%) for six genes with at least 10 carriers in the UKBB.

Conclusion: In this population study, rare variants in *MC4R*, *PCSK1*, and *POMC* contribute to childhood and adult obesity risk. The incomplete obesity penetrance observed for reported experimentally characterised loss-of-function variants highlights the ongoing challenges in interpreting the pathogenicity of variants in monogenic disease.

Grants: This work was funded by the Medical Research Council

Conflict of Interest: None declared

P15.040.A Detection of germline copy number variations in Inborn Errors of Immunity using Ion AmpliSeq™ custom panel in genetic diagnostic clinic

Mohammad Sina[1], *Seyedeh Zahra Mousavi*[2], *Rosalba Monica Ferraro*[1], *Elena Laura Mazzoldi*[1], *Keivan Majidzade-A*[3], *Shiva Zarinfam*[2;3], *Silvia Clara Giliani*[1]

[1]*A. Nocivelli Institute for Molecular Medicine, Department of Molecular and Translational Medicine, University of Brescia, Brescia, Italy;* [2]*Department of Molecular Genetics, Faculty of Biological Sciences, Tarbiat Modares University, Tehran, Iran;* [3]*Motamed Cancer Institute, Breast Cancer Research Center, ACECR, Genetics Department, Tehran, Iran*

Background/Objectives: Inborn Errors of Immunity (IEIs) are rare monogenic disorders that impair human immune system functions. More than 450 genes are analyzed by next generation sequencing (NGS), and analysis of copy number variations (CNVs) could enhance the diagnostic performance by approximately 12%. Identifying CNVs remained a persistent challenge, especially with the Ion Torrent PGM sequencing platforms

Methods: A cohort of 189 patients with IEIs, negative for small variants using AmpliSeq™ PGM NGS platform, underwent CNV analysis using the HMZDelFinder and GATK-gCNV. A search was conducted to identify a specific 23-base pair Alu insertions within the panel gene.

Results: HMZdefinder found 54 homozygous deletions, six oft hem were selected for wet lab validation; none were confirmed. Mappability analysis disclosed 80 targets (1.2%) of 6478 were not unique, primarily in the *PMS2* gene. There was no correlation between false-positive detection of homozygous deletion and mappability. GATK-gCNV identified 500 copy number variations across 181 samples, 106 occurrences were unique, including 88 heterozygous and six homozygous deletions, and 12 with an increase in copy number. Depth of coverage analysis by IGV and and Samtools was applied on these areas and regions with low consistency were excluded. A total of Seven regions were selected for real-time PCR analysis, and the mutation in the *CLBP* gene was confirmed. A tool to visually represent exons in consecutive sequences alongside their respective peers was developed.

Conclusion: Validating predicted variations through alternative methods is crucial, given the false-positive rate that exceeds 90%.

Grants:
The present work was supported by funding from the Angelo Nocivelli Foundation

Conflict of Interest: None declared

P15.041.B Systematic analysis of homozygous copy number losses in exomes enriched for consanguinity improves diagnostic yield and uncovers multiple novel ultra-rare and unique autosomal recessive disorders

Ankur Chaurasia[1;2], *Anju Shukla*[1], *Shruti Pande*[1], *Greeshma Purushotham*[1], *Akhil Kanathay Ashokan*[1], *Purvi Majethia*[1], *Namanpreet Kaur*[1], *Priyanka Upadhyai*[1], *Neha Quadri*[1], *Gandham Sri Lakshmi Bhavani*[1], *Dhanya Lakshmi Narayanan*[1], *Shalini Nayak*[1], *Andrew Fry*[3;4], *James Poulter*[5], *Verity Hartill*[5;6], *Emma Hobson*[6], *Alistair Pagnamenta*[7;8], *William Newman*[2;9], *Paul R. Kasher*[10], *Sonal Nagarkar-Jaiswal*[11], *Siddharth Banka*[2;9], *Katta Girisha*[1;12]

[1]*Kasturba Medical College, Manipal, Manipal Academy of Higher Education, Department of Medical Genetics, Manipal, India;* [2]*School of Biological Sciences, Faculty of Biology, Medicine and Health, University of Manchester, Division of Evolution, Infection and Genomics, Manchester, United Kingdom;* [3]*University Hospital of Wales, Heath Park, All Wales Medical Genomics Service, Cardiff, United Kingdom;* [4]*School of Medicine, Cardiff University, Division of Cancer and Genetics, Cardiff, United Kingdom;* [5]*University of Leeds, Leeds Institute of Medical Research, Leeds, United Kingdom;* [6]*Chapel Allerton Hospital, Yorkshire Regional Genetics Service, Leeds, United Kingdom;* [7]*University of Oxford, Centre for Human Genetics, Oxford, United Kingdom;* [8]*Oxford University Hospitals NHS Foundation Trust, NIHR Oxford Biomedical Research Centre, Oxford, United Kingdom;* [9]*St Mary's Hospital, Health Innovation Manchester, Manchester University NHS Foundation Trust, Manchester Centre for Genomic Medicine, Manchester, United Kingdom;* [10]*School of Biological Sciences, Faculty of Biology, Medicine and Health, Manchester Academic Health Science Centre, The University of Manchester, Division of Neuroscience and Experimental Psychology, Manchester, United Kingdom;* [11]*Centre for Cellular & Molecular Biology, Hyderabad, India;* [12]*College of Medicine and Health Sciences, Sultan Qaboos University, Department of Genetics, Muscat, Oman*

Background/Objectives: Large-scale CNVs analysis in exomes remains challenging.

Methods: A novel pipeline for homozygous CNV analysis from exomes was developed. Relevant calls were validated. Clinical and functional studies were performed for novel disorders.

Results: From a phenotypically heterogeneous cohort of 2,021 exomes enriched for consanguinity we called 42,386 homozygous losses and filtered 1,224 calls, which we prioritised to identify 20 diagnoses resulting from homozygous chromosome deletions involving known recessive disease-genes.

We also identified 9 possibly novel recessive ultra-rare single gene disorders and detected further patients with variants in 4 of these genes in additional datasets to confirm: *VPS36*-related neurodevelopmental disorder; *FAM177A1*-related motor disorder; *TFCP2L1*-related renal tubulopathy; and *FILIP1*-related arthrogryposis with microcephaly. RT-PCR from patient fibroblasts showed that the *VPS36* deletion results in a truncated transcript at a lower abundance than in controls. RNAi-mediated *vps36* knockdown in developing *drosophila* larvae caused developmental delay, early lethality, and locomotion defects. Further work with mutant transgenic lines and crispant zebrafish models of other selected genes is ongoing.

We also identified 2 potentially unique recessive contiguous gene deletion disorders: 14q32.33 *IGHV* genes cluster knockout in an individual with palmoplantar keratoderma; and 6p22.2 knockout including a histone genes cluster in a patient with microcephaly, seizures, and white matter abnormalities.

Conclusion: Our pipeline significantly reduces analytical burden, improves diagnosis, shows that disorders of ~2% probands in our cohort are attributable to homozygous chromosome deletions, and leads to discovery of novel ultra-rare and unique recessive diseases.

Grants: UKRI Global Challenges Research Fund, IA/CRC/20/1/600002, 1R01HD093570-01A1, BT/PR40007/MED/97/490/2020, IA/CPHE/20/1/505226, 5/7/1740/CH/Adhoc/2021-RBMCH.

Conflict of Interest: None declared

P15.042.C Development of external quality assessment for trio exome sequencing

Dave Cregeen[1], *Julia Baptista*[2], *Karen Stals*[3], *Hannah Robinson*[3], *Zandra Deans*[1]

[1]*GenQA, NINE, Edinburgh BioQuarter, Edinburgh, United Kingdom;* [2]*Synnovis, King's College Hospital, London, United Kingdom;* [3]*Exeter Genomics Laboratory, Royal Devon and Exeter University Healthcare Foundation Trust, Exeter, United Kingdom*

Background/Objectives: Trio exome sequencing analysis is performed routinely by diagnostic genomics laboratories for rare monogenic disorders which can be phenotypically and genetically heterogeneous. Genomics Quality Assessment (GenQA) has provided external quality assessment (EQA) for exome sequencing since 2021 to assess the quality of analysis, interpretation, and reporting. In 2021 the EQA comprised data analysis of single nucleotide variants (SNVs), in 2022 this was expanded to include data analysis of copy number variants (CNVs), and in 2023 DNA samples were included.

Methods: Exome sequencing results (or DNA samples) and clinical information were provided to participants who were required to analyse and report clinically relevant variants using their routine format. Variants reported (including classification), clinical interpretation and clerical accuracy was assessed by a panel against peer-reviewed marking criteria.

Results: Participation has increased to 64 laboratories in 2023 with the majority able to analyse both SNVs and CNVs. Variant classification and the evidence utilised varied considerably although clinically relevant variants are reported in most cases. Most participants included all results in a single report however many provided three separate reports. Clinical interpretation for the index case was generally good, however the implications for future offspring and the wider family were often omitted.

Conclusion: EQA for trio exome sequencing has been developed and run successfully for 3 years. Most participants can interpret both SNV and CNV results however variant classification can be inconsistent. This EQA will promote improvements to reporting in the clinical setting and will be provided as a DNA-based scheme going forwards.

Grants:

Conflict of Interest: None declared

P15.043.D EMQN best practice guideline update for genetic testing in hereditary breast and ovarian cancer

Stacey Hume[1], *Trudi McDevitt*[2], *Miranda Durkie*[3], *Norbert Arnold*[4], *George Burghel*[5], *Samantha Butler*[6], *Kathleen Claes*[7], *Peter Logan*[8], *Rachell Robinson*[9], *katie sheils*[10], *Nicola Wolstenholme*[10], *Helen Hanson*[11], *Clare Turnbull*[12]

[1]*Children's Hospital of Eastern Ontario, Newborn Screening Ontario, Ottawa, Canada;* [2]*Children's Health Ireland at Crumlin, Department of Clinical Genetics, Crumlin, Ireland;* [3]*Sheffield Children's NHS Foundation Trust, Sheffield Diagnostic Genetics Service, Sheiffield, United Kingdom;* [4]*Institut of Clinical Chemistry, Institut of Clinical Molecular Biology, Gynecology and Obstetrics, Kiel, Germany;* [5]*Manchester University NHS Foundation, North West Genomic Laboratory Hub, Manchester, United Kingdom;* [6]*Central and South Genomic Laboratory, West Midlands Regional Genetics Laboratory, Birmingham, United Kingdom;* [7]*Ghent University Hospital, Center for Medical Genetics, Gent, Belgium;* [8]*HSCNI / Belfast Trust Laboratories, Regional Molecular Diagnostics Service, Belfast, Ireland;* [9]*Leeds Teaching Hospitals NHS Trust, Genetics Department, Leeds, United Kingdom;* [10]*EMQN, Manchester, United Kingdom;* [11]*St George's University Hospitals NHS Foundation Trust, Clinical Genetics, London, United Kingdom;* [12]*Institute of Cancer Research, London, United Kingdom*

Background/Objectives: The past decade has brought about significant changes to hereditary breast and ovarian cancer (HBOC) diagnostic testing therefore we sought to update the previous EMQN testing guidelines published in 2007.

Methods: To achieve broad expert consensus, laboratory representatives from centres across the UK, EU and Canada met via teleconferences to generate a first guideline draft. These were further consolidated with a final draft circulated to the broad EMQN community for feedback. The comments were considered and the final guidelines approved in November 2023.

Results: The guidelines summarise cancer risk data from recent studies for the most commonly tested high and moderate risk HBOC genes for laboratories to refer to as a guide. Furthermore, 50 recommendations are provided for somatic and germline testing services with regards to clinical referral, laboratory analyses, variant interpretation, and reporting. The guidelines present recommendations where '**must**' is assigned to advocate that the recommendation is essential; and '**should**' is assigned to advocate that the recommendation is highly advised but may not be universally applicable. Finally, for the purposes of encouraging standardisation and aiding implementation of recommendations, example report wording covering the essential points to be included is provided for the most common HBOC referral and reporting scenarios.

Conclusion: These guidelines are aimed primarily at genomic scientists working in diagnostic testing laboratories. In addition to the summarising cancer risk data from recent studies, the 50 recommendations alongside the example report wording encourages international HBOC test standardization.

Grants: Supported by the EMQN

Conflict of Interest: Stacey Hume EMQN, Trudi McDevitt: None declared, Miranda Durkie AstraZeneca speaker honorarium,

Norbert Arnold: None declared, George Burghel: None declared, Samantha Butler: None declared, Kathleen Claes: None declared, Peter Logan: None declared, Rachell Robinson: None declared, katie sheils Work for EMQN, Nicola Wolstenholme Work for EMQN, Helen Hanson: None declared, Clare Turnbull Money for speaking, Advisor

P15.044.A Inter laboratory variability in DNA extraction from fresh tissue – A 2023 EQA review

Fiona Moon[1], *Zandra Deans*[1]

[1]*GenQA, Edinburgh, United Kingdom*

Background/Objectives: With advances in cancer genomic testing technologies, the requirement to optimise extraction methods to produce DNA of high quality is increasingly crucial. However, many laboratories are unable to benchmark their extraction protocols due to the variability of tissue routinely received within diagnostic laboratories.

Methods: To allow laboratories to evaluate the quality and quantity of DNA they can extract from fresh tissue, and benchmark their performance, GenQA provides a DNA extraction from fresh tissue EQA. Participating laboratories are provided with standardised fresh tissue samples and asked to extract DNA using their routine extraction protocol. Prior to extractions, laboratories could either snap freeze, or extract the tissue fresh.

Results: Laboratories returned the extracted DNA to GenQA, which was then assessed using droplet digital PCR (ddPCR) and weight to determine the quantity of DNA extracted. The quality of DNA extracted was determined by assessing the DNA integrity numbers (DIN) using TapeStation.

Nine different extraction methods were used by the 23 participating laboratories. There was no correlation between the weight of tissue supplied and the resulting mass of DNA extracted. The yield of DNA was variable across laboratories, ranging from 1.6 to 220µg. The DIN was consistently high, with an average DIN of 7.8 out of 10, which supports the evidence that fresh tissue is a good source of high molecular weight DNA.

Conclusion: The variability in mass extracted reaffirms the importance of EQA in supporting laboratories to highlight where improvements should be made to get the most from their samples.

Conflict of Interest: None declared

P15.045.B The association between Whole Exome Sequencing and Sanger sequencing in the discovery and (re)classification of gene variants in patients suggestive of cytogenomic alteration and normal results

Giovana Toccoli[1], *Henrique Garcia Silveira*[1], *Beatriz Carvalho Nunes*[1], *Priscila Soares Rodrigues*[1], *Maria Isabel Melaragno*[1]

[1]*Universidade Federal de São Paulo, Department of Morphology and Genetics, São Paulo, Brazil*

Background/Objectives: The investigation of genetic alterations using different methodologies allows the identification of the etiology of the disease with greater resolution. In this project, patients with a phenotype suggestive of cytogenomic rearrangement presenting normal karyotype and chromosome microarray results were referred for investigation of the diagnosis using Whole Exome Sequencing (WES). Thus, the determination of the variant' pathogenic mechanisms and relationship with the phenotype can benefit the patient, the genetic counseling of the family, and also the scientific knowledge.

Methods: A sample of 30 patients with a phenotype suggestive of cytogenomic rearrangement who have normal cytogenomic tests were studied by WES. The detected gene variants were classified following the recommendations of the American College of Medical Genetics (ACMG), with subsequent genotype-phenotype correlation.

Results: WES results showed pathogenic or likely pathogenic variants in 11 patients and a variant of uncertain significance in one patient. A variant classified as likely pathogenic was reclassified as pathogenic due to a de novo presentation, revealed by parental segregation analysis using Sanger sequencing. This variant was correlated with the phenotype of the patient, confirming the Xia-Gibbs syndrome, which is a rare syndrome with around 300 cases reported worldwide. Sanger sequencing for other cases will help in the reevaluation of the variant pathogenicity.

Conclusion: WES can assist in the diagnosis of patients with an altered phenotype and normal cytogenomic tests. The paternal segregation of the variants determined by Sanger sequencing allows their reclassification, thus completing the diagnostic investigation.

Grants: CNPq, CAPES and FAPESP.

Conflict of Interest: None declared

P15.046.C Whole exome sequencing revealed variants in the *KMT2A* gene responsible for Wiedemann-Steiner Syndrome in three individuals with initial suspicion of 22q11.2 deletion syndrome

Henrique Garcia Silveira[1], *Carlos Eduardo Steiner*[2], *Giovana Toccoli*[1], *Luise Longo Angeloni*[2], *Júlia Lôndero Heleno*[2], *Samira Spineli-Silva*[2], *Ana Mondadori dos Santos*[2,3], *Társis Paiva Vieira*[2], *Maria Isabel Melaragno*[1], *Vera Lucia Gil-da-Silva-Lopes*[2]

[1]*Universidade Federal de São Paulo, Departament of Morphology and Genetics, São Paulo;* [2]*Universidade Estadual de Campinas, Genética Médica e Medicina Genômica, Departamento de Medicina Translacional, Campinas;* [3]*Faculdade São Leopoldo Mandic, Campinas*

Background/Objectives: 22q11.2 deletion syndrome (22q11.2DS) presents high phenotypic variability with no exclusive clinical features, resulting in phenotypic overlap with other conditions. *KMT2A* variants are associated with Wiedemann-Steiner syndrome (WDSTS) and result in variable phenotypes. Here we describe three patients with initial clinical suspicion of 22q11.2DS and later identification of variants in the *KMT2A*. For the first time in literature, it was possible to compare differences and similarities between both disorders.

Methods: The patients were evaluated by a checklist for 22q11.2DS. Karyotype, MLPA, FISH, and chromosomal microarray analysis were used to investigate the 22q11.2 locus. Whole exome sequencing (WES) was performed to identify sequence variants. Sanger sequencing was performed on one patient's parents to verify the segregation of the variant. The interpretation of the variants was conducted according to recommendations of the ACMG, followed by genotype-phenotype correlation.

Results: All patients showed clinical features compatible with 22q11.2DS. All cytogenomic tests were normal. WES revealed three different heterozygous pathogenic variants in *KMT2A*. One patient had a de novo variant confirmed by Sanger sequencing of the parents. The comparison of two large cohorts of both syndromes from the literature indicated a phenotypic overlap concerning neuropsychiatric disorders and some facial features. However, hypertrichosis and thick eyebrows were described in WDSTS, while congenital heart diseases and velopharyngeal

insufficiency were described in 22q11.2DS. The three patients have a combination of phenotypes from both syndromes.

Conclusion: We suggest that WDSTS and 22q11.2DS should be included in each other's differential diagnoses.

Grants: FAPESP, CNPq, and CAPES

Conflict of Interest: None declared

P15.047.D Developing and scaling BeginNGS, an artificial intelligence-enabled genome sequencing system for newborn screening, diagnosis, and precision medicine for 412 childhood genetic diseases.

Katarzyna Ellsworth[1;2], *Meredith Wright*[1;2], *Lauren Olsen*[1;2], *Mei Baker*[3;4], *Sara Caylor*[1;2], *Thomas Defay*[5], *Annette Feigenbaum*[1;2;6], *Lucia Guidugli*[1;2], *Soofia Khan*[1;2], *Yong Kwon*[1;2], *Alisa Lam*[1;2], *Jennie Le*[1;2], *Jerica Lenberg*[1;2], *Yupu Liang*[5], *Lakshminarasimha Madhavrao*[1;2], *Rebecca Mardach*[1;2;6], *Bill Mowrey*[5], *Daeheon Oh*[1;2], *Liana Protopsaltis*[1;2], *Muhammed Saad*[1], *Erica Sanford*[1], *Gunter Scharer*[1], *Jennifer Schleit*[1;2], *Brandan Schultz*[1;2], *Laurie Smith*[1], *Mari Tokita*[1;2], *Lucitia Van Der Kraan*[1], *Kristen Wigby*[1;2;6], *Mary Willis*[1], *Mark Yandell*[7], *Charlotte Hobbs*[1;2;6], *Stephen Kingsmore*[1;2]

[1]*Rady Children's Institute for Genomic Medicine, San Diego, United States;* [2]*Rady Children's Hospital - San Diego, San Diego, United States;* [3]*State Hygiene Laboratory, Madison, United States;* [4]*University of Wisconsin School of Medicine and Public Health, Madison, United States;* [5]*Alexion Pharmaceuticals, Inc., Boston, United States;* [6]*University of California San Diego, Department of Pediatrics, La Jolla, United States;* [7]*Fabric Genomics, Oakland, United States*

Background: Newborn genome sequencing (GS) can build upon the success of existing newborn screening (NBS) in severe, childhood disorders by identification and treatment at/before symptom onset. BeginNGS is a precision medicine delivery platform for treatable/preventable genetic childhood diseases, based on rapid GS, artificial intelligence, and a precision medicine guidance system. We developed and tested version 2 of BeginNGS in preparation for a large, adaptive clinical trial.

Methods:54,168 pathogenic (P) and likely pathogenic (LP) variants from ClinVar and Mastermind (Genomenon) associated with 412 disorders and 341 genes were selected for this phase. These disorders were associated with 1,722 effective therapeutic interventions. The sensitivity and specificity of the platform were tested in diverse populations: ~450,000 UK Biobank, ~140,000 Mexico City Prospective Study subjects, and 7,575 individuals who had received diagnostic rGS.

Results: In 3,118 children with suspected genetic disorders diagnostic rGS had reported 187 variants in 152 BeginNGS disorders. BeginNGS identified 78.6% of these variants and 81.5% of these disorders. With the addition of Transformer (Fabric), BeginNGS identified 98.4% of diagnostic rGS-reported variants and 98.7% of rGS-positive probands. Among 3,519 family members of probands who received diagnostic rGS, BeginNGS identified 3.6% actionable findings. In archived newborn DBS from 705 infant deaths, BeginNGS identified 57 positive subjects, with 18% of these having effective therapies.

Conclusion: These studies suggest a generalizable approach to validation of pathogenicity assertions associated with rare/ultra-rare genetic disorders. BeginNGS has sufficient analytic performance to proceed to a large, adaptive clinical trial of BeginNGS as a supplement to standard NBS.

Grants:R01HD101540 and UM1TR004407

Conflict of Interest: None declared

P15.048.A Finding answers for clinical exome-negative patients: The University of Wisconsin Undiagnosed Disease Program

Stephen Meyn[1;2], *Bryn Webb*[1;2], *Derek Pavelec*[3], *Heather Heilmann*[1], *Katheryn Hayes*[1], *Hieu Nguyen*[4], *Xiangqiang Shao*[3;4], *Leah Frater-Rubsam*[4], *Kim Keppler-Noreuil*[2], *Vanessa Horner*[4;5], *April Hall*[1;2]

[1]*University of Wisconsin - Madison, Center for Human Genomics and Precision Medicine, Madison, United States;* [2]*University of Wisconsin - Madison, Department of Pediatrics, Madison, United States;* [3]*University of Madison - Wisconsin, Biotechnology Center, Madison, United States;* [4]*Wisconsin State Laboratory of Hygiene, Cytogenetics and Molecular Genetics Services, Madison, United States;* [5]*University of Wisconsin - Madison, Department of Pathology and Laboratory Medicine, Madison, United States*

Background/Objectives: Just over half of 9,000+ rare genetic disorders have a known cause and clinical exome sequencing only diagnoses 35-40% of patients tested. To address these issues we created the University of Wisconsin Undiagnosed Disease Program (UW UDP), whose goals are to: 1) provide patients with actionable diagnoses; 2) discover new disease genes and mechanisms; and 3) evaluate emerging technologies.

Methods: Our standard workflow begins with clinical WES reanalysis, followed by trio genome sequencing, supplemented by long-read WGS, optical mapping, and/or RNA-Seq.

Results: To date, the UW UDP has enrolled 113 probands and 172 relatives. >85% of probands had prior clinical WES. We identified candidate causal variants for 9 of our first 22 probands, including detecting a deletion and an instance of chromoanasynthesis missed by clinical testing, identifying new candidate disease genes, using RNA-Seq to rule out a clinical diagnosis of Aicardi Goutieres syndrome as well as determining that a patient's novel phenotype is the likely result of synergy between two rare disorders. We recently switched to Nanopore long-read genome sequencing as our first-tier test and are investigating the diagnostic utility of routine methylome analysis.

Conclusion: Our initial results suggest that an undiagnosed disease program can be an important component of a comprehensive center for rare diseases, as augmenting clinical testing with comprehensive research evaluations and access to emerging technologies can yield overall diagnostic rates exceeding 55% while advancing our understanding of rare genetic disorders.

Grants: Funding provided by the UW Center for Human Genomics and Precision Medicine

Conflict of Interest: Stephen Meyn I own a 1% stock interest in Gene42 and we use their phenotyping software (PhenoTips) in our Undiagnosed Disease Program., Bryn Webb: None declared, Derek Pavelec: None declared, Heather Heilmann: None declared, Katheryn Hayes: None declared, Hieu Nguyen: None declared, Xiangqiang Shao: None declared, Leah Frater-Rubsam: None declared, Kim Keppler-Noreuil: None declared, Vanessa Horner: None declared, April Hall: None declared

P15.049.B Clinician access to genomic data improves diagnostic rate by 10%: experience of pitfalls of variant prioritization

Adrienne Elbert[1], *Indhu Rajan Babu*[2], *Nan Zhang*[3], *Cuong Bui*[3], *Karar Al-Mamaar*[1], *SHELIN ADAM*[2], *Stephanie Huynh*[1], *Shila Padhi*[3], *Hyun Lee*[1], *Jan Friedman*[2], *Cornelius Boerkoel*[1], *Laura Li*[3]

[1]*Provincial Medical Genetics Program, Medical Genetics, Vancouver, Canada;* [2]*The University of British Columbia, Medical Genetics, Vancouver, Canada;* [3]*Breakthrough Genomics, Irvine, United States*

Consortium: CAUSES

Background/Objectives: Clinical genomic and exome sequencing are often conducted through proprietary, variant prioritization tools and analytic pipelines that are not transparent for biases

and weakness. We hypothesized that re-analysis through alternate variant prioritization tools could improve diagnostic rates where clinical genomic testing was uninformative. We question the analytic, clinical and cognitive factors leading to missed molecular diagnoses.

Methods: We use an artificial intelligence platform Virtual Geneticist, as well as Exomiser and LIRICAL, in a cohort of solved research exome cases (positive control). Then we utilized these tools to perform a re-analysis within the clinic in two cohorts (n = 163 undiagnosed research cases and n = 71 undiagnosed clinical cases) ascertained for suspected genetic disease. We quantify the number of new diagnoses that result from factors internal to the process of variant prioritization, with reflection on the contributing factors for each solved case.

Results: AI-based variant prioritization in positive controls ranks the diagnostic variant among the top 10 in 99% of solved cases. Further clinical work-up and collaboration between the clinicians, laboratory and research resulted in new diagnoses in 10% of the cases. We identify increased phenotype complexity (dual genetic diagnoses and phenotype expansion) in the newly solved cases. Conflicting interpretation of disease mechanism, in particular for genes with multiple gene-phenotype dyads, and variant mechanism data (amorph, hypomorph, antimorph), are a major factor contributing to non-reported variants.

Conclusion: Clinician access to non-diagnostic exome data for the purpose of variant re-prioritization through alternate analytic pipelines improves diagnostic rates in suspicious cases by 10%.

Grants: N/A

Conflict of Interest: None declared

P15.050.C Using episignatures for reclassifying variants of uncertain significance: A real world systematic analysis and guidelines development

Christopher Campbell[1], *Siddharth Banka*[1,2], *Bekim Sadikovic*[3], *Marielle Alders*[4], *Matthew Tedder*[5]

[1]*Manchester University NHS Foundation Trust, Manchester Centre for Genomic Medicine, Manchester, United Kingdom;* [2]*University of Manchester, Division of Evolution, Infection and Genomics, Manchester, United Kingdom;* [3]*London Health Sciences Centre, Verspeeten Clinical genome Centre, Ontario, Canada;* [4]*Amsterdam Reproduction and Development Research Institute, Department of Clinical Human Genetics, Amsterdam, Netherlands;* [5]*Greenwood Genetic Center, Greenwood, United States*

Consortium: Manchester Centre for Genomic Medicine

Background/Objectives: Episignatures are being increasingly used to assess variant pathogenicity. However, guidelines for application of episignatures to reclassify VUS are required.

Methods/Results: From England's national diagnostic episignature service database, we identified 89/309 individuals who underwent testing for VUS interpretation. To ensure uniformity, we classified all variants using the ACMG guidelines.

EpiSign™ episignature analysis was positive in 45/89 (51%) cases. The prior Bayesian pathogenicity probabilities of 38/45 of these variants were ≥0.675 (warm VUS) (range 0.325-0.812). Adding a moderate (PP4/PS3_Mod) or strong (PP4/PS3_Strong) weighted pathogenic evidence criterion enabled reclassification of 38 or 45 variants respectively to Likely Pathogenic.

An episignature was not present in 44/89 (49%) cases. The prior Bayesian probabilities of 27/41 of these variants were ≤0.5 (tepid VUS) (0.1-0.812). Adding a moderate (BS3_Mod) or strong (BS3_Strong) weighted benign evidence criterion enabled reclassification of 2 or 4 variants respectively to Likely Benign. The remainder remained classified as a VUS, but with reduced posterior probability of pathogenicity.

These results were used to develop internal laboratory guidelines for VUS reclassification using episignatures. In brief, we recommend adding PP4/PS3_Strong to VUSs with positive episignatures; and BS3 to VUSs with negative episignatures with a prior probability of ≤0.5, and where the variant attributes fit the properties of the variants included in the episignature.

Conclusion: Episignature results correlate with prior Bayesian pathogenicity probabilities and are effective in reclassification of VUSs. These guidelines are being validated through the international Episign™ Clinical Testing Network (ECTN) expert group to help consolidate clinical reporting of episignature results in VUS reclassification across ECTN.

Conflict of Interest: None declared

P15.051.D Trio-genome analysis followed by subsequent targeted deep sequencing as diagnostic strategy for very low-grade mosaic detection

Maj-Britt Salewski[1], *Inga Nagel*[1], *Kirstin Hoff*[1], *Caroline Utermann-Thuesing*[1], *Daniel Kaschta*[1], *Franziska Gaass*[2], *Yorck Hellenbroich*[2], *Malte Spielmann*[1,2], *Inga Vater*[1]

[1]*University Hospital Schleswig-Holstein, Campus Kiel, Institute of Human Genetics, Kiel;* [2]*University Hospital Schleswig-Holstein, Campus Lübeck, Institute of Human Genetics, Lübeck*

Background/Objectives: Trio genome sequencing (GS) is increasingly supplanting diagnostic standard care strategies in human genetics. The advantages of this technique are not only the streamlined workflow of elucidating the segregation patterns of potentially disease causing variants, it also enables the identification of germline mosaics with low frequencies. The detection of such mosaics plays a pivotal role in assessing the likelihood of variant recurrence in offspring and thus has a significant impact on genetic counseling practices.

Methods: Short-read GS was performed in a patient with non-syndromic intellectual disability and gait disturbances and his unaffected parents. PCR amplification of the affected *ZNF711* sequence was required for subsequent in-depth sequencing.

Results: A hemizygous variant, NM_001330574.2:c.2299C>T p.(Arg767*), in *ZNF711* was identified, which likely caused the patient's symptoms. Trio GS revealed only one single read in the maternal dataset (Total depth: 37), that matched the variant of the index. Targeted deep sequencing confirmed a low-grade mosaic with an allele frequency of 2.1% (Total depth > 400,000).

Conclusion: The detection of low-frequency mosaic variants represents a challenge, but is crucial for the diagnosis and genetic counseling of affected families. Conventional Sanger sequencing lacks the sensitivity required for reliable mosaic detection and moreover is limited to a specific short sequence. With particular attention to evaluation, trio GS enables the detection of low-frequency mosaics on a genome-wide basis. Followed by in-depth sequencing this promises to be a highly suitable method for uncovering very low-frequency germline mosaics in health care.

Conflict of Interest: Maj-Britt Salewski provision of free of charge sequencing reagents and software by Illumina within the framework of a collaborative research study, Inga Nagel provision of free of charge sequencing reagents and software by Illumina within the framework of a collaborative research study, Kirstin Hoff provision of free of charge sequencing reagents and software by Illumina within the framework of a collaborative research study, Caroline Utermann-Thuesing provision of free of charge sequencing reagents and software by Illumina within the framework of a collaborative research study, Daniel Kaschta provision of free of charge sequencing reagents and software by Illumina within the framework of a collaborative research study, Franziska Gaass provision of free of charge sequencing reagents and software by Illumina within the framework of a collaborative research

study, Yorck Hellenbroich provision of free of charge sequencing reagents and software by Illumina within the framework of a collaborative research study, Malte Spielmann provision of free of charge sequencing reagents and software by Illumina within the framework of a collaborative research study, Inga Vater provision of free of charge sequencing reagents and software by Illumina within the framework of a collaborative research study

P15.052.A Preparing for paediatric screening: Targeted next-generation sequencing of saliva DNA from patients with Familial Hypercholesterolemia

Mosekunola Oladapo[1], *Niloofar Shirvanizadeh*[1], *Kristina Duvefelt*[1], *Peter Benedek*[1]

[1]*Karolinska University Hospital, Translational Analysis in Molecular Medicine, Cancergenomics and Proteomics, Pathology and Cancer-diagnostics, Medical Diagnostics Karolinska, Stockholm, Sweden*

Background/Objectives: Familial hypercholesterolemia (FH) is the most common autosomal dominant inherited disease, with a prevalence of 1/300. The disease causes highly elevated LDL cholesterol levels and left untreated it is strongly associated with premature atherosclerotic cardiovascular disease and premature death. FH is predominantly caused by heterozygous mutations in one of three genes: *LDLR*, *APOB*, or *PCSK-9*. DNA extracted from saliva is easy to obtain, non-invasive and stable at room temperature, and therefore suitable for paediatric genetic testing.

The aim of this study was to evaluate the suitability of using DNA prepared from saliva for DNA sequencing utilizing the Devyser FH NGS V2 kit to facilitate future paediatric screening.

Methods: Paired blood and saliva samples were obtained from twenty patients with suspected FH. DNA from blood was extracted with the QiAsymphony automated method, saliva DNA was collected and prepared using Oragene OG-610 kits. The total DNA concentration of all samples was measured using Picogreen. The DNA content of the saliva samples was analyzed using qPCR.

Sequencing was performed with Devyser FH-v2 kit according to their protocol on a MiSeq instrument.

Results: All QC parameters for the sequencing were within specifications from the vendor. The SNV data concordance was 100% between the paired blood and saliva samples, this applied both to common SNVs and FH mutations. The results were 100% concordant between replicates. A study concerning CNV detection is ongoing.

Conclusion: Saliva samples can be used for detection of FH mutations using the Devyser FH-v2 kit, and could facilitate testing through easier, non-invasive sampling.

Grants: -

Conflict of Interest: Mosekunola Oladapo Full, Niloofar Shirvanizadeh Full, Kristina Duvefelt Full, Peter Benedek Full

P15.053.B Integration of updated ACMG/AMP criteria for solving VOUS: a comprehensive review of past results in diagnostic tests

Thaís Córdova[1], *gui rosa*[1], *isa salgado*[1], *cintia ventura*[1], *joaquim sá*[1;2], **marisa teixeira**[1], *rita cerqueira*[1]

[1]*CGC Genetics, Unilabs, Porto, Portugal;* [2]*ULS de Coimbra, Coimbra, Portugal*

Background: In 2015, the American College of Medical Genetics and Genomics/Association for Molecular Pathology (ACMG/AMP) published their first guidelines for variant interpretation. However, Variants of Unknown Clinical Significance (VOUS), as defined by this system, pose a significant challenge in clinical settings. As such, a series of subsequent updates have been produced to improve criteria accuracy. Here, we revisited VOUS reported by our laboratory to assess whether the three latest updates (PP3/BP4, PVS1, PP1/PP4/BS4) could help solve their clinical significance.

Methods: Non-CNV VOUS reported in WES-based panels and single gene analysis for Alport Syndrome, CADASIL, Paraganglioma/Pheochromocytoma, Tuberous Sclerosis, Hereditary Hemorrhagic Telangiectasia and Noonan Syndrome, performed between 2020-2023, were filtered from our in-house data. Variants were reassessed leveraging the above-mentioned updated ACMG/AMP criteria. We also updated allele frequencies and constraint metrics according to GnomAD version 4.

Results: 131 VOUS were filtered, of which three (2.3%) were solved to Pathogenic, and twenty (15.3%) to Likely Pathogenic. No reported variant was reclassified to Likely Benign, but six (4.6%) gained benignity and/or lost pathogenicity criteria. The diagnostic yield virtually increased 17.6%, but the rate of solved VOUS was uneven between the chosen disorders.

Conclusion: We performed a comprehensive analysis of VOUS reported by our laboratory in the past four years for six different disorders. Integrating the most recent variant interpretation guidelines into our workflow improved VOUS resolution. Lack of curated disease-specific information and insufficient clinical information were the most important causes of an uneven diagnostic yield between the chosen disorders.

Conflict of Interest: None declared

P15.054.C A highly specific molecular diagnostic pipeline designed to sequence PKD1, a GC-rich gene partially existing in multiple copies, using a single round amplicon-based next-generation sequencing workflow

Maria Pozova[1], *Grigoris Constantinou*[1], *George Michael*[1], *Avgousta Petrou*[1], *Margarita Kazdagli*[1], *Apostolos Malatras*[1], *Alkis Pierides*[1], *Christoforos Stavrou*[2], *christiana polydorou*[1], *Constantinos Deltas*[1;3], *Gregory Papagregoriou*[1]

[1]*University of Cyprus, biobank.cy Center of Excellence in Biobanking and Biomedical Research, Nicosia, Cyprus;* [2]*Evangelismos Private Hospital, Nephrology Department, Paphos, Cyprus;* [3]*University of Cyprus, Medical School, Nicosia, Cyprus*

Background/Objectives: Autosomal dominant polycystic kidney disease (ADPKD) is the most common inherited kidney disease and is primarily caused due to mutations in genes *PKD1* and *PKD2*. Existing in multiple copies, *PKD1* remains a technical challenge for standard and next-generation sequencing (NGS) approaches. Hence, a cost-effective and robust pipeline was developed and optimized to sequence *PKD1*.

Methods: Sequence selection for *PKD1* was achieved by highly specific long-range PCR amplification of the gene in five sections, followed by amplicon-based NGS on the S5 GeneStudio system. To validate this method, 48 patients with previously described cystic kidney disease were screened for *PKD1* pathogenic variants. A complementary amplicon-based panel was used to sequence 23 additional genes, including *PKD2, PAX2*, *DNAJB11*, *GLIS2*, *JAG1*, *TSC1*, *TSC2*, *SEC63*, *GANAB*, *ALG5*, *ALG8*, *MUC1*, *UMOD*, *REN*, *HNF1B* and *SEC61A1*. Variants were called and annotated using the IonReporter V5.12 platform and validated by orthogonal methods.

Results: Quality checks of NGS runs confirmed the validity and high performance of the pipeline. Moreover, patient analysis revealed 40 pathogenic variants in *PKD1* and 4 in *PKD2* (13 missense, 23 nonsense/frameshift, 3 splicing, 3 large indels, 2 in-frame deletions), and 10 in other genes tested.

Conclusion: We have developed a first-in-line diagnostic tool for fast, confident, and specific clinical screening of *PKD1* by NGS, paired with a comprehensive analysis of additional genes explaining chronic renal failure due to cystic kidneys or tubular defects.

Grants:
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Conflict of Interest: None declared

P15.055.D Beat the clock: Exome reanalysis based on the new genes described during the ESHG 2023 conference.

Núria Martínez-Gil[1;2], *Amaia Lasa-Aranzasti*[1;2], *Jordi Leno*[1;2], *Irene Valenzuela Palafoll*[1;2], *Laura Trujillano*[1;2], *Anna Maria Cueto-González*[1;2], *Barbara Masotto*[1;2], *Eulàlia Rovira-Moreno*[1;2], *Anna Abulí*[1;2], *Patricia Muñoz-Cabello*[1;2], *Miriam Masas*[1], *anna tenes*[1], *Elena García-Arumí*[1;2], *Eduardo Tizzano*[1;2], *Marta Codina*[1;2]

[1]*Vall d'Hebron University Hospital, Department of Clinical and Molecular Genetics, Barcelona, Spain;* [2]*Vall d'Hebron Research Institute, Medical Genetics Group, Barcelona, Spain*

Background/Objectives: Whole Exome Sequencing (WES) is used for the diagnosis of genetic disorders, with a diagnostic yield of approximately 20-50%. Data reanalysis increase the diagnosed cases by 15-20%, thanks to new clinical information and novel causative genes. Every year a plethora of new articles are published describing new gene-disease associations. Although the information is eventually included in databases such as OMIM or HPO, the time lag between the publication and inclusion of information eventually result in a diagnostic delay.

Methods: To overcome this drawback we investigated a quicker diagnosis method, taking advantage of the knowledge shared between researchers at the ESHG 2023 conference. In particular, we have collected all novel genes described in oral and poster sessions and the phenotypes coded in HPO terms. With this data, we retrospectively searched through an in-house pipeline for potential pathogenic variants among more than 1300 exomes of rare disease patients followed at the Vall d'Hebron university hospital.

Results: Overall, we have compiled 83 new gene-disease associations, 46 with a recessive inheritance and 37 with a dominant inheritance. After variant prioritization, we identified in our series 102 candidate variants in 30 genes. Comparing the phenotype of our patients with the newly described entity, we found 12 variants in 10 different genes that could lead to the patient's diagnosis.

Conclusion: This approach may be applied to routine clinics as it helps to accelerate the diagnosis process and provides new information in the description of a novel gene, reducing the genetic diagnostic odyssey of undiagnosed cases.

Grants: FJC2021-046715-I

Conflict of Interest: None declared

P15.056.A CYP21A2 variant detection for molecular diagnosis of Congenital Adrenal Hyperplasia from Whole Genome Sequencing

Kevin Savage[1], *Louise McClelland*[2], *Gavin Ryan*[2], *Piers Fulton*[2], *Vitor Onuchic*[3], *Eric Roller*[3], *Susan Walker*[1], *Katrina Stone*[1], *David Bick*[1], *Matt Brown*[1], *Ellen Thomas*[1], *Richard Scott*[1], *Sue Hill*[2], *Dalia Kasperaviciute*[1]

[1]*Genomics England, London, United Kingdom;* [2]*NHS England, London, United Kingdom;* [3]*Illumina, San Diego, United States*

Background/Objectives: Congenital Adrenal Hyperplasia (CAH), prevalence 1:15,000, can be caused by CYP21A2 variants. High homology with paralog CYP21A1P complicates analysis using short-read sequencing.

Methods: We investigated performance of Dragen v4's CYP21A2 caller for clinical diagnosis of CAH, using WGS (150bp paired-end) across four cohorts:

Controls (n = 4644): no expected CAH.
CAH cases (n = 6): explicit CAH phenotype.
Potential CAH cases (n = 2080): compatible phenotype including ambiguous genetalia and salt wasting.
PCR genotyped (n = 9): with additional PCR test results. 6/9 had no pathogenic variants.

The caller only reports variants from known variants included in software configuration. We compared configuration against diagnostic variants reported by current standard of care testing in England (527 alleles covering 43 variants).

Results: In controls 2% (101/4644, CI 0.17-0.26%) had combined CYP21A2/CYP21A1P copy number = 2 (reference = 4), indicating common paralog deletion. 0.2% (10/4644, CI 0.1%-0.3%) had two pathogenic variants. Only 0.08% (4/4644, CI 0.02%-0.2%) had two predicted severe variants.

4/6 CAH cases had two pathogenic variants detected, showing strong enrichment. No enrichment of variants was seen among potential CAH cases (4/2080, 0.2%, CI 0.05%-0.4%).

From the PCR cohort, we detected 2/3 positive cases with no false positives. The variant in the remaining positive case was absent from caller configuration. 92% (488/527) of diagnostic alleles reported by current PCR testing were in caller configuration.

Conclusion: The CYP21A2 caller accurately detects known variants, improving clinical WGS for CAH. However, novel variant detection remains challenging.

Grants:

Conflict of Interest: Kevin Savage: None declared, Louise McClelland: None declared, Gavin Ryan: None declared, Piers Fulton: None declared, Vitor Onuchic Illumina, Eric Roller Illumina, Susan Walker: None declared, Katrina Stone: None declared, David Bick: None declared, Matt Brown: None declared, Ellen Thomas: None declared, Richard Scott: None declared, Sue Hill: None declared, Dalia Kasperaviciute: None declared

P15.057.B Diagnostic efficacy of WGS data reanalysis in a clinical setting

Majbritt Busk Madsen[1], *Andreas Ørslev Rasmussen*[1], *Magnus Gislason*[1], *Luca Robinson*[1], *Frederik Otzen Bagger*[1], *Maria Rossing*[1]

[1]*Copenhagen University Hospital, Rigshospitalet, Department of Genomic Medicine, Copenhagen, Denmark*

Background/Objectives: The use of whole genome sequencing (WGS) in clinical diagnostics has increased rapidly, and one of the advantages of WGS is that it allows for revisiting data when new knowledge occur. Thus, initially, we determined the diagnostic success rate in a patient cohort analyzed with WGS as part of their diagnostic work-up. Subsequently, we aim to reprocess and reanalyze the WGS data applying the latest knowledge of the genetic background of their respective diseases, hopefully increasing the diagnostic yield.

Methods: All clinical WGS samples sequenced at the Department of Genomic Medicine, Copenhagen University Hospital, from Dec 2017 to Nov 2022 were included in the study (n = 2990). The original diagnostic reports were curated, and reporting of a likely

pathogenic or pathogenic variant in a disease-relevant gene was regarded as a solved case (n = 405). The cohort consist of 2318 patients with various diseases, assessed either as single probands (n = 2141) or as part of a pedigree (n = 177). Samples were reprocessed with the latest version of the Danish national bioinformatic pipeline, including analysis of large structural variants, and reinterpreted with updated gene-lists and knowledge of respective disease mechanisms.

Results: Based on the curation of the clinical reports, preliminary results show a positive diagnostic yield of 17.5%, thus 1913 samples were eligible for WGS data reprocessing and reinterpretation. Sixty percent (1386/2318) had not previously been analyzed for large genomic rearrangements.

Conclusion: These preliminary results of a large-scale reevaluation project show great promise for increasing the diagnostic yield in a clinical diagnostic setting.

Grants: N/A

Conflict of Interest: None declared

P15.058.C Craft genetics: the impact of manual data curation in genome wide approaches

Melik Malek Khelifa[1], *Corinna Hendrich*[1], *Sandra von Hardenberg*[1], *Alisa Förster*[1], *Jana Lentes*[1], *Bernd Auber*[1], *Antje Schmidt*[2], *Torsten Kaussen*[3], *Nataliya Di Donato*[1], *Hans-Jürgen Christen*[4], *Tim Ripperger*[1], *Anke Katharina Bergmann*[1]

[1]*Hannover Medical School, Human Genetics, Hannover, Germany;* [2]*Children- and Teenager Hospital Auf der Bult, Departement of Neuropediatrics, Hannover, Germany;* [3]*Hannover Medical School, Department of Pediatric Cardiology, Hannover, Germany;* [4]*Children- and Teenager Hospital Auf der Bult, Departement of, Hannover, Germany*

Background/Objectives: The crucial impact of re-assessment of the genetic data by medical geneticist in the era of artificial intelligence and automated variant filtering and interpretation is underestimated but remains essential in difficult cases. Demonstrating the clinical utility of craft genetics, we report on four clinical scenarios from our outpatient clinic in which manual reevaluation together with joint clinical and genetic data interpretation were essential to make the diagnosis. We highlight aspects being important to consider in clinically highly suspicious cases with negative routine test results.

Methods: We report four families with a strong suspicion of a rare monogenic disease. Array-CGH, short-read exome or genome sequencing were performed. Automated routine diagnostic workflows were applied and reevaluated.

Results: Routine diagnostic workflows failed to identify causative variants in all scenarios. However, various cases-specific reassessment approaches, such as re-annotation of non-coding DNA regions or data complementation from a second tissue, led to firm diagnoses. By reevaluation, we identified (likely) pathogenic variants and established the diagnoses of Wieacker-Wolff syndrome (*ZC4H2*), mosaic Cornelia de Lange syndrome (*NIPBL*), alveolar capillary dysplasia with misalignment of pulmonary veins (*FOXF1*), and distal hereditary motor neuronopathy type 8 (*SORD*).The automated workflows were unsuccessful in finding a causative variant on account of the existence of multiple transcripts, low allele fraction, deletion in an enhancer region and a novel disease association absent from databases, respectively.

Conclusion: In modern genetic diagnostics, craft genetics stays invaluable and can significantly increase the diagnostic yield. The inclusion of novel assessment strategies is a prerequisite to develop more intelligent analysis tools.

Grants: None.

Conflict of Interest: None declared

P15.059.D A *COL4A4* novel founder mutation causes autosomal recessive Alport syndrome in the Cypriot population

christiana polydorou[1], *Gregory Papagregoriou*[1], *Despina Hadjipanagi*[1], *George Michael*[1], *lakis yioukkas*[2], *maria kkolou*[3], *androulla pastelli*[4], *panayiota loukaidou*[4], *Chrysoula Pipili*[5], *kypros constantinou*[6], *toumasi elpida*[6], *isavella savva*[6], *andreas soloukides*[7], *Constantinos Deltas*[1;8]

[1]*University of Cyprus, biobank.cy Center of Excellence in Biobanking and Biomedical Research, Nicosia, Cyprus;* [2]*Paphos General Hospital, Nephrology Department, Paphos, Cyprus;* [3]*Nephrology Department, Larnaca General Hospital, Larnaca, Cyprus;* [4]*Larnaca General Hospital, Nephrology Department, Larnaca, Cyprus;* [5]*Famagusta General Hospital, Nephrology Department, Paralimni, Cyprus;* [6]*Nicosia General Hospital, Transplant Center, Nicosia, Cyprus;* [7]*Nicosia General Hospital, Transplantation Center, Nicosia, Cyprus;* [8]*University of Cyprus, University of Cyprus Medical school, Nicosia, Cyprus*

Background/Objectives: Alport syndrome (AS) is a rare genetically heterogeneous inherited glomerulopathy, which is characterized by persistent microscopic hematuria (MH) since childhood, progressing to proteinuria and chronic kidney function decline, leading to kidney failure.

Methods: In this study we studied seven patients from four Cypriot families with collagen IV nephropathy, using next generation sequencing (NGS) analysis.

Results: Patients in all four families, which originate from geographically different parts of the island, inherited a founder novel pathogenic variant in the *COL4A4* gene, substituting arginine for glycine at amino acid residue 1248 (COL4A4:p.-Gly1248Arg). This variant was never reported in genomic or exomic databases or the Human Gene Mutation Database (HGMD), while it has high scores of pathogenicity in prediction algorithms. The variant has not been detected in 1000 exomes of the Cypriot general population (CYPROME) and according to ACMG-AMP criteria, it is classified as a variant of uncertain significance leaning to likely pathogenic (VUS-Franklin by Genoox). A homozygous patient for COL4A4:c.3742G>C, (p.Gly1248Arg) variant, reached end-stage renal disease (ESRD) and was transplanted at the age of 46-yrs, whereas another heterozygous relative in the same family reached ESRD at 58-yrs. All other heterozygous patients presented a spectrum of clinical symptoms, always including MH.

Conclusion: This is strong evidence that the variant should be classified as pathogenic/likely pathogenic, establishing a clinical diagnosis, and enabling correct counseling and treatment.

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Conflict of Interest: None declared

P15.060.A Implementation of External Quality Assessment for Inherited Retinal Disorders: Assuring Test Standards to Support the Identification of Patients Eligible for Therapeutic Intervention

Rachel Taylor[1], *Lonneke Haer-Wigman*[2], *Lies Hoefsloot*[3], *Anne-Françoise Roux*[4], *Hanno Bolz*[5], *Victoria Williams*[1], *Nicola Wolstenholme*[1], *Simon Patton*[1]

[1]*EMQN CIC, Manchester, United Kingdom;* [2]*Radboud University Medical Center, Nijmegen, Netherlands;* [3]*Erasmus MC, Clinical*

Genetics, Rotterdam, Netherlands; [4]Hospital Center University De Montpellier, Montpellier, France; [5]Bioscientia Labor Ingelheim, Ingelheim am Rhein, Germany

Background/Objectives: Inherited retinal disorders (IRDs) represent an important cause of blindness. Pathogenic variants in >250 genes have been identified as causal. The clinical utility of a precise genetic diagnosis is well documented. Gene augmentation therapy for patients with IRD due to biallelic variants in *RPE65* is now available, and several clinical trials for gene-specific therapies are underway. Thus, diagnostic proficiency is vital for improved patient outcomes. The aim of this work was to implement an external quality assessment (EQA) scheme for IRDs, to ensure laboratories correctly identify patients eligible for therapeutic intervention.

Methods: Thirty laboratories from 17 countries were selected to participate in a pilot EQA scheme. Four DNA samples and corresponding mock clinical case scenarios were supplied for testing. Three samples were provided for analysis of genes associated with approved/in trial therapies (e.g.- *RPE65*, *RPGR*, *RPGR ORF15*) and one for gene panel analysis. Clinical reports were assessed for genotyping, interpretation, and accuracy by an independent team of experts.

Results: Twenty-six laboratories returned clinical reports. Preliminary data indicates on average, 83% of laboratories received full marks for genotyping, 27% received full marks for interpretation and 65% received full marks for clerical accuracy. Laboratories performed least well in the case involving RPGR ORF15 testing.

Conclusion: EQA for IRDs has enabled a thorough assessment of laboratory performance, including the reporting of variants associated with crucial clinical interventions. We anticipate that continued provision of this scheme will serve to raise and maintain testing standards and facilitate standardisation of clinical reporting.

Grants: N/A

Conflict of Interest: None declared

P15.061.B Family study of the distribution pattern of whole-genome-based polygenic risk scores in families with ulcerative colitis

Ingrid Lojova[1;2;3], Jaroslav Minárik[1;3], Zuzana Pös[1;2], Mario Hlavacka[4], Adam Hurtuk[5], Oliver Kubicka[5], Natalia Forgacova[1;2;3], Silvia Bokorová[2], Lýdia Lukyová[2], Zuzana Holesova[6], Andrea Zatkova[1], Tatiana Sedlackova[2], Juraj Gazdarica[2;7], Jaroslav Budiš[2;7], Tomas Szemes[2;3], **Jan Radvanszky**[1;2;3]

[1]Institute of Clinical and Translational Research, Biomedical Research Center of the Slovak Academy of Sciences, Department of Human Genetics, Bratislava; [2]Comenius University Science Park, Bratislava; [3]Department of Molecular Biology, Faculty of Natural Sciences, Bratislava; [4]Faculty of Mathematics, Physics and Informatics, Comenius University, Department of Applied Informatics, Bratislava; [5]Institute of Informatics, Information Systems and Software Engineering, Faculty of Informatics and Information Technologies of Slovak University of Technology, Bratislava; [6]Geneton Ltd., Bratislava; [7]Slovak Center of Scientific and Technical Information, Bratislava*

Background/Objectives: Genomic predispositions for inflammatory bowel disease (IBD), including ulcerative colitis (UC), may have complex patterns, including both monogenic, oligogenic and highly polygenic forms. We present our findings on: 1) the effect of four different selection strategies for choosing potentially relevant genomic positions for polygenic risk score (PRS) calculations; 2) the patterns of PRS distributions among UC affected and unaffected family members; and 3) the results of screening for potential monogenic pathogenic variants.

Methods: WGS data were generated for 62 individuals, including 7 UC patients, 7 family members (three families) and 48 controls. Risk positions were extracted from GWAS Catalog. PRSs were calculated using the linear weighted sum of risk alleles. Monogenic causes were screened using a commercial annotation tool.

Results: We proved the potential of PRSs in identifying at-risk individuals, but also in selecting patients for screening of monogenic/oligogenic UC. Considering the different variant sets tested, those obtaining the widest range of genomic positions (i.e. positions associated with traits IBD) were found to have the largest discriminatory power between patients and controls. Family members have not always increased PRSs. We also identified a novel variant/gene-phenotype association, possibly causing mono-/oligogenic UC. The significance of the identified *ZBP1* variant (NM_030776.3:c.3G>A) will require further studies.

Conclusion: PRSs promise breakthroughs in prevention, diagnostics and therapy of complex diseases, especially when combined with screening for monogenic genetic causes.

Grants: Scientific Grant Agency (VEGA_2/0146/23, VEGA_2/0114/24), SAS grants for PhD students (APP0509), Slovak Research and Development Agency (APVV-21-0296_INCAM; APVV-20-0472_Sepmin) and Horizon Europe Framework Programme Grant (ID: 101087124 (ADDIT-CE)).

Conflict of Interest: None declared

P15.062.C STRavinsky STR database and PGTailor PGT tool, demonstrating superiority of CHM13-T2T over hg38 and hg19 for STR-based applications

Noam Hadar[1], **Amit Safran**[1]*, ginat narkis[1;2], Shirly Amar[2], Marina Varnavsky[2], Glenda Calniquer Palti[2], Ohad Shmuel Birk[1;2]*

[1]Morris Kahn Laboratory of Human Genetics, NIBN and Faculty of Health Sciences, Ben Gurion University of the Negev, Beer Sheva, Israel; [2]Genetics Institute, Soroka Medical Center, Beer Sheva, Israel

Background/Objectives: The possible involvement of Short-Tandem-Repeats (STRs) in various biological processes has been a subject of extensive research. STRs are significant in diverse fields, including forensics, evolutionary biology, and pre-implantation-genetic-testing (PGT). Commonly used reference genomes such as GRCh37/hg19 and GRCh38/hg38, primarily constructed through short-read sequencing, have been limited in STR mapping. However, advancements in long-read sequencing and the creation of the CHM13-T2T reference genome have now allowed for an accurate and comprehensive integration of STRs into the human genome framework.

Methods: We developed *STRavinsky*, a detailed and extensive STR database, compatible with hg19, hg38, and T2T genomes, using high performance computing. Using *STRavinsky*, we developed *PGTailor*, an interactive web-application designed for PGT assay creation.

Results: Our analysis reveals that the T2T genome outperforms its predecessors, hg19 and hg38, by identifying a significantly higher number of STRs. Notably, we observed a pronounced frequency of TGGAA repeat sequences in the short arms of acrocentric chromosomes, supporting earlier hypotheses about their role in the genesis of Robertsonian translocations. Intriguingly, we pinpointed a unique concentration of these repeats in specific regions, such as 16q11.2 and 9q12, hinting at their potential involvement in prevalent cytogenetic abnormalities. Finally, leveraging the enhanced capabilities of T2T and *STRavinsky*, we introduced *PGTailor* - an innovative freely-available

online tool designed to streamline and simplify the creation of STR-focused PGT assays in mere minutes: https://fohs.bgu.ac.il/birklab/PGTailor.

Conclusion: *STRavinsky* leverages the comprehensive nature of the T2T genome to offer a complete STR mapping database, essential for both research and clinical purposes, enabling novel insights to aberrations in human genetics.

Conflict of Interest: None declared

P15.063.D Trio whole exome sequencing in pediatric intensive care unit patients: Illuminating the clinical utility

Hannah Wallaschek[1], *Nadia Meyer*[1], *Joelle Ronez*[1], *Manuela Richter*[2], *Jost W. Richter*[3], *Alexander von Gise*[2;4], *Gunnar Schmidt*[1], *Nataliya Di Donato*[1], *Bernd Auber*[1], *Sandra von Hardenberg*[1]

[1]*Hannover Medical School, Department of Human Genetics, Hannover, Germany;* [2]*Children's Hospital Auf der Bult, Department of Neonatology, Hannover, Germany;* [3]*Klinikum Braunschweig, Department of Neonatology, Braunschweig, Germany;* [4]*Hannover Medical School, Department of Pediatric Cardiology and Intensive Medicine, Hannover, Germany*

Background/Objectives: Rare genetic disorders often present as severe multisystemic conditions and are a significant cause of pediatric hospitalization in intensive care units (ICU). Diagnosis can be challenging and often includes a variety of invasive procedures. Whole exome sequencing (WES) including parent sequencing (trio-WES) is a potent tool to detect the genetic causes of rare diseases. Here we report on the utility of trio-WES from the clinicians' perspective and the position of trio-WES in the diagnostic pathway.

Methods: The diagnostic yield and clinical utility was evaluated via the C-GUIDE™ questionnaire in 45 pediatric ICU patients in which trio-WES had been performed. Furthermore, operations and procedures (OPS) during the ICU stay were analyzed.

Results: The 45 patients received 331 OPS before initiating trio-WES, another 109 OPS were performed while waiting for the genetic results. The average turnaround time (TAT) for genetic results was 25 days. Genetic diagnosis was provided for 37.8% of the children. Clinical utility was considered generally high with a mean score of 7.34 in the C-GUIDE, and even higher when a genetic diagnosis was confirmed (14.07 vs 3.42).

Conclusion: Trio-WES is an effective diagnostic tool. The high C-Guide score underline its clinical utility. Our results should encourage clinicians to consider genetic testing during ICU stays as a first-tier diagnostic to avoid invasive OPS. Duration of WES is still time-consuming. Numerous additional tests were performed during TAT, and some children passed away in the meantime. In the future, rapid sequencing can address this issue.

Grants: None.

Conflict of Interest: None declared

P15.064.A COL4A5 p.(Gly743Ala) missense substitution identified in a large Italian family. Is this variant truly likely pathogenic, or could it be considered a likely predisposing allele?

Sergio Daga[1;2], *Lorenzo Loberti*[1;2;3], *Riccardo Pracella*[4], *Anna Carrer*[1;2;3], *Pietro Ilardi*[1;2;3], *Giulia Rollo*[1;2], *Kristina Zguro*[1;2], *Andrea Guarnieri*[5], *Guido Garosi*[5], *Alessia Giorli*[6], *Marco Mandalà*[6], *Francesca Ariani*[1;2;3], *Rossella Tita*[3], *Marco Castori*[4], *Alessandra Renieri*[1;2;3], *Maria Antonietta Mencarelli*[3]

[1]*Medical Genetics, University of Siena, Siena, Italy;* [2]*Med Biotech Hub and Competence Center, Department of Medical Biotechnologies, University of Siena, Siena, Italy;* [3]*Genetica Medica, Azienda Ospedaliero-Universitaria Senese, Siena, Italy;* [4]*Division of Medical Genetics, Poliambulatorio "Giovanni Paolo II", Fondazione IRCCS-Casa Sollievo della Sofferenza, Viale Padre Pio 7, 71013, San Giovanni Rotondo, Italy;* [5]*Department of Medical Sciences, Nephrology, Dialysis and Transplantation Unit, University Hospital of Siena, Siena, Italy;* [6]*Department of Otolaryngology Unit, University of Siena, Azienda Ospedaliero Universitaria Senese, Siena, Italy*

Background/Objectives: *COL4A5* gene variants cause X-Linked Alport Syndrome (AS) with males exhibiting severe renal impairment while females present only microhaematuria. Most of the deleterious substitutions, affecting Glycines in the collagenous domain, significantly alter the protein, impact the folding capacity of the heterotrimer, and often lead to end-stage kidney disease. Nevertheless, is it possible that some *COL4A5* variants are not associated with typical AS?

Methods: Employing Clinical Exome Sequencing (CES) we identified a p.(Gly743Ala) *COL4A5* variant, classified as likely pathogenic according to ACMG guidelines, as an incidental finding in a 6-years-old boy without microhaematuria/proteinuria.

Results: We ascertained the germinal origin, identifying it in 12 out of 15 family members screened (80%), unusually without AS signs except for two which reported eGFR decrease and transplantation *COL4A5*-unrelated. No variants in modifier genes allowing to mitigate or reduce the pathogenic impact of p.(Gly743Ala), have been identified. The in-silico comparison on the prediction of mutant stability suggests destabilizing impact and disfunctionalization, however these data are in contrast with the genotype/phenotype correlation so much so this variant seems to not cause renal-extrarenal manifestations.

Conclusion: The ever-increasing use of CES opens up new chapters in the diagnostic field, in particular in late-onset or chronic diseases that are not devoid of difficulties in interpretation in the clinical context. A crucial role is taken on by variants such as the p.(Gly743Ala) that can be classified as a "likely predisposing allele" assuming a new role in follow-up, therapeutic interventions and psychological management of patients and families.

Conflict of Interest: Sergio Daga CO-PI of PRIN 2022 Italian grant, Full time, Lorenzo Loberti Full-time, Riccardo Pracella Full-time, Anna Carrer Full-time, Pietro Ilardi Full-time, Giulia Rollo Full-time, Kristina Zguro Full-time, Andrea Guarnieri Full-time, Guido Garosi Full-time, Alessia Giorli Full-time, Marco Mandalà Full-time, Francesca Ariani Full-time, Rossella Tita Full-time, Marco Castori Full-time, Alessandra Renieri Full-time, Maria Antonietta Mencarelli Tuscany region PART project, Full-time

P15.065.B Flexible and rapid validation of structural variation using nanopore adaptive sequencing

Aida Paivandy[1], *Felix Lenner*[1], *Tord Jonson*[2], *Hans Ehrencrona*[2], *Anna Lindstrand*[3], *Jennifer Howe*[4], *Stephen Scherer*[4], **Lars Feuk**[1]

[1]*Uppsala University, Immunology, Genetics and Pathology, Uppsala, Sweden;* [2]*Lund University, Department of Laboratory Medicine, Lund, Sweden;* [3]*Karolinska Institute, Department of Molecular Medicine and Surgery, Stockholm, Sweden;* [4]*The Hospital for Sick Children, The Centre for Applied Genomics, Toronto, Sweden*

Background/Objectives: Identification of genomic rearrangement by microarrays or shortread sequencing often lacks information about the exact architecture and breakpoints of variants due to technical limitations. In addition, validation of complex structural variants (SVs) is often performed using custom assays, complicating confirmation of relevant findings. In this study we evaluate nanopore adaptive sequencing for flexible and rapid validation and characterization of complex genomic rearrangements.

Methods: Adaptive sequencing is an in silico target enrichment, where continued sequencing or ejection of a fragment is based on whether it matches a defined reference sequence. Using adaptive sequencing, we targeted 10 regions of different SV types, including deletions, translocations and complex rearrangements. Each sample was analyzed on a MinION flow-cell, and to evaluate multiplexing, four samples were run together on flow-cell on the P2 device.

Results: MinION sequencing resulted in between 13.5-17.8 Gb of data per flow cell, with mean on-target coverage of 18-34X and an off-target read depth average of 4.6X. We were able to validate all targeted SVs, and identify breakpoints at nucleotide resolution with one exception. We also show that background reads can be used to detect SVs in non-targeted regions of the genome.

Conclusions: Our results show that adaptive sequencing represents a robust, flexible and rapid strategy for validation of clinically relevant complex genomic rearrangements in patient samples. By providing sequence information, read depth and methylation data, nanopore adaptive sequencing has advantages over other validation assays used in diagnostic labs today.

Grant references: Swedish Research Council (2017-01861) and Hjärnfonden (FO2022-0207).

Conflict of Interest: None declared

P15.066.C Comprehensive evaluation of AlphaMissense predictions by evidence quantification for variants of uncertain significance

Amina Kozaric[1;2], *Lejla Delalic*[1], *Belma Mutapcic*[1;2], *Lejla Comor*[1], *Eric Siciliano*[1], *Mark Kiel*[1]

[1]*Genomenon, Inc, Ann Arbor, United States;* [2]*International Burch University, Ilidža, Bosnia and Herzegovina*

Background: Accurate variant classification is critical for genetic diagnosis. Variants without clear classification, known as variants of uncertain significance (VUS), pose a significant diagnostic challenge. The AI-based AlphaMissense algorithm, correlating with known variants, has not been evaluated for VUS. This study examines AlphaMissense performance in variant classification, specifically for VUS.

Methods: A systematic comparison between AlphaMissense predictions and ACMG/AMP classification was conducted for 5845 missense variants in 59 genes associated with representative Mendelian disorders. A framework for quantifying and modeling VUS pathogenicity was used to facilitate comparison. Sensitivity, specificity, density distribution, heatmaps, Sankey plot, and statistical analyses were performed using R studio.

Results: Manual review classified 5845 variants as 4085 VUS, 1576 pathogenic/likely_pathogenic, and 184 benign/likely_benign. AlphaMissense prediction and ACMG classification were concordant for 1887 variants (1352 pathogenic, 132 benign, and 403 VUS/ambiguous). The sensitivity and specificity of AlphaMissense predictions for pathogenicity were 92% and 78%. Quantitation of VUS evidence and heatmaps weakly correlated with AlphaMissense score. For VUS without computational evidence, AlphaMissense incorporation changed VUS quantification for 878 variants, while 56 were reclassified to likely pathogenic. When AlphaMissense replaced existing computational evidence for all VUS, 1709 variants changed quantified criteria, while 63 were reclassified to likely pathogenic.

Conclusion: AlphaMissense, while promising for predicting known pathogenicity, currently lacks reliability suitable for predicting classification of clinically encountered VUS. Our research suggests augmenting AlphaMissense with empirical evidence may improve performance through incorporation of a quantitative framework aiding in VUS classification.

Grants: This study was funded by NHGRI R44HG010446.

Conflict of Interest: Amina Kozaric Genomenon, Lejla Delalic Genomenon, Belma Mutapcic Genomenon, Lejla Comor Genomenon, Eric Siciliano Genomenon, Mark Kiel Genomenon, Genomenon

P15.067.D Increasing Diagnostic Yield from Short-Read WGS Data

Maria Lachgar Ruiz[1], *Natalie J Chandler*[1], *Britt Hanson*[1], *Ben Paternoster*[1], *Cristina Magro*[1], *Ashley Pritchard*[1], *Graham Rose*[1], *Sarah Nesbitt*[1], *Patrick Lombard*[1], *Gregory Costain*[2;3;4], *Vann Chau*[5;6], *Katherine Howell*[7;8], *Ingrid Scheffer*[7;8;9;10], *J Helen Cross*[11;12], *Alissa D'Gama*[13;14], *Annapurna Poduri*[13;14], *Dame Lyn Chitty*[1;15], *Amy McTague*[11;16]

[1]*North Thames Genomic Laboratory Hub, Great Ormond Street NHS Foundation Trust, London, United Kingdom;* [2]*SickKids Research Institute, Program in Genetics and Genome Biology, Toronto, Canada;* [3]*University of Toronto, Temerty Faculty of Medicine, Department of Molecular Genetics and Department of Paediatrics, Toronto, Canada;* [4]*Hospital for Sick Children, Division of Clinical and Metabolic Genetics, Department of Paediatrics, Toronto, Canada;* [5]*Hospital for Sick Children, Division of Neurology, Department of Paediatrics, Toronto, Canada;* [6]*University of Toronto, Department of Paediatrics, Temerty Faculty of Medicine, Toronto, Canada;* [7]*Murdoch Children's Research Institute, Melbourne, Australia;* [8]*Royal Children's Hospital, Department of Neurology, Melbourne, Australia;* [9]*University of Melbourne, Department of Paediatrics and Department of Medicine, Melbourne, Australia;* [10]*Austin Health, and Florey Institute of Neuroscience and Mental Health, Melbourne, Australia;* [11]*Great Ormond Street Hospital, Department of Neurology, London, United Kingdom;* [12]*UCL Great Ormond Street Institute of Child Health, Developmental Neurosciences, London, United Kingdom;* [13]*Boston Children's Hospital, Epilepsy Genetics Program, Division of Epilepsy and Neurophysiology, Department of Neurology and Division of Newborn Medicine, Department of Pediatrics, Boston, United States;* [14]*Harvard Medical School, Department of Pediatrics, Boston, United States;* [15]*UCL Great Ormond Street Institute of Child Health, Genetic and Genomic Medicine, London, United Kingdom;* [16]*UCL Great Ormond Street Institute of Child Health, Zayed Centre for Research into Rare Disease in Children, London, United Kingdom*

Consortium: International Precision Child Health Partnership (IPCHiP)

Background/Objectives: GeneSTEPS is a multi-centre study examining the diagnostic yield and clinical utility of rapid trio whole genome sequencing (WGS) in epilepsy patients <12 months old. Current analysis strategies show a 48% diagnostic yield. However, bioinformatic filtering strategies face several limitations, such as variants in non-coding regions, calling of mosaic variants and detection and filtering of structural and mitochondrial variants. Here, we describe initial results of deeper interpretation strategies which have increased diagnostic yield.

Methods: Four strategies have been applied 1) gene agnostic analysis was used to look for potential novel genes, 2) all known pathogenic variants in the ClinVar database were called, 3) de novo non-coding variants within epilepsy genes were investigated, 4) non-coding variants were investigated in cases with a single hit in an autosomal recessive gene.

Results: Two novel genes have been identified as potential candidate genes; work is being undertaken to investigate these further. A novel *DNM1* de novo deep intronic variant near other variants with proven effect on splicing was detected in one patient and a previously published likely pathogenic variant in the *UFM1* promoter region was identified that had not been called by the pipeline due to being non-coding.

Conclusion: Deeper interpretation approaches improve diagnostic yield from short-read WGS. Other approaches we are applying include improving the detection and filtering of structural and mitochondrial variants, detection of mosaic variants and repeat expansions and further analysis of non-coding regions.

Grants: GeneSTEPS grant & NIHR Biomedical Research Centre at Great Ormond Street Hospital, London, UK.

Conflict of Interest: None declared

P15.068.A Statistical results from a six-year whole exome analysis of patients with rare or undiagnosed diseases in a single department.

Tadashi Kaname[1], *Kumiko Yanagi*[1], *Arisa Igarashi*[1], *Taiga Aoki*[1], *Takaya Iida*[1], *Masahiko Yamamori*[1], *Kazuhito Satou*[1], *Koh-ichiro Yoshiura*[2], *Nobuhiko Okamoto*[3], *Yoichi Matsubara*[4]

[1]National Center for Child Health and Development, Dept Genome Medicine, Tokyo, Japan; [2]Nagasaki University, Dept Human Genetics, Nagasaki, Japan; [3]Osaka Women's and Children's Hospital, Dept Medical Genetics, Osaka, Japan; [4]National Center for Child Health and Development, Tokyo, Japan

Consortium: the IRUD-NCCHD-GM consortium

Background/Objectives: Comprehensive genome analysis for diagnosis and cause identification of undiagnosed diseases is useful and national projects are underway in many countries. We have been participating in a major project in Japan, 'The Initiative on Rare and Undiagnosed Diseases', since its launch in summer 2015, and are performing a comprehensive genome analysis. Here we report on the statistical results of 1560 undiagnosed cases in which whole exome analysis was performed on patients and/or their families in a single department.

Methods: Undiagnosed cases were enrolled at IRUD-NCCHD-GM consortium hospitals where no diagnosis had been made after prior direct sequencing or panel analysis of candidate genes for the suspected disease. All patients, or their family members, were subjected to whole exome analysis after written informed consent was obtained. We performed in-house CNV analysis in addition to the usual variant calling and filtering.

Results: The yield of reaching a conclusive diagnosis, including re-analysis, in these 1560 cases (2015-2021) was 45.9%. Re-analysis in 2022 of 1560 cases from 2015 to 2021, when all exome analyses were conducted, led to the identification of a cause and diagnosis in 100 new cases (6.4%). In addition to the diagnosis of ultra-rare diseases, novel diseases such as MAPK8IP3-related disease and STAT6-gain-of-function disorder were found.

Conclusion: Whole exome analysis is currently cost-effective and is the first choice for cause identification of undiagnosed diseases. Re-analysis should also be performed on a regular basis, as it is certain to increase the rate of identification of the cause of undiagnosed cases.

Grants: AMED(22ek0109549s0202)

Conflict of Interest: None declared

P15.069.B Evaluation of quality of WGS and WES results using validated protocols

Katerina Kvapilova[1;2], *Pavol Misenko*[3], *Ján Radvánszky*[3], *ondrej brzon*[1], *Jaroslav Budiš*[3], *Juraj Gazdarica*[3], *Ondrej Pos*[3], *Marie Korabecna*[4], *Martin Kasny*[1], *Tomas Szemes*[3], *Petr Kvapil*[1], *Jan Pačes*[5], *Zbynek Kozmik*[6]

[1]Institute of Applied Biotechnologies a.s., Praha 10; [2]Charles University, Faculty of Science, Prague, Czech Republic; [3]Geneton s.r.o., Bratislava, Slovakia; [4]Institute of Biology and Medical Genetics, First Faculty of Medicine, Charles University and General University Hospital in Prague, Prague, Czech Republic; [5]Institute of Molecular Genetics of the Czech Academy of Sciences, Laboratory of Genomics and Bioiformatics, Prague, Czech Republic; [6]Institute of Molecular Genetics of the Czech Academy of Sciences, Laboratory of Transcriptional Regulation, Prague, Czech Republic

Background/Objectives: Whole exome sequencing (WES) and whole genome sequencing (WGS) are standards in human clinical diagnostics and population genomics. We evaluated the quality and genotype concordance of single nucleotide variants (SNVs) and small insertions/deletions (indels) in paired blood and saliva genomic DNA (gDNA) isolates for WES and WGS protocols using reference-validated protocols.

Methods: The NIST standard Coriell NA12878 was repeatedly sequenced to validate WGS and WES protocols. NA12878 data calls were compared to the truth dataset published by the Genome in a Bottle Consortium. A paired comparison of blood-derived gDNA and saliva-derived gDNA was performed for SNVs and indels for both, WES and WGS. The level of genotype concordance was studied using ACMG SF v 3.2. gene set in two settings: exonic regions only/complete genes.

Results: The F1 score of ten paired blood-saliva samples range between 0.8030-0.9998 for SNVs and between 0.8883-0.9991 for indels in the WGS, and between 0.8643-0.999 for SNVs and between 0.7781-1.000 for indels in WES. The quality pattern of called variants obtained from genomic-reference-based technical replicates correlates with data calls of paired blood–saliva-derived samples in all levels of tested examinations.

Conclusion: The resulting data shows a clear advantage of the WGS approach over WES; especially for indels analysis while confirming no significant differences between blood and saliva samples.

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Conflict of Interest: None declared

P15.071.D SpadaHC: a database to improve and homogenize the classification of variants in hereditary cancer genes in the Spanish population

José M. Moreno-Cabrera[1;2], **Lidia Feliubadaló**[1;2], *Marta Pineda*[1;2], *Patricia Prada-Dacasa*[1;2], *Mireia Ramos*[1;2], *Jesús del Valle*[1;2], *Joan Brunet*[1;2], *Bernat Gel*[3], *María Currás-Freixes*[4], *Bruna Calsina*[4], *Milton E. Salazar-Hidalgo*[4], *Marta Rodriguez-Balada*[5], *Barbara Roig Bourgine*[5], *Sara Fernández*[5], *Mercedes Durán*[6], *Mónica Arranz Ledo*[6], *Mar Infante Sanz*[6], *Adela Castillejo*[7], *Estela Dámaso*[7], *Jose Luis Soto*[7], *Montserrat de Miguel*[8], *Beatriz Hidalgo Calero*[8], *José Manuel Sánchez Zapardiel*[8], *Teresa Ramon y cajal*[9], *Adriana Lasa*[10;11], *Alexandra Gisbert-Beamud*[12], *Anael Lopez-Novo*[13], *Clara Ruiz-Ponte*[10;13], *Miriam Potrony*[10;14], *Maria Isabel Alvarez*[10;14], *Ana Osorio*[10;15], *Isabel Lorda*[10;15], *Mercedes Robledo*[10;16], *Alberto Cascón*[10;16], *Anna Ruiz*[17], *Nino Spataro*[17], *Imma Hernan*[18], *Emma Borràs Angosto*[18], *Alejandro Moles-Fernandez*[19;20], *Julie Earl*[21], *Juan Cadiñanos*[22], *Ana B. Sánchez-Heras*[23], *Anna Bigas*[2;24;25], *Gabriel Capellá*[1;2], *Conxi Lázaro*[1;2]

[1]Catalan Institute of Oncology IDIBELL-ONCOBELL, Hereditary Cancer Program, L'Hospitalet de Llobregat; [2]Instituto de Salud Carlos III, CIBERONC, Madrid; [3]PMPPC-IGTP, Hereditary Cancer Group, Badalona; [4]CNIO, Familial Cancer Clinical Unit, Human Cancer Genetics Program, Madrid; [5]HUSJR, Institut d'Investigació Sanitària Pere Virgili (IISPV) - Universitat Rovira i Virgili (URV), Institut d'Oncologia de la Catalunya Sud (IOCS), Reus; [6]IBGM, UVa-CSIC, Cancer Genetics Group, Unit of Excellence, Valladolid; [7]Hospital General Universitario de Elche - FISABIO, Unidad de Genética Molecular, Elche; [8]Hospital Universitario 12 de Octubre, Laboratorio de cáncer hereditario,

Servicio de Bioquímica Clínica-Análisis clínicos, Madrid; [9]Hospital de la Santa Creu i Sant Pau, Familial Cancer Clinic, Medical Oncology, Barcelona; [10]Instituto de Salud Carlos III, CIBERER, Madrid; [11]Hospital de la Santa Creu i Sant Pau, Genetics Department, Barcelona; [12]Institut de Recerca Sant Pau-CERCA Center (IR Sant Pau), Barcelona; [13]FPGMX, Instituto de Investigación Sanitaria de Santiago, Grupo de Medicina Xenómica-USC, Santiago de Compostela; [14]Hospital Clinic of Barcelona and FRCB - IDIBAPS - University of Barcelona, Biochemistry and Molecular Genetics Department, Barcelona; [15]Hospital Universitario Fundación Jiménez Díaz, IIS-FJD, Departamento de Genética y Genómica, Madrid; [16]CNIO, Hereditary Endocrine Cancer Group, Human Cancer Genetics Program, Madrid; [17]Center for Genomic Medicine, Parc Taulí Hospital Universitari, I3PT-CERCAUniversitat Autònoma de Barcelona, Genetics Laboratory, Sabadell; [18]Consorci Sanitari de Terrassa, Molecular Genetics Unit, Terrassa; [19]Vall d'Hebron Hospital Universitari, Department of Clinical and Molecular Genetics, Barcelona; [20]VHIR, Vall d'Hebron Hospital Universitari, Medicine Genetics Group, Barcelona; [21]Biomarkers and Personalized Approach to Cancer Group (BioPAC), Madrid; [22]Fundación Centro Médico de Asturias, Oviedo; [23]Elche General University Hospital, Cancer Genetic Counseling Unit, Medical Oncology Department, Elche; [24]Institut Hospital del Mar d'Investigacions Mèdiques, Program in Cancer Research, Barcelona; [25]Josep Carreras Leukemia Research Institute, Barcelona

Consortium: The SpadaHC Consortium

Background/Objectives: The accurate classification of genetic variants is crucial for clinical decision-making in hereditary cancer. In Spain, genetic diagnostic laboratories have traditionally approached this task independently due to the lack of a dedicated resource. Here we present SpadaHC, a web-based database for sharing variants in hereditary cancer genes in the Spanish population.

Methods: SpadaHC is implemented using a three-tier architecture consisting of relational database, web tool and bioinformatics pipeline. Spanish genetic diagnostic laboratories can share variant classifications and variants from individuals in VCF format.

Results: The platform supports open-restricted access, flexible dataset submissions, automatic pseudo-anonymization, VCF quality control, variant normalization, and liftover between genome builds. Users can explore and search data flexibly, receive automatic discrepancy notifications, and access SpadaHC population frequencies based on many criteria. Current version of SpadaHC (v1.58.0, February 2024) includes 17 laboratory members, storing 1.17 million variants (20908 unique) from 4306 patients and 14834 laboratory classifications. We analyzed classification discrepancies from SpadaHC v1.21.0 (July 2023), where a total of 2469 unique variants were classified by at least two laboratories, and 84 of these had clinically relevant discrepancies, between class 1-2-3 and class 4-5. We addressed these diescrepancies through a 3-phase resolution strategy, which resolved 80 of them.

Conclusion: This work highlights the importance of data sharing to allow discussion and promote consistency in variant classifications among laboratories, so patients and family members can benefit from more accurate clinical management.

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Conflict of Interest: None declared

P15.072.A Development of an RNA-sequencing service within an NHS Diagnostic Laboratory

Claire Kyle[1], *Sam Kidman*[1], *Ciaron McAnulty*[1], *Brian Wilson*[2]

[1]*Newcastle Genetics Laboratory, Newcastle Hospitals NHS Foundation Trust, Newcastle upon Tyne, United Kingdom;* [2]*Northern Genetics Service, Newcastle Hospitals NHS Foundation Trust, Newcastle upon Tyne, United Kingdom*

Background/Objectives: The growing volume and complexity of DNA-based genetic testing has increased detection of variants of uncertain significance (VUS). Determining the splicing impact of VUSs is predicted to increase diagnostic yield by >35%. At present, RNA-sequencing is almost exclusively used in a research setting, and guidance on how this data should be analysed and reported in a diagnostic setting is not well developed. Our aim is to develop diagnostic-standard RNA-sequencing for both identified VUSs and as an independent test. Here we describe our experience with a sub-cohort of 75 individuals harbouring a potentially spliceogenic VUS, underpinning our approach to diagnostic RNA-sequencing.

Methods: RNA was extracted from blood or fibroblast cultures from participants, as part of a broader RNA-sequencing project. Short read RNA-sequencing and multiple bioinformatics analyses were undertaken and compared.

Discussion: Challenges to the diagnostic evaluation of RNA-sequencing data include thresholds for sequencing depth and quality, appropriateness of the tissue tested (e.g. similarity of splicing/expression profiles), evaluation of 'leaky' splicing or intron retention, and the potential impact of nonsense-mediated decay for incomplete mRNAs. We describe development of a novel bioinformatics analysis pathway to accurately detect and quantify all types of splicing variant (exon skipping, cryptic splicing and intron retention) with appropriate quality thresholds, and creation of a variant assessment pathway, illustrated by key cases.

Conclusion: RNA-sequencing can be an effective tool to increase diagnostic yield. We propose an RNA-sequencing analysis and reporting pathway to support reclassification of known DNA variants in a diagnostic laboratory setting.

This work was funded by NHS England as a Transformation Project.

Conflict of Interest: None declared

P15.073.B Targeted NGS sequencing of RT-PCR products: a powerful diagnostic tool for hereditary diseases

Alexandra Filatova[1], *Daria Akimova*[1], *Ksenia Davydenko*[1], *Evgeniya Osipova*[1], *Iuliia Viakhireva*[1], *Peter Sparber*[1], *Mikhail Skoblov*[1]

[1]*Research Centre for Medical Genetics, Moscow*

Background/Objective: An increasing number of studies emphasize the importance of RNA-analysis in the diagnosis of inherited diseases. In this study, we used targeted NGS of RT-PCR products to conduct a comprehensive RNA-analysis.

Methods: Total RNA was isolated from patients' clinically available tissue samples (predominantly fibroblasts and PBMC, occasionally other tissue types: muscle biopsy, intestinal organoids, etc.). RNA underwent DNaseI treatment, followed by reverse transcription and PCR of targeting loci. PCR-products were sequenced using various NGS platforms and processed via an in-house pipeline.

Results: We analyzed over 200 patient samples using targeted NGS of RT-PCR products and investigated dozens of genes associated with various hereditary diseases. This approach provided mean coverage of the loci of interest ranging from 5'000x to 300'000x (with an average of ~100'000x), enabling the detection of minor splicing events (subject to NMD degradation or residual expression of the reference isoform) and quantification of allele expression imbalances. Unlike gel electrophoresis and Sanger sequencing, targeted NGS allows for the analysis of multiple isoforms and their proportions. Sequencing of different gene loci in a single run made our approach cost-effective for

routine use. As a result, our comprehensive RNA-analysis identified mRNA structure/expression changes and associated nucleotide variants in many patients where pathogenic variants were previously unidentified. For many VUS variants, we confirmed their effect on splicing and mRNA structure. In some cases, our analysis clarified the clinical features of the disease.

Conclusion: The use of targeted NGS during RNA-analysis is a very effective approach for diagnosing patients with hereditary diseases.

Conflict of Interest: None declared

P15.074.C Improvement of histopathological & genetic diagnostics of vascular anomalies using non-destructive 3Dhistopathology

Rene Hägerling[1;2]

[1]*Charité - Universitätsmedizin Berlin, Institute of Medical and Human Genetics, Berlin, Germany;* [2]*Berlin Institute of Health, BIH Center for Regenerative Therapies, Berlin, Germany*

Background/Objectives: For histopathological analysis and subsequent diagnosis of diseased tissues a rapid, accurate histopathological and genetic analysis of biopsied tissues are essential. However, the 2-dimensional slide-based histopathological analysis does not represent tissue structures and molecular targets sufficiently, which aggravates an accurate and spatial analysis of tissue.

Methods: To overcome these limitations, we have established a 3D-histopathology approach based on non-destructive volumetric lightsheet-microscopy, which bears the potential to revolutionize the way pathological analysis are done. In comparison to conventional 2D-pathology, non-destructive 3D-histopathology allows rapid slide-free histological imaging of a whole tissue sample, volumetric analysis of diagnostic relevant structures such as immune cells, vessels and an improved spatial analysis of cell distribution relevant to study e.g. the microenvironment.

Results: To achieve this, we have successfully established a fluorescence-based analogue of the histological gold-standard (hematoxylin&eosin), suitable for lightsheet-microcopy. In combination with codon-reassignment-technology and nanobody-based staining protocols detecting relevant markers such as immune cells, vessels or proliferation state, this approach offers cutting-edge diagnostic phenotyping of the specimen. Due to non-destructive nature of the optical-sectioning methodology, the sample is available for downstream applications such as molecular diagnostics of the previously imaged specimen. Using this approach we were able to identify genetic alterations in combination with new vascular phenotypes, which will set the basis for future improved histological & genetic classification.

Conclusion: In summary, this innovative approach for 3D-histopathology allows more detailed diagnosis of tissue samples from patients with vascular anomalies and has the potential to become translated to tumor tissue as well.

Grants: ERC-StG PREVENT.

Conflict of Interest: Rene Hägerling ERC-StG PREVENT

P15.075.D The clinical genome: validation data beyond clinical exome

Kornelia Tripolszki[1], *Melina Bilinski*[1], *Matthias Bieg*[1], *Anett Marais*[1], *Catarina Pereira*[1], *Javier Martini*[1], *Vasiliki Karageorgou*[1], *Mukunth Sadagopan*[1], *Divine Lawir Fondzenyuy*[1], *Swathi Chinnappa*[1], *Stephanie Weissgraeber*[1], *Malgorzata Bochinska*[1], *Thomas Hackenberg*[1], *Katja Bruesehafer*[1], *Chris Junghans*[1], *Johannes Numrich*[1], *Krishnakumar Kandaswamy*[1], *Jorge Pinto Basto*[1], *Peter Bauer*[1], *Omid Paknia*[1]

[1]*CENTOGENE GmbH, Rostock, Germany*

Background/Objectives: Difficult-to-sequence regions are usually neglected by exome sequencing. Here, we report the establishment of a PCR-free genome sequencing (GS) based assay, that systematically screens hard-to-sequence regions such as repeat expansion (RE), SMN1/SMN2, GBA1, and in addition detects structural rearrangements.

Methods: For the PCR-free GS library preparation gDNA extracted from blood was used. After genome sequencing, the reads were aligned to the hg19 reference genome. A bioinformatics pipeline that integrates the DRAGEN pipeline (Illumina) and in-house algorithms was used. Throughout the validation, 52 positive control cases were utilized (thirty-five RE, six SMN1/SMN2, six GBA1/GBAP1 conversion and five cases with structural variants (SV)). In 2269 samples, we performed post-launch monitoring and inspected the performance of the bioinformatic pipeline. The identified variants were confirmed through orthogonal methods.

Results: All 52 positive control samples were successfully detected by our combined assay. In the subset of 2269 post-launch monitoring patients, four individuals were detected to have 0 copies of SMN1, one patient was detected to carry a recombinant variant in GBA1, and a total of 24 patients had positive RE findings that were considered to be clinically relevant. Regarding the complex SV results, seven different variants were detected by SV calling in eight patients.

Conclusions:

In our study, we present a validated GS assay, that leverages the benefits of the PCR-free GS technology and seamlessly integrates the secondary DRAGEN pipeline and our in-house pipeline. This comprehensive workflow not only increases the diagnostic yield, advances genomic research, but also substantiates our understanding of genetic variation in rare disease patients.

Conflict of Interest: Kornelia Tripolszki CENTOGENE GmbH, Melina Bilinski CENTOGENE GmbH, Matthias Bieg CENTOGENE GmbH, Anett Marais CENTOGENE GmbH, Catarina Pereira CENTOGENE GmbH, Javier Martini CENTOGENE GmbH, Vasiliki Karageorgou CENTOGENE GmbH, Mukunth Sadagopan CENTOGENE GmbH, Divine Lawir Fondzenyuy CENTOGENE GmbH, Swathi Chinnappa CENTOGENE GmbH, Stephanie Weissgraeber CENTOGENE GmbH, Malgorzata Bochinska CENTOGENE GmbH, Thomas Hackenberg CENTOGENE GmbH, Katja Bruesehafer CENTOGENE GmbH, Chris Junghans CENTOGENE GmbH, Johannes Numrich CENTOGENE GmbH, Krishnakumar Kandaswamy CENTOGENE GmbH, Jorge Pinto Basto CENTOGENE GmbH, Peter Bauer CENTOGENE GmbH, CENTOGENE GmbH, Omid Paknia CENTOGENE GmbH

P15.076.A Catalogue of alternative splicing events of the NBN gene

Vita Setrajcic Dragos[1], *Srdjan Novaković*[2]

[1]*Institute of Oncology Ljubljana, Ljubljana, Slovenia;* [2]*Institute of Oncology Ljubljana, Department of Molecular Diagnostics, Ljubljana, Slovenia*

Background/Objectives: Nearly all human multi-exon genes are involved in alternative splicing. RNA splicing defects are a common molecular mechanism of disease, and RNA sequencing (RNAseq) considerably improves diagnostics yield. Understanding the naturally occurring alternative splicing isoforms of clinically relevant genes is of great importance for correct interpretation of

RNA splicing assays and also for determining the strength level of ACMG/AMP PVS1 evidence.

Methods: Control samples harboring only undoubtedly benign variants in the coding region and 25 nt of intronic sequence of *NBN* gene were selected for alternative splicing isoform discovery. Blood samples were sequenced using an in-house developed RNAseq protocol.

Results: We have identified all 10 previously described alternative splicing events as well as 49 previously undescribed alternative splicing events. The highest expressed alternative splicing event was cryptic exon 2 inclusion. No exon skipping event was observed at high percentage, suggesting all exons are crucial for normal protein function.

Conclusion: While alternative transcripts expressed in blood for the *NBN* gene had only been evaluated in the literature using RT-PCR and Sanger sequencing, we were the first to determine alternative transcripts for this gene using the NGS method. We have gathered all known and newly discovered alternative transcripts in an annotated catalog of alternative transcripts of the *NBN* gene.

Grants:

Conflict of Interest: None declared

P15.077.B Adaptive sampling long-read sequencing to improve repeat expansion and methylation detection in neurological disease genetic diagnostics

Eddy de Boer[1;2], Arjen Scheper[1;2], Dennis Hendriksen[1;2], Bart Charbon[1;2], Laura Bosscher[1;2], Danielle Schoonhoven[1;2], Joukje Kloosterman[1;2], Lennart Johansson[1;2], Henny Lemmink[1;2], Jan Jongbloed[1;2], B. Sikkema-Raddatz[1;2], **Cleo van Diemen**[1;2]

[1]*University Medical Center Groningen, Genetics;* [2]*University of Groningen, Groningen*

Background/Objectives: Though short-read sequencing is increasingly becoming the standard method in genome diagnostic workflows, numerous other methods are still required. The detection of repeats, pseudogenes, complex genomic rearrangements and also important phasing, orientation and methylation information are difficult, or even impossible, to acquire using just short-read sequencing. This makes diagnostics time-consuming, expensive, difficult to maintain, and limited. To overcome this, we aim to introduce ONT to streamline and improve our diagnostic workflow.

Methods: We have designed a neurological disease gene panel for ONT adaptive sampling to simultaneously analyze genes carrying repeat regions, genomic regions where methylation status affects pathogenicity (e.g. *FRM1*) and genes with pseudogenes (e.g. *PMS2*). We optimized the laboratory process by altering various technical variables, such as short-read elimination, shearing methodologies and flowcell loading conditions. We used an in-house developed variant interpretation pipeline (https://github.com/molgenis/vip/). For validation we retrospectively sequenced twenty patients with a known variant in one of the included genes of the ONT adaptive sampling gene panel and determined the coverage needed for accurate variant calling.

Results: Our optimized technical workflow reaches an output of approximately 15 Gb (13-18 Gb) with a mean read-length of 10 Kb, resulting in 95% 30x coverage of 455/471 genes of our gene panel.

Currently, the validation is still ongoing, but first results show that repeat length determination and methylation status are fully concordant with diagnostic test results.

Conclusion: ONT seems a relevant technique in a diagnostic workflow and the possibilities of adaptive sampling are promising.

Grants:

Conflict of Interest: None declared

P15.078.C Involvement of *GSPT2* variants in a new X-linked inherited syndrome with developmental delay and epileptic encephalopathy

Helga Westers[1], *Jelkje de Boer-Bergsma[1], Elles Wierenga[1], Jasmine Kiers[1], Gea Beunders[1;2], Cleo van Diemen[1]*

[1]*University of Groningen, University Medical Centre Groningen;* [2]*Amsterdam UMC*

Background/Objectives: Here we describe a family with X-linked inheritance with one affected index boy and two presumably affected uncles with partly overlapping phenotypes, including central and obstructive apneas, severe epilepsy, profound developmental delay, a secondary microcephaly, mild nonspecific dysmorphic features and edema. We identified a VUS in the GTPase domain of *GSPT2*. *GSPT2* encodes eRF3b, which forms a complex with eRF3a (encoded by *GSPT1*). This complex is involved in translation termination. Here we describe the clinical and functional follow-up to prove causality.

Methods: New index patients were searched for via GeneMatcher. Wildtype and mutant eRF3b proteins were generated via an in vitro transcription translation kit followed by GTPase activity assays. Changes in translation termination efficiency were investigated via read-through assays in cell lines.

Results: A GeneMatcher query led us to five patients with similar phenotypes with variants in *GSPT2*. Using our newly developed cell-free assay we were able to generate wildtype and mutant eRF3b proteins. Preliminary data suggest impaired GTPase activity of at least one of the variant proteins. The read-through assay did not provide evidence that termination efficiency is impaired by this particular variant.

Conclusion: eRF3b missense variants are implicated in severe X-linked epileptic encephalopathy. We hypothesize that these variants disrupt GTPase activity of the eRF1/eRF3 complex which might affect polypeptide release from the ribosome. Ongoing functional studies aim to confirm this hypothesis. Furthermore, our cell-free GTPase activity assay has the potential to be broadly used for functional assessment of VUS located in GTPase domains of any protein.

Conflict of Interest: None declared

P15.079.D Benefits of the analysis of a complex chromosomal rearrangement in the implementation of long read approaches

Mathilde Quibeuf[1], Kevin Cassinari[1], Oriane Mercati[1], Anne-Marie Guerrot[1], Claude Houdayer[1], Geraldine Joly-Helas[1], **Pascal Chambon**[1]

[1]*Rouen Normandie Univ, Inserm U1245, CHU Rouen, Department of Genetics and reference Center for Developmental Disorders, Rouen, France*

Background/Objectives: The detection of structure variants by long-read approaches is one of the keys to the management of patients in diagnostic deadlocks. This work will show how the analysis of a highly complex chromosome rearrangement, such as a chromoanagenesis, helps to acquire new benchmarks and new interpretation logics specific to the different types of alterations that are now detectable by these techniques, especially by Optical Genome Mapping (OGM).

Method:

Results: A chromoanagenesis was identified in a 3-month-old girl presenting with mild dysmorphic elements, minor

malformations and a significant motor delay. This diagnosis was based on (i) the results of array-CGH showing a 8,3Mb 6q15q16.3 deletion, associated with seven smaller deletions and restricted to a limited number of chromosomes and (ii) the analysis of the karyotype showing an additional complex reciprocal translocation between chromosomes 5, 6 and 12. Long read analysis was performed by OGM for further characterization of the anomaly. Interestingly, OGM data helped new users to apprehend the different algorithms and tools. New users were guided to retrieve (i) all the CNVs detected by array CGH, (ii). the translocation identified by karyotyping, and also (iii) additional balanced structural variants. Both the graphical interface and the raw text result files were needed to fully characterize the chromoanagenesis.

Conclusion: This work illustrates the interest of analyzing a complex chromosomal anomaly in order to quickly apprehend the whole spectrum of anomalies identifiable by long read approaches. Sharing such data on complex rearrangements could help the community to improve the understanding and the use of long-read approaches.

Grants: None

Conflict of Interest: None declared

P15.080.A Highly sensitive variant detection in VNTR is possible with boosted exome sequencing: the example of MUC1-related nephropathy

Hassan Saei[1], *Vincent Moriniere*[2], *Laurence Heidet*[3], *Bertrand Knebelmann*[4], *Patrick Nischke*[5], *Corinne ANTIGNAC*[3], *Guillaume Dorval*[3]

[1]*Imagine Institute, Labortory of Hereditary Kidney Diseases, INSERM U1163, Paris, France;* [2]*Necker Children Hospital, Genetic Diagnosis Department, Paris, France;* [3]*Imagine Institute, Labortory of Hereditary Kidney Diseases, INSERM U1163, paris, France;* [4]*Necker Children Hospital, Nephrology Department, Paris, France;* [5]*Imagine Institute, Bioinformatics Department, Paris, France*

Background/Objectives: Tandem repeats in the human genome can be associated with significant and yet underdiagnosed genetic disorders. Frameshift variations in the *MUC1* coding-VNTR cause autosomal dominant tubulointerstitial kidney disease (ADTKD-*MUC1*). Short-read sequencing (SRS) is hindered by poor read mappability and motif complexity for discovering these variations. We developed a computational pipeline for precise variant discovery in the *MUC1* VNTR region from SRS data. We aimed to improve *MUC1* VNTR coverage in exomes since regular exome sequencing proved ineffective in this context.

Methods: Our pipeline utilizes k-mer frequency-based genotyping method and proven to be effieicnt in diagnosing 44 overlooked patients in a cohort of 3735 patients. We improved *MUC1* gene VNTR capture in exome sequencing by incorporating spike-in probes, which include probes with 4x tiling of the VNTR region, using the Twist custom panel protocol. We compared regular (six samples) and boosted exome data (16 samples) from known *MUC1*-positive and negative controls.

Results: The enhanced exome achieves a coverage of 144x for *MUC1* VNTR, compared to 72.1x in the regular exome. As expected, our genotyping tool missed pathogenic variations in 2 out of 3 true positives in the standard exome. However, we successfully detected all 13 true positives using the enhanced exome. Our pipeline identifies coverage below 100 as insufficient for VNTR genotyping in panel data, even with a mean coverage of 700x achieved after downsampling trials.

Conclusion: Adequate coverage is crucial for accurate VNTR genotyping. This approach could improve the diagnostic yield of the VNTR-associated diseases.

Grants: This work was supported by state funding (ANR-10-IAHU-01).

Conflict of Interest: Hassan Saei PPU-Imagine PhD Fellowship, PPU-Imagine PhD Fellowship, Vincent Moriniere Nephrologist, Necker Children Hospital, Paris, France, Laurence Heidet Nephrologist, Necker Children Hospital, Paris, France, Bertrand Knebelmann Nephrologist, Necker Children Hospital, Paris, France, Patrick Nischke: None declared, Corinne ANTIGNAC Principal investigator, Group leader, Guillaume Dorval Principal investigator

P15.081.B Dual molecular diagnoses of Cutis Laxa and Noonan Syndrome in Rwandan Individuals: A differential analysis of the facial dysmorphism with GestaltMatcher

Alexej Knaus[1], *Tzung-Chien Hsieh*[1], *Meghna Ahuja Bhasin*[1], *Nobert Dukuze*[2], *Olivier Hakizimana*[2], *Shahida Moosa*[3;4], *Uwe Kornak*[5], *Peter Krawitz*[1], *Annette Uwineza*[6]

[1]*University Hospital Bonn, Institute for Genomic Statistics and Bioinformatics, Bonn, Germany;* [2]*University of Rwanda, College of Medicine and Health Sciences, Kigali, Rwanda;* [3]*Stellenbosch University, Division of Molecular Biology and Human Genetics, Stellenbosch, South Africa;* [4]*Tygerberg Hospital, Medical Genetics, Stellenbosch, South Africa;* [5]*University Medical Center Göttingen, Institute for Human Genetics, Göttingen, Germany;* [6]*University Teaching Hospital of Kigali, Department of Clinical Biology, Kagali, Rwanda*

Background/Objectives: The detection of multiple Mendelian disorders in a single individual is complicated by the overlap or interference of gene-specific phenotypes. Here, we use next-generation phenotyping (NGP) to dissect the dual diagnosis of Cutis Laxa and Noonan Syndrome. Further we assessed the prediction of the disease-causing genes in published cases with various dual molecular diagnoses.

Methods: Exome sequencing was performed on a cohort of individuals with facial dysmorphisms from Rwanda, and data was prioritized with PEDIA (Prioritization of Exome-Data by Image Analysis). Additionally, multiple cases with diverse dual diagnoses, identified through a literature review, were subjected for facial analysis with GestaltMatcher.

Results: In two siblings from Rwanda, presenting with large fontanelles, developmental delay, and cardiac anomalies, pathogenic multilocus variations (PMVs) in *ATP6V0A2* (c.1935+1G>C and c.688dup) and in *PTPN11* (c.145G>A) were prioritized on the top ranks, confirming dual diagnosis with autosomal-recessive Cutis Laxa type IIa and Noonan syndrome 1.

Exemplarily, facial analysis of published cases revealed that coexistence of characteristic phenotypes with high similarities to both disorders is rare, but signals can be detected in the lower ranks of the GestalMatcher analysis. However, in some instances, PMVs seem to result in novel facial dysmorphism with little similarity to known disease patterns.

Conclusion: NGP is a promising technology for differential analysis of the facial gestalt complementing comparisons that are based on clinical features. GestaltMatcher may potentially identify novel genetic interactions. A practical implication is particularly significant for guiding the correct diagnosis and prediction of genes associated with rare diseases, especially in low-resource settings.

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declared, Shahida Moosa: None declared, Uwe Kornak: None declared, Peter Krawitz GeneTalk GmbH, Annette Uwineza: None declared

P15.082.C Beta evaluation of the AmplideX® PCR enrichment technology with ONT long-read DNA sequencing of a panel of eleven challenging genes

Laura Feyereisen[1], *Tony Yammine*[1], *Emilie Landais*[1], *Hélène Moret*[2], *Corinne Metay*[3], *Serge Pissard*[4;5], *Soumeya Bekri*[6;7], *Christine Bellanne-Chantelot*[8], *Anne-Sophie Lèbre*[9;10]

[1]*CHU de Reims, Laboratoire de Génétique, Reims, France;* [2]*CHU de Reims, Plateforme PFBM, Reims, France;* [3]*AP-HP Sorbonne Université, Hôpital Salpêtrière, Centre de Génétique Moléculaire et Chromosomique, UF de Cardiogénétique et Myogénétique Moléculaire et Cellulaire, Paris, France;* [4]*GHU Henri Mondor, Département de Génétique, Créteil, France;* [5]*Université Paris-Est Créteil (UPEC), Département de Génétique, Créteil, France;* [6]*CHU Rouen Normandie, Laboratoire de Biochimie Métabolique, Rouen, France;* [7]*Université Rouen Normandie, UNIROUEN, INSERM U1245, Rouen, France;* [8]*AP-HP Sorbonne Université, Hôpital Salpêtrière, Département de Génétique Médicale, Paris, France;* [9]*Université Reims Champagne-Ardenne (URCA), Reims, France;* [10]*Université Paris Cité, Institute of Psychiatry and Neuroscience of Paris (IPNP), INSERM U1266, [Kreb's team], Paris, France*

Background/Objectives: Short-read DNA sequencing is the main technical approach used in clinical diagnostics. However, for some challenging genes, additional non-NGS methods are required, such as PCR/CE, qPCR, MLPA or Sanger sequencing. To simplify user workflow, eleven traditionally hard-to-decipher genes (*CFTR, FMR1, HBA1/2, HBB, SMN1/2, F8, GBA, CYP21A2* and *TNXB)* have been pooled in a new prototype Asuragen kit. In this beta program, we evaluated the workflow on clinical samples and reviewed genotype agreement between the new assay and classical methods.

Methods: We evaluated the prototype Asuragen AmplideX Nanopore Carrier Plus kit, combining the PCR enrichment technology with Nanopore DNA sequencing (Oxford Nanopore Technologies; ONT). Target genes were amplified by LR-PCR from positive clinical samples. Libraries were prepared, pooled, and loaded on a MinION R10.4.1 flow cell. Automated analysis and reporting was performed using the prototype Asuragen AmplideX Reporter software.

Results: The eleven candidate genes were sequenced and multiple variant types were assessed, including STR, SNV, Indel, CNV or SV. Variant allele phasing was determined for multiple genes including *CFTR, HBA1/2, HBB, SMN1/2, GBA, CYP21A2* and *TNXB*. Variant zygosity, sequencing accuracy, and average error rate of ONT sequencing were evaluated.

Conclusion: We have been satisfied by the usability and performance of this assay, including the purpose-built software to enable accurate genotyping and reporting of challenging targets. We anticipate this assay will streamline testing conducted for screening as well as for diagnostic purposes.

Grant: Asuragen (PCR reagents, analysis software and laptop, library prep reagents) ONT (Mk1B, Flow cells, library prep reagents)

Conflict of Interest: None declared

P15.083.D Pilot project: Neonatal screening using genomic methods

Kristína Valovičová[1;2], *Iveta Valášková*[1;3;4]

[1]*Sophgena a.s., Praha 3, Czech Republic;* [2]*Masaryk University, Faculty of Science, Department of Experimental Biology, Section of Genetics and Molecular Biology, Brno, Czech Republic;* [3]*University Hospital Brno, Center of Molecular Biology and Genetics, Internal Hematology and Oncology Clinic, Brno, Czech Republic;* [4]*Masaryk University, Faculty of Medicine, Institute of Medical Genetics and Genomics, Brno, Czech Republic*

Background/Objectives: Rare illnesses are the primary contributors to infant death and lifelong disability. Genomic sequencing has revolutionized the diagnostic approach, offering many individuals a swift, precise, and economically viable genetic diagnosis. The use of genomic sequencing for neonatal screening has the ambition to improve and expand the early detection of treatable rare diseases, while the genomic data stored can potentially contribute to improved health care across the lifespan and support further research.

Methods: DNA was isolated from a dried blood drop and a buccal swab from newborns. KAPA HyperPETE Newborn Screening Panel commercial kit (Roche) was used for library preparation. Samples were also subjected to WES and subsequently analysed using the enhanced virtual panel of genes associated with rare diseases. Illumina DNA Prep with Enrichment and Twist Alliance VCGS were used. Libraries were sequenced using NovaSeq X Plus (Illumina).

Results: Using both approaches, we were able to capture pathogenic variants in control samples (CF, SMA, immunodeficiencies, CAH, PHE). These pathogenic variants in control samples were previously detected using NGS, Sanger sequencing, MLPA or allele specific PCR and qPCR assays and were interpreted based on relevant information in integrated databases of genomic variants and relevant scientific literature.

Conclusion: We tested DNA samples from newborns using a commercially available panel kit and WES to detect rare diseases. Both approaches seem to be suitable method for neonatal screening for rare diseases, but further studies are needed to compare the benefits and challenges (scientific, ethical and social aspects) of these two approaches.

Grants: MUNI/A/1556/2023

Conflict of Interest: None declared

P15.084.A Genome sequencing outperforms karyotyping, chromosomal microarray and exome sequencing for structural variants detection in prospective head-to-head comparison

Franziska Gaass[1], *Christina Post*[1], *Daniel Kaschta*[1], *Bianca Greiten*[1], *Anna-Sophie Liegmann*[1], *Rebecca. Gembicki*[1], *Jelena Pozojevic*[1], *Katharina Schau-Römer*[1], *Maj-Britt Salewski*[1], *Franka Rust*[1], *Christine Zühlke*[1], *Eva Maria Murga Penas*[1], *Nadine Hornig*[1], *Kirstin Hoff*[1], *Juliane Köhler*[1], *Vincent Arriens*[1], *Caroline Utermann-Thuesing*[1], *Varun Sreenivasan*[1], *Kristian Händler*[1], *Valerie Katharina Berge*[1], *Saranya Balachandran*[1], *Neseebullah Kakar*[1], *Maike Dittmar*[1], *Britta Hanker*[1], *Yorck Hellenbroich*[1], *Irina Hüning*[1], *Monika Kautza-Lucht*[1], *Almuth Caliebe*[1], *Inga Vater*[1], *Inga Nagel*[1], *Malte Spielmann*[1]

[1]*Institute of Human Genetics, University Hospital Schleswig-Holstein, University of Lübeck and Kiel University, Lübeck, Germany, Lübeck, Germany*

Consortium: UKSH Genom Konsortium

Background/Objectives: Genome Sequencing (GS) represents a powerful tool to detect all genetic variants in a single test including structural variants (SVs). However, karyotyping, chromosomal microarray (CMA) and exome sequencing (ES) are still part of standard diagnostic care in many European countries. Here we set out to conduct a prospective study to compare karyotyping, chromosomal CMA and ES for SVs detection with single GS and trio GS.

Methods: We prospectively recruited 345 individuals affected with developmental disorders and/or congenital anomalies and performed karyotyping, CMA and ES and in parallel short read single GS and trio GS. Three independent scientific teams then performed SVs analysis and compared results.

Results: We identified 34 diseases associated SVs (10,83%: 24 deletions, 7 duplications, 2 translocations, 1 mosaic-trisomy). All 34 variants were detected by single GS and trio GS, whereas 3 were missed by karyotyping, CMA and ES (3 deletions). The diagnostic gap was caused by single exon deletions with deep intronic breakends.

Conclusion: In our prospective head-to-head comparison GS outperformed karyotyping, CMA and ES for SVs detection. Based on our results, GS could replace karyotyping and CMA for SVs detection and improve care for rare diseases.

Grants:
Illumina supplied sequencing reagents for the study.

Conflict of Interest: Franziska Gaass provision of free of charge sequencing reagents and software by Illumina within the framework of a collaborative research study, Christina Post provision of free of charge sequencing reagents and software by Illumina within the framework of a collaborative research study, Daniel Kaschta provision of free of charge sequencing reagents and software by Illumina within the framework of a collaborative research study, Bianca Greiten provision of free of charge sequencing reagents and software by Illumina within the framework of a collaborative research study, Anna-Sophie Liegmann provision of free of charge sequencing reagents and software by Illumina within the framework of a collaborative research study, Rebecca. Gembicki provision of free of charge sequencing reagents and software by Illumina within the framework of a collaborative research study, Jelena Pozojevic provision of free of charge sequencing reagents and software by Illumina within the framework of a collaborative research study, Katharina Schau-Römer provision of free of charge sequencing reagents and software by Illumina within the framework of a collaborative research study, Maj-Britt Salewski provision of free of charge sequencing reagents and software by Illumina within the framework of a collaborative research study, Franka Rust provision of free of charge sequencing reagents and software by Illumina within the framework of a collaborative research study, Christine Zühlke provision of free of charge sequencing reagents and software by Illumina within the framework of a collaborative research study, Eva Maria Murga Penas provision of free of charge sequencing reagents and software by Illumina within the framework of a collaborative research study, Nadine Hornig provision of free of charge sequencing reagents and software by Illumina within the framework of a collaborative research study, Kirstin Hoff provision of free of charge sequencing reagents and software by Illumina within the framework of a collaborative research study, Juliane Köhler provision of free of charge sequencing reagents and software by Illumina within the framework of a collaborative research study, Vincent Arriens provision of free of charge sequencing reagents and software by Illumina within the framework of a collaborative research study, Caroline Utermann-Thuesing provision of free of charge sequencing reagents and software by Illumina within the framework of a collaborative research study, Varun Sreenivasan provision of free of charge sequencing reagents and software by Illumina within the framework of a collaborative research study, Kristian Händler provision of free of charge sequencing reagents and software by Illumina within the framework of a collaborative research study, Valerie Katharina Berge provision of free of charge sequencing reagents and software by Illumina within the framework of a collaborative research study, Saranya Balachandran provision of free of charge sequencing reagents and software by Illumina within the framework of a collaborative research study, Neseebullah Kakar provision of free of charge sequencing reagents and software by Illumina within the framework of a collaborative research study, Maike Dittmar provision of free of charge sequencing reagents and software by Illumina within the framework of a collaborative research study, Britta Hanker provision of free of charge sequencing reagents and software by Illumina within the framework of a collaborative research study, Yorck Hellenbroich provision of free of charge sequencing reagents and software by Illumina within the framework of a collaborative research study, Irina Hüning provision of free of charge sequencing reagents and software by Illumina within the framework of a collaborative research study, Monika Kautza-Lucht provision of free of charge sequencing reagents and software by Illumina within the framework of a collaborative research study, Almuth Caliebe provision of free of charge sequencing reagents and software by Illumina within the framework of a collaborative research study, Inga Vater provision of free of charge sequencing reagents and software by Illumina within the framework of a collaborative research study, Inga Nagel provision of free of charge sequencing reagents and software by Illumina within the framework of a collaborative research study, Malte Spielmann provision of free of charge sequencing reagents and software by Illumina within the framework of a collaborative research study

P15.085.B Integration of ExpansionHunter into the clinical variant interpretation platform EVAdb

Erik Tilch[1;2], *Martin Pavlov*[1;2], *Mohsen Shakibafar*[2], *Ivana Dzinovic*[1;2], *Michael Zech*[1;2], *Juliane Winkelmann*[1;2], *Christian Mertes*[1;2], *Riccardo Berutti*[1;2;3]

[1]*Institute of Neurogenomics, Computational Health Center, Helmholtz Munich, Neuherberg, Germany;* [2]*Institute of Human Genetics, Klinikum rechts der Isar, Technical University of Munich, Munich, Germany;* [3]*NGS Bioinformatics, Center for Advanced Studies, Research and Development in Sardinia, Pula, Italy*

Background/Objectives: More than 50 short tandem repeats (STR) expansion loci have been associated to monogenic disorders and most of them are detectable with ExpansionHunter on whole genome sequencing data. We implemented ExpansionHunter and a tool for clinical interpretation into the open source EVAdb Project. EVAdb is the raw data processing pipeline, variant call database, and diagnostic expert system in use at the Institute of Human Genetics of the University Hospital Klinikum rechts der Isar in Munich, Germany.

Methods: We examined the available literature, original and reviews, and OMIM to collect reliable information on pathogenic ranges of the STR expansions detectable with ExpansionHunter. We aggregated this information in an annotation table. Finally, we run our analysis on a cohort of more than 2500 genomes.

Results: We created an easily maintainable and expandable, literature-based annotation database that collapses distinct repeat count effect ranges from diverse sources into a single range, which can account for different phenotypes for the same locus and discrepancies between locus definition in ExpansionHunter and the literature. Additionally, we provided the system with a resource of clinical repeat count distributions, which increases with each sample analyzed. Z-scores for a patient's repeat counts are generated on the fly by the EVAdb, facilitating the detection of outliers e.g. based on the disease prevalence.

Conclusion: We introduced ExpansionHunter in EVAdb and created an expandable literature annotation resource and a resource of clinical distributions of repeat expansions for more than 2500 samples. These resources are available for clinicians and collaborators using EVAdb.

Conflict of Interest: None declared

P15.086.C Gene-Curator.org: A user-centric tool for managing gene disease associations

*Constantin Wolff[1], Nina Rank[1], Jan Halbritter[1], **Bernt Popp**[2]*

[1]Charité – Universitätsmedizin Berlin, Department of Nephrology and Internal Intensive Care Medicine, Berlin, Germany; [2]Berlin Institute of Health, Center of Functional Genomics, Berlin, Germany

Background/Objectives: ClinGen and other large expert groups are curating gene-disease entities, but still few genes have reliable gene-disease associations. We created "Gene-Curator.org" to simplify gene disease curation for genetic researchers by providing a user-friendly interface and a comprehensive database.

Methods: Utilizing a web-based platform developed with Vue, JavaScript, and Firebase as a backend, Gene-Curator provides functionalities for uploading gene lists and performing gene-disease curation. It is fully customizable, displays important genetic information for curation, and allows manual and automated annotations with a JSON configuration file-based workflow. The platform emphasizes user-friendly and automated interfaces to improve curation efficiency and accuracy. Freely available code makes the tool easy to install standalone.

Results: We used ClinGen recommendations to simplify the curation workflow for kidney disease (KD) diagnostic genes. The workflow includes pre-curation, curation, and a curated entity repository. Lumping and splitting create gene-disease entities during pre-curation. In the curation section, entities are assigned to KD groups (e.g., syndromic vs. isolated; adult vs. pediatric) and given a point-score which results in four confidence levels. Tracking of curation workflow versions, users involved in the curation, and a second look system enable reproducibility and quality control. The design makes curation easy, fast, intuitive, reproducible, and shareable.

Conclusion: Gene-Curator is a versatile tool that significantly aids researchers in gene data analysis and curation, thanks to a strong and intuitive software design that prioritizes user engagement and data accuracy. Future developments include a versioned curation workflow repository, data sharing with PanelApp, and federated instance communication.

Grants: None.

Conflict of Interest: Constantin Wolff: None declared, Nina Rank DFG, Clinician scientist program Charité, Charité, Jan Halbritter DFG, Charité, Bernt Popp DFG, Charité, Labor Berlin

P15.087.D High prevalence of GAA-*FGF14*-related late-onset spinocerebellar ataxia in a Dutch cohort

Ana Ignjatijevic[1;2], *Elles Wierenga[1;2], Femke Boorsma[1;2], Fleur Vansenne[1;2], Corien Verschuuren[1;2], Dineke Verbeek[1;2], Helga Westers[1;2], Cleo van Diemen[1;2]*

[1]University Medical Centre Groningen, Genetics, Groningen; [2]University of Groningen, Groningen, Netherlands

Background/Objectives: A pathogenic GAA repeat expansion in the first intron of the fibroblast growth factor 14 gene (*FGF14*) has been recently identified as a highly prevalent cause of spinocerebellar ataxia 27B (SCA27B) in various populations. We screened 262 Dutch cases with late-onset spinocerebellar ataxia for *FGF14* repeat expansions using a combination of long-range PCR (LR-PCR) and repeat-primed PCRs.

Methods: Genomic DNA samples were first tested by LR-PCR spanning the GAA-expansion to determine the presence of expanded alleles. Next, the presence of the correct repeat motif was verified by repeat-primed PCR of the GAA and another prevalent GAAGGA repeat. Several samples were validated by Oxford Nanopore Technologies long read sequencing.

Results: Overall, 48/262 patients had an expanded *FGF14* repeat based on LR-PCR. Of these, 34 were identified to have a likely pathogenic expanded allele of $(GAA)_{>250}$, resulting in a total diagnostic yield of 13%. Long read sequencing confirmed the sizes and sequence of the repeats.

Conclusion: We conclude that *FGF14* GAA repeat expansions are the most common known genetic cause of late-onset spinocerebellar ataxia in the Dutch population, which is comparable to studies in other populations.

Conflict of Interest: None declared

P15.088.A lonGER - German National initiative for clinical long-read sequencing

Nadja Ehmke[1], *Florian Kraft[2], Gunnar Schmidt[3], Elena Buena-Atienza[4], Nicolas Casadei[4], Casper Groß[4], Sebastian Giesselmann[2], Thomas Eggermann[2], Benedikt Schnur[3], Manuel Holtgrewe[5], Ingo Kurth[2], Janine Altmüller[6], Stephan Ossowski[4], Tobias Haack[4], Bernd Auber[3]*

[1]Charité - Universitätsmedizin Berlin, Institute of Medical and Human Genetics; [2]RWTH Aachen University Hospital, Institute for Human Genetics and Genomic Medicine; [3]Hannover Medical School, Department of Human Genetics; [4]University of Tübingen, Institute of Medical Genetics and Applied Genomics; [5]Berlin Institute of Health, Core Unit Bioinformatics; [6]Berlin Institute of Health, Core Unit Genomics

Background/Objectives: With over 50% of rare disease patients unresolved after short-read genome sequencing (SR-GS), 'solving the unsolved' genetic diseases is arguably the largest unmet need in clinical genetics. Streamlined diagnostic-grade implementation of long-read (LR) GS is expected to improve accurate variant detection and interpretation. We founded the lonGER consortium to evaluate the clinical utility of LR-GS and provide a scalable framework with harmonized data background and statistical methodology for data interpretation.

Methods: To demonstrate increased diagnostic sensitivity of LR-GS, we are sequencing a multi-center cohort of unsolved patients with neurological, neurodevelopmental, and imprinting disorders that is expectedly enriched for complex genomic variation. We will implement, for the first time, LR-GS in the diagnostic work-up of a prospective cohort of patients (250 trios and 250 singletons) with a broad range of clinical indications including rare diseases and cancer predisposition. Sequencing is performed on PromethION instruments at 4 sequencing centers: Aachen, Berlin, Hannover, and Tübingen. A pilot study was conducted to demonstrate their ability to generate high quality datasets and apply appropriate bioinformatics pipelines.

Results: 80 reference and patient samples were sequenced with LR- and SR-GS and prospective inclusion of patient samples is ongoing. Our benchmarking studies support the increased sensitivity of LR-GS to detect different types of (epi-)genetic variation.

Conclusion: The lonGER pilot study has demonstrated the advantages of LR-GS in routine clinical diagnostics. Over the next two years, lonGER will provide a unique data set for the evaluation of LR-GS in clinical diagnostics through LR-GS of 1000 deeply phenotyped individuals.

Conflict of Interest: None declared

P15.089.B Genetic mosaicism: an important element to take under consideration for accurate genetic counselling

Sofia Ourani[1;2;2], *Katherine Anagnostopoulou*[3], *Nikolaos Marinakis*[4], *Emily Athanasiou*[1], *christiana polydorou*[2;2;5], *Avraam Elia*[6], *Jan Traeger-Synodinos*[4], *Constantinos Deltas*[2;5], *Athina Ververi*[7;8;8]

[1]*Hospital Archibishop Makarios III, Clinical Genetics, Nicosia, Cyprus;* [2]*University of Cyprus, School of Medicine, Nicosia, Cyprus;* [3]*Neoscreen Laboratory, Athens, Greece;* [4]*St. Sophia's Children's Hospital, National and Kapodistrian University of Athens, Laboratory of Medical Genetics, Medical School, Athens, Greece;* [5]*University of Cyprus, 4. biobank.cy, Center of Excellence in Biobanking and Biomedical Research, Nicosia, Cyprus;* [6]*Hospital Archibishop Makarios III, Paediatric Department, Nicosia, Cyprus;* [7]*Papageorgiou Hospital, Genetic Unit, Thessaloniki, Greece;* [8]*Aristotle University of Thessaloniki, School of Medicine, Thessaloniki, Greece*

Background/Objectives: Somatic mosaicism refers to the presence of two or more populations of genetically distinct cells within an individual. Current literature estimates the percentage of parental mosaicism ranging from 0.33 to 25.9%.

Methods: We describe three unrelated patients in whom parental mosaicism was proved through detailed investigation.

Results: The first patient is a boy diagnosed with a pathogenic variant in *GRID2* gene due to motor delay and ataxic gait, which was considered to be of de novo origin at first hand. The couple had a second pregnancy and through the prenatal testing which showed the same mutation in the fetus, low level of mosaicism for the causative variant in the father's sample of DNA extracted from peripheral blood was identified. The second child is a boy studied for epilepsy and neurodevelopmental disorder. Trio Whole exome sequence revealed a pathogenic variant in *YWHAG* gene, which was also identified in DNA of leukocytes of the mother in a mosaic state (~18%). Our third case is an adult patient who was studied after his brother died of complications of vascular Ehlers Danlos syndrome due to a pathogenic variant in *COL3A1* gene. Mosaicism was shown in the maternal sample.

Conclusions: Recent data support the hypothesis that somatic and/or germinal mosaicism should be considered in the pathogenicity of inherited human diseases. New advanced diagnostic tools give us the opportunity to detect mosaicism more easily and accurately than before, thus facilitating precise genetic counseling, prenatal diagnosis as well as creating appropriate plans for pregnancy and delivery.

Conflict of Interest: None declared

P15.090.C 1 Sequencing per life

Filip Zembol[1], *Martina Bittoova*[1], *Monika Koudová*[1], *Petr Klempt*[1], *Simona Suhajova*[1], *Hana Dvorackova*[1], *Barbora Honysová*[1]

[1]*GNT Labs by GENNET, Bioinformatics*

Background/Objectives: The 1 sequencing per life project is designed to improve genetic care for patients and also reduce the costs associated with sequencing and the need to resample the patient. The goal is to create a sufficiently robust sequencing panel and workflow to get all important genetic information about patients.

Methods: The first phase was the creation of a robust LIMS (GLIS) to automatically pass samples from the requisition to the laboratory. The next phase was the creation of a robust bioinformatics analysis from sample prioritization and selection, through registration and evaluation environment to report generation. The last phase is the automation and proper interfacing of the sub-pipelines backed by CEIVD software.

Results: Creating a robust LIMS with the right database structure ensured an automated system for receiving requests into the lab and prioritizing them for deployment for sequencing. Automation and integration of multiple bioinformatics approaches ensured correctness of outputs. The next phase was sub-panelisation and panel-specific bioinformatics analyses. The automatically linked reporting system generated panel-specific reports after manual validation.

Conclusion: This project has simplified the process of passing the sample from Doctor to patient, with faster and more accurate genetic outputs. Automation has also prevented human error with such a large volume of samples. Furthermore, the large database of exome variants used for population studies is a great advantage.

Grants: No grants.

Conflict of Interest: Filip Zembol fulltime, Martina Bittoova fulltime, Monika Koudová: None declared, Petr Klempt: None declared, Simona Suhajova: None declared, Hana Dvorackova: None declared, Barbora Honysová: None declared

P15.091.D A multimodal approach to molecular diagnosis in NF1 highlights the need for bespoke analysis in undiagnosed patients

Ellie McAleese-Park[1], *Emily Whittle*[2], *Laura Weir*[2], *Claire Kyle*[2], *Giles Holt*[3], *Brian Wilson*[2;3]

[1]*Newcastle University, Faculty of Medical Sciences, Newcastle upon Tyne, United Kingdom;* [2]*Northern Genetics Service, Newcastle-upon-Tyne, United Kingdom;* [3]*Newcastle University, Biosciences Institute, Newcastle upon Tyne, United Kingdom*

Background/Objectives: Neurofibromatosis type 1 (NF1) is a complex autosomal dominant disorder, characterised by a susceptibility to developing nerve sheath tumours and a range of potential multisystemic complications. Genetic confirmation of the diagnosis can provide prognostic information, and enable reproductive decision making and definitive cascade testing in relatives. Diagnostic testing in NHS England involves DNA/RNA analysis and copy number evaluation of the *NF1* gene. The molecular diagnostic rate among clinically affected individuals is ~90% with DNA analysis, and ~95% with RNA analysis. As NF1 is a monogenic disorder with a highly specific phenotype, this suggests that some causative variants are missed using standard diagnostic approaches. We aimed to explore the use of non-standard techniques to achieve a molecular diagnosis in this scenario.

Methods: We adopted a multimodal approach to further investigation in a patient with classical NF1, using techniques not available in standard NHSE workflows, including Bionano optical genome mapping, long-range PCR and Nanopore Flongle sequencing

Results: Bionano analysis identified a 6kbp insertion into intron 1 of the *NF1* gene. Long-range PCR was used to generate a 12kb fragment for Nanopore Flongle sequencing, allowing us to identify the breakpoints.

Conclusion: We achieved a molecular diagnosis of NF1 using a multimodal approach to investigation in this patient, allowing definitive testing of at risk relatives. This highlights the need for diagnostic laboratories to have a spectrum of technologies at their disposal to further investigate those with a firm clinical, but not molecular, diagnosis.

Grants: This work was supported by the Newcastle Hospitals Charity.

Conflict of Interest: None declared

P15.092.A EVAdb: a comprehensive clinical variant interpretation platform incorporating automated scalable analysis workflows

Martin Pavlov[1;2], *Erik Tilch*[1;2], *Mohsen Shakibafar*[1], *Matias Wagner*[1;2], *Michael Zech*[1;2], *Elisabeth Graf*[1], *Thomas Meitinger*[1],

Thomas Wieland[2], Thomas Schwarzmayr[2], Holger Prokisch[1;2], Juliane Winkelmann[1;2], Christian Mertes[1;2], Tim Strom[1], Riccardo Berutti[1;2;3]

[1]Klinikum rechts der Isar, Institute of Human Genetics, München, Germany; [2]Helmholtz Munich, Institute of Neurogenomics, Oberschleißheim, Germany; [3]Center for Advanced Studies, Research and Development in Sardinia, NGS Bioinformatics, Pula, Italy

Background/Objectives: Next-Generation Sequencing (NGS) has catalysed a paradigm shift in genomics, advancing clinical and research frontiers. The vast data volumes generated by NGS platforms demand advanced data management solutions, intricate analysis pipelines, and a meaningful interpretation platform to bridge genomic findings with clinical utility. The EVAdb framework is developed to address these challenges, offering a unified platform that streamlines NGS data processing, ensures analytical and statistical rigour, and translates complex genomic datasets into actionable insights for diagnostics and research.

Methods: EVAdb employs a modular implementation approach, integrating a lab inventory and sample management system, NGS processing and analysis pipelines, a variant interpretation and evaluation platform, as well as visualisation and quality assessment utilities. The platform supports a growing range of analyses, such as exome, genome, RNA-, ChIP-, and ATAC-sequencing. It is designed for accredited diagnostic purposes and is expanding with additional analysis workflows and cloud-ready capabilities (open-source, GitHub). EVAdb accommodates sample submission, library preparation, sequencing monitoring, and downstream analysis, all through a specialised web interface.

Results: To date EVAdb has processed 40,312 exomic, 5,909 genomic, and 31,810 RNA sequences of diverse backgrounds, aiding the molecular diagnosis of 3,409 rare genetic disorder patients and the identification of over 100 novel disease genes. Hence, it has been selected as the basis for various large-scale collaborative local and international efforts.

Conclusion: Established as a comprehensive framework for NGS analysis, EVAdb significantly enhances molecular diagnostics and the study of rare genetic disorders, making a case for its wider adoption in the genomic community.

Grants: Bavarian Genomes, GENOMIT, DigiMed, mitoNET.

Conflict of Interest: None declared

P15.094.C Diagnosis journey and main determinants of diagnosis delays of people living with a rare disease in Europe: a Rare Barometer retrospective patient survey

Jessie Dubief[1], *Fatoumata Faye[1]*

[1]EURORDIS-Rare Diseases Europe, Rare Barometer, Paris, France

Background/Objectives: Identifying the main steps of the diagnosis journey and the main determinants of the time to diagnosis for people living with a rare disease in Europe.

Methods: Retrospective survey of 6,507 (family members of) people diagnosed with 1,675 different rare diseases.

Results: Descriptive analysis of the number of years between symptom onset and each step of the diagnosis journey:

	Mean	Lower quartile	Median	Upper quartile	Maximum	N
First medical contact	0.4	0.0	0.0	0.3	45.0	5,762
First symptomatic treatment	3.4	0.0	0.5	3.3	60.6	5,535
Initial diagnosis	3.4	0.0	0.3	3.3	70.0	6,232
First referral to a Centre of Expertise	3.6	0.0	0.3	3.0	70.0	3,420
Confirmed diagnosis	4.7	0.1	0.8	5.0	71.2	6,507

Main determinants of diagnosis delays (ordinal logistical regression):

	OR (95% CI)
Number of healthcare professionals consulted	5.15 (4.1–6.4)
Symptom onset at adolescence (10-19 years old)	4.79 (3.7–6.2)
Symptom onset during childhood (2-9 years old)	3.11 (2.4-4.0)
Misdiagnosis	2.48 (2.1–2.9)
Living in Northern Europe	2.15 (1.8–2.6)
Patient is a woman	1.22 (1.1–1.4)
Living in Western Europe	1.96 (1.6–2.3)
Family history of the rare disease	1.36 (1.1–1.6)
Needs for psychological support unmet	1.34 (1.2–1.5)
Genetic disease	1.33 (1.1–1.5)
Referral to a Centre of Expertise	1.17 (1.0-1.3)
Needs for financial support unmet	1.16 (1.0-1.3)

Conclusion: These results can inform policies and actions to improve access to diagnosis for all people living with a rare disease.

Grants: Operating Grants 2022 and 2023 from the European Union's Health Programme (EU4Health, 2021-2027).

Conflict of Interest: None declared

P15.095.D Evaluating global metabolomics profiling as a diagnostic tool for rare diseases

Adam Kennedy[1], *Karen DeBalsi[1], Kaisa Teele Oja[2], Karit Reinson[2], Mihkel Ilisson[2], Katrin Ounap[2], Laura Sommerville[1], Matt Mitchell[3], Anne Evans[4]*

[1]Metabolon, Research and Development, Morrisville, United States; [2]Institute of Clinical Medicine, University of Tartu and Genetics and Personalized Medicine Clinic, Tartu, Estonia; [3]Metabolon, Data Sciences and Statistics, Morrisville, United States; [4]Metabolon, Platform Operations, R&D, Morrisville, United States

Background/Objectives: Here, we evaluated global metabolomics profiling as a diagnostic tool for rare diseases.

Methods: We analyzed the plasma metabolomic profiles of 133 persons suspected of having a rare disease but without a conclusive diagnosis. Metabolite raw peak intensities were normalized, log-transformed, and Z-scored. Metabolite concentrations higher than the 97.5th percentile or lower than the 2.5th percentile of the normal range were flagged as potential diagnostic markers.

Results: We detected 1237 compounds in the cohort, covering 9 super pathways and 120 sub-pathways. Every patient demonstrated metabolic perturbations, the majority of which could be linked to disease. For example, one patient demonstrated

significantly elevated carnitines and long-chain fatty acids, which are characteristic of very-long-chain acyl-CoA dehydrogenase deficiency. Another patient showed significant decreases in carnitine and acylcarnitines, suggesting systemic primary carnitine deficiency. In a third example, a set of twins both demonstrated a significant increase in arginine and citrulline, a decrease in ornithine, and the presence of argininosuccinate, which altogether, suggest dysfunctional urea cycle metabolism. In cases where validated metabolic signatures of disease were not observed, perturbations aligned with patients' symptomologies, suggesting that global metabolomics profiling can elucidate novel disease signatures and inform on the patient's clinical phenotype.

Conclusion: While further studies are needed to confirm the suspected diagnoses and characterize novel disease signatures, this study supports the hypothesis that global metabolomics can narrow the focus of diagnostic testing for rare diseases to ultimately reduce the time-to-diagnosis and improve patient outcomes.

Grants: PRG471, PRG2040

Conflict of Interest: None declared

P15.096.A DNA methylation profiling in Kabuki syndrome: reclassification of germline *KMT2D* VUS and sensitivity in validating postzygotic mosaicism

Marcello Niceta[1], *Andrea Ciolfi*[1], *Marco Ferilli*[1;2], *Lucia Pedace*[3], *Camilla Cappelletti*[1], *Claudia Nardini*[1], *Mathis Hildonen*[4], *Luigi Chiriatti*[1], *Evelina Miele*[3], *Maria Lisa Dentici*[5], *Maria Gnazzo*[6], *Claudia Cesario*[6], *Elisa Pisaneschi*[6], *Anwar Baban*[7], *Antonio Novelli*[6], *Maitz Silvia*[8], *Angelo Selicorni*[9], *Gabriella Maria Squeo*[10], *Giuseppe Merla*[10;11], *Bruno Dallapiccola*[1], *Zeynep Tümer*[4;12], *Maria Cristina Digilio*[5], *Manuela Priolo*[13], *Marco Tartaglia*[1]

[1]*Bambino Gesù Children's Hospital, IRCCS, Molecular Genetics and Functional Genomics Research Unit, Rome;* [2]*Sapienza University of Rome, Department of Pediatric Hematology/Oncology, Roma, Italy;* [3]*Bambino Gesù Children's Hospital, IRCCS, Department of Pediatric Hematology/Oncology, Cell and Gene Therapy, Rome;* [4]*Copenhagen University Hospital, Rigshospitalet, Department of Clinical Genetics, Kennedy Center, København, Denmark;* [5]*Bambino Gesù Children's Hospital, IRCCS, Medical Genetics Unit, Rome;* [6]*Bambino Gesù Children's Hospital, IRCCS, Laboratory of Medical Genetics, Rome;* [7]*Bambino Gesù Children's Hospital, IRCCS, Department of Pediatric Cardiology and Cardiac Surgery, Rome;* [8]*Ospedale San Gerardo, Genetica Clinica Pediatrica, Monza, Italy;* [9]*Sant'Anna Hospital - Como, Pediatria, Como, Italy;* [10]*Casa Sollievo della Sofferenza, Laboratory of Regulatory and Functional Genomics, San Giovanni Rotondo, Italy;* [11]*University of Naples Federico II, Department of Molecular Medicine and Medical Biotechnology, Napoli, Italy;* [12]*The University of Copenhagen, Faculty of Health and Medical Sciences, Department of Clinical Medicine, København, Denmark;* [13]*Antonio Cardarelli Hospital, Medical and Laboratory Genetics, Napoli, Italy*

Consortium:

Abstract text

Background/Objectives: Autosomal dominant Kabuki syndrome (KS) is a rare neurodevelopmental disorder caused by heterozygous inactivating variants of the lysine-specific methyltransferase 2D (*KMT2D*) gene. Despite the distinctive gestalt, the disorder is clinically variable, and a phenotypic scoring system has been introduced to help clinicians reach a clinical diagnosis. The phenotype, however, can be less pronounced in some patients, including those carrying postzygotic mutations. The full spectrum of pathogenic variation in *KMT2D* has not been fully characterized, which may hamper the clinical classification of a portion of these variants.

DNA methylation (DNAm) profiling has previously been used as a tool to classify variants in genes associated with several neurodevelopmental disorders, including KS.

Methods: We applied a KS-specific DNAm signature, optimized by a machine learning based classifier, in a cohort of 30 individuals with *KMT2D* variants and clinical features of KS.

Results: We succeeded in correctly classifying all the tested individuals. Three *KMT2D* variants were upgraded to disease-causing, while for 10 variants, a pathogenic role in the context of KS was rejected.

By testing five individuals with postzygotic pathogenic *KMT2D* variants, we also provided evidence that DNAm profiling has power to recognize pathogenic variants at different levels of mosaicism, identifying 15% as the minimum threshold.

Conclusion: The use of DNAm profiling applied for functional classification of *KMT2D* variants and postzygotic mosaicism in the context of KS demonstrates that it is a robust and reliable tool to rapidly rule out or confirm a clinical suspicion of this disorder.

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Conflict of Interest: None declared

P15.097.B Assessing the clinical utility of a breakpoint analysis method in identification of structural variants in targeted sequencing genetic tests

Lotta Koskinen[1], *Margarita Andreevskaya*[1], **Antti Väisänen**[1], *Mikko Muona*[1], *Tuuli Pietilä*[1], *Janica Djupsjöbacka*[1], *Ville Kytölä*[1], *Kati Kämpjärvi*[1], *Samuel Myllykangas*[1], *Pertteli Salmenperä*[1], *Juha Koskenvuo*[1], *Miko Valori*[1]

[1]*Blueprint Genetics, Espoo, Finland*

Background/Objectives: Structural variants (SVs) are a diverse group of genetic alterations impacting gene copy number, structure, and regulation. Despite their clinical relevance certain SV types are typically not detected by standard short-read variant calling pipelines. We assessed the frequency and types of clinically relevant SVs in targeted NGS genetic testing using a breakpoint analysis method.

Methods: Samples referred to clinical genetic testing were sequenced using whole or clinical exome assay and a short-read Illumina platform at Blueprint Genetics laboratory. SV calling was done using an in-house developed read breakpoint method that combines read-pair (RP) orientation-based and split-read(SR)/clipped-read based approaches to detect deletions, tandem duplications, inversions, novel insertions and retrotransposon (RTE) insertions such as Alu-insertions. Only variants undetected by standard variant calling methods and those classified as pathogenic, likely pathogenic, or of uncertain significance were considered.

Results: In the analysis of ~80,000 samples, we detected 162 clinically relevant SVs from which 79% were diagnostic and 21% were considered a carriership in a recessive disorder. RTE insertions, specifically Alu-insertions, were the most common type of SV. The second most identified type was deletions.

Conclusion: SV detection based on read breakpoints enables the detection of clinically relevant SVs that are not recognized by standard NGS variant callers, increasing diagnostic yield even in targeted sequencing. Besides detecting additional SVs, breakpoint analysis results can be used to assist design of orthogonal confirmation of copy-number variants and can decrease the need for confirmation tests.

Grants:

Conflict of Interest: Lotta Koskinen Blueprint Genetics Ltd, Margarita Andreevskaya Blueprint Genetics Ltd, Antti Väisänen

Blueprint Genetics Ltd, Mikko Muona Blueprint Genetics Ltd, Tuuli Pietilä Blueprint Genetics Ltd, Janica Djupsjöbacka Blueprint Genetics Ltd, Ville Kytölä Blueprint Genetics Ltd, Kati Kämpjärvi Blueprint Genetics Ltd, Samuel Myllykangas Blueprint Genetics Ltd, Blueprint Genetics Ltd, Pertteli Salmenperä Blueprint Genetics Ltd, Juha Koskenvuo Blueprint Genetics, Blueprint Genetics, Miko Valori Blueprint Genetics Ltd

P15.099.D Diagnostic genome sequencing improves genetic testing workup in imprinting disorder phenotypes: a retrospective study

Luise Kessler[1], Jeremias Krause[1], Florian Kraft[1], Asmaa Kenawy[2], Gyorgy Fekete[3], Anna Lengyel[3], Cordula Knopp[1], Ingo Kurth[1], Miriam Elbracht[1], **Thomas Eggermann**[1]

[1]RWTH Aachen, Human Genetics and Genomic Medicine, Aachen; [2]Alexandria University, Human Genetics; [3]Semmelweis University, Budapest, 2nd department of pediatrics

Background: The molecular confirmation of imprinting disorders (ImpDis) is challenging due to their clinical and molecular heterogeneity and overlap to monogenic differential diagnoses (DD). Therefore, many patients do not receive a diagnosis with the current stepwise and targeted diagnostic procedure. To circumvent the limitations, we assessed the suitability of whole genome sequencing (WGS) as diagnostic step after methylation testing and exclusion of ImpDis-specific alterations.

Methods: WGS was conducted in 46 clinically diagnosed patients with Silver-Russell- (SRS n = 35) and Beckwith-Wiedemann syndrome (BWS n = 11). Parental samples were available in 27 cases for trio-analysis. Data processing was based on an integrated AI pipeline (emedgene/ILMN). Targeted variant types included SNVs, CNVs, uniparental disomy, and repeats.

Results: 14 pathogenic variants and 4 VUS could be identified. The detection rate differed markedly between the ImpDis. Whereas 37% referred as SRS were diagnosed with SRS-specific monogenic alterations or DD, no pathogenic variants were detected in the clinically suspected BWS-cohort.

Conclusions: WGS significantly improves the diagnostic yield in patients with phenotypically suspected SRS, broadens the spectrum of overlapping disorders and allows further insights into the etiology of this heterogeneous disorder. A precise molecular diagnosis provides the basis for personalized clinical management. The negative results in the BWS-cohort might be explained by the lower threshold of clinical criteria, and the mosaic occurrence of overgrowth-causing alterations. However, WGS should be implemented as the second-tier diagnostic tool, as it addresses all currently known variant types in differential diagnoses of ImpDis and shortens the diagnostic odyssey.

Grant: In-kind reagents provided by Illumina.

Conflict of Interest: None declared

P15.101.B Advantages of genome sequencing in a large cohort of exome-negative cases

Konrad Platzer[1], Eva Berger[1], Brandon Bresack[1], Luise Kulosik[1], Christian Stockhaus[1], Monica Wojcik[2;3], Dagmar Wieczorek[4], Steffen Syrbe[5], Vincent Strehlow[1], Heidi Rehm[3], Maximilian Radtke[1], Denny Popp[1], Tilman Polster[6], Henry Oppermann[1], Johannes Lemke[1], Gabrielle Lemire[3], Anne O'Donnell-Luria[3], Andreas Merkenschlager[7], Julia Hentschel[1], Janina Gburek-Augustat[7], Tobias Bartolomaeus[1], **Rami Abou Jamra**[1]

[1]University of Leipzig Hospitals, Institute of Human Genetics, Germany; [2]Divisions of Newborn Medicine and Genetics and Genomics, Boston Children's Hospital, United States; [3]Broad Center for Mendelian Genomics, Broad Institute of MIT and Harvard, United States; [4]University of Duesseldorf Hospitals and Clinics, Institute of Human Genetics, Germany; [5]Department of Pediatrics, University of Heidelberg Hospitals and Clinics, Germany; [6]University of Bielefeld Hospitals, Department of Pediatrics, Mara Hospital, Germany; [7]University of Leipzig Hospitals, Department of Pediatrics, Germany

Background/Objectives: Half of rare disorder remain elusive after exome sequencing (ES). However, the benefit of genome sequencing (GS) is still poorly defined.

Methods: We performed GS (trio in 90%) using standard wet-lab techniques for 220 individuals (n = 350 in June), who remained undiagnosed after ES. We used the software Emedgene, Varvis, and ExpansionHunter to analyze single nucleotide, small indel, repeat expansion, mitochondrial and copy number variants.

Results: We successfully solved 9% of the cases. We partially or possibly solved further 9%. Careful evaluation showed that a third of the above-mentioned solved cases clearly required GS and that ES is not sufficient to identify the relevant variant. Identified variants were small copy number variants, repeat expansions and (relatively few) non-coding variants. In the other two thirds, GS solved the cases due to trio design or as it uncovered human errors in the initial ES analysis. Further reasons were progress in public databases and a better software. We observe that GS depends more on having differential diagnoses. Two major, but solvable inconveniences are the difficulty of evaluating non-coding variants, and structural variants without gain or loss of DNA. We joined forces with Wojcik, O'Donnell-Luria, et al., medRxiv, who obtained similar results of 744 GS cases.

Conclusion: GS clearly outperforms ES in identifying copy number variants and repeat expansions. Further benefits with room for improvements are non-coding variants and structural variants. GS is the better first line analysis as it has a higher yield and it avoids time-consuming step-by-step diagnostics.

Grants: none

Conflict of Interest: None declared

P16

BIOINFORMATICS, MACHINE LEARNING AND STATISTICAL METHODS

P16.002.A Integrative multi-omics analysis leverage molecularly-defined subgroup identification: A novel subgroup of UCHL1-related cancers is associated with genomic instability and sensitivity to DNA-damaging treatment

Sebastian Burkart[1;2], *Christopher Weusthof[2], Karam Khorani[2], Sonja Steen[2], Jochen Heß[2;3]*

[1]Institute of Human Genetics, University Heidelberg, Heidelberg; [2]Section Experimental and Translational Head and Neck Oncology, Heidelberg University; Departement of Otolaryngology, Heidelberg; [3]Research Group Molecular Mechanisms of Head and Neck Tumors, German Cancer Research Cencer, Heidelberg

Background/Objectives: Identification of molecularly-defined cancer subgroups and targeting tumor-specific vulnerabilities have the potential to improve treatment response and patient outcomes, but remain an unmet challenge of high clinical relevance.

The establishment of a comprehensive multi-omics analysis approach allows versatile molecular subgroup characterization in head and neck squamous cell carcinoma (HNSC), which is easily adaptable to multiple clinical questions.

Methods: We established an UCHL1-related gene-set to identify and molecularly characterize an UCHL1-related subgroup by

integrative analysis of multi-omics data. A xgbTree-model was trained with TCGA-HNSC based on GSVA scores for gene-sets of MSigDB to robustly predict UCHL1-related cancers. Potential vulnerabilities of UCHL1-related cancer cells were elucidated by an in-silico drug screening approach.

Results: This study describes a versatile integrative multi-omics workflow for identification of molecularly-defined subgroups in cancer.

UCHL1-related HNSC were characterized by higher frequencies of genomic alterations, which was also evident for UCHL1-related cancers of other solid tumors predicted by the classification model. These data indicated an impaired maintenance of genomic integrity and vulnerability for DNA-damaging treatment, which was supported by a favorable prognosis of UCHL1-related tumors after radiotherapy, and a higher sensitivity to irradiation or DNA-damaging compounds (e.g. Oxaliplatin).

Conclusion: Our study established UCHL1-related cancers as a novel subgroup across most solid tumor entities with a unique molecular phenotype and DNA-damaging treatment as a specific vulnerability.

The described workflow of integrative multi-omics analysis is a versatile tool for molecularly-defined subgroup identification and elaboration of treatment targets. It might be transferable to different clinical questions to leverage translational research in the field of multi-omics.

Grants: NA

Conflict of Interest: None declared

P16.003.B Integration of variant annotations using deep set networks boosts rare variant association genetics

*Brian Clarke[1;2], **Eva Holtkamp**[3;4], Hakime Öztürk[1], Marcel Mück[2], Magnus Wahlberg[2], Kayla Meyer[2], Felix Munzlinger[2], Felix Brechtmann[3], Florian R. Hölzlwimmer[3], Julien Gagneur[3;4;5;6], Oliver Stegle[1;7;8;9]*

[1]German Cancer Research Center, Division of Computational Genomics and Systems Genetics, Heidelberg, Germany; [2]German Cancer Research Center, AI Health Innovation Cluster, Heidelberg, Germany; [3]Technical University of Munich, School of Computation, Information and Technology, Garching bei München, Germany; [4]Helmholtz Munich, Helmholtz Zentrum München, Neuherberg, Germany; [5]Technical University of Munich, Klinikum rechts der Isar, Institute of Human Genetics, München, Germany; [6]Munich Center for Machine Learning, Munich; [7]European Molecular Biology Laboratory, Genome Biology Unit, Heidelberg, Germany; [8]EMBL's European Bioinformatics Institute (EMBL-EBI), Wellcome Genome Campus, Hinxton, United Kingdom; [9]Wellcome Sanger Institute, Trust Genome Campus, Hinxton, United Kingdom

Background/Objectives: Rare genetic variants can strongly predispose to disease, yet accounting for rare variants is statistically challenging. Meta-analysis methods over different test types and annotations have demonstrated the added value of incorporating a broad spectrum of annotations. However, methods integrating variant annotations in a data-driven manner are lacking.

Methods: Here, we propose DeepRVAT (Deep Rare Variant Association Testing), a deep set model that learns gene impairment scores from rare variants, annotations, and phenotypes. DeepRVAT infers the relevance of different annotations and their combination directly from data, eliminating ad hoc modeling choices that characterize existing methods. DeepRVAT yields a single, trait-agnostic gene impairment score, enabling both risk prediction and gene discovery in a unified framework and seamless integration into established association testing frameworks.

Results: We apply DeepRVAT on 34 quantitative and 26 binary traits across 167,245 UK Biobank (UKBB) whole-exome-sequenced (WES) individuals. We integrate state-of-the-art annotations, including AlphaMissense, PrimateAI, AbSplice, DeepRipe, and DeepSEA, and find a substantial increase in gene discoveries and improved replication rates on held-out individuals over previous methods. Furthermore, we significantly improve detection of individuals at high genetic risk by combining common variant polygenic risk scores with DeepRVAT. We demonstrate the applicability of pre-trained DeepRVAT models to new traits, facilitating the study of disease cohorts with limited training data, and finally apply DeepRVAT to the latest 500k UKBB WES release.

Conclusion: DeepRVAT combines a flexible deep neural network with a rigorous statistical testing framework, thereby contributing to the understanding of human trait biology and boosting the identification of genetically predisposed individuals.

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Conflict of Interest: Brian Clarke German Cancer Research Center (DKFZ), Heidelberg, Eva Holtkamp Helmholtz Munich, Neuherberg, Hakime Öztürk German Cancer Research Center (DKFZ), Heidelberg, Marcel Mück German Cancer Research Center (DKFZ), Heidelberg, Magnus Wahlberg German Cancer Research Center (DKFZ), Heidelberg, Kayla Meyer German Cancer Research Center (DKFZ), Heidelberg, Felix Munzlinger German Cancer Research Center (DKFZ), Heidelberg, Felix Brechtmann Technical University of Munich, Garching, Florian R. Hölzlwimmer Technical University of Munich, Garching, Julien Gagneur Technical University of Munich, Garching, Oliver Stegle German Cancer Research Center (DKFZ), Heidelberg European Molecular Biology Laboratory, Heidelberg, Oliver Stegle is a paid advisor of Insitro Inc

P16.004.C REEV - Novel platform for variant evaluation

Dzmitry Hramyka[1], Henrike Sczakiel[2;3;4], Felix Boschann[2;4], Mikko Petteri Nieminen[1], Max Xiaohang Zhao[1;4], Lara Einicke[4], Stefan Mundlos[3;4], Dominik Seelow[1], Nadja Ehmke[4], Dieter Beule[1], Manuel Holtgrewe[1]

[1]Berlin Institute of Health, Core Unit Bioinformatics, Berlin, Germany; [2]Berlin Institute of Health at Charité - Universitätsmedizin Berlin, BIH Biomedical Innovation Academy, Clinician Scientist Program, Berlin, Germany; [3]Max Planck Institute for Medical Genetics, RG Development & Disease, Berlin, Germany; [4]Charité - Universitätsmedizin Berlin, Freie Universität Berlin and Humboldt-Universität zu Berlin, Institute of Medical Genetics and Human Genetics, Berlin, Germany

Background/Objectives: The rapid advancement in broad genetic testing, especially through exome sequencing (ES) and genome sequencing (GS), has resulted in a significant surge in the detection and analysis of genetic variations. This advancement brings the significant challenge of distinguishing the causal variant(s) among numerous non-pathogenic variants. Here, we introduce REEV (Review, Evaluate, and Explain Variants), a novel web-based platform aiming to streamline the variant evaluation process, focusing on sequence and structural variants in the context of rare diseases.

Methods: We implemented REEV as a web server that provides integrated data from various free and public domain sources, providing comprehensive genetic information analysis. The platform integrates external tools such as InterVar and AutoCNV to

allow semi-automatic assessment of variants using ACMG criteria. REEV is implemented in the TypeScript and Python programming languages.

Results: REEV facilitates both casual and registered users in generating and sharing public permanent links for specific variants and allows for the submission of these variants to ClinVar. Additionally, the platform offers functionality to bookmark particular variants and store their associated ACMG assessments, thereby enhancing collaborative research and facilitating more efficient data exchange.

Conclusion: REEV supports genetic diagnostics with a user-friendly platform that uniquely integrates diverse data sources and adheres to ACMG criteria, becoming essential for rare disease research.

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Conflict of Interest: None declared

P16.005.D EAGLE-MUT: Predicting and evaluating per-nucleotide somatic mutation susceptibility

Mario Aguilar[1;2], *Yana Vassileva*[1], *Melissa Sanabria*[1], *Anna Poetsch*[1;2]

[1]*TU Dresden, Dresden, Germany;* [2]*National Center for Tumor Diseases, Partner site Dresden (NCT), Dresden, Germany*

Background/Objectives: Mutagenesis distributes heterogeneously over the genome and depends on different mutational processes. Distribution is dependent on the direct sequence context and larger range influences, such as the epigenome and genome functionality. As a consequence, each nucleotide in the genome has a distinct context-dependent probability to mutate. We estimate somatic mutation probabilities genome wide. By interrogating specific loci, such as cancer genes, we can investigate the mutation probability alone, as opposed to mutation rates that depend on selective processes.

Methods: We developed EAGLE-MUT (Efficient Analysis with a Genome-wide LSTM to Evaluate per-nucleotide MUTation susceptibility), a Long Short-Term Memory (LSTM) recurrent neural network model, that infers the susceptibility to somatic mutagenesis genome-wide in nucleotide resolution. It uses 500 nt windows, the human reference sequence and single-base-substitutions in esophageal adenocarcinoma ($n = 423$).

Results: EAGLE-MUT achieves a 12-fold increase in correct predictions compared to a random model and learns DNA trinucleotide content (cosine similarity $= 0.99$), but utilizes a broader context that reveals motifs of > 100 nt in size. Applying EAGLE-MUT to individual patients underscore individual differences in somatic mutation probabilities. We highlight divergent susceptibilities for the same driver genes throughout the cohort (e.g., TP53, SMAD5, ARID1A).

Conclusion: EAGLE-MUT captures the specificity of mutagenesis in WGS data and derives per-nucleotide probabilities of mutagenesis through a bigger sequence context. EAGLE-MUT can be applied for individual patients to personalize the assessment of mutagenesis and can support the investigation of evolutionary trajectories in individual tumor samples.

Grants: NCT Proof-of-Concept Trial Program "DECISIONS"
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Conflict of Interest: None declared

P16.006.A Face Mesh Detection and Annotation for Genetic Disorders with Facial Phenotypes

Fabio Hellmann[1], *Marc Lorenz*[1], *Leonhard Okrusch*[1], *Dominik Schiller*[1], *Tobias Baur*[1], *Silvan Mertes*[1], *Daniela Angelova-Toshkina*[2], *Michaela Kuhlen*[2], *Elisabeth André*[1]

[1]*University of Augsburg, Chair for Human-Centered Artificial Intelligence, Augsburg, Germany;* [2]*University of Augsburg, Pediatrics and Adolescent Medicine, Augsburg, Germany*

Background/Objectives: Facial dysmorphism does often manifest in syndromic conditions that are genetically related. These dysmorphisms exemplify facial feature abnormalities, which are difficult or impossible to represent by today's facial landmark algorithms due to their sometimes complexity in detail. Adapting the facial landmark algorithms to faces affected by syndromic conditions is challenging as, to date, no dataset with those faces and their corresponding landmarks is available. Therefore, we propose a workflow to semi-automatically annotate faces with dysmorphism and fine-tune a facial landmark detection model during the annotation process. Furthermore, we will provide the manually annotated landmarks.

Methods: We propose a workflow to enhance the adaptability and robustness of the state-of-the-art face landmarks detection model introduced by Kartynnik et al. [1]. To fine-tune the model on faces with genetic syndromes, we incorporated the cooperative machine learning paradigm into our workflow to annotate the facial landmarks semi-automatically.

Results: Preliminary results of our method indicate that the fine-tuned model on the manually annotated landmarks based on the syndromic faces increases the automatic detection.

Conclusion: This research aims to improve the accuracy of facial landmark detection for faces affected by genetic syndromes. Our method uses the landmark detection model by Kartynnik et al. [1] as a basis and fine-tuned it on semi-automatedly annotated syndromic images with facial dysmorphism.

Grants: None.

[1] Kartynnik, Yury, et al. "Real-time facial surface geometry from monocular video on mobile GPUs." arXiv preprint arXiv:1907.06724 (2019).

Conflict of Interest: None declared

P16.007.B A regional nonsense constraint score offering clinical and biological insights into genetic disorders.

Alexander Blakes[1;2], *Nicola Whiffin*[3;4;5], *Colin Johnson*[6], *Jamie Ellingford*[1;2], *Siddharth Banka*[1;2]

[1]*Manchester Centre for Genomic Medicine, Division of Evolution and Genomic Sciences, School of Biological Sciences, Faculty of Biology, Medicine and Health, University of Manchester, Manchester, United Kingdom;* [2]*Manchester Centre for Genomic Medicine, Manchester University NHS Foundation Trust, Health Innovation Manchester, Manchester, United Kingdom;* [3]*Big Data Institute, University of Oxford, Oxford, United Kingdom;* [4]*Wellcome Centre for Human Genetics, University of Oxford, Oxford, United Kingdom;* [5]*Program in Medical and Population Genetics, Broad Institute of MIT and Harvard, Cambridge, United States;* [6]*Leeds Institute of Medical Research, Faculty of Medicine and Health, University of Leeds, Leeds, United Kingdom*

Background: Nonsense-mediated decay (NMD) critically influences the molecular and clinical impact of protein-truncating variants (PTVs). PTVs in some regions may escape NMD. The accurate interpretation of these variants is a major challenge in clinical genomics. However, existing constraint scores do not comprehensively account for NMD escape.

Methods: Canonical protein-coding transcripts were partitioned into regions likely to trigger or escape NMD, and exome sequencing data from 730,947 individuals in gnomAD v4 was used to calculate regional nonsense constraints therein. These data were compared with distributions of de novo PTVs (*dn*PTVs) in 44,966 rare disease trios.

Results: 1,947/19,694 transcripts (10%) have significant regional nonsense constraint. Of these, 625 transcripts (32%) have weak pLI and LOEUF annotations, suggesting that our regional score may prioritise disease-relevant genes missed by transcript-level metrics.

Bases in constrained regions are more highly conserved (phyloP) and have higher AlphaMissense scores than unconstrained regions ($p < 10^{-8}$).

In 44,966 rare disease trios, *dn*PTVs are 4- to 7-fold enriched in constrained regions versus unconstrained regions. In 13,921 trios with clinical outcome data, *dn*PTVs in a constrained region are more likely to be diagnostic than those in unconstrained regions (OR 4.6-6.5, $p < 0.01$).

Finally, we identified 31 genes with at least three unique *dn*PTVs in a constrained region and no disease association in OMIM, highlighting the potential of our metric for prioritising novel disease-gene associations. Work to identify new diagnoses and validate our metric against variants in ClinVar is ongoing.

Conclusions: Our results will improve diagnosis, gene discovery, and mechanistic insights in genetic disorders.

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Conflict of Interest: Alexander Blakes: None declared, Nicola Whiffin: None declared, Colin Johnson: None declared, Jamie Ellingford Part-time employee of Genomics England., Siddharth Banka: None declared

P16.008.C NanopoReaTA: Real-Time RNA-Sequencing Analysis for time-efficient Decision-Making

Stefan Pastore[1], *Anna Wierczeiko*[2], *Stefan Mündnich*[1], *Anne Busch*[2], *Vincent Dietrich*[2], *Mark Helm*[1], *Tamer Butto*[1], *Susanne Gerber*[2]

[1]*Johannes-Gutenberg-University, Institute of Pharmaceutical and Biomedical Sciences, Mainz, Germany;* [2]*University Medical Center of the Johannes Gutenberg University, Institute of Human Genetics, Mainz, Germany*

Consortium: Landes Initiative Rheinland-Pfalz and the Resilience, Adaptation, and Longevity (ReALity) initiative of the Johannes Gutenberg University of Mainz, Deutsche Forschungsgemeinschaft (DFG)

Background/Objectives: Oxford Nanopore Technologies' (ONT) sequencing platform offers an excellent opportunity to perform real-time analysis during sequencing. This feature allows for early insights into experimental data and accelerates a potential decision-making process for further analysis, which can be particularly relevant in the clinical context. Here, we present NanopoReaTA (Nanopore-seq real time transcriptional analysis), an innovative real-time transcriptional analysis toolbox designed for the Oxford Nanopore Technologies (ONT) sequencing platform.

Methods: Functioning directly on the data output from MinKnow, the tool integrates a Nextflow pipeline, allowing comprehensive analyses for both genome wide and gene-specific expression patterns. Additionally, an Rshiny based GUI offers an interactive experience, guiding users through configuration settings and allowing them to extract valuable information from sequencing runs in progress.

Results: By having access to real-time insights into both transcriptome-wide and gene-specific data, users can enhance decision-making in scientific experiments and diagnostic investigations, potentially leading to significant time and resource savings.

Conclusion: With NanopoReaTA, differential expression analysis for ONT based RNA sequencing experiments can be performed in real-time. The interactive graphical user interface allows to visualize results on demand with the opportunity to gain genome-wide and gene-specific insights on gene and transcript expression patterns.

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Conflict of Interest: None declared

P16.009.D Contributing to genetic diagnostic effectiveness with heterogeneous graph neural networks

Jose L. Mellina-Andreu[1], *Luis Bernal*[1], *Antonio Gómez-Skarmeta*[1], *Mina Ryten*[2], *Sara ALVAREZ*[3], **Alejandro Cisterna García**[1], *Juan Botia*[1,4]

[1]*Faculty of Informatics, Universidad de Murcia, Department of Information and Communications Engineering, Murcia, Spain;* [2]*UK DRI Centre at Cambridge;* [3]*NIM Genetics, Genómica y Medicina S.L., Spain;* [4]*University College London, Department of Neurodegenerative Diseases, London, United Kingdom*

Contributing to genetic diagnostic effectiveness with heterogeneous graph neural networks

Background/Objectives: We are concerned with predicting novel causal associations between genes and phenotypes to increase the yield of genetic diagnosis. A comprehensive gene-phenotype associations catalogue is paramount for effective diagnosis. Yet unknown phenotype-gene associations may contribute to a significant percentage of undiagnosed patients.

Methods: We redefined the prediction of novel gene-phenotype associations as a link prediction task between nodes on a heterogeneous graph. We modelled the graph with machine learning starting from a graph of phenotypes and genes based on HPO. Phenotypes were encoded using BERT, a transformer-based deep learning network. Genes were characterized using information from various sources (Gnomad, GTEx, STRING amongst others). With this we then created a deep heterogeneous graph neural network called PhenoLinker.

Results: PhenoLinker outperforms cutting edge methods (HPODNets, GeneMANIA and 6 other) in AUCPR (0.503 vs 0.357) and F1 (0.643 vs 0.433). When trained on an HPO version and tested on associations added in later versions, the average AUCPR is of 0.73. When tested on the Gene2Phenotype associations not in HPO, PhenoLinker predicted that 58% should be added to HPO, 14 fold over random associations. Moreover, PhenoLinker improved a genetic diagnostic software. After reanalysing a battery of undiagnosed cases using the software empowered with PhenoLinker predictions, 13 new variants helped nominally diagnosing subjects through new gene-phenotype associations like ARHGEF10 and peripheral neuropathy (HP:0009830) and OPA1 and Microcephaly (HP:0000252).

Conclusion: PhenoLinker can help increasing diagnostic yield of genetic diseases. Predictions and their corresponding in-silico explanations are available at https://huggingface.co/spaces/Melland/PhenoLinker.

Grants:

Conflict of Interest: Jose L. Mellina-Andreu: None declared, Luis Bernal: None declared, Antonio Gómez-Skarmeta: None declared,

Mina Ryten: None declared, Sara ALVAREZ NIM Genetics, Genómica y Medicina SL, Alejandro Cisterna García: None declared, Juan Botia: None declared

P16.010.A Decoding the intercellular communication of immune cells in neuromyelitis optica and multiple sclerosis

Maria Jose Cardenas Espinosa[1;2], *Anika Witten*[1], *Catharina Groß*[2], *Lisa Lohmann*[2], *Eva Dawin*[2], *Heinz Wiendl*[2], *Monika Stoll*[1], *Luisa Klotz*[2]

[1]*University of Münster, Institute of Human Genetics, Münster, Germany;* [2]*University of Münster, Department of Neurology, Münster, Germany*

Background/Objectives: Neuromyelitis optica (NMO) and multiple sclerosis (MS) are neuroinflammatory and neurodegenerative diseases characterized by infiltration of immune cells in the central nervous system (CNS). Despite of inherent similarities, the identification of antibodies targeting water channel aquaporin-4 (APQ4) and myelin oligodendrocyte glycoprotein (MOG) in NMO has differentiated them as two distinct diseases. The specific molecular mechanisms involving autoreactive immune cells in both disorders remain to be fully elucidated.

Methods: Bulk RNA- sequencing was performed on sorted PBMCs (B cells, $CD4^+$T cells and monocytes) from 22 NMO patients (AQP4/MOG), 11 MS patients and 11 healthy donors (HD). Sample were processed using HISAT2 and Stringtie pipeline. Subsequently, differential gene expression analysis was performed using DESeq2, and cell-cell communication analysis via CellChat.

Results: In monocytes, differential gene expression analysis comparing NMO and HD (criteria: padj < 0.05 and $\log_2$FC >1) showed a higher number of upregulated genes (IL1A, TGIF1, CSF1) associated with pro-inflammatory responses than B cells and CD4 T^+ cells. Moreover, TGF-β and MAPK signaling pathways were among the top enriched pathways, suggesting a prominent inflammatory role of monocytes in the pathology of NMO.

Further analysis of ligand-receptor interactions identified dysregulated-signaling pathways including CCL, CXCL, MHC-II in NMO and IL-16, CXCL and NRXN in MS, particularly in interactions between monocytes and CD4 T^+ cells.

Conclusion: Further analysis of ligand-receptor interactions identified dysregulated-signaling pathways including CCL, CXCL, MHC-II in NMO and IL-16, CXCL and NRXN in MS, particularly in interactions between monocytes and CD4 T^+ cells.

Conflict of Interest: None declared

P16.011.B A novel life-time risk model to estimate the age-dependent penetrance of rare and common variants in complex diseases and application to Alzheimer disease

Catherine Schramm[1], *Emmanuelle Génin*[2], *Olivier Quenez*[3], *Gaël Nicolas*[3], *Camille Charbonnier*[4]

[1]*Univ Rouen Normandie, Inserm U1245, Normandie Univ, F-76000, Rouen, France;* [2]*Inserm UMR1078, Brest, France;* [3]*Univ Rouen Normandie, Inserm U1245, Normandie Univ, CHU Rouen, Department of genetics, F-76000, Rouen, France;* [4]*Univ Rouen Normandie, Inserm U1245, Normandie Univ, CHU Rouen, Department of biostatistics, F-76000, Rouen, France*

Consortium: ADES consortium

Background/Objectives: Recent association studies on exome sequencing datasets unveiled rare genetic risk factors with stronger levels of association with complex diseases than most of their common counterparts. Although each of them are rare or ultra-rare, together they affect a non-negligeable part of the population. It is thus essential to accurately estimate their absolute penetrance before putative use in a clinical setting.

Methods: We developped a model combining information from prospective cohorts and case/control sequencing data into a Kaplan-meier-like estimator to assess age-dependent penetrance of different rare variant categories, stratified for major common risk factors and/or genetic risk score (GRS) strata. When cumulative frequencies allowed for it, we tested for epistasy between common and rare variants via bootstrap. After validation through extensive simulation studies, we applied our model to Alzheimer disease, integrating the ADES-ADSP case-control sequencing dataset with large published prospective data.

Results: We derived Alzheimer disease penetrance curves for rare but recurrent *TREM2*-p.R47H, *TREM2*-p.R62H, *ATP8B4*-p.G395S and *ABCA7* truncating variants, stratified for the common APOEe4 allele and GRS tertiles. Cumulated effects of *TREM2*-p.R47H and APOEe4/e4 led to almost complete penetrance by age 85, although *TREM2*-p.R47H alone and APOEe4/e4 alone confered less than 30% and 50% risk by age 85, respectively.

Conclusion: Our model offers a perspective to estimate the penetrance of combinations of risk factors integrating prospective and case/control data. Results on Alzheimer disease data show the importance of accounting for digenic dimensions, at least, and that the background effect of GRS appears negligeable, in comparison.

Conflict of Interest: None declared

P16.012.C Mind the ancestry gap: Improving the genetic prediction of psychiatric disorders

Jette Steinbach[1;2], *Emil Michael Pedersen*[1;2], *Florian Privé*[1;2], *Andrew Schork*[3;4;5], *Bjarni Jóhann Vilhjálmsson*[1;2;6;7]

[1]*The National Centre for Register-based Research, The National Centre for Register-based Research, Aathus BSS, Aarhus University, Denmark, Aarhus V, Denmark;* [2]*Lundbeck Foundation Initiative for Integrative Psychiatric Research, Aarhus V, Denmark;* [3]*Institute of Biological Psychiatry, Mental Health Center Sct Hans, Roskilde, Denmark;* [4]*The Translational Genomics Research Institute (TGEN), Neurogenomics Division, Phoenix, AZ, United States;* [5]*GLOBE Institute, Faculty of Health and Medical Sciences, Copenhagen University, Section for Geogenetics, Copenhagen, Denmark;* [6]*The Broad Institute of MIT and Harvard, Novo Nordisk Foundation Center for Genomic Mechanisms of Disease, Cambridge, MA, United States;* [7]*Bioinformatics Research Centre (BiRC), Bioinformatics Research Centre (BiRC), Aarhus University, Aarhus, Denmark*

Background/Objectives: Existing polygenic scores (PGS) are not transferable between genetic ancestries. A decrease in PGS prediction accuracy by up to 78% in individuals of African genetic ancestry relative to individuals of European genetic ancestry can introduce bias and increase health disparities in clinical practice. Even though this problem is well known for PGS, we lack information about the transferability of alternative measures capturing genetic (or environmental) information. This study will evaluate and compare the impact of genetic heterogeneity on the prediction accuracy of PGS, family history (FH) and electronic health records (EHR) for psychiatric disorders.

Methods: I will predict risk in > 650.00 individuals using data from the iPSYCH2015 case-cohort study, Danish registers and the UK Biobank. The outcome will be the partial correlation between the observed and predicted case-control status as stratified by genetic ancestry.

Results: Our preliminary results, obtained in the iPSYCH2015 sample, show that FH is more robust than PGS for

predicting the case-control status in individuals of non-European genetic ancestry. We expect to replicate our findings in data from the UK Biobank. We also hypothesize that the accuracy of EHR-based prediction models may differ significantly between Denmark and the UK.

Conclusion: By evaluating the prediction accuracy of three commonly used measures capturing genetic and environmental information, this study provides valuable insight into the development of unbiased and transferable prediction models. Our results will help decrease health disparities and social injustice present in current clinical prediction models.

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Conflict of Interest: Jette Steinbach This work was supported by Danish Data Science Academy (2023-1420), which is funded by the Novo Nordisk Foundation (NNF21SA0069429) and VILLUM FONDEN (40516), Emil Michael Pedersen: None declared, Florian Privé: None declared, Andrew Schork Andrew Schork is funded by a Lundbeck Foundation Fellowship., Bjarni Jóhann Vilhjálmsson B.J. Vilhjálmsson is funded by a 2019 fellowship from the Lundbeck Foundation (R335-2019-2339) and a grant from the Independent Research Fund Denmark (2034-00241B).

P16.013.D OFF-PEAK: Detection of copy number variants using noncoding and off-target NGS reads in Mendelian conditions and in cancer

Mathieu Quinodoz[1;2;3], *Karolina Kaminska*[1;2], *Francesca Cancellieri*[1;2], *Ji Hoon Han*[1;2], *Virginie Peter*[1;2;4], *Elifnaz Celik*[1;2], *Lucas Janeschitz-Kriegl*[1;2], *Nils Schärer*[1;2], *Daniela Hauenstein*[1;2], *Bence Gyorgy*[1;2], *Giacomo Calzetti*[1;2], *Vincent Hahaut*[1;2], *Sónia Custódio*[5], *Ana Sousa*[5], *Yuko Wada*[6], *Yusuke Murakami*[7], *Almudena Avila Fernández*[8;9], *Cristina Rodilla*[8;9], *Pablo Mínguez*[8;9], *Carmen Ayuso*[8;9], *Koji Nishiguchi*[10], *Cristina Santos*[11;12], *Luisa Coutinho Santos*[12], *Viet H Tran*[13;14], *Veronika Vaclavik*[13], *Hendrik Scholl*[1;2], *Carlo Rivolta*[1;2;3]

[1]*Institute of Molecular and Clinical Ophthalmology Basel (IOB), Ophthalmic Genetic Group, Basel, Switzerland;* [2]*University of Basel, Department of Ophthalmology, Basel, Switzerland;* [3]*University of Leicester, Department of Genetics and Genome Biology, Leicester, United Kingdom;* [4]*Bern University Hospital, Department of Ophthalmology, Bern, Switzerland;* [5]*Centro Hospitalar Universitário Lisboa Norte (CHULN), Department of Medical Genetics, Hospital Santa Maria, Lisboa, Portugal;* [6]*Yuko Wada Eye Clinic, Sendai, Japan;* [7]*Kyushu University, Dept of Ophthalmology, Graduate School of Medical Sciences, Fukuoka, Japan;* [8]*Universidad Autónoma de Madrid (IIS-FJD, UAM), Department of Genetics & Genomics, Instituto de Investigación Sanitaria-Fundación Jiménez Díaz University Hospital, Madrid, Spain;* [9]*Centre for Biomedical Network Research On Rare Diseases (CIBERER), Madrid, Spain;* [10]*Nagoya University Graduate School of Medicine, Department of Ophthalmology, Nagoya, Japan;* [11]*Universidade NOVA de Lisboa, NOVA4Health, NOVA Medical School, Faculdade de Ciências Médicas, Lisbon, Portugal;* [12]*Instituto de Oftalmologia Dr Gama Pinto (IOGP), Lisbon, Portugal;* [13]*Hôpital ophtalmique Jules-Gonin, Université de Lausanne, Unité d'oculogénétique, Lausanne, Switzerland;* [14]*King's College London, Centre for Gene Therapy and Regenerative Medicine, London, United Kingdom*

Background/Objectives: Copy-number variants (CNVs) play a substantial role in the molecular pathogenesis of many hereditary diseases, in cancer, as well as in normal human interindividual variation. Yet, they are still rather difficult to identify in mainstream sequencing projects, especially involving Whole-Exome Sequencing (WES), because they tend to occur in DNA regions that are not routinely chosen for sequence analysis.

Methods: We discovered that DNA reads from uncaptured sequences, present as contaminants in WES procedures, could be used to identify elusive pathogenic CNVs, especially when 'off-target' reads can be mapped back to the noncoding regions of the genome. We therefore developed OFF-PEAK, a user-friendly tool that harnesses the enormous, although neglected power of such reads.

Results: We tested OFF-PEAK on sequencing data from 96 cancer samples, as well as 130 exomes of individuals with Mendelian diseases. For both sets of data, OFF-PEAK identified an unexpectedly large number of novel and bona-fide pathogenic CNVs, which were then validated experimentally by standard molecular biology techniques,. Specifically, our tool demonstrated a very high performance (97% sensitivity and 87% specificity), when compared to values obtained experimentally in the wet lab.

Conclusion: In conclusion, our data show that CNVs represent an underestimated element of pathogenicity in both Mendelian conditions and in cancer, and that such events can be easily identified by the analysis of off-target reads from NGS procedures.

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Conflict of Interest: None declared

P16.014.A Exploration of the mutational landscape in Clinvar & Gnomad for the functional effect in (over-) lapping short open reading frames (sORFs)

Jeremias Krause[1], *Martin Danner*[1;2], *Lars Perchalla*[2], *Florian Kraft*[1], *Matthias Begemann*[1], *Miriam Elbracht*[1], *Ingo Kurth*[1]

[1]*University hospital Aachen, Institute for Human Genetics and Genomic Medicine, Aachen, Germany;* [2]*scieneers GmbH, Karlsruhe, Germany*

Background/Objectives: Short open reading frames (sORFs) are a class of genomic elements which recently gained attention because sORFs may encode functionally relevant micropeptides. These sORFs are dispersed across the whole genome and may overlap with canonical coding regions. Such overlapping sORFs are usually neglected in genetic analysis because it is difficult to distinguish their effect from effects on the respective canonical transcript.

Methods: Using the custom annotations feature of the ensembl variant effect predictor (VEP) and the genome analysis toolkit (GATK) we implemented a pipeline, which enabled us to annotate a potential impact on sORFs versus the main coding region of an overlapping gene, in which the sORF is embedded. We utilized this pipeline to visualize the landscape of variants present in ClinVar and gnomAD for a consensus set of sORFs, previously published by gencode. Using ESMFold we subsequently generated 3D Structures of the observed variants and their corresponding wild type micropeptides to train a deep learning model to predict potentially deleterious variants.

Results: A noticeable proportion of variants present in ClinVar affect sORFs, most of which are currently classified as variants of uncertain significance (VUS). Most variants in ClinVar that fall into sORFs and that are predicted to have a functional impact on the sORF are missense variants.

Conclusion: sORF affecting variants might depict an overlooked disease mechanism. To prove this hypothesis, future functional evidence beyond bioinformatic predictions is required.

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Conflict of Interest: None declared

P16.015.B Establishing the impact of genome and transcriptome reference on gene co-expression

Ariela Buxbaum Grice[1], *Noam Beckmann*[1], *Anina Lund*[1], *Ryan Thompson*[1]

[1]*Icahn School of Medicine at Mount Sinai, Genetics and Genomics/ Neuroscience, New York, United States*

Background/Objectives: Since 2003, six genome assemblies (NCBI34, NCBI35, NCBI36, GRCh37/hg19, GRCh38/hg38, T2T-CHM13) and a myriad of transcriptome assemblies (GENCODE v1 to v45) have been released, each filling in gaps or correcting errors in its predecessors. Due to time and resource constraints, research is often performed using non-up-to-date references, with an implicit assumption that reference choice has a negligible impact on findings. Here, using five references spanning multiple genome and transcriptome assemblies, we characterized the effect of reference usage on gene-gene correlations.

Methods: Raw reads from 2 large Alzheimer's disease gene expression datasets of the human prefrontal cortex (ROSMAP, n = 633; MSBB, n = 246) were mapped and quantified using 5 references (NCBI36, GRCh37/hg19, GRCh38.12[v30], GRCh38.13[v43], T2T-CHM13). Counts were processed independently for each reference using the same procedure: removal of low count genes, raw counts normalization (*voomWithDreamWeights*), and residualization using previously defined covariates. For every pair of genes, Pearson correlations were compared for each pair of references. Additionally, WGCNA coexpression network module memberships were compared across assemblies.

Results: Although gene-gene correlations and module membership were generally consistent between assembly pairs, they were never identical (e.g., range of differences between correlations: -1.72-1.46). Consecutive references were most similar, with GRCh38 (v30, v43) and T2T-CHM13 closest. Interestingly, gene-gene correlation differences altered coexpression module memberships significantly (mean Jaccard index = 74.7%).

Conclusion: While gene-gene correlations and coexpression network structure are generally replicated between references, reference usage plays an important role for a non-trivial number of genes, thereby impacting coexpression structure.

Grants: None

Conflict of Interest: Ariela Buxbaum Grice Full-time PhD student, Noam Beckmann Assistant Professor at Icahn School of Medicine at Mount Sinai, Anina Lund Full time PhD student, Ryan Thompson Assistant Professor

P16.016.C Efficient and local pleiotropy-robust omic-wide mendelian randomization with LaScaMolMR.jl

Samuel Mathieu[1], *Louis-Hippolyte Minvielle-Moncla*[1], *Mewen Briend*[1], *Valentine Duclos*[1], *Anne Rufiange*[1], *Patrick Mathieu*[1,2]

[1]*Institut Universitaire de Cardiologie et de Pneumologie de Québec, Québec, Canada;* [2]*Laval University, Medecine Faculty, Surgery Department, Québec, Canada*

Background: The recent rise of large genetic cohorts led to an increase in scale of molecular Quantitative Trait Loci studies, leading to more extensive Mendelian randomization (MR) investigations aiming to identify causal relationships between molecular traits and diseases. This increase led to the identification of multiple causal candidates and potential drug targets over time. However, the increase in scale of such studies and more complex study designs come with computational challenges.

Methods and Results: We present "LArge SCAle MOLecular Mendelian Randomization with Julia" (LaScaMolMR.jl), an integrated all-Julia package optimized for Omic-wide Mendelian Randomization (OWMR) Studies. This versatile package eliminates the two-language problem and implements fast algorithms for instrumental variables selection approaches with both cis and trans instruments and perform the most popular regression estimators for MR studies with molecular exposures. It reduces the compute time via metaprogramming allowing easy deployment of multi-threaded approach and the internalization of linkage disequilibrium (LD) investigation of potential instrumental variables. Via its integrated approach and high-computational performance, LaScaMolMR.jl allows users who have minimal programming experience to perform large scale OWMR studies. We developed the "No Local Pleiotropy" (NoLoP) approach that allows the user to further investigate potential pleiotropic effects local to the exposure dataset. We further show the NoLoP approach can identify causal genes when there are multiple potential targets in the same locus compounded by the LD structure in case studies.

Conclusion: LaScaMolMR.jl is an easy to implement and computationally performant package that allows control of local pleiotropy in OWMR.

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Conflict of Interest: None declared

P16.017.D An explainable, ontology-based genetic algorithm for diagnosis and outcome prediction using patient phenotypic data

Elena Casiraghi[1], *Nariman Ammar*[2], *Bryan Laraway*[3], *Corneliu Antonescu*[4], *Julius Jacobsen*[5], *Yasemin Bridges*[5], *Harry Caufield*[6], *Harshad Hegde*[6], *Carlo Kroll*[5], *Kevin Schaper*[3], *Madan Krishnamurthy*[3], *Melissa Haendel*[3], *Christopher Mungall*[6], *Peter Robinson*[7], **Justin Reese**[6]

[1]*University of Milan, AnacletoLab, Computer Science Department, Milano, Italy;* [2]*Illinois State University, Information Technology, Normal, United States;* [3]*University of Colorado Anschutz Medical Campus, Aurora, United States;* [4]*Banner Health, Colorado Medical Center, Greeley, United States;* [5]*William Harvey Research Institute, Queen Mary University of London, Barts and the London School of Medicine and Dentistry, London, United Kingdom;* [6]*Lawrence Berkeley National Laboratory, Division of Environmental Genomics and Systems Biology, Berkeley, United States;* [7]*Berlin Institute of Health, Charité Universitätsmedizin, Berlin, Germany*

Background/Objectives: Rich phenotypic data from electronic health records and clinical case reports can be used for computational phenotyping of rare disease and to predict clinical outcomes. For these use cases, explainability and accuracy of predictions are particularly important.

Methods: We describe a genetic algorithm to learn a set of phenotypic features (an "HPO profile") that can diagnose patients, characterize important phenotypic features, and predict clinical outcomes. While traditional genetic algorithms use mutation operations (adding, removing, and crossing over of "genes") on chromosome representations of features, we have adapted this strategy to use mutation operations on the HPO graph. In addition, we developed a fitness function that uses semantic similarity of the HPO profile to a patient to determine the fitness of each profile.

Results: We applied this algorithm to learn HPO profiles to predict the correct diagnosis for 8,171 synthetic disease cases and 3,305 case reports from literature. Performance as measured by test AUPRC was excellent in predicting the presence of Marfan Syndrome (1.0 and 0.802 on synthetic and real cases, respectively). We next applied this strategy to identify phenotypic features that predict important sequelae after acute COVID-19 infection. The genetic algorithm predicted myocardial infarction and kidney disease with a test AUPRC of (0.215 and 0.208, respectively, vs. an expected value of 0.09 for a random classifier).

Conclusion: Our genetic algorithm provides a method to directly learn explainable phenotypic features with diagnostic and prognostic value.

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Conflict of Interest: None declared

P16.018.A A three level modeling for identifying important predictor variables in genome-wide association studies suffering from p >> n.

Jakub Liu[1;2], *Joanna Szyda*[1;3], *Paula Dobosz*[3;4]

[1]*Wroclaw University of Environmental and Life Sciences, Department of Genetics, Wrocław, Poland;* [2]*Freie Universitaet Berlin, Berlin, Germany;* [3]*Poznan University of Medical Science, University Cancer Diagnostic Center, Poznań, Poland;* [4]*University of Warsaw, Institute of Genetics and Biotechnology, Warsaw, Poland*

Background/Objectives: Many genomic datasets are characterized by a much greater number of explanatory variables (p) than the number of observations (n). As a consequence, it is not possible to fit all the variables into a single statistical model.

Methods: A three-level approach was applied to the association analysis of COVID-19 susceptibility with SNPs identified in whole genome sequences of 1222 individuals. The first stage consisted of fitting k logistic regression models with subsets of SNPs that guarantees $p < n$. Each SNP was present in at least one of the k models. In the second stage, goodness-of-fit of each model was used as a dependent variable in a single Elastic Net Regression model that incorporated effects of all explanatory variables estimated in the first stage. In the third step, the effects of the p explanatory variables were clustered using the k-means clustering algorithm to identify SNPs that are important predictors of the susceptibility to COVID-19.

Results: For the original set of ~42 million SNPs, two groups representing SNPs important and irrelevant to being resistant or susceptible to COVID-19 infection were identified.

Conclusions: This approach enables the selection of explanatory variables for the situation of $p < n$, for which fitting all variables in the same model would enforce shrinkage while fitting a multitude of single SNP models poses problems with multiple testing. Even though the method is computationally intensive, it is well suited for parallelization and, hence, computationally feasible.

Conflict of Interest: None declared

P16.019.B Evaluation of structural variants calling performances using short and long reads sequencing

Valentina Andrioletti[1], *Federica De Paoli*[1], *Ivan Limongelli*[1], *Susanna Zucca*[1], *Ettore Rizzo*[1]

[1]*enGenome srl, Pavia, Italy*

Background/Objectives: In recent years, the scientific community has shown a growing interest in structural variants, genetic alterations involving more than 50 bp, as they are involved in the mechanisms of many genetic diseases. Illumina-based NGS technologies face challenges in identifying such complex events, due to the nature of their short reads, even if variant callers are becoming more and more sensitive. Third-generation platforms promise to overcome this issue by sequencing long DNA fragments up to 20 Kbps, spanning the break ends of these structural variants. To assess the real potential of both approaches, we benchmarked the "best in class" solutions, Illumina-Dragen and PacBio on the well characterized Coriell samples.

Methods: We evaluated the structural variants calling performances of Illumina and PacBio sequencing platforms on HG002 and HG001 WGS Coriell samples, comprising more than 14K deletions and 2K duplications. To be as comprehensive as possible, the variants have been stratified by length and type, and compared using both "truvari" and "witty.er", tools able to determine the overlap of complex structural events.

Results: Long-read technology outperforms short-reads in terms of sensitivity (average improvement: 20%, maximum for PacBio: 98.5%, maximum for Illumina: 69.2%), with deletions being the most detectable type of variant, but precision decreases on average by 10%, affecting mostly duplications.

Conclusion: Our analysis confirms the increase in detection power of long-read sequencing which comes at the cost of precision, suggesting that this technology requires even more stringent filtering and prioritization steps.

Grants: EIC Accelerator 190164416

Conflict of Interest: Valentina Andrioletti enGenome srl, Federica De Paoli enGenome srl, Ivan Limongelli enGenome srl, enGenome srl, Susanna Zucca enGenome srl, enGenome srl, Ettore Rizzo enGenome srl

P16.020.C Extracting phenotypes from clinical descriptions using large language models: a comparison between automated and manual approach.

Silvia Berardelli[1;2], *Andrea Gazzo*[3], *Federica De Paoli*[1], *Ivan Limongelli*[1], *Ettore Rizzo*[1], *Paolo Magni*[2], *Susanna Zucca*[1]

[1]*enGenome srl, Pavia, Italy;* [2]*University of Pavia, Department of Electrical, Computer and Biomedical Engineering, Pavia, Italy;* [3]*Memorial Sloan Kettering Cancer Center, Department of Pathology and Laboratory Medicine, New York, United States*

Background: Standardized phenotypic descriptions are essential in healthcare. Clinicians struggle to manually extract and convert them from literature or medical records. The advent of large language models has the potential to streamline the extraction of clinical descriptions into standardized Human Phenotype Ontology (HPO) terms.

