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AICARDI GOUTIERES SYNDROME: FROM NATURAL HISTORY TO DESIGN OF DISEASE-SPECIFIC OUTCOME MEASURES

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DISCLOSURES

The present work represents part of the research work that I've conducted during my PhD program

CHAPTER 1: AICARDI GOUTIERES SYNDROME

1.1 HISTORICAL BACKGROUND AND EPIDEMIOLOGY OF AICARDI GOUTIERES SYNDROME

In 1984 two French pediatric neurologists, Jean Aicardi and Françoise Goutières, described in the paper "A Progressive familial encephalopathy in infancy with calcifications of the basal ganglia and chronic cerebrospinal fluid lymphocytosis" the neurological profile of eight children, belonging from five different families, affected with a severe early onset progressive encephalopathy (described as occurred within the first year of life).

This phenotype was associated with neuroradiological abnormalities such as diffuse cerebral calcifications (localized prominently in the basal ganglia), diffuse white matter abnormalities, and loss of brain volume mass.

Laboratory studies conducted on the cerebrospinal fluid (CSF) documented the concomitant presence of chronic lymphocytosis.

The recurrence this phenotype between siblings within consanguineous families and the concomitant presence of affected subjects of both sexes led to the hypothesis of a genetic origin of the disorder through autosomal recessive inheritance.

Taking into consideration the aforementioned sign and symptoms, the authors warned again the risk of a misdiagnosis of this disease as the sequelae of congenital infections.

In 1988 Professor P. Lebon (Professor Emeritus at the Université René Descartes at Paris), identified a new characteristic element of the pathology, which will later prove to be extremely useful in the diagnostic definition of the syndrome: the occurrence of increased levels of circulating IFNα in both the serum and the cerebrospinal fluid, in the absence of demonstrable cerebral infections.

The official label of the disease as "Aicardi-Goutières Syndrome" was introduced in 1992 (Bönnemann CG et al, 1992) and the first eight cases described were followed by numerous other ones (Goutieres F et al, 1998; Lanzi G et al, 2002; Lanzi G et al, 2005; Goutieres F et al, 2005).

In 2001, the time of the first International Meeting on the Syndrome, 50 cases were known, with a geographic distribution mainly in Europe and America.

Following the discovery of the first disease-causing gene (TREX1) in 2000 by Prof. YJ Crow, in-depth genetic studies have been conducted in the genetic field with the discovery, to date, of other 6 genes whose mutations associated with the occurrence of Aicardi Goutieres Syndrome (RNASEH2B, RNASEH2A, RNASEH2C, SAMHD1, ADAR1, IFIH1).

Aicardi-Goutières Syndrome is currently defined as an inflammatory genetic disease with prevalently autosomal recessive transmission, with prevalent manifestations affecting the central nervous system and skin, belonging to the category of interferonopathies (Eleftheriou D et al, 2017, Lee-Kirsch MA, 2017).

1.2 NATURAL HISTORY OF THE DISEASE

The classic phenotype of Aicardi Goutieres Syndrome has been described as characterized by the presence of microcephaly, neurological signs of involvement of the pyramidal and extrapyramidal pathways (Rice G et al, 2007), cerebral calcifications, alterations of the white matter (leukodystrophy), cerebral atrophy (Livingston JH et al, 2016), increase in CSF cellularity (lymphocyte component), increase in circulating levels of CSF interferon alpha (IFNα) and increase in the expression of genes regulated by IFNα (the so-called positive "interferon signature", Rice G et al, 2013).
The phenotypic spectrum appears widen progressively, thanks to better accuracy of the diagnosis (Crow Y et al, 2015, Livingston JH et al, 2016)

1.2.1 DISEASE ONSET

Classic form onset

The onset of the disease typically occurs within the first year of life (often within the first trimester), following an uneventful pregnancy and delivery. The literature does not report outstanding psychomotor abnormalities before the disease onset, even if authors describe the possible occurrence of a delay of the acquisition of milestones, not better quantified to date.

Extreme irritability, sleep-wake rhythm disturbances (which range from trouble falling asleep to complete rhythm reversal) and feeding difficulties are the most common symptoms observed and reported by families, with abrupt onset often described (parents are often able to report the precise date on which the symptoms began to manifest).

The presence of minor febrile elevations (37.5-38°C), not attributable to manifest infection, is very common at the clinical onset. The trend of hyperpyrexia has sometimes the characteristic of being intermittent-remitting.

With the progression of this first phase, developmental delay (delayed acquisition of motor, social and communicative milestones when compared to a neurotypical child) or psychomotor regression (defined as a process characterized by the loss of some previously achieved milestones) become evident.

These findings are associated with the progressive onset of neurological impairment (characterized by pyramidal and extrapyramidal signs and symptoms) and occurrence of secondary microcephaly (defined as head circumference below the 3rd percentile observed after birth).

After this first phase, which has a duration of a few months, authors reported a slackening of the progression of the disorder, with clinical stabilization.

The disease trigger event has not been definitively clarified yet. However, it has been hypothesized that an early and intense activation of the immune system (more frequently by infectious agents of viral origin) may play the role of triggering factor of the disorder.

Neonatal onset

In 10-20% of individuals, the disease onset occurs during the neonatal phase (defined as within the first 28 days of life).

The clinical presentation is similar to that of congenital viral infections, characterized by a pathological neurological objectivity (with an extremely poor and stereotyped motor repertoire, and slow or inexistent acquisition of the first psychomotor milestones) associated with primary microcephaly (often noticed before birth), transient hepatosplenomegaly, alteration of hepatic index values (particularly, transaminases and gamma-GT), transient thrombocytopenia and anemia.

The phenotype characterizing early onset children is usually very severe, with important neurological impairment and a greater risk of death within the first two years of life.

The genotypes most frequently associated with neonatal onset are those related to mutations in the TREX1 and RNASEH2A genes.

Late onset

With the diffusion of increasingly accurate diagnostic techniques and the description of ever wider phenotypic spectrum it has been observed that, in some subjects, the clinical onset of the syndrome may occur later than what initially reported in the literature (Orcesi S et al, 2008).

The phenotype of these subjects usually appears to be milder than what is typically associated with