Methods: We evaluated GPT-4 (openai.com) and PhenoBERT (PMID:35471885) for their ability to extract and convert clinical descriptions into HPO terms. GPT-4 excels in extracting and analyzing phenotypic descriptions in text but produces hallucinations when converting to HPO terms. Conversely, PhenoBERT is effective in conversion but often misses phenotypic descriptions in the raw text. To optimize the process, we adopted a two-steps method. Initially, GPT-4 analyzes textual, tabular or image-based input to generate a phenotypic description. Subsequently, PhenoBERT converts it into the corresponding HPO terms. This approach was tested on a benchmark of patients carrying oligogenic variants (PMID:35411390) described in the literature and lacking HPO terms. Its accuracy was evaluated by comparison with expert-driven manual curation of phenotyping.

Results: On 488 digenic and oligogenic combinations, an average of 4 phenotypes per case (median = 3, max = 47, min = 1) were extracted, with execution times ranging from 5 to 10 minutes. Compared to manual curation, we obtained complete concordance in 92% of cases. Unrecorded phenotypes were observed in 5% of cases, while extra unnecessaries phenotypes in 3%.

Conclusions: In conclusion, our LLM-based approach enables the automated extraction of phenotypes. Applied to the genomic context, it can contribute to speed up the diagnostic process.

Grants: EIC Accelerator 190164416

Conflict of Interest: Silvia Berardelli: None declared, Andrea Gazzo enGenome srl, Federica De Paoli enGenome srl, Ivan Limongelli enGenome srl, enGenome srl, Ettore Rizzo enGenome srl, Paolo Magni enGenome srl, Susanna Zucca enGenome srl, enGenome srl

P16.021.D In-depth variant interpretation: AI-powered tools for advancing genomic understanding

Federica De Paoli[1], *Silvia Berardelli*[1;2], *Giovanna Nicora*[1;2], *Ettore Rizzo*[1], *Ivan Limongelli*[1], *Susanna Zucca*[1]

[1]*enGenome srl, Pavia, Italy;* [2]*University of Pavia, Department of Electrical, Computer and Biomedical Engineering, Pavia, Italy*

Background/Objectives: Identifying causative variants is a major challenge in genomics and there's an urgent need to uplift the diagnostic yield of Rare Diseases. Envisioning a solution, we developed a suite of AI-driven tools to support the genomic community.

Methods: We developed the "Suggested Diagnosis" AI system, using pathogenicity, phenotypes, and familial inheritance to prioritize causative variants. A layer of Explainable-AI elucidates the rationale behind results and identifies potential Incidental Findings.

Beyond monogenic analysis, we developed diVas, an AI-model to identify digenic/oligogenic variants, leveraging gene-gene interaction evidence.

Finally, riding the wave of generative AI, we released VarChat (varchat.engenome.com, 10.20944/preprints202311.1130.v1), to synthesize scientific literature into clinically relevant summaries.

Results: The "Suggested Diagnosis", awarded as "Best performing Genome Interpretation" model within the CAGI6 challenge (https://doi.org/10.1101/2023.08.02.23293212), was benchmarked on 220 real samples from 6 different cohorts (SNV/INDELs and CNVs). Causatives were ranked in the top 20 in 90% of cases. This model is integrated in the eVai platform (evai.engenome.com). IF identification reduces the list of candidate variants by 80%.

DiVas was benchmarked on 600 manually curated combinations (sensitivity: 81%) and 11 real samples (sensitivity 73%). Digenic predictions are available at oliver.engenome.com (https://doi.org/10.1101/2023.10.02.560464).

VarChat validation is ongoing through manual curation by an expert.

Conclusion: We benchmarked our AI models on heterogeneous cohorts, and we made our predictions explainable, thus promoting human trust in AI predictions. We released two new open tools for the genomic community, to further boost the diagnostic yield of rare diseases.

Grants: EIC Accelerator 190164416

Conflict of Interest: Federica De Paoli Inventor of the digenic method, enGenome srl, Silvia Berardelli: None declared, Giovanna Nicora Inventor of the digenic method, Ettore Rizzo enGenome srl, Ivan Limongelli enGenome srl, enGenome srl, Susanna Zucca enGenome srl, enGenome srl

P16.022.A A novel VEP plugin to annotate Short Tandem Repeats with HGVS nomenclature

Giovanni Cerchia[1], *Federica De Paoli*[1], *Valentina Andrioletti*[1], *Susanna Zucca*[1], **Ettore Rizzo**[1], *Ivan Limongelli*[1]

[1]*enGenome srl, Pavia, Italy*

Background/Objectives: Short Tandem Repeats (STR) play a pivotal role in unraveling the genetic underpinnings of some genetic conditions. As their significance grows, it becomes increasingly essential to establish a standardized HGVS notation for STR. The "STRike" plugin supports the annotation process directly with the Variant Effect Predictor (VEP) tool, enabling a correct application of HGVS rules.

Methods: Following the standard guidelines outlined by the HGVS consortium, the plugin generates STR HGVS notation at both genomic and coding level, directly leveraging VEP annotations. It was developed using the Perl API offered by VEP.

The plugin was tested on a dataset of variants obtained by considering STR events submitted in ClinVar (release 2023-09) overlapping a manually curated catalog of clinically relevant STR, for a total of 331 expansions and 90 contractions. The concordance between the newly generated notation and the one reported in this database was evaluated.

Results: During the testing phase, VEP release 97.4 was run in offline mode within a dedicated containerized environment. Tested on the 421 STR events submitted in ClinVar, the plugin reaches a 98.81% concordance in HGVS notation generation. Among the tests performed, only 5 mismatches were found and manually investigated, highlighting an error during the submission process to the database.

Conclusion: The "STRike" plugin is publicly available and its use can be a step forward for standardizing STR annotation. This could support the analysis of these events and their proper disclosure in the literature.

Grants: EIC Accelerator 190164416

Conflict of Interest: Giovanni Cerchia enGenome srl, Federica De Paoli enGenome srl, Valentina Andrioletti enGenome srl, Susanna Zucca enGenome srl, enGenome srl, Ettore Rizzo enGenome srl, Ivan Limongelli enGenome srl, enGenome srl

P16.023.B LncRNAs as biomarkers for pediatric medulloblastoma classification of novel Gr4 molecular subgroups

ivan martinez de estibariz[1], *Miguel García-Ariza*[2;3], *Laura Zaldumbide*[4], *Lide Alaña*[2], *Itziar Astigarraga*[2;3;5], *Angela Gutierrez-Camino*[2], *daniel sinnett*[6], *Lorena Mosteiro González*[4], *Elixabet Lopez-Lopez*[2;7], *Idoia Martin-Guerrero*[1;2], **Nerea Bilbao Aldaiturriaga**[2;7]

[1]*University of the Basque Country (UPV/EHU), Genetics, Physical Anthropology and Animal Physiology, Leioa, Spain;* [2]*Biocruces Bizkaia Health Research Institute, Pediatric Oncology Group, Barakaldo, Spain;* [3]*Hospital Universitario Cruces, Pediatrics, Pediatric Hematology and Oncology Unit, Barakaldo, Spain;* [4]*Hospital Universitario de Cruces, Pathology, Barakaldo, Spain;* [5]*University of the Basque Country (UPV/EHU), Pediatrics Faculty of Medicine and Nursing, Leioa, Spain;* [6]*CHU Sainte-Justine Research Center, Hematology-Oncology, Montreal, Canada;* [7]*University of the Basque Country (UPV/EHU), Biochemistry and Molecular Biology, Leioa, Spain*

Background/Objectives: Medulloblastoma (MB) is an aggressive brain tumor accounting for 30% of all malignant central nervous system cancers in childhood. As the molecular subtype classification (WNT, SHH, Gr3 and Gr4) does not account for all existing heterogeneity, we aimed to stablish the role of long non-coding RNAs (lncRNAs), which may play a pivotal role in brain tumors, in the classification of MB, especially of Gr3/Gr4 subtypes.

Methods: Fifty-five pediatric patients and 5 controls from 25 hospitals classified upon their clinical immunochemical (IHC) and methylation status (MS-MIMIC profiling) were studied. LncRNA expression profile was performed by total RNA paired-end sequencing, aligning with STAR v2.5.3a, using FeatureCounts for quantification (lncKB hg38), and performing non-supervised clustering and batch effect correction.

Results: The IHC and methylation-based classification and lncRNA expression-based clustering showed subtype coincidence of 78% of patients. The lncRNAs profiling showed Gr3, Gr4 and previously non-classified (n/c) patients on a unique cluster, suggesting a relevant expression continuum of those subtypes. A specific hierarchical clustering of that group revealed 5 clusters: one for Gr3 (7 patients) and 4 for Gr4/n/c patients (26 Gr4 and 12 n/c patients). One of the Gr4 clusters showed the highest median age at diagnosis, *SNCAIP* overexpression and chromosome 8p loss, common genetic features of the subgroup 4β. All n/c patients located on a specific Gr4 subgroup.

Conclusion: LncRNAs may be key biomarkers for MB classification, unveiling the molecular landscape of Gr4 subtype.

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Conflict of Interest: None declared

P16.025.D A user-centric approach in the development of a decision support system for whole-genome-sequenced (WGS) cancer cases

Clare Kennedy[1], *Syrus Nourbakhsh[1], Alona Sosinsky[1], Liz Edwards[1], Kristof Goossens[1], Laura Valis[1], Jamie Trotman[2;3], Patrick Tarpey[2;3], Dorte Wren[2;4], Laura Ions[2;5], Alistair Reid[2;6], Christopher Wragg[2;7], Gareth Gerrard[2;8], Azim Mohamedali[2;8], Steven Best[2;8], Helen Warren[2;5], Eleanor Baker[2;6], Jonathan Williams[2;9], Natasha Vafadar[2;9], Noha Eltawil[2], Mikel Valganon[2;10], Carryl Dryden[2;4], Angharad Goodman[2;5], Kate Moloney[2;9], Katharine Jones[2;4], Polly Talley[2], Angela Hamblin[2], Sandra Hing[2], Joo Wook Ahn[2;3]*

[1]*Genomics England, London, United Kingdom;* [2]*NHS England, United Kingdom;* [3]*Cambridge University Hospital NHS Foundation Trust, East Genomic Laboratory Hub, Cambridge, United Kingdom;* [4]*Great Ormond Street Hospital for Children NHS Foundation Trust, North Thames Genomic Laboratory Hub, London;* [5]*Newcastle upon Tyne Hospitals NHS Foundation Trust, North East and Yorkshire Genomic Laboratory Hub, Newcastle, United Kingdom;* [6]*Manchester University NHS Foundation Trust, North West Genomic Laboratory Hub, Manchester, United Kingdom;* [7]*North Bristol NHS Trust, South West Genomic Laboratory Hub, Bristol, United Kingdom;* [8]*Guy's and St Thomas' NHS Foundation Trust, South East Genomic Laboratory Hub, London, United Kingdom;* [9]*Birmingham Women's and Children NHS Foundation Trust, Central and South Genomic Laboratory Hub, Birmingham, United Kingdom;* [10]*The Royal Marsden NHS Foundation Trust, North Thames Genomic Laboratory Hub, London, United Kingdom*

Background/Objectives: While landmark studies have demonstrated the potential therapeutic and prognostic utility of WGS for cancer, case analysis represents a bottleneck threatening such benefits to the patient. Highly skilled Clinical Scientists undertake the challenging and intricate task of variant interpretation. Reducing the time resource required at the analysis stage could significantly improve case turnaround times, enabling faster patient care and actionability of findings. Here, we present a decision support system (DSS) to fulfil an unmet need for solutions to streamline WGS case analysis and thus maximise the clinical utility of WGS to cancer patients.

Methods: A user-centric approach supported the identification of functional and non-functional requirements. Platform architecture and infrastructure was developed by a collaborative team of expert data architects, software engineers and bioinformaticians utilising engineering and software best practices and harnessing the power of cloud computing.

Results: In close collaboration with NHS England (NHSE), Genomics England (GEL) have custom-built a DSS for the analysis of NHS cancer referrals. WGS referral data is automatically loaded into the DSS, and to date, Clinical Scientists have access to >1,700 cases and can visualise genome-wide changes, prioritise variants with advanced filtering, access database annotations and record variant interpretations.

Conclusion: Embedding the DSS into analysis workflows will eventually contribute to reducing turnaround times and through ongoing collaboration with NHSE, GEL will continue to implement new DSS features. Focusing our attention to aligning with newly emerging UK Somatic Variant Interpretation Guidelines will further support Clinical Scientists and maximise the clinical utility of WGS in the patient pathway.

Grants: N/A

Conflict of Interest: None declared

P16.026.A Indiscriminate usage of surrogate variables leads to overfitting in differential gene expression analyses

Dung Hoang[1], *Anina Lund[1], Ryan Thompson[2], Noam Beckmann[2]*

[1]*Icahn School of Medicine at Mount Sinai, Neuroscience, New York, United States;* [2]*Icahn School of Medicine at Mount Sinai, Medicine, New York, United States*

Background/Objectives: Surrogate variable analysis (SVA) aims to reduce heterogeneity in high-throughput experiments by identifying and accounting for latent confounders (surrogate variables, SVs). This approach, essential for certain analyses (e.g., eQTL), is becoming increasingly used for differential expression analyses (DEA). This study evaluates the impact of SVA on DEA, using simulated and published datasets.

Methods: Expensive simulations (20,000 genes for 1,000 samples) with varying signal (known and unknown) to noise (2.9×10^6 parameters) were performed to characterize the impact of SVA on DEA where the true differentially expressed genes (DEGs) are known. Five DEA approaches were employed: oracle (all parameters are known), no SV adjustment, SV adjustments for: known number of SVs and number of SVs estimated by SVA "BE" and "leek" algorithms. Effect of SVA was assessed in real data by evaluating the reproducibility of DEA results (performed with limma) with and without SVA in two large Alzheimer's disease (ROSMAP, MSBB) and schizophrenia (MSSM-Penn-Pitt, NIMH-HBCC) gene expression datasets.

Results: Overall, accounting for SVs in DEA led to overfitting in simulation analyses, with a high false positive rate of DEG identification (mean = 0.22, standard deviation = 0.26). When SVA was applied to real data, the reproducibility of DEG signatures dropped substantially for Alzheimer's disease (Spearman's correlations pre-SVA rho = 0.479 and post-SVA rho = 0.127) and for schizophrenia (pre-SVA rho = 0.383 and post-SVA rho = 0.126).

Conclusion: The use of SVA in DEA requires careful consideration to avoid overfitting, cautioning against its indiscriminate usage.

Grants:

Conflict of Interest: None declared

P16.027.B Comprehensive network modeling approaches unravel dynamic enhancer-promoter interactions across neural differentiation

Anat Kreimer[1]

[1]*Rutgers University, Piscataway,NJ, United States*

Background/Objectives: Increasing evidence suggests that a substantial portion of disease-associated mutations occur in enhancers, cis-regulatory regions essential to gene regulation. Understanding the structure and mechanism of regulatory programs this variation affects can shed light on disease mechanisms.

Methods: Here, we collected epigenetic and gene expression datasets from seven time points during neural differentiation. Focusing on this model system, we constructed intricate networks of enhancer-promoter interactions at individual stages of neural induction. These networks functioned as the base of a rich series of analyses, through which we demonstrated the network's temporal dynamics and enrichment for various disease-associated variants. We applied the Grivan-Newman bi-clustering algorithm to these networks to reveal biologically relevant sub-structures of gene regulation. We also validated our predicted enhancer-promoter interactions using transcription factor overexpression datasets and massively parallel reporter assays.

Results: Our results suggest a general framework for exploring gene regulation programs and their dynamics across developmental processes and a comprehensive approach to exploring the effects of disease-associated variants on transcriptional networks. The techniques applied to our networks have been published alongside our findings as a computational tool, E-P-INAnalyzer. Our approach is general and can be applied across different cellular contexts and disorders.

Conclusion: Analysis of enhancer promoter intecation networks can shed light on mechanisms of gene regulation underlying most biological processes and can suggest how genetic variants disrupt gene transcription networks.

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Conflict of Interest: None declared

P16.028.C SMapper: Visualising spatial prevalence data while accounting for data sparseness and incompleteness

Lynn Khellaf[1], arwin ralf[2], Khanh Toan Nguyen[1], Manfred Kayser[2], **Michael Nothnagel[1]**

[1]University of Cologne, Cologne Cener for Genomics, Cologne, Germany; [2]Erasmus University Medical Center, Department of Genetic Identification, Rotterdam, Netherlands

Background/Objectives: Available spatial prevalence data are often limited by sparse geographic coverage and insufficient sample size. Not accounting for sparseness and incompleteness in the data visualization, as done by most previously developed bioinformatic tools, carries the risk of severe misinterpretation e.g., concluding an apparently restricted occurrence of a feature of interest, such as disease prevalence, allele or Y-haplogroup frequency, while the true occurrence region maybe much larger.

Methods: To overcome these limitations, we developed the SMapper bioinformatic tool to visualize spatial prevalence data including those that are sparse and incomplete. In its spatial visualization at the worldwide level, SMapper clearly separates geographic regions where the feature of interest was observed at some frequency, from those regions not covered by the data, and those where, although covered, the feature was not observed (zero frequency). Moreover, the uncertainty of a zero-frequency observation is visualized by taking the respective sample size into account.

Results: This way, SMapper overcomes interpretational issues known for existing tools that are caused by data limitations. Here, we introduce SMapper and present data and applications in fields such as epidemiology, anthropology and forensic genetics.

Conclusion: For widespread applications, we made SMapper publicly available as a web service as well as a native Linux installation and a singularity container at https://rhodos.ccg.uni-koeln.de/smapper/.

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Conflict of Interest: None declared

P16.029.D STIGMA: Single cell tissue-specific gene prioritization using machine learning

Saranya Balachandran[1], *Cesar A. Prada-Medina[2;3], Martin Atta Mensah[4;5;6], Juliane Glaser[6], Naseebullah Kakar[1;7], Inga Nagel[1], Jelena Pozojevic[1], Enrique Audain Martinez[8;9;10], Marc Phillip Hitz[8;9;10], Martin Kircher[1], Varun Sreenivasan[1], Malte Spielmann[1;3;6]*

[1]Institute of Human Genetics, University Hospital Schleswig-Holstein, University of Lübeck and Kiel University, Lübeck, Germany; [2]Novo Nordisk Research Centre Oxford, Innovation Building, Oxford, United Kingdom; [3]Human Molecular Genetics Group, Max Planck Institute for Molecular Genetics, Berlin, Germany; [4]Institut für Medizinische Genetik und Humangenetik, Charité - Universitätsmedizin Berlin, corporate member of Freie Universität Berlin and Humboldt-Universität zu Berlin, Berlin, Germany; [5]BIH Charité Digital Clinician Scientist Program, BIH Biomedical Innovation Academy, Berlin, Germany; [6]RG Development & Disease, Max Planck Institute for Molecular Genetics, Berlin, Germany; [7]Department of Biotechnology, BUITEMS, Quetta, Pakistan; [8]Institute of Medical Genetics, Carl von Ossietzky University, Oldenburg, Germany; [9]DZHK e.V. (German Center for Cardiovascular Research), Partner Site Hamburg/Kiel/Lübeck, Germany; [10]Department of Congenital Heart Disease and Pediatric Cardiology, University Hospital of Schleswig-Holstein, Kiel, Germany

Background/Objectives: Clinical exome and genome sequencing transformed disease genetics, but many genes lack functional annotation and characterization, hindering interpretation of genetic variants underlying human genetic disorders. However, tissue-specific gene expression information can be used to identify candidate genes for rare congenital diseases.

Methods: Here, we introduce Single cell TIssue-specific Gene prioritization using MAchine Learning (STIGMA), an approach that leverages single-cell RNA-seq (scRNA-seq) data. While existing scoring methods fail to capture expression heterogeneity within tissues, STIGMA prioritizes genes by learning the temporal expression dynamics across cell types during healthy organogenesis. By supervised machine learning, it predicts the probability of pathogenicity for all genes from scRNA-seq expression and intrinsic gene properties.

Results: STIGMA's performance, was evaluated using mouse limb and human fetal heart scRNA-seq datasets. In individuals with congenital limb malformations, STIGMA prioritized 469 variants in 345 genes, with *UBA2* as a notable example. For congenital heart defects, we detected 34 genes harboring nonsynonymous de novo variants in two or more individuals from a set of 7,958 individuals, including *Prdm1*, which is associated with hypoplastic left ventricle and hypoplastic aortic arch.

Conclusion: We demonstrate that STIGMA effectively prioritizes tissue-specific candidate genes from scRNA-seq. The ability to capture the heterogeneity of gene expression across cell populations makes STIGMA a powerful tool for the discovery of disease-associated genes and facilitates the identification of causal variants.

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Conflict of Interest: Saranya Balachandran Universitätsklinikum Schleswig-Holstein, Cesar A. Prada-Medina: None declared, Martin Atta Mensah: None declared, Juliane Glaser: None declared, Naseebullah Kakar: None declared, Inga Nagel: None declared, Jelena Pozojevic: None declared, Enrique Audain Martinez: None declared, Marc Phillip Hitz: None declared, Martin Kircher: None declared, Varun Sreenivasan: None declared, Malte Spielmann: None declared

P16.031.B Integrating single-cell with bulk transcriptomics combined with machine learning identifies metabolic pathway epithelial-enriched gene signature predictive of 5-year survival in breast cancer patients

Chengyi Wang[1], Samuel Chong[2], **Caroline Lee**[1;3;4]

[1]Duke-NUS Medical School, Singapore; [2]National University of Singapore, Pediatrics, Singapore, Singapore; [3]National University of Singapore, Biochemistry, Singapore; [4]National Cancer Centre, Singapore, Singapore

Background/Objectives: Breast cancer's (BC) cellular heterogeneity complicates prognosis. Single-cell transcriptomics (SCT) can assess specific cell-types' roles in prognosis, but their clinical implications in BC are not fully understood. This study integrates single-cell with bulk transcriptomics to analyze the clinical relevance of cell-type enriched genes (CEGs) identified from SCT, developing a machine-learning (ML) based prognosis predictive model.

Methods: CEGs, identified from SCT data of 81 BC patients, were defined as those expressed in significantly greater proportions (percentage difference > 0.3) and at higher levels (fold change > 1.5) in specific cell types (e.g., epithelia, immune, stroma). These genes were trained on The Cancer Genome Atlas (TCGA) dataset (701 patients) using about 100 ML algorithms and validated on TCGA test datasets (300 patients) and the METABRIC dataset (over 2000 patients).

Results: Epithelial-enriched CEGs predicted lower 5-year survival in BC. ML models achieved an AUC of 0.75 in TCGA training and 0.71 in METABRIC validation. TCGA testing confirmed the models' robustness (AUC 0.63). Gene analysis showed significant metabolic pathway enrichment in these genes, especially glycolysis and the Pentose Phosphate Pathway.

Conclusion: This study underscores the prognostic value of epithelia-enriched gene signatures in BC. The ML models demonstrated strong predictive capabilities, linking specific metabolic pathways to prognostic outcomes. These findings enhance understanding of BC cellular heterogeneity and support precision treatment strategies. By integrating SCT with advanced ML techniques, our approach refines prognostic stratification and enhances clinical decision-making in BC management.

Grants: None

Conflict of Interest: None declared

P16.032.C HerediClassify: Automated variant classification on a hereditary breast and ovarian cancer variant database

Anna-Lena Katzke[1], *Marvin Döbel[2], Jan Hauke[3], Marc Sturm[2], Gunnar Schmidt[1]*

[1]Hannover Medical School, Department of Human Genetics, Hannover, Germany; [2]University Hosptial Tübningen, Institute of Medical Genetics and Applied Genomics, Tübingen, Germany; [3]University Hosptial Cologne, Center for Familial Breast and Ovarian Cancer, Center for Integrated Oncology (CIO), Cologne, Germany

Background/Objectives: The progressively more detailed guidelines for variant interpretation in combination with the increase in samples and the introduction of whole genome sequencing pose a challenge to manual variant classification. Variant interpretation has become a bottleneck in genetic diagnostics.

Especially challenging is the occurrence of variants of uncertain significance (VUS). The German Consortium of Hereditary Breast and Ovarian Cancer alone encounters an estimated 1,600 VUS every year. In order to aid in the classification and reclassification of these variants, we are developing a tool for automated variant classification.

Methods: To develop HerediClassify, we implemented (1) ACMG/AMP guidelines along with the most recent SVI recommendations and (2) gene specific guidelines for ATM, BRCA1, BRCA2, CDH1, PALB2, PTEN and TP53. 20/28 ACMG rules were implemented. Data availability limits application of ACMG rules that rely on functional or segregation data.

Results: We present an open-source variant classification algorithm designed for modularity and extensibility. This does not only allow for easy switching between, for instance, prediction tools but also provides the option to choose between different rule specifications. Additionally, the clear separation between annotation and rule assessment allows for the easy implementation of new rules, rule specifications and classification frameworks.

Conclusion: Whilst HerediClassify is not yet at a stage where all variants can be classified, it already can be used for the prioritization of variants for classification or reclassification

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Conflict of Interest: Anna-Lena Katzke Hannover Medical School, Marvin Döbel: None declared, Jan Hauke: None declared, Marc Sturm: None declared, Gunnar Schmidt: None declared

P16.033.D *comorbidPRS*: an R package assessing shared predisposition between correlated traits using polygenic scores

Vincent Pascat[1;2;3], Liudmila Zudina[1;3], Anna Ulrich[1;3], Jared Maina[2], Marika Kaakinen[1;3;4], Igor Pupko[1], Amélie Bonnefond[2;3], Ayse Demirkan[1;4], Zhanna Balkhiyarova[1;3;4], Philippe Froguel[2;3], **Inga Prokopenko**[1;2;4]

[1]University Of Surrey, Department of Clinical and Experimental Medicine, United Kingdom; [2]European Genomic Institute for Diabetes, INSERM UMR1283, CNRS UMR 8199, Lille, France; [3]Imperial College London, Department of Metabolism, Digestion and Reproduction, United Kingdom; [4]University Of Surrey, People-Centred Artificial Intelligence Institute, United Kingdom

Background/Objectives: Polygenic (Risk) Score (PRS) is a useful approach in evaluation of the estimated genetic liability to a given outcome or genetic variability contributing to a quantitative trait level. While PRSs for complex diseases and traits are very popular, there a limited implementation of PRSs to uncover the shared genetic predisposition between phenotypes, i.e. when genetic variants influence more than one phenotype. We developed an *R* package, *comorbidPRS*, enabling a systematic evaluation of shared genetic effects between (cor)related phenotypes through the use of PRSs.

Methods: As input, *comorbidPRS* uses a set of SNPs with their effects on original phenotype (P_o) as P_o-PRS. *comorbidPRS* outputs

a summary of associations between P_o-PRS and target phenotype (P_t) with customisable graphical features. We applied *comorbidPRS* to dissect the shared genetic predisposition between elevated blood pressure (systolic, diastolic, pulse pressure [SBP/DBP/PP = SBP − DBP]) and several cancers (Kidney Cancer, Prostate Cancer, Colorectal Cancer [KidC/PrC/CrC]) using the UK Biobank and GWAS summary statistics.

Results: We confirmed a nominal higher risk of KidC for individuals with genetic predispositions to elevated DBP ($OR_{PRS-DBPtoKidC} = 1.04\,[1.00 - 1.08]$, $P - value_{PRS-DBPtoKidC} = 4.34 \times 10^{-2}$). We report an association between elevated DBP and the genetic risk of PrC ($OR_{PRS-PrCtoDBP} = 1.06\,[1.03 - 1.10]$, $P - value_{PRS-PrCtoDBP} = 9.64 \times 10^{-5}$), as well as associations between CrC PRS and decreased SBP ($OR_{PRS-CrCtoSBP} = 1.06\,[1.03 - 1.10]$, $P - value_{PRS-CrCtoSBP} = 9.64 \times 10^{-5}$), DBP($OR_{PRS-CrCtoDBP} = 1.06\,[1.03 - 1.10]$, $P - value_{PRS-CrCtoDBP} = 9.64 \times 10^{-5}$), and PP ($OR_{PRS-CrCtoPP} = 1.06\,[1.03 - 1.10]$, $P - value_{PRS-CrCtoPP} = 9.64 \times 10^{-5}$).

Conclusion: Using *comorbidPRS*, we highlight mechanistic relationships between regulation blood pressure traits and susceptibility to three comorbid malignancies. *comorbidPRS* provides the evaluation of shared genetic susceptibility between (cor)related phenotypes through PRS.

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Conflict of Interest: None declared

P16.034.A A new forensic DNA phenotyping tool that improves intermediate eye colour prediction in Brazilians

Thassia Mayra Telles Carratto[1], *Chris Phillips*[2], *Maria Angeles Casares de Cal*[3], *Antonio Gomez Tato*[3], *Ana Freire-Aradas*[2], *Aguinaldo Simões*[4], *Heather Norton*[5], *Esteban Parra*[6], *Denise Syndercombe Court*[7], *Celso Teixeira Mendes-Junior*[8]

[1]*University of São Paulo, Department of Chemistry, Faculty of Philosophy, Sciences and Letters at Ribeirão Preto, Ribeirão Preto, Brazil;* [2]*University of Santiago de Compostela, Forensic Genetics Unit, Institute of Forensic Sciences, Santiago de Compostela, Spain;* [3]*University of Santiago de Compostela, Faculty of Mathematics, Santiago de Compostela, Spain;* [4]*University of São Paulo, Ribeirão Preto Medical School, Ribeirão Preto, Brazil;* [5]*University of Cincinnati, Department of Anthropology, Cincinnati, United States;* [6]*University of Toronto at Mississauga, Department of Anthropology, Mississauga, Canada;* [7]*King's College London, King's Forensics, Department of Analytical, Environmental and Forensic Sciences, Faculty of Life Sciences and Medicine, London, United Kingdom;* [8]*University of São Paulo, Faculty of Philosophy, Sciences and Letters at Ribeirão Preto, Ribeirão Preto, Brazil*

Background/Objectives: In forensic DNA phenotyping, accurately predicting intermediate eye colour remains a challenge. While IrisPlex falls short in this regard, the Snipper system, although capable, exhibits lower accuracy compared to blue and brown eyes. Given the prevalence of intermediate eye colour in Brazil, this project aims to develop a more accurate predictive tool tailored for Brazilians and suitable for forensics.

Methods: DNA extracted from 501 individuals from Ribeirão Preto, Brazil, was subjected to next-generation sequencing and microarray assays. SNP genotypes from 504 genes were obtained, with missing markers imputed. Divergence values were calculated for each marker, and the top 21 were selected for further evaluation. Using the Naive-Bayes algorithm, we incrementally added markers, selecting the model with the best accuracy across three eye colour categories. IrisPlex and Snipper were also employed for comparison.

Results: The optimal model, consisting of 14 markers, achieved a sensitivity of 72.82% for intermediate eye colour, surpassing IrisPlex (0.00%) and Snipper (36.89%). For blue and brown categories, the 14-marker model demonstrated sensitivities of 93.18% and 81.64%, respectively, with an overall accuracy of 80.84%. Notably, only one SNP (rs1129038) from the 14 used had been employed by other tools for eye colour prediction.

Conclusion: The preliminary results indicate the potential for enhancing intermediate eye colour prediction. However, evaluating the model's performance in independent samples is crucial to ensure its forensic applicability.

Grants: CNPq/Brazil, FAPESP/Brazil, CAPES/Brazil (Finance Code 001), NSERC/Canada, US National Institute of Justice.

Conflict of Interest: None declared

P16.035.B Performance of deep-learning based approaches to improve polygenic scores

Martin Kelemen[1], *Yu Xu*[1;2], *Michael Inouye*[1;2]

[1]*British Heart Foundation Cardiovascular Epidemiology Unit, University of Cambridge, Department of Public Health and Primary Care, Cambridge, United Kingdom;* [2]*Cambridge Baker Systems Genomics Initiative, University of Cambridge, Department of Public Health and Primary Care, Cambridge, United Kingdom*

Background/Objectives: Polygenic risk scores (PRS) have the potential to become part of genomic healthcare initiatives across the developed world over the coming decade. To maximise their clinical relevance, PRS models will need to incorporate environmental risk factors and also leverage the latest methodological improvements. Neural-network (NN) based deep learning has emerged as the premier method to model complex, non-linear phenomena, which may be used to exploit gene-gene (G*x*G) and gene-environment (G*x*E) interactions to improve the accuracy of phenotype prediction.

Methods: We present a framework for using NNs to infer the amount of non-linearity present in a phenotype, which also controls for the confounding effect of LD. We fit NN models to both simulated and 28 real disease and anthropometric traits in the UK Biobank.

Results: Simulation results confirmed that our framework could adequately control for LD and may be used to infer non-linear effects, when such effects genuinely exist. Using this approach on real data, we found evidence for small amounts of non-linearity due to G*x*G and G*x*E at ~7% and ~4% difference in r^2, respectively. Despite this evidence for non-linear effects, the NN models were outperformed by linear regression models for both genetic-only and gene+environmental input scenarios by ~7% and ~5% difference in r^2, respectively.

Conclusion: Despite NNs being the state-of-the-art method for modelling DNA sequence features, our results indicate that similar success in predicting phenotypic variance should not be expected. Therefore, the usefulness of NNs for genomic medicine through PRS generation may be limited.

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Conflict of Interest: Martin Kelemen: None declared, Yu Xu: None declared, Michael Inouye Trustee of the Public Health Genomics (PHG) Foundation., Research collaborations with AstraZeneca, Nightingale Health, and Pfizer., Scientific Advisory Board of Open Targets.

P16.038.B Enhancing genomic data sharing with blockchain-enabled dynamic consent in Beacon v2

Leman Binokay[1;2], *Hamit Mervan Çelik*[3], *Gültekin Gürdal*[3;4], *Tolga Ayav*[3;5], *Tugkan Tuglular*[3;5], *Yavuz Oktay*[6;7;8], *Gokhan Karakulah*[1;2;8]

[1]Izmir International Biomedicine and Genome Institute, Molecular Biology and Genetics, Izmir, Türkyie; [2]Izmir International Biomedicine and Genome Center, Bioinformatics Platform, Izmir, Türkyie; [3]Research Ecosystems Araştırma Geliştirme Yazılım Sanayi ve Ticaret A.Ş., Izmir, Türkyie; [4]Izmir Institute of Technology, Library, Izmir, Türkyie; [5]Izmir Institute of Technology, Computer Engineering, Izmir, Türkyie; [6]Dokuz Eylül University School of Medicine, Medical Biology, Izmir, Türkyie; [7]Izmir International Biomedicine and Genome Center, Neurogenomics, Izmir, Türkyie; [8]Solaris Genomics Arge ve Sağlık Teknolojileri A.Ş., Izmir, Türkyie

Background/Objectives: Genomic data sharing faces the significant challenge of aligning individual consent preferences with data accessibility. This study aims to integrate dynamic consent within the Beacon v2 framework, enhanced with blockchain technology, to address these challenges and improve the ethical management of genomic data.

Methods: We developed a federated search and analytics platform, incorporating blockchain technology to secure and streamline consent management processes. The platform follows FAIR (Findable, Accessible, Interoperable, Reusable) data principles, ensuring efficient utilization of genomic and associated clinical data. The integration of blockchain technology with dynamic consent mechanisms was meticulously designed to complement Beacon v2's existing structure.

Results: The implementation of blockchain-enabled dynamic consent in Beacon v2 provided a more secure and transparent framework for genomic data sharing. This approach enhanced participants' ability to manage their consent preferences dynamically, aligning with ethical and legal standards. The initiative, primarily focused on rare diseases, demonstrated increased data utility and participant trust in the data sharing process.

Conclusion: The incorporation of blockchain technology with dynamic consent in Beacon v2 signifies a substantial step forward in genomic data sharing. This approach balances individual privacy and preferences with the need for scientific advancement, potentially serving as a model for future research practices in genomics. The broader implications of this study extend to enhancing data security and ethical management in various areas of genomic research and patient care.

Grants: This study was supported by the TÜBİTAK TEYDEB 1507 Program, under project number 7220189.

Conflict of Interest: None declared

P16.039.C Distinctive genotype-driven approach to panel analysis and reporting

Dina Yagel[1;2], *Rotem Greenberg*[1;2;3], *Michal Naftali*[1], *naama elefant*[1], *Ofer Isakov*[1;2;4], *Shay Ben-Shachar*[1;2;3;4]

[1]Clalit Health Services, Genomic Center, Petach Tikva, Israel; [2]Clalit Health Services, Berkowitz Family Precision Medicine Clinic, Clalit Research Institute, Ramat Gan, Israel; [3]Clalit Health Services, Clalit Research Institute, Ramat Gan, Israel; [4]Tel Aviv University, Faculty of Medicine, Tel Aviv, Israel

Background: Target gene panels (GPs) are used to focus a genetic analysis around the primary phenotype presented by the patient. Accurate extraction of additional phenotypic details from clinical summaries is challenging and time intensive. Therefore, a phenotype-driven analysis may result in exclusion of causative variants.

Consequently, a standardized genotype-driven approach is essential to alleviate the workload for Variant Scientist (VS) and ensure consistent, reliable reporting.

Methods: GPs' genes are divided into "core" or "extended" lists based on the strength of phenotypic association. Variants within these genes are reported solely based on their likelihood of being deleterious (according to ACMG-guidelines), without additional assessment of the observed phenotype. Standardized reporting thresholds are determined by the variant's posterior probability of pathogenicity, which is more stringent for the "extended" set compared to the "core".

Results: A comprehensive Standard Operating Procedure (SOP) was drafted and followed by all VS. The SOP encompasses analysis filter-sets, variant classification rules, and reporting policies. The VS maintain a dedicated emphasis on variant classification and remain undistracted by phenotypic matching, resulting in a reduced turnaround time. Consistency was achieved through the examination of the same case by three VSs.

Conclusion: This approach is designed to ensure consistent and unified reporting of variants, leaving the responsibility for clinical decisions to the treating physicians. Although, this method might sometimes report more variants to physicians, it also ensures that variants are reported consistently and can help with diagnoses that might otherwise be missed by referring physicians.

Grants: The authors declare no conflict of interest

Conflict of Interest: None declared

P16.040.D Identifying regulatory variation in the human brain through single-cell multiomics and whole genome sequencing

Alexandra Pančíková[1;2;3], *Olga Sigalova*[2;3], *Koen Theunis*[2;3], *Gert Hulselmans*[2;3], *Julie De Man*[2;3], *Florian De Rop*[2], *Sara Salama*[4], *Katy Vandereyken*[4], *Shinjini Mukherjee*[4], *Thierry Voet*[4;5], *Jonas Demeulemeester*[1], *Stein Aerts*[2;3]

[1]VIB-KU Leuven Center for Cancer Genomics, Laboratory of Integrative Cancer Biology, Leuven, Belgium; [2]VIB-KU Leuven Center for Brain & Disease Research, Laboratory of Computational Biology, Leuven, Belgium; [3]VIB.AI, Leuven, Belgium; [4]KU Leuven Institute for Single Cell Omics (LISCO), Leuven, Belgium; [5]KU Leuven, Laboratory of Reproductive Genomics, Center for Human Genetics, Leuven, Belgium

Background/Objectives: The genetic causes of sporadic Parkinson's disease remain poorly understood. This is primarily due to the fact that the majority of SNPs identified in genome wide association studies implicate non-coding parts of the genome. Non-coding variation may lead to cell-type specific changes in gene expression that contribute to disease development, but this remains challenging to study.

Methods: To comprehensively identify all types of genetic variation and their impact on gene regulation in the brain, we are generating a large single nuclei multiomic atlas and matched long-read bulk whole-genome sequencing data from 190 donors (both patients and controls). We developed in-house pipelines to analyse these data and obtain comprehensive variant calls, epigenetic states (endogenous DNA methylation and exogenously labelled open-chromatin; Fiber-seq) and personalised diploid genome for each donor. We are now combining these personal genomes with donor-matched multiome data to identify allele-specific signals and reveal regulatory variation.

Results: Our genomes have average coverage of 28X, and we detect 32,991 structural variants 4,504,259 SNPs, and 295,560 and 338,821 small insertions and deletions per genome on average. Our atlas currently consists of over 1.2M RNA and 1.5M ATAC high quality nuclei from substantia nigra and cingulate gyrus. We can

identify expected cell types and assign pooled nuclei to corresponding donors.

Conclusion: Uniquely combining population-scale matched long read genomes and single-cell multiome atlassing is revealing the extent of regulatory variation in human brains and will implicate currently cryptic causal variation underpinning Parkinson's Disease.

Grants:
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Conflict of Interest: None declared

P16.041.A Interpreting artificial neural networks for detecting genome-wide association signals for complex diseases

Burak Yelmen[1], *Maris Alver*[1], *Silva Kasela*[1], *Lili Milani*[1]

[1]*University of Tartu, Institute of Genomics, Tartu, Estonia*

Background/Objectives: Investigating the genetic architecture of complex diseases is challenging due to the highly polygenic and interactive landscape of genetic and environmental factors. Although genome-wide association studies have identified thousands of variants for multiple complex phenotypes, conventional statistical approaches can be limited by simplified assumptions such as linearity and lack of epistasis models.

Methods: In this work, we trained artificial neural networks for predicting complex diseases using both simulated and real genotypes from the Estonian Biobank. We assessed the weights and features learned by these models and extracted feature importance scores to identify potential genomic loci associated with the target phenotype. We used simulated data under various scenarios to test the capacity of our approach to detect causal loci.

Results: Simulation results demonstrated that causal loci can be detected with good precision using strict selection criteria but downstream analyses are required for fine-mapping the exact variants due to linkage disequilibrium. By applying our approach to the schizophrenia cohort in Estonian Biobank (1664 cases), we were able to detect multiple novel loci related to this highly polygenic and heritable disorder. We also performed enrichment analyses with detected loci in genic regions, which predominantly identified terms associated with neurological processes.

Conclusion: With further improvements in model optimization and confidence measures, nonparametric artificial neural network models can be utilised for identifying genomic loci associated with complex diseases, serving as initial screening tools for subsequent functional studies.

Grants: This work received funding from the European Union's Horizon 2020 programme under grant agreements No. 847776 (CoMorMent) and 964874 (Realment).

Conflict of Interest: None declared

P16.042.B Novel genetic insights into human facial variation: integrating AI-enhanced phenotyping with C-GWAS

Xianjing Liu[1;2], *Ziyi Xiong*[3;4], *Fan Liu*[3], *Eppo Wolvius*[2], *Manfred Kayser*[3], *Gennady Roshchupkin*[1;4]

[1]*Erasmus MC, Department of Radiology and Nuclear Medicine, ROTTERDAM, Netherlands;* [2]*Erasmus MC, Department of Oral & Maxillofacial Surgery, ROTTERDAM, Netherlands;* [3]*Erasmus MC, Department of Genetic Identification, ROTTERDAM, Netherlands;* [4]*Erasmus MC, Department of Epidemiology, ROTTERDAM, Netherlands*

Background/Objectives: Genome-wide association studies (GWAS) are powerful tools for identifying genetic links to phenotypic traits, but they face significant computational hurdles when analyzing complex traits derived from medical imaging. To tackle this challenge, we have developed a sophisticated algorithmic pipeline: First utilizes AI-based techniques to derive phenotypes, in a federated learning (FL) setting, maintaining data privacy, thus eliminating the need to share raw data, particularly 3D facial images; Second, applies combined-GWAS (C-GWAS) to enrich genetic signal due to the similarity between imaging traits.

Methods: The study utilized two cohorts: 3,314 children from the Generation R Study and 3,995 elderlies from the Rotterdam Study. Facial morphology was encoded into a low-dimensional representation using a 3D mesh autoencoder, an advanced AI model, and trained via NVIDIA Flare, a federated learning open-source library, yielding 200 AI-based facial traits. Subsequent analyses included EasyQC, single-trait GWAS, standard meta-analysis, and C-GWAS.

Results: C-GWAS identified 43 genomic loci, including 12 novel ones. Facial heatmaps visualized the AI-based facial traits linked to these loci, such as rs4648379 (*PRDM16*), rs3122635 (*CRB1*), and rs752172 (*PAX3*), affecting nose shape, chin dimples, and nasal bridge structure, respectively. These loci also showed associations with various health conditions, including asthma, insomnia, snoring, dental development, brain morphology, and total ventricular volume.

Conclusion: Our study's findings of known and novel genetic loci linked to facial variation underscore the efficacy of our proposed methodological pipeline in studying image-based complex traits within the GWAS framework. This approach is promising for broader applications in large-scale consortia facilitating advanced research while maintaining data privacy

Grants: None.

Conflict of Interest: None declared

P16.043.C Modelling genetic effects on gene expression dynamics in B cells

Daniela Zanotti[1;2], *Alessandro Raveane*[1], *Matiss Ozols*[3], *Marc Jan Bonder*[4], *Francesca Ieva*[2;5], *Nicole Soranzo*[1;3;6], *Nicola Pirastu*[1], *Blagoje Soskic*[1]

[1]*Human Technopole, Genomics Research Centre, Milano, Italy;* [2]*Politecnico di Milano, MOX - Laboratory for Modeling and Scientific Computing, Department of Mathematics, Milano, Italy;* [3]*Wellcome Sanger Institute, Human Genetics Department, Hinxton, United Kingdom;* [4]*University of Groningen, University Medical Center Groningen, Department of Genetics, Groningen, Netherlands;* [5]*Human Technopole, Health Data Science Centre, Milano, Italy;* [6]*University of Cambridge, British Heart Foundation Centre of Research Excellence, Cambridge, United Kingdom*

Background/Objectives: During activation and differentiation B cells go through large changes in gene expression. Previous studies have shown that expression quantitative trait loci (eQTLs) can have dynamic effects in B cells undergoing differentiation. Some of these studies cluster cells along pseudotime trajectories and are dependent upon the binning choice, while other cluster-free methods are too computationally intensive to be applied to large-scale settings. We aimed to address both these limitations by modelling gene expression as a continuous function of pseudotime, and investigating allelic effects in differentiating B cells.

Methods: Using B cells from the OneK1K cohort, we estimated pseudotime trajectories and then modelled the gene expression of 8,324 genes along them using splines. Finally, we applied Functional Principal Component Analysis (FPCA) to the generated

curves and their derivatives and used the scores for cis-eQTL analysis.

Results: We detected 2,022 and 236 genes with significant dynamic cis-eQTLs using the FPCA scores of the original curves and the derivatives, respectively. In the first case, many functions showed differences in height between genotypes, while the derivatives capture effective differences in the functions' shape. The identified genes were enriched for specific immune cell signatures such as response to vaccination.

Conclusion: Our approach demonstrated high efficacy in detecting genetic loci which regulate dynamic gene expression in B cells. Moreover, we were able to detect both dynamic and static eQTLs, without relying on prior assumptions such as clustering while retaining low computational costs. This suggests that our method could be efficiently used in large-scale single-cell cohorts.

Conflict of Interest: None declared

P16.044.D GestaltGAN: Artificially synthesizing photorealistic portraits of rare genetic disorders that preserve characteristic clinical features

Aron Kirchhoff[1], *Alexander Hustinx*[1], *Behnam Javanmardi*[1], *Tzung-Chien Hsieh*[1], *Fabian Brand*[1], *Peter Krawitz*[1]

[1]*Institute for Genomic Statistics and Bioinformatics, Bonn, Germany*

Background/Objectives: Medical images are often sparsely available due to privacy reasons or limited number of cases, but they are needed for research and teaching. Especially training deep learning models requires large amounts of training images, one example is GestaltMatcher which predicts the most likely syndrome for a given dysmorphic patient portrait.

Methods: Generative AI is increasingly used in many different fields, one example is medicine where it can be used to generate artificial medical images. We applied Generative Adversarial Networks (GAN) to dysmorphic portraits. The StyleGAN network was trained on images from the GMDB. We used ESRGAN to increase the resolution of portraits from the training data with low quality. Then the training data was aligned&cropped to achieve a uniform format. The 20 most frequent syndromes from the GMDB were included in the training of our conditional StyleGAN model. To give the model access to more features of human faces an unaffected class was added to the training data. The unaffected faces from the FFHQ dataset share many features like hair and skin with the dysmorphic faces and facilitate the learning effort of the deep learning model.

Results: We show that the model is capable of producing high-quality dysmorphic portraits that contain the characteristic features of the disorders. At the same time, we show that the persons in the synthesized images look distinct from the patients in the training data.

Conclusion: The images synthesized by our method can be used for publications, data sharing, and training of deep learning models while highly preserving patients' privacy.

Grants:

Conflict of Interest: None declared

P16.045.A Development of a polygenic risk score for metabolic dysfunction-associated steatotic liver disease prediction in UK Biobank

ioanna gavra[1], *Panagiota Giardoglou*[1], *Athina Amanatidou*[1], *Ioanna-Panagiota Kalafati*[1;2], *Maria Kafyra*[1], *Evangelia Mentsiou-Nikolaou*[1], *Despoina Brekou*[1], *panagiotis moulos*[3], **George Dedoussis**[1]

[1]*Harokopio University, Kallithea, Greece;* [2]*University of Thessaly, Department of Nutrition & Dietetics;* [3]*B.S.R.C. Alexander Fleming, Fundamental Biomedical Research (IFBR), Vari, Greece*

Background/Objectives: Metabolic dysfunction-associated steatotic liver disease (MASLD) is the leading cause of liver-related morbidity and mortality. Although, the invasive liver biopsy remains the golden standard for MASLD diagnosis, MRI-derived Proton Density Fat Fraction (MRI-PDFF) is an accurate, non-invasive method for the assessment of treatment response. This study aimed at developing a Polygenic Risk Score (PRS) to improve MRI-PDFF prediction using UK Biobank data to assess an individual's genetic liability to MASLD.

Methods: We iteratively sequestered 10% of MRI-PDFF samples as a validation set and split the rest of each dataset into base and target partitions, containing GWAS summary statistics and raw genotype data, respectively. PRSice2 was deployed to derive PRS candidates. Based on the frequency of SNP appearances along the PRS candidates, we generated different SNP sets according to variable frequency cutoffs. By applying the PRSs to the validation set, we identified the optimal SNP set, which was then applied to a Greek Nonalcoholic fatty liver disease (NAFLD) study.

Results: Data from 3,553 UK Biobank participants yielded 49 different SNP sets. After calculating the PRS on the validation set for every SNP set, an optimal PRS with 75 SNPs was selected (incremental $R^2 = 0.025$, p-value $= 0.00145$). Interestingly, 43 SNPs were successfully mapped to MASLD-related known genes. The selected PRS could predict traits, like LDL cholesterol and diastolic blood pressure in the UK Biobank, as also disease outcome in the Greek NAFLD study.

Conclusion: Our findings provide strong evidence that PRS is a powerful prediction model for MASLD, while it can also be applied on populations of different ethnicity.

Conflict of Interest: None declared

P16.046.B PANGEN: an online platform for the comparison and creation of diagnostic gene panels

Ofer Isakov[1;2;3;4], *Dina Yagel*[2], *Rotem Greenberg*[2], *Michal Naftali*[2], *Shay Ben-Shachar*[2;3;4]

[1]*Rabin medical center, Raphael Recanati Genetic Institute, Petah Tikva, Israel;* [2]*Clailt Research Institute, Ramat Gan, Israel;* [3]*Harvard, The Ivan and Francesca Berkowitz Family Living Laboratory Collaboration, United States;* [4]*Tel Aviv University, Faculty of medicine, Tel Aviv, Israel*

Background/Objectives: Targeted gene panel sequencing is used to limit the search for causative genetic variants solely to genes with established phenotypic association. Gene panel design is challenging due to the lack of consensus regarding phenotypic associations for some genes, which increases variation in gene composition for the same panel offered by different laboratories. We developed PANGEN, a platform that provides a centralized resource for gene panel information, with the ability to compare and generate new intelligent diagnostic panels.

Methods: Gene-phenotype associations were collected from 12 public and commercial sources (Blueprint, Cegat, Centogene, ClinGen, Fulgent, GeneDx, Health in Code, Human Phenotype Ontology (HPO), Invitae, PanelApp, Prevention genetics and Pronto diagnostics). Associations were stratified by levels of confidence. Pairwise similarity was calculated for every panel combination. Difficult to map regions in the panel were collected

from the Genome in a bottle (GIAB) stratifications dataset and the UCSC database.

Results: Overall, 1,533 panels, 9,759 phenotypes and 6,979 genes were collected. The platform provides an interface to (i) explore and compare collected panels, (ii) find similar panels, (iii) identify genes with high GC content or duplication levels, (iv) generate gene panels by combining various sources and (v) stratify a generated panel into genes with a strong phenotype association ("core") and those with a weaker association ("extended").

Conclusion: The presented platform represents a unique resource for gene panel exploration and comparison that facilitates the generation of tailored diagnostic panels through a public online web server (https://clalit-gc.shinyapps.io/gene_panel_generator_app/)

Conflict of Interest: None declared

P16.047.C Gen-toolbox prompts potential oligogenic patterns in a clinical neuromuscular disease cohort

Villem Pata[1;2], *Markus Marandi*[3], *Katrin Ounap*[1;4], *Sander Pajusalu*[1;4]

[1]*University of Tartu, Department of Clinical Genetics, Institute of Clinical Medicine, Tartu, Estonia;* [2]*Tartu University Hospital, Anesthesiology and Intensive Care Clinic, Tartu, Estonia;* [3]*University of Tartu, Institute of Computer Science, Tartu, Estonia;* [4]*Tartu University Hospital, Genetics and Personalized Medicine Clinic, Tartu, Estonia*

Background/Objectives: We propose a framework to investigate oligogenic patterns in clinical sequencing cohorts, aiming to explore whether some phenotypes in undiagnosed cohorts result from the combined effect of multiple variants across biologically relevant genes.

Methods: Gen-toolbox, a novel Hail-based toolkit, categorizes high-quality filtered variants by Ensembl VEP-predicted variant impact and allele frequency, assessing variant burden in subgroups. Summary variant statistics are calculated in each subgroup (N = 12) for corresponding genes. Monte-Carlo simulations compare sums of variant burdens of gene sets (N = 5) between phenotype positive and negative cohorts with an arbitrarily large number of random (N = 6e6) combinations. This large number of simulations causes most ratios to approach 1 after data normalization. We hypothesize non-Gaussian distribution and gene grouping above the 99th percentile to correlate to potential oligogenic patterns. Highlighted genes are further studied with gene ontology enrichment analysis using g:Profiler. Gen-toolbox works with different generation (g)VCF files, including Dragen NovaSeq X Plus output.

Results: Analyzing 4462 samples sequenced in Tartu University Hospital between 2015-2022 sent for various conditions (285 with neuromuscular disorders, 4177 otherwise afflicted), non-Gaussian distribution was noted in the modifier, AF < 0.05 subgroups. In other subgroups with normal distribution, high-burden gene enrichment analysis included the terms "signal receptor binding", "collagen-containing extracellular matrix", "tissue development" with 2.5e-14 < p-value <5e-6.

Conclusion: Gen-toolbox may detect potential oligogenic patterns for neuromuscular disorders in clinical cohorts. Further ontological and expression analyses are warranted to validate associations.

Grants: Estonian Research Council grants PSG774, PRG471 and PRG2040.

Conflict of Interest: None declared

P16.048.D In silico design of a recombinant multi-epitope vaccine against non-small cell lung cancer (NSCLC) via advanced immunoinformatics and reverse vaccinology approaches

Ali Alishavandi[1], *Tara Shekari*[2], *Mohammad Hossein Yazdi*[3]

[1]*Student Research Committee, Iranshahr University of Medical Sciences, Iranshahr, Iran;* [2]*Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran, Department of Pharmacutics, Tehran, Iran;* [3]*Biotechnology Research Center, Tehran University of Medical Sciences, Tehran, Iran., Tehran, Iran*

Background/Objectives: Non-small cell lung cancer (NSCLC) is the most common form of lung cancer. Nowadays, cancer vaccines offer promising prospects in treatment of malignancies. This study aims to develop a chimeric vaccine against NSCLC via immunoinformatics and reverse vaccinology methods.

Methods: Mutated amino acid sequences of KRAS, EGFR, and MUC1 were retrieved from the UniProt database. Epitope prediction for binding to the MHCI/II, B cells, and T cells was cunducted by IEDB and CTLpred servers. The semi-final vaccine construct was assembled, and its antigenicity, immunogenicity, allergenicity, and physicochemical properties were examined by respective tools.Thereafter, the secondary and tertiary structures of the vaccine were visualized by PSIPRED and RaptorX web servers, respectively. Docking of the vaccine/TLR-4 complex was performed using ClusPro2.0, followed by molecular dynamic (MD) simulation through normal mood analysis (NMA) using iMODS server.

Results: After multiple screenings, 18 T cell and 7 continuos/discontinuos B cell epitopes were identified. The final cunstruct consisted of 477 residues, and the docked vaccine/TLR-4 showed robust interactions. Also, Ramachandran plot demonstrated that over 96% of the residues were located in the favorable and permitted regions. MD simulation and physycochemical assessments, including B-factor, covariance matrix reported promising results with a neutral PH. C-ImmSim server immune simulation confirmed secreation of IFN-γ, IL-12, and IgG with a tendency toward Th1. Finally, IEDB estimated the vaccine's population coverage over 90% worldwide.

Conclusion: Our proposed chimeric vaccine showed favorable physicochemical properties and produced robust immune responses as well. Future research should assess the efficacy of this vaccine in animal experiments.

Grants: Recombinant vaccines research center

Conflict of Interest: None declared

P16.049.A Computing constraint metrics in gnomAD v4

Kristen M. Laricchia[1;2], *Julia Goodrich*[1;2], *Michael Wilson*[1;2], *Avery Wang*[1], *Katherine Chao*[1;2], *Grace Tiao*[1;2], *F. Kyle Satterstrom*[1;2], *Mark Daly*[1;2;3], *Heidi Rehm*[1;2], *Kaitlin Samocha*[1;2], *Konrad Karczewski*[1;2]

[1]*Broad Institute of MIT and Harvard, Cambridge, United States;* [2]*Massachusetts General Hospital, Boston, United States;* [3]*Institute for Molecular Medicine Finland, Helsinki, Finland*

Consortium: gnomAD Consortium

Background/Objectives: The Genome Aggregation Database (gnomAD) provides many metrics that aid with variant classification, including gene constraint, which measures how tolerant a gene is to different classes of variation. Less tolerant genes are often implicated in rare disease, so constraint metrics can help clinicians with gene and variant prioritization. Here, we utilized the increased scale and diversity of gnomAD v4 to recompute constraint metrics previously calculated on gnomAD v2.

Methods: We restructured the v2 constraint pipeline and rebuilt the mutational models utilizing over 800,000 individuals (76,215 genomes and 730,947 exomes) in v4. We calculated updated counts of observed and expected synonymous, missense, and predicted loss-of-function (pLoF) variants, and used these counts to refine the LOEUF (loss-of-function observed/expected upper bound fraction) scores.

Results: We generated constraint metrics using gnomAD v4 for 16,862 MANE Select transcripts, of which ~86% are well powered for detecting constraint against pLoF variants (expected pLoF ≥ 10). The larger sample size in v4 increased the AUC for distinguishing haploinsufficient genes from 0.87 in v2 to 0.89, continuing to outperform RVIS, and an analysis comparing 31,058 development disorder cases to 5,492 controls revealed a stronger enrichment of de novo pLoFs in cases in the most constrained LOEUF deciles in v4 than in v2.

Conclusion: The addition of updated constraint metrics in gnomAD v4 provides a resource that can aid in gene and variant analyses and help identify novel gene candidates for rare disease, as well as inform variant pathogenicity.

Grants: NA

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P16.051.C GenoMed4All,a federated learning platform for clinical and omics data

Davide Piscia[1;2], Patricia A. Apellániz[3], Borja Arroyo[4], Santiago Barrio[4], Francisco Moreno[4], Juan Parras[3], Silvia Uribe[5], Federico Alvarez[4], **Sergi Beltran**[1;2;6]

[1]Centro Nacional de Análisis Genómico - CNAG, Barcelona, Spain; [2]Universitat de Barcelona, Barcelona, Spain; [3]Information Processing and Telecommunications Center, Universidad Politécnica de Madrid, Madrid, Spain; [4]GATV, Escuela Técnica Superior de Ingenieros de Telecomunicación, Universidad Politécnica de Madrid, Madrid, Spain; [5]Escuela Técnica Superior de Ingeniería de Sistemas Informáticos, Universidad Politécnica de Madrid, Madrid, Spain; [6]Facultat de Biologia - Universitat de Barcelona, Departament de Genètica, Microbiologia I Estadística, Barcelona, Spain

Consortium: GenoMed4All consortium

Background/Objectives: Machine learning (ML) models have the potential to assist healthcare professionals in tasks such as diagnostics and predictive analysis. However clinical and genomics data are often siloed and difficult to access due to legal, ethical and technical reasons.

The emerging paradigm of data federation enables the use of data at different levels across resources. To take advantage of federated datasets it is necessary to develop adequate federated infrastructures enabling the training of ML models and conducting federated analyses.

Methods: Within the EU funded project Genomed4All we developed a platform enabling its users to run ML training on federated data. The platform is built on open source technologies such as Mlflow, Keycloak and Flower. The platform was developed to support Haematological diseases (Myelodysplastic syndromes, Multiple Myeloma and Sickle Cell), but can be adapted for other diseases.

The platform is composed of a central node and of a worker node module which has to be deployed in the different hospitals. The data includes clinical and omics data (genomics, transcriptomics and radiomics data) and FHIR is used as data model schema.

Results: Preliminary results with synthetic data show that the platform is able to support federated learning on distributed clinical and genomics datasets. In the next phase of the project several hospitals will connect to the central node.

Conclusion: The platform developed through Genomed4All paves the way to use federated learning as a solution to exploit data in settings where data is siloed and data privacy is of paramount importance.

Grants: Genomed4all, Horizon 2020

Conflict of Interest: None declared

P16.052.D Accelerating variant interpretation with the Ensembl Variant Effect Predictor

Sarah Hunt[1], Ola Austine Orimoloye[1], S. Nakib Hossain[1], Diana Lemos[1], Nuno Saraiva-Agostinho[1], Likhitha Surapaneni[1], **Jamie Allen**[1]*, Andrew Yates[1]*

[1]European Molecular Biology Laboratory, European Bioinformatics Institute, Wellcome Genome Campus, Hinxton, Cambridge, United Kingdom

Background/Objectives: Genome sequencing to identify likely disease-associated variants is common in clinical research and diagnostic settings. Robust tools are needed to annotate the large number of variants found and identify a priority subset for further investigation. The Ensembl Variant Effect Predictor (VEP) is a powerful toolset for variant annotation and prioritisation.

Methods: Ensembl VEP's core algorithms predict variant molecular consequence and it integrates a wide range of tools and reference data, including ClinVar pathogenicity assertions and gnomAD population frequencies. We have recently extended the available functionality by the integration of new data and analysis methods.

Results: MaveDB scores, which report the cellular phenotypes observed via multiplexed assays of variant effect, are now available through Ensembl VEP. Ribosome profiling has revealed thousands of open reading frames within long non-coding RNAs and the UTRs of protein coding genes, some of which may be functional and of clinical importance. Ensembl VEP can now predict when variants impact such ORFs. Predictions of variant deleteriousness have recently been integrated from tools including: AlphaMissense and VARITY for missense variant tolerance, Enformer for impact on gene expression and SpliceVault for impact on splicing. Other improvements to reporting include flagging de novo genotypes when trios are analysed.

Conclusion: Ensembl VEP provides an extensive range of analysis options, choice of interfaces and a flexible filtering tool which together enable detailed annotation and accelerate variant interpretation.

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Conflict of Interest: None declared

P16.053.A Genomic and metabolomic biomarkers for episodic memory

Anni Malmberg[1], *Matti Pirinen*[2;3;4], *Johannes Kettunen*[5], *Katri Räikkönen*[6], *Johan Eriksson*[7;8;9], *jari lahti*[6]

[1]*University of Helsinki, Department of Psychology and Logopedics, Finland;* [2]*University of Helsinki, Department of Mathematics and Statistics, Helsinki, Finland;* [3]*Institute for Molecular Medicine Finland, Helsinki Institute of Life Science, University of Helsinki, Helsinki, Finland;* [4]*University of Helsinki, Department of Public Health, Helsinki, Finland;* [5]*University of Oulu, Oulu, Finland;* [6]*University of Helsinki, Department of Psychology and Logopedics, Helsinki, Finland;* [7]*University of Helsinki, Helsinki, Finland;* [8]*Folkhälsan Research Center, Helsinki, Finland;* [9]*National University of Singapore, Singapore, Singapore*

Background/Objectives: Episodic memory (EM) is an endophenotype for dementia. We studied to what extent metabolomic and genomic biomarkers predict EM over and above traditional epidemiological markers in late adulthood and if the same markers predict EM in children.

Methods: With LASSO we selected 39 SNPs from 13M genomic variants and 14 metabolites from 137 NMR-derived metabolites associated with EM in the Helsinki Birth Cohort Study (HBCS) comprising ~61-year-old Finns (N = 711). We constructed a Cardiovascular Risk Factors, Aging, and Dementia (CAIDE) score from traditional risk factors for dementia: age, sex, education, systolic blood pressure, total cholesterol, BMI and physical inactivity. Finally, we used linear regression to test if CAIDE-score and biomarkers associated with EM in an independent HBCS-derived test sample (N = 101) and if the genome score predicted EM in the Glaku sample of 354 ~12-year-old children.

Results: In the HBCS test sample, the CAIDE-score, genome and metabolome scores explained 0.002% (p-value = 0.27), 4.6% (p-value = 0.017), and 0.012% (p-value = 0.14) of the variance in EM, respectively. After accounting for the CAIDE-score, the genome and metabolome scores explained 4.3% (p-value = 0.04), and 1.2% (p-value = 0.23) of the variance, respectively. The three scores together explained 5.4% (p-value = 0.039) of the variance. In the Glaku sample, the genome score showed no association with EM (p-value = 0.91).

Conclusion: Model predictability improved when accounting for genome score in addition to the CAIDE-score. However, the CAIDE-score and metabolome scores performed poorly in the independent HBCS test sample, and results for the genome score were not generalizable to children.

Grants: University of Helsinki Research Foundation

Conflict of Interest: None declared

P16.055.C Identification of cell type specific transcript isoforms by integration of bulk short-read, long-read and single cell RNA-seq

Ryo Yamamoto[1], *Noah Zaitlen*[1], *Xinshu Xiao*[1]

[1]*University of California, Los Angeles, Los Angeles, United States*

Background/Objectives: Alternative splicing profoundly influences the functional diversity of cell types, impacting up to 95% of multi-exon genes in humans. While bulk RNA-seq has advanced our understanding in diverse functions of transcript isoforms, it is imperative to scrutinize isoform abundances at a finer granularity than bulk tissues. However, accurately estimating isoform expression at a cell level remains challenging due to sparsity and 3′ bias inherent in single cell RNA-seq data. Moreover, few attempts have been made to study cell type specific isoforms at a population level.

Methods: We introduce Besciege, a Bayesian inference method that integrates scRNA-seq with bulk long-read and short-read RNA-seq to estimate isoform abundance across different cell types. Given single-cell and bulk RNA-seq in the same tissue, we first estimate sample level cell fractions and isoform abundance which are then used to estimate the population mean of isoform abundance for each cell type using non-negative least squares. Finally, we employ the hierarchical Bayesian model to estimate sample level cell type isoform abundance, which accounts for co-variability between isoforms within the same gene.

Results: Rigorous simulations demonstrate that Besciege performs accurate estimation of isoform abundance at a population level (mean Pearson's correlation = 0.92). Applied to the GTEx dataset, Besciege identifies more than 5,000 genes that exhibit isoform switching across cell types in 7 tissues, providing evidence for prevalent differential isoform usage at a cellular level.

Conclusion: Besciege is a powerful tool to quantify isoform abundance in cell types, enabling studies to characterize diverse functions of cell type specific isoforms.

Grants:

Conflict of Interest: None declared

P16.056.D Privacy-preserving evaluation of polygenic risk scores using fully homomorphic encryption

Jiaqi Li[1], *Elizabeth Knight*[1], *Matthew Jensen*[1], *israel Yolou*[1], *Mark Gerstein*[1]

[1]*Yale University, New Haven, United States*

Background/Objectives: Polygenic risk scores (PRS), which are statistical models that utilize genetic variants to predict an individual's risk of having a particular trait or condition, can be valuable in various clinical applications. However, their calculation involves both patients' genomic data and PRS models, posing privacy concerns. Here we apply fully homomorphic encryption (FHE) to PRS calculation. FHE permits PRS calculations directly on both encrypted genomes and models, and allows only the genomic data owner with the secret key to decrypt the results.

Methods: We apply the CKKS FHE scheme implemented within the Lattigo library to perform encrypted PRS calculations. We demonstrate the effectiveness of our method using both models derived from synthetic datasets and a real PRS model for schizophrenia risk.

Results: The application of our FHE-based protocol to a 110k-SNP PRS model for schizophrenia demonstrates high accuracy and negligible statistical difference from non-encrypted methods. We demonstrate that a decrease in mean square error between results from our encrypted method and the optimal LDPred2 model correlates with an increased polynomial modulus degree, showing how enhanced encryption parameters reduce computational error. Running our program with 1000 genotypes on the 110k-SNP model can take less than 5 minutes on a personal laptop, underscoring the scalability and efficiency of our method.

Conclusion: Our work successfully applies FHE to PRS calculation without compromising data security, accuracy, or computational integrity, showcasing a promising tool for clinical genomic settings where data privacy is paramount.

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Conflict of Interest: None declared

P16.057.A Assess individual risk scores for rare variants in stratifying epilepsy cases

Maryam Erfanian Omidvar[1;2], *Emadeldin Hassanin*[2;3], *Dheeraj Bobbili*[2], *Holger Lerche*[1], *Patrick May*[2]

[1]*Department of Neurology and Epileptology, Hertie Institute for Clinical Brain Research, University of Tübingen, Tübingen, Germany;*

[2]LCSB - Luxembourg Centre for Systems Biomedicine, University of Luxembourg, Esch-sur-Alzette, Luxembourg; [3]Institute for Genomic Statistics and Bioinformatics, University of Bonn, Bonn, Germany

Consortium: Epi25 Collaborative

Background/Objectives: The study investigates the potential of using individual rare genetic variant profiles to improve the classification and stratification of patients with epilepsy. Previous research has shown that rare genetic variants in specific genes are associated with different types of epilepsy, and this study aims to explore the cumulative contribution of these variants to create an individual risk score (IRS) that can better identify and stratify patients with epilepsy.

Methods: The study analyzed whole-exome sequencing data from 7,589 individuals with epilepsy (from the Epi25 dataset) and 3,962 ancestry-matched controls. We employed different allele frequency cutoffs and functional genomic annotations to evaluate the impact of the IRS on epilepsy stratification within genes associated with specific phenotypes.

Results: The findings revealed that all functional variants combined with AF ≤ 0.00001 exhibit the highest efficacy in distinguishing individuals with genetic generalized epilepsy (GGE) (OR: 1.4, $P = 5.73 \times 10^{-14}$) and developmental and epileptic encephalopathies (DEE) (OR: 1.3, $P = 5.91 \times 10^{-5}$) from controls. While for non-acquired focal epilepsy (NAFE), missense and splicing variants with AF ≤ 0.00001 showed strong discriminatory power (OR:1.4, $P = 2.86 \times 10^{-14}$), suggesting the insignificant role of LoF variants in improving the power to distinguish patients in this subphenotypes. Loss of function-based score could only distinguish DEEs (OR: 1.2, $P = 4.74 \times 10^{-2}$).

Conclusion: Overall, the study suggests that rare variant-individual risk scores could be a useful tool for improving the classification and stratification of patients with epilepsy.

Grants: DFG Research Unit FOR-2715 (Germany), FNR (Luxembourg)

Conflict of Interest: None declared

P16.058.B ExHap: visualising shared homozygosity haplotypes in distant individuals from exome variants

Shahryar Alavi[1;2], *Reza Maroofian*[2]

[1]Palindrome, Isfahan, Iran; [2]UCL Queen Square Institute of Neurology, Department of Neuromuscular Diseases, London, United Kingdom

Objectives: Understanding the prevalence and distribution of homozygous pathogenic variants in recessive disease-causing genes is essential. We aimed to develop a tool, ExHap, to identify the founder/recurrent status of shared homozygous variants in patients' exome data, shedding light on the extent of variant spread within populations.

Methods: Genomic VCF files (GVCFs) were utilized to jointly call exome variants in patients and controls. ExHap, developed using R, organised individuals' homozygosity haplotypes based on the length of runs of homozygosity (ROH) surrounding the variant. A colour plot visualised WT/heterozygous/homozygous variant positions, facilitating the inspection of flanking haplotypes.

Results: ExHap successfully generated homozygosity haplotype maps for shared pathogenic variants in DPM3, TOR1A, MED27, HPCA, WBP4, and TMEM67. Measuring shared ROH lengths between distantly related patients allowed us to calculate the most recent common ancestor (MRCA) age. Notably, a rare pathogenic MED27 variant in two unrelated Pakistani families was identified as an ancient founder mutation.

Conclusion: Analysing homozygosity haplotypes of pathogenic variants in recessive disorders enhances our understanding of their distribution in populations. Calculating MRCA age enables the anticipation of founder variant frequency, improving genetic counselling. This approach has the potential to minimise the risk of genetic disorders in offspring from distantly related couples. For recurrent variants, assessing the burden of mutational hotspots proves informative in understanding variant dynamics.

Grants:

Conflict of Interest: None declared

P16.059.C Guideline for assessing phenotypic evidence (PM7) by computational facial analysis in ACMG/AMP variant classification framework for variant of unknown significance

Tzung-Chien Hsieh[1], *Hannah Klinkhammer*[1], *Simona Capponi*[2;3], *Elaine Marchi*[4], *Gholson Lyon*[4], *Peter Krawitz*[1]

[1]Institute for Genomic Statistics and Bioinformatics, University Hospital Bonn, Bonn; [2]German Cancer Consortium (DKTK), partner site Freiburg, German Cancer Research Center (DKFZ), Germany; [3]Department of Urology, Medical Faculty-University of Freiburg, Germany; [4]Department of Human Genetics, NYS Institute for Basic Research in Developmental Disabilities, United States

Background/Objectives: Streamlining the classification for variants of unknown significance (VUS) is vital for patients with undiagnosed conditions. While next-generation phenotyping techniques like GestaltMatcher have significantly contributed to diagnosing rare disorders, the lack of computational facial analysis support in current ACMG/AMP guidelines has prompted this study to propose its inclusion. This study introduced new criteria, PM7 for the moderate level of pathogenicity, alongside existing PP4, integrating GestaltMatcher within a Bayesian framework and aims to aid the reclassification of VUS.

Methods: In the Bayesian framework of ACMG/AMP guidelines, we used GestaltMatcher scores to evaluate phenotypic match strength across syndromes, calculating associated likelihood ratios (LR). GestaltMatcher was trained on 8,547 images representing 244 disorders. We validated it on 1,499 images of 321 syndromes not in the training set. For each image, gestalt scores were computed across all disorders. Five-fold cross-validation was performed to establish thresholds aligning with Bayesian framework. Ultimately, we tested the thresholds on 32 patients with mutations in *TAF1*.

Results: We determined thresholds of 0.56 and 0.47, resulting in LR of 18.6 and 4.29, respectively. These values correspond to PM7 for moderate evidence and PP4 for supporting evidence when the gestalt score for a disorder exceeds these thresholds. When testing *TAF1* patients, nine satisfied PM7 and 16 fulfilled PP4 criteria, and calculating LR against randomly selected patients yielded 18.2 and 4.28, aligning with ACMG/AMP Bayesian framework. Additionally, facial analysis reclassified three out of eight VUS patients as likely pathogenic.

Conclusion: We demonstrated that incorporating GestaltMatcher into PP4 and PM7 criteria in ACMG/AMP guidelines enhances VUS classification.

Grants:

Conflict of Interest: None declared

P16.060.D A robust liability scale coefficient of determination

Emil Pedersen[1], *Jette Steinbach*[1], *andrew schork*[2], *Morten Krebs*[2], *Esben Agerbo*[1], *Florian Privé*[1], *Bjarni Jóhann Vilhjálmsson*[1;3;4]

[1], national centre for register-based research, Aarhus, Denmark; [2]institute of Biological Psychiatry, Mental Health services, Sct. Hans, Roskilde, Denmark; [3]Bioinformatics Research centre, Aarhus university, Aarhus, Denmark; [4]Broad Institite of MIT and Harvard, Novo

Nordisk foundation Center for Genomics, Mechanisms of Diseases, Cambridge, United States

Background/Objectives: The liability-scale coefficient of determination (R-squared) is a popular measure for assessing the accuracy of prediction models, allowing for comparison between studies. Lee et al. (Gen Epi, 2012) provided the standard transformation of the observed-scale R-squared to liability-scale that accounts for case ascertainment. However, Krebs et al. (medRxiv, 2023) recently showed that the standard transformation is unsuitable for some types of sample ascertainment and predictors.

Methods: We compare the standard liability-scale transformation to a R-squared based on a weighted probit regression. Using simulation, we compare R-squared measures to the true liability-scale R-squared. Finally, we will assess the differences between R-squared measures in the UK biobank and iPSYCH cohort. We compare three predictors; polygenic scores (PGS), binary family history indicator, and LT-FH + +.

Results: Preliminary simulations have been performed. Without ascertainment, we find the standard transformation is biased for family-based measures and unbiased for PGS. The weighted probit model is within 5% of the true R-squared for all predictors. With ascertainment, the standard transformation underestimates the true R-squared by 20%, while the weighted probit R-squared underestimates the true R-squared by 9 % on average across all predictors.

Conclusion: Traditional liability-scale transformations of the coefficient of determination are significantly impacted by case ascertainment and deviations in distribution assumptions. The weighted probit regression is less biased. Hence, existing evaluations of prediction models may be biased and comparisons may be unreliable.

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P16.061.A Validation of Twist CNV backbone panels at different probe densities for large pathological CNV detection

Ivan Limongelli[1], *Valentina Andrioletti*[1], *Tina Han*[2], *Ettore Rizzo*[1], *Alonzo Lee*[2], *Alessandro Davassi*[2], *Tanya Tannous*[2], *Susanna Zucca*[1]

[1]*enGenome srl, Pavia, Italy;* [2]*Twist Bioscience, South San Francisco, United States*

Background/Objectives: Accurate identification and annotation of pathogenic copy number variants (CNVs) is essential for providing proper diagnosis and treatment. Although array-based tests have been the gold-standard for detecting large CNV events, with the widespread use of NGS, a streamlined workflow with whole exome sequencing is becoming more appealing for many laboratories. Here we present a validation of an optimized method to detect large CNVs with ad-hoc designed Twist panels.

Methods: Twist CNV backbone panel was mixed with Twist Exome 2.0 plus Comprehensive Exome Spike-in and tested on 43 Coriell samples that had CNV previously confirmed by array. Three different probe densities were tested: 25kb, 50kb and 100kb. The 43 test and 12 additional reference samples were sequenced on Illumina NovaSeq 6000 and CNVs were called using the enGenome-eVai-Secondary Workflow, employing a read-depth approach to identify events. To benchmark our NGS results, a positive set of 52 CNVs was selected and the performance of each probe design was evaluated.

Results: All the samples except one had a great correlation with the baseline (>0.97). On average 345 filtered CNV variants could be identified per sample, with the 25kb design having the highest number of variants called (358). For all three designs, the sensitivity was 98.08%, with only one variant missed. We also determined a false positive rate (FPR) of <1% in all three designs.

Conclusion: Altogether these results support the use of these CNV backbone panels with exome to identify large CNVs with a sensitivity comparable to array based methods, without compromising SNV/INDEL calling.

Grants: EIC Accelerator 190164416

Conflict of Interest: Ivan Limongelli enGenome srl, enGenome srl, Valentina Andrioletti enGenome srl, Tina Han Twist Bioscience, Ettore Rizzo enGenome srl, Alonzo Lee Twist Bioscience, Alessandro Davassi Twist Bioscience, Tanya Tannous Twist Bioscience, Susanna Zucca enGenome srl, enGenome srl

P16.062.B Exploring the miRNA-Mediated Regulation of ERO1L as a Potential Prognostic Biomarker in Cervical Cancer

Havva Ozgen Eyupoglu[1;2], *erol eyupoglu*[2], *Şükrü Tüzmen*[1;3]

[1]*Eastern Mediterranean University, Faculty of Dentistry, via Mersin 10, Türkyie;* [2]*Marmara University, Medical Biology and Genetics, Istanbul, Türkyie;* [3]*GenBiomics R&D, Techno Park, Eastern Mediterranean University, via Mersin 10, Türkyie*

Background/Objectives: Cervical cancer is one of the most common malignancies among women. While screening programs are available, molecular biomarkers for disease prognosis are needed. Endoplasmic Reticulum Oxidoreductase 1 Alpha (ERO1L/ERO1A) has recently been studied as a potential biomarker in distinct malignancies. However, detailed molecular/cellular analyses for cervical carcinoma remain to be elucidated. To illustrate ERO1L's prognostic potential in cervical cancer, comprehensive in silico analyses have been performed.

Methods: The mRNA expression profile of cervical cancer patients and control samples from the TCGA databank was examined using the GEPIA2 platform. Gene alteration profiles were assessed by cBioPortal.Protein expression was determined by the Human Protein Atlas. Survival graphs were depicted by GEPIA2 and Kaplan-Meier(KM) Plotter. miRNA analyses were performed by the Cancer MIRNome platform.

Results: Gene expression analyses revealed that ERO1L was significantly upregulated in TCGA cervical carcinoma patients.These patients also had ERO1A gene amplification compared to healthy donors. High expression at the mRNA level was also consistent with the protein expression data both from patients and cervical cell lines. Notably, higher expression of ERO1L was correlated with worse survival in cervical cancer patients. Target analyses showed that ERO1L is a potential target of miR-218-5p.As expected, miR-218-5p was downregulated in cervical carcinoma patients and diminished miR-218-5p expression is correlated with worse survival.

Conclusion: To our knowledge, this study is the first to reveal the regulation of ERO1L, an important gene in cervical cancer prognosis, through miR-218-5p.We further aim to examine ERO1L;the work is in progress involving population-based mutation screening from patient biopsies.

Grants:

Conflict of Interest: None declared

P16.063.C The German Human Genome-Phenome Archive (GHGA) – Enabling secondary use of omics data in Germany by building a national secure infrastructure

Anna Benet-Pages[1;2], *Christian Mertes*[3], *Jan Eufinger*[4], *Ulrike Traeger*[4], *Julien Gagneur*[5], *Juliane Winkelmann*[6;7;8;9]

[1]*Helmholtz Research Center Munich, Institute of Neurogenomics, Neuherberg, Germany;* [2]*Medical Genetics Center Munich, Bioinformatics, Munich, Germany;* [3]*Technical University of Munich, Department of Informatics, Garching, Germany;* [4]*German Cancer Research Center (DKFZ), Computational Genomics and Systems Genetics, Heidelberg, Germany;* [5]*Technical University of Munich, Computational Genomics and Systems Genetics, Garching, Germany;* [6]*Helmholtz Research Center Munich, Institute of Neurogenomics, Neuherbergg, Germany;* [7]*Technical University of Munich, Institute of Human Genetics, München, Germany;* [8]*Munich Cluster for Systems Neurology, Munich, Germany;* [9]*Deutsches Zentrum für Psychische Gesundheit, Munich, Germany*

Consortium: The GHGA Consortium

Background/Objectives: The German Human Genome-Phenome Archive (GHGA) is building a secure national omics data infrastructure, as part of the National Research Data Infrastructure (NFDI) Initiative and as member of the federated European Genome-Phenome Archive (FEGA). This secure infrastructure will allow German institutions to share genetic data under controlled access, enabling GHGA's main goal: the facilitation of secondary use of human omics data for research purposes.

Methods: We are currently testing the core business logic and functionalities of the first public version of the GHGA Archive which include submission of research and metadata, handling of data access requests, and granting requesters access to data. The already publicly available GHGA Metadata Catalog collects and displays archived human omics data sets since 2022. It allows the search for non-personal information as defined in the GHGA Metadata Model using established ontologies and vocabularies describing the submitted data sets.

Results: We showcase GHGA's approach in development, deployment, and operation of a sustainable and secure infrastructure for data access embedded in an ethico-legal framework to handle omics data in a data-protection-compliant manner (open and FAIR).

Conclusion: GHGA is going to host a standardized and harmonized collection of human omics data in Germany with an ethico-legal framework for data sharing that helps to overcome challenges around consent and GDPR-compliance. Further integration into international collaborative efforts are ensured through interoperability with the German "Modellvorhaben" and with the participating countries of the European Genomic Data Infrastructure (GDI) project.

Grants: German Research Foundation (DFG) 441914366 (NFDI 1/1).

Conflict of Interest: None declared

P16.064.D Variant effect predictors: a systematic review and practical guide

Cristian Riccio[1;2], *Max L. Jansen*[1;2], *Linlin Guo*[3;4], *Andreas Ziegler*[1;2;3;4;5]

[1]*Cardio-CARE, Medicine Campus Davos, Davos, Switzerland;* [2]*Swiss Institute of Bioinformatics, Lausanne, Switzerland;* [3]*University Center of Cardiovascular Science & Department of Cardiology, University Heart and Vascular Center Hamburg, University Medical Center Hamburg-Eppendorf, Hamburg, Germany;* [4]*Center for Population Health Innovation (POINT), University Heart and Vascular Center Hamburg, University Medical Center Hamburg-Eppendorf, Hamburg, Germany;* [5]*School of Mathematics, Statistics and Computer Science, University of KwaZulu-Natal, Pietermaritzburg, South Africa*

Background/Objectives: Large-scale association analyses using whole-genome sequence data have become feasible, but understanding the functional impacts of these associations remains challenging. Although many tools are available to predict the functional impacts of genetic variants, it is unclear which tool should be used in practice. This work provides a practical guide to assist in selecting appropriate tools for variant annotation.

Methods: We conducted a MEDLINE search up to November 10, 2023, and included tools applicable to a broad range of phenotypes that can be used locally and have been recently updated. Tools were categorized based on the types of variants they accept and the functional impacts they predict. Sequence Ontology terms were used for standardization.

Results: We identified 118 databases and software packages, encompassing 36 variant types and 161 functional impacts. Combining only three tools, namely SnpEff, FAVOR, and SparkINFERNO, allows for the prediction of 99 (61%) distinct functional impacts. Thirty-seven tools predict 89 functional impacts that are not supported by any other tool, while 75 tools predict pathogenicity and can be used within the ACMG/AMP guidelines. We launched a website allowing researchers to select tools based on desired variants and impacts.

Conclusion: More than 100 tools are already available to predict approximately 160 functional impacts. About 60% of the functional impacts can be predicted by the combination of three tools. Unexpectedly, recent tools do not predict more impacts than older ones. Future research should allow predicting the functionality of unsupported variant types, such as gene fusions.

URL: https://cardio-care.shinyapps.io/VEP_Finder/

Conflict of Interest: None declared

P16.065.A The STOP g.APP: visualising the impact of stop-loss variants to aid variant interpretation

Alexandra Geary[1], *Elston N. D'Souza*[1], *Nicola Whiffin*[1]

[1]*Nuffield Department of Medicine (NDM), University of Oxford, United Kingdom*

Background/Objectives: Variants intersecting stop codons are linked to a broad range of both rare, and common human genetic conditions, including cardiomyopathies, and diabetes. However, interpreting their impact is not straightforward as (1) the effect of addition of amino acids to the C-terminus on protein function is difficult to predict, and (2) if there is no alternative stop codon upstream of the polyadenylation signal the transcript may be degraded by non-stop decay. Differentiating likely deleterious variants from those that are likely tolerated often requires specialist analysis and skills and, at present, no tools are available to assist in the interpretation of variants of this kind.

Methods: To address this issue, we have developed 'the STOP g.APP', an R/RShiny web app that allows users to visualise potential effects of stop disrupting variants in Ensembl v110 transcripts, providing information on downstream alternative stops, extension statistics, and whether non-stop decay is predicted. Importantly, the tool accounts for a change in frame when predicting the effect of insertion and deletion variants.

Results: As a validation of our approach, we show that variants predicted to cause non-stop decay are under significantly higher levels of constraint and cross-species conservation than variants predicted to result in small additions to the protein C-terminus.

Conclusion: The STOP g.APP is a freely available open-source resource that can be used by both research and clinical communities to

aid in the interpretation of stop-intersecting variants in human genes, and supplement interpretation using the ACMG-AMP guidelines.

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Conflict of Interest: Alexandra Geary: None declared, Elston N. D'Souza: None declared, Nicola Whiffin Partner is an employee and owns stock options in Vertex pharmaceuticals, Receives research funding from Novo Nordisk, Has consulted for ArgoBio

P16.067.C Solve-RD experience in structural variant calling and clinical interpretation in 12,500 unsolved rare disease exomes

german demidov[1], *Steven Laurie*[2], *Stephan Ossowski*[3]

[1]*Institute of Medical Genetics and Applied Genomics, University of Tübingen, Tübingen, Germany, Germany;* [2]*Centre Nacional d'Anàlisi Genòmica (CNAG), Baldiri Reixac 4, 08028 Barcelona, Spain; Universitat de Barcelona (UB), Barcelona, Spain, Spain;* [3]*Institute of Medical Genetics and Applied Genomics, University of Tübingen, Tübingen, Germany, Germany*

Consortium: the Solve-RD Consortium

Background: Structural variants (SVs) including large deletions and duplications (CNVs), inversions, translocations, and complex SVs have the potential to disrupt gene function resulting in rare disease. Nevertheless, current pipelines and clinical decision support systems for exome sequencing (ES) tend to focus primarily on SNVs and short insertions and deletions. Solve-RD aims to identify disease-causing variants in previously undiagnosed rare disease (RD) families. We reanalysed exomes from 12,014 families and 12,520 affected individuals to detect overlooked causal copy-number and structural variants.

Methods: A comprehensive pipeline of three read-depth based tools, ClinCNV, Conifer, ExomeDepth, together with Manta for identification of paired-end and split-read signatures, was used to detect CNV/SVs across all 12,500 ES datasets in Solve-RD.

Results: Analysis of the first ~6,000 families resulted in a new molecular diagnosis as a result of CNV identification for 51 families (1). We also identified pathogenic CNVs that are partially explanatory of the proband's phenotype in a further 34 individuals. In addition, 8 structural variants detected solely by Mantat were considered causal (2). Here we present the findings of the analysis of the complete cohort with ~6,000 additional families.

Conclusions: CNV and SV reanalysis in exomes can be performed at large scale and provide diagnosis to patients which were considered unsolved for many years. Improved pipelines for CNV and SV detection as well as comprehensive visualization of variants can reduce the time required for the genetic data interpreters to reach the diagnosis.

Funding: EU H2020 (779257)

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1. medRxiv, https://doi.org/10.1101/2023.10.22.23296993
2. medRxiv, https://doi.org/10.1101/2023.10.28.23297720

Conflict of Interest: None declared

P16.068.D Implementation of RNA-seq analysis in patients with rare diseases

Lucía Peña-Pérez[1;2;3], *Anders Jemt*[2;4], *Håkan Thonberg*[3], *Jesper Eisfeldt*[2;3;4], *Henrik Stranneheim*[1;2;4], *Anna Wedell*[1], *Anna Lindstrand*[3], *Valtteri Wirta*[2;4;5]

[1]*Karolinska University Hospital, Centre for Inherited Metabolic Diseases, Stockholm, Sweden;* [2]*SciLifeLab, Department of Microbiology, Tumour and Cell Biology, Stockholm, Sweden;* [3]*Karolinska Institute, Department of Molecular Medicine and Surgery, Stockholm, Sweden;* [4]*Karolinska University Hospital, Genomics Medicine Center Karolinska, Stockholm, Sweden;* [5]*SciLifeLab, School of Engineering Sciences in Chemistry, Biotechnology and Health, Stockholm, Sweden*

Background/Objectives: Whole genome sequencing (WGS) is one of the most comprehensive techniques for rare disease diagnosis. However, many patients remain undiagnosed even after having had their genome analyzed. One of the main reasons is the bottleneck in variant interpretation, often caused by the inability to predict the effects of non-coding/deep intronic variants on protein. Performing RNA-seq can allow us to see the effects directly on the transcripts, potentially increasing the diagnostic yield. Herein we present our findings and experiences from analyzing a cohort of 126 RNA-seq samples through a newly developed bioinformatic pipeline called tomte.

Methods: Blood samples from 126 rare disease patients were collected in PAXgene tubes and RNA was isolated, sequenced, and processed through tomte (https://github.com/genomic-medicine-sweden/tomte). Tomte is a pipeline written in Nextflow that processes FASTQ files, performs alignment, transcript quantification, allele-specific variant calling, and variant annotation. Furthermore, it detects mono-allelic expression, aberrant expression, and aberrant splicing to aid in rare disease diagnostics.

Results: In our cohort of 126 samples, we could identify 18 aberrant splicing and 38 aberrant expression events, which we could correlate to potentially causative genetic variants previously identified with WGS.

Conclusion: RNA-seq has the potential of increasing the diagnostic yield for rare disease patients by directly showing the effects of variants on transcripts.

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Conflict of Interest: None declared

P16.069.A Novel discoveries and enhanced genomic prediction from modelling genetic risk of cancer age-at-onset

Ekaterina Maksimova[1], *Sven Erik Ojavee*[2;3], *Marie Sadler*[2;3;4], *Reedik Mägi*[5], *Zoltan Kutalik*[2;3;4], *Matthew Robinson*[1]

[1]*Institute of Science and Technology Austria (ISTA), Klosterneuburg, Austria;* [2]*UNIL - Université de Lausanne, Department of Computational Biology, Lausanne, Switzerland;* [3]*SIB Swiss Institute of Bioinformatics, Lausanne, Switzerland;* [4]*University of Lausanne, University Center for Primary Care and Public Health, Lausanne, Switzerland;* [5]*Institute of Genomics, University of Tartu, Tartu, Estonia*

Background/Objectives: Inference in case-control genome-wide association studies on late-onset diseases suffers from the uncertainty that control group subjects might be diagnosed later in life. Time-to-event analysis treats controls as right-censored, without additional assumptions about future disease occurrence, representing a more sound inference alternative.

Methods: We extensively compared the discovery and genomic prediction performance of the time-to-event model GMRM-BayesW to GMRM-BayesRR-RC and other state-of-the-art methods on simulated data and data on 11 cancers from the UK and Estonian Biobanks.

Results: In simulation analyses, we demonstrate that modern association testing methods are generally unable to recover the genetic basis of later-life infrequently occurring phenotypes within biobank data. Nevertheless, we show that GMRM-BayesW yields a better understanding of the polygenicity and genetic architecture

of the 11 cancers recorded in the UK Biobank and highlights that heritable genetic risk is attributable to many thousands of genomic regions. We replicate previous findings from large-scale case-control studies and find 25 previously unreported genomic regions, out of which 5 replicated in independent data. Finally, we show that Bayesian modelling utilizing time-to-event data increases prediction accuracy by on average 20% compared to the recent summary statistic approach LDpred-funct.

Conclusion: We provide empirical evidence that discovery, genomic prediction, and understanding of genetic architecture of traits are greatly improved by analyzing age-at-diagnosis. Thus, as sample sizes increase, incorporating time-to-event data should be commonplace, improving case-control studies by using richer information about the disease process.

Grants:
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Conflict of Interest: Ekaterina Maksimova: None declared, Sven Erik Ojavee MSD, Marie Sadler: None declared, Reedik Mägi: None declared, Zoltan Kutalik: None declared, Matthew Robinson Research funding from Boehringer Ingelheim

P16.070.B CCUT - a versatile deep learning toolbox to enhance chromatin capture contact maps

Stanislav Sys[1], *Susanne Gerber*[1], *Karin Everschor-Sitte*[2]

[1]*University medicine at the Johannes Gutenberg University in Mainz, Human Genetics, Mainz, Germany;* [2]*University of Duisburg-Essen, Faculty of Physics, Duisburg, Germany*

Consortium: Mainz Institute of Multiscale Modeling (M3ODEL)

Background/Objectives: Chromatin Capture Experiments as Hi-C and Micro-C have become a popular method for genome architecture exploration. Nevertheless, these techniques come with the drawback of high sequencing cost and problems in mapping low coverage libraries to high resolution genomic structures. While there have been impressive breakthroughs in the field of pairwise contact matrix reconstruction, major challenges remain due to the high computational demands as well as the complexity and specificity of modern deep learning methods. Here we present the Chromatin Capture Upsampling Toolbox (CCUT) designed as a framework for deploying state-of-the-art pre-built and custom deep learning methods for matrix reconstruction. CCUT is a versatile toolbox not only capable of matrix reconstruction, but also offering a standardized way to transform, manipulate and visualize chromatin data.

Methods: CCUT consists of prebuilt models which can be directly used to enhance the quality of low-resolution matrices. All prebuilt models, which also include state-of-the-art models from other publications such as ESR-GAN, SWIN-Transformer and HiCARN by Hicks et al, were evaluated based on standardized metrics. Pre-built models were trained on the highest standard open datasets available (HiC & Micro-C @ 4DN).

Results: Our Toolkit provides excellent reconstruction results and offers the versatility needed to tackle noisy genomic data, while offering computationally efficient and best performance models at the same time.

Conclusion: CCUT offers a novel and user-friendly approach to designing and deploying deep learning models for chromatin matrix reconstruction, while allowing standardized and easy model evaluation.

Grants:

Conflict of Interest: None declared

P16.071.C Automatic identification of pathogenic rearrangements in the CFH region using long-read Nanopore sequencing

Marie Mille[1], **Denis Bertrand**[1], *Carine el Sissy*[2], *Nadhir Yousfi*[3], *Paula Vieira-Martins*[2], *Abderaouf Hamza*[4], *Sophie Vandermeersch*[3], *Sacha Beaumeunier*[1], *Michael Blum*[5], *Nicolas Philippe*[5], *Véronique fremeaux-bacchi*[2], *Laurent MESNARD*[3;6;7]

[1]*SeqOne, Bioinformatics, Montpellier, France;* [2]*APHP, Immunologie biologique, Paris, France;* [3]*INSERM-Sorbonne Université, UMRS 1155, Paris, France;* [4]*Institut Curie, Service de génétique, Paris, France;* [5]*SeqOne, R&D, Montpellier, France;* [6]*APHP, CNR-MAT, Paris, France;* [7]*APHP, Service de Soins Intensifs Néphrologique et Rein Aigu, Paris, France*

Background/Objectives: Genomics has the potential to reshape the management of Thrombotic microangiopathy (TMA) which encompasses various genetically-driven diseases. Complement-mediated thrombotic microangiopathy (cTMA) can be associated with complex structural variants (SV) rearrangement operating at the CFH-CFHRs locus. Gene fusions in the CFH-CFHR region is a specific molecular diagnostic bottleneck associated with 5% of c-TMA cases.

Methods: The highly repetitive character of sequences in this region makes it difficult to detect structural variants even with long-read sequencing. These events are occuring in two 30kb regions crossing partially CFH and CFHR1 genes that differ only by 500 single nucleotide variations (SNV).

To overcome this issue, we designed a bioinformatic pipeline that can detect gene fusions in the CFH-CFHR1 region. Our method uses SNV differences between the 2 regions to detect chimeric reads and breakpoint areas. Read mapping to these regions are extracted and mapped back to the CFH region to identify chimeric reads. According to the breakpoint location and the change of region identified in the chimeric reads we infer the structural variation in the CFH-CFHR1 region.

Results: We tested our approach on twenty patients for the detection of CFH-CFHR1 classic hybrids, CFHR1-CFH reverse, and homozygous deletions of CFHR1-CFHR3. In four samples, Multiplex Ligation Probe Amplification confirmed that the hybrids were detected correctly.

Conclusion: This novel SV detection method is able to call variations on highly homologous regions for long-read sequencing. This opens the way to molecular characterization of complex rearrangement structures including pathogenic structural variations associated with microangiopathy.

Conflict of Interest: Marie Mille SeqOne, SeqOne, Denis Bertrand SeqOne, SeqOne, Carine el Sissy: None declared, Nadhir Yousfi: None declared, Paula Vieira-Martins: None declared, Abderaouf Hamza: None declared, Sophie Vandermeersch: None declared, Sacha Beaumeunier SeqOne, SeqOne, Michael Blum SeqOne, Nicolas Philippe SeqOne, SeqOne, Véronique fremeaux-bacchi: None declared, Laurent MESNARD: None declared

P16.072.D Ratio-based alternative splicing for assessing and enhancing the quality of transcriptomic data using Quartet RNA reference materials

Ying Yu[1], *Qingwang Chen*[1], *Leming Shi*[1;2], *Yuanting Zheng*[1]

[1]*Fudan University, State Key Laboratory of Genetic Engineering, Human Phenome Institute, School of Life Sciences, and Shanghai Cancer Center, Shanghai, China;* [2]*The International Human Phenome Institutes, Shanghai, China*

Background/Objectives: Certified RNA reference materials play a crucial role in evaluating the reliability of RNA sequencing (RNA-seq) for detecting subtle biological differences in clinical settings, such as molecular subtyping of diseases. In our previous work, we established Quartet RNA reference materials and created gene-level ratio-based transcriptomics-wide reference datasets (Yu Y et al., Nature Biotechnology, 2023). However, another analysis type in transcriptomics data is alternative splicing (AS), which holds significant potential for disease research. Unfortunately, objective assessment at the AS level is currently lacking.

Methods: To address this gap, we quantified AS of Quartet RNA reference materials using 21 batches of multi-laboratory RNA-seq datasets generated with diverse protocols. We then processed the data using three bioinformatics pipelines. Based on these multi-batch and multi-analysis datasets, we constructed ratio-based reference datasets at the AS level, serving as the "ground truth" for benchmarking. Subsequently, we employed the signal-to-noise ratio (SNR) metric and consistency with reference datasets (RC) to evaluate the RNA-seq quality of AS quantification across batches.

Results: Our findings revealed substantial variation in RNA-seq quality for AS quantification across different batches and analysis pipelines, as assessed by SNR and RC measurements. Additionally, combining multi-batch and multi-analysis data introduced batch effects that obscured the differentiation of biologically distinct groups. Notably, the ratio-based approach, *i.e.* scaling study samples' absolute values relative to concurrently profiled reference material(s), yielded reproducible and comparable data.

Conclusion: Our study provided a valuable resource for assessing and enhancing the quality of transcriptomic data at AS level.

Grants: National Key R&D Project of China (2023YFC3402501 and 2021YFF1201305)

Conflict of Interest: None declared

P16.073.A Identification of DNA methylation signatures for Kleefstra syndrome type 2 caused by KMT2C variants

Karim Karimi[1], *Michael Levy*[1], *Raissa Relator*[1], *Jennifer Kerkhof*[1], *Jessica Rzasa*[1], *Carolyn Lauzon-Young*[1], *Haley McConkey*[1], *Alexandra Afenjar*[2], *Marie Vincent*[3], *Benedicte Duban-Bedu*[4], *Nicolas Chatron*[5], *Catherine Vincent-Delorme*[6], *Anne-Marie Guerrot*[7], *Eric Bend*[8], *Peter Henneman*[9], *Liselot van der Laan*[9], *Michael J. Lyons*[10], *Anne O'Donnell-Luria*[11], *Slavica Trajkova*[12], *Alfredo Brusco*[12], *Giovanni Battista Ferrero*[12], *Olivier Vanakker*[13], *Jo Sourbron*[13], *Marielle Alders*[9], *Matthew Tedder*[10], *Brittany Simpson*[14;15], *Charles Schwartz*[10], *Bekim Sadikovic*[1;16]

[1]*Verspeeten Clinical Genome Centre, London Health Sciences Centre, London, Canada;* [2]*Armand Trousseau Hospital, AP-HP Sorbonne Université, Department of Genetics, France;* [3]*Centre Hospitalier Universitaire Nantes, France;* [4]*Centre de génétique chromosomique, Lille, France;* [5]*Service de Génétique, Hospices Civils de Lyon, Lyon, France;* [6]*University Hospital of Lille, Clinical Genetics Unit Guy Fontaine, Lille, France;* [7]*Rouen University Hospital, Department of Neonatal Medicine, Rouen, France;* [8]*Prevention Genetics, The University of Utah, Marshfield, WI, United States;* [9]*Amsterdam Reproduction & Development Research Institute, Department of Human Genetics, Netherlands;* [10]*Greenwood Genetic Center, Greenwood, United States;* [11]*Broad Institute, Program in Medical and Population Genetics, Cambridge, United States;* [12]*University of Turin, Department of Medical Sciences, Turin, Italy;* [13]*Ghent University Hospital, Center for Medical Genetics, Ghent, Belgium;* [14]*St Jude Children's Research Hospital, Memphis, Tennessee;* [15]*University of Tennessee Health Science Center, Le Bonheur Children's Hospital, Department of Pediatrics, Memphis, Tennessee;* [16]*Western University, Department of Pathology and Laboratory Medicine, London, Canada*

Background/Objectives: Kleefstra syndrome 2 (KLEFS2) is a rare neurodevelopmental disorder characterized by a spectrum of features ranging from mild to severe intellectual disability, delayed psychomotor development, and distinctive facial dysmorphisms. This study aimed to identify a specific episignature associated with KLEFS2 arising from variants in *KMT2C*, elucidating its diagnostic potential and molecular distinctions from related syndromes.

Methods: DNA samples were extracted from peripheral blood of 22 individuals with genetic variants in *KMT2C* and clinical features consistent with KLEFS2. DNA methylation analysis was performed using the Illumina Infinium Methylation EPIC Bead Chip microarrays. Controls were randomly selected from EpiSign Knowledge Database (EKD) at the London Health Sciences Centre (LHSC). The methylation levels were fitted in a multivariate linear regression model to identify differentially methylated probes (DMPs). A support vector machine (SVM) classifier was constructed to train the model.

Results: In total, 225 DMPs were selected to construct the model. DNA methylation analyses indicated a clear and robust separation between patients carrying truncating variants and controls. The SVM classifier demonstrated robustness and specificity in distinguishing KLEFS2 from controls and other neurodevelopmental disorders, particularly KLEFS1, Rubinstein-Taybi and Kabuki syndromes. Additionally, identification of differentially methylated regions (DMRs) revealed several genes potentially associated to neurological functions observed in KLEFS2.

Conclusion: This study presents a distinct epigenetic signature specific to KLEFS2, providing a foundation for early diagnosis and understanding its molecular underpinnings, crucial for advancing clinical genetic practices and potential therapeutic interventions.

Grants: This study was supported by the Government of Canada through Genome Canada and the Ontario Genomics Institute (OGI-188).

Conflict of Interest: None declared

P16.075.C Leveraging ATAC-seq with transformers for context-sensitive cis-regulatory modeling

Panos N. Firbas[1], *Carolina Gonçalves*[1], *Gonzalo de Polavieja*[1]

[1]*Champalimaud Foundation, Lisboa, Portugal*

Background/Objectives: Transformers are emerging as a promising tool in cis-regulation and chromatin modeling, but the typical design of relying solely on genomic sequences as input severely limits their applicability in biological research.

Methods: We introduce a transformer model that integrates genomic sequences with ATAC-seq signal to model individual cis-regulatory elements (CREs). ATAC-seq allows the identification of CREs to use as samples, but also facilitates the modeling of Transcription Factor binding sites (TFbs) through the 'shadows' created by DNA-bound proteins. Our approach allows the model to make context-dependent predictions, unlocking its potential for real-world applications.

Results: The model, which consists of a convolutional tokenizer, followed by a transformer-encoder body and the task heads, was trained with a masking task and fine-tuned with a nucleotide classification task for CTCF, H3k27ac, and H3k4me3. A small version of the model achieves F1 scores of over 0.9 at the center of CTCF targets and over 0.83,0.87 per nucleotide for the other two classes. Analysis of its attention mechanism reveals its ability to pinpoint CTCF binding sites with nucleotide-level precision. We present evidence that the predictions are protein-DNA binding events, even when those have not been captured by the ChIPseq assay.

Conclusion: We introduce a novel approach in modeling cis-regulatory elements and TFbs with transformers and provide an implementation that yields high-quality context-sensitive predictions. Researchers can use a single molecular assay to identify CREs and classify them for H3k27ac and H3k4me3, but also to locate CTCF and other TFbs with nucleotide resolution.

Grants: FindingPheno_H2020_No952914

Conflict of Interest: None declared

P16.076.D A fine-mapping knockoff filter approach revealed association between multiple sclerosis and SNPs in 3'UTR of MYO1D and intronic region of ASIC2 (17q11.2-q12) in a study involving trios from Sardinian population.

GIULIA NICOLE BALDRIGHI[1], *Claus Thorn Ekstrøm*[2], *andrea nova*[1], *teresa fazia*[1], *valeria saddi*[3], *maria luisa piras*[3], *luisa bernardinelli*[1]

[1]*University of Pavia, Brain and Behavioural Sciences;* [2]*University of Copenhagen, Public Health;* [3]*Presidio Ospedaliero S. Francesco, ASL*

Background/Objectives. Multiple sclerosis (MS) is a progressive autoimmune disease affecting the central nervous system, leading to diverse physical and cognitive impairments. Although its aetiology remains undefined, factors such as genetics and familial predisposition play pivotal roles. Here, a fine-mapping approach was applied to the region where ASIC2 gene, known for its association to MS, is located (17q11.2-q12). Moreover, the genetically isolated Sardinian population was employed because MS prevalence in this population is higher compared to the national mean, 330 per 100,000 inhabitants, 95%CI: [321, 338].

Methods. A multi-step analysis was used to find MS-associated genetic variants in a sample of 157 trios. Firstly, genotypes were imputed, through haplotype reconstruction and 2537 single nucleotide polymorphisms (SNPs) were obtained after post-imputation quality controls. Secondly, genotype matrix was augmented with knockoff variants and family-based association test was performed to study conditional associations of genetic segments at multiple resolutions. Eventually, consistencies between knockoff filter results and original non-imputed variants were evaluated.

Results/Conclusion. The alternative allele of variant rs756787 (involving CDK5R1 and the 3'UTR of MYO1D) consistently showed statistical significance in increased MS risk. This SNP was revealed to be associated with autoimmune traits and smoking initiation. Conversely, two intronic regions of ASIC2, marked by rs56175840 and rs12936220, reported significance in reduced MS risk. They were found to be associated with BMI and cerebellum cortex volume from literature. Since for these two SNPs the recessive homozygotes occur less frequently in Sardinian MS cases than in controls, this might suggest their involvement in pathways conferring a protection against MS.

Conflict of Interest: None declared

P16.077.A Accurate identification of pathogenic structural variants guided by multi-platform comparison

Nico Alavi[1], **M-Hossein Moeinzadeh**[1], *Jakob Hertzberg*[1], *Uira Melo*[2], *Maryam Ghareghani*[1], *Lion Ward Al Raei*[1], *Eldar Abdullaev*[1], *Malte Spielmann*[3], *Stefan Mundlos*[2], *Martin Vingron*[1]

[1]*Max Planck Institute for Molecular Genetics, Department of Computational Molecular Biology, Berlin, Germany;* [2]*Max Planck Institute for Molecular Genetics, Research Group Development and Disease, Berlin, Germany;* [3]*University of Lübeck, Institute of Human Genetics, Lübeck, Germany*

Background/Objectives: Structural variants (SVs) are a common cause of human diseases and contribute to inter-individual variability. However, their detection remains a challenge, especially when relying on short-read sequencing. To investigate and overcome these challenges, we analyzed a cohort of 9 individuals genotyped by combining short-read, long-read, and linked-read sequencing as well as optical mapping.

Methods: Our dataset combined with novel tools for scalable manual variant inspection allowed us to create a comprehensive benchmark. Utilizing this benchmark, we evaluated how each technology and their corresponding SV callers perform across various settings. Guided by our comparison, we designed a short-read-based machine learning method to identify true-positive SVs using genomic annotations and alignment features. We demonstrate the method's applicability in a clinical scenario on a cohort of limb malformation patients.

Results: Our curated benchmark contains 79,645 deletions, 68,900 insertions, 16,506 duplications, and 331 inversions. Long-read-based SV detection outperforms all other surveyed methods, especially in detecting small to medium-sized deletions and insertions. To mitigate that, we demonstrate that our machine-learning method identifies 10% more true-positive deletions than state-of-the-art short-read-based methods while maintaining an accuracy above 84%. We further show the potential of our method in a clinical context, identifying 40% more manually confirmed candidates than a consensus approach.

Conclusion: While long-read-sequencing exhibits superior SV detection performance, short-read-sequencing remains more accessible, scalable, and less costly. We demonstrate that machine learning can push the boundaries of current short-read-based methods and drastically reduces the need for time-consuming manual inspection of variants in the context of clinical diagnostics.

Grants: BMBF - iGenVar FKZ 031L0169A

Conflict of Interest: None declared

P16.078.B Exploring the Impact of GLP1R Coding Variants on Type 2 Diabetes and Depression through a Multi-phenotype approach.

Zhanna Balkhiyarova[1;2;3], *Giuseppe Deganutti*[4], *Christopher Reynolds*[4], *Ben Jones*[3], *Inga Prokopenko*[1;2]

[1]*University of Surrey, 1 Department of Clinical and Experimental Medicine, School of Biosciences and Medicine, Guilford, United Kingdom;* [2]*University of Surrey, People-Centred Artificial Intelligence Institute, Guilford, United Kingdom;* [3]*Imperial College London, Section of Metabolism, Digestion and Reproduction, Department of Medicine;* [4]*Coventry University, Centre for Health and Life Sciences, United Kingdom*

Background/Objectives: In this study we investigate multi-phenotype association between type 2 diabetes (T2D) and depression, two conditions imposing a considerable burden on global healthcare systems. The bidirectional link between T2D and depression, contributing to adverse microvascular and macrovascular outcomes, is a focal point.

Methods: Leveraging phenotypic data from the UK Biobank, including whole exome sequencing for 467,836 individuals, we concentrated on the GLP1R gene and its 200kb flanking region. Analysing six key phenotypes in 70,001 individuals, including random glucose, HbA1c, BMI, T2D, major depressive disorder, and depressive symptoms, we employed plink for single-phenotype association analysis and SCOPA software, using a reverse regression approach for multi-phenotype analysis.

Results: Our meticulous approach, adjusting for demographic variables, revealed significance in multi-phenotype analysis for 19

variants. Notably, a rare GLP1R gene variant (6:39073639:G:A) exhibited significant associations with both glucose metabolism and depression ($p = 4.28 \times 10^{-9}$). Furthermore, a previously reported rare variant linked to random glucose, rs146868158, demonstrated significance in the model with metabolic phenotypes ($p = 0.026$).

Conclusion: These findings illuminate shared mechanisms in the development of type 2 diabetes and depression, providing insights for targeted interventions and personalized treatment strategies.

Grants: Diabetes UK (BDA number: 20/0006307), LONGITOOLS, H2020-SCI-2019- 874739.

Conflict of Interest: None declared

P16.079.C Copy number variations mapping in a cohort of 1860 asymptomatic individuals improving interpretation

Virginie Bernard[1], *Quentin Charret*[1], *Clément Lionnet*[1], *Maëlle Martinet Gerphagnon*[1], *Valentin Klein*[1], *Laura Fancello*[1], *Anthony Ferrari*[2], *Anne Sophie Sertier*[2], *Elise Dugas*[2], *Groupêment de Coopération Sanitaire Auragen*[3], *Jean François Scariot*[4], *Alain Viari*[4], *Julien Thevenon*[1]

[1]*Centre Hospitalier Universitaire de Grenoble, La Tronche, France;* [2]*Léon Bérard Center, Lyon, France;* [3]*Edouard Herriot Hospital, Lyon, France;* [4]*Inria Centre at the University Grenoble Alpes, Montbonnot-Saint-Martin, France*

Background/Objectives: Genome sequencing enables the fine detection of copy number variations (CNVs) and individual structural variations (SVs). This approach allows for the discovery of a wide variety and complexity of variations not accessible through previous techniques. CNV and SV detection limitation on short-read sequencing data are known. Clear variation visualization is crucial for confident interpretation and robust identification of differentiating predictive factors between true and false positives.

Methods: To improve CNV interpretation, a cohort of 1860 genome sequencings from asymptomatic individuals representing 2025 France Genomic Medicine Initiative sequencing AURAGEN routine is usefull. Cohort CNVs and SVs mapping, coupled with international dbVAR data are accessible. For each patient, a dynamic pan-genomic representation of CNV and LoH through a dynamic interface comparing to dbVar population frequency and local cohort are available.

Results: The regions observed in more than 1% of this population include 1,637 Mb. Additionally, we add 377 Mb losses and 380 Mb gains specific to the AURAGEN routine, for 178 Mb intervals dbVar absent. Those are intervals poorly covered at 40X depth or with high variability. This helps interpretation. Among mappable autosomal regions, it identifies 943 Mb of non-variable regions possibly under selection pressure. The 1,103 Mb of regions without loss concentrates 9339 genes for which no sequence in the coding sequence is deleted. Among them, 1576 genes have a pLI > 0.9, and 1510 genes have no loss in the cohort.

Conclusion: This mapping of CNV and LoH helps genome interpretation for diagnostic and research.

Grants:
PFMG2025 (https://pfmg2025.aviesan.fr/)

Conflict of Interest: None declared

P16.080.D Differences in cell type composition in the liver by the linear mixed model in the deconvolution method under thermal stress conditions

Krzysztof Kotlarz[1;2], *Bartłomiej Hofman*[1], *Magda Mielczarek*[1;2], *Yachun Wang*[3], *Joanna Szyda*[1;2]

[1]*Wrocław University of Environmental and Life Sciences, Wrocław, Poland;* [2]*Poznan University of Medical Sciences, Poznań, Poland;* [3]*China Agricultural University, Beijing, China*

Background/Objectives: In recent years, the impact of environmental stressors on cellular composition has become a key point in biological research. Therefore, in our study, we were interested in checking whether thermal stress impacts the proportions of particular cell types in a given tissue. For this purpose, we used a custom-written program implementing a novel approach based on Linear Mixed Model (LMM).

Methods: Bulk tissue expression from the liver was measured under two environmental conditions where available for 10 individuals. Additionally, the control cell-type specific gene expression profiles from the liver were utilised as a reference. In this study, we suggested the LMM with expression cell type profile modelled as a random effect to estimate the proportion of particular cell types expressed under two conditions. Two LMMs were considered: (i) model assuming no-covariance between cell-types specific expressions, (ii) model including covariance between cell-types specific expressions quantified by Jaccard similarity coefficient. Different environmental conditions were expressed by thermal stress and rats were chosen as the experimental organisms by their physiological similarities to humans.

Results: Significant differences were estimated in most cell populations except B cells. Endothelial cells, immune cells, hepatocytes, cholangiocytes, and plasmocytes exhibited altered proportion levels under thermal stress conditions.

Conclusion: These findings highlight a systemic impact of thermal stress on cellular dynamics, suggesting potential implications for immune responses, inflammatory cascades, and hepatic function.

Grants: Calculations were carried out at the Wroclaw Centre for Networking and Supercomputing.

Conflict of Interest: None declared

P16.081.A An end-to-end workflow to diagnose patients with rare diseases using only open-source components in a clinical setting.

Ramprasad Neethiraj[1], *Anders Jemt*[1], *Henrik Stranneheim*[1], *Daniel Nilsson*[1], *Måns Magnusson*[1;2], *Chiara Rasi*[1], *Valtteri Wirta*[1]

[1]*SciLifeLab, Clinical Genomics, Stockholm, Sweden;* [2]*Klarna, Stockholm, Sweden*

Background/Objectives: A rare disease diagnostic pipeline consists of four important steps: alignment, variant calling, annotation, and variant prioritization. While there are open-source tools available for the first two steps, the annotation and variant prioritization stages are more complex and require financial and personnel investment to develop and establish the necessary infrastructure. As a result, rare disease diagnosis typically relies on expensive commercial solutions for variant annotation and prioritization. Hence, there is a need to develop open-source alternatives that are easy to set up and customize according to local needs and, at the same time, do not compromise on the diagnostic yield.

Methods: We utilize tools like CADD, Variant Effect Predictor (VEP), and Vcfanno to combine annotations from several publicly available databases, like ClinVar and ClinGen, and in-house allele frequency databases like Loqusdb (https://github.com/Clinical-Genomics/loqusdb). For variant prioritization, we utilize Genmod,

which uses a customizable weighted sum rank model to score individual variants based on their annotations, disease models and inheritance patterns (https://github.com/Clinical-Genomics/genmod).

Results: Our system is currently in use to diagnose patients with rare diseases in the Stockholm healthcare region, and has a diagnostic yield of around 40%. Additionally, our results are fully compatible with Scout, an open-source web-based decision support tool that can be used in the clinic to visualize, share, and solve cases in a collaborative manner (https://github.com/Clinical-Genomics/scout).

Conclusion: Our approach provides an entirely open-source solution that is easy to set up and customize according to local needs. Importantly, it does not compromise on the diagnostic yield.

Grants:

Conflict of Interest: None declared

P16.082.B Optimised variant filtering specificity improves the efficiency of diagnostic genomic testing in rare disease

Rebecca Locke[1;2], Christopher Pyatt[1;2], Jethro Rainford[1;2], Gavin Fuller[1;2], Isabelle Delon[1;2], Kirsty Bradshaw[2;3], Elizabeth Johnston[2;3], Kate Downes[1;2], Joo Wook Ahn[1;2], Matthew Garner[1;2]

[1]Cambridge Genomics Laboratory, Cambridge University Hospitals NHS Foundation Trust, Cambridge, United Kingdom; [2]NHS East Genomic Laboratory Hub, East Genomics, United Kingdom; [3]Genomics and Molecular Medicine Service, Nottingham University Hospitals NHS Trust, Nottingham, United Kingdom

Background/Objectives: Variant classification presents a major bottleneck for diagnostic genomic testing in rare disease. Stringent variant filtering can reduce the classification workload but must be balanced with the requirement for sensitivity to maintain diagnostic yield. Increasing the number of cases where no variants require interpretation also facilitates automated reporting of these cases, enhancing service delivery efficiency. We assessed current filtering approaches and identified opportunities for improvement, which will enable scalability as testing demand increases.

Methods: Our new approach annotates a virtual gene panel-filtered VCF with mode of inheritance (MOI) information. This maps gene-level MOI to all rare diseases from PanelApp which allows gene-disease specific MOI thresholds to be applied. These thresholds were incorporated into our filtering rules along with other improvements, and their effectiveness was assessed against a set of 3184 previously reported cases.

Results: 17% of cases receive a positive report ($n = 527$) and 83% ($n = 2657$) receive no molecular diagnosis (NMD), of which 9% ($n = 234$) have no variants presented for review or classification with the current approach. Improved filtering has increased this to 53% ($n = 1403$) which has a significant impact on the workload required to review and exclude these variants to reach the same NMD conclusion. The optimised approach reduced the total number of variants requiring analysis by 61% compared to the current approach. Importantly, no pathogenic variants were filtered out.

Conclusion: We have optimised our genomic variant filtering which improves diagnostic specificity without impacting sensitivity. We expect this will reduce turnaround times for patients.

Grants:
N/A

Conflict of Interest: None declared

P16.083.C Advancing Therapeutic Insights in Cancer Biology through Integration of cBioPortal and Open Targets

Mirella Kalafati[1], Anika Bongaarts[1], Jessica Singh[1], Laura Varela[1], Zeynep Karagöz[1]

[1]The Hyve, Utrecht, Netherlands

Objectives: The growth of curated public data within cBioPortal presents an opportunity to unravel the intricate associations between genetic variants and cancer types. Open Targets (OT) stands out as a tool by integrating various datasources, including genomic data, to assess target-disease associations and the validity of therapeutic targets. While cBioPortal manages processed genomics data at the patient level, OT focuses on target-disease relations derived from these data without dealing with individual patient details. This project aims to develop an automated pipeline for generating OT-compatible summary/meta-data from cBioPortal and seamlessly integrating it into OT.

Methods: A scalable pipeline, written in Scala and Python, was developed at The Hyve to handle the generated evidence from the large amount of genomic data in cBioPortal, to be uploaded in OT. This pipeline includes an algorithm to transform the evidence derived from cBioPortal data into a score between 0 and 1, where each type of cBioPortal evidence will be weighted for its significance and relevance for the gene-disease association.

Results: The automated pipeline successfully extracts relevant data from cBioPortal, transforms and standardizes it to align with OT format, computes scores, and integrates evidence into OT. A dedicated section within OT for cBioPortal evidence is also developed, with an intuitive user interface.

Conclusions: The integrated platform allows users to easily rank drug targets for cancer based on advanced cancer genomics data. This automated integration significantly benefits the genomics and molecular data field of cancer biology, creating a more interconnected ecosystem for researchers and clinicians alike.

Conflict of Interest: None declared

P16.084.D Integrating high-throughput assays and deep learning for characterization of human *cis*-regulatory elements

Mohan Dash[1], Chengyu Deng[2;3], Jay Shendure[4;5], Nadav Ahituv[2;3], Martin Kircher[1;6], Max Schubach[1]

[1]Berlin Institute of Health at Charité – Universitätsmedizin, Berlin, Berlin, Germany; [2]University of California San Francisco, Department of Bioengineering and Therapeutic Sciences, San Francisco, United States; [3]University of California San Francisco, Institute for Human Genetics, San Francisco, United States; [4]University of Washington, Department of Genome Sciences, Seattle, United States; [5]Howard Hughes Medical Institute, Seattle, United States; [6]University Medical Center Schleswig-Holstein, University of Lübeck, Institute of Human Genetics, Lübeck, Germany

Consortium: Impact of Genomic Variation on Function (IGVF)

Background:

Nucleotide variants in *cis*-regulatory elements (CREs) are major drivers of phenotypic differences and human disease. However, their regulatory code remains allusive, thus requiring the development of deep-learning approaches that can predict CRE-activity only by their DNA sequence. These approaches have been either focused on biochemical features of a single cell-type (i.e., small-scale with underlying sampling bias) or predict thousands of features in parallel, thereby raising issues in terms of multi-collinearity and interpretability.

Methods: To better benchmark and improve these methods, we characterize >60,000 proximal regulatory CREs in diverse human cell-types with massively parallel reporter assays (MPRAs). We designed our MPRA library using a convolutional neural

network (CNN) architecture trained on sequences of accessible and closed chromatin in multiple cell-types. The library was tested in human hepatocytes and kidney epithelial cells, and data processed to compute MPRA-activity. Furthermore, we trained regression models to identify key features for MPRA-activity.

Results: MPRA experiments resulted in good replicate Pearson correlations of ~70% in both cell-types and our initial CNN model predicted chromatin-accessibility with a Pearson correlation of 35%. From the regression analysis of MPRA data, chromatin-accessibility (DNase-seq), boundary-element (CTCF), histone-modifications (H3K27ac), variant-density, and conservation (PhyloP) were found to be key features, with a Pearson correlation to 44%.

Conclusion: We identified features in addition to DNA sequence that led to better prediction of MPRA activity and more specific motif enrichment. We believe that the models and datasets generated here will increase our genome-wide interpretation of functional CREs and variation within them.

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Conflict of Interest: None declared

P16.085.A OncoClassify: Somatic Mutation Classification in Cancer Genomics

Claire Morgan[1;2], *Leslie Matalonga*[1;2], *Joel Dieguez*[1;2], *Raul Tonda*[1;2], *Genis Parra*[1;2], *Ivo Gut*[1;2], *Sergi Beltran*[1;3]

[1]*Centre Nacional d'Anàlisi Genòmica (CNAG), Barcelona, Spain;* [2]*Universitat de Barcelona (UB), Barcelona, Spain;* [3]*Universitat de Barcelona (UB), Departament de Genètica, Microbiologia I Estadística, Facultat, de Biologia, Barcelona, Spain*

Background/Objectives: Accurate classification of somatic mutations and subsequent assignment of actionability is crucial for data-driven cancer treatment. While joint recommendations have been proposed for the classification of oncogenic mutations by the cancer genomics community (ClinGen, CGC and VICC), there remains a gap in implementing these proposals within a next-generation sequencing context.

Methods: We introduce OncoClassify, a command-line tool designed to rapidly classify somatic mutations as benign or oncogenic in accordance with joint recommendations. OncoClassify leverages information from cancer mutation and biomarker databases and can be easily updated and harmonized. Notably, OncoClassify can be customized to utilize specific data sources based on the analysis objectives and the licenses of the data sources. Actionability is assigned at multiple levels including exact amino acid match, amino acid positional match, exonic region, and gene level for SNVs, indels, CNVs, and gene fusion events.

Results: OncoClassify generates an oncogenicity score for each mutation, which determines its classification. The tool also provides the underlying source data that contributes to the score. Benchmarking demonstrates that OncoClassify either outperforms existing tools or performs comparably. It efficiently processes annotated whole-genome sequencing (WGS)-derived VCF files in seconds. Furthermore, OncoClassify is containerized and can be deployable on local or high-performance computing (HPC) machines to be integrated in existing analysis workflows.

Conclusion: OncoClassify provides a robust and efficient solution for the classification of somatic mutations, thereby facilitating personalized cancer treatment strategies. The application will be improved and updated to ensure adherence to the latest guidelines by the cancer genetics community.

Grants:

Conflict of Interest: None declared

P16.087.C Unveiling Methylation Patterns: a novel workflow to identify and visualize differentiallymethylated regions and individual cytosines

Celeste Moya-Valera[1], *Francisco Lara-Hernández*[1], *Elena Quiroz*[1], *Veronica Lendinez*[1], *Sergio Valdes*[2;3], *Ana Lago-Sampedro*[2;3], *Pablo Marin-Garcia*[4], *Vicente Arnau*[5;6;7], *Gemma Rojo*[2;8], *Javier Chaves*[1;9], *Ana Barbara Garcia-Garcia*[1;9]

[1]*INCLIVA Biomedical Research Institute, Genomics and Diabetes Unit, Valencia, Spain;* [2]*Biomedical Research Institute of Malaga (IBIMA), Malaga, Spain;* [3]*Regional University Hospital of Malaga, Endocrinology and Nutrition Department, Malaga, Spain;* [4]*Mgviz.org;* [5]*University of Valencia, Valencia, Spain;* [6]*Spanish Research Council (CSIC), Spain;* [7]*Institute of Integrative Systems Biology (I2Sysbio), Valencia, Spain;* [8]*Regional University Hospital of Malaga, Malaga, Spain;* [9]*CIBERDEM, ISCIII, Madrid, Spain*

Background/Objectives: DNA methylation (mDNA) plays a crucial role in genome regulation and gene expression. The main method to study mDNA is bisulfite-treated DNA sequencing, focused on CpG sites in regulatory regions. Several bioinformatic methods are available for analysing and visualising differentially methylated regions (DMRs), but the complete study and representation of results remain complex and unresolved. We have developed a complete workflow for DMRs and methylated cytosines detection and visualization.

Methods: The workflow is divided into four modules that can be run individually, enabling a customised analysis. These modules include: read trimming and quality control; processed read alignment; cytosine clustering and DMR annotation; and DMR and cytosine visualization. The workflow was tested on 24 bisulphite-treated exome DNA samples selected from the Egabro-Pizarra study (general Spanish population from Andalucia), focused on Type 2 Diabetes Mellitus (T2D) development.

Results: Our workflow can identify altered exome mDNA with a high sensitivity and robustness. Furthermore, it has detected mDNA alterations in some genes (*AKT1*, *GNAS*, *DVL1*, *TCF7*, *IRS2*, and *SOCS2*) that could be involved in T2D development.

Conclusion: This workflow enables exploration of the epigenetic landscape, revealing patterns and trends in mDNA that may indicate regulatory processes or disease associations.

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Conflict of Interest: None declared

P16.088.D Rigorous screening for splice-altering mutations improves the identification of potential disease-causing variants in whole genome sequencing data.

Christian W Remmele[1;2;3], *Michaela AH Hofrichter*[1], *Daniel Bengl*[1], *Wahyu Prastyo*[1], *Asuman Koparir*[1], *Shoko Komatsuzaki*[1], *Thomas Haaf*[1], *Tobias Müller*[4], *Marcus Dittrich*[1;4]

[1]*Institute of Human Genetics, Julius-Maximilians-University, Würzburg, Germany;* [2]*Center for Rare Diseases, University Hospital Würzburg, Würzburg, Germany;* [3]*Bavarian Genomes Network for Rare Diseases, Germany;* [4]*Department of Bioinformatics, Julius-Maximilians-University, Würzburg*

Consortium: Bavarian Genomes Network for Rare Diseases

Background/Objectives: In whole genome sequencing (WGS) analyses, intronic regions of genes yield many times more rare single nucleotide variants (SNVs) and short insertions/deletions (INDELs) than exonic regions. Although variants in exons exhibit more pronounced deleterious effects on average, intron variants have also been demonstrated to be disease causing, particularly when they affect splice mechanisms. These intronic variants, constituting the majority of rare variants in genes, are often disregarded, difficult to classify, and underreported.

Methods: WGS datasets consisting of an inhouse cohort and public datasets were analyzed with an inhouse pipeline. Potential splice effects were predicted utilizing SpliceAI with optimized parameters for all identified SNVs and INDELs in the gene body (5′ to 3′ transcript borders) as well as for SNVs and INDELs in ClinVar.

Results: High splice prediction scores (>= 0.5, n = 125) for intronic variants in ClinVar were strongly overrepresented for known (likely) pathogenic (71/115) compared to known (likely) benign variants (26/6,865). Read mapping and variant calling of the WGS datasets yielded 3.9/0.9 million SNVs/INDELs per sample, of which 72.6/21.0 thousand rare variants were located in the gene body. A median of 12 intronic variants with high scores for splice-altering effects were predicted. Even though deep intronic variants exhibited on average lower splice prediction scores than canonical splice sites, we were able to validate the effect on splicing for a deep intronic variant experimentally and demonstrate its pathogenicity.

Conclusion: This study illustrates the advantage of screening intronic variants by predicting their potential splice-altering effects, thereby aiding in the classification of patients' mutations for pathogenicity.

Grants:

Conflict of Interest: None declared

P16.089.A Preliminary studies of human Pseudogene and Homology Database (hPHD)

Bilge Nihan Satkın[1;2], *Ayhan Kütükçü*[1], *Ece ÇİÇEK*[1], *Guzin Tunca Alparslan*[1]

[1]*Trakya University, Faculty of Engineering, Department of Genetics and Bioengineering, Edirne, Türkyie;* [2]*Trakya University, Faculty of Medicine, Department of Medical Genetics, Edirne, Türkyie*

Background/Objectives: The human genome harbors an estimated 20,000 pseudogenes, serving as archival records offering insights into evolutionary trajectory. Pseudogenes, arising from retrotransposition or duplication events, lack protein-coding capacity due to pathogenic variants. Variant calling in molecular diagnostics faces challenges due to high homology of pseudogenes with functional genes. Proper primer usage is crucial for accurate variant detection.

Methods: We aim to develop a publicly available repository of PCR primers tailored for disease-related genes with homology to pseudogenes or other genomic sequences. This repository will include validated primer sets from in-house data and literature. Users can search using NCBI gene symbols, accessing primer pairs with exon numbers and literature references. Future plans involve implementing a curation system for user-submitted primers.

A customized pipeline, derived from the Psi-Phi program suite, has been integrated for identifying pseudogenes. This pipeline utilizes tblastn analysis within clusters of closely related genomes. It accepts complete or draft genome files in *.fasta or *.fas formats. The pipeline identifies sequence homology and positions of homolog regions, highlighting differences.

Results: Access to this open source will be available with a domain address based on Trakya University's server for users.

Conclusions: The developed repository of PCR primers addresses challenges in variant calling due to pseudogene homology. Integration of the Psi-Phi program suite pipeline enhances identification and understanding of pseudogenes and high-grade homology. This comprehensive resource will facilitate accurate variant detection in molecular diagnostics and support evaluating homolog regions in the human genome.

References:

Grants: None

Conflict of interest: None declaired

Conflict of Interest: None declared

P16.090.B Benchmarking deep learning-based retinal biomarkers against tangible features reveals their distinct potentials for systemic disease prediction

Michael Beyeler[1], *Sofia Ortin Vela*[1], *Sven Bergmann*[2]

[1]*UNIL - Université de Lausanne, Lausanne, Switzerland;* [2]*UNIL, Lausanne, Switzerland*

Background/Objectives: Colour fundus images (CFIs) allow for non-invasive assessment of retinal features providing important information on health. This study benchmarks 17 tangible vascular traits (TVTs) against "deep" retinal features (DRFs) from self-supervised deep learning models, aiming to enhance systemic disease prediction, interpret deep learning models, understand genetic disease mechanisms, and ease manual annotation workload.

Methods: Analysing retinal images from a cross-sectional dataset of 22k UK Biobank participants, we compared TVTs extracted and characterised using our in-house annotation pipeline (https://doi.org/10.1101/2023.07.07.23292368) with 1024 DRFs corresponding to the latent space embeddings from the recently published RETFound model trained on 1.6 million images in the Moorfields AlzEye study. We performed GWAS on these DRFs, as well as their 17 leading principal components. To compare TVTs and DRFs, we used simple Pearson correlation analysis and LD score regression to quantify phenotypic and genotypic correlations, and Cox models to explore disease associations.

Results: TVTs outperformed RETFound in predicting cardiovascular outcomes like myocardial infarction (HR = 1.20). Further, they exclusively predicted age at death (HR = 1.17). In contrast, DRFs more accurately predicted diabetes (HR = 1.27) and cataracts (HR = 1.32). Lastly, a DRF emerged as the most heritable feature across methods and as an analogue to vessel tortuosity (h2 = 0.26, genetic correlation = 0.9).

Conclusion: We conclude that TVTs and DRFs provide complementary characterisations of CFIs calling for more integrative methods to maximise their potential for early disease diagnosis and advancing our understanding of disease mechanisms through genetics. RETFound demonstrates the ability to learn classic features in a self-supervised manner, suggesting an avenue for reducing manual analysis in retinal imaging.

Grants:
https://data.snf.ch/grants/grant/209510

Conflict of Interest: None declared

P16.091.C Reducing re-identification risk of genomic data using k-mer approach

Remy Costa[1;2;3], *William Ritchie*[2], *Alban Mancheron*[1;3]

[1]*Université de Montpellier, Montpellier, France;* [2]*Institute of Human Genetics, Montpellier, France;* [3]*LIRMM - Laboratoire d'informatique, de robotique et de microélectronique de Montpellier, Montpellier, France*

Background/Objectives: Advancements in genomics have greatly increased the volume of available genomic data, offering significant potential for disease understanding and personalized medicine. However, this also raises privacy risks due to the sensitive nature of genomic information. Addressing this challenge, our study explores innovative methods to minimize privacy vulnerabilities in genomic data sharing.

Methods: We propose an approach based on k-mers to estimate re-identification risks. K-mers, short DNA sequences, inherently reduce privacy risks by minimizing direct associations with identifiable genomic features such as Short Tandem Repeats or transposons. By analyzing Single Nucleotide Polymorphisms (SNPs) within these k-mers against public databases like dbSNP, we identify potential privacy vulnerabilities. The novelty lies in developing a risk score based on the SNP analysis within k-mers, aiming to assess re-identification risks. We aim to develop a risk score indicating the likelihood of re-identification from shared k-mer sets, offering a novel approach to mitigate privacy concerns.

Results: We expect to produce a risk score that enables precise evaluation of re-identification risks in genomic data sharing. This tool will facilitate the selective sharing of genomic data by filtering out sensitive k-mers, reducing privacy risks and supporting the safe advancement of genomics research.

Conclusion: By balancing genomic research benefits with privacy protection, our study contributes to safer data sharing practices. The development of a re-identification risk metric and data filtering tool represents a significant step towards secure, open science and research reproducibility.

Grants:
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Conflict of Interest: None declared

P16.092.D SvirlPool. Long, lifted, pooled: Reliable structural variant detection

Vinzenz May[1], *Manuel Holtgrewe*[1], *Dieter Beule*[1]

[1]*Berlin Institute of Health, Core Unit Bioinforamtics, Berlin, Germany*

Background/Objectives: Reliable structural variant (SV) detection is integral to rare disease diagnostics and cancer genomics, as well as genome variation in populations. Existing methods of structural variant detection with long reads usually use read-to-reference alignments to detect SVs on abstractions of SV signals. Typical challenges are the detection of very large or complex SVs as well as SVs within difficult regions, such as repeats or low complexity regions.

Methods: We present a novel method to combine alignment- and assembly-based methods. We identify candidate regions in read to reference alignments and then assemble clusters of relevant parts of aligned reads. The resulting assembled fragments are then aligned to a reference sequence of choice and used for SV-calling. The pooling of sequencing samples in our workflow allows us to very exactly match SVs that are shared between several samples.

Results: On standard benchmark datasets for long-read whole genome sequencing, we achieve results that are at least as competitive as existing methods. A strong advantage of our approach is the reliable detection of SVs across samples, e.g., as needed for pedigree and matched tumor-normal analysis.

Conclusion: Our method will allow very precise structural variant calling with long reads well suited for rare disease research and diagnostics use case, as well as in cancer genomics research. Furthermore, our new method can take full advantage of the rapid advancements of new reference genomes.

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Conflict of Interest: None declared

P16.093.A Structure analysis of missense variants in MYBPC3

Ana Gomes[1], *luis enrique gomez*[1;2], *agustín izquirdo*[1;3;4], *héctor martin cifuenes*[1], *sofia de la fuente*[1], *Marisol Delea*[1], *Carlos David Bruque*[1;5]

[1]*Hospital de Alta Complejidad El Calafate - SAMIC, Unidad de Conocimiento Traslacional Hospitalaria Patagónica, El Calafate, Argentina;* [2]*Hospital General de Agudos Dr. Juan A. Fernández., Division de cardiologia., Buenos Aires, Argentina;* [3]*Centro de Investigaciones Endocrinológicas "Dr. César Bergadá" (CEDIE) CONICET – FEI, División de Endocrinología., Buenos Aires, Argentina;* [4]*Hospital de Niños Ricardo Gutiérrez, Unidad de Medicina Traslacional. Servicio de Inmunología, Buenos Aires, Argentina;* [5]*Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Buenos Aires, Argentina*

Background/Objectives: *MYBPC3*, encoding cardiac myosin binding protein C (MyBP-C), emerges as the second candidate gene to study genetic cases of hypertrophic cardiomyopathy (HCM). We thoroughly analyzed a dataset comprising pathogenic *MYBPC3* variants from the literature between 1995 and 2017, focusing on missense variants and their possible stability effect.

Methods: We generated a database of pathogenic variants from the literature and only missense variants (40%, 246 of 603) were considered in this study. Regarding the protein structure, we selected the structure defined by AlphaFold2. Stability was evaluated using the FoldX Program. Protein stability was calculated using FoldX's stability command and ΔΔG values were estimated as the difference between the energy of the wild-type protein and the average of five replicates for each missense variant. A threshold of 1,6 kcal/mol was considered, and values above this threshold significantly destabilize a protein.

Results: A subset of missense variants in the database was systematically classified and grouped based on the protein position, residue type, interactions with other proteins, correlation with protein domains, and stability. We found a significant stability concern in 39% (97 of 246) of missense variants across the protein domains/regions. In some cases, the analysis set revealed a possible effect on the pathogenicity of the missense variants.

Conclusion: Our results provide insights into the impact of *MYBPC3* missense variants on energy stability and structural characterization of the MyBP-C protein, contributing to a better understanding of the disease mechanisms in HCM.

Conflict of Interest: None declared

P16.094.B Enhancing Detection of Low-Frequency SNPs in SARS-CoV-2 through Integrated Sequencing and umiVar Variant Calling

Victoria Cepeda Espinoza[1], *Bryant Gilot*[2], *Casper Groß*[1], *Leon Schütz*[1], *Stephan Ossowski*[1]

[1]*Institute for Medical Genetics and Applied Genomics, Medicine, Tübingen, Germany;* [2]*Wilhelm Schickard Institute for Computer Science, Computer Science, Tübingen, Germany*

Background/Objectives: The rapid emergence of new variants in SARS-CoV-2 highlights the importance of capturing intra-host diversity. Challenges in detecting low-frequency single nucleotide variants (SNVs) arise from errors introduced during reverse transcription, amplification, and sequencing. To overcome these challenges, we utilize an integrated framework that combines (i) Twist BioScience probes with magnetic bead capture for targeted enrichment of SARS-CoV-2, (ii) unique molecular identifiers (UMIs)

for precise PCR and sequencing error correction, (iii) ultra-deep short-read sequencing for high sensitivity, and (iv) our newly developed UMI-aware variant caller, umiVar.

Methods: We simulated clinical samples using binary mixtures of controls synthesized by Twist BioScience, spiked with ultra-high resolution (UHR) material. The experimental process involved active target capture/enrichment, reverse transcription, ligation of UMIs, and Illumina short-read sequencing. The computational process involved error correction using PCR duplicates, a nucleotide-exchange specific error model, and variant calling. UmiVar distinguishes PCR copies of the same DNA molecule based on barcodes and mapping positions, with 3-4 copies needed for good error correction.

Results: Compared to existing variant calling methods, umiVar demonstrates superior performance in identifying SNVs at extremely low allele frequencies. Its UMI correction mechanism significantly enhances mapping coverage and accuracy, enabling the detection of newly emerging mutations in the viral intra-host population.

Conclusion: By integrating an advanced sequencing approach with the innovative variant caller umiVar, we have significantly improved the accuracy of low-frequency SNV detection in SARS-CoV-2 compared to existing methods, which promises to advance our understanding of viral evolution and potentially improve public health responses.

Conflict of Interest: None declared

P16.096.D Epigenetics in Multiple Sclerosis: a multi-level analysis combining DNA methylation pattern, age acceleration, epigenetic drift using public datasets

rebecca cavagnola[1], *GIULIA NICOLE BALDRIGHI*[1], *davide sacco*[1], *Davide Gentilini*[1;2]

[1]*Università di Pavia, Department of Brain and Behavioral Sciences, Pavia, Italy;* [2]*IRCSS, istituto Auxologico Italiano, Bioinformatics and Statistical Genomics Unit, Milano*

Background: Multiple Sclerosis (MS) is a complex trait disease, causing chronic inflammation of the central nervous system (CNS). Although its etiology remains unknown, it is widely proven that innate and adaptive immune processes contribute to its pathogenesis, interacting with genetic, environmental, and epigenetic factors. Epigenetic alterations might impact on the regulation of gene expression, interfering with biological pathways for brain and immunological development.

In this work, the role of epigenetics in the molecular mechanism underlined by MS was investigated by combining results obtained from public datasets.

Methods: Methylation profiles obtained through Illumina HumanMethylation450K chips and EPIC 850K were downloaded by GEO database, for a total of 439 subjects. For each dataset, genome-wide analysis was performed to identify DNA methylation differences, age acceleration between MS cases and controls. Subsequently, results were combined in a meta-analytical approach. Eventually, stochastic epigenetic mutations were evaluated as an indicator of epigenetic drift and regions characterized by enrichment of methylation defects were prioritized and studied.

Results/Conclusion: Results from differential methylation analyses were meta-analyzed to increase the power of the study. Although no significant genome-wide findings emerged from the aggregation, methylation patterns aligned with specific genes previously associated with MS were observed. Analysis of rare epivariation reveled interesting insights to understand plausible pathogenesis factors and therapeutic targets. This research might help in understanding the mechanism under the regulation of the transcription in such complex trait diseases.

Conflict of Interest: None declared

P16.097.A TandemTwister, a rapid and universal tool for genotyping tandem repeats across all long-read sequencing technologies.

Lion Ward Al Raei[1;1], *Maryam Ghareghani*[1], *M-Hossein Moeinzadeh*[1], *Nico Alavi*[1], *Martin Vingron*[1], *Jakob Hertzberg*[1]

[1]*Max Planck institute for molecular genetics, computational molecular biology, Berlin, Germany*

Background: Tandem repeats (TRs) are parts of the genome sequence consisting of consecutively repeated units with polymorphic copy numbers and mutations in repeat units. They have been used in DNA fingerprinting for their hyper variability in the population. They have also been associated with complex traits and various genetic disorders, including neurodegenerative disease and developmental disorders. Having a fast and scalable tool for accurately genotyping these variants is indeed essential for understanding their functional impacts, phenotype and disease-association studies.

Methods: We developed TandemTwister, a novel algorithm written in c++, as a highly scalable and parallelized tool for the genotyping of copy numbers in tandem repeats.

Results: We evaluated the performance of TandemTwister on different sequencing technologies in the Ashkenazim trio on a set of ~900k GIAB's tandem repeat region annotations. For each Hifi sample, TandemTwister ran in ~5 minutes on 32 cpu cores, with 99,5% recall and 93.1% mendelian consistency and 99.1% considering an off-by-one error. In addition, we genotyped tandem repeat profiles of the HGSVC2 cohort of 35 assembled genomes from different populations and analysed inheritance patterns and haplotype blocks of tandem repeats in the three trio sets. Furthermore, we validated the ability of TamdemTwister to detect pathogenic repeat expansions in a cohort of 31 samples with neurodegenerative and developmental disorders.

Conclusion: TandemTwister is an accurate scalable tool for genotyping tandem repeats. It is the fastest genotyping tool up to date suited for all long-read sequencing technologies as well as assembled genomes.

Conflict of Interest: None declared

P16.098.B Removing array-specific batch effects in GWAS mega-analyses by applying a two-step imputation workflow

Mohammed Nasr[1;2], *Alexander Teumer*[1;2], *Eva König*[3], *Christian Fuchsberger*[3], *Sahar Ghasemi*[4], *Grabe Hans*[1;5], *Henry Völzke*[5;6], *Uwe Völker*[2;7]

[1]*University Medicine Greifswald, Department of Psychiatry and Psychotherapy, Greifswald, Germany;* [2]*Deutsches Zentrum für Herz-Kreislauf-Forschung (DZHK) e.V., Berlin, Germany;* [3]*Institute for Biomedicine, Eurac Research, Bolzano, Italy;* [4]*Institute of Genetic Epidemiology, Medical Center – University of Freiburg;* [5]*German Center for Neurodegenerative Diseases (DZNE), Greifswald, Germany;* [6]*Institute for Community Medicine, University Medicine Greifswald, Greifswald;* [7]*Interfaculty Institute for Genetics and Functional Genomics, University Medicine Greifswald, Greifswald*

Background/Objectives: Combining individual-level data in genetic association studies (mega-analyses) increases statistical power for identifying genetic effects underlying diseases. Batch effects occurring when combining imputed genotypes of different

array-type are a major limitation. Here, we developed a two-step imputation workflow, to overcome the array-type bias.

Methods: We included 8189 individuals genotyped altogether on three different array types (Illumina Omni 2.5, Illumina GSA, and Affymetrix SNP 6.0). In the two-step imputation, array-specific reference panels were designed for the first imputation step and subsequently imputed against the 1000GenomesPhase3 reference panel. We compared the results of the combined standard array-specific imputation (reference) against a one-step imputation using the intersection of variants of the included array and our two-step imputation. Batch effects were assessed using the first 20 genetic principal components of the combined sample after the final imputation. The performance of the different approaches was evaluated by comparing the imputation quality R2 value and the deviation of minor allele frequency (MAF).

Results: The one-step approach included 58,091 genotyped variants for imputation. In the two-step design, the intermediate imputation resulted in 2,128,598 autosomal variants used as input for the 1000Genomes imputation. Unlike the standard imputation, no batch patterns occurred in both the one-step and the two-step imputation workflow. Our two-step imputation outperformed the one-step approach by an 18% higher median R2 and a lower median MAF deviation.

Conclusion: Our new workflow removing batch effects will foster mega-analysis of different arrays for analysing genome-wide data and genetic scores using common and low-frequency variants.

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Keywords: genetic imputation, association studies, mega-analysis

Conflict of Interest: None declared

P16.099.C Assessing the relative power of constraint metrics in variant prioritisation for rare-variant association testing

Barney Hill[1], *Nikolas Baya*[1], *Frederik Lassen*[1], *Duncan Palmer*[1], *Cecilia Lindgren*[1]

[1]*University of Oxford, Big Data Institute*

Background/Objectives: Recent rare-variant association studies have unveiled numerous gene-phenotype associations within the human exome. These associations are enabled by set-based testing applied to prioritised collections of variants with evidence of pathogenicity. As such, our power to detect significant associations is limited by our ability to accurately predict such damaging variations.

Methods: We aim to map missense variants in the UK Biobank onto their canonical protein sequences to then apply protein variant effect predictors such as EVE and Tranception to classify damaging missense variants. First, we evaluated the performance of these classifiers by comparing the number of significant burden tests across selected phenotypes in the UK Biobank 450K exome sequence dataset. Second, we compared the overlapping annotation sets with the existing ClinVar pathogenic labeled missense variants.

Results: EVE and Tranception successfully replicated almost all existing associations and increased the total number of associations across a wide range of phenotypes. The precision of both EVE and Tranception, when compared against ClinVar, was 68% and 57% respectively, significantly outperforming the 47% precision achieved by using PP3 moderate and/or SpliceAI > 0.2.

Conclusion: Our findings demonstrate that the use of protein fitness scores can significantly improve the prediction accuracy of damaging missense variants, enabling more powerful gene association tests. We anticipate that the continued development of methods integrating protein structural prediction into variant classification will further enhance accuracy and boost our ability to detect gene-phenotype associations.

Grants:

Conflict of Interest: None declared

P16.100.D Metadata-guided Feature Disentanglement for Training Robust Functional Genomics Models

Remo Monti[1;2], *Alexander Rakowski*[1], *Viktoriia Huryn*[2], **Marta Lemanczyk**[1], *Uwe Ohler*[2;3], *Christoph Lippert*[1;4]

[1]*Hasso Plattner Institute, University of Potsdam, Digital Engineering Faculty, Potsdam, Germany;* [2]*Max-Delbrück-Center for Molecular Medicine in the Helmholtz Association, Berlin Institute for Medical Systems Biology, Berlin, Germany;* [3]*Humboldt University, Departments of Biology and Computer Science, Berlin, Germany;* [4]*Hasso Plattner Institute for Digital Health, Icahn School of Medicine at Mount Sinai, New York, United States*

Background/Objectives: With high-throughput technologies, genomics datasets grow rapidly in size, including functional genomics datasets. This allows training large Deep Learning (DL) models to predict epigenetic readouts, such as protein bindings or histone modifications, from genome sequences. Large datasets come at a price of data consistency, aggregating results from studies with varying experimental conditions. While this can be beneficial, as it allows studying the effects of different biological conditions, it can also introduce unwanted biases from confounding experimental factors.

Methods: We propose Metadata-guided Feature Disentanglement (MFD) - a DL approach to disentangle biologically relevant features from potential technical biases. MFD incorporates target metadata into model training by conditioning weights of the model output layer on different experimental factors. It then separates the factors into disjoint groups and enforces independence of the corresponding feature subspaces with an adversarially learned penalty. We train MFD on human genome data to predict peak calls from ENCODE experimental tracks, and we demonstrate its impact on model interpretability and downstream task performance on independent data.

Results: Disentangling biological from technical MFD features improves classification of enhancer sequences on FANTOM5 and Vista data, achieving comparable performance to a non-interpretable baseline. Furthermore, MFD features lead to higher enrichment of rare and eQTL variants in gnomAD and GTEx datasets than the baseline.

Conclusion: The metadata-driven approach combined with disentanglement of feature subspaces allows for better model introspection, by connecting latent features to experimental factors, without compromising, or even improving performance in downstream tasks.

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Conflict of Interest: None declared

P16.101.A COMET: A comprehensive Python module for community detection in biological networks

Nicolas Ruffini[1], *Susanne Gerber*[2]

[1]*Leibniz-Institut für Resilienzforschung, Mainz, Germany;* [2]*Institute of Human Genetics, University Medical Center Mainz, Mainz, Germany*

Background:

Complex biological systems are characterized by intricate networks of protein-protein interactions (PPIs) that underlie

various physiological and pathological processes. Understanding the organization and function of these networks requires advanced computational tools that can dissect these interactions into manageable subcommunities.

Methods: COMET, a comprehensive Python module, has been developed to meet this need. It incorporates the Louvain and Leiden algorithms for efficient subcommunity detection within biological networks across different organisms. The module utilizes the STRING database to access extensive gene network connections, offering researchers the flexibility to analyze PPIs based on physical interactions, textual evidence, or user-defined thresholds.

Results: COMET enables a nuanced exploration of multi-omics data, providing insights into the intricate organization of biological networks. Through its advanced algorithms and the integration of the STRING database, it allows for the detailed analysis of PPIs. The addition of plotting functions significantly aids in the interpretation of data, offering clear, visual representations of the detected subcommunities and their interactions. This feature not only simplifies the complexity inherent in biological data but also aids in the dissemination of findings to a broader audience.

Conclusion: The COMET module represents a powerful tool for the analysis of biological networks, facilitating the detection of subcommunities and enhancing the interpretability of complex biological data through comprehensive plotting capabilities. By offering a user-friendly approach to dissecting multi-omics data, COMET aids researchers in uncovering specific molecular pathways and mechanisms, advancing our understanding of complex biological systems.

Conflict of Interest: None declared

P16.102.B GHGA: Standardizing and harmonizing NGS analysis workflows to create a unified omics data resource for Germany

Zehra Hazal Sezer[1;2], *Kübra Narci*[3;4], *Florian Heyl*[5], *Paul Merges*[5], *Luiz Gadelha*[5], *Sameesh Kher*[5], *Andrew Behrens*[6;7], *Jens Krueger*[8;9], *Julien Gagneur*[7;10;11], *Christian Mertes*[12;13]

[1]*High Performance and Cloud Computing Group and Applied Bioinformatics, Eberhard Karls Universität Tübingen, Tübingen, Germany, Tuebingen, Germany;* [2]*German Human Genome-Phenome Archive (GHGA), Tübingen, Germany;* [3]*German Human Genome-Phenome Archive (GHGA), DKFZ, Heidelberg, Germany, Heidelberg, Germany;* [4]*Computational Oncology Group (CO), Molecular Precision Oncology Program, National Center for Tumor Diseases (NCT) Heidelberg and German Cancer Research Center (DKFZ), Heidelberg, Germany, Germany;* [5]*German Human Genome-Phenome Archive (GHGA), DKFZ, Heidelberg, Germany;* [6]*School of Computation, Information and Technology, Technical University of Munich, Garching, Munich, Germany;* [7]*Munich Data Science Institute, Technical University of Munich, Garching, Germany;* [8]*High Performance and Cloud Computing Group and Applied Bioinformatics, Eberhard Karls Universität Tübingen, Tuebingen, Germany;* [9]*German Human Genome-Phenome Archive (GHGA), Tuebingen, Germany;* [10]*Munich Data Science Institute, Technical University of Munich, Garching, Germany;* [11]*Institute of Human Genetics, School of Medicine, Technical University of Munich, Munich, Germany;* [12]*School of Computation, Information and Technology, Technical University of Munich, Garching, Germany;* [13]*Institute of Human Genetics, School of Medicine, Technical University of Munich, Germany*

Consortium: The German Human Genome-Phenome Archive

Background: As human omics data expands, the requirement for data management is to facilitate secondary use. Therefore the German Human Genome-Phenome Archive (GHGA), within the federated European Genome-Phenome Archive (FEGA), is developing a scalable and secure IT infrastructure for Germany, an ethico-legal framework to handle omics data in a data-protection-compliant but open and FAIR manner, a harmonized metadata schema, and standardized workflows to process all incoming omics data.

Methods: We are building upon the nf-core and nextflow community to build NGS analysis workflows for all incoming data modalities such as nf-core/SAREK. GHGA enables the creation of harmonized and standardized NGS resources across datasets and projects by maintaining and developing workflows, creating runtime configurations for each data modality with stable identifies, and continuously evaluating the performance.

Results: In collaboration with the nf-core community and beyond, GHGA is maintaining and co-developing six workflows. To evaluate the performance of the workflows GHGA together with the German NGS Competence-Network developed a continuous benchmarking framework: NCBench. GHGA is creating a runtime configuration to uniformly process NGS data while guaranteeing the highest standards and quality of the workflows.

Conclusion: By maintaining scalable, reproducible, and continuously benchmarked workflows, GHGA will create a harmonized and standardized NGS data resource ready to be used by the German research community. Such a harmonized resource will enable cross-analysis of projects and population-scale studies, promote new collaborations and research projects, and establish the foundation for developing a German-based variant frequency database.

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Conflict of Interest: None declared

P16.103.C Assessment of next-generation sequencing-based polygenic risk score genotyping for breast cancer risk prediction

Alexandra Baumann[1;2;3], *Christian Ruckert*[4], *Christoph Meier*[5], *Tim Hutschenreiter*[1;2], *Robert Remy*[6], *Benedikt Schnur*[7], *Marvin Döbel*[8], *Rudel Nkouamedjo Fankep*[6], *Dariush Skowronek*[9;10], *Oliver Kutz*[1;2;11], *Norbert Arnold*[12], *Anna-Lena Katzke*[7], *Michael Forster*[12], *Anna-Lena Kobiela*[6], *Katharina Thiedig*[13], *Andreas Zimmer*[14], *Julia Ritter*[15], *Bernhard Weber*[5;16], *Ellen Honisch*[17], *Karl Hackmann*[1;2], *Gunnar Schmidt*[7], *Marc Sturm*[8], *Corinna Ernst*[6]

[1]*Institute for Clinical Genetics, University Hospital Carl Gustav Carus and Faculty of Medicine of TUD Dresden University of Technology, Dresden, Germany; ERN GENTURIS, Hereditary Cancer Syndrome Center Dresden, Germany; National Center for Tumor Diseases (NCT), NCT/UCC Dresden, a partnership between German Cancer Research Center (DKFZ), Faculty of Medicine and University Hospital Carl Gustav Carus, Dresden, Germany;* [2]*German Cancer Consortium (DKTK), Dresden, Germany; German Cancer Research Center (DKFZ), Heidelberg, Germany; Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany;* [3]*Department of Systems Biology and Bioinformatics, University of Rostock, Rostock, Germany;* [4]*Institute of Human Genetics, University of Münster, Münster, Germany;* [5]*Institute of Human Genetics, University of Regensburg, Regensburg, Germany;* [6]*Center for Familial Breast and Ovarian Cancer, Center for Integrated Oncology (CIO), Medical Faculty, University of Cologne and University Hospital Cologne, Cologne, Germany;* [7]*Department of Human Genetics, Hannover Medical School (MHH), Hannover, Germany;* [8]*Institute of Medical Genetics and Applied Genomics, University Hospital Tübingen, Tübingen, Germany;* [9]*Interfaculty Institute of Genetics and Functional Genomics, University of Greifswald, Greifswald, Germany;* [10]*Department of Human Genetics, University Medicine Greifswald, Greifswald;* [11]*Department of Gynecology and Obstetrics, University Hospital Carl*

Gustav Carus at TUD Dresden University of Technology and Faculty of Medicine of TUD Dresden University of Technology, Dresden, Germany; [12]Department of Gynecology and Obstetrics, Institute of Clinical Chemistry Institute of Clinical Molecular Biology, University Hospital Schleswig-Holstein, Campus Kiel, Kiel, Germany; [13]Division of Gynaecology and Obstetrics, Klinikum rechts der Isar der Technischen Universität München, München, Germany; [14]Institute for Human Genetics, Medical Center University of Freiburg, Faculty of Medicine, University of Freiburg, Freiburg, Germany; [15]Department of Human Genetics, Labor Berlin – Charité Vivantes GmbH, Berlin, Germany; [16]Institute of Clinical Human Genetics, University Hospital Regensburg, Regensburg, Germany; [17]Department of Gynaecology and Obstetrics, University Hospital Düsseldorf, Heinrich-Heine University Düsseldorf, Düsseldorf, Germany

Consortium: Bioinformatics Working Group of the German Consortium for Hereditary Breast & Ovarian Cancer

Background/Objectives: The influence of numerous genetic loci on cancer risk can be represented as a polygenic risk score (PRS). The breast cancer (BC) PRS is included in the individual BC risk prediction via the CanRisk interface. Reproducibility of expected allele frequencies (AFs) at the respective loci is essential to calculate a legitimate PRS. Next-generation sequencing (NGS) is often used for genotyping, which revealed several challenges to be considered.

Methods: Five centers of the German Consortium on Hereditary Breast and Ovarian Cancer (GC-HBOC) provided NGS data of 324 unique BC PRS loci (PRS sets BCAC 313, BRIDGES 306). PRS loci were analyzed with different variant callers and settings, and the AFs per GC-HBOC center were determined. We compared expected AFs of the BC PRS sets against GC-HBOC center-observed and population database-derived AFs. Potential work-arounds such as alternative alleles in linkage disequilibrium were evaluated.

Results: In gnomAD v3, 4 of 324 BC PRS loci were missing, AFs of further 24 loci deviated from the PRS-expected AF, and for 39 loci, technical artifacts were reported. Data from GC-HBOC centers showed remarkably deviating AFs for 16 to 22 loci, thereby affecting final BC risk prediction. Both sequencing approach and variant caller, including mode of calling, influenced the results.

Conclusion: Prior to diagnostic use, sequencing quality of PRS loci and associated AFs need to be evaluated and optimized. Further, PRS sets developed based on SNP array data have to be re-evaluated in regards to the PRS loci and reproducibility of expected AFs.

Grants: Multiple

Conflict of Interest: None declared

P16.104.D Integrating Nanopore Sequencing: Unveiling High-Dimensional Chromatin Dynamics through Epigenetic Assays

Alejandro Ceron Noriega[1], *Anne Julia Sarah Kerber[1], Stanislav Sys[1], Nicolò Alagna[1], Tamer Butto[1], Susanne Gerber[1]*

[1]JGU- University Medicine, Institute of Human Genetics, Mainz

Consortium: SFB1551

Background/Objectives: Our research delves into the regulation of gene function within chromatin structures by integrating epigenetic assays and Nanopore sequencing, overcoming limitations associated with short-read technologies. Nanopore's unique ability to conduct three experiments simultaneously in a single flow cell contributes to comprehending high-dimensional chromatin folding in genomics and epigenetics. Despite the growing momentum in epigenetic sequencing assays, the integration of Nanopore sequencing remains unestablished. Our goal is to bridge this gap, merging epigenetic assays with Nanopore sequencing, a crucial step for understanding chromatin dynamics in a high-dimensional context.

Methods: We propose an integrated approach that combines library preparation with Nanopore sequencing for ATAC-seq, CUT&tag, and Pore-C assays. Leveraging our expertise, we focus on developing a workflow that integrates this information. Our methodology involves coupling neural differentiation cell populations with Pore-C, ATAC-seq and CUT&tag assays, combined with machine learning approaches that address challenges such as upsampling low-quality matrices and cell type identification. Integrating data from three trials significantly contributes to understanding high-dimensional chromatin folding.

Results: Successful implementation of the initial trials of ATAC-seq, CUT&tag, and Pore-C has been achieved. Ongoing work involves fine-tuning the pipeline for real-time data analysis of selected epigenetic assays.

Conclusion: Our approach demonstrates the feasibility of long-read epigenetic sequencing assays, advancing our understanding of chromatin dynamics in a high-dimensional genomic and epigenetic context.

Grants: Emergent algorithmic intelligence, EAI. Carl-Zeiss-Stiftung, SFB1551 and Mainz Institute of Multiscale Modeling

Conflict of Interest: None declared

P16.105.A Multi omics data integration approaches to investigate Pediatric Multiple Sclerosis

Martina Tosi[1], *Alen Zollo[2], Andrea Corona[2], Nicola Pomella[1], Nadia Barizzone[1], Fjorilda Caushi[1], Parsa Khoshgoftar[1], Loredana Martignetti[3], Ferdinando Clarelli[4], Elisabetta Mascia[4], Massimo Filippi[5], FEDERICA ESPOSITO[6], Maria Trojano[7], Maria Pia Amato[8], Eleonora Cocco[9], Roberto Giuseppe Ernesto BERGAMASCHI[10], Maura Pugliatti[11], Angelo Ghezzi[1], MARTINELLI BONESCHI FILIPPO GIOVANNI[2], sandra d'alfonso[1]*

[1]University Of Eastern Piedmont, Department Of Health Sciences, Novara, Italy; [2]ASST Santi Paolo Carlo, Department Of Health Sciences, Neurology Unit And Multiple Sclerosis Center, Milano, Italy; [3]Institut Curie, INSERM U900, Paris, France; [4]IRCCS San Raffaele Scientific Institute, Laboratory of Human Genetics of Neurological Disorders, Institute of Experimental Neurology, Division of Neuroscience, Milan, Italy; [5]IRCCS San Raffaele Scientific Institute, Neurology and Neurorehabilitation Unit, Neurophysiology Service, Neuroimaging Research Unit, Division of Neuroscience, Vita-Salute San Raffaele University, Milano, Italy; [6]IRCCS Ospedale San Raffaele, Laboratory of Human Genetics of Neurological Disorders, Institute of Experimental Neurology, Division of Neuroscience, Neurology and Neurorehabilitation Unit, Milan, Italy; [7]University of Bari "Aldo Moro", Italian Multiple Sclerosis and Related Disorders Registry, School of Medicine, Bari, Italy; [8]Università Degli Studi Di Firenze, Neurologia, Firenze, Italy; [9]Centro Regionale Sclerosi Multipla, Ospedale Binaghi, Cagliari, Italy; [10]IRCCS Fondazione Mondino, Pavia, Italy; [11]Azienda Ospedaliero-Universitaria Di Ferrara, Ferrara, Italy

Consortium: PEDIGREE

Background: Multiple Sclerosis (MS) is a multifactorial disease. Only 5% of MS patients develop the disease before 18 years (Pediatric MS). PedMS offers a unique opportunity to gain clinical, biological, environmental information in proximity to the actual disease onset. Our study aimed to identify genetic and environmental components associated to PedMS by performing multi omics data integration.

Methods: After recruiting an Italian multicentric cohort, we performed GWAS (Illumina GSA and HRC imputation), metagenomic (16S sequencing) and methylation (Illumina Epic Array v2) analyses on PedMS (up to 460) and matched healthy controls (HC,

up to 1693). We integrated data following either an early strategy with MOFA (omics matrices are concatenated in a single one containing features from all omics for co-occurrent samples) and a late one (each omics is analysed independently, and the most significant results are merged in the end).

Results: Late integration of the most significantly associated data from GWAS (1916 SNPs with $p < 1 \times 10^{-5}$) and 1382 differentially methylated regions from epigenetic analyses identified an overlap between 14 SNPs and 4 significant DMRs. We also investigated the co-localization of those SNPs and DMRs with 200 MS associated loci known from literature, identifying 2 significant overlaps for each omics.

Conclusion: We have analysed several omics individually underlining differences between PedMS and HC. We have already performed a preliminary late integration analysis and we are combining genetic, epigenetic and metagenomic data by an early integration strategy to identify latent factors that could explain PedMS pathogenesis.

Grants: 2022/R-Multi/013

Conflict of Interest: None declared

P16.106.B Enhancing variant prioritisation pipeline - Integrating PEDIA with VarFish via plugin-driven approach

Meghna Ahuja Bhasin[1], *Pietro Incardona*[1;2], *Alexej Knaus*[1], *Alex Schmid*[1], *Peter Krawitz*[1], *Tzung-Chien Hsieh*[1], *Manuel Holtgrewe*[3], *Miriam Elbracht*[4]

[1]*Institute for Genomic Statistics and Bioinformatics, Bonn, Germany;* [2]*Core Unit for Bioinformatics Data Analysis, Bonn, Germany;* [3]*CUBI – Core Unit Bioinformatics, Berlin Institute of Health, Berlin, Germany;* [4]*Institute for Human Genetics and Genomic Medicine, RWTH Aachen*

Background: Variant prioritisation is a critical step in genomic analysis, crucial for identifying disease-associated genetic variations. Case Annotation and Disorder Annotation (CADA) and GestaltMatcher algorithms quantify phenotypic similarity throughout clinical features (HPO) and portrait photo analysis providing the user with applicable scores. PEDIA combines these scores with molecular information into a gene-based score that can be used for variant prioritisation. Here, we integrate PEDIA into VarFish using a plugin-based approach and improve the PEDIA score to be variant-based rather than gene-based. Performance was assessed in a multi-center study of TNAMSE.

Methods: Whole exome sequencing data from 163 patients with rare diseases were imported into VarFish and filtered by frequency, impact, and quality. Molecular pathogenicity was weighted using CADD scoring, while GestaltMatcher provided facial scores. Phenotypic scoring was performed with the latest CADA version with improved performance. Various score combinations were evaluated. The integration was made extensible using Django CMS plugin-based methodology. Further benchmarking was done with tools like LIRICAL, Amely, and Exomizer.

Results: PEDIA achieved a top-10 accuracy of 83%, significantly higher than CADD and CADA scores alone. Integrating GestaltMatcher and PEDIA into VarFish demonstrated the platform's capability to analyse medical images alongside variants. Extensive benchmarking showed superior performance of PEDIA over other freely available tools like LIRICAL, Amely and Exomiser.

Conclusion: The integration of PEDIA into VarFish offers a potent synergy for comprehensive variant prioritisation, with variant-based scores improving usability and efficiency for clinicians.

Grants: No external grants or funding were received for this study.

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P16.107.C Multi-omics integration reveals (patho)physiological gene regulation in human neural crest cell differentiation

Michael Eibl[1], *Susanne Theiss*[1], *Ayat Ahmed*[1], *Hjorleifur Einarsson*[2], *Robin Andersson*[2], *Magdalena Laugsch*[1]

[1]*Institute of Human Genetics, Transcriptional Regulation in Developmental Disorders, Heidelberg, Germany;* [2]*Copenhagen Biocenter, Department of Biology, University of Copenhagen, Computational and RNA biology, København, Denmark*

Background/Objectives: Several neurodevelopmental disorders are associated with dysregulation of both the central nervous system and neural crest cells (NCC). This also applies to Bosch-Boonstra-Schaaf Optic Atrophy Syndrome (BBSOAS), caused by mutations in the transcription factor NR2F1, which is a driver of neurodevelopment and NCC differentiation. We aim to elucidate the global transcriptional landscape governed by NR2F1 within the overall regulatory circuitry of NCC by multi-omics integration and network analysis to help us understand pathomechanisms and advance molecular diagnosis.

Methods: We modelled neural crest development by differentiating human induced pluripotent stem cells (hiPSC) into NCC. We then performed Cap Analysis of Gene Expression (CAGE)-, Assay for Transposase-Accessible Chromatin (ATAC)-sequencing and NR2F1 chromatin immunoprecipitation followed by DNA-sequencing (ChIP-seq) at four time points of the differentiation. Subsequently, regulatory networks were inferred from paired expression and chromatin accessibility data (PECA2).

Results: We reproduced known and revealed novel transcription factor (TF)-target gene (TG) relationships underlying NCC differentiation. Predicted NR2F1 target genes were validated with corresponding NR2F1 ChIP-seq data. In addition, we identified active enhancers among the predicted regulatory elements (REs) through enhancer RNA (eRNA) expression analysis. Several SNPs associated with diseases such as orofacial clefting could be mapped to these REs.

Conclusion: Our multi-omics integration approach allows prediction of high-confidence gene regulatory relationships in the course of human NCC differentiation. We demonstrated the power of this approach by validating these networks with ChIP-seq data for the key driver of the neurodevelopmental disorder BBSOAS and showing that several disease variants can be mapped to the network.

Conflict of Interest: None declared

P16.108.D Bringing together next-generation association studies and fine-mapping by leveraging genetic variant pleiotropy to improve our understanding of human complex traits

Martin Tournaire[1], *Yves Rozenholc*[1], *Marie Verbanck*[1]

[1]*Université Paris Cité, UR 7537 BioSTM, Biostatistique, Traitement et Modélisation des données biologiques, Paris, France*

Background/Objectives: Using molecular phenotypes (*e.g.* expression quantitative trait loci) to fine-map the associations between genetic variants and complex traits from genome-wide association studies (GWAS) seems highly limited because of their very small overlap. Taking the opposite view to current methods, we propose to leverage pleiotropy, occurring when a genetic

variant affects at least two traits, which is thought to play a central role in the genetic architecture of complex traits.

Methods: By conceptualizing 5 distinct biological mechanisms leading to observed pleiotropy (direct, polygenicity-mediated, confounding-mediated, trait-mediated, linkage disequilibrium), we have developed PleioVar, an algorithm to detect both direct and pleiotropic effects of genome-wide variants. PleioVar derives variant effects from an integrative Mendelian Randomization method (*i.e.* LHC-MR) modeling pleiotropy and allows to disentangle the variant-level effects using Gaussian mixture models. To assess the performances of PleioVar, we built a comprehensive network GWAS simulation framework encompassing multiple complex scenarios. Then, using GWAS summary statistics from UK Biobank, we used PleioVar to disentangle the variant effects on a set of heritable traits and diseases.

Results: On simulations, PleioVar successfully predicted variant effects with high accuracy, and simulated genetic variants were correctly clustered as displaying the proper type of pleiotropy. Using GWAS summary statistics from the UK Biobank, genetic variant effects were identified by PleioVar, leading to a genome-wide map of variant-trait association with promising results.

Conclusion: This method, designed to map genetic variants to traits by leveraging pleiotropy, could help better understand the human genetic architecture of complex traits and diseases.

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Conflict of Interest: None declared

P16.109.A GROVER: Building a language model of the human genome

Melissa Sanabria[1;1], *Jonas Hirsch*[1], *Anna Poetsch*[1]

[1]*Technische Universität Dresden, Dresden, Germany*

Background/Objectives: Large Language Models trained without explicit supervision, have enabled the generation of coherent and syntactically accurate text in natural language. While DNA is very analogous to language through sequence structure that resembles grammar, syntax, and semantics, a key distinction lies in the absence of a conventional concept of words.

Methods: We have built GROVER (Genome Rules Obtained Via Extracted Representations), a foundation DNA language model with an optimized vocabulary for the human genome, trained with no additional information besides sequence.

Results: Analyzing GROVER's learned representations, we observed that at the word level it primarily learns information related to their frequency, GC content, AG content, AC content, and length. Some tokens are almost exclusively localized in repeats, while the vast majority are distributed over different genome elements. The model also learns context and lexical ambiguity. Annotation of repeats and chromatin colors shows the ability to extract functional annotations from the genome such as orientation of LINEs and ALUs, satellites, and promoters. We also show possible applications on a selection of different tasks, including prediction of DNA-protein binding ($F1 = 0.75$ identifying CTCF binding sites across different cell types).

Conclusion: DNA language models like GROVER can be used to investigate individual predisposition to disease and treatment responses, which to a large extent is encoded in the patients' general and somatic genome. It has the potential to push progress in personalized medicine and its transparent design allows insight into sequence dependent molecular biological mechanisms.

Grants: German Cancer Aid, MSNZ DresdenP2

Conflict of Interest: None declared

P16.110.B Identifying non-coding somatic cancer driver mutations using sequence-based models

Carlos Urzua-Traslaviña[1;2], *Tijs van Lieshout*[1;2], *Lucía Barbadilla-Martínez*[2;3], *Noud Klaassen*[2;4], *Vinícius Franceschini-Santos*[2;4], *Jeroen de Ridder*[2;3], *Bas van Steensel*[2;4], *Lude Franke*[1;2]

[1]*University Medical Center Groningen, Groningen, Netherlands;* [2]*Oncode Institute, Utrecht, Netherlands;* [3]*UMC Utrecht, Utrecht, Netherlands;* [4]*The Netherlands Cancer Institute (NKI), Amsterdam, Netherlands*

Background/Objectives: With pending availability of large quantities of whole genome sequencing data, there is an urgent need to identify functional non-coding somatic variants in cancer. The indistinguishability of passenger variants pushes existing methods to require large sample-sizes to identify regions enriched for somatic mutations. Recently, several sequence-based models have become available that can make inferences on the functional consequences of non-coding single nucleotide variants (SNVs). We hypothesize the promoter regions of potential driver genes could be enriched for such 'functional' somatic variants in tumor genomes.

Methods: We collected publicly available somatic SNV calls from ICGC located around the transcription start sites of protein coding genes. Using two-tailed Wilcoxon rank test, we then compared the predictions of observed mutations against two background models: 1) alternative alleles at the same base pair positions and 2) nearby mutations (15bp) in the same region. We evaluated regions with the following strategy: We first used a new fast sequence-based model, trained on promoter activity assay data of K562 cells to generate a discovery set of regions which we then replicated with state-of-the-art model Borzoi.

Results: The well-known TERT promoter showed the strongest 'functional' non-coding somatic mutation enrichment with both models. We also identified several promotor regions of other genes that showed strong mutation enrichment. We are currently following up on these findings.

Conclusion: By applying sequence-based models to WGS data it is possible to identify regions enriched for non-coding somatic mutations.

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P16.111.C More accurate and faster expression and splicing methods improve DROP, the Detection of RNA Outliers Pipeline

Felix Brechtmann[1], *Andrew Behrens*[1], *Ines Scheller*[1], *Cristian Sandu*[1], *Nicholas Smith*[1], *Vicente Yépez*[1], *Julien Gagneur*[1;2], *Christian Mertes*[1;2]

[1]*TUM School of Computation, Information and Technology (CIT), Department Informatik und Mathematik, Garching bei München, Germany;* [2]*Klinikum rechts der Isar der Technischen Universität München, München, Germany*

Consortium: German Human Genome-Phenome Archive (GHGA)

Background/Objectives: RNA-seq is a powerful approach to prioritise candidate genes for individuals affected with a rare genetic disorder and increases diagnostic yield by approximately 15%. DROP is a widely used workflow for RNA-seq-based diagnostics with a growing user base worldwide. Learning from

its application in many large-scale projects, such as Solve-RD, we updated DROP and integrated recently developed approaches, accelerating the detection of aberrant events in RNA-seq data.

Methods: We benchmarked the optimal hard thresholding procedure to determine the optimal latent space dimension, introduced by Gavish and Donoho, on several RNA-seq datasets and implemented it in OUTRIDER. We further integrated FRASER 2.0 for splicing analyses and extended QC measures, including a sex verification step. In addition, we are porting DROP to the workflow framework, Nextflow, adhering to the nf-core standards ensuring portability, reproducibility, and GA4GH compliance.

Results: DROP accelerates the adoption of RNA-seq in human genetic centres and has demonstrated success in expediting rare disease diagnoses. OUTRIDER's updates reduce the compute time for the expression outlier analysis by 15-fold. FRASER 2.0 uses the new intron Jaccard index that reduces the false positives by 8-fold while maintaining high sensitivity and simplifying the splicing analysis overall. By porting DROP to nf-core/Nextflow, we co-develop with the German Human Genome-Phenome Archive (GHGA) a containerized, scaleable, and cloud-ready workflow.

Conclusion: These improvements to DROP, and its integration as a Nextflow pipeline into GHGA's workflows, enable healthcare professionals and researchers to fully utilise RNA sequencing in rare disease diagnostics.

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Conflict of Interest: None declared

P16.112.D Integrating multigenerational sequencing data to enhance de novo variant detection

Laurens Hannes[1;2], *Kato Milis*[2], *Jeroen Breckpot*[1;2], *Alejandro Sifrim*[2]

[1]*UZ Leuven, Center for Human Genetics, Leuven, Belgium;* [2]*KU Leuven, Department of Human Genetics, Leuven, Belgium*

Background/Objectives: Adoption of genome sequencing in clinical practice has steadily increased, bringing forth a 60-fold increase in detected variant numbers compared to exome sequencing. As a result, there is a proportional increase in false variant calls, complicating the seemingly straightforward detection of de novo variants.

Methods: We present SeptoDeNovo, an adapted version of the TrioDeNovo model, integrating grandparental sequencing data to re-evaluate de novo variant calls and detect parental mosaicism. We benchmarked our model using simulated genome sequencing data at 30X coverage for 12 pedigrees with each 4 grandparents, 4 parents and an index, adding a known set of de novo variants to each index. To evaluate the robustness of the technique we also downsampled grandparental data to 15X coverage. We also compared the performance of SeptDeNovo to other tools, such as TrioDeNovo, DeNovoGear and DeNovoCNN, estimating the true and false positive rates of each approach.

Results: SeptoDeNovo outperformed TrioDeNovo and other widely used de novo callers, DeNovoGear and DeNovoCNN in recalling true variants while producing less false positive calls. This increased performance was also observed in scenarios of lower coverage of grand parental data, as well as in cases where less than 4 grandparents were available.

Conclusion: As a higher proportion of the general population will have their genome sequenced over their lifetime, we envision the usage of models like SeptoDenovo to fully leverage the available information and as such improve diagnostic performance.

Grants: KU Leuven internal fund (C24M/19/075)

Conflict of Interest: None declared

P16.113.A Novel multi-omics deconfounding variational autoencoders can obtain meaningful disease subtyping

Zuqi Li[1], *Sonja Katz*[2;3], *Vitor Martins Dos Santos*[3;4], *David Fardo*[5], *Peter Claes*[1;6;7], *Edoardo Saccenti*[3], *Kristel Van Steen*[1;8], *Gennady Roshchupkin*[2;9]

[1]*KU Leuven, Human Genetics, Leuven, Belgium;* [2]*Erasmus MC, Radiology and Nuclear Medicine, Rotterdam, Netherlands;* [3]*Wageningen University & Research, Wageningen, Netherlands;* [4]*LifeGlimmer GmbH, Berlin, Germany;* [5]*University of Kentucky, Lexington, United States;* [6]*UZ Leuven, Medical Imaging Research Center, Leuven, Belgium;* [7]*KU Leuven, Electrical Engineering, Leuven, Belgium;* [8]*University of Liège, GIGA-R Medical Genomics, Liège, Belgium;* [9]*Erasmus MC, Epidemiology, Rotterdam, Netherlands*

Consortium: TranSYS

Background/Objectives: Unsupervised learning, particularly clustering, is crucial for disease subtyping and patient stratification. With the availability of large-scale multi-omics data, clustering algorithms can be empowered by deep learning models, e.g. variational autoencoder (VAE), to exploit the between-individual heterogeneity. However, the impact of confounders—external factors unrelated to the condition, e.g. batch effect and age—on clustering is often overlooked, introducing bias and spurious biological conclusions.

Methods: We proposed four VAE-based deconfounding approaches utilizing multi-omics data: including i) removal of latent features correlated with confounders (XVAE + FS), ii) a conditional variational autoencoder (cXVAE), iii) adversarial training (adv-XVAE), and iv) adding a regularisation term to the loss function (cr-XVAE). Based on real-life multi-omics data from The Cancer Genome Atlas, we simulated various confounding effects (linear, non-linear, categorical, combination) and evaluated each model's performance across 50 repetitions based on reconstruction error, clustering stability, and deconfounding effectiveness ($ARI_{cancerType}$).

Results: We demonstrate a substantial impact of the artificially introduced confounder effect on patient clustering ($ARI_{cancerType} = 0.33$), yet various proposed models effectively mitigated this effect, with cXVAE clearly outperforming other frameworks ($ARI_{cancerType} = 0.66$). cXVAE not only accurately recovers true patient labels but also reveals meaningful pathological associations among cancer types, reinforcing deconfounded representation validity.

Conclusion: The multi-omics deconfounding conditional autoencoder presented in this study has big potential in delivering accurate patient subgrouping in the presence of confounders.

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Conflict of Interest: None declared

P16.115.C Context-Adjusted Proportion of Singletons (CAPS): A robust metric for assessing negative selection and benchmarking variant pathogenicity predictors

Mikhail Gudkov[1], *Loic Thibaut*[2], *Steven Monger*[1], *Debjani Das*[1], *David Winlaw*[3], *Sally Dunwoodie*[1], **Eleni Giannoulatou**[1]

[1]*Victor Chang Cardiac Research Institute, Darlinghurst, Australia;* [2]*Institute for Molecular Bioscience, Saint Lucia, Australia;* [3]*Ann & Robert H. Lurie Children's Hospital of Chicago, Chicago, United States*

Consortium: Congenital Heart Disease Synergy Study group

Background/Objectives: Interpreting genetic variants remains challenging, especially for understudied variant classes. Population genetics methods, like the Mutability-Adjusted Proportion of Singletons (MAPS) metric, assess variant effects through population distributions. However, MAPS' sensitivity to the singletons-by-mutability model calibration may lead to biased estimates for specific variant sets. This study introduces Context-Adjusted Proportion of Singletons (CAPS), a novel metric refining MAPS methodology by eliminating the mutability layer, enhancing robustness in assessing negative selection in the human genome. Here, CAPS was employed to benchmark pathogenicity predictors without relying on known pathogenic variant sets.

Methods: CAPs derives the expected level of rare variation on a per-context basis from synonymous variants. For missing contexts, expected proportions of singletons were approximated from intronic variants using probit regression. Leveraging gnomAD genomic data, we benchmarked pathogenicity predictors using CAPS and compared our estimates to ClinVar variant classifications.

Results: CAPS outperforms MAPS, yielding more accurate negative selection estimates. Using CAPS, poorly calibrated predictors were identified, highlighting CADD and REVEL as the best-performing methods, with REVEL exhibiting better calibration. Our findings underscore the importance of cautious predictor integration into variant interpretation pipelines and emphasise biases associated with variant classification.

Conclusion: CAPS emerges as a promising metric for studying negative selection, serving as a valuable benchmarking tool for pathogenicity predictors. This study underscores the need for careful predictor integration and recognition of biases in variant classification, contributing to advancements in genetic variant assessment and interpretation.

Grants: National Health and Medical Research Council (Synergy Grant 1162878, Investigator Grants 2018360 and 2007896), NSW Health.

Conflict of Interest: None declared

P16.116.D Pipeline for proteomic in silico cell-type deconvolution

Tuguldur Tumurbaatar[1;2], *Helena Pelin*[3], *Lisa Barros de Andrade e Sousa*[3], *Nishant Chintalagiri*[1;2], *Johannes B, Müller-Reif*[4;5], *Philipp E Geyer*[4;5], *Orsolya Genzel-Boroviczény*[1], *Matthias Mann*[4;6], *Marie Piraud*[3], *Christoph Klein*[1], *Claudia Nussbaum*[1], *Susanne Pangratz Fuehrer*[1], *Sarah Kim-Hellmuth*[1;2]

[1]*University Hospital LMU Munich, Department of Pediatrics, Dr. von Hauner Children's Hospital, Munich, Germany;* [2]*Helmholtz Munich, Institute of Translational Genomics, Department of Computational Health, Neuherberg, Germany;* [3]*Helmholtz Munich, Helmholtz AI, Neuherberg, Germany;* [4]*Max Planck Institute of Biochemistry, Proteomics and Signal transduction, Martinsried, Germany;* [5]*OmicEra Diagnostics GmbH, Planegg, Germany;* [6]*Novo Nordisk Foundation Centre for Protein Research, University of Copenhagen Faculty of Health and Medical Sciences, Proteomics Program, København, Denmark*

Background/Objectives: Analysing the human blood proteome grants us an in-depth look into the health of an individual and opens horizons for the development of novel diagnostic and therapeutic methods. However, affinity-based techniques such as ELISA, which are used for single-protein quantifications, are being replaced by mass spectrometry (MS)-based methods that provide comprehensive results in even small sample volumes. Despite the attractiveness of MS-based workflows, there are prominent issues that need to be addressed, most notably, the abundance of missingness in MS datasets and the lack of cell-type resolution in bulk tissue MS. Therefore, our goal is to develop a pipeline that can impute these missing values and perform cell-type deconvolution from MS blood proteomics data.

Methods: Based on pre-existing MS proteomics datasets and comparative studies on the effectiveness of various mathematical imputation and deconvolution methods, we developed an R-based pipeline built on existing methods, capable of imputing missing protein expression values and with the help of a signature matrix, calculate the proportions of specific cell-types within a given sample.

Results: The pipeline is modular, allowing for customization and sample-specific optimization. Various normalization and data preparation workflows are included. Accuracy can be assessed by comparing with flow cytometry data of the same samples.

Conclusion: Implementing multiple imputation and deconvolution methods, our pipeline can serve as a valuable tool for blood MS proteomics. This enables the characterization of protein abundance in immune cells without needing to isolate them or in samples where sample volume is a limiting factor.

Grants: DFG TRR359 PILOT

Conflict of Interest: None declared

P16.118.B In silico study on the mutation induced conformational changes of vasopressin receptor 2 from a clinical nephrogenic diabetes insipidus case

Chun-Chun Chang[1], *Ting-Yu Hu*[2], *Hao-Jen Hsu*[2]

[1]*Hualien Tzu Chi Hospital, Buddhist Tzu Chi Medical Foundation, Department of Laboratory Medicine, Hualien, Taiwan;* [2]*Tzu Chi University, Department of Biomedical Sciences and Engineering, Hualien, Taiwan*

Background/Objectives: Congenital nephrogenic diabetes insipidus (CNDI) is a genetic disorder caused by mutations in arginine vasopressin receptor 2 (AVPR2), rendering collecting duct cells insensitive to the peptide hormone arginine vasopressin stimulation for water reabsorption. The CNDI condition of an 8-month-old male patient was identified with a Valine 279 deletion–mutation in the *AVPR2* gene according to symptoms, family history, and DNA sequence analysis. Mutation in AVPR2 can lead to kidney-related diseases. However, the signaling differences and conformational changes induced by these mutations in AVPR2 remain unclear.

Methods: We utilized molecular dynamics simulations to construct distinct mutant AVPR2 systems, both with and without AVP bound. This approach allowed us to explore how these mutations affect structural dynamics, molecular switches, and internal signal transmission.

Results: Our investigation revealed that mutated systems exhibit dynamic activity that is fundamentally distinct from the wild type. These differences included conformational changes in the transmembrane region and alterations in the molecular switch mechanism. The analysis of signal transduction pathways reveals that the presence or absence of AVP binding introduces variations in signal transmission. The amino acids identified may play a pivotal role in the functional deficits observed in mutations of AVPR2. Our results are also consistent with previous experiments that Tolvaptan may correct the M272R mutation.

Conclusion: Our findings provide a preliminary insight into the activation and inactivation mechanisms of AVPR2 under various mutations, which are valuable insights for future drug development targeting AVPR2.

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Conflict of Interest: None declared

P16.119.C Detection of tissue-border-specific marker genes using spatially resolved transcriptomics

Vincent Hammer[1], *Stephan Ossowski*[1]

[1]*Universitätsklinikum Tübingen – Institut für Medizinische Genetik und angewandte Genomik, Tübingen, Germany*

Background/Objectives: Direct cell-to-cell contact can trigger import developmental processes during organogenesis. Some of the short distance signalling pathways triggered by cell-to-cell contact mediate cell differentiation, proliferation, and structure of whole tissues. A better understanding of the gene expression patterns in these edge regions could improve our understanding of organogenesis and pathogenesis. Using untargeted spatially resolved transcriptomics data, we introduce a pipeline for identifying marker genes for tissue-to-tissue contact, and visualizing the gene expression changes across different timepoints.

Methods: Stereo-seq data of mouse embryos was obtained through the public CNGB sequence archive. Preprocessing was performed using the SAW pipeline. Binning, quality control, clustering, and annotation were performed with Stereopy.

The pipeline first filters the annotated regions by size to reduce noise. Then, spots with different types of tissue in direct contact are detected. These spots are expanded and linked to form edges. The resulting edges are filtered by their length and annotation robustness. Next, the average expression of a gene as a function of distance from these edges are calculated. These expression profiles are then filtered for correlation across replicates. Finally, these expression profiles are visualized and sorted by pattern.

Results: We identified genes with increased transcriptional activity at tissue edges. Sets of genes specific to individual tissue types could be defined. Additionally, changes in the expression patterns of these genes across different timepoints of organogenesis could be traced.

Conclusion: This pipeline offers new insights into the gene activity at tissue borders, and helps understand the transcriptional activity in these regions across distance and time.

Grants:

Conflict of Interest: None declared

P16.120.D Deep Generative Models to improve Polygenic Risk Scores' accuracy and portability across ancestries and admixtures

Andrea Lampis[1;2], *Michela Carlotta Massi*[1], *Andrea Mario Vergani*[1;2], *Matteo Matteucci*[2], *Francesca Ieva*[1;2], *Emanuele Di Angelantonio*[1;3]

[1]*Human Technopole, Italy;* [2]*Politecnico di Milano, Milano, Italy;* [3]*University of Cambridge, United Kingdom*

Background/Objectives: Polygenic Risk Scores (PRSs) hold potential for precision medicine but face limitations, especially in non-Europeans due to historical biases towards European genetic studies. Addressing the challenge of PRS transferability, this study leverages Deep Learning-based generative models to enhance PRS accuracy across diverse populations. We propose a novel approach employing Latent Diffusion Models (LDMs) for generating realistic genetic data across ethnicities, aiming to improve PRS calculation for underrepresented groups and admixed individuals, and offer new insights into disease etiology by analyzing the obtained latent space.

Methods: We trained a LDM with a Variational AutoEncoder (VAE) and a UNet-based denoising model, conditioned on disease presence. Utilizing Coronary Artery Disease (CAD) GWAS summary statistics to select SNPs of interest across populations, we developed population-specific models and a combined model for multiple ethnicities. We assessed model performance by comparing PRS accuracy using generated and actual genetic data, versus state-of-the-art approaches that only combine summary statistics from different cohorts. Additionally, we applied Machine Learning explainability and latent space arithmetic to investigate population-specific disease etiologies.

Results: Analyzing data from 449,557 controls and 37,541 CAD subjects from UKBiobank, our models achieved high reconstruction quality, with an average error rate of 20/2048 SNPs. Traditional PRSs calculated with our method showed improved performance over baseline methods, especially in non-European and admixed populations, while enriching results with insights from latent space analyses.

Conclusions: Our approach improves PRS transferability and accuracy across ethnicities, addressing privacy concerns and enhancing genetic research inclusivity, laying groundwork for disease genetic exploration in diverse groups.

Conflict of Interest: None declared

P16.121.A GWAS of longitudinal changes in bodysize and their relationships with childhood and adulthood adiposity in UK Biobank

Yelyzaveta Poliakova[1;2], *Vincent Pascat*[3], *Yevheniya Sharhorodska*[4;5], *Zhanna Balkhiyarova*[4;6;7], *Marika Kaakinen*[2], *Marjo-Riitta Järvelin*[2;8], *Inga Prokopenko*[4;6]

[1]*Lviv State Regional Clinical Perinatal Center, Department of Newborn Screening, Lviv, Ukraine;* [2]*Imperial College London, School of Public Health, London, United Kingdom;* [3]*University of Lille, EGID, Lille, France;* [4]*University of Surrey, Department of Clinical & Experimental Medicine, Guildford, United Kingdom;* [5]*Imperial College London, Vascular Science of the National Heart and Lung Institute, London;* [6]*University of Surrey, People-Centred Artificial Intelligence Institute, Guildford, United Kingdom;* [7]*Imperial College London, Department of Metabolism, Digestion and Reproduction, London;* [8]*University of Oulu, Oulu, Finland*

Background. Genome-wide association studies (GWAS) identified hundreds of DNA variants contributing to higher child-/adulthood overall body adiposity, body-mass index (BMI) and central obesity, waist-to-hip ratio (WHR) in adults. Such cross-sectional estimates overlook regulation of longitudinal bodysize changes (LBC) through the lifecourse.

Methods. We performed GWAS of LBC from childhood (10y.) to adulthood BMI, grouped into thin, average, and plump/obese. We implemented linear mixed-models (BOLT-LMMv2.3) in 400,429 UK Biobank Europeans with both non-missing measures. Hierarchical clustering was employed to group the associated loci in relation to respective cross-sectional adiposity measures and WHR, using GWAS meta-analyses (GIANT consortium).

Results. We identified 111 independent significant LBC signals (P-value$\leq 5 \times 10^{-8}$). Of those, 66 LBC signals overlapped with established adulthood BMI loci, including *FTO, SEC16B, ADCY3, TMEM18, MC4R, GPRC5B, GPR139, RPL31P12, MAP2K5*. Additional 24 LBC signals were shared with childhood bodysize only, while further 18 LBC loci affected all three phenotypes. All three novel loci, at *RFPL4AP3, PCLO*, and *RASA4*, and six shared with BMI were established for educational attainment, bipolar or major depressive disorder, and T2D. Clustering highlighted that most LBC loci had "set-point" body size effect, where higher overall adiposity was related to lower central adiposity, while only 14 related to change from "thinner" childhood to "plumper/obese" adulthood.

Conclusion. Our investigation transcends traditional analyses of cross-sectional BMI and offers new insights into LBC trajectories genetics. We report that genetic predisposition to LBC from

childhood to adulthood does not fully overlap with respective cross-sectional measures. LBC processes highlight enrichment with mental health.

Grants: British Academy fellowship RaR\100571; LongiTools, H2020-SC1-2019-874739.

Conflict of Interest: None declared

P16.122.B Unraveling complex chromosomal rearrangements: a clinical perspective on efficient enumeration in karyotype graphs

Barbara Poszewiecka[1], *Krzysztof Gogolewski*[1], *Anna Gambin*[1]

[1]*Faculty of Mathematics, Informatics, and Mechanics, University of Warsaw, Institute of Informatics, Warszawa, Poland*

Background/Objectives: With the evolution of sequencing technologies, the need to decipher genome plasticity resulting from various chromosomal rearrangements, particularly those involving multiple breakpoints, has become increasingly important. The main objective is to address the challenge of efficiently enumerating the rearrangements scenarios of Karyotype Graphs (PMID: 31842730), crucial for understanding complex chromosomal rearrangements observed in genomes.

Methods: To handle complex chromosomal rearrangements, we have developed an algorithm leveraging the Karyotype Graph model. This multigraph provides an efficient representation of sets of breakpoints and genomic segments. In scenarios lacking telomere-free connected components, the decomposition yields exclusively linear chromosomes. Our method ensures a structured enumeration of diverse chromosomal rearrangements offering a concise yet comprehensive understanding of potential scenarios with minimal cardinality.

Results: Our approach, utilizing connectivity and witness certificates representing the current state of the recursion, efficiently handles edge multiplicity decrements, avoiding undesirable graph disconnections. With a polynomial time complexity, it facilitates the exploration of diverse rearrangement scenarios, as illustrated by a case study involving a proband with congenital chromoanasynthesis in a patient harboring such alterations from Nazaryan-Petersen et al. (2018) study (PMID: 30419018). Notably, our algorithm identifies additional rearrangement scenarios beyond those initially presented, underscoring its effectiveness in handling complex genomic data.

Conclusion: Our algorithm offers a promising path for further development and clinical application, including enhancements for circular derivative chromosomes and integration of optical genome mapping data. It contributes to understanding genomic rearrangements and their clinical implications in various pathological conditions, particularly in complex cancer genomes.

Grants: -

Conflict of Interest: None declared

P16.123.C A machine learning tool to predict gene-phenotype associations

Ana Santos-Pereira[1;2], *Joana Vilela*[1;2], *Ana Rita Marques*[1;2], *João Xavier Santos*[1;2], *Célia Rasga*[1;2], *Astrid Vicente*[1;2], **Hugo Martiniano**[1;2]

[1]*Instituto Nacional de Saúde Doutor Ricardo Jorge, Departamento de Promoção da Saúde e Prevenção de Doenças não Transmissíveis, Lisboa, Portugal;* [2]*Instituto de Biosistemas & Ciências Integrativas, Faculdade de Ciências da Universidade de Lisboa, LIsboa, Portugal*

Background/Objectives: Driven by rapid advancements in DNA sequencing techniques, both speed and cost have plummeted, propelling a significant shift in clinical diagnosis from targeted gene panels to whole-exome and whole-genome sequencing (WES/WGS). However, a major hurdle persists: effectively interpreting the vast pool of variants generated by WES/WGS, which presents a multitude of potential diagnostic candidates. While recent approaches using gene-phenotype association algorithms offer promise, they often struggle with limited data, hindering their effectiveness. To address these limitations, we developed a novel machine learning-based method to determine gene-phenotype associations leveraging data from large-scale, publicly available databases.

Methods: Our methodology consists on a data-driven approach, based on the use of graph machine learning methods. Graph Machine learning techniques were be used to predict ranked gene-disease associations for all combinations of genes and Human Phenotype Ontology (HPO) terms. These rankings form the basis for assessing the putative association of genetic variants to the disease or phenotype of interest.

Results: We show that our method improves the identification of pathogenic variants in the ClinVar database, when compared to recent tools, such as Exomiser, GADO, Phen2gene and Phenolyzer.

Conclusion: We developed a machine learning based method to determine gene-phenotype associations using data from publicly available databases. This allows us to prioritize candidate variants with greater accuracy and interpretability, potentially leading to faster and more precise diagnosis.

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Conflict of Interest: None declared

P16.124.D Integrating proteomic networks for comprehensive health assessment: a novel approach using the UK Biobank cohort

Alessia Mapelli[1;2], *Francesca Ieva*[1;2], *Emanuele Di Angelantonio*[1]

[1]*Human Technopole, Milan, Italy;* [2]*Politecnico di Milano, Milan, Italy*

Background/Objectives: The progression of diseases induces changes in both molecular and observable traits within the human body. Proteins, acting as crucial mediators of gene functions, influence trait determination, providing valuable insights for data-driven approaches to diagnosis, prevention, and monitoring. Understanding the functional interdependencies among molecular components becomes essential, as health conditions frequently arise from disturbances in the complex intracellular network rather than isolated gene abnormalities. Existing methods in network medicine often rely on either general biological graph databases or exclusively on data-driven approaches. However, protein-protein interaction databases tend to favor well-studied proteins, impacting the thoroughness of the analysis, and confounding factors may influence co-expression networks, leading to biased associations.

Methods: In this study, a novel representation method utilizes networks to integrate biological knowledge from databases into an estimation-based approach, minimizing noise in co-expression network construction and addressing the impact of confounding factors.

Results: The method was applied to the UK Biobank cohort, comprising 54,219 participants recruited in the United Kingdom, and 2,923 unique proteins were measured using antibody-based Olink technology. The data's high dimensionality in proteins and

samples contributes to the comprehensive description of the proteomes. This unique representation allows the capture of individualized imprints of current health status and can simultaneously represent behavioral traits such as smoking and the potential risk of diseases like diabetes.

Conclusion: This work aims to represent proteomes reliably within a network framework. This serves as a quantitative health assessment tool for simultaneously evaluating multiple health conditions - an emerging concept.

Conflict of Interest: None declared

P16.125.A Detection of pathogenic structural variation from whole genome sequencing data by applying bioinformatic SV algorithms and comparing their performance

Lubomir Balabanski[1], *Maya Atanasoska*[1;2], *Slavyana Yaneva Staykova*[1], *Spasimir Shishinyov*[1], *Irena Bradinova*[1;3;4], *Daniela Avdjieva-Tzavella*[5], *Katya Kovacheva*[6], *Daniela Mircheva*[1], *Radoslava Vazharova*[1;7], *Draga Toncheva*[1]

[1]*Genetic and Medical Diagnostic Laboratory "CellGenetics", Sofia, Bulgaria;* [2]*Sofia University "St. Kliment Ohridski", Faculty of Biology, Department of Genetics, Sofia, Bulgaria;* [3]*National Genetic Laboratory, University Hospital for Obstetrics and Gynaecology "Maichin Dom", Sofia, Bulgaria;* [4]*Medical University - Sofia, Faculty of Medicine, Department of Obstetrics and Gynaecology, Sofia, Bulgaria;* [5]*Paediatric Hospital „Prof. Dr. Ivan Mitev", Sofia, Bulgaria;* [6]*Medical University - Pleven, Centre of Competence in Personalised Medicine, 3D and Telemedicine, Robotic and Minimally Invasive Surgery, Pleven, Bulgaria;* [7]*Sofia University "St. Kliment Ohridski", Faculty of Medicine, Department of Biology, Medical Genetics and Microbiology, Sofia, Bulgaria*

Background: Whole genome sequencing (WGS) is currently the most comprehensive genetic analysis as it enables the identification of different types of variations (SNPs, indels, copy-number variants, structural variation) through application of different specialised bioinformatic algorithms.

Methods: Whole genome sequencing was performed in collaboration with BGI Genomics on a large cohort of over 230 patients from Bulgaria with different rare diseases or partner pairs for carrier screening. Variant calling was performed applying the GATK toolkit for SNP/indel detection, algorithm *CNVnator* for copy-number variation and *BreakDancer* for structural variation. An in-house strategy based on genomic architecture and single-read analysis was developed to filter out the large number of false-positive findings for each patient and to map the potential breakpoints down to single-base-pair resolution.

Results: We detected in total 7 pathogenic/likely pathogenic structural variants of different types summarised in the table below (five causative variants, one incidental finding and one carrier status):

Variant	Type	Zygosity	Diagnosis
FANCA:c.190-256_283+1690delinsC	deletion-insertion	homozygous	Fanconi anaemia
CACNA1A:c.4250+814_*3032del	deletion	heterozygous	Episodic ataxia
EDA:c.654_742-382delinsCCA	deletion-insertion	hemizygous	Ectodermal dysplasia
SMN1:c.[343_367dup;407_421del; 450_461del;474 + 19_474+23del]	complex rearrangement	heterozygous	Spinal muscular atrophy
PSEN1:c.-51425_-136+5312inv	inversion	heterozygous	Alzheimer's disease
ABCC6:c.2996-1724_4209-479del	deletion	heterozygous	/Carrier screen/
FARS2:c.613-6799_904+27484del	deletion	heterozygous	/Incidental finding/

Comparing bioinformatic algorithm sensitivity and specificity, we concluded that *CNVnator* has an advantage at detecting CNVs based on coverage fluctuations, while *BreakDancer* performs better at detecting breakpoint-evident SVs with unmapped read fragments.

Conclusion: Applying a combination of different bioinformatic algorithms for SNV/CNV/SV detection on whole-genome sequencing data provides the highest diagnostic yield combining several methodologies in a single test.

Conflict of Interest: None declared

P16.126.B Developing a bioinformatic pipeline to analyse a given disease-associated repeat locus using long-read PacBio HiFi sequencing data

Anita Szabo[1], *Egor Dolzhenko*[2], *Christina Zarouchlioti*[1], *Ismail Moghul*[1], *Petra Liskova*[3], *Pavlina Skalicka*[3], *Stephen Tuft*[4], *Guilherme De Sena Brandine*[2], *Janet Aiyedun*[2], *Valeriya Gaysinskaya*[2], *Tom Mokveld*[2], *Michael Eberle*[2], *Duncan Kilburn*[2], *Sarah Kingan*[2], *Alice Davidson*[1;4]

[1]*University College London Institute of Ophthalmology, Department of Ophthalmology, London, United Kingdom;* [2]*Pacific Biosciences (PacBio), Menlo Park, United States;* [3]*Charles University of Medicine and General University Hospital in Prague, Prague, Czech Republic;* [4]*Moorfields Eye Hospital, London, United Kingdom*

Background/Objectives: Currently >50 monogenic human disorders are associated with short tandem repeat (STR) expansions. STRs are somatically unstable elements that can contract/expand in age and tissue specific manners. Somatic instability, repeat length variation, methylation status and sequence structure can modify disease severity and onset. Here we describe a workflow developed to investigate disease-associated STRs across the human genome. The workflow utilises a repeat expansion associated with an age-related eye disease, Fuchs Endothelial Corneal Dystrophy (OMIM # 613267; termed CTG18.1), as an exemplar locus to showcase the broad utility and outputs of the pipeline developed.

Methods: The workflow first genotypes a given tandem repeat and phases reads spanning it in a specified set of PacBio HiFi samples using the tandem repeat genotyping tool (TRGT). It then generates and visualizes the distribution of allele lengths and sequence composition across all samples. Then it performs an automated repeat interruption analysis and visualizes sequencing data containing repeats with abnormal lengths, sequence composition, or repeat interruptions.

Results: Using HiFi data generated from genomic DNA, we developed a workflow to interrogate and visualise the allelic structure, repeat length, instability and repeat interruption profiles. This workflow has been documented as a Jupyter Notebook to enable researchers to apply and analyse their repeat loci of interest.

Conclusion: Here we describe a computational workflow to enable researchers to interrogate their STR of interest using HiFi data. The generated outputs offer broad utility to the STR biology community.

Grants: Moorfields Eye Charity PhD studentship GR001395; UKRI Future Leader Fellowship MR/S031820/1

Conflict of Interest: None declared

P16.127.C Advancing precision medicine: A ML-based recommender system for accelerated diagnostics in rare hereditary diseases using next-generation sequencing data

Matthias Bieg[1], Vivi Lieu Mertes[1], Jorge Pinto Basto[1], Omid Paknia[1], Peter Bauer[1], **Krishnakumar Kandaswamy**[1]

[1]*CENTOGENE GmbH, R&D Bioinformatics, Rostock, Germany*

Background/Objectives: Diagnosing rare hereditary diseases through the molecular analysis of next-generation sequencing (NGS) data, encompassing both Whole Exome Sequencing (WES) and Whole Genome Sequencing (WGS) cases, involves collaboration across disciplines from NGS wet lab scientists to clinical and medical experts. Despite advancements of variant annotation and prioritization, the interpretation remains labor-intensive, requiring a substantial team of clinical specialists. To improve efficiency, we implemented a recommender system that calculates the likelihood of identifying a causal variant for a given patient. This system enables strategic case assignments to clinical specialists based on their experience and expertise, significantly expediting the diagnostic process.

Methods: We utilize our Centogene Biodatabank, incorporating clinically diagnosed in-house patients from WES/WGS data—11,829 without a causal variant and 17,066 with a confirmed clinically relevant DNA variant. Employing a gradient boosting model, we train the system using features like HPO terms, ethnicity, and family medical history, enhancing our binary classification for identifying the presence or absence of causal variants with high recall.

Results: The model was benchmarked against 3,211 previously diagnosed in-house patients. It achieves a recall of 82%, at a precision of 69%, while the AUC of the ROC curve is at 74%.

Conclusion: The AI model accurately determines the probability of identifying a causal variant based on specific patient details, including HPO terms, ethnicity, and family medical history. This information serves as a valuable recommendation tool for clinical specialists, qualifying the a-priori probability of a positive diagnostic finding and thereby contributing to a substantial acceleration of the diagnostic process.

Conflict of Interest: Matthias Bieg employed at CENTOGENE GmbH, Vivi Lieu Mertes employed at CENTOGENE GmbH, Jorge Pinto Basto employed at CENTOGENE GmbH, Omid Paknia employed at CENTOGENE GmbH, Peter Bauer employed at CENTOGENE GmbH, Krishnakumar Kandaswamy employed at CENTOGENE GmbH

P16.128.D Bridging the performance gap for underrepresented populations: Anchor regression for polygenic risk scores

Hannah Klinkhammer[1;2], Carlo Maj[3], **Peter Krawitz**[2]*, Christian Staerk[1], Andreas Mayr[1]*

[1]*Institute for Medical Biometry, Informatics and Epidemiology, Medical Faculty, University of Bonn, Bonn, Germany;* [2]*Institute for Genomic Statistics and Bioinformatics, Medical Faculty, University of Bonn, Bonn, Germany;* [3]*Center for Human Genetics, University of Marburg, Marburg, Germany*

Background/Objectives: Polygenic risk scores (PRS) lack performance in populations that are under-represented in the training data. To account for population structure, genetic principal components (PCs) are included in the derivation of PRS and in the subsequent analysis. In this study we propose a new approach to make use of the PCs aiming for more robust PRS performance across populations.

Methods: We implemented anchor regression in the statistical boosting framework snpboost. Anchor regression is a technique that transfers ideas from causality into predictive modelling and interpolates between two stage least squares regression, ordinary least squares regression and residual regression via its tuning parameter γ. Here, as a proxy for ancestry, we consider the first 10 PCs as anchors, comparable to instrumental variables that influence the genetic variants. Models for BMI and LDL were trained on data from the UK Biobank and evaluated on independent test sets from different populations (EUR, AFR, SAS, EAS).

Results: Depending on a suitable choice of its tuning parameter γ anchor regression could improve the predictive performance in terms of R^2 compared to the classical approach of including the PCs and PRS in a multiple regression. The largest potential gain in R^2 was found in individuals of EAS ancestry (from 3.3% to 5.6%). Noteworthy, the optimal performance was reached for different tuning parameters in the different populations.

Conclusion: Preliminary results suggest that using PCs as anchors in the anchor regression framework can help to bridge the transferability gap. Further research is warranted, e.g. regarding the choice of the tuning parameter.

Grants:

Conflict of Interest: None declared

P16.129.A AlphaMissense is better correlated with functional assays of missense impact than earlier prediction algorithms

Alicia Ljungdahl[1;2]*, Sayeh Kohani[1], Nicholas Page[1;2], Eloise Wells[1], Emilie Wigdor[1], Shan Dong[1;2], Stephan Sanders[1;2]*

[1]*University of Oxford, Paediatrics, Oxford, United Kingdom;* [2]*University of California, San Francisco (UCSF), Psychiatry, San Francisco, United States*

Background/Objectives: Missense variant interpretation remains a major challenge in functional genomics and medicine. Numerous different in silico approaches have been developed to predict missense impact. We performed an independent assessment, including AlphaMissense, using functional data generated from previously published multiplexed assays of variant effect (MAVE) of 17,696 missense variants in five genes.

Methods: For each gene, all possible missense variants from single nucleotide substitutions were predicted from the Matched Annotation from NCBI and EMBL-EBI (MANE) isoform. Functional data were downloaded from the published manuscripts and annotated against 36 prediction algorithms using ANNOVAR (dbnsfp42a). Missense severity scores were added from EVE, PrimateAI-3D, and AlphaMissense, along with pathogenicity, as defined by ClinVar. Correlation between functional scores and each predictor was assessed using absolute Spearman correlation.

Results: AlphaMissense was the highest correlated algorithm to the functional data for *PTEN* and *BRCA1*, 2nd for *MSH2*, 3rd for *DDX3X*, and 5th for *KCNQ4*. PrimateAI-3D ranked in the top five for four and ranked first for two genes (*MSH2, KCNQ4*). In four of the five genes, AlphaMissense scores show a modestly higher correlation than REVEL (an algorithm widely used for clinical predictions).

Conclusion: AlphaMissense represents the current best-in-class predictor by this metric; however, the improvement over other algorithms is modest. Across missense predictors, many variants are predicted to impact function despite minimal consequences observed with MAVE assays. ClinVar classification of these discordant variants is variable, agreeing with AlphaMissense in *DDX3X* and MAVE data in *MSH2*; training data quality is likely the limiting factor.

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P16.130.B AI reclassification of pancreatic cancer variants in MMR genes

Raul Valin[1], *Laura Martinez Gomez*[1], *Nooshin Bayat*[1], *Serban Georgescu*[2], *Shuya Abe*[3], *Fuji Masaru*[3], *Shinichirou Tago*[3], *Ceres Fernandez-Rozadilla*[4], *Ana Fernandez-Montes*[5], *Jesus Garcia-Mata*[5]

[1]*Fujitsu Research of Europe, Computing Research Group, Madrid, Spain;* [2]*Fujitsu Research of Europe Ltd, Computing Research Group, Slough, United Kingdom;* [3]*Fujitsu Research, Fujitsu Ltd., Japan;* [4]*Foundation Health Research Institute of Santiago de Compostela (IDIS), Cancer Predisposition and Biomarkers lab, Santiago de Compostela, Spain;* [5]*Complexo Hospitalario Universitario de Ourense - CHUO, Medical Oncology Department, Ourense, Spain*

Background/Objectives: Several works have highlighted the role of germline mutations in the MMR genes for hereditary pancreatic cancer [1]. However, the recent implementation of NGS technologies into genetic diagnosis have resulted in a high rate (40%) of detection of variants of unknown significance (VUS). In this work, we present an AI tool for classification of germline VUSs, which we validate using known pathogenic germline variants from ACMG guidelines. The tool is then tested in a dataset of germline variants found in pancreatic cancer patients from Japan.

Methods: A graph-based deep learning model (GDLM) has been used to classify the pathogenicity of germline variants [2]. The model was validated by comparing the variant classification in ClinVar 2020 and the capacity of GDLM to reclassify variants with this information, with that of ClinVar 2023, considered as the ground truth for the comparison. Once validated, we tested GDLM in a clinical dataset of pancreatic patients from Japan and evaluated the potential to reclassify clinical VUSs [3].

Results: The obtained results show that GDLM classifies, in average, 98% of the variants classified as benign in ClinVar 2023 and 100% of the pathogenic variants correctly in the validation set.

For the pancreatic cancer dataset, GDLM reclassifies as pathogenic 3 of the 5 (60%) VUSs found in patients with pancreatic cancer. The other two are classified as benign variants.

Conclusion: These results support the use of GDML as a helpful tool for interpretation of VUSs in MMR genes.

References:

[1] https://doi.org/10.3390/ijms23031205
[2] https://doi.org/10.3390/cancers15041118
[3] https://doi.org/10.1097/CEJ.0000000000000787

Conflict of Interest: Raul Valin Full-time employed by Fujitsu, Laura Martinez Gomez Full-time employed by Fujitsu, Nooshin Bayat Full-time employed by Fujitsu, Serban Georgescu Full-time employed by Fujitsu, Shuya Abe Full-time employed by Fujitsu, Fuji Masaru Full-time employed by Fujitsu, Shinichirou Tago Full-time employed by Fujitsu, Ceres Fernandez-Rozadilla Fujitsu consultant, Ana Fernandez-Montes Fujitsu consultant, Jesus Garcia-Mata Fujitsu consultant

P16.131.C Unveiling Common Genes in Alpha-Synucleinopathies Through Weighted Gene Co-expression Network Analysis

Omer Faruk Duzenli[1], *Medinenur Yozlu*[1], *Duygu Gezen-Ak*[2], *Emrah Yucesan*[1]

[1]*Istanbul University-Cerrahapasa Institute of Neurological Sciences, Department of Neurogenetics, Istanbul, Türkyie;* [2]*Istanbul University-Cerrahapasa Institute of Neurological Sciences, Department of Neuroscience, Istanbul, Türkyie*

Background/Objectives: Parkinson's disease (PD), Dementia with Lewy bodies (DLB) and Multiple system atrophy (MSA) are classified as alpha-synucleinopathies accompanied by Lewy bodies (LB) or glial cytoplasmic inclusions (GCI). Although these diseases show alpha-synuclein (α-syn) pathology, they affect different brain regions and cells and present with various clinical symptoms. The main focus of this study is to identify the common genes and pathways functioning in the mentioned diseases. Our goal is to enable a more rational evaluation of the diagnosis and treatment of the disease based on our findings.

Methods: For this purpose, three data set were utilized using the NCBI-GEO database, GSE134472 for MSA, GSE193438 for DLB, and GSE205450 for PD respectively obtained from post-mortem brain tissues. The analysis of Differentially Expressed Genes in these data sets was performed using the Weighted Gene Co-expression Network Analysis (WGCNA) tool in the R Studio program. The Metascape interface was employed for functional enrichment analysis.

Results: A total of 148 associated genes ($p < 0.001$) were identified as common in the diseases. Enrichment analysis revealed that these genes are associated with various pathways, e.g. regulation of cytoskeletal organization, membrane trafficking. The *ACTR2*, *CLTC*, *COPA* and *TUBB4A* are common hub genes related to three diseases.

Conclusion: Our findings have demonstrated the association between the failure of intracellular transport and neurodegenerative diseases, consistent with the literature. Consequently, research focusing on the diagnostic and therapeutic aspects of the intracellular transport system may contribute to the early detection and the exploration of potential therapeutic strategies for the presented diseases.

Grants: There is no grant received.

Conflict of Interest: None declared

P16.132.D Evaluation of graph-based explainable AI in pathogenicity estimation using clinically relevant variants.

Laura Martinez Gomez[1], *Raul Valin*[1], *Serban Georgescu*[2], *Nooshin Bayat*[1], *Fuji Masaru*[3], *Shuya Abe*[3], *Shinichirou Tago*[3], *Ana Fernandez-Montes*[4], *Ceres Fernandez-Rozadilla*[5], *Jesus Garcia-Mata*[4], *Salvador Capella-Gutierrez*[6], *José María Fernández González*[6], *Anna Redondo Guitarte*[6], *Miguel Vazquez*[6]

[1]*Fujitsu Research of Europe, Computing Research Group, Madrid, Spain;* [2]*Fujitsu Research of Europe, Computing Research Group, Slough, United Kingdom;* [3]*Fujitsu Research, Fujitsu Ltd., Japan;* [4]*Complejo Hospitalario Universitario de Ourense – CHUO, Department of Medical Oncology, Ourense, Spain;* [5]*Instituto de Investigacion Sanitaria de Santiago, Cancer Predisposition and Biomarkers, Santiago de Compostela, Spain;* [6]*Barcelona Supercomputing Center (BSC), Life Sciences Department, Barcelona, Spain*

Background: Advancements in low-cost, high-throughput DNA sequencing and computing technologies have rapidly expanded the scope of genetic testing, leading to a substantial increase in the number of identified DNA variants per test. This inevitably culminates in the detection of variants that remain undefined and unclassified, referred to as variants of unknown significance, that comprise approximately 40% of all identified variants. We

developed a graph-based deep learning model that classifies the pathogenicity of germline variants using explainable artificial intelligence (XAI) which combines various pathogenicity prediction algorithms with data from multiple databases considering neighbouring variants and their potential clinical implications to identify potentially significant domains. Here we validate its performance using a set of clinically validated variants.

Methods: A clinically representative dataset of variants (n = 1757), including those identified from families in the Deciphering Developmental Disorders (DDD) study, that have been through multiple rounds of variant filtering and clinical evaluation was used to evaluate the performance of our algorithm. Integrating the dataset as a benchmarking event within the 'AMetaPred' community of ELIXIR's platform OpenEBench, we compared the performance of our tool with that of other available metapredictors, including REVEL, AlphaMissense and MetaRNN.

Results: The results show that the graph-based explainable AI consistently ranks within the top 5 across a comprehensive set of performance metrics, including precision, recall, accuracy and other additional relevant parameters.

Conclusions: The robust performance shown in this dataset underscores its efficacy and establishes it as a reliable choice in comparison to other algorithms relevant in the field.

Conflict of Interest: Laura Martinez Gomez Full-time employed by Fujitsu, Raul Valin Full-time employed by Fujitsu, Serban Georgescu Full-time employed by Fujitsu, Nooshin Bayat Full-time employed by Fujitsu, Fuji Masaru Full-time employed by Fujitsu, Shuya Abe Full-time employed by Fujitsu, Shinichirou Tago Full-time employed by Fujitsu, Ana Fernandez-Montes Fujitsu Research consultant, Ceres Fernandez-Rozadilla Fujitsu Research consultant, Jesus Garcia-Mata Fujitsu Research consultant, Salvador Capella-Gutierrez: None declared, José María Fernández González: None declared, Anna Redondo Guitarte: None declared, Miguel Vazquez: None declared

P16.133.A MaLiPre - machine-learning-based estimation of lymphocyte infiltration in tumor tissues

Dennis Witt[1], *Axel Gschwind*[1], *Vincent Hammer*[1], *Andrea Forschner*[2], *Michael Bitzer*[3], *Irina Bonzheim*[4], *Janina Beha*[5], *Cristiana Roggia*[1], *Olaf Riess*[1], *Christopher Schroeder*[1], *Christian Müller-Kett*[6], *Stephan Ossowski*[1]

[1]*Universitätsklinikum Tübingen – Institut für Medizinische Genetik und angewandte Genomik, Tübingen, Germany;* [2]*Universitäts-Hautklinik Tübingen, Tübingen, Germany;* [3]*Innere Medizin I Tübingen - Gastroenterologie, Hepatologie, Infektionskrankheiten, Tübingen, Germany;* [4]*Universitätsklinikum Tübingen Institut für Pathologie, Tübingen, Germany;* [5]*Universitätsklinikum Tübingen - Zentrum für personalisierte Medizin, Tübingen, Germany;* [6]*IU International University of Applied Sciences - Campus Bad Honnef, Bad Honnef, Germany*

Background/Objectives: Next-generation sequencing forms the foundation of personalized cancer treatment decisions, as comprehensive genetic information allows for a better understanding of tumor processes. However, interpreting these OMICS data is complex, and the number of accessible samples is often too small to apply machine learning (ML) techniques successfully.

Methods: This explorative study used tumor RNA-seq data to assess if lymphocyte fractions in tumor samples could be efficiently predicted using gene expression data. Thousands of representative virtual tissues were generated from openly accessible single cell data, and several machine learning models were trained. Results were validated with pairs of histological slices and RNA-seq data taken from publicly available GDC-data and an unpublished in-house cohort of 130 Melanoma/CCC tumor samples. The ML prototypes were evaluated for their predictive accuracy in both regressive and classification contexts.

Results: Twenty-four prototypes were developed. Significant advantages were made in training by virtual tissues, achieving AUC of up ato 0.96 in classification contexts (low vs. high lymphocyte infiltration; deep learning model). In regression, the mean absolute error was as low as 0.02 (MSE: 0.002, RMSE: 0.045) with a corresponding R^2 of up to 0.449 for a generalized linear model.

Conclusion: The study demonstrates the potential of using ML methods to predict real-world lymphocyte fractions in several tumor entities from RNA-expression data. With a high AUC of up to 0.96 for significant lymphocyte infiltration, this approach can reliably differentiate patients susceptible to immunotherapy

Conflict of Interest: None declared

P16.134.B ExonViz – A web application to generate and visualize transcripts including variants

Redmar R. van den Berg[1], **Marlen C. Lauffer**[1], *Jeroen Laros*[1;2]

[1]*Leiden University Medical Center (LUMC), Department of Human Genetics, Leiden, Netherlands;* [2]*National Institute of Public Health and the Environment (RIVM), Department of Bioinformatics and Computational Services, Bilthoven, Netherlands*

Background/Objectives: Visualization of transcripts, including features like coding regions and mutational landscape, is crucial within the field of human genetics. Apart from diagnostics, these illustrations can also be used to assess potential genetic treatment options, in teaching settings, and for genetic counseling. For certain types of genetic treatments, like canonical exon-skipping, these illustrations should also include information about the reading frames of exons.

To date, no application is available that visualizes transcripts including reading frame information, so clinicians and researchers must resort to manually drawing transcripts with tools like Illustrator, Photoshop, BioRender, PowerPoint, etc.

Results: Here, we present ExonViz, a simple application to draw transcripts that include coding and non-coding regions. All exons are to scale and the reading frames of the exons are visible. Single or multiple variants can be illustrated along the transcript and are shown in the correct location. Further, we allow the user to generate their own transcripts to incorporate features like poison or cryptic exons. ExonViz can be accessed as a web application via https://exonviz.rnatherapy.nl or as a Python package from https://pypi.org/project/exonviz/.

Conclusion: ExonViz is the first publicly accessible application that allows the user to draw transcripts including additional features such as coding regions, exon reading frames, and the mutational landscape along the transcript. ExonViz can be used for illustrations within publications, assessment of treatment options, teaching purposes, or genetic counseling.

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Conflict of Interest: Redmar R. van den Berg: None declared, Marlen C. Lauffer DFG Walter-Benjamin Fellowship, Speaker honoraria for Recordati Rare Disease Foundation, Jeroen Laros: None declared

P16.135.C Extraction of symptoms and HPO codes from Spanish health records generated by GPT

Nooshin Bayat[1], *Nuria García-Santa*[1], *Ander Martinez*[1], *Raul Valin*[1], *Laura Martinez Gomez*[1], *Serban Georgescu*[2], *Ceres Fernandez-Rozadilla*[3], *Ana Fernandez-Montes*[4], *Jesus Garcia-Mata*[4]

[1]Fujitsu Research of Europe, Computing Research Group, Pozuelo De Alarcon (Madrid), Spain; [2]Fujitsu Research of Europe Ltd, Computing Research Group, Slough, United Kingdom; [3]Foundation Health Research Institute of Santiago de Compostela (IDIS), Cancer Predisposition and Biomarkers lab, Santiago de Compostela, Spain; [4]Complexo Hospitalario Universitario de Ourense - CHUO, Medical Oncology Department, Ourense, Spain

Background/Objectives: The human phenotype ontology (HPO) [1] is being widely used to unify the terms related to patient phenotypes and symptoms which can be further used for diagnosis, decision support, or variant interpretation, amongst others. So far, there have been some studies related to HPO coding of natural language texts in English. In this study we describe a machine learning method that recognizes the symptoms from Spanish clinical records and extracts the related HPO code.

Methods: GPT-3.5 Turbo 16k was used to simulate clinical records related to colon cancer in Spanish. The Named Entity Recognition (NER) method was used to automatically label the symptoms from texts [2]. This was followed by the Entity Linking (EL), using knowledge graph [3], to extract the relevant HPO term for each symptom. Manual annotation of each step was used to evaluate the results.

Results: The NER system used in this study recognized 256 (94%) symptoms from a total of 273 manually labelled terms. Of these, 168 (66%) were correctly linked to their HPO code, while 88 (34%) were incorrect or missing.

Conclusion: As far as we know, this is the first report of a machine learning-based automatic extraction of both symptoms and HPO codes from Spanish clinical records. The patient-described symptoms were recognized with high accuracy (94%). However, further improvements are needed to reach the higher accuracy for HPO linking (higher than 66%).

References:

[1] https://doi.org/10.1093/nar/gkaa1043

[2] https://zenodo.org/records/10103882

[3] https://ceur-ws.org/Vol-2696/paper_111.pdf

Conflict of Interest: Nooshin Bayat Fujitsu employee (full time), Nuria García-Santa Patent: EP3637431A1, Fujitsu employee (full time), Ander Martinez Fujitsu employee (full time), Raul Valin Fujitsu employee (full time), Laura Martinez Gomez Fujitsu employee (full time), Serban Georgescu Fujitsu employee (full time), Ceres Fernandez-Rozadilla Fujitsu consultant, Ana Fernandez-Montes Fujitsu consultant, Jesus Garcia-Mata Fujitsu consultant

P16.136.D Aberrant expression prediction across human tissues

Florian Hölzlwimmer[1], *Jonas Lindner*[1], *Nils Wagner*[1;2], *Francesco Paolo Casale*[3;4;5], *Julien Gagneur*[1;6;7]

[1]Computational Molecular Medicine, School of Computation, Information and Technology, Technical University Munich, Garching, Germany; [2]Munich School for Data Science (MUDS), Helmholtz Association, Oberschleißheim, Germany; [3]Institute of AI for Health, Helmholtz Munich, Neuherberg, Germany, Oberschleißheim, Germany; [4]Helmholtz Pioneer Campus, Helmholtz Munich, Oberschleißheim, Germany; [5]School of Computation, Information and Technology, Technical University Munich, Garching, Germany; [6]Institute of Computational Biology, Helmholtz Munich, Oberschleißheim, Germany; [7]Institute of Human Genetics, School of Medicine, Technical University of Munich, München, Germany

Background/Objectives: Despite the frequent implication of aberrant gene expression in diseases, algorithms predicting aberrantly expressed genes of an individual are lacking.

Methods: To address this need, we compiled an aberrant expression prediction benchmark covering 8.2 million rare variants from 633 individuals across 48 tissues. Using this benchmark, we developed AbExp, a specialized predictor combining in a tissue-specific fashion expression variability with variant effects on isoforms and on aberrant splicing.

Results: While not geared toward aberrant expression, the deleteriousness score CADD and the loss-of-function predictor LOFTEE showed mild predictive ability (1-1.5% average precision). Leveraging these and further variant annotations, AbExp yielded 10% average precision, significantly outperforming both CADD and LOFTEE. Integrating AbExp with expression measurements from clinically accessible tissues led to another two-fold improvement. Furthermore, we show on UK Biobank blood traits that performing rare variant association testing using the continuous and tissue-specific AbExp variant scores instead of LOFTEE variant burden increases gene discovery sensitivity and enables improved phenotype predictions.

Conclusion: The development of a DNA-sequence-based method for predicting aberrant gene expression in multiple tissues, which can also generalize to unseen variants, represents a significant advancement in the ability to identify and understand the genetic underpinnings of human traits and diseases.

Grants: GHGA (#1 - NFDI - DFG)

Conflict of Interest: None declared

P16.137.A Pathway Analysis in Cognitive Decline using Longitudinal Data with Respect to Common Variants

Elaheh Vojgani[1;2], *Kumar Parijat Tripathi*[3], *Luca Kleineidam*[4], *Alfredo Ramirez Zuniga*[3], *Michael Nothnagel*[1;2]

[1]University of Cologne, Cologne Center for Genomics (CCG), Köln, Germany; [2]Cologne University Hospital, Köln, Germany; [3]University of Cologne, Department of Psychiatry, Medical Faculty, Köln, Germany; [4]University Hospital Bonn, 3 Department of Neurodegenerative Diseases and Geriatric Psychiatry, Bonn, Germany

Background/Objectives: Biological pathways intricately govern cellular processes, and understanding their role in neurological disorders' cognitive decline is paramount. Alzheimer's disease (AD) is a multifaceted neurological disorder characterized by cognitive decline, often manifesting early in life. Investigating the underlying biological mechanisms triggering cognitive impairment before dementia onset is crucial.

Methods: In this study, we employ a pathway-driven statistical approach, considering common genetic variants across pathways, to generate pathway scores. These scores aim to assess their interaction with time in driving cognitive decline, utilizing longitudinal data with temporal cognitive scores. Our approach involves two key components: first, developing a pipeline for pathway score generation with simulation studies involving various degrees of variant effect sizes within a pathway as well as different proportions of protective variants within a pathway to evaluate its efficacy, and second, applying the scores in a linear mixed model to examine their association with cognitive decline using real data from available cohorts.

Results: Preliminary results indicate that with large proportions of deleterious variants in a pathway and a strong correlation between genetic effect size and CADD score of a variant, the linear

model is very well able to predict the phenotype, with coefficients of determination consistently exceeding 0.5.

Conclusion: The proposed model captures phenotypic variation in simulated data effectively through pathway scores. This approach promises to enhance the understanding of biological processes driving the neurological disorders. In future, we will implement an R-package pipeline to enable users to obtain CADD-based pathway scores.

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Conflict of Interest: None declared

P16.138.B Integrating genomics and transcriptomics for the identification of novel genetic bases of Inborn Errors of Immunity

Nerea Moreno-Ruiz[1;2], *Laura Batlle-Masó*[3;4], *Laith Moushib*[5;6], *Andrea Martin-Nalda*[4;7], *Romina Dieli-Crimi*[5;6], *Anna Esteve-Codina*[8], *Hafid Laayouni*[1;9], *Roger Colobran*[5;6;10], *Ferran Casals*[2;11;12]

[1]Institute for Evolutionary Biology (UPF-CSIC), Department of Medicine and Life Sciences, Universitat Pompeu Fabra, Barcelona, Spain; [2]Universitat de Barcelona, Departament de Genètica, Microbiologia i Estadística, Facultat de Biologia, Barcelona, Spain; [3]Universitat Pompeu Fabra, Barcelona, Spain; [4]Vall Hebron Research Institute (VHIR), Infection and Immunity in Pediatric Patient, Barcelona, Spain; [5]Vall Hebron University Hospital (VHUH), Immunology Division, Barcelona, Spain; [6]Vall Hebron Research Institute (VHIR), Translational Immunology, Barcelona, Spain; [7]Vall Hebron Univeristy Hospital (HUVH), Pediatric Infectious Diseases and Immunodeficiencies Unit, Barcelona, Spain; [8]Centre Nacional D'Anàlisi Genòmica (CNAG), Barcelona, Spain; [9]BarcelonaBeta Brain Research Center, Pasqual Maragall Foundation, Barcelona, Spain; [10]Vall Hebron Univeristy Hospital (HUVH), Clinical and Molecular Genetics Department, Barcelona, Spain; [11]Institut de Biomedicina de la Universitat de Barcelona (IBUB), Barcelona, Spain; [12]Centro de Investigaciones Biomédicas en Red de Enfermedades Raras (CIBERER), Instituto de Salud Carlos III (ISCIII), Madrid, Spain

Background/Objectives: Inborn errors of immunity (IEI) comprise a diverse group of severe disorders affecting the immune system. While the genetic foundations of IEI have been under study for decades, a significant percentage of patients remain undiagnosed following conventional genetic testing. In this project, we employed a combination of genome and transcriptome sequencing in 26 IEI patients with previous nonconclusive genetic testing and their families with the aim of establishing a molecular diagnosis.

Methods: Initially, we analyzed WGS data prioritizing variants with potentially high impact on the phenotype, this step included WGS for available relatives (26 patients plus 49 relatives). Next, RNA-seq data from peripheral blood was analyzed using the DROP pipeline (Yépez V. et al 2021) (27 patients plus 48 age and gender matched controls), yielding results for aberrant expression, aberrant splicing, and mono-allelic expression.

Results: The combination of both datasets led to the identification of 55 genes with aberrant expression, 516 genes with aberrant splicing, and 613 variants with mono-allelic expression. Among these, two particularly promising candidates are currently undergoing validation: a substantial reduction in CD40L expression in a young adult and a splicing alteration in STAT2 caused by two intronic variants.

Conclusion: We demonstrated that the integration of genomic and transcriptomic profiling has the potential to increase the diagnostic yield in complex cases of IEIs.

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Conflict of Interest: None declared

P16.139.C cfCNV: an optimized pipeline for detecting copy number variations for cell free DNA data

Fahimeh Palizban[1], *Sina Majidian*[1]

[1]University of Lausanne, Department of Computational Biology, Lausanne, Switzerland

Background/Objectives: Cell-free DNA (cfDNA) analysis has emerged as a promising approach for non-invasive detection of genomic alterations, particularly copy number variations (CNVs). CNVs are involved in many diseases, including cancer, developmental, and genetic disorders and identified utilizing short and long-read sequencing. However, no CNV caller has been developed for cfDNA data specifically, and the reliability and accuracy of existing CNV callers for cfDNA data is unknown. This study presents a benchmarking approach to evaluate the performance of different CNV callers on cfDNA short and long reads.

Methods: Benchmarking process assesses sensitivity, specificity, precision, and computational efficiency metrics. We evaluated the pipelines' robustness to varying sequencing depths, fragment sizes, and allelic imbalances, reflecting real-world challenges encountered in cfDNA analysis.

Results: Here, by reviewing around 30 tools we have selected five of the most related tools considering the low concentration of cfDNA data. Real and simulated cfDNA data were used to investigate the ability of different tools to identify CNVs in a diverse range of scenarios and the minimum coverage at which these tools can function was measured at approximately 5x depth of sequencing. In addition, the optimal window size for each read-depth-based method at different read coverages was obtained as 100Kb-1Mb showing more accurate results for short read sequencing.

Conclusion: This newly developed pipeline facilitates the broad applications of cfDNA CNV caller for clinical and research contexts, ultimately advancing the precision and reliability of cfDNA-based genomic analysis for potential diagnosis and monitoring of diseases. Availability: https://github.com/FPalizban/cfCNV.

Grants: This research was supported by Research Partnership Grants from ZHAW.

Conflict of Interest: Fahimeh Palizban This research was supported by Research Partnership Grants from ZHAW, Sina Majidian This research was supported by Research Partnership Grants from ZHAW

P16.140.D Consistency of the facial phenotype in Koolen-de Vries and Jansen-de Vries syndrome

Jolijn Verseput[1], *Nina Claessens*[2], *Michiel Vanneste*[2;3], *Guido de Jong*[4], *Leonie de Vries*[1], *Sanne de Vries*[1], *Thomas Maal*[4], *Peter Claes*[2;3], *Bert de Vries*[1]

[1]Radboud University Medical Center, Human Genetics Department, Nijmegen, Netherlands; [2]UZ Leuven, Medical Imaging Research Center, Leuven, Belgium; [3]KU Leuven, Human Genetics Department, Leuven, Belgium; [4]Radboud University Medical Center, 3D Lab Radboudumc, Nijmegen, Netherlands

Background/Objectives: Facial shape modelling techniques can assist clinicians in determining the presence of a facial gestalt for a specific neurodevelopmental syndrome, and which facial features are most distinguishable. We used three-dimensional facial shape analysis to evaluate consistency in facial features for two neurodevelopmental disorders, namely Koolen-de Vries syndrome

(Koolen, 2012; Zollino, 2012; OMIM #610443) and Jansen- de Vries syndrome (Jansen, 2017; OMIM #617450).

Methods: 3D scans were made from 16 individuals with KDVS and 9 individuals with JDVS. These images were compared to 3D scans of the face of age and sex matched healthy controls based on craniofacial growth curves (Matthews, 2021). First, facial variation compared to the controls was displayed in terms of direction and severity for each individual (Matthews, 2023). These new age- and sex-normalized features were used in a subsequent analysis to determine phenotypic consistency in terms of variation in the direction of these features. Results were compared to a database of twenty well-known syndromes.

Results: The first analysis showed limited phenotypic consistency in the full face for both syndromes. The experiment was repeated for eight separate facial regions which revealed more consistency in specific features compared to others, fitting with the existing knowledge on the gestalt.

Conclusion: We show that 3D dense surface scans allow for improved facial characterization of both Koolen-de Vries and Jansen-de Vries syndrome when divided into separate facial regions contributing to the existing knowledge of the facial gestalt.

Grants: This work was supported by the Koolen-De Vries and Jansen-de Vries syndrome foundations, and the FWO (G0D1923N)

Conflict of Interest: None declared

P16.141.A Extending DeepTrio for sensitive detection of complex de novo mutation patterns.

Fabian Brand[1], *Jannis Guski*[1], *Peter Krawitz*[1]

[1]*University Hospital Bonn, Institute for Genomics Statistics and Bioinformatics, Bonn, Germany*

Background/Objectives: De novo mutations (DNMs), and among them clustered DNMs within 20 base pairs of each other (cDNMs) are known to be a potential cause of genetic disorders. However, identifying DNM in whole genome sequencing (WGS) data is a process that often suffers from low specificity. We propose a deep learning framework for DNM and cDNM detection in WGS data based on Google's DeepTrio software for variant calling, which considers regions of 110bp up- and downstream from possible variants to take information from the surrounding region into account

Methods: We trained a model each for the DNM and cDNM detection tasks and tested it on data generated on the HiSeq and NovaSeq platforms. In total, the model was trained on 82 WGS trios generated on the NovaSeq and 16 on the HiSeq.

Results: For the DNM detection task, our model achieves a sensitivity of 95.7% and a precision of 89.6%. The extended model adds confidence information for cDNMs, in addition to standard variant classes and DNMs. While this causes a slight drop in DNM sensitivity (91.96%) and precision (90.5%), on HG002 cDNMs can be isolated from other variant classes in all cases (5 out of 5) with a precision of 76.9%.

Conclusion: Since the model emits confidence probabilities for each variant class, it is possible to fine-tune cutoff thresholds to allow users to select a desired trade-off between sensitivity and specificity. These results show that DeepTrio can be retrained to identify complex mutational signatures with only little modification effort.

Conflict of Interest: None declared

P16.142.B GenoPhenoCorr: Simplifying the characterization of genotype-phenotype correlations

Lauren Rekerle[1], *Daniel Danis*[1], *Peter N. Robinson*[1;2]

[1]*The Jackson Laboratory For Genomic Medicine, Farmington, United States;* [2]*Berlin Institute of Health, Berlin, Germany*

Background/Objectives: Understanding the intricate relationship between genotypes and phenotypes is a fundamental pursuit in genetics. Although many other areas of genetics research have been optimized through new computational technologies, genotype-phenotype correlations have remained tedious and time consuming for researchers. We present GenoPhenoCorr, a Python package that is designed to expedite the identification of genotype-phenotype correlations.

Methods: GenoPhenoCorr takes Global Alliance for Genomics and Health (GA4GH) phenopacket data as input. A typical use case investigates a cohort of individuals with pathogenic variants in a given gene and compares different classes of variants, different locations, or different variants. Each phenopacket represents a single patient with a list of recorded phenotypes and at least one variant. The software organizes the data into a table, showcasing all the recorded phenotypes, variants, affected transcripts, and the corresponding proteins along with the frequency of each within the cohort. Users can then run different comparisons based on the information in the table. Genotype phenotype correlations are then calculated by analyzing the counts of individuals with vs. without annotations to Human Phenotype Ontology (HPO) terms.

Results: We evaluated GenoPhenoCorr on ten cohorts of individuals with diseases including KBG Syndrome and Isolated Sulfite Oxidase Deficiency. We could replicate results of some published genotype phenotype correlation studies and will present new results on other cohorts.

Conclusion: GenoPhenoCorr enables interactive exploration of results, provides a library of functions for investigating correlations, and can be easily extended. By integrating novel algorithms, comprehensive data management, and interactive visualization, GenoPhenoCorr simplifies the identification of genotype phenotype correlations.

Grants: 5U24HG011449

Conflict of Interest: None declared

P16.143.C A novel and highly customisable pipeline for genome-wide detection of SNP-by-methylation interaction effects

Ellisif Nygaard[1], *Julia Romanowska*[1;2], *Astanand Jugessur*[1;2], *Miriam Gjerdevik*[2;3], *Øystein A. Haaland*[1]

[1]*University of Bergen, Department of Global Public Health and Primary Care, Bergen, Norway;* [2]*Norwegian Institute of Public Health, Centre for Fertility and Health, Oslo, Norway;* [3]*Western Norway University of Applied Sciences, Department of Computer Science, Electrical Engineering and Mathematical Sciences, Bergen, Norway*

Background/Objectives: Although many cohorts have both genotype and DNA methylation (DNAm) data, few tools are designed for genome-wide integrative analysis of such datasets. We aim to develop a robust and user-friendly pipeline to perform genome-wide scans for SNP-by-DNAm interaction effects.

Methods: We constructed a pipeline by substantially extending and refining our previously published candidate-SNP framework1 for integration of SNP and DNAm data. Our development process included devising and optimising the placement of omic-specific and cross-omics quality-control steps, and creating an updated approach for combining SNPs with aggregated DNAm data.

Results: Our pipeline transforms vast numbers of SNPs and DNAm probes into a manageable, analysis-ready structure. We define each contiguous genomic interval annotated with a specific

full-stack ChromHMM chromatin state as a 'state locus' (SL). DNAm probes are assigned to the SL they are located within, effectively grouping them into biologically meaningful categories. Each SL is then paired with SNPs according to genomic proximity, and based on within-SL aggregated DNAm levels, the individuals are divided into SL-specific strata. Finally, association analyses are performed for each SNP-SL pairing, testing for interaction effects across strata. The pipeline supports alternative quality-control preferences and user-supplied annotations.

Conclusion: Our pipeline introduces a novel genome-wide integration protocol for pairing SNPs with biologically meaningful groupings of DNAm probes, facilitating a genome-wide screening for SNP-by-DNAm interaction effects. This comprehensive toolkit is highly customisable in terms of user needs, research questions, and data characteristics.

References: 1. PMID32678018

Grants: Research Council of Norway (grant 262700); Biobank Norway 4 (grant 322672)

Conflict of Interest: None declared

P16.144.D Adapting fine-mapping for GWAS meta-analyses with missing information

Joonas Kartau[1;2], *Matti Pirinen*[1;2;3]

[1]*University of Helsinki, Department of Mathematics and Statistics, Helsinki, Finland;* [2]*University of Helsinki, Institute for Molecular Medicine Finland, Institute of Life Science, Helsinki, Finland;* [3]*University of Helsinki, Department of Public Health, Helsinki, Finland*

Background/Objectives: Fine-mapping is a statistical approach to identify causal variants from the summary data of a Genome-Wide Association Study (GWAS). To improve statistical power, data from multiple cohorts are routinely combined in a meta-analysis before fine-mapping, often leading to sample size mismatches due to variant exclusions in certain studies. Current methods like FINEMAP use computational approximations that ignore missing information, producing unreliable results. We have developed FINEMAP-MISS, an extension to the FINEMAP model that incorporates missing information.

A potential alternative to FINEMAP-MISS is GWAS summary statistics imputation (SSI). While SSI works well in some scenarios, we show that under realistic conditions SSI can lead to unreliable fine-mapping results.

Methods: We compare the performance of SSI with FINEMAP-MISS in different scenarios. Using dosage data from the UK Biobank we repeatedly generated two sets of GWAS summary statistics with one causal variant and set 50% of variants missing in one set. We vary causal variant effect size, sample size, or the selection of missing variants (systematic or random) and measure fine-mapping quality.

Results: We show that FINEMAP-MISS is well calibrated, but SSI results in an increase in posterior inclusion probabilities (PIPs) for non-causal variants. For example, with a sample size of 20,000 both methods perform similarly, but when increasing sample size to 200,000 SSI assigned at least one non-causal variant a PIP > 0.9 in 23% of simulations, while FINEMAP-MISS assigned none.

Conclusion: We recommend using FINEMAP-MISS when performing fine-mapping on meta-analysis data to enable fine-mapping on datasets of greater scale and power.

Grants: Academy of Finland

Conflict of Interest: None declared

P16.145.A VarFish annotation capabilities extended for large-scale whole genome sequencing

Lara Einicke[1;2], *Max Xiaohang Zhao*[1;2], *Oliver Stolpe*[3], *Angela Abad Perez*[1], *Ronja Adam*[1;2], *Felix Boschann*[1;2;4], *Magdalena Danyel*[1;4], *Nadja Ehmke*[1;2], *Martin Atta Mensah*[1;4], *Sarina Schwartzmann*[1], *Henrike Sczakiel*[2;4;5], *Dieter Beule*[3], *Manuel Holtgrewe*[3]

[1]*Charité – Universitätsmedizin Berlin, Institute of Medical Genetics and Human Genetics, Berlin, Germany;* [2]*Berlin Institute of Health at Charité – Universitätsmedizin Berlin, Berlin, Germany;* [3]*Berlin Institute of Health at Charité – Universitätsmedizin Berlin, Core Unit Bioinformatics – CUBI, Berlin, Germany;* [4]*Berlin Institute of Health at Charité - Universitätsmedizin Berlin, BIH Biomedical Innovation Academy, Clinician Scientist Program, Berlin, Germany;* [5]*Max Planck Institute for Molecular Genetics, FG Development & Disease, Berlin, Germany*

Background/Objective: The interpretation of genetic variants from whole genome sequencing (WGS) requires the integration of many data sources. This includes population frequencies, variant pathogenicity predictions and per-gene intolerance scores, literature reference, and functional assay data. Fast and user-friendly presentation of this information is essential for enabling effective analysis of WGS variant data.

Methods: We have integrated many new and updated data sources into our existing web-based variant interpretation platform VarFish. Based on the key-value store RocksDB and a microservice-based architecture, the software supports users in the analysis of sequence and structural variants from short- and long-read sequencing data.

Results: We present an updated, user-friendly interface for VarFish. A large number of additional data sources and prediction scores have been integrated, including Genotype-Tissue Expression project (GTEx), dbNSFP and AlphaMissense. Additionally, support for multiple genome assemblies have been implemented. We also present a long-term approach to tracking and validating usability changes.

Conclusion: Through major architectural changes and the use of modern database software, we have been able to greatly enhance the annotation capabilities in VarFish.

Grants: none

Conflict of Interest: None declared

P17
LARGE SCALE ASSOCIATION STUDIES (GWAS)

P17.001.B A functional schizophrenia-associated genetic variant near the *TSNARE1* and *ADGRB1* genes.

Rachel Boyd[1], *Marah Wahbeh*[1], *Christian Yovo*[1], *Bailey Rike*[1], *Andrew McCallion*[1], *Dimitrios Avramopoulos*[1]

[1]*The Johns Hopkins University School of Medicine, Genetic Medicine, Baltimore, United States*

Background/Objectives: Large-scale genome wide association studies (GWAS) have identified >200 independent loci contributing to schizophrenia risk. Overlap with epigenetic marks suggest that most GWAS-implicated variants are regulatory; however, there is no direct evidence that individual variants are functional or regulate genes involved in disease-relevant pathways. Based on the strength of association with schizophrenia and the presence of regulatory epigenetic marks, we chose a strongly associated variant (rs4129585) near *TSNARE1*, tested its functional potential, and assayed differences that may drive risk allele pathogenicity.

Methods: To test rs4129585 and the surrounding sequence for regulatory function, we utilized a transgenic zebrafish reporter assay. Next, we introduced the risk and non-risk alleles into human-derived iPSCs, differentiated isogenic clones into neural precursor cells (NPCs), and performed RNA-sequencing.

Results: We observed that rs4129585 and the surrounding sequence drives reporter expression in schizophrenia-relevant neuronal populations in zebrafish, including regions involved in auditory processing, anxiety/fear, and aggression. In NPCs, the two alleles generated significant transcriptional differences in the expression of 109 genes at FDR <0.05 and 259 genes at FDR <0.1. We show that these genes are highly interconnected in pathways enriched for synaptic proteins, axon guidance, and regulation of synapse assembly.

Conclusion: These results suggest that rs4129585 does not regulate *TSNARE1* transcripts in NPCs, as previously thought, but may regulate the neighbouring *ADGRB1*, a regulator of synaptogenesis. Our results suggest that rs4129585 is a functional, common variant that functions in specific pathways involved in schizophrenia risk.

Grants: Canadian Institutes of Health Research (CIHR-DFD-181599). National Institutes of Health (R01MH113215, R21MH122936, R01HG010480, R21NS128604, T32GM007814-40).

Conflict of Interest: None declared

P17.002.C Genome-wide association study reveals autoimmune basis of idiopathic achalasia

Sandeep Grover[1], *Carlo Maj*[1], *Pouria Dasmeh*[1], *Johannes Schumacher*[1]

[1]*Center for Human Genetics, Universitatsklinikum Giessen und Marburg - Standort Marburg, Marburg, Germany*

Consortium: Achalasia Risk Consortium (ARC)

Background/Objectives: Achalasia is a rare disorder of the digestive tract characterized by the failure of the lower esophageal sphincter to relax resulting in massive dilation and irreversible impairment of the esophagus. It results from the degeneration of neurons in the myenteric plexus; however, the exact cause of this degeneration is unknown. Previous targeted gene association studies have implicated several *HLA* alleles, suggesting an autoimmune etiology.

Methods: We undertook a comprehensive meta-analysis of genome-wide association studies across eight datasets spanning ten countries, encompassing a cohort of 4,602 cases and 10,766 controls of European ancestry.

Results: Analyzing approximately 11 million SNPs, we identified 28 independent genome-wide significant risk SNPs (lead SNPs) across six genomic loci, five of which were previously unidentified (*PTPN22*, *UGT1A8*, *TNFSF8*, *ZNF365*, *CASC20*). Notably a heritability estimate of 12.1% (SE = 3.6%) was observed. The most significant risk factor identified was the previously reported eight-amino-acid residue insertion within the HLA-DQβ1, encoded by the rs28688207 C allele (OR = 2.54, 95% CI = 2.36-2.86, p = 2.33E-55). Pleiotropic analysis highlighted the involvement of *TNFSF8* in the proliferation of CD4 + T-cells and *PTPN22* in predisposition to various autoimmune diseases. Furthermore, we identified 72 potential causal genes in non-HLA regions, notably enriched in the immune system-related genes, particularly those implicated in autoimmunity. Phenome-wide correlation analysis showed the strongest correlation of achalasia with Crohn's disease, an autoimmune disorder (rg = 0.309 (SE = 0.093), p = 9.85E-04).

Conclusion: These results strongly suggest that achalasia has an autoimmune origin, providing clear directions for further scientific experiments to understand its mechanisms.

Conflict of Interest: None declared

P17.003.D Interactions between maternal genomics and human milk fatty acids are associated with milk microbiota and childhood atopy

Zhi Yi Fang[1], *Sara Stickley*[1], *Joel Sagman*[1;2], *Kelsey Fehr*[3;4;5], *Shirin Moossavi*[3;4;5], *Kozeta Miliku*[6], *Catherine Field*[7], *Piushkumar Mandhane*[8], *Elinor Simons*[5;9], *Theo Moraes*[10], *Malcolm Sears*[11], *Stuart Turvey*[12], *Padmaja Subbarao*[10;13;14], *Meghan Azad*[3;4;5], *Qingling Duan*[1;2]

[1]*Queen's University, Dept. of Biomedical and Molecular Sciences, Kingston, Canada;* [2]*Queen's University, School of Computing, Kingston, Canada;* [3]*University of Manitoba, Dept. of Pediatrics and Child Health, Winnipeg, Canada;* [4]*University of Manitoba, Dept. of Food and Human Nutritional Sciences, Winnipeg, Canada;* [5]*Children's Hospital Research Institute of Manitoba, Manitoba Interdisciplinary Lactation Centre (MILC), Winnipeg, Canada;* [6]*University of Toronto, Dept. of Nutritional Sciences, Toronto, Canada;* [7]*University of Alberta, Department of Agricultural, Food and Nutritional Science, Edmonton, Canada;* [8]*University of Alberta, Dept. of Pediatrics, Edmonton, Canada;* [9]*University of Manitoba, Section of Allergy and Immunology, Winnipeg, Canada;* [10]*The Hospital for Sick Children, Dept. of Pediatrics, Toronto, Canada;* [11]*McMaster University, Division of Respirology, Dept. of Medicine, Hamilton, Canada;* [12]*University of British Columbia, Dept. of Pediatrics, British Columbia Children's Hospital, Vancouver, Canada;* [13]*McMaster University, Dept. of Medicine, Hamilton, Canada;* [14]*University of Toronto, Dalla Lana School of Public Health, Toronto, Canada*

Background/Objectives: Breastfeeding and human milk have many health benefits in infants. Earlier work by our team identified that maternal genomics and human milk components such as fatty acids (HMFAs) were associated with human milk microbiota (HMM). In this study, we determined if interactions between maternal genomics and HMFAs impact HMM and risk of childhood atopy among human milk-fed children.

Methods: HMM and HMFAs were analyzed using 16S rRNA-sequencing and gas-liquid chromatography, respectively, in breastmilk of 726 mothers from the CHILD Cohort Study. Maternal and infant genomics were obtained using the Illumina HumanCoreExome BeadChip. Linear regression analysis determined main and interaction effects of HMM and infant polygenic risk scores (PRSs) on childhood atopy (assessed by skin prick tests at ages 3-5 years). We also identified HMFAs that interact with maternal genomics to modulate HMM.

Results: Among infants with high PRSs, those exposed to elevated *Acinetobacter* in human milk had lower atopy prevalence ($P_{Bonferroni} = 0.05$). *Acinetobacter* abundance, in turn, were associated with interactions between maternal polymorphisms on chromosome X (e.g., rs3747309, P = 2.1e-8) and docosapentaenoic acid concentrations. Moreover, increased alpha-diversity in human milk was associated with reduced atopy prevalence as well as interactions between maternal loci on chromosome 18 and increased hexadecenoic acid (e.g., rs57924617, P = 6.81e-11).

Conclusion: Our findings suggest that interactions between maternal genomics and HMFAs may influence the microbial community in human milk, which may modulate risk of atopy. Understanding gene-by-exposure interaction may guide early-life prevention strategies for childhood atopy.

Grants: Canadian Institutes of Health Research (MRT-168044, PJT-178390, FBD-181414).

Conflict of Interest: None declared

P17.004.A Landscape of effects of gene expression on immune-mediated diseases.

Sodbo Sharapov[1], *Arianna Landini*[1], *Nicole Soranzo*[1;2;3], *Nicola Pirastu*[1]

[1]*Human Technopole, Milan, Italy;* [2]*Wellcome Sanger Institute, Human Genetics Department, Hinxton, United Kingdom;* [3]*University*

of Cambridge, British Heart Foundation Centre of Research Excellence, Cambridge, United Kingdom

Background/Objectives: Most genome-wide association signals are suggested to act through gene expression. However, despite the extensive expression catalogs it has been difficult to attribute disease loci to these eQTLs. Here we aimed at exploring the contribution of eQTLs to disease in terms of number, primary vs secondary hit, and effect size distribution.

Methods: To test for pleiotropy between eQTLGen cis-eQTLs and immune-mediated disease's loci, we used leave-one-out conditional analysis coupled with Bayesian colocalization. We applied Mendelian randomisation to estimate the effect of gene expression on disease.

Results: Of the 139 significant disease loci overlapping with at least one eQTL we were able to colocalise 51 (36,7%). We compared if the colocalization could be attributed to the top hit or to secondary conditionally independent ones, the latter were 3.61x more likely to be responsible. Despite eQTLs of large effect have been described, when colocalized with a disease, the effect was generally below 0.4 standard deviations of gene expression, suggesting that they may be constrained due to their effect on health. Finally, we have identified several loci where eQTLs for multiple genes simultaneously colocalised with the disease locus, suggesting a common local regulation of their expression.

Conclusion: We show that disease loci are most likely driven by secondary association signals which can be detected when properly accounted for. The distribution of eQTLs puts an upper boundary to the disease relevant effect sizes that will be useful to plan future, larger eQTL studies.

Grants:

Conflict of Interest: None declared

P17.005.B A loci in *LINC01708* is associated with left ventricular outflow tract obstruction

Martin Broberg[1;2], *FinnGen Consortium*[3], *Emmi Helle*[1]

[1]*University of Helsinki, Stem Cells and Metabolism Research Program, Helsinki, Finland;* [2]*FIMM, FIMM, Helsinki, Finland;* [3]*FinnGen, FinnGen, Helsinki, Finland*

Consortium: FinnGen

Background/Objectives: Left ventricular outflow tract obstruction (LVOTO) are structural congenital heart defects (CHD) affecting the left side of the heart and the aorta. LVOTO defects are associated with aortic insufficiency (AI) and aortic stenosis (AS). However, AS and AI are also found in older individuals (>50 years of age) without CHDs. Our aim was to elucidate whether there are similar genetic determinants for congenital LVOTO defects and late onset AS/AI.

Methods: We performed a genome wide association study (GWAS) in FinnGen release 12 for broad LVOTO, including individuals diagnosed with congenital LVOTO defects (ICD-10 codes Q23-25) and early onset AS/AI (ICD-10 codes I35 in patients <50 years of age) cases = 2656. A separate GWASs were done for patients with congenital LVOTO defects (cases = 1734), early onset AS/AI (cases = 922), and late onset AS/AI (those diagnosed with an I35 code at >50 years of age, cases = 18,106). The number of controls was 475,604.

Results: We discovered a genome-wide significant loci associated with broad LVOTO group in rs35046143 (Chr1, *LINC01708*, beta = 0.21, stderr = 0.027, $p = 2.1 \times 10^{-14}$). This signal persisted in the congenital LVOTO cohort (beta = 0.21, stderr = 0.034, $p = 4.4 \times 10^{-10}$). A nearby signal in rs11166276 (Chr1, *LINC01708*, beta = 0.26, stderr = 0.025, $p = 7.6 \times 10^{-25}$) was significant in the late onset AS/AI cohort.

Conclusion: Our results suggest that congenital LVOTO defects share partially similar genetic risk loci with late onset aortic valve disease (AS/AI) not associated with CHD.

Grants:

Conflict of Interest: None declared

P17.006.C Genome-wide association analyses identify candidate genes for residual disease in ovarian cancer

Dhanya Ramachandran[1], *jonathan tyrer*[2], *Stefan Kommoss*[3], *Anna DeFazio*[4], *Marjorie Riggan*[5], *Peter Schürmann*[1], *Lisa-Marie Speith*[1], *Tjoung-Won Park-Simon*[1], *Peter Hillemanns*[1], *Jacobus Pfisterer*[6], *Andrew Berchuk*[5], *Sharon Johnatty*[7], *Penelope Webb*[7], *Susan Ramus*[8], *Georgia Chenevix-Trench*[7], *paul pharoah*[9], *Thilo Dörk*[1], *Florian Heitz*[10;11]

[1]*Hannover Medical School, Gynaecology Research Unit, Hannover, Germany;* [2]*Centre for Cancer Genetic Epidemiology, Department of Oncology, Cambridge, United Kingdom;* [3]*Universitäts-Frauenklinik, Tübingen, Germany;* [4]*The Westmead Institute for Medical Research, Westmead, Australia;* [5]*Duke University, Durham, United States;* [6]*Gynecologic Oncology Center, Kiel, Germany;* [7]*QIMR Berghofer Medical Research Institute, Herston, Australia;* [8]*UNSW Sydney, Sydney, Australia;* [9]*Cedars-Sinai Medical Center, Department of Computational Biomedicine, Los Angeles, United States;* [10]*KEM | Evang. Kliniken Essen-Mitte gGmbH, Department of Gynecology, Essen, Germany;* [11]*Charité – Universitätsmedizin Berlin, Department of Gynecology, Berlin, Germany*

Consortium: The Ovarian Cancer Association Consortium (OCAC), Arbeitsgemeinschaft Gynäkologische Onkologie (AGO) Study Group

Background/Objectives: Survival from ovarian cancer strongly depends on the resection status after primary surgery. We aimed to identify genomic factors that may influence resection status.

Methods: We performed case-only GWAS analyses in the Ovarian Cancer Association Consortium to identify genetic variants associated with residual disease (complete resection RD = 0 cm versus any residual disease RD>0 cm). Logistic regression analyses were performed for overall (n = 7705) or restricted to invasive (n = 7099) or high-grade serous (n = 4954) ovarian carcinomas, and also with adjustment for FIGO stage (n = 7111, 6540, 4564, respectively). The most strongly associated variants were subsequently tested for their effect on methylation and mRNA expression in an independent set of 378 ovarian tumours from the German AGO-OVAR 11 study.

Results: A genome-wide significant association with resection status was observed for rs72845444, a variant upstream of *MGMT*, in HGSOC (MAF = 0.02; OR = 0.75, 95%CI = 0.57-0.97, $p = 3.9 \times 10$-8). In gene-based analyses, *PPP2R5C* was the most strongly associated gene in HGSOC after stage adjustment ($p = 4.8 \times 10$-6). *MGMT* encodes a DNA repair enzyme, and *PPP2R5C* encodes the B56γ subunit of the PP2A tumour suppressor. In ovarian tumours from the AGO-OVAR 11 study, variants near *MGMT* and *PPP2R5C* correlated with methylation and transcript levels, and *PPP2R5C* mRNA levels predicted progression-free survival in patients with residual disease.

Conclusion: Our results suggest that heritable variation at the *MGMT* and *PPP2R5C* loci impact resection status and progression-free survival in high-grade serous ovarian cancer.

Grants: Supported by the DFG (Do761/15-1). The OCAC was funded through the ORCF, NIH, FP7-HEALTH, and diverse grants at single consortium centers.

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Peter Schürmann: None declared, Lisa-Marie Speith: None declared, Tjoung-Won Park-Simon: None declared, Peter Hillemanns: None declared, Jacobus Pfisterer Jacobus Pfisterer received honoraria from Roche Pharma AG, AstraZeneca, Amgen, Clovis Oncology, MSD Oncology, GSK, Chugai Pharma, Teva, Medupdate, SAI MedPartners, Decision Resources, Simon-Kucher and partners, Juniper, Bionest partners, Vox Bio, Axiom healthcare strategies, Prosapient, iMed Institut, Lilly., Jacobus Pfisterer is a consultant/advisor for AstraZeneca, Roche, Pharma AG, Tesaro, Clovis Oncology, MSD Oncology., Andrew Berchuk: None declared, Sharon Johnatty: None declared, Penelope Webb: None declared, Susan Ramus: None declared, Georgia Chenevix-Trench: None declared, paul pharoah: None declared, Thilo Dörk: None declared, Florian Heitz: None declared

P17.007.D A cross-population genome-wide association study of sleep duration

Isabelle Austin-Zimmerman[1], *daniel levey*[2;3], *Joseph Deak*[2;3], *marco galimberti*[2;3], *Keyrun Adhikari*[2;3], *murray stein*[4;5], *Marta Di Forti*[1;6], *joel gelernter*[2;3]

[1]*King's College London, Social, Genetic, and Developmental Psychiatry Centre, IoPPN, London;* [2]*Yale University School of Medicine, Division of Human Genetics, Department of Psychiatry, New Haven, United States;* [3]*VA Connecticut Healthcare System, Department of Psychiatry, West Haven, United States;* [4]*University of California San Diego, Departments of Psychiatry and Herbert Wertheim School of Public Health, La Jolla, United States;* [5]*VA San Diego Healthcare System, Psychiatry Service, San Diego, United States;* [6]*South London and Maudsley NHS Foundation Trust, United Kingdom*

Consortium: The VA Million Veteran Program

Background/Objectives: Differences in sleep duration, quality, and timing are associated with variation in health outcomes. Genetic studies may help explain the underlying mechanisms of sleep and its relationships to other conditions.

Methods: Using data from the UK Biobank and the Million Veteran Program we have conducted a cross-population genome wide association study of sleep duration (0-23hrs). We investigate the genetic correlation between sleep duration other traits, and use genomic structural equation modelling to consider causal relationships.

Results: We identify 363 (260 novel) independent loci for sleep duration. Genetic correlation analysis shows sleep duration is largely distinct from other measures of sleep or sleep disorders. We see several examples of negative corelation between a given trait and sleep duration, but positive associations to long (>9hrs) and short sleep (<6hrs) (e.g., depression, ADHD, cannabis use disorder, smoking). This supports clinical observations of a non-linear relationship between sleep duration and various other traits.

A sensitivity analysis of sleep duration restricted to 6-9hrs is >97% genetically correlated to the full sleep duration measure, demonstrating that a GWAS on sleep duration largely captures variation within the healthy range. This has implications for future work investigating sleep genetics.

Conclusion: This is the largest available GWAS of sleep duration, and the first to extend analyses outside of European ancestry populations. We identify novel loci for sleep duration and provide insight to the shared and unique genetic architecture across multiple sleep and neuropsychiatric traits.

Grants: Department of Veterans Affairs Office of Research and Development, USVA, grant I01CX001849; VA Cooperative Studies Program study, no. 575B.

Conflict of Interest: None declared

P17.008.A Genetic architecture of fat and lean mass across body compartments in men and women

Alice Williamson[1;2], *Laura Wittemans*[2;3;4]

[1]*Precision Healthcare University Research Institute (PHURI), Queen Mary University of London, London, United Kingdom;* [2]*MRC Epidemiology Unit, Institute of Metabolic Science, University of Cambridge, Cambridge, United Kingdom;* [3]*Big Data Institute at the Li Ka Shing Centre for Health Information and Discovery, University of Oxford, Oxford, United Kingdom;* [4]*Nuffield Department of Women's and Reproductive Health, Medical Sciences Division, University of Oxford, Oxford, United Kingdom*

Consortium: The Body Composition Genomics Consortium

Background: Genetic analyses of overall adiposity and fat distribution have primarily focused on simple measures of body size (e.g., body mass index; BMI) or fat distribution (e.g., BMI-adjusted waist-hip ratio; WHR), important contributors to cardiometabolic disease risk. However, these simple measures do not explain the full spectrum of fat distribution across all body compartments nor consider lean mass. Dual X-ray absorptiometry scans (DEXA) provide greater insight into regional body composition.

Methods: We conducted fixed-effect genome-wide association study (GWAS) meta-analysis for 31 DEXA-derived regional fat and lean mass traits, including 74,554 individuals of European ancestry across 7 studies. Sex-stratified GWAS analyses allowed identification of sex-dimorphic loci. Cross-ancestry comparison included an additional 4,701 individuals of Qatari ancestry.

Results: We identified 679 independent signals ($P < 5 \times 10^{-8}$) after cross-trait fine mapping. Of these, 384 were specific to fat mass compartments, with limited overlap with signals for commonly used measures such as BMI (8%) or BMI adjusted WHR (48%), despite their much larger sample sizes. In Qatari-only analyses, 372 of these loci also had a significant signal ($P < 5 \times 10^{-5}$). We identified substantial differences in genetic effects between sexes on large body compartments, specifically peripheral fat distribution in the legs. Deep molecular and clinical phenotyping identified subsets of loci with distinct metabolic effects on human health.

Conclusion: This is the first large-scale study to identify the complex genetic architecture and sex differences of both fat and lean mass. This study demonstrates the value in going beyond simple indices to investigate the mechanisms influencing body composition.

Grants:

Conflict of Interest: Alice Williamson: None declared, Laura Wittemans Laura BL Wittemans is now an employee of Novo Nordisk.

P17.009.B Genome-wide aggregated *trans*-effects analysis on the risk of polymyalgia rheumatica and giant cell arteritis in UK Biobank

Amarachukwu Nwagbata[1], *Andrii Iakovliev*[1;2], *ann w morgan*[3], *Paul McKeigue*[1], *Athina Spiliopoulou*[1;2]

[1]*Usher institute, University of Edinburgh, Edinburgh, United Kingdom;* [2]*Institute for Genetics and Cancer, University of Edinburgh, Edinburgh, United Kingdom;* [3]*Leeds Institute of Cardiovascular and Metabolic Medicine and NIHR Leeds Biomedical Research Centre, University of Leeds, Leeds, United Kingdom*

Background/Objectives: Polymyalgia rheumatica (PMR) and giant cell arteritis (GCA) are frequently overlapping age-associated

systemic inflammatory diseases. We investigated the genetic architecture of PMR/GCA by integrating genotype, gene expression and proteins to identify "core" disease genes.

Methods: Using summary statistics for eQTLs (eQTLGen) and pQTLs (deCODE, UKB-PPP), we performed genome-wide aggregation of *trans*-effects (GATE) to compute genotypic scores for *trans*-regulated gene expression or protein level in 5673 PMR/GCA cases and 458530 controls in UK-Biobank. Core genes were identified by testing association between PMR/GCA and GATE scores, with subsequent validation using measured protein levels in 668 PMR/GCA cases and 50626 controls in UK-Biobank.

Results: We detected seven putative core genes at $P < 10^{-05}$: *ANKRD55* and *LEF1* from the eQTL-GATE analysis, and *CXCL9*, *PLG*, *IL13RA1*, *NRSN1*, *ASCC1* from the pQTL-GATE analysis. *ANKRD55* and *PLG* are GWAS hits for PMR/GCA. *ANKRD55* was additionally validated by a *cis*-score association. Protein-level measurement was available for *CXCL9*, *IL13RA1* and *PLG*. All three proteins were associated with PMR/GCA at $P < 10^{-03}$.

Conclusion: Using GATE analysis, we detected core genes for PMR/GCA pointing to relevant pathways: four genes are involved in cytokine/chemokine signalling pathways (*CXCL9, ANKRD55, ASCC1, IL13RA1*), and one (*PLG*) in blood coagulation. The *CXCL9-CXCR3* chemokine axis has been previously associated with recruitment and organisation of B-cells in the vessel wall in GCA. *PLG* encodes for plasminogen, which when activated degrades many blood plasma proteins, including fibrin-containing blood clots. The detected core genes present opportunities as drug targets or for drug repurposing once clinically validated.

Grants: AMS Springboard [SBF006/1109]

Conflict of Interest: None declared

P17.010.C Investigating evidence for interactions between *FLG* and other genes contributing to atopic dermatitis

Ashley Budu-Aggrey[1;2], Katie Watts[1;2], Hansjoerg Baurecht[3], Ben Brumpton[4;5;6], Laurent Thomas[4;6;7;8], Herman den Dekker[9], Luba Pardo Cortes[10], John Curtin[11], Mariona Bustamante[12;13;14], Klaus Bonnelykke[15], Marie Standl[16;17], Carol Wang[18;19], Bjarke Feenstra[20], Suzanne Pasmans[10], Tamar Nijsten[10], Liesbeth Duijts[21;22], Franz Rueschendorf[23], Stephan Weidinger[24], David P Strachan[25], Mari Løset[4;26], Kristian Hveem[4;5], Sara Judith Brown[27], **Lavinia Paternoster**[1;2]

[1]*Medical Research Council Integrative Epidemiology Unit, Bristol Medical School, University of Bristol, Bristol, United Kingdom;* [2]*Population Health Sciences, Bristol Medical School, University of Bristol, Bristol, United Kingdom;* [3]*Department of Epidemiology and Preventive Medicine, University of Regensburg, Regensburg, Germany;* [4]*K.G. Jebsen Center for Genetic Epidemiology, Department of Public Health and Nursing, NTNU, Norwegian University of Science and Technology, Trondheim, Norway;* [5]*HUNT Research Centre, Department of Public Health and Nursing, NTNU, Norwegian University of Science and Technology, Levanger, Norway;* [6]*Clinic of Medicine, St. Olavs Hospital, Trondheim University Hospital, Trondheim, Norway;* [7]*Department of Clinical and Molecular Medicine, NTNU, Norwegian University of Science and Technology, Trondheim, Norway;* [8]*BioCore - Bioinformatics Core Facility, NTNU, Norwegian University of Science and Technology, Trondheim, Norway;* [9]*The Generation R Study Group and department of Pediatrics, Erasmus MC, University Medical Center Rotterdam, Rotterdam, Netherlands;* [10]*Department of Dermatology, Erasmus MC, University Medical Center Rotterdam, Rotterdam, Netherlands;* [11]*Division of Immunology, Immunity to Infection and Respiratory Medicine, School of Biological Sciences, The University of Manchester, Manchester Academic Health Science Centre, and Manchester University NHS Foundation Trust, Manchester, United Kingdom;* [12]*ISGlobal, Institute for Global Health, Barcelona, Spain;* [13]*Universitat Pompeu Fabra (UPF), Barcelona, Spain;* [14]*CIBER Epidemiología y Salud Pública, Madrid, Spain;* [15]*COPSAC, Copenhagen Prospective Studies on Asthma in Childhood, Herlev and Gentofte Hospital, University of Copenhagen, Copenhagen, Denmark;* [16]*Institute of Epidemiology, Helmholtz Zentrum München, German Research Center for Environmental Health, Neuherberg, Germany;* [17]*German Center for Lung Research (DZL), Munich, Germany;* [18]*School of Medicine and Public Health, University of Newcastle, Newcastle, Australia;* [19]*Hunter Medical Research Institute, Newcastle, Australia;* [20]*Department of Epidemiology Research, Statens Serum Institut, Copenhagen, Denmark;* [21]*Department of Pediatrics, division of Respiratory Medicine and Allergology, Erasmus MC, University Medical Center Rotterdam, Rotterdam, Netherlands;* [22]*Department of Pediatrics, division of Neonatology, Erasmus MC, University Medical Center Rotterdam, Rotterdam, Netherlands;* [23]*Max Delbrück Center for Molecular Medicine in the Helmholtz Association, Berlin, Germany;* [24]*Department of Dermatology, Allergology and Venereology, University Hospital Schleswig-Holstein, Kiel, Germany;* [25]*Population Health Research Institute, St George's, University of London, London, United Kingdom;* [26]*Department of Dermatology, Clinic of Orthopaedy, Rheumatology and Dermatology, St. Olavs Hospital, Trondheim University Hospital, Trondheim, Norway;* [27]*Centre for Genomic and Experimental Medicine, Institute for Genetics and Cancer, University of Edinburgh, Edinburgh, United Kingdom*

Consortium: the EAGLE Eczema Consortium

Background/Objectives: Atopic dermatitis (AD) is a common inflammatory disorder. Filaggrin (*FLG*) loss-of-function mutations are the strongest genetic risk factor for AD, and are involved in skin barrier dysfunction. However, there is evidence for residual genetic modifier effects on AD risk. We explored gene-gene interactions with *FLG* on a genome-wide scale.

Methods: A genome-wide association (GWAS) meta-analysis was performed for 395,508 European individuals across 16 cohorts. For each cohort, GWAS was performed after stratifying individuals according to their *FLG* genotype (determined by the presence of any one of the most prevalent null mutations: R501X, 2282del4, R2447X, S3247X). Fixed effects meta-analysis was performed in individuals with *FLG* null mutations: 3,360 cases; 33,366 controls, the '*FLG* positive stratum', and those without *FLG* null mutations: 19,759 cases; 339,023 controls, the '*FLG* negative stratum'. An interaction Z-test was used to investigate differences in SNP associations between each stratum.

Results: The strongest signal for differential association was found for rs74445319 mapping close to *UBE2E3* ($P = 9.01 \times 10^{-7}$), which was more strongly associated with AD in the *FLG* positive stratum ($P = 3.42 \times$ 10-6; OR(95% CI) = 0.72(0.63-0.83)) compared to the negative stratum ($P = 0.09$; OR(95% CI) = 1.06(0.99-1.14)). Among 91 variants previously reported for AD risk, none showed strong evidence of interacting with *FLG* after correcting for multiple testing ($P < 5.49 \times 10^{-4}$).

Conclusion: We found limited evidence of *FLG*UBE2E3* interactions explaining AD risk. However stronger signals may be uncovered with the addition of larger sample sizes.

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Wellcome Trust Senior Research Fellowship to SJB (220875/Z/20/Z).

Conflict of Interest: None declared

P17.011.D Genetic Determinants and Polygenic Score Assessment of Insulin Resistance

Usama Aliyu[1], *Umm-Kulthum Ismail Umlai*[1], *Salman Toor*[1], *Asma Anwar Fathi Elashi*[1], *Yasser Al-Sarraj*[2], *Abdul-Badi Abou-Samra*[3], *Karsten Suhre*[4,5], *Omar Albagha*[1]

[1]Hamad Bin Khalifa University (HBKU), Doha, Qatar; [2]Qatar Genome Program (QGP), Doha, Qatar; [3]Hamad Medical Corporation, Doha, Qatar; [4]Weill Cornell Medicine, New York, United States; [5]Weill Cornell Medicine - Qatar, Doha, Qatar

Background: Insulin resistance (IR) and beta cell dysfunction (BCF) are the major drivers of type 2 diabetes (T2D). Various common and rare genetic variants have been associated with IR and T2D in European populations. However, the genetic architecture of IR in Middle Eastern (ME) populations remains unexplored.

Methods: We conducted a comprehensive Genome Wide Association Study (GWAS) on the indices of IR (HOMA2-IR) and BCF (HOMA2-%B) in 6,217 non-diabetic individuals from the Qatar Biobank (QBB; Discovery cohort; n = 2170, Replication cohort; n = 4047) with and without body mass index (BMI) adjustment. We also developed polygenic scores (PGS) for HOMA2-IR and compared their predictive performance with European-derived PGS (PGS000877).

Results: We replicated 11 HOMA-IR-associated and 24 HOMA-%B-associated loci at nominal statistical significance. We also identified a novel locus associated with BCF near the *VEGFC* gene, tagged by rs61552983 ($P = 4.38 \times 10^{-8}$). Our top PGS performed similar to PGS000877 in predicting HOMA2-IR (adjusted R^2; $AdjR^2 = 0.229 \pm 0.014$; $P = 5.4 \times 10^{-4}$ versus $AdjR^2 = 0.231 \pm 0.014$; $P = 5.0 \times 10^{-7}$) and IR (area under the reciever operating characteristic curve; AUC = 0.552 versus AUC = 0.553).

Conclusion: This is the first GWAS on HOMA2 and the first GWAS focusing on IR and BCF in the ME. We reported a novel locus in *VEGFC* implicated in IR and BCF, highlighting the importance of population genetics on deciphering the genetics of complex humain traits.

Funding: Qatar National Research Fund (QNRF; Grant no. NPRP11C-0115-180010).

Conflict of Interest: None declared

P17.012.A Exploring the genetic landscape of pregnancy-related phenotypes and complications through low-pass whole genome sequencing in over 100,000 Chinese pregnancies

Huanhuan Zhu[1], *linxuan li*[1], **Xinyi Zhang**[1], *Xin Jin*[1]

[1]BGI Research, Shenzhen, China

Background: Over the last decade, significant advancements in Genome-wide Association Studies (GWAS) have been made. A primary challenge is the underrepresentation of non-European populations and limited availability of pregnancy phenotypic studies. Non-Invasive Prenatal Testing (NIPT) for fetal trisomies, utilizing maternal plasma cell-free DNA sequencing, has emerged as a globally adopted molecular test with over 10 million participants. Our previous research demonstrated the potential of NIPT data for human genetic studies.

Methods: We broadened this approach to encompass a range of maternal and neonatal phenotypes and common pregnancy complications. We conducted a genetic analysis of 104 pregnancy-related phenotypes from over 100,000 Chinese women, marking this as the largest such genetic study in the Asian population.

Results: Our GWAS revealed 410 genome-wide trait-locus associations, with 71.71% previously reported. Among the 116 novel hits, 83 were reproduced from independent replication studies. We discovered a significant association of ESR1 with fasting glucose, hemoglobin, hematocrit, and leukocyte counts. Considering the dramatic fluctuations in estrogen levels during pregnancy, we propose these ESR1- associations may be pregnancy-specific and merit further exploration. We investigated the genetic background of Gestational Diabetes Mellitus (GDM) and Intrahepatic Cholestasis of Pregnancy (ICP). For GDM, we replicated two known genes, CDKAL1 and MTNR1B. For ICP, we discovered a novel association SLC39A9. We established a website for offering all the GWAS results.

Conclusion: Our findings underscore the power of leveraging NIPT data to gain genetic insights into pregnancy-related phenotypes, accelerating future mechanistic studies into complex pregnancy traits and diseases.

Grants: PMID: 30290141, Cell 2018.

Conflict of Interest: None declared

P17.013.B Short tandem repeats and breast cancer risk

Joe Dennis[1], *Kyriaki Michailidou*[2], *Georgia Chenevix-Trench*[3], *Thilo Dörk*[4], *Douglas F. Easton*[1]

[1]Cambridge University, Centre for Cancer Genetic Epidemiology, Cambridge, United Kingdom; [2]The Cyprus Institute of Neurology & Genetics, Biostatistics Unit, Nicosia, Cyprus; [3]QIMR Berghofer Medical Research Institute, Herston, Australia; [4]Hannover Medical School, Gynaecology Research Unit, Hannover, Germany

Background/Objectives: Common genetic variants in approximately 150 regions are associated with breast cancer risk. These were identified through genotyping and imputation to reference panels of SNPs and indels but not short tandem repeats (STRs). We imputed STR genotypes from a panel phased onto SNP haplotypes for 133,000 breast cancer cases and 108,000 controls of European ancestry from the Breast Cancer Association Consortium (BCAC).

Methods: STRs at 446,000 loci were imputed using Beagle v5.4. We tested for associations using logistic regression, considering STRs in the model as: (a) a continuous covariate based on allele length, (b) each allele against all other alleles, (c) all alleles in a single model. We adjusted for country and ten principal components and ran conditional analyses adjusting for previously identified lead SNPs/indels.

Results: In the analysis by STR length, we identified significant associations ($P < 5 \times 10E\text{-}8$) at 1,164 STR loci. 1,083 of these were located in 106 of the previously established regions. Following conditional analyses, 122 STRs in 27 regions were associated at $P < 0.001$. 51 of the 161 strong SNP/Indel signals tested were no longer significant after conditional analysis, indicating that STRs may be driving these signals. Outside the known regions, novel associations driven by rare STR alleles were observed at seven loci.

Conclusion: A large proportion of established breast cancer associated variants are correlated with STRs, some of which may be causally associated with risk. Novel associations were observed for a small number of STR loci outside the previously known regions.

Grants: NIHR Cambridge Biomedical Research Centre (NIHR203312)

Conflict of Interest: None declared

P17.014.C Defining the genetic basis of asthma requiring high treatment burden and loss of control

Noemi Nicole Piga[1], *Michael Portelli*[2], *Sharon Lutz*[3,4], *Ann Chen Wu*[3], *Ian M. Adcock*[5], *Glenda Lassi*[6], *Ian Sayers*[2], *Katherine Fawcett*[1]

[1]Genetic Epidemiology Group, Department of Population Health Sciences, University of Leicester - Leicester (United Kingdom); [2]Centre for Respiratory Research, NIHR Respiratory Biomedical Research

Centre, School of Medicine, Biodiscovery Institute, University of Nottingham - Nottingham (United Kingdom); [3]PRecisiOn Medicine Translational Research (PROMoTeR) Center, Department of Population Medicine, Harvard Medical School and Harvard Pilgrim Health Care Institute, Boston, MA, USA; [4]Department of Biostatistics, Harvard T.H. Chan School of Public Health, MA, USA; [5]National Heart & Lung Institute, Imperial College London – London (United Kingdom); [6]Translational Science and Experimental Medicine, Research and Early Development, Respiratory & Immunology, R&D BioPharmaceuticals, AstraZeneca - Cambridge (United Kingdom)

Background/Objectives: Approximately 10% of asthma patients remain uncontrolled despite maximal treatment and represent an unmet clinical need. We performed a genome-wide association study (GWAS) with moderate-severe asthma population to identify genetic risk factors.

Methods: UK Biobank participants with asthma were identified based on questionnaire data and electronic health records. Cases were further defined as having either: primary care prescriptions within stages 4-5 of British Thoracic Society guidelines, hospitalisation, or asthma-associated deaths. Controls were sex-matched, free from asthma, asthma-relevant prescriptions, and lung diseases. A GWAS was performed with ~9.8 million high-quality common variants, followed by a sensitivity analysis excluding allergic conditions. A meta-analysis of three independent studies was carried out for replication. Validated sentinel variants and their genomic loci (+/-1Mb) were fine-mapped to generate 95% credible sets, and linked to genes using variant/gene-annotation and colocalisation with molecular quantitative trait loci.

Results: We identified 7,681 cases (mean age of 57 years and 64% female) and 38,405 controls of European ancestry-like. The GWAS revealed 67 signals (p-value <= $5 \times 10\text{-}6$). Sensitivity analysis showed comparable results and replication in similarly defined subjects (11,862 cases and 30,377 controls) validated 21 signals all previously described. Fine-mapping revealed 615 putative causal variants in 16 different genomic loci. Variant-to-gene mapping analysis highlighted 90+ genes, including eight genes with at least three evidences of association (*BACH2*, *D2HGDH*, *IL4R*, *IL1RL1*, *NSMCE1*, *RORA*, *RPS26*, *SMAD3*).

Conclusion: Our GWAS identified 21 reproducible genetic signals associated with moderate-severe asthma. We generated hypotheses about which variants and genes are involved as a basis for future functional work.

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Conflict of Interest: Noemi Nicole Piga: None declared, Michael Portelli: None declared, Sharon Lutz: None declared, Ann Chen Wu: None declared, Ian M. Adcock: None declared, Glenda Lassi Glenda Lassi is a full time employee at AstraZeneca UK, Ian Sayers: None declared, Katherine Fawcett: None declared

P17.015.D Impact of control selection strategies on GWAS study of Schizophrenia and Bipolar disorder in UK Biobank

Jingzhan Lu[1], *Johan Thygesen*[2], *HARRY GREEN*[1]

[1]*University of Exeter, Medical School, Clinical and Biomedical Sciences, Exeter, United Kingdom;* [2]*University College London, Institute of Health Informatics, London, United Kingdom*

Background/Objectives: An essential part of a GWAS study is determining which control samples to use. Control selection is further complicated in extensive population datasets with longitudinal health records, as some choices must be made as to who classifies as healthy controls. The impact of these choices remains largely unexplored. This investigation provides insights into control selection strategies for GWAS.

Methods: We assessed the impact of three control selection strategies on GWAS in schizophrenia(SCZ) and bipolar disorder(BD) considering covariates: age, gender and deprivation. These were S1:random selection without covariates, S2:case/control matched analysis with covariates and S3:random selection with covariates. The study included 1,988 SCZ and BD cases of European ancestry from the UK Biobank, with a 1:4 case-to-control ratio. Individual GWAS were conducted under each strategy using regenie. Results were compared with the Psychiatric Genomics Consortium(PGC 2022), the largest GWAS meta-analysis(PGC: mean β/p-value difference −0.002/0.434) to date, which was considered the gold standard.

Results: The GWAS results showed that S2(matched) had smaller deviation from the PGC results(mean β/p-value difference −0.003/0.386) than S1(mean β/p-value difference 0.001/0.383) and S3(mean β/p-value difference −0.010/0.378). These results show that while including covariates improved concordance with the gold standard GWAS, case-control matching on covariates yielded optimal performance.

Conclusion: The strongest matching strategy ensures a more balanced control group selection in GWAS studies, as it maintains a close similarity in covariate levels between case and control groups. To ensure generalisability of these results, future studies will test the effectiveness of these three strategies on alternative cohorts and phenotypes.

Grants: None

Conflict of Interest: None declared

P17.016.A Genome-wide association study of hemoglobin regulation and anemia during pregnancy in 96,558 Chinese pregnant women

Xinxin Guo[1], *Siyang Liu*[1]

[1]*Sun Yat-sen University, School of Public Health (Shenzhen)*

Background/Objectives: Gestational anemia is a global concern affecting about one-third of pregnant women, linked to various adverse birth outcomes. Despite its significance, our understanding of the genetic basis underlying gestational anemia remains limited. Our objective is to identify key genetic factors associated with gestational anemia and investigate whether there is a causal relationship between gestational anemia and adverse birth outcomes, as well as long-term health status.

Methods: We utilized data from 96,558 Chinese pregnant women and conducted separate Genome-Wide Association Studies (GWAS) in two distinct hospital cohorts and subsequently a fixed-effect meta-analysis encompassing five biomarkers (total hemoglobin (N = 96,558), hemoglobin A1 (N = 42,118), hemoglobin A2 (N = 42,128), fetal hemoglobin (N = 42,118), and ferritin(N = 53,000)) and two types of anemia (anemia (N = 96,546) and iron deficiency anemia (N = 32,578)). Pleiotropy and pathway enrichment analysis were conducted to interpret the genetic variants. Finally, two-sample Mendelian randomization studies between gestational anemia traits and 7 adverse birth outcomes, as well as 183 long-term health outcomes were conducted.

Results: We identified 175 signals at 89 loci, including 60 novel loci. We demonstrated that ESR1 may be a crucial regulator of hemoglobin during pregnancy by influencing the hypoxic response process mediated by EPAS1. Finally, we suggested the negative effect of gestational anemia on stillbirth and ovarian cancer.

Conclusion: This study deepens our understanding of the genetic basis of gestational anemia, offering insights into potential treatment strategies.

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Conflict of Interest: None declared

P17.017.B Identification of novel variants associated with LDL-C levels and evaluation of polygenic scores: a multi-ancestry Genome Wide Association Study

Umm-Kulthum Ismail Umlai[1], Salman Toor[1], Yasser Al-Sarraj[2], Shaban Mohammed[3], Moza Al-Hail[3], Ayman El-Menyar[3], Mohammed Gomaa[3], Amin Jayyousi[3], Nadeem Qureshi[4], Jassim AlSuwaidi[3], **Omar Albagha**[1]

[1]Hamad Bin Khalifa University (HBKU), Ar Rayyan, Qatar; [2]Qatar Foundation, Ar-Rayyan, Qatar; [3]Hamad Medical Corporation, Doha, Qatar; [4]University of Nottingham, United Kingdom

Background/Objectives: The genetic determinants of low-density lipoprotein cholesterol (LDL-C) levels in blood have been predominantly explored in European populations and remain poorly understood in Middle Eastern populations. We investigated the genetic architecture of LDL-C variation in Qatar.

Methods: Whole genome sequencing data of 13,701 individuals (discovery; n = 5,939, replication; n = 7,762) from the population-based Qatar Biobank (QBB) cohort was used to conduct a genome-wide association study (GWAS) on serum LDL-C levels. We also performed a multi-ancestry meta-analysis with the largest LDL-C GWAS conducted by the Global Lipids Genetics Consortium (GLGC). Lastly, we developed Qatari-specific polygenic score (PGS) panels and tested their performance against PGS derived from other ancestries.

Results: We replicated 165 previously reported loci that showed high correlation in allele frequency ($R^2 = 0.77$) and effect size ($R^2 = 0.53$) with the GLGC study (Graham *et. al*., Nature 2021). The multi-ancestry meta-analysis identified 2 novel LDL-C-associated loci; rs10939663 (*SLC2A9;* $P = 1.05 \times 10^{-8}$) and rs12783213 (*INPP5A;* $P = 3.17 \times 10^{-9}$). Moreover, the multi-ancestry derived PGS (PGS000889) performed best at predicting LDL-C levels, while the Qatari-derived PGS panels also showed comparable performance.

Conclusion: We report novel genetic variants associated with LDL-C levels, which may be explored further to decipher their potential roles in the etiopathogenesis of cardiovascular diseases. Moreover, our findings also highlight the importance of including underrepresented populations in GWAS to improve genetic insights into various traits/diseases.

Grants: This study was supported by a grant from the Qatar National Research Fund (QNRF; Grant No. PPM 03-0324-190038).

Conflict of Interest: None declared

P17.018.C Variants across the allelic spectrum make important contributions to cystic kidney disease

Omid Sadeghi-Alavijeh[1], *Melanie Chan[1], Gabriel Doctor[1], Catalin Voinescu[1], Alex Stuckey[2], Alexander Ho[2], Horia Stanescu[1], Detlef Bockenhauer[1], Adam Levine[1], Daniel Gale[1]*

[1]University College London, Renal Medicine, United Kingdom; [2]Genomics England, United Kingdom

Consortium: Genomics England Research Consortium

Background/Objectives: Cystic kidney disease (CyKD) gene discovery has been led by family/candidate gene studies, limiting the ascertainment of additional genome-wide variants involved. Using whole genome sequencing data from the 100,000 Genomes Project (100KGP), we sought to systematically characterize CyKD across allele types and frequency.

Methods: We performed a genome-wide association analysis involving 1,209 unrelated CyKD patients and 26,096 ancestry-matched unaffected controls. We examined all types of variants across the allelic frequency, both genome-wide and per gene, using a generalized linear mixed model. Heritability and meta-analysis were conducted, including cohorts in the Finnish, UK, and Japanese biobanks.

Results: 995/1209 (82.30%) cases had an explaining monogenic variant. Gene-based analysis of rare damaging variants revealed *PKD1* ($P = 1.13 \times 10^{-309}$), *PKD2* ($P = 1.96 \times 10^{-150}$), *DNAJB11* ($P = 3.52 \times 10^{-7}$), *COL4A3* ($P = 1.26 \times 10^{-6}$) and truncating mono-allelic *PKHD1* ($P = 2.98 \times 10^{-8}$) variants to be significantly associated with CyKD. Removing "solved" cases revealed an association at *IFT140* ($P = 3.46 \times 10^{-17}$) and strengthened the *COL4A3* ($P = 9.27 \times 10^{-7}$) signal.

Genome-wide structural variant associations included deletions in *PKD1* ($P = 2.17 \times 10^{-22}$), *PKD2* ($P = 7.48 \times 10^{-12}$) and the 17q12 locus containing *HNF1–* ($P = 4.12 \times 10^{-8}$).

Meta-analysis of 2,923 cases and 900,824 controls across 6,641,351 common variants did not reveal significant associations. CyKD common variant heritability was 3-9% across three European cohorts.

Conclusion: This represents the first whole genome examination of a national CyKD cohort using robust statistical methodology. We confirm the recently described *IFT140* association, highlight CyKD as a presenting feature of collagen IV-related nephropathy and expand the spectrum of abnormalities associated with *PKHD1*. Common/structural variants play an under-recognized and important role in CyKD heritability. Further work is needed to understand the underlying biological mechanisms.

Grants:

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P17.019.D genome-wide association studies of thyroid-related hormones, dysfunction, and autoimmunity among 85,421 Chinese pregnancies

Yuandan Wei[1], *yuqin Gu[1], Yanhong Liu[1], Xinxin Guo[1], Siyang Liu[1]*

[1]Sun Yat-Sen University

Background/Objectives: To identify the missing genetic components related to thyroid function traits and thyroid autoimmunity, and to gain a deeper understanding of the genetic architecture of thyroid function in pregnant women and its impact on birth outcomes.

Methods: Utilizing hospital electronic medical records and non-invasive prenatal testing data from 85,421 Chinese pregnancies, we conducted the most comprehensive GWAS studies of thyroid traits during pregnancy. The comprehensive thyroid-related traits include thyroid-stimulating hormone (TSH)/ free thyroxine (FT4) levels, 4 thyroid dysfunctions, and 2 thyroid autoimmunity phenotypes related to thyroperoxidase antibodies. We explored the associations between 8 maternal thyroid traits and 6 fetal birth outcomes (birth gestational days, preterm birth, birth weight, birth length, low birth weight, and Macrosomia) through a combination of observational analysis of cohort data and Mendelian randomization (MR).

Results: We identified 141 genome-wide loci, of these 98 genome-wide associations that have not been previously reported for the 8 thyroid-related traits. 13 loci that contained more than one independent signal suggests allelic heterogeneity at the thyroid-associated loci. In MR analysis, we discovered a significant potential causal effect of elevated TSH levels during pregnancy on greater birth gestational days after Bonferroni correction (β95% CI = 0.07[0.05-0.09], P = 5.54E-4).

Conclusion: This study greatly improved our understanding of the genetic basis of thyroid traits during pregnancy. And MR studies offer evidence that maternal TSH levels during pregnancy may be a potential causal factor for gestational duration.

Grants: The study was supported by the Shenzhen Basic Research Foundation (20220818100717002), and the National Natural Science Foundation of China (31900487).

Conflict of Interest: None declared

P17.020.A Determining the genetic correlation of glioma and five primary extracranial cancers which metastatise to the central nervous system

Zak Thornton[1], *jamie robinson*[1], *kathreena kurian*[1], *Lavinia Paternoster*[1]

[1]*University of Bristol, MRC Integrative Epidemiology Unit, Bristol, United Kingdom*

Background/Objectives: There are five extra-cranial cancers which are commonly metastasise to the central nervous system (CNS); breast, colorectal, kidney, lung, and skin melanoma. The aim of this study is to investigate whether there is an increased genetic correlation between these cancer types and glioma, compared to other cancers that don't typically metastasise to the CNS.

Methods: We used IEU OpenGWAS, NHGRI-EBI GWAS catalogue and dbGaP to retrieve GWAS summary statistics from 20 different cancer types. We used Bivariate linkage disequilibrium score regression (LDSC) to estimate global cross-trait genetic correlation (r_g) between various cancer types and glioma to determine if CNS metastasising cancers are more highly correlated with glioma compared to other cancer types. Additionally, we will use Local Analysis of Variant Annotation (LAVA) to estimate genetic correlation within smaller genomic loci, to highlight genomic regions of shared genetic aetiology between traits.

Results: Using LDSC, we found that five cancer types; breast, lung, non-small cell lung cancer (NSCLC), skin melanoma and squamous cell carcinoma had some evidence for global genetic correlation with glioma ($p < 0.05$). Skin melanoma had the highest genetic correlation with glioma ($r_g = 0.3175$, $p = 0.0013$). We found that NSCLC was significantly correlated in the non-glioblastoma caseload ($p = 0.0233$) but not in the glioblastoma caseload. We will also show results from our regional correlation analysis.

Conclusion: We have found three of the five CNS metastasising cancers of interest had positive global genetic correlation with glioma. These analyses shed light on the shared genetic aetiology between some cancers, which may help to identify common causes and drug repurposing opportunities.

Grants:

Conflict of Interest: Zak Thornton: None declared, jamie robinson JWR now works for Boehringer-Ingelheim full-time, kathreena kurian: None declared, Lavinia Paternoster LP has undertaken research supported by Takeda Pharmaceuticals and is part of a Innovative Medicines Initiative-European funded consortia (BIOMAP-IMI.eu) with multiple industry partners

P17.021.B Evaluating sex differences in the ultra-rare variant contribution to autism and their effects on liability.

Mahmoud Koko[1], *Varun Warrier*[2], *Hilary Martin*[1]

[1]*Wellcome Sanger Institute, Human Genetics, Hinxton, United Kingdom;* [2]*Autism Research Centre, University of Cambridge, Department of Psychiatry, Cambridge, United Kingdom*

Consortium: APEX Consortium

Background/Objectives: We examined the burden of de novo mutations (DNM) and ultra-rare inherited variants in 26,908 autistic children from the SPARK study, asking whether they contribute to the sex bias in autism prevalence.

Methods: DNM rates were compared between autistic children (10,482 males and 2,991 females) and their sex-matched siblings (3,305 males and 3,458 females). Over-transmission from 40,356 parents to 20,893 autistic males and 6,015 females was measured using the Transmitted/Untransmitted allele ratio. Rate differences were converted to the liability scale.

Results: Deleterious protein truncating and missense variants increased autism liability without significant sex differences, both when inherited (Z ~ 0.01 - 0.08) or occurring de novo (Z ~ 0.25 - 0.5). DNM variant liability in individuals with cognitive or motor impairment was two-fold higher than in individuals with autism only. The liability conferred by deleterious variants in established autism genes did not reveal significant sex differences in either phenotypic group. Genes that are more highly expressed in male than female fetal brain tissues were enriched for deleterious variants in the motor/cognitive impairment group, also without a significant sex bias. A Bayesian TADA meta-analysis of SPARK trios and published summary statistics (27,092 autistic individuals) identified 84 genes associated with autism (FDR < 0.1%) - an increase from 72 published, which increased further when SPARK was stratified to individuals with/out motor/cognitive impairment.

Conclusion: While they confer higher liability to comorbid motor and cognitive impairment, damaging rare variants are unlikely to mediate sex-differential risk.

Grants: SFARI Grant RNAG/669 G10 9280

Conflict of Interest: None declared

P17.023.D Genetic risk for psychiatric disorders is associated with measurable differences in brain volume and cerebrospinal fluid volume at birth

Harriet Cullen[1;2], *Konstantina Dimitrakopoulou*[3], *Hamel Patel*[4], *Charles Curtis*[4], *Joseph Hajnal*[1], *A David Edwards*[1]

[1]*King's College London, Perinatal Imaging & Health Department, London, United Kingdom;* [2]*King's College London, Department of Medical and Molecular Genetics, London, United Kingdom;* [3]*Guy's and St Thomas' NHS Foundation Trust and King's College London, Translational Bioinformatics Platform, NIHR Biomedical Research Centre, London, United Kingdom;* [4]*King's College London, NIHR Maudsley Biomedical Research Centre, London, United Kingdom*

Background/Objectives: Case-control studies, predominantly in adults and adolescents, have consistently identified differences in brain morphology in individuals affected with psychiatric disorders. The genetic architecture of psychiatric disorders is polygenic and many of the genes identified through genome-wide association studies show elevated expression during foetal and early neonatal life. Here we focus on this early timepoint and

ask if polygenic risk for psychiatric disorders is associated with measurable differences in the infant brain at birth.

Methods: Our term-born European subsample comprises 267 infants with MRI volumetric and genetic data. Genotype data were used to compute polygenic risk scores (PRS) for five psychiatric disorders (ADHD, ASD, Major Depressive Disorder, Bipolar Disorder and Schizophrenia) and a Cross-Disorder risk (eight psychiatric disorders combined). Estimates of the total brain volume (TBV), cerebrospinal fluid (CSF) volume, white and grey matter volumes, ventricular volume, and volumes of five subcortical structures were extracted from T2-weighted MRI images at term-equivalent age. The relationship between infant brain volume and psychiatric PRS was explored using linear regression models with appropriate covariates.

Results: Greater genetic risk for ADHD ($p = 1.12 \times 10^{-3}$, $\beta = 0.13 \pm 0.04$) and Cross-disorder ($p = 1.44 \times 10^{-3}$, $\beta = 0.13 \pm 0.04$) were associated with larger CSF volume. Greater genetic risk for Cross-disorder was also associated with smaller white matter volume ($p = 1.06 \times 10^{-3}$, $\beta = -0.07 \pm 0.02$) and smaller total brain volume ($p = 3.22 \times 10^{-3}$, $\beta = -0.11 \pm 0.04$).

Conclusion: This work identified several statistically significant associations between genetic susceptibility for psychiatric disorders and both brain and CSF volumes at birth. It adds to the literature addressing the largely unexplored role of common genetic variation in shaping the developing brain.

Conflict of Interest: None declared

P17.024.A Beyond Volume: Unraveling the Genetics of Human Brain Geometry

Sabrina Primus[1;2], *Felix Hoffstaedter*[3;4], *Federico Raimondo*[3;4], *Simon Eickhoff*[3;4], *Juliane Winkelmann*[2;5;6], *Konrad Oexle*[1;2;5], *Kaustubh Patil*[3;4]

[1]*Neurogenetic Systems Analysis Group, Institute of Neurogenomics, Neuherberg, Germany;* [2]*Institute of Neurogenomics, Helmholtz Munich, Neuherberg, Germany;* [3]*Institute of Neuroscience and Medicine, Brain and Behaviour (INM-7), Research Centre Jülich, Jülich, Germany;* [4]*Institute of Systems Neuroscience, Medical Faculty, Heinrich Heine University, Düsseldorf, Germany;* [5]*Institute of Human Genetics, School of Medicine, Technical University Munich, München, Germany;* [6]*Munich Cluster for Systems Neurology (SyNergy), München, Germany*

Background/Objectives: Laplace-Beltrami (LB) eigenmodes of brain structures allow for reconstructing brain activity, indicating a close relation between brain geometry and function[1]. Further, the spectrum (set of eigenvalues) of the associated LB operator can be regarded as an encoding of form ("Shape-DNA")[2]. The LB spectra of subcortical brain structures have been shown to have considerable heritability[3].

1.Pang, J. C. *et al*. Geometric constraints on human brain function. *Nature* **618**, 566–574 (2023).

2.Reuter, M., Wolter, F.-E. & Peinecke, N. Laplace–Beltrami spectra as 'Shape-DNA' of surfaces and solids. *Computer-Aided Design* **38**, 342–366 (2006).

3.Ge, T. *et al*. Multidimensional heritability analysis of neuroanatomical shape. *Nat Commun* **7**, 13291 (2016).

Methods: We examined the genetics of subcortical brain structure shapes in 19,862 healthy White-British UK Biobank participants by multivariate GWAS (MOSTest) on their LB spectra while controlling for global form descriptors. Moreover, a Canonical Correlation Analysis (CCA) was conducted to investigate the relationship between shape and polygenic risk scores of brain-related diseases and traits.

Results: We identified several independent variants being associated with the shapes of one or various brain structures at a genome-wide significance level. The CCA revealed significant correlations between brain structure shapes and polygenic risk scores of several traits.

Conclusion: We delineate the genetics of brain structure shapes beyond global attributes.

Grants: Helmholtz Imaging (HIP) – NImRLS Project

Conflict of Interest: Sabrina Primus HIP - NImRLS Project, Felix Hoffstaedter HIP - NImRLS Project, Federico Raimondo HIP - NImRLS Project, Simon Eickhoff HIP - NImRLS Project, Juliane Winkelmann HIP - NImRLS Project, Konrad Oexle HIP - NImRLS Project, Kaustubh Patil HIP - NImRLS Project

P17.025.B Host genomics and interactions with environmental exposures are associated with the early-life gut microbiota and atopic diseases in the CHILD Cohort Study

Sara Stickley[1], *Zhi Yi Fang*[1], *Amirthagowri Ambalavanan*[1], *Yang Zhang*[2], *Charisse Petersen*[3;4], *Darlene Dai*[3;4], *Bianca Robertson*[5], *Chloe Yonemitsu*[5], *Lars Bode*[5], *Piushkumar Mandhane*[6], *Elinor Simons*[7], *Jeffrey Brook*[8], *Theo Moraes*[9], *Malcolm Sears*[10], *Meghan Azad*[7;11], *Stuart Turvey*[3;4], *Padmaja Subbarao*[9], *Qingling Duan*[1;2]

[1]*Queen's University, Department of Biomedical and Molecular Sciences, Kingston, Canada;* [2]*Queen's University, School of Computing, Kingston, Canada;* [3]*University of British Columbia, Division of Allergy and Immunology, Department of Pediatrics, Vancouver, Canada;* [4]*Child and Family Research Institute and British Columbia Children's Hospital, Department of Pediatrics, Vancouver, Canada;* [5]*University of California San Diego, Department of Pediatrics and Larsson Rosenquist Foundation Mother Milk Infant Center of Research Excellence (MOMI CORE), La Jolla, United States;* [6]*University of Alberta, Department of Pediatrics, Edmonton, Canada;* [7]*University of Manitoba, Section of Allergy and Immunology, Department of Pediatrics and Child Health, Winnipeg, Canada;* [8]*University of Toronto, Occupational & Environmental Health Division, Dalla Lana School of Public Health, Toronto, Canada;* [9]*Hospital for Sick Children and University of Toronto, Department of Pediatrics, Toronto, Canada;* [10]*McMaster University, Department of Medicine, Hamilton, Canada;* [11]*Children's Hospital Research Institute of Manitoba, Manitoba Interdisciplinary Lactation Centre (MILC), Winnipeg, Canada*

Background/Objectives: Environmental exposures such as diet and antibiotics are known to influence the gut microbiota. In comparison, less is known about the role of host genomics on gut microbial composition, especially during early-life. In this study we integrate host genomics with exposures to investigate their main and interaction effects on the gut microbiota during the first year of life, and identify microbes associated with risk of childhood atopy.

Methods: We leveraged microbiome data from the CHILD Cohort Study generated from 16S rRNA sequencing of stool samples collected at 3 months ($N = 747$) and 1 year ($N = 766$), genomic profiles from the Illumina HumanCoreExome Bead Chip, and questionnaire data on environmental exposures. Genome-wide gene-environment interactions were assessed using linear regression models.

Results: *Megasphaera micronuciformis* abundance at 1 year was associated with variants on chromosome 6 (e.g., rs7771791) among breastfed children exposed to low concentrations of the human milk oligosaccharide disialyl-lacto-N-hexaose ($P = 6.4 \times 10^{-9}$). Moreover, *Clostridium sensu stricto sp. 4* abundance at 3 months was associated with loci on chromosome 1 (e.g., rs6696969) in males born during the winter months ($P = 1.4 \times 10^{-9}$). These microbial abundances were correlated with allergic sensitizations ($P_{Benjamini\text{-}Hochberg} = 3.7 \times 10^{-2}$) and atopic dermatitis ($P_{Benjamini\text{-}}$

$_{Hochberg} = 8.8 \times 10^{-3}$) in children between ages 3-5 years, respectively.

Conclusion: Our findings suggest that interactions between host genomics and environmental exposures influence the early-life gut microbiota of children, which may impact their susceptibility to atopic diseases. Improved understanding of factors influencing gut dysbiosis and childhood diseases will inform early-life biomarkers and interventions.

Grants: Canadian Institutes of Health Research (MRT-168044, PJT-178390), Ontario Graduate Scholarship.

Conflict of Interest: None declared

P17.026.C Changes in the genetic architecture of SARS-CoV-2 infection observed for the Omicron variant

Frank Geller[1], *xiaoping wu*[1], *Vilma Lammi*[2], *Erik Abner*[3], *Ole Pedersen*[4], *Erik Sørensen*[5], *Lampros Spiliopoulos*[1], *Peter Bager*[1], *Anders Hviid*[1], *Hanna Ollila*[2], *Sisse Rye Ostrowski*[5], *Bjarke Feenstra*[1]

[1]*Statens Serum Institut, Copenhagen, Denmark;* [2]*Institute for Molecular Medicine, Helsinki, Finland;* [3]*Estonian Genome Centre, Tartu, Estonia;* [4]*Zealand University Hospital, Køge, Denmark;* [5]*Copenhagen University Hospital, Copenhagen, Denmark*

Background/Objectives: The genetics of SARS-CoV-2 infection has been investigated by the Host Genetics Initiative (HGI) in about 220,000 cases diagnosed before the predominance of the Omicron variant in late 2021. We performed a genome-wide association study in 22,041 cases infected with the Omicron variant and 24,841 controls with no known SARS-CoV-2 infection to investigate potential differences between associated SNPs for Omicron compared to previously reported SNPs identified by the HGI for earlier SARS-CoV-2 variants.

Methods: Cases of SARS-CoV-2 infection with Omicron and controls were identified through the Danish EFTER-COVID project and linked to genetic data from the Danish Blood Donor Study and Copenhagen Hospital Biobank.

Results: The SNPs identified in our GWAS only partly overlapped with SNPs previously identified by the HGI. The most remarkable findings were (1) a locus on chromosome 3q27.3 (a known QTL for BMI-adjusted adiponectin), and (2) drastic changes in associations at the AB0 locus, where a protective effect was no longer seen for the SNP associated with blood group 0 and where the SNP with the strongest association suggests a protective effect of blood group B against Omicron infection. We decided to extend our study to a meta-analysis with more than 150,000 cases from international cohorts, and preliminary results confirm our findings and show additional new associations.

Conclusion: The Omicron variant has a large number of mutations compared to the original Wuhan strain, which is probably a main reason for the observed changes in the genetics of SARS-CoV-2 infection.

Conflict of Interest: None declared

P17.027.D Whole-exome sequencing association study for the identification of genetic modifying factors of lung and liver disease in Alpha-1 Antitripsin Deficiency

Nerea Matamala[1], *Sara Gil-Martin*[1;2], *Guillermo Pita*[3], *Anna Gonzalez-Neira*[3], *Myriam Calle-Rubio*[4], *Juan Luis Rodriguez-Hermosa*[4], *otto hagen*[1], *Gema Gomez-Mariano*[1], *Francisco Javier Alonso*[1;2], *Beatriz Martinez-Delgado*[1]

[1]*Institute of Health Carlos III (ISCIII), Rare Disease Research Institute (IIER), Majadahonda;* [2]*Institute of Health Carlos III (ISCIII), Biomedical Research on Rare Diseases Network Centre (CIBERER), Majadahonda;* [3]*Spanish National Cancer Research Centre (CNIO), Human Genotyping-CEGEN Unit, Madrid, Spain;* [4]*Hospital Clínico San Carlos (HCSC), Madrid, Spain*

Background/Objectives: Alpha-1 antitrypsin deficiency (AATD) is a genetic condition that can cause lung and/or liver damage. Although environmental factors are known to influence on clinical phenotype, not all the phenotypic variation in AATD can be explained by them, suggesting the contribution of genetic factors. The aim of this study was to identify genetic modifying factors of lung and liver disease in patients with AATD and PiZZ genotype.

Methods: We have performed a genetic association study with whole exome sequencing data from 72 patients with PiZZ genotype, 13 of them had liver damage (ZZ-HIG) while 59 developed lung disease (ZZ-PUL). Variants significantly associated with ZZ-HIG or ZZ-PUL cases were functionally annotated.

Results: We have identified 537 genes with variants significantly associated with one of the groups, 664 variants in 412 genes presented a higher frequency in ZZ-HIG cases and 270 variants in 125 genes appeared more frequently in ZZ-PUL cases. Fourteen percent (14%) of the significant variants are coding, while 86% are intronic variants that could have an effect on gene regulation. The functions of the genes associated with liver disease (ZZ-HIG) reveal involvement in transport pathways and lipid metabolism mainly. In the case of variants associated with lung disease (ZZ-PUL), they are related to specific functions such as neutrophil recruitment, surfactant production and lung function.

Conclusion: Although further validation is needed, the results suggest that these variants could be useful biomarkers for the stratification of patients with AATD, providing information about the risk of developing lung or liver disease.

Grants: SEPAR-1114, AESI PI20CIII/00015

Conflict of Interest: None declared

P17.028.A Genome-Wide Polygenic Models for 2,922 Plasma Protein Levels from Over 50,000 UK Biobank Participants

Pouria Dasmeh[1], *Hannah Klinkhammer*[2;3], *Gabriel Schweizer*[1], *Peter Krawitz*[3], *Johannes Schumacher*[1], *Christian Staerk*[2], *Andreas Mayr*[2], **Carlo Maj**[1]

[1]*Philipps-University Marburg, Center for Human Genetics, Marburg, Germany;* [2]*Institut für Medizinische Biometrie, Informatik und Epidemiologie (IMBIE), Bonn, Germany;* [3]*Institute for Genomic Statistics and Bioinformatics, Bonn, Germany*

Background/Objectives: Protein expression is significantly influenced by genetics, with regulatory variants typically located within or close to their encoding genes (cis-pQTLs). The study of distant trans-pQTLs has been limited due to smaller effect sizes and also power and computational issues in analyzing genome-wide variant-gene pairs.

Methods: Using snpboost framework we derived genome-wide sparse polygenic models for 2,922 plasma proteins from 52,682 UK Biobank samples (60% training, 20% validation, 20% testing). Model performances were assessed by considering the incremental-R^2 with respect to covariates-only models (i.e., sex, age, and 10 PC). Cis-models were derived by filtering for variants within 500 kb of gene flanking regions.

Results: Polygenic models showed strong (mean incremental R^2 of 0.103) but heterogeneous performances (500 proteins with incremental-$R^2 < 0.01$, 68 proteins showing a predominant genetic component with incremental-$R^2 > 0.5$) and genetic architectures (from 1 to 3,211 variants, mean 405). On average cis-pQTL effect-sizes are 4.64 times stronger than trans-pQTL and represent only 5.78% of the PRS models but the corresponding cis-models reach on average 55.63% of the genome-wide models' performances. Noteworthy, predicted proteins among 14 pairs of genomic-

coordinates overlapping genes showed lower Pearson correlations in the genome-wide models than in the cis-models (average 0.126 vs 0.242).

Conclusion: Snpboost genome-wide polygenic models for plasma protein levels corroborated the presence of stronger oligogenic cis-regulatory effects but also revealed substantial distant-regulatory polygenic components which can also be less influenced by co-regulation or coincidental associations among overlapping genes. The models are available at https://proteinprs.com/.

Conflict of Interest: None declared

P17.029.B New findings in the genetics of eating behaviour (EB): Genome-Wide Association Studies (GWAS) on taste perception (TP) and food liking (FL) in Italian cohorts

Maria Pina Concas[1], Sara Spinelli[2], Silvia Camarda[3], Erminio Monteleone[2], Alessandro Pecori[1], Caterina Dinnella[2], **Paolo Gasparini**[1;3]

[1]Institute for Maternal and Child Health IRCCS "Burlo Garofolo", Trieste, Italy; [2]University of Florence, Department of Agriculture, Food, Environment and Forestry (DAGRI), Florence, Italy; [3]University of Trieste, Department of Medicine, Surgery and Health Sciences, Trieste, Italy

Background/Objectives: Both TP and FL play a fundamental role in EB and, consequently, in health status. Despite moderate/high heritability, little is known about genes involved. Therefore, this study aimed to investigate the molecular bases of 1) TP and 2) FL in Italian cohorts.

Methods: The following individuals were enrolled in the study: 1354 individuals from the Italian Taste Project (ITP), 729 from Friuli-Venezia Giulia (FVG), and 701 from Val Borbera (VB).

1)GWAS on TP were conducted on the perceived intensity of eight different substances, in ITP subjects and replicated in FVG and VB.

2)GWAS on FL were performed testing 100 foods in FVG and replicated in VB.

Results: 1)A unique clear signal ($p < 5 \times 10^{-8}$) was detected in correspondence to the *TAS2R38* gene for bitterness, and for the first time, saltiness, sourness, and astringency. In addition, we identified suggestive loci ($p < 10^{-6}$) for sweetness, umami and pungency in regulator of G-proteins and potassium channel genes.

2)GWAS on FL led to an interesting signal for milk chocolate. The best SNP ($p = 1.02 \times 10^{-9}$) is an eQTL for the *LARP1* gene in Nucleus Accumbens (NAc), a central driver of reward response controlling food gratification and regulating behaviour. Subjects carrying the minor allele showed a reduced liking for sweet foods, and a reduced impulsivity ($p < 0.05$).

Conclusion: This study provided new findings in the genetics of EB that highlight both the role of *TAS2R38* not only in bitter perception, and that of NAc in regulating FL and impulsive behaviour. Further studies are needed to better elucidate these results.

Grants: NA

Conflict of Interest: None declared

P17.030.C Phenotypic and genome-wide association studies on dicarbonyls

Philip Harrer[1;2], *Julica Inderhees[3;4;5], Chen Zhao[1;6], Barbara Schormair[1;2], Erik Tilch[1;6], Christian Gieger[7;8], Annette Peters[7;9;10], Olaf Jöhren[3;4], Thomas Fleming[11], Peter P. Nawroth[11], Klaus Berger[12], Marco Hermesdorf[12], Juliane Winkelmann[1;2;13;14], Markus Schwaninger[3;5], Konrad Oexle[1;2;6]*

[1]Helmholtz Munich, Institute of Neurogenomics, Oberschleißheim, Germany; [2]Technical University of Munich, Institute of Human Genetics, München, Germany; [3]University of Lübeck, Institute for Experimental and Clinical Pharmacology and Toxicology, Lübeck, Germany; [4]University of Lübeck, Bioanalytic Core Facility, Lübeck, Germany; [5]German Centre for Cardiovascular Research, Hamburg-Lübeck-Kiel, Germany; [6]Helmholtz Munich, Neurogenetic Systems Analysis Group, Oberschleißheim, Germany; [7]Helmholtz Munich, Institute of Epidemiology, Oberschleißheim, Germany; [8]Helmholtz Munich, Research Unit of Molecular Epidemiology, Oberschleißheim, Germany; [9]German Center for Diabetes Research, Neuherberg, Germany; [10]Ludwig Maximilian University of Munich, Chair of Epidemiology, Institute for Medical Information Processing, München, Germany; [11]Heidelberg University, Department of Internal Medicine, Heidelberg, Germany; [12]University of Münster, Institute of Epidemiology and Social Medicine, Münster, Germany; [13]Munich Cluster for Systems Neurology (SyNergy), München, Germany; [14]German Centre for Mental Health, München-Augsburg, Germany

Background/Objectives: Phenotypic and genome-wide association studies (GWAS) examining the dicarbonyl compounds methylglyoxal (MG), glyoxal (GO), and 3-deoxyglucosone (3-DG) in a disease-independent context have not been provided yet.

Methods: LC-MS was employed to measure MG, GO, and 3-DG in 1,304 serum samples collected from two distinct populations, KORA ($n = 482$) and BiDirect ($n = 822$). Subsequently, these measurements were analyzed for associations with genome-wide SNPs (GWAS) and phenome-wide traits. To identify major independent trait associations, redundancy analysis (RDA) was applied.

Results: The correlation between dicarbonyls was significant, notably stronger for MG and GO ($\rho = 0{\cdot}6$) than for 3-DG and MG or GO ($\rho = 0{\cdot}4$). Phenotypic findings encompassed significant associations of all dicarbonyls with sex, waist-to-hip ratio, glomerular filtration rate (GFR), gamma-glutamyltransferase (GGT), and hypertension. Additionally, MG and GO were significantly associated with age and C-reactive protein, GO and 3-DG with glucose and antidiabetics, MG with contraceptives, GO with ferritin, and 3-DG with smoking. RDA identified GFR, GGT, and glucose as major explanators of dicarbonyl variance. Genome-wide association studies (GWAS) did not yield significant loci. However, limiting multiple testing correction to lead SNPs from GWASs on RDA-selected traits revealed a significant association ($p = 2{\cdot}3 \times 10\text{-}5$) between 3-DG and rs1741177, an eQTL of NF-κB inhibitor NFKBIA.

Conclusion: This first large-scale, population-based study has identified numerous phenotypic associations, with GFR and GGT being of pivotal importance, providing unbiased and novel perspectives on dicarbonyls beyond the current state. GWAS power will profit from meta-analysis with analogous studies.

Grants: Deutsche Forschungsgemeinschaft, Helmholtz Munich, German Centre for Cardiovascular Research (DZHK), German Federal Ministry of Research and Education (BMBF).

Conflict of Interest: None declared

P17.031.D Enhanced insights into the genetic architecture of 3D cranial vault morphology using pleiotropy-informed conditional GWAS

Seppe Goovaerts[1], *Hanne Hoskens[1], Noah Herrick[2], MENG YUAN[1], Mark Shriver[3], John Shaffer[4], Susan Walsh[2], Seth Weinberg[4], Peter Claes[1]*

[1]KU Leuven, Department of Human Genetics; [2]Indiana University Indianapolis, Department of Biology, Indianapolis, United States; [3]Pennsylvania State University, Department of Anthropology; [4]University of Pittsburgh, Department of Oral and Craniofacial Sciences

Background/Objectives: Large-scale GWAS studies have identified several hundred genomic loci associated with typical-range variation in facial and brain shape, but only tens of loci associated with cranial vault shape, leaving it an understudied component of the craniofacial complex. Surrounding and protecting the neocortex, the cranial vault plays a central role during craniofacial development, and its morphogenesis and growth are intimately tied to adjacent tissues and organs. Hence, an improved genetic understanding of its development is key to understanding the coordinated development of craniofacial structures and the etiology of craniofacial anomalies. Here, we test the hypothesis that leveraging genetic data from related traits can improve the discovery of SNPs associated with cranial vault shape.

Methods: We first show that the genomic architectures of brain shape, facial shape, and bone mineral density are strongly enriched for SNPs associated with cranial vault shape due to genomic and structural correlations. Based on GWAS summary statistics from independent cohorts, these auxiliary traits were then leveraged to re-analyze a recent cranial vault GWAS using a conditional false discovery rate (FDR).

Results: This yielded 120 independent genomic loci at 1% FDR, almost three-fold that of unconditioned analysis. Genes near these loci implicate key craniofacial transcription factors and signaling pathways, including genes involved in FGF, BMP, Wnt, Hedgehog, and retinoic acid signaling, most of which were not previously identified as contributors towards vault shape.

Conclusion: Altogether, our improved GWAS discovery substantially enhances the genetic understanding of typical-range variation in cranial vault shape and craniofacial development more broadly.

Grants: R01DE027023

Conflict of Interest: None declared

P17.032.A A genome-wide association study of mortality in the Canary Islands (Spain)

Silvia Gonzalez-Barbuzano[1], *Eva Suarez-Pajes*[1], *Mª del Cristo Rodríguez Pérez*[1], *Almudena Corrales*[1;2], *Antonio Cabrera de León*[1;3], *Carlos Flores*[1;2;4], *Itahisa Marcelino-Rodriguez*[1;3]

[1]Hospital Universitario Nuestra Señora de Candelaria, Santa Cruz de Tenerife, Spain; [2]CIBER de Enfermedades Respiratorias (CIBERES), Instituto de Salud Carlos III, Madrid, Spain; [3]Preventive Medicine and Public Health Area, Universidad de La Laguna, San Cristóbal de La Laguna, Spain; [4]Genomics Division, Instituto Tecnológico y de Energías Renovables, Granadilla, Spain

Background/Objectives: Canary Islanders have one of the highest incidence and mortality rates for cardiovascular disease and cancer in Spain. This study aimed to identify risk variants for mortality in this population using a genome-wide association analysis.

Methods: The study is a cross-sectional analysis of increased mortality risk assessing 9.6 million TOPMed-imputed genetic variants in 1,259 Canary Islanders individuals (394 deaths) from the CDC cohort. The region around 2 Mb of the significant variants, identified using logistic regression models, was then subjected to Cox regressions for sensitivity analyses. Significance was declared at $p < 5.0 \times 10^{-8}$. Furthermore, fine mapping and functional analyses of the significant locus were performed to prioritise the variants implicated.

Results: We identified three genome-wide significant variants located at 17q21.32 ($p_{lowest} = 3.36 \times 10^{-8}$). Cox regression analysis prioritised the *NFE2L1*, *CBX1*, and *SNX11* genes. These genes have previously been associated with several types of cancer. This is consistent with more than a third of deaths in this study were due to this condition.

Conclusion: We performed the first survival analysis in Canary Islanders and identified risk variants and genes of interest at 17q21.32. Rare variant analyses in this region are underway.

Grants: Grant PMP22/00033 funded by ISCIII, and by the European Union NextGenerationEU, Mecanismo para la Recuperación y la Resiliencia; Fundación Canaria Instituto de Investigación Sanitaria de Canarias (ST23/04); ITER agreement (OA23/043); Gobierno de Canarias, Social European Fund "Canarias Avanza con Europa" (TESIS2022010042).

Conflict of Interest: None declared

P17.033.B Exploring the genetic risk of childhood daytime urinary incontinence

Anders S. Breinbjerg[1;2], *Cecilie Siggaard Jørgensen*[1;2], *Gudmundur Bragi Walters*[3;4], *Jakob Grove*[5;6;7], *Thomas Damm Als*[5;6], *Konstantinos Kamperis*[1;2], *Lilja Stéfansdóttir*[3], *Janne Pia Thirstrup*[5;6], *Britt Borg*[1;2], *Clara Albiñana*[8], *Bjarni Jóhann Vilhjálmsson*[5;7;8;9], *Viðar Ö Eðvarðsson*[4;10], *Hreinn Stefansson*[3], *Preben Bo Mortensen*[5;8;11], *Esben Agerbo*[5;8;11], *Thomas Werge*[5;12;13], *Anders Børglum*[5;6], *Ditte Demontis*[5;6], *Kári Stefánsson*[3;4], *Søren Rittig*[1;2], **Jane Hvarregaard Christensen**[5;6]

[1]Aarhus University Hospital, Department of Pediatrics and Adolescent Medicine, Aarhus, Denmark; [2]Aarhus University, Department of Clinical Medicine, Aarhus, Denmark; [3]deCODE genetics, Reykjavík, Iceland; [4]University of Iceland, Faculty of Medicine, Reykjavík, Iceland; [5]iPSYCH, The Lundbeck Foundation Initiative for Integrative Psychiatric Research, Aarhus and Copenhagen, Denmark; [6]Aarhus University, Department of Biomedicine, Aarhus, Denmark; [7]Aarhus University, Bioinformatics Research Centre, Aarhus, Denmark; [8]Aarhus University, NCRR - National Centre for Register-based Research, Aarhus, Denmark; [9]Broad Institute of MIT and Harvard, Novo Nordisk Foundation Center for Genomic Mechanisms of Disease, Cambridge, United States; [10]Landspitali University Hospital, Children's Medical Center, Reykjavík, Iceland; [11]Aarhus University, CIRRAU - Center for Integrated Register-based Research, Aarhus, Denmark; [12]Copenhagen University Hospital, Institute of Biological Psychiatry, Mental Health Services, København, Denmark; [13]Copenhagen University, Department of Clinical Medicine, København, Denmark

Background/Objectives: Childhood incontinence is stigmatized and underprioritized, and a basic understanding of its pathogenesis is missing. Our goal was to identify risk-conferring genetic variants in daytime urinary incontinence (DUI).

Methods: We conducted a genome-wide association study in the Danish iPSYCH2015 cohort. Cases (3,024) were identified through ICD10 diagnosis codes and redeemed prescriptions for DUI in children aged 5 to 20. Controls (30,240) were matched on sex and psychiatric disorders and downscaled to a 1:10 ratio. Replication was performed in the Icelandic deCODE cohort (5,475 cases/287,773 controls). Single-nucleotide polymorphism heritability was calculated using the genome-based restricted maximum likelihood method. Cross-trait genetic correlation was estimated using linkage disequilibrium score regression. Polygenic risk scores generated with LDpred2-auto were assessed for association.

Results: Variants on chromosome 6 (rs12210989, OR = 1.24 [95% CI: 1.17-1.32], $P = 3.21 \times 10^{\div 12}$) and 20 (rs4809801, OR = 1.18 [95% CI: 1.11-1.25], $P = 3.66 \times 10^{\div 8}$) reached genome-wide significance and implicated the *PRDM13* and *RIPOR3* genes. Chromosome 6 findings were replicated ($P = 0.024$, OR = 1.09 [95% CI: 1.01-1.16]). Liability scale heritability ranged from 10.20% (95% CI: 6.40-14.00%) to 15.30% (95% CI: 9.66%-20.94%). DUI and nocturnal enuresis showed positive genetic correlation

($r_g = 1.28 \pm 0.38$, $P = 0.0007$). Significant associations were established between DUI and attention-deficit/hyperactivity disorder (OR = 1.098 [95% CI: 1.046-1.15], $P < 0.0001$) and body mass index (OR = 1.129 [95% CI: 1.081-1.18], $P < 0.0001$) polygenic risk.

Conclusion: Genetic variants associated with childhood DUI were identified and genes important in balancing excitatory and inhibitory neurons in the central nervous system and detrusor smooth muscle activity were implicated. These findings may help guide identification of new treatment targets.

Conflict of Interest: Anders S. Breinbjerg Kimberley-Clark, Kimberley-Clark, Cecilie Siggaard Jørgensen: None declared, Gudmundur Bragi Walters deCODE genetics/Amgen, Jakob Grove: None declared, Thomas Damm Als: None declared, Konstantinos Kamperis Ferring Pharmaceuticals

Novo Nordisk, Kimberley-Clark, Lilja Stéfansdóttir deCODE genetics/Amgen, Janne Pia Thirstrup: None declared, Britt Borg Ferring Pharmaceuticals, Clara Albiñana: None declared, Bjarni Jóhann Vilhjálmsson Allelica, Viðar Ö Eðvarðsson: None declared, Hreinn Stefansson deCODE genetics/Amgen, Preben Bo Mortensen: None declared, Esben Agerbo: None declared, Thomas Werge: None declared, Anders Børglum: None declared, Ditte Demontis Medice Nordic, Kári Stefánsson deCODE genetics/Amgen, Søren Rittig Kimberley-Clark, Jane Hvarregaard Christensen: None declared

P17.034.C Exploring genetic factors influencing apolipoprotein A-IV: a genome-wide association meta-study

Adriana Koller[1], *Claudia Lamina*[1], *Barbara Kollerits*[1], *Florian Kronenberg*[1]

[1]*Medical University of Innsbruck, Institute of Genetic Epidemiology, Innsbruck, Austria*

Consortium: ApoA-IV GWAS Consortium

Background/Objectives: Apolipoprotein A-IV (apoA-IV) is a major component of high-density lipoprotein and chylomicron particles. Primarily produced in enterocytes, apoA-IV has antioxidative properties and is associated with lipid phenotypes and kidney function. We aimed to identify genetic variants associated with apoA-IV levels and to explore the relevance of these findings in relation to known association between apoA-IV and different phenotypes.

Methods: Quantification of apoA-IV from plasma samples was performed using a double-antibody ELISA. Using our nf-gwas pipeline, we conducted a GWAS on HRC-imputed data to identify variants associated with apoA-IV concentrations in ten population-based studies (n~25,000).

Results: Mean apoA-IV concentrations ranged from 15.3 to 17.6 mg/dl between the studies. Our meta-GWAS (adjustment: age, sex, four principal components) identified 89 genome-wide significant variants within the *APOA5-A4-C3-A1* cluster (lead SNP: rs2849167, ß = 0.151, $p = 1.48 \times 10^{-47}$ near *APOA4*) and 55 variants around the *KLKB1* gene (lead SNP: rs4253311, ß = 0.086, $p = 6.74 \times 10^{-17}$), along with rare variants associated with apoA-IV concentrations. Sex-stratified analyses revealed similar results in men and women. We explored the impact of various covariates (e.g. kidney function and cholesterol efflux capacity) on the results. Additionally, we employed mediation analysis and a Mendelian randomization approach to assess the causal relationships between potential phenotypes and variants associated with apoA-IV concentrations.

Conclusion: This study represents the largest GWAS conducted on apoA-IV levels to date, revealing several significant loci associated with apoA-IV concentrations and providing insights into the genetic determinants of apoA-IV levels and phenotypes of interest.

Grants: The Austrian Research Fund (FWF, W-1253 DK HOROS) has supported this project.

Conflict of Interest: None declared

P17.035.D Dissecting the biomedical and genetic structure of a Southern Italian cohort: the Moli-sani study

Federica Santonastaso[1], *Simona Costanzo*[2], *Edoardo Giacopuzzi*[1], *Alessandro Raveane*[1], *Chiara Chiereghin*[1], *Arianna Landini*[1], *Sodbo Sharapov*[1], *Soumick Chatterjee*[1], *Davide Bolognini*[1], *Manuel Tardaguila*[1], *Michela Colombo*[1], *Ilaria Gori*[1], *Alessandro Gialluisi*[2;3], *Amalia De Curtis*[2], *Emanuele Di Angelantonio*[4;5], *Giovanni de Gaetano*[2], *Maria Benedetta Donati*[2], *Nicola Pirastu*[1], *Licia Iacoviello*[2;3], *Nicole Soranzo*[1;6]

[1]*Human Technopole, Genomics Research Centre, Milan, Italy;* [2]*IRCCS Neuromed, Department of Epidemiology and Prevention, Pozzilli, Italy;* [3]*LUM University, Department of Medicine and Surgery, Bari, Italy;* [4]*University of Cambridge, British Heart Foundation Centre of Research Excellence, Cambridge, United Kingdom;* [5]*Human Technopole, Health Data Science Centre, Milan, Italy;* [6]*Wellcome Sanger Institute, Human Genetics Department, Hinxton, United Kingdom*

Background/Objectives: Human genetic diversity in Southern Europe has been historically undersampled owing to limited genomic initiatives. In Italy, complex demographic events and a unique geography contributed to high genetic heterogeneity. To characterise the architecture of biomedical traits and diseases, we carried out the largest to date genomic survey in Italy using Moli-sani, a prospective cohort of approximately 25,000 individuals (age ≥ 35, 49.3% men) recruited from city hall registries in the Molise region.

Methods: We profiled genome-wide SNPs to (i)address genetic structure vis-a-vis other Italian and European populations; (ii) describe genetic contributions to over fifty biomedical traits relevant for chronic-degenerative diseases; (iii)assess the PRS portability estimated from other European populations in South Italy.

Results: While confirming a broad genetic overlap with Northern European populations, our GWASs have identified 73 new SNPs and 10 new loci associated with 7 biomedical traits. For example, we have identified different variants near the *HBB* gene showing greater allelic frequencies and per-allele effect size in Moli-sani than UK Biobank. Among these, we observe *rs11549407* and *rs35004220*, known to be involved in beta-thalassemia disorders in South Europe and likely reflecting past historical events. We estimate that PRSs, developed from European summary statistics, explain ~20% less variance than estimates in Northern populations.

Conclusions: The genetic profiling of the Moli-sani cohort, coupled with its large size, depth of phenotyping, and prospective design, provides a unique resource to study genetic contributions to common diseases and traits, and to which extent these are modulated in the context of South Italy's unique history and environments.

Conflict of Interest: None declared

P17.036.A Genome-wide association study of Borderline Personality Disorder accounting for age at diagnosis and family history

Alisha Hall[1;2], *Jette Steinbach*[3], *Emil Michael Pedersen*[3], *Jessica Rose Mundy*[1;2], *Esben Agerbo*[3], *Soren Dinesen Ostergaard*[1;2], *Jean-*

Christophe Philipp Debost[1;2], Bjarni Jóhann Vilhjálmsson[3], Isabell Brikell[4], Katherine Musliner[1;2]

[1]Aarhus University, Department of Clinical Medicine, Aarhus, Denmark; [2]Aarhus University Hospital - Psychiatry, Department of Affective Disorders, Aarhus, Denmark; [3]Aarhus University, National Centre for Register-Based Research, Aarhus, Denmark; [4]Karolinska Institute, Department of Medical Epidemiology and Biostatistics, Stockholm, Sweden

Background/Objectives: Borderline Personality Disorder (BPD; ICD-10 F60.3x) is characterized by marked instability in emotions, self-image, and interpersonal relationships. Individuals with BPD make up a large proportion of the patient population in psychiatric hospital services. To develop more effective treatment options and aid early intervention, we must improve our understanding of BPD etiology. Previous studies have reported a heritability of 46–75%, but previous genome-wide association studies (GWAS) of BPD have been underpowered to detect significantly associated genetic variants.

Methods: We conducted a GWAS of BPD liability in the Lundbeck Foundation Initiative for Integrative Psychiatric Research (iPSYCH) sample, accounting for age at diagnosis and family history. iPSYCH is a nationally representative case-cohort study of all individuals born in Denmark 1981–2008. The sample for this study consisted of ~7K individuals with BPD from the entire iPSYCH sample and ~46K population representative controls. We applied the extended liability threshold model conditioned on age, sex, and family history (LT-FH + +) to estimate each individual's liability to develop BPD in the study sample and calculated a linear mixed model GWAS of BPD liability with the first ten genetic principal components and genotyping array type as covariates.

Results: Variants at the 5q21 locus between *NIHCOLE* and *RNU6-334P* showed genome-wide significant associations with BPD liability.

Conclusion: We conducted the largest single-sample GWAS of BPD to date and identified SNPs significantly associated with BPD. Future studies are needed to replicate these findings.

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Conflict of Interest: Alisha Hall: None declared, Jette Steinbach: None declared, Emil Michael Pedersen: None declared, Jessica Rose Mundy: None declared, Esben Agerbo: None declared, Soren Dinesen Ostergaard SDØ owns/has owned units of mutual funds with stock tickers DKIGI, IAIMWC, SPIC25KL and WEKAFKI, and owns/has owned units of exchange traded funds with stock tickers BATE, TRET, QDV5, QDVH, QDVE, SADM, IQQH, USPY, EXH2, 2B76, IS4S and EUNL., SDØ received the 2020 Lundbeck Foundation Young Investigator Prize., SDØ is supported by the Novo Nordisk Foundation (grant number: NNF20SA0062874), the Lundbeck Foundation (grant numbers: R358-2020-2341 and R344- 2020-1073), the Danish Cancer Society (grant number: R283-A16461), the Central Denmark Region Fund for Strengthening of Health Science (grant number: 1-36-72-4-20), The Danish Agency for Digitisation Investment Fund for New Technologies (grant number 2020–6720), and Independent Research Fund Denmark (grant number: 7016-00048B and 2096-00055A)., Jean-Christophe Philipp Debost: None declared, Bjarni Jóhann Vilhjálmsson BJV is a member of the scientific advisory board for Allelica., Isabell Brikell: None declared, Katherine Musliner: None declared

P17.038.C Deep-learning powered GWAS on T1 brain MRI data

Alexander Rakowski[1], *Remo Monti[1;2], Christoph Lippert[1;3]*

[1]Hasso-Plattner Institut, Digital Health & Machine Learning, Potsdam, Germany; [2]Max-Delbrück-Center for Molecular Medicine in the Helmholtz Association, Berlin, Germany; [3]Hasso Plattner Institute for Digital Health at Mount Sinai, New York, United States

Background/Objectives: GWAS studies traditionally analyzed single traits e.g., disease diagnoses or biomarkers. Nowadays, large-scale cohorts such as UK Biobank (UKB) collect imaging data with sample sizes large enough to perform genetic association testing. Typical approaches extract predefined features, e.g., volumes of regions of interest. This limits the scope of such studies to predefined traits and can ignore novel patterns present in the data. TransferGWAS employs deep neural networks to extract low-dimensional representations of imaging data for GWAS, eliminating the need for predefined biomarkers. We apply TransferGWAS on T1 brain MRI data from UK Biobank.

Methods: We encoded 36,000 T1 brain MRI scans from UKB, using models trained on MRI scans from the Alzheimer's Disease Neuroimaging Initiative and natural images from the ImageNet dataset, and performed a multivariate GWAS on the resulting features. Furthermore, we fitted Polygenic Scores (PGS) of the deep features and computed genetic correlations between them and a range of selected phenotypes.

Results: We identified 289 independent loci, associated mostly with bone density, brain, or cardiovascular traits, and 14 having no previously reported associations. We evaluated the PGS in a multi-PGS setting, improving predictions of several traits. By examining clusters of genetic correlations, we found novel links between diffusion MRI traits and type 2 diabetes.

Conclusion: Using deep learning-based phenotypes allowed us to identify novel loci, compute predictive PGS, and enhanced understanding of the genetic basis of type 2 diabetes.

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Conflict of Interest: Alexander Rakowski European Union's Horizon 2020 research and innovation programme under grant agreement No 101016775., Remo Monti European Union's Horizon 2020 research and innovation programme under grant agreement No 101016775., Christoph Lippert European Union's Horizon 2020 research and innovation programme under grant agreement No 101016775., Hasso-Plattner Institut

P17.039.D Converging rare and common variant evidence drives discovery of genes associated with central obesity

Nikolas Baya[1;2], *Frederik Lassen[1;2], Barney Hill[2], Samvida Venkatesh[1;2], Duncan Palmer[2], Cecilia M. Lindgren[1;2]*

[1]Centre for Human Genetics, University of Oxford, United Kingdom; [2]Big Data Institute, University of Oxford, United Kingdom

Background/Objectives: Obesity, a pervasive health challenge, demands a comprehensive understanding of its genetic underpinnings. Our goal was to leverage UK Biobank's whole-exome sequencing data to unravel obesity's causal mechanisms and provide novel insights into its genetic architecture.

Methods: We ran mixed-model gene- and variant-level association tests for three obesity-related traits across >395,000 individuals with European ancestry in the UK Biobank using SAIGE.

We performed variant-level association tests on rare (MAF < 0.01) and common (MAF>0.001) variants using exome sequencing data and imputed genotypes, respectively.

We tested gene-by-gene for an enrichment of damaging rare variation for each obesity-related trait, accounting for nearby common variant genetic association signals. Statistical fine-mapping to define variant conditioning was performed with FINEMAP.

We tested for significantly higher polygenic priority scores (PoPS) in genes with significant rare variant burden versus non-significant genes.

Results: Burden of rare damaging variation in 19 genes was significantly associated with at least one obesity-related trait ($P < 1.58 \times 10^{-7}$, Bonferroni corrected).

We identified six genes with evidence for allelic series, where the effect size of variants monotonically increased with the predicted severity of the variant.

We found that genes with significant association between rare variant burden and obesity had significantly higher common-variant PoPS compared to genes without significant association, demonstrating convergent common and rare variant signals in obesity genetics.

Conclusion: Our study furthers the understanding of the genetic architecture of obesity and uses multiple lines of evidence to nominate genes with potential therapeutic significance.

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Conflict of Interest: None declared

P17.040.A Common and rare variant association studies of left and right ventricular phenotypes identify novel genetic loci and therapeutic targets for heart failure

Hannah Nicholls[1], *Jose Vargas*[1;2;3], *Anwar Chahal*[4;5;6], *Mohammed Khanji*[1;4], *Steffen Petersen*[1;4], *Patricia Munroe*[1], *Nay Aung*[1;4]

[1]*William Harvey Research Institute, London, United Kingdom;* [2]*Veterans Affairs Medical Center, Washington;* [3]*Georgetown University, Washington, United States;* [4]*Barts Heart Centre, St Bartholomew's Hospital, London, United Kingdom;* [5]*WellSpan York Hospital, Center for Inherited Cardiovascular Diseases, York, United States;* [6]*Mayo Clinic, Department of Cardiovascular Diseases, Rochester, United States*

Background/Objectives: Left and right ventricular (LV and RV) structure and function measured from cardiovascular magnetic resonance (CMR) imaging are pivotal for understanding complex cardiovascular diseases including heart failure (HF). The genetic contributions to LV and RV phenotypes have not been comprehensively explored.

Methods: We performed genome-wide association studies (GWAS) and rare-variant collapsing analyses of twenty clinically relevant LV and RV volumetric/functional phenotypes from 56,509 European UK Biobank participants using ~8.9 million TOPMed imputed variants and whole-exome sequencing data. Bioinformatic examination, including colocalisation, polygenic priority scoring, druggability via Open Targets and DrugnomeAI, was performed.

Results: Single and multi-trait GWAS identified 310 loci associated with at least one phenotype ($P < 5 \times 10^{-8}$), 262 loci across >1 phenotype, and 84 novel loci across CMR traits. Rare variant analysis found associations with 13 genes (*RBM20, TTN, EFCAB3, FHOD3, FLNC, CORO6, CYP3A4, DUSP13, SDCCAG8, GAA, GIP, CSRP3*, and *SIRT4*) ($P < 2.5 \times 10^{-6}$). These rare variant associations were independent of nearby common variants in conditional analyses. Five variants (in *LSM3, MICB*, *LOC101928505, IGF1R, KANSL1* loci) colocalised with loci from a recently reported large HF case-control GWAS meta-analysis. From 462 prioritised genes, 15 were Mendelian cardiomyopathy genes and 54 interact with drugs across any disease indication. *DGAT1 and CDK12* genes were identified through multiple evidence streams as promising HF targets with roles in cardiac lipid metabolism and stress-induced transcription activation, respectively.

Conclusion: These findings substantially enrich our knowledge of the genetics of ventricular measurements, providing opportunities for identifying and repurposing drugs for cardiovascular diseases.

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P17.043.D Massively Parallel Reporter Assay (MPRA) combined with bayesian fine mapping approaches prioritize variants and their possible functional mechanism associated with Multiple Sclerosis (MS) from Genome Wide Association Studies (GWAS)

Endri Visha[1], *Nicola Pomella*[2], *Ferdinando Clarelli*[3], *Lucia Corrado*[2], *Alessandro Pizzino*[4], *Fjorilda Caushi*[2], *Beatrice Piola*[2], *Diego Cotella*[2], *alaa habeeb*[2], *Muralidharan Thavamani*[2], *Laura Follia*[2], *Martina Tosi*[2], *domizia vecchio*[5], *Massimo Filippi*[6], *Maurizio Leone*[7], *MARTINELLI BONESCHI FILIPPO GIOVANNI*[8], *FEDERICA ESPOSITO*[3], *Nadia Barizzone*[2], *sandra d'alfonso*[2]

[1]*Novara, Medical Biotechnologies, Novara, Italy;* [2]*Province of Novara, Dipartimento di scienza della Salute, Novara, Italy;* [3]*IRCCS San Raffaele Scientific Institute, Division of Neuroscience, Milan, Italy;* [4]*Novara, Dipartimento di scienza della Salute, Novara, Italy;* [5]*Province of Novara, Department of translational medicine, Novara, Italy;* [6]*Milan, Division of neuroscience, Milan, Italy;* [7]*San Giovanni Rotondo, Scienze mediche, San Giovanni Rotondo, Italy;* [8]*Milano, Dipartimento di scienza della salute, Neurology unit and multiple sclersis center ASST, Santi Paolo Carlo, Milan, Italy*

Background/Objectives: Multiple Sclerosis (MS) is an autoimmune multifactorial disease affecting central nervous system. Large-scale GWAS detected 200 MS risk loci, but linkage disequilibrium represents limitation in pinpointing likely causative variants, thus functional studies are required.

Methods: We applied functionally informed fine-mapping (Paintor and CaviarBF) on 36 MS regions, replicating significantly in an Italian cohort (5903 individuals, 4259 MS,1644 HC) and overlapping with at least one drug target gene (DGIdb). Variant annotation was done using GWAVA, CADD, and FINSURF scores. For variant -to-gene mapping, we employed Open Targets Genetics. For 5 regions, we functionally analysed all the SNPs in LD ($r2 > 0.75$) with the lead SNP using Massively Parallel Reporter Assay (MPRA).

MPRA significative variants were analysed with mpralm tool, and annotated with motifbreakR for their TF selective binding properties. Results were confirmed with RegulomeDB,GTEX.

Results: For 11 regions, Paintor and CaviarBF prioritized at least 1 SNP with evidence of causality PIP >0.75, and 5 of these SNPs target a drug target gene.

For 4 regions, MPRA analyses identified at least one SNP influencing gene expression of a drug target gene, with a statistically significant effect in two independent replicates. Rs72924108 emerges above all with selective binding in the presence of the C allele of the ZNF754 TF a paralog of the YY1, which is involved in the regulation of transcription of RNA-polymerase II.

Conclusion: Although these findings need validation through in vitro methods, they potentially affect our understanding of disease mechanisms and their drug targets

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Conflict of Interest: None declared

P17.044.A The contribution of MODY genes in type 1 diabetes susceptibility

Eulalia Catamo[1], Andrea Conti[1], Luana Aldegheri[1;2], Davide Tinti[3], Klemen Dovc[4;5], Riccardo Bonfanti[6], Roberto Franceschi[7], Ivana Rabbone[8], Tadej Battelino[4;5], Dario Iafusco[9], Elena Faleschini[1], Gianluca Tornese[1;2], **Antonietta Robino**[1]

[1]Institute for Maternal and Child Health – IRCCS Burlo Garofolo, Trieste, Italy; [2]University of Trieste, Department of Medical Sciences, Trieste, Italy; [3]Center for Pediatric Diabetology, A.O.U. Città della Salute e della Scienza, Torino, Italy; [4]University of Ljubljana, Faculty of Medicine, Ljubljana, Slovenia; [5]University Children's Hospital, University Medical Centre Ljubljana, Department of Endocrinology, Diabetes and Metabolism, Ljubljana, Slovenia; [6]Diabetes Research Institute, IRCCS San Raffaele Hospital, Department of Pediatrics, Milano, Italy; [7]S. Chiara General Hospital, APSS, Division of Pediatrics, Trento, Italy; [8]University of Eastern Piedmont, Division of Pediatrics, Department of Health Sciences, Novara, Italy; [9]Università degli Studi della Campania "Luigi Vanvitelli", Department of Woman, Child and of General and Specialized Surgery, Napoli, Italy

Background/Objectives: Type 1 diabetes (T1D) is a polygenic disease caused by the autoimmune destruction of pancreatic beta cells. Genome-wide association studies have identified over 60 loci associated with T1D, but this knowledge has not advanced precision medicine. In contrast, the precision medicine approach has been a great success in monogenic forms of diabetes, such as Maturity-Onset Diabetes of the Young (MODY).

Although monogenic and polygenic diabetes are traditionally considered distinct diseases, recent data support the hypothesis of a genetic continuum between MODY and polygenic forms of diabetes.

Methods: In the present work, 383 T1D young individuals (mean age:13.9 ± 3.4, 52% females) and 187 healthy controls (mean age:12.8 ± 3.4, 56% females) were recruited. Participants were genotyped using Illumina Infinium Global Screening Array. A Polygenic Score (PGS) based on Single Nucleotide Polymorphisms (SNPs) in 16 genes responsible for MODY was developed and its association with T1D and related clinical characteristics (i.e., age at diagnosis, glycated haemoglobin, autoantibody positivity) was tested by regression analysis.

Results: The PGS obtained by a Bayesian regression approach using 334 SNPs performed best. This PGS was associated with higher T1D prevalence (beta = 5.14, p-value = 1.1×10^{-3}) and higher presence of Zinc transporter 8 (ZnT8) autoantibodies (beta = 12.58, p-value = 1.6×10^{-2}). No association emerged with other clinical characteristics.

Conclusion: In conclusion, our study showed a polygenic contribution of MODY variants in T1D susceptibility, suggesting the existence of shared genetic characteristics between different forms of diabetes.

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Conflict of Interest: None declared

P17.045.B Genome-wide association study of SARS-CoV-2 neutralising antibody titres after COVID-19 vaccination reveals associations in HLA class I and II regions

Barbara Puzek[1;2], *Paul R. Wratil[3;4], Thu Giang Le Thi[1], Marcel Stern[3], Gaia Lupoli[3], Andreas Osterman[3;4], nadine fricker[5], Ana Zhelyazkova[6], Leandra Koletzko[7], Tarek Jebrini[8], Alexander Choukér[9], Berthold Koletzko[1], Helga P. Török[7], Sibylle Koletzko[1;10], Kristina Adorjan[11;12;13], Veit Hornung[14], Oliver T. Keppler, Prof. Dr. med.[3;4;14], Sarah Kim-Hellmuth[1;2]*

[1]Department of Pediatrics, Dr. von Hauner Children's Hospital, LMU University Hospital Munich, Munich, Germany; [2]Institute of Translational Genomics, Department of Computational Health, Helmholtz Munich, Munich, Germany; [3]Max Von Pettenkofer Institute and Gene Center, Virology, National Reference Center for Retroviruses, LMU Munich, Munich, Germany; [4]German Centre for Infection Research (DZIF), Partner Site, Munich, Germany; [5]Institute of Human Genetics, Division of Genomics, Life & Brain Research Centre, University Hospital of Bonn, Bonn, Germany; [6]Institute of Emergency Medicine and Management in Medicine, LMU University Hospital Munich, Munich, Germany; [7]Department of Medicine II, LMU University Hospital Munich, Munich, Germany; [8]Department of Psychiatry and Psychotherapy, Munich, Germany; [9]Department of Anesthesiology, Laboratory of Translational Research Stress and Immunity, LMU University Hospital Munich, Munich, Germany; [10]Department of Pediatrics, Gastroenterology and Nutrition, School of Medicine Collegium Medicum University of Warmia and Mazury, Olsztyn, Poland; [11]University Hospital of Psychiatry and Psychotherapy, University of Bern, Bern, Switzerland; [12]Institute of Psychiatric Phenomics and Genomics (IPPG), LMU University Hospital Munich, Munich, Germany; [13]Center for International Health (CIH), LMU Munich, Munich, Germany; [14]Gene Center and Department of Biochemistry, LMU Munich, Munich, Germany

Consortium: Riscoin Study Group

Background/Objectives: Understanding the genetics of the response to COVID-19 vaccines is crucial for comprehending immune response to SARS-CoV-2 and for developing future vaccination strategies. Recently, several studies reported genetic factors that contribute to the variability in the levels of antibodies against the SARS-CoV-2 spike protein produced after a COVID-19 vaccination. We investigated the genetic basis of the neutralising capacity against SARS-CoV-2 developed after receiving two doses of an mRNA COVID-19 vaccine.

Methods: We conducted a genome-wide association study of neutralising capacity against SARS-CoV-2 variant Omicron-BA.1 in the cohort of 3881 individuals fully vaccinated against COVID-19. In the analysis, we accounted for population structure, relatedness, age, sex, and medical history.

Results: Our study identified significant genetic associations with neutralising antibody titres in the human leukocyte antigen (HLA) class I and II regions. We found that, in particular, carrying the HLA-A*03 allele is associated with higher and carrying the HLA-DQB1*05, respectively, with lower neutralising capacity in our cohort.

Conclusion: Our study revealed genetic contributions to the neutralising capacity against Omicron-BA.1 developed after a COVID-19 vaccination. By highlighting the potential role of specific HLA alleles in neutralising titre variation, these findings contribute to our understanding of SARS-CoV-2 susceptibility, disease progression, and the potential for targeted interventions based on genetic profiles.

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Reckitt, Helga P. Török Abbvie, Biogen, BMS, Falk Foundation, Galapagos, Janssen-Cilag Pfizer, Pharmacosmos, Takeda Pharma, AbbVie, Calypso Biotech, Immunic Janssen-Cilag, Pharmacosmos, Sibylle Koletzko Abbvie, Danone, Janssen, Mead Johnson, Nestle Nutrition, Pfizer, Sanofi, and Takeda, Mead Johnson, Kristina Adorjan: None declared, Veit Hornung: None declared, Oliver T. Keppler, Prof. Dr. med.: None declared, Sarah Kim-Hellmuth: None declared

P17.046.C Mendelian randomization analysis provides evidence for causal relationships between retina image-derived phenotypes and non-ocular diseases and risk factors

Ilaria Iuliani[1;2], *Sofia Ortin Vela*[1;2], *Michael Beyeler*[1;2], *Olga Trofimova*[1;2], *Mattia Tomasoni*[3], *David Presby*[1;2], *Florence Hoogewoud*[3], *Sven Bergmann*[1;2;4]

[1]*University of Lausanne, Computational Biology, LAUSANNE, Switzerland;* [2]*Swiss Institute of Bioinformatics, LAUSANNE, Switzerland;* [3]*Jules-Gonin Eye Hospital, FAA, University of Lausanne, LAUSANNE, Switzerland;* [4]*University of Cape Town, Integrative Biomedical Sciences, Cape Town, South Africa*

Background/Objectives: While characterizing vasculatures is generally challenging, retinal imaging offers a cost-effective and noninvasive method for assessing the vascular system. Recent research suggests retinal vascular properties as potential biomarkers for systemic non-ocular diseases. Here we explore causal relationships between retinal vascular properties and various diseases and risk factors.

Methods: The UK Biobank dataset comprises more than 130k Color Fundus Images (CFIs) from nearly 72k genotyped subjects, each with information on the status of various diseases and their risk factors. We previously conducted an extensive analysis to extract image-derived phenotypes (IDPs) and performed genome-wide association studies (GWAS) of these phenotypes. Here we use bidirectional two-sample Mendelian Randomisation (MR) analysis to evaluate causal relationships between the vascular IDPs and non-ocular diseases such as hypertension, angina and diabetes along with their associated risk factors, including blood pressure, cholesterol level and BMI.

Results: Our MR analyses provide highly significant evidence that cardiovascular disease (CVD) risk factors have a causal impact on the retinal vasculature. Specifically, we reveal that CVD risk factors exert stronger effects on vascular IDPs than the reverse, underlining the utility of IDPs for early CVD diagnosis. Consistent with previous findings, we observe that individuals with genetically elevated blood pressure tend to have lower retinal vascular density. Other IDPs were also affected, indicating broader implications of blood pressure on retinal vasculature remodeling.

Conclusion: Our study demonstrates the potential of MR to identify causal links between retinal image features and CVD, emphasizing the broader applications of these features as potential disease biomarkers beyond ocular health.

Grants: SNSF 209510

Conflict of Interest: None declared

P17.047.D Genetic determinants of the plasma lipidome and relations to cardiometabolic risk in children and adolescents

Yun Huang[1], *Sara Stinson*[1], *Malte Thodberg*[1], *Louise Aas Holm*[1;2], *Roman Thielemann*[1], *karolina sulek*[3], *Morten Lund*[2;4], *Cilius Esmann Fonvig*[1;2;5], *Min Kim*[3], *Kajetan Trost*[1], *Helene Bæk Juel*[1], *Trine Nielsen*[5;6], *Peter Rossing*[3;5], *Maja Thiele*[7;8], *Aleksander Krag*[7;8], *Cristina Legido-Quigley*[3;9], *Jens-Christian Holm*[1;2;5], *Torben Hansen*[1]

[1]*Novo Nordisk Foundation Center for Basic Metabolic Research, University of Copenhagen, Denmark;* [2]*The Children's Obesity Clinic, Department of Pediatrics, Copenhagen University Hospital Holbæk, Denmark;* [3]*Steno Diabetes Center Copenhagen, Denmark;* [4]*Department of Biomedical Sciences, Faculty of Health and Medical Sciences, University of Copenhagen, Denmark;* [5]*Department of Clinical Medicine, Faculty of Health and Medical Sciences, University of Copenhagen, Denmark;* [6]*Medical Department, Zeeland University Hospital, Denmark;* [7]*Center for Liver Research, Department of Gastroenterology and Hepatology, Odense University Hospital, Denmark;* [8]*Department of Clinical Research, Faculty of Health Sciences, University of Southern Denmark, Denmark;* [9]*Institute of Pharmaceutical Science, King's College London, United Kingdom*

Background/Objectives: Several lipid species have been associated with type 2 diabetes, cardiovascular disease, and steatotic liver disease. While the genetic architecture of the lipidome has been investigated in adults, the genetic determinants of lipid species and their association with cardiometabolic risk in children and adolescents remains understudied.

Methods: We measured 227 plasma lipid species in 1,149 children and adolescents (44.8% boys) with a median age of 11.2 years from an obesity clinic and from the general population. We performed genome-wide association analyses to identify genetic variants influencing circulating lipids and assessed the causal impact of these lipids on cardiometabolic risk profiles using Mendelian randomization (MR).

Results: We identified 37 independent genome-wide significant loci ($P < 5 \times 10^{-8}$) for 52 lipid species, including loci associated with sphingolipids (*SPTLC3*, *SYNE2*, *ATP10D*), phosphatidylethanolamine (*LIPC*), phosphatidylinositol (*MBOAT7*), and 9 previously unreported loci. One-sample MR analysis using 5 loci with biological plausibility on individual-level data identified positive causal associations between ceramide and liver enzymes, sphingomyelin and hemoglobin A1c (HbA1c), and phosphatidylethanolamine and high-sensitivity C-reactive protein in children and adolescents. Two-sample MR using summary statistics showed consistent results with one-sample MR, and indicated additional causal links, specifically between ceramide and higher HbA1c levels, and phosphatidylinositol with higher liver enzymes.

Conclusion: Our results provide insights into the genetic determinants of plasma lipid species in children and adolescents, emphasizing causal links between specific risk lipids and cardiometabolic risk profiles, which may provide further insights into targeted intervention strategies.

Grants: The Innovation Fund Denmark (0603-00484B), The Novo Nordisk Foundation (NNF15OC0016544).

Conflict of Interest: None declared

P17.048.A Exploring the genetic architecture of Lipoedema.

Sara Dobbins[1], *Alan Pittman*[1], *Pia Østergaard*[1], *Ege Sackey*[1], *Vaughan Keeley*[2], *Peter Mortimer*[3], *Kristiana Gordon*[3], *Dionysios Grigoriadis*[4]

[1]*St. George's University of London, london;* [2]*University Hospitals of Derby and Burton NHS Foundation Trust, Derby, United Kingdom;* [3]*St George's Universities NHS Foundation trust, London, United Kingdom;* [4]*Constructive Bio, Cambridge, United Kingdom*

Background: Lipoedema is a chronic adipose tissue disorder mainly affecting women, causing excess subcutaneous fat deposition on the lower limbs with pain and tenderness. Whilst

the family history of patients suggests a strong genetic component, little is understood about the genetics underlying the susceptibility to this disease.

Methods: A tightly phenotyped cohort of 200 lipoedema patients from two UK specialist clinics was recruited. Firstly, to understand the impact of common variation on disease, we performed a genome wide association study (GWAS) on 130 cases with publicly available control data. Secondly, we performed whole-exome sequencing on over 100 patients (including 36 extended families). We employed family-based and gene-burden approaches to identify candidate genes with an excess of rare impactful variants in lipoedema patients compared to controls.

Results and Conclusions: Our analysis has identified a common variant upstream of the *LHFPL6* gene suggestively associated with lipoedema (rs1409440, ORmeta ≈ 2.01, Pmeta ≈ 4 × 10-6). This gene is involved with lipoma formation but exactly how this relates to lipoedema is not yet understood. Our gene burden and segregation analysis of the exome and whole genome sequencing data has identified several interesting candidate genes. We are in the process of performing a replication study of both our GWAS and gene-based findings on a well-phenotype Spanish cohort. Whilst further replication of these findings are required in different populations, we hope that this work will lead to new avenues of research in therapeutics and diagnostics, ultimately improving the lives of people with lipoedema.

Grants: Lipoedema Foundation

Conflict of Interest: None declared

P17.049.B postpartum psychosis: could chronic sleep deprivation be protective?

Chiara Petrosellini[1], *Sofia Eriksson*[1], *olivia protti*[1], *dimitrios siassakos*[1], *Andrew McQuillin*[1]

[1]*UCL, United Kingdom*

Background/Objectives: Postpartum Psychosis (PP) is a severe but treatable perinatal mental illness. It is a medical emergency due to the associated risks of suicide and infanticide.

Women with pre-existing Bipolar Affective Disorder (BPAD) have a significant risk of PP following childbirth, but prediction is challenging.

Sleep disturbance is a well-established trigger of psychosis and one of the earliest manifestations of PP. A relationship between sleep disruption and PP is assumed but poorly understood.

Methods: Women with BPAD were recruited from general practices, hospitals and community mental health teams in England. Participants who have given birth were screened for perinatal psychiatric complications. 150 women with BPAD who developed PP and 262 women who did not develop PP following childbirth were included.

Polygenic Risk Scores (PRS) were computed using summary statistics of Genome-Wide Association Studies of Insomnia (INS) and Short Sleep (SS). Logistic regression was used to model the effect of each PRS on PP following childbirth.

Results: Logistic regression analyses showed significant associations between sleep-related PRS scores and a diagnosis of PP.

INS PRS and SS PRS were significantly lower in women who developed PP compared to those who did not develop PP following childbirth ($p = 0.0035$ and $p = 0.000794$ respectively).

Conclusion: Genetic vulnerability to insomnia and short sleep contributes to an increased risk of chronic sleep deprivation. This may foster a heightened tolerance to sleep disruption, mitigating the impact of childbirth on mental health. A higher individual PRS for INS and/or SS may therefore be protective against PP in women with BPAD.

Grants: Rosetrees Trust
EGA Hospital Charity

Conflict of Interest: None declared

P17.050.C Identification of genetic regulation at the proteomic level in the long-term stroke outcome: A Proteome-Wide Association study

Natalia Cullell[1;2], *Cristina gallego*[2], *jara carcel-marquez*[2], *elena muiño*[2], *laia llucià-carol*[2], *miquel lledós*[2], *jesus martin-campos*[2], *Israel Fernandez-Cadenas*[2], *jerzy krupinski*[1]

[1]*fundacio docencia i recerca mutuaterrassa, neuroscience, 08221, Spain;* [2]*Biomedical Research Institute Sant Pau (IIB Sant Pau), Stroke Pharmacogenomics and Genetics group, Barcelona, Spain*

Background/Objectives: The stroke outcome is highly variable and depends on genetic. This study aims to pinpoint potential targets for enhancing long-term stroke outcomes integrating genomics with proteomics (PWAS) and transcriptomics (TWAS).

Methods: We followed a four-stage PWAS with FUSION to identify genetically regulated proteins impacting the modified Rankin scale at three months (mRS3). In the discovery, we merged brain proteomes from the ROS/MAP ($N = 376$;1443 proteins) and a mRS3 GWAS from the GODS cohort ($N = 1225$). We validated the causality with Bayesian colocalization and Summary-based-data Mendelian Randomization(SMR). In the replication1 and 2 the brain proteomic dataset from the Banner Sun Health Research Institute and a new GWAS of mRS3 (not published;$N = 688$), respectively, were analyzed. We meta-analyzed the results using the adaptively weighted Fisher's method. We conducted a TWAS to explore the association of 5,420 transcripts from the CommonMind Consortium (452 brain samples) with mRS3. To identify potential drugs modulating mRS3, we utilized Trans-Phar.

Results: Three proteins were significantly associated with mRS3 ($P < 0.05$) in the discovery with a posterior probability4 > 0.5. The three proteins were significant in the Replication1 and one in the replication2. Two out of the three proteins were significant after multiple testing in the meta-analysis, in the TWAS and had confirmed causality by SMR. Using Trans-Phar, we identified three compounds previously studied in stroke and predicted to modulate gene expression positively for the stroke outcome.

Conclusion: Using a multi-omic approach, we have identified potentially genetically-regulated drug targets whose modulation could lead to the improvement of long-term stroke outcome.

Grants: EPIGENESIS-PI17/02089,EPINEXO-PI20/00678(CarlosIIIInstitute/FEDER),RICORS Stroke network

Conflict of Interest: None declared

P17.051.D Investigating the association between atopic dermatitis severity and known susceptibility variants.

Katie Watts[1;2], *Samuel Lessard*[3], *Maris Teder-Laving*[4], *Karin Ytterberg*[5], *Laurent Thomas*[6], *Ben Brumpton*[6], *Kristian Hveem*[6], *Emanuele de Rinaldis*[3], *Katherine Klinger*[3], *Shameer Khader*[3], *Johannes Kettunen*[7], *Kaisa Tasanen-Määttä*[8;9], *Laura Huilaja*[8;9], *Chengliang Dai*[10], *Jake Saklatvala*[11], *Ravi Ramessur*[11], *Nick Dand*[11], *Sinead Langan*[12], *Külli Kingo*[13], *Clement Chatelain*[3], *Alexandra Hicks*[3], *pol Solé-Navais*[5], *Mari Løset*[6], *Catherine Smith*[11], *Sara Judith Brown*[14], *Lavinia Paternoster*[1;2]

[1]*University of Bristol, Medical Research Council Integrative Epidemiology Unit, Bristol Medical School, Bristol, United Kingdom;* [2]*University of Bristol, Population Health Sciences, Bristol Medical School, United Kingdom;* [3]*Sanofi US, Precision Medicine & Computational Biology, Cambridge, MA, United States;* [4]*University of Tartu,*

Institute of Genomics, Estonia; [5]University of Gothenburg, Department of Obstetrics and Gynecology, Institute of Clinical Sciences, Sahlgrenska Academy, Sweden; [6]Norwegian University of Science and Technology, K.G. Jebsen Center for Genetic Epidemiology, Department of Public Health and Nursing, Norway; [7]University of Oulu, Biocenter Oulu and Center for Life Course Health Research, Finland; [8]University Hospital of Oulu, Finland; [9]University of Oulu, Research Unit of Clinical Medicine, Finland; [10]UCB S.A, Slough, United Kingdom; [11]King's College London, St John's Institute of Dermatology, School of Basic & Medical Biosciences and Department of Medical & Molecular Genetics, United Kingdom; [12]London School of Hygiene and Tropical Medicine, Faculty of Epidemiology and Population Health, United Kingdom; [13]Faculty of Medicine, Institute of Clinical Medicine, University of Tartu, Estonia; [14]University of Edinburgh, Centre for Genomic and Experimental Medicine, United Kingdom

Consortium: BIOMAP consortium, FinnGen

Background/Objectives: Atopic dermatitis is a complex inflammatory skin and systemic disease that affects up to 10% of adults. Approximately 30% of cases can be classified as moderate or severe and for these patients, treatment is not always effective. Currently, there are no established genetic markers of atopic dermatitis severity, but these could be helpful to identify patients at risk of severe disease and in possibly directing new therapeutic strategies.

Methods: To identify such markers, we performed a meta-analysis of >11 million genetic variants using 20,275 patients with moderate-severe atopic dermatitis and 59,002 patients with non-moderate-severe atopic dermatitis from 5 European cohorts.

Results: Two previously established atopic dermatitis susceptibility loci (lead SNP rs558269137 in *FLG* and lead SNP rs1365121 in *PRR5L*) were associated at a genome-wide significance threshold. More broadly, we found that of the 81 known European susceptibility loci 14 showed an association with severity after correction for multiple testing ($p < 6.2 \times 10^{-4}$) and a further 29 at $p < 0.05$. After adjusting for the power differential between studies, these numbers increased to 15 and 43 loci respectively. Regression analysis also confirmed a positive relationship between severity betas and reported susceptibility betas across the 81 loci (beta = 0.36, 95% confidence intervals = 0.32-0.40).

Conclusion: Overall, these findings provide insight into the genetic basis of atopic dermatitis severity.

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Conflict of Interest: Katie Watts Part of a Innovative Medicines Initiative-European funded consortia (BIOMAP-IMI.eu) with multiple industry partners, Samuel Lessard Has stock or shares in Sanofi, Employee at Sanofi, Maris Teder-Laving: None declared, Karin Ytterberg: None declared, Laurent Thomas: None declared, Ben Brumpton: None declared, Kristian Hveem: None declared, Emanuele de Rinaldis Has stock or shares in Sanofi, Employee at Sanofi, Katherine Klinger Has stock or shares in Sanofi, Employee at Sanofi, Shameer Khader Has stocks or shares in Sanofi, Employee at Sanofi, Johannes Kettunen: None declared, Kaisa Tasanen-Määttä: None declared, Laura Huilaja: None declared, Chengliang Dai Employee at UCB, Jake Saklatvala Part of a Innovative Medicines Initiative-European funded consortia (BIOMAP-IMI.eu) with multiple industry partners, Ravi Ramessur Part of a Innovative Medicines Initiative-European funded consortia (BIOMAP-IMI.eu) with multiple industry partners, Nick Dand Part of a Innovative Medicines Initiative-European funded consortia (BIOMAP-IMI.eu) with multiple industry partners, Sinead Langan Part of a Innovative Medicines Initiative-European funded consortia (BIOMAP-IMI.eu) with multiple industry partners, Külli Kingo: None declared, Clement Chatelain Has stocks or shares in Sanofi, Employee at Sanofi, Alexandra Hicks Has stocks or shares in Sanofi, Part of a Innovative Medicines Initiative-European funded consortia (BIOMAP-IMI.eu) with multiple industry partners, Employee at Sanofi, pol Solé-Navais: None declared, Mari Løset: None declared, Catherine Smith Part of a Innovative Medicines Initiative-European funded consortia (BIOMAP-IMI.eu) with multiple industry partners, Sara Judith Brown Part of a Innovative Medicines Initiative-European funded consortia (BIOMAP-IMI.eu) with multiple industry partners, Lavinia Paternoster Part of a Innovative Medicines Initiative-European funded consortia (BIOMAP-IMI.eu) with multiple industry partners. Has undertaken research supported by Takeda Pharmaceuticals.

P17.052.A GWAS of neuropathic pain detects putative causal signal in *NCAM1*

Richard Packer[1;2], *Nick Shrine[1], Alexander T Williams[1], Jing Chen[1], Abril Izquierdo[1], Kayesha Coley[1], Mikko Martilla[3], William Hennah[3], Martin Tobin[1;2]*

[1]Department of Population Health Sciences, University of Leicester, Leicester; [2]Leicester NIHR Biomedical Research Centre, Glenfield Hospital, Leicester; [3]Orion Pharma, Espoo, Finland

Background/Objectives: Neuropathic pain (NP) is a chronic debilitating condition affecting 7-8% of the population. Currently therapy is limited with side-effects. Human genetic studies can identify putative causal genes to better identify new targets for drug development. However, genetic studies have reported only one locus, SLC25A3, at genome-wide significance and have not exploited the primary care records in large biobank studies to increase case ascertainment and power for detection.

Methods: We combined primary care prescription records, primary and secondary care clinical and operation codes and self-report data from UK Biobank and used DeepPheWAS to define 38 NP phenotypes, our primary aim was to perform a GWAS of all cause NP (34,278 cases, 140134 controls), in exploratory analysis we also performed GWAS on a further 23 NP phenotypes. We used SAIGEgds for association testing and a variant-to-gene mapping framework to identify putative causal genes.

Results: For all cause NP a single novel genome-wide ($< 5 \times 10^{-8}$) significant signal was found in *NCAM1* (rs12277922 risk-allele T). *NCAM1* met three variant-to-gene criteria, (nearest gene, pQTL colocalization and polygenic priority score). The same SNP in FinnGen was associated with increased risk of multiple back pain traits, pain in joint and analgesic medication use. Exploratory analysis revealed 5 genes with >1 variant-to-gene criteria: *MICU1*, *ASCC1*, *ROR1* in sciatica traits; *KCNN2* in post-surgical NP and; *HLA-DRB5* for central NP.

Conclusion: Our study provides robust evidence implicating several genes including *NCAM1* in NP risk warranting further investigation as potential drug targets.

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P17.054.C Genome-wide association study of dental caries and correlations with cardiometabolic traits

Evelina Stankevic[1], *Malte Thodberg[1], Frank Geller[2;3], Lars Ängquist[1], Christina Mikkelsen[1;2], Nikoline Nygaard[4], Merete Markvart[4], Jonas Ghouse[5;6], Karina Banasik[7], Erik Sørensen[2], Kári*

Stefánsson[8;9], Hreinn Stefansson[8], Niels Grarup[1], Daniel Belstrøm[4], Anja Olsen[10;11], Henning Bundgaard[12;13], Søren Brunak[7], Christian Erikstrup[14;15], Sisse Rye Ostrowski[2;12], Bjarke Feenstra[2;3;16], Ole Pedersen[12;17], Torben Hansen[1]

[1]Novo Nordisk Foundation Center for Basic Metabolic Research, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark; [2]Department of Clinical Immunology, Copenhagen University Hospital, Rigshospitalet, Copenhagen, Denmark; [3]Department of Epidemiology Research, Statens Serum Institut, Copenhagen, Denmark; [4]Department of Odontology, Section for Clinical Oral Microbiology, University of Copenhagen, Copenhagen, Denmark; [5]Laboratory for Molecular Cardiology, Department of Cardiology, Rigshospitalet, Copenhagen University Hospital, Copenhagen, Denmark; [6]Laboratory for Molecular Cardiology, Department of Biomedical Sciences, University of Copenhagen, Copenhagen, Denmark; [7]Translational Disease Systems Biology, Novo Nordisk Foundation Center for Protein Research, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark; [8]deCODE genetics/Amgen, Inc., Reykjavík, Iceland; [9]Faculty of Medicine, University of Iceland, Reykjavík, Iceland; [10]Department of Public Health, Aarhus University, Aarhus, Denmark; [11]Danish Cancer Society, Copenhagen, Denmark; [12]Department of Clinical Medicine, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark; [13]Department of Cardiology, Rigshospitalet, Copenhagen University Hospital, Copenhagen, Denmark; [14]Department of Clinical Immunology, Aarhus University Hospital, Aarhus, Denmark; [15]Department of Clinical Medicine, Aarhus University, Aarhus, Denmark; [16]Department of Biology, University of Copenhagen, Copenhagen, Denmark; [17]Department of Clinical Immunology, Zealand University Hospital, Køge, Denmark

Background/Objectives: Recent studies indicate a potential link between dental caries (DC) and cardiometabolic diseases, suggesting that shared genetic factors may play a role in influencing the development and progression of both conditions. Our objective was to map the genetic variations associated with DC and investigate the shared genetic influences between DC and cardiometabolic diseases.

Methods: We conducted a genome-wide association study of objectively measured childhood DC in permanent dentition in 72,139 individuals from the Danish Blood Donor Study Cohort. We used LD score regression to examine genetic correlations across cardiometabolic, oral, psychiatric and lifestyle traits. We performed fine-mapping to pinpoint causal variants, followed by colocalization to determine overlapping genetic signals between DC and correlated traits.

Results: We found eight genetic loci associated with DC ($P < 5 \times 10^{-8}$), six of which were previously unreported. Genetic correlations were identified between DC and several cardiometabolic traits, including type 2 diabetes ($R_g = 0.27$, $P = 3.4 \times 10^{-8}$), body mass index ($R_g = 0.24$, $P = 1.0 \times 10^{-7}$), coronary artery disease (CAD, $R_g = 0.18$, $P = 1.7 \times 10^{-4}$) and high-density lipoprotein (HDL, $R_g = -0.26$, $P = 3.1 \times 10^{-7}$). Colocalization suggested functional links between DC and CAD (a locus on chromosome 3 with PP.H4 = 0.97), and HDL (loci on chromosomes 4 and 22 with PP.H4 > 0.92).

Conclusion: We highlight the polygenic nature of DC and through identified genetic correlations and colocalization patterns suggest shared pathophysiological mechanisms between DC and cardiometabolic traits.

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Conflict of Interest: Evelina Stankevic: None declared, Malte Thodberg: None declared, Frank Geller: None declared, Lars Ängquist: None declared, Christina Mikkelsen: None declared, Nikoline Nygaard: None declared, Merete Markvart: None declared, Jonas Ghouse: None declared, Karina Banasik: None declared, Erik Sørensen: None declared, Kári Stefánsson Employee of deCODE Genetics/Amgen Inc., Hreinn Stefansson Employee of deCODE Genetics/Amgen Inc., Niels Grarup: None declared, Daniel Belstrøm: None declared, Anja Olsen: None declared, Henning Bundgaard: None declared, Søren Brunak: None declared, Christian Erikstrup: None declared, Sisse Rye Ostrowski: None declared, Bjarke Feenstra: None declared, Ole Pedersen: None declared, Torben Hansen: None declared

P17.055.D GWAS meta-analysis on Atrial Septal Defect identifies 7 novel loci

Sofie Dannesbo[1], Søren Rand[2], Oliver Vad[1], Jonas Ghouse[1], Kasper Iversen[1], Henning Bundgaard[3], Lars Allan Larsen[4], **Morten Olesen**[5]

[1]Copenhagen University Hospital, Rigshospitalet, Department of Cardiology; [2]Copenhagen University Hospital, Rigshospitalet; [3]Copenhagen University Hospital, Rigshospitalet, Department of Cardiology, København, Denmark; [4]Copenhagen University; [5]Copenhagen University, Department of Biomedical Sciences

Background: Atrial septal defect (ASD) is one of the most common types of congenital heart defects, occurring in about 25% of children. ASD patients have an increased risk of cardioembolic stroke, atrial fibrillation, heart failure, and increased long-term mortality.

Material and Methods: We performed the largest GWAS meta-analysis of ASD to date using 2,763 ASD cases from five patient cohorts and more than 1,000,000 controls. Genetic correlation was assessed using linkage disequilibrium score regression (LDSC).

Results: Our analysis identified nine genome-wide significant loci ($P < 5 \times 10^{-8}$) that are associated with

ASD, of which seven were novel. The top hit loci were located near NSG1 (rs870142, $P = 2.0 \times 10^{-24}$). The second most significant hit was rs76774446 near the gene WNT3 ($P = 2.3 \times 10^{-19}$). This gene plays a critical role in development of the heart before birth. We also identified a locus on chr 8 close to the transcription factor SOX17 ($p = 8.8 \times 10^{-10}$) and a loci on chr 10 ($p = 5.6 \times 10^{-09}$) close to NRP1, encoding a VEGF receptor. Haploinsufficiency of VEGF related signaling genes predisposes to the development of Tetralogy of Fallot.

Using LDSC we found significant genetic correlation between ASD and right ventricular end-systolic volume ($rg = 0.42$, $P = 6.8 \times 10^{-5}$) and cardioembolic stroke ($rg = 0.50$, $p = 3.6 \times 10^{-3}$). In line with this a ASD PRS-cs score was associated with dysregulation of more than 10 proteins in the blood ($p < 1 \times 10^{-8}$).

Conclusion: This GWAS meta-analysis identified seven novel and two known genetic variants associated with ASD. Our findings contribute to understanding the genetic basis of ASD and underlying molecular mechanisms.

Conflict of Interest: None declared

P17.056.A Clinical and etiological differences between alopecia areata patients with and without nail abnormalities

Yasmina Gossmann[1], *Marisol Herrera Rivero[2], swapnil Awasthi[3], stephan ripke[3], Silke Redler[4], Markus Nöthen[1], Regina C. Betz[1], F. Buket Basmanav[1]*

[1]Institut für Humangenetik der Universität Bonn, Universitätsklinikum Bonn, Bonn, Germany; [2]Klinik für Psychiatrie und Psychotherapie, Universitätsklinikum Münster, Münster, Germany; [3]Klinik für Psychiatrie und Psychotherapie, Charité Universitätsmedizin Berlin, Berlin, Germany; [4]Institut für Humangenetik und Anthropologie, Universitäts-Klinikum Düsseldorf, Düsseldorf, Germany

Background/Objectives: Alopecia areata (AA) is a common autoimmune hair loss disease with a lifetime prevalence of 2%. AA can be accompanied by nail abnormalities in 10-66% of patients according to previous studies. In the current study, we investigated i) the prevalence of nail abnormalities in AA patients, ii) associations between the presence of a nail phenotype and clinical parameters of AA and iii) genetic factors associated with the manifestation of nail phenotypes in AA patients using our own cohort data from about 3,000 patients.

Methods: We stratified our patients based on the presence of nail abnormalities and analyzed whether clinical parameters of AA differs between patients with vs. without nail abnormalities using binary logistic regression analysis. The genome-wide association study (GWAS) was performed by Plink 1.9 on a set of 7,202,815 variants using a logistic regression model.

Results: In our cohort, 762 patients (43%) presented with nail abnormalities compared to 1,131 who did not have any nail phenotype. Onset and severity of AA and comorbidity status of AA patients were significantly associated with the manifestation of nail abnormalities. Our GWAS resulted in 12 suggestive loci ($p < 10^{-5}$; top-SNP = 7.4e-7) associated with developing nail phenotypes in AA, implicating altogether 20 genes based on physical mapping and expression quantitative trait loci annotations.

Conclusion: Our results suggest that the clinical presentation of AA shows differences between patients with and without nail abnormalities and that genetic factors play a role in the risk of AA patients to develop an accompanying nail phenotype.

Conflict of Interest: None declared

P17.058.C Genetic architecture of adipose tissue fatty acids identifies local regulatory effects

Xinyu Yan[1], *Sergio Villicaña*[1], *Maxim Freidin*[2], *Amy L. Roberts*[1], *Max Tomlinson*[1], *Julia El-Sayed Moustafa*[1], *Jordana T Bell*[1], *Kerrin S Small*[1]

[1]*King's College London, Department of Twin Research & Genetic Epidemiology, London, United Kingdom;* [2]*Queen Mary University of London, Department of Biology, London, United Kingdom*

Background: Fatty acids (FAs) are essential molecules, which function as structural components, energy sources, and signaling mediators. Subcutaneous adipose tissue (SAT) is the largest fat depot and plays a crucial role in maintaining health and homeostasis. While genetic regulation and health impacts of circulating FA levels are well established, less is known about the genetic regulation of FA levels within adipose tissue itself.

Methods: To address this, we measured 18 FAs in SAT from 569 female twins in the TwinsUK cohort.

Results: Heritability of FAs ranged from 5% to 59%, indicating a substantial genetic regulation of adipose FA levels, with arachidonic acid showing the highest heritability. GWAS identified 10 FA-associated *loci* across 13 fatty acids (i.e. palmitic acid/palmitoleic acid - *SCD*, dihomo-γ-linolenic acid/arachidonic acid - *FADS2*). Using concurrently measured adipose gene expression and methylation, we identified colocalizations between FA GWAS *loci* and expressions of *SCD, FADS1* and *FEN1* and methylation of 17 CpGs, suggesting these FAs are regulated by molecular processes within SAT. To explore links between genetic regulation of adipose FAs and cardio-metabolic traits, we 1) performed PheWAS for the 10 GWAS lead SNPs, and identified strong associations to diabetes, body fat percentage, and cardiovascular disease and 2) tested associations between polygenic scores (PGSs) of cardiometabolic traits and FA levels, identifying strong associations between PGSs of BMI, body-fat distribution and triglycerides and a range of FAs like lauric acid, palmitoleic acid and arachidonic acid (FDR 5%).

Conclusion: Overall, our results identify local genetic regulation of these key molecules within an important metabolic tissue.

Grants: No.

Conflict of Interest: None declared

P17.059.D isGWAS: ultra-high-throughput, scalable and equitable inference of genetic associations with disease

Chris Foley[1;2], **Zhana Kuncheva**[1;2], *Soroosh Afyouni*[1;2], *riccardo marioni*[3], *Heiko Runz*[4], *Benjamin B. Sun*[4]

[1]*Optima Partners, United Kingdom;* [2]*The Bayes Centre, The University of Edinburgh, United Kingdom;* [3]*University of Edinburgh, United Kingdom;* [4]*Biogen, Cambridge, United States*

Background/Objectives: Genome-wide association studies (GWAS) have proven a powerful tool for human geneticists to generate biological insights or hypotheses for drug discovery. Nevertheless, a dependency on sensitive individual-level data together with ever-increasing cohort sample sizes, numbers of variants and phenotypes studied put a strain on existing algorithms, limiting the GWAS approach from maximising potential.

Methods: Here we present in-silico GWAS (isGWAS), a uniquely scalable algorithm to infer regression parameters in case-control GWAS from cohort-level summary data.

Results: For any sample size, isGWAS computes a variant-disease association parameter in ~1 millisecond, or ~11m variants in UK-Biobank within ~4 minutes (~1500-fold faster than state-of-the-art). Extensive simulations and empirical tests demonstrate that isGWAS results are highly comparable to traditional regression-based approaches. We further introduce a heuristic re-sampling algorithm, leapfrog re-sampler (LRS), to extrapolate association results to semi-virtually enlarged cohorts.

Conclusion: Owing to significant computational gains we anticipate a broad use of isGWAS and LRS which are customizable on a web interface.

Grants:

Conflict of Interest: None declared

P17.060.A Association between rare coding variant burden and participation in cognitive testing in the UK Biobank

Eilidh Fenner[1], *Elliott Rees*[1], *James Walters*[1]

[1]*Cardiff University, Centre for Neuropsychiatric Genetics and Genomics, Cardiff, United Kingdom*

Background/Objectives: The UK Biobank (UKBB) is subject to participation bias, whereby individuals who took part in optional components of the study, such as online questionnaires, are healthier and more educated than those who did not complete these components. Here, we analysed exome sequencing data from 200,625 UKBB samples to examine whether damaging rare coding variant (RCV) burden is associated with likelihood of participation in cognitive testing.

Methods: We investigated 3 phenotypes reflecting increasing levels of participation, examining individuals who: participated in baseline cognitive testing (n = 168,767); completed at least one cognitive test from an online test battery (n = 47,318); completed sufficient cognitive tests for the derivation of a generalised cognition score (*g*)(n = 30,481).

Results: For each phenotype, individuals who participated carried significantly fewer RCVs in constrained (pLI ≥ 0.9), brain-expressed, and schizophrenia-associated genes than those who did not participate. Additionally, individuals with high levels of participation (those for whom *g* could be derived) displayed

significantly higher scores on a cognitive test measuring fluid intelligence than individuals with lower levels of participation (those for whom *g* could not be derived).

Conclusion: Our results demonstrate that RCVs contribute to participation in cognitive testing in the UKBB, with individuals who completed optional cognitive tests carrying significantly fewer damaging RCVs. This effect may confound genetic studies of cognition in UKBB, where false negative associations may be reported for variants with strong effects on cognition. Our study underscores the need for careful consideration of participation-related factors in genetic studies of cognition in UKBB.

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Conflict of Interest: Eilidh Fenner: None declared, Elliott Rees ER and JTRW reported receiving grants from Akrivia Health outside the submitted work. JTRW reported receiving grants from Takeda Pharmaceutical Company Ltd outside the submitted work. Takeda and Akrivia played no part in the conception, design, implementation, or interpretation of this study., James Walters ER and JTRW reported receiving grants from Akrivia Health outside the submitted work. JTRW reported receiving grants from Takeda Pharmaceutical Company Ltd outside the submitted work. Takeda and Akrivia played no part in the conception, design, implementation, or interpretation of this study.

P17.061.B Genome-wide landscape of blood traits pleiotropy

Arianna Landini[1], *Sodbo Sharapov*[1], *Nicola Pirastu*[1], *Nicole Soranzo*[1;2;3]

[1]*Human Technopole, Italy;* [2]*British Heart Foundation Centre of Research Excellence, University of Cambridge, Cambridge, United Kingdom;* [3]*Human Genetics Department, Wellcome Sanger Institute (WT), Hinxton, United Kingdom*

Background/Objectives: The regulation of blood cell counts results from the complex interplay between genetics and physiological status. Despite previous studies uncovered numerous genetic associations, distinguishing between cell type-specific and shared signal remains unclear. We address this gap by exploring the pleiotropic landscape of 29 blood cell count traits.

Methods: We performed GWAS of TOPMed-imputed genotypes from ~350k UK Biobank (UKBB) individuals of European ancestry. We explored the landscape of pleiotropy by using leave-one-out conditional approach, obtaining signals with a single underlying causal SNP, followed by Bayesian colocalisation analysis.

Results: We identified 13,027 significant trait-loci pairs (p-value < 5e-8, MAF > 1e-4), corresponding to 1328 overlapping genomic regions. Among these, 247 were trait-specific, while the remaining were associated with multiple traits, up to 13 linked to all 29 traits. Conditional analysis revealed 23,904 independent association signals, which were grouped in 4435 colocalisation groups containing few signals specific to a single trait (3283). Despite the high level of pleiotropy, about half (42.5%) of these groups involved only two traits, while few (1.3%) encompassed over half of the 29 blood traits. Individual signals near *ABO* and *SLC7A5* influenced 25 traits with different patterns. While the firsts showed concordant effects across most traits, the seconds showed divergent effects (increasing red blood cells and platelets while decreasing white blood cells), suggesting different roles in blood cells regulation.

Conclusion: These results deepen our understanding of blood cell genetic regulation, highlighting gene and locus pleiotropy and intricate local regulation, likely reflecting differences in variant activity across cell types and along the differentiation process.

Conflict of Interest: None declared

P17.062.C Genome-wide association study for resting electrocardiogram traits using whole genome sequence in Middle Eastern populations

Muhammad Khan[1], *Abdullah Shaar*[1], *Khalid Kunji*[1], *Mohamed Elshrif*[1], *Ehsan Ullah*[1], *Dr. Georges Nemer*[2], *Mohammed Ali*[3], *Mohammed Bashir*[3], **Mohamad Saad**[1]

[1]*Qatar Computing Research Institute, Hamad Bin Khalifa University;* [2]*College of Health and Life Sciences, Hamad Bin Khalifa University;* [3]*Hamad Medical Corporation*

Background/Objectives: Electrocardiogram (ECG) measures are important to ensure individuals receive appropriate treatment for life threatening conditions (e.g. long QT syndrome). In genomic medicine, Middle East (ME) populations are underrepresented. Our aim here is to study the genetic architeture of ECG traits in the Middle East.

Methods: Genome-wide association study was conducted for ECG traits (i.e., RR, PR, QRS, QTc, JT, and P wave) in an ME cohort of ~14,000 Qataris with whole genome sequence data. The performance of existing polygenic risk scores (PRS) was evaluated, and novel PRS were developed.

Results: Seventy-four independent loci were obtained ($P < 5 \times 10^{-8}$): RR (1 locus), PR (19 loci), QRS (8 loci), QTc (21 loci), JT (21 loci), and P wave (4 loci). Out of 74 loci, 6 (8.1%) were novel: PR (*STAC* and *CSMD1*), QRS (*ANK1* and *NCOA2*), QTc (*LSP1*), and P wave (*MKLN1*). All PRS were significantly associated with the corresponding ECG traits, with the least significant showing a $P = 3.89 \times 10^{-15}$. PGS002121 was the best PRS for RR ($R^2 = 0.008$, $P = 8.54 \times 10^{-27}$), PGS000905 for PR ($R^2 = 0.053$, $P = 2.57 \times 10^{-165}$), PGS002166 for QRS ($R^2 = 0.024$, $P = 1.53 \times 10^{-75}$), PGS002276 for QTc ($R^2 = 0.059$, $P = 4.83 \times 10^{-185}$), and PGS001902 for P wave ($R^2 = 0.089$, $P = 4.19 \times 10^{-25}$). Local PRSs substantially outperformed the existing ones (e.g., 1.3% increase of R^2 for QTc).

Conclusion: In this study, we confirmed many known ECG loci, and identified novel loci for ME populations. "European" PRS performed well in our cohort, but local PRS showed the best performance.

Grants: PPM 06-0511-230027

Conflict of Interest: None declared

P17.063.D Dissecting the *LCT* selective sweep: insights into the genetics of T2D heterogeneity

Bethany Quinton[1], *Philip Zahariev*[1], *winston lau*[1], *Toby Andrew*[2], *dallas swallow*[1], *nikolas maniatis*[1]

[1]*University College London, GEE, United Kingdom;* [2]*Imperial College London, Metabolism, Digestion, and Reproduction, London, United Kingdom*

Background/Objectives: The region surrounding the *LCT* gene shows the strongest signature of positive selection in Europeans due to selection for lactase persistence (LP-rs4988235). A non-coding microRNA (miRNA-128) concealed behind this sweep has been associated with metabolic diseases. Others have associated rs4988235, or additional SNPs in this long region of strong linkage disequilibrium (LD), with obesity or more controversially with type 2 diabetes (T2D). miRNA-128, 186kb away from LP-rs4988235, is claimed to have a functional role in T2D but GWAS meta-analyses support no such association.

Methods: We shed light on the genetics of T2D in the LCT region and identify target cis-genes by studying the haplotype structure in T2D cases/controls with recorded BMI, stratifying by

LP haplotypes, and mapping directly on LD maps which can precisely locate and integrate disease and eQTL mapping results.

Results: Using European LD maps shows no strong signal for either T2D or BMI when comparing all T2D cases against controls, but crucially a significant signal was detected when comparing obese and lean diabetics. It is located 850kb from rs4988235 and is enriched with significant SNPs conferring risk (OR>2) for high BMI in diabetics. Furthermore, haplotypes carrying the risk allele from these SNPs and the LP allele confer an increase in BMI in diabetics. In silico analysis identified a number of cis-genes using adipose and skeletal muscle expression.

Conclusion: LP-rs4988235 is not causal. Instead, the identified T2D-associated eQTLs are implicated in dysregulating expression levels of cis-genes which appear to play a role in the disease pathway.

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Conflict of Interest: Bethany Quinton: None declared, Philip Zahariev: None declared, winston lau University College London, Toby Andrew Medical Research Council, Imperial College London, dallas swallow: None declared, nikolas maniatis Medical Research Council, University College London

P17.064.A Epigenetics of the circulating proteome in Generation Scotland

Josephine Robertson[1], *Spyros Vernardis*[2;3], *Camilla Drake*[4], *danni gadd*[1], *Archie Campbell*[1], *David Porteous*[1], *andrew mcintosh*[1;5], *Jamie Pearce*[6], *Tom Russ*[5;7], *Simon Cox*[8], *caroline hayward*[1;4], *Aleksej Zelezniak*[2;3], *daniel mccartney*[1], *Markus Ralser*[2;3], *riccardo marioni*[1]

[1]*University of Edinburgh, Centre for Genomic and Experimental Medicine, Institute of Genetics and Cancer, United Kingdom;* [2]*The Francis Crick Institute, London, United Kingdom;* [3]*Eliptica Limited, London;* [4]*University of Edinburgh, MRC Human Genetics Unit, Institute of Genetics and Cancer, United Kingdom;* [5]*University of Edinburgh, Division of Psychiatry, Centre for Clinical Brain Sciences, United Kingdom;* [6]*University of Edinburgh, Centre for Research on Environment, Society and Health, School of Geosciences, United Kingdom;* [7]*University of Edinburgh, Alzheimer Scotland Dementia Research Centre, Department of Psychology, United Kingdom;* [8]*University of Edinburgh, Lothian Birth Cohorts, Department of Psychology, United Kingdom*

Background/Objectives: The molecular architecture of proteome regulation is incompletely understood. Epigenetic changes such as DNA methylation are likely to play a crucial role; this can be explored via epigenome-wide association studies (EWAS) of the circulating proteome[1, 2]. To date, no large-scale EWASs have included proteins measured by mass spectrometry (MS).

Methods: An EWAS of 439 circulating MS proteins and peptides was performed in 14,761 individuals from Generation Scotland[3]. MS proteomics data were generated using a redesigned high-throughput approach[4]. Methylation was measured in baseline blood samples, using the Infinium HumanMethylationEPIC BeadChip[5]. An EWAS for each protein was carried out using OmicS-data-based Complex trait Analysis to perform iterative linear regression with covariates including technical factors, age, sex, white cell proportions, body mass index and smoking[6].

Results: 133 of the 439 signals were mapped to individual proteins, predominantly involved in inflammatory and immune responses, blood coagulation and lipid metabolism. Iterative linear models resulted in a full EWAS model, revealing 113,114 significant CpG – protein (p-Bonferroni < 8.2e-11) associations for 120 mapped proteins, with a median genomic inflation factor of 1.3.

Conclusion: Our large-scale EWAS of the circulating proteome, as measured by MS, reveals complex relationships between the epigenome and circulating proteome. We will further refine the model, trialling different methods to account for CpG intercorrelations, prior to gene/pathway enrichment analysis and causal inference analyses with disease outcomes.

Grants: ECAT-Plus/Wellcome Trust PhD Training Fellowship for Clinicians

Conflict of Interest: None declared

P17.066.C Novel loci associated with increased adiposity and lower cardio-metabolic risk in the UK Biobank

Nathalie Chami[1;2], *Zhe Wang*[1;2], *Alexander Rauch*[3;4], *Michael Preuss*[1], *Roelof A.J. Smit*[1;5], *Ruth Loos*[1;2;5]

[1]*Icahn School of Medicine at Mount Sinai, New York, United States;* [2]*The Mindich Child Health and Development Institute, Icahn School of Medicine at Mount Sinai, New York, United States;* [3]*Functional Genomics & Metabolism Research Unit, Department of Biochemistry and Molecular Biology,University of Southern Denmark, Odense, Denmark;* [4]*Molecular Endocrinology & Stem Cell Research Unit, Department of Endocrinology and Metabolism, Odense University Hospital and Steno Diabetes Center Odense and Department of Clinical Research,University of Southern Denmark, Odense, Denmark;* [5]*The Novo Nordisk Foundation Center for Basic Metabolic Research, Faculty of Health and Medicine, University of Copenhagen, København, Denmark*

Background/Objectives: Individuals with obesity are at a higher risk of metabolic outcomes, such as type 2 diabetes and cardiovascular disease. However, some do not suffer from these conditions. We aimed to identify genetic loci that contribute to the uncoupling of obesity from cardiometabolic co-morbidities.

Methods: We leveraged individual-level data of the UK Biobank and performed GWAS analyses on 35 adiposity and cardiometabolic risk factors.

Results: We identified 266 variants associated with increased adiposity, but with protective cardiometabolic effects. We replicate previously known loci including *IRS1, PPARG, VEGFA, and KLF14*. In a PheWAS analysis, a GRS of the 266 variants ($GRS_{uncoupling}$) was associated with increased risk of obesity ($OR = 1.16$, $P < 4 \times 10\text{-}108$), but a decreased risk of cardiometabolic outcomes, including diabetes ($OR = 0.94$, $P = 5.56 \times 10\text{-}21$) and ischemic heart disease ($OR = 0.96$, $P = 7.38 \times 10\text{-}11$). The 266 loci were validated in the Bio*Me* Biobank ($N_{samples} = 23k$); The $GRS_{uncoupling}$ successfully identified individuals with higher BMI and better cardiometabolic profile in Bio*Me*. Specifically, individuals in the top decile of the $GRS_{uncoupling}$ had higher BMI but lower triglycerides, LDL and HbA1C and higher HDL compared to individuals in the lowest decile. Clustering analyses grouped the 266 variants into eight clusters, each with a distinct association signature. Our gene prioritization analyses prioritized genes that play a role in metabolic regulation and adipogenesis, signaling and developmental processes, and intracellular transport and cholesterol regulation.

Conclusion: In summary, we identified 266 variants that uncouple adiposity from cardiometabolic outcomes and cluster into eight subsets with distinct cardiometabolic risk profiles.

Conflict of Interest: None declared

P17.067.D Genetic analysis of fibrotic multi-morbidity across multiple ancestry groups

Ebrima Joof[1], *Georgie Massen*[2], *Olivia C. Leavy*[1], *Gina Parcesepe*[1], *Iain Stewart*[3], *Guruprasad Aithal*[4], *Chris J. Scotton*[5], *Dorothee Auer*[4],

Susan Francis[4], Gisli Jenkins[3], Jennifer Quint[2], Louise Wain[1], Richard Allen[1]

[1]University of Leicester, Department of Population Health Sciences, Leicester, United Kingdom; [2]Imperial College London, School of Public health, London, United Kingdom; [3]Imperial College London, National Heart & Lung Institute, London, United Kingdom; [4]University of Nottingham, NIHR Nottingham Biomedical Research Centre, Nottingham, United Kingdom; [5]University of Exeter, Respiratory Medicine, Exeter, United Kingdom

Consortium: The DEMISTIFI consortium

Background/Objectives: Fibrosis is a major factor in global mortality that can affect most organ systems. The incidence of fibrotic diseases in many different organ systems hinders the development of targeted therapies. A potential strategy to overcome this challenge is to target genetic mechanisms and pathways associated with shared fibrosis pathology or fibrotic multi-morbidity (FMM).

Methods: We included unrelated individuals of four ancestry groups (European, African, East-Asian and South-Asian) in UK Biobank (a population cohort of 500,000 individuals). Fibrotic diseases, as defined by a published consensus list, were assigned to one of 11 organs or systems (Respiratory, Liver, Biliary, Cardiovascular, Intestinal-Pancreatic, Integumentary, Skeletal, Systemic, Reproductive, Urinary and Diabetes). Individuals with records of fibrotic disease in more than one organ/system were defined as FMM cases, and all others defined as controls. We then performed a genome-wide association study to test the association between each genetic variant across the genome with FMM separately for each ancestry group. Genetic signals that reached genome-wide significance ($p < 5 \times 10^{-8}$) were considered associated with FMM.

Results: There were 11 independent signals associated with FMM susceptibility in Europeans, including one located near the *DCLRE1B* gene which is linked to telomere maintenance and protection. One signal was associated with FMM in Africans, two signals in East-Asians and no signals reached genome-wide significance in South-Asians.

Conclusion: Functional follow-up of these FMM signals may reveal potential causal pathways of general fibrosis which can be leveraged in the development of new therapies for those affected by fibrosis.

Grants: The DEMISTIFI consortium; Grant No: MR/W014491/1.

Conflict of Interest: None declared

P17.068.A Imaging genetics of language network functional connectivity reveals links with language-related abilities, dyslexia and handedness

Jitse S. Amelink[1], *Merel C. Postema[1], Xiang-Zhen Kong[2], Dick Schijven[1], Amaia Carrion Castillo[1], Sourena Soheili-Nezhad[1], Zhiqiang Sha[1], Barbara Molz[1], Marc Joliot[3], Simon E. Fisher[1], Clyde Francks[1]*

[1]Max Planck Institute for Psycholinguistics, Language & Genetics, Nijmegen, Netherlands; [2]Zhejiang University, Psychology, Hang Zhou Shi, China; [3]University of Bordeaux, Bordeaux cedex, France

Background/Objectives: Language is supported by a distributed network of brain regions with a particular contribution from the left hemisphere. A multi-level understanding of this network requires studying its genetic architecture.

Methods: We used resting-state imaging data from 29,681 participants (UK Biobank) to measure connectivity between 18 left-hemisphere regions involved in multimodal sentence-level processing, as well as their right-hemisphere homotopes, and interhemispheric connections. We used multivariate genome-wide association analysis, polygenic scores and a gene-based exome-wide association analysis to study the genetic architecture of these traits.

Results: Multivariate genome-wide association analysis of this total network, based on genetic variants with population frequencies > 1%, identified 14 genomic loci, of which three were also associated with asymmetry of intrahemispheric connectivity. Polygenic dispositions to lower language-related abilities, dyslexia and left-handedness were associated with generally reduced leftward asymmetry of functional connectivity. Exome-wide association analysis based on rare, protein-altering variants (frequencies ≤ 1%) suggested 7 additional genes.

Conclusion: These findings shed new light on genetic contributions to language network organization and related behavioural traits. We would like to thank the research participants and employees of 23andMe, Inc. for providing the dyslexia genome-wide summary statistics.

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Conflict of Interest: None declared

P17.069.B Unique genome-wide methylation patterns within HIV disease

Thilona Arumugam[1], *Veron Ramsuran[1], Werner Smidt[1]*

[1]University of KwaZulu-Natal, Durban, South Africa

Background/Objectives: DNA methylation-mediated gene regulation has been recently associated with host-virus interaction. Previously, HIV was shown to alter DNA methylation profiles of the host. However, these studies examined unrelated individuals which failed to take into consideration the effects of genetic and lifestyle factors on DNA methylation. Thus, this aimed to determine the effects of DNA methylation on HIV pathophysiology in a longitudinal cohort.

Method: In this study we explored a rare cohort of females ($n = 120$) followed up over 15 years from pre-HIV infection to post-HIV infection. Using this unique cohort, we explored the role genome-wide methylation has on HIV infection and disease progression, using the Infinium MethylationEpic v2.0 array.

Results: We report unique DNA methylation patterns from the HIV-negative timepoint compared to the HIV-positive timepoint in the same patients. We identified 8 DMPs across pre-infection and post-infection and 14,274 DMPs between pre-treatment and post-treatment timepoints. Gene Ontology enrichment was performed on this module using the genes linked to the CpG probes, which revealed associations with Interleukin-1 regulation, viral entry/viral processes, histone modifications and B-cell proliferation.

Conclusion: Data from this study shows that genome methylation patterns vary across different stages of HIV infection and that these methylation patterns might have an impact on controlling HIV infection.

Grants:

1. South African Medical Research Council
2. Royal Society
3. Loreal Unesco Women in Science

Conflict of Interest: None declared

P17.070.C A phenome-wide association study of tandem repeat variation in 168,554 individuals from the UK Biobank

Celine Manigbas[1], *Bharati Jadhav*[1], *Paras Garg*[1], *Mariya Shadrina*[1], *William Lee*[1], *Andrew Sharp*[1], *Alejandro Martin Trujillo*[1]

[1]*Icahn School of Medicine at Mount Sinai, Department of Genetics and Genomic Sciences and Mindich Child Health and Development, New York, United States*

Most genetic association studies focus on binary variants, typically ignoring other variant types that could contribute to the "missing heritability" of the genome. We hypothesized that common length polymorphisms in TR tandem repeats (TRs), a multi-allelic variant not assayed in standard genetic studies, can modulate phenotypic variation of human traits. To investigate this, we performed direct TR genotyping and phenome-wide association studies for 30,291 traits and 36,085 TRs in 168,554 individuals with genome sequencing from the UK Biobank.

We identified 47 TRs that showed causal associations with 73 traits based on fine-mapping and conditional analysis. We replicated 23 of 31 (74%) of these causal associations with matching traits in the All of Us cohort. While this set included several known repeat expansion disorders, novel associations we identified were attributable to common polymorphic variation in TR length, rather than rare expansions. Notable findings include a coding polyhistidine motif in *HRCT1* influencing risk of hypertension which showed a strong negative causal association with the incidence of high blood pressure ($p = 4.1 \times 10^{-24}$) and the use of blood pressure medications ($p = 2.9 \times 10^{-14}$). Causal TRs were strongly enriched for associations with local gene expression and DNA methylation, supporting their causality and providing insights into the molecular mechanism by which TRs impact the associated phenotype.

In conclusion, our study provides a comprehensive evaluation of the impact of TR variation on human traits along with highlighting the importance of including TRs in future genetic association studies.

Conflict of Interest: None declared

P17.071.D Identifying and mapping genetic determinants associated with hepatitis B virus infection susceptibility in Uganda

IRENE NAMARA[1;2;3], *Allan Kalungi*[1;2], *Tafadzwa Machipisa*[1;4;5;6;7], *Richard Mayanja*[1;2;3], *Christopher Kintu*[1;2;3], *Daudi JJingo*[2;3], *Ronald Galiwango*[2;3], *Manuel Corpas*[8;9;10], *Segun Fatumo*[1;11;12;13]

[1]*MRC/UVRI & LSHTM Uganda Research Unit, The African Computational Genomics (TACG) Research Group, Entebbe, Uganda;* [2]*Makerere University, College of Health Sciences, Kampala, Uganda;* [3]*Makerere University, African Centre of Excellence in Bioinformatics and Data science, Kampala, Uganda;* [4]*University of Pennsylvania, Department of Genetics, Perelman School of Medicine, Philadelphia, United States;* [5]*University of Pennsylvania, Penn Center for Global Genomics & Health Equity, Perelman School of Medicine, Philadelphia, United States;* [6]*David Braley Cardiac, Vascular and Stroke Research Institute, Clinical Research Laboratory-Genetic and Molecular Epidemiology Laboratory (CRLB-GMEL), Population Health Research Institute (PHRI) & McMaster University, Hamilton, Ontario, Canada;* [7]*University of Cape Town & Groote Schuur Hospital, Department of Medicine, Capetown, South Africa;* [8]*University of Cambridge Biomedical Innovation Hub, Cambridge Precision Medicine Limited, ideaSpace, Cambridge, United Kingdom;* [9]*University of Cambridge, Institute of Continuing Education, Madingley Hall, Cambridge, United Kingdom;* [10]*Universidade Internacional de La Rioja, Facultad de Ciencias de la Salud, Madrid, Spain;* [11]*MRC/UVRI and LSHTM, Entebbe, Uganda., MRC/UVRI and LSHTM, Entebbe, Uganda., Entebbe, Uganda;* [12]*London School of Hygiene and Tropical Medicine, London School of Hygiene and Tropical Medicine, London, United Kingdom;* [13]*Centre for Genomics Research and Innovation, NABDA/FMST, H3Africa Bioinformatics Network (H3ABioNet) Node, Abuja, Nigeria*

Background/Objectives: Hepatitis B is a major global health problem particularly in the WHO Western Pacific and African Regions, where 116 and 81 million people, respectively, are chronically infected. The impact of HBV exposure varies significantly, ranging from resistance and spontaneous viral clearance to development of chronic HBV infection. This variability is influenced by host genetics, viral characteristics, and environmental elements, however, little is known about it's host genetics in Africa. In an effort to bridge this gap, our study sought to identify the genetic determinants that shape distinct susceptibility patterns in HBV infection amongst Ugandans.

Methods: We conducted a GWAS of HBV susceptibility among 6,399 Ugandans from the Uganda Genomic Resource (UGR). To ensure robustness of our findings, we examined replication across independent studies. We performed Bayesian fine mapping to identify potential causal variants, functional mapping and annotation using FUMA (Functional Mapping and Annotation), and PheWAS analyses using the GWASATLAS to understand the relationship between our lead SNPs and variation across different phenotypes.

Results: We identified 78 significant SNPs, mapping to 22 independent novel loci associated with HBV infection susceptibility. Subsequently, we also replicated SNP rs6457709 previously reported to be associated with HBV infection susceptibility in Europeans by Tian C et al, 2017.

Conclusion: Our study identified 22 independent novel loci associated with HBV infection susceptibility and validated a previously published SNP (rs6457709), that was reported to be associated with HBV infection susceptibility in Europeans. Ultimately, additional GWASs are required to represent the impact of diverse African genomes on disease susceptibility.

Grants: Wellcome Trust [grant number: 220740/Z/20/Z].

Conflict of Interest: None declared

P17.072.A Ribosomal DNA Copy Number Associates with Hematological Profiles and Renal Function

Francisco Rodriguez-Algarra[1], *David Evans*[2], *Vardhman Rakyan*[1]

[1]*Blizard Institute, London, United Kingdom;* [2]*Frazer Institute, Brisbane, Australia*

Background/Objectives: The phenotypic impact of variation in repetitive genomic features, such as the multi-copy, multi-locus 47S ribosomal DNA (rDNA), is poorly understood. Despite its key role and displaying copy number (CN) variation in humans (~100-600 copies), strong evidence for rDNA-associated variation impacting human traits is still lacking.

Methods: We estimate rDNA CN on 157,227 whole genome sequences from white British UK Biobank (UKB) participants using a proxy method we develop and validate. We then conduct a phenome-wide screen of rDNA CN, address the causality of the top associations, and replicate key results.

Results: Phenotypes in the blood "counts" and "biochemistry" categories appear enriched in a phenome-wide screen of rDNA CN associations. rDNA CN and systemic inflammation markers, including the neutrophil-to-lymphocyte ratio, strongly associate in targeted models. Further analyses suggest rDNA CN causally influences blood composition, showing no association between rDNA CN and factors known to alter blood composition

measurements. Stringent population filtering retains the associations. Similar results replicate in other ethnicities and the second UKB sequencing release (further 209,681 participants). rDNA CN also associates significantly with renal function biomarkers. Estimates of Glomerular Filtration Rate derived from these associate negatively with rDNA CN, suggesting higher CN corresponds with worse renal function, leading to higher kidney failure risk.

Conclusion: We find that rDNA CN associates with hematological profiles and renal function, highlighting a novel source of genetic variation influencing phenotype, and providing a broader explanation for the genetic basis of human trait variance.

Grants: Barts Charity (MGU0390), Rosetrees Trust (100182)

Conflict of Interest: None declared

P17.073.B New genetic modifiers of age of onset in ATTRV30M amyloidosis

Estefânia Carvalho[1;2], *Andreia Dias*[1;2], *Márcia Almeida Liz*[3], *Teresa Coelho*[4], *Alda Sousa*[1;2], *Miguel Alves-Ferreira*[1;2;5], *Mariana Santos*[1;2;3], *Carolina Lemos*[1;2]

[1]*i3S - Instituto de Investigação e Inovação em Saúde da Universidade do Porto, Porto, Portugal;* [2]*Abel Salazar Biomedical Sciences Institute - University of Porto, Porto, Portugal;* [3]*Instituto de Biologia Molecular e Celular, Porto, Portugal;* [4]*Centro Hospitalar Universitário de Santo António, Porto, Portugal;* [5]*Centro de Genética Preditiva e Preventiva, Instituto de Biologia Molecular e Celular (CGPP-IBMC), Porto, Portugal*

Background/Objectives: Transthyretin (ATTR) amyloidosis is a severe and fatal genetic disorder caused by *TTR* variants that lead to amyloid fibril accumulation in multiple organs. Globally and in Portugal, the most prevalent pathogenic variant is Val30Met (ATTRV30M). Importantly, there is significant diversity in phenotype, severity, and age of onset (AO) among affected individuals. Our research focuses on uncovering genetic variants linked to ATTRV30M amyloidosis that could explain the AO variability.

Methods: DNA samples were gathered from 175 individuals diagnosed with ATTRV30M amyloidosis, including 92 early-onset (AO < 40yrs) and 83 late-onset cases (AO>50yrs), along with 187 controls. We used Precision Medicine Diversity Arrays and analyzed data using Axiom Analysis Suite. Two methodologies were employed: a GWAS and a candidate gene analysis, the latter focusing on genes previously identified by our group.

Results: Our GWAS approach unraveled genes potentially associated with AO of ATTRV30M amyloidosis including RGS6, KCTD5 and ARHGEF10. In the candidate gene approach, we investigated several genes related to the Rho GTPase pathways and found a genetic variant that showed a marked association with late-onset of the disease.

Conclusion: We uncovered new genetic modifiers potentially associated with ATTRV30M amyloidosis and that may act as modulators of AO. Interestingly, we found, in both approaches, genes related to the Rho GTPase pathways. Potential genetic modifiers will be assessed bioinformatically to predict deleteriousness. The most promising genes will be studied functionally, and the results replicated in a bigger cohort.

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Conflict of Interest: None declared

P17.074.C DNA methylation in blood and essential hypertension in African-Brazilian quilombo populations

camila cristina avila martins[1], *Mariana Maschietto*[2], *lilian kimura*[1], *lucas alvizi*[3], *vinícius magalhães borges*[4], *Ana Krepischi*[1], **Regina Mingroni-Netto**[1]

[1]*Universidade de São Paulo, Centro de Pesquisa sobre o Genoma Humano e Células Tronco, Instituto de Biociências, são paulo, Brazil;* [2]*Hospital Infantil Boldrini, Centro de Pesquisa, Campinas, Brazil;* [3]*University College London, Cell & Development Biology, London, United Kingdom;* [4]*Joan C. Edwards School of Medicine, Department of Biomedical Sciences, Huntington, United States*

Background/Objectives: This study aimed to investigate if there is an association between DNA methylation changes in blood and essential hypertension in Afro-Brazilian rural populations (*quilombo* populations in Vale do Ribeira - São Paulo, Brazil).

Methods: We evaluated DNA methylation in peripheral blood samples from normotensive (39) and hypertensive (41) individuals using the Infinium Methylation EPIC BeadChip platform. Data were analyzed using RnBeads and ChAMP packages and cross-referenced with information obtained from databases such as EWAS Atlas, GWAS catalog, GeneCards, literature data and tools such as VarElect and EWAS Toolkit.

Results: The comparison between normotensive and hypertensive individuals identified 190 differentially methylated CpG sites (DMPs, adj.P.Val < 0.05) and 46 differentially methylated regions (DMRs, p.valueArea <0.05). Among the DMRs, those located in or near *MIR539*, *TSHBS1*, *CERS3* and *MIR487B* were highlighted, as genes that interfere with vascular function; *GABBR1*, that affects pressure regulation by the nervous system, and *ABAT*, which is connected with the function of GABA neurotransmitter and *GABBR1*; *PON3* and *IL5RA*, as genes that are related to inflammation and kidney function; *RNF39*, *HLA-DQB2*, *NUDT12* and *KCNH2*, that have shown genetic or epigenetic associations with blood pressure by unknown mechanisms. We also found DMRs located in or near genes related to pathways such as transcription, translation, proliferation, adhesion, apoptosis, differentiation and protein ubiquitination, but without previous data on association with hypertension.

Conclusion: : The study provides valuable information about the genetic and epigenetic underpinnings of essential hypertension and could contribute to developing personalized treatments.

Grants: Funding Agencies CNPq and CEPID-FAPESP (2013/08028-1).

Conflict of Interest: None declared

P18
GENETIC EPIDEMIOLOGY AND PHARMACOGENOMICS

P18.001.D Participation bias in the Estonian biobank

Ivan Kuznetsov[1], *Vasili Pankratov*[1;2]

[1]*Institute of Genomics, University of Tartu, Tartu, Estonia;* [2]*Bioinformatics research institute, Aarhus University, Aarhus, Denmark*

Consortium: Estonian Biobank Research Team

Background/Objectives: Large-scale biobanks are a unique resource for genetic epidemiological studies due to their exceptional sample sizes. Recent studies show biobanks to be biased in terms of allele frequencies as well as demographic and socioeconomic characteristics, which can lead to discoveries irrelevant to the general population. Our study analyzes the Estonian Biobank (EstBB) comprising more than 20% of the country's adult population. We ask if sampling such a big fraction

of the general population makes the EstBB robust to the participation bias. Next, we explore the driving factors and consequences of such bias. Finally, we look for the best weighting procedure to correct the sample for the participation bias.

Methods: We use data from the EstBB (~212,000 individuals). To detect participation bias we compare demographic characteristics of EstBB to census data. Then we compare mean polygenic scores for various traits between subcohorts of the EstBB based on sex and recruitment strategy. Weighting is performed using characteristics available in the EstBB and census data (e.g. sex, year of birth, place of birth/residence).

Results: We find that the EstBB is not perfectly representative of the general population of Estonia. Significant differences exist in polygenic scores for educational attainment, body mass index and height between the EstBB subcohorts by recruitment strategy and sex. Weighting improves representativeness but results in a substantial reduction in the effective population size.

Conclusion: The EstBB is affected by multiple aspects of participation bias similar to other well-known biobanks despite the recruitment of more than 20% of the population.

Conflict of Interest: None declared

P18.002.A Utility of Polygenic Risk Scores for Prediction of Incident Type 2 Diabetes

Dariusz Ratman[1], *Jiayi Sun*[1], *Placede Tshiaba*[1], *Tate Tunstall*[1], *Premal Shah*[1], *Matthew Rabinowitz*[1], *Akash Kumar*[1], *Kate Im*[1]

[1]*MyOme, Inc., Menlo Park, United States*

Background/Objectives: The clinical utility of genetic information in assessing Type 2 Diabetes (T2D) risk remains uncertain. Our study evaluates the added value of T2D polygenic risk scores (PRS) compared to established risk factors.

Methods: Utilising multi-ancestry GWAS summary statistics and five multiethnic cohorts, we developed a cross-ancestry PRS (caPRS) model for T2D. This model was assessed against two models based on established risk factors: a simple model (age, sex, family history, BMI, waist circumference, blood pressure, smoking) and a clinical model (additionally including fasting glucose, triglycerides, HDL-C). We tested the caPRS's association with T2D incidence in three multiethnic validation cohorts (ARIC, MESA, UKB subset) and compared its discriminatory power to the simple and clinical models.

Results: Our results show that the caPRS is associated with T2D incidence across all cohorts. Adjusting for clinical covariates, the hazard ratio per standard deviation for European, South Asian, and African populations was 1.48 (1.43-1.53), 1.33 (1.09-1.62), and 1.18 (1.08-1.29), respectively. Adding caPRS to the simple and clinical model significantly improved the C-index.

The difference in absolute risk across caPRS strata was most pronounced for individuals in the highest clinical risk quartile, showing a 10-year cumulative incidence of 6.4% and 17.8% for those in the top and bottom 20% of caPRS distribution. Conversely, this difference was minimal in the lowest clinical risk quartile.

Conclusion: Our findings demonstrate that genetic information can provide more personalised T2D risk prediction and would be most informative for those with elevated clinical risk.

Conflict of Interest: Dariusz Ratman MyOme, Inc., MyOme, Inc., Jiayi Sun MyOme, Inc., MyOme, Inc., Placede Tshiaba MyOme, Inc., MyOme, Inc., Tate Tunstall MyOme, Inc., MyOme, Inc., Premal Shah MyOme, Inc., MyOme, Inc., Matthew Rabinowitz MyOme, Inc., Natera, Inc., MyOme, Inc.

Natera, Inc., Akash Kumar MyOme, Inc., MyOme, Inc., Kate Im MyOme, Inc., MyOme, Inc.

P18.003.B Sequencing synergy: integration of short and long reads for comprehensive pharmacogenetics testing

Sònia Font Tellado[1], *Patrick Brennan*[1], *Birgit Busse*[1], *Leila Keskic*[1], *Sophie Gentili*[1], *Steffen Lott*[1], *Oliver Wachter*[1]

[1]*MVZ Martinsried GmbH, Planegg, Germany*

Background/Objectives: We present the development and validation of a Pharmacogenetics (PGx) pipeline for star allele calling, annotation and reporting. We combine short read sequencing of 42 clinically relevant PGx genes with long read sequencing of CYP2D6. Our method enables the analysis of single nucleotide variants (SNVs) in all target genes and copy number variants (CNVs) in CYP2D6. We use a custom bioinformatics pipeline to process long and short read data, call star-alleles and provide clinically relevant drug recommendations in a single report.

Methods: a custom amplicon panel was ordered from Illumina and sequenced on a NovaSeq6000. CYP2D6 sequencing (whole gene) was performed on a Sequel II (Pacific Biosciences). SNV calling was done with Illumina DRAGEN. A custom bioinformatics pipeline combining pbaa, pbmm2 and pangu was used to call SNVs and CNVs in CYP2D6. The open-source tool PharmCAT was used for star allele calling, variant annotation and drug recommendations. 7 Genome in a Bottle (GIAB) samples and 15 Coriell samples with known star-alleles were used as benchmarking datasets for full workflow validation.

Results: SNV calling in short reads showed a recall of 98% and precision of 98% on the GIAB dataset. Star allele calling on the Coriell dataset showed a recall of 93% and a precision of 96% (including CYP2D6). Recall and precision for CYP2D6 only were 79% and 73%, respectively (SNV and CNV).

Conclusion: our PGx pipeline enables cost-effective star allele calling with high recall and precision by combining short read sequencing, long read sequencing and state-of-the-art bioinformatics tools.

Grants:

Conflict of Interest: None declared

P18.004.C Polygenic and metabolomic scores in predicting disease prognosis

Sini Kerminen[1], *Luke Jostins-Dean*[1], *Kirsten Schut*[1], *Juhi Somani*[1], *Peter Wurtz*[1], **Jeffrey Barrett**[1]

[1]*Nightingale Health Plc., Helsinki, Finland*

Consortium: Nightingale Health Biobank Collaborative Group

Background/Objectives: Polygenic scores have been shown to have limited utility in predicting progression to disease-specific death. Metabolomic scores, however, can be strong predictors of both incident disease and mortality. Here we compare polygenic and metabolomic scores for predicting disease progression.

Methods: We used 460,000 samples with metabolomic and genetic information from the UK Biobank. For chronic kidney disease (CKD), chronic obstructive pulmonary disease (COPD) and rheumatoid arthritis (RA), we built metabolomic scores to predict disease incidence and progression (end-stage renal disease for CKD, hospitalisation or death from acute exacerbation for COPD, and corrective joint surgery for RA). We used previously published susceptibility polygenic scores.

Results: CKD patients in the highest decile of metabolomic progression score were 50.2-times (HR; 30.5-82.5 95% confidence interval) more likely to develop end-stage renal disease than the

rest. For COPD the equivalent HR was 3.2 (3.5-4.2) and for RA 2.4 (1.7-3.5). Metabolomic scores for disease incidence and progression were highly correlated, and both predicted progression equally well. Conversely, polygenic scores showed predictive ability only for COPD with HR of 1.9 (1.4-2.6) for the top decile. Further, we show by simulation that metabolomic scores can enhance the number of cases for trials to increase power.

Conclusion: Metabolomic scores can predict disease progression, and aid in selecting participants for detailed follow-up, such as enriching trials for progression. By contrast, extant polygenic risk scores predict disease incidence but usually have weak prediction of disease progression.

Grants: Nightingale Health.

Conflict of Interest: Sini Kerminen Stock options: Nightingale Health Plc, Nightingale Health Plc, Luke Jostins-Dean Stock options: Nightingale Health, Nightingale Health, Kirsten Schut Stock options: Nightingale Health, Nightingale Health, Juhi Somani Nightingale Health, Peter Wurtz Stocks and stock options: Nightingale Health, Nightingale Health, Jeffrey Barrett Stocks and stock options: Nightingale Health, Nightingale Health

P18.005.D Phenotypic and metabolomic landscape of *FAM47E-SHROOM3* exonic haplotypes

Dariush Ghasemi-Semeskandeh[1;2], Eva König[1], Luisa Foco[1], Nikola Dordevic[1], Martin Gögele[1], Johannes Rainer[1], Markus Ralser[3], Dianne Acoba[4;5], Dorien Peters[2], Peter Pramstaller[1], **Cristian Pattaro**[1]

[1]Eurac Research, Institute for Biomedicine, Bolzano, Italy; [2]Leiden University Medical Center, Department of Human Genetics, Leiden, Netherlands; [3]Charité - Universitätsmedizin Berlin, Institute of Biochemistry, Berlin, Germany; [4]AstraZeneca, Clinical Renal, Late-stage Development, Cardiovascular, Renal and Metabolism (CVRM), BioPharmaceuticals R&D, Gothenburg, Sweden; [5]Université Paris Cité, Institut Necker Enfants-Malades (INEM), INSERM U1151, Paris, France

Background/objectives: Genome-wide association studies identified a chromosome 4q21.1 locus spanning *FAM47E*, *STBD1*, *CCDC158*, and *SHROOM3*, prominently associated with kidney function markers but also with electrolytes, hematological and cardiovascular traits. Functional studies linked *SHROOM3*, encoding an actin-binding protein involved in cell shaping, with kidney damage. However, systematic explorations of functional variants of all genes at the locus are lacking. We thus conducted phenome-wide, metabolomic and proteomic analyses of exonic haplotypes spanning the four genes.

Methods: We reconstructed haplotypes for 12,834 individuals of the Cooperative Health Research in South Tyrol (CHRIS) study, following genotype imputation from a whole-exome sequencing reference panel of a 3422 CHRIS sample subset. Haplotypes, including 146 exonic and intronic variants over the four genes, were tested for association with 74 serum, urine, and anthropometric traits, 172 serum metabolites, and 148 plasma proteins concentrations using linear models.

Results: The 11 identified haplotypes (frequency: 2% to 24%) showed both shared and specific characteristics. Compared to the most common haplotype, most haplotypes were jointly associated with kidney function and serum magnesium levels. The second most common haplotype (12% frequency) was associated with better kidney function and lower dodecanoyl-, hydroxyvaleryl- and tiglyl-carnitine concentrations. A haplotype of 4% frequency was associated with better kidney function and lower hematological marker levels. A haplotype of 2% frequency was associated with lower magnesium, glutamine, and putrescine levels. Cluster analysis revealed six distinct trait groups and two distinct haplotype groups.

Conclusion: *FAM47E-SHROOM3* functional haplotypes exhibit marked pleiotropic effects and identify population subgroups with distinct biomarker profiles.

Grants: H2020-MSCA-ITN-2019 ID:860977 (TrainCKDis).

Conflict of Interest: Dariush Ghasemi-Semeskandeh: None declared, Eva König: None declared, Luisa Foco: None declared, Nikola Dordevic: None declared, Martin Gögele: None declared, Johannes Rainer: None declared, Markus Ralser: None declared, Dianne Acoba: None declared, Dorien Peters: None declared, Peter Pramstaller: None declared, Cristian Pattaro Quotient Therapeutics

P18.006.A Genetic insights in the cardiovascular multimorbidity of psoriasis

Charalabos Antonatos[1], **Alexandros Pontikas**[1]*, Adam Akritidis[1], Katerina Grafanaki[2;3], Sophia Georgiou[2], Yiannis Vasilopoulos[1]*

[1]University of Patras, Biology, Rion, Greece; [2]Medical School, Dermatology-Venereology, Rion, Greece; [3]Medical School, Biochemistry, Rion, Greece

Background/Objectives: We performed genome-wide pleiotropy analyses to unveil common risk factors and implicated pathways mediating cardiovascular multimorbidity (CVM) in psoriasis, including coronary artery disease (CAD), heart failure (HF) and myocardial infarction (MI).

Methods: Utilizing GWAS data from European cohorts with psoriasis ($n = 44164$), CAD ($n = 184305$), HF ($n = 977323$), and MI ($n = 395795$), we assessed global genetic correlations and explored pleiotropy by integrating common, bi-allelic variants excluding the major histocompatibility complex under a composite null hypothesis. Gene-level and multi-trait colocalization analyses were conducted to investigate the biological implications of pleiotropic loci. Bi-directional and drug-based Mendelian Randomization were employed to examine the causal relationship of psoriasis with CVMs, and the impact of lipid-lowering drugs on psoriasis risk with the largest GWAS for low-density lipoprotein (LDL) measurement in Europeans.

Results: Psoriasis and CVMs show a modest genome-wide correlation ($0.15 < r_g < 0.3$, $P < 1 \times 10^{-6}$), with 61 overlapping risk loci reporting significant pleiotropic associations (38 unique), including 28 in psoriasis-CAD. Gene-based analyses documented significant enrichment in T-helper 17 pathways in all CVMs, LDL metabolism in psoriasis-CAD and psoriasis-MI, while 5 loci were reported as causal. Psoriasis was also a causal factor for all CVMs except MI. Excluding pleiotropic variants, genetically proxied inhibition of *PCSK9* ($\beta = -0.23$, $P = 0.001$) and *LDLR* ($\beta = -0.44$, $P = 1.31 \times 10^{-8}$) significantly reduced psoriasis risk, thus unveiling the implication of the LDL receptor-mediated pathway in psoriasis and CAD comorbidity.

Conclusion: By triangulating genetic evidence, we prioritize genes and pathways for the comorbid mechanisms in psoriasis and CVMs, facilitating the development of personalized therapeutic strategies.

Grants:

Conflict of Interest: None declared

P18.007.B Estimating polygenic score effects in non-European populations for improved lifetime disease risk estimation

Brooke Wolford[1]*, Diana Dunca[2], Veronica Tozzo[3], Kristina Zguro[4], Masahiro Kanai[5], Ying Wang[5], Pedro De Macedo Gomes[6], Eleni Fthenou[7], Radja Badji[7], Kuang Lin[8], Kristi Läll[9], Aoife McMahon[10], Yue Ji[10], Kristian Hveem[1], Yukinori Okada[11], Bogdan Pasaniuc[3], Robin G Walters[8], Derrick Bennett[8], David van Heel[2], Henrike Heyne[6], Alicia*

1700

Martin[5], Nina Mars[12], Andrea Ganna[12], Samuli Ripatti[12], Stravroula Kanoni[2]

[1]Norwegian University of Science and Technology, Department of Public Health and Nursing, Trondheim, Norway; [2]Queen Mary University of London, William Harvey Research Institute, Faculty of Medicine and Dentistry; [3]David Geffen School of Medicine at UCLA, Department of Computational Medicine; [4]University of Siena, Med Biotech Hub and Competence Centre, Department of Medical Biotechnologies; [5]Massachusetts General Hospital, Analytic and Translational Genetics Unit; [6]Hasso Plattner Institute for Digital Engineering, University of Potsdam; [7]Qatar Biobank, Qatar Precision Health Institute; [8]University of Oxford, Nuffield Department of Population Health; [9]University of Tartu, Estonian Genome Centre, Institute of Genomics; [10]European Molecular Biology Laboratory, European Bioinformatics Institute, Wellcome Genome Campus; [11]Osaka University School of Medicine Graduate School of Medicine, Department of Statistical Genetics; [12]University of Helsinki, Institute for Molecular Medicine Finland, FIMM, HiLIFE

Consortium: INTERnational consortium of integratiVE geNomics prEdiction

Background/Objectives: Age- and sex-specific polygenic score (PGS) effects have been previously reported in European ancestry individuals, but it is unknown to what extent these apply across ancestry groups.

Methods: Within the INTERnational consortium of integratiVE geNomics prEdiction (INTERVENE), we leveraged information from 11 biobanks with inclusion of East and South Asian, African, Middle Eastern, and admixed American ancestry groups. In a combined sample of over 1 million (40% non-European), we calculated MegaPRS-derived PGSs and assessed incident events from health registries. We employed Cox proportional hazards models and Global Burden of Disease baseline risks to estimate PGS effects and lifetime risks for 18 high burden diseases.

Results: PGS had a larger effect for coronary heart disease (CHD) in South-Asian men compared to women ($HR_{male} = 1.34$ (95% CI: 1.29 - 1.39) vs $HR_{female} = 1.20$ (95% CI: 1.14 - 1.27), $PGS*Sex_{Interaction}$ P-value $= 7.92 \times 10^{-4}$), whereas for T2D the effect was larger in East Asian women compared to men ($HR_{male} = 1.53$ (95% CI: 1.50 - 1.55) vs $HR_{female} = 1.62$ (95% CI: 1.59 - 1.66), $PGS*Sex_{Interaction}$ P-value $= 1.76 \times 10^{-5}$). These trends were consistent in Europeans. Heterogeneous effect sizes by age were detected for CHD, T1D and T2D in South Asians, whereas T2D and prostate cancer exhibited age-specific effects in East Asians. We are replicating in the additional ancestry groups.

Conclusion: Our results demonstrate variable sex- and age-specific PGS associations across ancestries and emphasize the importance of country- and ancestry-calibrated lifetime risk estimates for global utility of PGS.

Grants: Horizon 2020 (101016775), MSCA Postdoctoral Fellowship (101110878)

Conflict of Interest: Brooke Wolford: None declared, Diana Dunca: None declared, Veronica Tozzo: None declared, Kristina Zguro: None declared, Masahiro Kanai: None declared, Ying Wang: None declared, Pedro De Macedo Gomes: None declared, Eleni Fthenou: None declared, Radja Badji: None declared, Kuang Lin: None declared, Kristi Läll Kristi Läll has participated as an analyst in a collaboration research project at the Institute of Genomics, University of Tartu, which was funded by Geneto OÜ, Aoife McMahon: None declared, Yue Ji: None declared, Kristian Hveem: None declared, Yukinori Okada: None declared, Bogdan Pasaniuc: None declared, Robin G Walters: None declared, Derrick Bennett: None declared, David van Heel: None declared, Henrike Heyne: None declared, Alicia Martin: None declared, Nina Mars: None declared, Andrea Ganna Andrea Ganna is the founder of Real World Genetics Oy., Samuli Ripatti: None declared, Stravroula Kanoni: None declared

P18.008.C Investigating the Utility of Pharmaceutically Informed, Type-2 Diabetes, Partitioned Polygenic Scores in the UK Biobank

Luke Marques[1], *jonathan coleman[1], Cathryn Lewis[1], Gerome Breen[1]*

[1]Social Genetic and Developmental Psychiatry Centre, London, United Kingdom

Background/Objectives: Drug repositioning (DR) is a cost-effective approach to complement traditional, lengthy and expensive drug discovery methods. We explore the utility of pharmaceutically informed partitioned polygenic scores (*p*PGS) for this effort, through enrichment analysis, aiming to identify target-indication pairs enriched for Type-2 Diabetes (T2D) signal. We further investigate the relationship between these *p*PGSs and clinically relevant outcomes in the UK Biobank (UKB) using Primary Care records.

MAGMA gene-set enrichment analysis was previously used to suggest DR opportunities for schizophrenia, using a large collection of gene-sets, describing drug target genes. The limitation of this method is that it provides no individual level information. Conversely, a *p*PGS approach has the advantage of providing individual level scores for each gene-set.

Methods: We developed *p*PGSs for T2D across the aforementioned gene-sets, using PRSet. We used weights from a large T2D GWAS and built *p*PGSs for 412,765 UKB enrolled individuals. PRSet's enrichment analysis was employed and compared with an enrichment analysis using MAGMA of the same gene-sets.

Results and Conclusions: PRSet's implementation resulted in 602 gene-sets showing nominal significance, including a *p*PGS for sulfonylureas (A10BB, $p = 2.9997 \times 10^{-4}$), and 108 which reached the minimal permuted competitive *p*-value ($p = 10^{-4}$). However, enriched *p*PGSs lacked specificity towards expected gene-sets and the leading results were not supported by the MAGMA analysis. We further observed individuals in the cohort who have combinations of bottom and top decile *p*PGSs, suggesting the underlying heterogeneity of T2D may relate to the drug gene-sets. Work on clinical outcomes analyses in the UKB using *p*PGSs is ongoing.

Grants:

Conflict of Interest: None declared

P18.009.D Tandem repeat polymorphisms as genetic modifiers of brain structure

Richard Mantey[1], *santiago estrada[1;2], kristian haendler[3;4], Marc Beyer[3;5], Monique M.B. Breteler[1;6], N. Ahmad Aziz[1;7]*

[1]Deutsches Zentrum für Neurodegenerative Erkrankungen e. V. (DZNE), Population Health Sciences, Bonn, Germany; [2]Deutsches Zentrum für Neurodegenerative Erkrankungen e. V. (DZNE), AI in Medical Imaging, Bonn, Germany; [3]Deutsches Zentrum für Neurodegenerative Erkrankungen e. V. (DZNE), Platform for Single Cell Genomics and Epigenomics (PRECISE), Bonn, Germany; [4]University Hospital Schleswig-Holstein, Institute for Human Genetics, Lübeck, Germany; [5]Deutsches Zentrum für Neurodegenerative Erkrankungen e. V. (DZNE), Systems Medicine, Bonn, Germany; [6]University of Bonn, Institute for Medical Biometry, Informatics and Epidemiology (IMBIE), Faculty of Medicine, Bonn, Germany; [7]University of Bonn, Department of Neurology, Faculty of Medicine, Bonn, Germany

Background/Objectives: Genomic tandem repeats, often overlooked in genome-wide studies, may contribute to the 'missing heritability' conundrum. Recently, we demonstrated that intermediate and pathological range cytosine-adenine-guanine(CAG) repeats in polyglutamine disease-associated genes were associated with cognitive function and depression risk in the general population. However, the impact of repeat polymorphisms in these and other disease-causing genes on brain structure remains unknown. Therefore, we aimed to assess the relation between size polymorphisms in repeat expansion disease-associated genes and brain structure.

Methods: We used data from the first 2912 participants (aged 30 to 94) of the Rhineland Study, a prospective cohort study in Germany. Employing amplicon-based targeted sequencing (Illumina NovaSeq 6000), we estimated the diploid genotypes in 21 loci with ExpansionHunter. Brain imaging features were derived from 3T MRI scans using FreeSurfer, and multiple linear regression models were used to examine the association between tandem repeat sizes and brain imaging features, adjusting for relevant factors.

Results: Larger expansions in a dodecamer repeat-containing region in the cystatin B*(CSTB)* gene were associated with reduced total grey matter volume (standardized β[95% confidence intervals]: -1.53[-2.41 to -0.65]). Moreover, the aggregated repeat expansion burden, defined as the mean standardized allele size across all the investigated genes, was significantly associated with a marked reduction in both total brain volume (-0.37[-0.70 to -0.04]), and total grey matter volume (-0.24[-0.44 to -0.04]).

Conclusion: Our results implicate repeat polymorphisms as important genetic modifiers of brain structure, and warrant further investigation of their role as genetic modifiers of brain structure and function in health and disease.

Grants: European Research Council Starting Grant(Number:101041677)
DZNE institutional-funds.

Conflict of Interest: None declared

P18.010.A Causal Effect of puberty time on the Risk for Depression in females, but not in males: Results From a Two-Sample Multivariable Mendelian Randomization Study

Triinu Peters[1], *Raphael Hirtz*[2], *Anke Hinney*[1], *Jochen Antel*[1], *Corinna Grasemann*[3], *Heike Hölling*[4], *Björn-Hergen von Holt*[5], *Johannes Hebebrand*[1], *Nicola Albers*[1], *Christine Hars*[2]

[1]University Hospital Essen, Department of Child and Adolescent Psychiatry, Psychosomatics and Psychotherapy, Essen, Germany; [2]University Hospital Essen, Division of Pediatric Endocrinology and Diabetology, Department of Pediatrics II, Essen, Germany; [3]Ruhr-University Bochum, St. Josef-Hospital, Department of Pediatrics, Division of Rare Diseases and CeSER; [4]Robert Koch Institute, Department of Epidemiology and Health Monitoring, Berlin, Germany; [5]Universität zu Lübeck, Institut für Medizinische Biometrie und Statistik, Lübeck, Germany

Background/Objectives: A fair number of epidemiological studies suggest that age at menarche (AAM) is associated with depression, but there is evidence of residual confounding. This also applies to the relationship between male puberty time (MPT) and depression, even though less thoroughly studied. Previous Mendelian randomization (MR) studies on AAM have provided mixed findings, MR studies on MPT are lacking.

Methods: We performed two-sample MR analyses. For AAM, we used 360 single-nucleotide polymorphisms (SNPs) as instrumental variable. For males, we used 71 SNPs related to pubertal timing defined by the age at voice break. For the broadly defined depression, we used the latest GWAS on 246,363 cases and 561,190 controls. Multiple methods to account for heterogeneity of the instrumental variable, systematic and idiosyncratic pleiotropy, and horizontal pleiotropy were used. Body mass index (BMI), educational attainment, and total white blood count for AAM and BMI for MPT were considered as pleiotropic phenotypes in the multivariable MR analysis.

Results: A causal effect of early AAM on higher depression risk was found in the univariable (inverse-variance weighted (IVW): OR = 0.96, p = 0.0003) and in the multivariable MR analysis (IVW: OR = 0.96, p = 0.007). In males, univariable MR indicated a causal effect (IVW: OR = 0.93, p = .03). However, this was not confirmed by multivariable MR (IVW: OR = 0.95, p = .13).

Conclusion: Considering the adverse consequences of earlier AAM on mental health, this finding should foster efforts to address risk factors promoting earlier menarcheal age. There is currently no evidence that MPT is related to MDD risk

Grants: none

Conflict of Interest: Triinu Peters University Hospital Essen, Raphael Hirtz University Hospital Essen, Anke Hinney University Duisburg-Essen, Jochen Antel University Hospital Essen, Corinna Grasemann Ruhr-University Bochum, Heike Hölling Robert-Koch-Institute, Berlin, Björn-Hergen von Holt Universität zu Lübeck, Johannes Hebebrand. He is listed as an inventor in three patent applications of the University of Duisburg-Essen for the use of leptin analogues for treatment of anorexia nervosa and depression., Univerity Hospital Essen, speech honoraria from Novo Nordisk and Amryt Pharmaceuticals in the past three years, Nicola Albers University hospital Essen, Christine Hars University hospital Essen

P18.011.B Cross-Population Comparison of Aptamer and Antibody Proteomics Measures

Jayna Nicholas[1], *Daniel Katz*[2], *Usman A. Tahir*[3], *Catherine L. Debban*[4], *Francois Aguet*[5], *Thomas Blackwell*[6], *Russell P Bowler*[7], *Jingsha Chen*[8], *Clary B. Clish*[5], *Josef Coresh*[9], *Elaine Cornell*[10], *Daniel E. Cruz*[3], *Rajat Deo*[11], *Margaret F. Doyle*[10], *Peter Durda*[10], *Lynette Ekunwe*[12], *James S. Floyd*[13], *Xiuqing Guo,*[14], *Ron C. Hoogeveen*[15], *Craig Johnson*[13], *Leslie A. Lange*[16], *Yun Li*[1], *Alisa Manning*[5], *James Meigs*[17], *Josyf C. Mychaleckyj*[4], *Nels C. Olson*[10], *Katherine A. Pratte*[7], *Bruce M. Psaty*[13], *Alexander P. Reiner*[13], *Peifeng Ruan*[18], *Magdalena del Rocio Sevilla Gonzalez*[17], *Amil M. Shah*[19], *Quan Sun*[1], *Russell P. Tracy*[10], *Jia Wen*[1], *Alexis C. Wood*[14], *James G. Wilson*[3], *Bing Yu*[20], *Mary R. Rooney*[8], *Ani W Manichaikul*[4], *Ruth Dubin*[18], *Stephen S. Rich*[4], *Jerome I. Rotter*[14], *Peter Ganz*[21], *Robert E. Gerszten*[3], *Kent d. Taylor*[14], *Laura M. Raffield*[1]

[1]University of North Carolina, Chapel Hill, United States; [2]Stanford University, Stanford, United States; [3]Beth Israel Deaconess Medical Center, Boston, United States; [4]University of Virginia, Charlottesville, United States; [5]Broad Institute, Cambridge, United States; [6]University of Michigan, Ann Arbor, United States; [7]National Jewish Health, Denver, United States; [8]Johns Hopkins Bloomberg School of Public Health, Baltimore, United States; [9]Institute for Optimal Aging, New York, United States; [10]Larner College of Medicine at University of Vermont, Burlington, United States; [11]University of Pennsylvania, Philadelphia, United States; [12]University of Mississippi Medical Center, Jackson, United States; [13]University of Washington, Seattle, United States; [14]Lundquist Institute, Torrance, United States; [15]Baylor College of Medicine, Houston, United States; [16]School of Medicine, Colorado Anschutz Medical Campus, Aurora, United States; [17]Massachusetts General Hospital, Boston, United States; [18]UT Southwestern, Dallas, United States; [19]Harvard Medical School, Boston, United States; [20]UT Health, School of Public Health, Houston, United States; [21]University of California, San Francisco, San Francisco, United States

Consortium: Cohorts for Hearts and Aging Research in Genetic Epidemiology, Trans-Omics for Precision Medicine Program

Background/Objectives: Protein-altering variants (PAVs) may alter affinity-reagent binding to protein targets, contributing to inter-assay measurement differences. As allele frequencies differ between populations, PAVs may further contribute to inter-population heterogeneity.

Methods: We explored the impact of PAVs on the correlation of 2,157 plasma proteins measured with SomaScan 7k (ANML-RFU) and Olink Explore 3072 (NPX values) in 2,080 Multi-ethnic Study of Atherosclerosis participants [n = 884 White, 513 Black, 399 Admixed Americans, 289 East Asian].

Results: The median Pearson's correlation between inverse-normalized measures was 0.26 [Q1 = 0.0094, Q3 = 0.61], with 23% of proteins exhibiting heterogeneity between correlation estimates by ancestry group (Cochran's Q p-value < 0.05). Of the 2,157 proteins, we identified significant associations with genetic variants ±1MB of the protein-encoding gene (*cis*-pQTL) for 776 proteins on SomaScan and 846 on Olink. Similar proportions of SomaScan (23%) and Olink (25%) *cis*-pQTL SuSiE credible sets contained PAVs, with 33 SomaScan and 31 Olink credible sets containing PAVs common (EAF>0.10) in African, but rare (EAF < 0.01) in European ancestry. Of the 453 proteins with platform-overlapping credible sets, 19 proteins exhibited platform-discordant *cis*-pQTL effect sizes -- all containing PAVs in the credible set. For these proteins, adjustment for the associated PAV improved protein measure correlation across platforms (see table for examples). Notably, adjustment for ancestry-differentiated PAV rs399145 (*PLAUR* locus, EAF = 0.12 in Africans, EAF < 0.002 in others) improved cross-platform correlation in participants of African ancestry from 0.27 to 0.59.

	Pearson's Correlation	
	PILRA	suPAR
Pre-PAV Adjustment	-0.35	0.44
Post-PAV Adjustment	0.27	0.57

Conclusion: PAV-adjustment may offer a novel strategy for harmonizing cross-platform, cross-population differences in affinity-based protein measurements.

Grants: NIH T32-GM135123

Conflict of Interest: None declared

P18.012.C Bias from heritable confounding in Mendelian randomization studies

Eleanor Sanderson[1], *Tom Palmer*[1], *Kate Tilling*[1], *George Davey Smith*[1], *Gibran Hemani*[1]

[1]*MRC Integrative Epidemiology Unit, University of Bristol, Bristol, United Kingdom*

Background/Objectives: Mendelian randomization (MR) is an approach to causal inference that uses genetic variants to test and estimate the effect of an exposure on an outcome of interest. MR relies on a set of assumptions, including that there is no pathway from the genetic variants to the outcome that does not act through the exposure. Genetic variants for MR studies are often selected through genome-wide association studies (GWAS). The sample size of GWAS studies has been increasing rapidly, increasing the number of genetic variants selected as being associated with the exposure of interest and how distally mediated those that are selected are from the exposure.

Methods: We use theoretical analysis and simulation studies to explore how increasing GWAS sample size will affect MR estimation and propose estimation approaches to mitigate the resulting bias.

Results: We show through a plausible network simulation model that more distally mediated variants are more likely to act through confounders of a hypothesised exposure-outcome relationship. We show when this can result in bias in an MR estimation that is larger than the bias in linear regression and propose solutions to identify such bias in an MR study. We illustrate our approach through estimation of the causal effect of age at menarche on type 2 diabetes, hypothesising that it may be biased by confounding from adiposity.

Conclusion: As more genetic variants are included in MR there is greater potential for confounding bias, approaches should be taken to mitigate that bias.

Conflict of Interest: None declared

P18.014.A Long-term health outcomes of bilateral salpingo-oophorectomy in BRCA1 and BRCA2 pathogenic variant carriers with personal history of breast cancer

Hend Hassan[1], *Isaac Allen*[1], *Tameera Rahman*[2], *Andrew Bacon*[3], *Craig Knott*[4], *Catherine Huntley*[5], *Lucy Loong*[6], *Yvonne Walburga*[1], *Katrina Lavelle*[2], *Eva Morris*[7], *Steven Hardy*[2], *beth torr*[8], *Fiona McRonald*[9], *Sally Vernon*[9], *Margreet Lüchtenborg*[9], *Joanna Pethick*[9], *Francesco Santaniello*[4], *Shilpi Goel*[4], *Sophie Allen*[8], *Diana Eccles*[10], *Clare Turnbull*[11], *marc tischkowitz*[12], *paul pharoah*[13], *Antonis C. Antoniou*[1]

[1]*Centre for Cancer Genetic Epidemiology, Public Health and Primary Care, Cambridge, United Kingdom;* [2]*National Disease Registration Service, NHS Digital, Leeds, United Kingdom;* [3]*National Cancer Registration and Analysis Service (NCRAS), Cambridge, United Kingdom;* [4]*Health Data Insight CIC, Cambridge, United Kingdom;* [5]*The Institute of Cancer Research, London, United Kingdom;* [6]*Institute of Cancer Research, Oxford, United Kingdom;* [7]*University of Oxford, United Kingdom;* [8]*The Institute of Cancer Research, United Kingdom;* [9]*National Disease Registration Service, National Health Service England, London, United Kingdom;* [10]*Department of Cancer Sciences, Faculty of Medicine, University of Southampton, United Kingdom;* [11]*Division of Genetics and Epidemiology, Institute of Cancer Research, Sutton, United Kingdom;* [12]*Cambridge Biomedical Research Centre, National Institute for Health Research, Department of Medical Genetics, United Kingdom;* [13]*Department of Computational Biomedicine, Cedars-Sinai Medical Center, Los Angeles, United States*

Background/Objectives: Women with pathogenic variants in *BRCA1* and *BRCA2* have high risk of developing ovarian cancer after breast cancer diagnosis. Bilateral salpingo-oophorectomy (BSO) is recommended for ovarian cancer risk reduction. We used novel linked electronic health records data to examine the association between BSO after breast cancer diagnosis i*n BRCA1/2* carriers and long-term health outcomes.

Methods: We identified breast cancer patients diagnosed in England between 1995 and 2019 from the National Cancer Registration Dataset (NCRD). Data were linked to genetic testing labs to identify *BRCA1/2* carriers and to the Hospital Episode Statistics (HES) to identify BSO delivery. Outcomes were ascertained from HES and NCRD. Follow-up started at the latest of the BC diagnosis date plus one year and the genetic test dates. The associations were examined using multivariable Cox-regression, with BSO modelled as a time-dependent covariate.

Results: A total of 33,377 women with personal history of breast cancer had a *BRCA1/2* genetic test, 1,709 carried a *BRCA1* and 1,788 carried a *BRCA2* pathogenic variant. 859 *BRCA1* and 1,013 *BRCA2* carriers had BSO after breast cancer diagnosis. BSO was

associated with a significant reduction in all-cause mortality in both *BRCA1* and *BRCA2* carriers with hazard ratios of 0.61 (95%CI: 0.44-0.86) and 0.42 (95%CI: 0.31-0.57). There was no association between BSO and cardiovascular diseases or contralateral breast cancer risk. Ongoing analyses are examining the associations with neuropsychiatric outcomes and second non-breast cancers.

Conclusion: BSO after breast cancer diagnosis in *BRCA1/2* carriers is associated with a significant reduction in all-cause mortality.

Grants: CRUK Catalyst Award CanGene-CanVar (grant number: C61296/A27223)

Conflict of Interest: None declared

P18.015.B Point-of-care-testing of pharmacogenetic variants using a portable nanopore sequencer

Satomi Mitsuhashi[1], *Takahiro Shimizu[1], Takehiro Ichikawa[1], Masahiro Hoshino[1], Yu Suzuki[1], Takayuki Fukano[1], Hisanao Akiyama[1], Kazuhiko Hanzawa[2], Yoshihisa Yamano[1]*

[1]St. Marianna University School of Medicine; [2]Niigata University School of Medicine and Dental Sciences

Background: Clopidogrel sulfate (CLP), an antiplatelet drug, is metabolized into its active form by cytochrome P450 enzyme encoded by the *CYP2C19* gene. Approximately 20% of Japanese individuals carry a loss-of-function polymorphism of this gene, resulting in reduced efficacy of CLP in preventing ischemic stroke. Although it is preferrable to select drugs based on *CYP2C19* polymorphisms for precision medicine, there is a hurdle for rapidly determine the polymorphism in clinical setting. The Flongle is a small and portable nanopore DNA sequencing apparatus capable of real-time analysis of DNA sequences, potentially applicable for genetic analysis in clinical settings such as Emergency Unit. Therefore, we aim to conduct a proof-of-conceptual study to establish a method for identifying *CYP2C19* polymorphisms as a point-of-care testing (POCT).

Methods: DNA samples of 48 stroke patients who consented to participate in the study were used. Target regions were amplified by PCR, and DNA sequencing was performed using flongle. Results were confirmed by Sanger sequencing. Whole-genome sequencing detection were also evaluated for the polymorphism. Additionally, a rapid analysis system using direct PCR from saliva samples was developed.

Results: High-accuracy detection of *CYP2C19* polymorphisms was achieved for all blood-derived DNA samples. Furthermore, polymorphism identification from saliva samples was possible within 3 hours using the rapid analysis system.

Conclusion: The method using Flongle may be useful in selecting antiplatelet drugs and can be cost-effectively applied to various pharmacogenetic analyses beyond CYP2C19 at the bedside. It is anticipated to be a widely available POCT tool for pharmacogenetic analysis in the future.

Conflict of Interest: None declared

P18.016.C Metabolic profiling of different glycemic traits: a two-sample Mendelian randomization study

Jacky Mo[1], *Tommy Wong[1], Mingqi Zhou[2], Jie V. Zhao[1], Catherine Schooling[1], Annabel He[1], April Luo[1], Shiu Lun Au Yeung[1]*

[1]The University of Hong Kong; [2]University of California Irvine

Consortium: the Meta-Analyses of Glucose and Insulin-related traits Consortium

Background/Objectives: Clarifying the similarities and differences in the association with downstream metabolites (e.g., lipid subfractions and amino acids) of glycemic traits and liability to type 2 diabetes may help clarify the pathophysiology of these inter-related glycemic traits in various diseases.

Methods: We assessed the causal relation of four glycemic traits and type 2 diabetes liability with 167 metabolites using Mendelian randomization (MR) with various sensitivity analyses and a reverse Mendelian randomization analysis. We extracted instruments for fasting glucose, 2-hour glucose, fasting insulin, and glycated hemoglobin from the Meta-Analyses of Glucose and Insulin-related traits Consortium ($n = 200,622$), and those for type 2 diabetes liability from a meta-analysis of multiple cohorts (148,726 cases, 965,732 controls) in Europeans. Outcome data were from summary statistics of 167 metabolites from the UK Biobank ($n = 115,078$).

Results: Fasting glucose and 2-hour glucose were not associated with any metabolite. Higher glycated hemoglobin was associated with higher free cholesterol in small low-density lipoprotein. Type 2 diabetes liability and fasting insulin were inversely associated with apolipoprotein A1, total cholines, lipoprotein subfractions in high-density-lipoprotein and intermediate-density lipoproteins, and positively associated with aromatic amino acids.

Conclusion: These findings indicate hyperglycemia-independent patterns and highlight the role of insulin in type 2 diabetes development. Further studies should evaluate these glycemic traits in type 2 diabetes diagnosis and clinical management.

Grants: n/a

Conflict of Interest: None declared

P18.017.D Improving the quality of pharmacogenetic testing and reporting: standardising nomenclature through External Quality Assessment (EQA)

Rebecca Goodall[1], *Michael Woodcock[1], Henry Thomas[1], Nicola Wolstenholme[1], Simon Patton[1]*

[1]EMQN CIC, Manchester, United Kingdom

Background/Objectives: Pharmacogenetic laboratory tests provide patients with individual therapeutic profiles, these can be preserved in their records, guiding current and future treatments. In this rapidly developing field, it is essential that results remain clear to clinicians, with stable, unambiguous nomenclature. The EMQN pharmacogenetics panel EQA scheme has been running for 4 years, providing a programme including expert review of genotyping and interpretation and evaluation of nomenclature. We present a summary of nomenclature identified through EQA.

Methods: Participant laboratories were sent mock patient pharmacological case scenarios alongside DNA samples (sourced from the Coriell Institute for Medical Research) and invited to test and submit pdf reports, including relevant clinical interpretation according to their routine practices. Reports were independently assessed by an EMQN group of peer reviewers against robust marking criteria. Following assessment and an appeals process, laboratories were informed of their performance.

Results: Participation has increased since 2019, with seventy five laboratories assessed in 2023. Improvement was observed in the use and consistency of pharmacogenetic nomenclature. By 2023, 96% (72/75) laboratories used star allele nomenclature for all or some of the genes in their panel, 92% (69/75) reported dbSNP RefSNP ID and 93% (70/75) reported variants using HGVS nomenclature. Errors included transcription errors and out-of-date nomenclature.

Conclusion: Laboratories are increasingly using standardised nomenclature systems for reporting. Continued participation in EQA can improve the quality of reporting using standardised

nomenclature to ensure that best practices are embedded in laboratory practice, enabling clear communication to patient, clinician and pharmacist.

Grants: None

Conflict of Interest: None declared

P18.018.A Cost-Effectiveness of a Polygenic Risk Score Based Breast Cancer Screening Program in Estonia

*Filipa Sampaio[1], **Peeter Padrik**[2], Krista Kruuv-Käo[2], Katrin Koiduaru[3], Neeme Tonisson[4,5], Inna Feldman[1]*

[1]Uppsala University, Department of Public Health and Caring Sciences, Uppsala, Sweden; [2]OÜ Antegenes, Tartu, Estonia; [3]University of Tartu, Institute of Family Medicine and Public Health, Tartu, Estonia; [4]University of Tartu, Institute of Genomics, Tartu, Estonia; [5]Tartu University Hospital, Genetics and Personalized Medicine Clinic, Tartu, Estonia

Consortium: BRIGHT - Be RIGHT with breast cancer management

Background/Objectives: This study assessed the cost-effectiveness of risk-stratified breast cancer (BC) screening in Estonia for women starting at age 35 versus standard screening for ages 50-69, using the polygenic risk score (PRS) test's isolated impact.

Methods: A Markov model was used to estimate the costs and health outcomes of the current age-based screening compared with a PRS tailored approach, adapted to each woman's risk. The model estimated the cumulative costs and health outcomes of a cohort of 1000 women aged 35 as they moved between the states healthy/at risk, cancer stages 0, I, II, III, IV, and dead, over a lifetime horizon. Costs were estimated from a health-payer perspective, in 2022 €. Model parameters were sourced from national sources and literature. Costs and health outcomes were discounted at 5% annually. An incremental cost-effectiveness ratio (ICER) was calculated as the difference in costs divided by the difference in quality-adjusted life-years (QALY) between both strategies.

Results: Risk-stratified screening led to a redistribution of BC stages, with more early (0-I) and fewer advanced stages (II-IV) and averted 1.5 BC deaths per 1,000 women. Risk-stratified screening resulted in larger net costs of €145,235 (mainly related to PRS test and counselling costs), and a gain of 3.85 QALYs, with an ICER of €37,755 per QALY gained. The findings were sensitive to the uncertainty around risks estimates of developing BC and in variations in discount rate.

Conclusion: A PRS-tailored BC screening has clinical benefit and is potentially cost-effective in Estonia.

Grants: European Commission via EIT Health BRIGHT innovation activity (project #220720, #230121).

Conflict of Interest: Filipa Sampaio: None declared, Peeter Padrik Ownership in OÜ Antegenes, Employment in OÜ Antegenes, Krista Kruuv-Käo Option in OÜ Antegenes, Full time employment in OÜ Antegenes, Katrin Koiduaru: None declared, Neeme Tonisson: None declared, Inna Feldman: None declared

P18.020.C Multiple genetic control of anti-COVID-19 vaccine response by HLA locus

Martina Esposito[1], *Francesca Minnai[1], Massimiliano Copetti[2], Giuseppe Miscio[2], Rita Perna[2], Ada Piepoli[2], Gabriella De Vicentis[2], Mario Benvenuto[2], Paola D' Addetta[2], Susanna Croci[3], Margherita Baldassarri[3], Mirella Bruttini[3], Chiara Fallerini[3], Raffaella Brugnoni[4], Paola Cavalcante[4], Fulvio Baggi[4], Elena Maria Grazia Corsini[4], Emilio Ciusani[4], Francesca Andreetta[4], Tommaso A. Dragani[5], Maddalena Fratelli[6], Massimo Carella[2], Renato Mantegazza[4], Alessandra Renieri[3], Francesca Colombo[1]*

[1]National Research Council, Institute for Biomedical Technologies, Segrate, Italy; [2]Fondazione IRCCS Casa Sollievo della Sofferenza, San Giovanni Rotondo, Italy; [3]University of Siena, Medical Genetics, Siena, Italy; [4]Fondazione IRCCS Istituto Neurologico Carlo Besta, Milano, Italy; [5]Aspidia S.r.l, Milano, Italy; [6]Istituto di ricerche farmacologiche "Mario Negri" IRCCS, Milano, Italy

Consortium: GEN-COVID

Background. Since the beginning of the anti-COVID-19 vaccination campaign, it has become evident that vaccinated subjects exhibit considerable inter-individual variability in the response to the vaccine that could be partly explained by host genetic factors. A recent study reported that the immune response elicited by the vaccine was influenced by a specific allele of the human leukocyte antigen gene *HLA-DQB1*.

Methods. We carried out a genome-wide association study to investigate the genetic determinants of the antibody response to the Pfizer-BioNTech vaccine in an Italian cohort of 1,351 subjects recruited in three centers. Linear regressions between normalized antibody levels and genotypes of >7 million variants were performed, using sex, age, centers, days between vaccination boost and serological test, and five principal components as covariates. We also analyzed the association between normalized antibody levels and 204 HLA alleles, with the same covariates as above. **Results**. Our study confirms the involvement of the HLA locus and shows significant associations with variants in *HLA-A*, *HLA-DQA1*, and *HLA-DQB1* genes. In particular, the HLA-A*03:01 allele is the most significantly associated with serum levels of anti-SARS-CoV-2 antibodies. Other alleles, from both major histocompatibility complex class I and II are significantly associated with antibody levels.

Conclusions. These results support the hypothesis that HLA genes modulate the response to Pfizer-BioNTech vaccine and highlight the need for genetic studies in diverse populations and for functional studies aimed to elucidate the relationship between HLA-A*03:01 and CD8+ cell response upon Pfizer-BioNTech vaccination.

Grants. 8 × 1000 Istituto Buddista Italiano Soka Gakkai (ID 2020-2016_RIC_3).

Conflict of Interest: None declared

P18.021.D A genome-wide association study in European advanced cancer patients treated with opioids identified variants regulating the expression of OPRL1 as possible modulators of pain intensity

Francesca Minnai[1,2], *Morena Shkodra[3,4], Sara Noci[5], Cinzia Brunelli[3], Alessandra Pigni[3], Ernesto Zecca[3], Martina Esposito[1], Pål Klepstad[6,7], Augusto Caraceni[8,9], Marco Cesare Maltoni[10], Stein Kaasa[4,11], Frank Skorpen[6,12], Maria Caterina Pallotti[13], Oscar Corli[14], Francesca Colombo[1]*

[1]Institute for Biomedical Technologies, National Research Council, Department of Biomedical Sciences, Segrate (MI); [2]Università degli Studi di Milano, Department of Medical Biotechnology and Translational Medicine (BioMeTra), Segrate (MI), Italy; [3]Fondazione IRCCS Istituto Nazionale dei Tumori, Palliative care, Pain therapy and Rehabilitation Unit, Milan, Italy, Italy; [4]University of Oslo, Oslo, Norway; [5]Fondazione IRCCS Istituto Nazionale dei Tumori, Genetic Epidemiology and Pharmacogenomics Unit, Milan, Italy; [6]Norwegian University of Science and Technology, Department of circulation and medical imaging, Trondheim, Norway; [7]St Olavs University Hospital, Department of Anesthesiology and Intensive Care Medicine, Trondheim, Norway; [8]Fondazione IRCCS Istituto Nazionale dei

Tumori, Palliative care, Pain therapy and Rehabilitation Unit, Milan, Italy; [9]Università degli Studi di Milano, Department of Clinical Sciences and Community Health, Milan, Italy; [10]University of Bologna, Medical Oncology Unit, Department of Medical and Surgical Sciences, Bologna, Italy; [11]Oslo University Hospital, Department of Oncology, Oslo, Norway; [12]Norwegian University of Science and Technology, Department of Clinical and Molecular Medicine, Faculty of Medicine and Health Sciences, Trondheim, Norway; [13]IRCCS Istituto Romagnolo per lo Studio dei Tumori (IRST) "Dino Amadori", SS Cure Palliative, Terapia del dolore e Medicina Integrativa, Meldola (FC), Italy; [14]Istituto di Ricerche Farmacologiche Mario Negri - IRCCS, Milan, Italy

Background/Objectives: Cancer pain causes an unbearable suffering and lowered quality of life, thus advanced cancer patients often require analgesic therapy. Opioids of step III of the WHO-defined analgesic ladder are the standard of care for the treatment of cancer pain. However, 20-30% of patients do not receive benefits from therapy or have a low response. Literature suggests that genetics plays a role in predisposing patients to a good or poor response to opioids and, herein, we investigated it by performing a genome-wide association study (GWAS).

Methods: We individually genotyped 2,060 European advanced cancer patients treated with morphine, buprenorphine, fentanyl, and oxycodone. We performed a whole-genome regression model (using REGENIE software) between genotypes and the opioid response phenotype, defined as a numerical score measuring pain intensity based on patients' responses to the Brief Pain Inventory Questionnaire.

Results: The GWAS identified five non-coding variants on chromosome 20 at *P*-value 5.0×10^{-8}. For all of them, the minor allele was associated with a lower pain intensity. These variants were intronic of *PCMTD2* gene and were 200 kbp downstream of *OPRL1*, the Opioid Related Nociceptine Receptor 1. Interestingly, four of them acted as expression quantitative trait loci, modulating the expression of *OPRL1*, according to eQTLGen database.

Conclusion: This is the largest GWAS performed in this field, so far. Our promising results strengthen the role of genetics in opioid response. Further functional analyses are needed to validate the results obtained and to understand the biological mechanism underlying the association observed.

Grants: Associazione Italiana per la Ricerca sul Cancro (MFAG-22970).

Conflict of Interest: None declared

P18.023.B Causal Evidence of the Association between Alcohol Consumption and Cardiovascular Disease in East Asian

Keumji Jung[1], *Sun Ha Jee*[1]

[1]*Yonsei University, Department of Epidemiology and Health Promotion, Seoul, Korea, Rep. of South*

Background/Objectives: Light-moderate alcohol intake has been associated with lower cardiovascular disease, however the association varies across different subtypes. The aim of this study is to investigate the causal association between alcohol consumption and vascular disease in east Asian.

Methods: We conducted both a conventional observational study and genetic study among 125,727 participants. We performed a GWAS analysis, and selected SNPs (ALDH2, ADH1B) highly related to alcohol as instrumental variables. We used Cox proportional hazards models to estimate the relationship between vascular disease and both self-reported alcohol consumption, genetically determined alcohol amount. To confirm the results, we conducted the two sample mendelian randomization (2SMR) using Korean and Japan data.

Results: Among men, observational epidemiological study showed that self-reported alcohol amount had J-shaped associations with the incidence of stroke. In contrast, genetic epidemiological study shows genotype-predicted mean alcohol intake had a continuously positive association with stroke. For myocardial infarction (MI), both observational epidemiological studies and genetic epidemiological studies have shown an inverse association with alcohol intake. This same result was observed in a 2SMR using the BBJ and KCPS-II.

Conclusion: Our study provides causal evidence for the association between alcohol consumption and vascular disease in the Asian population using genetic epidemiology. These suggest that reducing alcohol consumption could be an effective preventive strategy for stroke. However, the effect of alcohol intake on MI needs further evaluation.

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Conflict of Interest: None declared

P18.024.C Challenges to improve the diversity in population genomics to enhance genomics discoveries

Ben Hollis[1], *Jen Harrow*[1], *Karyn Mégy*[1], *Ali Abassi*[1], *Amanda O'Neill*[1], *Shikta Das*[1], *Xiao Jiang*[1], *Katherine Smith*[1], *Stewart MacArthur*[1], *Sean O'Dell*[1], *Sebastian Wasilewski*[1], *Quanli Wang*[2], *Slavé Petrovski*[1]

[1]*AstraZeneca, Centre for Genomics Research, Discovery Sciences, BioPharmaceuticals R&D, Cambridge, United Kingdom; [2]AstraZeneca, Centre for Genomics Research, Discovery Sciences, BioPharmaceuticals R&D, Waltham, United States*

Early-stage incorporation of human genomic data into the assessment of drug targets has been shown to significantly increase drug pipeline success rates. Genome-wide association studies have uncovered thousands of common variants associated with human disease, but the contribution of highly penetrant rare variants that demonstrate strong association with clinically meaningful outcomes in common diseases remains relatively unexplored. The UK Biobank (UKB) study comprises of phenotypic data linked to medical records for approximately 500,000 participants, that in conjunction with whole genome sequences, is offering an unprecedented opportunity to evaluate the effect of rare genetic variants on a broad collection of traits. However, historically the percentage of ancestry population on large-scale genomics studies was overwhelmingly European (78%), so more effort is needed to ensure the data reflects the diversity within the human population. To answer this need, AstraZeneca's Centre for Genomics Research (CGR) is establishing one of the world's most comprehensive and diverse genetic resource and makes gene-phenotype associations publicly available through the AZ PheWAS Portal (https://azphewas.com/). We are also addressing the challenge of harmonising health information coming for a range of geographies and healthcare systems, so cohorts and controls can be built across all biobanks. We demonstrate a range of clinical data harmonisation approaches developed by CGR and applied to diverse population genetic data and highlight the pharmacogenomic important gene CYP2D6, which has high allelic variation between populations and affects the way some drugs are metabolized.

Conflict of Interest: None declared

P18.025.D Real-world impact of exome sequencing: a single center's experience

Georgia Christopoulou[1], Stavroula Samara[1], Aikaterini Oikonomaki[1], Katerina Roumelioti[1], Eleni Katsoni[1], Olga Dimou[1], Andromachi Vagena[1], Stavros Bournazos[1], **Pantelis Constantoulakis**[1]

[1]*Genotypos M.S.A., Genetics and Genomics, Athens, Greece*

Background/Objectives: Exome sequencing (ES) has become a valuable tool in the genetic diagnostic setting. We aim to present the landscape of ES based on our laboratory's 5-year experience with real-world referrals and to highlight the diagnostic yield and challenges faced in our actual day-to-day practice.

Methods: During 2017-2023, 4.288 patients were referred to our center. ES was applied on DNA extracted from peripheral blood or buccal swabs: library preparation was performed with Clinical Exome (Sophia Genetics) or Whole Exome (Twist Bioscience) panels and sequenced on Illumina NextSeq-550/550 (Illumina). Bioinformatics analyses were conducted by SOPHiA DDM® bioinformatics pipelines.

Results: 1.811/4.288 (42.23%) of data sets required variant interpretation while the remaining were NGS services. Of the 1.811 cases, 802 (44,28%) were pre-conceptual carrier screenings of different levels and 1.009 (55.72%) were patients with suspected genetic conditions. In the latter, 307 patients had causative mutations (30,42% overall diagnostic yield). The majority were SNV/Indels, while 41 clinically significant CNVs (13,35%) were detected; 37 were directly related to the phenotype, and 4 were incidental findings. 234/307 (76,22%) were patients who received a definite genetic diagnosis and the rest were hematology patients with actionable genomic profiles. Of the rare disease diagnoses, the most prevalent were nephrology (51), neuromuscular (27), ophthalmology (26), and cardiology (25) cases. Diagnostic yield was substantially higher in subgroups where clinical description, medical and family history were detailed.

Conclusion: Real-world private sector ES has a key role in genomic diagnosis. Detailed patient information is essential to further increase the diagnostic yield and decipher more cases.

Grants:

Conflict of Interest: None declared

P18.026.A Pleiotropic genetic architecture and causal estimates of eosinophils and atopic dermatitis

Charalabos Antonatos[1], *Alexandros Pontikas[1], Adam Akritidis[1], Katerina Grafanaki[2,3], Sophia Georgiou[2], Aikaterini Zacharopoulou[4], Ileana Afroditi Kleidona[4], Stamatis Gregoriou[4], Yiannis Vasilopoulos[1]*

[1]*University of Patras, Biology, Rion, Greece;* [2]*Medical School, Dermatology-Venereology, Rion, Greece;* [3]*Medical School, Biochemistry, Rion, Greece;* [4]*Medical School, Dermatology-Venereology, Athens, Greece*

Background/Objectives: Here, we calculated the causal estimates between blood cell traits (BCTs) and atopic dermatitis (AD), a chronic inflammatory skin disease, and characterized the pleiotropic genetic architecture of AD to unveil novel risk loci.

Methods: We leveraged genome-wide association studies on AD and BCTs in European participants. We employed single-variable, two-sample mendelian randomization and linkage disequilibrium score regression to compute causal estimates and genetic correlations between BCTs and eczema. We next performed pleiotropy analysis under the composite null hypothesis (PLACO) excluding major histocompatibility complex (MHC) and a series of in silico analyses, including gene-based analyses and colocalization to investigate novel risk loci mediating AD risk in BCTs.

Results: Eosinophil count (EC) and eosinophil percentage (EP) were associated with increased AD risk, while lymphocyte percentage (LP) was inversely associated per 1-standard deviation. However, only EC and EP showed significant genome-wide correlations. Prioritizing EC for PLACO analysis based on the larger number of shared non-MHC variants ($n = 8547984$), we identified 132 independent risk loci and 275 genes enriched in leukocyte proliferation and interleukin signaling. Twenty-four pleiotropic loci (18%) shared causal variants between AD and EC, where 12 mapped genes have not been previously identified including *CCR7* and *SH2B3*.

Conclusion: By triangulating genomic evidence, our preliminary data provide significant insights in the causal role of increasing eosinophils in the AD risk. We prioritize 24 loci (12 novel), which may serve as potential therapeutic targets and facilitate drug repurposing.

Grants: This investigation was supported by a research grant from the National Eczema Association NEA23-CRG186.

Conflict of Interest: None declared

P18.027.B Epigenetic biomarkers of asthma exacerbations identified in European populations

Elena Martin-Gonzalez[1], *Javier Perez-Garcia[1], Esther Herrera-Luis[2], Mario A. González-Carracedo[1], simon kebede merid[3], Natalia Hernandez-Pacheco[3], Fabian Lorenzo-Diaz[1], Ruperto González-Pérez[4], Olaia Sardón[5], José M. Hernández-Pérez[6], Paloma Poza-Guedes[4], Inmaculada Sánchez-Machín[4], Elena Mederos-Luis[4], Paula Corcuera[5], berta román bernal[7], Uroš Potočnik[8], Michael Kabesch[9], Anke-Hilse Maitland-van der Zee[10], Jesús Villar[11], Erik Melen[3], Maria Pino-Yanes[1]*

[1]*Universidad de La Laguna (ULL), Genomics and Health Group, Department of Biochemistry, Microbiology, Cell Biology and Genetics, La Laguna, Spain;* [2]*Johns Hopkins University, Department of Epidemiology, Bloomberg School of Public Health, Baltimore, United States;* [3]*Karolinska Institutet, Department of Clinical Science and Education, Södersjukhuset, Stockholm, Sweden;* [4]*Hospital Universitario de Canarias, Allergy Department, La Laguna;* [5]*Hospital Universitario Donostia, Division of Pediatric Respiratory Medicine, San Sebastián, Spain;* [6]*Hospital Universitario de N.S de Candelaria, Department of Respiratory Medicine, Santa Cruz de Tenerife, Spain;* [7]*, Hospital Dr. José Molina Orosa, Crta. Arrecife-San Bartolomé, Km 1, 3, Arrecife, Respiratory Medicine, Las Palmas, Spain;* [8]*University of Maribor, Taborska ulica 8, 2000, Faculty of Medicine, Maribor, Slovenia;* [9]*University Children's Hospital Regensburg (KUNO), Department of Pediatric Pneumology and Allergy, Regensburg, Germany;* [10]*Amsterdam UMC, Location AMC, University of Amsterdam, Meibergdreef 9, 1105 AZ, Pulmonary Medicine, Amsterdam, Netherlands;* [11]*Instituto de Salud Carlos III, CIBER de Enfermedades Respiratorias (CIBERES), Madrid, Spain*

Background/Objectives: Despite several genetic markers for asthma exacerbations have been identified in the past years, the role of epigenetics remains uncovered. Our objective was to evaluate whether DNA methylation (DNAm) is associated with asthma exacerbations.

Methods: The DNAm profile of 406 blood samples from Spanish individuals was determined with the Infinium MethylationEPIC microarray (Illumina). The association of DNAm with asthma exacerbations was tested adjusting for age, sex, batch effect, genetic ancestry, and tissue heterogeneity at different levels: differentially methylated positions, regions, and epigenetic modules determined with weighted gene co-expression network analysis. Suggestive CpGs identified using a false discovery rate (FDR)<0.1 were validated in blood samples from 222 European individuals and nasal epithelia from 111 Spanish individuals. Finally, cis-methylation quantitative trait loci (meQTLs) analysis and enrichment analyses for CpGs with $p < 1 \times 10^{-4}$ in signals

identified in previous studies focused on other phenotypes were performed.

Results: We found 4 CpGs (FDR < 0.1), 4 differentially methylated regions, and one module related to environmental exposures to toxic compounds were significantly associated with asthma exacerbations (FDR < 0.05). Additionally, 3 CpGs (cg25345365, cg06710464, and cg15508223) were validated in Europeans and reached the genomic significance threshold ($p < 9 \times 10^{-8}$). Furthermore, the CpG cg25345365 was cross-tissue validated in nasal epithelial ($p = 0.001$) and was regulated by 6 independent genetic variants (FDR < 0.05). Finally, enrichment analyses revealed an overrepresentation of prior associations with immune-mediated diseases, mortality, and prenatal and environmental exposures.

Conclusion: We identified differentially methylated positions, regions, and epigenetic modules associated with asthma exacerbations in European populations.

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Conflict of Interest: None declared

P18.028.C Identification of proteins for stroke progression using Mendelian Randomization

Andrew Elmore[1], *Nimish Adhikari*[2], *Gina Marie Peloso*[2], *Lavinia Paternoster*[1], *Daniel Posner*[3], *Juan P Casas*[3], *April Hartley*[1], *Kelly Cho*[3], *Gibran Hemani*[1], *Kate Tilling*[1], *George Davey Smith*[1], *Tom Gaunt*[1]

[1]*University of Bristol, Medicine, United Kingdom;* [2]*Boston University, Boston, United States;* [3]*Harvard Medical School, Boston, United States*

Background/Objectives: Individuals who have experienced a stroke, or transient ischemic attack, face a heightened risk of future cardiovascular events. Identification of genetic and molecular risk factors for subsequent cardiovascular outcomes may identify effective therapeutic targets to improve prognosis after an incident stroke.

Methods: We performed genome-wide association studies (GWAS) for subsequent major adverse cardiovascular events (MACE) ($N_{cases} = 51{,}929$, $N_{cntrl} = 39{,}980$) and subsequent arterial ischemic stroke (AIS) $N_{cases} = 45{,}120$, $N_{cntrl} = 46{,}789$) after first incident stroke within the Million Veteran Program and UK Biobank. We then used genetic variants associated with proteins (pQTLs) to determine the effect of 1,463 plasma protein abundances on subsequent MACE using Mendelian randomization (MR).

Results: Two variants were significantly associated with subsequent cardiovascular events: rs76472767 (OR = 0.75, 95% CI = 0.64-0.85, $p = 3.69 \times 10^{-08}$) with subsequent AIS and rs13294166 (OR = 1.52, 95% CI = 1.37-1.67, $p = 3.77 \times 10^{-08}$) with subsequent MACE. Using MR, we identified 2 proteins with an effect on subsequent MACE after a stroke: *CCL27* (effect OR = 0.77, 95% CI = 0.66-0.88, adj. p = 0.05), and *TNFRSF14* (effect OR = 1.42, 95% CI = 1.24-1.60, adj. p = 0.006). These proteins are not associated with incident AIS and are implicated to have a role in inflammation.

Conclusion: We found evidence that two proteins with little effect on incident stroke appear to influence subsequent MACE after incident AIS. These associations suggest that inflammation is a contributing factor to subsequent MACE outcomes after incident AIS and highlights potential novel targets.

Grants: None

Conflict of Interest: Andrew Elmore: None declared, Nimish Adhikari: None declared, Gina Marie Peloso: None declared, Lavinia Paternoster: None declared, Daniel Posner: None declared, Juan P Casas: None declared, April Hartley AEH started working for Novo Nordisk after contributing to this manuscript, Kelly Cho: None declared, Gibran Hemani: None declared, Kate Tilling: None declared, George Davey Smith: None declared, Tom Gaunt TRG receives funding from Biogen and GSK for unrelated work

P18.029.D Limited overlap between genetic effects on disease susceptibility and disease survival: evidences from seven biobanks and ten common diseases

Zhiyu Yang[1], *Fanny-Dhelia Pajuste*[2], *Kristina Zguro*[3], *Yipeng Cheng*[4], *Danielle Kurant*[5], *Andrea Eoli*[6], *Julian Wanner*[6], *Bradley Jermy*[1], *Stravroula Kanoni*[7], *David van Heel*[7], *Caroline Hayward*[4], *riccardo marioni*[4], *daniel mccartney*[4], *Alessandra Renieri*[3], *simone furini*[3], *Reedik Mägi*[2], *Alexander Gusev*[5], *Petros Drineas*[8], *Peristera Paschou*[9], *Henrike Heyne*[6], *Samuli Ripatti*[1], *Nina Mars*[1], *Andrea Ganna*[1]

[1]*University of Helsinki, Institute for Molecular Medicine Finland, Helsinki, Finland;* [2]*University of Tartu, Estonian Genome Centre, Tartu, Estonia;* [3]*University of Siena, Med Biotech Hub and Competence Center, Siena, Italy;* [4]*University of Edinburgh, Centre for Genomic and Experimental Medicine, Edinburgh, United Kingdom;* [5]*Harvard Medical School, Medical Oncology, Boston, United States;* [6]*University of Potsdam, Hasso Plattner Institute, Potsdam, Germany;* [7]*Queen Mary University of London, William Harvey Research Institute, London, United Kingdom;* [8]*Purdue University, Department of Computer Science, West Lafayette, United States;* [9]*Purdue University, Department of Biological Sciences, West Lafayette, United States*

Consortium: FinnGen, Genes & Health Research Team, Genomics England Research Consortium, INTERVENE consortium

Background/Objectives: Understanding disease progression is of a high biological and clinical interest. Unlike disease susceptibility whose genetic basis has been abundantly studied, less is known about the genetics of disease progression and its overlap with disease susceptibility.

Methods: Considering ten common diseases (N cases ranging from 17,152 to 99,666) across seven biobanks, we systematically compared the genetic architecture of susceptibility and progression, defined as disease-specific mortality.

Results: We identified only one locus significantly associated with disease-specific mortality and show that, at a similar sample size, more genome-wide significant loci can be identified in a GWAS of disease susceptibility. Variants that were significantly affecting disease susceptibility were weakly or not associated with disease-specific mortality. Moreover, susceptibility polygenic scores (PGSs) were weak predictor of disease-specific mortality while a PGS for general lifespan was significantly associated with disease-specific mortality for five out of ten diseases. We used theoretical derivation and simulation to propose plausible explanations for our empirical observations and account for potential index-event bias.

Conclusion: Overall, our findings point to little similarity in genetic effects between disease susceptibility and disease-specific mortality and suggest that either larger sample sizes or different measures of progression are needed to identify the genetic underpinning of disease progression.

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Conflict of Interest: Zhiyu Yang: None declared, Fanny-Dhelia Pajuste: None declared, Kristina Zguro: None declared, Yipeng Cheng: None declared, Danielle Kurant: None declared, Andrea

Eoli: None declared, Julian Wanner: None declared, Bradley Jermy: None declared, Stravroula Kanoni: None declared, David van Heel: None declared, Caroline Hayward: None declared, riccardo marioni: None declared, daniel mccartney: None declared, Alessandra Renieri: None declared, simone furini: None declared, Reedik Mägi: None declared, Alexander Gusev: None declared, Petros Drineas: None declared, Peristera Paschou: None declared, Henrike Heyne: None declared, Samuli Ripatti: None declared, Nina Mars: None declared, Andrea Ganna AG is Founder of Real World Genetics

P18.030.A Amplification and buffering of disease risk alleles by metabolic reaction fluxes

Carles Foguet Coll[1;2], *Xilin Jiang*[1;2;3], *Scott C. Ritchie*[1;2;4], *Samuel Lambert*[1;2], *Yu Xu*[1;2], *Emanuele Di Angelantonio*[1;2;5], *John Danesh*[1;2], *Adam Butterworth*[1;2], *Michael Inouye*[1;2;4]

[1]*British Heart Foundation Cardiovascular Epidemiology Unit, Department of Public Health and Primary Care, University of Cambridge, Cambridge, United Kingdom;* [2]*Victor Phillip Dahdaleh Heart and Lung Research Institute, University of Cambridge, Cambridge, United Kingdom;* [3]*Department of Epidemiology, Harvard T.H. Chan School of Public Health, Boston, United States;* [4]*Cambridge Baker Systems Genomics Initiative, Baker Heart and Diabetes Institute, Melbourne, Australia;* [5]*Human Technopole, Fondazione Human Technopole, Milan, Italy*

Background/Objectives: Our previous research demonstrated that the stochiometric determinants of metabolic networks can be used to simulate the metabolic effects of genetically driven variations in transcript levels and predict personalized organ-specific reaction flux values. Here we use genetically-predicted reaction fluxes to perform a systematic search for instances where fluxes can function as buffers or amplifiers of coronary artery disease (CAD) susceptibility in individuals carrying known risk alleles.

Methods: Genetically personalized organ-specific reaction fluxes were simulated for 459,902 genotyped UK Biobank participants of European ancestry. Within this cohort, we evaluated the capacity of 9,984 reaction fluxes to buffer or amplify the effect of 5,852 known CAD risk variants on disease susceptibility. We measured buffering/amplification between a reaction and a variant as the interaction between reaction flux value and risk allele dosage in CAD risk. The significance of this interaction was evaluated using COX proportional hazards models for CAD events.

Results: Our analysis identified hundreds of instances where reaction fluxes can amplify or buffer the effect of genetic variants on CAD susceptibility. Notably, a majority of these variants are in well-established CAD loci, such as LPA and LDLR. Furthermore, many of the implicated reactions are linked to processes with putative roles in the disease such as inflammation, hypertension, or lipid metabolism.

Conclusion: This work establishes proof of concept that biochemical reaction fluxes can alter the penetrance of disease risk alleles. This analysis can be extended to other diseases and contribute to improving our understanding of variant-to-function (V2F) and disease biology.

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Conflict of Interest: Carles Foguet Coll: None declared, Xilin Jiang: None declared, Scott C. Ritchie: None declared, Samuel Lambert: None declared, Yu Xu: None declared, Emanuele Di Angelantonio: None declared, John Danesh J.D. has received multiple grants from academic, charitable and industry sources outside of the submitted work., J.D. serves on scientific advisory boards for AstraZeneca, Novartis, Our Future Health and UK Biobank., Adam Butterworth A.B. has received grants unrelated to this work from AstraZeneca, Bayer, Biogen, BioMarin, Bioverativ, Novartis and Sanofi., Michael Inouye M.I. is a trustee of the Public Health Genomics (PHG) Foundation, a member of the Scientific Advisory Board of Open Targets., M.I. has a research collaboration with AstraZeneca that is unrelated to this study.

P18.031.B Pharmacogenetics for cardiovascular drugs: Lost in translation

Benoît Delabays[1], *Katerina Trajanoska*[1], *Joshua Walonoski*[2], *Vincent Mooser*[1]

[1]*McGill Genome Centre, Human Genetics, Montréal, Canada;* [2]*Medeloop Inc., Canada*

Consortium: N/A

Background/Objectives: Pharmacogenetics (PGx) aims at optimizing drug efficacy and safety. Cardiovascular (CV) drugs are among the most prescribed drugs. We wondered how many findings from genome-wide association studies (GWAS) of marketed CV drug response have already translated into PGx prescribing recommendations.

Methods: We identified marketed CV drugs from the PharmGKB database. For each of them, we retrieved and curated GWAS from the GWAS Catalog and extracted PGx information from their labels approved by European, Japanese and American regulatory agencies and related recommendations from expert PGx panels (CPIC and DPWG).

Results: Out of 221 marketed CV drugs we identified, 27 had been studied in 101 GWAS, and 40 had PGx recommendations from ≥1 regulatory agencies or expert panels. Genome-wide significant associations were reported for 48 PGx traits, mapping to 306 genes. Six of these genes were endorsed in PGx recommendations for 4 drugs (clopidogrel, rosuvastatin, simvastatin, and warfarin). For the remaining 36 drugs, PGx information was based on candidate-gene analyses or other studies. Overall, the level of recommendations varies substantially between agencies and expert panels, except for clopidogrel.

Conclusion: Only a small fraction of GWAS findings on marketed CV drug response have been translated yet into PGx recommendations. Consistent evidence supporting PGx testing is limited to very few CV drugs. Substantial additional work is warranted to identify and validate PGx variants for more marketed CV drugs and to evaluate their clinical utility. Preemptive PGx offers unprecedented opportunities to achieve these goals.

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Conflict of Interest: Benoît Delabays: None declared, Katerina Trajanoska: None declared, Joshua Walonoski Medeloop Inc., Medeloop Inc. (full time), Vincent Mooser Medeloop Inc., Medeloop Inc.

P18.032.C Powering Genomic Research through *All of Us*

Anjene Musick[1], *Melissa Basford*[2], *Paul Harris*[3], *David Glazer*[4], *Dan Roden*[5], *Anthony Philippakis*[6], *Eric Banks*[6], *Mine Cicek*[7], *Kimberly Doheny*[8], *Stacey Gabriel*[9], *Richard Gibbs*[10], *Gail Jarvik*[11], *Evan Eichler*[12], *Eric Boerwinkle*[13], *Heidi Rehm*[9], *Stephen Thibodeau*[7], *Scott Topper*[14], *Robert Meller*[15]

[1]*National Institutes of Health, All of Us Research Program, Bethesda, United States;* [2]*Vanderbilt University Medical Center, Vanderbilt Institute of Clinical and Translational Research, Nashville, United*

States; [3]Vanderbilt University Medical Center, Department of Biomedical Informatics, Nashville, United States; [4]Verily Life Sciences, South San Francisco, United States; [5]Vanderbilt University Medical Center, Department of Clinical Pharmacology, Nashville, United States; [6]Broad Institute of MIT and Harvard, Data Sciences Platform, Cambridge, United States; [7]Mayo Clinic, Department of Laboratory Medicine and Pathology, Rochester, United States; [8]Johns Hopkins University School of Medicine, Department of Genetic Medicine, Baltimore, United States; [9]Broad Institute of MIT and Harvard, Cambridge, United States; [10]Baylor College of Medicine, Human Genome Sequencing Center, Houston, United States; [11]University of Washington School of Medicine, Department of Medicine, Division of Medical Genetics, Seattle, United States; [12]University of Washington School of Medicine, Department of Genome Sciences, Seattle, United States; [13]Baylor College of Medicine, Human Genome Sequencing Center, Houston, United States; [14]Color Health, Burlingame, United States; [15]Morehouse School of Medicine, Neuroscience Institute, Atlanta, United States

Consortium: The All of Us Research Program Genomics Investigators

Background/Objectives: The *All of Us* (AoU) Research Program is creating one of the world's largest, most diverse datasets by combining multiple data types from one million diverse participants from across the United States. Here we describe the program's most recent genomics data release which includes >245k whole genome sequences (>11k with structural variant calls), >315k arrays, >1k long read sequences, as well as auxiliary data and variant annotations.

Methods: Biospecimens are collected from program participants and undergo a process of DNA extraction for sequencing and genotyping. Four genome centers used identical library construction protocols, arrays, sequencers, software, and software configuration. The AoU Data and Research Center (DRC) performed additional QC and joint calling. To lower the compute cost and the barrier of entry to use genomic data, the DRC also provided data for common downstream use cases, including predicted continental ancestries, variant annotations, relatedness, and additional conversions into other variant formats (eg, plinkbed, bgen, Hail).

Results: *All of Us* identified >1 billion genetic variants, including >275 million previously unreported genetic variants, >3.9 million in coding regions. Principal component analysis identified almost half of participants from non-European ancestries. Summary level data are publicly available (https://databrowser.researchallofus.org/), and individual-level data can be accessed by researchers through the *All of Us* Researcher Workbench, a secure, cloud-based platform..

Conclusion: The *All of Us* Research Program allows researchers world-wide to access an expansive dataset containing longitudinal health data combined with genomic data, enabling novel health research and medical breakthroughs.

Grants: NIH Office of Director 3OT2OD035404 and others.

Conflict of Interest: None declared

P18.033.D Beyond the genome: a reference map of biological influences on the human plasma proteome

Julia Carrasco-Zanini[1;2;3], *Eleanor Wheeler*[1], *Burulça Uluvar*[3], *Nicola Kerrison*[1], *Mine Koprulu*[1], *Nicholas J. Wareham*[1], *Maik Pietzner*[1;2;3], *Claudia Langenberg*[1;2;3]

[1]*MRC Epidemiology Unit, University of Cambridge, Cambridge, United Kingdom;* [2]*Precision Healthcare University Research Institute, Queen Mary University of London, London, United Kingdom;* [3]*Computational Medicine, Berlin Institute of Health at Charité-Universitätsmedizin, Berlin, Germany*

Background/Objectives: Broad-capture proteomic platforms now enable assessment of thousands of plasma proteins, many of which are not actively secreted, and their origins are largely unknown. Identification of their major determinants, beyond genetic variation, can provide clues to their origins and strong links to diverse diseases.

Methods: We integrated genomic with deep phenomic information to identify modifiable and non-modifiable determinants of 4,775 plasma proteins in healthy individuals (N = 8350). We create the first map of the human plasma proteome based on shared determinants of protein variance and demonstrate clear segregation into clusters of genetic and non-genetic influences.

Results: For over a third (N = 1575) of protein targets, biological influences explained 10-77% of the variation (median = 19.88%, IQR: 14.01 – 31.09%), independent of the relatively strong influence of technical factors (median = 2.48%, IQR: 0.78 – 6.41%). While non-modifiable factors (genetic variation, age, sex) best explained most protein targets (N = 3242); modifiable factors best explained a considerable number (N = 1737). The proteome map, together with genetic and disease follow-up analyses, highlights 1) biological influences that shape the plasma proteome including important drug targets, 2) causal, modifiable determinants of proteins, 3) potential drivers underlying hundreds of protein-disease associations, and 4) molecular mediators linking modifiable risk factors to diseases.

Conclusion: We provide the first human reference map that identifies and contextualizes major biological and technical influences on the plasma proteome and helps to translate findings from proteomic studies for a large range of diverse diseases.

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Conflict of Interest: Julia Carrasco-Zanini: None declared, Eleanor Wheeler E.W. is now an employee at AstraZeneca., Burulça Uluvar: None declared, Nicola Kerrison: None declared, Mine Koprulu: None declared, Nicholas J. Wareham: None declared, Maik Pietzner: None declared, Claudia Langenberg: None declared

P18.034.A Exploring the causal effects of circulating galectin-3 and ST2 on 11 sleep-related traits and 8 cardiometabolic traits: A Bidirectional Mendelian Randomization Study

Chun Hei Wu[1], *Man Yung Cheung*[1;2;3]

[1]*The University of Hong Kong, Department of Medicine, Hong Kong, China;* [2]*State Key Laboratory of Pharmaceutical Biotechnology, The University of Hong Kong, Hong Kong, China;* [3]*Institute of Cardiovascular Science and Medicine, The University of Hong Kong, Hong Kong, China*

Background/Objectives: Both Galectin-3 (gal-3) and ST2 proteins are cardiovascular proteins associated with inflammation and immune responses. Observational studies have reported associations between gal-3 or ST2 protein and sleep-related traits as well as cardiometabolic traits. Our study aims to evaluate the causal links between gal-3 or ST2 protein and these traits.

Methods: We conducted a comprehensive two-sample bidirectional Mendelian randomization analysis, utilizing genome-wide association study summary statistics from UK Biobank and other large consortia to investigate the causal links between them.

Results: Genetically predicted gal-3 was causally associated with short sleep duration (Odd Ratio (OR): 1.007, [95% CI, 1.002-1.011]), frequent insomnia symptoms (OR: 1.017, [95% CI, 1.011-1.024]), sleep duration (OR: 0.988, [95% CI, 0.978-0.998]), napping (OR: 0.993, [95% CI, 0.988-0.999]), and body mass index (BMI) (beta: 0.015, [95% CI, 0.007-0.023]). In the reverse analysis, triglyceride levels (beta: 0.104, [95% CI, 0.043-0.165]), fasting

insulin levels (beta: -0.447, [95% CI, -0.791–-0.101]), HbA1c levels (beta: 0.235, [95% CI, 0.054-0.417]), and waist-hip ratio adjusted for BMI (beta: -0.075, [95% CI, -0.14–-0.008]) were associated with genetically predicted gal-3. Furthermore, genetically predicted ST2 was found to be causally associated with sleep duration (OR: 1.009, [95% CI, 1.000-1.019]) and BMI (beta: 0.009, [95% CI, 0.001-0.017]). In the reverse analysis, triglyceride levels (beta: 0.065, [95% CI, 0.009-0.120]) and sleep duration (beta = 0.196, [95% CI, 0.017-0.375]) were associated with genetically predicted ST2.

Conclusion: Overall, our study provides evidence supporting the causal associations between genetically predicted gal-3 or ST2 and sleep-related traits and cardiometabolic traits.

Grants: None

Conflict of Interest: None declared

P18.035.B Examining the link between 179 lipid species and 8 disease endpoints

Linda Ottensmann[1], *Rubina Tabassum*[1], *Sanni Ruotsalainen*[1], *Mathias Gerl*[2], *Christian Klose*[2], *Daniel McCartney*[3], *Elisabeth Widén*[1], *Kai Simons*[2], *Samuli Ripatti*[1;4;5], *Veronique Vitart*[6], *caroline hayward*[6], *Matti Pirinen*[1;7]

[1]*Institute for Molecular Medicine Finland (FIMM), University of Helsinki, Finland;* [2]*Lipotype GmbH, Dresden, Germany;* [3]*Centre for Genomic and Experimental Medicine, Institute of Genetics and Cancer, University of Edinburgh, Edinburgh;* [4]*University of Helsinki, Department of Public Health, Clinicum, Faculty of Medicine, Helsinki, Finland;* [5]*Broad Institute of the Massachusetts Institute of Technology and Harvard University, Cambridge, United States;* [6]*Medical Research Council Human Genetics Unit, Institute of Genetics and Cancer, University of Edinburgh, Edinburgh, United Kingdom;* [7]*University of Helsinki, Department of Mathematics and Statistics, Helsinki, Finland*

Consortium: FinnGen

Background/Objectives: Human plasma lipidome captures risk for cardiovascular diseases and beyond. Genome-wide association studies of individual lipid species have identified many lipid-associated loci, however, the influence of associated genetic variants on disease risk remains poorly understood.

Methods: Regional heritability for 179 lipid species from the GeneRISK cohort was estimated with FINEMAP. For lead variants and top variants from FINEMAP in high heritability regions, association analyses of 8 disease endpoints were performed in FinnGen (N = 500,348), UK Biobank White British subset (N = 408,959), and Generation Scotland (N = 20,032). We performed univariable Mendelian randomization (MR) analysis of lipid species and the disease endpoints. Statistically significant results were explored further using multivariable MR (MVMR).

Results: We identified 11 loci (*ABCG8*, *AL161670.1*, *APOE*, *CERS4*, *FADS2*, *GLTPD2*, *LINC01722*, *LIPC*, *NTAN1*, *TMC4*, *ZPR1*) with regional heritability values > 2%. Variants in these high-impact loci are associated (P < 6.25e-9) with Alzheimer's disease, Cholelithiasis, Type 2 diabetes, Ischemic heart disease (IHD), Statin medication, Pure hypercholesterolemia, and Age-related macular degeneration (AMD). Univariable MR analysis linked 20, 45, and 49 lipid species to AMD, IHD, and Cholelithiasis, respectively. The genetic instruments contributing to the significant effect of Phosphatidylethanolamines (PE) on AMD are located within the *LIPC* locus. For IHD, MVMR analysis supports a causal role of ApoB.

Conclusion: We identified 11 loci with high regional heritability. Lipid species-associated variants within these loci revealed significant associations with disease endpoints. Variants within *LIPC* influence PE levels and AMD risk. MVMR supports a causal role of ApoB on IHD.

Conflict of Interest: Linda Ottensmann: None declared, Rubina Tabassum: None declared, Sanni Ruotsalainen: None declared, Mathias Gerl employee of Lipotype GmbH, Christian Klose shareholder of Lipotype GmbH, Daniel McCartney: None declared, Elisabeth Widén: None declared, Kai Simons shareholder of Lipotype GmbH, CEO of Lipotype GmbH, Samuli Ripatti: None declared, Veronique Vitart: None declared, caroline hayward: None declared, Matti Pirinen: None declared

P18.036.C Impact of *CYP2C19* variants on clinical efficacy of doubled maintenance dose of clopidogrel in coronary artery disease Syrian patients undergoing percutaneous coronary intervention

Nour Haj Saleh[1], *Lama Youssef*[1]

[1]*Faculty of Pharmacy, Damascus University, Department of Pharmaceutics & Pharmaceutical Technology, Damascus, Syria, Arab Rep*

Background/Objectives: Variability in the response to clopidogrel antiplatelet therapy is influenced by pharmacogenomics, particularly the cytochrome P450 (CYP) enzyme encoded by the *CYP2C19* gene. This study aimed to determine the frequencies of clinically relevant *CYP2C19* alleles and assess their impact on the clinical efficacy of a double maintenance dose of clopidogrel in Syrian patients undergoing percutaneous coronary intervention (PCI).

Methods: This prospective cohort study enrolled 50 Syrian patients with coronary artery disease (CAD) who underwent PCI and received a double maintenance dose of clopidogrel. *CYP2C19* polymorphisms were assessed using polymerase chain reaction (PCR) followed by amplicon Sanger sequencing. Patients were followed up after 18-24 months to evaluate their clinical response to clopidogrel.

Results: Allelic frequencies of *CYP2C19*2*, *CYP2C19*3*, and *CYP2C19*17* were 8%, 0%, and 17%, respectively. The distribution of our study participants by CYP2C19 predicted phenotype was as follows: 56% were normal metabolizers (NMs), 26% were intermediate metabolizers (IMs), 12% were rapid metabolizers (RMs), and 2% were ultra-rapid metabolizers (UMs). Our study did not find a correlation between the *CYP2C19*2* allele and recurrence of cardiac ischemic events in patients receiving a double maintenance dose of clopidogrel. Similarly, no correlation was observed between the *CYP2C19*17* allele and bleeding event rate in our study population.

Conclusion: Our findings demonstrate that a doubled maintenance dose of clopidogrel (150 mg/day) could mitigate the variability in clopidogrel antiplatelet efficacy between *CYP2C19*2* carriers and non-carriers in Syrian patients undergoing PCI.

Grants: The Ministry of Higher Education and Scientific Research - Fund to support scientific research and technological development & Damascus University.

Conflict of Interest: None declared

P18.037.D To screen or not to screen: evaluating the costs and benefits of genomic risk pre-screening in public health

Katie Saxby[1], *Christopher Nelson*[1;2], *Alex Sutton*[3], *Nicola Cooper*[3]

[1]*University of Leicester, Department of Cardiovascular Sciences, United Kingdom;* [2]*University of Leicester, NIHR Biomedical Research Centre, Leicester, United Kingdom;* [3]*University of Leicester, Department of Population Health Sciences*

Background/Objectives: There are high hopes for the use of polygenic risk scores (PRS) in population healthcare. One such hope is to utilise PRS to identify people at elevated risk of disease to focus prevention strategies. For diseases that do not have a screening programme in place, the patient level benefits are clear. But what is the impact when screening is already offered at the

population level and incorporating genomic risk leads to a reduction in the number screened?

Methods: Using a PRS for abdominal aortic aneurysm disease, the cost-effectiveness of the current UK screening program has been compared with an intervention where genetic pre-screening determines which patients are invited to the screening programme.

Results: Pre-screening offers an economically cheaper alternative to population wide screening in a scenario where genomic risk information is freely available. However, on average, this results in a reduction in benefits to patients with aneurysms due to worse outcomes from missed cases.

Conclusion: Pre-screening will result in a loss of benefits to AAA patients unless genetic prediction is 100% accurate. Better disease prediction, especially identifying patients with poor outcomes, can limit the loss of these benefits and maximise the cost-effectiveness of the programme. Money saved from screening fewer patients could provide opportunity cost to improve overall patient benefits in the health service by freeing up financial resource to fund other services or interventions that offer better value for money.

Grants: This research was funded by the Wellcome Trust 222959/Z/21/Z.

Conflict of Interest: None declared

P18.038.A Exploring the causal link between type 2 diabetes and Alzheimer's disease: a systematic review and meta-analysis of mendelian randomization studies

Si Han[1], *Tom Lelieveldt*[2], *Fariba Ahmadizar*[1], *Miriam Sturkenboom*[1], *Geert Jan Biessels*[3]

[1]*UMC Utrecht, Department of Data Science and Biostatistics, Utrecht, Netherlands;* [2]*University College Utrecht, Department of Biomedical Science, Utrecht, Netherlands;* [3]*UMC Utrecht, Department of Neurology, Utrecht, Netherlands*

Background/Objectives: The causal relationship between type 2 diabetes (T2D) and Alzheimer's disease (AD) remains controversial. In this study, we assessed evidence from mendelian randomization (MR) studies to provide a less biased estimate of any association between T2D and AD through a meta-analysis.

Methods: We searched PubMed, Web of Science and EMBASE databases from inception until Oct 2023. We included two sample MR studies investigating the association between T2D and AD. Our study only included MR studies with unique exposure/outcome cohorts to reduce the bias of duplicate cohorts. The combined odds ratio (OR) with a 95% confidence interval (CI) of AD risk was calculated using a random-effects model. Heterogeneity was evaluated using I^2 statistic. Two investigators independently extracted the data and appraised the risk of bias by using the Newcastle–Ottawa Scale (NOS).

Results: Among 274 identified publications, 7 two sample MR studies with unique exposure/outcome cohorts were included in our meta-analysis. All of the included population were of European ancestry. The pooled risk estimate revealed that genetic predisposition to T2D is significantly associated with an increased risk of AD (OR: 1.08; 95% CI:1.01 to 1.16; I^2:68%; $p < 0.01$).

Conclusion: Our study reveals a statistically significant association between a genetic predisposition to T2D and an elevated risk of AD. While our findings highlight a notable statistical relationship, further research and consideration of potential confounding factors are essential to fully understand the complex interplay between genetic factors associated with T2D and the risk of developing AD.

Grants: Si Han is granted by the China Scholarship Council.

Conflict of Interest: None declared

P18.039.B Causal relationship between serum uric acid and eGFR and chronic kidney disease: Mendelian randomization using polygenic risk scores

Kai-Yi Chen[1], *Yung-Feng Lin*[2], *Chien-Hsiun Chen*[3], *Hui-Ying Weng*[2;4], *Shih-Feng Tsai*[2;4;5], *Ming-Wei Lin*[1;4]

[1]*National Yang Ming Chiao Tung University, Institute of Public Health, Taipei, Taiwan;* [2]*National Health Research Institutes, Institute of Molecular and Genomic Medicine, Miaoli, Taiwan;* [3]*Academia Sinica, Institute of Biomedical Sciences, Taipei, Taiwan;* [4]*National Yang Ming Chiao Tung University, Biomedical Industry PhD Program, Taipei, Taiwan;* [5]*National Yang Ming Chiao Tung University, Department of Life Sciences and Institute of Genome Sciences, Taipei, Taiwan*

Background: Chronic kidney disease (CKD) is ranked as the 9th cause of mortality in Taiwan. Previous studies reported that uric acid (UA) is a potential risk factor of CKD, but the causal relationship between UA and renal function remains inconsistent. The study used polygenic risk score of uric acid as instrumental variable (IV) in Mendelian randomization analysis, to explore the causal relationship between UA and estimated glomerular filtration rate (eGFR) and CKD.

Methods: A total of 102,536 Han Chinese subjects with the genome-wide genotypes of the TWB2.0 chip were obtained from the Taiwan Biobank. After quality control, a genome-wide association study (GWAS) of uric acid with 87,654 participants and 445,874 genetic variants was performed. The weighted PRS, C + T, LDpred, PRS-CS, and DBSLMM methods were used to calculate the polygenic risk scores. Mendelian randomization using the two-stage least squares method was conducted with polygenic risk score of UA as IV, and eGFR and CKD as the outcome variables.

Results: After adjusting for age and sex, a polygenic risk score consisting of 114,073 genetic variants from the GWAS results of the Taiwan Biobank was used as an IV in MR analysis. Significant causal relationship between UA and eGFR (β = -2.54, 95% C.I.=-2.64 to -2.44) as well as UA and CKD (OR=2.47, 95% C.I.=2.26 to 2.71) was found.

Conclusion: Our results confirmed the causal relationships between uric acid and eGFR as well as CKD.

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Conflict of Interest: Kai-Yi Chen: None declared, Yung-Feng Lin Taipei Medical University Hospital, Chien-Hsiun Chen Academia Sinica, Taipei, Taiwan, Hui-Ying Weng: None declared, Shih-Feng Tsai National Health Research Institutes, Miaoli, Taiwan, Ming-Wei Lin National Yang Ming Chiao Tung University, Taipei, Taiwan

P18.040.C Genomic analyses of bullying victimisation in children and adolescents

Tracy Odigie[1], *Leonard Frach*[1], *Andrea Allegrini*[1], *Wikus Barkhuizen*[1], *Laura Hegemann*[2;3], *Laurie J. Hannigan*[2;4;5], *Alexandra Havdahl*[2;3;5], *Jean-Baptiste Pingault*[1;6]

[1]*University College London, Department of Clinical, Educational and Health Psychology, Division of Psychology and Language Sciences, London, United Kingdom;* [2]*Nic Waals Institute, Lovisenberg Diaconal Hospital, Oslo, Norway;* [3]*University of Oslo, Department of Psychology, Oslo, Norway;* [4]*University of Bristol, Medical Research Council Integrative Epidemiology Unit, Bristol, United Kingdom;* [5]*Norwegian Institute of Public Health, Center for Genetic Epidemiology and Mental Health, Oslo, Norway;* [6]*King's College London, Social,*

Genetic and Developmental Psychiatry Centre, Institute of Psychiatry, Psychology and Neuroscience, London, United Kingdom

Background/Objectives: Bullying victimisation is a phenomenon experienced by over 65% of children and adolescents globally. Quantitative methodical approaches have been used to identify profiles of bullying behaviours. However, these approaches require summary data from genome-wide association studies (GWAS's), which is currently unavailable for bullying victimisation. This paper aims to conduct a GWAS of bullying victimisation and perform downstream genetic analyses.

Methods: We use genotyped data from the Norwegian Mother, Father and Child Cohort Study (MoBa) (~N = 43,000). Bullying victimisation is measured at ages 5, 8 and 14. The GWAS summary statistics of bullying victimisation is used to explore genetic correlations across 21 psychiatric conditions and associated traits. Polygenic scores of the traits are generated and used to predict bullying victimisation. Mendelian randomisation analyses will check for causality.

Results: Significant genetic correlations (ranging from 0.21-0.70) were identified between 15 traits and the exposure to childhood bullying victimisation. For adolescence bullying, high significant genetic correlations were identified in 6 traits (ranging from 0.40 to 1.28). Genetic correlation and polygenic score analyses show consistent significant results between bullying victimisation and traits. Small causal effects on psychiatric and associated traits in children and adolescents are identified.

Conclusion: In line with evidence of pervasive pleiotropy between psychiatric and associated traits, bullying victimisation and mental health related phenotypes are genetically correlated. This study will contribute to our understanding of the implications pre-existing heritable mental health vulnerabilities have on exposure to bullying victimisation.

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Conflict of Interest: None declared

P18.041.D Revising clinical pharmacogenomics: panel-based targeted haplotyping using Oxford Nanopore Technologies long-read sequencing

Koen Deserranno[1], *Laurentijn Tilleman*[1], *Kaat Rubben*[1], *Dieter Deforce*[1], *Filip Van Nieuwerburgh*[1]

[1]*Ghent University, Department of Pharmaceutics - Lab of Pharmaceutical Biotechnology, Gent, Belgium*

Background/Objectives: Current pharmacogenomics (PGx) testing strategies are mainly based on microarray, polymerase chain reaction (PCR), or short-read sequencing. Despite their value in identifying single nucleotide variants (SNVs) and insertion/deletions (INDELs), these assays do not permit to identify large structural variants nor allow unambiguous haplotype phasing for star-allele assignment. To address these issues, we assessed the performance of long-read sequencing in PGx while enriching for a panel of 1,036 genes from the Pharmacogenomics Knowledge Base (PharmGKB).

Methods: Germline DNA of five Genome in a Bottle (GIAB) reference samples was used for Oxford Nanopore Technologies PCR-free library preparation on R10.4.1 PromethION flow cells. Target enrichment was performed real-time during sequencing using adaptive sampling. To increase cost-effectiveness, three samples were multiplexed on one flow cell, decreasing the cost to € 320 per patient.

Results: By evaluating concordance with existing truth sets, we demonstrate accurate variant and star-allele calling for five GIAB samples. We show that three samples can be multiplexed on one flow cell, resulting in 99.35% and 99.84% recall and precision for the targeted variants. We illustrate that the long reads allow for better haplotype discrimination and improved star-allele calling, demonstrated by the enhanced calls for *CYP1A2*, *CYP2B6*, *CYP2C9*, *CYP2E1*, *CYP3A4*, *DPYD*, *GSTP1*, *SLCO1B1* and *UGT1A1*.

Conclusion: Targeted nanopore sequencing delivers superior PGx information for variant calling and haplotype phasing, with the potential for better dosing regimens. PGx based on targeted long-read sequencing is a valuable tool to advance the implementation of personalized medicine.

Grants: Special Research Fund, Ghent University, BOF21/DOC/042

Conflict of Interest: Koen Deserranno In the past, KD has received free congress access from Oxford Nanopore Technologies (ONT) to present prior research findings at their scientific meeting., Laurentijn Tilleman: None declared, Kaat Rubben: None declared, Dieter Deforce: None declared, Filip Van Nieuwerburgh: None declared

P18.042.A Two drifted variants account for over 90% of pathogenic BRCA alleles in Orkney and Shetland genomes

James F. Wilson[1;2], *Shona Kerr*[2], *Lucija Klaric*[2], *Marisa Muckian*[1;3], *Emma Cowan*[4], *Lesley Snadden*[4], *Gannie Tzoneva*[5], *Alan R Shuldiner*[5], *Zosia Miedzybrodzka*[4;6]

[1]*University of Edinburgh, Usher Institute, Edinburgh, United Kingdom;* [2]*University of Edinburgh, MRC Human Genetics Unit, Edinburgh, United Kingdom;* [3]*London School of Hygiene and Tropical Medicine, Department of Infectious Disease Epidemiology, London, United Kingdom;* [4]*NHS Grampian, Department of Medical Genetics, Aberdeen, United Kingdom;* [5]*Regeneron Genetics Center, Tarrytown, NY, United States;* [6]*University of Aberdeen, Medical Genetics Group, Aberdeen, United Kingdom*

Background/Objectives: For breast and ovarian cancer risk assessment in the populations of Orkney and Shetland (the Northern Isles of Scotland) and their diasporas, it is important to quantify genetically drifted *BRCA1* and *BRCA2* pathogenic variants. Two actionable variants in these genes are observed recurrently at much higher frequencies than in cosmopolitan UK populations.

Methods: Here, we report a pathogenic *BRCA2* splice variant, c.517-2A>G, observed in four Shetland families to segregate with breast and ovarian cancer. We investigated the frequency and origin of this variant in exome sequences from a population-based research cohort of 2,108 volunteers with three or more Shetlandic grandparents, VIKING I.

Results: Nine research volunteers carry this variant, on a shared haplotype (carrier frequency of 0.4%, ~130-fold higher than in UK Biobank). Four of these volunteers, previously unaware that they carried the variant, sought confirmatory diagnostic testing, leading to cascade testing being offered to >20 further relatives. Vital records indicate genealogical linkage to a founder from the Isle of Whalsay, Shetland. This is similar to our observations for the *BRCA1* founder variant Val1736Ala from the Isle of Westray, Orkney, where return of results has led to 56 cascade tests, subsequent to five carriers being identified.

Conclusion: In total, 93.5% of pathogenic BRCA variant carriers in Northern Isles genomes are accounted for by these two drifted variants. We therefore recommend that those of Orcadian and Shetlandic origin be offered testing for the relevant drifted pathogenic variant, particularly those with Westray or Whalsay ancestry.

Grants: Wellcome Trust (222060/Z/20/Z -PIII031)

Conflict of Interest: James F. Wilson University of Edinburgh, Shona Kerr University of Edinburgh, Lucija Klaric BioAge Labs,

BioAge Labs, Marisa Muckian London School of Hygiene and Tropical Medicine, Emma Cowan NHS Grampian, Lesley Snadden NHS Grampian, Gannie Tzoneva Regeneron Genetics Center of Regeneron Pharmaceuticals, Regeneron Genetics Center of Regeneron Pharmaceuticals, Alan R Shuldiner Regeneron Genetics Center of Regeneron Pharmaceuticals, Regeneron Genetics Center of Regeneron Pharmaceuticals, Zosia Miedzybrodzka NHS Grampian

P18.043.B Genome-wide gene-environment interaction study uncovers 162 novel vitamin D status variants after precise estimation of individual ambient UV exposure

Rasha Shraim[1;2;3], *Jos van Geffen*[4], *Michiel van Weele*[4], *Ross McManus*[2], *Lina Zgaga*[1]

[1]*Trinity College Dublin, Public Health and Primary Care, Dublin, Ireland;* [2]*Trinity College Dublin, Clinical Medicine, Dublin, Ireland;* [3]*University of Galway, The SFI Centre for Research Training in Genomics Data Sciences, Galway, Ireland;* [4]*Royal Netherlands Meteorological Institute, De Bilt, Netherlands*

Background/objectives: Vitamin D status is influenced by genetic, lifestyle, and environmental factors, such as sun exposure. Recent genome-wide studies have identified over 140 variants linked to vitamin D status. However, the interaction effects remain largely unobserved in studies to date, which used a dichotomized summer/winter season variable as a proxy of environmental exposure.

Methods: We used an exact measure of ambient UV dose to approximate environmental exposure for each participant in the UK Biobank, based on their place of residence and date of blood sampling. UV dose was adjusted for weather, ozone, altitude and other key factors, and the final estimate accounted for accumulation/decay of vitamin D in the body. We carried out a genome-wide joint test of marginal and interaction effects in 25-hydroxyvitamin-D (25OHD) in 338,977 White British individuals, adjusting for other key covariates.

Results: Our findings identify over 160 novel independent variants significantly associated with 25OHD concentration. Downstream functional analysis of these variants supports vitamin D involvement in metabolic pathways and hormone and skin phenotypes, and identifies novel genes linked to 25OHD. Based on these results, we also evaluate the change in the SNP-based heritability of 25OHD associated with ambient UV.

Conclusion: Our findings provide new insights into the biology of vitamin D status. These results highlight the importance of using accurate environmental exposure measures and accounting for interactions in uncovering the genetic architecture of complex traits.

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Conflict of Interest: None declared

P18.044.C Mitochondrial genome variability in COVID-19 patients

Alessia Fiorentino[1], *Claudio Fiorini*[1], *Danara Ormanbekova*[1], *Alessandro Mattiaccio*[2], *Edoardo Spagnolo*[2], *Maddalena Giannella*[3], *Pierluigi Viale*[3], *Zaira Palacios Baena*[4], *Tommaso Pippucci*[5], *Marco seri*[5], *Leonardo Caporali*[6], *Valerio Carelli*[1;6]

[1]*IRCCS Istituto delle Scienze Neurologiche di Bologna, Programma di Neurogenetica, Bologna, Italy;* [2]*Alma Mater Studiorum - University of Bologna, Department of Medical and Surgical Sciences (DIMEC), Bologna, Italy;* [3]*IRCCS Azienda Ospedaliero-Universitaria di Bologna, Infectious Diseases Unit, Bologna, Italy;* [4]*Institute of Biomedicine of Seville (IBIS)/CSIC - University Hospital Virgen Macarena, Unit of Infectious Diseases and Clinical Microbiology, Seville, Spain;* [5]*IRCCS Azienda Ospedaliero-Universitaria di Bologna, Medical Genetics Unit, Bologna, Italy;* [6]*Alma Mater Studiorum - University of Bologna, Department of Biomedical and NeuroMotor Sciences (DiBiNeM), Bologna, Italy*

Consortium: Orchestra Genomics group

Background: The main pathomechanism in severe COVID-19 is an immune imbalance and systemic inflammatory response. The clinical features of COVID-19, range from an asymptomatic state to pneumonia to death. These heterogeneous symptoms might be partly explained by the individual's hereditary susceptibility. In addition to their primary function as energy producers and regulators of cellular processes, mitochondria play an important role in the vertebrates innate immunity. The mitochondria innate antioxidant and immune capacities could also play a role in the susceptibility, and in this regard the mitochondrial DNA (mtDNA) variants might serve as susceptibility markers for severe COVID-19.

Methods: We collected DNA from 640 COVID-19 patients' whole peripheral blood and high-coverage Whole Genome Sequence (WGS) was performed. We retrieved mtDNA variants from the WGS VCF files and mtDNA haplogroup was assigned to each patient using HaploGrep v2.4.0.The mtDNA copy number was calculated using the nuclear and mitochondrial mean coverage. The patients have been categorised using the WHO Clinical Progression Scale (score 1 to 10) and divided into three groups: mild (1-4), moderate (5-7) and severe (8-10).

Results: We highlighted a significant difference in mtDNA copy number, which decreased in relation to the increase of disease severity, suggesting that most severe cases were relatively depleted of mtDNA, possibly reflecting into OXPHOS deficiency. Furthermore, the haplogroup H frequency resulted to be significantly higher in mild patients, protecting from severe forms of COVID-19.

Conclusion: Our data confirm mitochondria as a player in the COVID-19 infection and as a possible target for therapy.

Grants: H2020-ORCHESTRA

Conflict of Interest: None declared

P18.045.D High *IL1B* expession in the in vitro differentiated macrophages indicates poor clinical response to adalimumab in Crohn's disease patients

Boris Gole[1], *Cvetka Pernat*[2], *Gregor Jezernik*[1], *Larisa Goričan*[1], *Mario Gorenjak*[1], *Ursula Prosenc Zmrzljak*[3], **Uroš Potočnik**[1;4;5]

[1]*Faculty of medicine, University of Maribor, Centre for human molecular genetics and pharmacogenomics, Maribor, Slovenia;* [2]*Maribor University Medical Centre, Division of Internal Medicine, Department of Gastroenterology, Maribor, Slovenia;* [3]*BIA Separations CRO – Labena d.o.o., Ljubljana, Slovenia;* [4]*Faculty for Chemistry and Chemical Engineering, University of Maribor, Laboratory for Biochemistry, Molecular Biology and Genomics, Maribor, Slovenia;* [5]*Maribor University Medical Centre, Department for Science and Research, Maribor, Slovenia*

Background/Objectives: Molecular markers of anti-TNFα therapy response in Crohn's disease (CD) patients described in bulk peripheral blood immune cells suffer from poor reproducibility. Recent studies suggested that research of specific cell sub-types may yield superior biomarkers that could be translated into clinical practice. Peripheral blood monocytes and macrophages were previously indicated as key factors in both CD development and anti- TNFα therapy. Here we investigate monocyte to macrophage differentiation and expression of the inflammatory cytokines by

these cells in relation to the outcome of the adalimumab (anti-TNFα) therapy in CD patients.

Methods: CD patients responsive and non-responsive to adalimumab were recruited. Peripheral monocytes were isolated, differentiated into macrophages and exposed to inflammatory conditions with/without concurrent adalimumab treatment. We compared the in vitro outcomes to the clinical response of the donor patients. In parallel, we measured expression of the inflammatory cytokines in the in vitro monocytes/macrophages. Additionally, we analysed expression of the inflammatory cytokines in the peripheral monocytes using the single-cell RNA-sequencing.

Results: Monocytes and in vitro differentiated macrophages of adalimumab non-responders express more inflammatory cytokines than those of responders. The biggest differences are in the *IL1B* expressions. The *IL1B* expression in the in vitro differentiated macrophages of CD patients negatively correlates with their clinical response to adalimumab.

Conclusion: We propose *IL1B* expression in the macrophages as a potential biomarker for adalimumab response in CD patients.

Grants: Slovenian Research and Innovation Agency research core funding P3-0427 and research grant J3-9258. Labena d.o.o. co-financed the single-cell RNA-sequencing.

Conflict of Interest: Boris Gole: None declared, Cvetka Pernat: None declared, Gregor Jezernik: None declared, Larisa Goričan: None declared, Mario Gorenjak: None declared, Ursula Prosenc Zmrzljak U.P.Z. is full-time employed at Labena d.o.o., which co-financed the presented work, Uroš Potočnik: None declared

P18.047.B Systematic investigation of proteogenomic variation between sexes and its relevance for human diseases

Mine Koprulu[1], *Eleanor Wheeler*[1], *Nicola Kerrison*[1], *Spiros Denaxas*[2;3;4;5], *Julia Carrasco-Zanini*[1;6], *Harry Hemingway*[2;4;5], *Nicholas J. Wareham*[1], *Maik Pietzner*[1;6;7], *Claudia Langenberg*[1;6;7]

[1]*MRC Epidemiology Unit, University of Cambridge, United Kingdom;* [2]*UCL Institute Of Health Informatics, United Kingdom;* [3]*British Heart Foundation Data Science Centre, London, United Kingdom;* [4]*Health Data Research UK, London, United Kingdom;* [5]*National Institute of Health Research University College London Hospitals Biomedical Research Centre;* [6]*Precision Healthcare University Research Institute, QMUL, London, United Kingdom;* [7]*Computational Medicine, Berlin Institute of Health at Charité-Universitätsmediz, Berlin, Germany*

Background/Objectives: Many aspects of human development and health differ between sexes, but the underlying mechanisms or extent to which genetic factors contribute to any differences remain largely unknown. Understanding genetically driven sex-differences at the molecular level, specifically proteins as the biologically active entity between the genome and the phenome, is important for basic and translational genetic research.

Methods: To better understand sex differences in human health and disease, we conducted a systematic, large-scale investigation of sex differences in the genetic regulation of the plasma proteome (>5,000 targets), including their disease relevance.

Results: Plasma levels of two-thirds of protein targets differed significantly by sex. In contrast, genetic effects on protein targets were remarkably similar, with very few protein quantitative loci (pQTLs, n = 74) showing significant sex-differential effects (for 3.9% and 0.3% of protein targets from antibody- and aptamer-based platforms, respectively). Most of these 74 pQTLs represented directionally concordant effects significant in both sexes, with only 21 pQTLs showing evidence of sexual dimorphism, i.e. effects restricted to one sex (n = 20) or with opposite directions between sexes (n = 1 for CDH15). None of the sex-differential pQTLs translated into sex-differential disease risk.

Conclusion: Our results demonstrate strong similarity in the genetic regulation of the plasma proteome between sexes with important implications for genetically guided drug target discovery and validation.

Grants: Medical Research Council: MC_UU_12015/1, MC_PC_13046, MC_UU_00006/1

Conflict of Interest: Mine Koprulu: None declared, Eleanor Wheeler E.W. is now an employee of AstraZeneca., Nicola Kerrison: None declared, Spiros Denaxas: None declared, Julia Carrasco-Zanini: None declared, Harry Hemingway: None declared, Nicholas J. Wareham: None declared, Maik Pietzner: None declared, Claudia Langenberg: None declared

P18.048.C Implementation of Genetics-Based Precision Prevention in Breast Cancer: Results from the Estonian Arm of the BRIGHT Study

Madli Tamm[1], *Peeter Padrik*[2;3], *Anette Paas*[2], *Anni Lepland*[4], *Krista Kruuv-Käo*[2], *Siim Sõber*[2], *Laura Roht*[4;5], *Kristiina Ojamaa*[3], *Sander Pajusalu*[4;5], *Anna Padrik*[2], *Jagnar Pindmaa*[2], *Kadri Luga*[6], *Ly Rootslane*[7], *Anne Ilves*[8], *Sulev Ulp*[9], *Kersti Kallak*[3;5], *Ave-Triin Tihamäe*[10], *Liis Leitsalu*[1;4], *Neeme Tonisson*[1;2;4]

[1]*Institute of Genomics, University of Tartu, Tartu, Estonia;* [2]*OÜ Antegenes, Tartu, Estonia;* [3]*Haematology and Oncology Clinic, Tartu University Hospital, Tartu, Estonia;* [4]*Genetics and Personalized Medicine Clinic, Tartu University Hospital, Tartu, Estonia;* [5]*Institute of Clinical Medicine, University of Tartu, Tartu, Estonia;* [6]*OÜ Rapla Perearstikeskus, Rapla, Estonia;* [7]*AS Magnum, Laagri, Estonia;* [8]*Women's Clinic, Tartu University Hospital, Tartu, Estonia;* [9]*Radiology Clinic, Tartu University Hospital, Tartu, Estonia;* [10]*Surgery Clinic, Tartu University Hospital, Tartu, Estonia*

Consortium: Be RIGHT with breast cancer management (BRIGHT)

Background: Breast cancer (BC) is the leading cause of cancer-related deaths in women. Standard BC screening considers only age as a risk factor, excluding women under 50 with elevated risks. Our international BRIGHT study highlights the role of genetic predisposition with polygenic risk score (PRS) and monogenic pathogenic variant (MPV) testing. We hypothesise that genetics-based screening is feasible, acceptable, and has clinical utility.

Methods: BRIGHT study addresses 2400 healthy women aged 30–49 in Estonia, Sweden, and Portugal. In Estonia, four recruitment channels for personalised prevention with home sampling and digital counselling were tested with feedback collection from patients and medical professionals. The service model included a questionnaire for MPV testing and clinical PRS testing (2803 SNPs). Personalised screening and prevention recommendations were given based on the tests' results.

Results: The recruitment was effective across all channels. Amongst 800 participants aged 35–49, 72 had MPVs tested after consultations by clinical geneticists, resulting in 5 MPV diagnoses. PRS testing for all identified 124 women at higher risk than the average 50-year-old. They started personalised screening earlier than the national standard, resulting in one immediate BC diagnosis. Feedback on the implementation of precision prevention was very positive.

Conclusion: The BRIGHT study demonstrated the feasibility of genetics-based precision prevention, facilitating earlier BC screening for younger women with elevated genetic risks. The predominantly digital service minimised the burden on healthcare personnel.

Grants: Supported by the European Commission via EIT Health BRIGHT innovation activity (project #220720, #230121) and the Estonian Research Council PRG555 grant.

Conflict of Interest: Madli Tamm: None declared, Peeter Padrik OÜ Antegenes, Anette Paas OÜ Antegenes, Anni Lepland: None declared, Krista Kruuv-Käo OÜ Antegenes, Siim Sõber OÜ Antegenes, Laura Roht: None declared, Kristiina Ojamaa: None declared, Sander Pajusalu: None declared, Anna Padrik OÜ Antegenes, Jagnar Pindmaa OÜ Antegenes, Kadri Luga: None declared, Ly Rootslane: None declared, Anne Ilves: None declared, Sulev Ulp: None declared, Kersti Kallak: None declared, Ave-Triin Tihamäe: None declared, Liis Leitsalu: None declared, Neeme Tonisson OÜ Antegenes

P18.049.D Variation in the causal influence of body mass on common diseases across a lifetime

Torgny Karlsson[1], *Fatemeh Hadizadeh*[1], *Mathias Rask-Andersen*[1], *Daniel Schmitz*[1], *Åsa Johansson*[1]

[1]*Uppsala University, Immunology, Genetics and Pathology, Uppsala, Sweden*

Background/Objectives: Obesity is a major risk factor for most non-communicable diseases. The burden of disease is substantially higher in the older part of the population, but how obesity affects disease risk at different ages is largely unknown. To quantify the causal impact of obesity over the lifespan, we introduce a new method – Time-Resolved Mendelian Randomization – which estimates the time-dependent, cumulative effect of body mass index (BMI) on disease rate.

Methods: The method properly accounts for the time-dependence of the BMI instrument and relies only on patient register data to estimate effects, using Aalen's additive hazard model. We applied the method to determine life-course effects on four common diseases in the UK Biobank, including type 2 diabetes, coronary artery disease, atrial fibrillation, and osteoarthritis.

Results: Substantial differences in life-course effects were observed between diseases. Time-series trends of the causal estimates for atrial fibrillation and osteoarthritis increased with age in a highly non-linear fashion. In comparison, a significant trough in effect was found for coronary artery disease at around 60-70 years of age, in both sexes. A similar feature was found for type 2 diabetes in females. Besides osteoarthritis, obesity was a larger risk factor in males compared to females, at all ages above a critical time-point.

Conclusion: By resolving the causal effect of a lifelong exposure to high BMI in time, we can elucidate the temporal aetiology of obesity-related disease and find the appropriate age for introducing preventive interventions.

Grants: Swedish Research Council, Swedish Heart Lung Foundation, Novo Nordisk Foundation.

Conflict of Interest: None declared

P18.050.A An apolipoprotein L1 polygenic score is associated with susceptibility to giant cell arteritis

Natalie Chaddock[1], *Michal Zulcinski*[1], *Javier Martin*[2], *Anders Malarstig*[3], *James Peters*[4], *Mark M Iles*[1;5], *ann w morgan*[1;5]

[1]*University of Leeds, School of Medicine, Leeds, United Kingdom;* [2]*Institute of Parasitology and Biomedicine López-Neyra, CSIC, Spain;* [3]*Karolinska Institutet, Department of Medical Epidemiology and Biostatistics, Stockholm, Sweden;* [4]*Imperial College London, Department of Immunology and Inflammation, London, United Kingdom;* [5]*NIHR Biomedical Research Centre, Leeds, United Kingdom*

Consortium: UK GCA Consortium

Background/Objectives: Whilst glucocorticoid monotherapy remains the primary treatment for giant cell arteritis (GCA), concomitant adverse effects and toxicity underscores the necessity for targeted therapies and enhanced risk stratification. Prior work posits that evidence of genetic association can increase success rates in clinical trials and highlight biomarkers for risk assessment, particularly when aggregated with other 'omics data, including proteomics. However, relatively little is presently known about the genetic basis of GCA.

Methods: Polygenic risk scores (PRS) were developed for 169 circulating plasma proteins and tested for association with GCA susceptibility (cases $N = 729$, controls $N = 2{,}619$). Associated PRS were replicated in an independent dataset (cases $N = 1{,}129$, controls $N = 2{,}654$) and their respective proteins were assessed for causality with Mendelian randomization (MR). Finally, protein-protein interaction (PPI) network analysis was performed to evaluate relationships between proteins with associated PRS.

Results: The Apolipoprotein L1 (APOL1) PRS had a statistically significant protective GCA association ($P\text{-value}[P] = 1 \times 10^{-4}$), which replicated in an independent cohort ($P = 8.69 \times 10^{-4}$). This relationship was corroborated by evidence for causality in MR ($beta = -0.093$; $SE = 0.02$; $P = 4.42 \times 10^{-9}$). PPI network analysis revealed enrichment for gene ontology pathways "negative regulation of fibrinolysis" and "negative regulation of blood coagulation".

Conclusion: Genetically higher APOL1 protein levels are associated with lower GCA susceptibility, implicating a role of reverse cholesterol transport in GCA. PPI network analysis findings also suggest a contribution of fibrinolytic and coagulation cascades to GCA susceptibility, highlighting pathways of interest for future pharmaceutical targeting.

Grants: Supported by MRC DiMeN, NIHR Leeds BRC, and MRC TARGET Partnership awards.

Conflict of Interest: None declared

P18.051.B Systematic investigation of environmental factors and interplay with genetic risk reveals potential for improved prediction of male-pattern hair loss

Sabrina Henne[1], *Hannah Klinkhammer*[2;3], *Christian Staerk*[2], *Andreas Mayr*[2], *Markus Nöthen*[1], *Stefanie Heilmann-Heimbach*[1]

[1]*Institute of Human Genetics, University Hospital of Bonn & University of Bonn, Bonn, Germany;* [2]*Institute for Medical Biometry, Informatics and Epidemiology, Medical Faculty, University of Bonn, Bonn, Germany;* [3]*Institute for Genomic Statistics and Bioinformatics, Medical Faculty, University of Bonn, Bonn, Germany*

Background/Objectives: Male-pattern hair loss (MPHL) is highly heritable, yet existing prediction models are limited. Previous studies discussed the potential contribution of environmental factors and implicated smoking, alcohol consumption, and BMI as additional risk factors for MPHL. However, the evidence has been inconclusive. Here, we aimed to systematically investigate associations with environmental factors and assess their value in MPHL prediction using data from the UK Biobank.

Methods: Our analyses investigated associations between MPHL and environmental factors encompassing lifestyle, anthropometry, biological measurements, and medical history in 169,105 men. We performed age-adjusted linear and logistic regression, and cross-trait LD score regression. Integrative PRS models based on boosting techniques were tested using logistic regression, adjusted for age and 20 principal components.

Results: Results indicate that men with MPHL tend to smoke less, drink less, have a higher BMI, and possess fairer or red hair. Regarding blood-biomarkers, men with MPHL tend to have higher testosterone, IGF-1, and creatinine levels. Associated medical

conditions included a higher risk of diabetes, cardiovascular disease, and calcinosis cutis alongside a lower risk of lung disease and alcohol-related liver disease. Genetic correlations included aspartate aminotransferase and albumin levels. Incorporating environmental factors already marginally improved the prediction accuracy of PRS models, increasing the area-under-the-curve (AUC) from 0.75 to 0.76. Prediction accuracy notably improved via further inclusion of blood-biomarkers (AUC = 0.80), with proteomics (e.g. DSG4) having the greatest impact.

Conclusion: Our analyses identify environmental factors associated with MPHL and suggest improvability of predictive models, especially by integration of blood-biomarkers. Mendelian randomization analyses to clarify potential causal relationships are underway.

Grants:

Conflict of Interest: Sabrina Henne: None declared, Hannah Klinkhammer: None declared, Christian Staerk: None declared, Andreas Mayr: None declared, Markus Nöthen M.M.N. holds shares in Life & Brain GmbH., M.M.N. is a member of the excellence cluster ImmunoSensation2., M.M.N. receives salary payments from Life & Brain GmbH., M.M.N. has received fees for membership in an Advisory Board of HMG Systems Engineering GmbH, and for membership in the Medical-Scientific Editorial Office of the Deutsches Ärzteblatt., Stefanie Heilmann-Heimbach S.H.-H. receives salary payments from Life & Brain GmbH.

P18.052.C 100,000 Genomes of Europe: Unlocking genetic variability across Europe for science and health

Anthony Herzig[1], *Astrid Vicente*[2;3], *Hugo Martiniano*[2;3], *Emmanuelle Génin*[1;4], *Helen Ray-Jones*[5], *Jeroen van Rooij*[5], *André Uitterlinden*[5]

[1]*Inserm, Univ Brest, EFS, UMR 1078, GGB, Brest, France;* [2]*Instituto Nacional de Saúde Doutor Ricardo Jorge, Av Padre Cruz, Lisbon, Portugal;* [3]*Biosystems and Integrative Sciences Institute (BioISI), Faculdade de Ciências da Universidade de Lisboa, Lisboa, Portugal;* [4]*CHRU Brest, Brest, France;* [5]*Laboratory for Population Genomics, Erasmus MC, Rotterdam, Netherlands*

Consortium: The GoE/1 + **MG consortium**

Background/Objectives: As part of the 1 million genomes (1 + MG) initiative, the Genome of Europe (GoE) project will create a pan-European reference database comprising whole-genome sequencing (WGS; 30x) data from >100,000 European citizens. Spanning 51 contributing partners from 29 countries providing existing and de novo datasets (short-read and long-read WGS), GoE presents enormous potential for understanding the genetic dimension to public health in Europe; including through interactions with the European Rare Disease Research Alliance (ERDERA).

Methods: Challenges for GoE are evident across three axes: technological, statistical, and ethical, in order for GoE to provide wide-ranging utility. Here we present the scope of the "use-cases" of GoE, which will establish the envisaged applications of GoE.

Results: The following pilot studies are outlined: (1) Building a fine-scale map of genetic structure across Europe to inform aggregating individual-level data. (2) Creating a reference database for individual variant look-ups; with particular attention on clinically relevant variant frequencies in actionable-disease and pharmacogenetic genes. (3) Building reference panels for ancestry-informed genetic imputation and the required secure informatics environments. (4) Deriving distributions of polygenic (risk) scores, calibrated for ancestry gradients in Europe; with a focus on cancer phenotypes in collaboration with the Can.Heal project. (5) Understanding the added value of long-read sequencing in the general population and exploring the 'dark regions of the human genome'.

Conclusion: We lay out here the methodological advances and adjustments necessary to best unlock the potential of GoE; particularly in the context of the prospective GoE federated data embedding in 1 + MG.

Conflict of Interest: None declared

P18.053.D Comparison of disease prediction ability and transferability of polygenic scores vs. EHR-based score across three biobank studies, 1,034,311 individuals, and 18 diseases

Tuomo Hartonen[1], *Kira Detrois*[1], *Maris Teder-Laving*[2], *Kristi Läll*[2], *Zhiyu Yang*[1], *Reedik Mägi*[2], *Samuli Ripatti*[1], *Andrea Ganna*[1]

[1]*University of Helsinki, Institute for Molecular Medicine Finland, FIMM, HiLIFE, Helsinki, Finland;* [2]*University of Tartu, Estonian Genome Centre, Institute of Genomics, Tartu, Estonia*

Consortium: FinnGen, Estonian Biobank research team

Background/Objectives: Polygenic Scores (PGSs) have emerged as a promising tool to guide the screening and treatment of complex diseases. However, systematic comparisons with non-genetic risk factors are lacking. Within the INTERVENE consortium, we compared the ability of PGSs vs. electronic health record (EHR)-based scores to predict the onset of 18 diseases by creating PhenoScores comprising education, region of birth, Charlson comorbidity index, and diagnosis-based Phenotype Risk Scores (PheRSs). We included three biobank studies: FinnGen, UK Biobank (UKB), and Estonian Biobank (EstBB), totalling 1,034,311 individuals.

Methods: We calculated the PGSs using the latest publicly available genome-wide association study summary statistics. The PheRSs were based on elastic-net models, incorporating up to 164 diagnoses as predictors. We then fitted Cox proportional hazards models to predict the onset of each disease using the covariates age, sex, principal components, PGSs, and PhenoScores.

Results: In FinnGen, adding the PGS to a model with the PhenoScore improves onset prediction for 8/18 diseases. Similarly, the PhenoScores improve onset prediction over PGSs for 8/18 diseases. Interestingly, for type 2 diabetes, asthma, hip osteoarthritis, and atrial fibrillation, the two scores improve onset prediction independent of each other. PhenoScores trained on the external cohorts (UKB or EstBB), and tested in FinnGen reached similar c-indices to within-FinnGen-trained PhenoScores for 7/11 and 9/11 diseases, respectively.

Conclusion: PGSs and EHR-based PhenoScores have an independent effect on disease risk prediction and can be complementary. EHR-based scores transfer well between biobanks for the majority of the diseases considered.

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Conflict of Interest: Tuomo Hartonen: None declared, Kira Detrois: None declared, Maris Teder-Laving: None declared, Kristi Läll: None declared, Zhiyu Yang: None declared, Reedik Mägi: None declared, Samuli Ripatti: None declared, Andrea Ganna Founder of Real World Genetics

P18.054.A Genetic insights into coronary artery disease in underrepresented populations: assessing two-sample Mendelian randomization across diverse ancestry populations

Sarah Silva[1;2], *Segun Fatumo*[1;2], *Dorothea Nitsch*[1]

[1]*London School of Hygiene and Tropical Medicine, Department of Non-Communicable Disease Epidemiology, London, United Kingdom;* [2]*The African Computational Genomics Research Group (TACG), MRC/UVRI and LSHTM, Entebbe, Uganda*

Background/Objectives: Understanding the causal effect of modifiable risk factors on a disease is crucial for aiding and shaping public health policies, identifying targets of interventions and advancing our global understanding of health and diseases. With this however, the disproportionate representation of non-European ancestries in research has raised important questions regarding the transferability and reliability of genetic findings on a diverse global scale.

Methods: We investigated the feasibility of conducting a two-sample Mendelian randomization (MR) analysis in populations of diverse ancestries, focusing on methodological challenges and biologically differences when data from European, East Asian, South Asian and African ancestry populations were compared against each other. Employing data generated from large-scale genome-wise association studies (GWAS), we compared the causal effects of lipid traits, blood pressure, body-mass index, type-2 diabetes and kidney function on coronary artery disease.

Results: We found that allowing for a lenient alpha threshold in order to extend the MR methodology to be more inclusive of non-European data resulted in an increase in weak instrument bias, resulting in imprecise estimates and a reduced ability to detect true causal effects. Notably, our results showed causal associations known to be inconsistent with established findings, specifically between lipid traits in South Asian populations compared to European ancestry populations.

Conclusion: These findings reiterate the urgent need for independent large-scale GWAS in non-European populations, to improve the power and reliability of MR studies as well as to develop methods which take into account population-specific effects.

Grants: This work was self-funded.

Conflict of Interest: None declared

P18.055.B Longitudinal Proteomic Profiling Reveals Temporal Changes in Long-COVID

Xavier Farré[1], *Susana Iraola-Guzmán*[1], *Gemma Castaño Vinyals*[2], *Manolis Kogevinas*[2], *Carles Barceló*[3], *Israel Fernández–Cadenas*[4], *Rafael de Cid*[1]

[1]*IGTP Institut Germans Trias i Pujol, GCAT, Badalona, Spain;* [2]*ISGlobal, Barcelona, Spain;* [3]*Fundación Instituto de Investigación Sanitaria Islas Baleares (IdISBa), Palma, Spain;* [4]*Institut de Recerca - Hospital Santa Creu i Sant Pau, Stroke Pharmacogenomics and Genetics Laboratory, Barcelona, Spain*

Background/Objectives: Long-COVID, characterized by persistent health challenges post-SARS-CoV-2 infection, necessitates a thorough understanding of its complex pathogenesis, encompassing symptoms from fatigue to cognitive dysfunction. Proposed mechanisms include viral persistence, immune dysregulation, microbiota alterations, autoimmunity, clot formation, and neurological abnormalities. This study focuses on unraveling the molecular underpinnings through a protein analysis, aiming to identify biomarkers and therapeutic targets to mitigate long-term impacts.

Methods: In the COVICAT, COVID Cohort of Catalonia, over 10,000 participants underwent three follow-up assessments (2020, 2021, 2023). Long-COVID classification combined participant-reported symptoms and electronic health records. Longitudinal analysis of 1,467 proteins, related to cardiometabolic and inflammation processes, was conducted using the Olink proteomic assay. Paired samples from 146 Long-COVID cases and 25 COVID-19 matching controls, collected in 2020 and 2023, were examined.

Results: A significant decrease in the expression of over 500 proteins was noted in COVID-19 samples from 2023 compared to 2020, enriched for endoplasmic reticulum stress proteins, indicating impact from the initial SARS-CoV-2 encounter. Intriguingly, 354 proteins were uniquely expressed in Long-COVID samples. Functional enrichment revealed overrepresentation in blood coagulation, Insulin-like Growth Factor regulation, and conditions like cardiovascular disorders.

Conclusion: Our investigation into the proteomic landscape of Long-COVID has uncovered substantial alterations in protein expression, emphasizing the impact of the initial SARS-CoV-2 encounter. The identified proteins associated with endoplasmic reticulum stress and those unique to Long-COVID shed light on intricate mechanisms, offering potential therapeutic avenues for mitigating persistent impacts.

Grants: MaratóTV3-167/C/2021
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Conflict of Interest: None declared

P18.056.C Causal effects of the plasma proteome on childhood health

Roman Thielemann[1], *Sara Stinson*[1], *Yun Huang*[1], *Louise Aas Holm*[1,2], *Jens-Christian Holm*[2], *Simon Rasmussen*[1], *Torben Hansen*[1]

[1]*The Novo Nordisk Foundation Center for Basic Metabolic Research, København, Denmark;* [2]*The Children's Obesity Clinic, European Centre of Obesity Management, Holbæk Hospital, Department of Paediatrics, Holbæk, Denmark*

Background/Objectives: Protein quantitative trait loci (pQTL) studies in pediatric populations remain limited. PQTLs combined with Mendelian randomization can infer causal effects of proteins on health and disease. Here, we use genetic and proteomic data from The HOLBAEK Study, a cohort of 3,811 Danish children and adolescents, to 1) identify plasma pQTLs in childhood and adolescence and 2) examine changes in pQTLs across the lifespan.

Methods: We performed genome-wide association studies for 184 plasma proteins measured with Olink proteomics in 3,811 children and adolescents (44.2% boys) with a median age of 11.7 years. Participants were recruited from an obesity clinic ($n = 1,773$) and a population-based cohort ($n = 2,038$). We assessed gene-environment interactions with overweight and pubertal status. Lead pQTLs were matched and compared with adult data from the UK Biobank Pharma Proteomics Project.

Results: We identified 1,448 independent, genome-wide associations for 184 proteins in children and adolescents. For several variants, gene-environment interactions with overweight and pubertal status were observed. Matched pQTLs explained a similar proportion of variance (PVE) in The HOLBAEK Study and the UK Biobank, but for several proteins, PVE differed across age.

Conclusion: We performed a large-scale Olink pQTL study in children and adolescents. PQTLs explained similar proportions of variation in plasma protein levels in adults and children, but several proteins exhibited large differences in PVE across age. In ongoing studies, we use Mendelian Randomization to identify proteins with causal effects on cardiometabolic traits. This study will elucidate causal disease mechanisms of cardiometabolic risk and obesity in early life.

Conflict of Interest: None declared

P18.057.D Who benefit most from testing polygenic risk score (PRS) in panel-negative breast cancer families?

Teresa Ramon y cajal[1], *Adrià López-Fernández*[2], *Laura Duran-Lozano*[2], *Guillermo Villacampa*[2], *Victor Navarro*[2], *Eduard Perez*

Ballestero[2], Mònica Pardo Muñoz[2], Esther Darder[3], Ana Raquel Jimenez-Macedo[4], Mireia Cartro[5], Consol Lopez[1], Nuria Calvo[1], Mara Cruellas[2], Maite Torres[3], Ariadna Roque[3], Lidia Feliubadaló[6], Anna Vallmajo[7], Gemma Llort[8], Martín Espinosa-Bravo[9], Noemi Tuset[7], Conxi Lázaro[6], Joan Brunet[3], Judith Balmaña[2]

[1]Hospital Sant Pau, Familial Cancer Clinic. Medical Oncology, Barcelona, Spain; [2]Hospital Vall d'Hebron Institute of Oncology (VHIO), Hereditary Cancer Genetics Group, Barcelona, Spain; [3]Catalan Institute of Oncology, Genetic Counselling Unit. Hereditary cancer program, Girona, Spain; [4]Consorci Sanitari de Terrassa, Genetic Counseling Unit., Terrassa, Spain; [5]Corporació Sanitària Parc Taulí, Genetic Counselling Unit, Sabadell, Spain; [6]Catalan Institute of Oncology, Hereditary Cancer Program. Oncobell Program, IDIBELL., Spain; [7]Hospital Universitari Arnau de Vilanova, Genetic counselling in familial cancer Unit. Medical Oncology Service, Lleida, Spain; [8]Corporació Sanitària Parc Taulí, Genetic Counselling Unit. Medical Oncology, Sabadell, Spain; [9]Hospital Vall d'Hebron., Gynecology, Barcelona, Spain

Background/Objectives: Healthy women with familial breast cancer(BC) generally pursue early age-based screening based on family history(FH) alone. Previous results in PRiSma showed that one third of women changed their risk category after inclusion of breast density(BD) and PRS. Our aim was to identify those women who benefit most from PRS testing.

Methods: 417 healthy women (30-65y) were considered. Data on personal factors(PF), FH and BD were collected and PRS_{268} was analyzed. Women were classified into 10-y risk(NICE categories) based on CanRisk(v5). We compared the group distribution of women according to their baseline prediction (PF + FH+panel) and the estimate obtained after the incorporation of BD + PRS or PRS alone. We used descriptive statistics and chi square test.

Results: According to baseline prediction, the median 10-y risk was 3.8%(IQR3.1-4.7) and women were classified into moderate(75%), average(22%) and high(3%) risk. Overall, 144(34.5%) changed group after the addition of BD + PRS. Women in the 2-4% risk interval were more likely to shift group compared to those in 4-7% (40.2% vs 27.4%, $p = 0.01$). Additionally, 53/84(63%) of these women decreased to average risk. Similar results were found when we evaluated the contribution of adding PRS to baseline +BD prediction, leading to the re-assignment of 56% of women to average risk.

Conclusion: Most healthy women from panel-negative BC families are at moderate risk. PRS testing together with personal and family information would modify risk stratification and adjust screening recommendations towards population level in a significant proportion of women whose baseline predicted risk is close to the lower bound of moderate risk.

Grant: ISCIII-PI19/01195

Conflict of Interest: Teresa Ramon y cajal ISCIII-PI19/01195, Lilly, Adrià López-Fernández ISCIII-PI19/01195

ISCIII-PI23/01047, Laura Duran-Lozano ISCIII-PI19/01195, ISCIII-PI23/01047, BCRF, Guillermo Villacampa: None declared, Victor Navarro: None declared, Eduard Perez Ballestero: None declared, Mònica Pardo Muñoz ISCIII-PI19/01195, Esther Darder ISCIII-PI19/01195, Ana Raquel Jimenez-Macedo: None declared, Mireia Cartro: None declared, Consol Lopez: None declared, Nuria Calvo ISCIII-PI23/01047, Mara Cruellas ISCIII-PI23/01047, Maite Torres: None declared, Ariadna Roque: None declared, Lidia Feliubadaló: None declared, Anna Vallmajo: None declared, Gemma Llort: None declared, Martín Espinosa-Bravo: None declared, Noemi Tuset: None declared, Conxi Lázaro: None declared, Joan Brunet: None declared, Judith Balmaña ISCIII-PI19/01195, ISCIII-PI23/01047, Astra Zeneca, Astra Zeneca

P18.058.A Gene-based association study to identify radiogenomic markers of rectal bleeding in prostate cancer patients treated with radiotherapy

Olivia Fuentes-Ríos[1;2;3], *Miguel E. Aguado-Barrera[1;2], Carlos López[1;2], Javier Manuel Galego Carro[1;2;3], Ana Crujeiras[1;2], Carla Coedo-Costa[1;2], Antonio Gomez-Caamaño[4], Ana Vega[1;2;3]*

[1]Instituto de Investigación Sanitaria de Santiago de Compostela, Cancer genetics and rare diseases, Santiago de Compostela; [2]Fundación Pública Galega de Medicina Xenómica, Functional Genomics, Santiago de Copostela; [3]Universidad de Santiago de Compostela, Santiago de Compostela, Spain; [4]Hospital Clínico Universitario de Santiago de Compostela, Servicio de Oncología Radioterapica, Santiago de Compostela

Background/Objectives: Radiogenomics studies inherited genetic variations contributing to the inter-variability in response to radiotherapy. In prostate cancer patients, one of the most relevant adverse effects associated with radiotherapy is rectal bleeding, which in its most severe grades can compromise the patient's life. NGS technologies provide unprecedented and affordable access to genomic information for discovering novel biomarkers related to the phenotype. Our aim is to identify candidate genes associated with late rectal bleeding in patients with adenocarcinoma of the prostate treated with radiotherapy, using whole-exome sequencing.

Methods: We selected 141 patients, 46 with rectal bleeding (cases;CTCAE grade≥2) and 95 without any toxicity (controls;CTCAE = 0) from the RADIOGEN-prostate cohort who had at least 5 years of follow-up. DNA libraries were constructed from peripheral blood samples collected before radiotherapy using the SureSelect-All-Exon-V6 + UTR-kit(Agilent) and were sequenced on the NovaSeq6000(Illumina). Genomic variants were detected by GATKv.4.0 and annotated with ANNOVARv.2019, PLINK_v.1.90 was used for data quality control. Rare variants with frequency < 0.01 (GnomAD_AF_NFE_exome.v2.1.1) were prioritized. For the analysis, we carried out the Optimal Sequence Kernel Association test, age-at-radiotherapy and rectum-volume-(percent)-receiving-65Gy were included as covariates. Bonferroni correction was performed.

Results: Five genes, namely *NUP188, CRNKKL1, ZCCHC2, HIPK3*, and *MAP1A*, reached a nominal p-value < 9.1 × 10-04. Notably is that the circular RNA Circ-HIPK3 encoded by the *HIPK3* gene has been implicated in cellular radiosensitivity [PMID:37536436]. No genes remained significant after Bonferroni correction.

Conclusion: In this study, we identified new potential candidate genes linked to rectal bleeding in prostate cancer patients undergoing radiotherapy. These discoveries could be further investigated for validation in independent cohorts.

Conflict of Interest: None declared

P18.059.B High frequency of lobular breast cancer in *CDH1* germline carriers supports update of clinical guidelines for *CDH1* testing and pathological characterization

J. Garcia-Pelaez[1], *alexandre dias[2], parry guilford[3], carla oliveira[1]*

[1]i3S - Instituto de Investigação e Inovação em Saúde; IPATIMUP - Institute of Molecular Pathology and Immunology of the University of Porto and Faculty of Medicine, University of Porto, Porto, Portugal.; [2]i3S - Instituto de Investigação e Inovação em Saúde; IPATIMUP - Institute of Molecular Pathology and Immunology of the University of Porto, Porto, Portugal.; [3]Cancer Genetics Laboratory, Centre for Translational Cancer Research (Te Aho Matatū), Department of Biochemistry, University of Otago, Dunedin, New Zealand

Background/Objectives: *CDH1* germline Pathogenic/Likely Pathogenic variants (PV/LPV) predispose for Diffuse Gastric and Lobular Breast Cancer syndrome (DGLBCS). Sensitive and specific clinical criteria for testing, ensure clinical management of the correct population at risk. Herein, we systematically analysed the frequency of gastric and breast cancer in *CDH1*-PV/LPV carrier families, and itsrelationship with current clinical criteria, across different continents.

Methods: We compiled retrospective clinical information from 878 *CDH1*-PV/LPV carrier families, including 207 from internal communications (32 institutions) and 671 from a systematic review of the literature (151 original articles). Variants were classified with *CDH1* ACMG-AMP guidelines. Phenotypical presentation, fulfillment of clinical criteria and continent of origin were analysed.

Results: From 878 *CDH1* germline PV/LPV carrier-families, 314(35.8%) were European, 512(58.3%) American, 26(3.0%) Asian, 24(2.7%) from Oceania and 2(0.2%) were African. From 314 European families, 78% fullfiled clinical criteria, while only 59.6% of American families did. Among families lacking criteria, American families presented 2-fold more families with LBC involvement than European families (18.4% vs 10.2%; $p < 0.05$). Despite these discrepancies, 39.5% (124/314) of European families and 37.1% (190/512) of American families either fullfiled clinical criteria or lacked criteria but had LBC involvement. In Asia, only 2/26 families presented LBC involvement, and in Oceania none of the 24 families did. In these two continents, most families fulfilled criteria and were GC-only families.

Conclusion: This systematic collection of CDH1 PV/LPV carrier families across continents demonstrates higher frequency of LBC-associated cancer in the western world, strongly supporting further broadening of clinical criteria for CDH1 testing to LBC-families.

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Conflict of Interest: None declared

P18.060.C Diet-by-polygenic score interaction effects on kidney function

Giulia Barbieri[1;2], *Michele Filosi*[2], *Ryosuke Fujii*[3], *Dariush Ghasemi-Semeskandeh*[2], *Martin Gögele*[2], *Peter Pramstaller*[2], *Cristian Pattaro*[2], *Essi Hantikainen*[2]

[1]*University of Verona, Diagnostics and public health, Verona, Italy;* [2]*Eurac Research, Institute for Biomedicine, Eurac Research, Bolzano, Italy;* [3]*Fujita Health University School of Medical Sciences, Department of Preventive Medical Sciences, Toyoake, Japan*

Background/Objectives: Chronic kidney disease (CKD) affects 10% of the worldwide population. While diet plays a key role in CKD prevention and management, interaction between diet and CKD genetic predisposition is largely unexplored. We investigated the interplay between a polygenic score (PGS) and dietary patterns in determining the CKD-defining trait, the creatinine-based estimated glomerular filtration rate (eGFR), in the Cooperative Health Research In South Tyrol (CHRIS) study.

Methods: We derived eGFR on 5,975 individuals without CKD and related morbidities (median age: 40 years; 53% females; median eGFR: 101.6 ml/min/1.73m^2), who completed the GA^{2}LEN food frequency questionnaire. We estimated four CKD-relevant nutrients (protein, potassium, sodium, phosphorus) and the Dietary Approach to Stop Hypertension (DASH) index, reflecting overall diet quality. The PGS was derived from a large genome-wide association study meta-analysis ($n = 1{,}046{,}070$), applying the PRS-CS algorithm, controlling for population structure via continuous shrinkage correction (959,833 common variants with high imputation quality). We fitted multiply-adjusted generalized additive models incorporating tensor product smooths and diet-PGS interaction terms to model non-linear associations with eGFR.

Results: PGS and DASH index explained 5.6% and 2.3% of eGFR variance, respectively. eGFR resulted non-linearly associated with PGS ($P < 0.0001$) and DASH index ($P = 0.042$) and linearly associated with all nutrients but sodium. We observed significant interaction of the PGS with protein intake ($P = 0.028$) and protein-corrected phosphorus levels ($P = 0.007$): high protein intake was associated with lower eGFR in individuals with high PGS but not in those with low PGS.

Conclusion: Genetic predisposition may modify the association between protein intake and kidney function.

Conflict of Interest: None declared

P18.062.A Impact of CYP2C19 genetic variants on side effects of antidepressants leading to treatment discontinuation

Hanna Kariis[1], *Dage Särg*[1;2], *Kristi Krebs*[1], *Maris Alver*[1], *Kelli Lehto*[1], *Lili Milani*[1]

[1]*University of Tartu, Institute of Genomics, Tartu, Estonia;* [2]*University of Tartu, Institute of Computer Science, Tartu, Estonia*

Consortium: REALMENT Consortium (Horizon 2020)

Background/Objectives: Antidepressants are commonly prescribed for psychiatric disorders but often result in side effects which can lead to treatment discontinuation. Genetic variation in CYP2C19 can affect plasma drug concentration and has been linked to side effects, but the findings are not consistent.

We aim to investigate the associations between side effects leading to discontinuation and CYP2C19 metaboliser status separately for escitalopram, citalopram, sertraline, and amitriptyline.

Methods: We leveraged self-reported data from the Estonian Biobank Mental Health online Survey ($N = 86{,}244$), the Estonian Biobank Vaccines and Medications Adverse Effect study ($N = 49{,}366$) and used a natural language processing model to extract side effects from free text in electronic health records ($N = 445$). The sample included 7147 individuals (84,4% women, mean age 48,7 years) who had taken any of the four antidepressants: citalopram ($N = 988$), sertraline ($N = 1948$), escitalopram ($N = 3654$), and amitriptyline ($N = 871$). We used logistic regression, with discontinuation due to side effects as the outcome and CYP2C19 metaboliser status as the predictor.

Results: For most drugs, the effects between side effects and metaboliser status were in expected direction but there was some heterogeneity between drugs. Overall, side effect reporting largely increased with slower metabolism and decreased with faster metabolism.

Conclusion: Questionnaire and health record data from biobanks are a valuable resource for pharmacogenetic research. We are now developing a machine learning model for side effect annotation with sufficient accuracy to skip manual verification, thereby increasing the sample size. A better understanding of the underlying mechanisms could help to identify individuals at risk of side effects.

Grants: REALMENT (Horizon 2020)

Conflict of Interest: None declared

P18.063.B Identification of *RSAD2* as a Potential Biomarker for Omalizumab Response in Pediatric Asthma Patients

Larisa Goričan[1], *Boris Gole*[1], *Anja Bizjak*[1], *Gregor Jezernik*[1], *Vojko Berce*[1;2], *Korneliusz Golebski*[3], *Cornelis M van Drunen*[4], *Michael*

Kabesch[5], Anke H. Maitland-van der Zee[3;6], Erik Melen[7], Susanne Reinartz[8], Susanne J. H. Vijverberg[3;6], Uroš Potočnik[1;9;10]

[1]Faculty of Medicine, University of Maribor, Center for Human Molecular Genetics and Pharmacogenomics, Maribor, Slovenia; [2]University Medical Centre Maribor, Department of Pediatrics, Maribor, Slovenia; [3]Amsterdam University Medical Center, University of Amsterdam, Department of Respiratory Medicine, Amsterdam, Netherlands; [4]Amsterdam University Medical Center, University of Amsterdam, Department of Otorhinolaryngology, Amsterdam, Netherlands; [5]University Children's Hospital Regensburg (KUNO), Department of Pediatric Pneumology and Allergy, Regensburg, Germany; [6]Amsterdam University Medical Center, University of Amsterdam, Department of Pediatric Pulmonology, Amsterdam, Netherlands; [7]Karolinska Institutet, Department of Clinical Sciences and Education Södersjukhuset, Stockholm, Sweden; [8]Tergooi Medical Center, Department of Otorhinolaryngology, Hilversum, Netherlands; [9]Faculty for Chemistry and Chemical Engineering, University of Maribor, Laboratory for Biochemistry, Molecular Biology and Genomics, Maribor, Slovenia; [10]University Medical Center Maribor, Department for Science and Research, Maribor, Slovenia

Consortium: The PERMEABLE consortium

Background/Objectives: Biologics are becoming a key option for treating children with severe, corticosteroid-resistant asthma. However, reliable biomarkers for selecting the most effective treatment are missing. We utilized a functional ex vivo method to discover biomarkers that indicate the efficacy of omalizumab, an anti-IgE biological, in pediatric asthma patients.

Methods: We isolated peripheral blood immune cells from children/juveniles with moderate to severe asthma, naive to therapy with omalizumab. The cells were cultured in the presence of patient's own serum with/without omalizumab for 24 hours, followed by an in vitro simulation of an asthma attack using patient-specific allergens. Basophil activation test was used as readout to classify patients as good- or poor/non-responders to omalizumab. RNA-seq analysis of basophils from the good- vs the poor/non-responders identified several differentially expressed genes, predominantly associated with the response to virus and apoptosis.

Results: Notably, *RSAD2* expression decreased in the poor/non-responder group, showing a negative correlation with omalizumab response. The results were confirmed by qPCR on additional samples and in clinical setting, where pre-therapy *RSAD2* expression significantly negatively correlated with the FeNO levels at 3/6 months of therapy.

Conclusion: Our integrated approach combining in vitro cell model, transcriptomics, and clinical data underscores *RSAD2* as a promising biomarker for omalizumab response in pediatric asthma.

Grants: The PERMEABLE consortium is funded by ZonMW (PN 456008004), the Swedish Research Council (PN 2018-05619), the Slovenian Ministry of Education, Science and Sport (PN C3330-19-252012), and the German Ministry of Education and Research (BMBF, PN FKZ01KU1909A) as part of the ERA PerMed JTC 2018 Call.

Conflict of Interest: None declared

P18.064.C Preparing for Genomic Newborn Screening in Germany: lesson learned from comparative analysis of existing initiatives

Isabel Betzler[1], *Maja Hempel[1], Stefan Kölker[2], Eva Winkler[3], Sven Garbade[2], Christian Schaaf[1], Heiko Brennenstuhl[1]*

[1]Institute of Human Genetics, Heidelberg University and Heidelberg University Hospital, Heidelberg, Germany; [2]Centre for Paediatrics and Adolescent Medicine, Division of Paediatric Neurology and Metabolic Medicine, Heidelberg University and Heidelberg University Hospital, Heidelberg, Germany; [3]Section of Translational Medical Ethics, National Center for Tumour Diseases, Heidelberg University and Heidelberg University Hospital, Heidelberg, Germany

Background/Objectives: The feasibility of genomic newborn screening (gNBS) is growing as sequencing costs decline and the understanding of the impact of genetic variants on disease advances. A central task of ongoing projects revolves around which target diseases to incorporate in gNBS. Our study aims to analyze target gene-disease lists from six initiatives, providing insights for advancing and implementing gNBS in Germany.

Methods: Target gene-disease lists and inclusion criteria from six gNBS studies were analyzed regarding disease number, composition, group proportions, and ClinGen gene-disease validity curations.

Results: Despite similar selection criteria, our analysis revealed variations in total gene count (mean 488, range 237-889, SD ± 243.4) and disease group composition. A consensus emerged for 53 target diseases, predominantly associated with inborn errors of metabolism (83%, 44/53). Each study proposed an exclusive set of gene-disease pairs, positively correlating with total gene count per study. While most pairs received "Definitive" or "Strong" ClinGen curation, some pairs included on gNBS target lists were labeled as "Refuted" (n = 28) or "Disputed" (n = 5), particularly in genetic cardiac diseases. Notably, 17-48% of genes lacked ClinGen curation.

Conclusion: To date, no consensus has been reached on the selection of target diseases for gNBS. The diversity of proposed gene-disease targets and variation/lack in ClinGen curation emphasizes challenges in standardizing inclusion criteria for gNBS. Our study provides valuable insights into selection of target diseases for gNBS in Germany and advocates for collaboration working towards harmonization and consensus on criteria, aiming to ensure screening objectivity, integrity, and acceptance.

Grants: NEW_LIVES [BMBF Föderkennzeichen 01GP2201A]

Conflict of Interest: None declared

P18.065.D Addition of Genetically Predicted Plasma Lipids to Measured Levels Improves Cardiovascular Risk Stratification

Oliver Vad[1;2], *Christian Paludan-Müller[1;2], Rasmus Frosted[1;2], Jesper Hastrup Svendsen[1;3], Morten Olesen[2]*

[1]Copenhagen University Hospital, Rigshospitalet, Department of Cardiology, København, Denmark; [2]University of Copenhagen, Department of Biomedical Sciences, København, Denmark; [3]University of Copenhagen, Department of Clinical Medicine, København, Denmark

Background/Objectives: Dyslipidemia is a major risk factor for cardiovascular morbidity and mortality and has a substantial genetic component. This study aimed to explore whether genetic predisposition to plasma lipid levels contributes with an added risk independent of measured lipid levels.

Methods: Summary statistics for non-HDL cholesterol levels were obtained from non-UK Biobank individuals. A polygenic risk score (PRS-Cholesterol) was constructed using PRS-CS-auto and applied in 422,571 individuals of European ancestry from the UK Biobank. The cohort was stratified into three groups at baseline: Normal cholesterol (cholesterol <5.2 mmol/L and no cholesterol-lowering treatment), treated dyslipidemia (cholesterol-lowering medication), and untreated dyslipidemia (cholesterol >=5.2 mmol/L and no cholesterol-lowering medication). Using Cox-models, hazard ratios (HR) were estimated for a composite

endpoint of myocardial infarction, heart failure, and cardiovascular-related death. Models were adjusted for sex, age, hypertension, diabetes, body-mass index, measured plasma lipids, and ten first genotype principal components.

Results: One standard deviation increase in PRS-Cholesterol was associated with increased cardiovascular disease rate among individuals with normal cholesterol levels (HR: 1.14, 95% confidence interval [CI]: 1.10-1.18, $P = 1.16 \times 10^{-11}$) and among those with untreated dyslipidemia (HR: 1.09, 95% CI: 1.06-1.12, $P = 2.07 \times 10^{-10}$). There was no association with increased rate among individuals taking cholesterol lowering medication (HR: 0.98, 95% CI: 0.95-1.00, $P = 0.10$).

Conclusions: PRS-Cholesterol was independently associated with a higher rate of cardiovascular disease in individuals not receiving lipid-lowering therapy and may aid in the identification of individuals at an increased cardiovascular risk.

Grants: Research Foundation at Rigshospitalet; Skibsreder Per Henriksens R., og hustrus fond; The Novo Nordisk Foundation

Conflict of Interest: Oliver Vad Research foundation at Rigshospitalet; Skibsreder Per Henriksens R., og hustrus fond, Christian Paludan-Müller Research foundation at Rigshospitalet, Rasmus Frosted: None declared, Jesper Hastrup Svendsen JHS has received research grants (institutional) from Medtronic outside this work, Speaker fee from Medtronic, Member of a Medtronic advisory board, Morten Olesen Novo Nordisk Foundation, MSO have received speaker fee from Biosence webster not related to this work

P18.067.B Integration of single-cell transcriptomics and targeted genomics on peripheral blood cells identifies novel hub genes for anti-TNFα therapy response in Crohn's disease

Boris Gole[1], *Larisa Goričan*[1], *Mario Gorenjak*[1], *Gregor Jezernik*[1], *Ursula Prosenc Zmrzljak*[2], *Cvetka Pernat*[3], *Pavel Skok*[3], *Uroš Potočnik*[1;4;5]

[1]*Faculty of medicine, University of Maribor, Centre for human molecular genetics and pharmacogenomics, Maribor, Slovenia;* [2]*BIA Separations CRO – Labena d.o.o., Ljubljana, Slovenia;* [3]*Maribor University Medical Centre, Division of Internal Medicine, Department of Gastroenterology, Maribor, Slovenia;* [4]*Faculty for Chemistry and Chemical Engineering, University of Maribor, Laboratory for Biochemistry, Molecular Biology and Genomics, Maribor, Slovenia;* [5]*Maribor University Medical Centre, Department for Science and Research, Maribor, Slovenia*

Background/Objectives: Lack of reliable biomarkers of response to anti-TNFα biologicals hinders personalized therapy for Crohn's disease (CD) patients. Novel approaches to uncover reproducible biomarkers use transcriptomic-genomic integration and deconvoluted immune cell abundance in bulk peripheral blood cells (PBMCs). Motivation behind our study is to shift the paradigm of anti-TNFα biomarker discovery towards specific immune cell subpopulations using single-cell RNA-sequencing (scRNA-seq).

Methods: The scRNA-seq was performed on PBMCs samples from CD patients either naïve to biological therapy, in remission while on adalimumab (anti-TNFα therapy), or while on ustekinumab but previously non-responsive to adalimumab. Differentially expressed genes (DEGs) influenced by the two therapies were removed from analyses, thus identifying DEGs in adalimumab non-responders relative to the responders. These were further tested with gene ontology analysis, genomic profiling on two independent patient cohorts, gene expression on sorted immune cells, Mendelian randomization and oligogenic risk score calculation.

Results: The scRNAseq revealed 1353 DEGs in 6 PBMCs populations. 7 genes upregulated in CD4 + T-cells and 6 genes downregulated in double-negative T-cells were further confirmed through gene ontology and genomic profiling. Finally, data from independent cohorts, Mendelian randomization and oligogenic risk score identified DEGs highly relevant to anti-TNFα therapy response in CD.

Conclusion: The scRNA-seq enabled direct pinpointing of the causal PBMCs sub-populations. Jointly with an innovative study design it uncovered novel candidate genes in anti-TNFα response landscape of CD, potentially obscured by therapy or inflammation.

Grants: Slovenian Research and Innovation Agency research core funding P3-0427 and research grant J3-9258. Labena d.o.o. co-financed the single-cell RNA-sequencing.

Conflict of Interest: Boris Gole: None declared, Larisa Goričan: None declared, Mario Gorenjak: None declared, Gregor Jezernik: None declared, Ursula Prosenc Zmrzljak U.P.Z. is full-time employed at Labena d.o.o., which co-financed the presented work, Cvetka Pernat: None declared, Pavel Skok: None declared, Uroš Potočnik: None declared

P18.068.C Inherited lack of functional CCR5 is associated with increased risk of coronary heart disease

Matthew Traylor[1], *Dominik Pfister*[2], *Christina Mogensen*[2], *Ciara Vangjeli*[1], *Joanna Howson*[1]

[1]*Novo Nordisk Research Centre Oxford (NNRCO), United Kingdom;* [2]*Novo Nordisk A/S - site Måløv, Måløv, Denmark*

Background/Objectives: Inhibition of C-C chemokine receptor type 5 (*CCR5*) is used to prevent and treat human immunodeficiency virus infection and is being pursued in clinical trials as a treatment for inflammatory mechanisms in diseases such as metabolic dysfunction associated steatohepatitis and fatty liver disease. Carriers of a common deletion of *CCR5* (Δ32) in European populations do not express functional CCR5 on their cell surface and therefore have no functional CCR5 signalling. Given the role of inflammatory mechanisms in coronary heart disease (CHD), we explored whether carriers of Δ32 had altered risk of CHD and therefore whether CCR5 inhibition might alter CHD risk.

Methods: We meta-analysed GWAS summary data for CHD from the largest published GWAS (Aragam et al; Nat Genet 2022) with FinnGen R9 (I9_CHD) and assessed whether a) variants within 250kb either side of CCR5 were associated with disease and b) whether these associations could be attributed to CCR5 Δ32.

Results: Variants in the *CCR5* region were associated with CHD (smallest p-value, rs75719572, OR(95% CI) = 1.042(1.029-1.055), p = 1.9e-10). CCR5 Δ32 was not included in the Aragam et al. dataset because indels were removed in the original publication. However, all associated variants were in LD with Δ32 and their strength of association was proportional to LD with Δ32, consistent with the association being attributable to Δ32.

Conclusion: Inherited lack of functional *CCR5* is associated with an approximately 8.5% increase in risk of CHD, assuming additivity per allele. Ongoing clinical trials using CCR5 inhibitors should fully assess the potential for an increase in CHD in treated subjects.

Grants: None

Conflict of Interest: Matthew Traylor Full time employee of Novo Nordisk., Dominik Pfister Full time employee of Novo Nordisk., Christina Mogensen Full time employee of Novo Nordisk., Ciara Vangjeli Full time employee of Novo Nordisk., Joanna Howson Full time employee of Novo Nordisk.

P18.069.D An Evaluation of Age-varying Genetic Effects underlying Body-Mass Index and Blood Pressure in the UK Biobank

Genevieve Leyden[1], *Panagiota Pagoni*[1], *Tom G. Richardson*[1], *Gibran Hemani*[1], *George Davey Smith*[1], *Eleanor Sanderson*[1]

[1]*University of Bristol, MRC Integrative Epidemiology Unit, United Kingdom*

Background/Objectives: Cross-sectional genome-wide association studies (GWAS) assume that genetic effects are constant throughout one's lifetime. However, many traits have age-varying genetic associations which may have implications for their implementation in downstream analyses such Mendelian randomization (MR). In this study we conducted a series of age-stratified GWAS across the 40-69 year age-range in the UK Biobank (UKB) for body-mass index and 3 blood pressure traits (systolic, diastolic and pulsatile pressure (PP)) at 2-year intervals.

Methods: We used a meta-regression approach to systematically identify SNPs with evidence for age interaction effects among discovery hits (inclusion 1) and genome-wide (inclusion 2). We describe, using an MR framework (1) the association between these SNPs on secondary cardiovascular outcomes; and (2) the relationship between the genetically predicted SNP/age interaction on secondary outcomes.

Results: We identified differential enrichment of age-interaction effects which was trait dependent, e.g. 10.23% of BMI discovery SNPs had evidence of an age-interaction compared to 42.38% for PP ($P < 0.05$). Furthermore, the results of our MR-based analyses support a relevant role for SNP/age interaction effects underlying the relationship between PP and adverse cardiovascular outcomes (e.g. the PP (inclusion 1) SNP/age interaction was positively associated with peripheral artery disease in our MR-based framework: Beta = 0.99; $P < 1.82 \times 10^{-13}$; 95%-CI: 2.08-3.53). All summary data derived in this project has been made readily accessible to the research community.

Conclusion: This data generated provides a valuable resource for further exploration of mechanisms relevant to the genetic architecture of complex traits.

Grants: This research was funded by the Medical Research Council: MC_UU_00032/1

Conflict of Interest: Genevieve Leyden: None declared, Panagiota Pagoni: None declared, Tom G. Richardson TGR is employed by Glaxo Smith Kline outside the work presented in this abstract., Gibran Hemani: None declared, George Davey Smith: None declared, Eleanor Sanderson: None declared

P18.070.A Population-based Genomic Screening: Developing an Evidence-Based Tool for Comprehensive Gene Panel Selection

Ana Nyasha Zimani[1], *Borut Peterlin*[1]

[1]*Clinical Institute of Genomic Medicine, Ljubljana, Slovenia*

Background/Objectives: Implementation of population-based genomic screening requires specific inclusion criteria depending on screening aims in different populations. We have developed an up-to-date tool for a comprehensive approach to curation of tailored genomic screening panels.

Methods: To enable informed gene selection according to the criteria such as disease severity, disease burden, age of onset, actionable interventions, and gene-disease association, we integrated data on monogenic diseases from evidence-based resources, including Orphadata, HPO, and OMIM. Inclusion criteria were selected following traditional and modern screening recommendations. An algorithm for disease severity was adapted from a phenotype-based severity classification and actionability was assessed based on the availability of orphan drugs and other mechanism-based interventions sourced from Rx-Genes, Orphadata and NHGRI CGD datasets.

Results: We identified 7574 monogenic diseases with an OrphaCode or MIM number identifier, of which 6459 (85.3%) had evidence of gene-disease validity in ClinGen or were included in Genomics England PanelApp. Of 6187 (81.7%) disorders with sufficient data for a phenotype-based severity classification, 454 (7.3 %) disorders were classified as mild, 2824 (45.6%) as moderate, 2597 (42%) as severe and 312 (5%) as profound. Age of onset was reported for 4817 disorders, including antenatal (557, 9%) pediatric (3229, 52.2%), adult (851, 13.8%), and onset at all ages 401 (6.5%). Actionable interventions were available for 1505 (24.3%) of disorders.

Conclusion: We developed an evidence-based tool enabling a comprehensive approach to curation of gene panels depending on their screening aims. Its design facilitates rapid adaptation of genomic screening in population-based screening programs and clinical settings.

Grants: None.

Conflict of Interest: None declared

P18.071.B Pharmacogenomics in the Scottish Isles

David Twesigomwe[1], *Gannie Tzoneva*[2], *Alan R Shuldiner*[2], *James Wilson*[3,4]

[1]*Sydney Brenner Institute for Molecular Bioscience, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa;* [2]*Regeneron Genetics Center, Tarrytown, New York, United States;* [3]*MRC Human Genetics Unit, University of Edinburgh, Institute of Genetics and Cancer, Western General Hospital, Crewe Road, EH4 2XU, Edinburgh, United Kingdom;* [4]*Centre for Global Health Research, Usher Institute, University of Edinburgh, Teviot Place, EH8 9AG, Edinburgh, United Kingdom*

Background/Objectives: Genetic variation is partly responsible for variability in drug response across populations. However, the full catalogue of pharmacogenetic variants and their distribution are yet to be established, thus posing challenges in implementing individualised medicine in understudied populations. This study aimed to characterise variation in key drug metabolism and transporter genes across founder populations from the Northern and Western Isles of Scotland.

Methods: This study used genomic data from 4200 research volunteers (from Viking Genes studies). We implemented the StellarPGx pharmacogenomics pipeline to call star alleles (haplotypes and structural variants) in tier 1 Very Important Pharmacogenes (VIPs) from whole genome sequence (WGS) datasets (N = 1860 from Orkney and Shetland). In addition, we analysed 4000 whole exome (WES) datasets from these populations. As there was a sample overlap (N = 1860) with both WGS and WES, we used the WGS data to benchmark StellarPGx's utility for characterising pharmacogenetic variation based on WES.

Results: We present frequencies of known and potentially novel star alleles in 11 key cytochrome P450 genes (e.g. *CYP2D6*, *CYP2C19*) and other tier 1 VIPs for the Shetland and Orkney populations in this study and comparisons with the pharmacogenetic landscape across other European populations. Diplotype-phenotype translation for key genes such as *CYP2D6, CYP2C19*, and *SLCO1B1* showed that up to 40% of the study participants included in each analysis had phenotypes that might warrant treatment adjustments informed by pharmacogenetic testing.

Conclusion: Understanding the pharmacogenomic variation landscape across Shetland and Orkney populations is an important step towards precision medicine implementation in the Scottish isles.

Conflict of Interest: David Twesigomwe: None declared, Gannie Tzoneva GT is an employee and/or stockholder of Regeneron Genetics Center of Regeneron Pharmaceuticals, GT is an employee and/or stockholder of Regeneron Genetics Center of Regeneron Pharmaceuticals, Alan R Shuldiner ARS is an employee and/or stockholder of Regeneron Genetics Center of Regeneron Pharmaceuticals, ARS is an employee and/or stockholder of Regeneron Genetics Center of Regeneron Pharmaceuticals, James Wilson: None declared

P18.072.C GentroPy: the new Python package for Open Targets Genetics Pipelines

Daniel Considine[1;2], *Xiangyu Ge*[1;2], *Irene López*[1;3], *Kirill Tsukanov*[1;3], *Annalisa Buniello*[1;3], *Ellie McDonagh*[1;3], *Daniel Suveges*[1;3], *David Ochoa*[1;3], *Yakov Tsepilov*[1;2]

[1]*Open Targets, Hintxon, United Kingdom;* [2]*Wellcome Sanger Institute, Hintxon, United Kingdom;* [3]*European Bioinformatics Institute, European Molecular Biology Laboratory, Hintxon, United Kingdom*

Consortium: Open Targets

Background/Objectives: Open Targets Genetics (https://genetics.opentargets.org/) is a comprehensive tool for predicting causal genes in established human GWAS loci using a machine learning framework. Though effective, the original analysis pipeline faced computational challenges, including scalability to ever growing datasets, and was limited to GWAS of European ancestry. Here we present Gentropy, a toolkit optimised for scalable and reproducible genomic analysis, that encompasses the Open Targets Genetics pipelines for Locus-2-Gene prediction. This Python package enables ingestion of the ever-expanding volume of multi-omic datasets, and the ability to incorporate non-European ancestries.

Methods: The package employs PySpark for the ETL pipeline, with default utilisation of Google Cloud Platform for computational tasks, although local operations are possible. The package was applied to 15,987 GWAS Catalog studies, 2,408 FinnGen R10 studies, and 64,442 curated associations.

Results: The package includes the first optimised version of the genetic data ingestion and harmonisation pipelines, the clumping, PICS fine mapping, eCAVIAR colocalisation and L2G-pics computation pipelines. It uses GnomAD v2.1 as the LD reference for seven major population ancestries. From raw data to Locus-2-Gene prediction scores, the entire analysis now takes less than 24 hours to complete. Future releases will incorporate SuSiE fine-mapping and COLOC approaches.

Conclusion: The freely available package, accessible at https://github.com/opentargets/gentropy, facilitates the interpretation and analysis of GWAS and functional genomic studies for target identification.

Conflict of Interest: None declared

P18.073.D *DPYD* genotyping for fluoropyrimidine dose optimization: Population-based characterization and pharmacogenomic insights

Alicia Torres García[1;2], *Xando Díaz Villamarín*[1;2], *Rocío Morón Romero*[1;2], *Luis Javier Martínez-González*[3;4], *Maria Jesus Alvarez Cubero*[2;3;4], *Jose Cabeza*[1;2]

[1]*Hospital Universitario Clínico San Cecilio, Pharmacy Unit, Granada, Spain;* [2]*ibs.GRANADA Instituto de Investigación Biosanitaria, Granada, Spain;* [3]*Centro Pfizer-Universidad de Granada-Junta de Andalucía de Genómica e Investigación Oncológica (GENYO), Granada, Spain;* [4]*University of Granada (UGR), Biochemistry and Molecular Biology III, Faculty of Medicine, Granada, Spain*

Background/Objectives: *DPYD* variants (rs3918290, rs55886062, rs67376798 and rs56038477) genotyping has been implemented in our hospital for fluoropyrimidines dose tailoring in accordance with recommendations of European Medicines Agency (EMA) and Food and Drug Administration (FDA) approved drug labels. This study aims to characterize these *DPYD* variants in our population by describing genotypic, allelic, and phenotypic frequencies, conducting linkage disequilibrium (LD) analysis, studying the Hardy-Weinberg (H-W) equilibrium for each variant, and comparing with databases.

Methods: Genotyping of 1039 patients was performed for four *DPYD* gene polymorphisms (rs3918290, rs55886062, rs67376798, and rs56038477) using Real-time PCR (QuantStudio™ 12K-Flex, Applied Biosystems). Allelic frequencies were compared with 1000Genomes database, while phenotypic data were obtained from PharmGKB database. Statistical analysis was conducted using R-4.3.2. software.

Results: No significant differences were detected between our cohort and the 1000Genomes reference population (IBS), supporting the results obtained for our population. There were also no significant differences observed in terms of H-W equilibrium for any of the variants, nor LD between the variants. Significant differences were detected in rs3918290 (Granada: MAF = 0.1 vs Europe: MAF = 0.5; p = 0.041) and rs56038477 (Granada: MAF = 1.30 vs Europe: MAF = 2.4; p = 0.027). However, a higher allelic frequency was observed in Nordic population compared to Mediterranean population, which may explain the detected differences.

Conclusion: *DPYD* genotyping is particularly relevant in clinical practice for patients undergoing fluoropyrimidines treatment. Significant differences exist in the inheritance of these variants depending on the European population, with rs3918290 and rs56038477 being less prevalent in the studied cohort.

Grants: Hospital Pharmacy, Hospital Universitario Clínico San Cecilio (Granada)

Conflict of Interest: None declared

P18.074.A Leveraging whole genome sequencing data to model the expected number of people affected by repeat expansion disorders

Delia Gagliardi[1;2], *Kristina Ibañez*[2], *Matteo Zanovello*[1], *Valentina Escott-Price*[3], *Douglas Langbehn*[4], *John J. Hardy*[5], *Henry Houlden*[1], *Arianna Tucci*[2]

[1]*UCL Institute of Neurology, Department of Neuromuscular Diseases, London, United Kingdom;* [2]*Queen Mary University of London, William Harvey Research Institute, London, United Kingdom;* [3]*Cardiff University, Division of Neuroscience and Mental Health, School of Medicine, Cardiff, United Kingdom;* [4]*The University of Iowa, Departments of Psychiatry and Biostatistics, Iowa City, United States;* [5]*UCL Institute of Neurology, Department of Neurodegenerative Disorders, London, United Kingdom*

Consortium: Genomics England Research Consortium

Background/Objectives: Repeat expansion disorders (REDs) affect approximately 1/3000 people worldwide. Prevalence estimates are hampered by heterogeneous clinical presentation, geographic distributions, and technological limitations, likely leading to under-ascertainment. Novel bioinformatics tools have made possible to detect with high accuracy disease-causing repeat expansions from whole genome sequencing (WGS) data.

Methods: Using ExpansionHunter, we profiled WGS data of 82,176 individuals from 100,000 Genomes Project and Trans-

Omics for Precision Medicine. Then, we aimed to estimate the expected number of people affected by REDs based on disease allele frequency. To this aim, we tabulated the UK population's age distribution (based on the Office of National Statistics) to calculate the number of disease allele carriers by age for each RED, and we used corresponding age at onset distribution and survival length for disease modelling.

Results: We estimated that the number of people with C9orf72-Amyotrophic lateral sclerosis, HD in carriers with 40 CAG repeats and DM1 was over two, three and 1.5 times higher than respective literature reported figures based on disease observation, while C9orf72-Frontotemporal dementia estimate fell within the reported range. Moreover, we observed an unexpected increased prevalence of SCA1, 2 and 6.

Conclusion: These discrepancies indicate either underdiagnosis of mild and heterogenous phenotypes, or incomplete penetrance of REDs. Defining the global burden of REDs through prevalence and penetrance estimate is crucial to determine patient specific needs and secure resources for healthcare services. Further work in large population cohorts with detailed clinical information is needed to be able to address these crucial questions.

Conflict of Interest: None declared

P18.075.B The pharmacogenetic risk factors for apixaban associated bleeding and inefficacy

Jozefina Palic[1], *Ana Marija Slišković*[2], *Vedran Pašara*[2], *Lana Ganoci*[3], *Livija Šimičević*[3,4], *Martina Lovrić Benčić*[2,5], *Tamara Božina*[1]

[1]*University of Zagreb, School of Medicine, Department of Medical Chemistry, Biochemistry and Clinical Chemistry, Zagreb, Croatia;* [2]*University Hospital Centre Zagreb, Department of Cardiovascular Diseases, Zagreb, Croatia;* [3]*University Hospital Centre Zagreb, Department of Laboratory Diagnostics, Zagreb, Croatia;* [4]*University of Zagreb, School of Medicine, Department of Medical Chemistry, Biochemistry and Clinical Chemistry, Zagreb;* [5]*University of Zagreb, School of Medicine, Department of Internal Medicine, Zagreb, Croatia*

Background/Objectives: Apixaban has good safety and efficacy, however, there are cases of bleeding and thromboembolic events in practice. Apixaban is a substrate of CYP3A enzymes and ABCB1/ABCG2 transporters. The polymorphisms in the genes that encode them could affect apixaban pharmacokinetics. The objective of the study is to determine association between relevant polymorphisms and occurrence of apixaban-related adverse events.

Methods: These are preliminary results of the larger cohort PGx-CardioDrug (N = 1100). Adults with a new indication for apixaban treatment were genotyped for CYP3A4*22, CYP3A4*1B, CYP3A5*3, ABCG2c.421C>A, ABCB1c.1236C>T, c.2482-2236G>A, and c.3435C>T variants. Analysis was performed with specific TaqMan® Assays on ABI7500 Real-Time PCR. Clinical and laboratory data were collected. Associations between carriers/noncarriers of variant alleles or haplotypes/phenotype and bleeding/thromboembolic events were analysed. Statistical analysis was performed by JASP0.17.1 software.

Results: 140 patients (Mdn age = 66,5 (20-89); f = 59, m = 81) were treated in dosages of 2 × 5mg (n = 113) and 2 × 2,5mg (n = 27). Eleven patients had apixaban associated bleeding (serious = 3, anaemia = 3, epistaxis = 2, haematoma = 2, haematuria = 2, haemoptysis = 1) and six thromboembolic events (insult = 4, thrombosis = 2). Genotype distribution is consistent with Hardy Weinberg's equilibrium. Based on the univariate analysis, a significant association between ABCB1 c.1236C>T variant carriers and smaller bleeding occurrence (OR = 0.27, 95% CI: 0.06-1.13, p = 0.046) was found.

Conclusion: There is no association between the CYP3A4*22, CYP3A4*1B, CYP3A5*3, ABCG2c.421C>A, ABCB1c.2482-2236G>A, and c.3435C>T variants nor phenotype with the apixaban-related side effects. The results should be re-evaluated on the larger cohort.

Grants: This study is part of the project "Pharmacogenomics in Prediction of Cardiovascular Drugs Adverse Reaction - PGx-CardioDrug" supported by the Croatian Science Foundation (UIP-2020-02-8189).

Conflict of Interest: None declared

P18.076.C Unravelling the interplay between the microbiome and genetics in Inflammatory bowel diseases (IBD)

Angeliki Taliouraki[1], *michalis gkizis*[2], *Electra Sofou*[3], *Glykeria Gkoliou*[3], *Giorgos Bamias*[2], *Andreas Agathangelidis*[1], *Panagoula Kollia*[1]

[1]*National and Kapodistrian University of Athens, Department of Biology, Athens, Greece;* [2]*National and Kapodistrian University of Athens, GI Unit, 3rd Academic Department of Internal Medicine,Sotiria Hospital, Athens;* [3]*Centre for Research and Technology Hellas, Institute of Applied Biosciences, Thessaloniki*

Background/Objectives: Inflammatory bowel diseases (IBD) are considered intricate, multifactorial entities with a series of pathogenic factors. Against this background, we explored the interplay between genetic and environmental factors in IBD patients receiving different treatments, through assessing: (i) the diversity of the gut microbiome, and (ii) the immune response capacity.

Methods: DNA/RNA were isolated from affected and healthy ileal and colonic mucosa biopsies from 40 IBD patients. The V3-V4 region of the 16S rRNA gene was amplified, while libraries were prepared and paired-end sequenced on the MiSeq platform. Data were analysed using Qiime2. Sanger-based sequencing was performed for the detection of single nucleotide polymorphisms (SNPs), whereas the expression levels of genes associated with leukocyte migration were evaluated with qRT-PCR. Statistical analysis was performed in R.

Results: Analysis of microbiome diversity based on the Bray-Curtis index revealed three clusters. Interestingly, one cluster corresponded to patients treated with biological agents and exhibited a distinct microbiome composition, with an increase in the *Ruminococcaceae* and *Lachnospiraceae* species. These patients also displayed downregulation of genes involved in leukocyte migration and actin remodeling (namely, *RHOA*, *SELL* and *PECAM1*) and SNPs associated with xenophagy, the PRR immune response and the response to anti-TNF treatment.

Conclusion: Biological agents induce a distinct gut microbial signature in IBD patients and prevent inflammation by suppressing leukocyte migration, thus, allowing the proliferation of anti-infflammatory bacteria species despite the host's opposing genetic background.

Conflict of Interest: None declared

P18.077.D Partitioning the plasma proteome into genetically mediated causal signals and environmental sensors of complex disease risk

Kristin Tsuo[1,2], *danni gadd*[3,4], *Mitja I. Kurki*[1,2,5], *Zhili Zheng*[1,2,5], *Austin Argentieri*[1,2], *Zhana Kuncheva*[3,4], *Denis Baird*[6], *riccardo marioni*[3,4], *Chris Foley*[3,4], *Heiko Runz*[6], *Hailiang Huang*[1,2], *Benjamin B. Sun*[6,7], *Chia-Yen Chen*[6], *Mark Daly*[1,2,5], *Alicia Martin*[1,2]

[1]*Broad Institute of MIT and Harvard, Cambridge, United States;* [2]*Massachusetts General Hospital, Boston, United States;* [3]*Optima Partners, United Kingdom;* [4]*University of Edinburgh, United Kingdom;* [5]*Institute for Molecular Medicine Finland, Helsinki, Finland;* [6]*Biogen*

Inc., Cambridge, United States; [7]University of Cambridge, United Kingdom

The plasma proteome uniquely bridges the gap between inherited and modifiable risk factors for disease. We therefore aimed to characterize plasma proteins into mediators of genetic vs. environmental risk using the UK Biobank (UKB) Pharma Proteomics Project resource. Previous work identified thousands of associations between levels of ~3K proteins and onset of 20 age-related diseases, such as rheumatoid arthritis and chronic obstructive pulmonary disease (COPD). To interrogate which of these proteins have putative causal effects on incident disease, we first employed Mendelian Randomization (MR) using genetic instruments from the UKB. MR implicated more than 20 proteins in causal roles, with an enrichment of significant associations for autoimmune diseases.

We next investigated whether the proteins could serve as quantitative sensors of environmental risk factors. Using smoking as an example, we found that 64% of the disease-associated proteins measured in blood plasma were significantly associated with smoking status in the UKB. We developed a protein score for smoking, trained on the protein levels of the never and current smokers, which predicted smoking status with high accuracy (AUC = 0.95) and showed distributions in previous smokers that reflected time since smoking. Using Cox PH models to test for associations between this score and disease onset, we found that the score accurately predicts incident COPD even in never smokers, suggesting that it may capture a broader signature of smoking-related environmental exposures. Altogether, our study presents an integrative approach that combines large-scale MR and environmental exposure data to advance the discovery of protein biomarkers and predictors.

Conflict of Interest: Kristin Tsuo: None declared, danni gadd Optima Partners, Mitja I. Kurki: None declared, Zhili Zheng: None declared, Austin Argentieri: None declared, Zhana Kuncheva Optima Partners, Denis Baird Biogen Inc., riccardo marioni Optima Partners, Epigenetic Clock Development Foundation, Chris Foley Optima Partners, Heiko Runz Biogen Inc., Hailiang Huang: None declared, Benjamin B. Sun Biogen Inc., Chia-Yen Chen Biogen Inc., Mark Daly Founder of Maze Therapeutics, Alicia Martin: None declared

P18.079.B Multi-omic signatures of perfluoroalkyl exposure in a population cohort

Yasrab Raza[1], *Julia El-Sayed Moustafa[1], Ricardo Costeira[1], Jordana T Bell[1], Cristina Menni[1], Claire Steves[1], Kerrin S Small[1]*

[1]King's College London, Department Of Twin Research & Genetic Epidemiology, London, United Kingdom

Background/Objectives: PFOA (perfluorooctanoic acid) and PFOS (perfluorooctanesulphonic acid) are synthetic chemicals, with global ubiquitous exposure, resistant to degradation and environmentally persistent. These chemicals possess long half-lives and are linked to poor health outcomes, including hyperlipidaemia, thyroid disease and cancer, however the molecular response to exposure is less understood.

Methods: We aimed to understand the molecular effects of PFOA/PFOS exposure using two longitudinal sub-studies spanning 18 years (n = 2069, n = 335) within the TwinsUK cohort, by assessing multi-tissue associations between serum levels of PFOA/PFOS and the epigenome, transcriptome, and metabolome.

Results: Consistent with regulatory changes, PFOA/PFOS levels decreased over time in these sub-studies. Epigenetic associations in adipose (n = 184) and blood (n = 342) were observed, but not skin (n = 92), suggestive of long-term exposure impact and cross-tissue effect. CpG sites linked to PFOA/PFOS included CPT1A ($\beta = 0.69, p = 1.3e-07$) and NAT1 ($\beta = 0.96, p = 4.5e-07$). A longitudinal effect (3 timepoints) on blood gene expression was found; PFOA associated with 15 genes and PFOS associated with 953 genes. PPARD ($\beta = 0.38, p = 0.0014$) was amongst genes found to be associated. Cross-sectional gene expression associations demonstrate a transient effect with THRA ($\beta = 0.86, p = 2.5e-05$) and PPARA ($\beta = -0.70, p = 0.00039$), indicating time sensitive associations with the transcriptome. In contrast, associations with metabolites displayed a sustained effect both cross-sectionally and longitudinally. Metabolite associations were enriched for lipids and amino acids, including pathways involved with phospholipid metabolism, long chain fatty acids and tryptophan metabolism.

Conclusion: These findings indicate PFOA/PFOS exposure perturbs molecular mechanisms, particularly lipid and TCA cycle pathways. We demonstrate potential biological mechanisms through which PFOA/PFOS exposure may lead to adverse health outcomes.

Grants:

Conflict of Interest: None declared

P18.080.C A genome-wide association study of corticosteroid response in europeans hospitalized for COVID-19

Melody Ramirez-Falcon[1;2], *Eva Suarez-Pajes[1], Luis A. Rubio-Rodríguez[3], Silvia Diz-de Almeida[4;5], Silvia Gonzalez-Barbuzano[1], Eva Tosco-Herrera[1], José M. Lorenzo-Salazar[3], Raquel Cruz[4;5], José A. Riancho[6;7;8], Augusto Rojas-Martinez[9], Jesús Villar[2;10], Pablo LAPUNZINA[4;11;12], Angel Carracedo[4;5;13], Carlos Flores[1;2;3]*

[1]Research Unit, Hospital Universitario Ntra. Sra. de Candelaria, Santa Cruz de Tenerife, Spain; [2]Centro de Investigación Biomédica en Red de Enfermedades Respiratorias (CIBERES), Instituto de Salud Carlos III, Madrid, Spain; [3]Genomics Division, Instituto Tecnológico y de Energías Renovables, Granadilla de Abona, Spain; [4]Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER), Instituto de Salud Carlos III, Madrid, Spain; [5]Centro Singular de Investigación en Medicina Molecular y Enfermedades Crónicas (CIMUS), Universidade de Santiago de Compostela, Santiago de Compostela, Spain; [6]Instituto de Investigación de Valdecilla (IDIVAL), Cantabria, Spain; [7]Universidad de Cantabria, Cantabria, Spain; [8]Hospital Universitario Marqués de Valdecilla, Cantabria, Spain; [9]Tecnológico de Monterrey, Escuela de Medicina y Ciencias de la Salud, Monterrey, Mexico; [10]Research Unit, Hospital Universitario de Gran Canaria Dr. Negrín, Las Palmas de Gran Canaria, Spain; [11]Instituto de Genética Médica y Molecular (INGEMM), Hospital Universitario La Paz-IDIPAZ, Madrid, Spain; [12]ERN-ITHACA-European Reference Network; [13]Fundación Pública Galega de Medicina Xenómica, Instituto de Investigación Sanitaria (IDIS), Santiago de Compostela, Spain

Consortium: SCOURGE Cohort Group

Background/Objectives: Corticosteroids (CS) improve survival in severe COVID-19 patients, although with variable response. We performed a genome-wide association study (GWAS) of 90-day mortality in hospitalized COVID-19 patients treated with CS to identify genetic variants associated with CS response.

Methods: Data from the SCOURGE consortium patients enrolled before vaccination were included. We tested logistic regression models in ~9.5M TOPMed-imputed variants (significance set at $p = 5 \times 10^{-8}$). Sensitivity analyses were conducted among CS-untreated patients to assess robustness of the findings. We combined Bayesian fine mapping, colocalization with GTEx eQTLs, transcriptome-wide association studies (TWAS), and functional predictions to prioritize genes and evaluate the biological impact of the variants.

Results: Three variants of 16q23.3 were associated with 90-day mortality among CS-treated patients ($p_{lowest} = 2.79 \times 10^{-8}$). These variants did not associate with 90-day mortality among CS-untreated patients ($p = 0.497$). The analyses prioritized *PLCG2* around a credible set of 16 variants, one with a high predicted phenotypic impact, based on CADD>MSC95%. Colocalization analysis and TWAS did not reveal any significant association with eQTLs or tissue-gene expression.

Conclusion: We conducted the first GWAS of CS response in severe COVID-19 patients and identified association of variants in *PLCG2* gene, which has been previously associated with diverse immune disorders.

Grants: ERA PerMed (JTC_2021; AC21_2/00039 from Instituto de Salud Carlos III [ISCIII]) and funds from Next Generation EU as part of the actions of the Recovery Mechanism and Resilience; ISCIII (COV20_00622, PI20/00876) co-funded by EU 'A way of making Europe'; FIISC (PIFIISC23/05); Gobierno de Canarias, Social European Fund "Canarias Avanza con Europa" (TESIS2022010042 and TESIS2021010046).

Conflict of Interest: None declared

P18.081.D A Comprehensive Nutrigenomics Survey of Gene-Diet Interactions in the UK Biobank

Matteo Di Scipio[1;2], *Guillame Paré*[2;3;4;5]

[1], *Department of Medicine, Faculty of Health Sciences, McMaster University, Hamilton, ON, Canada;* [2], *Population Health Research Institute, David Braley Cardiac, Vascular and Stroke Research Institute, Hamilton Health Sciences and McMaster University, Hamilton ON, Canada;* [3], *Thrombosis and Atherosclerosis Research Institute, David Braley Cardiac, Vascular and Stroke Research Institute, Hamilton ON, Canada;* [4], *Department of Pathology and Molecular Medicine, McMaster University, Michael G. DeGroote School of Medicine, Hamilton ON, Canada;* [5], *Department of Health Research Methods, Evidence, and Impact, McMaster University, Hamilton, ON, Canada*

Background: Precision nutrition aims to offer genomic-guided dietary advice. Such advice depends on the presence of significant gene-diet interactions (G × D) for specific diet and outcome pairs. However, broad-sense GxD heritability across clinically-actionable traits has yet to be fully elucidated.

Methods: We applied MonsterLM, a method to estimate gene × environment interactions with minimal model assumptions, to assess the contribution of genome-wide G × D to major health outcomes in the UK Biobank ($N = 141{,}144$ – $325{,}989$). We tested 31 cardiometabolic disease-related outcomes with 23 dietary exposures totalling 713 outcome-exposure combinations. Dietary data comprised of single-nutrient intakes (SNIs) from 6 major nutritional classes (carbohydrates, protein, fats, vitamins, minerals, and discretionary), 6 dietary indexes (DASH, HEI, AHEI, MEDI, DII, ACS), and dietary pattern principal components (dPC) derived from food frequency questionnaires.

Results: We identified 20 significant G × D ($p < 7.0 \times 10^{-5}$) explaining 2.1 - 21.2 % of outcome variance: two with dPC1 (urea, BMI), one with the Mediterranean Diet Index (Apolipoprotein B), four with caffeine (Apolipoprotein A, Cystatin C, GGT, SHBG), and 13 with alcohol (Albumin, HbA1c, CRP, Cystatin C, Urate, Apolipoprotein A, HDL-cholesterol, Triglycerides, ALP, IGF-1, Total Bilirubin, BMI, and WHR). These significant interactions were further explored with sensitivity, gene-level G × D, canalization, and pathways analyses.

Conclusion: Our comprehensive nutrigenomics survey showed that significant G × D exist but tend to be specific, moderate, and occur much more frequently in discretionary diet components like alcohol and caffeine. These findings will help guide future nutrigenomics research in areas where genetics is likely to have a major impact.

Conflict of Interest: Matteo Di Scipio: None declared, Guillame Paré Novartis, Bayer, Amgen, Sanofi, Illumina

P18.082.A Evaluating health impacts: A framework for personalized prevention policies— A pilot study of pharmacogenetics in colorectal cancer treatment

Pragathy Kannan[1], *Helena Kääriäinen*[1], *Markus Perola*[1;2], *Alexandra Costa*[3;4], *Maria Luis Cardoso*[3;5], *Fatima Lopes*[3;5], *Astrid Vicente*[3;5], *angelica valz gris*[6], *angelo maria pezullo*[6], *Stefania Boccia*[6;7], *peter piko*[8], *roza adany*[8]

[1]*Finnish Institute for health and welfare, Department of Public health and welfare, Helsinki, Finland;* [2]*University of Helsinki, Faculty of Medicine, Helsinki, Finland;* [3]*National Health Institute Dr. Ricardo Jorge, Health Promotion and NCDs Prevention, Lisboa, Portugal;* [4]*University of Lisbon, Institute of Social and Political Sciences, Lisboa, Portugal;* [5]*University of Lisbon, BioISI–Biosystems and Integrative Sciences Institute, Lisboa, Portugal;* [6]*Università Cattolica del Sacro Cuore, Department of Life Sciences and Public Health, Roma, Italy;* [7]*Fondazione Policlinico Universitario A. Gemelli, Department of Woman and Child Health and Public Health, Roma, Italy;* [8]*University of Debrecen, HUN-REN-DE Public Health Research Group, Debrecen, Hungary*

Consortium: PROPHET consortium

Background/Objectives: Conducted within the PROPHET project, this study aims to develop a comprehensive Health Impact Assessment (HIA) framework for personalized prevention policies utilizing the WHO model (https://www.who.int/tools/health-impact-assessments). Specifically, pre-treatment Dihydropyrimidine dehydrogenase (DPYD) genotyping in Colorectal Cancer (CRC) patients undergoing Fluoropyrimidine (FP) chemotherapy will serve as a case study to pilot the proposed framework.

Methods: A literature review on pre-treatment DPYD-gene genotyping and the application of HIA in personalized prevention policies was conducted. Pseudoanonymized and aggregated data from cancer and healthcare registries will be collected for CRC patients undergoing FP chemotherapy in Finland, Italy, and Portugal. A "Markov" model will be utilized to simulate the health impacts of DPYD genotyping. National stakeholder committees have been established. We want to prioritize a comprehensive policy evaluation emphasizing literature reviews, statistical modelling and stakeholder consultations.

Results: The literature review identified gaps in applying HIAs to genomics, prompting further investigation into health impacts associated with genomic integration in personalized prevention policies. Qualitative insights from our national stakeholder committees, comprising experts from healthcare, patient organizations, and medicines agencies, enhance the study's scientific inquiry. The committees' enthusiasm aligns with PROPHET's goals, actively contributing to integrating public health approaches with personalized strategies.

Conclusion: Initial findings highlight the importance of an HIA framework for evaluating the value of genomics in personalized prevention, with enthusiastic participation from diverse stakeholders. Our final results aim to contribute to improved health outcomes and equal access across Europe.

Grants: PROPHET is funded by the European Commission under the Horizon Europe research and Innovation Programme, under Grant agreement ID: 101057721.

Conflict of Interest: None declared

P18.083.B Influence of mRNA expression variation on viral control in HIV-infected individuals

Sergey Oreshkov[1,2], *Christian Thorball*[3,4], *Jenny Meylan-Merlini*[2], *Maude Muriset*[2], *Jacques Fellay*[3,4], *Federico Santoni*[1,2]

[1]*UNIL - Université de Lausanne, Lausanne, Switzerland;* [2]*Lausanne University Hospital, Service d'endocrinologie, diabétologie et métabolisme, Lausanne, Switzerland;* [3]*Lausanne University Hospital, Unité de médecine de précision, Lausanne, Switzerland;* [4]*EPFL, Lausanne, Switzerland*

Background/Objectives: The interaction between HIV-1 and the host immune system plays a crucial role in the natural control and progression of the infection. Previous studies have identified number of HIV-1 restriction factors, which are counteracted by the viral proteins. The blood expression levels of these restriction factors vary among individuals, suggesting a contribution of this variability to interindividual differences in the spontaneous control of HIV infection.

Methods: Study enrolled 150 participants from the Swiss HIV Cohort Study, with provided genotypes, pre-antiretroviral treatment PBMC aliquots, and viral load measurements. Using BRB sequencing, we derived gene expression profiles and assessed their associations with viral load. Furthermore, Mendelian randomization studies were performed on genes exhibiting causal relationships within these associations.

Results: We employed a generalized linear model to assess the association between 792 genes and HIV viral load. Pathway analysis revealed upregulation in innate immune response and proteasome complex with increased viral load, alongside heightened mitochondrial activity and cell-cycle. Conversely, reductions were observed in ribosomal transcripts and downregulation of genes involved in cytokine-cytokine receptor interaction, including IL4R, IL7R, and TCF. Utilizing Egger Regression and Transcriptome-Wide Mendelian Randomization, we estimated both host-driven and pathogen-driven alterations in gene expression.

Conclusion: We performed a large transcriptomic analysis of PBMCs from PLWH without antiretroviral treatment at the time of sample collection. In an analysis comparing gene expression levels and HIV-1 VL, numerous genes with a significant negative or positive correlation with spontaneous viral control were identified.

Grants:

Conflict of Interest: None declared

P18.084.C Unveiling Genetic Predispositions in Pleural Mesothelioma: a GWAS and Polygenic Risk Score Approach

Khadija Sana Hafeez[1], *Carla Debernardi*[1], *Alessandra Allione*[1], *Simonetta Guarrera*[2], *Daniela Ferrante*[3], *Dario Mirabelli*[4], *elton jalis herman*[1], *Irma Dianzani*[5], *Corrado Magnani*[3], *Elisabetta Casalone*[1], *Giuseppe Matullo*[1]

[1]*University of Turin, Department of Medical Sciences, Turin, Italy;* [2]*Italian Institute for Genomic Medicine, Department of Medical Sciences, Turin, Italy;* [3]*University of Eastern Piedmont, Department of Translational Medicine, Novara, Italy;* [4]*CPO Piedmont, Department of Medical Sciences, Turin, Italy;* [5]*Università del Piemonte Orientale, Department of Health Sciences, Novara, Italy*

Background/Objectives: Pleural Mesothelioma (PM), a lethal cancer predominantly caused by asbestos exposure, has poor prognosis and limited treatment options. The role of genetic predisposition in PM is not well understood. This study leverages GWAS to identify genetic variants (out of 10 Million) linked to PM risk and develops a novel Polygenic Risk Score (PRS) to evaluate its predictive potential.

Methods: GWAS was conducted on 419 PM cases and 394 controls, with documented asbestos exposure. Rigorous genotype quality control was implemented, and imputation was performed using Michigan Imputation Server. We conducted single-variant association tests adjusting for age, sex, and principal components using SAIGE. Subsequently, the dataset was divided into training and testing sets for calculating effect sizes and PRS, respectively.

Results: We identified significant SNPs in novel loci, including *OAT, ITGBL1*, and *MICAL3* [$p < 5e-8$], alongside suggestive links to already reported PM risk in loci such as *ATG5, PVT1*, and *THRB*. Known PM drivers like *ZFHX3, SLIT3, DCLK1, PTPRD*, and *ZFPM2* were also identified. The PRS effectively distinguished cases from controls (Kolmogorov-Smirnov $p = 0.04604$), with significant ORs of 2.2 (95%CI 1.1-4.6) for ≥80th and 2.7 (95%CI 1.0-7.7) for ≥90th quantiles.

Conclusion: These findings are crucial for identifying high-risk individuals, guiding targeted prevention, and potentially uncovering protective factors against PM in asbestos-exposed populations. While the PRS shows promise, further refinement and validation are essential for its clinical application. Results will undergo validation in a prospective study to assess predictive ability.

Grants: AIRC under IG 2018—[ID.21390 project—P.I. GM]

Conflict of Interest: None declared

P18.085.D Systematic molecular analyses of the NAPKON cohorts - current state and overview

Bettina Lorenz-Depiereux[1], *Thomas Bahmer*[2], *Sandra Ciesek*[3], *Michael Mülleder*[4], *Stefanie Mücke*[5], *Bärbel Fösel*[1], *Verena Kopfnagel*[5], *Christoph Dolch*[5], *Matthias Nauck*[6], *Soni Savai Pullamsetti*[7], *Mario Looso*[8], *Markus Ralser*[4], *Christian Schäfer*[6], *Mario Schattschneider*[6], *Stefan Schreiber*[2], *Janne Vehreschild*[9], *Christian Gieger*[1], *Martin Witzenrath*[10], *Uwe Völker*[11], *Gabriele Anton*[12], *Thomas Illig*[5]

[1]*Helmholtz Munich, Institute of Epidemiology, Research Unit Molecular Epidemiology, Oberschleißheim, Germany;* [2]*Universitätsklinikum Schleswig-Holstein, Campus Kiel, Internal Medicine, Kiel, Germany;* [3]*University Hospital Frankfurt, Institute of Medical Virology, Frankfurt am Main, Germany;* [4]*Charité – Universitätsmedizin Berlin, Institute of Biochemistry, Berlin, Germany;* [5]*Hannover Medical School, Hannover Unified Biobank, Hannover, Germany;* [6]*University Medicine Greifswald, Institute for Clinical Chemistry and Laboratory Medicine, Greifswald, Germany;* [7]*University of Giessen, Centre for Infection and Genomics of the lung, Gießen, Germany;* [8]*Max Planck Institute for Heart and Lung Research, Bioinformatics Core Unit, Bad Nauheim, Germany;* [9]*Frankfurt University Hospital, Centre for Internal Medicine, Medical Department 2, Frankfurt am Main, Germany;* [10]*Charité – Universitätsmedizin Berlin, Department of Infectious Diseases, Respiratory Medicine, and Critical Care, Berlin, Germany;* [11]*Hospital of the University Greifswald, Interfaculty Institute for Genetics and Functional Genomics, Greifswald, Germany;* [12]*Bielefeld University, Central Biobank University Bielefeld, Bielefeld, Germany*

Consortium: NAPKON and NUKLEUS study group

As part of the German National Pandemic Cohort Network COVID-19 (NAPKON), a total of 7097 patients were recruited in 3 different cohorts (2753 SÜP, 710 HAP, and 3634 POP patients) by the end of 2023. Biosamples from 2520 patients (1217 SÜP, 300 HAP, and 1003 POP patients) for whom a complete set of biosamples was available, were comprehensively characterised using molecular techniques on 7 different analysis platforms.

Genotype data were generated, and proteome, metabolome, transcriptome and cytokine profiles were determined at up to

three time points (baseline, acute phase, 3-month follow-up) for each HAP and SÜP patient and at baseline for POP. In addition, epigenetic (methylation, protein-DNA interaction) and virological data, including SARS CoV-2 sequences were collected. The various analytical data are integrated into the NUKLEUS infrastructure (NUM clinical epidemiology and study platform) with direct links to the respective aliquots analysed. The systematic analysis data is now available for research projects via the NAPKON use and access process.

As part of a DFG application, exome sequence data is also being generated and integrated into NAPKON. In another follow-up project, SAPCRiN (Sample Analysis in Post COVID Research in NAPKON), the 12-month follow-up samples will be analysed using the above-mentioned methods and evaluated with a focus on post Covid.

Here we provide the current state as initial overview of the various methods and types of data generated in the context of systematic molecular analyses in NAPKON, with a focus on technical implementation and quality assurance. Both are essential for the provision and use of high-quality data.

Conflict of Interest: None declared

P18.086.A *HLA* combination analysis reveals differentially multiple sclerosis-associated combinations, suggesting differences in the underlying pathogenesis mechanism

Anna Serova-Erard[1;2], *Igor Faddeenkov*[1], *Sonia Bourguiba-Hachemi*[1], *Pierre-Antoine Gourraud*[1;3], *Nicolas Vince*[1], *François Cornélis*[1;2;4]

[1]*Nantes Université, INSERM UMR1064, Center for Research in Transplantation and Translational Immunology (CR2TI), Nantes, France;* [2]*Clermont-Ferrand University Hospital, Université Clermont-Auvergne, Génétique – Oncogénétique Adulte – Prévention (GENOAP), Clermont-Ferrand, France;* [3]*Nantes Université, Nantes University Hospital, Pôle Hospitalo-Universitaire 11: Santé Publique, Clinique des données, INSERM CIC 1413, Nantes, France;* [4]*Université Clermont-Auvergne, UFR de Médecine et des professions paramédicales, Clermont-Ferrand, France*

Background/Objectives: *HLA* association in multiple sclerosis (MS) involves 13 MS-alleles: *HLA-A*02:01*,

*HLA-B*38:01/*44:02/*55:01, LTA-H51P, HLA-DRB1*03:01/*08:01/*13:03/*15:01*,

*HLA-DQA1*01:01, HLA-DQB1*03:01/*03:02 and HLA-DPB1*03:01* (Moutsianas et al, 2015). Our objective was to identify *HLA*-wide combinations differentially associated with MS.

Methods: We analysed WTCCC *HLA* data for 11,376 MS-cases and 18,872 controls. We used principal component analysis (PCA) for genetic homogeneity selection. *HLA* alleles, imputed using the *HIBAG R* package or inferred with proxy SNPs (*rs2229092, rs9273912* and *rs9277565*), were recoded to consider only the 13 MS-associated alleles. We searched for MS-associated genotype combinations in a 20% sub-sample, nominal combinations being to be tested for replication in the remaining 80%. Confirmed combinations were investigated in the global sample (100%). *Haplomat* provided putative diplotypes.

Results: Following PCA analysis, we retained 9,024 MS and 13,923 controls. In the 20% sub-sample, we observed 776 different *HLA*-wide recoded genotype combinations, 41 were nominally

MS-associated ($P < 0.05$) and 22 of those were replicated (*P corrected* <0.05/41). The 22

HLA-combinations represent 27,9% of MS-patients, defining 4 groups with non-overlapping 95% confidence intervals of odds ratios (OR):

High-risk OR from 6.75 to 3.99
Medium-risk OR from 3.36 to 2.86
Low-risk OR from 2.56 to 1.83
Protective OR from 0.57 to 0.30

Genetic effect was provided by the recoded genotypes themselves, as, within

HLA-combinations, using unrecoded *HLA* typing, alleles and putative diplotypes counts were similar between MS and controls.

Conclusion: Such differentially associated *HLA*-combinations suggest a direct contribution to different *HLA*-associated pathogenesis pathways of potential relevance for personalized medicine in MS and other *HLA*-associated diseases.

Conflict of Interest: None declared

P18.087.B Precision Medicine in Treatment-Resistant Schizophrenia: Leveraging Pharmacogenetic Insights for Improved Outcomes

Iván Martín da Silva[1;2], *Pau Riera*[1;2], *Sara Bernal*[2;3], *Meritxell Cros*[2;3], *María Gámez-Lechuga*[1;2], *Iluminada Corripio*[2;4], *Josefina Pérez-Blanco*[2;5], *Almenta-Gallego David*[2;5], *Anna Díaz-Pérez*[2;5], *Dolors Puigdemont*[2;5], *Laia Arias i Queralt*[2;5], *Javier de Diego-Aledíño*[2;5], *Narcis Cardoner*[2;5]

[1]*Hospital de la Santa Creu i Sant Pau, Hospital Pharmacy, Barcelona, Spain;* [2]*Institut de Recerca - Hospital Santa Creu i Sant Pau, Barcelona, Spain;* [3]*Hospital de la Santa Creu i Sant Pau, Genetics, Barcelona, Spain;* [4]*Hospital Universitari de Vic, Psychiatry, Vic, Spain;* [5]*Hospital de la Santa Creu i Sant Pau, Psychiatry, Barcelona, Spain*

Background/Objectives: Antipsychotics' effectiveness is widely variable, with 30-50% of patients showing poor response and/or adverse reactions.

Our objective was to highlight the clinical significance of precision medicine in treatment-resistant schizophrenia, drawing from our experience with PGx-informed prescribing.

Methods: Schizophrenia patients resistant or intolerant to antipsychotic treatment were prospectively included between 2021 and 2024. CYP1A2, CYP2D6, CYP2C9, CYP2C19 and CYP3A4 were genotyped during hospital stay.

The following data were collected: sex, age, comorbidities, follow-up from first admission, study reason, hospitalizations, and side effects.

Results: A total of 21 patients (50.4% men) with a median age of 43.3 (IQR, 32.0-51.0) years and 2.3 comorbidities (IQR, 2.0-3.0) were included. They were administrated a median of 3.0 psychopharmacs/patient (range: 1-7 drugs), with 45% receiving 4 or more drugs.

Patients presented a therapeutic failure rate of 66.7% or intolerance in 33.3%. Median follow-ups were 16.1 and 1.4 years, before and after the genotyping, respectively.

Results justified therapeutic refractoriness in 52% of cases. PGx-informed prescribing resulted in dosage adjustment in 38.1% of patients, while 76.2% opted for antipsychotic switching. Non-responder patients had 2.7 (IQR, 1.0-4.0) psychiatric-hospitalizations per person from 2020 until pharmacogenetic analysis (mean length-of-stay: 95.8), compared to 0.36 (IQR, 0-1) psychiatric-hospitalizations per person after genotyping (mean length-of-stay: 32.0).

Preceding pharmacogenetic analysis, 14 patients (66.7%) had to discontinue antipsychotics due to side effects, especially risperidone and clozapine. After genotyping, only three patients (14.3%) presented side effects.

Conclusion: Our findings highlight pharmacogenetics' relevance in schizophrenia management, concerning efficacy and

safety. Longer-term follow-up and larger samples are warranted to reinforce our results.

Conflict of Interest: None declared

P18.088.C Hereditary causes of pediatric malignancies at Children's Clinical University Hospital: insights from WES implementation in diagnostics since 2022

Lauma Freimane[1], Dmitrijs Rots[1], Anna Janberga[1], Egija Berga Svitina[1], Līvija Bārdiņa[1], Gita Taurina[1], Rasa Insberga[1], Ieva Mičule[1], Santa Kursīte[1], Dace Enkure[1]

[1]Children's Clinical University Hospital, Riga

Background/Objectives: Pathogenic germline variants are identified in 8–10% of children with malignancies. The discovery of a genetic predisposition in a patient affected by a malignancy carries significant implications, impacting the patient's psychosocial well-being, family relationships, and influencing the type and intensity of treatment, as well as the risk of recurrence and secondary cancers.

Methods: We conducted germline exome sequencing with analysis of pediatric cancer panel with SNV and CNV for all pediatric hematological malignancies and solid malignancies diagnosed since 2022 in Latvia who consented for the analysis.

Results: Since 2022, a comprehensive analysis of germline predispositions was conducted for a total of 103 pediatric malignancies. The sample proportions for solid and hematological malignancies with germline predisposing variants were similar, accounting for 9.68% (6/62) and 9.52% (4/42), respectively. Notably, genetic findings of uncertain significance were identified in 6.8% of cases (n = 7). We confirmed Fanconi anemia in two patients due to compound heterozygous variants in *BRCA2* gene and two cases of Noonan syndrome due to pathogenic variants in *PTPN11* gene, also without prior suspicion for a monogenic condition. For hematological malignancies, germline variants were found in the *PTPN11* and *IKZF1* gene, as well as a CNV loss in 18p11.32p11.31. For solid pediatric malignancies, variants were found in the *BRCA2, RB1, PTEN, NF1* genes.

Conclusion: Germline cancer predisposition testing in pediatric patients is essential for establishing a precise diagnosis, explaining ~10% of cases. Our data shows that in 4 out of 10 cases, there were no suspicions for a genetic condition.

Conflict of Interest: None declared

P18.089.D Exploring the Interplay of Genetic Susceptibility, Lifestyle, and Clinical Factors in Cardiovascular Disease

Jeeeun Kim[1;2], Hyojin Pyun[1], Soo Ji Lee[1;3], Joohon Sung[1;3]

[1]Seoul National University, Graduate School of Public Health, Seoul, Korea, Rep. of South; [2]Procagen, R&D center, Gyeonggi-do, Korea, Rep. of South; [3]Seoul National University, Institute of Health & Environment, Seoul, Korea, Rep. of South

Background: Both genetic and lifestyle factors are known to be associated with cardiovascular disease (CVD). However, it is unclear whether the association between lifestyle and CVD risk depends on the individual's lipid-related genetic predisposition. This study investigates the complex relationships between genetic susceptibility to dyslipidemia, lifestyle behaviors, clinical risk factors, and CVD.

Methods: We analyzed a cohort of 72,239 individuals of East Asian descent (with 1931 CVD events) A genome-wide polygenic risk score, derived from over 6 million genetic variants, was used to estimate genetic predisposition to dyslipidemia Lifestyle was evaluated based on four modifiable components (diet, physical activity, drinking habit, smoking habit), while clinical risk factors were assessed using components of the metabolic syndrome.

Results: Participants with a high genetic risk for dyslipidemia are associated with an increased overall CVD risk, which individuals with a high genetic risk for dyslipidemia had a 35% higher risk of CVD compared to those with low genetic risk. Individuals with favorable lifestyles and metabolically healthy statuses demonstrated reduced risk of CVD events, regardless of their genetic susceptibility to dyslipidemia. Sex-specific interaction effects were also observed.

Conclusion: Adhering to a healthy lifestyle and maintaining metabolic health emerge as an important strategy for mitigating the risk of subsequent CVD beyond genetic predispositions for dyslipidemia. These findings highlight the importance of lifestyle modifications in CVD prevention, especially in individuals with genetic susceptibility to dyslipidemia.

Conflict of Interest: None declared

P18.090.B Elective genome screening, clinical experience in 1300 healthy individuals

Luis Izquierdo[1;1], Bibiana Palao[1], Miriam Leon[1], Elena Ordonez[2], Isabel Castilla[2], Mireia Lechuga[2], Maria Moreno[1], Irene Perea[1], Raquel Bernad[1], Enrique Fernandez[1], Nerea Lobato[1], Laura Garcia[2], Vincenzo Cirigliano[2]

[1]Veritas Intercontinental, Madrid, Spain; [2]Veritas Intercontinental, Barcelona, Spain

Background/Objectives: Elective genome sequencing (EGS) is the genome analysis of individuals without a medical indication. This screening strategy enables the study of actionable conditions with the goal of preventing. To provide clinical utility to this approach, all the variants found should be curated properly. We present the results of the first 1300 patients performed with the only EGS test available with clinical utility.

Methods: The test was performed in 1300 people after a medical consultation where myGenome (Veritas Intercontinental) test was prescribed. Extracted DNA from blood or saliva was sequenced at approximately 30X average coverage. Interpretation was performed with a proprietary genomic pipeline that analyzes 600 genes based on ACMG-SF list and ACOG recommendations, together with pharmacogenomics and multifactorial diseases. Clinical variants were curated by a team of expert geneticists.

Results: Clinically relevant genetic variants were identified in 21% of individuals. From all the variants detected, 18.5% are included in ACMG-SF v3.2 list. Divided by medical area, the variants were related to hemochromatosis (42.6%), hereditary cancer predisposition (26.6%), hereditary cardiovascular disease (12.8%) and other diseases (17.8%). Additionally, 64% patients were carriers of one or more recessive conditions, 87% presented risk alleles for multifactorial diseases and relevant pharmacogenomics associations were detected in 99% of the patients, 32% of them were severe gene-drug interactions.

Conclusion: The inclusion of EGS in the medical setting allows an effective identification of relevant genetic findings related to clinical diseases, carrier status, pharmacogenomics, and risk alleles, that provides an outstanding preventive approach for clinicians.

Grants: None significant to the purpose of this research.

Conflict of Interest: Luis Izquierdo full time, Bibiana Palao full-time, Miriam Leon full-time, Elena Ordonez full-time, Isabel Castilla full-time, Mireia Lechuga full-time, Maria Moreno full-time, Irene Perea full-time, Raquel Bernad full-time, Enrique Fernandez full-time, Nerea Lobato full-time, Laura Garcia full-time, Vincenzo Cirigliano full-time

P18.091.B Molecular biomarkers of risk and resilience in health from exposure to environmental stress: A systematic review

Carys Räsänen-Young[1], *Laura J. Smyth*[1], *Yogesh Gupta*[1], *Claire Hill, Frank Kee, Ruth Hunter*[1], *Bernadette McGuinness, Amy Jayne McKnight*[1]

[1]*Centre for Public Health, Queen's University Belfast, United Kingdom*

Background/Objectives: Environmental stress is associated with poor health outcomes. However, the underlying biological mechanisms mediating this relationship is poorly understood. This research aims to identify molecular biomarkers, including resilience markers, associated with environmental stressors and explore approaches to measure their relationship.

Methods: The electronic databases Medline, EMBASE, Scopus, Web of Science were searched for human studies, with an environmental exposure and a laboratory-derived molecular biomarker. Studies were screened using Covidence. Methodological rigour was assessed with Joanna Briggs criteria. A narrative review was conducted and meta-analysis on studies with similar data using R studio.

Results: 63,868 studies were identified; 1,313 studies passed criteria for the review.

21 molecular biomarkers were found associated with environmental exposures. Most, 655 studies, used DNA methylation. Cancer was the primary health outcome associated with any of the biomarkers and environmental stressors. Arsenic and tobacco smoke were the most measured exposures. Study characteristics varied with exposure. In genetic biomarkers, 128 studies looked at SNPs and socio-economic status, occupation, aromatic compounds, pesticides, and toxic elements. Many observations were found; 8 studies showed association of PON1 polymorphisms and pesticide level in blood, GSTT1 null genotype had an increased risk of hepatotoxicity in 5 studies from arsenic, benzene, or pesticides, 3 studies observed methylation in BDNF promoter sites and socio-economic status.

Conclusion: This systematic review provides evidence that may aid identifying vulnerable populations, support reasoning behind interventions, and improve understanding of the biological mechanisms underlying stress from the environment.

Grants: Science Foundation Ireland and the Department for the Economy, Northern Ireland Investigator Program Partnership Award (15/IA/3152); CRY is in receipt of PhD studentships from the NI Department for the Economy; UKRI Industrial Strategy Challenge Fund (ESRC, SBDRP, ES/V016075/), the US-Ireland R&D Partnership Programme by HSC R&D division (STL/5569/19) and UKRI (MRC MC_PC_20026).

Conflict of Interest: None declared

P18.092.C Mendelian randomization and bayesian colocalization analysis implicate GP6 as a potential drug target for cardioembolic stroke in south Asians

Devendra Meena[1], *Siwei Wu*[1], *James Yarmolinsky*[1], *Dipender Gill*[1], *Alexander Smith*[1], *Marie-Joe Dib*[2], *Ganesh Chauhan*[3], *Anand Rohatgi*[4], *Abbas Dehghan*[1], *Ioanna Tzoulaki*[1]

[1]*Imperial College London White City Campus, Department of Epidemiology and Biostatistics, London, United Kingdom;* [2]*Hospital of the University of Pennsylvania, Division of Cardiovascular Medicine, Philadelphia, United States;* [3]*Rajendra Institute of Medical Sciences, Department of Genetics & Genomics, Ranchi, India;* [4]*UT Southwestern Medical Center, Department of Medicine, Division of Cardiology, Dallas, United States*

Background/Objectives: Circulating plasma proteins are clinically useful biomarkers for stroke risk. We examined the causal links between 2,922 plasma proteins and stroke risk using Mendelian randomisation in individuals of South Asian ancestry.

Methods: We obtained genetic instruments for plasma proteins from the UK Biobank (N = 920). GWAS summary data for strokes (N ≤ 11,312) were sourced from GIGASTROKE. Our primary approach involved the Wald ratio or inverse-variance weighted (IVW) methods, with statistical significance set at FDR < 0.1. Additionally, a Bayesian colocalization approach was applied to minimize bias from linkage disequilibrium.

Results: We found evidence of a potential causal effect of plasma glycoprotein VI (GP6) levels on cardioembolic stroke ($OR_{Wald\text{-}ratio} = 2.53$; 95% CI 1.59-4.03, $P = 9.2 \times 10^{-5}$, FDR = 0.059). Generalised MR accounting for correlated SNPs showed consistent direction of effect ($OR_{gIVW} = 2.21$; 95% CI 1.46-3.33, $P = 1.6 \times 10^{-4}$). Colocalization analysis indicated that plasma GP6 levels colocalize with cardioembolic stroke (posterior probability [PP] = 91.4%). Multi-trait colocalization showed that these two traits colocalize with *GP6* expression in the coronary artery and brain tissues (PP > 50%). The potential causal effect of GP6 on cardioembolic stroke was not significant in Europeans ($OR_{IVW} = 1.08$; 95% CI 0.93-1.26, P = 0.29).

Conclusion: Our joint MR and colocalization analyses suggest that genetically predicted GP6 is potentially causally associated with cardioembolic stroke risk in individuals of South Asian ancestry. Future MR studies with larger sample size for plasma GP6 levels should be implemented to further validate our findings.

Grants: Imperial College British Heart Foundation Centre for Research Excellence (RE/18/4/34215)

Conflict of Interest: None declared

P18.093.D Phenotypic variability of CADASIL cerebral angiopathy in the presence of the NOTCH3 p.R1231C mutation frequently observed in the European population

Louis Lambert[1], *Veronica Sandroni*[1;2], *Jessica Lebenberg*[1;3], *Teresa Nutile*[4], *Daniela Ruggiero*[4], *Dominique Hervé*[1;3], *Hervé Perdry*[5], *Elisabeth Tournier-Lasserve*[1;3;6], *Hugues Chabriat*[1;3;7], *Marina Ciullo*[4;8], **Anne-Louise Leutenegger**[1]

[1]*Inserm Université Paris Cité, NeuroDiderot, Paris, France;* [2]*APHP, Paris, France;* [3]*APHP, Translational Neurovascular Centre and CERVCO, Hôpital Lariboisière, Paris, France;* [4]*Institute of Genetics and Biophysics A. Buzzati-Traverso, CNR, Naples, Italy;* [5]*Université Paris Saclay, Inserm CESP, Villejuif, France;* [6]*APHP, Service de génétique moléculaire Neurovasculaire, Hôpital Saint-Louis, Paris, France;* [7]*Université Paris Cité, FHU Neuro-Vasc, Paris, France;* [8]*IRCCS Neuromed, Pozzilli, Italy*

Background/Objectives: CADASIL (Cerebral Autosomal Dominant Arteriopathy with Subcortical Infarcts and Leukoencephalopathy) is a prevalent hereditary small cerebral vessel disease caused by mutations in the NOTCH3 gene. This study focused on the p.R1231C mutation, frequent in Europe and located in the EGFr 31 domain. The objective was to investigate the phenotypic variability associated with this mutation, as recent studies suggest severity differences based on the affected EGFr repeats (most severe for EGFr 1 to 6).

Methods: Clinical and imaging data were collected from two sources: a cohort of patients followed at CERVCO rare disease referral center in Lariboisière Hospital (LRB: EGFr1-6, N = 309; p.R1231C, N = 17) and an isolated population in southern Italy (ITA, N = 23).

Results: 1) In the LRB hospital cohort, after adjustment for age, sex and vascular risk factors (notably hypertension), no difference

was observed between EGFr1-6 and p.R1231C patients. 2) However, the p.R1231C cohort at LRB exhibited a notably more severe clinical and MRI phenotype compared to the ITA population, even with comparable vascular risk factors.

Conclusion: This suggests wide phenotypic variability associated with the p.R1231C mutation, influenced by patient recruitment mode and the presence of vascular risk factors. We are now looking for genetic risk factors associated with this variability.

Grants: AARG-NTF-20-683992, ANR-20-CE17-0031.

Conflict of Interest: None declared

P18.094.A Integrating genetic data in Trial Emulation: Exploring the potential of Polygenic Risk Scores

Jakob German[1;2], *Sarah Urbut*[2;3], *Anthony Philippakis*[2], *Andrea Ganna*[1;2]

[1]*Institute for Molecular Medicine Finland, Helsinki, Finland;* [2]*Broad Institute of MIT and Harvard, Cambridge, United States;* [3]*Massachusetts General Hospital, Boston, United States*

Background/Objectives: A central goal of pharmacogenomics is to use genetic information to identify individuals benefitting most from a pharmacological intervention. We investigate the utilization of polygenic-risk-scores (PRS) in trial-emulation designs using FinnGen (N = 429,191), a biobank-based study with comprehensively longitudinal drug purchases information from national registers.

Methods: We selected and emulated five trials. Two RCTs focused on T2D patients, three on cardiovascular diseases. We defined a list of eligibility criteria, specified a comprehensive list of confounding variables, and matched the cohorts using propensity-score matching to adjust for baseline confounding. 90 selected PRS were used to examine residual confounding. Average treatment effects were estimated using Cox-proportional-hazard-models.

Results: We achieved estimate agreement with the original RCT in all trials.

A trial-emulation approach results in reduced differences in PRS between treatment and control groups compared to a naïve approach (e.g. T2D-PRS; standardized-difference (SD) = 0.0042, p = 0.9089 vs SD = 0.0875, p = 8.75e-10). A genetically-informed causal framework identified residual confounding in the emulated trial. Using genome-wide-significant SNPs associated with BMI we demonstrated a causal relationship between BMI and both the outcome and treatment (beta = 0.0156, p = 3.27e-27 and beta = 0.0579, p = 8.20e-3) indicating residual confounding due to BMI. Investigations of the interaction between PRS for the trial outcome and the drug, aiming to identify subgroups with differential treatment response, showed no significance.

Conclusion: Our work highlights the importance of trial-emulation when studying genetic impact on differential treatment response in biobank studies, as genetic studies are not immune to traditional epidemiological bias. Furthermore, we showed how genetic information can be used to detect residual confounding in trial-emulation.

Grants: Broad-Institute-of-MIT-and-Harvard

Conflict of Interest: None declared

P18.095.B Genetic Determinants of Early Childhood Appetite and Their Impact on Obesity: a prospective study from the Norwegian Mother, Father, and Child cohort

Roya Karimi[1], *Jonas Sundfjord*[1], *Adina Lupu*[1], *Nicolas Bargas*[1], *Marc Vaudel*[1;2], *stefan johansson*[1;3]

[1]*Mohn Center for Diabetes Precision Medicine, Department of Clinical Science, University of Bergen, Bergen, Norway;* [2]*Department of Genetics and Bioinformatics, Norwegian Institute of Public Health, Oslo, Norway;* [3]*Department of Medical Genetics, Haukeland University Hospital, Bergen, Norway*

Background/Objectives: With global obesity rates rising, it's vital to explore the genetic underpinnings of early eating behaviors. This study aims to identify genetic variants affecting young children's appetite and their potential connection to childhood obesity.

Methods: This study utilized genome-wide association studies (GWAS) on three parental reports regarding their children's appetite including the child's eating adequacy and child's food fussiness. By applying genomic structural equation modeling (GenomicSEM) we quantified the genetic contributions to these three GWAS summary statistics, resulting in the construction of a latent variable that captures the essence of childhood appetite between 18 months and 3 years. We generated Polygenic Risk Scores (PRS) using LDpred2 from GenomicSEM results. Linear regression was then applied to assess the association between early appetite PRS and subsequent obesity risk in childhood.

Results: The GenomicSEM analyses show a slightly significant and moderate association between several genetic variants, most notably within the GLP1R gene and early childhood appetite. The other identified variants were linked with genes relevant to BMI and obesity. The genome-wide polygenic risk score accounted for 3 to 5% of the variance in BMI across timepoints from 18 months to 8 years old.

Conclusion: This research identifies genetic variations associated with early childhood appetite and their links with the risk of obesity, underscoring the genetic basis for early intervention strategies in the prevention of obesity.

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Conflict of Interest: None declared

P18.096.C Trans-ethnic Mendelian Randomization of Hematological Traits and Type-2 Diabetes Mellitus

Chisom Soremekun[1;2], *Daudi JJingo*[3], *David Patrick Kaateete*[2], *Oyekanmi Nash*[4], *Dorothea Nitsch*[5], *Moffat Nyirenda*[6], *Dipender Gill*[7], *Eleftheria Zeggini*[8;9], *Harald Grallert*[1;10], *Annette Peters*[1;10;11], *Tinashe Chikowore*[12;13], *Chiara Batini*[14;15], *Opeyemi Soremekun*[8], *Segun Fatumo*[5;6]

[1]*Helmholtz Zentrum München, German Research Center for Environmental Health (GmbH), Institute of Epidemiology, Neuherberg;* [2]*Makerere University, Immunology and Molecular Biology, Kampala, Uganda;* [3]*Makerere University, African Center of Excellence in Bioinformatics (ACE-B), Kampala, Uganda;* [4]*National Biotechnology Development Agency, Centre for Genomics Research and Innovation, Abuja, Nigeria;* [5]*London School of Hygiene and Tropical Medicine, Department of Non-Communicable Disease Epidemiology, London, United Kingdom;* [6]*Uganda Virus Research Institute, MRC/UVRI and LSHTM Uganda Research Unit, Entebbe, Uganda;* [7]*Imperial College London, Department of Epidemiology and Biostatistics, School of Public Health, London, United Kingdom;* [8]*Helmholtz Zentrum München – German Research Center for Environmental Health, Institute of Translational Genomics, Neuherberg;* [9]*TUM School of Medicine, Technical University of Munich and Klinikum Rechts der Isar, Munich, Germany;* [10]*German Center for Diabetes Research (DZD), Neuherberg, Germany;* [11]*Ludwig-Maximilians-Universität München, Institute for Medical Information Processing, Biometry and Epidemiology, Medical Faculty, Munich, Germany;* [12]*University of the Witwatersrand, Wits Donald Gordon Medical Centre, School of Clinical Medicine, Faculty of Health Sciences,*

Johannesburg, South Africa; [13]Harvard Medical School, Brighams Women Hospital, Boston, United States; [14]University of Leicester, Genetic Epidemiology Group, Department of Health Sciences, Leicester, United Kingdom; [15]Leicester National Institute for Health and Care Research, Biomedical Research Centre, Glenfield Hospital, Leicester, United Kingdom

Background/Objectives: Observational studies have identified associations between hematological traits and type-2 diabetes mellitus (T2D). However, it is difficult to infer causal effects due to the potential of confounding. Our study utilizes the Mendelian randomization (MR) approach to address the above limitation and investigate the causal effect of hematological traits such as white blood cell (WBC), platelets (PLT), and red blood cell (RBC) on T2D in individuals of African and European ancestry.

Methods: The cohorts included African and European ancestry participants in the Blood Cell consortium and the Million Veteran Program dataset. Using GWAS summary statistics, we applied Two-sample MR to estimate the causal relationship between hematological traits and T2D.

Results: In the main IVW MR estimates for the RBC genetically predicted mean corpuscular hemoglobin concentration (MCHC), mean corpuscular hemoglobin (MCH), and mean corpuscular volume (MCV) levels were associated with decreased risk of T2D in the African dataset. In the Europeans, MCH, MCHC, and MCV levels were also significantly associated with decreased risk of T2D, however, MCHC was not significant after correcting for multiple tests. For WBC traits, there was evidence of protective effects of total WBC count and neutrophil count (NEU) on T2D in the African Population. Contrary to our observation, there was no association of NEU, and WBC on T2D in European ancestry.

Conclusion: These consistent findings majorly support the causal association between hematological traits and T2D in the African and European populations. However, the contrasting result for the WBC might indicate potential population differences between these immune-related factors and T2D across distinct populations.

Conflict of Interest: None declared

P18.097.D The role of *SLCO1B1*, *ABCG2*, *CYP2C9*, *CYP2C19* and *CYP2D6* in medication adherence and discontinuation: a biobank-scale analysis in 412,181 individuals.

Mattia Cordioli[1], *Andrea Corbetta[2], Hanna Kariis[3], Sakari Jukarainen[1], Pekka Vartiainen[1], Tuomo Kiiskinen[1], Matteo Ferro[1], FinnGen Consortium[1], Estonian Biobank research team[3], Kelli Lehto[3], Mikko Niemi[4], Samuli Ripatti[1], Lili Milani[3], Andrea Ganna[1]*

[1]Institute for Molecular Medicine Finland, Helsinki, Finland; [2]Human Technopole, Italy; [3]Estonian Genome Center, University of Tartu, Tartu, Estonia; [4]University of Helsinki, Helsinki, Finland

Background/Objectives: While numerous pharmacogenetic variants have been linked to altered drug metabolism or adverse drug reactions, their impact on patients medication-taking behaviors has not been studied in large populations. We investigated the role of notable pharmacogenes in medication adherence and discontinuation by leveraging genetic and drug purchase data from the FinnGen study (n = 412,181).

Methods: We focused on well-established drug-gene pairs, including *SLCO1B1*, *ABCG2*, *CYP2C9* for, respectively, statins (all types, $n = 115{,}369$), rosuvastatins ($n = 5116$) and fluvastatins ($n = 2407$); *CYP2C19* and clopidogrel ($n = 6400$); *CYP2D6* and tamoxifen ($n = 2028$). For each gene, we included star allele diplotypes with established prescribing recommendations and grouped them based on their metabolic activity profile. We defined adherence as the ratio of total purchased quantity to total days of purchasing, and discontinuation as either early (after one purchase) discontinuation, or within the first year. We tested the association between each pharmacogene metabolic activity profile and the three drug-taking phenotypes.

Results: The *CYP2D6* poor metabolizer profile showed a significant association with increased odds of early discontinuation of tamoxifen (OR = 3.36 (1.36 - 8.29), $P = 0.009$), but not with 1-year discontinuation and adherence. No significant association was observed for the other pharmacogenes and the three drug-taking behaviors.

Conclusion: These findings suggest a marginal role of pharmacogenetic variants on drug adherence and discontinuation, with the exception of tamoxifen. Patients with a *CYP2D6* poor metabolizer profile showed increased risk of discontinuation and might benefit from preemptive pharmacogenetic testing in assessing a personalized treatment alternative before initiation.

Conflict of Interest: Mattia Cordioli: None declared, Andrea Corbetta: None declared, Hanna Kariis: None declared, Sakari Jukarainen: None declared, Pekka Vartiainen: None declared, Tuomo Kiiskinen: None declared, Matteo Ferro: None declared, FinnGen Consortium: None declared, Estonian Biobank research team: None declared, Kelli Lehto: None declared, Mikko Niemi: None declared, Samuli Ripatti: None declared, Lili Milani: None declared, Andrea Ganna AG is founder of Real World Genetics.

P18.098.A Modifiable pathways and epigenetic markers for the effects of childhood maltreatment on psychiatric disorders: Mendelian randomization study

Nicole Ying Ting Ng[1], *Jie V. Zhao[2], John Yen Tang[1], Martin Man Chun Chui[1], Christopher Chun Yu Mak[1], Brian Hon Yin Chung[1]*

[1]The University of Hong Kong (HKU), Department of Paediatrics and Adolescent Medicine, Hong Kong; [2]The University of Hong Kong (HKU), School of Public Health, Hong Kong

Background/Objectives: Childhood maltreatment (CM) is associated with increased risks of psychiatric disorders later in life. It is important to understand the mediating pathways for intervention. This Mendelian randomization (MR) study aims to confirm the relationship between CM and psychiatric disorders, explore the causal role of mediators, and identify the potential influence of DNA methylation (DNAm).

Methods: Two-Sample MR using summary data from European ancestry was performed to evaluate the causal effect of genetically predicted CM (n = 143,473) on the risk of different psychiatric disorders. Two-step, multivariable MR, and epigenetic MR were conducted to identify mediators of the relationship.

Results: CM was significantly associated with higher risk of attention-deficit/hyperactivity disorder (ADHD) (OR, 95% CI: 10.09, 4.76-21.40), major depressive disorder (MDD) (OR, 95% CI: 1.89, 1.32-2.70) and schizophrenia (OR, 95% CI: 5.89, 1.46-23.78). These relationships were mediated by addiction-related, cognitive, and socioeconomic traits. Following adjustment for all significant mediators, the direct effect of CM on ADHD attenuated substantially (OR, 95% CI: 2.39, 1.64-3.48), while the direct effect of CM on schizophrenia was slightly reduced (OR, 95% CI: 5.10, 2.71-9.60). Contrastingly, direct effect on MDD remained unchanged despite adjusting for mediators. DNAm levels at 11 unique CM-associated CpG sites were associated with psychiatric disorders.

Conclusion: This study provides novel insights into the mediators and biological markers between CM and psychiatric disorders in later life. Multi-modal preventative and responsive interventions are required to reduce CM prevalence and its long-term consequences.

Grants: Seed Funding for Basic Research (2202100680) from the University of Hong Kong

Conflict of Interest: None declared

P18.099.B MR-link-2: pleiotropy robust *cis* Mendelian randomization validated in four independent gold-standard datasets of causality provides human evidence for hypomyelating leukodystrophy-10

Adriaan van der Graaf[1], *Robert Warmerdam*[2], *Chiara Auwerx*[1;3], *Urmo Võsa*[4], *Carolina Borges*[5;6], *Lude Franke*[2;7], *Zoltan Kutalik*[1]

[1]*University of Lausanne, Department of Computational Biology, Ecublens, Switzerland;* [2]*University Medical Center Groningen, Department of Genetics, Groningen, Netherlands;* [3]*University of Lausanne, Center for Integrative Genomics, Ecublens, Switzerland;* [4]*University of Tartu, Estonian Genome Center, Tartu, Estonia;* [5]*University of Bristol, MRC Integrative Epidemiology Unit, Bristol, United Kingdom;* [6]*University of Bristol, Population Health Services, Bristol, United Kingdom;* [7]*Oncode Institute, Utrecht, Netherlands*

Consortium: eQTLGen Consortium

Background/Objectives: Mendelian randomization (MR) can identify causality from observational data but has increased type 1 error rates (T1Es) due to pleiotropy. In molecular exposures usually a single associated region is available making it difficult to address pleiotropy. We develop a pleiotropy robust *cis* MR method and test it on novel ground truth datasets.

Methods: We developed MR-link-2, an MR method using summary statistics and linkage disequilibrium information to estimate causality and pleiotropy from single associated regions. We compare MR-link-2 to 5 other *cis* MR and coloc methods in three validation datasets: i) Causality between 266 metabolites and 287 reactions from 4 studies and 3 pathway databases ii) canonical causality between 13 complex traits and iii) causality between 12 cell types and 93 of their marker genes.

Results: Metabolite analysis: MR-link-2 has superior area under the receiver operator characteristic curve (AUC) (max 0.80) compared to other methods. MR-link-2 has better precision across comparisons (up to 51-98% relative precision for others), alignment with *le Chatelliers* principle is 80% (max 63% for others). MR-link-2 exclusively identifies the causal link for hypomyelinating leukodystrophy-10 between glycine and pyrroline-5-carboxylate, previously only found in model systems. In complex traits, MR-link-2 has lower T1Es (0.09 vs 0.15, expectation: 0.05) and lower heterogeneity (Q statistic 583 vs min 1359). Testing for forward and reverse causality of blood cell type composition and expression of marker genes shows MR-link-2 has superior AUC 0.90 (best competing: 0.67).

Conclusion: In 3 human validation datasets, MR-link-2 is ideally suited for molecular phenotypes. Exhibiting good T1Es and superior AUC.

Grants:

Conflict of Interest: Adriaan van der Graaf: None declared, Robert Warmerdam: None declared, Chiara Auwerx: None declared, Urmo Võsa: None declared, Carolina Borges: None declared, Lude Franke: None declared, Zoltan Kutalik This study was funded by the Swiss National Science Foundation (310030_189147, Zoltan Kutalik) and the Department of Computational Biology of the University of Lausanne (Zoltan Kutalik)

P18.100.C Inference of causality between the vascular endothelial growth factor and ARDS: a Mendelian randomisation analysis

Eva Suarez-Pajes[1], *Nick Shrine*[2], *Eva Tosco-Herrera*[1], *Tamara Hernandez-Beeftink*[2;3], *Luis A. Rubio-Rodríguez*[4], *M. Isabel García-Laorden*[5;6], *Almudena Corrales*[1;6], *Miryam Prieto González*[7], *Aurelio Rodríguez-Pérez*[8;9], *Demetrio Carriedo*[10], *Jesús Blanco*[6;11], *Alfonso Ambrós*[12], *Elena González Higueras*[13], *Elena Espinosa*[14], *Arturo Muriel-Bombin*[11], *David Domínguez*[14], *Abelardo García de Lorenzo*[15], *Jose Manuel Añon*[6;15], *Marina Soro*[16], *Jesús Villar*[5;6], *Martin Tobin*[2;3], *Louise Wain*[2;3], *Carlos Flores*[1;4;6], *Olivia C. Leavy*[2;3], *Beatriz Guillen-Guio*[2;3]

[1]*Research Unit, Hospital Universitario Nuestra Señora de Candelaria, Santa Cruz de Tenerife, Spain;* [2]*Department of Population Health Sciences, University of Leicester, Leicester, United Kingdom;* [3]*NIHR Leicester Biomedical Research Centre, University of Leicester, Leicester, United Kingdom;* [4]*Genomics Division, Instituto Tecnológico y de Energías Renovables (ITER), Santa Cruz de Tenerife, Spain;* [5]*Research Unit, Hospital Universitario Dr. Negrín, Las Palmas de Gran Canaria, Spain;* [6]*CIBER de Enfermedades Respiratorias (CIBERES), Instituto de Salud Carlos III, Madrid, Spain;* [7]*Intensive Care Unit, Complejo Asistencial Universitario de Palencia, Palencia, Spain;* [8]*Department of Anesthesiology, Hospital Universitario de Gran Canaria Dr. Negrín, Las Palmas de Gran Canaria, Spain;* [9]*Department of Medical and Surgical Sciences, University of Las Palmas de Gran Canaria, Las Palmas de Gran Canaria, Spain;* [10]*Intensive Care Unit, Complejo Hospitalario Universitario de León, León, Spain;* [11]*Intensive Care Unit, Hospital Universitario Rio Hortega, Valladolid, Spain;* [12]*Intensive Care Unit, Hospital General de Ciudad Real, Ciudad Real, Spain;* [13]*Intensive Care Unit, Hospital Virgen de la Luz, Cuenca, Spain;* [14]*Department of Anesthesiology, Hospital Universitario N.S. de Candelaria, Santa Cruz de Tenerife, Spain;* [15]*Intensive Care Unit, Hospital Universitario La Paz, IdiPAZ, Madrid, Spain;* [16]*Anesthesiology and Critical Care Department, Hospital IMED Valencia, Valencia, Spain*

Background/Objectives: Acute respiratory distress syndrome (ARDS) commonly develops by sepsis. A genome-wide association study (GWAS) linked vascular endothelial growth factor pathway genes (*VEGFA* and *VEGFR1*) to post-sepsis ARDS risk. To infer the causal effect of VEGFA and VEGFR1 circulating levels in ARDS, we performed two-sample bidirectional Mendelian randomisation.

Methods: We evaluated the causal effect using inverse variance-weighted (IVW) estimates and performed sensitivity analyses with five other methods, based on strong (F-stat>10) instrumental variables (IV) that met genome-wide significance ($p < 5e\text{-}8$) in GWAS of VEGFA and VEGFR1 circulating protein levels, separately (UK Biobank, N = 46,836). For the opposite direction, we selected strong IVs reaching $p < 5e\text{-}6$ in post-sepsis ARDS GWAS. We evaluated absence of pleiotropy and outliers and performed MR-RAPS using weaker instruments (GWAS $p < 0.05$).

Results: We found no evidence to suggest that either VEGFA ($p_{IVW} = 0.992$) or VEGFR1 ($p_{IVW} = 0.924$) have a significant causal effect on ARDS risk. There was no evidence suggesting that ARDS has a causal effect on either VEGFA ($p_{IVW} = 0.487$) or VEGFR1 ($p_{IVW} = 0.168$) levels.

Conclusion: Our results do not support causality between circulating VEGFA and VEGFR1 levels and the development of post-sepsis ARDS. Further analyses are required to understand VEGF regulation in patients in acute phase and its role in ARDS causality.

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P18.101.D *NF2*-related Schwannomatosis: an updated epidemiological study.

Claire Forde[1], *Miriam Smith*[1], *George Burghel*[1], *Naomi Bowers*[1], *Nicola Roberts*[1], *Tim Lavin*[2], *Jane Halliday*[3], *Andrew King*[3], *Scott Rutherford*[3], *Omar Pathmanaban*[3], *Simon Lloyd*[4], *Simon Freeman*[4], *Dorothy Halliday*[5], *Patrick Axon*[6], *Juliette Buttimore*[6], *Shazia Afridi*[7], *Roger Laitt*[3], *Owen Thomas*[3], *D Gareth Evans*[1]

[1]*Manchester Centre for Genomic Medicine, Manchester, United Kingdom;* [2]*Salford Royal, United Kingdom;* [3]*Manchester Centre for Clinical Neurosciences (MCCN), United Kingdom;* [4]*Manchester Royal Infirmary, United Kingdom;* [5]*Nuffield Orthopaedic Centre, United Kingdom;* [6]*Cambridge University Hospitals, United Kingdom;* [7]*Guys & St Thomas Nhs Foundation Trust, United Kingdom*

Background/Objectives: New diagnostic criteria for *NF2*-related schwannomatosis (NF2) have been published (Genet Med. 2022). An updated UK prevalence has been generated with an emphasis on the rate of de novo NF2 (50% rate is widely quoted).

Methods: The UK National NF2 database identifies patients meeting updated NF2 criteria from a highly ascertained population cared through England's specialised service. Diagnostic prevalence was assessed on 01/02/2023.

Results: 1082 living NF2 patients were identified on prevalence day (equivalent to 1 in 61,445). The tables detail the rates of de novo, familial and mosaic NF2. The proportion with inherited NF2 from an affected parent was only 23% in England. If those without a confirmed molecular diagnosis or bilateral vestibular schwannoma (BVS) are excluded, the rate of de novo NF2 remains high (72%).

	Population (millions)	NF2 patients living	Prevalence per 1000	1 in x	Hetero-zygotes	Familial	%familial of total living
England	56.00	960	0.01714	58331	415	222	23.1
Wales	3.14	50	0.01594	62720	22	12	24.0
Scotland	5.45	51	0.00936	106863	21	6	11.8
N Ireland	1.90	21	0.01105	90476	14	5	23.8
Total	66.48	1082	0.01627	61445	472	245	22.6

	de novo	Mosaic	%mosaic of de novo	Heterozygote, proven mosaic, or BVS	% familial
England	738	204	27.6	787	28.2
Wales	38	9	23.7	44	27.3
Scotland	37	14	37.8	43	14.0
N Ireland	16	3	18.8	21	23.8
Total	829	230	27.7	895	27.4

Conclusion: This work confirms a higher rate of de novo NF2 than previously reported, and highlights the benefits of maintaining patient databases for accurate counselling.

Conflict of Interest: Claire Forde: None declared, Miriam Smith: None declared, George Burghel: None declared, Naomi Bowers: None declared, Nicola Roberts: None declared, Tim Lavin: None declared, Jane Halliday: None declared, Andrew King: None declared, Scott Rutherford: None declared, Omar Pathmanaban: None declared, Simon Lloyd: None declared, Simon Freeman: None declared, Dorothy Halliday: None declared, Patrick Axon: None declared, Juliette Buttimore: None declared, Shazia Afridi: None declared, Roger Laitt: None declared, Owen Thomas: None declared, D Gareth Evans AstraZeneca and Everything Genetic Ltd

P18.102.A Incorporating genetic and clinical predictors for antidepressant side effects in the Genetic Link of Anxiety and Depression Study

Danyang Li[1], *Yuhao Lin*[1], *Helena Davies*[1], *Gerome Breen*[1]

[1]*Social Genetic and Developmental Psychiatry Centre, United Kingdom*

Background/Objective: Antidepressants are the most common treatment for moderate or severe depression. Side effects are crucial indicators for antidepressants, but their expression varies widely among individuals.

Methods: In this study, we leveraged deep phenotyped data from self-reported questionnaires in the Genetic Link of Anxiety and Depression (GLAD) study to predict side effects and discontinuation across three antidepressant classes (SSRI, SNRI, tricyclic antidepressants (TCA)) at the first and the last (most recent) year. Over 260 predictors spanning genetic, clinical, comorbidity, demographic, and drug information were included. XGBoost, random forest, cubist, elastic net, and support vector machine (RBF and polynomial kernel) were trained and their performance were compared.

Results: The final dataset comprised 5358 individuals, with 4354 in the first and 3414 in the last prescription. The average prevalence of side effects and discontinuation was 74.1% and 28.7%, respectively. In the initial year, the best AUROC for predicting SSRI discontinuation and side effects were 0.66 and 0.60. In the last year of SSRI prescription, the highest AUROC reached 0.72 for discontinuation and 0.87 for side effects. Models for predicting discontinuation and side effects of SNRI and TCA showed comparable performance. The history of side effects and discontinuation in antidepressant use were the most influential predictors for the outcomes.

Conclusion: Our findings demonstrate the feasibility of predicting antidepressant side effects using self-reported questionnaire, particularly for the last prescription. These results contribute valuable insights for the development of clinical decisions aimed at optimizing treatment selection with enhanced tolerability.

Grants: National Institute for Health and Care Research BioResource [RG94028, RG85445]

Conflict of Interest: None declared

P18.103.B Investigating the comorbidity of schizophrenia and metabolic syndrome using real-world health and biobank data

Maris Alver[1], *Silva Kasela*[1], *Liina Haring*[2;3], *Laura Birgit Luitva*[1], *Estonian Biobank Research Team EstBB*[1], *Health Informatics Research Team University of Tartu*[4], *Krista Fischer*[1;5], *Märt Möls*[1;5], *Lili Milani*[1]

[1]*Estonian Genome Centre, Institute of Genomics, University of Tartu, Tartu, Estonia;* [2]*Psychiatry Clinic of Tartu University Hospital, Tartu, Estonia;* [3]*Department of Psychiatry, Institute of Clinical Medicine,*

University of Tartu, Tartu, Estonia; [4]Institute of Computer Science, University of Tartu, Tartu, Estonia; [5]Institute of Mathematics and Statistics, University of Tartu, Tartu, Estonia

Background/Objectives: Schizophrenia (SCZ) patients exhibit 30% higher prevalence of metabolic syndrome (MetS) compared to the general population with its suboptimal management contributing to increased mortality. Large-scale studies providing real-world evidence of the underlying causes remain limited.

Methods: To address this gap, we used real-world health data from the Estonian Biobank, spanning a median follow-up of ten years, to investigate the impact of genetic predisposition and antipsychotic treatment on the development of MetS in SCZ patients. Specifically, we set out to characterize antipsychotic treatment patterns, genetic predisposition of MetS traits, MetS prognosis, and body mass index (BMI) trajectories, comparing SCZ cases (n = 677) to age- and sex-matched controls (n = 2,708).

Results: SCZ cases exhibited higher genetic predisposition to SCZ (OR = 1·75, 95%CI 1·58–1·94), but lower polygenic burden for increased BMI (OR = 0·88, 95%CI 0·88–0·96) and C-reactive protein (OR = 0·88, 95%CI 0·81–0·97) compared to controls. While SCZ cases showed worse prognosis of MetS (HR 1·95, 95%CI 1·54–2·46), higher antipsychotic adherence within the first treatment year was associated with reduced long-term MetS incidence. Linear mixed modelling, incorporating multiple BMI timepoints, underscored the significant contribution of both, antipsychotic medication, and genetic predisposition to higher BMI, driving the substantially upward trajectory of BMI in SCZ cases.

Conclusion: These findings contribute to refining clinical risk prediction and prevention strategies for MetS among SCZ patients and emphasize the significance of incorporating genetic information, long-term patient tracking, and employing diverse perspectives when analyzing real-world health data.

Grants:

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P18.104.C Discovery of core genes for systemic lupus erythematosus via genome-wide aggregation of trans-effects.

*Andrii Iakovliev[1], **Buddhiprabha Erabadda**[2], Olivia Castellini-Pérez[3], Marta E Alarcón-Riquelme[3], Elena Carnero-Montoro[3], Paul McKeigue[2], Athina Spiliopoulou[2]*

[1]University of Edinburgh, Institute of Genetics and Cancer, United Kingdom; [2]University of Edinburgh, Usher Institute, United Kingdom; [3]Pfizer-University of Granada-Junta de Andalucía Centre for Genomics and Oncological Research, Spain

Background/Objectives: Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by upregulation of Type 1 interferon signaling and B-cell mediated autoimmunity. We test whether the genetic architecture of SLE fits an "omnigenic" sparse effector model and identify "core" effector genes using genome-wide aggregated *trans*-effects (GATE) analysis.

Methods: We used eQTLs from eQTLGen and pQTLs from deCODE and UKB-PPP to compute GATE scores in a case-control GWAS of SLE (5267/4909 cases/controls). We identified putative core genes based on strength of association of the GATE score with SLE, the effective number of *trans*-QTLs contributing to the score, and the specificity of the association (lack of correlation with scores for other genes).

Results: We detected a cluster of 11 co-regulated Type 1 interferon-stimulated genes (ISGs) strongly associated with SLE, one of which [*ISG15* (Log OR = 0.1, $P = 1 \times 10^{-6}$)] is a monogenic cause of lupus. From pQTL analyses, 12 putative core genes were identified at P value $< 10^{-6}$ including two of the three receptors that recognise B-cell activating factor (BAFF), *TNFRSF17*, *TNFRSF13B*, and two genes encoding immune checkpoints, *PDCD1*, *CD27*.

Conclusion: Our results suggest that SLE is associated with genetic upregulation of basal Type 1 interferon signaling. Core genes detected through our pQTL-GATE analysis are promising drug targets. BAFF is the only specific drug target for SLE, with a BAFF-inhibitor approved for its treatment. PD-1, encoded by *PDCD1*, is an attractive target for drug repositioning, with PD-1 agonists recently passing a Phase2 trial for refractory rheumatoid arthritis.

Grants: AMS-Springboard [SBF006/1109]; Cross-Disciplinary Fellowship (XDF) Programme [MC_FE_00035]

Conflict of Interest: None declared

P18.105.D Pharmacogemomic Landscape of TNF Inhibitors in the Middle Eastern Qatari Population

Zainab Jan[1], *Dinesh Velayutham[1], Borbala Mifsud[1], Puthen Veettil Jithesh[1]*

[1]Hamad Bin Khalifa University (HBKU), College of Health and Life Sciences, Ar Rayyan, Qatar

Background/Objectives: Autoimmune diseases are one of the leading causes of mortality and morbidity worldwide with a substantial economic burden. Tumor necrosis factor alpha (TNF-α) is an important cytokine that frequently contributes to the pathogenicity of autoimmune diseases and hence TNF inhibitors (TNFi) are widely used to treat several autoimmune diseases. However, 40% of the patients do not respond positively when treated with TNFi. Several pharmacogenomic variants affecting TNFi response have been identified. However, the prevalence of these variants in the Qatari population is still not known.

Methods: The distribution of known and novel variants in 116 genes associated with TNF inhibitors in 14,387 Qatari individuals was assessed using whole genome sequencing data. We calculated the cumulative allele probability score (CAP) to measure the risk of an individual in the population having functional variants in a particular gene.

Results: Out of the 176 known pharmacogenomic variants associated with response to TNF inhibitors, the majority (~90%) had different allele frequency distribution in the Qatari population compared to other world populations from the gnomAD dataset. High frequency of rs1800629 (TNF), rs1800896 (*IL10)*, and rs1143634 (*IL1B)* variants were observed, which are known to be associated with responses to etanercept and infliximab. Moreover, we identified that *CR1* and *GBP6* have the highest CAP scores that are associated with response to multi-TNF inhibitors.

Conclusion: The findings of this study could be the basis for developing and implementing pharmacogenetic testing in autoimmune disease patients being considered for TNF inhibitor therapy in Qatar and beyond.

Grants: QNRF-PPM

Conflict of Interest: None declared

P19
POPULATION GENETICS AND EVOLUTIONARY GENETICS

P19.001.A Genetic study of intrahepatic cholestasis of pregnancy in 101,023 Chinese women unveils East Asian-specific etiology linked to historic HBV infection

Yanhong Liu[1], *Yuandan Wei*[1], *yuqin Gu*[1], *Zijing Yang*[1], *Xinxin Guo*[1], *Hao Zheng*[1], *Siyang Liu*[1]

[1]*Sun Yat-sen University, School of Public Health (Shenzhen), Shenzhen*

Background/Objectives: Intrahepatic cholestasis of pregnancy (ICP) is the most common and high-risk liver disorder during human reproduction. This study delves into ICP genetic etiology in East Asians, drawing comparisons with Europeans to comprehend ICP etiology in the context of genetic background and evolution.

Methods: We conducted the hitherto largest-scale genome-wide association studies (GWAS) on total bile acid (TBA) and ICP among 101,023 Chinese pregnancies. Findings were replicated in two cohorts and compared with Europeans. Additionally, phenome-wide association and spatio-temporal evolution analyses were employed to understand the function and evolutionary pattern of ICP-associated sites.

Results: We identified eight TBA and five ICP loci, including ten novel loci. Notably, we found an East-Asian-specific genetic locus at 14q24.1, contributing to a 6.41 μmol/L increase in TBA and 15.23-fold higher risk of ICP per risk allele (95%*CI*: 15.10-15.36, $P = 9.23 \times 10^{-375}$). Phenome-wide association and spatial-temporal evolution analyses revealed that 14q24.1 ICP risk locus exhibits resistance to hepatitis B infection and became prevalent only within the last 3,000 years in East and Southeast Asia.

Conclusion: Our investigations unraveled distinct ICP etiology between Europeans and East Asians, and linked it with a historical HBV epidemic in East and Southeast Asia. These findings lay the groundwork for an improved understanding of ICP pathophysiology and emphasize the need for integrating population evolution into genetic medicine for personalized genomics and clinical guidance.

Grants: The study was supported by Shenzhen Science and Technology Program (20220818100717002), Guangdong Basic and Applied Basic Research Foundation (2022B1515120080, 2020A1515110859), and National Natural Science Foundation of China (31900487).

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P19.002.B Complications during pregnancy and delivery in patients with vascular Ehlers Danlos syndrome: A retrospective study in the Dutch cohort.

Lisa van den Bersselaar[1], **Ingrid MBH van de Laar**[1], *Marieke Baars*[2], *Annette Baas*[3], *Eelco Dulfer*[4], *Paula Helderman*[5], *yvonne hilhorst-hofstee*[6], *Martijn Kauling*[7], *Marlies Kempers*[8], *Martijn Oudijk*[9], *Alessandra Maugeri*[2], *Hennie Brüggenwirth*[1], *arjan houweling*[2], *S. Demirdas*[1]

[1]*Erasmus MC, University Medical Center, Department of Clinical Genetics, Rotterdam, Netherlands;* [2]*Amsterdam UMC, University of Amsterdam, Department of Human genetics, Amsterdam, Netherlands;* [3]*University Medical Center Utrecht, Department of Genetics, Utrecht, Netherlands;* [4]*University Medical Center Groningen, Department of Genetics, Groningen, Netherlands;* [5]*Maastricht University Medical Center, Department of Clinical Genetics, Maastricht, Netherlands;* [6]*Leiden University Medical Center, Department of Clinical Genetics, Leiden, Netherlands;* [7]*Erasmus MC, University Medical Center, Department of Cardiology, Rotterdam, Netherlands;* [8]*Radboud University Nijmegen Medical Center, Department of Human genetics, Nijmegen, Netherlands;* [9]*Vrije Universiteit Amsterdam, Department of Obstetrics, Amsterdam, Netherlands*

Background/Objectives: Pathogenic/ likely pathogenic (P/LP) variants in *COL3A1* cause vascular Ehlers Danlos Syndrome (vEDS). Women with vEDS have an increased risk for pregnancy and delivery related complications.

Description of the genotype and pregnancy-related outcomes of women with vEDS can contribute to establish guidelines for pregnancy and delivery management.

Methods: Female patients with a P/LP *COL3A1* variant were included in a multicentre cohort study from 2019 until 2021. Comprehensive genetic and clinical data was collected through retrospective analysis.

Results: Forty-three women with 121 pregnancies were included (53.5% with a glycine substitution, 20.9% with a haploinsufficient variant and 25.6% with a splice/delins/del variant). No uterine rupture or life threatening vascular events occurred in the perinatal period. Twenty-three pregnancies (23/121, 19.0%) ended in a miscarriage. Miscarriages were significantly more frequent in women with a glycine substitution compared to other *COL3A1* variant types (19/23, 82.6%, $p = 0.018$). Over ten percent of the women (13.8%) had a spontaneous preterm birth. Complications occurred in 14/94 deliveries (14.9%), including eight severe perineal tears and six postpartum haemorrhage.

Conclusions: No pregnancy-related deaths, arterial dissections or uterine ruptures occurred in our cohort. Severe perineal tears were seen ten times more often in our cohort compared to the general population. Miscarriage, spontaneous preterm births and preterm pre-labour rupture of membranes occurred twice as often compared to the general population. Since no life threatening events occurred during pregnancy and delivery, discouragement of pregnancy in all women with vEDS in current guidelines might be too strict. Based on these data, we propose a shared decision making process.

Conflict of Interest: None declared

P19.003.C Evolutionary insights from a population database: Limited consequences of archaic protein-altering variants in present-day humans

Barbara Molz[1], *Mikel Lana Alberro*[2], *Else Eising*[1], *Dick Schijven*[1], *Clyde Francks*[1,3,4], *Simon E. Fisher*[1,4]

[1]*Max Planck Institute for Psycholinguistics, Language & Genetics Department, Nijmegen, Netherlands;* [2]*Max Planck Institute for Evolutionary Anthropology, Department of Evolutionary Genetics, Leipzig, Germany;* [3]*Radboud University Medical Center, Department of Cognitive Neuroscience, Nijmegen, Netherlands;* [4]*Donders Institute for Brain, Cognition and Behaviour, Nijmegen, Netherlands*

Background/Objectives: The sequencing of archaic hominin genomes allowed the use of comparative approaches to assemble a catalogue of protein-altering changes that apparently became fixed on the lineage leading to *Homo sapiens*. These fixed changes have been seen as promising entry-points to further understanding of human-specific aspects of our biology. While prior experimental validation of such variants has been limited to in vitro assays and model organisms, large population databases now offer the unique opportunity to identify possible present-day

carriers of archaic variants at supposedly fixed sites, and examine their phenotypic consequences.

Methods: We used exome sequencing data of nearly 500,000 people in UK Biobank to identify carriers of 43 archaic protein-altering variants, including a variant in *TKTL1* for which substantial effects on cortical neurogenesis have been demonstrated in functional experiments. We contrasted phenotypic profiles of carriers with the sample norm.

Results: This study identified 165 unique carriers of archaic variants at 18 of the 43 interrogated positions, of whom 62 carriers could be attributed to *TKTL1*. A qualitative phenotypic analysis of an archaic variant in *SSH2* showed no divergence from the sample norm in health, psychological, or cognitive traits. A similar pattern was observable for brain imaging metrics and educational level for carriers of the archaic variant in *TKTL1*.

Conclusion: Our results highlight the importance of large, diverse databases to most accurately capture the genetic diversity in present-day humans, and suggest that experimental validation in cell-based/animal model systems is not by itself sufficient to fully capture the biological and evolutionary roles of putative fixed variants.

Grants:

Conflict of Interest: None declared

P19.004.D Validation of diagnostic rare pathogenic variants in 5 breast cancer genes by GSAMD and PMDA array platforms.

Jeroen van Rooij[1], Jard de Vries[1], Ans van de Ouweland[2], Maartje J Hooning[3], Linda Broer[1], Willemina Geurts-Giele[2], Robert van der Helm[2], Marieke van Dooren[2], Margriet Collée[2], Lies Hoefsloot[2], Joyce Van Meurs[1;4], André Uitterlinden[1], **Annemieke Verkerk**[1]

[1]Erasmus Medical Center, Internal Medicine, Rotterdam, Netherlands; [2]Erasmus Medical Center, Clinical genetics, Rotterdam, Netherlands; [3]Erasmus Medical Center Cancer Institute, Oncology, Rotterdam, Netherlands; [4]Erasmus Medical Center, Orthopedics, Rotterdam, Netherlands

Background/Objectives: At the Erasmus Medical Center we initiated the GOALL project (Genotyping On All Patients), in which we investigate the use of high throughput SNP arrays for improving clinical care and making personalized medicine available to a larger public. In this pilot we compared the performance of array-based genotyping with previously clinically determined pathogenic variants, for 240 breast cancer cases. The diagnostic results were based on Sanger sequencing (SNVs and indels ≤ 50 bp) for the genes BRCA1, BRCA2, CHEK2, PALB2 and ATM and/or MLPA (deletions or duplications > 50 bp) for BRCA1/BRCA2.

Methods: Carriers were selected with pathogenic variants that were present on the Illumina GSAMDv3 and/or Thermofischer PMDA arrays and then genotyped. After data processing and QC, samples were called, in a blinded fashion, for SNVs and indels, using the manufacturer's software, and analyzed by PENNCNV and NEXUS (Biodiscovery) to assess copy number variations (> 50 bp).

Results: Using standard processing in combination with zCall and manual reclustering for SNVs and indels, and specific QC tresholds for CNV calling, 98% of the diagnostic SNV and indels, and 90% of CNVs, were confirmed by array. Five false positive SNVs or indels, and four false positive CNVs were detected.

Conclusion: We demonstrate a high sensitivity and specificity for detection of rare pathogenic variants by genotyping array in 5 breast cancer genes. We conclude that arrays are a low cost and effective way to screen for pathogenic variants in larger populations.

Grants: Internal Koers23 program from the Erasmus Medical Center (project-no.109433).

Conflict of Interest: None declared

P19.005.A Denisovan admixture facilitated environmental adaptation in Papua New Guinean populations

Danat Yermakovich[1], *Mathilde André[1], Nicolas Brucato[2], Jason Kariwiga[3;4], Matthew Leavesley[3;5], Vasili Pankratov[1], Mayukh Mondal[1;6], Francois-Xavier Ricaut[2], Michael Dannemann[1]*

[1]Institute of Genomics, University of Tartu, cGEM, Tartu, Estonia; [2]Paul Sabatier University, Centre de Recherche sur la Biodiversité et l'Environnement, Toulouse, France; [3]The University of Papua New Guinea, Strand of Anthropology, Sociology and Archaeology, School of Humanities and Social Sciences, Port Moresby, Papua New Guinea; [4]The University of Queensland, School of Social Science, Saint Lucia, Australia; [5]James Cook University, Brisbane Campus, CABAH & College of Arts, Society and Education, Brisbane City, Australia; [6]University of Kiel, Institute of Clinical Molecular Biology, Kiel, Germany

Background/Objectives: As a result of past admixture between modern and archaic human groups, present-day Papua New Guineans (PNG) carry both Neandertal and Denisovan introgressed DNA. In this work, we investigate the distribution of archaic DNA within the genomes of two PNG populations residing in the highlands and lowlands of the region and how it contributed to adaptive processes to the unique environmental features these populations were exposed to.

Methods: We annotated Neandertal and Denisovan DNA in the genomes of 74 PNG from the lowlands of Daru Island and 54 highlanders from the Mt Wilhelm region. Then, we assessed the level of population differentiation for archaic segments between both PNG populations and compared them to those of random non-archaic sets of SNPs. Finally, we investigated genomic regions overlapping the most differentiated archaic haplotypes for their gene content and variants with phenotypic associations.

Results: We show that highly differentiated Denisovan-like haplotypes, more prevalent among PNG highlanders, were situated in genomic regions associated with brain development genes. Conversely, those with a higher frequency in lowlanders overlapped with genes enriched for immune response processes. Furthermore, Denisovan-like haplotypes displayed pronounced signatures of diversification within the major histocompatibility complex.

Conclusion: Our study suggests that the presence of Denisovan DNA in PNG genomes has contributed to adaptive processes to environmental challenges and served as a beneficial source of genetic variation.

Grants: Estonian Research Council, European Regional Development Fund, EU Horizon 2020, French National Research Agency, French Ministry of Foreign and European Affairs, Labex TULIP, Leakey Foundation.

Conflict of Interest: None declared

P19.006.B Distinct genetic profiles influence body mass index between infancy and adolescence

Geng Wang[1], *Samuel McEwan[1], Jian Zeng[1], Mekonnen Haile-Mariam[2], Loic Yengo[1], Michael Goddard[2;3], Kathryn Kemper[1], Nicole Warrington[1;4]*

[1]The University of Queensland, Institute for Molecular Bioscience, Saint Lucia, Australia; [2]Agriculture Victoria, AgriBio, Centre for AgriBioscience, Bundoora, Australia; [3]The University of Melbourne, Faculty of Science, Parkville, Australia; [4]The University of Queensland, Frazer Institute, Saint Lucia, Australia

Background/Objectives: Body mass index (BMI) changes throughout life with age-varying genetic contributions. We aimed to investigate the genetic contribution to BMI across early life using repeated measures from the Avon Longitudinal Study of Parents and Children (ALSPAC) cohort.

Methods: Random regression was used to estimate the genetic covariance matrix (G) of the BMI trajectory from ages one to 18 with 65,930 repeated BMI measurements from 6,291 genotyped ALSPAC participants. We estimated genetic correlations across early life and performed an eigenvalue decomposition of G analysis to identify age-varying genetic patterns of BMI. Finally, we investigated the impact of a polygenic score derived from adult BMI associated loci in the UK Biobank on the estimated genetic components across early life.

Results: The SNP-based heritability (h_{SNP}^2) was relatively constant across early life, between 25-30%. The genetic contribution to BMI in early childhood is different to that in later childhood, indicated by the genetic correlations of BMI across different ages. The eigenvalue decomposition revealed that the primary axis of variation (explaining 89% of G) consistently increases with age, while the second eigenfunction (explaining around 9% of G) represents factors with opposing impacts on BMI between early and later ages. Adjusting for the adult BMI polygenic score attenuated the h_{SNP}^2 from late childhood.

Conclusion: Although common genetic variation explains around 25-30% of BMI variability across early life, our findings indicate that there is a different genetic profile operating during infancy than later childhood.

Grants: National Health and Medical Research Council (Australia) Investigator grant (APP2008723)

Conflict of Interest: None declared

P19.008.D Evolutionary and expression analyses of *ATXN3* paralogs in primates support the functional relevance of *ATXN3L* retrocopy

Daniela Felício[1;2;3], *Maria Inês Martins*[2], *Andreia Pinto*[2], *Inês P. D. Costa*[1;2], *António Amorim*[1;2;4], *Alexandra Lopes*[1;2], *Susana Seixas*[1;2], *Sandra Martins*[1;2]

[1]*Instituto de Investigação e Inovação em Saúde (i3S), Porto, Portugal;* [2]*Institute of Molecular Pathology and Immunology of the University of Porto (IPATIMUP), Porto, Portugal;* [3]*Instituto Ciências Biomédicas Abel Salazar (ICBAS), University of Porto, Porto, Portugal;* [4]*Dep. Biology, Faculty of Sciences, University of Porto, Porto, Portugal*

Background/Objectives: Ataxin-3 gene (*ATXN3*; 14q32.1) encodes a ubiquitously expressed deubiquitinating enzyme that presents a polyglutamine tract, responsible for Machado-Joseph disease (MJD/SCA3 - spinocerebellar ataxia type 3) when expanded in humans. An *ATXN3* paralog called ataxin-3 like (*ATXN3L*; Xp22.2) encodes a protein shown to cleave ubiquitin substrates in vitro more efficiently than ATXN3. Previous studies on another polyglutamine disease, SCA1, demonstrated that *ATXN1* copy (*ATXN1L*) alleviated the neuropathology in mice. Thus, it is of upmost importance to understand whether *ATXN3* copies can have a role in MJD.

Methods: To identify and explore *ATXN3* copies, we retrieved highly homologous sequences from 33 primates and analysed their diversity, local synteny and selective constraints in reconstructed phylogenies. Additionally, due to *ATXN3L* conserved reading frame, we analysed protein domain conservation and mRNA expression by qPCR.

Results: Our results suggested three independent retrotransposition events: 1) in Haplorrhini (~70 MYA) for *ATXN3L*; 2) in a Platyrrhini-Catarrhini ancestor (~45 MYA) for *ATXN3L2* (LOC100132280; 8q23.2 in human); and 3) in Cercopithecidae (~20 MYA) for *ATXN3L3* (LOC699321; chr11 in Rhesus monkey). *ATXN3L* seems to be under selective constraints throughout primate evolution like the parental gene, whereas *ATXN3L2* and *ATXN3L3* are likely turning into inactive copies. Finally, we detected *ATXN3L* expression in human spleen, placenta, testis, and cerebral cortex.

Conclusion: Phylogenetic analyses support the functional relevance of *ATXN3L*, but further functional studies will be crucial to assess ATXN3L importance in MJD.

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Conflict of Interest: None declared

P19.009.A Homozygous of *OSMR* p.G513D predisposes to PCA in Chinese descent

Hui-Ying Weng[1], *Ding-Dar Lee*[2;3], *Yung-Feng Lin*[4], *Sheng-Hung Lee*[5], *Yun-Ting Chang*[2], *Chih-Chiang Chen*[2], *Yuan-Chia Chu*[6;7;8], *Ralph Kirby*[9], *Ming-Wei Lin*[1;10], *Shih-Feng Tsai*[1;5;9]

[1]*National Yang Ming Chiao Tung University, Biomedical Industry PhD Program, Taipei, Taiwan;* [2]*Taipei Veterans General Hospital, Department of Dermatology, Taipei, Taiwan;* [3]*National Yang Ming Chiao Tung University, Faculty of Medicine, Department of Dermatology, Taipei, Taiwan;* [4]*Taipei Medical University Hospital, Department of Medical Research, Taipei, Taiwan;* [5]*National Health Research Institutes, Institute of Molecular and Genomic Medicine, Miaoli, Taiwan;* [6]*Taipei Veterans General Hospital, Information Management Office, Taipei, Taiwan;* [7]*Taipei Veterans General Hospital, Big Data Center, Taipei, Taiwan;* [8]*National Taipei University of Nursing and Health Sciences, Department of Information Management, Taipei, Taiwan;* [9]*National Yang Ming Chiao Tung University, Department of Life Sciences and Institute of Genome Sciences, Taipei, Taiwan;* [10]*National Yang Ming Chiao Tung University, Institute of Public Health, Taipei, Taiwan*

Background/Objectives: Primary cutaneous amyloidosis (PCA) is a skin disorder and the clinical characteristic is amyloid deposited in the dermis. PCA has been commonly observed in Asian population, especially in Chinese descent. Mutations of *OSMR* and *IL31RA* genes have been identified in PCA families. In this study, we investigated the genome sequences of in a cohort of PCA patients in Taiwan and compared the *OSMR* genetic variants with other populations.

Methods: The genomic sequence of 91 PCA patients were conducted by whole exome sequencing and the whole genome genotyping data of 37,770 individuals were obtained from the Taipei Veterans General Hospital (TVGH) of Taiwan Precision Medicine Initiative (TPMI) in Taiwan (TVGH-TPMI). The *OSMR* genetic variants were investigated in different countries and ethnicities using our PCA data and public databases.

Results: A variant of *OSMR*, p.G513D was found in 24.18% (22/91) of the PCA cohort, as compared to 2.61% (39/1495) in the control group from Taiwan Biobank. The *OSMR* p.G513D variant was observed in 2.5% of TVGH-TPMI individuals and three out of seven individuals (42.86%) were homozygous who were diagnosed clinically as having PCA ($p = 0.0001$). This *OSMR* p.G513D variant had a highly variable frequency in various different human populations which is consistent with the high incidence rate of PCA in East Asians.

Conclusion: We identified the *OSMR* p.G513D in individuals of Chinese descent that predisposes to PCA and this common variant is an important cause of high incidence of PCA in East Asians.

Grants No: National Health Research Institutes, Taiwan (12A1-MGGP01-037)

Conflict of Interest: None declared

P19.010.B TP53 c.542G>A identified as a Swedish founder variant with a risk of predominantly breast cancer

Alexander Sun-Zhang[1], *Meis Omran*[1;2], *Yaxuan Liu*[1], *Emma Tham*[1;3], *Svetlana Bajlica Lagercrantz*[1;3]

[1]*Karolinska Institutet, Department of Oncology-Pathology, Stockholm, Sweden;* [2]*Karolinska University Hospital, Cancer Theme, Stockholm;* [3]*Karolinska University Hospital, Department of Clinical Genetics, Stockholm*

Consortium: Swedish Clinical TP53 Study Group (SweClinTP53)

Background/Objectives: Heritable *TP53*-related cancer syndrome (h*TP53*rc) is a cancer predisposition syndrome caused by germline *TP53* alterations. The genotype-phenotype correlation is used to guide genetic counseling and surveillance. In the first nationwide delineation of the Swedish h*TP53*rc-cohort we found a potential founder variant, c.542G>A/p.R181H. Thus, we aimed to characterize this potential founder and its phenotypical expression.

Methods: Genotype and phenotype data for all 83 families (176 individuals) with h*TP53*rc in Sweden were retrieved retrospectively. Data included *TP53* variant, variant classification, pedigrees, age at first tumor onset and tumor characteristics. Families were classified according to classic Li-Fraumeni syndrome (classic LFS), Chompret or hereditary breast cancer (HBC) criteria. Short tandem repeat (STR) markers flanking *TP53* were analyzed in six of the families with PCR and fragment length analysis.

Results: In total, 18 families (42 individuals) harbored the c.542G>A variant and 60% of the carriers of this variant had an HBC phenotype compared to only 14% for all other variants ($p < 0.001$). Age at first tumor onset was higher (40 vs. 33 years, $p = 0.009$) and no patients with c.542G>A developed childhood tumors. Three of the families shared a common haplotype including the *TP53* gene. Prevalence of c.542G>A is significantly higher in the Swedish cohort compared to other cohorts.

Conclusion: Our cohort with 18 families represents the largest characterization of c.542G>A and haplotype analysis shows that this is the first Swedish founder variant. Families with this variant are predominantly associated with a risk of breast cancer.

Grants: The King Gustaf V Jubilee Fund (201052)

Conflict of Interest: None declared

P19.012.D Computing ages of alleles associated with rare autoinflammatory diseases in Anatolia

Damla Karadavut[1], *Gulsah Merve Kilinc*[1], **idil yet**[1]

[1]*Graduate school of Health Sciences, Department of Bioinformatics, Ankara, Türkyie*

Background/Objectives: Understanding the population dynamics together with its structure requires certain parameters in population genetics. One is allele age estimation. It helps us to understand mutations in a deeper way although it's comparably new (Malaspinas et al., 2012). Allele age means the first occurrence time of the SNP in a population. With allele age and other parameters, mutations can be studied in certain time intervals.

Methods: Relate has a genealogy-based method to infer mutation ages from genome data. Using genealogy trees, Relate computes information about populations like allele ages. First, it builds genealogy trees via WGS, one for each genome region. Then, it maps the mutations to trees. The branch they are mapped on gives the time interval the mutation was emerged (Speidel et al., 2019). In this study, the parameters and pipeline advised in tool's article were used. 47 SNPs related to Behçet's Disease, FMF and TRAPS are studied to in Anatolia via samples from Ancient (6 individuals) - Modern Anatolia (16 individuals) and 1000 Genome Project.

Results: Age results of 25 SNPs were found in generation:

Disease	Age Interval
Behçet's Disease	981.452 - 74080.4
FMF	8050 - 17929.8
TRAPS	3263.58 - 8160.29

Conclusion: Since studied autoinflammatory diseases are important for Anatolia population, emergence times of related SNPs are beneficial to know. Those times may match with important events in the history. By knowing the mutation age, we may connect those events with the diseases and get a new perspective about the topic.

Grants: HACETTEPE UNIVERSITY BAP TEZ, ID:20649

Conflict of Interest: None declared

P19.013.A The Genome of Europe: A Reference Database of Genomes as part of the 1 Million Genomes initiative (1 + MG)

Jeroen van Rooij[1], *Mariliis Vaht*[2], *Helen Ray-Jones*[1], *Andres Metspalu*[2], *André Uitterlinden*[1]

[1]*Erasmus Medical Center, Internal Medicine, Rotterdam, Netherlands;* [2]*University of Tartu, Institute of genomics, Estonian Genome Centre, Tartu, Estonia*

Consortium: Genome of Europe, WG12 of the 1+ Million Genomes

Background/Objectives: The 1 million genomes (1 + MG) initiative as part of the Digital Europe Program (DEP), was declared in 2018 and includes 27 signatory countries aiming to make at least 1 million whole genome sequences (WGS) accessible for use in research, health care, and prevention. Working Group 12 (WG12) of the 1 + MG, named Genome of Europe (GoE), was started in 2019 to establish a European Reference Genome Database of >500k WGS (@30x coverage).

Methods: We have defined a strategy to collect the first 100k genomes to be representative of the diverse European population and integrate them into various relevant research applications, dubbed use cases. The data collection will be in line with the 1 + MG framework, adhere to various ELSI and ICT guidelines, and be made accessible via the Genomics Data Infrastructure (GDI) project in the DEP.

Results: We outline the planned activities in the GoE 100k project involving 51 institutes from 29 European countries. Objectives include integrating existing WGS datasets and de novo sequencing for diverse populations, addressing ethical and legal considerations, ensuring data security, and embedding GoE data into GDI. Activities include genetic diversity analyses, multi-ancestry imputation services, and recalibration of genetic risk profiles, and establish longer term (clinical) applications for GoE.

Conclusion: Beyond its immediate goals, GoE contributes to genetic-phenotypic discoveries, national genome programs, and integration into the European Health Data Space. Prioritizing public engagement, transparency, and adaptability, GoE will stimulate European genomic research competitiveness, advancing personalized medicine and broader scientific and healthcare objectives, and integrate with European initiatives in health care and prevention.

Grants:

Conflict of Interest: None declared

P19.014.B The shared ancestry between the *C9orf72* hexanucleotide repeat expansion and intermediate-length alleles using haplotype sharing trees and HAPTK

Osma Rautila[1], *Karri Kaivola*[1], *Harri Rautila*[1], *Laura Hokkanen*[1], *Jyrki Launes*[1], *Timo Strandberg*[1], *Hannu Laaksovirta*[1;2], *Johanna Palmio*[3;4], *Pentti Tienari*[1;2]

[1]*University of Helsinki;* [2]*Helsinki University Hospital;* [3]*Tampere University Hospital;* [4]*University of Tampere*

Background/Objectives: The C9orf72 hexanucleotide repeat expansion (HRE) is a common genetic cause of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). The inheritance is autosomal dominant, but a high proportion of cases are sporadic. One possible explanation is de novo expansions of unstable intermediate-length alleles (IAs). Given the hypothesis that HREs are formed from IAs, a significant amount of haplotype sharing should exist between the HRE and IA haplotypes.

Methods: Using haplotype sharing trees (HST) with the novel haplotype analysis toolkit (HAPTK) we quantified and analyzed the amount of haplotype sharing between the HRE and IA groups in two different Finnish datasets.

Results: From the HSTs, we derived majority-based ancestral haplotypes of HRE carriers and discovered that IAs containing ≥18-20 repeats share large haplotypes in common with the HRE. Using HSTs of HRE and IA carriers, we demonstrate that in contrast to the shorter IAs, the longer IA haplotypes are largely indistinguishable from HRE haplotypes.

Conclusion: Our results indicate that the haplotypes with longer IAs belong to the same pool of haplotypes as the HRE. This suggests that the longer IAs represent potential premutation alleles. In addition to these findings, we present a method to quantify all shared haplotypes at a given genomic locus and demonstrate a way to efficiently assess haplotype sharing between different cohorts. Lastly, we have published the anonymized HSTs to an open access repository, enabling the comparison of different HRE cohorts globally against the Finnish HRE haplotypes in the future.

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P19.015.C Epidemiological analysis of international ATTRv genetic testing data

Zornitsa Pavlova[1;2], *Tihomir Todorov*[1;2], *Albena Todorova*[1;2;3]

[1]*Independent Medico-diagnostic Laboratory "Genome Center Bulgaria", Sofia, Bulgaria;* [2]*Genetic Medico-diagnostic Laboratory Genica, Sofia, Bulgaria;* [3]*Medical University Sofia, Faculty of Medicine, Department of Medical Chemistry and Biochemistry, Sofia, Bulgaria*

Background/Objectives: Hereditary transthyretin amyloidosis (ATTRv) is multisystem condition affecting the peripheral nervous system, the heart, etc. It is caused by pathogenic variants in the TTR gene. The prevalence of ATTRv is variable and several endemic loci have been reported. We are currently presenting our data from TTR genetic testing from different regions of Europe and Asia.

Methods: Dried blood spot samples were obtained from patients from thirteen countries: Austria (416), Spain (304), Czech Republic (117), Poland (90), Ireland (6), Belgium (17), Italy (2), Luxembourg (12), Switzerland (178), Israel (156), Thailand (19), Hong Kong (13) and Malaysia (45). They were tested via Sanger sequencing of the TTR gene.

Results: The following TTR pathogenic variants were detected according to the country of origin (NM_000371.4): c.130C>T, p.Pro44Ser (Malaysia); c.148G>A, p.Val50Met (Spain, Switzerland, Malaysia, Luxemburg, Poland); c.155T>C, p.Val52Ala (Israel); c.157T>C, p.Phe53Leu (Poland); c.200G>A, p.Gly67Glu (Hong Kong); c.200G>T, p.Gly47Val (Malaysia); c.238A>G, p.Thr80Ala (Israel); c.290C>A, p.Ser77Tyr (Israel); c.337G>C, p.Val113Leu (Austria); c.340A>G,p.Val144Leu (Italy); c.349G>T, p.Ala117Ser (Malaysia, Thailand); c.424G>A, p.Val142Ile (Spain, Switzerland, Belgium, Malaysia). In two samples referred for testing from Czech Republic a variant of uncertain clinical significance was reported c.280G>C, p.Arg94His. The most frequent amyloidogenic variants were c.148G>A, p.Val50Met and c.424G>A, p.Val142Ile, which is consistent with the global epidemiologic data. For the regions of Israel and Malaysia endemic pathogenic variants were proved to be present.

Conclusion: The analysis of genetic epidemiologic data will continue to contribute to the accurate clinical interpretation of TTR genetic findings and to the timely diagnosis of ATTRv.

Grants: Funded by Pfizer

Conflict of Interest: None declared

P19.016.D Two major waves of dispersal explain higher levels of R1a in ancient tribes of India

Jaison Jeevan Sequeira[1], *Mohammed S Mustak*[1]

[1]*Mangalore University, Applied Zoology, Mangaluru, India*

Background/Objectives: Early human migrations were gender-neutral, but during the Bronze Age, migrations became male-dominated and were often for raiding. Nomads from the Eurasian Steppe carried paternal R1a and Q haplogroups. It is surprising to note that even south Indian tribes have a higher frequency of R1a. We conducted a study on Eurasian, North Indian, and South Indian populations using Y-STR profiles to understand this exception.

Methods: We analyzed the Y-chromosome STR profile of 85 Sarasvata Brahmins who claimed to have migrated from North to the South West coast of India. We also collected published data for Q and R1a samples from the Indian subcontinent and Eurasia. We conducted multivariate analysis, Fst analysis, and haplotype dating using rho statistics.

Results: The MDS plot indicates a more uniform cluster with both northern and southern Indian castes and tribes. However, the Neighbour Joining tree shows two separate clusters of castes and tribes. It is also evident that the caste group comprises both brahmins and non-brahmin groups. Sarasvata Brahmins clustered with Sourashtra brahmins. The haplotype dating of R1a and Q haplogroup reveals that there is no noticeable difference between their ages in Eurasian and Indian samples.

Conclusion: According to our research, it appears that there were at least two separate waves of migration of R1a and Q haplogroups into the Indian subcontinent. The first haplotype distinguishes the tribes from the caste groups. It is possible that two different migrations at different times could explain the moderate levels of R1a found in Indian tribes.

Grants:

Conflict of Interest: None declared

P19.017.A Characterisation of diverse global ancestries within participants of the UK Biobank

Fiona Pantring[1;2;3], *Gianpiero L. Cavalleri*[1;2;3], *Edmund Gilbert*[1;2;3]

[1]*Royal College of Surgeons in Ireland, School of Pharmacy and Biomedical Sciences, Dublin, Ireland;* [2]*The SFI Centre for Research Training in Genomics Data Science, Ireland;* [3]*The FutureNeuro Research Centre, Dublin, Ireland*

Background/Objectives: The UK Biobank (UKB) is a large dataset containing in-depth phenotype and genotype data of nearly 500,000 UK-based participants. Studies leveraging the UKB typically focus on a subset of participants with homogenous European ancestry, labelled as "White British" according to self-identification and genotype-based principal component analysis. Here we characterise global ancestries present in the UKB through the application of population genetic approaches to 78,573 participants without the "White British" label.

Methods: We developed a novel approach which uses machine learning to predict an individuals' continental ancestry from genetic references from the 1000 Genomes and Human Genome Diversity Projects. These clusters were further divided by applying community detection to a network of Identity-By-Descent sharing.

Results: We find that the UKB is a repository of diverse ancestries primarily from Europe, Africa, and South Asia. The over 150 communities within these continental ancestry groups, whilst capturing worldwide diversity, reflect the demographic history of Great Britain and its Commonwealth in the 20th century and are therefore not necessarily represented in other datasets. An example includes South Asian ancestry communities born in Southeast Africa, but resident today in Britain. Our communities also facilitate novel findings of community-specific genetic risk factors, such as one of the highest worldwide frequencies of an idiopathic pulmonary fibrosis risk variant in Maltese genotypes.

Conclusion: We characterised worldwide ancestries in the UKB by identifying continental-level ancestry clusters and fine-scale communities. Furthermore, we provide a framework for studies of health-related genetic variation specific to otherwise understudied genetic communities.

Grants: SFI [18/CRT/6214]

Conflict of Interest: None declared

P19.018.B A retrospective study on allelic distribution for recessive diseases in an Italian cohort

Pasquale Di Letto[1], *GIULIA NICOLE BALDRIGHI*[2], *Annalaura Torella*[1;3], *Mariateresa Zanobio*[1], *Roberta Zeuli*[1], *Margherita Scarpato*[1], *Margherita Russo*[1], *Marianthi Karali*[1], *Sandro Banfi*[1;3], *Vincenzo Vincenzo Nigro*[1;3]

[1]*Università degli Studi della Campania "Luigi Vanvitelli", Precision Medicine, Napoli, Italy;* [2]*Università di Pavia, Brain and Behavioral Sciences, Pavia, Italy;* [3]*Telethon Institute of Genetics and Medicine, Pozzuoli, Italy*

Background/Objectives: Consanguinity and the presence of recessive disease carriers are considered reproductive risk factors. In this retrospective study, we aim to investigate in our 7000 exomes database geographical and temporal pathogenic variants' distribution to evaluate putative mechanisms for detrimental alleles' dispersion and/or clusterization. Two main groups of recessive diseases have been analysed, inherited retinal diseases (IRDs) and neuromuscular diseases (NMDs) in the Italian population.

Methods: We selected 850 Italian patients with causative homozygous and compound heterozygous variants from the University of Campania "Luigi Vanvitelli" Medical Genetics Unit. We collected 573 patients affected by IRDs and 277 by NMDs. To investigate how the proportion of homozygous and compound heterozygous states vary over time a General Linear Model was used.

Results: Starting with patients born in 1940, we tried to find out whether homozygotes decreased in favour of heterozygotes. We detected a significant trend with a yearly decrease of 1% in the abundance of causative homozygous variants. Furthermore, in Campania, we observed an enrichment for IRD variants, c.14977_14978del (NM_206933.4) and c.286A>C (NM_000350.3) in *USH2A* and *ABCA4* respectively, which have very low frequency in general population databases. Moreover, a cluster in Salerno province for the variant c.-32-13T>G (NM_000152.5) in *GAA* was identified.

Conclusion: Our study unravels a link between homozygous variants' prevalence and time hinting a role for sociocultural behaviour in generating genetic isolates, whether geographical or not, in the past. On this basis, sociocultural changes could be a reliable index to study allelic distribution fluctuation in terms of time and space.

Conflict of Interest: None declared

P19.019.C The reliability of inferred archaic segments in human genomes

Nancy Bird[1], *Hrushikesh Loya*[2], *Leo Speidel*[1;3], *Simon Myers*[2], **Garrett Hellenthal**[1]

[1]*University College London, UCL Genetics Institute, London, United Kingdom;* [2]*University of Oxford, Department of Statistics, Oxford, United Kingdom;* [3]*Francis Crick Institute, Ancient Genomics Laboratory, London, United Kingdom*

Background/Objectives: Many or all present-day human genomes include segments of DNA inherited from archaic humans due to admixture events occurring >30,000 years ago. Several methods have been published to detect such segments. These variously require phased haplotypes, an archaic reference sequence and/or an outgroup with little related archaic introgression. Inference from these approaches have been used to (e.g.) document purifying selection of archaic segments and understand their influence on traits. However, the comparative accuracy of different methods at detecting archaic segments remains underexplored. Little is known about how genomic features correlate with assignment accuracy and the consequences of such non-random bias.

Methods: We used simulations to examine the accuracy in detecting archaic ancestry under four widely-used approaches: Sprime, diCal, IBDMix and HMMix, and two unpublished approaches, illustrating false positive and false negative rates. We also assess the consistency of archaic ancestry assignments across approaches when applied to real data, and how this consistency correlates with genomic features.

Results: In simulations we find substantial variation across approaches in the ability to identify archaic ancestry segments. We also demonstrate an inconsistency across approaches in identified archaic segments in modern humans. For example, out of all SNPs identified as Neanderthal in any of the published methods, only 29% of these are found in more than two methods.

Conclusion: Our benchmarking of different methods highlights the difficulties in robustly identifying archaic segments in modern human genomes. We discuss the implications for downstream analyses that make use of inferred archaic segments.

Grants: Wellcome Trust (224575/Z/21/Z)

Conflict of Interest: None declared

P19.021.A ERAP2 allotype divergence revealed by population-level exosome sequencing

Aroosha Raja[1], *Abdulrahman Alasiri*[2], *Marion van Vugt*[2], *Jessica Van Setten*[2], *Jonas Kuiper*[1]

[1]*UMC Utrecht, Centre For Translational Immunology, Department of Ophthalmology, Utrecht, Netherlands;* [2]*UMC Utrecht, Department of Cardiology, Utrecht, Netherlands*

Consortium: CAPSTONE

Background: Polymorphisms in immune genes impact immune responses across individuals. Endoplasmic reticulum aminopeptidases 2 (ERAP2) are major part of adaptive immunity involved in trimming of antigenic peptides presented on Major histocompatibility class-I molecules. ERAP2 is highly polymorphic showing recent marks of evolutionary adaptations. The coding variants of ERAP2 are shown to be protective against pathogenic infections but at the cost of onset of autoimmune diseases. Despite the peculiar evolutionary history and substantial implications for human health, diversity of ERAP2 allotypes in population remains uncertain.

Methods: We used exome sequencing data to explore protein-altering variants of ERAP2 in 160,000 individuals of European descent. Conservative filtering steps were applied for phasing and processing to characterize significant ERAP2 variants for estimation of allotypes. Linkage disequilibrium (LD), clinicopathological associations and docking profiles of allotypes were assessed.

Results: We identified 15 protein-altering variants in exon-2 through exon-18 alongside 2 essential splice variants in LD with protein-altering variants of ERAP2. Considering phased haplotypes of variants, we identified 27 ERAP2 haplotypes in the European population. Through phylogenetics, we observed three large clades corresponding to 3 major haplotypes (A-C) controlled by splice variants rs2248374 and rs17486481. Homozygosity for rs2248374's G allele results in nonsense-mediated decay while A allele homozygosity denotes functional allotypes. Docking simulations on 14 functional allotypes revealed differences in ligand binding efficacies.

Conclusion: Population diversity of ERAP2 alters structure resulting in functional implications important for drug targeting and precision medicine.

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Conflict of Interest: None declared

P19.022.B The Genetic Contribution to life satisfaction stability and change: A longitudinal twin study.

Charlotte Pahnke[1], *Marco Deppe*[2], *Carlo Maj*[3], *Rana Aldisi*[1;4], *Friederike David*[1;5], *Christian Kandler*[2], *Markus Nöthen*[1], *Andreas Forstner*[1;6]

[1]*University of Bonn, School of Medicine & University Hospital Bonn, Institute of Human Genetics, Bonn, Germany;* [2]*University of Bremen, Department of Psychology, Bremen, Germany;* [3]*University of Marburg & University Hospital of Marburg, Center for Human Genetics, Marburg, Germany;* [4]*University of Bonn, School of Medicine & University Hospital Bonn, Institute for Genomic Statistics and Bioinformatics, Bonn, Germany;* [5]*University of Marburg, Department of Psychiatry and Psychotherapy, Marburg, Germany;* [6]*Research Center Jülich, Institute of Neuroscience and Medicine (INM-1), Jülich, Germany*

Background/Objectives: Multi-trait analyses of genome-wide association studies led to the proposal of the Well-Being Spectrum, consisting of the genetically correlated traits depressivity, neuroticism, positive affect and life satisfaction (PMID30810878). Additionally, the inclusion of loneliness and self-rated health has been proposed (PMID30643256). Here, we perform longitudinal analyses of the genetic contribution to stability and change of life satisfaction based on polygenic scores.

Methods: Latent state-trait-change models were fitted to life satisfaction reports assessed on up to seven occasions in 4,566 participants of the German TwinLife study. Polygenic scores for the well-being spectrum and its individual traits were constructed using PRS-CS and included in full sample, age and twin-specific models. Finally, polygenic score results were compared with classical twin modelling.

Results: In the full model and most age groups, polygenic scores were significantly associated with stable trait scores of life satisfaction, whereas associations with linear and quadratic change were smaller and mostly not statistically significant. This pattern was reflected in twin-based estimates, except for the quadratic change component. The inclusion of loneliness and self-rated health in an extended well-being spectrum score consistently, but not significantly, increased associations with the life satisfaction components.

Conclusion: We demonstrated a greater additive genetic contribution to trait life satisfaction than to linear change. Results for the quadratic change component were mixed. The observed group differences highlight that genetic effects may be population-specific (e.g. with respect to age). Our findings encourage the use of longitudinal data in complex genetic research.

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Conflict of Interest: None declared

P19.024.D Model of stabilizing selection fit observed genetic architectures of human complex traits and common diseases

Evan Koch[1;2], **Noah Connally**[1], *Nikolas Baya*[3], *Mary Pat Reeve*[4], *Mark Daly*[3;4;5], *Benjamin Neale*[3;5], *Eric Lander*[3], *Alex Bloemendal*[3], *Shamil Sunyaev*[1;2]

[1]*Harvard Medical School, Department of Biomedical Informatics, Boston, United States;* [2]*Brigham and Women's Hospital, Boston, United States;* [3]*Broad Institute, Cambridge, United States;* [4]*Institute for Molecular Medicine Finland, Helsinki, Finland;* [5]*Massachusetts General Hospital, Boston, United States*

Background/Objectives: The question of how evolutionary forces maintain variation in complex traits is a mainstay of evolutionary genetics. There is a strong theoretical argument that quantitative traits experience stabilizing selection, while disease traits may, intuitively, experience directional selection. GWAS show that allele frequencies are negatively correlated with effect sizes, which is consistent with both stabilizing and directional selection.

Methods: To distinguish between different forms of selection, we compared the effect sizes and frequencies of the trait-increasing (or risk-increasing) allele at each site. For each trait, we evaluated that distribution's consistency with different evolutionary models: neutral evolution, directional selection, stabilizing selection, and a combination of directional and stabilizing selection.

Results: Across traits, plotting allele effect sizes and frequencies produced a characteristic U-shape. The symmetry of this distribution suggests stabilizing selection, and, in our statistical analysis, models of stabilizing selection were supported for all traits distinguishable from neutrality. For most traits, data were similarly consistent with stabilizing selection on one trait or on multiple traits simultaneously (pleiotropy).

Conclusion: Though it is difficult to infer the specific costs or benefits of different traits in human evolutionary history, the alleles contributing to many human traits show evidence of stabilizing selection. This is true both for quantitative traits and for

disease traits whose effects on modern humans are generally deleterious. This finding shows the need for further investigation into both the causes of stabilizing selection, and the extent to which it is influenced by pleiotropy.

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Conflict of Interest: None declared

P19.025.A Exploring evolutionary trajectories in ovarian cancer patients by longitudinal analysis of ctDNA

Oliver Kutz[1;2;3;4], *Stephan Drukewitz*[5;6], *Alexander Krüger*[6], *Daniela Aust*[2;4;6;7;8], *Doreen William*[1;2;4;6], *Sandra Oster*[7], *Evelin Schröck*[1;2;4;6], *Gustavo Baretton*[2;4;6;7;8], *Theresa Link*[2;3;4], *Pauline Wimberger*[2;3;4], *Jan Kuhlmann*[3;4]

[1]*Institute for Clinical Genetics, University Hospital Carl Gustav Carus and Faculty of Medicine of TUD Dresden University of Technology, Dresden, Germany; ERN GENTURIS, Hereditary Cancer Syndrome Center Dresden, Germany; National Center for Tumor Diseases (NCT), NCT/UCC Dresden, a partnership between German Cancer Research Center (DKFZ), Faculty of Medicine and University Hospital Carl Gustav Carus, Dresden, Germany;* [2]*German Cancer Consortium (DKTK), Dresden, Germany; German Cancer Research Center (DKFZ), Heidelberg, Germany; Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany;* [3]*Medical Faculty and University Hospital Carl Gustav Carus, Technische Universität Dresden, Department of Gynecology and Obstetrics, Dresden, Germany;* [4]*National Center for Tumor Diseases (NCT), NCT/UCC Dresden, a partnership between German Cancer Research Center (DKFZ), Faculty of Medicine and University Hospital Carl Gustav Carus, TUD Dresden University of Technology and Helmholtz-Zentrum Dresden-Rossendorf (HZDR), Germany; German Cancer Consortium (DKTK), Dresden, Germany; German Cancer Research Center (DKFZ), Heidelberg, Germany, Dresden;* [5]*Institute of Human Genetics University Hospital, Leipzig, Germany;* [6]*Core Unit for Molecular Tumor Diagnostics (CMTD), National Center for Tumor Diseases (NCT), NCT/UCC Dresden, a partnership between German Cancer Research Center (DKFZ), Faculty of Medicine and University Hospital Carl Gustav Carus, TUD Dresden University of Technology and Helmholtz-Zentrum Dresden-Rossendorf (HZDR), Germany; German Cancer Consortium (DKTK), Dresden, Germany;* [7]*University Hospital Carl Gustav Carus at the TU Dresden, Institute of Pathology, Dresden, Germany;* [8]*Tumor- and Normal Tissue Bank, National Center for Tumor Diseases (NCT), NCT/UCC Dresden, a partnership between German Cancer Research Center (DKFZ), Faculty of Medicine and University Hospital Carl Gustav Carus, TUD Dresden University of Technology and Helmholtz-Zentrum Dresden-Rossendorf (HZDR), Germany;, Dresden, Germany*

Background: We analysed, whether temporal heterogeneity of ctDNA mirrors evolutionary patterns in ovarian cancer.

Methods: Targeted sequencing of 275 cancer-associated genes was performed in primary tumor biopsies and in ctDNA of six longitudinal plasma samples from 15 patients, using the Illumina platform.

Results: While there was a low overall concordance between the mutational spectrum of the primary tumor biopsies vs. ctDNA, *TP53* variants were the most shared somatic alterations. Up to three clusters were detected in each tumor biopsy, likely representing predominant clones of the primary tumor. By tracing these clusters in ctDNA we revealed two evolutionary patterns. In pattern#1, clusters detected in the primary tumor biopsy were likely relapse seeding clones, as they contributed a major share to ctDNA at relapse. In pattern#2, similar clusters were present in tumors and ctDNA; however, they were cleared from liquid biopsy after chemotherapy and were undetectable at relapse. ctDNA private variants were present among both patterns with some of them mirroring possible subclonal expansions after chemotherapy.

Conclusion: We demonstrate that tracing the temporal heterogeneity of ctDNA could be a promising tool for deciphering evolutionary trajectories in ovarian cancer. Our findings could have important clinical implications as in patients with pattern#1, a single tumor biopsy may allow for predicting relapse eligible for targeted therapy. Further research will be necessary to predict at primary diagnosis whether a patient`s tumor will progress according to pattern#1 or #2.

Grants: The study was supported by Deutsche Krebshilfe (reference numbers: 70113616, 70113636).

Conflict of Interest: None declared

P19.027.C Novel Insights Into Lifetime Risk In HDGC: Lower Cancer Risk For The Most Recurrent *CDH1* Variant In Europe

Rita Barbosa-Matos[1], *João Fonseca*[1], *Luzia Garrido*[1], *Susana Seixas*[1], *João Parente Freixo*[1], *Renata Oliveira*[2], *Robert Hüneburg*[3], *Patrick Benusiglio*[4], *Karin Dahan*[5], *damien feret*[5], *Isabel Spier*[3], *Stefan Aretz*[3], *Camille Desseignes*[6], *Ana Rita Ferreira Pacheco Quental*[2], *Susana Fernandes*[2], *Susy Cabral Costa*[2], *Lúcia Vilarinho*[2], *Sérgio Castedo*[1;2], *Ana Grangeia*[2], *Alice Porto Guerra Vasconcelos*[2], *Diogo Rocha*[2], *Pedro Louro*[2], *Manuela Batista*[2], *Fátima Carneiro*[1;2], *Irene Gullo*[1;2], *Youenn Drouet*[7;8], *Carla Oliveira*[1]

[1]*i3S - Instituto de Investigação e Inovação em Saúde da Universidade do Porto, Porto, Portugal;* [2]*São João University Hospital, Porto, Portugal;* [3]*University Hospital Bonn, National Center for Hereditary Tumor Syndromes, Bonn, Germany;* [4]*IFSI the Pitie-Salpetriere Hospital Group AP-HP, Groupe Hospitalier Pitié-Salpêtrière, AP-HP, Sorbonne Université, Paris, France;* [5]*Institute Pathology And De Génétique, Charleroi, Belgium;* [6]*Assistance Publique – Hôpitaux de Paris, Medical Genetics Department, Paris, France;* [7]*Université de Lyon, Lyon, France;* [8]*Léon Bérard Center, Lyon, France*

Background/Objectives: The *CDH1* c.1901 C>T variant is the most recurrent in HDGC families across Europe, conferring susceptibility for Diffuse Gastric Cancer (DGC) and Lobular Breast Cancer (LBC). Despite lifetime risk estimates guide risk-reducing surgeries decision of individual families, current estimates used a combination of small families, cancer-enriched pedigree branches, different *CDH1* variants and lack histology confirmation of gastric and breast cancers.

Methods: Cancer status, histology, and age data were collected for 9 probands and 276 relatives. Cumulative risks of DGC and LBC were calculated relative to regional cancer incidence data using the genotype restricted likelihood (GRL) method. Clinical profiles and management strategies were also documented.

Results: Among 285 individuals, including 95 *CDH1* carriers, 26 had gastric cancer (all before 60 years), and 12 had breast cancer. Cumulative risks of DGC and LBC were estimated at 5.8% and 19.1% by age 60, respectively. Presence of gastric ulcer was significantly associated with DGC. No additional predictors of advanced disease were identified among carriers.

Conclusion: This is the largest cohort ever studied for the same *CDH1* variant present in a population sharing the same geographical area for centuries. Despite disease expressivity and early-onset of cancer is similar to families described worldwide, our data challenges all previous GC (>30% risk vs 6%) and BC (>40% vs 19.1%) risk estimations for *CDH1* carriers. We provide novel lifetime risk estimates considering specific cancer phenotypes associated with *CDH1* truncating variants and matched regional population incidence.

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2022-CARE-08;ERN Genturis Project:No.739547; SOLVE-RD Project:H2020-SC1-2017.

Conflict of Interest: None declared

P19.028.D Landscape of genetic variations from population-based whole genome sequencing in Indian population

Krithika Subramanian[1;2], *Bratati Kahali[1]*

[1]Centre for Brain Research, Bengaluru, India; [2]Manipal Academy of Higher Education, Bengaluru, Govindapura, India

Consortium: TATA Longitudinal Study of Aging (CBR-TLSA) consortium,

Srinivaspura Aging Neurosenescence and Cognition (CBR-SANSCOG) consortium

Background: The human genome, with 3.2 billion DNA base pairs, exhibits 0.1% genetic variation among individuals, mainly as single-nucleotide variant (SNV), short insertion and deletion (INDEL), and structural variant (SV). Despite extensive global genome sequencing, there's limited representation of the Indian population in genetic studies.

Methods: To address this gap, we analyzed 845 individuals from the TATA Longitudinal Study of Aging (CBR-TLSA) and Srinivaspura Aging Neurosenescence and Cognition (CBR-SANSCOG) cohorts in India. We utilized a standardized whole-genome sequencing (WGS) pipeline (mean 42X depth of coverage) to detect SNVs, INDELs, and SVs employing multiple algorithms.

Results: We identified and characterized 22.78 million SNVs (5.2% novel) and 2.28 million INDELs (5.4% novel) in 845 individuals. 1.22% SNVs and 0.6% INDELs are putative protein-disrupting variants. About 50 such variants are frequent in the dataset (n = 40). We identified 36,214 SVs (24,574 deletions, 2,913 duplications, 8,714 insertions, and 13 inversions) in 529 individuals, with 1.26% potentially impacting protein-coding regions. Seven rare SVs significantly impact the dosage-sensitivity of seven genes with autosomal dominant mode of inheritance implicated in several diseases. 56 SVs are highly conserved and exhibit high intolerance to loss-of-function.

Conclusion: This study highlights population-specific genetic variations in Indian cohorts. Rare variants predominated over common ones, with two-thirds of variations in <1% of individuals. Discovering fine-scale genetic variations in the underrepresented Indian population provides valuable insights for human genetic studies, potentially leading to improved diagnostic and treatment strategies for a substantial portion of the global population.

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Conflict of Interest: None declared

P19.029.A Using the ends of the polygenic risk score spectrum for schizophrenia to better capture anxiety-related brain structures – A UK Biobank study

Lisa Sindermann[1], *Christina Fell[2], Priyatam Dutta[1;3], Silvia Paracchini[3], Markus Nöthen[1], Andreas Forstner[1;4]*

[1]Institute of Human Genetics, University of Bonn, School of Medicine & University Hospital Bonn, Bonn, Germany; [2]School of Mathematics and Science, University of St Andrews, St Andrews, United Kingdom; [3]School of Medicine, University of St Andrews, St Andrews, United Kingdom; [4]Institute of Neuroscience and Medicine (INM-1), Research Center Jülich, Jülich, Germany

Background: Mental disorders present anxiety and overlapping genetic factors. The polygenic risk score for schizophrenia (SCZ PRS) is suggested to be linked to anxiety. In particular, the amygdala, insula, hippocampus, and frontal parts of the brain have been identified as anxiety-related regions. However, their link to the SCZ PRS is poorly understood, which we aim to investigate.

Methods: We stratified UK Biobank individuals in the 1st ($n = 2848$), 2nd ($n = 2746$), 9th ($n = 2578$), and 10th decile ($n = 2385$) of the SCZ PRS. Group differences in regional gray matter volumes (rGMV) of the amygdala, insula, hippocampus and medial frontal cortex (ROIs) and the fusiform gyrus as control region were examined. In an exploratory analysis, we tried to classify the 1st vs. 10th decile of the SCZ PRS spectrum via the ROIs using a machine learning approach.

Results: Results showed significantly reduced rGMV in the amygdala in the higher deciles compared to the lower deciles of the SCZ PRS spectrum. The classification accuracy of the machine learning approach was low at around 51.8%.

Conclusion: Differences in the amygdala volume may underlie the link between the genetic liability for SCZ and anxiety in the general population. Using the ends of the PRS spectrum may help in the future to improve personalized treatment options through better stratification of individuals.

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P20
FUNCTIONAL GENOMICS

P20.001.B Mutational signature of ionising radiation exposure in human fibroblasts

Lisa Hagenau[1], *Ana Tzvetkova[1;2], Lars Jensen[1], Matthias Port[3], Harry Scherthan[3], Andreas Kuß[1]*

[1]University Medicine Greifswald, Department of Functional Genomics, Greifswald, Germany; [2]University Medicine Greifswald, Institute of Bioinformatics, Greifswald, Germany; [3]Bundeswehr Institute of Radiobiology, affil. to the University of Ulm, München, Germany, München, Germany

Background/Objectives: DNA can be damaged in different ways by agents that prompt different DNA repair responses in the cell. Misrepair leads to mutations and for some agents, distinctive mutational signatures have been identified. Preliminary results of DNA damaged by ionising radiation (IR) indicate that exposure leads to an increase in somatic insertions/deletions (InDels), but not in somatic single-nucleotide variants. We use nanorate sequencing (NanoSeq), a modified duplex sequencing protocol, to identify and classify mutations in irradiated fibroblasts.

Methods: NanoSeq enables accurate identification of somatic mutations (SNVs and small InDels) from a mixed cell population, which saves time over subcloning single cell lines. Human gingival fibroblasts were irradiated with acute 2 and 10 Gy doses of ionising radiation followed by repair for 72h and preparation and

sequencing of NanoSeq libraries. The identified mutations were classified and compared with mutational signatures in the COSMIC database using the SigProfiler tools.

Results: High doses (10 Gy) of IR lead to an increase in InDels, particularly in microhomology-mediated deletions. This points to microhomology-mediated end joining (MMEJ) as major DSB repair mechanism. The number of SNVs does not increase significantly in irradiated samples and changes to the mutational profile vary between technical replicates. These results will be part of a larger study investigating mutational signatures in cells after treatment with IR, bleomycin and UV-B.

Conclusion: We show that IR exposure induces an increase in InDels. The InDel profile implicates MMEJ as principal DNA repair mechanism. For detecting IR-specific SNV-profiles, increased sequencing depth seems necessary.

Conflict of Interest: None declared

P20.003.D Testing the possibility of using DNA methylation arrays as first-tier test in individuals with neurodevelopmental problems, congenital anomalies or growth disorders

Tinatin Tkemaladze[1;2], *Christopher Campbell*[3], *Kakha Bregvadze*[1], *Eka Kvaratskhelia*[1], *Elene Abzianidze*[1], *Leigh Demain*[3], *Sarah Jenkinson*[3], *Sarah Hilton*[3], *Bekim Sadikovic*[4;5], *David Gokhale*[3], *Siddharth Banka*[3;6]

[1]*Tbilisi State Medical University, Department of Molecular and Medical Genetics, Tbilisi, Georgia;* [2]*Givi Zhvania Pediatric Academic Clinic, Department of Pediatrics, Tbilisi, Georgia;* [3]*Manchester University NHS Foundation Trust, Manchester, UK, St Mary's Hospital, Manchester, United Kingdom;* [4]*Western University, Department of Pathology and Laboratory Medicine, London, Canada;* [5]*London Health Sciences Centre, Verspeeten Clinical Genome Centre, London, Canada;* [6]*University of Manchester, Division of Evolution, Infection and Genomics, School of Biological Sciences, Faculty of Biology, Medicine and Health, Man, United Kingdom*

Background/Objectives: DNA methylation (DNAm) episignature analysis has emerged as valuable tool for evaluating variants of uncertain significance and for studying individuals with neurodevelopmental problems, congenital anomalies and growth disorders who remain unsolved after exome or genome sequencing. Thus far the possibility of using episignature analysis as first-line tests in such patients has not been evaluated systematically.

Materials and Methods: Molecular testing naïve patients suspected of genetic disorders underwent DNAm array followed by EpiSign™ episignature analysis and all positive results were orthogonally validated.

Results: We ascertained 62 individuals for the project. The diagnostic yield was 100% (5/5) for patients suspected of Fragile X syndrome. DNAm arrays confirmed diagnoses in 8/18 (44%) individuals with syndromic disorders which included conditions such as Coffin-Siris, Cornelia de Lange, Kabuki, Floating-Harbor, Williams-Beuren and Wolf-Hirschhorn syndromes. We established diagnoses in 6/24 (25%) individuals suspected of imprinting disorders including Silver-Russel, Beckwith-Wiedemann and Prader-Willi syndromes. No diagnoses were achieved in 15 individuals with non-syndromic neurodevelopmental problems. Notably, DNAm analysis identified alternative diagnoses in 4.8% (3/62) of cases.

Conclusion: Overall, we achieved diagnoses in 19/62 (30.6%) test-naïve patients and these results show that DNAm arrays could be a useful first-tier diagnostic tool for individuals with syndromic genetic or imprinting disorders. Our findings are especially relevant for resource limited healthcare systems. The diagnostic yield of this approach will increase in future with the development of combined copy number and methylation arrays and with identification of episignatures for more disorders, including for non-syndromic conditions.

Conflict of Interest: None declared

P20.004.A Genetic loci that disassociate obesity from abdominal fat distribution influence adipocyte differentiation and function

Jonathan Belanich[1;2], *Mario Urena*[1], *Aurore Hecq*[1;3], *Alizee Mosnier*[1], *Renee Gibson*[2], *Thiago Batista*[2], *Hesam Dashti*[2], *Brice Emmanuelli*[1], *Melina Claussnitzer*[2], *Tuomas Kilpeläinen*[1;2]

[1]*The Novo Nordisk Foundation Center for Basic Metabolic Research, København, Denmark;* [2]*Broad Institute, Cambridge, United States;* [3]*University of Mons - Campus Plaine Nimy, Mons, Belgium*

Background/Objectives: Obesity is negatively linked to cardiometabolic health, but this relationship can vary greatly among individuals. The biological mechanisms that regulate this variability remain incompletely understood. Intriguingly, some genetic loci have been found to show an inverse relationship between higher body mass index (BMI) and abdominal fat distribution, as indicated by a higher waist-hip ratio (WHR).

Methods: Initiating our search with the results from the largest GWAS for BMI and WHR, which included ~700,000 participants each, we identified >100 independent loci associated with higher BMI but lower WHR. Several of these loci were also found to associate with increased insulin sensitivity and a reduced risk of type 2 diabetes.

To unravel the biological mechanisms that separate obesity from abdominal fat distribution and poor cardiometabolic health, we focused on two previously uncharacterized loci, ADAMTS9 and EMILIN2. Knockdowns with siRNA in adipocytes led to decreased differentiation and function of the cells, affecting cellular metabolism and adipogenic marker genes.

Results: Further investigations using CRISPRi perturbation and the LipocyteProfiler for hundreds of image-based cellular features revealed that the perturbed cells experienced reduced lipid storage capacity and decreased glucose uptake, with no impact on physical markers such as proliferation and size. We have performed RNA-Seq on the same cells to deepen our analysis, allowing us to draw connections between the modified genetic functions and cellular phenotypes.

Conclusion: This study is investigating only two of a swathe of newly identified loci that may enhance the understanding of genes that uncouple obesity from abdominal fat distribution and poor cardiometabolic health.

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Conflict of Interest: None declared

P20.005.B Comparative 3D genome analysis between neural retina and RPE reveals differential *cis*-regulatory interactions at retinal disease loci

Eva D'haene[1;2], *Víctor López Soriano*[1;2], *Pedro Manuel Martinez-Garcia*[3], *Soraya Kalayanamontri*[3], *Alfredo Dueñas Rey*[1;2], *Ana Sousa-Ortega*[3], *Silvia Naranjo*[3], *Stijn Van de Sompele*[1;2], *Lies Vantomme*[1;2], *Quinten Mahieu*[1;2], *Sarah Vergult*[1;2], *Ana Bastos Neto*[3], *Jose Luis Gomez-Skarmeta*[3], *Juan Ramón Martínez Morales Martínez-Morales*[3], *Miriam Bauwens*[1;2], *Juan Jesus Tena-Aguilar*[3], *Elfride De Baere*[1;2]

[1]*Ghent University, Department of Biomolecular Medicine, Ghent, Belgium;* [2]*Ghent University Hospital, Center for Medical Genetics, Ghent, Belgium;* [3]*Universidad Pablo de Olavide, Centro Andaluz de Biología del Desarrollo, Sevilla, Spain*

Background/Objectives: Vision depends on the interplay between photoreceptors of the neural retina and the underlying retinal pigment epithelium (RPE). Genes involved in inherited retinal diseases (IRD) display specific spatiotemporal expression within these interconnected retinal components through local recruitment of *cis*-regulatory elements (CREs) in 3D nuclear space. Yet, the role of chromatin architecture in establishing these specific expression patterns remained unexplored.

Methods: To understand the role of differential chromatin architecture in establishing tissue-specific expression at IRD loci, we mapped genome-wide chromatin interactions using in situ Hi-C, and H3K4me3 HiChIP on neural retina and RPE/choroid from human adult donor eyes. Also, we functionally validated *cis*-regulatory interactions in the *ABCA4* locus using UMI-4C, multi-omic data integration and zebrafish enhancer assays.

Results: We determined chromatin looping between active promoters and 32,425 and 8,060 candidate CREs (cCREs) in the neural retina and RPE/choroid respectively. A comparative 3D genome analysis between these two retinal tissues revealed that 56% of 290 known IRD genes were marked by differential chromatin interactions. One of these was *ABCA4*, which is implicated in the most common autosomal recessive IRD. Integration of *ABCA4* promoter interactions and tissue-specific multi-omics data, as well as functional validation using zebrafish enhancer assays revealed multiple active *ABCA4*-CREs, likely achieving retina-specificity through a cooperative mechanism.

Conclusion: We have shown that gene regulation at key IRD loci is likely mediated by tissue-specific chromatin interactions. These findings do not only provide insight into tissue-specific regulatory landscapes at retinal disease loci, but also delineate the search space for non-coding genomic variation underlying unsolved IRD.

Conflict of Interest: None declared

P20.006.C Pinpointing candidate pathomechanistic actors of biglycan-related disease by mRNA-sequencing

Anne Hebert[1], *Josephina (Jeannette) Meester*[1], *Ligia Monica Mateiu*[1], *Maaike Bastiaansen*[1], *Aline Verstraeten*[1], *Bart Loeys*[1;2]

[1]*University of Antwerp, Center of Medical Genetics, Edegem, Belgium;* [2]*Radboud University Medical Center, Department of Clinical Genetics, Nijmegen, Netherlands*

Background/Objectives: Discrete pathogenic variants in biglycan (*BGN*) are associated with an aortopathy called Meester-Loeys syndrome (MRLS; loss-of-function truncating or splice site variants) and a skeletal dysplasia called X-linked spondylo-epimetaphyseal dysplasia (SEMDX; missense variants).

Methods: To gain insights into the pathomechanisms underlying these two divergent *BGN*-related phenotypes, mRNA-sequencing was performed on MRLS (n = 3), SEMDX (n = 2) and control (n = 2) skin fibroblasts.

Results: Comparative BioPlanet pathway enrichment analysis of mRNA-sequencing data from MRLS patient skin fibroblasts and matched controls revealed seventeen dysregulated genes associated with transforming growth factor-beta (TGF-β) regulation of the extracellular matrix (ECM). Gene ontology (GO) enrichment analysis indicated dysregulation of collagen-containing ECM, as well as adhesion- and cell-cycle-related processes. Given the increased TGF-β signaling in MRLS and the suggested role of BGN in regulating collagen fibril diameter, these mRNA-sequencing results support the importance of ECM dysregulation in MRLS.

Comparative GO enrichment analysis of mRNA-sequencing data from SEMDX patient skin fibroblasts and matched controls revealed a significant upregulation of inhibitors of the GDF5/BMP-pathway along with downregulation of the GDF5 ligand itself. This pathway controls chondrocyte proliferation, and pathogenic variants in *GDF5* have already been associated with skeletal dysplasias. We hypothesize that GDF5 could be an important binding partner or downstream effector of BGN and may, therefore, play a key role in SEMDX development.

Conclusion: Analysis of mRNA-sequencing data from MRLS and SEMDX patient skin fibroblasts identified intriguing candidate pathomechanistic actors or pathways for further investigation.

Grants:

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Conflict of Interest: None declared

P20.007.D Exploring the association between DNA methylation and asthma in the Canary Islands

Mario Martín Almeida[1], *Javier Perez-Garcia*[1], *Elena Martin-Gonzalez*[1], *Fabian Lorenzo-Diaz*[1;2], *José M. Hernández-Pérez*[3;4], *Paloma Poza-Guedes*[5;6], *Elena Mederos-Luis*[6], *berta román bernal*[7], *Inmaculada Sánchez-Machín*[6], *Jesús Villar*[8;9;10], *Ruperto González-Pérez*[5;6], *Maria Pino-Yanes*[1;11]

[1]*University of La Laguna, Genomics and Health Group, Department of Biochemistry, Microbiology, Cell Biology and Genetics, La Laguna, Tenerife, Spain;* [2]*Instituto Universitario de Enfermedades Tropicales y Salud Pública de Canarias (IUETSPC), La Laguna, Tenerife, Spain;* [3]*Hospital Universitario de N.S de Candelaria, Pulmonary Medicine Service, La Laguna, Tenerife, Spain;* [4]*Hospital Universitario de La Palma, Pulmonary Medicine Section, La Laguna, Tenerife, Spain;* [5]*Hospital Universitario de Canarias, Severe Asthma Unit, Allergy Department, La Laguna, Tenerife, Spain;* [6]*Hospital Universitario de Canarias, Allergy Department, La Laguna, Tenerife, Spain;* [7]*Hospital Dr José Molina Orosa, Pulmonary Medicine Service, Arrecife, Lanzarote, Spain;* [8]*Instituto de Salud Carlos III, CIBER de Enfermedades Respiratorias, Madrid, Spain;* [9]*Hospital Universitario Dr. Negrín, Multidisciplinary Organ Dysfunction Evaluation Research Network (MODERN), Research Unit, Las Palmas de Gran Canaria, Spain;* [10]*Li Ka Shing Knowledge Institute at the St. Michael's Hospital, Toronto, Ontario, Canada;* [11]*University of La Laguna, Instituto de Tecnologías Biomédicas (ITB), La Laguna, Tenerife, Spain*

Consortium: GEMAS
CONGENT

Background: Asthma is a complex respiratory disease that is three times more prevalent in the Canary Islands compared to other regions of Spain. Differences in genetic composition and environmental exposures have been proposed to explain these prevalence differences. In this sense, DNA methylation (DNAm) has been implicated in asthma pathogenesis, reflecting the interaction of gene-environmental factors. We aimed to identify DNAm markers associated with asthma susceptibility in the Canarian population.

Methods: Blood DNAm levels were analyzed at 823,755 CpG sites from 456 individuals (320 and 136 with and without asthma, respectively), using the Infinium MethylationEPIC microarray (Illumina). The association between DNAm at individual CpGs and asthma susceptibility was determined through an epigenome-wide association study (EWAS), using linear regression models adjusted for age, sex, ancestry, and tissue heterogeneity. Differentially methylated regions (DMRs) were examined using comb-p and *dmrff*, and enrichment analysis was performed using EWAS Toolkit and Enrichr databases. Multiple comparisons were adjusted with a false discovery rate (FDR)<5%.

Results: Although no CpG sites were significantly associated with asthma, 36 DMRs were associated with the disease (FDR < 5%). The top-hit region was annotated to *TRIM31* gene ($FDR = 1.39 \times 10^{-9}$), previously associated with other immune

system diseases and transcriptional regulation. CpGs suggestively associated in the EWAS ($p < 5 \times 10^{-4}$) showed enrichment in prior associations including ancestry, exposure to glucocorticoids, aging, and transcription factors involved in asthma development (i.e., NF-κB) (FDR < 0.05).

Conclusion: This study revealed new associations of DMRs with asthma susceptibility in the Canary Islands.

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Conflict of Interest: None declared

P20.008.A Genome-wide identification and functional assessment of non-coding variants in hypo-plastic left heart syndrome patients

Vartika Bisht[1], *Ludo Pagie*[1], *Miriam Smits*[1], *James Burgess1*[1;2], *Alexey Pindyurin*[1], *Wilhelmina S. Kerstjens-Frederikse*[3], *Cleo van Diemen*[3], *Joris Arensbergen*[1]

[1]*Annogen Bv, Amsterdam, Netherlands;* [2]*The Royal Children's Hospital Melbourne, Parkville, Australia;* [3]*University Medical Center Groningen, Groningen, Netherlands*

Consortium: ENHPATHY

Background/Objectives: Congenital heart disease (CHD) is one of the most common and severe birth anomalies with a prevalence of about 1% in the population. One specific type of CHD is hypo-plastic left heart syndrome (HLHS), which involves malformations in the left-sided structures of the heart. Since many HLHS patients do not have any pathogenic coding mutations, it is thought that non-coding mutations might explain some of these cases. However, assessing the functional impact of non-coding mutations from sequence alone is challenging given our limited understanding of how regulatory information is encoded in the genome.

Methods: We utilized Survey of Regulatory Elements (SuRE), a massively parallel reporter assay, to evaluate the functional impact of non-coding variants found in the genomes of five exome-negative HLHS patients on enhancer and promoter activity.

Results: Using SuRE, we have analyzed approximately 4.7 million non-coding single nucleotide substitutions, insertions, and deletions. Among these variants, we have identified about 18k variants which exhibit significant functional effects. Next, we prioritized 20 putative causal variants through comprehensive consideration of their effect size, population allele frequencies, overlap with known heart-specific regulatory regions and occurrence within the patient's family. We are currently validating the functional impact of these variants using enSERT, a CRISPR/Cas9-mediated site-specific transgenic mouse assay.

Conclusion: The integration of information about known regulatory elements with comprehensive functional assessment provided by SuRE has empowered us to identify potentially causal non-coding variants within the vast genomic landscape of HLHS. The ongoing validation in transgenic mice will show the strength of such an approach.

Conflict of Interest: None declared

P20.009.B High throughput functional assay to evaluate DNA polymerase epsilon exonuclease missense variants: a proof-of-concept

Albain Chansavang[1;2], *Bertrand Diebold*[1], *Ingrid Laurendeau*[2], *Eric Pasmant*[1;2], *Nadim Hamzaoui*[1;2]

[1]*Hôpital Cochin, AP-HP, Fédération de Génétique et Médecine Génomique APHP Centre Université Paris Cité, Département de Médecine Génomique des Tumeurs et Cancers, PARIS, France;* [2]*Institut Cochin, Inserm U1016, CNRS UMR8104, Université Paris Cité, CARPEM, PARIS, France*

Background/Objectives: DNA polymerase epsilon (POLE) is a replicative polymerase with an exonuclease-proofreading domain. Germline *POLE* exonuclease domain variants are responsible for hereditary tumor predisposition. Several somatic *POLE* variants are associated with high tumor mutational burden and good response to anti-PD1 treatment. It is therefore important to discriminate between pathogenic and benign variants. Our aim was to characterize the impact of polymerase epsilon exonuclease missense variants of the yeast *Pol2* gene (*POLE* ortholog) with a multiplexed assay of variant effect.

Methods: Every possible single nucleotide variant of two Pol2 residues (Pro301 and Met309) and an exo-null control were generated by oligonucleotide synthesis. Genomic edition of *S. saccharomyces* W303 was done by cotransformation of the oligonucleotide library and a CRISPR/Cas9 expression plasmid. Transformed cells were tested for genetic instability at the *URA3* locus by plating on 5FOA-containing medium. DNA was extracted from yeast cells before and after 5FOA selection and *Pol2* sequenced by NGS. Variants allelic frequencies were analyzed using Enrich2 pipeline.

Results: The exo-null and the known cancer driver p.Pro301Arg variants were enriched 50 and 500 fold, respectively, compared to wild-type. Pro301 was intolerant to mutations toward aminoacids with large side chains, which suggests a mechanism of steric hindrance to DNA binding in the exonuclease pocket. Met309 variants had a much less pronounced impact on genetic instability.

Conclusion: We successfully validated a multiplexed assay of the effect of *Pol2* exonuclease domain variants on genetic instability using a library of two saturation-mutated residues. This protocol is currently applied to the remaining exonuclease residues.

Grants: *Emergence 2020*, Cancéropôle Île-de-France

Conflict of Interest: None declared

P20.010.C CFAP99 deficiency leads to heterotaxy and scoliosis

Beril Ay[1], *Stephen Viviano*[2], *OZLEM AKGUN DOGAN*[3], *Yasemin Alanay*[3], *Engin Deniz*[2]

[1]*Acibadem Mehmet Ali Aydinlar University, School of Medicine, Istanbul, Türkyie;* [2]*Yale University, Department of Pediatrics, New Haven, CT, United States;* [3]*Acibadem Mehmet Ali Aydinlar University, School of Medicine, Department of Pediatrics, Division of Pediatric Genetics, Istanbul, Türkyie*

Background/Objectives: Birth defects affect 6% of births globally, leading to significant mortality and comorbidities. Current efforts focus on identifying underlying mechanisms for prevention. A 3-year-old female presented with congenital scoliosis, tethered spinal cord, hearing loss, and hypoplastic left heart syndrome resembling a heterotaxy type of cardiac defect. WES revealed a missense variant in *cilia and flagella-associated protein 99(CFAP99)*, which isn't associated with any disease.

Methods: We utilized loss-of-function assays using CRISPR/Cas9 mediated genome editing in the *Xenopus* model system for functional analysis. We designed two non-overlapping CRISPRs targeting two different exons, generated G0 tadpoles, and verified genomic edits using CRISPR edits analysis inference. Brightfield/fluorescence microscopy, immunohistochemistry, and optical coherence tomography imaging were utilized to detect cardiac and spinal cord defects.

Results: In *Xenopus, the cfap99* knockdown led to two significant phenotypes, heterotaxy and scoliosis, recapitulating

our patient's presentation. Mutant tadpoles had 25.2% cardiac looping vs 1.1% in wild types, suggesting defective left-right body patterning. Abnormal body axis formation was confirmed by examining the transcription factor *pitx2c* expression pattern, which is known to be asymmetric in *Xenopus*. Indeed, when *cfap99* was knocked down, *pitx2c* expression was abnormal, indicating the disrupted left-right patterning. Scoliosis was observed in *cfap99* knockout tadpoles 34.3% vs 1.2%. Further analysis revealed that cfap99 loss caused abnormal cilia development and impaired ciliary motion.

Conclusion: This is the first study that associated CFAP99 with multiple birth defects. Our findings in *Xenopus* model system suggest that *cfap99* variants lead to heterotaxy and scoliosis phenotype, primarily through disrupted cilia morphology and function.

Grants: E.D. - NIH/NICHD R01NS127879.

Conflict of Interest: None declared

P20.012.A Differential isoform regulation in the human brain across regions, development, and between neuropsychiatric disorder cases and controls.

Sofia Kudasheva[1;2], *Nicola Hall*[3], *Paul Harrison*[3;4], *Elizabeth Tunbridge*[3;4;5], *Wilfried Haerty*[1;2]

[1]*Earlham Institute (EI), Norwich, United Kingdom;* [2]*University of East Anglia, School of Biological Sciences, Norwich, United Kingdom;* [3]*University of Oxford, Department of Psychiatry, Oxford, United Kingdom;* [4]*Oxford Health NHS Foundation Trust, Warneford Hospital, Oxford, United Kingdom;* [5]*Boehringer Ingelheim, CNS Discovery Research, Biberach an der Riß, Germany*

Background/Objectives: Neurodevelopmental disorders (NDDs), including schizophrenia, are associated with dysregulated alternative splicing. However, NDD risk genes tend to be lowly expressed and have complex gene models making their isoform identification and quantification challenging with current approaches. This severely impedes the functional interpretation of their variation across tissues, and conditions.

Methods: We applied CaptureSeq target enrichment coupled with nanopore sequencing to characterise and quantify RNA isoform profiles of 1221 protein-coding and lncRNA genes across 52 human post-mortem brain samples: cortex, caudate, and hippocampus from 20 individuals (adult and fetal controls, and 12 adults with a neuropsychiatric diagnosis).

Results: We identified 44,624 unique isoforms (88.4% previously unannotated) and revealed 228 significant changes in isoform proportions (i.e. switches) between control adult brain regions, and 257 between adult and fetal cortex, with some switches predicted to impact the coding potential and protein structure. Out of 17 genes with isoform switches between controls and individuals with schizophrenia, we identify a novel isoform of schizophrenia risk gene CPEB1 that constitutes 94% of the gene's expression in schizophrenia cortex samples vs 47% in controls.

Conclusion: The previously overlooked brain isoform diversity and expression patterns provide us with an opportunity to characterise the functional implications of genetic variation associated with NDDs.

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Conflict of Interest: Sofia Kudasheva: None declared, Nicola Hall: None declared, Paul Harrison: None declared, Elizabeth Tunbridge Full-time employee of Boehringer Ingelheim, Wilfried Haerty: None declared

P20.013.B Uncovering preferential binding between putative promoters and enhancers using a combined capture Hi-C MPRA experiment

Pia Keukeleire[1], *Coline Arnould*[2;3], *Martin Kircher*[1;4], *Nadav Ahituv*[2;3]

[1]*University Hospital Schleswig-Holstein, University of Lübeck, Institute of Human Genetics, Lübeck, Germany;* [2]*University of California, Department of Bioengineering and Therapeutic Sciences, San Francisco, United States;* [3]*University of California, Institute for Human Genetics, San Francisco, United States;* [4]*Berlin Institute of Health at Charité, Exploratory Diagnostic Sciences, Berlin, Germany*

Background/Objectives: Massively Parallel Reporter Assays (MPRAs) have been employed to identify putative regulatory elements in the non-coding genome. While MPRAs offer flexibility in scale and elements tested, common approaches of designing such experiments have inherent shortcomings. Specifically, oligo synthesis inhibits the testing of large elements, and elements are frequently tested with a minimal promoter, disregarding the possibility that some enhancers may only pair with specific promoters.

Methods: To overcome both disadvantages, we designed a capture Hi-C (cHi-C) MPRA experiment that captures 1000 promoters and ~1500 putative enhancers in K562 cells to obtain conjugations of regulatory elements and their target promoters. Results of the cHi-C are sequenced using PacBio HiFi long reads and subsequently tested in a genome integrated manner using a lentivirus-based MPRA (lentiMPRA) in K562 cells.

Results: We were able to measure the activity of 982 promoter-enhancer, 3529 enhancer-enhancer and 16 promoter-promoter interactions. While most promoters interact with their closest enhancer, we found that a putative enhancer close to CUL1 causes high MPRA activity when combined with a more distal putative promoter (~15 kb) for the gene EZH2, as compared to the putative CUL1 promoter (~2kb). We note that both interactions show similar cHi-C abundance and that all three regions are in open chromatin.

Conclusion: Our initial experiment shows that the combination of cHi-C and lentiMPRA experiments overcomes the length limitations of state-of-the-art MPRAs, allows testing enhancers with their target promoter and can allow us to learn about specific enhancer and promoter pairings.

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Conflict of Interest: Pia Keukeleire: None declared, Coline Arnould: None declared, Martin Kircher: None declared, Nadav Ahituv Regel Therapeutics, Regel Therapeutics

P20.014.C Development of the central circadian clock sensitivity to glucocorticoids

Kristian Händler[1], *Varun Sreenivasan*[1], *Violetta Pilorz*[2], *Jon Olano Bringas*[3], *Laura Escobar Castañondo*[4], *Nora Bengoa-Vergniory*[3], *Henrik Oster*[2], *Malte Spielmann*[1;5;6], *Mariana Astiz*[2;7;8]

[1]*Universitätsklinikum Schleswig-Holstein (UKSH), University of Lübeck and University of Kiel, Institute of Human Genetics, Lübeck, Germany;* [2]*Center of Brain Behavior and metabolism (CBBM), University of Lübeck, Institute of Neurobiology, Lübeck, Germany;* [3]*Achucarro Basque Center for Neuroscience, Laboratory of Aggregation and Glial Response, Leioa, Spain;* [4]*Achucarro Basque Center for Neuroscience, Imaging Facility, Leioa, Spain;* [5]*Max Planck Institute for Molecular*

Genetics, Human Molecular Genetics, Berlin, Germany; [6]DZHK e.V. (German Center for Cardiovascular Research), Hamburg, Germany; [7]Achucarro Basque Center for Neuroscience, Laboratory of Circadian Physiology of Neurons and Glia, Leioa, Spain; [8]IKERBASQUE, Basque Foundation for Science, Bilbao, Spain

Background/Objectives: The circadian timing system allows the anticipation of daily recurring changes in the environment improving physiological fitness. In mammals, the circadian system is hierarchically organized, with a master pacemaker located in the hypothalamic suprachiasmatic nuclei (SCN). Glucocorticoid (GC) release from the adrenal gland is under SCN control, peaking before the active phase to synchronize systemically "awake" functions. While the mouse SCN is responsive to GCs during the perinatal period, the adult circuit seems to be resistant.

Methods: sn-RNAseq of mouse SCN at different developmental timepoints and validation via RNAscope and Proximity ligation assay (PLA)

Results: We show that the GC receptor (GR) is down regulated in neurons along SCN maturation, but astrocytes continue to express it. Indeed, astrocytic GR is activated by circadian GCs changes, demonstrating for the first time that the mature SCN receives direct GC-feedback.

Conclusion: Our data provides novel insight into the gain of cellular and transcriptional heterogeneity of the mouse SCN along development and the essential role of SCN astrocytes in timing physiology.

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Conflict of Interest: None declared

P20.015.D Targeting oncogenic *TERT* promoter variants by allele-specific epigenome editing

Alexandra Kouroukli[1], *Nivethika Rajaram[2], Pavel Bashtrykov[2], Helene Kretzmer[3], Reiner Siebert[1], Albert Jeltsch[2], Susanne Bens[1]*

[1]Institute of Human Genetics, Ulm University and Ulm University Medical Center, Ulm, Germany; [2]Institute of Biochemistry and Technical Biochemistry, Department of Biochemistry, University of Stuttgart, Stuttgart, Germany; [3]Computational Genomics, Max Planck Institute for Molecular Genetics, Department of Genome Regulation, Berlin, Germany

Background/Objectives: Silencing dominantly activated oncogenic alleles by addressing driver mechanisms (e.g. genomic alterations) is a promising cancer treatment strategy. Allele-specific epigenome editing (ASEE) reduces gene expression in an allele-specific manner (Rajaram et al. 2023). We investigated ASEE mediated oncogene silencing in cancer by targeting heterozygous *TERT* promoter (*TERT*p) variants (Kouroukli et al. 2023).

Methods: We screened 75 cell lines by Sanger sequencing for *TERT*p sequence variants. Next, we introduced sgRNA-guided dCas9-DNMT3A-3L (ASEE) complexes to selected cancer cell lines (CCLs) by transient transfection and investigated DNA methylation of 30 *TERT*p CpGs upon ASEE. *VEGFA* was investigated as off-target locus control. Afterwards, we assessed *TERT* RNA expression and cell viability.

Results: 53% of the CCLs contained at least one heterozygous variant allowing allele discrimination. We selected Hep-G2 and A-549 CCLs for this proof-of-principle which contained two different heterozygous variants; the activating mutation C228T (core *TERT*p) and the common SNP rs2853669 (THOR), respectively. These variants were targeted by the ASEE complexes. We introduced DNA methylation to the on-target allele in both CCLs with limited off-target allele and off-target locus methylation. We observed maximum CpG methylation gain of 39% and 76% after ASEE in Hep-G2 and A-549, respectively. The epigenome editing translated into reduced *TERT* RNA expression in Hep-G2. We did not observe a specific effect of ASEE on cell survival.

Conclusion: We successfully applied the concept of dominant oncogene inactivation by ASEE in cancer models. This new therapeutic strategy may be especially useful in reducing adverse side effects.

Grants: Baden-Württemberg Stiftung gGmbH ID09

Conflict of Interest: None declared

P20.016.A Exploring novel insights into parathyroid hormone receptors and their potential relevance to migraine

Andreia Dias[1;2;3], *Marta Ferreira[1;4], Mariana Santos[1;2;3], Alda Sousa[1;2;3], Carla Oliveira[1;4;5], Miguel Alves-Ferreira[1;2;3;6], Carolina Lemos[1;2;3]*

[1]i3S - Instituto de Investigação e Inovação em Saúde da Universidade do Porto, Porto, Portugal; [2]Abel Salazar Biomedical Sciences Institute - University of Porto, Porto, Portugal; [3]Instituto de Biologia Molecular e Celular, Porto, Portugal; [4]IPATIMUP - Instituto de Patologia e Imunologia Molecular da Universidade do Porto, Porto, Portugal; [5]Faculdade de Medicina da Universidade do Porto - FMUP, Porto, Portugal; [6]Centro de Genética Preditiva e Preventiva, Porto, Portugal

Background/Objectives: Migraine is a complex neurological disorder subdivided into migraine with aura (MA) or without aura (MO). Familial aggregation of migraine indicates a potential genetic predisposition, however environmental risk factors might contribute to migraine susceptibility. To investigate whether a specific pattern of RNA expression could distinguish between migraine cases (migraineurs) and migraine-free controls, we conducted a whole transcriptome analysis for the first time within a Portuguese cohort.

Methods: We performed total RNA sequencing on whole blood samples from 15 migraineurs and 12 age-matched controls. Differential expression analysis and gene set enrichment analysis (GSEA) were performed in different groups. Finally, we performed the protein-protein interaction (PPI) networks of differentially expressed genes.

Results: GSEA comparing migraineurs with controls highlighted upregulated pathways linked to metabolism, and downregulated immuno-inflammatory pathways. Moreover, the groups of female migraineurs and female MO patients emphasized significant upregulated pathways, including G protein-coupled receptors (GPCRs) signaling pathways, when compared with female controls. Interestingly, we found two important differentially expressed genes related to parathyroid hormone (PTH): *PTH1R* and *PTH2*. *PTH1R* was upregulated in female MO versus female controls, while *PTH2* was both upregulated between female migraineurs and female controls as well as between female MO and controls.

Conclusion: This study highlights an association of class B (secretin family) GPCRs, particularly PTH1R and PTH2, in female migraineurs. These molecules stand out as sturdy and promising biomarkers and/or targets for innovative therapeutic in female migraineurs.

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Conflict of Interest: None declared

P20.018.C Solving undiagnosed disease beyond the exome: Functional enhancers with medical relevance identified by ChIP-STARR-seq in neural cell models and integration with convolutional neural networks allows prioritizing functional non-coding variants from whole genome sequencing studies underlying missing heritability in neurogenetics

Ruizhi Deng[1], Elena Perenthaler[1], Anita Nikoncuk[1], Kristina Lanko[1], Wilfred F. van Ijcken[2], Gennady Roshchupkin[3], Eskeatnaf Mulugeta[4], **Stefan Barakat**[1]

[1]Erasmus MC University Medical Center Rotterdam, Department of Clinical Genetics, Rotterdam, Netherlands; [2]Erasmus MC University Medical Center Rotterdam, Biomics, Rotterdam, Netherlands; [3]Erasmus MC University Medical Center Rotterdam, Department of Epidemiology and Department of Radiology and Nuclear Medicine, Rotterdam, Netherlands; [4]Erasmus MC University Medical Center Rotterdam, Department of Cell Biology, Rotterdam, Netherlands

Consortium: Genomics England Research Consortium

Background/Objectives: Genome-wide assessment of genetic variation is becoming a routine in genetics, but functionally interpreting effects of non-coding variants both in common and rare disease remains extremely challenging.

Methods: Here we employ the massively parallel reporter assay ChIP-STARR-seq to functionally annotate non-coding regulatory element (NCRE) activity >140 thousand genomic sequences in human neural stem cells (NSCs) as brain developmental model.

Results: Highly active NCREs in NSCs show increased sequence constraint, are enriched for transcription factor (TF) binding motifs for YY1 and p53 family members and for primate-specific transposable elements, providing insights in gene regulatory mechanisms. Examination of the same genomic sequences in human embryonic stem cells (ESCs) identified differential NCRE-activity between both cell types, accompanied by rewiring of the epigenome landscape. Based on the experimentally measured NCRE-activity and the nucleotide composition of the assessed DNA sequences, we build a convolutional neural network (CNN) that allows the prediction of NCRE-activity solely based on DNA sequence composition, which can be used to identify functionally relevant nucleotides and TF motifs within each NCRE required for their NCRE function. The application of this detailed NCRE-atlas and its subsequent functional validation allowed prioritizing both SNPs underlying biologically relevant sequences of loci identified in genome-wide association studies for common neurological traits, as well as rare non-coding variants that might be causative in currently genetically unexplained individuals.

Conclusion: We foresee that the NCRE-atlas created here will help resolving missing heritability in human genetics and neurodevelopmental disorders, reducing the search space for functional relevant non-coding genetic variation.

Grants: ZonMw Vidi 09150172110002

Conflict of Interest: None declared

P20.019.D Loss-of-function *EHMT2* missense mutation as the cause of a Kleefstra-like syndrome

Beatriz Martinez-Delgado[1;2;3], *Estrella Lopez-Martín[1;2], Jennifer Kerkhof[4], Beatriz Baladron[1;2], Lidia Mielu[1;2], Diana Sanchez-Ponce[1], Ariadna Bada-Navarro[1], Marina Herrero-Matesanz[1], Lidia López[1;2], Jessica Rzasa[4], Dmitrijs Rots[5], Marta Fernandez Prieto[1], Esther Hernandez-San Miguel[1], Gema Gomez-Mariano[1;2], Purificación Marín Reina[6], Rosario Cazorla-Calleja[2;7], Francisco Javier Alonso[1;2;3], Tjitske Kleefstra[5], Manuel Posada[2], Eva Bermejo-Sanchez[1;2], Bekim Sadikovic[4], María J. Barrero[1;2]*

[1]Institute of Rare Diseases Research (IIER/ISCIII), Madrid, Spain; [2]Undiagnosed Diseases Program SpainUDP, Madrid, Spain; [3]Centro de Investigación Biomédica en Red de Enfermedades Raras, (CIBERER), Madrid, Spain; [4]Verspeeten Clinical Genome Centre, London Health Sciences Centre, Canada; [5]Radboud University Medical Center, Human Genetics, Nijmegen, Netherlands; [6]University and Polytechnic Hospital La Fe, Valencia, Spain; [7]Hospital Universitario Puerta de Hierro, Madrid, Spain

Consortium: SpainUDP

Background/Objectives: Dysregulation of the epigenetic machinery is associated with neurodevelopmental defects in humans. Kleefstra syndrome (KS) is a neurodevelopmental syndrome caused by heterozygous alterations in the gene *EHMT1* that cause loss-of-function. EHTM1 and EHMT2 are highly similar histone methyltransferases that play relevant roles in development. However, to date, *EHMT2* mutations has not been described to cause disease.

Methods: We describe a pediatric patient studied in the Undiagnosed cases Program, SpainUDP, with a KS overlapping phenotype and a single base de novo substitution in *EHMT2* that causes the amino acid change p.Ala1077Ser in the catalytic SET domain. Pathogenicity of this variant was functionally assessed by histone peptide binding, histone methyltransferase, and EpiSign™ episignature assays.

Results: This change was found to produce a reduction in the affinity of the EHMT2 catalytic domain for the H3 tail and in the activity of the enzyme by three- to five-fold. Alterations in histone methylation and gene expression profiles along with a positive EpiSign™ analysis suggest a significant overlap between the *EHMT2* p.Ala1077Ser variant and features described for *EHMT1* mutants causing KS.

Conclusion: We conclude that *EHMT2* haploinsufficiency likely causes a Kleefstra-like syndrome. Although we cannot rule out dominant negative effects caused by the *EHMT2* p.Ala1077Ser variant, our data suggest that loss of functional EHMT2 is probably more detrimental to cells than loss of EHMT1.

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Conflict of Interest: None declared

P20.020.A Functionally equivalent disease-relevant enhancers in the pancreas of Zebrafish and Human

Renata Bordeira-Carriço[1;2], *Joana Teixeira[1;2], Marta Duque[1;2], Mafalda Galhardo[1;2;3], Diogo Ribeiro[1;2], Rafael D. Acemel[4], Panos N. Firbas[4], Juan Jesus Tena-Aguilar[4], Ana Eufrásio[1;2], Joana Marques[1;2], Fábio Ferreira[1;2], Telmo Freitas[1;2], Fátima Carneiro[5;6], Jose Luis Gomez-Skarmeta[4], José Bessa[1]*

[1]Instituto de Biologia Molecular e Celular (IBMC), Porto, Portugal; [2]Instituto de Investigação e Inovação em Saúde (i3S), Porto, Portugal; [3]Instituto de Ciências, Tecnologias e Agroambiente (CIBIO), Porto; [4]Centro Andaluz de Biología del Desarrollo (CABD), Sevilla; [5]Faculdade de Medicina da Universidade do Porto (FMUP), Pathology Department, Porto, Portugal; [6]Instituto de Patologia e Imunologia Molecular da Universidade do Porto (IPATIMUP), Porto, Portugal

Background/Objectives: The pancreas is a central organ whose function, when impaired, is associated with high disease burden. Genome-wide association studies have identified various alleles of

pancreatic dysfunction located in non-coding sequences of DNA. Several of these regions contain epigenetic marks of cis-regulatory elements (CREs) active in pancreatic cells, suggesting that alterations in these regions might have a role in disease. The aim of this study was to find CREs located in non-coding regions of DNA that might be involved in pancreatic diseases.

Methods: To explore the transcriptional regulatory genome of zebrafish pancreas we combined epigenetic assays: ATAC-seq, ChIP-seq, 4C-seq and HiChIP-seq. We compared this data with ChIP-seq data from human pancreatic cell lines and performed reporter assays in both zebrafish and human pancreatic cell lines.

Results: We successfully identified interspecies equivalent CREs, overcoming the lack of sequence conservation of non-coding regions between species. We described a zebrafish *ptf1a* distal-enhancer whose deletion results in pancreatic agenesis during development, supporting the phenotype previously observed in humans and validating the causality of this condition in vivo. We also found a pancreatic enhancer of the tumor-suppressor *ARID1A*, whose deletion decreased ARID1A expression in pancreatic cell lines, potentially heightening the risk for pancreatic cancer.

Conclusions: Our findings demonstrate the evolutionary functional conservation of critical CREs and their essential role in the expression of key genes, with implications in pancreatic diseases. Therefore, this study emerges as a pivotal contribution to the study of the pancreas, supporting future innovative strategies for tackling pancreatic disorders.

Grants: ERC-2015-STG-ERC651602-BPD

Conflict of Interest: None declared

P20.021.B Use of transcriptome analysis as a complementary technology in genetic diagnosis

Marta Carreño[1], *Maria Segura-Puimedon*[1], *Marta de Castro-Miró*[2], *Jairo Rodríguez*[2], *Héctor Díez Nuño*[2], *LLuís Armengol*[1]

[1]*qGenomics, DryLab, Esplugues de Llobregat, Spain;* [2]*qGenomics, R + D, Esplugues de Llobregat, Spain*

Background/Objectives: Nowadays it has become evident that the combination of genomics, transcriptomics and proteomics can be useful to reach a definitive genetic diagnostic. In our work, we aimed at the assessment of the contribution of transcriptomics in reaching a diagnostic for patients with previous inconclusive exome or genome studies.

Methods: A total of eight individuals were evaluated using mRNAseq after an inconclusive result of a genomic analysis. RNA was obtained from different target tissues. In two cases, a heterozygous pathogenic variant had been detected in a gene associated with an autosomal recessive inheritance and the study was performed in search of a second hit. In the 6 remaining cases, the objective was to determine the functional effect of the candidate variants, located in genes with autosomal dominant or X-linked inheritance models: one likely pathogenic missense variant, one missense variant of uncertain significance (VUS), two non-canonical splice site VUS, one synonymous VUS and a complex rearrangement.

Results: In the two first cases no second variants were identified, but underexpression of the target genes was confirmed. Aberrant splicing isoforms were observed in the cases with synonymous and non-canonical splice site variants. The rearrangement causes the appearance of an aberrant isoform of the candidate gene. The remaining cases are still under study.

Conclusion: The application of transcriptomics is crucial to decrypt the effect of different types of variants on disease-associated genes and might help towards a definitive genetic diagnosis.

Grants: Not applicable.

Conflict of Interest: Marta Carreño Full-time, Maria Segura-Puimedon Full-time, Marta de Castro-Miró Full-time, Jairo Rodríguez Full-time, Héctor Díez Nuño Full-time, LLuís Armengol Full-time

P20.022.C Genetic drivers of transcriptomic variation in the individual sepsis response

Katie L Burnham[1], *Alex Tokolyi*[1], *Nikhil Milind*[1], *Wanseon Lee*[1], *Andrew Kwok*[2], *Kiki Cano-Gamez*[1,2], *Yuxin Mi*[2], *Cyndi G Geoghegan*[2], *Charles J Hinds*[3], *Julian C Knight*[2,4], *Emma Davenport*[5]

[1]*Wellcome Sanger Institute, Hinxton, United Kingdom;* [2]*Centre for Human Genetics, University of Oxford, Oxford, United Kingdom;* [3]*Centre for Translational Medicine and Therapeutics, William Harvey Research Institute, Faculty of Medicine and Dentistry, Queen Mary University of London, London, United Kingdom;* [4]*Chinese Academy of Medical Sciences (CAMS) Oxford Institute (COI), University of Oxford, Oxford, United Kingdom;* [5]*Wellcome Sanger Institute, Wellcome Genome Campus, Hinxton, United Kingdom*

Consortium: GAinS Investigators

Background/Objectives: Sepsis is a clinical syndrome caused by a dysregulated response to infection, where inter-individual heterogeneity is a major obstacle to understanding pathophysiological mechanisms and developing immunomodulatory treatments. Here, we aimed to investigate how host genetic variation affects the sepsis transcriptomic response.

Methods: Using genome-wide genotyping and gene expression data on 997 adult sepsis patients, we estimated the contribution of common genetic variation (GCTA) to previously defined sepsis endotypes. We mapped expression, splicing, and co-expression module quantitative trait loci (QTL), and through comparisons to healthy cohorts (mashr) identified sepsis-dependent regulatory effects, as well as colocalisation events with relevant GWAS associations. Finally, we tested QTLs for interactions between genotype and sepsis endotype, and incorporated transcription factor motifs (SNP2TFBS), predicted regulon activity (DoRothEA), and cell proportions (CIBERSORTx) to prioritise candidate upstream drivers.

Results: We found common variants were a significant contributor ($p = 0.019$) to sepsis endotype, and identified QTLs for 10,618 genes (eQTLs), 9,498 splice phenotypes (sQTLs), and 30 co-expression modules (modQTLs). We found 10.5% of eQTLs and 15.7% of sQTLs also tested in healthy cohorts had larger effects in sepsis, demonstrating the importance of context in regulatory variant interpretation. We additionally identified 1,578 genotype-endotype eQTL interactions, and from these inferred upstream regulators including HIF1A and CEBPB. These factors were correlated with progenitor and immature neutrophil subsets, further implicating glycolysis and emergency granulopoiesis in the poor outcome sepsis endotype.

Conclusion: Our combinatorial approach has identified putative mechanistic links between host genetic variation, cell subtypes, and the individual transcriptomic sepsis response.

Conflict of Interest: None declared

P20.025.B FOSL1 as a potential target in triple-negative breast cancer

Finja Hennig[1], *Thea Reinkens*[1], *Vera Schäffer*[1], *Marlies Eilers*[1], *Amelie Stalke*[1], *Brigitte Schlegelberger*[1], *Thomas Illig*[2], *Britta Skawran*[1]

[1]*Hannover Medical School, Department of Human Genetics, Hannover, Germany;* [2]*Hannover Medical School, Hannover Unified Bank (HUB), Hannover, Germany*

Background/Objectives: Breast cancer (BC) is the most common malignancy worldwide with the highest number of cancer-related deaths in women. Triple-negative breast cancer (TNBC) represents 15-20% of all BCs and is characterized by high aggressiveness, early onset and a poor prognosis. Aberrant expression of microRNAs (miRs), such as downregulated hsa-miR-449a-5p (miR-449a), plays a significant role in cancer. This study aimed to explore new target genes of miR-449a in TNBC and assess their therapeutic potential.

Methods: Ago2 RNA immunoprecipitation (Ago-RIP) followed by sequencing was performed to identify new target mRNAs of miR-449a. TNBC cell lines HCC38 and HCC1937 were transiently transfected with both miR-449a mimics and siRNA against the potential target gene FOSL1. Regulation of FOSL1 by miR-449a was validated by luciferase reporter assay and expression analyses. Tumor-relevant functional effects of FOSL1 were analyzed using in vitro assays.

Results: Ago-RIP-Seq revealed FOSL1 as a potential target gene of miR-449a. Treatment with miR-449a mimics reduced FOSL1 expression at both mRNA and protein levels, and the luciferase reporter assay validated regulation of FOSL1 by miR-449a. Knockdown of FOSL1 inhibited migration in both TNBC cell lines and increased apoptosis in one cell line.

Conclusion: FOSL1 emerges as a promising therapeutic target directly regulated by miR-449a, exhibiting a tumor-suppressive effect by mainly inhibiting migration. Further experiments will identify the cell signaling pathways altered by FOSL1.

Grants: -

Conflict of Interest: None declared

P20.026.C SUPT5H loss-of-function variants as modifying factor in beta-thalassemia trait

Tamara Koopmann[1], *Ahlem Achour*[2], *Nik Mohd Hasan*[1], *Jan Traeger-Synodinos*[3], *Jelmer Legebeke*[1], *Cornelis Harteveld*[1]

[1]*Leiden University Medical Center, Clinical Genetics, Leiden, Netherlands;* [2]*Charles Nicolle Hospital, Department of congenital and hereditary diseases, Tunis, Tunisia;* [3]*St. Sophia's Children's Hospital, Choremeio Research Laboratory, Athens, Greece*

Background/Objectives: Recently it was discovered that carriers of *SUPT5H* null-variants express a hematology resembling beta-thalassemia, including microcytic hypochromia and elevated HbA2. In this study we compare the hematologic data to carriers of beta-thalassemia and carriers of β-thalassemia with haplo-insufficiency of *SUPT5H*.

Methods: The Hb, MCV, MCH and HbA2 values of approx. 40 carriers of 22 different *SUPT5H* null-variants were compared to 15 carriers of beta0-thalassemia, 15 carriers of beta + -thalassemia and 9 beta-thalassemia intermedia patients, using one way ANOVA to determine whether there are significant statistical differences of each parameter between these 3 groups. The same was done between the group of compound heterozygotes SUPT5H/beta-thal (N = 3) with beta-thalassemia intermedia (N = 9).

Results: In the comparison between *SUPT5H* carriers vs Beta0 thal carriers, there are significant differences in terms of Hb, MCV and MCH, but no difference in HbA_2%. There are no mean differences between all groups for Hct and RBC level. In the comparison between *SUPT5H* vs beta + -thal there are no significant differences. Comparing beta-thal intermedia to compound heterozygotes *SUPT5H*/beta-thal it appears there is a higher Hb, but comparable Ht, MCV and MCH, suggesting a comparable hematology. However, the comparison of HbA_2 levels is significantly different.

Conclusion: By comparing red cell indices amongst different groups of beta0- and beta + -thal carriers and beta-thal intermedia patients, more insight into the exact clinical phenotype was obtained. The elevated HbA2 levels of 10.7% (SD = 2.0%) could facilitate the diagnosis of *SUPT5H*/beta-thal combinations in beta-thal carriers expressing clinically as beta-thalassemia intermedia and contribute to the discovery of new *SUPT5H* variants.

Conflict of Interest: None declared

P20.027.D Characterising the Genetic Basis of Neonatal Immune development through pQTL Mapping in Human Neonates.

Pauline Kuschel[1;2], **Alessandro Mattia**[1;2], *Barbara Puzek*[1;2], *Theresa Haslbeck*[1;2], *Tuguldur Tumurbaatar*[1;2], *Johannes B. Mueller-Reif*[3], *Lou Wackerbarth*[4], *Raffaele Conca*[1], *Matthias Mann*[3], *Markus Sperandio*[4], *Roland Immler*[4], *Christoph Klein*[1], *Susanne Pangratz Fuehrer*[1;3], *Claudia Nussbaum*[1], *Sarah Kim-Hellmuth*[1;2]

[1]*Dr. von Hauner Children's Hospital, University Hospital LMU Munich, Department of Pediatrics, Munich, Germany;* [2]*Institute of Translational Genomics, Helmholtz Munich, Department of Computational Health, Munich, Germany;* [3]*Max-Planck-Institute of Biochemistry, Proteomics and Signaltransduction, Martinsried, Germany;* [4]*Institute of Cardiovascular Physiology and Pathophysiology, Walter Brendel Center of Experimental Medicine, Ludwig-Maximilians University Munich, Munich, Germany*

Consortium: PILOT Consortium

Background/Objectives: The occurrence of complicated neonatal infections is still linked to long-term health complications and mortality. Addressing this, this study aims to fill the knowledge gap in the genetic basis of protein expression and its regulatory effect on the immune cell development, which has been extensively explored in adult populations but remains under-researched in neonates.

Methods: Protein quantitative trait loci (pQTL) were mapped in 383 samples of the Munich Preterm and Term Clinical (MUNICH-PreTCl) birth cohort. Mass spectrometry-based proteomic analysis was conducted from dried blood spots (DBS) to assess both, cellular and secreted, protein profiles. Genotyping was performed using the Illumina Global Screening Array chip. Missing protein expression values were imputed using the R package missForest, genotype imputation was performed via the Munich Imputation server, followed by QTL mapping using TensorQTL.

Results: Quality control procedures applied to genotype and proteomic data yielded 5,610,609 Single Nucleotide Polymorphisms and 964 proteins. 121 *cis* pQTLs were identified (p-value < 0.05), associated with biological processes such as neutrophil degranulation and complement activation. While pQTLs of secreted proteins could be replicated in existing plasma pQTL studies from adults, pQTLs of cellular proteins were often missed.

Conclusions: This study highlights the pivotal role of pQTL mapping in understanding the genetic influence on neonatal immune mechanisms and potential avenues for therapeutic interventions. Furthermore, it demonstrates the potential of DBS samples in functional genetics studies expanding the scope from plasma proteins to cellular protein profiles using miniscule sample volumes.

Grants: CRC/TRR 359 Perinatal Development of Immune Cell Topology

Conflict of Interest: None declared

P20.028.A Identification of *cis*-expression quantitative trait methylation (eQTMs) in saliva

Alba Hernangomez-Laderas[1;2], *Ariadna Cilleros-Portet*[1;2], *Sergi Marí*[1;2], *Bárbara P. González-García*[1;2], *Ane Arregi*[1;3;4], *Amaia Irizar*[1;3;4], *Iraia García-Santisteban*[1;2], *Corina Lesseur*[5], *Nora Fernandez-Jimenez*[1;2], *Jose Ramon Bilbao*[1;2;6]

[1]*University of the Basque Country (UPV/EHU), Department of Genetics, Physical Anthropology and Animal Physiology, Leioa, Spain;* [2]*Biobizkaia Health Research Institute, Barakaldo, Spain;* [3]*Biogipuzkoa Health Research Institute, Donostia, Spain;* [4]*Center for Biomedical Research in Epidemiology and Public Health Network (CIBERESP), Madrid, Spain;* [5]*Icahn School of Medicine at Mount Sinai, Department of Environmental Medicine and Public Health, New York, United States;* [6]*Center for Biomedical Research in Diabetes and Associated Metabolic Disorders (CIBERDEM), Madrid, Spain*

Background / Objectives: Saliva is an easily accessible tissue containing biomarkers that have proven useful for diagnosing both local and systemic diseases. *Cis*-expression quantitative trait methylation (*cis*-eQTM) studies, which identify the association between DNA methylation (DNAm) at CpG sites and the expression of nearby genes, may provide insights into the molecular mechanisms underlying variant associations with complex traits.

Methods: Using DNAm (Illumina EPIC array) and gene expression (exome capture RNA-seq) data from saliva of general population adult donors ($n = 100$) we calculated *cis*-eQTMs located 1 Mb up- and downstream gene start positions with the MatrixEQTL R package, including as covariates age, sex, RNA-seq batch, 5 genotype-PCs, 20 DNAm PCs and 8 expression PEER (probabilistic estimation of expression residuals) factors. Pathway enrichment analysis of the genes identified was performed using Enrichr.

Results: We identified 371 CpG-gene pairs ($P < 5.0 \times 10^{-8}$), involving 144 CpGs and 152 genes, with an average distance between CpG site and gene of 183 ± 240 Kb. CpGs are located upstream ($n = 168$) or downstream ($n = 203$) of the gene start site, as well as in gene body ($n = 48$). A positive correlation between DNAm and gene expression was observed in 198 eQTMs, while 173 show a negative association. Out of the 24 enriched pathways found "Antigen processing and presentation" was the most significant ($P = 1.09 \times 10^{-5}$).

Conclusion: We have constructed a useful catalog of saliva eQTMs to help uncover the biological mechanisms behind complex traits.

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00209.

Conflict of Interest: None declared

P20.029.B DNA methylation surrogates link childhood cognitive and non-cognitive development to socioeconomic status and lifespan: a multi-cohort epigenome-wide study

Giovanni Fiorito[1], *Giorgia Menta*[2], *Paolo Uva*[1], *Simone Ghislandi*[3], *Conchita D'Ambrosio*[4]

[1]*IRCCS Istituto Giannina Gaslini, Clinical Bioinformatics Unit, Genoa, Italy;* [2]*Luxembourg Institute of Socio-Economic Research (LISER), Luxembourg, Luxembourg;* [3]*Bocconi University, Department of Political Sciences, Milan, Italy;* [4]*Luxembourg University, Economics, Luxembourg, Luxembourg*

Background/Objectives: The accumulation of cognitive and non-cognitive skills over childhood and adolescence has been shown to predict better economic and health outcomes later in life. Accurately assessing developmental trajectories during childhood is thus essential for early identification and intervention on those experiencing slower development.

Methods: We leveraged DNA methylation data from 1,000 UK individuals across childhood (birth, 7 years, 15-19 years) to develop epigenetic surrogates for cognitive and non-cognitive pace of development (*DNAmPoD*). We validated its predictive power in independent childhood cohorts from the Netherlands, US, and Mexico (N>3,000 children) and adulthood cohorts from the UK and Italy (N>3,000 adults).

Results: *DNAmPoD* significantly associated with various cognitive and emotional outcomes in children. In adults, higher *DNAmPoD* scores predicted higher educational attainment, better job positions, and lower all-cause mortality. Gene set enrichment analysis revealed consistent involvement of pathways and gene ontologies crucial for brain development, including "*Regulation of neuron projection development*", "*Synapse organization*", and "*Dendrite development*". We also identified hub genes like *TIAM1*, *CYFIP1*, and *BAIAP2*, known for their roles in early-life brain development supporting the biomolecular relevance of the newly developed biomarker.

Conclusion: This large-scale study establishes *DNAmPoD* as a robust epigenetic surrogate for cognitive and non-cognitive development useful for early identification of children experiencing slower development. Unlike traditional methods requiring repeated measurements over time, *DNAmPoD* requires one data point per child, regardless of age. Eliminating the need for years of data collection, *DNAmPoD* potentially overcome traditional assessment of developmental trends.

Grants: Fonds National de la Recherche Luxembourg (Grant C19/SC/13650569/ALAC).

Conflict of Interest: None declared

P20.030.C Genetic regulation of single-cell personal gene correlations (co-eQTLs) is highly enriched for GWAS variants

Corinna Losert[1], *Dan Kaptijn*[2], *Maryna korshevniuk*[2], *Roy Oelen*[2], *Harm-Jan Westra*[2], *Monique van der Wijst*[2], *Marc Jan Bonder*[2], *Matthias Heinig*[1], *Lude Franke*[2]

[1]*Helmholtz Munich, Institute of Computational Biology, Oberschleißheim, Germany;* [2]*University Medical Center Groningen, Genetics Department, Groningen, Netherlands*

Consortium: sc-eQTLGen Consortium

Background/Objectives: Genes are known to operate in regulatory networks, and recently, we have shown that genetic variants can affect these in a cell-type specific manner through so-called co-expression QTLs (co-eQTLs). These co-eQTLs may help in interpreting the upstream regulators of genetic variants that have been associated with disease. However, identifying co-eQTLs requires large sample sizes which limited our previous study. Here we leverage the single-cell datasets consolidated by the sc-eQTLGen consortium to extend those analyses.

Methods: We developed a pipeline that enables efficient and systematic co-eQTL mapping in a federated and privacy sensitive way and applied it on 4 datasets of the sc-eQTLGen consortium including 1,188 PBMC samples.

Results: We identified co-eQTLs across major cell-types. In the largest dataset (oneK1K) we observed 1,045 co-eQTLs for CD4 + T cells involving 487 unique genes and 157 unique SNPs. Associated genes were enriched in coherent functions. Furthermore, co-eQTL SNPs were approximately twice as often associated with complex traits and diseases (from the GWAS catalog) as eQTL SNPs not showing a co-eQTL effect ($p = 0.001$). The other datasets showed high agreement of the identified co-eQTLs (Rb>0.67).

Conclusion: Our results indicate that there are wide-spread personal and cell-type specific differences in genetic regulation.

Application of our pipeline to all datasets of the sc-eQTLGen Consortium will enable further validation and extension of identified co-eQTLs laying the foundation for building cell-type specific gene regulatory networks. Additionally, the GWAS enrichment results suggest that co-eQTLs can be useful for the interpretation of disease mechanisms.

Grants:

Conflict of Interest: None declared

P20.031.D Unraveling the Role of Cytosolic tRNAs in Human Mitochondria

Sarah Emser[1;2], *Julia Arapovic*[3], *Felix Sternberg*[3], *Eva Millesi*[1], *Ralf Steinborn*[2]

[1]*University of Vienna, Department of Behavioural and Cognitive Biology, Vienna, Austria;* [2]*University of Veterinary Medicine Vienna, VetCore, Vienna, Austria;* [3]*University of Vienna, Department of Nutritional Sciences, Vienna, Austria*

Background/Objectives: Communication between the nucleus and mitochondria, mediated by anterograde and retrograde signalling, is vital for cellular function, with anterograde communication regulating nuclear genes encoding mitochondrial proteins and retrograde signaling influencing nuclear gene expression. One facet of the molecular interplay is the import of cytosolic tRNAs into mitochondria, yet its impact is not sufficiently substantiated. Here we investigated the presence and potential function of cytosolic tRNAs in human mitochondria by emphasising on their involvement in mitochondrial translation of Mitochondrial Derived Peptides (MDPs), resulting from an expanded coding repertoire of the mitochondrial genome.

Methods: To identify actively translated mitochondrial sequences, we screened public mitoplast-derived RNA-sequencing (RNA-seq) data for cytosolic tRNA(Arg) species, performed ribosome profiling and screened public mass spectrometry data to identify peptide fragments indicating an involvement of cytosolic tRNA in mitochondrial translation.

Results: The involvement of cytosolic tRNA(Arg) species in mitochondrial translation is highly indicated by RNA-seq data produced from isolated mitoplasts and non-canonical peptide fragments uncovered by mass spectrometry. In the near future, we will confirm this route of retrograde communication by running Reverse Transcription Quantitative PCR (RT-qPCR) assays and by identifying mitochondrial sequences being active on mitoribosomes.

Conclusion: The dynamic interplay between the nucleus and mitochondria is crucial for cellular function. The detection of cytosolic tRNAs in human mitochondria suggests their involvement in mitochondrial translation and MDP generation, sheds light on mitochondrial protein synthesis mechanisms and prompts further investigation into their role in cellular physiology and disease pathogenesis.

Grants: The study was supported by resources of the University of Vienna.

Conflict of Interest: None declared

P20.032.A Transcriptional regulation of genes throughout the cell cycle

Jennifer Herrmann[1;2], *Hangpeng Li*[1], *Nigel Roberts*[1], *Matthew Gosden*[1], *Kevin Clark*[1], *Emily Georgiades*[1], *Elisa Barrow-Molina*[1], *Simone Riva*[1], *James Davies*[1], **Robert Beagrie**[2], *Jim R Hughes*[1]

[1]*MRC Weatherall Institute of Molecular Medicine, Radcliffe Department of Medicine - Oxford University, Oxford, United Kingdom;* [2]*Centre for Human Genetics, University of Oxford, Oxford, United Kingdom*

Background/Objectives: The cell cycle is an essential and ubiquitous cellular process, and its disruption is linked to the pathogenesis of many common diseases, including cancer, cardiovascular and immunological conditions. Hundreds of mammalian genes exhibit periodic, cell cycle-dependent expression. We set out to ask which cis-regulatory elements control cell-cycle genes and whether mutations in these regions may contribute to the genetic basis of common disease.

Methods: We performed single-cell RNA-seq on mouse embryonic stem cells to identify genes whose expression varied through the cell cycle. We employed Capture-C to link these genes to putative regulatory elements and to examine the conservation of these elements between mouse and human. We use chromatin immunoprecipitation to follow the activity of regulatory elements through the cycle. Finally we use genome editing to validate non-coding GWAS variants that regulate expression of cell-cycle genes.

Results: We find hundreds of genes that exhibit periodic expression during the cell cycle, and show that these genes interact with distal enhancer elements. However, these enhancers do not exhibit periodic fluctuations in histone marks characteristic of enhancer activity, or periodic recruitment of transcription factors. Finally, we show that a SNP associated with a red blood cell phenotype (MCV) regulates the expression of the cell cycle gene *CCND3* in human red blood cells.

Conclusion: We show that distal enhancer elements are important regulators of cell cycle genes. Variants in these non-coding regions change expression of cell cycle genes in specific cell types and may thereby influence human phenotypes and diseases.

Grants: RAB (224135/Z/21/Z)
JRH (MC_UU_00016/14 and 225220/Z/22/Z)
JCH (108861/B/15/Z)

Conflict of Interest: Jennifer Herrmann: None declared, Hangpeng Li: None declared, Nigel Roberts: None declared, Matthew Gosden: None declared, Kevin Clark: None declared, Emily Georgiades: None declared, Elisa Barrow-Molina: None declared, Simone Riva: None declared, James Davies J.O.J.D. is a co-founder of Nucleome Therapeutics and he provides consultancy to the company. He also holds a patent for the MCC method, which is licensed to the company., Robert Beagrie R.A.B. holds a patent on 'Genome Architecture Mapping' (PCT/EP2015/079413)., Jim R Hughes J.R.H. is the founder, director, shareholder, and paid consultant of Nucleome Therapeutics. J.R.H. holds patents for Capture-C (WO2017068379A1, EP3365464B1, US10934578B2).

P20.033.B Uncovering early signs preceding disease-onset in a zebrafish model of rare tubulinopathy exhibiting untreatable neurodegeneration

Martina Venditti[1], *Graziamaria Paradisi*[1], *Giulia Fasano*[1], *Catia Pedalino*[1], *Marion Rosello*[2], *Filippo Del Bene*[3], *Marco Tartaglia*[1], **Antonella Lauri**[1]

[1]*Ospedale Pediatrico Bambino Gesù, IRCCS, Molecular genetics and functional genomics, Rome, Italy, Italy;* [2]*Institut Curie, PSL Research University, INSERM U934, CNRS UMR3215, Paris, France;* [3]*Sorbonne Université, INSERM, CNRS, Institut de la Vision, Paris, France*

Background/Objectives: Tubulinopathies are untreatable encephalopathies caused by mutations affecting microtubules dynamics, often showing early-onset neurodegeneration, progressive motor regression, spasticity and early death, representing a major burden for patients and society. However, contrary to well-recognized spinal muscular atrophy (SMA) and other motoneuron diseases (MNDs) with high incidence and decades of research,

these conditions remain overlooked. The absence of readily accessible in vivo models and thereby the lack of insights into the patho-mechanisms, leave them without effective drug policy. Within a functional genomics pipeline dedicated to orphan diseases, we established transient and stable genetic models in zebrafish recapitulating one of the condition caused by rare inherited loss of function (LoF) mutations affecting tubulin chaperons.

Methods: Here, we present the first results from setting-up gentle single-embryo genotyping to sub-cellular, cellular and whole-organism phenotyping in CRISPR-Cas9 mutants, obtained employing morphometric and behavioral analysis as well as super-resolution imaging.

Results: Our work ***a***. proposes the involvement of spinal motoneurons in the progressive manifestation of the disease; ***b***. demonstrates failure to thrive, progressive neuromuscular features resembling SMA; and ***c***. traces back the neurodegenerative traits to early signs already detectable in pre-symptomatic stages, including impaired microtubules dynamics in growing neurites.

Conclusion: By addressing a previously invisible disease, the work answers the EU-call for promoting equality with respect to access to diagnosis and offers a valuable in vivo vertebrate resource of a previously neglected MND, highlighting potential developmental windows for early diagnosis as well as targetable features preceding the disease onset.

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Conflict of Interest: None declared

P20.034.C Genetic, Molecular and Mouse Model Investigations of Neurodevelopmental Impact of Deleterious Variants of the TREX Transcription-mRNA Export Complex Subunits.

Jozef Gecz[1], *Rudrarup Bhattacharjee*[1], *Elizabeth Palmer*[2], *Alison Gardner*[1], *Renee Carroll*[1], *Ing Chee Wee*[1], *Danielle Mazurkiewicz*[1], *Luis Pérez-Jurado*[3], *Tarin Ritchie*[1], *Mark Corbett*[1], *Clare van Eyk*[1], *Sandra Piltz*[1], *Melissa White*[1], *Lyndsey Collins-Praino*[1], *Lachlan Jolly*[4], *Paul Thomas*[1], *Mike Field*[5], *Raman Kumar*[1]

[1]*University of Adelaide, Medicine, Adelaide, Australia;* [2]*University of New South Wales, Sydney, Australia;* [3]*Universitat Pompeu Fabra, Barcelona, Spain;* [4]*University of Adelaide, Biomedicine, Adelaide, Australia;* [5]*Hunter Genetics, Sydney, Australia*

Consortium: THOC Clinical Study Group

Background/Objectives: TREX (Transcription-mRNA Export) complex is a multi-subunit protein complex, conserved in all eukaryotes and implicated in neurodevelopmental disorders. We sought to identify the molecular, cell and developmental impacts of mutations of TREX subunits using patient cells, and a novel mouse model.

Methods: Through international collaboration we identified and studied 60 THOC2 variants, and novel variants in THOC6 (OMIM 615403), THOC7 and ALYREF (novel disease genes) that were predicted to be deleterious in children with variable clinical and neurodevelopmental outcomes. To gain further insight into the impact of TREX disruption on early neurodevelopment, we generated a mouse line with deletion of Thoc2 Exon37-38, modelled on a patient with ID, speech delay, hypotonia, and microcephaly.

Results: THOC2 protein stability was reduced in patient cells with missense variants, and splicing and deletion variants predominantly truncate the C-terminal RNA binding domain. Brain development, pre-natal survival and synaptic and electro-physiological properties of cortical neurons were impacted in the Thoc2 syndrome mouse. These were underlined by increased R-loop accumulation, DNA damage and consequent cell death that was comparable to the DNA damage of cells of patients with THOC2, THOC6, THOC7 and ALYREF variants.

Conclusion: Perturbed R-loop homeostasis, in stem cells and/or differentiated cells (i.e., neurons) in mice and human, and DNA damage-associated functional, i.e., trancriptional and proteomics alterations are at the root of THOC2/TREX syndromes. THOC2 and the TREX complex are therefore major, conserved players in the efficient maintenance of DNA integrity, a process which is essential for normal brain development and function in neurodevelopment and also neurodegeneration.

Grants: NHMRC 1163240

Conflict of Interest: None declared

P20.035.D Hypothalamic miRNA's profile revealing the pubertal onset control

Maria Morrou[1], *Pavlos Fanis*[1], *Marios Tomazou*[2], *Hend Abdulgadr M Alghol*[1], *Vassos Neocleous*[1], *Leonidas A Phylactou*[1]

[1]*The Cyprus Institute of Neurology and Genetics, Department of Molecular Genetics, Function and Therapy, Nicosia, Cyprus;* [2]*The Cyprus Institute of Neurology and Genetics, Department of Bioinformatics, Nicosia, Cyprus*

Background/Objectives: Puberty is defined as the transition period from childhood to adulthood. The onset of puberty is characterized by the pulsatile release of gonadotropin-releasing hormone (GnRH), marking the activation of the hypothalamic-pituitary-gonadal axis. Regulation of puberty onset includes a complex network of genetic factors, controlling the hypothalamic GnRH expression. MicroRNAs (miRNAs), small non-coding RNAs, are an emerging regulatory element of post-transcriptional activity on targeted mRNAs. In this study, we investigated in female mice during puberty the hypothalamic miRNA profile.

Methods: Female C57BL/6 mice of three ages of postnatal day (PND) – PND14, PND35, PND56 – representing the pre-pubertal, pubertal and post-pubertal developmental stage respectively, were used. Hypothalamic RNA isolation and miRNA's expression profile was acquired by next generation sequencing (NGS). Bioinformatic analysis was performed and pathway enrichment analysis was conducted as well as validation analysis by quantitative real-time PCR.

Results: Bioinformatic analysis revealed differential expression and occurred predominantly at the onset of puberty. Stabilized increased and decreased patterns had an upregulation and downregulation of expression respectively, between PND14 and PND56 stages, followed by stabilization of expression. Those two patterns included the majority of differentially expressed miRNAs. Validation experiments confirmed the expression patterns for selected miRNAs, while pathway enrichment analysis presented several transcriptional and post-transcriptional regulation processes.

Conclusion: A clear involvement of miRNAs in the switch from juvenile to puberty is observed and highlights the importance of epigenetic regulation. Further analyses are needed for the delineation of the signalling pathways in which miRNAs are implicated during several pubertal disorders.

Grants: Telethon Cyprus

Conflict of Interest: None declared

P20.036.A Widespread evolutionary conservation of gene regulatory elements in absence of direct sequence conservation

Mai Phan[1;2], Tobais Zehnder[2], Fiona Puntieri[2], Martin Vingron[2], **Daniel Ibrahim**[1;2]

[1]Berlin Institute of Health, Center for Regenerative Therapies, Berlin, Germany; [2]Max-Planck-Institut für molekulare Genetik, Berlin, Germany

Background/Objectives: Gene regulation is deeply conserved between species. Evolutionary conservation of non-coding elements (CNEs) is used to identify functional relevant regions, which when mutated in patients can underlie genetic disease.

However, many regulatory elements identified by chromatin marks are not conserved between mammals. This is particularly true for heart enhancers, even though the key genes driving heart development are conserved even between flies and human. This disparity could be attributed to algorithms based on sequence alignment, which might not faithfully detect functionally conserved regulatory sequences. Here, we develop a new algorithm and detect thousands of previously undetected CNEs.

Methods: We performed HiC-, ATAC-, ChIP- and RNA-seq from murine and chicken embryonic hearts and identified heart-specific cis-regulatory elements. A novel synteny-based algorithm, Interspecies Point Projection (IPP), identifies a novel class of indirectly conserved (IC) regions. We validate IC-regions using machine-learning models and binding site (TFBS) analysis. Finally, we perform in vivo enhancer reporter assays of IC-elements.

Results: IPP outperforms alignment-based methods, identifying 2-15 times more CNEs. Validation using epigenomic data shows that IC regions maintain active chromatin marks. Machine-learning models and TFBS-analysis show maintenance of shared TFBS despite high sequence divergence. In vivo validation of *all* tested enhancers confirms that IC-regions drive identical expression patterns and thereby represent bona fide homologous enhancers.

Conclusion: IPP dramatically increases the number of CNEs. IPP can be used to identify human-mouse conserved regions and explore which features (e.g. TFBS) are crucial to maintain functionality and guide interpretation of non-coding sequence variants in humans.

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Conflict of Interest: None declared

P20.037.B Cross-tissue cross-species analyses of epigenomes in *CHD8*-related neurodevelopmental disorder provides novel mechanistic insights

Evgenii Martirosian[1], *Terence Garner[2], Michael Levy[3], Conor Mohan[4], Molly Godfrey[5], Adam Stevens[2], Bekim Sadikovic[3;6], M. Albert Basson[4;7;8], Siddharth Banka[1;9]*

[1]The University of Manchester, Division of Evolution, Infection and Genomics, School of Biological Sciences, Faculty of Biology, Medicine and Health, Manchester, United Kingdom; [2]The University of Manchester, Division of Developmental Biology and Medicine, Faculty of Biology, Medicine and Health, Manchester, United Kingdom; [3]London Health Sciences Centre, Verspeeten Clinical Genome Centre, London, Canada; [4]King's College London, Centre for Craniofacial and Regenerative Biology, London, United Kingdom; [5]Manchester Centre for Genomic Medicine, St Mary's Hospital, Manchester University NHS foundation Trust, Health Innovation Manchester, Manchester, United Kingdom; [6]Western University, Department of Pathology and Laboratory Medicine, London, Canada; [7]University of Exeter Medical School, Clinical and Biomedical Sciences, Exeter, United Kingdom; [8]King's College London, MRC Centre for Neurodevelopmental Disorders, London, United Kingdom; [9]Manchester University NHS Foundation Trust, Manchester Centre for Genomic Medicine, St Mary's Hospital, Manchester, United Kingdom

Introduction: *CHD8*-related neurodevelopmental disorder (NDD) is amongst the commonest forms of monogenic autism. We have previously shown the diagnostic utility of *CHD8*-NDD episignature. Here, we examine if peripheral blood DNAm changes can provide mechanistic insights into a disorder that primarily affects the brain.

Methods/Results: From *CHD8*-NDD patient blood samples we identified 2,319 differentially methylated points (DMPs) representing 69 hyper- and 1,058 hypomethylated genes and found them to be enriched for gene ontology (GO) terms "Voltage-gated calcium channel complex" and "Calcium channel complex". To investigate if these results could be mechanistically relevant, we performed reduced representation bisulphite sequencing on *Chd8*$^{+/-}$ P5 mouse neocortex and identified 13,358 DMPs representing 2,311 hyper- and 1,665 hypomethylated genes and found them to be enriched for disease/tissue relevant GO terms "Synapse organisation", "Axon development" and "Regulation of trans-synaptic signalling". Comparison of human and mouse data showed that 114 genes had concordant hypo- or hypermethylated DMPs in both datasets. Further analysis revealed 65 differentially methylated CpG islands (CpGi) in *Chd8*$^{+/-}$ P5 mouse neocortex, including 17/65 on the X chromosome and 19/65 associated with OMIM-morbid genes (9 linked with autism). We did not find any probes in the corresponding human CpGis to be similarly differentially methylated in peripheral blood of patients.

Conclusion: These results indicate that *CHD8* haploinsufficiency disrupts epigenetic state of autism-associated genes; the epigenomic disruption of X chromosome may explain why published *CHD8*-NDD cohorts are enriched for males; and the peripheral blood epigenome may have some, but only limited, value in mechanistic studies of *CHD8*-NDD.

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Conflict of Interest: None declared

P20.038.C Assessing splicing related variants within *SDHB* using a minigene

Anni Köhler[1;2], *Alexandra A. Baumann[1;3], Andrés Cruz Garcia[1;2], Anja Richter[1;2], Susan Richter[4], Doreen William[1;5], Evelin Schröck[1;2;6], Arne Jahn[1;2;6]*

[1]Institute for Clinical Genetics, University Hospital Carl Gustav Carus at and Faculty of Medicine of TUD Dresden University of Technology, ERN-GENTURIS, Hereditary Cancer Syndrome Center, Dresden, Germany; [2]National Center for Tumor Diseases (NCT), NCT/UCC Dresden, a partnership between German Cancer Research Center (DKFZ), Faculty of Medicine and University Hospital Carl Gustav Carus, TUD Dresden University of Technology and Helmholtz-Zentrum Dresden-Rossendorf (HZDR), Dresden, Germany; [3]Department of Systems Biology and Bioinformatics, University of Rostock, Germany; [4]Institute for Clinical Chemistry and Laboratory Medicine, University Hospital Carl Gustav Carus at TUD Dresden University of Technology and Faculty of Medicine of TUD Dresden University of Technology, Dresden, Germany; [5]Core Unit for Molecular Tumor Diagnostics (CMTD) at National Center for Tumor Diseases (NCT), NCT/UCC Dresden, Germany; [6]German Cancer Research Center (DKFZ), Heidelberg, Germany

Consortium: German Cancer Consortium (DKTK), Dresden, Germany

Background/Objectives: Germline and somatic loss-of-function variants in Succinate dehydrogenase B (*SDHB*) are associated with paragangliomas and gastrointestinal stromal tumors. The lack of evidence for precise variant classification and increased high-throughput sequencing lead to a high number of variants of uncertain significance (VUS) with unclear clinical consequences. Despite the high performance of in silico splice prediction tools,

functional validation is required. Within this project, an *SDHB* minigene was used to investigate effects of selected variants on splicing in an in vitro system.

Methods: A minigene composed of four consecutive exons of *SDHB* was cloned and validated with controls on mRNA level using gel electrophoresis, Sanger sequencing and next-generation sequencing. Single nucleotide variants (SNVs,n = 28) were selected based on SpliceAI-prediction and mutated via site-directed mutagenesis. Mutated minigenes were transfected into HEK293T cells and RNA was analyzed.

Results: The transcriptional analysis revealed aberrant splicing for 18 out of 28 SNVs (64%) that were predicted to show an effect on splicing. The number of transcripts per SNV varied from one to six (mean = 2). For ten SNVs, at least one aberrant transcript led to a frameshift. The most prevalent effect observed was exon skipping (n = 11). Only four variants with aberrant splicing showed a significant proportion (>25%) of wildtype transcript.

Conclusion: The presented in vitro assay allows for detection of aberrant splicing for in silico prioritized SNVs of *SDHB* and the insights will be included for improved variant evaluation. VUS could be reclassified based on PVS1/BP7 criteria (ACMG), translating into direct clinical impact.

Grants: Mildred-Scheel-fellowship (German cancer aid)

Conflict of Interest: None declared

P20.039.D Single cell analysis of gut biopsy tissue reveals cell type- and inflammation-specific expression quantative trait loci in Crohn's disease

Roy Oelen[1], *Werna Uniken-Venema*[2], *Marjolein Dijkema*[1], *Emilia Bigaeva*[2], *Sofie de Jong*[2], *Maria Paraskevopoulou*[3], *Pratik Panchal*[3], *Xinghao Zhang*[3], *Eleonora A. M. Festen*[2], *Lude Franke*[1], *Monique van der Wijst*[1]

[1]*University Medical Center Groningen, Genetics, Groningen, Netherlands;* [2]*University Medical Center Groningen, Gastroenterology and Hepatology, Groningen, Netherlands;* [3]*Takeda Pharmaceuticals, GI Drug Discovery Unit, Cambridge, United States*

Background/Objectives: Genome wide associations studies have linked more than 320 genetic loci to IBD, however how these genetic risk factors lead to disease (progression) remains largely unknown. The advent of RNA-seq allowed us to link genetic variants to gene expression via expression Quantative Trait Loci (eQTLs), which have proven to be linked to disease (phenotypes). The overlap between genetic variants that are linked to IBD, and the ones that affect gene expression unfortunately is quite poor. We hypothesize that this happens because cell-specific effects in the bulk RNA data are masked.

Methods: We here employ single cell mRNA-sequencing of 159 gut biopsy samples (100 non-inflamed and 59 inflamed) and Global Screen Array genotyping from 151 corresponding individuals suffering from Crohn's Disease to observe 4149 eQTL effects at a cell-type-specific level.

Results: We identify 3396 independent loci, affecting the expression of 3231 unique genes, across 11 different cell types. 10 of these are within Crohn's disease risk loci. We have subsequently tested these independent effects together with differentially expressed genes, to see which eQTLs occur upon inflammation, and find that 298 of these effects are modulated by both cell type specificity and tissue status.

Conclusion: These results show how genetic variants can contribute to disease, and will be of help in developments for precision medicine in the field of IBD.

Grants: This work was supported by the Dutch Organisation for Scientific Research (NWO) Corona Fast-Track grant (440.20.001), an Oncode Senior Investigator grant, NWO VIDI grant (917.14.374) and NWO VICI grant (09150182010019) to LF.

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P20.040.A Decoding the genetic predisposition to asthma using epigenomics

Lianne Trap[1], *Bernard Stikker*[1], *Marjolein de Jong - de Bruijn*[1], *Anne Onrust-van Schoonhoven*[1], *Bahar Sedaghati-khayat*[1], *Emmely de Roos*[1], *Anneloes van Krimpen*[1], *Arfan Ikram*[1], *Guy Brusselle*[2], *Rudi W. Hendriks*[1], *Jeroen van Rooij*[1], *Ralph Stadhouders*[1]

[1]*Erasmus MC, Rotterdam, Netherlands;* [2]*Ghent University Hospital, Gent, Belgium*

Background/Objectives: Asthma, an inflammatory airway disease, results from intricate interactions between genetics and environmental factors. High medical costs are mostly incurred by the 5-10% of patients requiring intensive treatment due to poor symptom control. We aim to highlight targets for phenotype-specific treatment of asthma by studying the genetic variants underlying its predisposition.

Methods: Genome-wide association studies identify up to 179 asthma-associated loci, in which ~99% of putatively causal genetic variants reside in non-coding genomic regions— thus complicating direct identification of susceptibility genes. We apply active chromatin signatures and 3D chromatin folding to: 1) connect genetic variants to genes in a cell type-specific manner and to 2) partition a polygenic risk score (PRS) predicting childhood-onset asthma in a Dutch prospective cohort (The Rotterdam Study).

Results: Systematically intersecting asthma-associated genetic variants with features of gene regulatory activity of 47 cell types revealed clusters of genetic loci demonstrating cell-type specific regulatory patterns. These included well-known gene-cell type pairs, e.g. the interleukin 33 locus and epithelial cells, and new associations involving mast cells, B or T lymphocytes, and even early blood cell precursors. Epigenomic partitioning of a PRS for asthma identified 5 clusters of regulatory variants, with those linked to lymphocytes and lung epithelial cells contributing most to its predictive ability.

Conclusion: We present a systematic way to translate genetic associations into tractable hypotheses, uncovering potentially new biological mechanisms relevant for asthma pathogenesis and predictive risk assessment.

Grants: Dutch Lung Foundation grant 4.1.18.226

Conflict of Interest: None declared

P20.041.B Transcription termination failure is associated with altered DNA methylation and affects oncogenic pathways in high grade serous ovarian cancer (HGSOC)

Karolina Chwialkowska[1], *Magdalena Niemira*[2], *Agnieszka Bielska*[2], *Anna Zeller*[2], *Mariusz Kuzmicki*[3], *Jacek Szamatowicz*[3], *Joanna*

Reszeć- Giełażyn[4], Pawel Knapp[5], Marcin Moniuszko[6], Adam Kretowski[2], Miroslaw Kwasniewski[1]

[1]Medical University of Bialystok, Centre for Bioinformatics and Data Analysis, Bialystok, Poland; [2]Medical University of Bialystok, Clinical Research Centre, Bialystok, Poland; [3]Medical University of Bialystok, Department of Gynecology and Gynecological Oncology, Bialystok, Poland; [4]Medical University of Bialystok, Department of Medical Pathomorphology, Bialystok, Poland; [5]University Clinical Hospital in Bialystok, University Oncology Centre, Bialystok, Poland; [6]Medical University of Bialystok, Department of Regenerative Medicine and Immune Regulation, Bialystok, Poland

Background/Objectives: Readthrough transcription results from failure of canonical transcription termination and leads to downstream transcription (DoG – downstream of gene) causing read-in into next genes. Generated readthrough transcripts retain in the nucleus and may remain bound to the chromatin playing a role in regulating chromatin states and modulating gene expression. HGSOC mainly originates from the fallopian tube epithelium (FTE), and the one of the earliest molecular events occurring further past acquiring *TP53* mutations is the alteration of *PAX2*. The aim of the study was to evaluate the biological importance of readthrough transcription and its relationship with DNA methylation profile in HGSOC.

Methods: Stranded total RNA-Seq and genome-wide DNA methylation profiling (RRBS) were performed in the same specimens collected from ovarian tumours of 56 patients with HGSOC and 33 ovaries of non-cancer patients.

Results: Our results show statistical enrichment of DNA hypomethylation within DoG induction subset (p-value = 1.65×10^-4) as well as both DNA hypo- and hypermethylation within read-in upregulated genes (p-values = 6.06×10^-49). In the group of upregulated read-in genes exhibiting DNA demethylation, there was a significant over-representation (under FDR ≤ 0.05) of various pathways related to the epithelium development. Network-based modelling in this read-in genes set revealed *PAX2* and *EPCAM* within the top guide genes as well as *FOXD3* and *CDX2* as the top candidates.

Conclusion: Obtained data suggest that readthrough transcription induction in HGSOC is associated with altered DNA methylation level in a non-stochastic manner and might play biologically significant role during carcinogenesis affecting the loss of epithelial cell identity and driver/suppressor-related pathways.

Conflict of Interest: Karolina Chwialkowska IMAGENE.ME SA, Poland, IMAGENE.ME SA, Poland, Magdalena Niemira: None declared, Agnieszka Bielska: None declared, Anna Zeller: None declared, Mariusz Kuzmicki: None declared, Jacek Szamatowicz: None declared, Joanna Reszeć- Giełażyn: None declared, Pawel Knapp: None declared, Marcin Moniuszko: None declared, Adam Kretowski: None declared, Miroslaw Kwasniewski IMAGENE.ME SA, Poland, IMAGENE.ME SA, Poland

P20.042.C Biallelic loss-of-function of a tRNA modification gene leads to impaired shoaling behavior and decreased startle response in zebrafish

Seda Susgun[1;2;3;4], *Nguyen Quynh Nhu[1], Emrah Yucesan[5], Dilan Wellalage Don[1], Tae-Yoon Kim[1], Tae-Ik Choi[1], Sibel Aylin Ugur Iseri[2], Cheol-Hee Kim[1]*

[1]Chungnam National University, Department of Biology, Daejeon, Korea, Rep. of South; [2]Istanbul University, Aziz Sancar Institute of Experimental Medicine, Department of Genetics, Istanbul, Türkyie; [3]Istanbul University, Graduate School of Health Sciences, Istanbul, Türkyie; [4]Bezmialem Vakif University, Faculty of Medicine, Department of Medical Biology, Istanbul, Türkyie; [5]Istanbul University-Cerrahpasa, Institute of Neurological Sciences, Department of Neurogenetics, Istanbul, Türkyie

Background/Objectives: Recent studies indicate that aberrant tRNA modifications lead to several human diseases, particularly neurological disorders. Herein, we present a tRNA modification gene as a novel candidate for neurodevelopmental disorders (NDD) through a knock-out zebrafish model.

Methods: CRISPR-Cas9 mediated stable knockout zebrafish line was generated and functionally evaluated. Whole-mount in situ hybridization (WISH) was conducted to determine spatiotemporal expression profiles of target genes. EthoVision and DanioVision systems were used in behavioral assays.

Results: Knock-out larvae did not show any early morphological changes and were raised to adulthood. Adult knock-out zebrafish displayed behavioral alterations. In the startle response assay, knock-out zebrafish showed statistically significant decreased response compared to wild-type siblings. In addition, the knock-out zebrafish line exhibited impaired shoaling behavior. Post-behavioral WISH assessments in adult zebrafish brains revealed a significant reduction in c-fos positive cells indicating neural activity in the forebrain regions which explains altered behaviors at the molecular level.

Conclusion: Herein, we established the knock-out zebrafish model for a novel gene associated with NDD. Functional evaluations support that loss-of-function of the gene causes NDD phenotype.

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Conflict of Interest: None declared

P20.043.D Epigenetic, transcriptomic and genetic analyses of HR + /HER2- primary and metastatic breast cancer reveals heterogeneous mechanisms of endocrine therapy resistance

Jomar Sangalang[1;2], *Dlep Tran[1], Tony Sourisseau[3], Veronique Scott[1], Nusaïbah Ibrahimi[1], Alicia Tran-Dien[1], Nicolas Servant[4], Fabrice André[1], Sergey Nikolaev[1]*

[1]Institut Gustave Roussy, Villejuif, France; [2]Université Paris-Saclay - Faculty of Medicine, Le Kremlin-Bicêtre, France; [3]Institute Bergonié, Bordeaux, France; [4]Institut Curie, Paris, France

Background/Objectives: Endocrine therapy (ET) is the primary treatment for HR + /HER2- breast cancer patients; however, half of the patients exhibit ET resistance. Mechanisms of ET resistance are not fully understood, and require an integrated epigenetic, transcriptomic and genetic approach

Methods: We conducted ATAC, RNA, and whole-exome sequencing on primary and metastatic breast cancer lesions from 464 HR + /HER2- patients. The studied cohort represents primary breast cancers (pBC) derived from patients experiencing relapse or sustained remission within five years of resection and adjuvant ET, and ET-resistant metastatic breast cancers (mBC). Analysis of chromatin accessibility, gene expression, and mutations aimed to understand relapse mechanisms in pBC and resistance mechanisms in mBC. Cohort clustering utilized PAM50 probability scoring.

Results: Two PAM50 scoring-derived pBC groups exhibited different relapse risks, with high-risk cases showing increased chromatin accessibility and expression of E2F targets, MYC targets, and the JAK-STAT pathway. Low-risk cases were enriched in estrogen response (ER) pathways. ESR1 and TP53 driver alterations were enriched in mBC, while ER pathways and GATA3/FOXA1 transcription factors were more active in pBC. PAM50 scoring

revealed subgroups of mBC based on perceived transdifferentiation to other intrinsic subtypes. ESR1 mutations characterized "Luminal B" tumours and resembled pBC, "Her2" subtype exhibited ERBB2 alterations, and "Basal" subtype was associated with BRCA1/UBR5 alterations and poor survival.

Conclusion: This comprehensive analysis of HR + /HER2- breast cancer unveils key insights into ET resistance mechanisms. PAM50 scoring aids in understanding tumour heterogeneity, contributing to a deeper understanding of relapse and the molecular landscape of ET resistance.

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Conflict of Interest: None declared

P20.045.B Missing heritability in neurodegenerative diseases: application of "MaPSy" to uncover the impact of Variants of Uncertain Significance on splicing mechanism

Beatrice Piola[1], *Giovanni Papagni[1], Fjorilda Caushi[1], Nicola Pomella[1], Hesam Aminian[1], Francesco Favero[2], Davide Corà[3], Luca Magistrelli[4], Alfredo Brusco[5], Fabiola De Marchi[6], Letizia Mazzini[6], sandra d'alfonso[1], Lucia Corrado[1]*

[1]University of Eastern Piedmont, Department of Health Sciences, Novara, Italy; [2]University of Eastern Piedmont, Translational Research Centre for Autoimmune and Allergic Diseases (CAAD), Novara, Italy; [3]University of Eastern Piedmont, Department of Translational Medicine, Novara, Italy; [4]University of Eastern Piedmont, Maggiore Della Carità Hospital, Department of Neurology and ALS Centre, Novara, Italy; [5]University of Torino, Department of Medical Sciences, Torino, Italy; [6]University of Eastern Piedmont, Maggiore Della Carità Hospital, Novara, Italy

Background/Objectives: Neurodegenerative diseases are characterized by a genetic etiology either in the familial and the sporadic forms. Although NGS identified many new genetic causes, a missing heritability remains, which can be partially explained by Variants of Uncertain Significance (VUS) that could alter splicing mechanisms. The Massively Parallel Splicing Assay (MaPSy) was developed to test the impact on splicing mechanism of hundreds of variants. This study aims to analyze by a modified-MaPSy a subset of VUS identified by WGS in 140 neurodegenerative patients.

Methods: From the variants identified by WGS in a panel of 855 neurodegenerative disease genes, we prioritized those with a SpliceAI score >0,6 and Allele Frequency <0,001 (n = 146). An amplicon-based library was cloned into the pSPL3-plasmid and transfected into HEK293T cells. NGS analysis was performed, paired-end reads were aligned to a reference genome, and BAM files manually inspected.

Results: We performed MaPSy on 19 variants (7 exonic/ 6 intronic/ 6 splicing) representing those with the highest SpliceAI score among prioritized variants (SpliceAI >0.6). An aberrant splicing was detected in 15/19 variants. Analysis for the remaining four variants was impaired due to low NGS locus coverage. The aberrant splicing was predicted to generate an out-of-frame transcript for 12 variants, and in-frame for the remaining ones.

Conclusion: Our results confirm that VUS impact on splicing mechanism can be analysed by a massive approach and SpliceAI is an effective predictor for prioritizing splicing-altering variants. An ex-vivo analysis to comprehend the role of identified variants in disease mechanisms will be performed.

Grants: NEUrodevelopmental-Disorder-Genetics (NEUDIG)-PRIN20

Conflict of Interest: None declared

P20.046.C Deciphering the nucleotide code of diabetes-associated regulatory elements

Fábio Ferreira[1], *João Nogueira[1], Sofia Borges[1], Ana Eufrásio[1], Miguel Sousa[1], Mariana Cruz[1], Rui Macedo[1], José Bessa[1]*

[1]i3S - Instituto de Investigação e Inovação em Saúde da Universidade do Porto, Porto, Portugal

Background/Objectives: Many sequence variants associated to Type-2 Diabetes Mellitus (T2DM) overlap with non-coding cis-regulatory regions, such as enhancers, that regulate gene expression in endocrine pancreas. Understanding how variants and random mutations impact transcription factor (TF) binding and enhancer activity is essential to better understand endocrine pancreas function, predict T2DM progress, and improve therapeutics. Thus, we aim to decipher the nucleotide code of those genetic sequences and establishing a predictive model of variant effects.

Methods: Different enhancer reporter vector architectures for optimal enhancer activity assessment in mammal cells were tested. Data from GWA studies and human islets genomic features was used to select hundreds of candidate enhancers overlapping with or close to Diabetes-associated SNPs. Two top enhancer reporter vector architectures were used to test those putative enhancers, including some carrying relevant polymorphisms, and to establish endocrine pancreas reporter cell lines. Lentiviral sgRNA libraries are being used to perform random Cas9-mediated mutagenesis in those reporter lines, followed by assessment of enhancer activity. Machine learning tools will then be used to determine the sequence motifs impacting sequence activity.

Results: A reliable pipeline to identify active enhancer sequences in endocrine pancreas cells was defined. Hundreds of sequences were tested for enhancer activity and hundreds of reporter cell lines were established and validated. CRISPR-mediated mutagenesis is being performed in the validated sequences, to identify the nucleotides and TF binding sites required for normal activity.

Conclusion: We developed a pipeline to assess the impact of sequence variants and mutations in enhancer activity.

Grants: La Caixa Foundation grant HR21-01212

Conflict of Interest: None declared

P20.047.D The role of *ZFHX4* as orofacial clefting gene: a study involving human genetic data and corresponding zebrafish models

Nina Ishorst[1;2], *Selina Hölzel[1;3], Carola Greve[1;4], Öznur Yilmaz[2], Tobias Lindenberg[2], Jessica Lambertz[2], Dmitriy Drichel[5], Enrico Mingardo[3], Jeshurun Kalanithy[1;2], Khadija Channab[3], Franziska Degenhardt[1;6], Anna Siewert[1], Michael Dixon[7], Berina Zametica[1], Teresa Kruse[8], Edwin Ongkosuwito[9], Katta Girisha[10], Shruti Pande[10], Stefanie Nowak[1], Carine Carels[11], Iris van Rooij[12], Kerstin Ludwig[1], Benjamin Odermatt[2;3], Elisabeth Mangold[1]*

[1]Institute of Human Genetics, University of Bonn, School of Medicine & University Hospital Bonn, Bonn, Germany; [2]Institute of Anatomy, Division of Neuroanatomy, University of Bonn, School of Medicine & University Hospital Bonn, Bonn, Germany; [3]Institute of Anatomy and Cell Biology, University of Bonn, School of Medicine & University Hospital Bonn, Bonn, Germany; [4]Current address: LOEWE Centre for Translational Biodiversity Genomics, Frankfurt am Main, Germany; [5]Cologne Center for Genomics, University of Cologne, Cologne, Germany; [6]Current address: Department of Child and Adolescent Psychiatry, Psychosomatics and Psychotherapy, University Hospital Essen, University of Duisburg-Essen, Duisburg, Germany; [7]Faculty of Biology, Medicine & Health, University of Manchester, Manchester, United Kingdom; [8]University of Cologne, Faculty of Medicine and University Hospital Cologne, Department of Orthodontics, Cologne, Germany; [9]Department of Dentistry, Section of Orthodontics and

Craniofacial Biology, Radboud Institute for Health Sciences, Radboud university medical center, Nijmegen, Netherlands; [10]Department of Medical Genetics, Kasturba Medical College, Manipal, Manipal Academy of Higher Education, Manipal, India; [11]Department of Human Genetics, KU Leuven, Leuven, Belgium; [12]Department for Health Evidence, Radboud university medical center, Nijmegen, Netherlands

Background/Objectives: Orofacial clefting (OFC) is a frequent congenital anomaly and can occur either in the context of an underlying syndrome or isolated (nonsyndromic). The two common OFC subphenotypes are cleft lip with/without cleft palate (CL/P) and cleft palate only (CPO). In this study, we started out to identify de novo copy number variants (dnCNVs) that affect possible susceptibility genes for nonsyndromic CL/P (nsCL/P).

Methods: We reanalyzed exome sequencing data (50 nsCL/P trios) and called dnCNVs. For the herewith identified candidate gene we performed: targeted-sequencing in in-house nsCL/P patients, expression analysis in published single-cell RNA-sequencing data (mouse embryo, zebrafish larvae (zfl)) and knock out/down experiments in zfl including morphometric measurements of the craniofacial region at 4 days post-fertilization.

Results: We identified a heterozygous 86 kb de novo deletion affecting the Zinc Finger Homeobox 4 gene (*ZFHX4*), a suggested OFC candidate gene. Our targeted-sequencing and expression data at relevant time-points further support an important role of *ZFHX4* in OFC. The *zfhx4* knockout and knockdown zfl showed a statistically significant underdeveloped and abnormally shaped ethmoid plate and cartilaginous jaw, the latter resembling micrognathia.

Conclusion: Our findings add to current knowledge from the literature that *ZFHX4* mutations are involved in a much wider phenotypic spectrum leading to nonsyndromic and syndromic forms, not only of CL/P but also CPO. In conclusion, *ZFHX4* should be considered as a causative OFC gene.

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P20.048.A The Bone Regulome – a map of bone-specific enhancers for non-coding variant interpretation

Salaheddine Ali[1;2;3], **Regina Margarete Grün**[4], *Daniel Ibrahim[3;5], Robert Schöpflin[3], Sven Geißler[5], Stefan Mundlos[1;3;5], Uwe Kornak[1;3;4;5]*

[1]Charité – Universitätsmedizin Berlin, Institute for Medical Genetics and Human Genetics, Berlin, Germany; [2]Charité – Universitätsmedizin Berlin, Department of Hematology, Oncology and Tumorimmunology, Berlin; [3]Max Planck Institute for Molecular Genetics, Development and Disease Group, Berlin; [4]University Medical Center Göttingen, Institute of Human Genetics, Göttingen, Germany; [5]Berlin Institute of Health at Charité – Universitätsmedizin Berlin, BIH Center for Regenerative Therapies (BCRT), Berlin, Germany

Background/Objectives: The interpretation of sequence changes in the non-coding genome is challenging due to the limited knowledge on cell type- and differentiation-specific enhancer usage. Enhancers spatiotemporally control gene expression over large genomic distances and, thereby, regulate tissue development. We developed a map of bone-specific regulatory sequences for the interpretation of non-coding genetic variants in the context of rare as well as common bone disorders.

Methods: We performed HiC-, ATAC-, ChIP- and RNA-seq of in vitro differentiated osteoclasts, osteoblasts and their respective precursors. Regulatory sequences were called and assigned to regulated genes by the CRUP algorithm. For validation CRISPRa/i iPSC lines were used and expression was evaluated by qRT-PCR.

Results: Enhancer activity and chromatin loops broadly correlated with differential gene expression during osteoblast and osteoclast differentiation. However, also genes similarly expressed in both cell types (e.g. *NFATC1*) showed differential enhancer usage. Pre-formed chromatin loops were visible in absence of gene activity, e.g. in the *EN1* locus. In total, about 2% of the genome comprise potential bone-relevant enhancers. 33% of intergenic SNPs associated with bone mineral density overlapped with these regulatory sequences, of which the majority located in osteoblast-specific enhancers. The majority of enhancer candidates was positively validated using CRISPRa/i-iPSCs. An enhancer in the sclerostin (*SOST*) gene locus, which is deleted in van Buchem disease, increased expression 100-fold in CRISPRa-iPSCs, whereas neighboring genes remained unaffected.

Conclusion: Our map improves the understanding of bone cell biology and is a valuable resource for interpretation of non-coding variants in the era of whole genome sequencing.

Grants: DFG, Elsbeth Bonhoff Stiftung

Conflict of Interest: None declared

P20.049.B Functional characterization and transcriptional profiling of fibroblasts from patients with mutations in SLC16A2 gene

Letizia Esposito[1;2], *Federica Rey[2;3], Erika Maghraby[3;4], Letizia Messa[2;5], Fabio Bruschi[6;7], Gian Vincenzo Zuccotti[3;8], Davide Tonduti[6;7], Stephana Carelli[2;3], Cristina Cereda[2;8]*

[1]University of Milan, Pediatric Clinical Research Center "Romeo ed Enrica Invernizzi", Department of Biomedical and Clinical Sciences, University of Milan, Milan, Italy, Milan, Italy; [2]University of Milan, Center of Functional Genomics and Rare Diseases, Department of Pediatrics, Buzzi Children's Hospital, Milan, Italy, Milan, Italy; [3]University of Milan, Pediatric Clinical Research Center "Romeo ed Enrica Invernizzi", Department of Biomedical and Clinical Sciences, University of Milan, Milan, Italy, Milano, Italy; [4]University of Pavia, Department of Biology and Biotechnology "L. Spallanzani", pavia, Italy; [5]Politecnico of Milan, Department of Electronics, Information and Bioengineering (DEIB), Milan, Italy; [6]V. Buzzi Children's Hospital, Milan, Italy, Unit of Pediatric Neurology, C.O.A.L.A. (Center for Diagnosis and Treatment of Leukodystrophies), Milan, Italy; [7]University of Milan, Department of Biomedical and Clinical Sciences, Milano, Italy; [8]Children's Hospital "V. Buzzi, Department of Pediatrics, Milan, Italy

Background/Objectives: Genetics variants in *SLC16A2* gene encoding for the monocarboxylate transporter 8 (MCT8) cause a severe X-linked intellectual deficit known as Allan-Herndon-Dudley syndrome (AHDS). MCT8 promotes cellular uptake and efflux of thyroid hormones. Active T3 and retinoid X receptors

(RXR) can form heterodimer complexes which bind to hormone response elements (HREs) that leads to activate or repress transcription. Our aim is to investigate the impact of MCT8 mutations on the pathogenetic mechanisms of AHDS.

Methods: Fibroblasts were obtained from skin biopsies of 2 AHDS mutated patients and matched controls. RNA was extracted with TRIzoL™. Total RNA sequencing was performed with the CORALL Total RNA-Seq Library Prep Kit using Illumina NextSeq 500 Sequencing. Protein expression was valuated via western blot and immunofluorescence. MTT assay was used to compare cell viability. Live/dead assay discriminated live and dead populations. Lipids were detected via Oil Red O staining.

Results: A strong dysregulation in AHDS patients was highlighted by transcriptomic profiling, identifying 396 DEGs when comparing AHDS patients to controls. Moreover, MTT and Live/Dead assays demonstrated a reduced cell viability in AHDS_1 patient with a splicing variant [c.1690G>A (p.Gly564Arg)]. The C-terminal missense variant [c.1690G>A (p.Gly564Arg)] did not affect fibroblasts viability, challenging a personalized in vitro behavior. Target genes expression resulted upregulated in both patients. Furthermore, myelin related genes were significatively reduced. The lipid staining revealed an increasing presence of lipid droplets in AHDS fibroblasts.

Conclusion: Our preliminary data emphasize a mutation-specific impairment in patients' specific primary fibroblasts, used as pre-clinical experimental model of the disease.

Conflict of Interest: None declared

P20.050.C New insights in Aicardi-Goutières syndrome: characterization of novel players in a RNU7-1 mutation

Erika Maghraby[1;2], *Federica Rey*[2;3], *Letizia Esposito*[2;3], *Gian Vincenzo Zuccotti*[2;3], *Davide Tonduti*[3;4], *Stephana Carelli*[2;5], *Cristina Cereda*[5;6]

[1]*University of Pavia, Department of Biology and Biotechnology "L. Spallanzani", 27100, Italy;* [2]*University of Milan, Department of Biomedical and Clinical Sciences, Milano, Italy;* [3]*Buzzi Children's Hospital, Department of Pediatrics, Milano, Italy;* [4]*Buzzi Children's Hospital, Unit of Pediatric Neurology, C.O.A.L.A, Milano;* [5]*Center of Functional Genomics and Rare Diseases, Department of Pediatrics, Milano, Italy;* [6]*Buzzi Children's Hospital, Department of Pediatrics, 27100, Italy*

Background/Objectives: Aicardi-Goutières Syndrome (AGS) is a leukodystrophy majorly defined by raised levels of type I interferon-alpha (IFN-α) in absence of demonstrable infections. AGS is associated with mutations in 9 genes which all encode for proteins involved in metabolism of nucleic acids. Mutations in *RNU7-1* lead to the least characterized form of the disease. *RNU7-1* encodes for a small nucleolar RNA which is a member of the small nuclear ribonucleoprotein complex U7 (U7 snRNP). U7 snRNP leads to the cut of the Poly-A tail in replication-dependent histones (RDH) pre-mRNAs. The aim of this work is to dissect the role of *RNU7-1* mutation in AGS pathogenesis. We investigated canonical AGS features such as the upregulation of IFN-α, interferon-stimulated genes and specific outcomes of *RNU7-1* mutation in primary fibroblasts obtained from AGS patients and compared to healthy controls.

Methods: Total RNA was extracted using TRIzol reagent and the genes' expression levels were determined by Real-Time PCR. ELISA, Western Blot analysis and immunofluorescence were also performed to assess protein expression levels.

Results: Our results confirm the upregulation of ISGs and the typical "interferon signature" in AGS patients which cause an increase in IFN-α production. Moreover, we confirmed the modulation of this "interferon signature" through hydroxychloroquine treatment. Lastly, we assessed the increased presence of enriched Poly-A RDH transcripts confirming the role of U7 snRNP in the cleavage of Poly-A tail.

Conclusion: This work reports the molecular mechanisms involved in AGS9 mutation which lead to the upregulation of ISGs, IFN-α overproduction, and misprocessing of RDH mRNAs.

Conflict of Interest: None declared

P20.051.D Genetic variation at Krüppel-associated box (KRAB) domain-containing zinc-finger proteins may influence methylation variation at metastable epialleles

Julius Arthur Kuebel[1], *Noah Kessler*[2], *Maria Derakhshan*[1], *Andrew Prentice*[3], *Matt Silver*[1;3], *Garrett Hellenthal*[4]

[1]*London School of Hygiene & Tropical Medicine, Department of Population Health, London, United Kingdom;* [2]*University of Cambridge, Department of Genetics, Cambridge, United Kingdom;* [3]*Medical Research Council Unit The Gambia at The London School of Hygiene & Tropical Medicine, Fajara, London, Gambia;* [4]*University College London, Department of Genetics, Evolution & Environment, London, United Kingdom*

Background/Objectives: Metastable Epialleles (MEs) include CpGs that exhibit high interindividual methylation (DNAm) variation, while also showing within-individual consistency in methylation across tissues that suggests DNAm establishment in the early embryo. For this reason, MEs are promising candidate regions to explore the epigenetic links between early-life exposures and later health. However, little is known about the mechanisms – genetic, environmental, and stochastic – that drive DNAm variability at MEs. Interestingly, putative human MEs are enriched for proximity to certain subclasses of Retrotransposable Elements (REs) that are silenced via methylation by Krüppel-associated Box (KRAB) domain-containing Zinc-Finger Proteins (KZFPs). Here we explore whether genetic mutations in KZFPs partially drive methylation variability of putative human MEs.

Methods: We used a genome-wide scan to identify significant mQTLs (p-val < 1e-08) for each of 1128 putative human MEs, and matched control CpGs, using Agilent SureSelect Methyl-Seq Targeted Capture, and genotypes at >2M SNPs from 396 Gambian individuals. Next, we estimated the methylation variance explained by mQTLs in KZFPs as a proportion of that explained across all trans-mQTLs.

Results: We reveal that mQTLs in KZFPs explain >60% of the methylation variation explained by all trans-mQTLs in ~60% of putative MEs in Gambian individuals. By comparison, only 32% of matched controls had a trans-mQTL contribution by KZFPs >60%.

Conclusion: Our findings indicate a potential role of trans-mQTLs in KZFP genes maintaining interindividual methylation variation at some MEs, in addition to environmental and/or stochastic factors.

Grants: This work was supported by the Biotechnology and Biological Sciences Research Council [grant number BB/T008709/1].

Conflict of Interest: None declared

P20.052.A Single cell CRISPR screen reveals genome-wide regulatory landscape of coronary artery disease

Veronica Yumiceba[1], *Kristian Händler*[1], *Varun Sreenivasan*[1], *Saranya Balachandran*[1], *Tobias Reinberger*[2;3;4], *Philip Seibler*[5], *Jeanette Erdmann*[2;3], *Malte Spielmann*[1;3;6]

[1]*Institute of Human Genetics, University Hospital Schleswig-Holstein, University of Lübeck and Kiel University, Lübeck, Germany;* [2]*Institute for Cardiogenetics, University of Lübeck, Lübeck, Germany;* [3]*DZHK*

e.V. (German Research Centre for Cardiovascular Research), Partner Site Hamburg/Kiel/Lübeck, Lübeck, Germany; [4]University Heart Center Lübeck, Lübeck, Germany; [5]Institute of Neurogenetics, University of Lübeck, Lübeck, Germany; [6]Human Molecular Genetics Group, Max Planck Institute for Molecular Genetics, Berlin, Germany

Background/Objectives: Large and ethnically diverse GWAS studies have significantly contributed to the genetics of coronary artery disease (CAD). Most CAD variants have been found in the non-coding genome, which in combination with the sparsity genome annotations in relevant cellular context, impeded establishing a relationship between these variants and their pathology. It is known that the non-coding DNA contains regulatory elements which tune proper spatio-temporal transcription. We aim to quantify the transcriptional effects of these non-coding CAD-GWAS hits in one multiplexed experiment combining high-throughput CRISPR-Cas9 and single-cell technologies.

Methods: A 180 unique gRNAs library targeting 41 CAD non-coding SNVs distributed across 8 loci, plus controls was delivered to dCas9-expressing monocytes and primary endothelial cells. Single-cell RNA libraries from CRISPRa and CRISPRi screens, with around 20.000 cells input per experiment, were generated using 10x Chromium, and sequenced. Analysis was performed using SCEPTRE.

Results: Preliminary CRISPRa results aiming at cis effects identified 11 of the 41 non-coding variants to have regulatory functions on neighboring genes in a cell-type specific fashion. Apart from 2 variants which were active across both cell types. These 11 variants reside in 5 different loci. Interestingly, the lead variant was not often the functional one and rs1537373 appeared to regulate the expression of 3 genes.

Conclusion: The combination of single-cell phenotyping methods and high-throughput CRISPR-screens is an effective way to experimentally prioritize GWAS variants. We are currently scaling up the experiments to 290 loci, whose findings will impact our understanding of the functional non-coding loci and provide a baseline for enhancer therapies developments.

Grants:

Conflict of Interest: None declared

P20.053.B Unraveling mechanisms of TKI resistance in NSCLC: insights from PDX models

Naomi Kaste MFONFU[1;2;3], *Floryane Braye*[1], *Galina Boldina*[2], *Matteo Cesaroni*[2], *Luc Friboulet*[1], *Sergey Nikolaev*[1], *Andrey Yurchenko*[1]

[1]*Gustave Roussy, Villejuif, France;* [2]*Sanofi Vitry, Vitry-sur-Seine, France;* [3]*Université Paris-Saclay - Faculty of Medicine, Le Kremlin-Bicêtre, France*

Background/Objectives: Targeted therapies like tyrosine kinase inhibitors (TKIs) targeting EGFR and ALK are first-line treatments for Lung Cancer. However, after initial response, rare drug-tolerant cancer cells (persister cells) frequently remain and lead to the emergence of TKI resistance. Characterisation of this population of interest and investigation of the mechanisms of treatment evasion may improve understanding of resistance mechanisms.

Methods: Two patient derived PDX models from pretreated cancers were generated. One PDX model had EGFR and ALK mutations and responded to treatment with Osimertinib and Alectinib, and the other – had ALK mutations, and responded to Lorlatinib. Each tumor was grafted into 5 mice, 3 of which were treated till emergence of the stable residual disease, and 2 – used as a control without treatment. snRNA-seq was performed to characterize in comparative framework the untreated and persister cancer cells.

Results: Majority of persister cells were in the G1 phase of the cell cycle and suppressed most canonical cancer pathways, however a notable upregulation of the ciliated pathway was observed in both models. Transcription factors MEOX2 and MLX were identified as activated, indicating their roles in resistance mechanisms and inhibition of proliferation, mobility, and cell growth. Furthermore, distinct mechanisms were uncovered in different tumor models: the ALK PDX model exhibited enrichment of Epithelial-Mesenchymal Transition signatures in persister cells, while the EGFR ALK PDX model showed suppression of MYC and Interferon responses.

Conclusions: PDX models proved to be an informative tool to study cancer persistence. TKI treatment evasion mechanisms in lung cancer might be intermediated by activation of the ciliated pathway.

Conflict of Interest: None declared

P20.054.C Regulatory impact of rare genetic variants implicated in blood cell development and function

Manuel Tardaguila[1], *Dominique von Schiller*[2], *Michela Colombo*[1], *Ilaria Gori*[1], *Chiara Chiereghin*[1], *Dragana Vuckovic*[3], *Eve Coomber*[2], *Stephen Watt*[2], **Nicole Soranzo**[1;2;4]

[1]*Human Technopole, Italy;* [2]*Wellcome Sanger Institute, Hinxton, United Kingdom;* [3]*Imperial College London, United Kingdom;* [4]*University of Cambridge, United Kingdom*

Background/Objectives: Across human traits and diseases, resolving the path connecting genetic variants to molecular and cellular function and, ultimately, to disease, remains the biggest hurdle towards unlocking the investment of early genomic studies. Genetic variants discovered by genome-wide association studies have been shown to lie predominantly in non-coding regions of the genome, where for most traits and diseases we have a limited understanding of the impact of genetic effects on the regulatory code, and of the specific cellular, developmental and environmental contexts in which these variants act.

Methods: Here we deploy a multi-pronged approach to functionally characterise a set of high-priority non-coding variants impacting variation in blood cellular phenotypes. We focus on 94 rare variants displaying large phenotypic effects on different haematological indices. We use massively parallel reporter assays (MPRA) and arrayed CRISPR/Cas9 screens to assess their regulatory potential. We analyse gene expression and transcript usage in whole blood and immune cells from thousands of individuals.

Results: Our analyses reveal diverse modes of genetic regulation for approximately 75% of the rare variants tested. After careful curation using state-of-the-art annotation genomic metrics and phased WGS data, we identify 22 loci with strong evidence for genetic regulation impacting on blood cell development and biological function. Finally, using orthogonal experimental approaches, we identify a novel role for intronic variants in CUX1 in controlling megakaryocyte differentiation.

Conclusion: This systematic framework sheds light on the intricate mode of action of non-coding variants and advances the global effort to deliver on their translational potential.

Grants:

Conflict of Interest: None declared

P20.055.D Premature overexpression of miR-92a's target RBFOX2 impairs neural differentiation in the mouse cerebral cortex by hijacking the PTBP splicing program

Stephan Weißbach[1], *Hristo Todorov*[2], *Dewi Hartwich*[2], *Laura Schlichtholz*[2], *Sophie Muehlbauer*[2], *Dennis Strand*[3], *Susanne Strand*[3], *Tanja Vogel*[4], *Martin Heine*[1], *Susanne Gerber*[2], *Jennifer Winter*[2]

[1]*Johannes Gutenberg-Universität, Institute of Developmental Biology and Neurobiology (iDN), Mainz, Germany;* [2]*University Medical Center Mainz, Institute of Human Genetics, Mainz, Germany;* [3]*University Medical Center Mainz, Medizinische Klinik und Poliklinik, Mainz, Germany;* [4]*Universitätsklinikum Freiburg, Institut f.Anatomie u. Zellbiologie Abt. für Neuroanatomie, Freiburg im Breisgau, Germany*

Background: Alternative splicing is a pervasive phenomenon in the central nervous system that plays a crucial role in the transition from neural progenitor cells (NPCs) to neurons and additionally shapes the functional diversity of neuronal cell populations. The RNA-binding protein RBFOX2 is an indispensable mediator of alternative splicing in the developing neocortex. Furthermore, mutations in this gene are associated with several neurodevelopmental disorders.

Methods: To elucidate the specific role RBFOX2 plays in neural differentiation, we employed in-utero electroporation, thereby inducing *Rbfox2* overexpression in the mouse cerebral cortex at E13.5. Subsequently, we performed a comprehensive molecular characterization of RBFOX2 expression and splicing activity at E15.5.

Results: While RBFOX2 was absent from NPCs, it was already expressed in newly born neurons. Remarkably, overexpression resulted in impaired neuronal differentiation and aberrant migration phenotype. A genome-wide characterization of alternative splicing events uncovered a significant deviation from the normal splicing trajectory accompanied by a mixed NPC- and neuron-like splicing pattern compared to control samples. Furthermore, we identified a set of validated target exons that are normally repressed by PTBP2 to be significantly included upon *Rbfox2* overexpression, indicating an antagonistic relationship between these proteins. Finally, we showed that miR-92a repressed *Rbfox2* expression in vitro, whereas in vivo miR-92a-silencing in the cerebral cortex led to a significant up-regulation of *Rbfox2*.

Conclusion: Taken together, we demonstrated that RBFOX2 shapes neural differentiation through time-specific alternative splicing. Furthermore, we presented a novel post-transcriptional mechanism for regulating RBOFX2 during early cerebral cortical development via miRNA-silencing.

Conflict of Interest: None declared

P20.056.A JANUS - Joint analysis for augmentation of cluster specificity

Talip Yasir Demirtas[1], *Maximilian Billmann*[1]

[1]*Institute of Human Genetics, University of Bonn, School of Medicine and University Hospital Bonn, Bonn, Germany*

Background/ Objective: CRISPR screens systematically identify genes that impact various specific biological processes. To gain specificity of a screening experiment, the effect of a gene perturbation on a specific phenotype or the differential effect on cell fitness in a process-sensitizing context is measured. However, hit lists from these experiments typically include genes that have specific effects on the process as well as pleiotropic genes, which impact the process indirectly or directly.

Here, we develop an algorithm JANUS (Joint ANalysis for augmentation of clUSter specificity) that leverages public large CRISPR screening resources (The Cancer Dependency Map) to structure hit lists of phenotypes specific CRISPR screens and assign specificity and pleiotropy scores to each cluster.

Methods: JANUS jointly analyzes the screening set of interest and a selected reference data set such as the DepMap by quantifying how much the screening set of interest contributes to each gene cluster. Variance contributed by individual screens is quantified through partial correlation analysis. We used global and process-specific precision-recall analyses with gold standards to quantify functional information of gene-pairs and clusters.

Results: We apply JANUS to a set of 7 genome-wide CRISPR screens that investigate mitochondrial drug-gene interactions of energy metabolism. We show that JANUS increases the specificity and resolution of mitochondrial clusters. We applied JANUS to tissue-specific clustering, demonstrating that tissue-specific clusters connect both lowly mutated genes and cancer genes.

Conclusion: JANUS will help analyze and interpret complex phenotypic CRISPR screens by utilizing the rapidly growing number of simple fitness screens to systematically address the genotype-to-phenotype problem.

Grants: None.

Conflict of Interest: None declared

P20.057.B Tissue-specific mitochondrial regulation of gene expression

Xenofon Giannoulis[1;2], *Simon Wengert*[2;3], *Florin Ratajzak*[2;4], *Na Cai*[2;3]

[1]*Helmholtz Pioneer Campus, Helmholtz Zentrum München, Neuherberg, München, Germany;* [2]*Computational Health Centre, Helmholtz Zentrum München, Neuherberg, München, Germany;* [3]*Helmholtz Pioneer Campus, Translational Genetics, Neuherberg, München, Germany;* [4]*Institute of Computational Biology, Helmholtz Zentrum München, Neuherberg, München, Germany*

Background/Objectives: The mitochondrial DNA contains vital elements for oxidative phosphorylation and RNAs that are crucial for its translational machinery. However, gene regulatory effects resulting from the intricate interplay between nuclear and mitochondrial genomes have not been comprehensively explored.

Methods: We utilize cis- and trans-expression Quantitative Trait Loci (eQTL) analyses, employing whole-genome genetic data from 684 European individuals derived from the GTEx cohort across 48 tissues.

Results: First, we identify 309 significant mtDNA-cis-eQTLs (study-wide FDR 5%) for mtDNA-encoded genes across 38 out of 48 tissues tested, of which 108 are independent. Second, we identify 260 genome-wide significant associations ($P < 5 \times 10\text{-}8$) between nuclear-DNA-SNPs and mtDNA gene expression (mtDNA-trans-eQTL) at 210 unique nucDNA loci. Of these, 150 are cis-eQTLs for nucDNA encoded genes. Mediation analysis reveals that 117 out of 150 exhibit a cluster of nuclear-encoded genes, serving as mediators for the effects of mtDNA-trans-eQTLs. Third, we find 108 mtDNA-SNP associations ($P < 1.24 \times 10\text{-}09$) with 68 nucDNA-encoded genes (nucDNA-trans-eQTL). Among all the analyses, CNS tissues comprise 43% of all associations. Pathway and enrichment analyses reveal the broader biological relevance of our results. Specifically, the prominent enrichments include RNA polymerase II activity and mitochondria pathways, while mtDNA-trans-eQTLs overlap genes with significant GWAS hits on phenotypes such as BMI, and metabolic syndrome.

Conclusion: In summary, we establish the first comprehensive multi-tissue map of mtDNA and nucDNA crosstalk in gene expression regulation, advancing understanding of tissue-specific mito-nuclear interactions in health and disease.

Grants: The present contribution is supported by the Helmholtz Association under the joint research school Munich School for Data Science–MUDS.

Conflict of Interest: None declared

P20.058.C Searching for novel diabetes therapies by modulating glucose-induced epigenetic modifications in the zebrafish pancreas

Francisco Cadete[1;2], *Joana Teixeira*[1], *Joana Marques*[1;2], *Marta Duque*[1;2], *José Bessa*[1]

[1]*i3S - Instituto de Investigação e Inovação em Saúde da Universidade do Porto, Porto, Portugal;* [2]*Abel Salazar Biomedical Sciences Institute - University of Porto, Porto, Portugal*

Background/Objectives: Type 2 diabetes(T2D) is a major public health concern. It is partially characterized by endocrine pancreatic(EndoPc) transcriptional dysregulation. Short-term glucose exposure triggers insulin secretion while long-term hyperglycemia(LTH) causes irreversible EndoPc changes, rendering cells unresponsive to glucose. These dysfunctional cells present aberrant gene transcription, suggesting modifications to their regulatory elements. Enhancers, cis-regulatory elements, are marked by histone epigenetic marks and chromatin accessibility, defining their active/inactive state. A comprehensive histone epigenetic profiling of LTH-induced changes remains unexplored, along with strategies to restore these marks. Here, we aim to profile in vivo epigenetic modifications induced by LTH in EndoPc cells, and employ chromatin remodelling to rescue glucose homeostasis, using zebrafish as a model.

Methods: LTH was induced by subjecting zebrafish to extended overfeeding(8-weeks). LTH remodeled enhancers will be identified by profiling histone epigenetic marks(Chip-seq) and chromatin accessibility(ATAC-seq) in LTH EndoPc islets compared to normal feed fish. By taking advantage of chromatin remodelers guided by dCas9, we will induce specific and targeted epigenetic changes in these remodeled enhancers, potentially restoring EndoPc function.

Results: Overfeed animals exhibited higher glucose levels upon returning to normal feeding, indicating a constant state of hyperglycaemia. ATAC-seq results revealed differential chromatin accessibility between conditions, suggesting that EndoPc chromatin is remodeled differentially in response to LTH. To date, we have developed one transgenic zebrafish line that exhibit the expression of dCas9-p300 specifically within β-cells, under conditional activation of 4-hydroxytamoxifen.

Conclusion: This approach aims to unveil glucose-induced epigenetic changes responsible for the transcriptional dysregulation in LTH, offering new strategies for T2D treatment.

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Conflict of Interest: None declared

P20.059.D The Balancing Correlation Network (BaCoN) score improves prediction of gene buffering

Thomas Rohde[1], *Maximilian Billmann*[2]

[1]*University Hospital Bonn, Institute of Human Genetics, Bonn, Germany;* [2]*University Hospital Bonn, Institute of Human Genetics, Bonn*

Background/Objectives: Buffering between genes is a fundamental mechanism to achieve robust cellular functions. This mechanism can have various clinical implications, including cancer treatment resistance. Large gene perturbation and expression data have the potential to systematically predict gene buffering, but sophisticated computational methods are required to make precise, non-biased predictions. We aimed to systematically identify functional redundancy within the human genome.

Methods: Under the assumption that the buffering capacity of a gene depends on its expression level, we quantified about 150 million potential buffering relationships by correlating genome-wide CRISPR-Cas9-screening derived fitness scores with transcriptomic data across more than 1000 cell lines.

We developed a post-hoc correction method for Balancing Correlation Networks (BaCoN) to remove false positive correlations due to co-expression with causal genes. BaCoN amplifies highly specific signals by weighting each edge of a correlation-based network based on its rank among incident edges.

Results: BaCoN reduces the dominance of cell lineage-driven correlations, while increasing the amount of paralog pairs among the top predictions in general as well as pairs from a gold-standard paralog synthetic lethal set. BaCoN outperformed routinely used methods loke multiple linear regression and SVA-based batch correction, that are capable of correcting for confounding factors. Utilizing BaCoN, we identified the MFRN1-MFRN2 paralog gene pair as the top genetic vulnerability of chr8p depleted cancers and validated it in vivo by combinatorial perturbation experiments.

Conclusion: BaCoN is a novel method to balance correlation networks using expression and perturbation data to predict functional buffering between genes without prior knowledge of experimental confounding factors.

Grants: None.

Conflict of Interest: None declared

P20.060.A Replication origins are enriched at genes important for genome maintenance

Ipek Ilgin Gönenc[1], *Alexander Wolff*[1], *Christian Müller*[1], *Arne Zibat*[1], *Gökhan Yigit*[1;2], *Holger Bastians*[3], *Bernd Wollnik*[1;2;4]

[1]*University Medical Center Göttingen, Institute of Human Genetics, Göttingen, Germany;* [2]*German Center for Cardiovascular Research (DZHK), Partner Site Göttingen, Göttingen, Germany;* [3]*University Medical Center Göttingen, Department of Molecular Oncology, Section for Cellular Oncology, Göttingen, Germany;* [4]*University of Göttingen, Cluster of Excellence "Multiscale Bioimaging: from Molecular Machines to Networks of Excitable Cells" (MBExC), Göttingen, Germany*

Background/Objectives: DNA replication initiates in S-phase with the firing of replication origins at specific genomic regions. The precise regulation of origin firing is essential to ensure faithful DNA replication, as any alterations may predispose to genomic instability.

Methods: The cell cycle length of HCT116 cells was assessed by flow cytometry to analyze the replication origin firing at the beginning of the S-phase. Cells synchronized in mitosis were released into media containing EdU to label the newly synthesized DNA and hydroxyurea to arrest cells in early S-phase. The EdU-labeled DNA was subjected to click chemistry and NGS analysis. We upgraded the EdUseq analysis pipeline reported previously to a Python-based script enabling the identification of EdUseq peak-associated regions.

Results: The bin size for determining the replication origins required a novel peak-calling strategy using prominence values inspired by the topographical peak determination. We pinpointed 3989 replication origin bins largely overlapping with the early replicating DNA domains, revealing higher genic region presence. Evaluation of over 400 genes included in a comprehensive cancer panel revealed a significantly higher than expected number of genes positioned within the EdUseq peak-associated regions suggesting that genes with important roles in maintaining genomic stability, DNA repair, and cell cycle were likely to be located at replication origins in a non-stochastic way.

Conclusion: We identified replication origins by direct sequencing of newly synthesized DNA in early S-phase, which revealed the association of replication origins to genes important for maintaining genome stability.

Grants: Cluster of Excellence "Multiscale Bioimaging: from Molecular Machines to Networks of Excitable Cells"(MBExC; EXC 2067/1-390729940)

Conflict of Interest: None declared

P20.061.B The effect of miR-197-3p overexpression in familial mediterranean fever mouse macrophages

Emre Nalbant[1], *basak sen*[1], *tayfun hilmi akbaba*[1], *banu balci-peynircioglu*[1], *yeliz z. akkaya-ulum*[1]

[1]*Hacettepe University, Faculty of Medicine Department of Medical Biology, Ankara, Türkyie*

Background/Objectives: Familial Mediterranean Fever (FMF) is an autosomal recessive inflammasomopathy linked to the MEditerranean FeVer (*MEFV*) gene. Despite its monogenic origin, the correlation between genotype and clinical phenotype remains complex. Our studies highlighted the involvement of microRNAs (miRNAs), specifically miR-197-3p, in FMF pathophysiology. This study investigated the impact of miR-197-3p overexpression on macrophages derived from an FMF mouse model, focusing on its modulation of the pyrin inflammasome.

Methods: Bone marrow-derived macrophages (BMDMs) from FMF-KI mice were transfected with pre-miR-197-3p or mimic control using Lipofectamine 2000. After transfection, IL-1β secretion and pro-IL-1β levels were analyzed following LPS priming and inflammasome activator treatments (dsDNA, flagellin, ATP and C3 toxin). RNA isolation, RT-qPCR, immunoblot, and ELISA were conducted and results were analyzed with statistical analysis using Student's T-test.

Results: After pre-miR-197-3p transfection of mice BMDMs, successful overexpression was determined. Overexpression of miR-197-3p significantly reduced IL-1β secretion. IL-1β was found to be decreased significantly only in C3 treatment related with pyrin inflammasome activation.

Conclusion: This study provided insights into the regulatory role of miR-197-3p in inflammation pathways associated with FMF, particularly its impact on pyrin inflammasome activation. Further investigations are warranted to unravel the exact mechanisms underlying miR-197-3p's influence on inflammasome activation and priming in FMF and other autoinflammatory disorders

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P20.062.C New approach to deciphering complex cases of cystic fibrosis and its related disorders

clara blotas[1], *Mégane COLLOBERT*[1], *Anaïs Le Nabec*[1], *Emmanuelle Masson*[1,2], *Claude Férec*[1], **Stéphanie MOISAN**[1,2]

[1]*Univ Brest, Inserm, EFS, UMR 1078, GGB, F-29200 Brest, France;* [2]*Laboratoire de génétique moléculaire et d'histocompatibilité, CHRU Brest, Bretagne, France*

Background/Objectives: Cystic fibrosis (CF) is the most common severe inherited genetic disease in European populations, with 2114 reported variations within the *CFTR* (*Cystic Fibrosis Transmembrane Conductance Regulator*) gene. However, it is still challenging to understand the distinction between CF and CFTR-Related Disorders, as well as extreme phenotypes in CF. To explain these complex cases, our project aims to identify dysregulation of *CFTR* gene expression due to alterations in *cis*-regulatory elements (CREs).

Methods: We are using 4C (Circular Chromosome Conformation Capture) in intestinal, pancreatic and epididymal cells to map CRE-promoter interactions. Subsequently, we integrated epigenomic data as H3K27ac, H3K27me3 and CTCF (CCCTC-binding factor) marks to identify candidate CREs (cCREs). To functionally validate identified cCREs, we perform in vitro reporter gene assays. Targeted high-throughput sequencing is then applied to different patient cohorts to detect *cis*-regulatory variants.

Results: 4C analyses reveal several regions that interact with the *CFTR* promoter. Many interactions are conserved between cell-types, but specific peaks are also observed. Most of the regulatory regions have an enhancer effect. The first unique silencer of the *CFTR* gene was identified, located at +507,6 kb from the last codon. It is the first reported CRE outside the topologically associating domain of the *CFTR* locus. cCREs sequencing in patients revealed a dozen of SNPs with significantly different frequencies compared to the French population. Functional tests are carried out to validate their impact.

Conclusion: This work provides new elements for a better understanding of the *CFTR* locus organisation in a cell type-specific manner.

Grants: Vaincre la mucoviscidose

Conflict of Interest: None declared

P20.063.D A global, multi-project initiative to elucidate and annotate translation in 'non-coding' regions

Jonathan Mudge[1], *Jorge Ruiz-Orera*[2], *Eric Deutsch*[3], *Irwin Jungreis*[4], *Leron Kok*[5], *Ivo Fierro-Monti*[1], *Sonia Chothani*[6], *Robert Moritz*[3], *Jyoti Choudhary*[7], *Michal Bassani-Sternberg*[8], *Nicola Ternette*[9;10], *Owen Rackham*[11], *Juan Antonio Vizcaino*[1], *John Prensner*[12], *Sebastiaan van Heesch*[5], *Adam Frankish*[1]

[1]*EMBL's European Bioinformatics Institute (EMBL-EBI), Hinxton, United Kingdom;* [2]*Max Delbrück Center for Molecular Medicine, Berlin, Germany;* [3]*Institute For Systems Biology, Seattle, United States;* [4]*Massachusetts Institute of Technology, Cambridge, United States;* [5]*Princess Máxima Center for Pediatric Oncology, Utrecht, Netherlands;* [6]*Duke-NUS Medical School, Singapore, Singapore;* [7]*The Institute of Cancer Research, London, United Kingdom;* [8]*Ludwig Institute for Cancer Research, Agora Research Center, Lausanne, Switzerland;* [9]*Utrecht University, Utrecht, Netherlands;* [10]*University of Oxford, United Kingdom;* [11]*University of Southampton Highfield Campus, United Kingdom;* [12]*University of Michigan Medical School, Ann Arbor, United States*

Consortium: Ribo-seq annotation consortium

Background/Objectives: The non-coding portion of the human transcriptome is extensively translated. Especially, thousands of 'non-canonical' translations have been captured by Ribo-seq. Some represent missing microproteins with biological roles; others appear to be aberrant translation products which could have potential as diagnostic markers or therapeutic targets in cancer. Non-canonical translation can also be purely regulatory in nature, especially uORFs found in 5' UTRs, which are of emerging interest in disease. Clearly, there is an urgent need to elucidate the biology and implications of non-canonical translation. We have formed an international consortium to this end, centered around the creation of public Ensembl-GENCODE annotation resources.

Methods: Our consortium is sharing expertise in Ribo-seq, mass spectrometry-based proteomics and immunopeptidomics, molecular evolution, variant interpretation and gene and protein-annotation, in order to drive solutions for the identification, characterisation and standardized annotation of non-canonical translations.

Results: We will discuss the maturation of the Ensembl-GENCODE catalog of non-canonical translations as a resource to support human genetics. We will focus on (1) efforts to find peptide-level evidence for Ribo-seq ORF translation products, including immunopeptidomics data; (2) the development of evolutionary metrics to appraise ORF functionality; (3) the expansion of our non-canonical ORF resource via the incorporation of 'nanoORFs' and non-ATG translations.

Conclusion: As our resources are produced within the reference annotation 'ecosystem', this is the point at which non-canonical translation enters the mainstream of genomic and medical science. Nonetheless, these are early steps of a long-term, multi-faceted project, for which we are actively seeking deeper community engagement.

Conflict of Interest: None declared

P20.064.A LinkPrep™: a high-resolution, high-throughput, proximity ligation method that enables detection of structural variants and chromatin topology features in cancer.

*Miles Collier[1], Jonathon Torchia[1], Daniel Hwang[1], Mital Bhakta[1], **Cory Padilla**[1], Lisa Munding[1]*

[1]Dovetail Genomics, Scotts Valley, United States

Background/Objectives: Despite technological advances, including adoption of next-generation sequencing, many clinically relevant cancer driver mutations go undetected. The reasons are two-fold: 1) Structural variants (SVs) are challenging to detect using shotgun or long read approaches, especially heterogenous samples; 2) Epigenetic dysregulation, through mechanisms such as enhancer hijacking or changes to 3D genome organization are accepted to drive disease state. To address this, we developed LinkPrep: a proximity ligation protocol yielding high-quality, high-resolution, unbiased Hi-C libraries to detect SVs and chromatin topological features in under 8 hours – less than 50% of the time required for conventional Hi-C protocols. In our study, we demonstrate variant detection within the primary sequence (SVs, SNVs/Indels, and CNVs) and high-resolution 3D chromatin conformation. We benchmarked for genetic variant detection and chromatin topology in two lymphoblast cell lines (GM12878 and K562). LinkPrep detects interchromosomal translocations with 10-fold higher sensitivity over WGS. LinkPrep data shows uniform coverage and greater sensitivity over traditional Hi-C methods.

Importantly, LinkPrep libraries maintain topological features; TADs and chromatin loops, providing insight into novel epigenetic cancer drivers.

Methods:

Results:

Conclusion: LinkPrep enables higher-resolution assessments of chromatin topology, demonstrated by 50% more TADs and loops at 5-Kb resolution. We demonstrate LinkPrep is a next-generation Hi-C method enabling sensitive detection of clinically relevant SVs and high-resolution chromatin topology in a <8-hour workflow.

Grants:

Conflict of Interest: None declared

P20.065.B Epigenome-Wide Association Study of the Nasal Microbiome Composition Involved in Asthma Exacerbations

Javier Perez-Garcia[1], *Mario Martín Almeida[1], Elena Martin-Gonzalez[1], Ruperto González-Pérez[2], José M. Hernández-Pérez[3], Olaia Sardón[4;5], Paloma Poza-Guedes[2], Elena Mederos-Luis[6], Paula Corcuera[4], Inmaculada Sánchez-Machín[6], Leyre Lopez-Fernandez[4], Jesús Villar[7;8;9], Fabian Lorenzo-Diaz[1;10], Maria Pino-Yanes[1;7;11]*

[1]Universidad de La Laguna (ULL), Genomics and Health Group, Department of Biochemistry, Microbiology, Cell Biology and Genetics, La Laguna, Spain; [2]Hospital Universitario de Canarias, Severe Asthma Unit, Allergy Department, La Laguna, Spain; [3]Hospital Universitario de N.S de Candelaria, Pulmonary Medicine Service, La Laguna, Spain; [4]Hospital Universitario Donostia, Division of Pediatric Respiratory, Donostia, Spain; [5]University of the Basque Country (UPV/EHU), Department of Pediatrics, San Sebastián, Spain; [6]Hospital Universitario de Canarias, Allergy Department, La Laguna, Spain; [7]CIBER de Enfermedades Respiratorias, Instituto de Salud Carlos III, Madrid, Spain; [8]Hospital Universitario Dr. Negrín, Multidisciplinary Organ Dysfunction Evaluation Research Network (MODERN), Research Unit, Las Palmas, Spain; [9]Li Ka Shing Knowledge Institute at the St. Michael's Hospital, Toronto, Canada; [10]Instituto Universitario de Enfermedades Tropicales y Salud Pública de Canarias (IUETSPC), Universidad de La Laguna (ULL), La Laguna, Spain; [11]Instituto de Tecnologías Biomédicas (ITB), Universidad de La Laguna (ULL), La Laguna, Spain

Background: The upper-airway microbiome involved in asthma exacerbations is regulated by genetic variation. However, the influence of DNA methylation (DNAm) remains unknown. We aimed to identify epigenetic markers associated with airway bacteria involved in asthma exacerbations.

Methods: The nasal microbiome and methylome in 154 European asthma patients were profiled by targeted sequencing of the V3-V4 region of the 16S rRNA and using the Illumina MethylationEPICv2 array, respectively. The association of 715,588 CpGs with exacerbation-related microbiome traits was tested through microbiome epigenome-wide association studies (mbEWAS). Models were corrected for age, sex, ancestry, and tissue heterogeneity. Genomic significance was declared using $p < 9 \times 10^{-8}$. Sensitivity analyses of microbiome confounders were carried out. Differentially methylated regions (DMRs) were assessed and included in gene-set enrichment analyses.

Results: Among the evaluated bacterial genera, the CpG cg06651505 (*TENM2*) was associated with the presence of *Dialister* in the nasal microbiome ($p = 8.7 \times 10^{-9}$). A total of 68 DMRs associated with this genus were identified (false discovery rate[FDR]<0.05), with the top-hit located in *IGF2* (17 CpGs, $p = 1.30 \times 10^{-27}$). These associations remained significant after adjusting for antibiotics use, recent smoking, body mass index, and sequencing pool ($p_{CpG} = 8.7 \times 10^{-9}$ and $p_{DMR} = 4.37 \times 10^{-27}$). *TENM2* and *IGF2* have been previously associated with severe asthma and/or lung function. DMRs were enriched in genes regulating Toll-Like Receptor pathways, the first-line defense of the innate system against infections (FDR < 0.05).

Conclusions: The first mbEWAS in asthma identified methylation loci associated with *Dialister* presence in the nasal microbiome from asthma patients, with potential implications for the immune system in host-microbiome interactions.

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P20.066.C 3D genomics reveals tissue-specific regulatory networks associated with epilepsy and glioblastoma-linked Ptprz1

Miles Collier[1], *Cory Padilla*[1], *Myriam El Khawand*[1], *Jonathon Torchia*[1], *Lisa Munding*[1]

[1]*Dovetail Genomics, Scotts Valley, United States*

Background/Objectives: The three-dimensional (3D) organization of DNA has emerged as a crucial factor in gene regulation, impacting development and disease. Hi-C sequencing has been employed to investigate genomic interactions in the 3D space. However, its low resolution and accuracy, stemming from bias towards restriction site motifs have limited its adoption. Recent improvements, using sequence-independent endonucleases, including *MNase* (Micro-C) to generate nucleosome fragments have addressed this bias.

Methods: In this study, we present a novel method for isolating fixed chromatin from tissues without compromising the 3D structure. We sampled diverse tissue types from a singular mouse subject (including blood, confirming its clinical relevance), highlighting tissue-specific 3D features in liver and brain.

Results: 3D-genome differential analysis reveals subtle, yet significant variations between both tissues. A majority of topologically associating domains (TADs) (91%) and chromatin loops (85%) were conserved. Gene ontology (GO) analysis of chromatin loops (6% in brain and 9% in liver) indicates distinctions in functional categories closely linked to tissue-specific expression. Specifically, *Ptprz1* (Proteoglycan function), implicated in epilepsy and glioblastoma, exhibits brain-specific regulatory dynamics within TADs containing three looping interactions. A hybrid-capture targeting gene-promoters confirmed Ptprz1-specific interactions with increased sensitivity whilst massively reducing the sequencing burden (95-80%). The disruption of *Ptprz1*, and other tissue specific regulatory networks likely leads to pathologies.

Conclusion: In conclusion, integrating 3D genomics to uncover intricate chromatin architectures linked to diseases is proving to be increasingly important.

Grants:

Conflict of Interest: None declared

P20.067.D mEthAE: an explainable autoencoder for methylation data

Sonja Katz[1,2], *Vitor Martins Dos Santos*[3], *Edoardo Saccenti*[1], *Gennady Roshchupkin*[2]

[1]*Wageningen University & Research, Systems and Synthetic Biology, Wageningen, Netherlands;* [2]*Erasmus MC, Radiology, Rotterdam, Netherlands;* [3]*Wageningen University & Research, Biomanufacturing & Digital Twins, Wageningen, Netherlands*

Consortium: European Union's EU Framework Programme for Research and Innovation Horizon 2020, Grant Agreement No 860895 (TranSYS)

Background:

Despite insights from epigenome-wide association studies (EWAS), univariate linear analyses fall short in simultaneously considering spatial relationships among the 450.000 CpG sites, impeding a comprehensive understanding of CpG relationships. Deep learning algorithms, particularly autoencoders, are considered promising in capturing the complex patterns of DNA methylation data.

Methods: In this context, we propose mEthAE, a chromosome-wise autoencoder for interpretable dimensionality reduction of methylation data. Going beyond data compression, we developed a perturbation-based method for interpreting latent dimensions. Findings are compared to EWAS results, evaluating their predictive power, analyzing genomic location, associated biological pathways, correlation patterns, and genomic distances.

Results: mEthAE efficiently reduces 300,000 CpG sites into <1400 latent features - a 200-fold compression - without compromising on reconstruction accuracy or predictive power. Our interpretability approach uncovers clusters of CpGs with strong connections across all latent dimensions, significantly aligning with EWAS findings. Surprisingly, these connected CpGs lack correlation patterns or physical proximity on the chromosome.

Conclusion: Our analyses lead to an exciting conclusion: mEthAE uncovers largely uncharacterized long-range non-linear interaction patterns within DNA methylation data.

We believe this study is of great interest to the epigenetics community, demonstrating the utility of explainable deep learning algorithms to (i) provide a new perspective on our understanding of epigenetic interactions and (ii) reveal a sparsity of methylation signal which has strong implications for the design of novel DNA methylation arrays.

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Conflict of Interest: None declared

P20.068.A Novel bi-allelic TRMT1 variants perturb tRNA modifications expanding the clinical spectrum of intellectual disability

Stephanie Efthymiou[1], *Chenghong Deng*[2], *Cailyn Leo*[2], *Kejia Zhang*[2], **Renee Qing Lin**[1], *Irem Karagoz*[1], *Annarita Scardamaglia*[1], *Daniel Owrang*[3], *Reza Maroofian*[1], *Barbara Vona*[3], *Andrew Fry*[4], *Henry Houlden*[1], *Dragony Fu*[2]

[1]*UCL Queen Square Institute of Neurology, United Kingdom;* [2]*Center for RNA Biology, University of Rochester, Rochester, Department of Biology, New York, United States;* [3]*University Medicine Göttingen, Göttingen, Germany;* [4]*School of Medicine, Cardiff University, United Kingdom*

Consortium: TRMT1 Clinical Consortium

Background/Objectives: Genomic mutations in the *tRNA Methyltransferase 1* (*TRMT1*) gene have been implicated in certain cases of autosomal-recessive intellectual disability disorders. However, the underlying molecular mechanisms that cause the disease phenotypes remain elusive.

Methods: Here, we have identified 25 individuals from 20 families with bi-allelic variants in *TRMT1* presenting with developmental delay and intellectual disability. In addition, abnormal vision and/or hearing, progressive spasticity, choreoathetoid movements, refractory epilepsy, and brain atrophy were part of the core phenotype of this syndrome.

Results: We find that a subset of variants cause mis-splicing and loss of TRMT1 expression. Notably, patient cells with pathogenic *TRMT1* variants exhibited a deficiency in m2,2G modifications. We also dissect the molecular impact of each pathogenic variant on reconstitution of enzymatic activity and tRNA binding, thereby revealing novel aspects of TRMT1 structure.

Conclusion: Our studies elucidate the functional effects of novel pathogenic variants in *TRMT1* associated with a large number of autosomal-recessive cognitive disorders in the human population.

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Dystrophy United Kingdom (MDUK), Muscular Dystrophy Association (MDA USA)., full time, Dragony Fu NIH GR530882, full time

P20.069.B The Biology of Single Cell and Bulk Sequencing-Based CRISPR Screens

Angela Shaw[1], *Maximilian Billmann*[1]

[1]*Institute of Human Genetics, University of Bonn, School of Medicine and University Hospital Bonn, Bonn, Germany*

Background/Objectives: CRISPR screening, with high-content phenotypic readouts such as Perturb-Seq or more physiological culture conditions, carries great promise of assigning genes to functions where simple cell fitness screens fall short. A recent comprehensive study recorded single-cell RNA expression in 2.5 million cells, perturbing each transcribed gene individually. Here, we systematically compare the ability of these co-expression phenotypes with co-essentiality networks derived from the Cancer Dependency Map, comprising more than 1000 genome-wide CRISPR screens, to resolve functional neighborhoods within the cell.

Methods: For the comparative analysis of the two datasets, we leveraged FLEX, a tool to explore functional information and bias in genome-wide perturbation data. Global and process-resolved precision-recall analyses using gold standard functional databases CORUM, MsigDB Pathways and GO-BP were used to quantify the performance with which the data reconstruct known biology. Genetic interaction screens in standard conditions and on extracellular matrix components were conducted to determine whether simple fitness readouts can resolve functional modules required for 3D cell growth.

Results: We find that the majority of central cellular functions are resolved well by only one of the data sets. Single-cell expression phenotypes reconstructed the machinery facilitating transcription, translation and protein turnover in great detail, while the co-essentiality network better resolved chromatin remodeling, cell communication, and adhesion. Fitness readouts can resolve functional modules required for 3D cell growth, where expression phenotypes lack resolution.

Conclusion: Our study presents a comprehensive assessment of the strengths and weaknesses of CRISPR screening modalities, providing a compass for effectively screening for genetic modifiers of specific cellular processes.

Grants: None

Conflict of Interest: None declared

P20.070.C Comparative analysis of D4Z4 and D4Z4-like repeats in primate genomes: insights into FSHD and evolutionary dynamics

Joshua K Bopp[1], *Christian W Remmele*[2;3;4], *Thomas Haaf*[2], *Tobias Müller*[1], *Marcus Dittrich*[1;2]

[1]*Department of Bioinformatics at the University of Würzburg, Würzburg, Germany;* [2]*Institute of Human Genetics at the University of Würzburg, Würzburg, Germany;* [3]*Center for Rare Diseases at the University Hospital Würzburg, Würzburg, Germany;* [4]*Bavarian Genome Network for Rare Diseases, Germany*

Background/Objectives: Facioscapulohumeral muscular dystrophy (FSHD) is associated with the D4Z4 macrosatellite array on human chromosome 4, where repeat contraction combined with hypomethylation can lead to disease onset. Understanding evolutionary conservation and syntenic relationships of D4Z4 repeats in primates is important for deeper insight into pathogenesis. Prior methods encountered challenges in resolving macrosatellite repeats, while modern long-read techniques can achieve precise resolution while simultaneously enable methylation profiling. In this study publicly available ultra-long Nanopore sequencing data was used to bridge this gap by investigating methylation patterns within orthologous D4Z4 repeats of great apes and comparing their evolutionarily conservation.

Methods: Genome assemblies and nanopore long-read data of great apes were obtained from the T2T-Primates project and used for genomic analyses. The Nanopore ultra-long read data was accurately mapped to macrosatellite regions of the primate assemblies. Region specific methylation patterns were extracted. The study concentrated on syntenic D4Z4-containing regions of human chromosome 4 known for their involvement in FSHD.

Results: Here we focus on syntenic regions in primate genomes to investigate evolutionary relationships and conserved repeat

architecture. We identify complete and partial D4Z4 repeats and discuss their variability across primate species. Furthermore, we examine evolutionary distances within D4Z4 repeats across all samples. To elucidate epigenetic signatures, we analyse methylation patterns within these repeats. We investigate the conservation of methylation gradients along D4Z4 repeat arrays, indicating a shared epigenetic regulation of these elements.

Conclusion: This study delves into D4Z4 repeat variation and methylation patterns in primate genomes, providing insights into their evolutionary dynamics and the pathomechanism of FSHD.

Grants:

Conflict of Interest: None declared

P21
NEW TECHNOLOGIES

P21.001.D Nanopore long read whole genome sequencing in developmental disorders

Mathilde Geysens[1;2], *Benjamin Huremagic*[1], *Griet De Clercq*[3;4], *Erika Souche*[1], *Laurens Hannes*[1;2], *Annelies Dheedene*[3;4], *Björn Menten*[3;4], *Joris Vermeesch*[1;2], *Kris Van Den Bogaert*[1;2]

[1]*KU Leuven, Department of Human Genetics, Leuven, Belgium;* [2]*UZ Leuven, Center for Human Genetics, Leuven, Belgium;* [3]*Ghent University, Department of Biomolecular Medicine, Ghent, Belgium;* [4]*Ghent University Hospital, Center for Medical Genetics, Ghent, Belgium*

Background: Despite the implementation of massive parallel sequencing as standard of care, about half of patients with developmental disorders (DD) remain without genetic diagnosis. Structural and epigenetic variants have long been known to be involved in the pathogenesis of DD but remain challenging to map due to technological limitations. However, with dropping costs and increasing accuracy of long read sequencing (LRS) platforms, the concomitant assessment of single nucleotide, structural and epigenetic variation becomes feasible.

Methods: To evaluate the relevance of LRS clinical implementation for unsolved DD we performed whole genome nanopore sequencing in 50 patient-parent trios with intellectual disability and/or multiple congenital anomalies without molecular diagnosis after short read exome or genome sequencing. We optimized the nanopore sequencing technical workflow, developed an analytical pipeline and built a population reference set to assess structural variants (SVs). In addition, we validated phased methylation detection looking at imprinted regions as well as X-inactivation.

Results: We identify around 25.000 SVs per individual, of which 100 are classified as de novo. However, only 0.6 potential true de novo SV are retained upon manual curation and population filtering. On average, 2.1 X-linked inherited SVs are identified in males. SVs disrupting regulatory sequences of DD associated genes are further investigated and the haplotyped methylome is being scrutinized to study potential pathogenic mechanisms of non-coding variants.

Conclusion: LRS enables the detection of few, but potential disease-causing, hitherto unidentified SVs. We envision that the assessment of secondary but also primary epigenetic variants will further improve the diagnostic yield.

Grants: FWO-TBM-grant-T-003819N; FWO-grant-G0A2622N; KULeuven-grant-C1-C14/18/092; KULeuven-grant-C14/22/125; CDP-Horizon-2020-grant-F.7/KULEUVENB&G35332

Conflict of Interest: None declared

P21.002.A Variable Depth Whole Genome Sequencing: An Economical Approach for Genome-wide Detection of Clinically Significant Variants

Valery Ilinsky[1], *Anna Ilinskaya*[1]

[1]*Eligens, Riga, Latvia*

Background/Objectives: Traditional methods, such as microarrays and whole genome sequencing, either lack the resolution to effectively detect rare variants or are prohibitively expensive for widespread use. Our novel approach, "Variable Depth Whole Genome Sequencing" (VD-WGS), integrates low-coverage whole genome sequencing with targeted high-coverage sequencing of clinically significant rare variants. This method is especially beneficial for diagnosing polygenic diseases with multifactorial and monogenic nature.

Methods: VD-WGS involves preparing a single sequencing library, from which an aliquot undergoes targeted enrichment suitable for multiplexing up to 96 samples. This streamlined process significantly reduces both cost and complexity. For regions sequenced at low coverage, imputing is performed using reference panels.

Results: In low-coverage regions, using custom reference panel resulted in precision 99.4% with a recall of 95.7% for heterozygous variants, and a precision of 99% with a recall of 98.2% for homozygous variants. These results confirm the effectiveness of VD-WGS in accurately imputing genomic data at reduced coverage levels. In contrast, regions sequenced at high coverage displayed precision and recall rates up to 100%.

Conclusion: VD-WGS offers a cost-effective and efficient approach for detailed genomic analysis. With a cost as low as 20-30 EUR per sample, it is less expensive than both microarrays and traditional full genome or exome sequencing methods. This method provides a comprehensive analysis of clinically relevant genomic areas, combining the benefits of low-cost, broad coverage sequencing with the high accuracy of targeted high-coverage sequencing. VD-WGS has the potential to significantly advance genetic research and clinical diagnostics, making comprehensive genomic analysis more accessible and affordable.

Grants:

Conflict of Interest: Valery Ilinsky Valery is a stock owner of Eligens SIA., Anna Ilinskaya: None declared

P21.003.B T2T-CHM13 improves read mapping and detection of clinically relevant variation

Daniel Schmitz[1], *Adam Ameur*[1], *Åsa Johansson*[1]

[1]*Uppsala University, Department of Imunology, Genetics and Pahtology, Science for Life Laboratory, Uppsala, Sweden*

Background/Objectives: The new T2T-CHM13 assembly was released in March 2022, filling in the 8 % of the human genome that were not resolved in GRCh38. This new reference genome can be beneficial for population and clinical genomics, where whole genome sequencing (WGS) based approaches have become more common, but the extent of these benefits remains unclear. In this study, we aim to determine the increment of mapping quality and variant call performance with T2T-CHM13 as a reference.

Methods: We used a cross-sectional Swedish cohort (SweGen) comprising 1000 individuals with short-read Illumina (WGS) data available. Remapping and variant calling were performed using the nf-core/sarek pipeline.

Results: T2T-CHM13 improved a wide range of mapping and variant calling related metrics. A higher fraction of reads was properly paired and mismatch rate was lower, giving higher confidence in the mapping results. The fraction of ambiguous alignments was higher, indicating that alignments were falsely considered unique previously. Coverage of gene regions was more uniform. In comparison to GRCh38, we identified 10 million

additional variants, including 5.5 million singletons across the cohort, and observed an increased sensitivity for rare variants. SnpEff assigned impact ratings of moderate or high for 30 % more variants in T2T-CHM13.

Conclusion: T2T-CHM13 improves alignment metrics with higher mapping quality, better variant calling performance and confidence, especially for rare and deleterious variants. Using T2T-CHM13 as reference genome will enable the discovery of unknown disease-causing variants, benefiting especially rare-disease diagnostics.

Grants: Swedish Research Council (2019-01497), SciLifeLab

Conflict of Interest: None declared

P21.004.C TET Assisted Pyridine-Borane Sequencing version 1.0 - A rapid, scalable, and non-destructive sequencing approach to methylation profiling.

Lee French[1], *Craig Marshall[2], Travis Sanders[2], Sam Vogel[2], Aaron Garnett[2], Martin Ranik[2], Eduard Casas[2], Doug Wendel[2], Leo karamanof[1], Bjarne Faurholme[1], Abre de Beer[1], Ross Wadsworth[1], Kristina Giordia[2], Eric van der Walt[1], Brian Kudlow[2]*

[1]Watchmaker Genomics, Applications Development, Cape Town; [2]Watchmaker Genomics, Applications Development, Boulder, United States

Background/Objectives: Detection of 5-methylcytosine (5mC) serves as a powerful biomarker for studying and monitoring disease states. Bisulfite sequencing (BS-seq) converts unmethylated cytosine to uracil while 5mC is protected. This has limitations such as DNA destruction and the generation of low-complexity sequences making it sub-optimal for NGS. Here we introduce TET Assisted Pyridine-Borane Sequencing version 1.0 (TAPS v1.0), showcasing advancements to the technology originally introduced by Schuster-Böckler, Song, and their collaborators. TAPS v1.0 transforms 5mC into dihydrouracil (DHU) which is recognized as dU during PCR resulting in C-to-T conversions at 5mC sites. This leaves unmethylated cytosines unchanged. This nondestructive nature of 5mC conversion yields significantly higher library yield and complexity compared to BS-seq.

Methods: NA12878 human genomic DNA (containing spike-ins of variable methylated controls) along with cfDNA, FFPE breast DNA, and matched tumour and healthy breast DNA were prepared with the Watchmaker DNA Library Prep kit and subjected to either BS-seq (Zymo Research), EM-seq™ (NEB), or TAPS v1.0 treatment before bioinformatic analysis.

Results: TAPS v1.0 showed significantly higher final library yield and greater complexity than BS-seq and EM-seq with all sample types. TAPS-mediated 5mC detection was highly concordant with the other methods, yet preserved more of the underlying genomic information. Additionally, TAPS v1.0 preserved the disome in cfDNA and identified similar patterns of methylation, while improving CNV detection, in matched tumour-normal breast samples compared to BS-seq and EM-seq.

Conclusion: TAPS v1.0 shows great potential as a non-destructive, rapid, and scalable alternative for base-level methylation analysis.

Grants: None.

Conflict of Interest: Lee French Watchmaker Genomics, Watchmaker Genomics, Craig Marshall Watchmaker Genomics, Watchmaker Genomics, Travis Sanders Watchmaker Genomics, Watchmaker Genomics, Sam Vogel Watchmaker Genomics, Watchmaker Genomics, Aaron Garnett Watchmaker Genomics, Watchmaker Genomics, Martin Ranik Watchmaker Genomics, Watchmaker Genomics, Eduard Casas Watchmaker Genomics, Watchmaker Genomics, Doug Wendel Watchmaker Genomics, Watchmaker Genomics, Leo karamanof Watchmaker Genomics, Watchmaker Genomics, Bjarne Faurholme Watchmaker Genomics, Watchmaker Genomics, Abre de Beer Watchmaker Genomics, Watchmaker Genomics, Ross Wadsworth Watchmaker Genomics, Watchmaker Genomics, Kristina Giordia Watchmaker Genomics, Watchmaker Genomics, Eric van der Walt Watchmaker Genomics, Watchmaker Genomics, Brian Kudlow Watchmaker Genomics, Watchmaker Genomics

P21.005.D Surveying the RNA-seq landscape: A multi-workflow comparison for mRNA and whole transcriptome sequencing applications

Jennifer Pavlica[1], *Trishana Nundalall[1], Deelan Doolabh[1], Lee French[1], Ross Wadsworth[1], Ariele Hanek[1], Josh Haimes[1], Kailee Reed[1], Thomas Harrison[1], Rajat Roy[1], Heather Maasdorp[1], Giulia Corbet[1], David Gelagay[1], Travis Sanders[1], Eric van der Walt[1], Brian Kudlow[1]*

[1]Watchmaker Genomics

Background/Objectives: RNA-seq has become an essential method of studying the transcriptome. Unlike genomics, transcriptomics provides a snapshot of cellular activity at a specific point in time and in response to stimuli. Various approaches to RNA library preparation allow researchers to characterize protein-coding and non-coding transcripts, thus unraveling biological mechanisms, monitoring disease states, and validating biomarker and drug responses. There is a growing need for solutions that are sensitive, scalable, and provide robust performance especially with low inputs and challenging samples such as FFPE RNA. In addition, compatibility with alternate short-read sequencers is becoming critical as the sequencing landscape broadens.

Methods: Herein, we compare three commercially available stranded RNA library prep kits for both mRNA and whole transcriptome sequencing (WTS). Chemistries are evaluated across an input range of 1 to 1000 ng and include both high-quality RNA and degraded FFPE material.

Results: While performance across workflows is consistent at high inputs, the Watchmaker RNA library prep solutions outperform at low inputs with respect to genes detected, preservation of the gene expression profile, and fewer wasted bases. WTS of matched fresh frozen and FFPE samples further demonstrates the improved sensitivity of the Watchmaker solution with a higher percentage of gene overlap between the matched samples. Additionally, compatibility is demonstrated across both the Singular and Element short-read platforms – widening the utility of the Watchmaker workflows.

Conclusion: The Watchmaker RNA library prep solutions enable researchers to access more information from challenging samples with workflows that are compatible with multiple short-read sequencing platforms.

Grants: N/A

Conflict of Interest: None declared

P21.006.A Nanopore sequencing-based episignature detection

Benjamin Huremagic[1], *Mathilde Geysens[1;2], Erika Souche[1], Jeroen Breckpot[2], Koenraad Devriendt[2], Hilde Van Esch[2], Griet Van Buggenhout[2], Hilde Peeters[2], Kris Van Den Bogaert[1;2], Joris Vermeesch[1;2]*

[1]KU Leuven, Department of Human Genetics, Leuven, Belgium; [2]UZ Leuven, Centre for human genetics, Leuven, Belgium

Background/Objectives: A subset of developmental disorders is characterized by disease-specific genome-wide methylation changes. These episignatures inform about underlying pathogenic

mechanisms and can be used to assess the pathogenicity of genomic variants as well as confirm clinical diagnoses. Currently, episignature detection requires the use of indirect methylation profiling microarrays. As long-read-sequencing (LRS) enables the detection of single nucleotide variants (SNVs), structural variants (SVs), and base modifications, we aimed to assess its potential for simultaneous episignature and genomic variant detection.

Methods: Twenty patients, with thirteen distinct developmental disorders having known episignatures, were subjected to genome-wide nanopore sequencing. We first evaluated the compatibility of known episignatures with nanopore sequencing and subsequently developed a support vector machine for each developmental disorders.

Results: Methylation levels at episignature loci co-clustered disease samples and microarray-based disease reference. Our classifier identified the correct episignature in 17/20 blinded samples. The three remaining patients were classified as control by both our classifier and commercially available microarray-based episignature assay. All pathogenic SNVs and SVs detected by standard of care methods were identified. In addition, we were able to evaluate disease-associated imprinted regions as well as X-inactivation.

Conclusion: This study demonstrates nanopore sequencing is suitable for episignature detection and underscores the value of LRS for concurrent haplotyped genomic and epigenomic analyses.

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Conflict of Interest: None declared

P21.007.B Telomere length estimation using sequencing data of a large cohort

Lara E. Michels[1], Stephan Drukewitz[1], Rami Abou Jamra[1], Bernt Popp[1;2], **Isabell Schumann**[1]

[1]Institute of Human Genetics, University of Leipzig Medical Center, Leipzig; [2]Center of Functional Genomics, Berlin Institute of Health at Charité, Berlin, Germany

Background/Objectives: The current standard for telomere length (TL) analysis requires qPCR. Several bioinformatic tools can analyze short read genome sequencing (GS) telomere content. But they are not investigated for the widespread targeted sequencing (TS). Here, we present the results of bioinformatic TL analysis using GS and TS data.

Methods: We evaluated four tools (TelSeq, Computel, TelomereHunter, and Telomerecat) on a cohort of 11 families (38 individuals) with TS, GS, and established qPCR. Further, we have screened a cohort of 7500 exomes from different enrichment kits as well as 2500 large panel datasets.

Results: Using GS and TS, we were able to observe the expected phenomenon of a negative correlation with age, which was validated using qPCR. Using TelomereHunter, the estimation of TL with age was comparable for GS and TS. In contrast, TelSeq, Computel, and Telomerecat did not produce reliable TS results. Additionally, we discovered that issues such as establishing the workflow for a specific enrichment kit and the need to normalize with age-matched controls should be considered prior to TL measurement based on TS.

Conclusion: Aside from biological age, TL analysis is relevant for a variety of genetic disorders, including telomeropathies. Although our findings support GS as the best data for analyzing TL, our large cohort screening of TL in TS data yields promising results. This is important for the community because TS is widely used and most genetic institutions have access to TS but not GS.

Grants: None.

Conflict of Interest: None declared

P21.008.C Nanopore sequencing with unique molecular identifiers accurately determines SNP haplotypes and repeat number of the LPA KIV-2 VNTR

Stephan Amstler[1], *Gertraud Streiter[1], Cathrin Pfurtscheller[1], Lukas Forer[1], Silvia Di Maio[1], Hansi Weissensteiner[1], Bernhard Paulweber[2], Sebastian Schoenherr[1], Florian Kronenberg[1], Stefan Coassin[1]*

[1]Institute of Genetic Epidemiology, Department of Genetics, Innsbruck, Austria; [2]First Department of Internal Medicine I, Paracelsus Medical University, Salzburg, Austria

Background/Objectives: About 90% of lipoprotein(a) plasma variance is determined by a complex, haplotype-dependent interplay between *LPA* SNPs and the size of the *LPA* KIV-2 VNTR, with up to 40 KIV-2 repeats per allele (≈5.6 kb each). NGS has identified several modifier SNPs within KIV-2 but fails to capture SNP haplotypes. We evaluated nanopore sequencing with unique molecular identifiers (UMI-ONT-Seq) for detecting and haplotyping KIV-2 SNPs and determining KIV-2 repeat number.

Methods: UMI-ONT-Seq was performed on mixtures of two KIV-2 plasmids with 87 differences (mixture levels 1% to 5% and unmixed) and 63 multi-ancestry human samples with NGS reference data. We evaluated the impact of sequencing chemistries and basecalling algorithms, the minimal reads per UMI required for Q40 consensus sequence quality, error profiles and the accuracy of determining the KIV-2 repeat number via coverage-corrected haplotype quantification.

Results: Six reads per UMI were sufficient to achieve Q40 consensus sequence quality (i.e. the sequences of each KIV-2 unit). Variants were accurately detected down to 1% level. In plasmid samples, sensitivity, specificity, and precision were (mean ± SD) 0.998 ± 0.004, 1.0 ± 0.0, 0.995 ± 0.013. Variants from the same plasmid (being one haplotype) showed the same noise-free levels. Human variant levels were highly correlated with NGS ($R^2 = 0.983$). KIV-2 repeat number was in close agreement with ddPCR ($R^2 = 0.847$), with 44 of 63 samples within ddPCR confidence interval.

Conclusion: UMI-ONT-Seq supplies accurate full-length SNP haplotypes for each KIV-2 repeat and precisely quantifies repeat numbers. UMI-ONT-Seq provides benchmarking data for variant calling and haplotyping in genomic regions not resolvable with NGS.

Grants:
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P21.009.D Liquid biopsy in cancer: beyond the targeted analysis of single variants

Morghan Lucas[1;2], Thomas Keßler[1;3], Anke Arnold[1], Georg Beyer[4], Stefan Werner[3;5;6], Lina Bergmann[3;5], Gunhild von Amsberg[7], Rachel Würstlein[8], Robert Hüneburg[9;10], Aysel Ahadova[11;12], Lena Bohaumilitzky[11;12], Matthias Kloor[11;12], Verena Steinke-Lange[1;2;3], Elke Holinski-Feder[1;2;3], **Ariane Hallermayr**[1;2;3]

[1]MGZ – Medizinisch Genetisches Zentrum, Munich, Germany; [2]Medizinische Klinik und Poliklinik IV, Campus Innenstadt, Klinikum der Universität München, Munich, Germany; [3]European Liquid Biopsy

Society, Hamburg, Germany; [4]Department of Medicine II, University Hospital, Ludwig Maximilian University Munich, Munich, Germany; [5]Institute for Tumor Biology, University Cancer Center Hamburg, University Medical Center Hamburg-Eppendorf, Hamburg, Germany; [6]Mildred-Scheel-Nachwuchszentrum HaTRiCs4, Universitäres Cancer Center Hamburg, Hamburg, Germany; [7]University Medical Center Hamburg-Eppendorf, Department of Hematology and Oncology, Hamburg, Germany; [8]Breast Center, Department of Gynecology and Obstetrics and CCC Munich LMU, LMU University Hospital, Munich, Germany; [9]Department of Internal Medicine, University Hospital Bonn, Bonn, Germany; [10]National Center for Hereditary Tumor Syndromeheis, University Hospital Bonn, Bonn, Germany; [11]Department of Applied Tumor Biology, Institute of Pathology, Heidelberg University Hospital, Heidelberg, Germany; [12]Clinical Cooperation Unit Applied Tumor Biology, German Cancer Research Center (DKFZ), Heidelberg, Germany

Background/Objectives: Liquid biopsy diagnostics have predominantly focused on circulating tumor DNA (ctDNA) analysis in the context of actionable somatic hotspot variants for guiding treatment decisions. Our previous work introduced LIquid biopsy Fragmentation, Epigenetic signature, and Copy Number Alteration analysis (LIFE-CNA) utilizing whole-genome sequencing (WGS) in colorectal cancer (CRC) patients. Expanding on this strategy, our goal is to broaden the application of LIFE-CNA to other tumor entities.

Method: Plasma samples from patients diagnosed with diverse tumor types, including 2 breast cancer, 9 prostate cancer, and 2 Lynch Syndrome (LS)-associated cancer cases, as well as 5 intraductal papillary mucinous neoplasm (IPMN) cases presenting with a risk to develop pancreatic cancer, were sequenced using the LIFE-CNA method.

Results: Using 55 healthy controls, we established distinct cutoffs for the detection of ctDNA based on global and regional fragmentation patterns, transcriptionally active chromatin, and somatic copy number alterations (CNVs). All breast and prostate cancer cases exhibited significant alterations in both fragmentation profiles and CNVs. In addition, all 5 IPMN cases showed an overlap with a distinct epigenetic signature. In contrast, both LS cases showed no significant variations.

Conclusions: This proof-of-principle study demonstrates the potential of LIFE-CNA beyond CRC detection. However, cohort expansion is crucial to confirm the sensitivity of this approach across tumor entities. Considering functional differences between tumor entities could further improve the method's sensitivity. The study underscores the utility of employing multiple metrics in ctDNA analysis and highlights LIFE-CNA's adaptability to detect various cancer types in a minimally invasive manner.

Grants: N/A

Conflict of Interest: None declared

P21.010.A Integrating host genetic and gut metagenomic risk prediction for common diseases

Yang Liu[1;2;3;4;5], *Scott C. Ritchie[1;2;4;5;6;7], Shumei Teo[4;5;8], Matti O. Ruuskanen[9;10], Oleg Kambur[9], Qiyun Zhu[11;12], Jon Sanders[13], Yoshiki Vazquez-Baeza[14], Karin Verspoor[15;16], Pekka Jousilahti[9], Leo Lahti[10], Teemu Niiranen[9;17], Veikko Salomaa[9], Aki S. Havulinna[9;18], Rob Knight[14;19;20], Guillaume Méric[4;21;22;23;24], Michael Inouye[1;2;3;4;5;6;7]*

[1]University of Cambridge, Victor Phillip Dahdaleh Heart and Lung Research Institute, Cambridge, United Kingdom; [2]University of Cambridge, British Heart Foundation Cardiovascular Epidemiology Unit, Cambridge, United Kingdom; [3]The University of Melbourne, Department of Clinical Pathology, Melbourne, Australia; [4]Baker Heart and Diabetes Institute, Cambridge Baker Systems Genomics Initiative, Melbourne, Australia; [5]University of Cambridge, Department of Public Health and Primary Care, Cambridge, United Kingdom; [6]Wellcome Genome Campus and University of Cambridge, Health Data Research UK Cambridge, United Kingdom; [7]University of Cambridge, British Heart Foundation Cambridge Centre of Research Excellence, Cambridge, United Kingdom; [8]The University of Melbourne, Centre for Youth Mental Health, Melbourne, Australia; [9]Finnish Institute for Health and Welfare, Department of Public Health and Welfare, Helsinki, Finland; [10]University of Turku, Department of Computing, Turku, Finland; [11]Arizona State University, School of Life Sciences, Tempe, United States; [12]Arizona State University, Biodesign Center for Fundamental and Applied Microbiomics, Tempe, United States; [13]Cornell University, Department of Ecology and Evolutionary Biology, Ithaca, United States; [14]University of California San Diego, Center for Microbiome Innovation, La Jolla, United States; [15]RMIT University, School of Computing Technologies, Melbourne, Australia; [16]The University of Melbourne, School of Computing and Information Systems, Melbourne, United States; [17]Turku University Hospital and University of Turku, Division of Medicine, Turku, Finland; [18]University of Helsinki, Institute for Molecular Medicine Finland, Helsinki, Finland; [19]University of California San Diego, Department of Computer Science and Engineering, La Jolla, United States; [20]University of California San Diego, Department of Pediatrics, La Jolla, United States; [21]Monash University, Central Clinical School, Melbourne, Australia; [22]The University of Melbourne, Department of Cardiometabolic Health, Melbourne, Australia; [23]La Trobe University, Department of Cardiovascular Research, Melbourne, Australia; [24]Uppsala University, Department of Medical Science, Uppsala, Australia

Background/Objectives: While both genomics and gut metagenomics have shown promise in prediction of various diseases in the general population, the extent to which they can jointly predict future risk of common diseases remains unclear. In a prospective population-based multi-omics cohort, we investigate the individual and combined predictive capacity of host genetics and gut microbiome for multiple common diseases, as well as their additive values to conventional risk factors (CRFs).

Methods: Genotyping data and shotgun metagenomics sequencing of stool samples of 5,676 individuals aged 25 to 74 years at baseline were utilised for studying four incident diseases – coronary artery disease (CAD), Alzheimer's disease (AD), prostate cancer (PC) and type 2 diabetes (T2D) with ~18 years of follow-up. Prediction performance of disease-specific CRFs, polygenic risk scores (PRSs) calculated using large population-based external summary statistics and gut microbiome risk scores (GMSs) developed using machine learning were assessed separately and jointly.

Results: PRS and GMS individually achieved similar prediction performance to CRFs and were associated with all diseases studied. While baseline age was the strongest predictor for CAD, AD, and PC, adding PRS and GMS to age increased C-statistic by 0.049, 0.019 and 0.032, respectively. For all four diseases, the multi-omics model combining PRS, GMS and CRFs achieved higher prediction performance than models based on any risk factors separately, with ΔC-statistic over CRFs of 0.024 for CAD, 0.014 for T2D, 0.017 for AD, and 0.031 for prostate cancer.

Conclusion: Integrated PRS and GMS models improve the predictive value over CRFs for common chronic diseases.

Conflict of Interest: Yang Liu: None declared, Scott C. Ritchie: None declared, Shumei Teo: None declared, Matti O. Ruuskanen: None declared, Oleg Kambur: None declared, Qiyun Zhu: None declared, Jon Sanders: None declared, Yoshiki Vazquez-Baeza: None declared, Karin Verspoor: None declared, Pekka Jousilahti: None declared, Leo Lahti: None declared, Teemu Niiranen TN has received speaking honoraria from Servier Finland and AstraZeneca (not related to this study)., Veikko Salomaa VS has had research collaboration with Bayer Ltd (Outside this study)., Aki S. Havulinna:

None declared, Rob Knight: None declared, Guillaume Méric: None declared, Michael Inouye: None declared

P21.011.B Simultaneous Profiling of SNP Genotyping, Copy Number Variation and Fragment Length Determination Using Nanofluidic qPCR for Pharmacogenomics Studies

Jian Qin[1;1], Hui Ren[1], Amit Khanna[1], Win Hwang[1], Joel Brockman[1], Arnaldo Barican[1], Greg Harris[1], Tom Goralski[1], Julie Alipaz[1], Charles Park[1], David King[1], Naveen Ramalingam[1]

[1]Standard BioTools, South San Francisco, United States

Background/Objectives: Pharmacogenomics (PGx) testing evaluates a person's genetic variation to determine how the individual metabolizes or responds to medications. It is vital in identifying responders versus nonresponders to medications, optimizing drug doses, and mitigating the risk of adverse events. PGx tests interrogate single-nucleotide polymorphisms (SNPs), copy number variation (CNV), and microsatellite repeat numbers within specific genes associated with differential drug metabolism. However, the traditional qPCR-based and capillary electrophoresis-based methods use separate, cumbersome workflows for SNP genotyping, CNV determination, and fragment analysis for microsatellite markers. In this work, we report a single automated workflow using a qPCR-based nanofluidic system to simultaneously profile SNPs, CNVs, and microsatellite repeat numbers of DNA samples.

Methods: The PGx Assay Panel includes assays for SNPs, CNVs, and microsatellite regions of interest. DNA samples are subject to a targeted enrichment step through preamplification and analyzed on a nanofluidic chip (IFC) with the Biomark™ X9 System. 96 samples are interrogated simulatenously against 96 assays in nanoliter volumes. Analysis and interpretation of SNP genotyping, CNV determination, and fragment analysis of the microsatellite reagions is performed using our data analysis software. We also explored the possibility of using saliva samples directly without extraction.

Results: We have assessed this panel with Coriell DNA samples with known genotypes, copy numbers, and microsatellite repeat numbers. The average call accuracy for SNP and CNV was 99.9% and 98.0% respectively.

Conclusion: We demonstrated a streamlined workflow for the simultaneous testing of SNP, CNV, and microsatellite loci. This workflow can play a major role in PGx testing when implemented in clinical routines.

Conflict of Interest: None declared

P21.012.C Affordable CompleteWGS: Haplotype phased near perfect human WGS

Brock Peters[1], Sissi Zhang[1], Ying Cai[1], Siwei Liang[1], Lijing Pan[2], Bian Cai[2], Xigao Chen[2], Shengting Li[2], Radoje Drmanac[1]

[1]Complete Genomics, Research, San Jose, United States; [2]MGI, Research, China

DNA sequencing technology has been advancing rapidly since the early 2000s. However, experimental determination of the haplotypes of individual human genomes as a routine process is still largely overlooked. We describe a novel human NGS/MPS based WGS process, CompleteWGS, a combination of high-quality PCR free and DNA cobarcoding-based (stLFR) WGS. This enables reporting of all inherited variants (separated from somatic mutations) phased in chromosome arm-size contigs, almost no false positive errors, improved mapping and variant calling in difficult regions (*e.g.*, dark genes), and structural variant calling with targeted de novo assembly. Using samples HG001, HG002, and HG005, we show near perfect false positive and false negative calling rates of > 0.995, phased contig N50 sizes of > 10 Mb comprising almost all heterozygous SNPs and indels, and the successful assembly and placement of 100s of SVs. To achieve this a PCR free and a stLFR WGS libraries were prepared for each sample and sequenced to 30X coverage per library. To demonstrate the utility of this type of data we performed CompleteWGS on DNA from blood cells achieving the same result as for the cell lines and combined it with full length transcriptome analysis to accurately predict expression of each encoded protein allele and identify long range cis regulators of allele-specific expression. Importantly, using commercially available WGS, the total cost of automated preparation and sequencing of CompleteWGS is $200. This opens new frontiers in how research and clinical scale human genome sequencing should be done in the future.

Conflict of Interest: Brock Peters Complete Genomics, Complete Genomics, Sissi Zhang Complete Genomics, Ying Cai Complete Genomics, Siwei Liang Complete Genomics, Lijing Pan MGI, Bian Cai MGI, Xigao Chen MGI, MGI, Shengting Li MGI, MGI, Radoje Drmanac Complete Genomics, Complete Genomics

P21.013.D Cost-effective Cas9-mediated targeted sequencing of Spinocerebellar ataxia repeat expansions

Keiji Tachikawa[1], Satomi Mitsuhashi[1], Yoshihisa Yamano[1], Takahiro Shimizu[1], Takeshi Imai[1], Riyoko Ko[1], Yosuke Kawai[2], Yosuke Omae[2], Katsushi Tokunaga[2], Martin C Frith[3]

[1]St. Marianna University School of Medicine; [2]National Center for Global Health and Medicine; [3]University of Tokyo

Hereditary repeat diseases are caused by an abnormal expansion of short tandem-repeats in the genome. Among them, Spinocerebellar ataxia (SCA) is a heterogenous disease, and currently, 16 responsible repeats are known. Genetic diagnosis is obtained by analyzing the number of repeats through separate testing of each repeat. Although simultaneous detection of candidate repeats using current massively parallel sequencing technologies has been developed to avoid complicated multiple experiments, these methods are generally expensive. In this study, we developed a cost-effective SCA repeat panel (FLO-SCAp) using Cas9-mediated targeted long-read sequencing and the smallest long-read sequencing apparatus, "Flongle." This panel enabled the detection of repeat copy number changes, internal repeat sequences, and DNA methylation in seven patients with different repeat expansion diseases. The median (interquartile range) of coverage and on-target rate were 39.5 (12-72) and 11.6% (7.5-16.5), respectively. To validate our approach, we compared repeat copy number changes measured by FLO-SCAp and short-read whole-genome sequencing (SR-WGS). A high correlation was observed between FLO-SCAp and SR-WGS when the repeat length was 250 base pairs or less ($r = 0.98$, $p < 0.001$). Thus, FLO-SCAp represents the most cost-effective method for conducting multiplex testing of repeats and can serve as the first-line diagnostic tool for SCA. We will also include the result of the newest version of nanopore flowcell (R10.4).

Conflict of Interest: None declared

P21.014.A Enhancing STR detection in human genetics: A comparative study of Long-Read WGS and PCR-Based methods

Ning Wang[1], Yicong Wang[1], Wenxin Zhang[2], Jie Huang[2], Le Li[1], Lingna Xue[1], Tang Li[1], Fang Chen[1]

[1]MGI Tech, Shenzhen, China; [2]National Institutes for food and drug Control (NIFDC), Department of In Vitro Diagnostic Reagent, Beijing, China

Background/Objectives: Short tandem repeats (STRs) play an increasing role in genetics and disease research, especially in genome-wide cohort studies. However, few studies demonstrate the consistency between human whole genome sequencing (WGS) data and PCR-based technologies. In this study, we deployed a comparative study to evaluate the STR detection performance from WGS data with single-end 400bp (SE400) comparing to amplicon-based sequencing.

Methods: We conducted SE400 WGS on 157 male and 123 female samples and analyze a set of 129 STR loci including 54 autosome-STRs, 48 Y-STRs and 27 X-STRs, resulting 30,216 STR loci in total. Setting allele depth thresholds at 1×, 2×, 5× and 10× to evaluate the STR detection rate and compare the STR typing concordance with amplicon-based sequencing by massively parallel sequencing (mMPS) and capillary electrophoresis (CE) for performance comparison.

Results: The SE400 WGS averaged a depth of 24.69×, identifying 29,602, 19,854, 18,791, and 14,446 STR loci across allele depth thresholds of 1× to 10×. Concordance with mMPS and CE ranging from 93.56% to 97.75% and 93.71% to 97.78%, respectively. In 77 samples with WGS depth ≥30×, setting a 10× allele depth threshold for STRs resulted in notably high concordance rates of 99.01% with mMPS and 98.91% with CE. Furthermore, STRs characterized by a core repeat region between 24-176 bp and those with complex repeat motifs demonstrated improved detectability and concordance.

Conclusion: Single-end 400bp WGS, at an average depth of 30× and employing a 10× allele depth threshold, showcases exceptional STR detection and high concordance rates, highlighting its efficacy in genetic research.

Grants: None.

Conflict of Interest: None declared

P21.015.B Combining AmplideX PCR chemistries, nanopore sequencing, and automated analysis software to genotype complex, clinically-relevant variants in 11 challenging targets associated with hereditary diseases

John Milligan[1], *Bryan Killinger*[2], *Cody Edwards*[1], *Christopher Fraher*[2], *Jon Kemppainen*[1], *Monica Roberts*[2], *Jonathan Turner*[1], *Theodore Markulin*[2], *Brennan Greenlee*[1], *Eduardo Priego*[2], *Juliette Baker*[1], *Mahesh Yarasi*[2], *Connor Parker*[1], *Mia Mihailovic*[2], *Ashima Sharma*[1], *Brian Haynes*[1], *Bradley Hall*[1]

[1]*Asuragen, Inc. a Bio-Techne Brand, Research and Development, AUSTIN, United States;* [2]*Asuragen, Inc. a Bio-Techne Brand, Bioinformatics, AUSTIN, United States*

Background/Objectives: Detecting variants associated with severe genetic disorders is critical for diagnostic and screening applications. While NGS is often leveraged, it is frequently incapable of resolving complex pathogenic variants involving short tandem repeats, copy number/structural variation (CNV/SV), and pseudogenes. Here, we combine AmplideX PCR enrichment, nanopore sequencing, and bioinformatic analysis software to genotype 11 challenging targets (*CFTR, SMN1/2, FMR1, HBA1/2, HBB, F8* intron inversions*, GBA, CYP21A2, TNXB*).

Methods: We tested >300 unique samples, including cell-line genomic DNA (gDNA; n = 150) and whole blood gDNA from residual clinical samples (n = 175). Target regions were enriched in 4 PCR reactions, barcoded, pooled, and sequenced on MinION™ flow cells (R10.4.1) with a Mk1B or GridION™ (Oxford Nanopore Technologies). We developed software to automate analysis/reporting and compared results to those obtained from multiple orthogonal methods.

Results: Across all variant types, the assay was ≥95% concordant with orthogonal results. SNVs/indel concordance was >98%, including >50 unique *CFTR* variants. *SMN1/2* CNV (0 to >=3) showed ≥95% agreement. *FMR1* was >98% concordant for repeat sizing and AGG interrupt detection. *GBA* and *CYP21A2/TNXB* were differentiated from their pseudogenes, gene-pseudogene fusions were identified, and SVs were resolved. *F8* intron inversions showed 100% agreement.

Conclusion: Our results demonstrate the utility of combining nanopore sequencing with novel PCR chemistry and algorithms to detect challenging variants causing hereditary disorders. The assay accurately resolves complex genotypes insurmountable by routine NGS using affordable, scalable technologies in a common workflow suitable for any laboratory. The software enables custom variant reporting, ClinVar classification, and in-silico novel variant predictions to streamline interpretation.

Conflict of Interest: John Milligan Asuragen, a Bio-Techne brand, Asuragen, a Bio-Techne brand, Bryan Killinger Asuragen, a Bio-Techne brand, Asuragen, a Bio-Techne brand, Cody Edwards Asuragen, a Bio-Techne brand, Asuragen, a Bio-Techne brand, Christopher Fraher Asuragen, a Bio-Techne brand, Asuragen, a Bio-Techne brand, Jon Kemppainen Asuragen, a Bio-Techne brand, Asuragen, a Bio-Techne brand, Monica Roberts Asuragen, a Bio-Techne brand, Asuragen, a Bio-Techne brand, Jonathan Turner Asuragen, a Bio-Techne brand, Asuragen, a Bio-Techne brand, Theodore Markulin Asuragen, a Bio-Techne brand, Asuragen, a Bio-Techne brand, Brennan Greenlee Asuragen, a Bio-Techne brand, Asuragen, a Bio-Techne brand, Eduardo Priego Asuragen, a Bio-Techne brand, Asuragen, a Bio-Techne brand, Juliette Baker Asuragen, a Bio-Techne brand, Asuragen, a Bio-Techne brand, Mahesh Yarasi Asuragen, a Bio-Techne brand, Asuragen, a Bio-Techne brand, Connor Parker Asuragen, a Bio-Techne brand, Asuragen, a Bio-Techne brand, Mia Mihailovic Asuragen, a Bio-Techne brand, Asuragen, a Bio-Techne brand, Ashima Sharma Asuragen, a Bio-Techne brand, Asuragen, a Bio-Techne brand, Brian Haynes Asuragen, a Bio-Techne brand, Asuragen, a Bio-Techne brand, Bradley Hall Asuragen, a Bio-Techne brand, Asuragen, a Bio-Techne brand

P21.016.C Detection of mitochondrial heteroplasmy: A comparison between long nanopore reads and short illumina reads

Barbara Slapnik[1;2], *Robert Sket*[1;2], *Tine Tesovnik*[1;2], *Barbara Jenko Bizjan*[1;2], *Jernej Kovač*[1;2]

[1]*University Children's Hospital, University Medical Center Ljubljana (UMC), Ljubljana, Clinical Institute of Special Laboratory Diagnostics, Ljubljana, Slovenia;* [2]*University of Ljubljana, Faculty of Medicine, Ljubljana, Slovenia*

Background/Objectives: Mitochondrial diseases, marked by cellular energy deficiency from respiratory chain defects, pose diagnostic challenges due to genetic heterogeneity, heteroplasmy, and nuclear-mitochondrial segments (numts). Clinical symptoms manifest when heteroplasmy exceeds 60-90%, varying with mutation and tissue. However, precise diagnosis, prognosis, and counseling in medical genetics demand detecting low-level variants. Our aim was to establish heteroplasmy detection thresholds using Oxford Nanopore Technology (ONT) and compare with Next Generation Sequencing (NGS).

Methods: Bioinformatics simulations involved merging fastq files, introducing m.8993T>G heteroplasmy. Short and long reads were aligned to the hg38 reference genome using BWA and Minimap2, respectively. Mutserve2 was used for variant calling. Precise thresholds were determined through 999 repetitions for each heteroplasmy level.

Results: At low coverage depth (150x), Mutserve2 detects heteroplasmy as low as 3% with long reads and 1% with short reads, albeit inconsistently. ONT achieves over 90% reliability for levels exceeding 21%, contrasting with NGS at 9% heteroplasmy

level. Mutserve2 tends to underestimate ONT heteroplasmy but correlates strongly ($R^2 = 0.960$) with expected levels, while NGS exhibits a higher correlation ($R^2 = 0.998$).

Conclusion: Our findings highlight challenges and opportunities with nanopore sequencing for accurate heteroplasmy detection in mtDNA. The knowledge gained will contribute to better development of the diagnostic applications to detect heteroplasmic mitochondrial variants in human diseases. Furthermore, insights gained will aid in refining bioinformatics pipelines and inform future studies leveraging nanopore sequencing for comprehensive mtDNA analysis.

Grants: Young Research Fellowship SRA#56916; UMC-tertiary grants: TP20210119, TP20210130

Conflict of Interest: None declared

P21.017.D Towards improved Parsortix® efficiency for circulating tumor cell enrichment in pancreatic cancer

Nele Vandenbussche[1;2;3], *Béatrice Lintermans*[1;2;3], *Charlotte Fieuws*[1;2;3], *Tijs Merckaert*[1;2;4], *Kris Gevaert*[1;2;4], *Kathleen Claes*[1;2;3]

[1]*Ghent University, Department of Biomolecular Medicine, Ghent, Belgium;* [2]*Cancer Research Institute Ghent (CRIG), Ghent, Belgium;* [3]*Ghent University Hospital, Center for Medical Genetics, Ghent, Belgium;* [4]*Ghent University, VIB Center for Medical Biotechnology, Ghent, Belgium*

Background/Objectives: With its increasing incidence and dismal prognosis, pancreatic ductal adenocarcinoma (PDAC) is becoming a global oncologic problem. Circulating tumor cells (CTCs) could be an interesting biomarker for PDAC, since they contain genetic information from the tumor of origin. However, isolating CTCs from peripheral blood of PDAC patients remains challenging since they are rare and consist of various subtypes. The Parsortix® device captures CTCs from blood samples based on cell size and deformability. We evaluated its ability to enrich and capture different subtypes of pancreatic cancer cells.

Methods: Three pancreatic cancer cell lines, mimicking different CTC subtypes (epithelial, mesenchymal, and intermediate), were stained and spiked into healthy donor blood. These samples were processed using the Parsortix® and recovery rates were determined. Data-independent acquisition-mass spectrometry identified differentially expressed proteins and pathways between the cell lines.

Results: The mesenchymal cell line had a significant lower recovery rate ($32.8 \pm 10.2\%$) compared to the epithelial cell line ($62.6 \pm 18.5\%$). The Rho signaling pathway, responsible for cell contractility, was downregulated in the mesenchymal cells.

Conclusion: Parsortix® can capture epithelial pancreatic cancer cells from blood samples, but is less suitable for capturing mesenchymal cancer cells. We are currently exploring the hypothesis that introducing an activator of the Rho pathway into patient blood samples could stiffen CTCs. This could enhance recovery rates and facilitate downstream multi-omics analysis.

Grants: Ghent University Special Research Fund (BOF/24J/2023/165)

Conflict of Interest: None declared

P21.018.A Designing a single-cell RNA sequencing strategy for transcriptome-wide RNA analysis

Danique Berrevoet[1], *Koen Deserranno*[1], *Dieter Deforce*[1], *Filip Van Nieuwerburgh*[1]

[1]*Ghent University, Laboratory of Pharmaceutical Biotechnology, Ghent, Belgium*

Consortium: N/A

Background/Objectives: The current single cell RNA sequencing field has been dominated by focusing on messenger RNA. Consequently, valuable information present in long non-coding RNA (lncRNA) remains largely unexplored. To overcome these constraints, we developed a cost-effective approach based on the cutting-edge Smart-seq3xpress (SS3X) protocol. This approach aims to achieve two primary goals: (1) capturing the entire transcriptome and (2) eliminating the abundant but rather irrelevant ribosomal RNA.

Methods: The miniaturized SS3X protocol, as published by *Hagemann-Jensen et al*., was modified by adding a synthetic poly(A) tailing step between cell lysis and reverse transcription utilizing E. coli Poly(A) Polymerase. After oligo-dT priming, the poly(A) tailed RNA is reverse transcribed to cDNA and preamplified using PCR to obtain cDNA copies. This cDNA is tagmented, and barcoded in index PCR. Using the SEQuoia RiboDepletion Kit, the pooled sequencing library is ribodepleted. Finally, Illumina sequencing is performed.

Results: When comparing the standard SS3X protocol to the poly-adenylation protocol (SS3XA), the latter demonstrated significantly increased cDNA yields per cell. The capture of non-natively polyA-tailed molecules was successfully achieved, as illustrated by the retrieval of 5.8S, 18S, and 28S ribosomal RNA prior to ribodepletion. Moreover, 194 unique lncRNAs (>98% sequence identity) were identified.

Conclusion: The developed method demonstrates the successful capture of the total RNA content. These promising results underscore the potential of the SS3XA protocol as a valuable tool for transcriptome-wide RNA analysis. The next step encompasses ultimate verification through the comparison of the sequencing outcomes with and without polyadenylation.

Grants: Not applicable.

Conflict of Interest: None declared

P21.019.B Whole genome sequencing methods with low input DNA and their automation

Christian Daviaud[1], *Zuzana Gerber*[1], *Emmanuel Menard*[1], *Stephane Meslage*[1], *Florian Sandron*[1], *Vincent Meyer*[1], *Jean-François Deleuze*[1], *Francis Rousseau*[1]

[1]*CEA, Centre National de Recherche en Génomique Humaine, Évry-Courcouronnes, France*

Background/Objectives: With the recent drop in sequencing price, whole genome sequencing (WGS) becomes the method of choice to interrogate the human genome for medically relevant causal variants. However, for some applications such as cancer genetics, the current input requirement of 1µg DNA for PCR-Free WGS is not always attainable. In this study, we compared different PCR-Free WGS approaches compatible with low input gDNA.

Methods: NGS libraries were prepared from human DNA reference standard HG002 using five commercial kits adapted to low input WGS PCR-Free library prep. DNA fragmentation was either enzymatic or mechanic. The input ranged from 50ng to 500ng gDNA. We compared the library yield and quality control profiles for different inputs. All libraries were sequenced on NovaSeq6000 (Illumina) at 30×. We assessed the depth, breadth and uniformity of genome coverage among the different kits. We evaluated their capacity to detect SNVs and indels correctly compared to known GIAB reference sequence of HG002 individual.

Results: We observed that enzymatic fragmentation provides more homogeneous results than mechanical fragmentation. We noted that the lowest input usually yields libraries with the

shortest insert size, sometimes resulting in coverage below 30×. Finally, two protocols have been selected and automated at CNRGH.

Conclusion: All methods tested in this study yielded exploitable sequencing results with low-input gDNA. The coverage metrics were similar for inputs around 200 ng, while performance varied for inputs below 100 ng. The CNRGH now has a fully automated production platform for PCR-free WGS, enabling high-throughput projects to be conducted with low input DNA.

Grants: LabEx GENMED (ANR-10-LABX-0013).

Conflict of Interest: None declared

P21.020.C Development of miniaturized plate-based 10X assays for low-input samples

Elise Callens[1], *Koen Deserranno*[1], *Dieter Deforce*[1], *Filip Van Nieuwerburgh*[1]

[1]*Ghent University, Laboratory of Pharmaceutical Biotechnology, Ghent, Belgium*

Consortium: n/a

Background/Objectives: The development of next-generation sequencing (NGS)-based technologies, especially single cell RNA-sequencing (scRNA-seq), has significantly advanced our understanding of immunology and cancer genomics. The 10X Genomics (10X) platform is well established in the field of scRNA-seq. However, its high-throughput nature, requires an input of several thousands of cells, which is often unavailable in clinical settings. Therefore, we present a state-of-the-art plate-based 10X technique enabling single cell 10X analysis starting from low input samples.

Methods: This technique leverages the highly sensitive and miniaturized plate-based Smart-seq3xpress (SS3x) protocol and is downstream compatible with any 5′ 10X library preparation kit. The method was demonstrated using Jurkat T lymphoblasts sorted via the BD FACSAria Fusion. Sorted cells were then subjected to an adapted SS3x workflow enabling the generation of a cDNA pool. Subsequently, cDNA was subjected to a 5′ 10X library preparation kit, with or without an additional T cell receptor (TCR) enrichment phase, depending on the kit.

Results: The proficient generation of a cDNA pool from 96 Jurkat T lymphoblasts was achieved, followed by a successful library preparation using either the 5′ 10X GEX or VDJ kit. 70% of the sorted cells were recovered with >1000 reads, which is in line with the expectation considering sorting and library preparation efficiency. Additionally, TCR-enriched data revealed over 80% shared identical clonotypes, confirming their Jurkat T lymphoblast origin.

Conclusion: We believe that this cost-effective technique will become an established tool for immunological research in clinical cases with a low input number of cells.

- **Grants:** n/a

Conflict of Interest: None declared

P21.021.D Ultra-plex sequencing of single cells and limited samples

Ioanna Andreou[1], *Samuel Rulli*[2], *Katja Heitz*[1], *Silke Huebner*[1], *Christin Meerschiff*[1], *Eric Lader*[2]

[1]*QIAGEN GmbH, Hilden, Germany;* [2]*QIAGEN Sciences Inc, Germantown, United States*

Background/Objectives: Single-cell genome analysis enables researchers to gain novel insights into tumor heterogeneity. Conducting single-cell genomic analysis using next-generation sequencing (NGS) methods has traditionally been challenging since the amount of genomic DNA present in a single cell is limited. PCR-based amplification methods normally have high error rates, low coverage uniformity, and extensive allelic drop-outs making interpretation of SNVs and CNVs challenging.

Methods: We introduce a new whole-genome amplification method, which utilizes an optimized isothermal DNA amplification reaction with an error correcting polymerase and a downstream library preparation protocol to allow pooling of amplified DNA. The key DNA amplification steps use multiple displacement amplification (MDA) technology to amplify gDNA directly from single cells, nuclei, or previously isolated genomic DNA. During the MDA reaction, sample-barcodes are incorporated into the newly synthetized DNA, allowing the amplified DNA to be pooled and processed in to one ultra-plex library.

Results: Our current workflow allows 24 samples to be multiplexed in one library and 24 libraries to be produced for a total of 576 samples per sequencing run. The generated libraries are fully compatible to hybrid capture procedures, which will reduce the sequencing effort only to the regions of interest.

This method can be used to investigate chromosomal instability using low pass sequencing, and SNV or CNV following whole genome or exome-capture workflows.

Conclusion: The presented method allow single cell, nuclei, or other limited DNA sequencing in an ultra-plex mode, by introducing sample barcodes during the MDA. This method saves library preparation time, reduces DNA library costs, and limits plastic consumable usage.

Conflict of Interest: Ioanna Andreou full-time, Samuel Rulli full-time, Katja Heitz full time, Silke Huebner part-time, Christin Meerschiff part-time, Eric Lader full-time

P21.022.A Knock-in of disease-related variants in the zebrafish model organism

Michiel Vanhooydonck[1], *Elyne De Neef*[1], *Hanna De Saffel*[1], *Annekatrien Boel*[2], *Kathleen Claes*[1], *Andy Willaert*[1;3], *Bert Callewaert*[1]

[1]*Ghent University, Biomolecular medicine, Ghent, Belgium;* [2]*Ghent University, Reproductive Medicine, Ghent, Belgium;* [3]*Ghent University, Core Zebrafish Facility Ghent*

Background/Objectives: Introducing a precise disease-specific genetic variant in animal models allows for a more accurate representation of the molecular and physiological consequences. While generating knock-out lines is relatively straightforward, generating a knock-in (KI) model for specific base-pair variants remains challenging. This is mainly due to low editing efficiencies (EE) and potential occurrence of off-targets. Here, we optimize the KI EE of disease-related variants in zebrafish.

Methods: We introduced six base-pair substitutions in three different zebrafish genes by CRISPR/Cas9-mediated homology-directed repair (HDR). We used NGS analysis to assess the effect of four different Cas9 concentrations, improved thermostability of HDR templates, different micro-injection procedures, and the introduction of synonymous mutations in the PAM sequence, on KI EE in zebrafish larvae. In addition, we evaluated prime-editing as a potential alternative to conventional HDR-based genome editing.

Results: Increasing Cas9 concentrations augmented KI EE, with optimal injected amounts of Cas9 between 400-800 pg. The use of Thermostable Alt-R™ HDR templates (IDT) further increased KI EE, while PAM modifications did not. Injection of the components directly into the zygote was not superior to injections in the yolk. Prime-editing showed up to a fourfold higher EE than HDR and increased the F0 founder pool for four targets, while off-target

effects were only detected in conventional HDR-based genome editing.

Conclusion: Prime-editing is a promising alternative to conventional HDR-based knock-in with higher EE and less off-target effects. This methodology simplifies the creation of precise genomic mutations in zebrafish, facilitating the exploration of human diseases.

Funding: FWOOPR2020009501

Conflict of Interest: None declared

P21.023.B Comparison of Single-Cell Transcriptomic Technology: 5′ and 3′ scRNAseq of viable frozen tissues or single-cell transcriptomics of formalin-fixed paraffin-embedded tissues.

Bhuwan Khatri[1], Anna M Stolarczyk[1], Matthew Caleb Marlin[1], Miles Smith[1], Cherilyn Pritchett-Frazee[1], Maraget Beach[2], Eileen Pelayo[2], Zohreh Khavandgar[2], Paola Pérez[2], David E Kleiner[3], Stephen M Hewitt[3], Kevin Wei[4], Erin M Theisen[4], Kandice L Tessneer[1], Soumya Raychaudhuri[5], Michael B Brenner[4], Johann E Gudjonsson[6], Nir Hacohen[5], Judith A James[1;7], R Hal Scofield[1;7;8], Stephen Shiboski[9], Astrid Rasmussen[1], Alan N Baer[2;10], A Darise Farris[1;7], Caroline Shiboski[9], Blake M Warner[2], Joel M Guthridge[1;7], **Chris Lessard**[1;7]

[1]*Oklahoma Medical Research Foundation, Oklahoma City, United States;* [2]*National Institute of Dental and Craniofacial Research, National Institutes of Health, Bethesda, United States;* [3]*National Cancer Institute, National Institutes of Health, Bethesda, United States;* [4]*Harvard Medical School, Brigham and Women's Hospital, Boston, United States;* [5]*Broad Institute, Cambridge, United States;* [6]*University of Michigan, Ann Arbor, United States;* [7]*University of Oklahoma Health Sciences Center, Oklahoma City, United States;* [8]*Department of Veterans Affairs Medical Center, Oklahoma City, United States;* [9]*University of California San Francisco, San Francisco, United States;* [10]*Johns Hopkins University, Baltimore, United States*

Background/Objectives: Compare 3′ and 5′ scRNA-seq of viably frozen minor salivary gland (MSG) biopsies collected from Sjögren's disease (SjD) patients to the single-cell transcriptomics of formalin-fixed paraffin-embedded (scFFPE) MSG biopsies.

Methods: For 3′ and 5′ scRNA-seq, viably frozen MSG (2 SjD, 5 healthy controls (HCs)) were thawed, dissociated, counted and split (>90% viable cells, then captured targeting 8000-10000 cells. Following the 10X Flex/scFFPE protocol, 25µm sections from FFPE MSG were dissociated. Cells were counted, labelled, and captured (yield: 8000-20000 per tissue). Sequencing data were analyzed (CellRanger), ambient RNAs corrected (SoupX), and doublets detected (scDblfinder). Cells with feature counts <200 or >5000, mitochondrial percent >5%, and doublets were removed.

Results: scFFPE, 3′, and 5′ scRNA-seq yielded similar feature/gene counts per cell (median = 4571, 5186, 7452, respectively). scFFPE was superior in ambient RNA reduction and identifying discrete cell populations. An expanded scFFPE dataset (8 SjD^{Ro+}, 9 HCs) yielding 371,454 cells among 23 cell states after QC. Cells were separated into immune and non-immune clusters and annotated using reference data (CellTypist or MSG reference data), then differentially expressed (DE) genes ($p < 0.02$) were identified using a pseudobulk approach (DESeq2) and subjected to Ingenuity Pathway Analysis. Immune and glandular cell DE genes mapped to both common and different pathways in SjD^{Ro+} vs. HCs, including Type I interferon: downregulation (plasma cells, plasmacytoid DCs, naive B cells) upregulation (migratory DCs, DC1, seromucous and mucous acini, macrophages, mast cells).

Conclusions: scFFPE yielded superior single-cell data compared to 3′ and 5′ using viable cells. **Grants:** UC2AR081032&S1&02S1; UC2DE032254; NIDCR15-D-0051

Conflict of Interest: Bhuwan Khatri: None declared, Anna M Stolarczyk: None declared, Matthew Caleb Marlin: None declared, Miles Smith: None declared, Cherilyn Pritchett-Frazee: None declared, Maraget Beach: None declared, Eileen Pelayo: None declared, Zohreh Khavandgar: None declared, Paola Pérez: None declared, David E Kleiner: None declared, Stephen M Hewitt: None declared, Kevin Wei Received a sponsored research agreement from Gilead Sciences and 10X Genomics., Erin M Theisen: None declared, Kandice L Tessneer: None declared, Soumya Raychaudhuri: None declared, Michael B Brenner: None declared, Johann E Gudjonsson: None declared, Nir Hacohen: None declared, Judith A James: None declared, R Hal Scofield Received consulting fees from Johnson and Johnson Innovative Medicine (formerly Janssen) and Merk Pharmaceuticals., Stephen Shiboski: None declared, Astrid Rasmussen: None declared, Alan N Baer Received consulting fees from Bristol Myers Squibb (BMS) and iCell Gene Therapeutics., A Darise Farris Grant/research support from Johnson and Johnson Innovative Medicine (formerly Janssen; ended 12/31/2023)., Caroline Shiboski: None declared, Blake M Warner Receives funding to support research from Pfizer, Inc., and Mitobridge, Inc., a subsidiary of Astellas Bio., Joel M Guthridge: None declared, Chris Lessard Grant/research support from Johnson and Johnson Innovative Medicine (formerly Janssen; ended 12/31/2023).

P21.024.C Assessment of episignature analysis using PacBio long-read sequencing

Veronique Ivashchenko[1], *Juliet Hampstead[1], Ronny Derks[1], Amber den Ouden[1], Gelana Khazeeva[1], Simone Van Den Heuvel[1], Raoul Timmermans[1], Jordi Corominas Galbany[1], Rolph Pfundt[1], Tom Hofste[1], Helger Yntema[1], Lisenka Vissers[1], Alexander Hoischen[1;2], Christian Gilissen[1]*

[1]*Radboud university medical center, Department of Human Genetics, Nijmegen, Netherlands;* [2]*Radboud university medical center, Department of Laboratory Medicine, Translational Metabolic Laboratory, Nijmegen, Netherlands*

Background/Objectives: Whole-genome DNA methylation detection (5mC) is typically performed using short-read bisulfite sequencing procedures. Pacific Biosciences (PacBio) long-read sequencing (LRS) technology can also detect 5mC on native DNA. Here we assessed the ability of PacBio HiFi LRS to: (1) robustly identify genome-wide methylation, (2) identify differentially imprinted regions, and (3) identify genome-wide epigenetic signatures for genetic disorders.

Methods: We compared public data from the Genome In A Bottle HG002 cell line with two short-read whole-genome DNA methylation sequencing procedures to assess reproducibility and evaluated the detection of 41 known parentally imprinted regions using 8 in-house PacBio trios. For outlier detection we trained an HMM model on 15 samples and tested it for differentially methylated CpG islands on chromosome X when comparing one female sample against 15 males. Genome-wide episignatures were investigated using 12 patients with known *KMT2A* loss-of-function mutations and 39 control samples sequenced on the PacBio Revio.

Results: PacBio methylation calls were highly comparable to existing protocols with a Pearson correlation >0.9. Methylation probabilities on imprinted regions were close to 50% for 31 out of 41 (76%) regions. Our method for regional methylation patterns detection successfully reported 149 out of 168 (89%) differentially methylated CpG islands. Using published *KMT2A* signatures we were successfully able to distinguish *KMT2A* patients from controls.

Conclusion: Overall our results indicate that PacBio HiFi long-read sequencing can be reliably used for the investigation of genome-wide 5mC patterns and episignature analysis for patients.

Grants: Research Year Grant from the Toulouse University Hospital, Toulouse, France.

Conflict of Interest: None declared

P21.025.D Clinical utility of long read whole genome sequencing to confirm diagnosis in neonatal hypotonia

Hans Matsson[1], *Ida Höijer*[2], *Joakim Klar*[1], *Padraic Corcoran*[2], *Cecilia Soussi Zander*[3]

[1]*Uppsala university, Immunology, Genetics and Pathology, Uppsala, Sweden;* [2]*Science for Life Laboratory, Immunology, Genetics and Pathology, Uppsala, Sweden;* [3]*Uppsala University Hospital, Clinical genetics, Uppsala, Sweden*

Background/Objectives: Infants presenting with hypotonia often require rapid clinical assessment and genetic testing using several targeted assays and genome wide analyses to confirm a diagnosis. Here, we used PacBio Revio long read whole genome sequencing on DNA from floppy infants to confirm previously known diagnoses and to evaluate the workflow in a clinical diagnostics laboratory. One patient presented with Prader Willi-like features and a nerve conduction defect for which also spinal muscular atrophy was suspected. No deletion nor methylation defect in the Prader Willi region was detected using MLPA.

Methods: Genomic DNA was extracted from blood, size selected and prepared for PacBio whole genome sequencing on a Revio instrument. PacBio WGS Variant Pipeline was used for read alignment, variant calling, and phasing. Variant analyses and visualisation were performed using Alissa Interpret and IGV software.

Results: Analyses of small variants using DeepVariant verified a paternal inherited pathogenic variant NM_019066.5(MAGEL2):c.1966C>T p.(Gln656*), thus confirming Schaaf-Yang syndrome. We detected a heterozygous deletion of *SMN1* by analysis of realigned BAM file using Paraphase. The other *SMN1* allele was devoid of pathogenic variants thus ruling out spinal muscular atrophy. Sanger sequencing of *SMN1* verified the absence of pathogenic variants.

Conclusion: We show the potential of long read whole genome sequencing as an effective single diagnostic test in the clinic to confirm diagnoses in patients with neonatal hypotonia.

Grants: This study was supported by a grant from the Regional Research Council Mid Sweden.

Conflict of Interest: None declared

P21.026.A Pathological Short Tandem Repeats Analysis by Long-Read Sequencing in affected individuals

Hagar Mor-Shaked[1;2], *Rivka Birnbaum*[1], *Shira Silverstein*[2], *Chaggai Rosenbluh*[1], *Orly Elpeleg*[1;2], *Tamar Harel*[1;2]

[1]*Hadassah Medical Center, Department of Clinical Genetics, Jerusalem, Israel;* [2]*Faculty of Medicine, The Hebrew University of Jerusalem, Faculty of Medicine, Jerusalem, Israel*

Background/Objectives: Pathological short tandem repeats (STRs) are repetitive DNA sequences found throughout the genome that can expand and contract in length, resulting in various genetic diseases. Traditional short-read sequencing technologies have limitations in detecting STRs due to their inherent instability and repetitive nature. Long-read sequencing (LRS), can provide a potential solution for accurate and comprehensive STR analysis.

Methods: Sixteen affected individuals with known pathogenic expansions in ten different loci were sequenced on PromethION, to obtain long-read whole-genome sequencing (LR-WGS). STR genotyping was performed using the straglr software as bundled within Oxford Nanopore Technologies wf-human-variation workflow using default parameters and with the `--str` flag to specify STR genotyping. Straglr provided repeat unit counts from the phased reads that span the collection of pre-annotated disease-associated human STRs.

Results: Straglr software analysis on LR-WGS data detected all previously known repeat expansions, with almost the same allele sizes that were known from repeat-prime PCR, and provided sizing information for large loci, in which the size was unknown. In addition, when relevant, methylation analysis was successfully conducted to support the analysis.

Conclusion: As the cost of genome sequencing continues to drop and new technologies emerge that can detect all types of genetic variations in one test, we hypothesis it is likely that clinical STR detection will shift towards LR-WGS. This is supported by the ability to analyze DNA methylation from Oxford Nanopore data, which is relevant for several pathological STRs.

Before implementing LR-WGS for STR detection, further validation would be necessary, which includes testing affected individuals for each genetic locus.

Grants:

Conflict of Interest: Hagar Mor-Shaked Geneyx Analysis, Rivka Birnbaum: None declared, Shira Silverstein: None declared, Chaggai Rosenbluh: None declared, Orly Elpeleg: None declared, Tamar Harel: None declared

P21.027.B Assessment of read depth requirements for gene and isoform discovery: a comparative study of long-read and short-read RNA sequencing data in human heart

Nina Gonzaludo[1], *Jocelyne Bruand*[1], *Amy Klegarth*[1], *Jason Underwood*[1], *Elizabeth Tseng*[1], *Kimberly A. Aldinger*[2]

[1]*PacBio, Menlo Park, CA, United States;* [2]*Seattle Children's Research Institute, University of Washington Birth Defects Research Laboratory, Seattle, WA, United States*

Background/Objectives: Long-read sequencing (LRS) allows for full-length isoform capture without cDNA fragmentation or computational assembly, revolutionizing transcriptomics. However, determining optimal read depth for isoform discovery is challenging. We compared read depth requirements for gene and isoform discovery using long- versus short-read RNA-Seq.

Methods: For 4 developing heart samples, library prep was performed using PacBio full-length Kinnex RNA kits with sequencing on PacBio Revio (1 sample per SMRT Cell 25M), and analysis with PacBio SMRT Link v13.0 Iso-Seq workflow and Pigeon. Short read RNA-seq was performed using Illumina TruSeq Stranded mRNA kits, with sequencing on NovaSeq6000 and analysis with STAR, RNA-SeQC, and RSEM. To simulate lower read depths and isoform discovery sensitivity, long reads were downsampled. We compared known and all unique genes and isoforms at different depths across platforms.

Results: Read depths ranged from 31.9-36.8M reads for PacBio data, and 81.6-123M reads for Illumina data. The identification of known and unique genes plateaued (increased less than 0.1% per 100k reads) at 5.6-9.5M and 7.7-13.1M reads, respectively. Known and unique isoform discovery plateaued at 7.8-11.4M and 12.5-19.3M reads, respectively. We show corresponding values in short-read data with a lower amount of genes and isoforms discovered at plateaus. Above these thresholds, higher depths improved detection of novel isoforms and may improve accuracy of existing gene annotation.

Conclusion: As LRS gains traction in transcriptomics, this study demonstrates read depth requirements for gene and isoform

discovery in human heart tissue. This information can help optimize experimental designs and multiplexing of samples, and inform decisions around sequencing depth.

Grants:

Conflict of Interest: Nina Gonzaludo PacBio stock, employee of PacBio, Jocelyne Bruand PacBio stock, PacBio employee, Amy Klegarth PacBio stock, PacBio employee, Jason Underwood PacBio stock, PacBio employee, Elizabeth Tseng PacBio stock, PacBio employee, Kimberly A. Aldinger in-kind library prep and sequencing for PacBio data

P21.028.A Low variant mutation detection using InfiniSeq targeted sequencing

Nelly Frenkel[1], Adi Ben-Yehuda[1], Adi Danin[1], Tom Fleischer[2], Michael Gershovits[2], Yardena Brilon[2], Hadar Haham[2], Noa Tzunz-Henig[2], **Tamir Biezuner**[1]

[1]Sequentify, R&D, Rehovot, Israel; [2]Sequentify, Bioinformatics, Rehovot, Israel

Background/Objectives: The detection of low variant frequency (VAF) somatic mutations using Next Generation Sequencing (NGS) plays a critical role in early cancer detection. Current NGS library preparation methods built for such tasks are laborious, long and expensive. We set out to resolve this problem by developing a targeted NGS library preparation method that can detect low VAFs at high fidelity in less than 4 hours of preparation time and at a low cost.

Methods: We conducted a Limit of Detection (LOD) experiment using mutated DNA which was serially diluted with normal DNA. The InfiniSeq Myeloid Malignancy Panel (Sequentify™) was employed to construct NGS libraries targeting 18 distinct variants with known Variant Allele Frequencies (VAFs) ranging from 63% to 0.05%.

Results: The sensitivity was assessed by comparing the expected VAFs of each variant against the analyzed data, while the specificity was evaluated using data derived from normal DNA. We demonstrate a consistent VAF detection at 100% sensitivity and accuracy for any VAFs above 5%. For 2%-5% and 0.5%-2% VAF the sensitivity was 97% and 90% respectively, with correspondingly high accuracy rates of 100% and 99%. The overall panel specificity clocked-in at 100%.

Conclusion: In a rigorously controlled experiment, our findings demonstrate the capability of the InfiniSeq protocol to accurately identify all clinically significant SNVs and short indels at low VAFs. This, together with the scalability of the protocol, underscores its potential for widespread population screening of early malignancy development.

Grants:

Conflict of Interest: Nelly Frenkel Stock options in Sequentify, Sequentify full time, Adi Ben-Yehuda stock options, Sequentify full time, Adi Danin stock options, Sequentify full time, Tom Fleischer co-founder, Sequentify, full employment, Sequentify, Michael Gershovits stock options, Sequentify full time, Yardena Brilon stock options, Sequentify full time, Hadar Haham stock options, Sequentify full time, Noa Tzunz-Henig stock options, Sequentify full time, Tamir Biezuner co-founder, Sequentify, full employment, Sequentify

P21.029.D Evaluation of synthetic long reads technology

Zuzana Gerber[1], *Florian Sandron[1], Christian Daviaud[1], Naell Lenoir[1], Margaux Gras[2], Vincent Meyer[1], Robert Olaso[1], Jean-François Deleuze[1;2]*

[1]CEA, CNRGH, Evry, France; [2]CReflX, Evry, France

Background/Objectives: Human whole-genome sequencing (WGS) has become an important tool in diagnostics of cancer and rare diseases. Certain cases cannot be resolved by short-read WGS, whereas long-read sequencing may increase their diagnostic yield. However, long-read approaches are not widely used, because long-read sequencers are expensive, the cost-per-Gb is superior to short-read WGS, and/or the error rate is elevated for certain technologies. Here we evaluate the potential of synthetic long-read approach, which can reconstruct long-read-like data from short-reads in silico.

Methods: At CNRGH, we tested "Illumina Complete Long Reads" (ICLR) on the Ashkenazim trio reference gDNA (Coriell Institute) including HG002 individual characterised by the GIAB consortium for SNVs, indels and structural variants (SV). We compared the results to publicly available data from long-read technologies PacBio and Oxford Nanopore as well as short-reads from Illumina.

Results: The protocol is easy to follow and to implement in a high-throughput sequencing laboratory. The detection of small variants (SNPs and indels) is comparable to true long-read technologies but seems less efficient for SVs, including SVs implicated in known genetic disorders, for example in genes CBS or SMN1. The sequencing effort required for ICLR is very high, driving the cost at least as high as true long-read sequencing.

Conclusion: Unlike true long-reads, ICLR does not require high input material quantity or exceptional quality, nor expensive dedicated instruments besides common short-read sequencers. However, ICLR has little advantage of performance or cost compared to true long-reads. Furthermore, ICLR analyses are restricted to the cloud version of Dragen.

Grants: N/A

Conflict of Interest: None declared

P21.030.A Clinical utility of PacBio HiFi sequencing on Revio

Christian Betz[1], *Hanno Bolz[1], Malte Spielmann[2;3;4], Lars Feuk[5], Anna Lindstrand[6], Katharina Herzog[7], Johannes Zschocke[7], Christian Gilissen[8], Lisenka Vissers[8], Alexander Hoischen[8]*

[1]Bioscientia Institute for Medical Diagnostics GmbH, Human Genetics, Ingelheim, Germany; [2]University Hospital Schleswig-Holstein, University of Lübeck and Kiel University, Institute of Human Genetics, Lübeck/Kiel, Germany; [3]Max Planck Institute for Molecular Genetics, Human Molecular Genetics Group, Berlin, Germany; [4]DZHK e.V: (German Center for Cardiovascular Research), Partner Site Hamburg/Kiel/Lübeck; [5]Uppsala University, Department of Immunology, Genetics, and Pathology and Science for Life Laboratory, Uppsala, Sweden; [6]Karolinska University Hospital, Department of Molecular Medicine and Surgery, Karolinska Institute, and Department of Clinical Genetics, Stockholm, Sweden; [7]Medical University Innsbruck, Institute of Human Genetics, Innsbruck, Austria; [8]Radboud University Medical Center, Department of Human Genetics, Research Institute for Medical Innovation, Nijmegen, Netherlands

Consortium: on behalf of the HiFi Solves EMEA consortium

Background/Objectives: Long-read sequencing (LRS) technologies, such as PacBio HiFi sequencing on Revio, for the first time allow to assess complete human genomes at scale. This opens up the possibility of LRS as a comprehensive first-tier test for clinical genetics and rare disease diagnostics.

Methods: To determine the clinical utility of LRS, we performed Revio HiFi-LRS of 100 human genomes, comprising 5-15 positive controls each with mutations in 10 disease genes/loci with high sequence homology or pseudogene background, or within segmental duplications, including *ABCC6*, *CYP21A2, STRC, SMN1/2, PKD1, RPGR-ORF15*, and *OTOA*. These loci represent diagnostic challenges because they are difficult to analyze by standard

technologies and require custom-made test modalities. We utilized a novel tool, Paraphase (Cheng et al. AJHG 2023), that enables full haplotyping for homologous genes.

Results: Our study demonstrates that LRS can reliably identify most of the known mutations, including SNVs, InDels, structural variants, and gene conversions, in complex loci. The *PKD1* locus, containing very long AG-rich repeats, occasionally presented with low or lack of coverage, which was compensable by including 'non-HiFi/low-pass reads' for these loci.

Conclusions: Comprehensive analysis of some of the most challenging loci of the human genome by Revio LRS highlight its potential as a first-tier test for clinical genetics and rare disease diagnostics in the near future.

Full disclosure/COI: reagents used in this study by the HiFi Solves EMEA consortium members were in part provided by PacBio.

Conflict of Interest: Christian Betz reagents used in this study by the HiFi Solves EMEA consortium members were in part provided by PacBio., Hanno Bolz reagents used in this study by the HiFi Solves EMEA consortium members were in part provided by PacBio., Malte Spielmann reagents used in this study by the HiFi Solves EMEA consortium members were in part provided by PacBio., Lars Feuk reagents used in this study by the HiFi Solves EMEA consortium members were in part provided by PacBio., Anna Lindstrand reagents used in this study by the HiFi Solves EMEA consortium members were in part provided by PacBio., Katharina Herzog reagents used in this study by the HiFi Solves EMEA consortium members were in part provided by PacBio., Johannes Zschocke reagents used in this study by the HiFi Solves EMEA consortium members were in part provided by PacBio., Christian Gilissen reagents used in this study by the HiFi Solves EMEA consortium members were in part provided by PacBio., Lisenka Vissers reagents used in this study by the HiFi Solves EMEA consortium members were in part provided by PacBio., Alexander Hoischen reagents used in this study by the HiFi Solves EMEA consortium members were in part provided by PacBio.

P21.031.B Beyond the Genome: Identification of Protein Sequence and Post-Translational Modifications with Quantum-Si's Next-Generation Protein Sequencer™ Platinum®

Khanh D.Q. Nguyen[1], **Richard Broadhead**[1]

[1]*Quantum-Si, Branford, United States*

Background/Objectives: Protein sequencing advances genomics research by providing crucial insights into the proteins encoded by the genome. Additionally, it offers a comprehensive understanding of cellular processes and disease mechanisms by detecting amino acid substitutions and post-translational modifications (PTMs) that cannot be captured by genomics data alone.

Methods: In this study, we present the power of next-generation protein sequencing™ on Platinum®, where individual peptides are immobilized on a semiconductor chip and probed by dye-labeled N-terminal amino acid (NAA) recognizers, followed by aminopeptidase cleavage that exposes each NAA in the peptide for recognition. Fluorescent intensity, lifetime, and binding kinetics of each NAA binding event is collected and converted into kinetic signatures for each peptide, enabling the sequencing of peptides, inference of proteins in unknown samples, and detection of variants and PTMs.

Results: To demonstrate the versatility of this core methodology, we sequenced a mixture of ten proteins: CDNF, IL4, HSA, VIME, IL6, PDL1, APOE4, FGF2, AKT1, and H4. The resulting peptides generated distinct kinetic signatures aligned to their respective sequences, highlighting Platinum's efficacy in analyzing multi-protein mixtures. Additionally, we successfully detected citrullination and dimethylation of arginine, known to play key roles in cardiovascular disease, autoimmune disease, and cancer. Finally, recent developments in Platinum Analysis Software have allowed us to sequence proteins without prior knowledge of their identities, enabling inferences of proteins associated with biological pathways.

Conclusion: As we envision future enhancements to Platinum, we anticipate an expanded coverage of the proteome, thereby unraveling even more information often overlooked with genomics data.

Grants:

Conflict of Interest: None declared

P21.032.C QGenome: Bridging the interface between common conditions and rare diseases through an app-based clinical decision aid

Mohamed Wafik[1], *Anjana Kulkarni*[1], *Helena Carley*[1], *vishakha tripathi*[1], *charlotte tomlinson*[1], *Sophia Cross*[1], *Poppy Emmet*[1], *Muriel Holder*[1], *Shwetha Ramachandrappa*[1], *Leema Robert*[1], *Rachel Bastiaenen*[2], *Teofila Bueser*[3], *Alexander Kenney*[4]

[1]*Guy's and St Thomas' NHS Foundation Trust, Clinical Genetics, London, United Kingdom;* [2]*Guy's and St Thomas' NHS Foundation Trust, Cardiology, London, United Kingdom;* [3]*The NHS South East Genomic Medicine Service Alliance;* [4]*UBQO limited, http://www.ubqo.com/, United Kingdom*

Background/Objectives: Reducing the time patients wait for a rare disease diagnosis is one of the main priorities in the UK Genome strategy. To facilitate this need, QGenome, a smart clinical application has been developed.

QGenome is designed by the Guy's Hospital Regional Genetics service, in partnership with the South East Genomic Medicine Service Alliance (GMSA) and UBQO limited (http://www.ubqo.com/). The aims are to:

Facilitate streamlined genomic testing, risk assessment, and referral guidance to relevant healthcare professionals.
Provide equity of "Genomics care" for patients across the UK.

The current scope of QGenome includes pathways for cancer genomics, renal genomics, and inherited cardiac conditions (ICC).

Methods: For each clinical indication/condition, QGenome provides workflow assessments to determine eligibility for genomic testing and/or specialist tertiary referral, via a series of questions about their patient's personal, past medical, and family history.

The app can be used to find relevant forms, save reports, and activate referrals. QGenome is freely available on iOS, android and web-based platforms (https://qgenome.co.uk/).

Results

- QGenome offers a quick, efficient clinical decision tool for healthcare professionals in busy clinical settings.
- It contains workflows to perform risk assessments and determine genomic testing eligibility.

Conclusion: QGenome enables more patients with inherited genetic conditions to be identified, and improves the efficiency of Genomic testing services.

QGenome's content is currently relevant to the NHS South East, North Thames, and East GMSAs. We aim to expand this nationally,

to the 4 remaining NHS GMSAs, and internationally by creating custom made content.

Download QGenome here:





iOS Android

Conflict of Interest: None declared

P21.033.D The performance of AlphaMissense to identify genes causing disease

Yiheng Chen[1;2], *Guillaume Butler-Laporte*[2;3], *Kevin Liang*[2;4], *Yann Ilboudo*[2], *Summaira Yasmeen*[5], *Takayoshi Sasako*[2;6], *Claudia Langenberg*[5;7], *Celia Greenwood*[2;4], *Brent Richards*[1;2;3;8;9]

[1]*McGill University, Department of Human Genetics;* [2]*McGill University, Lady Davis Institute;* [3]*McGill University, Department of Medicine;* [4]*McGill University, Quantitative Life Sciences Program;* [5]*Berlin Institute of Health at Charité—Universitätsmedizin Berlin, Computational Medicine;* [6]*Tanaka Diabetes Clinic Omiya;* [7]*Queen Mary University of London, Precision Healthcare University Research Institute;* [8]*King's College London, Department of Twin Research;* [9]*5 Prime Sciences Inc*

Background/Objectives: A novel algorithm, AlphaMissense, has been shown to have an improved ability to predict the pathogenicity of rare missense genetic variants. However, it is not known whether AlphaMissense improves the ability of gene-based testing to identify disease-causing genes.

Methods: Using whole-exome sequencing data from the UK Biobank, we compared gene-based association analysis strategies including sets of deleterious variants: predicted loss-of-function (pLoF) variants only, pLoF plus AlphaMissense pathogenic variants, pLoF with missense variants predicted to be deleterious by any of five commonly utilized annotation methods (Missense (1/5)) or only variants predicted to be deleterious by all five methods (Missense (5/5)). We measured performance to identify 519 previously identified positive control genes, which can cause Mendelian diseases, or are the targets of successfully developed medicines.

Results: These strategies identified 850k pLoF variants and 5 million deleterious missense variants, including 22k likely pathogenic missense variants identified exclusively by AlphaMissense. The gene-based association tests found 608 significant gene associations (at $p < 1.67 \times 10^{-7}$) across 24 common traits and diseases. Compared to pLOFs plus Missense (5/5), tests using pLoFs and AlphaMissense variants found slightly more significant gene-disease and gene-trait associations, albeit with a marginally lower proportion of positive control genes. Nevertheless, their overall performance was similar. Merging AlphaMissense with Missense (5/5), whether through their intersection or union, did not yield any further enhancement in performance.

Conclusion: In summary, employing AlphaMissense to select deleterious variants for gene-based testing did not improve the ability to identify genes that are known to cause disease.

Grants: CIHR: 365825, 409511, 100558, 169303

Conflict of Interest: Yiheng Chen YC is an employee of 5 Prime Sciences., Guillaume Butler-Laporte GBL consults for 5 Prime Sciences., Kevin Liang: None declared, Yann Ilboudo: None declared, Summaira Yasmeen: None declared, Takayoshi Sasako: None declared, Claudia Langenberg: None declared, Celia Greenwood: None declared, Brent Richards J.B.R.'s institution has received investigator-initiated grant funding from Eli Lilly, GlaxoSmithKline, and Biogen for projects unrelated to this research., J.B.R is the CEO of 5 Prime Sciences (www.5primesciences.com), which provides research services for biotech, pharma, and venture capital companies for projects unrelated to this research., J.B.R. He has served as an advisor to GlaxoSmithKline and Deerfield Capital.

P21.034.A Multiplexed full-length single-molecule nanopore sequencing of the human mitochondrial genome and its application for rare neuromuscular diseases and chronic lymphocytic leukaemia

Ivo Gut[1], *Ieva Keraite*[1], *Davide Canevazzi*[1], *Cristina Frias*[1], *Ida Paramonov*[1], *Elena Garcia-Arumi*[2], *Rita Horvath*[3], *Ferran Nadeu*[4], *Elias Campo*[4], *Simon Heath*[1], *Marta Gut*[1]

[1]*Centro Nacional de Análisis Genómico (CNAG), Barcelona, Spain;* [2]*Vall Hebron Institut de Recerca, Clinical and Molecular Genetics and Rare Disease, Barcelona, Spain;* [3]*University of Cambridge, Clinica Neuroscience, Cambridge, United Kingdom;* [4]*IDIBAPS, Barcelona, Spain*

Mitochondria are organelles found in most eukaryotic cells containing several copies of a circular genome encoding for vestigial functions of what once used to be a free-living organism. Within the human population, heteroplasmy is common and can have substantial impact on human health depending on the variant position, frequency and endogenous or exogenous factors. Accurate detection and quantification of such variants is important for diagnosis, genetic counselling, and treatment of mtDNA diseases.

Methods to reconstruct the mitochondrial DNA sequence using short-read next-generation sequencing come with an inherent bias due to amplification by PCR and mapping. They can fail to determine the phase of variants, to capture multiple deletions and to cover the mitochondrial genome evenly. Here we describe a method to target, multiplex and sequence at high coverage full-length human mitochondrial genomes as native single-molecules, utilizing the RNA-guided DNA endonuclease Cas9 and nanopore sequencing. Combining Cas9 induced breaks, that define the mtDNA beginning and end of the sequencing reads, as barcodes, we achieve high demultiplexing specificity and delineation of the full-length of the mtDNA, regardless of the structural variant pattern. Long-read sequencing data is analysed with a pipeline where our custom-developed software, baldur, efficiently detects single nucleotide heteroplasmy to below 1%, physically determines phase and can accurately disentangle complex deletions. We show the application of our method for the investigation of the mitochondrial genome of patients suffering from rare neuromuscular disorders as well as the accumulation of somatic mutations in the mitochondrial genome of patients suffering from Richter syndrome a progressed form of chronic lymphocytic leukaemia.

Conflict of Interest: None declared

P21.036.C Efficient detection of multi-locus imprinting disorders: a novel NGS-based methodology for simultaneous detection of multiple DNA methylation defects in imprinting disorders.

Jet Bliek[1], *Martin A. Haagmans*[1], *Marielle Alders*[1], *Irène NETCHINE*[2], *Marcel Mannens*[1], *Peter Henneman*[1], *Frederic Brioude*[2]

[1]*Amsterdam UMC location AMC, University of Amsterdam, Amsterdam Reproduction & Development Research Institute, Department of Human Genetics, Amsterdam, Netherlands;* [2]*INSERM, Centre de recherche Saint Antoine, Sorbonne Université, Hôpital Armand Trousseau, Endocrinologie moléculaire et pathologies d'empreinte, Paris, France*

Background/Objectives: Imprinting disorders (IDs), characterized by abnormal methylation patterns of specific imprinted genes, comprise a spectrum of rare diseases associated with embryonic development, growth and metabolism. The molecular diagnosis of IDs is based on the analysis of methylation patterns in differentially methylated regions (DMRs), which present an opposite methylation pattern between the paternal and maternal alleles. One intriguing aspect of IDs is the potential existence of multilocus imprinting disorders (MLID). This disorder involves aberrant methylation patterns of multiple loci across the genome, presenting a complex and challenging diagnostic landscape. Understanding the mechanisms and implications of MLID is crucial for accurate diagnosis and effective disease management.

Methods: Our novel methodology was based on NGS after capturing (TWIST Bioscience®) differentially methylated regions (DMRs) associated with or without pathological conditions. Normal methylation values of 49 DMRs (including around 4,500 CpG islands) were established by examining leukocyte DNA from 30 control subjects.

Results: Our study confirmed previously identified anomalies in 14 individuals affected by IDs (Prader-Willi, Angelman, iPPSD, Silver-Russell, Beckwith-Wiedemann, or genome wide uniparental disomy). We confirmed the presence of MLID in three patients with previously identified MLID.

Conclusion: By integrating advanced sequencing methodologies with a comprehensive examination of methylation patterns, our study offers a valuable contribution to the field of IDs, with implications for improved patient care. Our study's approach enhances rare disease understanding, paving the way for improved diagnostics, marking a significant stride in addressing IDs complexities.

Grants: French ANR grant ID-MOM (ANR-22-CE14-0021). FB: recipient of a grant from the French Society of Pediatric Endocrinology and Diabetology (SFEDP).

Conflict of Interest: None declared

P21.037.D AI cloud-based end-to-end technology for accurate, fast & affordable diagnosis for Spinal Muscular Atrophy in Paraguay

Carmen Garrido Navas[1;2;3], *María Teresa Martínez de Filartiga*[4], *Marco Casartelli*[5], *Ruth Zarate*[4], *Rubén Darío Duré*[4], *Sara Chulián Prado*[1], *Karmele Alapont*[2], *Anne Kristine Schack*[2], *Marija Chaushevska*[2;6], *Gjorgji Madjarov*[2;6], *Zoran Velkoski*[2], *Chris Kyriakidis*[2]

[1]*Asesoramiento Genetico SL, Genetic Counselling Department, Granada, Spain;* [2]*gMendel, CSO, Copenhagen, Denmark;* [3]*University of Granada, Granada;* [4]*Laboratorio Curie SRL, Paraguay;* [5]*Hospital General Pediátrico Niños de Acosta Ñu, Pediatrics department, Paraguay;* [6]*Ss. Cyril and Methodius University, Skopje*

Background/Objectives: Bi-allelic mutations in the *SMN1* gene result in motor neuron degeneration and spinal muscular atrophy (SMA), a progressive, recessive neuromuscular disease with varying presentation in terms of onset and severity. Due to the clinical and genetic heterogeneity of SMA, which has a low prevalence (1/10,000 births), diagnosis and treatment are highly challenging. SMA severity is associated with the copy number of the survival motor neuron 2 gene (*SMN2*) that is almost identical, in genomic sequence, with *SMN1*. Diagnosis of SMA is usually approached by RT-PCR for the *SMN1* gene (sometimes including *SMN2*) after clinical symptoms suggest the condition. This approach is not only slow and costly but also inefficient because the clinical symptoms overlap with other neuromuscular diseases increasing the misdiagnosis rate.

Methods: We collected 30 samples from patients affected with SMA from Paraguay and prepared sequencing libraries for ONT sequencing using our standard operating procedure. We multiplexed all 30 samples into one R10.4 flowcell and sequenced them in a GridION device. The analysis based on artificial intelligence was performed on the CE-IVD Phivea® platform resulting in disease classification.

Results: We analyzed the samples from SMA patients using the Phivea® platform and we were able to identify a point mutation (C>T) that usually occurs in the telomeric region, as well as to count the number of copies for *SMN1* and *SMN2*.

Conclusion: The Phivea® platform can be used as a novel automated approach to study *SMN1* and *SMN2* mutations to improve the diagnostic yield of SMA for patients from Paraguay.

Grants: N/A

Conflict of Interest: Carmen Garrido Navas Founder of ConGen, Works at ConGen, CSO at gMendel, María Teresa Martínez de Filartiga: None declared, Marco Casartelli: None declared, Ruth Zarate: None declared, Rubén Darío Duré: None declared, Sara Chulián Prado Employed at ConGen, Karmele Alapont Works for gMendel, Anne Kristine Schack Works for gMendel, Marija Chaushevska PhD student co-supervised at gMendel, Gjorgji Madjarov IPR with gMendel, Chief Artificial Intelligence Officer at gMendel, Zoran Velkoski Co-founder of gMendel, Employee at gMendel, CTO at gMendel, Chris Kyriakidis Co-founder of gMendel, Employee at gMendel, CEO at gMendel

P21.040.C HiFi genomes in the clinic - one test to rule them all

Marjan Weiss[1;2], *Ronny Derks*[1;2], *Jordi Corominas Galbany*[1], *Amber den Ouden*[1;2], *Lot Snijders Blok*[1;2], *Raoul Timmermans*[1], *Erik-Jan Kamsteeg*[1], *Nicole de Leeuw*[1], *Marjolijn Ligtenberg*[1], *Tom Hofste*[1], *Arthur van den Wijngaard*[3], *Helger Yntema*[1], *Christian Gilissen*[1;2], *Alexander Hoischen*[1;2;4;5;6], *Lisenka Vissers*[1;2]

[1]*Radboud University Medical Center, Department of Human Genetics, Nijmegen;* [2]*Radboud University Medical Center, Radboudumc Research Institute for Medical Innovation, Nijmegen;* [3]*Maastricht University Medical Center, Department of Clinical Genetics, Maastricht, Netherlands;* [4]*Radboud University Medical Center, Department of Internal Medicine, Nijmegen;* [5]*Radboud University Medical Center, Radboud Expertise Center for Immunodeficiency and Autoinflammation, Nijmegen;* [6]*Radboud University Medical Center, Radboud Center for Infectious Disease (RCI), Nijmegen*

Long-read genome sequencing (LRS) allows to assess the full human genome for the first time comprehensively and could offer a first-tier test for clinical diagnostics. To determine the clinical utility, we performed LRS for 100 samples (HiFi genomes on Pacific Biosciences (PacBio) Revio instrument, ~30-fold coverage), with known clinically relevant variants that are challenging or

impossible to identify by short-read sequencing. A fully automated PacBio-based in-house pipeline was developed for quality control and sequence alignment, as well as the detection and phasing of all variant types including SNV/indels, STRs, SVs and methylation alterations.

LRS could readily identify the vast majority (95%) of known pathogenic variants. Of these, 87% were automatically called, including CNVs, translocations, inversions, STR expansions, de novo methylation defects and SNV/indels in homopolymer stretches and/or homologous sequences; 8% required manual curation such as the inspection of the aligned sequencing reads. A minority of variants (5%) posed systematic challenges, which included variants in very long AG-rich repeats and cases with cytogenetic aberrations affecting the repeat-rich regions of the Y-chromosome and/or acrocentric p-arms. In conclusion, LRS can identify the vast majority of pathogenic variants that are most challenging to detect with short-read technologies. Although our study identified some specific pitfalls, we expect that these can likely be resolved with further (bioinformatic) optimization. Most importantly, we show the potential to use a single technology to accurately identify all types of medically relevant genome variants, opening avenues to work towards a single generic test for germline testing in the future.

Funding: European Union's Horizon 2020 (779257)

Conflict of Interest: None declared

P21.041.D Targeted Iso-Seq for USH2A from human retina with Samplix Xdrop Sort

Nick Zomer[1], *Merel Stemerdink*[1;2;3], *Raoul Timmermans*[1], *Jaap Oostrik*[1], *Michael Kwint*[1], *Erwin van Wijk*[1;2;3], *Erik de Vrieze*[1;2;3], *Alexander Hoischen*[1;4]

[1]*Department of Human Genetics, Research Institute for Medical Innovation, Radboud university medical center, Nijmegen, Netherlands;* [2]*Donders Institute for Brain Cognition and Behaviour, Radboud University Medical Center, Nijmegen, Nijmegen, Netherlands;* [3]*Department of Otorhinolaryngology, Radboud University Medical Center, Nijmegen, Netherlands;* [4]*Department of Internal Medicine; Radboud Expertise Center for Immunodeficiency and Autoinflammation and Radboud Center for Infectious Disease (RCI), Radboud University Medical Center, Nijmegen, Netherlands*

Background/Objectives: Long-read sequencing is revolutionizing human genetics, and full-length isoform sequencing technologies like Iso-Seq allow for improved characterization of isoform landscapes, especially when studying specific cell-types. However, standard Iso-Seq methods also present with some shortcomings; a bias for transcripts <10kb in size and modest coverage for lowly abundant transcripts. Here, we applied 'indirect target enrichment' of cDNA using the Xdrop Sort (Samplix) for single molecule enrichment in double-emulsion droplets and showcase successful enrichment of *USH2A* isoforms – aiming at large transcripts, including the identification and quantification of known and novel isoforms.

Methods: In order to generate targeted high coverage long-read RNAseq data of *USH2A*, the Samplix Xdrop Sort was employed for cDNA obtained from human post-mortem retinas. Through the use of three detection sequences distributed across the full-length of *USH2A*, the gDNA method was used for indirect target enrichment with 3ng of cDNA input. The resulting pool of enriched transcripts was sequenced through HiFi long-read sequencing (PacBio) followed by cDNA analysis.

Results: A successful enrichment of *USH2A* transcripts was observed, resulting in up to 125-fold coverage. A subset of sequenced reads demonstrated successful capture of full-length 18.9kb transcripts. Additionally, sequencing results revealed the presence of a novel exon in ~15% of transcripts, potentially supporting a previously undescribed transcript based on its UTR-like motives.

Conclusion: The Xdrop Sort can successfully be employed for the targeted Iso-Seq of cDNA. The current study highlights the capability of detecting a multitude of isoforms and splicing events, even for transcripts as large as 18.9kb like *USH2A*.

Grants:

Conflict of Interest: None declared

P21.042.A BeSolveRD: The Belgian Genome Resource to Resolve Rare Diseases

Kris Van Den Bogaert[1], *Mathilde Geysens*[1], *Wouter Bossuyt*[1], *Sylvain Brohée*[2], *Elfride De Baere*[3], *Julie Desir*[2], *Annelies Dheedene*[3], *Boyan Dimitrov*[4], *Katrien Janssens*[5], *Aimé Lumaka*[6], *Jeroen Luyten*[7], *Gert Matthijs*[1], *Björn Menten*[3], *Marije Meuwissen*[5], *Olivier Monestier*[2], *Catharina Olsen*[4], *Leonor Palmeira*[6], *Nicole Revencu*[8], *Nishkala Sattanathan*[5], *Julie Soblet*[9], *Erika Souche*[1], *Yves Sznajer*[8], *Sylvie Taziaux*[6], *Geert Vandeweyer*[5], *Hilde Van Esch*[1], *Elise Vantroys*[4], *Catheline Vilain*[9], *Koenraad Devriendt*[1], *Joris Vermeesch*[1]

[1]*University Hospital Leuven/KU Leuven, Center for Human Genetics Leuven, Leuven, Belgium;* [2]*Institut de Pathologie et de Génétique, Centre de Génétique Humaine, Gosselies, Belgium;* [3]*Ghent University Hospital/Ghent University, Center for Medical Genetics Ghent, Ghent, Belgium;* [4]*Vrije Universiteit Brussel, Center of Medical Genetics, Brussels, Belgium;* [5]*Antwerp University Hospital/University of Antwerp, Center of Medical Genetics, Edegem, Belgium;* [6]*Centre Hospitalier Universitaire de Liège, Center for Medical Genetics, Liège, Belgium;* [7]*KU Leuven, Department of Public Health and Primary Care, Leuven, Belgium;* [8]*Université Catholique de Louvain, Center for Human Genetics, Brussels, Belgium;* [9]*Université Libre de Bruxelles, Center for Human Genetics, Brussels, Belgium*

Background/Objectives: Despite the diagnostic implementation of chromosomal microarrays/shallow whole genome sequencing and whole exome sequencing (WES) for patients with intellectual disabilities/developmental disorders (ID/DD), approximately half remain undiagnosed using this standard of care (SoC). Several international studies have demonstrated improvements in diagnostic yield with the use of whole genome sequencing (WGS) compared to SoC approaches. To (i) technically validate WGS at different genetic centers in Belgium, (ii) investigate the clinical utility of WGS for ID/DD diagnosis and (iii) to assess the health economic impact, the 8 Belgian genetic centers engaged in a multicentric prospective randomized control trial, called BeSolveRD.

Methods: A total of 800 patients and both parents are being recruited of which half will be processed by SoC and half by WGS. In order to streamline the WGS analysis across the different sites, we set up a harmonized variant interpretation workflow within the Emedgene platform (Illumina). We also created a federated database (WINGS) to share variant and sequence information.

Results: Currently, we are over halfway through the project and have prospective data on about 400 patients showing already non-inferiority of WGS as compared to SoC.

Conclusion: We will present the current status of the project and interesting clinical findings. We will also elaborate on how BeSolveRD will provide the necessary Belgium specific data on clinical and health economic aspects that can aid the decision on the implementation of WGS at a national level.

Grants: Illumina sponsored study

Conflict of Interest: None declared

P21.043.B Targeted sequencing of chromosomal rearrangements using nanopore sequencing.

Joakim Klar[1;2], *Ida Höijer*[2], *Hans Matsson*[1], *Malin Melin*[2], *Adam Ameur*[3]

[1]*Akademiska Sjukhuset, Clinical Genetics, Uppsala, Sweden;* [2]*Uppsala University, SciLifeLab Clinical Genomics, Uppsala, Sweden;* [3]*Uppsala University, SciLifeLab National Genomics Infrastructure, Uppsala, Sweden*

Chromosome karyotyping followed by metaphase FISH is still a robust way to map and identify chromosomal rearrangements. It can however be a tedious process making use of multiple FISH probes to correctly identify the breakpoints with a limited resolution. Whole-genome sequencing (WGS) using short-read sequencing can give a faster result but have difficulties to correctly annotate more complex rearrangement that includes multiple deletion/duplications together with inversions. Sequencing using longer reads overcome many of the limitations of short-read sequencing, such as the ability to reliably produce sequences of long repetitive regions and large segmental duplications. In addition, the ability to enrich for regions of interest (ROI) prior to sequencing is for many applications advantageous. In a clinical setting this means a cost-effective way to sequence only the loci implicated in a translocation identified by routine karyotyping, thereby replacing FISH.

We therefore investigate the possibility to implement adaptive sampling 'Read Until' using Nanopore for targeted sequencing of complex translocation breakpoints or rearrangements with multiple breakpoints such as chromoanagenes. In our first study, we identified a seemingly balanced translocation by using conventional karyotyping. We used both WGS using short-read sequencing and targeted nanopore sequencing to identify multiple breakpoints together with duplications and inversions at both chromosomes involved. The use of long-read sequencing produced more reliable results with far less background compared to short-read sequencing.

Conflict of Interest: None declared

P21.044.C Optimized library preparation kit and workflow for improving cfDNA sequencing sensitivity

Sean Tighe[1], *Owen Smith*[1], *Tong Liu*[1], *Tiffany Truong*[1], **Tina Han**[1], *Elian Lee*[1], *Ramsey Zeitoun*[1], *Siyuan Chen*[1]

[1]*Twist Bioscience, South San Francisco, United States*

Background/Objectives: Next-Generation Sequencing (NGS) of cell-free DNA (cfDNA) has emerged as a promising strategy for early detection, diagnosis, and monitoring of cancer progression. In this process, cfDNA is extracted from a patient's blood and undergoes library preparation prior to NGS. The challenge with cfDNA library preparation is reliably capturing and converting all the fragmented DNA, especially when present in low concentrations within biological samples. Attaining low variant detection thresholds and variant calling confidence demands high-performance NGS libraries and targeted sequencing protocols.

Methods: Presented here is a workflow leveraging the Twist cfDNA library preparation kit and an optimized target enrichment workflow to maximize the conversion of duplex, on-target, sequenceable sample molecules.

Results: It is demonstrated that by increasing target coverage we can increase detection sensitivity at low variant allele frequencies. In addition, this workflow improves detection of duplex-molecule families by an estimated 40% relative to comparable workflows due to more efficient four-point ligation and newly optimized target enrichment. It is also demonstrated that achieving such improvements in complexity does not necessitate compromising data fidelity by introducing artifacts or losing uniformity. This improved conversion and sensitivity is applicable to as low as 1ng input samples with both native cfDNA and control cfDNA.

Conclusion: In summary, our optimized library preparation kit and streamlined workflow emerges as a valuable asset for those deploying NGS-based liquid biopsy assays.

Grants: None

Conflict of Interest: Sean Tighe Twist Bioscience, Twist Bioscience, Owen Smith Twist Bioscience, Twist Bioscience, Tong Liu Twist Bioscience, Twist Bioscience, Tiffany Truong Twist Bioscience, Twist Bioscience, Tina Han Twist Bioscience, Twist Bioscience, Elian Lee Twist Bioscience, Twist Bioscience, Ramsey Zeitoun Twist Bioscience, Twist Bioscience, Siyuan Chen Twist Bioscience, Twist Bioscience

P21.046.A Comparative analysis of sequencing data of patients with inherited cardiac conditions: Adaptive sampling vs. Targeted sequencing

Aidana Gabdulkayum[1], *Asset Daniyarov*[1], *Zhanel Mirmanova*[1], *Ayaulym Chamoieva*[1], *Madina Zhalbinova*[1], *Makhabbat Bekbossynova*[2], *Dauren Yerezhepov*[1], *Dos Sarbassov*[1;3], *Ainur Akilzhanova*[1]

[1]*National Laboratory Astana, Nazarbayev University, Astana, Kazakhstan;* [2]*National Research Cardiac Surgery Center, Astana, Kazakhstan;* [3]*Nazarbayev University, School of Sciences and Humanities, Astana, Kazakhstan*

Background/Objectives: Adaptive sampling is a new approach designed by Oxford Nanopore Technologies (ONT) to enrich the regions of interest and deplete off-target regions in a whole genome sequencing. The purpose of this study was to conduct the comparative analysis of Illumina and ONT targeted sequencing methods for patients with Inherited Cardiac Conditions (ICCs). ICCs are genetic diseases, with structural or functional heart abnormalities.

Methods: gDNA was isolated from 6 whole blood samples of patients with ICCs by using the commercial kit. Samples were sequenced on Illumina using TruSight Cardio sequencing kit. The BED file for ONT Adaptive Sampling was generated by UCSC browser, containing the same genes as of TruSight Cardio. The comparative analysis of obtained data was performed using bioinformatic tools and epi2me labs platform.

Results: As a result of targeted sequencing, the mean depth of coverage for targeted regions was 77 for ONT and 10650 for Illumina. The proportion of bases covered in ONT and Illumina was 80% and 43%, respectively.

Conclusion: Illumina sequencing achieved higher depth of coverage in comparison with ONT. However, the longer reads used on ONT sequencing provides higher proportion of bases covered and yield additional data for the off-target regions, that allows identification of possible novel mutations.

Grants: This research has been funded by the Science Committee of the Ministry of Science and Higher Education of the Republic of Kazakhstan (Grant No. AP14869903).

Conflict of Interest: None declared

P21.047.B Tandem repeat genotyping using massively parallel second generation sequencing: comparison of short-read and long-read technologies

Jan Radvanszky[1;2;3;4], **Ingrid Lojova**[1;2;3], *Marcel Kucharik*[2;4], *Andrej Balaz*[4;5], *Katerina Kvapilova*[6;7], *Petr Kvapil*[7], *ondrej brzon*[7], *Martin Kasny*[7], *Terezia Duranova*[2], *Natalia Forgacova*[1;2;3], *Matej Hrnčiar*[4;8],

Zuzana Holesova[4], Jozef Martis[4], Jozef Sitarcik[4], Jaroslav Budiš[2;4;9], Tomas Szemes[2;3;4]

[1]Biomedical Research Center of the Slovak Academy of Sciences, Institute of Clinical and Translational Research, Bratislava Karlova Ves; [2]Comenius University Science Park, Comenius University Science Park, Bratislava Karlova Ves, Slovakia; [3]Faculty of Natural Sciences, Comenius University, Department of Molecular Biology, Bratislava Karlova Ves; [4]Geneton Ltd., Bratislava Karlova Ves; [5]Faculty of Mathematics, Physics and Informatics, Comenius University, Department of Applied Informatics, Bratislava; [6]Charles University, Faculty of Science, Prague, Czech Republic; [7]Institute of Applied Biotechnologies a.s., Prague, Czech Republic; [8]Institute of Informatics, Information Systems and Software Engineering, Faculty of Informatics and Information Technologies of Slovak University of Technology, Bratislava, Slovakia; [9]Slovak Center of Scientific and Technical Information, Bratislava, Slovakia

Background/Objectives: The evaluation of tandem-repeat (TR) motifs from massively parallel sequencing (MPS) data is rapidly gaining importance in modern genomics. We are reporting on specific qualitative and quantitative measures of TR genotyping when comparing short-read and long-read second generation sequencing technologies.

Methods: Four human samples were analyzed using three whole-genome-sequencing (WGS) protocols: Illumina-short-read with ~30x depth-of-coverage (WGS_SR_30x), Illumina-short-read with ~100x depth-of-coverage (WGS_SR_100x), and ~30x Illumina-long-read (WGS_LR_30x). TR genotypes were characterized for ~50 clinically relevant TR loci using the Dante tool. Qualitative and quantitative parameters, covering genotyping-accuracy, coverage and phasing issues, were compared between these datasets.

Results: Strong presence of partial reads were recorded in SR data, affecting the number of spanning reads usable for direct genotyping. This could, together with stutters, increase the requirements on depth of coverage for more consistent genotyping results. Such partial reads were not typical for LRs, however, larger numbers of non-specific spanning reads were recorded, which in certain cases also interfered with the genotyping possibilities. Phasing of complex motifs was possible also with WGS_SR_100x, although the LR protocol led to more reads spanning whole motifs.

Conclusion: Possibilities and reliability of TR analyses are affected by certain aspects of data structure, such as sufficient depth of locus coverage (TRs require more reads than SNVs), longer read lengths (which may aid complex repeat motifs) and the possibility of visual verification through dedicated tools (for locus structure visualization).

Grants: Scientific-Grant-Agency (VEGA_2/0146/23;VEGA_2/0114/24), SAS grants for PhD students (APP0509), Slovak-Research-and-Development-Agency (APVV-21-0296_INCAM;APVV-20-0472_Sepmin), Horizon-Europe-Framework-Programme-Grant (ID:101087124;ADDIT-CE).

Conflict of Interest: None declared

P21.048.C Nanopore Sequencing as a cutting-edge technology for medulloblastoma classification

Mathilde Filser[1], *Jacob Torrejon[2], Kevin Merchadou[3], Elodie Girard[4], Christine Bourneix[1], Elisa Lemaitre[1], Riwan Brillet[4], Jennifer Wong[1], David Gentien[5], Audrey Rapinat[5], Nicolas Servant[4], Alexandre Vasiljevic[6], Anne-Isabelle Bertozzi[7], Sandra Raimbault[8], Arnault Tauziede-Espariat[9], Benoit Lhermitte[10], Cecile Conter[11], Celine Icher[12], Claire Berger[13], Claude-Alain Maurage[14], Damien Bodet[15], David Meyronet[16], Emmanuelle Uro-Coste[17], Emilie De Carli[18], Fabien Forest[19], Gilles Palenzuela[20], Guillaume Chotard[21], Guillaume Gauchotte[22], Helene Sudour[23], Ludovic Mansuy[24], Mariana Deparis[25], Marie-Christine Machet[26], Maxime Faisant[27], Natacha Entz-Werlé[28], Pascale Varlet[9], Pierre Leblond[29], Sophie Michalak[30], Stephanie Proust Houdemont[31], Valérie Rigau[32], François Doz[33], Franck Bourdeaut[33], Olivier Delattre[1], Olivier Ayrault[2], Julien Masliah-Planchon[1]*

[1]Institut Curie, Genetics Department, Paris, France; [2]3Institut Curie, PSL Research University, CNRS UMR, INSERM, Orsay, France Université Paris Sud, Université Paris-Saclay, CNRS UMR 3347, INSERM U1021, Orsay, France; [3]Bioinformatics Clinic Unit, Institut Curie, Paris, France, France; [4]INSERM U900, Institut Curie, Paris, France, France; [5]Institut Curie, PSL Research University, Translational Research Department, Genomics Platform, Paris, F-75248 France, France; [6]Centre de Pathologie et Neuropathologie Est, Centre de Biologie et Pathologie Est, Groupement Hospitalier Est, Hospices Civils de Lyon, Bron 69677, France, France; [7]Pediatric Oncology Department, Children's Hospital, Toulouse University Hospital, France, France; [8]Department of Pediatric Oncology, Oscar Lambret Cancer Center, Lille, France, France; [9]Service de neuropathologie, GHU Paris psychiatrie et neurosciences, hôpital Sainte-Anne, Paris université, Paris, France, France; [10]Department of Pathology, Strasbourg University Hospital, Strasbourg, France, France; [11]Department of Pediatric Oncology, Institut d'Hemato-oncologie Pediatrique, Lyon, France, France; [12]Department of Pediatric Onco-Hematology, University Hospital, Bordeaux, France, France; [13]Department of Paediatric Haematology and Oncology, University Jean Monnet, University-Hospital of Saint-Etienne, Saint-Etienne, France, France; [14]Department of Pathology, Centre Biologie Pathologie, Lille University Hospital, Hopital Nord, Lille, France, France; [15]Department of Pediatric Hematology and Oncology, University Hospital of Caen, Caen, France; [16]Department of Neuropathology, East Hospital Group, Hospices Civils de Lyon, Bron, France, University Claude Bernard Lyon I, Villeurbanne, France, France; [17]Département d'anatomie et de cytologie pathologiques, faculté de santé, institut universitaire du cancer Toulouse - Oncopole, université Toulouse III Paul-Sabatier, Toulouse, France, France; [18]Department of Pediatric Oncology, University Hospital, Angers, France, France; [19]Departments of Pathology and Molecular Biology of Tumors, University Hospital of Saint Etienne, Saint Etienne, France, France; [20]Department of Pediatric Hematology-Oncology, Centre Hospitalo-Universitaire de Montpellier, Montpellier, France, France; [21]Department of Pathology, Pellegrin Hospital, Bordeaux, France; [22]23Department of Biopathology CHRU of Nancy, Institut de Cancérologie de Lorraine, CHRU Nancy, Université de Lorraine, 54511, Vandœuvre-lès-Nancy, France; [23]24Pediatric and Young Adults Oncology Department, Centre Oscar Lambret, Lille, France; [24]Department of Pediatric Hematology and Oncology, Nancy University Hospital, Vandœuvre-lès-Nancy, France; [25]CHU, service d'oncologie et hématologie pédiatrique, Caen, France; [26]Service d'anatomopathologie, Centre Hospitalier Régional Universitaire, Tours, France; [27]Department of Pathology, CHU de Caen, Caen, France; [28]Pediatric Onco-Hematology Unit, University Hospital of Strasbourg, Strasbourg, France; [29]Centre Léon Bérard, IHOPe, Lyon, France; Oscar Lambret Comprehensive Cancer Center, Pediatric Oncology Unit, Lille, France; [30]Angers University Hospital, Department of Pathology, Angers, France; [31]Unité d'Onco-hémato-immunologie Pédiatrique, CHU Angers, Angers, France; [32]Department of Pathology and Onco-biology, Gui de Chauliac University Hospital, Montpellier, France, Institute of Functional Genomics, Montpellier University, Montpellier, France; [33]Department of Pediatric Oncology, Institut Curie, Paris, France

Background/Objectives: Medulloblastoma (MB) is one of the most prevalent embryonal malignant brain tumors, divided into four distinct molecular subgroups (WNT, SHH, group 3, and group 4) with its individual prognosis. A more extensive classification of MB has been provided, identifying numerous subtypes, some with poor prognosis, leading to a significant number of patients

succumbing to the disease while survivors experience side effects, highlighting the need for efficient subgrouping methods.

Methods: We evaluated the ability of Nanopore sequencing to provide clinically relevant methylation and copy number profiles on a discovery cohort of 45 frozen MB samples, benchmarked against the gold standard EPIC array, and validated on a cohort of 116 MB samples. Distinct DNA methylation profiles characterize each MB subgroup; the advent of Nanopore sequencing has positioned itself as a reliable technique for comprehensive copy number and methylation profiling

Results: Most MB in both the discovery (43/45; 95.6%) and the validation cohort (110/116; 94.8%) were accurately subgrouped by Nanopore sequencing. Flongle flow cells for 18 MB allowed for a more rapid and cost-effective analysis, with 94.4% (17/18) being correctly classified. Nanopore sequencing allowed us to correctly subtype 24/30 (80.0%) within MB groups.

Conclusion: We provided for the first-time proof of concept that Nanopore sequencing can subgroup MB using DNA extracted from formalin-fixed paraffin-embedded (FFPE) samples and confirmed its use on cell-free DNA obtained from cerebrospinal fluid. Its ability to deliver quick and cost-effective results firmly establishes Nanopore sequencing as a game-changer in the field of MB classification.

Conflict of Interest: None declared

P21.049.D *RareGPT*: towards improving large language models with the knowledge of rare diseases. A proof of concept study for genetic skeletal disorders.

Behnam Javanmardi[1], *Peter Krawitz*[1]

[1]*University of Bonn, Institute for Genomic Statistics and Bioinformatics, Bonn, Germany*

Background/Objectives: Large Language Models (LLMs) are already in use in a range of biomedical contexts (Thirunavukarasu et al. 2023) and can be envisioned to play major roles in assisting clinicians in various tasks and also influence educational and research work in medicine. However, to date, the developed medical LLMs have focused mainly on general medical knowledge or common diseases (Singhal et al. 2022), and that makes them prone to wrong answers or "hallucinations" when asked about certain rare diseases. In this contribution, we present our first steps in developing *RareGPT* aiming at fine-tuning LLMs with the knowledge of rare diseases. As a proof of concept, we start with customizing GPT-4 on basic knowledge of rare bone diseases.

Methods: We created a structured "knowledge file" from the *Nosology of Genetic Skeletal disorders: 2023 revision* by Unger et al. (containing 771 entries associated with 552 genes) and fed that to the custom GPT builder provided by OpenAI followed by a set of custom instructions in the configuration step.

Results: We compared the performance of GPT-4 and *RareGPT* in answering 50 short questions about genetic skeletal disorders. Out of these 50 questions, GPT-4 provided 34 and *RareGPT* provided 47 correct answers.

Conclusion: The performance of LLMs, such as GPT-4, in answering questions about rare diseases can be considerably improved by further fine-tuning them on the public knowledge in this field. We continue to add more rare disease knowledge to *RareGPT* and also plan to make use of open-source LLMs.

Grants:

Conflict of Interest: None declared

P21.050.A CaseSharing: A system for sharing case information on rare diseases adopting a user-based approach

Toyofumi Fujiwara[1], *Jae-Moon Shin*[1], *Eisuke Dohi*[2], *Yasunori Yamamoto*[1], *Atsuko Yamaguchi*[3]

[1]*Research Organization of Information and Systems, Database Center for Life Science, Kashiwa, Japan;* [2]*National Center of Neurology and Psychiatry, Department of Disease Research III, Kodaira, Japan;* [3]*Tokyo City University, Setagaya, Japan*

Background: The cost of whole genome sequencing has been decreasing, making it more common in diagnosing rare diseases. Sharing case information about undiagnosed cases and those with rare variants is crucial for discovering novel disease-gene relationships. A federated approach, connecting national genome initiatives, has been adopted to promote the sharing of case information. However, much case information remains outside these initiatives, isolated within clinical settings. In this study, we propose a new system that adopts a user-based approach to directly share case information, aiming to bridge this gap.

Methods: We have developed CaseSharing (https://pubcasefinder.dbcls.jp/casesharing) that is a management system for case information related to rare diseases. Case information is stored locally in JSON format, rather than in the cloud. In addition, we have developed CaseSharingDB that collects the metadata of case information such as gene names, disease names, and phenotypes stored in CaseSharing by each user.

Results: There is no need to worry about case information crossing national borders since case information is managed in local storage. Only metadata, which does not contain personal information, is collected from the case information and used for matching between CaseSharing users. Users can output case information in Phenopackets format, which facilitates case information sharing.

Conclusion: We developed CaseSharing, coupled with CaseSharingDB, that adopts a user-based approach to share rare disease case information while ensuring data privacy. This approach enhances the potential for discovering novel disease-gene relationships by facilitating broader data sharing across clinical settings.

Grant References: JSPS KAKENHI Grant Number 23K11886

Conflict of Interest: None declared

P21.051.D Enrichment-based long-read panel sequencing in a diagnostic setting

Katharina Herzog[1], *Klaus Oberhuber*[1], *Martina Witsch-Baumgartner*[1], **Johannes Zschocke**[1]

[1]*Institute for Human Genetics, Innsbruck, Austria*

Background/Objectives: For many conditions, genetic analysis currently involves short-read exome sequencing and targeted examination of a limited number of relevant genes. However, this approach generally fails to identify pathogenic promoter, deep intronic or structural variants, leaving uncertainty in the case of negative results. Furthermore, there are complex or homologous "problem" genes that require separate specialized analysis methods. Long-read genome sequencing may overcome these challenges but is not usually feasible in a diagnostic setting.

Methods: We combined customized full-length target gene enrichment (Twist Bioscience) with multiplex HiFi long-read sequencing (PacBio) for comprehensive genetic analysis of selected common conditions. A single enrichment panel (approx. 100 genes) was used for several indications such as cystic kidney disease (including *PKD1*), hypercholesterolemia (including *LPA*), congenital adrenal hyperplasia (including *CYP21A2*), various tumour dispositions (including *PMS2*), and others, with variant

analysis of target genes only. Twelve or more samples were analyzed together on a single PacBio SMRT cell.

Results: Our one-for-all approach identified all tested variants including variants missed or not detectable by exome sequencing. The analysis of multiple samples for different indications in the same run secures cost-effectiveness without prolonged waiting times.

Conclusion: Long-read panel sequencing is a flexible, rapid and low-cost approach for comprehensive full-length gene analysis of conditions caused by a limited number of well-known genes. It has the potential of resolving cases left open after traditional short-read exome sequencing at low cost without resorting to genomes.

Grants: No Grants

Conflict of Interest: None declared

P21.052.C Objective 3D phenotyping uncovers subclinical facial features in a 3q26 deletion/KBG syndrome dual diagnosis.

Michiel Vanneste[1;2;3], *Elise Pelgrims*[1], *Laurens Hannes*[1;2], *Ann Swillen*[1;2], *Peter Claes*[1;3;4], *Jeroen Breckpot*[1;2], *Hilde Peeters*[1;2]

[1]*KU Leuven, Department of Human Genetics, Leuven, Belgium;* [2]*University Hospital Leuven, Centre for Human Genetics, Leuven, Belgium;* [3]*KU Leuven, Medical Imaging Research Centre, Leuven, Belgium;* [4]*KU Leuven, Department of Electrical Engineering, ESAT/PSI, Leuven, Belgium*

Background/Objectives: Co-existing Mendelian disorders can cause atypical phenotypes, challenging genetic diagnostics and clinical management. Deep familial phenotyping contributes to matching genomic and phenotypic data, but is particularly challenging for facial features. Here, we report an individual with a dual diagnosis of paternally inherited KBG syndrome and a maternally inherited 3q26 deletion. We performed three-dimensional (3D) facial phenotyping to investigate the presence of a subclinical dual phenotype.

Methods: We collected 3D facial photos of the index and his nuclear family, seven individuals with KBG syndrome and unaffected controls. We used dense surface registration to capture the facial shape and used craniofacial growth curves to assess facial features irrespective of age- and sex-related variation. We corrected the index's facial shape for the maternal (3q26del) and paternal (KBG) shape effects and performed principal component analysis and cosine distance-based analysis to objectively assess phenotypic similarity.

Results: Clinically, the index's facial gestalt is dominated by features associated with the 3q26 deletion. However, the cosine similarity to KBG syndrome is high, which is an objective indication of the presence of the KBG gestalt. Projecting the original phenotypes in a principal component space shows separate clustering of controls, KBG syndrome and both 3q26del carriers. After correcting the index for maternal shape effects, clustering reveals the corrected shape features the KBG gestalt.

Conclusion: We introduce objective 3D facial phenotyping to deconstruct facial features of major gene effects, unveiling subclinical facial features of KBG syndrome in an individual with a 3q26 deletion and KBG syndrome.

Grants: /

Conflict of Interest: None declared

P21.055.B Efficient genotyping of a full set of human inversions with nanopore ultra long reads and adaptive sampling

Konstantinos Karakostis[1;2], *Ricardo Moreira Pinhal*[1;2], *Oscar Conchillo-Solé*[1;3], *Illya Yakymenko*[1;2], *Miquel Angel Senar*[4], *Marta Puig*[1;3], *Mario Caceres*[1;2;5]

[1]*Institut de Biotecnologia i de Biomedicina, Universitat Autònoma de Barcelona, Bellaterra, Spain;* [2]*Research Programme on Biomedical Informatics (GRIB), Hospital del Mar Research Institute (IMIM), Barcelona, Spain;* [3]*Departament de Genètica i de Microbiologia, Universitat Autònoma de Barcelona, Bellaterra, Spain;* [4]*Departament d'Arquitectura de Computadors i Sistemes Operatius, Universitat Autònoma de Barcelona, Bellaterra, Spain;* [5]*ICREA, Bacelona, Spain*

Consortium: Invfest

Background/Objectives. Structural variants have important roles in gene regulation and pathologic conditions in humans, but a large fraction of them, including many inversions, are missed due to their kb-long size, their balanced nature and their proximity to complex repeat regions. New long read techniques offer a great opportunity to characterize completely these variants. Thus, the aim of this study is to develop a cost-efficient and high throughput method to genotype a wide range of human inversions using Oxford Nanopore Technologies (ONT) ultralong reads (50-100kb).

Methods. We tested a combination of different DNA extraction and library tagmentation protocols on four human cell lines, followed by whole-genome ONT sequencing or adaptive sampling (AS) targeting ~640 candidate inversion regions.

Results. A comparative analysis of the yield, the read lengths (with N50 between 50-80kb) and enrichment (4-5X), shows that the Monarch DNA purification and Ultra-long library kits, combined with adaptive sampling, provide an ideal setting for sequencing most human inversions. Around 22-52% of the inversions could be genotyped in a single MinION flow cell with high accuracy. Moreover, by merging all the data together, ~75% of the inversions could be identified in each sample. The majority of missing inversions corresponded to those mediated by the largest inverted repeats, which could be compensated by increasing N50 and coverage.

Conclusion. We provide valuable insight into the use of nanopore sequencing to genotype complex genomic regions, with potential applications in phenotype association studies and clinical research.

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Conflict of Interest: None declared

P21.056.C Nanopore sequencing enhances detection of the *PHOX2B* expanded short tandem repeat in Congenital Central Hypoventilation Syndrome patients (CCHS).

Samuel Wilkinson[1], *Andrew Fleming*[1], *Ali Awan*[2], *Chloe Fisher*[2], *Matthew Edwards*[1], *Deborah Morris-Rosendahl*[1;3]

[1]*Royal Brompton Hospital, Clinical Genetics and Genomics Laboratory, London, United Kingdom;* [2]*Guy's and St Thomas' NHS Foundation Trust, Genomics Innovation Unit, London, United Kingdom;* [3]*National Heart & Lung Institute, Genomic Medicine, London, United Kingdom*

Background/Objectives: Short tandem repeats (STRs) are DNA sequence motifs, typically 2-6 bp, repeated consecutively. The *PHOX2B* gene has two such repeats in exon 3, encoding polyalanine structures (GCA, GCC, GCG or GCT). Expansion of the second repeat (c.721-780) to more than 20 residues causes autosomal dominant congenital central hypoventilation syndrome (CCHS), characterised by autonomic nervous system dysregulation

with disordered respiratory control, occurring soon after birth. Current techniques used to detect the expansion repeats are prone to PCR errors and false negative results; and short read NGS has low sensitivity to detect the expansion. Here we use Oxford Nanopore long-read technology and adaptive sampling to accurately detect the repeat expansion in *PHOX2B* in patients with CCHS.

Methods: Samples with known *PHOX2B* expansion repeats were sequenced on the PromethION 2 (P2) using R10.4.1 flow cells. DNA was sheared to between 14-20kb in length (Covaris g-tubes) and prepped using the ligation sequencing kit v14 (SQK-LSK114) from ONT. Adaptive sampling was used to target *PHOX2B*. The ONT EPI2ME package was used to generate VCF and variant annotation files. Once validated, samples were multiplexed to reduce cost and increase throughput using the Native barcoding kit v14 (SQK-NBD114.96).

Results: Expansion of the *PHOX2B* repeat was confirmed on control samples. A sample previously sequenced using short read technologies with a false negative result, was re-run in parallel with other test cases and controls, and the known expansion was detected.

Conclusion: Nanopore sequencing allows for better detection of expansion repeats that cannot be accurately detected by short read technologies.

Conflict of Interest: None declared

P21.058.A PacBio Revio and ONT PromethION long-read sequencing in SV calling and variant phasing

Giancarlo Tomio[1], *Janine Meienberg*[1], *Timo Siegenthaler*[1], *Pamela Nicholson*[2], *Stefan Krebs*[3], *Gabor Matyas*[1;4]

[1]*Swiss Foundation for People with Rare Diseases, Center for Cardiovascular Genetics and Gene Diagnostics, Schlieren-Zurich, Switzerland;* [2]*University of Berne, Next Generation Sequencing Platform, Berne, Switzerland;* [3]*Ludwig Maximilian University of Munich, Laboratory for Functional Genome Analysis, Gene Center, Munich, Germany;* [4]*University of Zurich, Zurich Center for Integrative Human Physiology, Zurich, Switzerland*

Background/Objectives: Short-read sequencing (SRS) is the most used technology in clinical sequencing, but it has limitations in areas of the genome harbouring complex structural variants (SVs) or repetitive/homologous sequence, and variant phasing information is often lost. Long-read sequencing (LRS) technologies do not suffer these challenges and have improved to the point of feasible use in clinical sequencing. Thus, we compared the latest Pacific Bioscience (PacBio) and Oxford Nanopore Technologies (ONT) LRS technologies in their ability to detect a known set of challenging variants.

Methods: Four DNA samples with SVs and single nucleotide variants previously detected by SRS (PCR-free WGS, PE150) were sequenced on a PacBio Revio as well as on an ONT PromethION and analysed with their respective standard pipelines. Several further SV callers were applied, and regions of interest were manually reviewed using Integrative Genomics Viewer.

Results: Our data revealed differences in the performance of the tested LRS approaches as well as in the performance of SV callers for challenging variants, including complex SVs and SVs with breakpoints in non-unique sequence regions. Nevertheless, both LRS approaches were able to detect breakpoints missed by SRS and to phase sequence variants according to their respective read lengths.

Conclusion: LRS can be the solution for breakpoint identification in repetitive regions and for characterisation of complex SVs not exceeding their read length. Improved SV callers are vital to take full advantage of LRS in clinical genetic testing. Current LRS technologies achieve SRS-like error rates at affordable prices and so enable an improved future for clinical sequencing.

Conflict of Interest: Giancarlo Tomio: None declared, Janine Meienberg: None declared, Timo Siegenthaler: None declared, Pamela Nicholson: None declared, Stefan Krebs: None declared, Gabor Matyas PacBio and ONT provided reagents free of charge

P21.059.B Thymidylate synthase disruption to limit cell proliferation in cell therapies

Kirmo Wartiovaara[1;2], *Rocio Maldonado*[1], *hossam montaser*[1], *Timo Otonkoski*[1], *Inkeri Soppa*[1], *Jonna Saarimäki-Vire*[1]

[1]*University of Helsinki;* [2]*Helsinki University Hospital, Clinical Genetics*

Background/Objectives: Many diseases or genetic conditions could be envisioned to be treated with cell transplantations with or without gene therapy. However, the use of additional or genetically modified, such as CRISPRed cells carries a risk of malignant cell proliferation.

Methods: Here, we describe a novel metabolic safety system to control cell proliferation without added genetic elements. For this, we inactivated a key enzyme for nucleotide metabolism, *TYMS*, in several cell types, thus obtaining cells that proliferate only when supplemented with exogenous thymidine but fail to replicate in its absence.

Results: We show that under thymidine supplementation, *TYMS*$^{-/-}$ cell lines and pluripotent stem cells proliferate normally, produce teratomas and differentiate into potentially therapeutic cell types such as pancreatic beta cells. After differentiation, the postmitotic cells do not require thymidine to function, as seen by prolonged in vivo production of human insulin in implanted mice. Hence, this method allows robust cell culture and manufacture while mitigating the risk of uncontrolled growth of transplanted cells.

Conclusion: We describe engineering fully functional human cells, which can be cultured and manufactured robustly under supplementation of thymidine. This allows the genetic manipulation and production of therapeutic cells. However, since the cell proliferation can be controlled, the recipients of the cells are safe from possible oncogenic modifications.

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Conflict of Interest: None declared

P21.060.C Streamlined implementation of nanopore sequencing in clinical routine diagnostics within the IonGER consortium

Florian Kraft[1], *Stephan Ossowski*[2], *Nadja Ehmke*[3], *Gunnar Schmidt*[4], *Elena Buena-Atienza*[2], *Nicolas Casadei*[2], *Janine Altmüller*[5], *Manuel Holtgrewe*[6], *Benedikt Schnur*[4], *Casper Groß*[2], *Thomas Eggermann*[1], *Sebastian Giesselmann*[1], *Bernd Auber*[4], *Ingo Kurth*[1], *Tobias Haack*[2]

[1]*Institute for Human Genetics and Genomic Medicine, Medical Faculty, RWTH Aachen University Hospital, 52074 Aachen, Germany.;* [2]*Institute of Medical Genetics and Applied Genomics, University of Tübingen, 72076 Tübingen, Germany.;* [3]*Institute of Medical Genetics and Human Genetics, Charité - Universitätsmedizin Berlin, Corporate Member of Freie Universität Berlin, Humboldt-Universität zu Berlin, and Berlin Institute of Health, Berlin.;* [4]*Department of Human Genetics, Hannover Medical School, Hannover, Germany.;* [5]*Max Delbrück Center for Molecular Medicine in the Helmholtz Association (MDC), Berlin Institute of Health at Charité-Universitätsmedizin Berlin,*

Core Facility Genomics, Charitéplatz 1, 10117 Berlin, Germany.; [6]Berlin Institute of Health at Charité-Universitätsmedizin Berlin, Core Unit Bioinformatics (CUBI), Berlin 10117, Germany

Consortium: lonGER consortium

Background/Objectives: Short-read genome sequencing (SR-GS) has limitation regarding the detection of structural variations, homologous sequences, and low-complexity regions. Moreover, in SR-GS separate analyses are required to detect both genetic and epigenetic variants. Long-read genome sequencing (LR-GS) has the potential to overcome these limitations and to increase the diagnostic yield in rare diseases. The question is whether nanopore LR-GS is mature and accurate enough to be applied in clinical routine diagnostics.

Methods: The lonGER (German national initiative for clinical long-read sequencing) consortium was launched to evaluate the feasibility and advances of using nanopore genome sequencing in a routine clinical setup. Further, the lonGER consortium is established as a platform for the exchange of experience and to provide SOPs for wet lab and bioinformatic analysis to facilitate streamlined implementation in clinical laboratories.

Results: We provide insights into the lonGER wet lab workflows and bioinformatic analysis for routine clinical use. In addition, several example cases with SNV, SV and methylation alterations are presented that illustrate the utility of nanopore sequencing for the analysis of rare diseases patients. Our data highlight that nanopore sequencing is as accurate or more accurate than SR-GS for most variants.

Conclusion: Within the first year of the lonGER initiative, we were able to demonstrate the straightforward implementation of robust wet lab and bioinformatic workflows for nanopore sequencing. The data highlight the advancements of nanopore sequencing and its transformative impact on genomic medicine.

Conflict of Interest: Florian Kraft Sequencing reagents from Oxford Nanopore Technologies, Cloud Compute from Nvidia, Travel cost from Oxford Nanopore Technologies, Stephan Ossowski: None declared, Nadja Ehmke: None declared, Gunnar Schmidt: None declared, Elena Buena-Atienza: None declared, Nicolas Casadei: None declared, Janine Altmüller: None declared, Manuel Holtgrewe: None declared, Benedikt Schnur: None declared, Casper Groß: None declared, Thomas Eggermann: None declared, Sebastian Giesselmann: None declared, Bernd Auber: None declared, Ingo Kurth: None declared, Tobias Haack: None declared

P21.061.D NGS panel customization for comprehensive genomic profiling

Akanksha Khare[1], Nishita Matcha-Balagrurvappa[1], Linus Forsmark[1], Katie Wilkins[1], Swati Gupta[1], Mike Ruvolo[1], **Houda Hallay**[2]

[1]Agilent Technologies, Genomics R&D, Santa Clara, United States; [2]Agilent Technologies, Germany

Background/Objectives: We developed a custom design pipeline to generate target enrichment gene panels for comprehensive genomic profiling of cancer samples, by detecting single nucleotide variations (SNV/INDEL), gene-level copy number variations (CNV), and DNA translocations (TL); and content to enable assessment of microsatellite instability (MSI) and tumor mutational burden (TMB).

Methods: The design methodology allows for addition of content based on the biomarker type. Probes for target regions are selected using empirical data on probe performance. Pre-defined content for MSI measurement covering 288 microsatellite sites. The performance of these sites in determining MSI status was demonstrated by comparing to the MSI-PCR reference method. To assess the performance of the design methodology, designs of various sizes (50 to 650+ genes) and content type were generated and assayed on reference samples and FFPE with known biomarkers. Sequencing metrics were evaluated for capture specificity and coverage of the target regions.

Results: Biomarkers were reliably detected with SNV detection down to 1% allele frequency, detection of gene amplifications and translocations concordant with FISH, and MSI and TMB determination concordant with PCR and Exome sequencing respectively. The design workspace was implemented in the SureDesign platform, providing a web-based interface for creating the designs.

Conclusion: We demonstrated full customization capability of the SureSelect Cancer target enrichment platform that pairs user-defined content with an optimized probe design methodology to enable the sensitive detection of the common types of DNA alterations found in FFPE samples.

Grants:

Conflict of Interest: Akanksha Khare Agilent Technologies, Nishita Matcha-Balagrurvappa Agilent Technologies, Linus Forsmark Agilent Technologies, Katie Wilkins Agilent Technologies, Swati Gupta Agilent Technologies, Mike Ruvolo Agilent Technologies, Houda Hallay Agilent Technologies

P21.063.B A novel assay for potential mass screening of Turner syndrome based on Oxford Nanopore technology.

Gjorgji Madjarov[1;2], *Marija Chaushevska[1;2], Anne Kristine Schack[2;3], Karmele Alapont[2], Carmen Garrido Navas[2;4;5], Zoran Velkoski[2], Chris Kyriakidis[2]*

[1]Ss. Cyril & Methodius University, Skopje, Macedonia; [2]gMendel, Copenhagen, Denmark; [3]University of Copenhagen, Copenhagen, Denmark; [4]Asesoramiento Genetico SL, Granada, Spain; [5]University of Granada, Granada, Spain

Background/Objectives: Turner syndrome (TS) is characterized by a complete or partial absence or as mosaic of one X chromosome; leading to the karyotype of either complete, 45,X0, or partial 46,XXq or 46,XXp or mosaic 45,X0/46XX. Depending on the genotype, patients with deletions on Xp have the features of a short stature and congenital malformations, whereas Xq-deletions have gonadal dysfunctions. TS represents the only common chromosomal monosomic syndrome with the prevalence of 5.6/1000 in newborns, yet only with an estimated survival of 0.3% of monosomy X conceptions survive to term. Approximately 45% of TS cases involves non-mosaic monosomy of chromosome X. Detecting and monitoring TS and its variations within mosaicism accommodates tailored therapeutic interventions.

Methods: Our research demonstrates a cost-effective, end-to-end assay with potential mass screening of Turner Syndrome (TS) based on Oxford Nanopore technology. The DNA library was prepared in <8 hours prior to loading on the GridIONx5. Our Phivea®, platform allows for real-time detection of chromosomal aberrations based on Machine Learning technologies designed for targeted sequencing.

Results: Our approach allows simultaneous assessment of 384 samples, equivalent to 96 subjects, each in 4 technical replicates, reaching analytical specificity and sensitivity-score at 99.06% and 98.43%, respectively. The experimental evaluation bases on Turner, Trisomy 21, Trisomy 18, Trisomy 13 and control female samples.

Conclusion: Phivea® platform can be used as a mass screening tool for detecting Turner syndrome in a rapid, economic and accessible manner.

Grants: N/A

Conflict of Interest: Gjorgji Madjarov IPR for gMendel and warrants from gMendel, Chief AI Officer at gMendel, Marija Chaushevska gMendel, Anne Kristine Schack gMendel, Karmele Alapont gMendel, Carmen Garrido Navas Founder Asesoramiento Genetico SL (Congen), Asesoramiento Genetico SL (Congen), CSO at gMendel, Zoran Velkoski Co-founder at gMendel, gMendel, Chris Kyriakidis Co-founder at gMendel, gMendel

P21.065.D Advancing Genetic Diagnostics: Optical Genome Mapping's Efficacy in Identifying Structural Variants for Autosomal Recessive Diseases

Ozge Beyza Gundogdu Ogutlu[1], *Mehmet Burak Mutlu*[2], *mehmet ali ergün*[3], *Sengül Ozdek*[4], *Gülsüm Kayhan*[3]

[1]*Erzurum City Hospital, Department of Medical Genetics, Erzurum, Türkyie;* [2]*Detagen Genetic Diseases Evaluation Center, Department of Medical Genetics, Kayseri, Türkyie;* [3]*Gazi University Faculty of Medicine, Department of Medical Genetics, Ankara;* [4]*Gazi University Faculty of Medicine, Department of Ophthalmology, Ankara*

Background/Objectives: Determining the genetic etiology is of great importance for many disease groups today. However, studies have shown that the etiology cannot be determined in 30-60% of patients with genetic diseases. One of the reasons for this is the limitations in the detection of structural variants. Optical genome mapping (OGM) is a new and effective method for detecting structural variants.

Methods: In this study, OGM was performed in 7 patients with a single heterozygous pathogenic variant detected for a recessive disease related to clinical findings as a result of routine molecular analyses.

Results: A structural variant was detected in the region associated with the target gene in two (29%) of seven patients. In one of these two patients with cone-rod dystrophy, an insertion of 976 bp in the 12th intron of the *TTLL5* gene, in which the likely pathogenic variant, as a result of WES analysis, was detected by OGM. The other patient, who was examined with a prediagnosis of Leber congenital amaurosis, a 7.5 kb deletion was detected 400 kb proximal to *NMNAT1* gene, in which a heterozygous pathogenic variant was detected in the WES analysis, covering the regulatory region of the gene.

Conclusion: This study supports the success of OGM in detecting structural variants. Few studies in the literature use the OGM method to diagnose monogenic diseases. This study is particularly important in terms of detecting the second variant with the OGM method in autosomal recessive diseases.

Grants: Supported by Scientific Research Projects Unit of Gazi University (TTU-2022-7742)

Conflict of Interest: None declared

P21.066.A A blended genome and exome sequencing method captures genetic variation in an unbiased, high-quality, and cost-effective manner

Toni Boltz[1], *Benjamin Chu*[2], *Julia Sealock*[1], *Calwing Liao*[1], *Lingyu Zhan*[3], *Jack Fu*[1], *Robert Ye*[1], *Lerato Majara*[1], *Sinéad Chapman*[1], *Matthew DeFelice*[1], *Jonna Grimsby*[1], *Simone Rubinacci*[1], *Dickens Akena*[4], *Elizabeth Atkinson*[5], *Lukoye Atwoli*[6], *Tim Bigdeli*[7], *Nelson Freimer*[3], *Michael Gatzen*[1], *Hailiang Huang*[1], *Christopher Kachulis*[1], *Symon Kariuki*[8], *Soyeon Kim*[1], *Karestan Koenen*[9], *Carlos Lopez Jaramillo*[10], *Justin McMahon*[9], *Sarah Medland*[11], *Charles Newton*[8], *Ana Maria Olivares Veira*[1], *Carlos Pato*[12], *Michele Pato*[12], *Chiara Sabatti*[2], *Dan Stein*[13], *Anne Stevenson*[9], *Erik Stricker*[5], *Rocky Stroud*[9], *Solomon Teferra*[14], *David Whiteman*[11], *Mary Yohannes*[1], *Susan Service*[3], *Roel A. Ophoff*[3], *Michael Talkowski*[1], *Daniel Howrigan*[1], *Loes Olde Loohuis*[3], *Benjamin Neale*[1], **Alicia Martin**[1]

[1]*Broad Institute of MIT and Harvard, United States;* [2]*Stanford University, United States;* [3]*University of California, Los Angeles, United States;* [4]*Makerere University, Uganda;* [5]*Baylor College of Medicine, United States;* [6]*Aga Kahn University, Kenya;* [7]*SUNY, United States;* [8]*KEMRI-Wellcome Trust Research Program, Kenya;* [9]*Harvard T.H. Chan School of Public Health, United States;* [10]*University of Antioquia, Colombia;* [11]*QIMR Berghofer Medical Research Institute, Australia;* [12]*Rutgers University, United States;* [13]*University of Cape Town, South Africa;* [14]*Addis Ababa University, Ethiopia*

Background/Objectives: Microarrays have been the primary technology used in GWAS to date because of their low cost and genomic coverage with imputation. However, custom content biases ascertainment of genetic variants and arrays do not capture novel variants.

Methods: We developed and scaled a blended genome exome (BGE) sequencing approach that combines a whole genome and exome capture library at a 67:33 blending ratio in a single tube to sequence the genome to 2-3X depth and the exome to 30-40X depth in a single CRAM file.

Results: We imputed common variants throughout the whole genome and called rare coding variants from 47,264 participants that met quality control from the Populations Underrepresented in Mental illness Association Studies (PUMAS) Project including African, African American, and Latin American populations. Target metrics included >9.5 Gb total bases/sample, >90% of exome bases at 10X, and 10-65% exome selected bases. We found high concordance ($r^2 > 0.9$) between well-imputed BGE variant calls (INFO>0.8) and orthogonal variant calls from the Illumina GSA, when MAF >1%. We also called copy number and structural variants using GATK-gCNV and VISTA, respectively, then compared them to high coverage "truth" data. Deletion and duplication CNVs called from gCNV spanning >4 exons have precision and recall close to 0.9 for both. SV calls have sensitivity and precision both >99% at >1kb but lower for shorter events.

Conclusion: BGE has nearly all the benefits of deep whole-genome sequencing at <20% of the cost, empowering genomic discoveries, particularly in underrepresented populations.

Grants: U01MH125047; U01MH125045; U01MH1250452; U01MH125049

Conflict of Interest: None declared

P21.067.B Reassessment of long-read technology performance

Erika Souche[1], *Benjamin Huremagic*[1], *Joris Vermeesch*[1]

[1]*KULeuven, Department of Human Genetics, Leuven, Belgium*

Background/Objectives: Long-read sequencing technologies are increasingly used in human genomics to characterise human population variation. Their rapid evolution requires a reassessment of their performance, including each technology's latest developments, namely data from the Revio of PacBio, high duplex flowcells from Oxford Nanopore (ONT) and Illumina Complete Long Read (ICLR) technology.

Methods: HG002 long reads from all technologies have been processed using a common pipeline to detect Single Nucleotide Variants (SNVs), insertions/deletions (indels) and Structural Variants (SVs). Coverage of clinically relevant genes with pseudogenes and phasing were assessed. Small variants and SVs were compared to GIAB benchmark datasets using hap.py and Truvari, respectively.

Results: The performance of long-read technologies to cover clinically relevant genes with pseudogenes was variable. A similar proportion of heterozygous variants could be phased by all technologies. ICLR outperformed both PacBio and ONT on SNV and indel calling but performed the least on SV calling. Interestingly, at equal coverage, SV calling performance was more affected by the SV detection tool than by the technology (PacBio/ONT). Although PacBio and ONT showed precision and recall above 0.9, only up to 40% of detected SVs could be benchmarked.

Conclusion: ICLR performs as well as short reads on small variants but still cannot compete with PacBio and ONT on SV calling. Only a small portion of detected SVs can be assessed using the currently available benchmark datasets, emphasing the needs for updating these benchmarks and/or creating new ones.

Grants: FWO-TBM grant T-003819N, FWO grant G0A2622N and KU Leuven grant C14/22/125.

Conflict of Interest: None declared

P21.068.C RNA-sequencing as a broadly applicable diagnostic test: experience from a cohort of ~350 individuals suspected of different genetic disorders

Janna Hol[1], *Camilla Calandrini*[1], *Hannie Douben*[1], *Monique M van Veghel-Plandsoen*[1], *Esmee Kasteleijn*[1], *Peter Elfferich*[1], *Leontine M A van Unen*[1], *Mark Nellist*[1], *Jordy Dekker*[1], *Rachel Schot*[1], *Evelien Kroon*[1], *Herma C van der Linde*[1], *Martina Wilke*[1], *Marjon van Slegtenhorst*[1], *Marjolein Weerts*[1], *Frank Sleutels*[1], *Marianne Hoogeveen-Westerveld*[1], *Lies Hoefsloot*[1], *Grazia Mancini*[1], *Tjakko Van Ham*[1]

[1]*Erasmus MC, University Medical Center Rotterdam, Department of Clinical Genetics, Rotterdam, Netherlands*

Background/Objectives: For genetic disorders, establishing the molecular diagnosis is paramount for disease management, counseling and to enable predictive genetic testing. Yet, DNA-based diagnostic techniques, such as exome sequencing, often fail to identify causative variants. RNA-sequencing (RNA-seq) is a promising tool to complement DNA-based approaches, but its potential as a widely applicable diagnostic test remains to be fully explored. Here, we report our findings for ~350 individuals suspected of a range of different genetic disorders and subjected to RNA-sequencing after DNA-based diagnostics failed to identify a causal variant.

Methods: RNA-seq was performed on RNA isolated from skin fibroblasts, blood or amniotic fluid cells, and analyzed by targeted, disease-panel or open transcriptome approaches. Abnormal gene expression and splicing were detected using z-scores, as described previously (Dekker et al., Am J Hum Genet, 2023). Data collected included clinical characteristics, types of analysis performed, variants identified and resulting diagnoses.

Results: RNA-seq resulted in a molecular diagnosis in ~14% of the cohort and the diagnostic yield varied significantly among different disease categories. RNA-seq enabled the identification of variants that alter gene expression and/or splicing in various ways, including unconventional variants affecting splice enhancers or branchpoints, as well as copy number variants and complex structural variants. Moreover, possibly pathogenic changes were identified in novel candidate disease genes.

Conclusion: Our findings demonstrate that RNA-seq can help identify a range of pathogenic variant types, thereby improving the diagnostic yield for genetic disorders. These results support the widely applicable use of RNA-seq in genome-diagnostic laboratories to complement genomic sequencing.

Conflict of Interest: None declared

P21.069.D Nanopore sequencing resolves challenging medically relevant genes (CMRG), focusing on SMN

Heather Jeffery[1], *Edmund Day*[1], *Sergey Nurk*[1], *Hayley Greenfield*[1], *Sissel Juul*[1], *Philipp Rescheneder*[1], *Phillip James*[1]

[1]*Oxford Nanopore Technologies, Oxford, United Kingdom*

Background/Objectives: The "Dark genome" is a term often used to describe genomic regions that are difficult to resolve using traditional sequencing methods. Currently there are over 5000 identified challenging medically relevant genes (CMRGs), such as the repeat expansion in the *FMR1* gene, and the 99.9% homologous *SMN1* and *SMN2* genes. We aim to show how long read sequencing can be used to resolve CMRGs such as the *FMR1* repeat expansion and *SMN1/SMN2* copy numbers.

Methods: Whole genome long read sequencing was performed on the Oxford Nanopore PromethION device using Coriell cell lines derived from subjects with fragile-x syndrome and spinal muscular atrophy. The Epi2Me human variation workflow was used to enumerate the *FMR1* repeat expansion motif. *SMN1* and *SMN2* copy numbers were calculated by aligning all relevant reads to the *SMN1* locus, then phased using discriminatory SNPs prior to generating normalised phased read counts.

Results: *SMN1* and *SMN2* copy numbers were correctly identified in the 12 Spinal Muscular Atrophy (SMA) samples analysed. We also show that longer read lengths facilitate improved alignment and detection of structural variants, including the *SMN1* exon 7-8 deletion known to be in one of the samples. Additionally, nanopore native long read sequencing was used to correctly enumerate the CGG repeat expansion in the *FMR1* gene as well as characterise methylation of the *FMR1* promotor in a haplotype resolved manner.

Conclusion: Nanopore sequencing can identify single haplotype variation in repeat regions, detecting both copy number and structural variations in Dark Genes previously unable to be resolved.

Grants: N/A

Conflict of Interest: Heather Jeffery Oxford Nanopore Technologies, Edmund Day Oxford Nanopore Technologies, Sergey Nurk Oxford Nanopore Technologies, Hayley Greenfield Oxford Nanopore Technologies, Sissel Juul Oxford Nanopore Technologies, Philipp Rescheneder Oxford Nanopore Technologies, Phillip James Oxford Nanopore Technologies

P21.071.B 3D Genomics: Unlocking the Secrets of the Non-Coding Genome

Nic Walker[1], *Kishen Chahwala*[1], *Hazel Jones*[1], *Dan Turner*[1], *Stefan Schoenfelder*[1], *Peter Fraser*[1]

[1]*Enhanc3D Genomics Ltd, United Kingdom*

Background/Objectives: Genomics-based approaches such as GWAS analyse genomes from a linear perspective and ignore the complex 3D structure. Most disease-associated GWAS variants are found in non-coding regions, areas highly enriched in elements that control gene expression. These regulatory elements can be located thousands to millions of base pairs from their target genes. Folding of the genome results in promoter-enhancer interactions that cannot be detected in linear DNA.

Methods: The GenLink3D™ pipeline uses promoter-capture Hi-C to identify long-range interactions for all annotated promoters in a given cell type in a single experiment. Combining our advanced

algorithms and machine-learning capability allows us to understand the 3-dimensional organisation of the entire genome with high-resolution, enabling us to decode and exploit novel targets for disease diagnosis and treatment, with high throughput and precision.

Results: Our analyses show that hundreds of thousands of regulatory elements are active in each cell type, some of which are ubiquitous and others cell-type specific. These elements interact with their target promoters, driving cell-type-specific expression. Systematically connecting disease-specific variants to their target genes can enable the discovery of novel disease-susceptibility genes, illuminate new drug targets and allow patient stratification. Specifically, we demonstrate how this approach can be applied to find novel SNP-associated genes in lupus and MS, and in patient stratification for SNP risk load variation across ethnically distinct populations.

Conclusion: We aim to provide a universal discovery tool to unlock any genetic disease by identifying the right genes, in the right cell types, and to de-risk the drug discovery process.

Grants: None

Conflict of Interest: Nic Walker Employed full-time by Enhanc3D Genomics, Kishen Chahwala Employed full-time by Enhanc3D Genomics, Hazel Jones Employed full-time by Enhanc3D Genomics, Dan Turner Employed full-time by Enhanc3D Genomics, Stefan Schoenfelder Employed full-time by Enhanc3D Genomics, Peter Fraser Employed full-time by Enhanc3D Genomics

P21.072.C Let your data make more FAIR impact using MOLGENIS EMX2

Joeri van der Velde[1], *Esther van Enckevort*[1], *Gerieke Been*[1], *Eleanor Hyde*[1], *Morris Swertz*[1]

[1]*University Medical Center Groningen, Genomics Coordination Center, Groningen, Netherlands*

Consortium: The MOLGENIS Team

Background/Objectives: Your data in the life sciences can have far more impact when it is findable and (re)usable by a larger audience.

Methods: To achieve this, we have developed MOLGENIS EMX2, the 5th generation of the MOLGENIS open source scientific data platform with 100+ active installations powering a variety of life science web applications including catalogues, research portals, omics data and patient mutation registries. Importing and exporting of data is easy and achieved through Excel and CSV files. Advanced users adopt our dedicated Python client, export RDF, use GraphQL, or R.

Results: For humans, we have developed attractive data displays, dashboards, query screens, data entry forms and tools to manage fully flexible data structures and user permissions. For machines, interfaces such as FAIR Data Point and Beacon v2 can be registered at search portals to make the contents of these applications known to the wider world. Interoperability is driven by pre-defined best-practice semantic datamodel templates for biobank-, cohort- and harmonisation-catalogues, FAIR Genomes, JRC Common Data Elements, DCAT, Beacon v2, SolveRD-RD3, all based on popular resources including MIABIS, NCIT, HPO, ORDO and DUO. Datamodels are easily extended, e.g. there is a metabolomics add-on for X-Omics.

Conclusion: We are delighted to present EMX2, the next generation of the MOLGENIS FAIR data platform. MOLGENIS EMX2 is open-source (GNU LGPL v3.0) and available at https://github.com/molgenis/molgenis-emx2. Install it yourself or contact us for hosting options.

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Conflict of Interest: None declared

P21.074.A Deciphering the epigenetics behind different clinical disorders associated with Xq27.1 duplications involving *SOX3* using Nanopore long read sequencing.

Mathilde Filser[1], **Nicolas Rive Le Gouard**[1], *Capucine Rossi*[1], *Victor Gravrand*[1], *Sandra Chantot-Bastaraud*[1], *Aurelie Waernessyckle*[2], *Euphrasie Servant*[2], *Boris Keren*[2], *Marie Legendre*[3], *Laurence Cuisset*[4], *Valerie Koubi*[5;6], *Catherine Vincent-Delorme*[7], *Jean-Pierre Siffroi*[1], *Laïla El Khattabi*[1;8]

[1]*Chromosomic Genomic Fonctional Unit, Medical Genetics Department, Armand Trousseau Hospital, AP-HP Sorbonne University, Paris, France;* [2]*Development Genomics Fonctional Unit, Medical Genetics Department, Pitié-Salpêtrière Hospital, AP-HP Sorbonne University, Paris, France;* [3]*Molecular Genetics Fonctional Unit, Medical Genetics Department, Armand Trousseau Hospital, AP-HP Sorbonne University, Paris, France;* [4]*Medical Genomic Unit of Systemic and Organic Diseases, Cochin Hospital, AP-HP.Centre, Paris Cité University, Paris, France;* [5]*Genetics Unit, Eylau-Unilabs Laboratories, Neuilly-sur-Seine, France;* [6]*Genetics Unit, CHRU de Tours, Tours, France;* [7]*Genetics Unit, Guy Fontaine Clinic, Jeanne de Flandre Hospital, Lille, France;* [8]*« Brain Development » Team, ICM Brain Institute – Pitié-Salpêtrière, Paris, France*

Introduction: *SOX3* (SRY-related HMG box-containing gene 3) is a SOX family gene located in Xq27.1. *SOX3* duplications are usually associated with panhypopituitarism for 46,XY individuals and variations in genital development for 46,XX individuals but also unaffected females. The molecular mechanism behind this variability in 46,XX individuals may be related to X inactivation bias but this has not been demonstrated.

Methods: We report three patients carrying *SOX3* duplications detected by gene panel sequencing or DNA microarray, one of whom was further investigated by long-read sequencing using Oxford Nanopore following an adaptive sampling targeted enrichment protocol. X inactivation was studied using a methyl-sensitive PCR.

Results: Individual 1 was referred for growth retardation with bilateral cryptorchidism and micropenis. His karyotype revealed a 46,XY formula and gene panel analysis revealed a 1.2 Mb duplication involving *SOX3* which was inherited from his unaffected mother (individual 2). X inactivation study showed no bias (49%/51%).

Individual 3 was referred for azoospermia. Karyotyping and FISH analysis revealed a 46,XX.ish Yp11.2(SRYx0) chromosomal formula. DNA microarray detected a 1.2 Mb *SOX3* duplication with similar breakpoints. X-inactivation was significantly biased (90%/10%). Nanopore sequencing showed that the normal allele was hypermethylated and uncovered a more complex rearrangement.

Discussion: X inactivation study, when performed, does not provide allele-specific information. Using Nanopore technology long-read sequencing, we were able to phase methylation and structural variation. This allowed us to demonstrate that *SOX3* duplication in females leads to a genital development disorder when the duplicated allele is predominantly hypomethylated while unbiased methylation prevents from this disorder.

Conflict of Interest: None declared

P22

TREATMENTS FOR GENETIC DISORDERS

P22.001.B Real-world safety and effectiveness of vosoritide: Results from an early access program in France

Valérie Cormier-Daire[1], *Thomas Edouard*[2], *Bertrand Isidor*[3], *Shelda Cohen*[4], *Swati Mukherjee*[4], *Jeanne Pimenta*[4], *Leila Lhaneche*[5],

Massimiliano Rossi[6], Elise Schaefer[7], Erin Goodman[8], Sabine Sigaudy[9], Genevieve Baujat[1]

[1]Paris cité university, Necker hospital, Imagine institute, Centre of Reference for Constitutional Bone Diseases (MOC), Paris, France; [2]Toulouse University Hospital, Endocrine, Bone Diseases and Genetics Unit, Toulouse, France; [3]Centre Hospitalier Universitaire de Nantes, Genetics, Nantes, France; [4]BioMarin (UK) Limited, London, United Kingdom; [5]BioMarin (FR) Limited, Paris, France; [6]Hospices Civils de Lyon, Service de génétique, Lyon, France; [7]CHU de Strasbourg - Hôpital de Hautepierre, Genetics, Strasbourg, France; [8]BioMarin Pharmaceutical Inc, Novato, United States; [9]Hôpital Timone Enfant, Département de Génétique Médicale, Marseille, France

Background/Objectives: Achondroplasia is the most common skeletal dysplasia with short stature. Vosoritide is currently approved in Europe for patients aged ≥ 4 months until closure of epiphyses. We report the experience of early access to vosoritide in France, led by the constitutional bone diseases (MOC) network.

Methods: In 2021, early access to vosoritide was initiated in patients aged ≥5 years. The national center of MOC network (Necker hospital) and five referral centers assessed patients for treatment and follow-up at month 1, 3, 6 and every 6 months. Data on patient characteristics, treatment compliance, safety and effectiveness (height, CDC height Z-score, annualized growth velocity (AGV) are reported.

Results: Sixty-two patients were enrolled, of whom 57 started early access treatment with vosoritide by December 2022. The mean age at treatment start was 8.6 years (min-max: 5-13 years) and 51% were male. Mean (range) exposure to treatment was 277 (32–443) days.

After 6 months treatment, the mean (SD) AGV was 5.9 (1.65) in males (n = 20) and 6.5 (2.64) cm/year in females (n = 16).

Among 22 patients who completed 12 months treatment (10 males, 12 females), mean (SD) height increase from baseline was 5.8 (1.71) cm and 6.5 (1.28) cm with a Z-score (SD) improvement of 0.3 (0.28) and 0.4 (0.37), respectively.

In total, 21 adverse events were reported, most of them mild (injection site reactions and vomiting).

Conclusions: Patients with achondroplasia under real-world conditions showed a safety and effectiveness profile consistent with vosoritide clinical trials.

Grants: The study was sponsored by BioMarin.

Conflict of Interest: Valérie Cormier-Daire Biomarin, Thomas Edouard: None declared, Bertrand Isidor: None declared, Shelda Cohen: None declared, Swati Mukherjee: None declared, Jeanne Pimenta: None declared, Leila Lhaneche: None declared, Massimiliano Rossi: None declared, Elise Schaefer: None declared, Erin Goodman: None declared, Sabine Sigaudy: None declared, Genevieve Baujat: None declared

P22.004.A Using odevixibat to treat recurrent cholestasis in a patient with bi-allelic UNC45A mutations causing osteo-oto-hepato-enteric syndrome

Floris Imhann[1], *Chantal Malcolm[2], Zhe Zhou[3], Henkjan J. Verkade[4], Sven van IJzendoorn[3], Hans Blokziji[2]*

[1]University Medical Center Groningen, Department of Genetics; [2]University Medical Center Groningen, Department of Gastroenterology and Hepatology; [3]University Medical Center Groningen, Department of Biomedical Sciences; [4]University Medical Center Groningen, Department of Pediatrics

Background/Objectives: Osteo-oto-hepatic-enteric syndrome (O2HE) is a rare genetic syndrome leading to neonatal cholestasis, congenital diarrhea, sensorineural hearing loss and/or bone fragility. Osteo-oto-hepatic-enteric syndrome is caused by bi-allelic mutations in the gene Unc-45 Myosin Chaperone A (UNC45A), which leads to a reduction in Myosin Vb (encoded by the MYO5B gene). MYO5B mutations can lead to progressive familial intrahepatic cholestasis type 10 (PFIC10). Therefore, we suspected a similar disease mechanism.

In this case report, we describe trying the novel PFIC drug odevixibat for the first time in an O2HE patient with episodes of severe cholestasis and pruritis.

Methods: The treating gastroenterologist, a paediatric gastroenterologist and the clinical geneticist in training diagnosed the patient and performed a brief literature study to investigate whether new drugs could work.

Results: A 37-year old man, with episodes of diarrhea and cholestasis during his entire life was seen at our out-patient clinic. Whole Exome Sequencing showed c.292C>T p.(Arg98Trp) and c.592C>T p.(Gln198*) mutations in UNC45, after which O2HE was diagnosed. After the literature study, the ileal bile acid transporter (IBAT) inhibitor odevixibat, which interrupts the entero-hepatic cycle of bile acids was tried. The patient's pruritis quickly disappeared and liver enzyme, bilirubin and blood bile acid levels all decreased rapidly. The patient remains without cholestasis after a one year follow-up.

Conclusion: Here, we present the first case of O2HE with cholestasis that has been successfully treated with odevixibat. We recommend that the UNC45A gene be added to cholestasis WES panels. When O2HE is diagnosed, odevixibat can be considered for cholestasis treatment.

Grants: None

Conflict of Interest: None declared

P22.005.B Finding a therapeutic molecule for ADLD: keep calm and never give up!

Mariia Zadorozhna[1;2], *Emanuela Pesce[3], Anna Basile[2], Erica Bascetta[2], Ilaria Cani[4;5], Foteini-Dionysia Koufi[6], Stefano Ratti[4], Pietro Cortelli[4;5], Nicoletta Pedemonte[3], Elisa Giorgio[1;2]*

[1]IRCCS Mondino Foundation, Neurogenetics Research Centre, Pavia; [2]University of Pavia, Department of Molecular Medicine, Pavia; [3]IRCCS Istituto Giannina Gaslini, UOC Genetica Medica, Genova, Italy; [4]Università di Bologna, Dipartimento di Scienze Biomediche e Neuromotorie, Bologna, Italy; [5]IRCCS Istituto delle Scienze Neurologiche di Bologna, Bologna, Italy; [6]University of Bologna, Department of Biomedical and NeuroMotor Sciences (DIBINEM), Cellular Signalling Laboratory, Bologna, Italy

Background/Objectives: Autosomal Dominant adult-onset LeukoDystrophy (ADLD) is a slowly progressive disease with CNS demyelination caused by Lamin B1 (LMNB1) overexpression due to gene duplications/noncoding deletions located upstream the *LMNB1* gene. Currently, there is no effective therapy to cure or slow down the progression of ADLD. The reduction of LMNB1 protein represents a promising strategy for treating ADLD. Drug-repositioning approach, using a dual-reporter stable CHO cell line, allowed our group to identify Alvespimycin (Alv) as positive hit. Alv is a HSP90 inhibitor under investigation for solid tumor treatment. Unfortunately, it has not yet been approved by FDA or EMA, limiting its exploitation for ADLD. We aim at testing FDA-approved Alv analogues as ready-to-use therapeutic solutions for our patients.

Methods/Results: We selected nine Alv functional analogs with different properties (targeting the carboxy-terminal domain; deriving from natural compounds; or synthetic molecules) and tested their efficacy in reducing LMNB1 protein levels by western blotting on ADLD fibroblasts. Unfortunately, none of these molecules was able to mimic Alv effect on LMNB1. To maximize the identification of other effective drugs for ADLD, we decided to develop a disease-relevant dual-reporter cell line, exploiting the

immortalized human oligodendrocyte line MO3.13, resembling the most affected cell type in ADLD.

Conclusion: Alv-mediated LMNB1 modulation was not confirmed by other Hsp90 inhibitors tested, suggesting a molecule-specific effect. However, the creation of an innovative disease-relevant cellular model will increase the chance of identifying effective molecules by drug-repositioning, paving the way for developing an effective ADLD treatment.

Grants: "Ricerca Finalizzata GR-2021-12373348" from the Italian Ministry of Health to EG.

Conflict of Interest: None declared

P22.006.C Growth hormone treatment in children with KBG syndrome: novel insights, challenges and recommendations from 6 novel patients and literature review

Sietse Aukema[1], *Kim Vandenput*[2], *Emanuela Scarano*[3], *Himanshu Goel*[4,5], *Lily Guo*[6], *Michiel Vanneste*[7], *Koenraad Devriendt*[7], *Nitash Zwaveling-Soonawala*[8], *Cordula Kiewert*[9], *Alma Küchler*[10], *Ilaria Parenti*[10], *Siddharth Banka*[11,12], *Elizabeth Wall*[13], *Gholson Lyon*[14,15,16], *Karen Low*[17], *Joyce Geelen*[18], *Yvonne van der Zwan*[19,20], *Charlotte Ockeloen*[1]

[1]*Radboud University Medical Center, Department of Human Genetics, Nijmegen, Netherlands;* [2]*Radboud University Medical Center, Nijmegen;* [3]*Universitaria di Bologna, Pediatrics Unit, IRCCS Azienda Ospedaliero, Bologna, Italy;* [4]*Hunter Genetics, Waratah, Australia;* [5]*University of Newcastle, Callaghan, Australia;* [6]*Chicago Medical School, Chigago, United States;* [7]*University Hospitals Leuven, KU Leuven, Center for Human Genetics, Leuven, Belgium;* [8]*Emma Children's Hospital, Amsterdam UMC, Department of Pediatric Endocrinology, Amsterdam, Netherlands;* [9]*University Hospital Essen, University of Duisburg-Essen, Division of Paediatric Endocrinology and Diabetes, Essen, Germany;* [10]*Institut für Humangenetik, Universität Duisburg-Essen, Essen, Germany;* [11]*University of Manchester, Manchester, United Kingdom;* [12]*St Mary's Hospital, Manchester Centre for Genomic Medicine, Manchester, United Kingdom;* [13]*West Midlands Regional Genetics Laboratory, Central and South Genomic Laboratory Hub, Birmingham, United Kingdom;* [14]*New York State Institute for Basic Research in Developmental Disabilities, Department of Human Genetics, New York, United States;* [15]*New York State Institute for Basic Research in Developmental Disabilities, George A. Jervis Clinic, New York, United States;* [16]*The City University of New York, Biology PhD Program, The Graduate Center, New York, United States;* [17]*University Hospital Bristol and Weston NHS Foundation Trust, Department of Clinical Genetics, Bristol, United Kingdom;* [18]*Radboud University Medical Center, Department of Pediatrics, Amalia Children's Hospital, Nijmegen, Netherlands;* [19]*Radboud University Medical Center, Department of Pediatric Endocrinology, Amalia Children's Hospital, Nijmegen, Netherlands;* [20]*Isala, Department of Paediatrics, Zwolle, Netherlands*

Background/Objectives: KBG syndrome is a neurodevelopmental disorder caused by pathogenic *ANKRD11* variants. (Postnatal) short stature is a major feature observed in 10-60% of patients. Recombinant human growth hormone (rhGH) treatment is available for patients with growth hormone deficiency (GHD). Additionally, rhGH is also effective in patients with syndromes associated with short stature including Prader-Willi, Noonan and Turner syndrome. For patients with KBG syndrome, there is little evidence available. However, it suggests that growth hormone (GH) treatment could be beneficial. Systematic analysis and detailed phenotyping are lacking.

Methods: MEDLINE/PubMed was searched for patients with KBG syndrome treated with GH. Relevant auxological-, endocrine and rhGH-treatment related data were collected from the article and/or corresponding author(s). In addition, unpublished patients were identified through our international network of collaborators.

Results: Literature review identified 22 patients with KBG syndrome who received rhGH treatment. Data of 6 novel patients was added to the retrospective cohort. Full GHD was observed only in a minority. Before start of rhGH treatment, the median height was -3.1 SDS and 88% had short stature. Patients who received treatment for at least one year showed a median height gain of 1.3 SDS and 72% of them reached a normal height at most recent measurement.

Conclusion: In this large cohort of 28 patients first analyses suggest a benefit of GH therapy for (a subset of) patients with KBG syndrome. Further (subgroup)analyses are ongoing.

Grants:

Conflict of Interest: None declared

P22.008.A Investigation of the Regulatory Role of Lysosomal Cathepsin A in the Autophagy

Selman Yanbul[1;1], *Volkan Seyrantepe*[1]

[1]*Izmir Institute of Technology, Molecular Biyology and Genetics, Izmir, Türkyie*

Background/Objectives: Lysosomal Cathepsin A is serine carboxypeptidase with catalytic functions and it complexes with Neu1 and ß-gal enzymes to protect them from hydrolytic degradation. Deficiency of Cathepsin A causes Galactosialidosis with secondary Neu1 and ß-Gal deficiencies. Previously, we generated a $CathA^{S190A}$ mouse which has a point mutation on the catalytic site to investigate its role in the processing of bioactive peptides. In this study, we aimed to elucidate whether Cathepsin A has a role in the regulation of autophagy in cell lines derived from $CathA^{S190A}$ mice.

Methods: Neuroglia and fibroblast cells were isolated from $CathA^{S190A}$ mice and an age-matched control group. The cells were cultured in the following conditions: (i) none, (ii) treated with 200 mM rapamycin for 1h, (iii) starvation medium for 2h, and (iv) starvation medium for 2h with 200 mM rapamycin treatment in last 1h. Autophagy markers were analyzed by qRT-PCR, Western Blot, and Immunocytochemistry.

Results: We showed alterations in *Beclin-1, Atg7, Atg9, p62*, and *Lamp2* gene expression levels in neuroglia and fibroblast cells from $CathA^{S190A}$ mice using qRT-PCR analysis. Western Blot and Immunocytochemistry analysis demonstrated increased levels of LC3-II, p62, and LAMP1 proteins in both cell lines derived from $CathA^{S190A}$ mice. Consistently, we showed accumulated LC3-II, p62, and LAMP1 protein levels significantly decreased after starvation with or without rapamycin treatment.

Conclusion: Overall, our results suggest that the unprocessed bioactive peptide accumulation due to catalytically inactive CathA in mice may contribute to the regulation of autophagic flux, and also starvation condition may induce reversion of dysregulated autophagy.

Conflict of Interest: None declared

P22.009.B Therapeutic Efficacy of Intrathecal Administration of AAVrh10-mHexa Vector in a Mouse Model of Tay-Sachs Disease

Melike Can Özgür[1], *Hatice Hande Basırlı*[1], *Jérôme Ausseil*[2], *Volkan Seyrantepe*[1,3]

[1]*Izmir Institute of Technology, Molecular Biology and Genetics, Izmir;* [2]*Université Paul Sabatier Toulouse, Centre de Physiopathologie de Toulouse Purpan, INSERM U1043, Toulouse, France;* [3]*Izmir Institute of Technology, IYTEDEHAM, Izmir*

Background/Objectives: Tay-Sachs disease, a lysosomal storage disorder, is caused by mutations in the *HEXA* gene. *HEXA* gene encodes for the α-subunit of the enzyme β-hexosaminidase A which degrades GM2 ganglioside in the brain and peripheral nervous system. The loss of the β-hexosaminidase A activity causes pathological accumulation of GM2 ganglioside in patients. Interestingly, disruption of HEXA leads to a mild phenotype in Hexa-deficient mice. Our group generated *Hexa-/-Neu3-/-* mice which showed severe neuropathological indications and survived until 20 weeks of age. Recently, Adeno-associated virus (AAV)-based gene therapy has been shown as an efficient delivery system for human studies. AAVrh10 is especially used in AAV-based therapeutics to target the central nervous system. Although there are several therapeutic approaches for lysosomal storage disorders, there is no cure for TSD. Here, we aimed to investigate the therapeutic efficacy of the AAVrh10-mHexa expressing mouse HEXA in the *Hexa-/-Neu3-/-* mice.

Methods: Molecular biological, immunohistochemical, and behavioral analyses were used to study the therapeutic efficacy of the AAVrh10-mHexa in the *Hexa-/-Neu3-/-* mice.

Results: We discovered that the life span of *Hexa-/-Neu3-/-* mice extended up to 28 weeks of age after intrathecal administration of AAVrh10-mHexa. Our data also revealed the clearance of accumulating GM2 ganglioside, increased Hexa enzyme activity, and decreased neuroinflammation in the cortex of *Hexa-/-Neu3-/-* mice. Consistently, motor activity was also improved in AAVrh10-mHexa-administered *Hexa-/-Neu3-/-* mice.

Conclusion: Our data suggest that AAVrh10-based intrathecal administration is a promising therapeutic strategy for the treatment of Tay-Sachs disease.

Grants: This study was funded by TUBITAK-Bosphorus (Grant No: 120N552).

Conflict of Interest: None declared

P22.010.C Human Neu3 Sialidase Reduces GM2 Ganglioside Accumulation in Neuroglia Cells of Tay-Sachs Disease Mice Model

Hatice Hande Basırlı[1], *Melike Can Özgür*[1], *Volkan Seyrantepe*[1;2]

[1]*Izmir Institute of Techology, Molecular Biology and Genetics, Izmir, Türkyie;* [2]*Izmir Institute of Techology, IYTEDEHAM, Izmir, Türkyie*

Background/Objectives: Tay-Sachs disease, a type of GM2 gangliosidoses, is a severe neurodegenerative disorder caused by a mutation in the HEXA gene encoding α subunit of β-hexosaminidase A enzyme. HexA enzyme is responsible for the degradation of GM2 ganglioside. The deficiency of the HexA enzyme causes the excessive accumulation of GM2 ganglioside resulting in progressive neurodegeneration in Tay-Sachs patients. Surprisingly, *Hexa-/-* mice showed mild neuropathological and clinical signs. We recently generated *Hexa-/-Neu3-/-* mice as an early onset Tay-Sachs disease mouse model that mimics neuropathological abnormalities in patients. Our group also showed impaired autophagic flux in the brains of *Hexa-/-Neu3-/-* mice. Although the murine Neu3 sialidase was shown to facilitate GM2 degradation with a bypass mechanism in *Hexa-/-* mice, the contribution of human Neu3 in GM2 degradation remains unclear. Here, we aimed to elucidate the efficacy of human Neu3-mediated gene therapy using AAVrh10-hNeu3 in neuroglia cells.

Methods: We evaluated the therapeutic effectiveness of AAVrh10-hNeu3 expression in neuroglia cells of 5-month-old *Hexa-/-Neu3-/-* mice using molecular biological and immunocytochemical analyses.

Results: We found significant clearance of accumulating GM2 ganglioside in AAVrh10-hNeu3 treated neuroglia cells of 5-month-old *Hexa-/-Neu3-/-* mice. Our data also showed the altered autophagic flux in neuroglia cells after AAVrh10-hNeu3 treatment.

Conclusion: Our data suggest that human Neu3 might be a potential therapeutic strategy to reduce GM2 ganglioside accumulation in Tay-Sachs disease.

Grants: This study was funded by TUBITAK-Bosphorus (Grant No: 120N552).

Conflict of Interest: None declared

P22.011.D Allele-Specific Antisense Oligonucleotides for the Treatment of BEST1-Related Dominantly Inherited Retinal Diseases: An In Vitro Model

Beren Karaosmanoglu[1], *gozde imren*[1], *Gulen Eda Utine*[2], *Hande Taylan Sekeroglu*[3], *Ekim Taskiran*[1]

[1]*Hacettepe University Faculty of Medicine, Medical Genetics, Ankara, Türkyie;* [2]*Hacettepe University Faculty of Medicine, Department of Pediatrics, Department of Pediatric Genetics, Ankara, Türkyie;* [3]*Hacettepe University Faculty of Medicine, Department of Ophthalmology, Ankara, Türkyie*

Background/Objectives: Inherited retinal diseases (IRDs) are a common and incurable health problem characterized by progressive vision loss caused by photoreceptor cell (PRC) dysfunction. Anti-sense oligonucleotides (ASOs), which manipulates unwanted mRNAs, have been clinically deployed for the treatment of several diseases. Effective treatments for a broad range of IRDs remain limited. The aim was to establish an in-vitro model utilizing induced PRCs for assessing the efficacy and safety of ASOs targeting *BEST1*, and to develop an in-vitro model for evaluating RNA-based therapeutics, for the treatment in IRDs.

Methods: A cell culture model was established by induction of PRCs from dermal fibroblasts via direct programming. A common single nucleotide polymorphism (SNP) was identified in *BEST1* (rs1800007) for targeting with GapmeR ASOs for multiple alleles of this SNP. The efficacy and possible off-target effects of these ASOs were analyzed in PRCs.

Results: The selected ASOs achieved allele-specific mRNA degradation with virtually no off-target effects on the global transcriptome profile. The presented in vitro model is a valuable platform for testing personalized IRD treatments and should inspire further research. To the best of our knowledge this study is the first to test RNA-based therapeutics involving the use of ASOs in an induced PRC model.

Conclusion: It will be possible to establish an ex-vivo disease model using dermal fibroblast samples from affected individuals. The disease model and ASOs, that are safe and effective therapeutic agents, can serve as a useful platform for the testing of personalized treatments for IRDs.

Grants: Supported by Hacettepe University (TSA-2021-19012).

Conflict of Interest: None declared

P22.012.A A comprehensive personal omics-based clinical interpreter based on genomic and transcriptomic profiles

Qingwang Chen[1], *Yaing Liu*[1], *Li Guo*[2], *Ying Yu*[1]

[1]*Fudan University, State Key Laboratory of Genetic Engineering, Human Phenome Institute, School of Life Sciences, and Shanghai Cancer Center, Shanghai, China;* [2]*Institute of Process Engineering, Chinese Academy of Sciences, State Key Laboratory of Multiphase Complex Systems, Beijing, China*

Background/Objectives: Precision medicine depends on the proper identification and interpretation of genomic variants to achieve personalized diagnosis, prognosis, and treatment

selection. However, to achieve precision medicine, one major challenge is lack of tools to integrate therapeutic alterations from various knowledgebases and provide interpretations based on personal multi-omics profiles.

Methods: We developed Personal Omics Interpreter (POI), a user-friendly web-based tool that jointly provides therapeutic interpretations, including targeted drugs, immunological drugs, and chemotherapeutic drugs, by leveraging personal genomic and transcriptomic profiles. Extensive validations were conducted using three large-cohort datasets consisting of over 22,000 cancer patients, including the Cancer Genome Atlas (TCGA) multi-cancer datasets, the MSK-IMPACT datasets, and a breast cancer dataset.

Results: The POI can provide comprehensive interpretation. First, POI is designed to accommodate multi-dimensional alterations as input, including somatic variants, germline variants and expression alterations. Secondly, POI utilizes the rich knowledge ofPrecision Medicine Knowledgebase (PreMedKB), an updated harmonized knowledgebase that we previously developed. Thirdly, POI uses a predictive algorithm to expand the therapeutic options based on gene-gene interactions. Fourthly, POI facilitates chemotherapy drug suggestions and phenotypic response predictions by resolving germline diplotypes through our previously developed tool *PAnno*. Based on validation using three large-cohort datasets, POI demonstrates a consistently high matching rate (94.7 ~ 95.6%) of patients and potential therapeutic interpretations.

Conclusion: POI provides comprehensive therapeutic interpretations at the personal genomics and transcriptomics level, which has the potential to assist researchers and clinicians in making informed treatment decisions. POI is available at https://premedkb.cn/poi/#/.

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Conflict of Interest: None declared

P22.013.B Preclinical evaluation in patient-derived cells of gene editing as a therapeutic approach for IQSEC2 syndrome

*Susanna Croci[1;2], **Alessandra Russo**[1;2], Sergio Daga[1;2], Sara Pastore[1;2], Francesca Mari[1;2;3], Maria Antonietta Mencarelli[3], Nicola Alessio[4], Sura Hilal Ahmed Al-Sammarraie[4], Anna Calarco[5], Gianfranco Peluso[5], Alessandra Renieri[1;2;3], Ilaria Meloni[1;2]*

[1]University of Siena, Medical Genetics, Italy; [2]University of Siena, Med Biotech Hub and Competence Center, Department of Medical Biotechnologies, Italy; [3]Azienda Ospedaliera Universitaria Senese, Genetica Medica, Siena, Italy; [4]Campania University, Experimental Medicine Department, Luigi Vanvitelli, Naples, Italy; [5]UniCamillus-Saint Camillus International University of Health Science, Rome, Italy

Background/Objectives: The X linked *IQSEC2* gene encodes a protein of 1488 amino acids located at the postsynaptic density of both excitatory and inhibitory neurons throughout the brain. The IQSEC2 protein plays a major role in controlling synaptic transmission, E/I balance and memory consolidation. Pathological mutations in *IQSEC2* account for ~2-5% of all children presenting with frequent seizures, severe intellectual disability and autism spectrum disorder. Currently there are no treatments for IQSEC2 syndrome and gene editing represents an extremely promising therapeutic approach since it allows maintaining the endogenous regulatory framework. To develop an innovative therapeutic approach, we successfully tested the efficacy of gene editing with CRISPR/Cas9 for the correction of *IQSEC2* mutations directly in neurons.

Methods: In collaboration with the Italian patient association AMA.le we collected several patient-specific cells used to generate iPSCs and neurons. We have engineered a two-plasmid system to correct two mutations located on the *IQSEC2* gene: c.3613_3613delC (p.(Leu1205Trpfd*92)) and c.4110_4111del (p.(Tyr1371Glnfs*15)). Mutation-specific sgRNAs and donor DNAs have been selected and cloned together with a GFP reporter system. Cas9 flanked by sgRNA recognition sequences for auto-cleaving has been cloned in a second plasmid.

Results and Conclusion: results analyzed by Next Generation Sequencing have shown efficient gene editing based on Homology-Directed Repair (HDR) in fibroblasts and neurons with up to 35% of corrected allele, outlining the relevant potentiality of the approach for this syndrome.

Grant: NA

Conflict of Interest: None declared

P22.014.C Trametinib's efficacy in bone involvement in two pediatric cases with severe oculoectodermal syndrome associated with KRAS mosaic mutations

Marie Vincent[1;2], *Soizic Tiriau[3], Marine Fouillet Desjonqueres[4], Cyrille Decante[5], Sébastien Barbarot[6], Pierre Corre[7], Nadir Benbrik[8], Guylene Le Meur[9], Bertrand Isidor[1], Solène Conrad[1], Genevieve Baujat[10], Pierre Vabres[11], Aymeric Rouchaud[4], Alice Fassier[12], Wassim Ouchetto[13], Alicia Besson[13], Marion Delous[13], Massimiliano Rossi[13;14]*

[1]CHU de Nantes, medical genetics, Nantes, France; [2]Institut du Thorax, INSERM, CNRS, UNIV Nantes, CHU de Nantes, Nantes, France; [3]CHU de Nantes, pediatrics, Nantes, France; [4]Hospices civils de Lyon, pediatrics, Lyon, France; [5]CHU de Nantes, pediatric surgery, Nantes, France; [6]CHU de Nantes, dermatology, Nantes, France; [7]CHU de Nantes, maxillofacial surgery, Nantes, France; [8]CHU de Nantes, pediatrics cardiology, Nantes, France; [9]CHU de Nantes, ophtalmology, Nantes, France; [10]Hôpital Necker enfants malades, medical genetics, Paris, France; [11]CHU Dijon Bourgogne, dermatology, Dijon, France; [12]Hospices civils de Lyon, radiology, Lyon, France; [13]University Lyon, INSERM U1028, CNRS UMR5292, Lyon Neuroscience Research Centre, GENDEV Team, Claude Bernard Lyon 1 University, Bron, France; [14]Hospices civils de Lyon, medical genetics, Lyon, France

Background/Objectives: Oculoectodermal syndrome (OES) results from somatic mosaic mutations in the KRAS proto-oncogene, with clinical manifestations affecting ocular, dermatological, cardiovascular, and neurological aspects. Bone involvement typically manifests around age 5, presenting as non-ossifying fibromas and giant cell granulomas.

Trametinib, a MEK protein inhibitor routinely used in oncology, has demonstrated efficacy in certain patients with RASopathies, notably on lymphatic and arteriovenous malformations, cardiac involvement, and dermatological lesions.

Methods: We present two 8-year-old children with severe OES, experiencing recurrent long bone fractures leading to significant functional impairment. Skin biopsies revealed KRAS variants (c.38G>A, p.Gly13Asp; c.436G>A, p.Ala146Thr) in 25% of fibroblasts from fresh skin biopsies.

To evaluate the impact of these mutations on KRAS function, we conducted transient KRAS overexpressions in HEK293 cells.

Results: Experiments revealed that the mutated versions induce hyperphosphorylation of the Erk1/2 protein, a phenomenon alleviated by treatment with Trametinib.

Based on isolated reported observations and considering the severity of these two patients, we initiated trametinib at 0.02 mg/kg/day around age 6, resulting in significant clinical improvement within six months. No fracture recurrences were noted, ambulation was restored, and notable bone remineralization occured. Side effects included skin-related issues (acne-like lesions, delayed wound healing, xerosis), managed with topical emollients.

Conclusion: In conclusion, trametinib proved well-tolerated and effective for severe OES bone involvement in these two

children. A therapeutic trial is warranted for confirmation in a larger patient cohort.

Grants: No

Conflict of Interest: None declared

P22.015.D The antioxidant L-Ergothioneine as a preventive treatment and biomarker for cystine stones

Clara Mayayo-Vallverdú[1;2], *Esther Prat[1;2], Marta Vecino-Pérez[1], Miguel Lopez de Heredia[3], Virginia Nunes[1;2]*

[1]IDIBELL Institut d'Investigació Biomèdica de Bellvitge, Human Molecular Genetics, L'Hospitalet de Llobregat, Spain; [2]Universitat de Barcelona - Campus Bellvitge, Physiological Sciences, L'Hospitalet de Llobregat, Spain; [3]Instituto de Salud Carlos III, CIBERER, Madrid, Spain

Background/Objectives: Cystinuria is a rare inherited disease caused by mutations in *SLC3A1* and *SLC7A9* genes, which encode the $b^{0,+}$AT transport system. Its main clinical manifestation is the presence of cystine stones in the urinary system. Current therapeutic and pharmacological approaches are not effective for all patients and/or have side effects. Therefore, we evaluated L-Ergothioneine as a new potential treatment to prevent cystine stones.

Methods: Two different doses of L-Ergothioneine were administered preventively and chronically to the *Slc7a9*$^{-/-}$ mouse model of cystinuria. During treatment, stone occurrence and growth were assessed by X-ray imaging, and metabolic parameters were obtained using metabolic cages. L-Ergothioneine metabolism, oxidative status and mitochondrial function were also evaluated at the renal level.

Results: Both treatments led to an above 50% decrease in the number of mice that developed stones, as well as a delay in their onset. L-Ergothioneine did not affect urinary cystine levels, but increased its solubility by 50%, preventing its precipitation. At the renal level, we detected an improvement in oxidative status and a recovery of mitochondrial function. Furthermore, we observed that the urinary ratio between L-Ergothioneine and its metabolite S-Methyl-L-Ergothioneine allows us to distinguish between stone-former and non-stone-former mice, suggesting its potential as a biomarker.

Conclusion: These results, together with the FDA's consideration of L-Ergothioneine as a safe compound at doses higher than those tested, support the need for a clinical trial to test L-Ergothioneine as a new potential treatment for cystinuria and to validate its role as a biomarker.

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Conflict of Interest: Clara Mayayo-Vallverdú: None declared, Esther Prat: None declared, Marta Vecino-Pérez: None declared, Miguel Lopez de Heredia: None declared, Virginia Nunes AVEROA SAS

P22.016.A Safety of Reduced Infusion Time of Agalsidase Beta in the Treatment of Fabry Disease: An Observational Study

Dominique P. GERMAIN[1;2], *Alice Porto Vasconcelos[2], Hakima Dahmani[2], Najya Bedreddine[3], Wenting Trang[2], Ye Fan[2], Thomas Ghafari[2]*

[1]University of Versailles, Division of Medical Genetics, Montigny-Le-Bretonneux, France; [2]APHP, French Referral Center for Fabry disease, Garches, France; [3]Association des Patients de la Maladie de Fabry (APMF), Perrigny-lès-Dijon, France

Background/Objectives: Fabry disease is an X-linked inborn error of metabolism caused by lysosomal alpha-galactosidase deficiency. Enzyme Replacement Therapy (ERT) with agalsidase beta was approved in 2001 and has demonstrated clinical benefits. The conventional lifelong biweekly 3-hour long infusions regimen is associated with a significant burden on patients' quality of life, but very few studies have explored the optimal duration of agalsidase beta infusions.

Methods: The safety of a 90-minute infusion time (mentioned in the Summary of Product Characteristics) of agalsidase beta without premedication was studied in 35 consecutive adult patients (18 males, 17 females) with Fabry disease (26 different GLA pathogenic variants) for a total of 72 infusions. The study population included patients naïve to the enzyme ($n = 6$) and with significant comorbidities (17/35). Upon their request and after informed consent, 9 patients were infused in 60-80 minutes. Physical examination was performed and vital signs were measured before and after each infusion.

Results: Agalsidase beta was infused without premedication at a constant rate between 0.78 and 1.17mg/min in all 72 infusions. Five patients received 105mg of agalsidase in 90 minutes (1.17mg/min). None of the 35 patients complained of any discomfort at any time, no single adverse effect was reported and the vital signs did not significantly change in any patient during any infusion.

Conclusion: The infusion of agalsidase beta in 90 minutes or less in 35 adult patients with Fabry disease for 72 infusions was well-tolerated, with no adverse effects. The optimized regimen reduces the burden of biweekly infusions and improves patients' quality of life.

Conflict of Interest: None declared

P22.017.B Ketogenic diet therapies for drug-resistant epilepsy might affect ion channels activity through the combination of both epigenetic changes and splicing events

Alessia Mauri[1;2], *Letizia Messa[2;3], Roberto Previtali[1;4], Davide Biganzoli[2], Clarissa Berardo[1;2], Sara Olivotto[4], Maria Cristina Visceglia[2], Andrea Meta[2], Alessandra Vasco[2], Stephana Carelli[2], Pierangelo Veggiotti[1;4], Cristina Cereda[2]*

[1]University of Milan, Department of Biomedical and Clinical Sciences, Milan, Italy; [2]Buzzi Children's Hospital, Center of Functional Genomics and Rare Diseases, Milan, Italy; [3]Politecnico di Milano, Department of Electronics, Information and Bioengineering, Mllan, Italy; [4]Buzzi Children's Hospital, Pediatric Neurology Unit, Milan, Italy

Background/Objectives: Increasing evidence suggests that epigenetic modifications are associated with ketogenic diet (KD) therapies. These are considered an effective treatment for drug-resistant epilepsy, such as GLUT1-deficiency syndrome (GLUT1-DS), whereas its underlying mechanisms remain elusive.

Confirmed the metabolic status, we aimed to explore the transcriptional dysregulations of two *SLC2A1*-mutated pediatric patients before and after KD treatment and to also assess alterations in the methylome profiling.

Methods: FIA-MS/MS platform was performed for the analysis of amino acids and acylcarnitines. Bulk RNA-sequencing was performed using Illumina Total RNA kit and sequenced on NS500. Differential expression analysis was conducted using DESeq.2 and gene set enrichment analysis (GSEA) was performed using ClusterProfiler. Analysis of alternative splicing and isoform switches was implemented using IsoformSwitchAnalyzeR. Methylation sequencing was performed on PromethION2 Solo and analysis was showed using Remora.

Results: Metabolite analysis has confirmed the ketosis status in two patients in diet (increase of C2 and C4OH). RNA-seq data highlighted a significant splicing change in GLUT1-DS children under KD treatment identifying the snRNA U1 up-regulated before the therapy. In addition to amino acids metabolism, translation and mitochondrial activity deregulated, when performing GSEA

with Reactome a strong epigenetic signature was observed: histones deacetylation and DNA methylation resulted down-regulated. Molecular function analysis showed a down-regulation in voltage-gated ion channel and passive transmembrane transporter activities. Moreover, analysis for 5mC and 5hmC profiles identified differentially methylated CpG sites before and after KD.

Conclusions: The observed epigenetic changes related to KD treatment might influence the alternative RNA processing of voltage-gated ion channels also by epigenetic splicing code.

Conflict of Interest: None declared

P22.018.C Development of a functional platform for the characterization of MCT8 mutants associated with Allan-Herndon-Dudley Syndrome and suitable to identify new pharmacological modulators by a drug repositioning approach

Cristina Pastorino[1], *Valeria Capurro*[1], *Valeria Tomati*[1], *Emanuela Pesce*[1], *Mariateresa Lena*[2], *Renata Bocciardi*[1;2], *Nicoletta Pedemonte*[1]

[1]*Istituto Giannina Gaslini, UOC Genetica Medica, Genova, Italy;* [2]*Università degli Studi di Genova, Department of Neurosciences, Rehabilitation, Ophthalmology, Genetics, Maternal and Child Health (DINOGMI), Genova, Italy*

Background/Objectives: Thyroid hormones (THs) are important in the development of the nervous system. Monocarboxylate transporter 8 (MCT8), encoded by the X-linked *SLC16A2* gene, transports THs across the blood-brain barrier. Hemizygous *SLC16A2* variants cause Allan-Herndon-Dudley Syndrome (AHDS), characterized by intellectual disability, spastic paraplegia and abnormal THs profile. Causative variants lead to MCT8 loss-of-function by affecting protein expression, maturation/trafficking and/or activity, through different mechanisms. Our work aims to design a functional platform to evaluate both wild-type and mutant MCT8 that can be applied for the identification of pharmacological modulators of this THs transporter.

Methods: We generated MDCK1 cell lines stably expressing wild-type and two MCT8 mutants. Fluorescent dyes were used to identify new substrates of MCT8 mimicking THs, suitable to monitor the MTC8-mediated transport by a high-throughput functional assay.

Results: We have obtained a *cell-based* functional assay exploiting the uptake of fluorescent dyes mimicking THs to evaluate the wild-type and the MCT8 mutants activity. Moreover, expression vectors used to generate the cell lines are tagged with an epitope suitable to evaluate subcellular localization and biochemical profile of MCT8 proteins.

Conclusion: We developed an innovative functional platform designed to characterize MTC8 mutants associated with AHDS. Our assays will be also applied for a high troughput screening to identify MCT8 modulators by a drug repositioning approach as a starting point for the development of possible targeted therapies for patients affected by AHDS, currently without effective therapeutic options.

Grants: Supported by the Italian Ministry of Health (grant SG-2021-12375569).

Conflict of Interest: None declared

P22.019.D A Novel Antisense Oligonucleotide for the Treatment of Early Onset *SCN2A* Developmental and Epileptic Encephalopathy: A First-in-Patient Report in a Preterm Infant with Refractory Status Epilepticus

Matias Wagner[1;2], *Claudia Nussbaum*[3], *Geza Berecki*[4], *Andreas W. Flemmer*[3], *Silvana Frizzo*[5], *Farina Heer*[2], *Florian Heinen*[2], *Robert Horton*[5], *William Motel*[5], *Brian Spar*[5], *Siegel Corinna*[6], *Christoph Hübener*[7], *Sophia Stoecklein*[8], *Marco Paolini*[8], *Martin Staudt*[9], *Moritz Tacke*[2], *Markus Wolff*[10], *Walid Fazeli*[11], *Steven Petrou*[5], *Marcio Souza*[5], *Ingo Borggräfe*[2]

[1]*Technical University of Munich, Institute of Human Genetics, Munich;* [2]*Ludwig Maxmilians University Hospital, Munich, Germany, Department of Pediatrics and Comprehensive Epilepsy Center, Munich;* [3]*Ludwig Maximilians University Hospital, 4Department of Neonatology, Munich;* [4]*The Florey Institute of Neuroscience and Mental Health, Ion Channels and Human Diseases Group, Parkville;* [5]*Praxis Precision Medicines, Boston;* [6]*Center of Human Genetics and Laboratory Diagnostics, Martinsried;* [7]*University Hospital, Ludwig Maximilian University, Department of Obstetrics and Gynecology, Munich;* [8]*University Hospital, Ludwig Maximilian University, Department of Radiology, Munich;* [9]*Department of Pediatric Palliative Care, Ludwig Maximilians University Hospital, Munich, Germany, Munich;* [10]*Klinik Lengg AG, Swiss Epilepsy Center, Zürich;* [11]*Children's Hospital, University Hospital Bonn, Department of Pediatric Neurology, Bonn*

Background: SCN2A-DEE is a severe disorder caused by gain-of-function (GoF) mutations in SCN2A encoding NaV1.2. Patients have a high risk of premature death and high burden of epileptic seizures, typically beginning within days of birth and difficult to control with anti-seizure medications (ASMs). Comorbidities include profound global developmental impairment as well as movement disorders, gastrointestinal symptoms, severe irritability, variable sleep problems and frequent hospitalization. Drug development efforts for early onset SCN2A-DEE have been scarce, however, preclinical findings suggest that gapmer antisense oligonucleotides (ASOs) that down regulate *SCN2A* expression may have potential to alter the disease course in patients.

Methods: A preterm infant was diagnosed prenatally with the *SCN2A* variant c.3986C>A p.(Ala1329Asp) and postnatally presented with status epilepticus. Standard of care treatment could not break the status epilepticus. Eligibility for PRAX-222 treatment was evaluated using in silico protein structural modeling and in vitro electrophysiology studies.

Results: Voltage clamp experiments confirmed structural modeling predictions that the p.Ala1329Asp variant interferes with binding of the inactivation motif that would lead to GoF via impaired inactivation and increased persistent current. Treatment with PRAX-222 was initiated when patient was 1 month and 2 weeks old, with 7 doses (Fig 4A) administered to date that were well-tolerated. Eight days after first administration, SE was interrupted intermittently and ultimately ceased following continued dosing. A >50% reduction in seizure frequency was observed during follow-up. Despite some neurodevelopmental progress, neurodevelopment was severely affected.

Conclusion: First-in-patient findings highlight the potential for PRAX-222 to be the first disease-modifying treatment for early onset GoF SCN2A-DEE.

Conflict of Interest: Matias Wagner: None declared, Claudia Nussbaum: None declared, Geza Berecki: None declared, Andreas W. Flemmer: None declared, Silvana Frizzo Employee of Praxis Precision Medicine, Farina Heer: None declared, Florian Heinen: None declared, Robert Horton Employee of Praxis Precision Medicine, William Motel Employee of Praxis Precision Medicine, Brian Spar Employee of Praxis Precision Medicine, Siegel Corinna: None declared, Christoph Hübener: None declared, Sophia Stoecklein: None declared, Marco Paolini: None declared, Martin Staudt: None declared, Moritz Tacke: None declared, Markus Wolff: None declared, Walid Fazeli: None declared, Steven Petrou Employee of Praxis Precision Medicine, Marcio Souza CEO of Praxis Precision Medicine, Ingo Borggräfe: None declared

P22.020.A Genetic newborn screening for treatable rare diseases: the Screen4Care project TREAT panel

Alessandra Ferlini[1], *Marianna Farnè*[1], *Fernanda Fortunato*[1], *Alice Margutti*[1], *Rita Selvatici*[1;2], *Antonio Novelli*[3], *Emanuele Agolini*[3], *Silvia Ottombrino*[3], *Enrico Bertini*[3], *Aldona Zygmunt*[4], *Michela Zuccolo*[5], *Markos Mihalatos*[5], *Matt Rodesch*[5], *Moshe Einhorn*[6], *Sergi Beltran*[7], *Leslie Matalonga*[7], *Stefaan Sansen*[8], *Christina Saier*[9], *Janbernd Kirschner*[9]

[1]*University of Ferrara, Unit of Medical Genetics, Dept Medical Sciences, Ferrara, Italy;* [2]*Consorzio Futuro in Ricerca, Ferrara, Italy;* [3]*Bambino Gesù Pediatric Hospital, Medical Genetics, Roma, Italy;* [4]*Pfizer Inc., Collegeville, Pennsylvania, Collegeville, United States;* [5]*F. Hoffmann-La Roche AG, Basel, Switzerland;* [6]*Genoox, GENOOX LTD, Tel Aviv-Yafo, Israel;* [7]*Centro Nacional de Análisis Genómico - CNAG, Centro Nacional de Análisis Genómico, Barcelona, Spain;* [8]*Sanofi Belgium, Sanofi, Diegem, Belgium, Machelen, Belgium;* [9]*University of Freiburg, Department of Neuropediatric and Muscle Disorders, Medical Center, Faculty of Medicine, University of Freiburg, Freiburg, Germany, Freiburg im Breisgau, Germany*

Consortium: Screen4Care EU-IHI Consortium

Background/Objectives: EU Screen4Care has designed a NGS gene panel (TREAT panel) for genetic newborn screening (gNBS) in up to 25.000 neonates in Italy, Germany, France, and Greece for early identification of rare diseases (RDs) with approved treatments.

Methods: The criteria and scoring adopted include mendelian diseases, natural history knowledge, clinical utility, amenability of NGS, and treatment. Both small and copy number variants will be called.

Results: We selected by cut off-narrowed scores 245 genes, which are associated with the following phenomics: 106 metabolic, 33 blood and coagulation, 29 endocrine, 26 immunological, 25 neurological and neuromuscular, 9 renal, 6 mendelian syndromes, 4 cardiological, and 7 other phenotypes. Among them, 168 have autosomal recessive, 24 autosomal dominant, 20 X-linked, and 33 both AD and AR inheritance. In infants screened by TREAT panel but resulted negative and became symptomatic during the first year of life we offer WGS in a two-tier, post-NBS, enrolment approach. This will allow us to identify the 245 gene-related diseases and many other RDs that can be picked at the neonatal age by WGS, thus allowing to know the frequency of early symptomatic RDs escaping the TREAT panel detection. The 24 European Reference Networks (ERNs) will be involved for the diagnostic work-up, treatment referral, and follow-up.

Conclusion: S4C would propose and possibly translate its research output into the diverse European health systems for an equal EU gNBS approach (1).

1-Garnier et al., PLoS One. 2023 Nov 22;18(11):e0293503

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Conflict of Interest: Alessandra Ferlini Screen4Care EU-IHI grant Coordinator grant number No 101034427, Marianna Farnè: None declared, Fernanda Fortunato: None declared, Alice Margutti: None declared, Rita Selvatici: None declared, Antonio Novelli: None declared, Emanuele Agolini: None declared, Silvia Ottombrino: None declared, Enrico Bertini: None declared, Aldona Zygmunt Pfizer Ltd US, Michela Zuccolo F Hoffmann-La Roche, Basel, Switzerland, Markos Mihalatos F Hoffmann-La Roche, Basel, Switzerland, Matt Rodesch F Hoffmann-La Roche, Basel, Switzerland, Moshe Einhorn GENOOX TEL AVIV, Sergi Beltran: None declared, Leslie Matalonga: None declared, Stefaan Sansen SANOFI GENZYME BELGIUM, Christina Saier: None declared, Janbernd Kirschner: None declared

P22.021.B Genotyping in paediatric vascular anomalies: the impact of molecular diagnosis on clinical practice

Sinead O'Sullivan[1], *Maria Shilova*[2], *Romi Das Gupta*[2], *Roy Kimble*[2], *Christopher Richmond*[1]

[1]*Genetic Health Queensland, Herston, Australia;* [2]*Queensland Children's Hospital, South Brisbane, Australia*

Background/Objectives: Vascular anomalies (VA) are a heterogenous group of conditions characterised by abnormal growth and/or development of blood and/or lymphatic vessels. Despite the diverse nature of these lesions, there is considerable phenotypic overlap, complicating precise stratification and accurate diagnosis. Increasing uptake and availability of next generation sequencing (NGS) has the potential to transform the diagnosis and management of VA.

Methods: A 46-month retrospective analysis of paediatric patients offered targeted NGS for VA was undertaken. We measured the clinical utility of genotyping by comparing pre-test and post-test diagnostic and management outcomes.

DNA was extracted from biopsied vascular lesions for all patients (N = 242), with additional germline confirmatory testing arranged for those with a phenotype and variant allele frequency compatible with a heritable VA.

Results: A molecular diagnosis was secured for 183 of 242 patients tested (76%). A somatic cause for VA was confirmed for 70% and a germline cause for 6%. Genetic testing supported the pre-existing clinical diagnosis for 52% of all individuals tested and revised the clinical diagnosis for 24%. For patients with a revised clinical diagnosis, a management change occurred for 49%. Overall, 27% of patients offered genetic testing for VA had a resultant change of management, with 17% referred to haematology for active consideration of molecularly targeted therapy (MTT).

Conclusion: Increasingly, the clinical utility of genotyping this cohort is being recognised. Our large cohort study findings support the view that genotyping patients with VA aids clinical care.

Grants: nil

240 words

Conflict of Interest: None declared

P22.023.D Two new cases of adult onset pulmonary arterial hypertension (PAH)associated with TBX4 pathogenic variants. The importance of clinical exam and theunveiling of imatinib as a possibile drug inducing PAH.

Fabio Sirchia[1], *Anna Meroni*[1], *alessandra greco*[2], *Laura Scelsi*[2], *annalisa turco*[2], *sandra schirinzi*[2], *Stefano Ghio*[2], *mauro acquaro*[2], *davide colombo*[2], *simone carbonera*[1], *Enza Maria Valente*[1]

[1]*The University of Pavia, Human Genetics, Pavia, Italy;* [2]*Fondazione I.R.C.C.S. Policlinico San Matteo, Pavia, Italy*

Background/Objectives: Heterozygous pathogenic loss-of-function variants in TBX4 gene are associated with "ischio-coxo-podo-patellar syndrome with or without pulmonary arterial hypertension (PAH)". This syndrome is a rare autosomal dominant disorder characterized by hypo/aplasia of the patellae, various anomalies of the pelvis and feet and onset of PAH in the neonatal period or in childhood.

Methods: We describe two patients with rare adult-onset presentation of TBX4-related PAH. One patient is a 64-year-old woman diagnosed with GastroIntestinal Stromal Tumor (GIST) in 2019 for which she undertook imatinib. After 2 years of treatment the patient developed signs of pulmonary hypertension, precapillary phenotype. Right cardiac catheterization confirmed precapillary PAH and elevated pulmonary vascular resistance. The diagnosis was, therefore, drug-induced PAH. Imatinib therapy was suspended and endothelin receptor antagonist therapy was started. There were no improvements at the echocardiographic

and hemodynamic control at 4 months. Thus, a phosphodiesterase 5 inhibitor was added to the therapy and Whole-Exome Sequencing was performed.

Results: Genetic analysis showed a heterozygous pathogenic loss-of-function variant in TBX4 gene in both our patients. Further clinical and radiologic investigations detected in our patients a mild skeletal dysplasia confirming the diagnosis of ischio-coxo-podo-patellar syndrome.

Conclusion: We highlight the importance of pre-test clinical examination and reverse phenotyping in patients with PAH in order to look for signs of TBX4-related skeletal dysplasia. To date imatinib is still used as a potential Tyrosine-kinase inhibitor for therapeutic trials but we suggest that in a subset of patients (e.g. in genetic predispositions) it could lead to the onset or worsening of PAH instead.

Grants: none.

Conflict of Interest: None declared

P22.025.B Elesclomol copper (ES-Cu) treatment for patients with Menkes disease: a named patient program.

Stephanie Lotz-Esquivel[1;2], *Natalia Serrano[2;3], Elena Godoy-Molina[4], Rosa Marqués Perez-Bryan[5;6], Alba Hevia[7], Aquilina Jiménez González[8], Vishal Gohil[9], Michael Petris[10], Nina Horn[11], Víctor Mangas-Sanjuan[12;13], Inés Medina[14], Miquel Villaronga[15], Rosa Farré[15], Rubén Varela[8], Mónica Sáenz[7], Rafael Artuch[16], Cristina Jou[17], Jordi Pijuan[2], Janet Hoenicka[2], Francesc Palau[1;2]*

[1]Hospital Sant Joan de Déu, Genetics and Molecular Medicine Department, Barcelona, Spain; [2]Institut de Recerca Sant Joan de Déu, Neurogenetics and Molecular Medicine Laboratory (IPER), Barcelona, Spain; [3]Engrail Therapeutics, Inc., San Diego, United States; [4]Málaga Regional University Hospital, Complex Chronic Children and Palliative Care Unit, Department of Pediatrics, Malaga, Spain; [5]Dr. Miguel de Linares Prezzi Primary Health Care Centre, Malaga, Spain; [6]Dulce Nombre de Maria Psychopedagogic Institute, Málaga, Spain; [7]Leon University Assistance Complex, Pharmacy Department, León, Spain; [8]Leon University Assistance Complex, Pediatric Neurology Department, León, Spain; [9]Texas A&M University, Department of Biochemistry & Biophysics, College Station, United States; [10]University of Missouri, Department of Biochemistry, Columbia, United States; [11]The Kennedy Centre and Copenhagen University Hospital, Rigshospital, Department of Genetics, Copenhagen, Denmark; [12]University of Valencia, Department of Pharmacy, Pharmaceutical Technology and Parasitology, Valencia, Spain; [13]Interuniversity Research Institute for Molecular Recognition and Technological Development, Valencia, Spain; [14]Hospital Sant Joan de Déu, Department of Pediatric Neurology, Neuropsychology Unit, Barcelona, Spain; [15]Hospital Sant Joan de Déu, Pharmacy Department, Barcelona, Spain; [16]Hospital Sant Joan de Déu, Department of Biochemistry, Inborn Errors of Metabolism Section, Barcelona, Spain; [17]Hospital Sant Joan de Déu, Department of Pathology, Barcelona, Spain

Background/Objectives: Menkes disease (MD) is a rare genetic neurodegenerative disorder of copper metabolism caused by pathogenic variants in the X-linked *ATP7A* gene. Treatment is mainly supportive involving subcutaneous copper histidine (Cu-His) administration, which often does not prevent progression and death before third year of life. Preclinical studies have shown that elesclomol-copper (ES-Cu) prevents neurodegeneration and improves survival in the mottled-brindled mouse model of MD. We report 2 patients with MD, as we aim to repurpose ES-Cu to improve their clinical condition and modify the natural history of the disease.

Methods: Es-Cu was injected subcutaneously once a week, following a careful dose-escalating scheme developed by an international committee of experts. Daily Cu-His was administered subcutaneously as standard of care on the days ES-Cu was not administered. Safety parameters were strictly surveilled, and efficacy was monitored thoroughly. The AEMPS authorized this exceptional treatment.

Results: No systemic adverse effects presented during the treatment periods.

Conclusion: ES-Cu exceptional treatment has shown an adequate safety profile and promising results at a neurological level in patients affected by a rare disease, with repurposing of a drug based on scientific knowledge, demonstrated in a disease model, pending clinical trial.

Grants: Menkes International Association (MIa) and Ramón Areces Foundation.

Patient	MD1	MD2
Genetic variant	ATP7A:c.3556delG	ATP7A:c.2626+1G>A
ATP7A expression (mRNA)	Reduced	Reduced
ATP7A protein	Undetectable	Undetectable
Cu-His	250 mcg/day	300 mcg/day
ES-Cu	150 mcg/dose (24 months of treatment)	140 mcg/dose (12 months of treatment)
Plasma copper and ceruloplasmin levels	636 ug/L (620-1544) 240 mg/L (200-470) (07/11/2023)	108 mcg/dL (90-190) 28 mg/dL (20-60) (16/08/2023)
Bayley-III Scale	**Percentile**	**Percentile**
Cognitive	37	63
Receptive language	63	37
Expressive language	37	25
Fine Motor	50	25
Gross Motor	2	<1
	(20/10/2023)	(22/09/2022)
Hair microscopy	Pili torti (18/01/2023)	Melanin granules, incomplete torsions (14/09/2023)

Conflict of Interest: Stephanie Lotz-Esquivel: None declared, Natalia Serrano NLS has signed a consultancy agreement with Engrail Therapeutics, Inc. after finalizing one year of treatment of ES-Cu., Elena Godoy-Molina EGM is a consultant to Engrail Therapeutics, Inc., Rosa Marqués Perez-Bryan: None declared, Alba Hevia: None declared, Aquilina Jiménez González: None declared, Vishal Gohil VMG is a consultant to Engrail Therapeutics, Inc., Michael Petris: None declared, Nina Horn: None declared, Víctor Mangas-Sanjuan: None declared, Inés Medina: None declared, Miquel Villaronga: None declared, Rosa Farré: None declared, Rubén Varela: None declared, Mónica Sáenz: None declared, Rafael Artuch: None declared, Cristina Jou: None declared, Jordi Pijuan: None declared, Janet Hoenicka: None declared, Francesc Palau FP is a scientific and medical advisor of Engrail Therapeutics, Inc.

P22.026.C Towards Targeted Therapeutics for PEBEL2 (early-onset progressive encephalopathy with brain edema and/or leukoencephalopathy-2).

Nicole Van Bergen[1;2], Adhish Walvekar[3], Tim Sikora[2], Myrto Patraskaki[3], Henry Beetham[4], Alejandro Hidalgo-Gonzalez[4], Carole Linster[3], **John Christodoulou**[1;2]

[1]University of Melbourne, Department of Paediatrics, Melbourne, Australia; [2]Murdoch Children's Research Institute, Brain and Mitochondrial Research Group, Melbourne, Australia; [3]University of Luxembourg, Enzymology and Metabolism Group, Luxembourg Centre for Systems Biomedicine, Belvaux, Luxembourg; [4]Murdoch Children's Research Institute, Stafford Fox Drug Discovery Facility, Melbourne, Australia

Background/Objectives: PEBEL2 is a severe neurodegenerative disorder often provoked by intercurrent febrile illnesses, caused by biallelic loss of function variants in *NAXD*, encoding a key NADH metabolite repair enzyme. Anecdotal evidence suggests that niacin-based therapies may be of benefit. We aimed to expand our clinical understanding of this disorder, and to more precisely ascertain the pathways and processes impacted, with a view to identify novel therapeutic interventions.

Methods: We examined the clinical features of published PEBEL2 patients, aiming to draw potential phenotype-genotype correlations. We used cultured mammalian cells to develop reliable assays that would be amenable to high-throughput drug screening (HTS), with subsequent high-impact hits to be further validated in iPSC-derived neuron and animal models we have developed.

Results: We found distinct patterns in clinical presentations depending on which subcellular compartment is affected by NAXD deficiency. Patient fibroblast and HAP1 knockout cell studies revealed mitochondrial OXPHOS impairment, reduced cell viability and increased superoxide production, which were adapted to allow HTS of a large compound/drug library, which are currently under way. Metabolomic studies revealed abnormalities in serine metabolism, which were ameliorated by inosine supplementation.

Conclusion: Our deep phenotype-genotype studies have allowed more accurate prognostication, and our robust HTS assays have given us an opportunity to identify novel targeted therapies which will be further validated in our iPSC and zebrafish models. With early diagnosis and prompt introduction of these precision therapies, we envisage that the clinical course of this devastating disorder can be reversed.

Grants: Luxembourg National Research Fund, Australian Mito Foundation

Conflict of Interest: None declared

P22.027.D Treatment with a MEK Inhibitor in patients with hypertrophic cardiomyopathy in Noonan syndrome

Andrea Gazzin[1;2], *Federico Fornari[3], Anna Maria Villar[4], Chiara Riggi[4], Diana Carli[5], Simona Cardaropoli[6], Claudio Isella[7;8], Alfredo Brusco[9], Enzo Medico[7;8], Giovanni Battista Ferrero[10], Alessandro Mussa[2;6]*

[1]Department of Biomedical Sciences and Oncology, University of Turin, Turin, Italy; [2]Clinical Pediatric Genetics Unit, Regina Margherita Children's Hospital, Turin; [3]Postgraduate School of Pediatrics, University of Turin, Turin, Italy; [4]Cardiology Department, Regina Margherita Children's Hospital, Turin, Italy; [5]Department of Medical Sciences, University of Turin, Turin, Italy; [6]Department of Public Health and Pediatrics, University of Turin, Turin, Italy; [7]Laboratory of Oncogenomics, Candiolo Cancer Institute, FPO-IRCCS, Candiolo, Italy; [8]Department of Oncology, University of Turin, Turin, Italy; [9]Department of Neurosciences Rita Levi-Montalcini, University of Turin, Turin, Italy; [10]Department of Clinical and Biological Sciences, University of Turin, Orbassano, Italy

Background/Objectives: Hypertrophic cardiomyopathy (HCM) is the major contributor to morbidity and mortality in Noonan Syndrome (NS) and carries a high risk of heart failure and arrhythmias. In recent studies, MEK inhibitors have shown a potential effectiveness. This study aims to describe the monocentric experience of the off-label use of the MEK inhibitor Trametinib for treating NS-associated HCM.

Methods: Five patients (4F, 1M; 1.5 months-14.5 years) with NS who had HCM and a *RAF1* germline pathogenic variant received an oral dose of 0.001-0.025mg/kg/day for 1-15 months. Patients were evaluated at $0, +1, +3, +6, +12$ months to measure the thickness of the left ventricular walls, ventricular outflow obstruction, diastolic function, levels of NT-proBNP, changes in the gene expression in the RAS/MAPK pathway and any adverse events that occurred.

Results: All patients showed a decrease in NT-proBNP levels, and four had a significant reduction in ventricular outflow obstruction (> 20%) as well as in septal thickness (>1SD). The treatment also resulted in consistent and long-lasting changes in gene expression. Some mild to moderate adverse events occurred, including retinopathy, hypoglycaemia, hyperCKemia, acneiform dermatitis, atopic dermatitis, arthralgia and alopecia. One newborn with severe health issues and premature birth passed away due to extracardiac causes, and one patient withdrawn from the study due to hair loss.

Conclusion: Our initial data on the use of Trametinib to treat HCM in individuals with NS are promising and encourage additional studies to confirm and strengthen the results.

Grants: Supported by a research fund from the Italian Society of Pediatrics (SIP) to AM.

Conflict of Interest: None declared

P22.029.B Transcriptomic changes in the hypothalamus upon AAV-Dio2-Mct8 gene therapy for X-linked Allan-Herndon-Dudley syndrome in a mouse model

Finja Sygo[1], *Adriana Arrulo Pereira[2], Varun Sreenivasan[1], Kristian Händler[1], Nathalie Kruse[1], Malte Spielmann[1], Markus Schwaninger[2]*

[1]Institute of Human Genetics, University Medical Center Schleswig-Holstein, University of Lübeck & Kiel University, Lübeck, Germany; [2]Institute of Experimental and Clinical Pharmacology and Toxicology, University of Lübeck, Lübeck, Germany

Background/Objectives: Allan-Herndon-Dudley syndrome (AHDS) is a rare X-linked disorder characterized by hypotonia, weakness, and delay of developmental milestones, with an associated increase in serum T3 levels. Inactivating mutations in a thyroid hormone transporter (MCT8), has been associated with

AHDS. In tanycytes, MCT8 plays a role in the hypothalamus-pituitary-thyroid axis, explaining the excess peripheral and central lack of thyroid hormones in the disease. We developed a gene replacement therapy using AAV-Dio2-*Mct8* to deliver Mct8 to tanycytes in a mouse model of AHDS. Here, we investigated the effect of this gene therapy on the hypothalamic transcriptome.

Methods: Double knockout mice (*Mct8/Oatp1c1*-DKO) were used as a disease model, since *Oatp1c1*, another thyroid hormone transporter, is suggested to compensate for the loss of *Mct8* in mice. The changes to the hypothalamic transcriptome upon AAV-Dio2-*Mct8* injection to tanycytes in *Mct8/Oatp1c1*-DKO mice was analyzed by RNA-sequencing, with wildtype and *Oatp1c1*-knockout mice as additional controls. Differential expression analysis was performed across 34 samples.

Results: Preliminary analysis revealed thousands of differentially expressed genes, when the knockout and AAV-Dio2-*Mct8*-treated mice were compared to the wildtype. However, only 14 genes appear to have altered expression in the *Mct8/Oatp1c1*-DKO mice upon treatment.

Conclusion: These results indicate significant differences between the wildtype mice and all other conditions. However, the effect of AAV-Dio2-*Mct8* treatment is rather small, suggesting that this gene replacement therapy does not rescue the entire molecular phenotype. Further analysis is needed to fully assess the effect of the treatment on the hypothalamic transcriptome.

Grants: DFG (CRC/TR296)

Conflict of Interest: None declared

P23
GENETIC COUNSELLING / SERVICES / EDUCATION

P23.001.C Development and psychometric evaluation of a questionnaire to measure university students' knowledge about the effects of alcohol use during pregnancy

Guilherme Petek Ramos Leite[1], *Beatriz Caroline dos Santos Pereira*[1], *Lucimar Retto da Silva de Avó*[1], *Carla Maria Ramos Germano*[1], *Débora Gusmão Melo*[2]

[1]*UFSCar - Federal University of São Carlos, Department of Medicine, São Carlos, Brazil;* [2]*UNIFESP - Federal University of São Paulo, Department of Morphology and Genetics, São Paulo, Brazil*

Background/Objectives: This study aimed to develop and validate a questionnaire to assess the knowledge of university students regarding the effects of alcohol consumption during pregnancy.

Methods: We chose to design an instrument with true-false-don't know questions. Following ethical approval, 45 true statements were formulated and subjected to content validation by 19 experts. Based on Content Validity Index (CVI), 17 statements were selected. The instrument, named Fetal Alcohol Consequences Test (FACT), was assessed by 31 university students for the level of understanding. Then, the questionnaire was administered to a national sample of university students, and an Exploratory Factor Analysis (EFA) was conducted. Each FACT correct answer valued 1 point, and the knowledge was categorized as high (total score ≥80%), moderate (score between 60-79%), and low (score ≤59%).

Results: During the development of the questionnaire, the CVI values ranged from 0.779 to 1.0, and all statements were considered suitable by the target audience. For psychometric evaluation, 768 students from 24 Brazilian states participated. In the EFA, 5 statements were removed, revealing a tool with 12 questions and two latent factors: "fetal alcohol spectrum disorders" and "conceptions and guidance on alcohol consumption during pregnancy". The mean FACT score was 7.71 ± 2.98, with a median of 8; 32.03% of the students had high (10-12 points), 24.09% moderate (8-9 points), and 43.88% low knowledge (<8 points). The questionnaire proved reliable: floor effect 1.17%, ceiling effect 9.25%, and Cronbach's alpha index of 0.798.

Conclusion: The FACT is useful and can contribute to health education on the subject.

Grants: FAPESP (2022/05564-9).

Conflict of Interest: None declared

P23.002.D Development and implementation of a mainstreaming Genomic Associate role

Kylie Morgan[1;2], *vishakha tripathi*[1], *Ashleigh Butler*[1]

[1]*Guy's & St Thomas' Trust, Clinical Genetics, London, United Kingdom;* [2]*Peter MacCallum Cancer Centre, Familial Cancer Centre, Melbourne, Australia*

Background/Objectives: The expansion of genomic testing by non-genetic professionals through mainstreaming pathways is being spearheaded within the United Kingdom (UK) through National Health Service (NHS) genomics initiatives. However, the substantial demand upon clinical time and resources pose as significant barriers to successful and timely implementation. The 'Genomics Associate' role was established in the UK NHS in 2021. The scope and utility of this new role within Clinical Genetics service provision is still in development. One suggested function is to facilitate and support mainstreaming initiatives, however, the value of this approach has not been evaluated.

Methods: Here we describe the development of a Genomic Associate role within a novel prostate cancer mainstreaming project developed between the Uro-Oncology and Clinical Genetics departments at Guy's and St Thomas' Trust, UK.

Results: We demonstrate that the Genomics Associate position adds significant value to the implementation of mainstream genetic testing in the uro-oncology setting. To highlight the utility of this role and its importance in facilitating mainstreaming we will present it's impact across four key domains: multi-disciplinary communication and networking, support of mainstreaming processes, supporting clinical activities of Genetic Counsellors and, auditing.

Conclusion: We highlight the effiacy and value of a Genomics Associate role in supporting development and implementation of the mainstreaming pathway, and the potential for further development of this role to assist Clinical Genomics service provision both with the UK NHS and beyond.

Grants:

Conflict of Interest: None declared

P23.003.A Developing a Genomic Medicine elective course enhancing communication, patients' perspective, and ethical understanding

Celia Azevedo Soares[1;2;3;4;5;6], *Lucia Lacerda*[1;2;3;7], *Paula Jorge*[1;2;3;7]

[1]*Instituto de Ciências Biomédicas Abel Salazar, Departamento de Microscopia;* [2]*Universidade do Porto, Unidade Multidisciplinar de Investigação Biomédica;* [3]*Universidade do Porto, Laboratory for Integrative and Translational Research in Population Health;* [4]*Universidade de Aveiro, Departamento de Ciências Médicas;* [5]*Universidade do Porto, i3S - Instituto de Investigação e Inovação em Saúde;* [6]*Unidade de Saúde Local de Sto António, Serviço de Genética Médica;* [7]*Unidade Local de Saúde de Sto António, Serviço de Genética Laboratorial*

Background/Objectives: Genomic Medicine is at the forefront of modern healthcare, revolutionizing diagnostics and personalized treatment. The rapid advancements in genomics demand that medical students not only comprehend the science but also acquire skills in ethical considerations, effective communication, and navigating the complex patient diagnostic odyssey. To address this educational need, we developed an innovative elective course for medical students.

Methods: Our Genomic Medicine elective at Abel Salazar Biomedical Sciences Institute - University of Porto integrates a multifaceted approach, offering comprehensive insights into the field including five main perspectives: 1) basic concepts in Genomic Medicine, 2) ethics in Genomic Medicine, 3) patient diagnostic odyssey, 4) national and international initiatives, and 5) communication skills. One unique aspect of our course is the inclusion of non-health professional guest lecturers: a patient association's representative/caregiver of a patient with a rare genetic disorder, as well as a health journalist.

Results: Our evaluation process focuses on the students' ability to convey complex Genomic Medicine concepts to three distinct audiences: a family, a patient organization member, and a health specialized journalist. We reported improvements in students' knowledge and communication skills. The caregiver's personal account provided valuable insight into the emotional and practical aspects of genetic/genomic conditions, enhancing students' empathetic understanding. Media-led sessions improved students' ability to translate intricate genomic information into layman's terms.

Conclusion: We have championed a curriculum that emphasizes empathy, ethics, and communication skills to prepare students as knowledgeable and compassionate practitioners in Genomic Medicine.

Grants: NA

Conflict of Interest: None declared

P23.004.B Engagement of patients and the public in personalised prevention using genomic information

Loes Lindiwe Kreeftenberg[1;2], Lidewij Henneman[1;2], Valeria Fava[3], Claudia Louati[4], Daniela Quaggia[3], Yasemin Zeisl[4], Martina Cornel[1;2], Carla van El[1;2]

[1]Amsterdam UMC, Vrije Universiteit Amsterdam, Human Genetics, Amsterdam, Netherlands; [2]Amsterdam Public Health Research Institute, Amsterdam, Netherlands; [3]Cittadinanzattiva APS, Rome, Italy; [4]European Patients' Forum (EPF), Etterbeek, Brussels, Belgium

Consortium: PROPHET

Background/Objectives: Personalised prevention using genomic information requires active involvement from the public and patients, who need to be well-informed and empowered to make decisions reflecting their personal values in deciding on health care and sharing data. This underscores the importance of their active participation in care, research, education, and governance for meaningful engagement. We aimed to map engagement practices, and assess the extent and types of engagement methods utilized in the field of personalised prevention of common chronic conditions.

Methods: A scoping review selected literature (in Medline, Embase, Scopus, Web of Science, APA PsycINFO and IBSS) from 2015 to 2023 was performed. Articles included were practices of patient and public engagement in personalised prevention and genomics conducted in Europe focussing on cancer, cardiovascular diseases and neurodegenerative disorders.

Results: 23 engagement practices were selected. Analysis revealed diverse engagement levels, the majority falling into the low to medium engagement category, and showed mainly one directional methods of engagement, including dissemination and consultation. Most engagement activities related to cancer, and none to neurodegenerative diseases. The care domain exhibited the most publications, followed by research, research combined with care, and governance combined with education.

Conclusion: By elaborating on and implementing practices that engage and empower the patients and public at all levels of the engagement spectrum, fostering a more inclusive and participatory approach to personalised prevention, ultimately leads to improved health outcomes for individuals and communities.

Grants: A product of the PROPHET project, EU Horizon Europe Research and Innovation Programme (Grant No. 101057721).

Conflict of Interest: None declared

P23.005.C Strategies to improve implementation of cascade testing in hereditary cancer syndromes

Jianbang Chiang[1], Zi Yang Chua[1], Jia Ying Chan[2], Elaine Lum[3], Joanne Ngeow[1;2]

[1]National Cancer Centre Singapore, Cancer Genetics Service, Singapore, Singapore; [2]Lee Kong Chian School of Medicine, Singapore, Singapore; [3]Duke-NUS Medical School, Health Services & Systems Research, Singapore, Singapore

Background/Objectives: Hereditary cancer syndromes constitute approximately 10% of all cancers. Cascade testing involves testing of at-risk relatives to determine if they carry the familial pathogenic variant. Despite growing efforts targeted at improving cascade testing uptake, current literature continues to reflect low uptake, typically below 30%. This study aims to systematically review current literature on intervention strategies to improve cascade testing, assess the quality of intervention descriptions and evaluate the implementation outcomes of listed interventions.

Methods: We searched major databases using keywords and subject heading of "cascade testing". Interventions proposed in each study were classified according to the Effective Practice and Organisation of Care (EPOC) taxonomy. Quality of intervention description was assessed using the TIDieR checklist, and evaluation of implementation outcomes was performed using Proctor's Implementation Outcomes Framework.

Results: Improvements in rates of genetic testing uptake was seen in interventions across the different EPOC taxonomy strategies. The average TIDieR score was 7.3 out of 12. Items least reported include modifications (18.5%), plans to assess fidelity/adherence (7.4%) and actual assessment of fidelity/adherence (7.4%). An average of 2.9 out of 8 aspects of implementation outcomes were examined. The most poorly reported outcomes (3.7%) were cost, fidelity and sustainability.

Conclusion: Most interventions have demonstrated success in improving cascade testing uptake. Uptake of cascade testing was highest with delivery arrangement (68%). However, the quality of description of interventions and assessment of implementation outcomes are often suboptimal, hindering their replication and implementation downstream. Therefore, formal assessment of implementation outcomes may help promote translation of these interventions into routine practice.

Conflict of Interest: Jianbang Chiang - Singapore Ministry of Health National Medical Research Council

- National Cancer Centre Research Fund, Zi Yang Chua: None declared, Jia Ying Chan: None declared, Elaine Lum: None declared, Joanne Ngeow National Research Foundation Singapore, Clinician Scientist Award (NMRC/CSA-INV/0017/2017, and MOH-000654), Singapore Ministry of Health's National Medical Research Council, National Cancer Centre Research Fund (NCCRF-YR2018-NOV-1) and the Terry Fox Foundation, Canada

P23.006.D Development of a digital risk-prediction tool for cardiovascular diseases based on family health history for use in the general population

Tetske Dijkstra[1], Lieke van den Heuvel[2], Jacobien Niebuur[1], Peter van Tintelen[2], Christian van der Werf[3], Boudien Sieperda[4], Jacoliene Zaal[4], Anneke Lucassen[5], Irene van Langen[1], Corrette Ploem[6], Els Maeckelberghe[7], **Imke Christiaans**[1]

[1]University Medical Centre Groningen/University of Groningen, Genetics, Groningen; [2]University Medical Center Utrecht/Univerity Utrecht, Biomedical Genetics, Utrecht, Netherlands; [3]Amsterdam University Medical Centers/University of Amsterdam, Heart Center, Department of Clinical and Experimental Cardiology, Amsterdam Cardiovascular Sciences, Amsterdam, Netherlands; [4]University Medical Centre Groningen, Legal Affairs, Groningen, Netherlands; [5]Wellcome Trust Centre for Human Genetics/University of Oxford, Clinical Ethics, Law and Society (CELS), Oxford, United Kingdom; [6]Amsterdam University Medical Centers/University of Amsterdam, Ethics, Law and Medical Humanities, Amsterdam, Netherlands; [7]University Medical Centre Groningen/University of Groningen, Beatrix Children's Hospital, Groningen, Netherlands

Background/Objectives: In inherited and familial cardiovascular diseases (CVDs) relatives can be at risk for early and preventable cardiovascular events. We developed an online risk-assessment tool based on family health history to help people evaluate their potential risk of CVD.

Methods: We started with a critical appraisal of legal and ethical challenges associated with such a tool. Using a focus group and Delphi procedure with clinical experts in inherited CVDs we developed family criteria for use by laypeople in the tool and evaluated them in different cohorts. Finally, two prototypes of the tool, with differences on data storage and risk prediction facilities, were developed and subjected to usability testing in 1000 laypeople using questionnaires.

Results: Legal challenges were mainly found in the European Union regulations on data protection and medical devices. Five expert-based family criteria focusing on cardiovascular events at young age in first-degree relatives were derived and showed substantial diagnostic accuracy. The two tool prototypes got similar evaluations on usability and tool results on CVD-risk did not lead to substantial anxiety.

Conclusion: The final tool for risk-assessment aligns with European Union regulations by not storing family health information and have users themselves determine their CVD risk based on their answers on the family criteria. Using the expert-based family criteria this tool can serve as a first tier for laypeople to easily determine if they have a potentially increased risk of CVD based on their family health history to facilitate early detection and improve public health.

Grants: Dutch Heart Foundation (2019T111)

Conflict of Interest: None declared

P23.007.A Evaluation of a self-test to screen for tumor risk syndromes revealed a good comprehensibility for laypersons and a high positive predictive value

Beate Vajen[1], *Paula Memenga[2], Johanna Tecklenburg[1], Caroline Scholz[1], Petra Anders[1], Scarlett Winterfeld[1], Brigitte Schlegelberger[1], Anke Katharina Bergmann[1], Eva Baumann[2], Susanne Morlot[1], Christian Landgraf[1]*

[1]Hannover Medical School, Department of Human Genetics, Hannover; [2]Hanover University of Music, Drama and Media, Department of Journalism and Communication Research, Hannover, Germany

Background: The identification of tumor risk syndromes (TRS) allows personalized therapy, preventive measures and identification of further affected family members. Despite their significance, TRS are underrecognized. We developed a screening tool with 5 questions for laypersons to identify an increased risk for TRS. Here, we present the evaluation of this self-test concerning 1) comprehensibility and 2) specificity, sensitivity and the positive predictive value (PPV).

Methods: 1) To assess the comprehensibility, an online survey was conducted anonymously from July to November 2023 in Germany. Participants ($N = 106$) indicated the extent to which they agreed with four statements on the ease of cognitive access (5-point Likert-type scale). 2) To evaluate the specificity, sensitivity and the PPV a cohort of 177 cancer patients with a suspicion of a TRS, who had received a genetic counselling between 2021-2023 at the Department of Human Genetics at Hannover Medical School, Germany, was analyzed retrospectively.

Results: 1) Overall, the self-test revealed a good comprehensibility ($M = 4.21$). 2) The sensitivity (true positive/true positive + false negative x100) was 92,6%. The specificity (true negative/true negative + false positive x100) was 37,9% and the PPV with a prevalence of 10% was 73%.

Conclusion: Here, we present a self-test for laypersons, that has the ability to screen for TRS due to its good comprehensibility and its high sensitivity and PPV. Although, we think that this tool can help to identify more TRS, we present also the limitations, especially the cancer diagnoses that are not covered by the self-test.

Conflict of Interest: None declared

P23.008.B The potential for chatbots in genetic counselling

Elen Siglen[1;2], *Hildegunn Høberg Vetti[1;2], Vidar Martin Steen[1;3], Tone Dahl-Michelsen[2], Cathrine Bjorvatn[1;2]*

[1]Haukeland University Hospital, Western Norway Familial Cancer Center/ Department of Medical Genetics, Bergen, Norway; [2]VID Specialized University, Faculty of Health Studies, Norway; [3]University of Bergen, Department of Clinical Science, Bergen, Norway

Background/Objectives: We have developed a health chatbot, named Rosa, that addresses questions about hereditary breast and ovarian cancer. The experiences from building Rosa were published in 2022 and have previously been presented at the ESHG meeting in 2019. We would now like to present our overall findings after building, testing, evaluating, and implementing Rosa chatbot in the clinic.

Methods: Healthy individuals at risk of hereditary cancer were invited to test Rosa during 2020/2021, and 16 of them shared their experiences through semi-structured interviews focusing on usability, usefulness, and trust. During winter 2023/2024 we have implemented Rosa as an information tool for breast and ovarian cancer patients during mainstream genetic testing. A selection of both physicians and patients are participating in in-depth interviews focusing on losses and gains when digitalizing health care tasks, such as information giving through chatbots.

Results: Rosa was described as accessible, trustworthy and a valuable supplement to genetic counselling by healthy, at-risk individuals. For some it could have replaced genetic counselling, but not if hereditary cancer was detected. Preliminary results from the 2023/2024 interviews indicate that the chatbot is also well received by cancer patients and does not cause anxiety, however both patients and physicians share concerns about the consequences digitalisation may have on the patient-physician relationship.

Conclusion: A chatbot is a valuable supplement to genetic counselling and may even replace counselling for selected groups. However, digitalization may lead to less human encounter with a potential of adverse effects on the health services.

Grants: This study is funded by the Norwegian Cancer Society.

Conflict of Interest: None declared

P23.009.C Integration of Genetic Counsellors into multi-professional Genetics Teams in the German-speaking Countries

Gunda Schwaninger[1], *Sabine Rudnik*[1], *Johannes Zschocke*[1]

[1]*Medical University of Innsbruck, Institute of Human Genetics, Innsbruck, Austria*

Background: The establishment of the genetic counsellor (GC) profession in German-speaking countries reached a significant milestone with the graduation of the inaugural cohort of Genetic and Genomic Counselling MSc students from the Medical University of Innsbruck in spring 2022. To share the knowledge gained from the experiences with these first students, an online workshop on GC training and integration was organized. Session 1 covered the education and professional profile of GCs, addressing challenges in integration. Session 2 explored experiences of GC students and providers during clinical placements, emphasizing the roles of GCs in interprofessional teams.

Methods: Online workshop with fifty-five participants, including 22 medical geneticists from Germany, Austria, and Switzerland, three senior GCs, six GC-graduates, ten GC-students, 12 prospective students, and two related professionals.

Results: The workshop revealed growing demand for GCs and positive experiences within participating institutes. Challenges included the GC scope of practice, legal aspects, remuneration, billing and supervision time. The workshop participants highlighted the need for increased numbers of trained GCs in the region. The MSc program team emphasized the importance of comprehensive clinical placements and standardized training. Experiences from different genetic centers showcased varying approaches to integrate GCs into clinical teams, indicating a positive shift in the perception of their role.

Conclusion: The workshop underscored the challenges and opportunities in integrating GCs into the healthcare systems of the German-speaking countries. Presently, the first group of GCs is dedicated to overcome obstacles of integration by closely collaborating with the regional societies and professional associations of human genetics.

Grant No external funding used.

Conflict of Interest: None declared

P23.010.D Is there an impact of Chromosomal Structural Rearrangements on IVF laboratory outcomes in PGT-SR cycles? A propensity score matching-based study

Daria Marzanati[1;2], *Sara D'Alessandro*[3], *Davide Gentilini*[1;4], *Elisa Rabellotti*[2], *Laura Privitera*[3], *Sonia Faulisi*[3], *Francesca Spinella*[5], *Anil Biricik*[5], *Ettore Cotroneo*[5], *Luca Pagliardini*[2], *Enrico Papaleo*[3], *Alessandra Alteri*[2]

[1]*University of Pavia, Department of Brain and Behavioral Sciences, Pavia, Italy;* [2]*IRCCS San Raffaele Scientific Institute, Reproductive Sciences Laboratory, Obstetrics and Gynaecology Unit, Milan, Italy;* [3]*IRCCS San Raffaele Scientific Institute, Obstetrics and Gynaecology Unit, Milan, Italy;* [4]*Istituto Auxologico Italiano IRCCS, Bioinformatics and Statistical Genomics Unit, Cusano Milanino,Milan, Italy;* [5]*Eurofins GENOMA Group, Molecular Genetics Laboratories, Rome, Italy*

Background/Objectives: Patients with Chromosomal Structural Rearrangements (CSR) frequently face fertility challenges due to abnormal gametogenesis. For affected couples, In Vitro Fertilization (IVF) with Preimplantation Genetic Testing for Structural Rearrangements (PGT-SR) is recommended to select transferable blastocysts with a normal or balanced karyotype. This retrospective cohort study evaluates the impact of CSR on IVF laboratory outcomes in PGT-SR patients.

Methods: IVF Laboratory outcomes of IVF cycles with PGT-SR (N = 136) were compared to those with preimplantation genetic testing for aneuploidy (PGT-A) (N = 422) using propensity score matching and a logistic regression model. Within the PGT-SR population, the association between transferable blastocysts rate, carrier's gender and CSR type was evaluated through a logistic regression model.

Results: There was no significant difference in the fertilization rate between the PGT-A and PGT-SR groups. However, the PGT-SR group presents a lower blastulation rate compared to the PGT-A group (OR = 0.76, 95% CI 0.63-0.93, p-value = 0.03). Similar trends were noted in the top-quality blastocyst rate (OR = 0.45, 95% CI 0.29-0.69, p-value < 0.01). In the PGT-SR cohort, no significant association was observed between the transferable blastocyst rate and the carrier's gender. Nevertheless, a notable association (OR = 0.16, 95% CI = 0.09-0.29, p-value < 0.01) was observed based on CSR type. Patients who carried reciprocal translocations had a lower transferable blastocyst rate compared to those with CSR other than reciprocal.

Conclusion: These findings reveal that patients with CSR showed a lower blastulation rate compared to those undergoing PGT-A, showing a reduced rate of transferable blastocysts in cases of reciprocal translocations. Consequently, providing counseling and managing expectations for couples undergoing PGT-SR is essential.

Grants: No grants

Conflict of Interest: None declared

P23.011.B Information comic as a pre-test educational tool about preconception carrier screening in German language

Christina Wölwer[1], *Gunda Schwaninger*[1], *Sabine Rudnik*[1]

[1]*Medical University Innsbruck, Institute of Human Genetics, Innsbruck, Austria*

Background/Objectives: Preconception carrier screening (PCS) allows screening for multiple recessive and X-linked diseases simultaneously and aims to identify carrier couples at an increased risk of having severely affected children. In Austria, preparations are underway to implement population-based PCS.

It has been debated how valid informed consent can be achieved considering the large number of rare diseases addressed. Academic societies in the United States (ACMG) and in Europe (ESHG) recommend a more generic consent for multiplex genetic testing. To help ensure that interested couples can make autonomous decisions, the ESHG suggests the usage of patient-tailored information materials, e.g. smartphone apps (PMID 29023436). In an American study comparing a brochure, video, and comic about PCS on knowledge and decision making, the comic scored best in terms of patient understanding (PMID 33135283). Thus, we developed an information comic about PCS in German language.

Methods: We created an educational comic using StoryboardThat presenting a couple considering a pregnancy who questions a gynaecologist about PCS. During the development of the comic, a team of clinical geneticists, genetic counsellors, and genetic counsellor trainees met several times to discuss and refine its contents.

Results: We developed an educational comic to inform future parents about PCS in German language. Main contents are basic genetics (different modes of inheritance), the purpose, benefits, limitations, possible outcomes, and potential reproductive options of PCS. The comic is accessible anytime via smartphone or computer through a QR code.

Conclusion: Our comic will improve awareness and genetic literacy about PCS in the general population.

Grants:

Conflict of Interest: None declared

P23.013.B Genomic Return of Results at the National Level: The United States' *All of Us* Research Program

Scott Topper[1], *Alicia Zhou*[1], *Cynthia Neben*[1], *Richard Gibbs*[2], *Stacey Gabriel*[3], *Heidi Rehm*[3], *Kimberly Doheny*[4], *Gail Jarvik*[5], *Chia-Lin Wei*[5], *Tara Dutka*[6], *Rhonda Moore*[6], *Anjene Musick*[6], *Anastasia Wise*[6]

[1]*Color Health, United States;* [2]*Baylor College of Medicine, United States;* [3]*Broad Institute of MIT and Harvard, United States;* [4]*Johns Hopkins University, United States;* [5]*University of Washington, United States;* [6]*NIH All of Us Research Program, United States*

Consortium: NIH All of Us Research Program

Background/Objectives: *All of Us* (the US Precision Medicine Initiative) will advance precision medicine by developing an open database of 1 million+ health-contextualized genome sequences, derived from a uniquely diverse population. Longitudinal participant engagement is a core aim, and direct-to-participant return of genetic results is an essential engagement strategy.

Methods: Since 2020, the program has offered >270,000 participants access to genetic ancestry, "traits", and health-related results including hereditary disease risk and pharmacogenomics (PGx). Participants opt-in and view results through an online, digital platform. Genetic counseling is available to all participants. The return-of-results protocol was developed under IRB and FDA oversight.

Results: To date, >70% of eligible participants have engaged with the return-of-results program. Of those, about 70% were individuals who have been underrepresented in biomedical research. 3% of result recipients have a disease-causing variant in one of the ACMG Secondary Findings v2 Gene List and were offered free clinical confirmation testing. 91% of PGx result recipients have a non-standard phenotype in one of seven genes (CPIC Level A, FDA). Participant feedback about the results return process was overall positive via Likert-scaled questions and free text responses.

Conclusion: *All of Us* has established a new standard for human subjects research and has directly returned results to one of the largest and most diverse cohorts to date. Participants are highly engaged with their research results, which can help drive recruitment and retention and ultimately increases the value of the program's research database.

Grants: NIH OT2OD028251

Conflict of Interest: None declared

P23.014.C The British Columbia Hereditary Cancer Follow-Up Initiative: Supporting hereditary cancer patients across the life-span

Melanie O'Loughlin[1], *Sze Wing Mung*[1], *Shanzhao Wang*[2], *Mandy Jevon*[1], *Eugene Cheung*[1], *Mary-Jill Asrat*[1;3], *Rebecca Morash*[4], *Kelsey Hamilton*[1], *Katherine Blood*[1;3], *Rona Cheifetz*[4;5], *Jennifer Nuk*[1;3], *Sophie Sun*[1;6], *Kasmintan Schrader*[1;3;7]

[1]*BC Cancer, Hereditary Cancer Program, Vancouver, Canada;* [2]*University of British Columbia, Dept of Gynecology and Obstetrics, Vancouver, Canada;* [3]*University of British Columbia, Department of Medical Genetics, Vancouver, Canada;* [4]*BC Cancer, High Risk Clinic, Vancouver, Canada;* [5]*University of British Columbia, Department of Surgery, Vancouver, Canada;* [6]*University of British Columbia, Department of Medical Oncology, Vancouver, Canada;* [7]*University of British Columbia, Department of Molecular Oncology, Vancouver, Canada*

Background/Objectives: The Hereditary Cancer Follow-up Initiative (HCFI) is a clinical service implemented at BC Cancer in July 2020 with the aim of ascertaining and addressing unmet clinical and psychosocial needs for individuals living with hereditary cancer conditions.

Methods: Adults in British Columbia or Yukon with a pathogenic variant in a hereditary cancer gene are invited to complete an annual survey. The survey collects information about cancer risk management practices, family communication and support needs. Completed surveys are triaged for phone follow-up with a genetic counsellor if the respondent indicates a support need, or if reported risk management practices are discordant with clinical recommendations.

Results: 5570 individuals have been identified as eligible, with 2304 completing a baseline survey to date (41%). Analysis of the demographics of invited participants and respondents with attention to self-reported ethnicity to evaluate equity is underway.

Analysis of early survey responses found 61% were triaged for genetic counsellor follow-up. 27% of participants reported risk management practices discordant with current clinical recommendations. A strong majority (97%) of participants reported speaking with relatives about the gene. A minority (6%) requested help with family communication, primarily regarding speaking with children. Further analysis will be completed on all baseline survey responses, with a focus on family communication practices across self-reported ethnicities.

Conclusion: The HCFI elucidates the longitudinal support needs of hereditary cancer pathogenic variant carriers in British Columbia and Yukon. Additional analysis will inform program planning to support effective hereditary cancer follow-up and family communication across ethnicites.

Conflict of Interest: None declared

P23.015.D From Estonian biobank to practice: undiagnosed adult CNV carriers express contentment to learn about their genetic finding

Marili Palover[1], *Margit Nõukas*[1], *Anu Reigo*[1], *Helene Alavere*[1], *Liis Leitsalu*[1;2], *Alexandre Reymond*[3], *Neeme Tonisson*[1;2], *Katrin Männik*[4]

[1]*University of Tartu, Institute of Genomics, Estonian Biobank, Tartu, Estonia;* [2]*Tartu University Hospital, Genetics and Personalized Medicine Clinic, Tartu, Estonia;* [3]*University of Lausanne, Center for Integrative Genomics, Lausanne, Switzerland;* [4]*Health 2030 Genome Center, Foundation Campus Biotech Geneva, Geneva, Switzerland*

Background/Objectives: While pioneering national genomic medicine initiatives focus on returning individual risk predictions for medically actionable monogenic and common diseases,

evidence-based recommendations on reporting genetic findings associated with high risk of complex clinical phenotypes (e.g. genomic disorder CNVs), to biobank volunteers are lacking.

Methods: We selected carriers of 16p11.2 and 16p12 CNVs of the Estonian biobank (EstBB) to establish a framework for returning CNV findings to population biobank participants and to evaluate psychosocial impact of receiving this information.

Results: 76 of 169 CNV carriers (45%) consented for return of results. The majority (89%) of CNV carriers presented health conditions characteristic of the associated genomic disorders, i.e. disorders of female reproductive health (53%), mental disorders (43%), gastrointestinal disease (67.1%) and speech disorders (51.3%). Preliminary analysis from receiving unexpected genetic information (data from 72 out of 76 participants) showed that almost all carriers (97.2%) appreciated being contacted. The participants felt that the information received was sufficient (98.6%), gave them more clearance (96%) and relief (86.1%). They thought that it did not increase their worry (93%) or anxiety (90.1%). Evaluation of long-term impact is planned to follow in 6 months.

Conclusion: Undiagnosed CNVs affect health and socio-economic outcomes in the general population. Carriers of such rearrangements who choose to consent for receiving the information appreciate being contacted and feel relieved knowing the origin of their health problems in spite of the severity or characteristics of their CNV related clinical outcomes.

Grants: Jacobs Foundation Research Fellowship (#2016 1217 09), Swiss National Science Foundation (#31003A_182632), Estonian Research Council (#PRG555).

Conflict of Interest: None declared

P23.016.A Bridging the Gap: Accelerating Genetic Disorder Diagnosis in Rio Grande's Hispanic Communities via Innovative Tele-Health Solutions

Daniel Brooks[1;2], *Blake Vuocolo*[2;3], *Robert Sierra*[3], *Christopher Holder*[3], *Lauren Urbanski*[3], *Keila Rodriguez*[4], *Jose Gamez*[5], *Surya Mulukutla*[5], *Lori Berry*[4], *Ana Herndandez*[4], *Segundo Lizardo-Guzman*[6], *Humberto Hidalgo*[4], *Alberto Allegre*[4], *Sandy Magallan*[4], *Sarah Rodriguez*[4], *Hongzeng Dai*[3;7], *Kent Carter*[4], *Claudio Soler-Alfonso*[2;3], *Brendan Lee*[3], *Seema Lalani*[2;3]

[1]*Baylor College of Medicine, Department of Pediatrics; Department of Genetics, Houston, United States;* [2]*Texas Children's Hospital - West Tower, Houston, United States;* [3]*Baylor College of Medicine, Department of Genetics, Houston, United States;* [4]*The University of Texas Rio Grande Valley, Department of Pediatrics, Edinburg, United States;* [5]*DHR Health Diabetes & Endocrinology Institute Pediatric and Young Adult Clinic, Edinburg, United States;* [6]*Kaizen Clinical Partners (Mercedes Children's Clinic), Mercedes, United States;* [7]*Baylor Genetics, Houston, United States*

Background/Objectives: In the medically underserved Rio Grande Valley (RGV), Project GIVE aims to mitigate disparities in genomic healthcare for a predominantly Hispanic population with high poverty and uninsured rates. This NIH-funded study leverages the Consultagene platform for genetic evaluations and whole genome sequencing (WGS).

Methods: Healthcare providers refer patients to Project GIVE via Consultagene. The process includes a virtual genetic evaluation and subsequent collection of buccal samples for trio genome sequencing, with results returned during a follow-up visit. Providers in the RGV are also educated on genetics through CME events. Data collection is ongoing to assess the impact on provider competency and patient outcomes.

Results: Of 147 referrals, 102 were accepted, and 49 completed their initial visit. A significant portion of these families fall below the poverty line. Diagnoses were made in 34% of cases with returned WGS results, and further gene-disease associations are being explored. Surveys indicate family satisfaction with the telemedicine approach and WGS results. Post-CME, providers showed increased understanding of when to refer and how to interpret genetic data.

Conclusion: Project GIVE demonstrates a successful model of using tele-genetics to improve access to genomic healthcare in under-resourced communities. Enhanced provider education and virtual engagement are pivotal to this model, which holds promise for replication in similar regions to address genomic health inequities.

Conflict of Interest: None declared

P23.017.C Why people misunderstand heritability and why it matters

Yulia Kovas[1], *Fatos Selita*[2], *Alice Gregory*[1], *Gabriela Amaral*[1], *Emma Greenwood*[1], *Sever Sava*[1], *Maxim Likhanov*[3]

[1]*Goldsmiths, University of London, Psychology, London, United Kingdom;* [2]*Goldsmiths, University of London, London, United Kingdom;* [3]*Beijing Normal University, Beijing, China*

Background/Objectives: Heritability is a non-intuitive concept and is often misunderstood. Many people do not know that heritability refers to genetic contribution to variation in the population, rather than to an individual's traits; and that heritability is not constant within and across populations.

Methods: We administered an in-depth on-line survey to adult participants (N = 780) from the general population. Participants selected a definition of heritability from four options; provided estimates of heritability for 11 traits; and answers to 14 items tapping into understanding of heritability, e.g. its changeability across developmental, educational and cultural contexts. Participants' other genetic knowledge was assessed with 20 items.

Results: The results confirmed a striking lack of understanding of heritability. Only about 20% of participants could identify the correct definition of heritability; and heritability estimates varied from 0% to 100%. Most participants thought that heritability cannot differ across countries; but thought that heritability can differ in the same country at different historical periods. Around 60% of participants were unsure or thought that a policy change (e.g. vaccination; compulsory education) can not affect heritability. Understanding heritability was associated with participants' education, occupation, genetic knowledge; and assumptions about the effectiveness of different treatments, views on agency and responsibility in the justice context, and beliefs about the appropriate use of genetic information.

Conclusion: Many people have an inaccurate understanding of heritability. Assumptions about the aetiology of different traits may influence views, decisions and choices. These results call for new approaches to making the central genetic concept of heritability more accurately understood.

Grants: n/a

Conflict of Interest: None declared

P23.018.B First Experiences with the Introduction of Genetic Counselors in Human Genetic Services in the German-speaking Countries

Simone Heidemann[1], *Johannes Zschocke*[2], *Gunda Schwaninger*[2]

[1]*Institute of Tumorgenetics North, Kiel, Germany;* [2]*Medical University Innsbruck, Institute of Human Genetics, Innsbruck, Austria*

Background/Objectives: In 2019, the Medical University of Innsbruck introduced the first Genetic and Genomic Counseling master's program in the German-speaking countries. A major challenge of this process was the absence of practicing Genetic Counselors (GC) in these countries, leading to a lack of experience with GCs in medical genetic practices and the absence of a legal framework for the profession. Consequently, student placements within the program commenced with neither the students nor their supervising consultants having any previous experience of collaborative teamwork between clinical geneticists and GCs.

Methods: To share insights from the initial implementation phase, supervising consultants from the seven student placement institutes were invited to participate in semi-structured interviews guided.

Results: From these interviews, three models of interprofessional teamwork between GCs and clinical geneticists emerged: (1) the alternating or tandem model, (2) qualified preliminary and follow-up patient interviews and (3) the provision of genetic services without direct genetic counseling. In addition, the interviewees provided recommendations for addressing legal concerns and for the effective establishment of appropriate compensation structures for GCs within the German-speaking countries.

Conclusion: Clinical geneticists taking part in the study estimated that the integration of GCs could potentially enhance counseling capacity by as much as 50%. Importantly, they did not foresee any reduction in counseling quality caused by the inclusion of GCs. This study provides evidence that the GC profession can provide additional skills to human genetic services and positively impact both patient support and overall capacity of genetic services also in the German-speaking countries.

Grants:

Conflict of Interest: Simone Heidemann: None declared, Johannes Zschocke founder

of the Masters course genetic and genomic counselling at the Medical University of Innsbruck, Gunda Schwaninger head of the Masters course genetic and genomic counselling at the Medical University of Innsbruck

P23.019.D Factors influencing pregnant women's decision to accept or decline prenatal diagnosis - a qualitative study

Charlotta Ingvoldstad[1;2;3], *Susanne Georgsson*[4], *Ove Axelsson*[5;6], *Ellen Ternby*[7]

[1]*Karolinska University Hospital, Centre for Fetal Medicine, Stockholm, Sweden;* [2]*Karolinska Institutet, Department of Molecular Medicine and Surgery, Stockholm, Sweden;* [3]*Uppsala University, Uppsala, Centre for Research and Bioethics, Uppsala;* [4]*The Swedish Red Cross University, Stockholm, Sweden;* [5]*Uppsala University, Centre for Clinical Research Sörmland, Eskilstuna, Sweden;* [6]*Uppsala University, Department of Women's and Children's Health, Uppsala;* [7]*Uppsala University, Department of Women's and Children's Health, Uppsala, Sweden*

Background/Objectives: Prenatal diagnosis for chromosomal anomalies is commonly offered worldwide. It is important that pregnant women receive adequate counselling to make informed decisions regarding prenatal diagnosis. The aim was to explore what factors influence pregnant women's decision-making process to accept or decline prenatal diagnosis.

Methods: A qualitative study using inductive qualitative content analysis. Individual, semi-structured phone interviews with 24 pregnant women in the first trimester, living in a medium-sized Swedish city, were carried out during a five-month period in 2016-2017.

Results: Two main themes emerged; (1)*"Individual factors - The women's experiences, perceptions and values"* with the three categories "Attitude towards anomalies", "Worry and need for reassurance", "Self-perceived risk" and (2)*"External factors - The women's perception of the test and others' views"* with the two categories "Test characteristics" and "Influence from others".

Conclusion: Pregnant women's decision-making process regarding prenatal tests is multidimensional, affected by both individual factors like experiences, perceptions and values and external factors like test characteristics and influence from others. Information about both test characteristics and the conditions tested for could help expectant women in the decision-making process by having better understanding of how a child with the condition in question could affect them and their family. Healthcare professionals giving information about and offering prenatal tests for chromosomal anomalies should be aware of how their attitudes can influence women's decisions.

Grants: This research was supported by funding from the Center for clinical research Sörmland and by ALF compensation from Region Uppsala and Uppsala University, Sweden.

Conflict of Interest: None declared

P23.020.A The establishment of a German Reference Network for Hereditary Tumor Syndromes to implement the European Reference Network (ERN) achievements into national health care systems

Stefan Aretz[1;2;3], *Nadine Weinstock*[4], *Christian Kratz*[5], *Alexander Volk*[3;6], *Isabel Spier*[1;2;3], *Miriam Elbracht*[7], *Christian Sutter*[8], *Christopher Schroeder*[9], *Bernd Wollnik*[10;11;12], *Reiner Siebert*[13], *Nicola Reents*[14], *André Reis*[3;15;16], *Evelin Schröck*[3;17;18], *Lorenz Grigull*[4], *Tim Ripperger*[3;19], *Verena Steinke-Lange*[3;20;21], *Elke Holinski-Feder*[3;20;21]

[1]*Institute of Human Genetics, Medical Faculty, University of Bonn, Bonn, Germany;* [2]*Center for Hereditary Tumor Syndromes, University Hospital Bonn, Bonn, Germany;* [3]*European Reference Network on Genetic Tumour Risk Syndromes (ERN GENTURIS);* [4]*Centre for Rare Diseases, University Hospital Bonn, Bonn, Germany;* [5]*Pediatric Hematology and Oncology, Hannover Medical School, Hannover, Germany;* [6]*Institute of Human Genetics, University Medical Center Hamburg-Eppendorf, Hamburg, Germany;* [7]*Institute for Human Genetics and Genomic Medicine, Medical Faculty, RWTH Aachen University, Aachen, Germany;* [8]*Institute of Human Genetics, University Hospital Heidelberg, Heidelberg, Germany;* [9]*Institute of Medical Genetics and Applied Genomics, University Hospital Tübingen, Tübingen, Germany;* [10]*Institute of Human Genetics, University Medical Center Göttingen, Göttingen, Germany;* [11]*DZHK (German Center for Cardiovascular Research), Partner Site Göttingen, Göttingen, Germany;* [12]*Cluster of Excellence "Multiscale Bioimaging: from Molecular Machines to Networks of Excitable Cells" (MBExC), University of Göttingen, Göttingen, Germany;* [13]*Institute of Human Genetics, Ulm University and Ulm University Medical Center, Ulm, Germany;* [14]*SemiColon – Lynch-Syndrome Patient Network, Mönchengladbach, Germany;* [15]*Institute of Human Genetics, University Hospital Erlangen, Friedrich-Alexander Universität Erlangen-Nürnberg, Erlangen, Germany;* [16]*Centre for Rare Disorders Erlangen (ZSEER), University Hospital Erlangen, Erlangen, Germany;* [17]*Translational Medical Oncology, Faculty of Medicine and University Hospital Carl Gustav Carus, Technische Universität Dresden, Dresden, Germany;* [18]*Institute for Clinical Genetics, University Hospital Carl Gustav Carus, Technische Universität Dresden and Hereditary Cancer Syndrome Center Dresden, Dresden, Germany;* [19]*Department of Human Genetics, Hannover Medical School, Hannover, Germany;* [20]*MGZ - Medical Genetics Center, München, Germany;* [21]*Arbeitsgruppe erbliche gastrointestinale Tumore, Medizinische Klinik und Poliklinik IV – Campus Innenstadt, Klinikum der Universität München, München, Germany*

Consortium: Deutsches Referenz-Netzwerk Erbliche Tumor-Syndrome (DRN ETS)

Background/Objectives: The foundation of European Reference Networks (ERN) represents a major step to improve patient care and research for rare and complex diseases. Families with Hereditary Tumor Syndromes (HTS) are a relevant target group and a paradigm for preventive and precision oncology. However, it is suspected that the majority of patients is still not diagnosed or experience a substantial delay in diagnosis, which results in inadequate medical care. The ERN GENTURIS aims to substantially improve medical care of families with GENetic TUmour RIsk Syndromes across Europe. Nevertheless, the achievements will only improve oncological care, if they are implemented in national health care systems, which is regarded as a major shortcoming.

Methods: Therefore and in line with the EU requirements, a German Reference Network for Hereditary Tumor Syndromes (GRN HTS) was established as one of 12 GRNs to date (www.se-atlas.de/drn).

Results: Currently, the GRN HTS consists of 11 centers (ERN members and / or specialized B centers of Centers for Rare Diseases. The network offers expertise for a broad spectrum of rare HTS and involves patient representatives. The website acts as an information platform for relevant areas and sources regarding clinical and research aspects of HTS.

Conclusion: Challenges are the recruitment of expert clinicians for specific areas and the extension of specialized outpatient clinics. Future goals of the network include the implementation of registries for rare HTS, training programs for organ-specific cancer centers, cross linking with other initiatives in the field, and ensuring the interoperability between local and ERN data structures.

Conflict of Interest: None declared

P23.021.B Reflexive predictions in genetic counselling: when prediction alters the future

Angus Clarke[1], *Mayli Mertens*[2]

[1]*Cardiff University, Cancer & Genetics, School of Medicine, Cardiff, United Kingdom;* [2]*Centre for Ethics, Department of Philosophy, University of Antwerp, Antwerpen, Belgium*

Background/Objectives: How can a predictive genetic test be, appear to be, or become, a self-fulfilling or self-defeating prophecy? How can the result of the test influence what then happens to the patient's health? The umbrella term for self-fulfilling and self-defeating prophecies is reflexive prediction, where prediction affects the predicted outcome. Some of these effects are unsurprising as they are the primary goal of testing, as when those at risk of inherited cancers use this knowledge to manage their risk. We focus on the less expected effects of predictive genetic testing and how they may be important in genetic counselling as preparation for genomic tests.

Methods: The methods employed have been literature review drawing on a theoretical model for analysis of reflexive prediction, reflection on professional practice, and discussion between the authors and with colleagues.

Results: We have identified several categories of reflexive prediction that are encountered in genetic counselling practice, where awareness of this reflexivity can yield important insights. Other categories have been identified where there is potential for feedback between genetic susceptibility and expectations held by the self or others. These effects will become more likely if/when genomic testing is more broadly applied to complex diseases, especially psychiatric illness and to cognitive and behavioural traits.

Conclusion: Learning to recognise the scope for reflexive prediction is already useful in genetic consultations; it will increase in importance as the scope of genomic testing broadens to more complex diseases and non-disease traits.

Grants: MM: Marie Skłodowska-Curie Postdoctoral Fellowship "Responsible prediction of gene expression: mitigating genetic risk profiling" 2023-25

Conflict of Interest: Angus Clarke Medical Research Council grant (co-investigator), Emeritus status, Mayli Mertens Marie Skłodowska-Curie Postdoctoral Fellowship 2023-2025, Research Fellow, Center for Ethics, Department of Philosophy, University of Antwerp.

P23.022.C Motivational text-messages increase compliance to *BRCA1/2* screening test

Rotem Greenberg[1], *Tom Muskat*[1], *Adi Berliner*[1], *Shay Ben-Shachar*[1,2]

[1]*Clalit Health Services, Clalit Research Institute, Ramat Gan, Israel;* [2]*Tel Aviv University, Faculty of Medicine, Tel Aviv, Israel*

Background: In 2020, Israel initiated *BRCA1/2* carrier screening program for Ashkenazi Jewish women. Despite its medical importance, the compliance was relatively low (2.4% of the eligible population) during the first year. To improve compliance, we implemented a motivational text messaging strategy.

Methods: We randomly sent five distinct motivational messages annually to all eligible women aged 25-50. Each message included a link to schedule an appointment and provided test information. Intervention impact was assessed by test performance at 3-6 months after receiving the messages, considering the time required to performing the testing.

Results: A 233,711 messages were sent, and 18,281 (8.2%) eligible women underwent testing after the intervention began. Within 3-6 months of receiving the message 10,478 (57.3%) women were tested. After 3 month the discontinuity was significant ($\beta = 0.26$, $p = 0.032$), inferring a significant 0.26% increased compliance. The increased ratio was 0.1% as the intervention continued, indicating a cumulative effect on compliance.

The message encouraging "immediate action" had the most significant impact (OR = 1.11, 95% CI [1.04, 1.18] (on compliance, while messages emphasizing "social norm" and "family responsibility" had the lowest compliance rates and were significantly less effective than the baseline message. The message focused on "health control" did not differ significantly from the baseline message.

Conclusion: Utilizing motivational messages offers a simple and cost-effective intervention with low associated costs. Tailoring messages based on various behavioral and socio-demographic characteristics may further enhance compliance.

Grants: This study was supported by the Ministry of Health, Digital Health Division

Conflict of Interest: Rotem Greenberg This study was supported by the Ministry of Health, Digital Health Division, Tom Muskat This study was supported by the Ministry of Health, Digital Health Division, Adi Berliner This study was supported by the Ministry of Health, Digital Health Division, Shay Ben-Shachar This study was supported by the Ministry of Health, Digital Health Division

P23.023.D Predictors of use of digital health tools in accessing genetic services: findings from the BRIDGE trial

Lingzi Zhong[1], *Molly Volkmar*[1], *Jemar Bather*[2], *Lauren Kaiser-Jackson*[1], *Richard Bradshaw*[3], *Rachelle Chambers*[4], *Daniel Chavez-*

Yenter[1;5], Amanda Gammon[1], Adrian Harris[2], Wendy Kohlmann[1], Rachel Monahan[4], Meenakshi Sigireddi[4], Ophira Ginsburg[6], Saundra Buys[1], Kensaku Kawamoto[3], Melody Goodman[2], Guilherme Del Fiol[3], **Kimberly Kaphingst**[1;5]

[1]University of Utah, Huntsman Cancer Institute, Salt Lake City, UT, United States; [2]NYU, School of Global Public Health, New York, NY, United States; [3]University of Utah, Department of Biomedical Informatics, Salt Lake City, UT, United States; [4]NYU Langone Health, Perlmutter Cancer Center, New York, NY; [5]University of Utah, Department of Communication, Salt Lake City, UT, United States; [6]National Cancer Institute, Rockville, MD, United States

Background/Objectives: Increasing use of digital health tools may affect access to genetic services. We examined whether patient engagement and sociodemographic characteristics predicted use of two digital health tools (patient portal and chatbot) and compared predictors of using pre-test genetic services via chatbot vs. standard of care (SOC).

Methods: We contacted 3,073 unaffected patients aged 25-60 years eligible for cancer genetic evaluation at University of Utah Health (UHealth) and NYU Langone Health (NYULH). Patients were randomized to receive either a patient portal messages with a hyperlink to a pre-test genetics education chatbot or information about scheduling a pre-test SOC appointment. Multivariable models identified predictors of patient portal use and pre-test genetic services use, stratifying by study arm and site.

Results: Opening study patient portal messages were significantly associated with having an assigned primary care provider (UHealth: SOC; NYULH: both arms); number of patient portal logins (UHealth and NYULH: both arms) and number of primary care visits in the previous three years (NYULH: SOC). Opting for pre-test genetic services was significantly associated with number of past patient portal logins (UHealth: chatbot; NYULH: both arms) and with having an assigned primary care provider (NYULH: SOC). Patient sociodemographic characteristics were not significantly associated with either outcome.

Conclusion: Patients' prior engagement with primary care and technology were related to their use of digital health tools to access genetic services, while their sociodemographic characteristics were not. Addressing general barriers to accessing healthcare may be important in interventions to increase access to genetic services.

Grants: U01CA232826, National Institutes of Health

Conflict of Interest: None declared

P23.024.A How people from migrant and refugee backgrounds experience perinatal genetic screening: a scoping review

Anaita Kanga-Parabia[1;2], *Belinda McClaren[1;2], Laura Biggs[1], Sharon Lewis[1;2], Erin Tutty[1;2], Alison Archibald[1;2]*

[1]Murdoch Children's Research Institute, Parkville, Australia; [2]The University of Melbourne, Paediatrics, Parkville, Australia

Background/objectives: People from migrant and refugee backgrounds often have poor healthcare experiences and outcomes. Understanding how this manifests in the perinatal genetic screening setting is a crucial step toward equitable care. We aimed to review evidence of perinatal genetic screening experiences of people from migrant and refugee backgrounds.

Methods: A scoping review was conducted using Joanna Briggs Institute framework. Search strategies were applied to Medline, Embase, and CINAHL databases to identify articles about experiences of perinatal genetic screening for people of migrant and refugee backgrounds. Evidence was synthesised using descriptive and content analysis.

Results: Of 6,925 unique articles identified, twenty-six articles were eligible and included for analysis. Most data were collected in Western countries, and participants were primarily born in Asia, South America, or Africa. Studies indicated that people from migrant and refugee backgrounds had varying awareness, knowledge and uptake of screening in different contexts. Several studies provided evidence of individuals being well informed and making decisions aligned with their values. However, there were examples of low awareness, knowledge, and uptake. Barriers and proposed strategies related to patient education, perceptions of autonomy, poor provider-patient interactions, and structural barriers to screening.

Conclusion: This scoping review revealed common barriers encountered in perinatal genetic screening care and related strategies to address these for people of migrant and refugee background. Understanding perspectives of people from migrant and refugee backgrounds experiences is foundational to ensuring equitable information and support about perinatal genetic screening for all.

Grants: Australian Government Research Training Program Scholarship

Conflict of Interest: None declared

P23.025.B Fragile X syndrome in Thailand: 30 years of experience

Pornprot Limprasert[1;2], *Oradawan Plong-On[2], Chariyawan Charalsawadi[1;2], Areerat Hnoonual[1;2]*

[1]Genomic Medicine Center, Faculty of Medicine, Prince of Songkla University, Songkhla, Thailand; [2]Department of Pathology, Faculty of Medicine, Prince of Songkla University, Songkhla, Thailand

Background/Objectives: Fragile X syndrome (FXS) is the most common form of X-linked intellectual disability (ID). This study aimed to share our 30 years of experience on the diagnosis of FXS and to determine the frequency of FXS in Thailand.

Methods: We reviewed our 3 cohort studies (1991-1999) and performed a retrospective review of 1,098 Thai patients (1,005 males and 93 females) with ID and developmental delay (DD) who had been referred for FXS DNA testing at Songklanagarind Hospital during 2000-2021. The DNA samples were analyzed using PCR to determine the number of CGG repeats and methylation status in the *FMR1* gene was used to confirm the diagnosis.

Results: Full mutation FXS was confirmed in 71/1,005 males (7.1%) and 3/93 females (3.2%). To our knowledge, this is the largest case of molecular genetic FXS testing from Thailand. The frequencies of FXS from 3 different cohorts of Thai male patients and periods of time were approximately 7.9%, 7.5% and 6.8% in Northern (2005-2008), Central (1997-1999) and Southern (1991-1999) Thailand, respectively. The overall frequency of FXS in male patients from our 30 years of experience was 7.1% (100/1,399, 1991-2021). Our earlier study found a frequency of fragile X carriers of 0.27% (1/359) in random blood donors of Thai women.

Conclusion: A review of the literature on worldwide frequencies of FXS showed that Thailand was among the countries with a high frequency of FXS, suggesting that molecular genetic screening for FXS (i.e., newborn or carrier screening) should be considered as a national policy in Thailand.

Conflict of Interest: None declared

P23.026.D Uptake of genetic counselling and multigene-panel testing among East Bavarian families at risk for hereditary breast and ovarian cancer with previous uninformative BRCA1/2 results

Heidi Stöhr[1], *Michaela Schläger*[2], *Christina Kiel*[1], *Ivana Holzhauser*[2], *Johannes Zschocke*[3], *Bernhard Weber*[1;2]

[1]*Universität Regensburg, Institut für Humangenetik, Regensburg, Germany;* [2]*Universitätsklinikum Regensburg, Institut für Klinische Humangenetik, Regensburg, Germany;* [3]*Medizinische Universität Innsbruck, Institut für Humangenetik, Innsbruck, Austria*

Background/Objectives: Genetic follow-up testing that comprises the analysis of newly identified high and moderate penetrance tumor susceptibility genes is increasingly integrated into clinical care for families at risk for hereditary breast and ovarian cancer with previous uninformative BRCA1/2 results. This study presents the implementation of a recontact program for follow-up multigene panel testing (MGPT) in East Bavarian families.

Methods: An invitation for pre-test counseling and MGPT was mailed to eligible individuals with a personal or family history of breast and ovarian cancer who had non-informative BRCA1/2 testing between 2008 and 2015. Factors influencing patient's receptivity to recontacting and genetic testing uptake were examined.

Results: Invitation letters were sent to 219 individuals, 41 (18.7%) were returned to sender and 111 addressees (50.7%) gave feedback. Of these, 10 (9%) were reported as deceased; 62 (56%) and four (4%), respectively, were scheduled for, or expressed interest in, a pre-test counseling appointment; 35 (31%) declined the offer for counseling or MGPT. Reminder letters positively influenced the response rate. A quarter of those contacted proceeded with MGPT. While demographic characteristics, tumor type and family cancer burden showed no effect on the response choices, attitudes towards science and research, previous unclear test results and recurrent tumor disease tended to influence the response rate. Family responsibility was the most notable motivator, difficult life situations, issues with health insurance and doubts about benefits were identified as barriers for MGPT.

Conclusions: This study shows that well-maintained databases and a low-barrier recontact approach supported by coordinators and genetic counsellors contribute to optimize future genetic follow-up programs.

Conflict of Interest: None declared

P23.027.A Capturing interdisciplinary clinicians' psychosocial and psychological experiences providing care for children with severe neurogenetic conditions

Suzanne Nevin[1;2], *Fleur Le Marne*[2], *Erin Beavins*[2], *Rebecca Macintosh*[2], *elizabeth pamer*[2], *rani sachdev*[2], *kenneth nunn*[3], *Annie Bye*[1;2]

[1]*UNSW Sydney, school of clinical medicine, Sydney, Australia;* [2]*Sydney Children's Hospital, Randwick, Randwick, Australia;* [3]*The Children's Hospital at Westmead, Westmead, Australia*

Consortium: ClinEquip Advisory group

Background/Objectives: Severe neurogenetic conditions present with heterogenous aetiologies. Clinicians exert strenuous effort undertaking complex prognostication and management, in the face of an unprecedented explosion of genetic diagnoses with limited natural history data for newly-described conditions. Despite this burgeoning scale and density of neurogenetic clinical care, supports for clinicians navigating ongoing uncertainties and balancing families' realistic hopes are lacking. This can impart unabating professional and intrapersonal stress on clinicians over substantial stages of their career.

The primary aim of this study was to investigate paediatric clinicians' psychosocial and psychological experiences navigating care for children with severe neurogenetic conditions. The secondary aim was to identify clinician preferences for tailored and codesigned supports.

Methods: We conducted a qualitative descriptive study with interdisciplinary clinicians using a purposeful sampling recruitment strategy. Twenty-four participants with expertise caring for children with severe neurogenetic conditions completed in-depth, semi-structured interviews. Interviews were transcribed, deidentified and an inductive thematic analysis was performed.

Results: Thematic analysis elicited interrelated themes. Clinicians experienced immense professional barriers providing patient-centred care across fragmented healthcare contexts. Physical, emotional, and psychological impacts were attributed to inadequate reflective practice training and a paucity of integrated resources. Multi-pronged strategies incorporating psychoeducation, interdisciplinary peer mentorship opportunities, and psychological resources to build clinician reflective practice skills providing complex care in an advancing era of medicine, were prioritised.

Conclusion: This study provides novel and in-depth insight into clinicians' experiences navigating care for children with severe neurogenetic conditions. Results will be used to inform the co-development of multipronged resources tailored for interdisciplinary clinicians.

250/250

Conflict of Interest: None declared

P23.028.B Genetic healthcare professionals' experiences with the communication about inherited genetic conditions from parents to their young children: a scoping review

Catarina Seidi[1;2;3], *Liliana Sousa*[1], *Álvaro Mendes*[2;3]

[1]*University of Aveiro, Aveiro, Portugal;* [2]*Centro de Genética Preditiva e Preventiva, Porto, Portugal;* [3]*Instituto de Biologia Molecular e Celular, Porto, Portugal*

Background/Objectives: Discussing inherited genetic conditions (IGCs) with young children poses significant challenges, and parents often seek guidance from genetic healthcare professionals (GHPs) on the best approach. Despite this communication being a key aspect of GHPs' roles, there is limited research exploring their experiences. This scoping review aims to map the evidence on GHPs' experiences in parent-young children (≤24 years old) communication about IGCs.

Methods: This review followed the JBI and PRISMA guidelines (https://inplasy.com/inplasy-2023-11-0009/). It included peer-reviewed primary research articles published in English since 1997. Studies that reported GHPs' perspectives on parent-children communication about IGCs were included. Four databases (Web of Science, PubMed, PsycINFO, and Scopus) were searched, and five articles met the eligibility criteria. These were qualitative, using interviews and focus-groups, and published from 2010-2020 in Australia (2), Belgium, USA, and the UK.

Results: The analysis suggested that GHPs generally feel responsible for facilitating parent-children communication about IGCs. They reported guiding parents in informing children, emphasizing early and age-appropriate communication. Their approach involves considering parents' and children's needs, IGCs' characteristics, and contextual factors. Strategies to facilitate communication include assessing family dynamics through focused questioning and providing parents with reliable educational materials. GHPs perceive their roles according to their professional background and guidelines; however, they

report constraints such as time limitations and insufficient training.

Conclusion: This review provides insight that may help to clarify GHPs' scope of practice, roles and needs in facilitating parent-children communication about IGCs. Further research is needed to understand how to improve practice and provide better support for these families.

Conflict of Interest: None declared

P23.029.C Acceptability of cascade-testing approaches for familial hypercholesterolaemia (FH): qualitative study of health-care professional & patient experiences

Laura Condon[1], *Nadeem Qureshi*[1], *Ralph Akyea*[1], *Kate Haralambos*[2], *Barbara Iyen*[1], *Phil Rowlands*[3], *Melanie Watson*[4], *Joe Kai*[1]

[1]*University of Nottingham;* [2]*University Hospital of Wales;* [3]*Patient Representative;* [4]*University Hospital Southampton NHS Foundation Trust*

Background/Objectives: In the UK, direct and indirect methods of contacting relatives for FH testing vary with lack of evidence on patient and relative experiences with regard to uptake of genetic testing. This study explores the experiences and perspectives of index patients, cascade relatives, and health professionals, to assess the acceptability of both contact approaches.

Methods: Patients, relatives and health professionals were purposefully sampled across three UK regions (Urban/rural/mixed) to reflect: 1) sociodemographic range, 2) different FH assessment & testing experiences, 3) contact tracing within or outside geographic region.

Semistructured one-to-one interviews explored the views and experiences of all respondents following informed consent. Data was transcribed verbatim and framework analysis was used to derive themes across all respondents and regions.

Results: A direct & indirect 'hybrid' model of communication taken on a case-by-case basis for each family was regarded most effective by patients and professionals. A FH specialist nurse-led model with enhanced communication and continuity of care throughout the cascade-testing pathway was strongly supported.

Professionals stated family communication should focus on FH as treatable to prevent major cardiac disease. Patients and professionals suggested barriers to uptake of testing could be reduced by removing the requirement for GP referral, enabling self-referral, and limiting the number of appointments. Continuity of care with a single FH professional for ongoing family support was valued particularly by parents of young children who underwent testing.

Conclusion: A service-led direct-contact approach was more reliable, but combining direct and indirect approaches, guided by index patients and family relationships, may be more acceptable.

Grants: NIHR-HTA programme

Conflict of Interest: None declared

P23.030.D Compliance and satisfaction with BRCA surveillance in specialized clinics vs. community follow-up.

Moran Echer[1;1], *Rita Vortman*[2], *Amihood Singer*[3], *Lena Sagi-Dain*[1]

[1]*Carmel Medical Centre, Haifa, Israel;* [2]*Rambam Health Care Campus, Haifa, Israel;* [3]*Israel Ministry of Health, Jerusalem, Israel*

Background/Objectives: Recommended surveillance for BRCA1/2 carriers manual breast exams, imaging, and gynecological follow-up, alongside the consideration of prophylactic mastectomy and salpingo-oophorectomy. This study aimed to assess adherence to these protocols among BRCA1/2 carriers and compare it between dedicated carrier clinics and community healthcare.

Methods: Conducted as a cross-sectional study, we distributed an anonymous questionnaire within the "Good BRCA Genes - Support and Information Group for BRCA Carriers" association, evaluating adherence to recommended surveillance and satisfaction with follow-up aspects.

Results: Among the 682 surveyed BRCA carriers, 68.5% reported full adherence to recommended medical follow-up. Non-adherence reasons included bureaucracy challenges, scheduling difficulties, timing uncertainties, and difficulty remembering examination dates. Satisfaction levels with appointment availability, scheduling, contact persons, and general practitioners' knowledge of BRCA mutations were below 50%. The 417 women monitored in dedicated breast clinics reported significantly higher optimal adherence (78.3% vs. 53.6%, $p < 0.0001$) and greater satisfaction with appointment availability (63.7% vs. 25.0%, $p < 0.0001$), appointment scheduling process (58.1% vs. 24.7%, $p < 0.0001$), availability of breast surgeons/gynecology specialists (67.4% vs. 50.8%, $p < 0.0001$), and availability of a contact person for consultations between appointments (53.5% vs. 20.8%, $p < 0.0001$).

Conclusion: These findings underscore the benefits of surveillance in dedicated BRCA1/2 clinics, emphasizing closer monitoring and increased satisfaction. Given the limited availability of such clinics and the rising number of BRCA1/2 carriers, our findings suggest the necessity for additional dedicated clinics and consideration of alternative solutions to enhance surveillance, such as healthcare professional training, digital tools, and artificial intelligence.

Grants: None

Conflict of Interest: None declared

P23.031.A Comparing experiences and enhancing communication: a study of BRCA1/2 carriers identified through screening versus genetic counseling

Rotem Greenberg[1], **Amir Peleg**[2], *Moran Echer*[2], *Amihood Singer*[3], *Lena Sagi-Dain*[2]

[1]*Clalit, Tel Aviv-Yafo, Israel;* [2]*Carmel Medical Centre, Haifa, Israel;* [3]*Israel Ministry of Health, Jerusalem, Israel*

Background/Objectives: This study aimed to evaluate the experiences of women carrying BRCA1/2 mutations identified through population-based screening, in comparison to women diagnosed following conventional genetic counseling, either due to personal or familial history of cancer. The evaluated outcomes included quality of explanations given before the genetic testing and following positive test result.

Methods: This cross-sectional survey employed an anonymous questionnaire distributed through the "Good Genes - a support and information group for BRCA carriers" association from January to March 2023. The questionnaire included Likert scale and open-ended questions.

Results: The questionnaire was completed by 110 respondents who had been diagnosed through BRCA screening, and 444 women who had undergone genetic testing after conventional genetic counseling based on personal or familial cancer history. Among those in the screening group, 45.5% rated the quality of pretest explanations as unsatisfactory, compared to 27.4% in the genetic counseling group ($p = 0.0005$). In terms of result delivery, the screening group reported higher instances of inappropriate timing (61.8% vs. 40.3%, $p < 0.0001$), suboptimal mode of delivery

(55.5% vs. 37.5%, p = 0.0008) and suboptimal quality in post-test explanations (51.4% vs. 33.9%, p = 0.0006), as well as elevated stress levels (74.3% vs. 64.3%, p = 0.043).

Conclusion: The findings of this survey underscore the pressing need for enhancements in pre-test explanation, as well as the post-test counseling for positive results, within the realm of BRCA screening.

Grants: None

Conflict of Interest: None declared

P23.032.B VisGen: A web-based approach for personal polygenic score calculation and visualization of absolute risk prediction for 18 different diseases

Nicolas Walk[1], Julian Wanner[1;2], Asma Aborobb[1], Mohammad Abdullah[1], Christoph Lippert[1;3], Samuli Ripatti[2], Henrike Heyne[1;2;3]

[1]Hasso Plattner Institute for Digital Engineering, Potsdam, Germany; [2]Institute for Molecular Medicine Finland, Helsinki, Finland; [3]Hasso Plattner Institute for Digital Health, Icahn School of Medicine at Mount Sinai, New York, United States

Consortium: INTERVENE

Background/Objectives: Polygenic scores (PGSs) have become an important part of precision medicine in diagnosis and prevention. However, their application and implementation are complex. Their availability to interested individuals e.g. as personal health information is thus limited.

Methods: Here we present VisGen, a secure, web-based application that enables users to upload their personal genotype data and visualize absolute risk estimates for 18 diseases without prior knowledge. In our tool, we integrate genotype imputation, PGS calculation and ancestry normalization. Calculated PGSs are translated into personal absolute disease risk estimates using a framework described in Jermy et al., medRxiv, 2023 taking into account overall disease prevalence, sex, age, ancestry, and country of origin (with five available options). VisGen integrates a React front-end with a Spring Boot Java backend application, offering asynchronous REST endpoints to initiate and manage PGS calculations and imputation. For imputation, Beagle is used along with bcftools. For PGS calculation and ancestry normalization, pgsc_calc (https://github.com/PGScatalog/pgsc_calc) is incorporated. Visualization and absolute risk estimation are performed by VisGen.

Results: We validated and tested various different methods e.g. for ancestry normalization and imputation using the UK Biobank cohort with approximately 550,000 individuals. We find that BEAGLE imputation improves PGS significantly more than mean imputation by PLINK2.

Conclusion: We present a user friendly web tool that enables interested individuals to easily calculate and visualize their PGSs and absolute disease risks making research results more accessible to the public.

Grants: Horizon 2020 (101016775), German Research Foundation (516649954)

Conflict of Interest: None declared

P23.034.D Unanticipated genotypes identified in a healthy population cohort

Yasmin Bylstra[1;2], weng khong lim[1;3;4], Jing Xian Teo[1], Melody Menezes[2;5], Jan Hodgson[2], saumya shekhar jamuar[1;6;7], Patrick Tan[4;8;9], David Amor[2;10]

[1]SingHealth Duke-NUS Institute of Precision Medicine, Singapore; [2]The University of Melbourne, Department of Paediatrics, Australia; [3]Duke-NUS Medical School, Cancer and Stem Cell Biology, Singapore; [4]Genome Institute of Singapore, Singapore; [5]Monash Ultrasound for Women, Australia; [6]KK Women's and Children's Hospital, Genetics Service, Singapore; [7]Duke-NUS Medical School, Paediatric Academic Clinical Programme, Singapore; [8]Duke-NUS Medical School, Singapore; [9]Precision Health Research Singapore, Singapore; [10]Murdoch Children's Research Institute, Australia

Background/Objectives: The expansion of genomics provides opportunity to screen individuals beyond clinical indication, yet the classification of genomic variants and implications for health outcomes in this context are still emerging. We analysed clinically relevant variants and expected clinical impact in a healthy population.

Methods: Whole genomes from 9637 healthy unrelated research-consented participants in Singapore were analysed, focusing on 1619 genes associated with severe paediatric disease and 73 clinically actionable genes. Associations between variants and expected phenotypes were assessed, including medical and family histories where available.

Results: After considering protein impact, clonal haematopoiesis, inheritance and participant demographics for 336 unique variants, 288 variants occurring in 529 participants were analysed further. Most identified variants were associated with incomplete penetrance (e.g. dilated cardiomyopathy), mild phenotype (e.g. cystinuria), late onset (e.g. Fabry disease) or potentially missed phenotype (e.g. Hajdu-Cheney syndrome). However, 22 participants had variants associated with highly penetrant disease (*APC*, *BRCA1*, *MEN1*, *MLH1*, *MSH2*, *TP53*, *VHL*) and 11 with severe paediatric disease predicted to be symptomatic (e.g. muscular dystrophy).

Conclusion: Despite a cohort selected for absence of pre-existing health conditions, individuals were identified carrying variants associated with highly penetrant or severe paediatric conditions. Further work is required to examine for subtle clinical signs. This study revealed the challenge of predicting clinical outcomes from genotype-derived screening and emphasises the importance of expanding phenotype characterisation which is highly relevant in population genomic and carrier screening.

Grants: Singapore National Precision Medicine Industry Alignment Fund, EYE ACP-PRISM Precision Medicine Initiative Fund

Conflict of Interest: None declared

P23.035.A ERN ITHACA, a European network with a focus on educational activities on rare neurodevelopmental disorders and multiple congenital malformations.

Klea Vyshka[1], Jolanda Van Golde[2], Anne Hugon[1], Marianne Le Dref[1], Nicholas Szeto[1], Gerieke Been[3], Sofia Douzgou HOUGE[4], Andreas Dufke[5], Agnies van Eeghen[6], Laurence Faivre[7], Jean-Marie Jouannic[8], Tjitske Kleefstra[2], Giovanni Mosiello[9], Alessandra Renieri[10], Gijs Santen[11], Morris Swertz[3], Katalin Szakszon[12], Marco Tartaglia[13], Zeynep TÜMER[14], Giuseppe Zampino[15], Christiane Zweier[16], Alain Verloes[1]

[1]ERN ITHACA, University Hospital Robert Debré, Clinical Genetics Department, Paris, France; [2]Department of Genetics, Erasmus Medical Centre, Rotterdam, Netherlands; [3]Genomics Coordination Center, University Medical Centre Groningen, Groningen, Netherlands; [4]Haukeland University Hospital / Health Bergen, Avdeling for medisinsk genetikk, Haukeland, Norway, Haukeland, Norway; [5]University Hospital Tübingen, Institut für Medizinische Genetik und angewandte Genomik, Tübingen, Germany; [6]Department of Pediatrics, Emma Children's Hospital, Amsterdam University Medical Center, Amsterdam, Netherlands; [7]Dept of Genetics and Centres of Reference for Development disorders and intellectual disabilities, FHU TRANSLAD and GIMI Institute, University Hospital Dijon, Dijon, France; [8]Hôpital Armand-Trousseau, Fetal Medicine, Paris, France; [9]Bambino Gesù Pediatric Hospital, Surgery, Urology and Neuro-Urology, Rome, Italy; [10]Med Biotech Hub and Competence Center,

Università di Siena, Medical Biotechnologies, Siena, Italy; [11]Department of Clinical Genetics Leiden University Medical Center, Leiden, Netherlands; [12]Faculty of Medicine, Departament of Pediatrics, University of Debrecen, Debrecen, Hungary; [13]Genetics and Rare Diseases Research Division, Ospedale Pediatrico Bambino Gesù, Rome, Italy; [14]Copenhagen University Hospital, Rigshospitalet, Clinical Genetics, Copenhaguen, Denmark; [15]Università Cattolica del Sacro Cuore, Department of Woman and Child Health, Center for Rare Diseases and Birth Defects, Institute of Pediatrics, Fondazione Policlinico Universitario Agostino Gemelli IRCCS, Rome, Italy; [16]Universitätsklinik und Poliklinik für Medizinische Onkologie, Universitätsspital Inselspital, Bern, Switzerland

Consortium: ERN ITHACA

Background/Objectives: ERN ITHACA is the European Reference Network for Intellectual disability, TeleHealth, Autism and Congenital Anomalies. One of ERN ITHACA's main objectives is to develop educational activities on rare neurodevelopmental disorders (NDD) and multiple congenital malformations by supporting conferences dedicated to developmental anomalies.

Methods: To achieve this objective, ERN ITHACA organises three flagship events, dedicated to health professionals and lay persons alike. EuroDysmorpho series bring together young clinical geneticists and trained dysmorphologists to share their experiences and present clinical challenges (co-organised with ESHG-Y). This conference is open to any presentation in the field of human development. EuroNDD is a 2-day workshop on care and research of genetic neurodevelopmental disorders. It focuses on covering themes like pathways, mechanisms of disease, emerging therapies and diagnostics. The Winter School on Fetal Diagnosis aims to address a learning gap in fetal phenotyping, genetic analysis, and data interpretation. As a single-day course it includes basic lectures and in depth-workshops on phenotyping, prenatal genomic testing, and the implications for genetic counselling.

Results: The EuroDysmorpho series has brought together more than 100 participants from all over Europe in its last edition, held in September 2023 in Lisbon. EuroNDD has brought together in April 2023 around 250 participants, whereas the Winter School is available to 60 participants only to preserve the interactive learning experience.

Conclusion: EuroDysmorpho and the Winter School yearly events. The next editions take place respectively in September 2024 and December 2024. EuroNDD is a biennial event. Follow ern-ithaca.eu for more information.

Grants: Grant Agreement 101085231.

Conflict of Interest: None declared

P23.036.B Facilitators and Barriers to family communication about Li-Fraumeni Syndrome

Florentia Fostira[1;2], *Marion McAllister*[2], *Frauke Pelz*[2]

[1]NCSR 'Demokritos', Human Molecular Genetics Laboratory, Athens, Greece; [2]Cardiff University, School of Medicine, Centre for Medical Education, Cardiff, United Kingdom

Background/Objectives: Effective communication of the genetic information related to cancer predisposition within families is important for cancer screening and prevention, yet numerous factors can impede this process. This study aimed to investigate the facilitators and barriers to genetic information communication among Greek families with Li-Fraumeni syndrome (LFS).

Methods: In-depth, semi-structured interviews were conducted, followed by thematic analysis to identify emerging facilitators and barriers to communication. Patients with LFS were recruited after invitation from treating physicians in Greece.

Results: Six patients were included. Four communication facilitators were identified and explored: (a) the desire to protect relatives from cancer, (b) support from family and health professionals, (c) positive relationships with specific family relatives and (d) the close-knit nature of the core Greek family, sculptured by strong cultural factors. Conversely, barriers included: (a) the physical and emotional burden specifically associated with LFS, (b) reluctance to cause distress or harm, (c) difficulty in conveying complex information and (d) practical concerns and issues. The strong familial bonds, characteristic of Greek families emerged as a significant facilitator, not described in previous studies, potentially applicable to other Mediterranean populations. A notable barrier to communication was the burden associated specifically with LFS, a novel finding with potential syndrome-specific implications.

Conclusion: This study contributes valuable insights to the literature on family communication in hereditary cancer syndromes, highlighting the unique challenges faced by individuals with LFS, for which the literature is scarce. Understanding the complexities of disseminating genetic information within families can inform strategies for improving communication and support.

Grants: N/A

Conflict of Interest: Florentia Fostira Research Grant received from AstraZeneca; not related to this work, AstraZeneca, GSK, Roche; not related to this work, AstraZeneca ; not related to this work, Marion McAllister: None declared, Frauke Pelz: None declared

P23.037.C Clinical utility of testing a large gene panel for hereditary breast cancer in different ancestry groups

Tabitha Osler[1;2], *Mardelle Schoeman*[3], *Jean-Tristan Brandenburg*[1;4], *Wenlong Carl Chen*[1;4;5], *Michael Urban*[2;3], *Christopher Mathew*[1;2]

[1]University of the Witwatersrand, Sydney Brenner Institute for Molecular Bioscience, Faculty of Health Science, Johannesburg, South Africa; [2]University of the Witwatersrand, Division of Human Genetics, Faculty of Health Science, Johannesburg, South Africa; [3]University of Stellenbosch, Division of Molecular Biology and Human Genetics, Faculty of Medicine and Health Sciences, Cape Town, South Africa; [4]University of the Witwatersrand, Strengthening Oncology Services Research Unit, Faculty of Health Sciences, Johannesburg, South Africa; [5]National Health Laboratory Service, National Cancer Institute, National Institute of Communicable Diseases, Johannesburg, South Africa

Background/Objectives: The optimal gene panel size for hereditary breast cancer testing remains uncertain, with larger panels detecting more variants of unknown significance (VUS), and pathogenic variants (PVs) in genes lacking management guidelines. We evaluated the impact of expanding the gene panel on testing outcomes across three ancestry groups.

Methods: 331 South African females of African, European, or Mixed ancestry with breast cancer had genetic testing. Initially, all participants were tested with a 9 gene panel containing established breast cancer susceptibility genes. Those without detected PVs underwent testing of an additional 75 genes. The prevalence of PVs and VUS between the panels was compared across the three ancestries.

Results: The 9 gene panel yielded 58 PVs with a further 12 detected by the 75 gene panel. The prevalence of PVs was similar in the three ancestry groups for both panels. Only 2/12 PVs in the 75 gene panel were in genes with confirmed associations with breast cancer and management guidelines for the disease. In total, 90 VUS were detected with the 9 gene panel, and 524 using the 75 gene panel. The mean number of VUS per participant in the 75 gene panel was 2.23 in Mixed Ancestry and 1.70 in African

ancestry participants, significantly higher than the mean of 0.95 found in those with European ancestry.

Conclusion: Expansion of the gene panel yielded a limited number of clinically actionable variants and a substantial increase in VUS detection, particularly in African and Mixed ancestry populations.

Grants: Cancer Association, South Africa Research Grant
REACCT-CAN, Science for Africa Foundation

Conflict of Interest: Tabitha Osler University of the Witwatersrand

MADIVA Research Study, Mardelle Schoeman: None declared, Jean-Tristan Brandenburg: None declared, Wenlong Carl Chen: None declared, Michael Urban: None declared, Christopher Mathew: None declared

P23.038.D Identification of pediatric cancer predisposition syndromes through genetic tumor testing and the role of molecular tumor boards (MTB)

Estela Carrasco López[1;2], *Marta Sese Faustino*[3], *Adrià López-Fernández*[1;2], *Anna Maria Cueto-González*[4], *Laura Trujillano*[4], *Berta Campos*[5], *Alejandro Moles-Fernandez*[5], *Elena Martinez Saez*[3], *Marta Garrido Pontnou*[3], *Rosa Somoza López de Haro*[3], *Raquel Hladun Alvaro*[6], *Lorena Valero Arrese*[6], *Constantino Sábado*[6], *Amaia Lasa-Aranzasti*[4], *Mara Cruellas*[1;2], *Aroa Soriano*[7], *Javier Hernandez Losa*[3], *Judith Balmaña*[1;2], *Lucas Moreno Martin Retortillo*[1]

[1]*Children's Hospital and Vall d'Hebron Women's Hospital, Medical Oncology, Barcelona, Spain;* [2]*VHIO Institut d'Oncologia, Medical oncology, Barcelona, Spain;* [3]*Vall d'Hebron University Hospital, Pathology Departament, Barcelona, Spain;* [4]*Vall d'Hebron University Hospital, Genetics, Barcelona, Spain;* [5]*Vall d'Hebron University Hospital, Genetic molecular, Barcelona, Spain;* [6]*Children's Hospital and Vall d'Hebron Women's Hospital, Pediatrics Oncology and Hematology, Barcelona, Spain;* [7]*VHIR - Vall d'Hebron Institut de Recerca, Pediatric Oncology Precision Medicine, Barcelona, Spain*

Background/Objectives: Identifying genetic variants in pediatric cancer predisposition syndromes (PCPS) is crucial for prognosis, treatment decisions and family counseling. Somatic tumor testing through NGS platforms and multidisciplinary MTBs in daily routine facilitate patient access to targeted therapies. The study's goal is to evaluate the role of somatic NGS testing and MTBs to identify potential PCPS.

Methods: Tumor genetic alterations in 170 pediatric cancer samples were assessed from november/21-december/23 with Oncomine Childhood Cancer Research Assay panel from ThermoFisher Scientific. After MTB review, pathogenic variants with a VAF ≥ 30% in a PCPS-associated gene were identified for further analysis through the Hereditary Plus OncoKitDx germline panel.

Results: Overall, 13.5% (23/170) of patients harbored a tumor genetic alteration potentially associated with a PCPS: *TP53 (10), DICER1 (2), SMARCB1 (2), PTEN (2), CDKN2A (1), NF1 (1), PTCH1 (1), RB1 (1), RET (1), TSC2 (1), WT1 (1)*. Among them, in 8 cases there were not possibility to test, and 15 underwent germline testing. Of those tested, 33% (5/15, 5/170, 3%) of cases tested were positive: *RB1* (1), *RET* (1) *TP53* (2 and 1 mosaicism). Cascade testing in relatives revealed de novo variants in 83% (5/6 cases). Parents or patients in reproductive ages received reproductive counseling.

Conclusion: Somatic genetic tumor testing identified almost 3% of patients with germline variants associated with PCPS in the absence of family history. MTB provide a multidisciplinary resource to cope with these findings and influence in their medical management.

Grants:

Conflict of Interest: None declared

P23.039.A Enhancing the communication in genetic counseling: The impact of educational videos on doctor-patient relationship.

Sebastian Sailer[1], *Julia Mahal*[2], *Carlotta Mayer*[2], *Maja Hempel*[1], *Hannah Wallaschek*[3], *Johanna Tecklenburg*[4], *Melanie Wittenberg-Marangione*[3], *Christian Schaaf*[1], *Beate Ditzen*[2]

[1]*Heidelberg University Hospital, Institute of Human Genetics, Heidelberg, Germany;* [2]*Heidelberg University, Institute of Medical Psychology, Heidelberg, Germany;* [3]*Medizinische Hochschule Hannover, Institute of Human Genetics, Hannover, Germany;* [4]*Bioscientia Institut für med. Diagnostik GmbH, Ingelheim, Germany*

Background: The importance of genomic data in patients care procure an increasing need in genetic analysis. According to German law, informed consent must be obtained prior to genetic testing. Our study dives into using educational videos to aid and improve doctor-patient communication in genetic counseling.

Objectives: Develop topic-driven video sequences and assess their impact on patient satisfaction and doctor-patient relationship by providing upfront information.

Methods: We produced five animated, short educational videos. Topics covered: 'Genetic Consultation Process,' 'Informed Consent,' 'Breast Cancer,' 'Genome Sequencing,' and 'Face2Gene.' Subjects included patients with consultations for 1. suspected genetic susceptibility to breast cancer (BC) or 2. with syndromic conditions warranting exome sequencing (SYN). Participants were randomly assigned to either an experimental group, exposed pre-consultation to the videos at home, or a control group without video exposure. Post-consultation, an assessment of patients and doctors was conducted, to gauge satisfaction levels with the consultation process and quality of doctor-patient relationship.

Results: Patients rated the videos highly understandable and useful. Overall, no significant differences were shown in patient satisfaction and doctor-patient relationship between experimental and control group. However, the BC subgroup displayed increased satisfaction and physicians noted reduced workload. The SYN subgroup reported decreased satisfaction and a less positive doctor-patient relationship.

Conclusion: Our educational videos can improve patient satisfaction and relieve doctors' workload in standardized consultation settings such as predisposition to BC. However, in more complex consultations, like those involving patients with syndromic conditions, the individual doctor-patient conversation appears to take precedence.

Grants: German Federal Ministry of Health

Conflict of Interest: None declared

P23.040.B Psychological impact of personalized breast cancer (BC) risk estimation and comparison of two results delivery models (PRiSma study)

Adrià López-Fernández[1], *Eduard Perez*[1], *Esther Darder*[2], *Consol Lopez*[3], *Adriana Bareas*[4], *Mireia Cartro*[5], *Guillermo Villacampa*[6], *Estela Carrasco López*[1], *Ariadna Roque*[2], *Rosa Alfonso*[3], *Ana Raquel Jimenez-Macedo*[7], *Mara Cruellas*[1], *Noemi Tuset*[8], *Monica Pardo*[9], *Maite Torres*[1], *Anna Vallmajo*[8], *Laura Duran-Lozano*[4], *Gemma Llort*[5], *Teresa Ramon y cajal*[3], *Joan Brunet*[2], *Judith Balmaña*[1]

[1]*Vall d'Hebron University Hospital, Medical Oncology, Barcelona, Spain;* [2]*Catalan Institute of Oncology, Hereditary Cancer Program, Girona, Spain;* [3]*Hospital Santa Creu i Sant Pau, Medical Oncology, Barcelona, Spain;* [4]*Vall d'Hebron Institute of Oncology, Hereditary Cancer Group, Barcelona, Spain;* [5]*Consorci Corporació Sanitària Parc*

Taulí, Medical Oncology, Sabadell, Spain; [6]Vall d'Hebron Institute of Oncology, Biostatistics Group, Barcelona, Spain; [7]Consorci Sanitari de Terrassa, Medical Oncology, Terrassa, Spain; [8]Hospital Universitari Arnau de Vilanova, Medical Oncology, Lleida, Spain; [9]Hospital del Mar, Medical Oncology, Barcelona, Spain

Background/Objectives: In women with a family history of BC without identified genetic alterations, the BOADICEA model estimates BC risk considering multiple factors. The objectives are: to (1) examine acceptance of personalized risk estimation disclosure, (2) investigate its psychological impact, (3) compare satisfaction between two results delivery methods.

Methods: Assessment of 10-year BC risk with BOADICEA model and classification as high, moderate, or average. Satisfaction, psychological impact (MICRA scale), cancer worry, and anxiety were assessed in all groups. Moderate and average risk women were randomly assigned to results disclosure in-person or by video.

Results: Out of 663 participants, 93% accepted results disclosure. Seven percent were high risk, 56% moderate, and 37% average. High-risk individuals had higher total MICRA scores compared to moderate and average (mean = 29, 16.7, 13.4, respectively,$p < 0.05$). Results were consistent in distress, positive experiences, and cancer worry. However, no significant differences were observed in uncertainty, anxiety, or perceived benefit.

In-person disclosure was associated with higher satisfaction (95% vs. 71%) and fulfillment of expectations (mean = 4.3 vs. 3.9) than video (all $p < 0.01$). Video disclosure resulted in higher total MICRA scores (mean = 17.7 vs. 13.2), distress and uncertainty, and lower positive experiences scores (all $p < 0.05$). No significant differences were found in cancer worry, anxiety or perceived benefit.

Conclusion: A high acceptance of personalized breast cancer risk estimation was observed, with higher psychological impact and cancer worry in high-risk individuals. In-person disclosure proved more satisfying and aligned with expectations than video delivery. However, video disclosure remains a viable approach to be considered for large-scale implementation.

Grants: Funded by ISCIII (PI19/01195)

Conflict of Interest: None declared

P23.041.C Well-being and self-care strategies for cardiovascular genetic counsellors: a qualitative study

Laura Yeates[1;2;3], *Lucas Mitchell*[4;5], *Ivan Macciocca*[6;7], *Helen Mountain*[8;9], *Mary-Anne Young*[4;5], *Colleen Caleshu*[10], *Alison McEwen*[9], *Jodie Ingles*[1;3;11]

[1]Garvan Institute of Medical Research, Genomics and Inherited Disease Program, Sydney, Australia; [2]The University of Sydney, Faculty of Medicine and Health, Sydney, Australia; [3]Royal Prince Alfred Hospital, Cardiology Department, Sydney, Australia; [4]Garvan Institute of Medical Research, Clinical Translation and Engagement Platform, Sydney, Australia; [5]St Vincent's Healthcare Clinical Campus, School of Clinical Medicine, Sydney, Australia; [6]Victorian Clinical Genetics Service, Melbourne, Australia; [7]University of Melbourne, Melbourne, Australia; [8]Genetics Services of Western Australia, Perth, Australia; [9]University of Technology Sydney, Graduate School of Health, Sydney, Australia; [10]Genome Medical, United States; [11]University of New South Wales, UNSW Medicine and Health, Sydney, Australia

Background: Repeated exposure to challenging clinical situations impacts healthcare professionals' well-being. Genetic counsellors assist individuals to understand and adapt to difficult medical information, which often has implications for close family members. In the cardiac setting, managing families with profound grief and trauma following sudden cardiac death can be difficult, with potential for burnout and compassion fatigue.

Aim: To explore the impact of cardiovascular genetic counselling practice on genetic counsellor well-being and describe self-care practices.

Methods: Participants were recruited through the Australasian Society of Genetic Counsellors. Semi-structured interviews were conducted exploring challenges in cardiovascular genetic counselling practice, supervision and self-care. Interviews were recorded, transcribed and analysed using reflexive thematic analysis. Self-reported demographics, psychological well-being and burnout measures were collected.

Results: Eighteen genetic counsellors participated. Median interview length was 54 minutes (range 40-74). All participants were female and 83% of European ethnicity. Few reported mild or moderate depression (17%), mild or moderate anxiety (22%) and none (0%) had scores indicating stress. Three (17%) had scores indicating burnout. Thematic analysis identified three themes: (1) cardiac genetic counselling is different, not harder or easier; (2) workplace pressures affect well-being; (3) a self-care "tool kit" is necessary with supervision a key component.

Conclusion: Cardiovascular genetic counsellor practice and workload can affect well-being. Genetic counsellors have a self-care 'tool kit' that includes supervision, to help maintain well-being.

Grants: LY receives a co-funded National Heart Foundation of Australia/ National Health and Medical Research Council (NHMRC) PhD Scholarship (#102568/#191351). JI receives a National Heart Foundation of Australia Future Leader Fellowship (#106732).

Conflict of Interest: Laura Yeates LY is the recipient of a co-funded National Heart Foundation of Australia/ National Health and Medical Research Council (NHMRC) PhD Scholarship (#102568/#191351)., Genetic Heart Disease Clinic, RPAH/ Centenary Institute, Lucas Mitchell Employee of the Garvan Institute, Ivan Macciocca Employee of VCGS, Helen Mountain Employee of UTS & GSWA, Mary-Anne Young Employee of the Garvan Institute, Colleen Caleshu CC is and employee of an has stock options in Genome Medical, Genome Medical, Alison McEwen Employee of UTS, Jodie Ingles JI receives research grant support from Bristol Myers Squibb unrelated to this work, JI is the recipient of a National Heart Foundation of Australia Future Leader Fellowship (#106732)., Employee of the Garvan Institute

P23.042.D Tuning into British South Asian voices on health and genetics - a springboard for effective educational intervention.

Shwetha Ramachandrappa[1], *Robin Lerner*[2], *Shapna Hussain*[2], *Nishat Safa*[2]

[1]King's College London Guy's Campus, United Kingdom; [2]Queen Mary University of London, Blizard Institute, United Kingdom

Consortium: The Genes and Health research team

Background/Objectives: British South Asians have consistently been shown to have poorer health outcomes than other ethnic groups. Engagement with health services and with health research is poor. Low levels of health awareness are repeatedly cited as a critical modifiable factor within the British South Asian community. Having a detailed understanding of key knowledge gaps within the community will be foundational to the success of efforts to address health inequalities.

Methods: Semi structured interviews and focus groups were conducted with participants in the Genes and Health study, a large genetics research study which has recruited over 60K British South Asians in community settings.

Results: Even within this research aware subset of the community there was limited understanding of inheritance, little recognition of what DNA was, and minimal awareness of DNA as having an impact on health or disease. Moreover, it was clear that there was a degree of suspicion of individuals and institutions who offered scientifically derived explanations of these concepts. Views on sensitive issues, including termination of pregnancy and post mortem examination varied considerably, even amongst members of the same family.

Conclusion: This in-depth, real world account of health and genetic awareness within the British South Asian community will inform the development of an instrument measuring knowledge, ability to take on new information, and attitudes towards health and genetics. This has utility across community, clinical, and research settings in evaluating current information resources and directing future strategies towards improving health and genetic literacy within the community.

Grants: Wellcome Trust (210561)

Conflict of Interest: None declared

P23.043.A The DIANA study: Decisions, Information and Autonomy in Norwegian Antenatal Care

Andrea Fotland Krohn-Hansen[1], *Cathrine Bjorvatn*[1], *Ragnhild Sekse*[1], *Jörg Assmus*[2], *Henriette Karlsen*[3], *Ine Moen*[3], *Ida Sørensen*[1]

[1]*Haukeland University Hospital, Medical Genetics, Bergen, Norway;* [2]*Haukeland University Hospital, Centre for Clinical Research, Bergen, Norway;* [3]*Haukeland University Hospital, Women's Clinic, Bergen, Norway*

Background: In 2022, non-invasive prenatal testing (NIPT) became a part of publicly funded antenatal care for all Norwegian women aged 35 or older at term. Very little is known about Norwegian women's experiences with NIPT, and the quality of health care provided to them. The DIANA study aims to evaluate this.

Methods: Multidimensional Measure of Informed Choice (MMIC) includes three subscales (knowledge, attitude, test behavior) which are combined to determine whether an informed choice is made. The Decision Regret Scale (DRS) is a five-item scale measuring distress or remorse after health care decisions. In-depth interviews with women will provide qualitative insights into their experiences and attitudes towards the new NIPT-based screening program.

Pregnant women (over age 35) referred for routine antenatal care at the Women's Clinic at Haukeland University Hospital are invited to join the study and asked to complete the MMIC immediately and DRS after four weeks. We aim to recruit 500 women. In-depth interviews with women identified as high-risk on NIPT are carried out 1-2 months after results.

Results: As of February 2024, 437 have been invited to join the study, 167 have responded to MMIC, and 90 to DRS. Interviews with three women with high-risk NIPT results have been carried out.

Conclusion: The DIANA study will allow us to evaluate the new Norwegian NIPT program and will provide pregnant women with an opportunity to express opinions on NIPT, reproductive autonomy and to shape their antenatal healthcare.

Grants: This project is funded by Helse Vest, project number: F-12815

Conflict of Interest: None declared

P23.044.B Presenting personalized clinical information directly to parents via an interactive website – the Chromosome 6 Project

Eleana Rraku[1], *Tyler D. Medina*[1;2;3], *Aafke Engwerda*[1], *Morris Swertz*[1;2], *Lennart Johansson*[1;2], *Conny Van Ravenswaaij-Arts*[1;4], *Imke Christiaans*[1]

[1]*University of Groningen, University Medical Centre Groningen, Department of Genetics, Groningen, Netherlands;* [2]*University of Groningen, University Medical Centre Groningen, Genomics Coordination Centre, Groningen, Netherlands;* [3]*University of Galway, School of Mathematical and Statistical Sciences, Galway, Ireland;* [4]*Jonx/Lentis Psychiatric Institute, Autism Team Northern-Netherlands, Groningen, Netherlands*

Consortium: n/a

Background/Objectives: The scarcity of clinical information surrounding rare chromosome 6 disorders presents challenges for both parents and clinicians. The Chromosome 6 Project (C6P) addresses this information gap by collecting detailed genotype and phenotype data on affected individuals directly from parents. The C6P aims to provide comprehensive clinical information based on features seen in individuals with a similar aberration, directly to parents via an interactive website. With the involvement of stakeholders, we determined how clinical features can best be reported to parents via this website.

Methods: Using a Delphi method, a panel of 32 parents and 21 (healthcare) professionals was asked to complete a survey in three rounds, consisting of 13 statements on the reporting of certain feature categories. The aim was to reach consensus on the given statements, defined as 75% of the respondents providing the same answer.

Results: Consensus was reached on 12 statements. Most respondents agreed that features that impact health or well-being should always be reported, even when present in only one individual with a similar aberration. No type of feature should be omitted from the clinical overview according to parents, but a reporting threshold can be used for clinically non-relevant issues or feature sub-types. The prevalence of each feature should always be provided.

Conclusion: Parents would like to access as much information as possible about potential health issues associated with their child's aberration. When providing such information online, it is essential to include clear explanations and additional resources to ensure correct interpretation and maximize parental benefit.

Conflict of Interest: None declared

P23.045.C Engaging communities: a systematic review of community-based participatory research in international genetic services

Heather Zierhut[1], *Megan Kocher*[2], *Pranathi Kandikonda*[1], *Cameron Donarski*[1], *Emily Simon*[1], *Mariana Ramirez-Mantilla*[3], *Crystal Lumpkins*[4], *Elena Fisher*[1]

[1]*University of Minnesota, Genetics, Cell Biology, and Development, Minneapolis, United States;* [2]*University of Minnesota, Libraries, Minneapolis, United States;* [3]*The University Of Kansas Medical Center, Kansas City, United States;* [4]*University of Utah, Huntsman Cancer Institute, Salt Lake City, United States*

Background/Objectives: Genetic counseling (GC) services have the potential to improve health outcomes, yet there are documented disparities in the use of GC services amongst minoritized racial/ethnic populations. Community-based participatory research (CBPR) is a recommended approach to better integrate diverse patient perspectives to improve genetics service delivery. How CBPR is being used and ways to effectively incorporate the principles in genetics services is needed.

Methods: Five databases were searched for international articles published from 2006 to 2023. Articles in the review met the following inclusion criteria: 1) published in 2006 or later; 2) peer-reviewed publication; 3) primary research studies; 4) community-based and participatory research; 5) focused on research topics of GC, clinical genetics, testing, and genetic service delivery. Criteria for evaluating CBPR involvement used a synthesis of best processes framework including criteria for "Assessing Partnership Approaches to Improve Public Health" by Israel *et al*. 1998.

Results: Screening of 3,824 studies identified 16 papers (n = 12 qualitative; n = 1 quantitative; n = 3 mixed methods). Most studies were conducted within the U.S. (n = 12), focused on cancer (n = 5), involved the Asian/Pacific Islander community (n = 8), and centered around genetic education (n = 12) rather than counseling, testing, or service delivery. Seven studies involved the community partners/advisors in nearly all aspects of the research process.

Conclusion: Integration of CBPR is greatly needed within genetics and GC services research to engage diverse stakeholders, improve genetic services, and ultimately reduce health disparities in genetic services.

Grants: N/A

Conflict of Interest: Heather Zierhut GenomeMedical LLC, NHGRI R01 and R25, University of Minnesota, Megan Kocher RE-246347-OLS-20, University of Minnesota, Pranathi Kandikonda University of Minnesota, Cameron Donarski: None declared, Emily Simon University of Minnesota, Mariana Ramirez-Mantilla NHGRI R01, R25 and others, Kansas University, Crystal Lumpkins R01, R25 and others, University of Utah, Elena Fisher NHGRI RO1, University of Minnesota

P23.046.D View of healthcare professionals on ultra-rapid genome sequencing and its implementation in clinical routine for critically ill children

*Claire Caillot[1], Etienne JAVOUHEY[2], Stephane Hays[3], Evan Gouy[1], Pauline MONIN[1], Gaetan Lesca[1;4], **Damien Sanlaville**[1;4], Nicolas Chatron[1;4]*

[1]Hospices Civils de Lyon, Service de génétique, Lyon, France; [2]Hospices Civils de Lyon, Service d'urgences et de réanimation pédiatriques, Hôpital Femme Mère Enfant, Lyon, France; [3]Hospices Civils de Lyon, Service de néonatologie et de réanimation néonatale, Hôpital de la Croix-Rousse, Lyon, France; [4]Université Lyon 1, Institut Neuromyogène, Laboratoire Physiopathologie et Génétique du Neurone et du Muscle, Equipe Métabolisme énergétique et développement neuronal, CNRS UMR 5310, INSERM U1217, Lyon, France

Background/Objectives: The clinical utility of ultra-rapid genome sequencing (urGS) in neonatal and paediatric intensive care situations has been demonstrated, and barriers to its implementation in clinical practice studied. We carried out the first study to identify the expectations of French healthcare professionals concerned by this test.

Methods: A questionnaire with 38 questions was developed and distributed via medical professional learned societies to these healthcare professionals.

Results: A total of 116 complete responses were received, including 35% from clinical geneticists, 19% from laboratory geneticists, and 32% from paediatric or neonatal intensivists. 97% respondents agreed that healthcare professionals should receive specific training before a first prescription. 94% of professionals considered urGS useful, and 97% that the result would likely modify a decision to withdraw life-sustaining treatment. A multidisciplinary validation of the urGS request was necessary for 87% of respondents, and multidisciplinary discussion of the result for 84%. Joint reporting of results by a clinical geneticist and NICU/PICU doctor was considered ideal for 91% of participants. 78% were against additional findings being reported at the same time as the result. Almost all respondents (99%) thought that psychological assistance was crucial after the result.

Conclusion: We propose a workflow based on the consensus elements emerging from the expressed views of the different implicated medical health care professionals.

Grants:

Conflict of Interest: None declared

P23.048.B Patients' experiences of being recontacted with new clinical information in a clinical genetics setting

Tiffany O'Brien[1;2], *Katrina Monohan[1;2], Anishka Abeysinghe[1;3], Chloe Pike[4], Laura Forrest[1;2;3]*

[1]Peter MacCallum Cancer Centre, Parkville Familial Cancer Centre, Melbourne, Australia; [2]The Royal Melbourne Hospital, Parkville Familial Cancer Centre & Genomic Medicine, Parkville, Australia; [3]The University of Melbourne, Sir Peter MacCallum Department of Oncology, Parkville, Australia; [4]University of Technology Sydney, Graduate School of Health, Chippendale, Australia

Background/Objectives: In 2019, evidence confirmed ovarian cancer was associated with germline *PALB2* pathogenic variants. This prompted the need for clinical genetics services to recontact women who had previously been identified with a *PALB2* PV and informed only of the breast cancer risk. This research aims to examine the experience of women recontacted by the Parkville Familial Cancer Centre (PFCC), their uptake of risk reducing surgery, and clinical and psychosocial outcomes.

Methods: A sequential mixed-methods design was used to collect data and included a clinical audit, patient survey and semi-structured interviews. Descriptive analyses were undertaken for quantitative measures and deductive thematic analysis for qualitative data.

Results: Of 95 women who were recontacted, 57 (60%) were within the age recommended for BSO: 31 (54%) had a BSO, 12 (21%) had not, and the remaining 14 (25%) were unclear. Fifteen (16%) made further contact with the PFCC, with nine patients requesting a follow-up appointment. Forty-one (43%) completed the survey with 23 (56%) feeling there should be an ongoing relationship with the PFCC and 32 (78%) felt it was the genetic health professional's responsibility to keep them up-to-date with new information. Nineteen were interviewed with most expressing an eagerness to be recontacted with new information and some expected that the genetics service would keep them up-to-date. The majority did not experience being recontacted negatively and felt the mode of recontact was appropriate.

Conclusion: Being recontacted by the PFCC aligned with women's expectations that clinical genetics services are responsible for informing patients of new clinical cancer risk information.

Conflict of Interest: None declared

P23.049.C Getting hospital executives and clinicians on the same page: sparking conversations about hospital-wide implementation of genomics.

*Cate Kelly[1;2], Katie Arkell[1;3], Trang Do[3], Melissa Martyn[1;3;4], Belinda McClaren[3;4], **Clara Gaff**[1;3;4]*

[1]Melbourne Genomics Health Alliance, Parkville, Australia; [2]The University of Melbourne, Department of Medicine (RMH), Melbourne, Australia; [3]Murdoch Children's Research Institute, Parkville, Australia; [4]The University of Melbourne, Department of Paediatrics, Melbourne, Australia

Background/Objectives: Genomic medicine is increasingly being provided in specialist services outside of traditional clinical genetics. Yet national genomic strategies do not address the hospital-wide changes needed. Clinical services wishing to use genomics need support to get meaningful engagement and traction with busy hospital decision-makers/executives. A recent scoping review did not identify any frameworks to guide safe, effective use of genomics at a hospital level. We aimed to determine how hospitals can be supported to optimally implement genomic medicine across diverse clinical services.

Methods: Drawing on the Knowledge to Action framework, we conducted an iterative design process with hospital executives and subject matter experts. A multidisciplinary group (with hospital governance, genetic counselling, implementation science and evaluation expertise) synthesised common clinical governance frameworks to design novel tools for hospitals to self-assess their maturity in genomic medicine and guide implementation. Following iteration at a workshop of hospital stakeholders (n = 29), we tested its useability at five metropolitan and regional hospitals.

Results: Testing showed clinician innovators and executives lacked a common language to discuss genomics; one is now evolving. Executives found assessing maturity challenging as genomics expertise is in pockets; spreading expertise beyond specific clinical areas within a hospital was raised by participants. Challenges identified included managing unwarranted variation and understanding the value of genomic medicine interventions.

Conclusion: The suite of tools we developed fills the gap between overarching health system-focussed genomic maturity models and the detailed evidence needed to guide clinical services. Importantly, and unexpectedly, a major benefit already is productive dialogue between executives and clinicians.

Funding: Victorian Government

Conflict of Interest: None declared

P23.050.D Co-designing Clinical Educational Resources and National Guidelines to Address Inequity of Access to Rare Disease Care in Australia

Lauren McKnight[1], Natalie Roberts[1], Krista Recsei[1], Chinthaka Balasooriya[1], Michelle Farrar[1;2], Nicole Millis[3], Louise Healy[3], Gareth Baynam[4;5], Sian Gannon[4], Elizabeth Lissiman[6], Yvonne Zurynski[7], Adam Jaffe[1;2], **Elizabeth Palmer**[1;2]

[1]UNSW Sydney, Medicine and Health, Sydney, Australia; [2]Sydney Children's Hospitals Network Randwick, Sydney, Australia; [3]Rare Voices Australia, Melbourne, Australia; [4]Rare Care Clinical Centre of Expertise for Rare and Undiagnosed Diseases, Perth Children's Hospital, Perth, Australia; [5]Western Australian Register of Developmental Anomalies and Genetic Services of WA, Perth, Australia; [6]Western Obstetrics, Balcatta, Australia; [7]Australian Institute of Health Innovation, Macquarie University, Sydney, Australia

Consortium: RArEST consortium

Background/Objectives: The 2021 UN Resolution *Addressing the Challenges of Persons Living with a Rare Disease and their Families* highlights that the >300 million people living with rare disease (RD), >70% of which are of genetic origin, face common challenges including inequitable access to evidence-based integrated care.

The Rare Disease Awareness, Education, Support, and Training (RArEST) Project, a 3-year partnership between the national peak body for Australians living with a RD, Rare Voices Australia, clinicians, and researchers, aims to support the implementation of the Australian Government's National Strategic Action Plan for RD and includes a clinical educational stream.

Methods: Multistakeholder consultations in the clinical educational stream included a learning needs analysis survey (n = 183 health professionals), mixed-methods consultation (n = 12 people living with RD and n = 18 multidisciplinary clinical working group), and health professional evaluation surveys.

Results: The consultation informed the co-production of (i) a suite of multi-modal rare disease educational resources (ii) the establishment of a multidisciplinary clinical community of RD practice (Rare Disease ECHO®) and (iii) the first National Recommendations for RD Health Care, launched in the Australian National Parliament in 2024. Resources are endorsed by national clinical professional bodies and accredited for continuing professional development.

Conclusion: Feedback from endorsing organisations and people living with RD was overwhelmingly positive, demonstrating the value of co-production to deliver educational resources that support a more equitable, person-centred, evidence-based, and integrated model of RD care aligning with the needs and preferences of people living with RD.

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P23.051.A Approach to pathological ultrasound findings and invasive diagnostic test results: Istanbul experience

MELDA ERDOGDU[1], *Tugba Kalayci[2], Tuğba Saraç Sivrikoz[3], Ayça Dilruba aslanger[2], Gülhanım Memiş[2], Birsen Karaman[2;4]*

[1]Istanbul University, Institute of Aziz Sancar Experimental Medicine (ASDETAE), Department of Genetics, Fatih, Türkyie; [2]Istanbul University, Istanbul Faculty of Medicine, Department of Medical Genetic, İstanbul, Türkyie; [3]Istanbul University, Istanbul Faculty of Medicine, Department of Obstetrics and Gynecology, Division of Perinatology, İstanbul, Türkyie; [4]Istanbul University, Child Health Institute, Pediatric Basic Science, Medical Genetic Division, İstanbul, Türkyie

Background/Objectives: There are social differences in the approach to prenatal testing and test results. In this study, we aimed to evaluate the attitudes of families who were offered the option of termination based on the results of prenatal invasive diagnostic tests or because of an unfavorable perinatal prognosis and the factors influencing their decision-making.

Methods: Face-to-face interviews were conducted with 280 families referred for fetal ultrasound abnormalities. Of the 217 families who agreed to participate in the survey, 147 underwent at least one of the invasive prenatal diagnostic tests (transabdominal chorionic villus biopsy, amniocentesis, and fetal blood sampling). Karyotype analysis, fluorescence in-situ hybridization (FISH), and array-CGH studies were performed.

Results: A chromosomal abnormality was detected in 21 (14.2%) of 147 families who underwent invasive prenatal diagnostic testing. While 15 (71.4%) of the anomalies were numerical, 6 (28.6%) were structural anomalies. Of the 70 families who were offered the option of termination according to the invasive prenatal diagnostic test results and/or because of unfavorable perinatal prognosis, 64 (91.4%) decided to terminate the pregnancy. The most influential factor in the decision to

continue the pregnancy was 'religious reasons'. In contrast, the most influential factor in the decision to terminate the pregnancy was 'information received from doctors/midwives/nurses.'

Conclusion: Awareness and use of prenatal invasive diagnostic tests and attitudes towards termination of pregnancy vary between countries depending on socio-economic, cultural, educational and religious characteristics.

Grants: This project was supported by the Istanbul University Scientific Research Projects (BAP) project no: TYL-2023-39547.

Conflict of Interest: None declared

P23.053.C A post-natal diagnosis of X-linked centronuclear myopathy: The importance of genetic counselling after reproductive carrier screening

Melissa Graetz[1], *Renee Dow*[1], *Lilian Downie*[1;2]

[1]*Mercy Hospital for Women, Genetics, Heidelberg, Australia;* [2]*Victorian Clinical Genetics Service, Murdoch Children's Research Institute, Parkville, Australia*

Background/Objectives: To highlight the importance of education and infrastructure for high risk results in screening programs. This case report demonstrates how essential education and support is for clinicians ordering reproductive carrier screening (RCS). It also emphasises the importance of genetic counselling and ongoing access to a genetics service for families identified as high risk after RCS.

Methods: This case report describes the postnatal diagnosis of X-linked centronuclear myopathy in a newborn male despite a high-risk carrier RCS result previously reported for the family. A male infant born prematurely at 30 weeks gestation with severe hypotonia requiring invasive respiratory support was referred to genetics. The family had a healthy 18 month old daughter. Clinical evaluation, chromosome microarray, spinal muscular atrophy screening and a targeted exome were arranged. Initially the mother disclosed no family history or RCS, but subsequently recalled having RCS in her first pregnancy. RCS had been arranged privately in her first pregnancy and she was not referred to a genetics service at time as her baby was female.

Results: A maternally inherited pathogenic variant in the *MTM1* gene associated with X-linked centronuclear myopathy was confirmed. Palliative care was offered and the infant died at 28 days of life. Genetic counselling resulted in referral to an IVF service and feedback to the private obstetrician and RCS provider.

Conclusion: Access to genetic counselling is essential as access to RCS increases. RCS reports need to be clear and concise, referral pathways readily available and education mandatory for clinicians ordering RCS.

Conflict of Interest: None declared

P23.054.D References to genetic testing in clinical guidelines

Anna Rehhahn[1], *Jörg Schmidtke*[2;3]

[1]*Hannover Medical School, Center for Rare Diseases, Hannover, Germany;* [2]*amedes MVZ wagnerstibbe, Hannover, Germany;* [3]*Hannover Medical School, Institute of Human Genetics, Hannover, Germany*

Background/Objectives: Genetic tests may confirm a suspected clinical diagnosis, accelerate the diagnostic process and influence prognosis and therapy, and hence are becoming an increasingly important part of diagnostic strategies in everyday clinical practice. Referrals to or consultations with a clinical geneticist are often not feasible due to limited resources. It has, however, not been systematically assessed, whether or not clinical guidelines themselves provide sufficient information to a clinician on which genetic tests to order in a particular clinical context.

Methods: In a first step, clinical guidelines published by the German Association of Scientific Medical Societies (AWMF) were examined for recommendations on genetic testing using a systematic and software-supported keyword search. Each finding was assessed for its relevance using a scoring model with 18 categories. We currently assess international clinical guidelines using the same methodological approach.

Results: Of the 563 currently valid AWMF clinical guidelines, covering well over 500 different disease groups, 200 (36%) recognize a genetic component in disease etiology, 75 of these broadly recommend genetic testing, 54 of these provide specific justifications for genetic testing, but only 9 contain enough information that would inform the clinician to directly order a genetic test.

Conclusion: The results indicate that the clinical relevance of genetics in everyday clinical practice is well recognized, but that the clinical-scientific societies are rarely sufficiently specific to provide a clinician with information that would empower him or her to order genetic tests directly.

Grants:

Conflict of Interest: None declared

P23.055.A X-linked Alport syndrome, UPD(X)pat and Ullrich-Turner mosaicism – solving a pedigree puzzle

Svenja Vishnolia[1], *Berenike Bögeholz*[1], *Loukas Argyriou*[1], *Silke Kaulfuß*[1], *Peter Burfeind*[1], *Alexandr Kuranov*[1], *Silke Pauli*[1], *Uwe Kornak*[1]

[1]*University Medical Center Göttingen, Institute of Human Genetics, Germany*

Background/Objectives: In a family with X-linked Alport syndrome, segregation analysis of at-risk family members presented us with a puzzle. Next generation sequencing (NGS) in a female patient, daughter of father carrying a pathogenic *COL4A5* variant hemizygous and a non-carrier mother, showed homozygosity for the known *COL4A5* variant. Possible causes were maternal sample mix-up, monosomy X (Ullrich-Turner syndrome) and paternal isodisomy of the X chromosome (UPD(X)pat). A step-by-step investigation was performed.

Methods: The tests that led to the correct diagnosis included NGS-based *COL4A5* analysis to genotype the mother, multiplex ligation probe amplification (MLPA) of *COL4A5* and *SHOX*, chromosomal analysis to test for monosomy X, and a SNP array to investigate UPD(X)pat.

Results: Initially, repeated sequencing of the mother confirmed her non-carrier status. However, MLPA of *COL4A5* and *SHOX* showed no abnormality consistent with an XX karyotype, leaving us with a puzzling pedigree. To rule out monosomy X completely using a second method, chromosome analysis revealed monosomy X mosaicism with 19% of cells affected. As this degree of mosaicism contradicted the unambiguous NGS results, a SNP array was performed and confirmed UPD(X)pat.

Conclusion: We present a healthy female patient carrying a homozygous pathogenic *COL4A5* variant due to UPD(X)pat combined with 19% monosomy X mosaicism. We hypothesise that the underlying mechanism is post-zygotic monosomy rescue of a monosomy X zygote. This unique case shows that it is often only the combination of different methods and the use of second methods to compensate for methodological limitations that can provide a complete picture.

Grants: None.

Conflict of Interest: None declared

P23.057.C Extending the known clinical spectrum of IPEX syndrome with a case report of a mildly affected young man

Berenike Bögeholz[1], *Alexandr Kuranov*[1], *Silke Kaulfuß*[1], *Silke Pauli*[1]

[1]*University of Göttingen, Universitätsmedizin Göttingen, Institut für Humangenetik, Göttingen, Germany*

Background/Objectives: Our patient, a 29-year-old young man, suffered from steroid-sensitive recurrent nephrotic syndrome with more than 20 relapses and first manifestation at the age of about 6 years. Specialists diagnosed the patient with focal segmental glomerulosclerosis (FSGS). According to the patient's parents, the patient did not show any particular susceptibility to infections; only atopic eczema had been observed repeatedly. During periods of stress, there were recurrent episodes of unexplained intestinal symptoms, intermittently accompanied by elevated body temperature.

Methods: Whole exome sequencing was performed for a suspected inherited kidney disease.

Results: Coincidentally, a pathogenic variant in *FOXP3* was identified. This variant had previously been described in patients with IPEX syndrome. In our case, the variant was inherited from a healthy mother. IPEX syndrome is a rare x-linked monogenic disorder of immune regulation. Pathogenic variants in the *FOXP3* gene have been identified to cause loss of regulatory T cell function. Dysfunction of the immune system leads to autoimmune and/or inflammatory diseases. The prognosis is very poor. Affected boys usually die in early childhood. They present with a triad of enteropathy, dermatitis and autoimmune endocrinopathy, typically diabetes type 1 or thyroiditis.

Conclusions: The case of the mildly affected young man who was diagnosed as an adult shows that the clinical spectrum of the IPEX syndrome is not yet fully understood. Many cases can be misdiagnosed, in our case as FSGS. However, it is important to note that primary immunodeficiencies can be the cause of many inconclusive cases with atypical symptoms.

Grants: None.

Conflict of Interest: None declared

P23.058.D The challenges of pre-symptomatic testing for late-onset neurological diseases: lessons learned from over 25 years of practice.

Milena Paneque[1;2;3], *Álvaro Mendes*[1;2], *Joana Damásio*[1;2;4], *Susana Lêdo*[1;2;5], *Jorge Sequeiros*[1;2;3], **João Parente Freixo**[1]

[1]*CGPP – Centre for Predictive and Preventive Genetics, IBMC – Institute for Molecular and Cell Biology, Univ. Porto, Portugal, Porto, Portugal;* [2]*UnIGENe, i3S – Instituto de Investigação e Inovação em Saúde, Univ. Porto, Portugal, Porto, Portugal;* [3]*ICBAS School of Medicine and Biomedical Sciences, Univ. Porto, Portugal, Porto, Portugal;* [4]*Serviço de Neurologia, Hospital de Santo António, Unidade Local de Saúde de Santo António, Porto, Portugal;* [5]*Instituto Universitário de Ciências da Saúde, CESPU, Gandra, Portugal, Porto, Portugal*

A program for genetic counselling (GC) and pre-symptomatic testing (PST) for late-onset neurological diseases (LONDs) was initiated in 1995, beginning with Machado Joseph disease (MJD), and later including Huntington disease (HD) and hereditary amyloidosis (ATTRh), all with high relative prevalence in Portugal.

This work aimed at outlining the challenges encountered in GC and PST for LONDs after over 25-years' practice at our outpatient genetics clinic.

We conducted a retrospective analysis of clinical records from 1,653 consultands seeking PST for a LOND since 1995. We reflected on the main challenges faced; effective means of supporting affected families; and the evolution of the program over time.

Psychosocial "catastrophic events" were extremely rare. Levels of depression and anxiety tended to decrease, while overall well-being improved. The finding of atypical, later-onset cases of ATTRh resulted in delayed diagnosis of probands and challenged PST within affected families. Conversely, the availability of clinical trials and new therapeutical options for ATTRh increased PST requests among younger individuals at risk. Uptake of PST also enabled several pre-natal diagnoses (PND) in LONDs. Notably, several young couples sought simultaneous PST and PND, which was a major challenge for GC.

As a consequence of the results, we simplified our protocol for GC and psychosocial assessment and support for consultands. Additionally, we propose further research into intra-familial communication of genetic-risk information and decision-making regarding PST. These insights may be applicable to other contexts, emphasizing the importance of continuous education for health-care professionals and ongoing improvement in quality of genetics services'.

Conflict of Interest: None declared

P23.059.A Empowering the Next Generation of Geneticists: Collaborations and Achievements of the ESHG-Young Committee

Mridul Johari[1;2;3], *Delia Sabau*[3;4], *Raquel Gouveia Silva*[3;5], *Silvia Kalantari*[3;6], *Ana Rita Matos*[3;7], *Rhys Dore*[3;8], *Magdalena Mroczek*[3;9], *Juliana Miranda Cerqueira*[3;10], *European Society of Human Genetics - Young Committee*[3]

[1]*Harry Perkins Institute of Medical Research, QEII Medical Centre, Nedlands, Western Australia, Australia and Centre for Medical Research, The University of Western Australia, Perth, Australia;* [2]*Folkhälsan Research Center and Department of Medical and Clinical Genetics, University of Helsinki, Helsinki, Finland;* [3]*European Society of Human Genetics - Young Committee, European Society of Human Genetics, Vienna, Austria;* [4]*Personal Genetics, Bucharest, Romania;* [5]*Serviço de Genética Médica, Departamento de Pediatria, Unidade Local de Saúde Santa Maria, Lisboa, Portugal;* [6]*Department of Molecular Medicine, University of Pavia, Pavia, Italy;* [7]*i3S - Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Porto, Portugal;* [8]*Barts Health NHS Trust, London, United Kingdom;* [9]*Department of Neurology, University Hospital of Basel, Basel, Switzerland;* [10]*Coeliac Disease Research Center, Faculty of Medicine and Health Technology, University of Tampere, Tampere, Finland*

Background/Objectives: The ESHG-Young (ESHG-Y) Committee stands at the forefront of representing and advancing young researchers and specialists in Human Genetics across Europe, by fostering connections and spearheading educational and professional development initiatives.

Methods: ESHG-Y representatives, as members of the ESHG Scientific Programme Committee (SPC), help in developing platform and plenary sessions. Our board members have also been representing and helping in Educomm and ERN-Ithaca activities. Additionally, we collaborate with African Society of Human Genetics (AfSHG), American Society of Human Genetics (ASHG), Human Genetics Society of Australasia (HGSA) and Junge Humangenetik.

Results: In 2023, the ESHG-Y organized six symposia, two educational sessions, and a workshop at the ESHG Annual Conference. Furthermore, the ESHG-Y was instrumental in organizing the Eurodysmorpho annual meeting and a forthcoming webinar on Non-Invasive Fetal Autopsy in collaboration with ERN-Ithaca. The committee's dedication resulted in an

enhanced presence in ESHG subcommittees, ASHG and HGSA working committees, and increased visibility through active participation in the ESHG social media team. Collaboration with the AfSHG through the Young Investigators Forum (YIF) led to the organization of a manuscript writing webinar, drawing on the knowledge of experienced professors. A landmark achievement was the publication of the YIF manuscript in the European Journal of Human Genetics, a significant contribution to the field. Additionally, the committee conducted a survey through its newsletter, targeting the need and presence of national Young Genetics Societies.

Conclusion: The ESHG-Y Committee's commitment to initiating innovative projects and nurturing international collaborations continues to support and develop the future leaders in Human Genetics.

Grants: ESHG Support

Conflict of Interest: None declared

P23.060.B Worldwide perspectives on genetic counselling supervision: an in-depth exploration of implementation strategies, Global practices and future directions across continents

Lídia Guimarães[1;2;3], *Bibiana Ribeiro*[3], *Mariangels Ferrer*[4], *Margarida Rangel Henriques*[5;6], *Marina Lemos*[5;6], *Milena Paneque*[1;2;3;7]

[1]*i3S - Institute for Research and Innovation in Health. University of Porto, Porto, Portugal;* [2]*CGPP - Center for Predictive and Preventive Genetics. University of Porto, Porto, Portugal;* [3]*ICBAS. School of Medicine and Biomedical Sciences, University of Porto, Porto, Portugal, Porto, Portugal;* [4]*Faculty of Biology, Medicine, and Health, University of Manchester, Manchester, United Kingdom;* [5]*FPCEUP - Faculty of Psychology and Educational Sciences. University of Porto, Porto, Portugal;* [6]*CPUP - Center of Psychology. University of Porto, Porto, Portugal;* [7]*IBMC - Institute of Molecular and Cellular Biology. University of Porto, Porto, Portugal*

Background/Objectives: Genetic Counselling Supervision (GCS) plays a crucial role in ensuring the quality and effectiveness of genetic counselling services worldwide. This research conducts a comprehensive investigation into the global situation of GCS practices, engaging key stakeholders who have been instrumental in both successfully establishing GCS and actively advocating for its integration as a routine of genetics healthcare.

Methods: The study spans diverse geographic regions, with representatives from each continent sharing valuable insights. Key inquiries focus on the following aspects: (1) existence of GCS in the countries represented by the interviewees, providing a snapshot of the current global distribution of GCS; (2) format characteristics: frequency, mandatory nature of supervision, funding mechanisms and possible regulation; (3) implementation processes: providing detailed accounts of how the implementation process of GCS unfolded in their respective countries, offering insights into successful strategies and challenges for countries where GCS is still not available.

Results: The on-going research has involved professionals representing nine countries: Greece, Romania, Austria, Brazil, Spain, India, Iceland, Germany, and Malta. The outcomes, explored via thematic analysis, suggest a progressive awareness and acceptance of GCS. Notably, the predominant strategy identified is the early initiation of GCS, establishing it as a regular practice for junior professionals.

Conclusion: These findings underscore a promising shift towards heightened awareness and incorporation of GCS as a routine and contribute valuable knowledge for policymakers, healthcare professionals, and genetic counsellors, offering a starting point for informed decision-making, collaborative efforts, and the advancement of GCS practices on an international scale.

Grants: Fundação para a Ciência e Tecnologia (FCT), Portugal

Conflict of Interest: None declared

P23.061.C The role of genetic counsellors in rare disease registries

Kathrin Taxer[1], *Georg Göbel*[2], *Gunda Schwaninger*[1], *Johannes Zschocke*[1]

[1]*Medical University of Innsbruck, Institute of Human Genetics, Innsbruck, Austria;* [2]*Medical University of Innsbruck, Institute of Medical Statistics and Informatics, Innsbruck, Austria*

Background/Objectives: Rare diseases affect approximately 350 million people globally with a prevalence of less than 1 in 2000 and present challenges in both diagnosis and treatment. Registries play a crucial role in advancing comprehension, research, and patient outcomes. Genetic Counsellors, as integral members of registry teams, can significantly enhance the efficacy of these registries.

Methods: A qualitative study approach involved conducting semi-structured interviews with stakeholders in rare disease clinics, including physicians specializing in rare diseases and patient representatives. The participants were queried about their expectations regarding a registry. Data was analyzed through a six-step thematic analysis process. Subsequently, attention was directed towards identifying specific tasks that Genetic Counsellors could undertake in the operations of registries.

Results: Stakeholders acknowledged the value of rare disease registries, underscoring advantages in study participation, epidemiological data, and research opportunities. Genetic Counsellors have the potential to optimize team efficiency, emerging as contributors who play a vital role in educating stakeholders, guiding discussions on informed consent, overseeing data management, and maintaining connections with patient advocates. This active engagement addresses operational challenges and ensures that the registry becomes a collaborative, patient-centered initiative with broad inclusivity and impact within the rare disease community.

Conclusion: Genetic Counsellors can significantly contribute to the success of rare disease registries by bridging the gap between the medical, scientific, and patient communities. Their expertise in genetics, communication, and patient interaction positions them as valuable team members, ensuring an effective, ethically sound, and patient-centered approach for the organization of rare disease registries.

Grants: No external grants were utilized for this project.

Conflict of Interest: None declared

P23.062.D European and African Society of Human Genetics-a collaboration that continues

Magdalena Mroczek[1;2], *Ana Rita Matos*[2;3], *Samuel Adadey*[4], *Mohamed Alimohamed*[5;6;7], *Patracia Nevondwe*[8], *Abbas Abel Anzaku*[9], *Wilson Mupfururirwa*[10], *Abdoulaye Yalcouyé*[11], *Silvia Kalantari*[2;12], *Ileana - Delia Săbău*[2;13], *Raquel Gouveia Silva*[2;14], *Rhys Dore*[2;15], *Juliana Miranda Cerqueira*[2;16;17], *Mridul Johari*[2;18;19], *Khutala Mnika*[20], *European Society of Human Genetics - Young Committee*[2], *African Society of Human Genetics-Young Investigator Forum African Society of Human Genetics*[21]

[1]*University Hospital of Basel, University of Basel, Department of Neurology, Basel, Switzerland;* [2]*European Society of Human Genetics*

- Young Committee, European Society of Human Genetics, Vienna, Austria; [3]University of Porto, i3s- Institute for Research and Innovation in Health, Porto, Portugal; [4]University of Ghana, WestAfrican Centre for Cell Biology of Infectious Pathogens (WACCBIP), Accra, Ghana; [5]Muhimbili University of Health and Allied Sciences, Department of Biochemistry and Molecular Biology, Dar es salaam, Tanzania; [6]Tanzania Human Genetics Organization, Dar es salaam, Tanzania; [7]University Medical Center Groningen, Department of Genetics, Groningen, Netherlands; [8]The University of the Witwatersrand, Division of Human Genetics, National Health Laboratory Services, and School of Pathology, Faculty of Health Sciences, Johannesburg, South Africa; [9]Institute of Human Virology, Department of Clinical Laboratory Services, Abuja, Nigeria; [10]University of Cape Town, Health Sciences Campus, Division of Human Genetics, Department of Pathology, Cape Town, South Africa; [11]Johns Hopkins University, United States; [12]University of Pavia, Department of Molecular Medicine, Pavia, Italy; [13]Personal Genetics, Bucharest, Romania; [14]Unidade Local de Saúde Santa Maria, Serviço de Genética Médica, Departamento de Pediatria, Lisbon, Portugal; [15]Barts Health NHS Trust, London, United Kingdom; [16]University of Tampere, Coeliac Disease Research Center, Faculty of Medicine and Health Technology, Tampere, Finland; [17]Universidade do Porto, Faculdade de Ciências da Nutrição e Alimentação, Porto, Portugal; [18]University of Helsinki, Folkhälsan Research Center and Department of Medical and Clinical Genetics, Helsinki, Finland; [19]The University of Western Australia, Harry Perkins Institute of Medical Research, QEII Medical Centre, Nedlands, Western Australia, Australia and Centre for Medical Research, Perth, Australia; [20]University of Cape Town, Health Sciences Campus, Division of Human Genetics, Department of Pathology, Cape Town, South Africa; [21]African Society of Human Genetics, Cape Town, South Africa

Background/Objectives: Advances in human genetics research rely heavily on international collaborations. However, most of the genetic studies in Europe are Eurocentric. Here, we present a story of a successful bottom-up intracontinental collaboration of young geneticists representing the European Society of Human Genetics - Young Committee (ESHG-Y) and the African Society of Human Genetics Young Investigator Forum (AfSHG-YIF).

Methods: Our collaboration started in 2022 during the International Federation of Human Genetics Meeting in Austria. This led to the organization of a successful YIF Meeting on February 21, 2023, at the University of Cape Town as a preceding event to the 14th International Congress of Human Genetics (ICHG) in 2023.

Results: The YIF meeting, our first event in person, gathered 143 participants from 28 countries, with a majority (112 participants) from Africa. The program included six sessions covering various topics: medical genetics, molecular diagnostics, cancer genetics and genomics, pharmacogenomics, genetic therapies, genetic counseling, and population genetics. Following our first successful meeting, we extended our collaboration to organize online training courses. An online grant writing workshop and a two-day training webinar on manuscript writing were jointly organized in January and June 2023, with several experienced professors as trainers. Currently, we are preparing a webinar on functional genomics in collaboration with The Jackson Laboratory (JAX).

Conclusion: Through collaborative efforts, young generations are organizing various intercontinental educational resources, fostering unity and enriching a new generation of individuals passionate about genetics.

Grants: none

Conflict of Interest: None declared

P23.063.A Analysis of intrafamilial communication degree and its influencing factors in patients with hereditary cancer predisposition syndromes

Lorena Moreno[1], *Elia Grau Garces*[1], *Fernando Yelamo*[1], *Aurora Sánchez Díaz*[2], *Clara López Solà*[3], *Ines Martin Villalba*[3], *Teresa Ocaña*[1], *Adela Rodriguez*[4], *Aleix Prat*[4], *Francesc Balaguer*[1]

[1]*Hospital Clínic Barcelona, Gastroenterology, Barcelona, Spain;* [2]*Hospital Clínic Barcelona, Biochemistry and Molecular Genetics, Barcelona, Spain;* [3]*Hospital Clínic Barcelona, Psychiatry and Clinical Psychology, Barcelona, Spain;* [4]*Hospital Clínic Barcelona, Medical Oncology, Barcelona, Spain*

Background/Objectives: When a germline mutation is identified, it's important to communicate this information to at-risk family members. This communication does not always occur, and several influencing factors have been described. The aim of our study is to analyze the communication level and the factors which may influence it.

Methods: In this retrospective study, 115 families with pathogenic or probably-pathogenic variants in hereditary cancer genes diagnosed between 2008-2021 were included.

Results: Participation rate stood at 62.61%. Seventy-nine percent of participants had explained information about the genetic variant to all at-risk family members, while 20.4% had not informed at least one family member. The degree of closeness between the proband and the at-risk family member was the only factor showing statistically significant differences ($p = 0.02$).

The main facilitators of communication were family support (48.3%), considering oneself a communicative person (34.5%), and genetic counselling (17.2%).

The primary reasons for not communicating included: no contact (50%), the family member being a minor (16.6%), not knowing how to communicate (16.6%), and believing the family member does not want to know (16.6%).

Seventy-six percent would accept the option for genetic counsellors to directly contact family members to convey this information to them, especially in cases where the patient faces obstacles in fulfilling this role.

Conclusion: These findings suggest a high level of intrafamilial communication. The closeness degree is the factor significantly influencing it.

Family support, considering oneself communicative, and genetic counselling have aided communication.

The option for genetic counsellors to directly inform family members, particularly when communication is challenging, is highly accepted.

Grants:

Conflict of Interest: None declared

P23.064.B Advancing Biobanking in Europe to foster Genomic Medicine

Manuela Pausan[1], *Saba Abdulghani*[1], *Petr Holub*[1], *Michaela Mayrhofer*[1], *Jana Pavlic-Zupanc*[1], *Eleanor Shember*[1], *Thomas Steger*[1], *Andrea Wutte*[1], **Jens Habermann**[1]

[1]*BBMRI-ERIC, Graz, Austria*

Consortium: On behalf of the BBMRI-ERIC community

Background/Objectives: BBMRI-ERIC is the European research infrastructure for biobanking and biomolecular resources. BBMRI has been granted ERIC-status by the EC in 2013. Currently, 24 countries and the World Health Organisation's International Agency for Research on Cancer constitute with its National Nodes and affiliated biobanks BBMRI-ERIC as one of the largest infrastructures in health and life sciences. BBMRI-ERIC's mission is to facilitate access to sample, data, and biomolecular resources to enable high-quality research, particularly personalized medicine.

Methods/Results: This is facilitated through services and advancements in the areas of Ethical, Legal and Societal Implications, Quality Management, Information Technology, Biobank Development, Public Affairs, Outreach, Education & Communications, and Finance- and Project Management. Hereby, BBMRI-ERIC engages its community and partners in more than 24 active EU projects fostering different domains towards genomic medicine including e.g. rare diseases, cancer, pediatrics, and infectious diseases. Our federated access and analysis platform has not only become a strong asset for major EU initiatives (e.g., EHDS, GDI, EUCAIM) but helps scientists and clinicians to accelerate their genomic medicine research: BBMRI's fully GDPR-compliant pipeline enables access to samples and data of over 5.1 Mio. patients facilitated by over 400 biobanks across 32 countries. The data comprise 7,834 distinct OMOP variables with biochemical measurements plus different types of OMICs data including e.g., over 200,000 genomes.

Conclusion: We will showcase how to access and make use of the richness of BBMRI-ERIC's resources and how to collaborate with our wider community on different topics through in-house or EU funding to foster genomic medicine.

Conflict of Interest: Manuela Pausan Employed by BBMRI-ERIC, MICROBE project, Saba Abdulghani: None declared, Petr Holub: None declared, Michaela Mayrhofer: None declared, Jana Pavlic-Zupanc: None declared, Eleanor Shember: None declared, Thomas Steger: None declared, Andrea Wutte: None declared, Jens Habermann: None declared

P23.065.C Internal data plays a crucial role in variant classification: a *MYH7 VUS* reclassification

Ana Patrícia Jesus[1], *Rafael Graça*[1], *Diana Antunes*[1;2;3], *Catarina Silveira*[1], *Yuri Chiodo*[1], *Maria Carmo-Fonseca*[4]

[1]*GenoMed - Diagnósticos de Medicina Molecular, S.A, Lisboa, Portugal;* [2]*Santa Marta Hospital, Lisboa, Portugal;* [3]*Dona Estefânia Hospital, Lisboa, Portugal;* [4]*Instituto de Medicina Molecular (IMM), Lisboa, Portugal*

Background/Objectives: The ultimate goal of genetic testing in inherited cardiomyopathies is to improve patient outcomes. The American College of Medical Genetics and Genomics and the Association for Molecular Pathology (ACMG/AMP) published general guidelines for the classification of genetic variants (2015), which comprises both laboratory-based and clinical evidence, but fails to encompass some specificities of certain diseases, namely cardiomyopathies. To tackle this, specific classification guidelines for *MYH7* were published by the ClinGen's Inherited Cardiomyopathy Variant Curation Expert Panel (VCEP), in 2018.

Methods: Reclassification of a *MYH7* gene variant c.5342G>A, p.(Arg1781His), found in individuals with hypertrophic cardiomyopathy, which the VCEP classifies as a variant of uncertain significance (VUS) (ACMG/ClinGen *MYH7* 2018: PS4_Moderate, PM2, PP3).

Results: With our internal data (6 families), and following the specific *MYH7* guidelines, we were able to increase the strength of the PS4 criteria to strong, thus reclassifying this variant as likely pathogenic (ACMG/ClinGen *MYH7* 2018: PS4, PM2, PP3). The clinicians of patients testing positive for this variant were informed about its reclassification, so that direct relatives could be screened. Variant segregation among families with affected members is another interesting information that can be used to reclassify a VUS to likely pathogenic.

Conclusion: This case highlights the importance of internal records and data sharing in variant classification and interpretation. Moreover, sharing this data in proper platforms can help diagnostic laboratories to make a better variant pathogenicity assessment and, thus, provide the chance for patients and their family members to have better genetic counselling.

Grants:

Conflict of Interest: None declared

P23.066.D Variant reclassification and clinical implications

Nicola Walsh[1], *Aislinn Cooper*[2], *Adrian Dockery*[2], *James O'Byrne*[3]

[1]*Children's Health Ireland (CHI) at Crumlin, Department of Clinical Genetics, Dublin, Ireland;* [2]*Mater Misericordiae University Hospital, Next Generation Sequencing Lab, Dublin, Ireland;* [3]*Mater Misericordiae University Hospital, National Centre for Inherited Metabolic Disorders, Dublin, Ireland*

Background/Objectives: Accurate molecular genetic diagnoses are important in genomic medicine. Variant classification (benign – pathogenic), is fundamental to these tests. Variant reclassification is the process of reassigning the pathogenicity of variants over time that challenges diagnostic legitimacy. With the mainstreaming of genetic testing and increasing use of Next Generation Sequencing (NGS) for clinical diagnosis, variant reclassification is becoming an issue within clinical genetics. This review explores the medical and scientific literature available on variant reclassification, focusing on its clinical implications.

Methods: We reviewed the literature that describes gene sequence variant reclassification. A thorough PubMed search was performed using the MeSH terms ["variant" AND "reclassification"] returning 358 results (accessed 29.06.2023). Abstracts were selected that examined variant reclassification with clinical implications published between January 2015 – June 2023.

Results: Factors affecting reclassification include standardised guidelines, clinical phenotype-genotype correlations through deep phenotyping and ancestry studies, large-scale databases and bioinformatics tools. Addressing VUS reclassification is essential to enhance the credibility of genetic testing and the clinical impact. As genomic databases grow and knowledge advances, reclassification rates are expected to change, reducing discordance in future classifications.

Variant reclassification frequency ranges from 3.6% to 58.8%. Recent studies have shown that significant reclassification can occur within one year of initial classification.

Conclusion: Variant reclassification affects patient diagnosis, precision therapy, family screening. The exact patient impact is yet unknown. Understanding influencing factors and adopting standardised guidelines are vital for precise molecular genetic diagnoses, ensuring optimal patient care and minimising clinical risk.

Grants: N/A

Conflict of Interest: None declared

P23.067.A A family case of OXTR gene related autism spectrum disorders

Joanna Karwowska[1], *Renata Posmyk*[1]

[1]*Medical University of Białystok, Department of Clinical Genetics, Białystok, Poland*

Background/Objectives: OXTR gene (locus 3p25.3) encodes the oxytocin receptor and its polymorphisms contribute to behavioural development as well as development of the nervous system. Increasing number of research suggest OXTR takes part in etiopathogenesis of autism, ADHD, depression and anxiety but still the exact clinical meaning and the inheritance mode of the gene

associated disorders remain unknown. OXTR is not affiliated with any record in OMIM database.

Methods: We present a 4-year-old boy with autism spectrum disorder. He was born in 34 hbd from second pregnancy, complicated with genitourinary infections, asthma and hypothyroidism in mother. After birth mild breathing difficulties occured. No congenital defects were detected, the motor development was normal but speech was delayed and still remains slurred with odd answers. His prematurly born brother suffers from Asperger syndrome and ADHD. The physical examination revealed height and weight within a normal range, two minor hipopigmentation macules and one cafe-au-lait spot on the skin, discrete dysmorphic features (prolonged face, high forehead, low set ears, downslanting palpebral fissures). FRAX syndrome was excluded and the NGS panel for autism and intellectual disability was performed.

Results: The test revealed a variant of unknown significance c.347_367dup in OXTR inherited from a normal mother. This variant is also present in patient's brother.

Conclusion: It is the next evidence of OXTR gene abnormalities taking part in autism development. This case suggest X-linked recessive inheritence since the affected boy and his brother received the variant from the healthy mother.

Grants:

Conflict of Interest: None declared

P23.068.B Declining genetic testing uptake among individuals from hereditary breast and ovarian cancer and Lynch syndrome families

*Ronit Tsemach[1;2], Hagit Baris Feldman[3;4], Elizabeth E. Half[5;6], Sue Kim[7], Maria Katapodi[8], **Sivia Barnoy**[1], Efrat Dagan[9]*

[1]Tel Aviv University, Nursing, Tel Aviv, Israel; [2]Tel-Aviv Sourasky Medical Center, Israel; [3]Tel-Aviv Sourasky Medical Center, Genetics Institute, Tel Aviv, Israel; [4]Tel Aviv University, Faculty of Medicine, Tel Aviv, Israel; [5]Rambam Health Care Campus, GI Malignancy Unit, Familial Cancer Syndromes, Haifa, Israel; [6]Technion Institute of Technology, The Ruth and Bruce Rappaport Faculty of Medic, Haifa, Israel; [7]Yonsei University, College of Nursing, Seoul, Korea, Rep. of South; [8]University of Basel, Department of Clinical Research, Basel, Switzerland; [9]University of Haifa, Nursing, Haifa, Israel

Background: Cascade genetic testing for individuals at high-risk for hereditary breast-ovarian cancer (HBOC) and Lynch syndrome (LS) enables individualized risk-management programs, including surveillance and/or risk-reduction surgeries. Despite the benefits, some at-risk individuals choose to postpone/decline genetic testing.

Objective: We evaluated narratives of individuals from HBOC and LS families who chose to decline/postpone cascade testing.

Methods: The iCascade study was conducted among Israeli HBOC and LS families, as part of a larger international study in collaboration with Switzerland and Korea. As part of the questionnaire, participants were asked to share their experiences about their decision to undergo or to postpone/decline genetic testing. Narrative data were analyzed using thematic content analysis.

Results: The Israeli cohort consists of 50-HBOC and 33-LS index-cases, and 99-HBOC and 96-LS relatives. Of 195 relatives, 38(19.5%) declined cascade testing, with 17/99(17.2%) from HBOC and 20/96(20.8%) from LS relatives. Decliners mean age was 34 ± 17 years. Most were females (53%), married (55%) and had children (76%). Narratives revealed three distinct levels of needs: Individual, family, and healthcare system. Individual concerns ($n = 18$) included fear of the results, feeling too young, and having no children. Family concerns ($n = 20$) reflected conflicts with mothers regarding testing. Healthcare system concerns ($n = 23$) pointed to insufficient medical support.

Conclusion: Decliners of cascade testing experienced a sense of coping alone after being informed by the mutation carrier in the family. The conflict with the mothers regarding genetic testing requires clear directions about the ways relatives can receive support from healthcare providers and how to approach genetic services.

Conflict of Interest: Ronit Tsemach PhD candidate, Hagit Baris Feldman Israel Cancer Research Fund (#2020PG-0000000034)., Elizabeth E. Half Israel Cancer Research Fund (#2020PG-0000000034)., Sue Kim: None declared, Maria Katapodi: None declared, Sivia Barnoy Israel Cancer Research Fund (#2020PG-0000000034)., Efrat Dagan Israel Cancer Research Fund (#2020PG-0000000034).

P23.069.C The need for young geneticist societies - a survey by the European Society of Human Genetics - Young Committee (ESHG-Y)

Raquel Gouveia Silva[1;2], Rita Barbosa-Matos[2;3], Mridul Johari[2;4;5;6], Rhys Dore[2;7], Juliana Miranda Cerqueira[2;8;9], Silvia Kalantari[2;10], Ileana - Delia Săbău[2;11], Magdalena Mroczek[2;12]

[1]Serviço de Genética Médica, Departamento de Pediatria, Unidade Local de Saúde de Santa Maria, Lisboa, Portugal; [2]European Society of Human Genetics - Young Committee; [3]i3S - Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Porto, Portugal; [4]Folkhälsan Research Center, Helsinki, Finland; [5]Department of Medical and Clinical Genetics, Medicum, University of Helsinki, Helsinki, Finland; [6]Harry Perkins Institute of Medical Research, Centre for Medical Research, University of Western Australia, Nedlands WA, Australia; [7]Guy's and St Thomas' NHS Foundation Trust, London, UK; [8]Coeliac Disease Research Center, Faculty of Medicine and Health Technology, University of Tampere, Tampere, Finland; [9]Faculty of Nutrition and Food Sciences, University of Porto, Porto, Portugal; [10]Department of Molecular Medicine, University of Pavia, Pavia, Italy; [11]Personal Genetics, Bucharest, Romania; [12]Department of Neurology, University Hospital of Basel, University of Basel, Basel, Switzerland

Background: Young geneticist societies (YGS) work within their respective national societies of human genetics, supporting young members in the genetics' field. The ESHG-Young, under the guidance of the *ESHG*, aimed to better understand the needs and efforts of other human geneticists' young committees.

Methods: Data were collected, using an online survey, from November 16th to December 15th, 2023. We further disseminated the survey, through social media platforms, to all ESHG trainee members. The YGS's roles were evaluated using likert scales.

Results: 78 participants completed the survey with the top 3 most represented countries being Portugal, Turkey and France. 24,4% (19/78) already have a YGS established in their home country. YGSs are already established in France, Italy, Turkey, Germany, Netherlands, Portugal, and Denmark with 10 participants being actively involved with them.

From the 59 participants who do not have a YGS, 95% (56/59) highlighted the need to have one. Most participants ($n = 49$) would be involved in establishing a YGS in their country and to collaborate with the ESHG-Young for this purpose.

YGSs are considered to have a moderately/very important role in: 'education and training for young members' and 'collaboration and networking' (93,6%; 73/78); 'acting as a voice for young members' (90,9%; 70/77); 'funding opportunities' (89,7%; 70/78); 'leadership opportunities' (85,7%; 66/77) and 'public education' (79,5%; 62/78).

Conclusion: This survey reinforced the importance of ESHG-Young in supporting the future connections of established YGS and the creation of new ones. We also identify the need to continue our mission, building a community that facilitates communication within young generations.

Conflict of Interest: None declared

P23.070.D Recontacting in clinical genetics: mission (im) possible?

Abduallah Jabori[1], *Elizabeth Loehrer*[1], *Sam Riedijk*[1], *Judith Verhagen*[1]

[1]*Erasmus MC, Department of Clinical Genetics, Rotterdam, Netherlands*

Background/Objectives: Despite a growing need, there is a lack of consensus on recontacting former patients about new genetic information. Previous studies revealed various perspectives of stakeholders, but often focussed on one stakeholder group each. However, to ensure support for recontacting practices, exchanging views among stakeholders is essential. This study explores to what extent stakeholders (dis)agree regarding recontacting and proposes recontacting strategies.

Methods: We performed a systematic review to identify key stakeholders and issues. In parallel, we recruited genetic counsellors, laboratory specialists, and patients for a three-round Delphi study. In the first round, participants expressed their views about recontacting in a questionnaire, which is based on the literature review. In the second round, participants evaluated statements that are derived from the first-round responses. Lastly, participants reevaluated the statements with insights from the second-round responses.

Results: The literature search has identified 3500+ records, and we have included 100+ reports. Based on findings from the literature, the questionnaire focussed on attitudes and ethical, legal, and psychosocial issues of recontacting. We will present the key findings of the literature and the (preliminary) outcomes of the Delphi study. This includes practical matters, such as whether burdens of recontacting can be mitigated for patients and providers, and whether patients should be recontacted if they did not consent?

Conclusion: To advance the practice of recontacting, research and dialogues among stakeholders are needed to help stakeholders navigate the manifold issues of recontacting. This study aims to serve as a stepping stone for recontacting strategies in clinical genetics.

Grants:

Conflict of Interest: None declared

P23.071.A Navigating the landscape of reproductive genetic literacy: insights from healthcare professionals

Marjan Naghdi[1], *Malou Heijligers*[1], *Ron van Golde*[2], *Aspasia Destouni*[3], *Maryn Reyneke*[1], *Anouk Janssen*[1], *Rick Essers*[1], *Aimee D.C. Paulussen*[1], *Arie van der Lugt*[4], *Rob Ruiter*[4], *Masoud Zamani Esteki*[1]

[1]*Maastricht University Medical Center MUMC+, Department of Clinical Genetics, Maastricht, Netherlands;* [2]*Maastricht University Medical Center MUMC+, Department of Obstetrics and Gynecology, Maastricht, Netherlands;* [3]*School of Medicine - Aristotle University of Thessaloniki, 3rd Obstetrics and Gynecology Department- "Ippokratio General Hospital", Larissa, Greece;* [4]*Maastricht University, Faculty of Psychology and Neuroscience, Maastricht, Netherlands*

Background/Objectives: Advances in genetic testing have given prospective parents valuable information for family planning, enabling timely interventions for special needs. However, this transformation has placed patients in the challenging role of decision-makers, adding stress due to the complexity of genetic information. Pregnancy-related stress carries risks such as miscarriage and premature delivery, making patient education crucial. While traditional healthcare prioritizes professional education, this study explores professionals' perspectives on educating patients. This shift aims to promote equal access to reproductive genetic options and enhance family planning and reproductive health.

Methods: This qualitative study conducted 14 semi-structured interviews with reproductive healthcare professionals from Maastricht University Medical Center in the Netherlands. Participants included genetic counsellors, gynaecologists/obstetricians, midwives, and psychologists/social workers. Thematic analysis, aided by Atlas.ti software, identified patterns within the data and organized codes into potential themes to capture key findings.

Results: Professionals emphasize the significance of prior knowledge in enabling patients to actively participate in genetic counselling, make informed decisions, and manage stress. While understanding the testing procedure, its safety, and potential results are crucial, professionals caution against overwhelming patients with excessive information. Five main challenges to effective counselling are identified: the complexity of genetic information, language barriers, untailored counselling approaches, occasional time constraints, and difficulties in interpreting uncertain test results without causing undue stress.

Conclusion: Professionals acknowledge the benefits of promoting literacy but report that achieving a balance between providing essential information and avoiding unnecessary anxiety-inducing details remains a challenge.

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Conflict of Interest: None declared

P23.072.B Evaluation of clinical utility of genetic and genomic testing using the Clinician-reported Genetic testing Utility InDEx (C-GUIDE) in the Sydney Children's Hospitals Network, Australia.

Ruvi Samarasekera[1;2], *Penny Xiao*[3], *Ryan Pysar*[1;2], *Stephanie Luca*[3], *Elizabeth Palmer*[1;2], *Robin Hayeems*[3], **Lisa Ewans**[1;2;4]

[1]*Sydney Children's Hospitals Network Randwick, Centre for Clinical Genetics, Randwick, Australia;* [2]*UNSW Sydney, School of Paediatrics and Child Health, Kensington, Australia;* [3]*The Hospital for Sick Children, Toronto, Canada;* [4]*Garvan Institute of Medical Research, Genomics and Inherited Diseases Program, Darlinghurst, Australia*

Background/Objectives: Value-based health care is increasingly important in the clinical environment. Demonstrating the clinical utility (CU) of genetic/genomic testing in rare disease remains a challenge due to clinical complexity and varied clinical and patient-reported outcomes, with a standardised approach lacking. The validated Clinician-reported Genetic testing Utility InDEx (C-GUIDE) was used to evaluate the CU of genetic/genomic testing in a rare disease population.

Methods: Convenience sampling identified genetic and non-genetic health professionals across the Sydney Children's Hospitals Network, Australia. Participants completed the C-GUIDE following the disclosure of genetic/genomic test results to 140 families. De-identified rater demographics and case information were captured. Using univariate statistics, mean and item-specific

C-GUIDE scores were determined; regression analysis identified factors predictive of utility in a rare disease context.

Results: Most individuals were paediatric (89%) with an even distribution of multi- and single system presentations. CU was higher for urgent compared to non-urgent testing (Mean = 15.89 vs. 11.62; p = 0.022). In univariate and multi-variate analysis, diagnostic results were associated with a significant increase in C-GUIDE score versus non-diagnostic (Mean = 15.89 vs. 11.62; p = 0.022 / multivariate 10.18) and was the most significant predictor of CU. Domain and item-based analyses showed higher CU for diagnostic results in: understanding diagnosis and prognosis, informing medical management, and family information. However, overall psychosocial wellbeing was similar regardless of whether a diagnosis was achieved.

Conclusion: From a clinician perspective, genetic/genomic testing has highest CU for diagnosed individuals. The C-GUIDE can be adopted as a means of demonstrating value in genetic/genomic testing.

Grants: Nil

Conflict of Interest: None declared

P23.073.C FindMe2care - a diagnosis-based contact platform for rare genetic conditions

Christian Gebhard[1], *Soeren Schumacher*[1], *Teresa Neuhann*[1], *Anna Teubert*[2], *Tom Kamphans*[3], *Ralf Glaubitz*[2], *Angela Abicht*[1], *Peter Krawitz*[4]

[1]*MGZ - Medizinisch Genetisches Zentrum, München, Germany;* [2]*Amedes Genetics, Hannover, Germany;* [3]*GeneTalk GmbH, Bonn, Germany;* [4]*Institute for Genomic Statistics and Bioinformatics, Bonn, Germany*

Background/Objectives: For research projects on rare disorders and clinical trials in particular, identifying suitable participants is a major challenge. Likewise, patients are navigating a complex and intricate landscape of information if they want to stay up to date about potential therapies for their genetic condition.

Methods: To address these needs, we initiated the contact platform FindMe2care. Patients who receive their molecular diagnosis of a monogenic disease are also provided with an individual QR code encrypting the details of their report as a PhenoPacket. This QR code can be used by a lay person to self-register on the platform without transmission errors. Upon successful registration users can receive information or inquiries from external entities through the platform. Such communications are customized to align with the individual's diagnosis and have undergone prior evaluation by an independent scientific advisory board.

Results: Despite self-registration and data submission by the patient, FindMe2care received phenotypic and genetic data of excellent quality fulfilling the requirements of international research networks. Preliminary data since the launch in January 2024 indicate that two out of three people take up this additional offer. Response rates and time to enrollment for an expected 1000 patients will be available for presentation at the end of May.

Conclusion: FindMe2care acts as a data trustee for patients who wish to participate in clinical research and benefit from personalized treatments, helping to improve care for rare genetic disorders. The platform is a non-profit initiative that all academic and non-academic genetic laboratories in Germany can join.

Grants:

Conflict of Interest: None declared

P23.074.A Using integrative psychotherapy as support for the genetic counseling process

Ljiljana Serman[1;2;3]

[1]*School of Medicine, University of Zagreb, Zagreb, Croatia, Department of Biology, Zagreb, Croatia;* [2]*School of Medicine, University of Zagreb, Zagreb, Croatia, Centre of Excellence in Reproductive and Regenerative Medicine, Zagreb, Croatia;* [3]*Everything for Her, Zagreb, Croatia*

Background/Objectives: Integrative psychotherapy comes from a synthesis of multiple theories: Humanistic Therapy, the Object Relations Theory and Self-psychology. Consequently, it is an important part of genetic counseling.

Methods: As evident across several clinical cases, psychotherapy methods of dream interpretation, countertransference and seff-reflection, resolve tension and doubts related to genetic testing for hereditary cancer.

Results: The results will be presented through a series of case presentations:

1. A 30-year-old patient is awaiting the results of genetic testing. Even though she denies being upset in any way, her dreams show us otherwise;
2. A 27-year-old patient is awaiting for her mother's genetic test results and is having a nightmare where a person experienced a car accident and doesn't know whether he/she made it alive;
3. A 35-year-old who is carrying BRCA mutation is considering bilateral mastectomy and is dreaming of losing her gloves;
4. A 50-year-old wants to get tested for hereditary cancer; however, she is so anxious that she speaks fast, causing the counselor to have a strong countertransference reaction in the form of body discomfort and the sense that something has been missed during the session.

Conclusion: Even though genetic counseling differs from psychotherapy, the above cases proved that psychotherapy is a crucial part of counseling. Integrative psychotherapy allows the therapist to integrate the best of all the psychotherapy approaches at the given moment, and most importantly, it considers the patient's needs. Furthermore, it is a very helpful tool in conflict resolution that happens as a consequence of genetic testing.

Conflict of Interest: None declared

P23.076.B Healthcare professionals' experiences with expanded non-invasive prenatal testing (NIPT) in Belgium: challenges and solutions

Zoë Claesen-Bengtson[1], *Karuna van der Meij*[2], *Joris Vermeesch*[3], *Lidewij Henneman*[2], *Pascal Borry*[1]

[1]*KU Leuven, Public Health and Primary Care, Leuven, Belgium;* [2]*Amsterdam UMC, Human Genetics, Amsterdam, Netherlands;* [3]*UZ Leuven, Human Genetics, Leuven, Belgium*

Background/Objectives: Genome-wide prenatal cell free DNA (cfDNA) screening can lead to the early detection of important health-related information for the fetus and pregnant woman. However, the technology-driven expanding scope of screening heightens information complexity and creates challenges for the clinical interaction. This study aimed to explore Belgian healthcare professionals' experiences to identify challenges and solutions to genome-wide cfDNA screening in practice.

Methods: We performed an in-depth interview study with 31 healthcare professionals including clinical geneticists, gynecologists, laboratory specialists, counselors, midwives, and a pediatrician in Belgium where genome-wide cfDNA

screening is publicly reimbursed. Interviews were recorded, transcribed verbatim, and analyzed inductively through an iterative process.

Results: Three key challenges to a responsible genome-wide cfDNA screening offer in a public healthcare setting were identified: (1) Information provision: the more we test, the more complex the information provision becomes; (2) Return of results: knowing more might be worse than knowing less; and (3) Competing interests complicate setting a (nation-wide) uniform scope. Solutions reported by participants included providing additional resources for counseling, implementing value-based counseling, and implementing a uniform scope of cfDNA screening.

Conclusion: To minimize potential harms and to maintain trust of NIPT-users, it is crucial that best practices for counseling and reporting cfDNA results from healthcare professional to expectant parent are more substantiated. From an equal access perspective, it is important to develop sustainable lines of communication among providers and between stakeholders to navigate the transparent implementation of technological developments in prenatal genetic screening.

Grants: No funding was received for this study.

Conflict of Interest: None declared

P23.077.C The role of genetic counsellors perceived by the patient- a qualitative assessment

Nicole Fife[1;2], *francesca catapano*[3], *Marie Met-Domestici*[1], *Francesca Mari*[2], *Christophe Cordier*[1;2]

[1]*Medisyn Sa, Genetics Department, Lausanne, Switzerland;* [2]*University of Siena, Department of Medical Biotechnologies, Siena, Italy;* [3]*University of Bologna, Department of Medical and Surgical Science (DIMEC), Bologna, Italy*

Background/Objectives: Genetic counsellors intend to educate and inform patients about their genetic condition in a patient-centered approach, using empathy, listening and communication skills. This research's aim was to assess how patients perceive and describe the role of genetic counsellors. It is imperative for genetic counsellors to receive their patients' feedback, to better accompany them and ultimately to improve the delivery of their sessions.

Methods: Between May 1st and June 30th, 2023, at the Genetics Department of SYNLAB Suisse SA (French-speaking part of Switzerland), every patient seen by both a genetic counsellor and the author was offered to participate. Patients were interviewed via phone call, ideally within five days of their genetic counselling session. They were asked six open-questions about how they felt before, during and after their session.

Results: 72 patients were interviewed for this research. Patients' responses were divided into four themes: a welcoming environment where words such as "positive" and "trust" were used; an ability to express oneself with words such as "free" and "at-ease"; an understanding of genetics with words such as "well-explained" and "clear"; and finally, an important familial aspect as the word "family" was used multiple times.

Conclusion: Overall, patients were satisfied by their genetic counsellor; however, a lack of detail and understanding was also perceived which emphasises how educating patients is a key part of the role of genetic counsellors. Future research should expand this project by interviewing a larger sample of patients in order to evaluate whether this perception is similar worldwide.

Grants:

Conflict of Interest: None declared

P24
ETHICAL, LEGAL AND PSYCHOSOCIAL ASPECTS IN GENETICS

P24.001.D Views on donation of genetic and genomic data for research in Latin America

Gabriela Chavarria-Soley[1], *Gabriel Macaya*[1], *Ronald Alfaro*[2], *Lauren Robarts*[3], *Richard Milne*[3;4], *Anna Middleton*[3;4], *Henriette Raventós*[1]

[1]*Universidad de Costa Rica, Centro de Investigación en Biología Celular y Molecular, San José, Costa Rica;* [2]*Universidad de Costa Rica, Centro de Investigación y Estudios Políticos, San José, Costa Rica;* [3]*Welcome Connecting Science, Engagement and Society, Hinxton, United Kingdom;* [4]*University of Cambridge, Kavli Centre for Ethics, Science, and the Public, Cambridge, United Kingdom*

Background/Objectives: The large amount of genomic data currently being generated can potentially be used by researchers worldwide, when the persons whose data is generated allow the sharing of their information. "Your DNA, Your Say" is an international anonymous online survey which covers attitudes towards genomic and medical data donation, as well as issues regarding trust.

Methods: We analyze the results of the survey for respondents from Argentina (N = 919), Costa Rica (N = 476), Mexico (N = 1347), and Brazil (N = 1349). Individuals were asked whether they would donate their medical and genetic information for use in research by medical doctors, non-profit researchers, and for-profit researchers.

Results: Responses can be grouped into three main categories: a) would share with all three types of users of the data, b) would share with medical doctors and non-profit researchers only, c) would not share with any type of researcher. A multinomial logistic regression shows that having a university education, being religious and living in Brazil all increase the odds of belonging to category a), while being 60 or older lowers those odds. Meanwhile, the odds of belonging to b) are higher in individuals older than 50. Regarding trust in different receptors of the information, respondents in all categories are likely to trust their own medical doctor and not trust any government. A tendency to trust researchers at universities in any country, or only in the respondent's own country is seen in categories a) and b), respectively.

Conclusion: This is one of the first explorations of views on genetic data sharing in Latin America.

Grants: Wellcome[206194]

Conflict of Interest: None declared

P24.002.A Consideration of return of incidental findings based on non-clinical benefits

Noriko Ohashi[1]

[1]*Osaka University, Ethical, Legal and Social Issues Core Institute for Datability Science, Suita, Japan*

Background/Objectives: The widespread use of comprehensive genome/genetic analysis presents a new problem, namely return of incidental findings (IFs). Clinical actionability (preventable or treatable) has been widely discussed as a standard for returning IFs, but there has been little discussion of returns based on non-clinical benefits which, for many, would be beneficial for e.g. life planning. This is considered here.

Methods: Investigation of research papers, guidelines, and legal regulations in Japan and abroad; interviews with experts; conducting and analyzing an online survey on public attitudes regarding IFs.

Results: Knowing in advance a disease identified as IFs, despite its being unpreventable or untreatable, may have the following benefits: 1) help in planning future life; 2) avoidance of a diagnostic odyssey on occurrence of an intractable disease that is difficult to diagnose; 3) obviation of the possibility that people with certain inherited arrythmias can, due to sudden onset syncope, endanger the lives of both themselves and others when driving.

Conclusion: There are ethical, legal, and social issues to consider when returning IFs. They relate to 1) to 3) above. The issue in case 1) is how to evaluate one's view of life. Case 3) concerns whether restrictions, such as refusing a driver's license, can be imposed on people who have not yet developed the disease, simply because of the possibility of developing it. In either case, many issues need to be considered, including how to obtain informed consent that reflects individual preferences.

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Conflict of Interest: Noriko Ohashi JSPS KAKENHI Grant Number JP21K10375

P24.003.B Prenatal diagnosis of variations of sex characteristics (VSC) /Intersex: Clinicians' experience and views about biological characteristics and sociocultural norms of gender and fertility

Iimor Meoded Danon[1], Noam Zaslansky[2], Hagit Daum[3], Tamar Paperna[4], **Shachar Zuckerman**[2]

[1]*Bar-Ilan University, Medical Sociology, The Azrieli Faculty of Medicine, Zefat, Israel;* [2]*Shaare Zedek Medical Centre, Medical Genetics Institute, Jerusalem, Israel;* [3]*Hadassah, Medical Genetics Institute, Jerusalem;* [4]*Rambam, Medical Genetics Institute, Haifa, Israel*

Background: Diagnosing VSC (bodies that differ genetically from typical normative male or female bodies, e.g. Klinefelter syndrome, Turner syndrome, Congenital adrenal hyperplasia, Androgen insensitivity syndrome, Kallman syndrome) by Prenatal diagnosis (PND) is a powerful social action that affects the social realities of patients, families and community. It is a process that embodies an imagined connection between various biological characteristics and sociocultural norms of gender and fertility. Recently, contradictorily changes occur concerning both self-determination and bodily autonomy of people with VSC, alongside a geneticization process that leads to PND practices, including termination of pregnancies and preimplantation genetic testing (PGT).

We examined how fertility and genetics professionals who encounter couples/families with a child with VSC and people with VSC undergoing various fertility processes, perceive VSC, how they perceive and manage preventive practices aimed at bodies with VSC, and how discourses on bodily autonomy take place in daily practice and interactions with patients and families in fertility and genetics settings.

Methods: In-depth interviews with 27 clinicians were conducted to learn about their perceptions, diagnosis practices, interactions with patients and families, and their understanding of bodily autonomy issues and practices related to them.

Results: Three dynamic themes emerged from our findings: controlling the diagnostic emergency of VSC, secrets and concealment in the genetic diagnostic process, and imagining and preventing the existence of fertile bodies.

Conclusion: While PND for VSC becomes widely acceptable, professionals involved in the process should be aware and address the specific issues and concerns of this group regarding medical, emotional, moral and psychosocial aspects.

Grants:

Conflict of Interest: None declared

P24.004.C Evaluating the psychosocial and clinical outcomes from the Australian TRACEBACK program

Laura Forrest[1]

[1]*Peter MacCallum Cancer Centre, Parkville Familial Cancer Centre, Melbourne, Australia*

Background/Objectives: The Australian TRACEBACK program retrospectively identifies predominantly deceased women with tubo-ovarian cancer who were not offered clinical genetic testing and performs germline panel sequencing. To date, TRACEBACK has notified 105 next-of-kin (NOK) or probands, about the identification of a pathogenic variant. Given the significant personal and familial implications of the unexpected information being communicated, the TRACEBACK evaluation (TRACEBACK.E) was conceptualised. TRACEBACK.E aims to examine the perceived value, and clinical and psychosocial outcomes of TRACEBACK for probands, NOK, and family members.

Methods: TRACEBACK.E uses a sequential, mixed-methods design consisting of an online prospective, longitudinal survey and semi-structured interviews. NOK/probands were invited to TRACEBACK.E after they had been notified of germline information by TRACEBACK. Codebook thematic analysis with inductive coding of qualitative data was used to generate findings.

Results: Twenty-six interviews were performed with most participants describing that the familial benefits outweighed the initial shock of receiving unexpected germline information. The majority valued TRACEBACK because they thought the opportunity for genetic testing had passed, instead this program enabled cascade testing thereby clarifying cancer risk in relatives. Further, TRACEBACK provided a definitive explanation for the ovarian cancer diagnosis, meaningfully excluding lifestyle or environmental causes. Participants suggested germline results should be returned to biologically related relatives to minimise the risk of information not being communicated by the next of kin to at-risk relatives.

Conclusion: The benefits of unexpectedly receiving genetic information from TRACEBACK outweighed any potential harms, but consideration about to whom notification occurs is needed.

Grants: Ovarian Cancer Australia

Conflict of Interest: None declared

P24.005.D Moral attitudes of key stakeholders on somatic gene editing for inherited cardiomyopathy: a qualitative interview study

Jannieke Simons[1], *Rieke van der Graaf*[1], *Pascal Borry*[2], *Hans van Delden*[1]

[1]*University Medical Center Utrecht, Julius Center for Health Sciences and Primary Care, Bioethics and Health Humanities, Utrecht, Netherlands;* [2]*KU Leuven, Centre for Biomedical Ethics and Law, Public Health and Primary Care, Leuven, Belgium*

Consortium: PSIDER Heart consortium, GEREMY consortium

Background/Objectives: This paper aims to unravel the array of moral attitudes among key stakeholders engaged in or affected by the development and potential future implementation of somatic gene editing therapy for inherited cardiomyopathy. Given

the early stage of therapy development, now is the right time for critical appraisal of, amendments and adaptations to these advancements, steering away from mere end-of-development-pipeline reflections.

Methods: We expect to conduct approximately 40 semi-structured in-depth interviews with stakeholders residing in the Netherlands, Belgium, and the United Kingdom, categorized into five groups: patients and carriers ("users"), bench researchers ("developers"); cardiologists ("providers"); pharmaceutical industry personnel ("suppliers"); and public health officials ("regulators"). Interviews explore the desirability of somatic gene editing therapy for inherited cardiomyopathy, conditions and considerations for potential first-in-human trials, and perspectives on fair access to potential future treatment. Thematic analysis identified recurring patterns and higher order themes through iterative refinement by constant comparison.

Results: Preliminary findings provide a nuanced understanding of the ethical landscape surrounding somatic gene editing therapy for inherited cardiomyopathy. Also, this study illuminates both shared and distinct underlying values among the identified stakeholder groups.

Conclusion: This qualitative in-depth interview study informs further therapy development, first-in-human trial design, and clinical implementation of a somatic gene editing therapy for inherited cardiomyopathy. Insights from this study will contribute to navigating ethical questions in the ever-evolving realm of genomics research.

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Conflict of Interest: None declared

P24.006.A Who guards the guardians? A U.S. national survey of institutional signing officials on ethical genomic data management and sharing practices

Vasiliki Rahimzadeh[1], *Jinyoung Baek*[2], *Jonathan Lawson*[2], *Edward Dove*[3]

[1]*Baylor College of Medicine, Houston, United States;* [2]*Broad Institute of MIT and Harvard, Cambridge, United States;* [3]*Edinburgh Law School, United Kingdom*

Background/Objectives: Data access committees (DAC) gatekeep access to secured genomic and related health data yet are challenged to keep pace with the rising volume and complexity of genomic data generation. Automated decision support (ADS) systems have been shown to support consistency, compliance, and coordination of genomic data sharing of data access review decisions. However, we lack understanding of how DAC members perceive the value add of ADS, if any, on the quality and effectiveness of their reviews

Methods: In this qualitative description study, we conducted 13 semi-structured interviews with DAC members from large, publicly funded genomic data repositories around the world to gather insights about current practices, challenges faced, and potential opportunities for implementing automated decision support tools. Participants included repository managers, bioinformaticians, ethicists, and other professionals involved in genomic data governance.

Results: Increasing genomic data generation was a powerful motivator for why DAC members would consider implementing ADS into their access workflows. Participants supported pilot studies that test ADS performance in cataloging data types, verifying user credentials and tagging datasets for use terms. Concerns related to over-automation, lack of human oversight, low prioritization, and misalignment with institutional missions tempered enthusiasm for ADS.

Conclusion: Future research is needed to build the evidence base around the comparative effectiveness and decisional outcomes of institutions that do/not use ADS into their workflows as more genomic data are made available for secure sharing.

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Conflict of Interest: Vasiliki Rahimzadeh Funded by the following grants on related topics: K01HG013112; U24HG011025, Funded by the following grants on related topics: K01HG013112; U24HG011025, Jinyoung Baek Employed by the Broad Institute of Harvard and MIT, and developers of the Data Use Ontology, Jonathan Lawson Lead software developer of the Data Use Ontology, Support for this study is provided by the National Human Genome Research Institute U24HG010262;, Employee of the Broad Institute of MIT and Harvard, lead developer of the Data Use Ontology, Edward Dove Support for this study is provided by the National Human Genome Research Institute U24HG010262;

P24.007.B Value-based governance frameworks for Prenatal Gene Editing

Rosario Isasi[1], *Kelly Ormond*[2], *Yvonne Bombard*[3], **Heidi Carmen Howard**[4], *Roel Feys*[5]

[1]*John P. Hussman Institute for Human Genomics, Dr. John T. Macdonald Foundation Department of Human Genetics, Miami, United States;* [2]*ETH Zurich, Health Ethics & Policy Lab, Department of Health Sciences and Technology;* [3]*Institute for Health Policy, Management and Evaluation, University of Toronto;* [4]*Lund University;* [5]*University of Miami Leonard M. Miller School of Medicine, Dr. John T. Macdonald Foundation Department of Human Genetics*

Consortium: PaSAGE: PAtient Supported Approaches to Gene Editing (PaSAGE) consortium

Prenatal gene editing (PGE) has yet to be introduced in clinical practice, yet it is foreseeable that it will be available soon in the research setting. As the potential translation of PGE develops, the policy landscape remains uncertain, as requirements differ based on criteria such as intended indication and technical approach. Reproductive technologies are one of the most variably governed areas of medicine, reflecting the broader social, moral, and political society in which they are embedded. However, policies often adopt a technocratic approach focusing on moratoria or other forms of control, while their underlying ethical normative underpinnings remain vague. Indeed, calls for assessing their implications are often performative with considerations for collective deliberation secondary. Thus, the need for values-based governance becomes ostensible.

To develop a values-based governance for PGE, we conducted a comparative policy study (e.g. soft and hard laws) in ~40 jurisdictions to identify the rationale and values supporting policy approaches to PGE. While across policies, autonomy and dignity are the most discernable principles, the notion of justice is also predominantly present albeit subject to heterogeneous conceptualizations. In our analysis, we paid special attention to emerging translational justice (TJ) and governance challenges. We maintain that robust values-based governance is predicated on meaningful inclusion of all stakeholders (e.g., patients/families, scientists, clinicians, funders and policy makers). We further outline a framework for incorporating TJ considerations within governance structures across the PGE clinical research continuum.

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Grant "PaSAGE: PAtient Supported Approaches to Gene Editing (PaSAGE)" National Human Genome Research Institute (grant R01HG011461), Roel Feys Research Associate in the study

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P24.008.C Judges' and Lawyers' insights into society's genomic readiness

Fatos Selita[1;2], *Yulia Kovas*[1], *Maxim Likhanov*[3]

[1]*Goldsmiths, University of London, United Kingdom;* [2]*Udayana University, Indonesia;* [3]*Beijing Normal University, China*

Background/Objectives: Judges' and lawyers' genetic literacy; and their views on the use of genetic information, and on the need for updated laws – have a significant impact on society. For example, these stakeholders need good genetic literacy to be able to combat deterministic views, assess genetics-related discrimination risks, develop the relevant jurisprudence influencing relevant laws, and mitigate risks for genetic services providers and consumers of such services.

Methods: We extended previous limited research into the genomic era readiness - exploring genetic knowledge and attitudes of 117 judges and lawyers. Participants completed an in-depth questionnaire (30 items), assessing their genetic knowledge, as well as views and attitudes on the use of genetic information. A small qualitative study provided further insights into the judges' and lawyers' views.

Results: Quantitative and qualitative analyses showed low knowledge of essential genetic concepts, including some striking cases of over- and under-estimation of the importance of both genetic and environmental factors. Participants' views on many issues were widely varied, from strong agreement to strong disagreement. Most judges and lawyers endorsed gene editing for prevention and treatment of disease, and the use of genetic information for crime prevention; but disagreed with the use of genetic information by insurers. Most participants thought that current laws are not sufficient to protect individuals from misuses of genetic data in insurance, education and employment contexts.

Conclusion: The results of this study call for efforts to improve readiness of the legal workforce for the genomic era, including relevant modules in lawyers' training.

Grants: n/a

Conflict of Interest: None declared

P24.009.D Who has the responsibility to inform relatives at risk of hereditary cancer? A population-based survey in Sweden

Kalle Grill[1], *Amicia Phillips*[2], *Barbro Numan Hellquist*[3], **Anna Rosén**[3]

[1]*Umeå University, Department of Historical, Philosophical and Religious Studies, Umeå, Sweden;* [2]*KU Leuven, Centre for Biomedical Ethics and Law, Department of Public Health and Primary Care, Leuven, Belgium;* [3]*Umeå University, Diagnostics and intervention, Oncology, Umeå, Sweden*

Background/Objectives: Results of hereditary cancer clinical investigations concern both patients and their at-risk relatives. Current standard practice for risk disclosure involves cooperation between the patients and the healthcare system, but responsibilities for each party are intertwined and sometimes unclear. We explored public attitudes on the different actors' moral and legal responsibilities to inform at-risk relatives.

Methods: We surveyed a random sample of the Swedish population in 2018. The online questionnaire probed attitudes on risk disclosure responsibilities in a hypothetical scenario where a patient was informed of an increased risk of colorectal cancer, meaning regular colonoscopy screening was recommended for them and their relatives.

Results: Of the 914 respondents (response rate 51%), 75% thought the healthcare system had a moral responsibility to inform relatives, while 59% ascribed moral responsibility to the patient. When asked who had the ultimate responsibility to inform, 71% thought it was the healthcare system. 66% thought the healthcare system should have a legal responsibility to inform and 21% thought the patient should be legally responsible. In the case of the patient actively objecting to disclosure, a majority (65% or 78%, depending on the degree of cancer risk for relatives) thought that the healthcare system should still inform relatives at risk.

Conclusion: Our study indicates that the Swedish general public ascribes moral responsibility to inform relatives to both patients and the healthcare system, but contrary to current practice, they believe the healthcare system has the ultimate responsibility. The majority supports disclosure against the patient's will.

Grants: FORTE, Cancer Research Foundation.

Conflict of Interest: None declared

P24.010.A Exploring the Public Health Impact of Direct-to-Consumer Approaches in Personalized Prevention

Pascal Borry[1], *Eva Van Steijvoort*[1], *Amicia Phillips*[1]

[1]*KU Leuven, Department of Public Health & Primary Care, Leuven, Belgium*

Consortium: PROPHET

Background/Objectives: Direct-to-consumer genetic testing offers that provide health information directly to the public have become widely available at a moderate cost. The rapid growth of this market has however raised concern with regard to the possible impact on the public health care system (e.g. downstream tests). This project aimed to assess current evidence on the public health impact of direct-to-consumer genetic testing within the context of personalized prevention.

Methods: We performed a critical review and analysis of the available literature to provide a comprehensive overview and to identify gaps for future research.

Results: Despite initial concerns surrounding DTC-GT and its potential impact on public health, many of the anticipated issues have not manifested as expected given the available data. It seems that merely one-third of DTC-GT consumers share their test results with a healthcare professional and primarily with primary care professionals. While occurrences of patients sharing test results only constitute a relatively small proportion of the total amount of patient visits, some referrals did prompt clinical actions (e.g. validation of test results, referrals to specialists). Considering the rapid growth of the DTC-GT market, there is the potential for a growing impact on the public healthcare system in the future.

Conclusion: Further international research exploring the impact of DTC-GT on the public health care sector would be highly beneficial to gain more insights as earlier research findings mostly represented early adopters of DTC-GT and might no longer represent the current population undergoing DTC-GT.

Grants: PROPHET, grant number 101057721

Conflict of Interest: None declared

P24.011.B Exploring attitudes and experiences with reproductive genetic carrier screening among couples seeking medically assisted reproduction: a longitudinal survey study

Eva Van Steijvoort[1], *Mathilde Cassou*[1], *Camille De Schutter*[1], *Eftychia Dimitriadou*[2], *Hilde Peeters*[2], *Karen Peeraer*[3], *Gert Matthijs*[2], *Pascal Borry*[1]

[1]*KU Leuven, Department of Public Health & Primary Care, Leuven, Belgium;* [2]*KU Leuven, Department of Human Genetics, Leuven, Belgium;* [3]*KU Leuven, Department of Development and Regeneration, Leuven, Belgium*

Background/Objectives: Reproductive genetic carrier screening (RGCS) can be performed to identify whether an individual carries pathogenic variants linked to one or more specific autosomal recessive or X-linked conditions. This study aimed to assess the attitudes and experiences of subfertile couples applying for medically assisted reproduction (MAR) using their own gametes towards RGCS for monogenic conditions.

Methods: A prospective survey study was conducted where subfertile couples were recruited from the fertility centre of a university hospital in Flanders, Belgium. Participants were offered RGCS free of charge and completed self-administered questionnaires at three different time points.

Results: Fifty five individuals, informed by their fertility specialists, willingly participated and completed the first questionnaire. Attitudes towards RGCS were positive and the intention to participate in RGCS was high. Twenty-six couples decided to participate in the follow-up study where the Belgian RGCS test was offered free of charge. Anxiety scores were only elevated and clinically relevant for a limited number of participants. A large proportion of participants would consider preventive reproductive options like prenatal diagnosis or IVF/ICSI combined with PGT-M in the event of an increased likelihood of conceiving a child with a hereditary condition. Participants were satisfied with their decision to undergo RGCS, and the majority would recommend RGCS to other couples.

Conclusion: This study provides valuable insights into the perspectives of subfertile couples applying for MAR using their own gametes, highlighting the need for appropriate counseling and timely information provision.

Grants: Research Fund Flanders (FWO) (G094518N)

Conflict of Interest: None declared

P24.012.C Standardising utility. What is the priority for genomic testing in rare disease?

Stephanie Best[1;2;3], *Zoe Fehlberg*[1;2], *Ilias Goranitis*[1;2], *Andrew Mallett*[2;4;5], *Zornitza Stark*[1;2;6]

[1]*The University of Melbourne, Parkville, Australia;* [2]*Australian Genomics, Parkville, Australia;* [3]*Peter MacCallum Cancer Centre, Melbourne, Australia;* [4]*JCU: James Cook University, Australia, Townsville, Bebegu Yumba campus, Douglas, Douglas, Australia;* [5]*Townsville University Hospital, Douglas, Australia;* [6]*Victorian Clinical Genetics Services, Parkville, Australia*

Background/Objectives: Without a standardised approach to generating evidence about the value of genomic testing, successful implementation of genomic medicine into healthcare remains challenging. Agreement about which indicators of utility to include is lacking. We aimed to identify and define priority indicators of utility for rare disease genomic testing amongst key stakeholder groups.

Methods: A modified-Delphi methodology was used to obtain consensus towards priority indicators of utility with the first round including predetermined indicators drawn from the literature and study team experience. Invited experts were selected for their leadership within policy, health services, research, and consumer advocacy. Quantitative and qualitative data were analysed to identify, define, and determine priority indicators.

Results: Two Delphi rounds were completed with 25 experts participating in Round 1 and 18 in Round 2. Consensus was achieved for 20 out of 40 proposed indicators e.g., prognostic information, timeliness of results, practical and health care outcomes, clinical accreditation, and diagnostic yield. Other indicators relating to topics such as discovery research, disutility, and factors secondary to primary reason for testing were not considered a priority by participants and were removed.

Conclusion: The findings align with a patient-centred approach to determining utility and incorporating the growing inclusion of patient/family measures and other contextual factors (e.g., costs to healthcare systems and accessibility) within overall utility. The outcomes of the study may inform a standardised approach to evidence generation and assessment to guide future research, decision-making, and implementation efforts.

Grants: The Australian Government's National Health and Medical Research Council through 'Australian Genomics Grant Program Proposal 2021-23'(Grant GNT2000001).

Conflict of Interest: None declared

P24.013.D Preconception carrier screening: implementation options in Austria

Sabine Rudnik[1], *Christina Wölwer*[1], *Klara Jetzinger*[1], *Johannes Zschocke*[1]

[1]*Medizinische Universität Innsbruck, Institute of Human Genetics, Innsbruck, Austria*

Background/Objectives: Preconception carrier screening (PCS) can identify high-risk couples for serious autosomal recessive or X-linked diseases in children. We present the results of a feasibility study in Innsbruck/Austria.

Methods: Following the recommendations of the ESHG (PMID 29023436) the following issues were addressed: 1) Which diseases/genes should be included for PCS? 2) How is informed consent achieved? 3) When is the best time point of introducing PCS? 4) How can results be returned?

Results:

1. In the Austrian population a pan-ethnic test is recommended. The underlying gene panels were adapted to the German background according to tier 1-3 as suggested by the ACMG (PMID 34285390).
2. New models to gain generic consent are required. Following encouraging studies in the US (PMID 33135283) we developed an information comic in German language which can be downloaded on every PC or mobile device.
3. PCS should best be offered to couples planning a pregnancy, but should be also made available in pregnancy. Eventually, we aim at a population-based screening through health care providers.
4. Return of results is a question of costs and resources. It should be generally consented couple based by the responsible physician or individually in case of carrier status

for X-linked diseases. Qualified genetic counseling must be offered to high-risk couples.

Conclusion: We evaluated the prerequisites for PCS in Austria, adapted gene panels for German speaking countries, and developed tools for an informed consent in German language. Nonetheless, there are still methodological issues and questions, how to implement PCS into the Austrian health care system.

Conflict of Interest: None declared

P24.014.A A collaborative working group of experts to define the list of diseases to be screened in the French genomic newborn screening project PERIGENOMED

Camille LEVEL[1], *Yannis Duffourd*[1], *Dominique Salvi*[2], *Frédéric Huet*[3], *Christine Binquet*[4], *Christophe Verny*[5], *David Cheillan*[6], *Jean-baptiste Arnoux*[6], *Philippe Charron*[7], *des portes vincent*[8], *Alexandre Belot*[9], *Eric Hachulla*[9], *Guillaume Jondeau*[10], *Olivier Chazouillères*[11], *Christine Bodemer*[12], *Martin Krahn*[13], *Jean-Plerre Hugot*[14], *Frédéric Gottrand*[14], *Jerome Bertherat*[15], *Peffault de la tour Regis*[16], *Frederic Galacteros*[17], *Sophie Susen*[18], *Denis Morin*[19], *Valérie Cormier-Daire*[20], *Agnès Linglart*[20], *Hélène Dollfus*[21], *Sylvie Odent*[22], *stéphane bezieau*[22], *Christel Thauvin-Robinet*[1], *Laurence Faivre*[1;23]

[1]*Centre de Génétique, CHU Dijon Bourgogne, Dijon, France INSERM UMR1231, Equipe GAD, Université de Bourgogne, Dijon, France FHU TRANSLAD, CHU Dijon Bourgogne, Dijon, France, Dijon, France;* [2]*Centre de Génétique, CHU Dijon Bourgogne, Dijon, France;* [3]*Pôle de Pédiatrie, CHU Dijon Bourgogne, Dijon, France;* [4]*INSERM CIC1432, Epidémiologie Clinique, Université de Bourgogne, Dijon, France FHU TRANSLAD, CHU Dijon Bourgogne, Dijon, France;* [5]*Brain Team Reference Network for Central nervous system;* [6]*G2M Reference Network for Metabolism disease;* [7]*Cardiogen Reference Network for Heart disease;* [8]*DéfiScience Reference Network for brain development and intellectual disability;* [9]*Fai2R Reference Network for Autoimmune/autoinflammatory diseases;* [10]*FAVA-Multi Reference Network for Vascular diseases;* [11]*FILFOIE Reference Network for Liver diseases;* [12]*FIMARAD Reference Network for Dermatology;* [13]*FILNEMUS Reference Network for Neuromuscular diseases;* [14]*FIMATHO Reference Network for Abdomino-thoracic diseases;* [15]*FIRENDO Reference Network for Endocrinology;* [16]*MaRIH Reference Network for Immuno-hematology;* [17]*MCGRE Reference Network for Red blood cells and dyserythropoiesis;* [18]*MHemo Reference Network for Hemorrhagic diseases;* [19]*ORKiD Reference Network for Renal Diseases;* [20]*OSCAR Reference Network for Bone-Calcium/Cartilage-Kidney;* [21]*SENSGENE Reference Network for Rare sensory diseases;* [22]*FHU GENOMEDS;* [23]*AnDDI-Rares Reference Network for Developmental abnormalities*

Consortium: International Consortium on Newborn Sequencing

Background: Global initiatives in genomics for newborn screening (gNBS) have emerged with advancing therapeutic methods and the cost-effective evolution of genetic technologies. Led by the French University Hospital Federation TRANSLAD, PERIGENOMED comprises PGC1, a feasibility study involving 2,500 newborns from 6 university hospitals; PGC2, a prefigurator covering all maternity units in a French region to assess nationwide utility and transferability. Ancillary studies explore psychosocial, budgetary, and regulatory impacts.

Method: A merged database contains the number of pilots screening each dyad among Generation Study, BabySeq, BeginNGS, GUARDIAN, NC Nexus, Baby Detect, Igenomix, NeoSeq, NeoExome and BabyScreen+ and metadata on dyad characteristics (age at onset, correlation level, ASQM score, etc.). In PGC1, we rely on the expertise of the 23 French Networks for Rare Disease (FNRD) to determine dyad inclusion in list 1 (treatable childhood disease), list 2 (actionable childhood disease, optional for parents). This approach ensures community acceptance, capitalizing on France's efficient rare disease care structure.

Results/Perspectives: Overall, 1,794 dyads (corresponding to 1,525 unique genes) are included by at least 1 out of 10 projects. Only 41 dyads are present in all 10 projects and 180 in 6 or more. Unless vetoed by the FNRD, these dyads will be included in the list 1. The 763 dyads in 2 to 5 projects will undergo FNRD evaluation, deciding inclusion in list 1 or 2. First FNRD results are expected in April 2024.

Grant: French Muscular Dystrophy Association (AFM-Téléthon)

Conflict of Interest: None declared

P24.015.B Genetic knowledge and genetic attributions for racial disparities in obesity

Matthew Lebowitz[1], *Rebecca Pearl*[2], *Carolyn Tucker*[2]

[1]*Columbia University, Department of Psychiatry, New York, United States;* [2]*University of Florida, Gainesville, FL, United States*

Background/Objectives: There are racial disparities in the prevalence and treatment of obesity, which is common and associated with multiple health risks. In 2013, the American Medical Association recognized obesity as a "disease," and increasingly biomedical conceptualizations have coincided with increased interest in the genetics of obesity. Genetic factors play a role in obesity, but repeated attempts at genetic explanations for racial health disparities are likely to be misguided and could lead to neglect of social determinants such as discrimination and unequal access to healthy foods/environments and healthcare. Healthcare providers with higher levels of genetic knowledge may be less likely to assume that racial disparities in obesity reflect genetic differences, but providers are often not knowledgeable about genetics.

Methods: Surveys measuring genetic knowledge, genetic attributions for obesity, and genetic attributions for racial disparities in obesity.

Results: In a pilot study among U.S. adults, endorsement of the notion that "patients' genes" are responsible for "differences in obesity rates between racial groups" was negatively associated with genetic knowledge and positively associated with genetic attributions for obesity. A newly funded study will investigate whether similar patterns emerge among a large sample of U.S. primary care physicians.

Conclusion: It is important to understand physicians' level of genetic knowledge and how it may relate to beliefs about racial disparities in obesity. As the presentation will discuss, these beliefs have important clinical and ethical implications, especially considering obesity's high population prevalence and the recent push to integrate genomics into obesity care.

Grants: R01HG012868 (US NIH).

Conflict of Interest: None declared

P24.016.C Disclosure of genetic risk in the family: Attitudes of the general Portuguese population

Iara Ribeiro[1;2], *João Parente Freixo*[3], *Liliana Sousa*[1;2], **Álvaro Mendes**[3]

[1]*University of Aveiro, Department of Education and Psychology, Aveiro, Portugal;* [2]*CINTESIS@RISE;* [3]*CGPP, IBMC, i3S - University of Porto, Porto, Portugal*

The expanding use of genetic and genomic testing poses challenges to the healthcare system in supporting patients in disclosing risk information to their relatives. This study aims to characterize the Portuguese public's attitudes towards the

disclosure of information about the risk of hereditary diseases, including preferences for service delivery and policy approaches.

We advertised an online survey in social media and public spaces. Data from 587 participants were analyzed through descriptive statistics.

The mean age of participants was 42.4 (range: 18-81, SD 14.22). The majority were female (77.9%), had children (56.9%), and had obtained higher education (77.3%). Participants wanted to be informed of their genetic risk by a clinician (92.6%) rather than a family member (85.2%), and to undergo genetic testing to confirm diagnosis (88.6%). Similarly, participants overwhelmingly believed that family members should be informed of their risk by clinicians (82.3%), rather than the participants themselves (70.8%). These preferences were observed in scenarios involving hereditary colorectal cancer and Huntington's disease. Participants preferred being informed by a clinician (92,5) or a distant family member (77.4%) rather than remaining uninformed, while 64.7% wished the first communication to come from a close family member. Most participants would support legislation binding patients to inform relatives (60.7%), and allowing clinicians to directly inform relatives (81.6%), even when the patient did not wish to disclose information (87.8%).

The Portuguese public prefers genetics services to directly contact relatives. Further discussion is needed on how to best cascade relevant risk information to relatives and the appropriate policy considerations.

Conflict of Interest: Iara Ribeiro: None declared, João Parente Freixo: None declared, Liliana Sousa Team member of the DECIDE project, funded by the FCT - the Portuguese Foundation For Science and Technology (2022.04025.PTDC), Álvaro Mendes Principal Investigator of the DECIDE project, funded by the FCT - the Portuguese Foundation For Science and Technology (2022.04025.PTDC)

P24.017.D Psychiatric Genetics in Europe: A Focus on Ethical Implications

Boris Chaumette[1;2], *Heidi Beate Bentzen*[3], *Filip Bialy*[4], *Virginia Bizzarri*[5], *Isabella Borg*[6], *Eline M. Bunnik*[7], *David Crepaz-Keay*[8], *Domenico Coviello*[9], *Ashok Handa*[10], *Maria Johansson Soller*[11;12], *Andrew McQuillin*[13], *Julia Perry*[14], *Marcella Rietschel*[15], *Kristiina Tammimies*[16], *Silke Schicktanz*[14], *Melanie Watson*[17], *Sarah L Wynn*[18], *Charlotta Ingvoldstad*[11;12;19]

[1]*Université de Paris, GHU Paris Psychiatrie et Neurosciences, Institute of Psychiatry and Neurosciences of Paris, Paris, France;* [2]*McGill University, Department of Psychiatry, Montreal, Canada;* [3]*Centre for Medical Ethics, University of Oslo, Oslo, Norway;* [4]*Adam Mickiewicz University Poznań, Poznan, Poland;* [5]*Complex Structure Neuropsychiatry of Childhood and Adolescence, ASL 3, Genova, Italy;* [6]*Pathology Department, University of Malta, Malta;* [7]*Erasmus MC, University Medical Centre Rotterdam, Department of Medical Ethics, Philosophy and History of Medicine, Rotterdam, Netherlands;* [8]*Mental Health Foundation, London, United Kingdom;* [9]*IRCCS Istituto Giannina Gaslini, Laboratory of Human Genetics, Genoa, Italy;* [10]*St Catherine's College, Collaborating Centre for values based practice, Oxford, United Kingdom;* [11]*Karolinska Institutet, Department of Molecular Medicine and Surgery, Stockholm, Sweden;* [12]*Karolinska University hospital, Department of Clinical Genetics, Stockholm, Sweden;* [13]*University College London, Division of Psychiatry, London, United Kingdom;* [14]*University Medical Center Göttingen, Department of Medical Ethics and History of Medicine, Göttingen, Germany;* [15]*Central Institute of Mental Health, Medical Faculty Mannheim, University of Heidelberg, Department of Genetic Epidemiology in Psychiatry, Mannheim, Germany;* [16]*Center of Neurodevelopmental Disorders (KIND), Centre for Psychiatry Research, Karolinska Institutet, Department of Women's and Children's Health, Stockholm, Sweden;* [17]*University Hospital Southampton NHS Foundation Trust, Wessex Clinical Genetics Service, Southampton, United Kingdom;* [18]*Unique - Rare Chromosome Disorder Support Group, Oxted, United Kingdom;* [19]*Uppsala University, Centre for Research ethics and Bioethics, Uppsala, Sweden*

Consortium: European Network on Ethics around Psychiatric Genetics (ENEPG)

Background: Psychiatric genetics raises unaddressed ethical issues. A COST Action – an EU funded collaboration in science and technology – dedicated to Enhancing Psychiatric Genetic Counselling, Testing, and Training in Europe (EnGagE) was established to build a network of European experts in the field.

Methods: COST EnGagE brought together 25 experts from 13 European countries to identify the main ethical issues related to psychiatric genetics. The issues were discussed by the experts in depth over a number of days, the summaries of each discussion session were transcribed and the main areas of ethical issues were explored and defined. the findings have been submitted to patient representatives and amended with their feedback.

Results: Four main ethical topics were identified: The problematic dualism between somatic and psychiatric disorders; The impact of genetic testing for psychiatric conditions on psychosocial experience, such as stigma; Professional responsibilities related to communication; Ethical issues in public health services. For each topic, we developed recommendations and points to consider in order to inform healthcare professionals involved in the clinical implementation of psychiatric genetics.

Conclusion: This European initiative draws attention to a set of ethical issues relevant for mental health and clinical genetics professionals, for patients and their family members, as well as society as a whole. This highlights the need for more interdisciplinary exchange to define standards in clinical implementation as well as in public communication. With this work, we launch the new European Network on Ethics around Psychiatric Genetics (ENEPG).

Grants: COST Action CA17130
Conflict of Interest: None declared

P24.018.A Attitudes and willingness toward sharing genomic data: Rare disease patient representatives differ from the Swedish general public

Maria Johansson Soller[1;2], *Virpi Töhönen*[1;2;3], *Anna Middleton*[4;5], *Richard Milne*[5;6], *Charlotte Ingvoldstad Malmgren*[1;2;7]

[1]*Stockholm, Clinical Genetics and Genomics, Stockholm;* [2]*Stockholm, Molecular medicine and surgery, Stockholm;* [3]*Stockholm, Center for metabolic diseases, Stockholm;* [4]*Cambridge, Kavli centre for ethics, science and the public, Cambridge, United Kingdom;* [5]*Hinxton, Engagement and Society, Wellcome connecting science, Hinxton, United Kingdom;* [6]*Cambridge, Centrum for research and Bioethics, Cambridge, United Kingdom;* [7]*Uppsala University, Centrum for research and Bioethics, Uppsala, Sweden*

Background/Objectives: Sharing of genetic data is pivotal for genomic research and development of precision medicine. Individuals with rare conditions benefit from precision medicine, as it aids in accurate diagnosis and treatment. This study explores the attitudes towards, and willingness of, patients with rare conditions to share their genomic data with research and medical professionals.

Methods: Employing a survey methodology as part of the international "Your DNA Your Say" study, the questionnaire incorporates nine short informative films elucidating the implications of DNA data sharing, followed by 29 questions. The cohort

comprises of 72 patients with rare conditions who were invited to participate via the Swedish association of Rare Disease. Their responses were compared to a matched control group of 144 individuals from the Swedish public.

Results: The patients demonstrate a strong comprehension of genetics and perceive DNA information as distinct from other medical data. The public also responded considerably more often an uncertainty for understanding of genetics. A significant majority of the patients expresses a willingness to anonymously donate genomic data, in contrast to the public. They also exhibit a heightened level of confidence and trust in healthcare providers and researchers, compared to the public.

Conclusion: Patients show a high willingness to donate their genomic data and a substantial trust in their doctors in contrast to the Swedish public. This emphasizes the imperative for public comprehension of the expanding landscape of data sharing and its implications for diagnosis and treatment in precision medicine.

Grants: This work was partly funded via the national initiative Genomic Medicine Sweden

Conflict of Interest: None declared

P24.019.C A systematic scoping review on public engagement regarding human germline gene editing

Wendy Geuverink[1;2], *Diewertje Houtman*[3], *Isabel Retel Helmrich*[3], *Lidewij Henneman*[1;2], *Martina Cornel*[1;4], *Sam Riedijk*[3]

[1]*Amsterdam UMC, location Vrije Universiteit Amsterdam, Department of Human Genetics, Amsterdam, Netherlands;* [2]*Amsterdam Reproduction and Development Research Institute, Amsterdam, Netherlands;* [3]*Erasmus Medical Center, Department of Clinical Genetics, Rotterdam, Netherlands;* [4]*Amsterdam Public Health Research Institute, Amsterdam, Netherlands*

Consortium: De DNA dialogen Consortium

Background/Objectives: Following the advent of the CRISPR-Cas technology in 2012, there has been a growing global call for public engagement regarding the potential use of human germline gene editing (HGGE). The need to include under-represented groups in these efforts is increasingly stressed, for example by the World Health Organization. The necessity to learn, not only about people's acceptance, but also about their underlying values regarding HGGE was emphasized as well. We performed a systematic scoping review on public engagement regarding HGGE to determine how these calls for public engagement have been answered so far. What methods were used and who was involved?

Methods: A systematic literature search was undertaken in December 2023 to identify articles that report on public and patient engagement studies regarding HGGE. Overall, 3464 records were retrieved from 12 databases. After title/abstract screening by two reviewers, 52 full-text articles were assessed for eligibility, resulting in a total of 35 articles.

Results: 68% of the included studies target the general public, with the majority using a low-level engagement method, namely a survey, to look at levels of HGGE acceptance. However, 2 public studies set up dialogues to enable a meaningful exchange of values, aiming to shape future policy using their results. Of the 6 studies that specifically engaged patient groups, 5 used interviews or focus groups to identify in-depth values and needs.

Conclusion: There is limited inclusion of traditionally under-represented groups, with the exception of some patient groups, and little effort on finding underlying values associated with HGGE.

Grants: [NWO/NWA.1389.20.075].

Conflict of Interest: Wendy Geuverink As of 1st September 2022, the authors are involved in a Netherlands Consortium "Public Realm Entrance of Human Germline Gene Editing" funded by The Netherlands Organization for Scientific Research (NWO NWA.1389.20.075)., Diewertje Houtman As of 1st September 2022, the authors are involved in a Netherlands Consortium "Public Realm Entrance of Human Germline Gene Editing" funded by The Netherlands Organization for Scientific Research (NWO NWA.1389.20.075)., Isabel Retel Helmrich As of 1st September 2022, the authors are involved in a Netherlands Consortium "Public Realm Entrance of Human Germline Gene Editing" funded by The Netherlands Organization for Scientific Research (NWO NWA.1389.20.075)., Lidewij Henneman As of 1st September 2022, the authors are involved in a Netherlands Consortium "Public Realm Entrance of Human Germline Gene Editing" funded by The Netherlands Organization for Scientific Research (NWO NWA.1389.20.075)., Martina Cornel As of 1st September 2022, the authors are involved in a Netherlands Consortium "Public Realm Entrance of Human Germline Gene Editing" funded by The Netherlands Organization for Scientific Research (NWO NWA.1389.20.075)., Sam Riedijk As of 1st September 2022, the authors are involved in a Netherlands Consortium "Public Realm Entrance of Human Germline Gene Editing" funded by The Netherlands Organization for Scientific Research (NWO NWA.1389.20.075).

P24.020.D Offering and regulating expanded carrier screening (ECS) in the UK: an exciting prospect in genetic testing or a 'can of worms'?

Cathy Herbrand[1], *Pascal Borry*[2], *Lorraine Culley*[3], *Kriss Fearon*[3], *Nicky Hudson*[3], *Zosia Miedzybrodzka*[4], *Sarah Norcross*[5], *Bronwyn Parry*[6], *Eva Van Steijvoort*[2], *Lucina Wilde*[3]

[1]*De Montfort University, Leicester, United Kingdom;* [2]*KU Leuven;* [3]*De Montfort University;* [4]*University of Aberdeen, United Kingdom;* [5]*Progress Educational Trust;* [6]*Australian National University*

Consortium: N/A

Background/Objectives: Expanded carrier screening (ECS) is increasingly being offered at preconception to identify whether people with no known risk may pass on recessive genetic conditions to their children. The lack of policy attention compared to PGT and newborn screening has left providers free to determine its cost, delivery and marketing. What practical and ethical complexities arise from the lack of regulation in the UK?

Methods: Using a scoping methodology (Arksey H & O'Malley L., 2005), this study situates ECS in the UK policy landscape. Semi-structured interviews were conducted with a purposive sample of 20 key policy, commercial, and clinical stakeholders with a remit or interest in regulating reproductive genomic testing in the UK to systematically capture emergent policy and practice issues.

Results: ECS does not appear on the agenda of several key UK policy organisations, and some policy stakeholders interviewed were unaware of the specific complexities it presents. The current lack of guidance has generated a range of practical and ethical issues for providers and users including:

- Inconsistent provision of genetic counselling about ECS's purpose, results and limitations;
- Confusion on using imported ECS-screened donated gametes;
- Lack of clarity regarding legal liability if a recessive condition is transmitted
- Challenges caused by the lack of communication and collaboration between the public and private sectors around ECS;
- Absence of clear, evidence-based information for test-takers.

Conclusion: This research shows the need for the development of appropriate policy, guidance and regulation and public discussion in the UK.

Grants: Economic and Social Research Council [ES/W012456/1]

Conflict of Interest: Cathy Herbrand PI of the ESRC grant, full time, Pascal Borry CI on the research project, FT, Lorraine Culley Consultant on the research project, Kriss Fearon FT, Nicky Hudson CI on the research project, FT, Zosia Miedzybrodzka CI on the research project, FT, Sarah Norcross FT, Bronwyn Parry CI, FT, Eva Van Steijvoort FT, Lucina Wilde PT

P24.021.A Views of the Swiss public towards Gene Editing

Kelly Ormond[1], *Sara Kijewski*[1], *Naomi Wyler*[1], *Agata Ferretti*[1], *Eirini Petrou*[1], *Claudia Reichmuth*[1], *Effy Vayena*[1]

[1]*ETH-Zurich, D-HEST, Switzerland*

Background/objectives: Given the paucity of country-specific data, understanding of the public's views on gene editing therapies remains limited.

Method: In September 2023 we used the Federal Register to conduct a random survey of the Swiss population, stratified by language region (German, French, Italian), gender and age.

Results: We present a representative sample of 3856 responses (26.5% response), including >2000 open-ended comments. When presented with 7 options for somatic editing, 7% disagreed with all therapeutic options, and 36% supported them all. Most agreed with somatic gene editing to cure life-threatening (76.3%) and debilitating diseases (69.5%). Fewer were supportive as severity decreased or with later onset; for example, 65.9% supported somatic editing to cure adult onset disease. Most were against using gene editing to enhance physical (79.4%) or cognitive (72.0%) abilities. In all scenarios, people were less likely to agree regarding embryo gene editing options- 58.1% supported it for life threatening disease and 49.1% for debilitating diseases. 44.8% felt parents do not have a right to edit their children's genes before birth. When asked what influenced their views, the most endorsed reasons for feeling positively were 'views towards what it means to have a good life' (59.8%) and 'views about illness and suffering' (58.5%); most selected "neutral" to describe religion's influence (68.9%). We will present final data and a content analysis of comments.

Conclusions: Uncovering systematic differences in the attitudes towards specific therapies and the values shaping them underscores the importance of including peoples' voices in policy decisions.

Conflict of Interest: Kelly Ormond NIH/NHGRI grants related to gene editing (PASAGE), not related to the current research, ETH-Zurich employee, Sara Kijewski ETH-Zurich employee, Naomi Wyler: None declared, Agata Ferretti ETH-Zurich employee, Eirini Petrou ETH-Zurich employee, Claudia Reichmuth: None declared, Effy Vayena ETH-Zurich employee

P24.022.B Public Resources for Public Good? Illuminating Power and Privilege in Global Scale Sequencing

Alice Popejoy[1], *Krystal Tsosie*[2], *Ann Mc Cartney*[3], *Mahsa Shabani*[4], *Nanibaa' Garrison*[5], **Robert Cook-Deegan**[6]

[1]*University of California, Davis (UC Davis Health), Public Health Sciences (Epidemiology Division), Davis, California, United States;* [2]*Arizona State University, School of Life Sciences, Tempe, Arizona, United States;* [3]*University of California, Santa Cruz, Genomics Institute, Santa Cruz, California, United States;* [4]*University of Amsterdam, Faculty of Law, Law Center for Health and Life, Amsterdam, Netherlands;* [5]*University of California, Los Angeles, Institute for Society & Genetics, Institute for Precision Health, Division of General Internal Medicine & Health Services Research, Los Angeles, California, United States;* [6]*Arizona State University, Consortium for Science Policy and Outcomes, Washington, DC, United States*

Consortium: Human Pangenome Reference Consortium (HPRC), Human Cell Atlas (HCA)

Background/Objectives: Future benefits to patients and populations are the presumptive justification for publicly funded resource-generating projects using advanced sequencing technologies. Evaluating the distribution of risks and benefits of sequencing, however, remains elusive. Global public resources designed with anticipated value for all confront distinct ethical issues related to data governance on an unprecedented scale.

Methods: We evaluated ethical considerations and governance trends in three global public resource-generating efforts. Projects evaluated share common elements including advanced sequencing technologies, multi-lateral funding, international research teams, and open data. Frameworks for control of samples, shared governance, and collective benefit (i.e., FAIR, CARE, and TRUST) offer lenses through which to evaluate foundational principles and practices of selected projects.

Results: We illuminate tensions between presumed benefits of global sequencing projects and data governance that is often controlled by an internal, privileged few, while community engagement is considered optional. These dynamics reflect historical trends of extractive biocolonialism, extending to biospecimens and sequence data. Violation of privacy is a risk deemed acceptable by those with the most privilege and power. Commercial ties between academic institutions and corporate sponsors involve agreements giving privileged access to health records and genomic data, whose terms are more private than the genome sequences being generated. Who benefits from this ecosystem remains an open question.

Conclusion: Public resource-generating projects must implement internal checks-and-balances on power dynamics, establish control mechanisms to respect sovereignty of Indigenous and other national entities, and design custom shared governance models that facilitate mutual respect for agreements.

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Conflict of Interest: Alice Popejoy: None declared, Krystal Tsosie CacheDNA Clinical Advisory Board, Ann Mc Cartney: None declared, Mahsa Shabani: None declared, Nanibaa' Garrison: None declared, Robert Cook-Deegan Unpaid board member for the Public Innovation Project, a nonprofit organization opposed to patent abuse

P24.023.C Evaluation of the information provision regarding health-related direct-to-consumer genetic testing for Dutch consumers: (un)informed decisions?

Danny Bruins[1], *Suzanne Onstwedder*[1;2], *Martina Cornel*[1], *Margreet G.E.M. Ausems*[3], *Marc van Mil*[4;5], **Tessel Rigter**[1;2]

[1]*VU University Medical Center, Department of Human Genetics, Amsterdam, Netherlands;* [2]*National Institute for Public Health and Environment, Center for Health Protection, Bilthoven, Netherlands;* [3]*UMC Utrecht, Department of Genetics, Utrecht, Netherlands;* [4]*UMC Utrecht, Center of Education and Training, Utrecht, Netherlands;* [5]*UMC Utrecht, Center for Molecular Medicine, Utrecht, Netherlands*

Background & Objectives: Previous studies have suggested that sellers of health-related direct-to-consumer genetic tests (DTC-GTs) often provide subpar information to potential consumers, hampering informed decision making. Provided information is

frequently incomplete, unbalanced, or too difficult for potential consumers to understand. It has not previously been studied whether these findings concerning information provision also hold true for health-related DTC-GT sellers accessible to Dutch consumers. To this end, the informational content on a selection of websites from several health-related DTC-GT sellers accessible to Dutch consumers was assessed in terms of completeness, balance, readability, and findability.

Methods: A recently published checklist outlining key items for policy guidance regarding DTC-GT services was utilized for an in-depth content analysis of selected sellers' websites.

Results (preliminary): Not all aspects relevant to health-related DTC-GT service provision were equally covered by the information provided by the sellers. Benefits of health-related DTC-GT usage were overemphasized compared to its risks and limitations, indicating information provision was unbalanced. College-level education was required for proper understanding of the provided information, implying substandard readability. Information concerning all themes was overall relatively evenly distributed across analyzed sellers' websites.

Conclusion (preliminary): Information provision regarding completeness, balance, and readability was suboptimal for analyzed health-related DTC-GT sellers. Industry-wide enhancement of information provision regarding health-related DTC-GT is warranted to better empower potential Dutch consumers for informed decision making concerning its usage.

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Conflict of Interest: None declared

P24.024.D Data sharing with research participants. Policy and process development for returning raw genomic data to parents/guardians of child participants in a pediatric cancer precision medicine trial.

Kristine Barlow-Stewart[1;2;3], *Eliza Courtney*[1;2], *Mark Cowley*[2], *Camron Ebzery*[4], *Noemi Fuentes-Bolanos*[1;2], *Andrew Gifford*[2;5;6], *Hazel Harden*[7], *Sarah Josephi-Taylor*[6;8], *Rishi Kotecha*[9;10;11], *Marion Mateos*[12], *Mitali Manzur*[13], *Di Milnes*[4], *Jane Nielsen*[14], *Matthew O'Connor*[12;15], *Bhavna Padhye*[16], *Catherine Pitman*[7], *Mark Pinese*[6;13], *Catherine Speechly*[17], *Ashleigh Sullivan*[18], *Toby Trahair*[12], *Katherine Tucker*[12;13], *Vanessa Tyrrell*[13], *Meera Warby*[12;13], *Andrew Wood*[19;20], *David Ziegler*[1;13], *Carolyn Johnston*[14]

[1]*Sydney Children's Hospitals Network, Kid's Cancer Centre, Randwick, Australia;* [2]*Children's Cancer Institute, Lowy Cancer Centre, UNSW, Randwick, Australia;* [3]*University of Sydney, Northern Clinical School, Faculty of Medicine and health, Sydney, Australia;* [4]*Royal Brisbane Women's Hospital, Genetic Health Queensland, Brisbane, Australia;* [5]*NSW Heath Pathology, Anatomical pathology, Randwick, Australia;* [6]*UNSW, School of Women's and Children's Health, Randwick, Australia;* [7]*Australian Families 4 Genomics, Brisbane, Australia;* [8]*Children's Hospital at Westmead, Clinical Genetics, Westmead, Australia;* [9]*Perth Children's Hospital, Department of Clinical Haematology, Oncology, Blood and Marrow Transplantation, Perth, Australia;* [10]*University of Western Australia, Leukaemia Translational Research Laboratory, Telethon Kids Cancer Centre, Telethon Kids Institute, Perth, Australia;* [11]*Curtin University, Curtin Medical School, Perth, Australia;* [12]*Sydney Children's Hospitals Network, Kid's Cancer centre, Randwick, Australia;* [13]*UNSW, Children's Cancer Institute, Lowy Cancer Centre, Randwick, Australia;* [14]*University of Tasmania, Faculty of Law, Hobart, Australia;* [15]*Women's and Children's Hospital, Adelaide, Australia;* [16]*The Children's Hospital at Westmead, Cancer Centre for Children, Westmead, Australia;* [17]*Prince of Wales Hospital, Hereditary Cancer Centre, Randwick, Australia;* [18]*Children's Health Queensland Hospital and Health Service, Department of Oncology, Brisbane, Australia;* [19]*Starship Child Health Hospital, Auckland, New Zealand;* [20]*University of Auckland, Department of Molecular Medicine and Pathology, Faculty of Medical and Health Science, Auckland, New Zealand*

Background: In pediatric cancer precision medicine clinical trials settings, parents proactively seeking treatment and answers to causation may request sharing of their child's raw genomic data and/or biospecimen. While data sharing with research participants is not universally mandated, the Australian ZERO Childhood Cancer Program required a Policy and Process document, ensuring such requests are met with ethical, timely and appropriate responses.

Method: Document development occurred iteratively: V1 from the literature; Vs2-4 following three Rounds of Delphi expert consultation; V5-6 following two rounds of consumer consultation; and ethically approved V7 - Policy (purpose; background; ethical considerations), Process (eight steps), leaflet for those making a request and consent form.

Results: 21 Delphi experts included high representation of those likely involved in meeting requests: 71% pediatric oncologists/hematologists and clinical geneticists/genetic counselors. Consensus (>80%) was achieved by Round 3: ethical considerations broadened, benefits and risks delineated, and process streamlined. Consumer consultations highlighted need for including anticipated timing of interpreted research findings report so parents could plan for potential sharing with other groups, providing empowerment; acknowledging that subsequent analysis and interpretation of raw genomic data may have great value; and facilitating a safe place to discuss requests and address tensions between clinicians and parents reported by those with experience of requesting.

Conclusion: Informed by expert consensus and consumer views, this guide to meeting data sharing requests in a spirit of reciprocity between the researchers who are the data custodians and adult or parents/guardians of child participants, may serve as a model for other research groups.

Conflict of Interest: None declared

P24.026.B Genetic therapies for cardiomyopathy: survey of attitudes of the patient community for the CureHeart project

Elizabeth Ormondroyd[1], *Wendy Borsari*[2], *Anuj Goel*[3], *Chris Grace*[4], *Barbara McDonough*[5], *Joel Rose*[6], *Christine Seidman*[5], *Hugh Watkins*[3]

[1]*University of Oxford, Radcliffe Department of Medicine, Oxford, United Kingdom;* [2]*Patient advisor, United States;* [3]*University of Oxford, United Kingdom;* [4]*University of Oxford, Oxford, United Kingdom;* [5]*Harvard Medical School, Boston, United States;* [6]*Cardiomyopathy UK, Amersham, United Kingdom*

Background/Objectives: Cardiomyopathies are a group of inherited heart muscle disorders. Expressivity is variable and complications can result in sudden cardiac death (SCD) at any age, heart failure and stroke. In around a third of patients a monogenic cause is identifiable, and development of genetic therapies that aim to correct the underlying genetic defect is underway. Here we describe results of a survey designed to understand preliminary views of the patient community about genetic therapies in the context of disease burden.

Methods: Internet survey publicised with bespoke information video via patient support groups in the UK and USA.

Results: 634 people responded of whom 96% had a personal and/or family history of cardiomyopathy. Concern about cardiomyopathy-related issues with a future dimension, such as disease progression, is significantly greater than concern about current

issues. 93.6% thought that genetic therapies should be developed for cardiomyopathy. In response to six different scenarios varying by age and clinical situation, a majority would consider participation in a genetic therapy trial, with significantly more for an adult with symptomatic disease and evident progression than an asymptomatic adult with SCD risk, or a child. In all scenarios, a majority said that the chance genetic therapy would stop or slow progression, and risk of serious adverse and unintended effects, were important considerations. Qualitative analysis of free-text responses found that concern was often informed by family experience.

Conclusion: Patient consideration of genetic therapy is likely to require individualised assessment of the benefits and risks.

Grants: BHF BBC/19/3/35106 and BBC/F/21/220106); Oxford NIHR Biomedical Research Centre; Cardiomyopathy UK.

Conflict of Interest: Elizabeth Ormondroyd: None declared, Wendy Borsari Employed by Tenaya Therapeutics, USA, Anuj Goel: None declared, Chris Grace: None declared, Barbara McDonough: None declared, Joel Rose: None declared, Christine Seidman: None declared, Hugh Watkins: None declared

P24.027.C More is more: balancing the need for data with the need for informed consent in genomics

Jonathan LoTempio[1;2]

[1]*University of Pennsylvania, Medical Ethics and Health Policy, Philadelphia, United States;* [2]*Institute for Advanced Studies, Wien, Austria*

Background/Objectives: There has been a proliferation of a projects aimed at making genomics data more interpretable through aggregating data together to better represent the human family. In this project, I examine key projects in genomics, including reference genome efforts from the Human Genome Project to the current pangenome efforts by the Human Pangenome Reference Consortium(1), the Genome Aggregation Database (GnomAD)(2), and the 1000 Genomes Project(3), as well as smaller efforts to create pangenomes from extant data(4). Through analysis of these projects, I aim to understand whether general research use consent is still fit for purpose in genomics.

Methods: This bioethics project leverages content analysis of management documents and bioinformatic comparison of the methodologies used within each project. This allows for the comparison of the design of projects and how the resources are used by researchers.

Results: There is a growing gap between the design of large-scale resource projects and the innovative uses of genomic data. This gap suggests a need for reframing of projects at the entry point to genomic studies. While experiments that are today achievable through bioinformatics do not yet cross the line from general research use to something else, the work already completed points to a future with much blurrier lines between consent to research projects or consent to be part of a larger resource.

Conclusion: An international workshop is urgently needed to discuss whether the distinction between research project and resource project needs to be explained during informed consent.

Grants: T32HG009496

Conflict of Interest: None declared

P24.028.D Ethical issues of prenatal whole exome sequencing : opinion statement of the French Federation of Human Genetics working group

Guillaume Cogan[1], *Marie-Bérengère Troadec*[2;3], *Françoise Devillard*[4], *Marie-Hélène Saint-Frison*[5], *David Geneviève*[6], *François Vialard*[7], *Delphine Heron*[8], *Tania Attie-Bitach*[9], *Alexandra Benachi*[10], *Pascale Saugier-Veber*[11]

[1]*Assitance Publique - Hopitaux de Paris, La Pitié-Salpêtrière, Medical genetics;* [2]*Université de Bretagne Occidentale;* [3]*, Brest, France;* [4]*Hôpital Couple-Enfant, Grenoble;* [5]*Assitance Publique - Hopitaux de Paris, Paris, France;* [6]*Université Montpellier, Montpellier, France;* [7]*Centre hospitalier de Poissy-Saint-Germain, Poissy, France;* [8]*Assitance Publique - Hopitaux de Paris, La Pitié-Salpêtrière, Medical genetics, Paris, France;* [9]*Assitance Publique - Hopitaux de Paris, Necker hôpital enfants malades, Paris, France;* [10]*Assitance Publique - Hopitaux de Paris, Hôpital Antoine Béclère, Clamart, France;* [11]*université Rouen Normandie, Paris, France*

Consortium: On behalf of the French Federation of Human Genetics

Background/Objectives: Exome sequencing (ES) was recently applied in prenatal medicine. It increased the diagnostic yield for fetuses with structural abnormalities, subsequently aiding in defining a prognosis and ultimately helps the decision on whether to continue or interrupt a pregnancy. Its utility is evident. However, prenatal ES (pES) raises ethical questions distinct from postnatal ES regarding (i) genetic departments organization to provide short turnaround time (ii) incomplete prenatal phenotype leading to biological interpretation difficulties (iii) report of secondary and incidental findings in the context of prenatal medicine, among others. Several societies such as the American College of Medical Genetics and Genomics, the Canadian College of Medical Genetics or the International Society for Prenatal Diagnosis established statements and recommendations for pES's clinical application. However, countries' medical practices and laws vary, impeding worldwide extension. For example, in France, termination of pregnancy is possible until full term and the healthcare system provides universal coverage for every user.

Methods: The French Federation of Human Genetics introduced an ethics working group composed of clinicians and biologists from all over France to address pES challenges.

Results: The outcome was the development of 16 suggestive proposals to improve prenatal care in France. Interestingly, some of these statements are not in line with other countries, notably regarding secondary and incidental findings report.

Conclusion: We believe this suggestions might not only serve France, but internationally, since pES is taking place in an increasing number of genetic departments worldwide.

Grants: No

Conflict of Interest: None declared

P24.029.A Reproductive decision making after a termination of pregnancy for foetal abnormalities (TOPFA).

Ileen Slegers[1;2], *Maaike Fobelets*[3], *Kim van Berkel*[1], *Boyan Dimitrov*[1], *Kathelijn Keymolen*[1], *Frederik Hes*[1]

[1]*Vrije Universiteit Brussel (VUB), Universitair Ziekenhuis Brussel (UZ Brussel), Clinical Sciences, Research group Genetics, Reproduction and Development, Centre for Medical Genetics, Brussels, Belgium;* [2]*Vrije Universiteit Brussel (VUB), Universitair Ziekenhuis Brussel (UZ Brussel), Faculty of Medicine and Pharmacy, Nursing and Midwifery Research Group (NUMID), Primary Care (PRIM), Brussel, Belgium;* [3]*Vrije Universiteit Brussel (VUB), Department of Public Health Sciences, Biostatistics and Medical Informatics (BISI) Research Group, Brussels, Belgium*

Background/objectives: Reproductive decision making is a complex and emotional process when having a genetic risk. On the other hand, the majority of pregnancies known with

congenital malformations remain currently unresolved. When a molecular genetic cause is not identified, proper genetic counselling regarding recurrence risk, and respectively prenatal and preimplantation genetic testing are not feasible in a next pregnancy. The purpose of this study is therefore to explore how future reproductive decisions are influenced by not having a molecular genetic diagnosis.

Methods: This qualitative descriptive study included 22 interviews with couples who terminated a pregnancy for foetal abnormalities (TOPFA). Data were collected through semi-structured interviews at the Centre of Medical Genetics (CMG), University Hospital, Brussels (UZ Brussel). All interviews were transcribed verbatim, pseudonymized and coded by thematic analysis.

Results: All couples described a TOPFA as one of the most difficult experience in their life. With a lack of molecular diagnosis in their TOPFA, couples anticipated that a new pregnancy would go hand in hand with feelings of anxiety, confrontation, uncertainty and insecurity; and a need for reassurance. But it did not hold them back to fulfil their wish for another child. Moreover, some couples described an even bigger desire explaining that not having a child after TOPFA would leave them with an emptiness.

Conclusion: These results show the need for extended psychological and medical follow-up for couples that are considering a next pregnancy after TOPFA.

Grants: This study is part of the Igencare study financed by Innoviris (2017-PFS-11e)

Conflict of Interest: None declared

P24.031.C Assessment of patient reported outcomes and measures in clinical trials investigating the impact of pharmacogenetic testing in psychiatry

Trif Stefan[1], *Andra Ciuca*[2], *Urs Heilbronner*[3], *Allan Young*[4], *Thomas G. Schulze*[3], *Roos van Westrhenen*[4;5;6], *Ramona Moldovan*[2;7;8]

[1]*Babes-Bolyai University, Psychology, Romania;* [2]*Babes-Bolyai University, Romania;* [3]*LMU University Hospital, Institute of Psychiatric Phenomics and Genomics, Germany;* [4]*King's College London, Department of Psychological Medicine, United Kingdom;* [5]*Parnassia Psychiatric Institute, Department of Psychiatry, Netherlands;* [6]*Maastricht University, Department of Psychiatry and Neuropsychology, Netherlands;* [7]*University of Manchester, Division of Evolution, Infection and Genomic Sciences, School of Biological Sciences, United Kingdom;* [8]*University of Manchester, Manchester Center for Genomic Medicine, St. Mary's Hospital, United Kingdom*

Consortium: The PSY-PGx Consortium

Background: Pharmacogenetics uses genetic testing to tailor medication, aid faster recovery, reduce side effects, improve treatment efficacy and lower healthcare costs. Patient Reported Measures (PROMs) are instruments designed to assess subjective experiences, or Patient Reported Outcomes (PROs). The objective of our study was to review the outcomes and measures used in clinical trials for psychiatric pharmacogenetics, with a focus on PROMs and PROs.

Methods: An electronic search was performed (PubMed, Scopus, and WoS) to identify all studies investigating the impact of pharmacogenetics in psychiatry. Inclusion criteria consisted of (1) RCTs published in English that (2) investigated the impact of pharmacogenetic testing and (3) included participants diagnosed with a psychiatric condition (e.g., depression, anxiety, bipolar affective disorder, schizophrenia).

Results: We identified 21 RCT reporting on 7,000 participants. Of the total 17 outcomes identified, the most common were clinical outcomes (n = 10) i.e. symptoms severity, side effects, remission and response; psychosocial outcomes (n = 4) i.e. treatment satisfaction or quality of life; and health economics outcomes (n = 3) i.e. hospitalization rate or sick leave. Out of the 35 outcome measures used in these RCTs, only 12 (34%) were PROMs; most were aimed at assessing depression and anxiety severity, or disability.

Conclusion: We provide an overview of the outcomes and the measures used in clinical trials exploring pharmacogenetic testing in psychiatry. Results show that only a third of the outcome measures included are patient-reported. We highlight the urgent need to prioritise PROMs in trial design in order to adequately support personalised medicine and patient-centred healthcare.

Conflict of Interest: None declared

P24.032.D Assessment of patient-reported outcomes and measures (PROs and PROMs) in advanced therapy medicinal products (ATMPs) trials for orphan conditions

Andra Ciuca[1], *Siddharth Banka*[2;3], *Tara Clancy*[2;3], *Jamie Kirkham*[4], *William Newman*[2;3], *Katherine Payne*[4], *Ramona Moldovan*[1;2;3]

[1]*Babes-Bolyai University, Department of Psychology;* [2]*Manchester Centre for Genomic Medicine;* [3]*Division of Evolution, Infection and Genomic Sciences, School of Biological Sciences, University of Manchester;* [4]*Centre for Biostatistics, The University of Manchester, Manchester Academic Health Science Centre*

Background: Advanced therapy medicinal products (ATMPs) are medicines based on genes or cells and can include gene therapy, somatic-cell therapy, and tissue-engineered medicines. Patient reported outcomes (PROs) are reports on health and wellbeing that come directly from the individual, without external interpretation. The aim of the present review is to assess the extent and quality of PROs and PROMs used in orphan ATMPs trials.

Methods: The database from NHS Special Pharmacy Service Horizon scanning was searched to include all ATMPs for orphan conditions. Clinical trial protocols were included if they investigated ATMPs for orphan conditions and were published in clinical trial databases.

Results: A total of 77 protocols were included. These accounted for a total of 68 unique ATMPs and 53 unique diagnoses. One third of the protocols included PROs (27/77, 35%). QoL was the most frequent PRO found (24/77, 31%). Other PROs such as pain and psychosocial outcomes were included in 11 studies (11/77, 14%). A total of 27 PROMs were identified. Of these, the majority were QoL related (17 Health-QoL measures, one general-QoL). Of the H-QoL measures identified, 11 were disease-specific and 6 were generic. We identified 4 PROMs designed to measure pain and 5 PROMs focused on other psychosocial aspects.

Conclusion: Results show that only a third of the outcomes and measures included are patient-reported. We highlight the urgent need to increase the number of PROs/PROMs as it is essential to include and reflect the patients' experience so that those intended to benefit from the research have the opportunity to influence its direction.

Conflict of Interest: None declared

P24.033.A What support do families find supportive on the path to a childhood-onset diagnosis?

Helen Dolling[1;2], *Kate Baker*[1], *Claire Hughes*[1], *Sara O'Curry*[3]

[1]*University of Cambridge, Psychology, Cambridge, United Kingdom;* [2]*Cambridge University Hospitals NHS Trust, Paediatrics, Cambridge, United Kingdom;* [3]*Cambridge University Hospitals NHS Trust, Psychology, Cambridge, United Kingdom*

Background/Objectives: The availability of whole exome and genome sequencing in the Neonatal and Paediatric Intensive Care Units have greatly expanded in recent years. The Next Generation Children Project (French et al., 2019, 2022) was the first large cohort of children (521 families) to have received rapid trio WGS analysis for any suspected single gene disorder. The Peregrin* follow-up study explores how early genomic testing and results impact parental experiences and early family relationships, to elucidate benefits or harms, decisional regret, and to identify risk and protective factors for family adjustment.

Methods: 96 parents participated in Peregrin* study, of whom 57% received a diagnosis of mostly rare/ultra-rare disease in their child. 58 mothers and 38 fathers completed online questionnaires, and 91 completed in-depth semi-structured interviews and the Five-Minute Speech Sample (FMSS). Interview transcripts were analysed by framework and content analysis methods and coded for narrative coherence (Sher-Censor, 2019) to explore parents' sense of competence, adaptive parenting, and parental attitudes regarding their child.

Results: Interviews illustrate the clinical and personal utility of genomic testing, but survey responses indicate ongoing parental anxiety and uncertainty about the future, coupled with contrasting attitudes and support needs over time and across parent gender. Condition-specific support groups are helpful to many, but parents' information needs and ability to cope with diagnosis and prognosis vary.

Conclusion: Families undergoing early genomic sequencing require holistic family support pre- and post-testing, as well as person-specific opportunities to make sense of their parenting journey from intensive care to long-term family life.

Grants: Rosetrees Trust & Isaac Newton Trust

Conflict of Interest: None declared

P24.034.B Exploring the complex attitudes of stakeholders to the inclusion of genes associated with non-syndromic hearing loss in reproductive genetic carrier screening

Lucinda Freeman[1;2], *Martin Delatycki*[3;4], *Jackie Leach Scully*[5], *Edwin Kirk*[2;6]

[1]*University of Technology Sydney, Graduate School of Health, Ultimo, Australia;* [2]*UNSW Sydney, School of Women's and Children's Health, Faculty of Medicine, Sydney, Australia;* [3]*Victorian Clinical Genetics Services, Parkville, Australia;* [4]*Murdoch Children's Research Institute, Parkville, Australia;* [5]*UNSW Sydney, Disability Innovation Institute, Sydney, Australia;* [6]*Sydney Children's Hospital, Randwick, Centre for Clinical Genetics, Randwick, Australia*

Background: For most children born with hearing loss, there are effective interventions and supports available. However, some couples may wish to avoid having a child born deaf. There is no consensus on whether deafness should be included in reproductive genetic carrier screening (RGCS). This is problematic as governments consider implementation of population RGCS programs.

Aim: This study explored views of multiple stakeholders regarding the inclusion of genes associated with non-syndromic hearing loss in RGCS.

Methods: This study used both quantitative and qualitative data collection methods to identify key themes across stakeholders. Collective data was analysed using stakeholder analysis theory.

Results: All stakeholder groups showed complex attitudes. A desire to support reproductive autonomy could be in tension with concerns about potential harms, such as sending negative messages about deafness. The findings suggest that inclusion of genes associated with NSHL could cause harm as the offer intrinsically may carry the message that a couple may wish to avoid having a child born deaf and secondly that this could lead to reduced empathy and acceptance for differences in society. Stakeholders however also recognised that inclusion of these genes supports access to information and choice. In addition, all stakeholders acknowledged the complexity of defining the severity of deafness, especially when compared to other conditions included in RGCS.

Conclusion: The findings provide some support for inclusion of genes associated with NSHL but identified strong concerns that need to be addressed in the development of a RGCS program.

Grants: Australian Government Research Training Program Scholarship and Mackenzie's Mission PhD stipend

250

Conflict of Interest: None declared

P24.035.C evaluating the effect of population rejuvenation and family support law in Iran on genetic screening and incidence of congenital abnormalities

Mohammad Mahdi Sajedifar[1;2], *massoumeh bolhassani*[3], *Maryam Radkish Saki*[4], *samineh sajedifar*[5], *sousan karimi*[6]

[1]*berlin school of business and innovation, Health Managment, Berlin, Germany;* [2]*international telematics university uninettono, Health Managment, rome, Italy;* [3]*semnan medical science university, Health, Iran;* [4]*gene exploration tech, Health, Iran;* [5]*mashhad university of medical science, vice chancellor for research, Iran;* [6]*anhalt university of applied science, molecular biotechnology, Germany*

Background/Objective: Some prenatal diagnosis tests in Iran have been performed for decades with many ups and downs due to the high prevalence of diseases. The recent intervention in the health system under the title of the Population and Family Rejuvenation Law can be examined from the point of view of influencing the increase in the number of births with birth defects due to the hindrance and limitation of genetic screening before birth.

Methodology: Investigating the increase in the population with genetic defects in the country by observing the most important principles affecting it, including "coverage of genetic screening" and the effect of the population rejuvenation law by intervening in genetic screening.

Results: By examining the field data, we came to the conclusion that the nature of the legislator's interventions in the field of health by preventing genetic screening, although it can increase the population, but it interferes with the basic principles of social and individual health.

Conclusion: Our investigations show that during the promulgation of the Population Rejuvenation Law in Iran, there is a possibility of an increase in the birth of sick babies due to reasons such as the fear of families and health workers of the intensification and criminalization of laws recommending genetic screenings.

Conflict of Interest: None declared

P24.036.D Ethical challenges for 13 years: The Japan Environment and Children's Study (JECS)

Kaori Muto[1;2], *Hideki Yui*[2;3], *Zentaro Yamagata*[3]

[1]*The University of Tokyo, Institute of Medical Science;* [2]*RIKEN, Center for Integrative Medical Sciences;* [3]*University of Yamanashi, Center for Birth Cohort Studies*

Background/Objectives: The Japan Environment and Children's Study (JECS) is a nationwide government-funded birth cohort

study since 2011. The primary goal of JECS is to investigate the impact of environmental factors on children's health and development. Approximately 100,000 children from prenatal stages have been recruited and followed until they reach 40 years of age, making it one of the largest birth cohort studies in the world. The aim of this paper is to reflect the past ethical issues that the JECS has faced during the inititial period for 13 years.

Methods: We conducted the content analysis of the minutes of two internal committtees of the JECS: the Ethics Committee and the Participant and Communication Committee between 2011-2023.

Results: The ten major ethical debates were extracted. (1) opting out of genomic analysis for parents and children and handling the results; (2) unreasonable inducements and incentives for continued participation; (3) establishing multiple options to withdraw consent; (4) establishing safe and pain-free blood collection methods for two-year-old children; (5) appropriate physical examination methods for secondary sexual characteristics; (6) completing the questionnaire by children and parental privacy; (7) crisis intervention when a child's response to the questionnaire contains bullying or suicide attempts; (8) extension of the follow-up period to 40 years; (9) shifting participation methods to apps; and (10) how to obtain consent when children reach the age of 16.

Conclusion: These ethical challenges should be shared with the other birth cohort studies worldwide.

Grants: Management Expense Grant of the University of Tokyo and RIKEN

Conflict of Interest: None declared

P24.038.B Taking the risk. A systematic review of ethical reasons and moral arguments in the clinical use of Polygenic Risk Scores

Lara Andreoli[1], *Hilde Peeters*[2], *Kristel Vansteen*[3], *Kris Dierickx*[1]

[1]*KU Leuven, Public Health and Primary care, Leuven, Belgium;* [2]*KU Leuven, Leuven, Belgium;* [3]*KU Leuven, Human Genetics, Leuven, Belgium*

Background/Objectives: Debates about the prospective clinical use of polygenic risk scores (PRS) have grown considerably in the last years. The potential benefits of PRS to improve patient care at individual and population level have been extensively underlined. Nonetheless, the clinical use of PRS presents a number of unresolved ethical challenges and normative gaps that hinder their optimal implementation. In our study, we investigate the ethical issues raised in the normative literature about the prospective implementation of PRS in the clinic for the prevention and treatment of common complex diseases.

Methods: We conducted a systematic review of reasons of the normative literature following the method outlined by Sofaer and Stretch (2012). The reporting strategy is based on the PRISMA checklist for systematic reviews.

Results: In total, we have included and thematically analyzed 34 records. The findings have been organized in 3 major themes: (1) 'Potential harms' outlines the harms of PRS to individuals and their kin; (2) 'Threats to health equity' considers ethical concerns of social relevance, with a focus on justice issues; (3) 'Towards best practices' collects a series of research priorities and provisional recommendations for an optimal clinical translation of PRS.

Conclusion: We conclude that the use of PRS in clinical care reinvigorates old debates in matters of health justice. However, open questions regarding best practices in clinical counseling, suggest that the ethical considerations and guidelines developed in monogenic contexts will not be sufficient to face PRS emerging challenges and fill the current 'normativity gap'.

Grants: H2020-MSCA-ITN-2019

Conflict of Interest: None declared

P24.039.C Negotiating severity behind the scenes: prenatal testing in Germany

Tamar Nov Klaiman[1], *Hilary Bowman-Smart*[2], *Ruth Horn*[3]

[1]*University of Augsburg, Ethics in Medicine, Augsburg, Germany;* [2]*University of South Australia, Australian Centre for Precision Health, Adelaide, Australia;* [3]*University of Augsburg, Ethics in Medicine, Augsburg, Germany*

Background/Objectives: Fetal-related severity is a key concept in policy and legislation relating to access to both reproductive technologies and selective abortions in many countries around the world, but not in Germany. This study sheds light on how 'severity' in the context of prenatal testing is understood and negotiated within the particular socio-cultural and legal context of Germany, where 'severity' relating to fetal clinical findings neither counts as a justification to implement population prenatal screening programs, nor as a legal ground to terminate pregnancy. It explores the views of professionals and women, showcases how they frame severity, and questions whether the existing framework addresses their concerns and needs.

Methods: 27 interviews were conducted with women who consume prenatal services and professionals who encounter them. The interviewees were probed to elaborate on their perceptions and concerns relating to prenatal testing and pregnancy management.

Results: The interviews reveal that despite it being legally outside the explicit reasons for testing and termination of pregnancy, both women and professionals negotiate severity behind the scenes. Their interpretation of severity is highly context-dependent and relies on clinical, social, and familial facets. Their perceptions of severity guide them in their decision-making around pregnancy management.

Conclusion: Acknowledging the personal nature of severity assessment and providing professional or legal guidance which explicitly mentions fetal anomaly as a legitimate factor in pregnancy management could provide healthcare professionals and patients with the room needed to manage the pregnancy favourably.

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Conflict of Interest: None declared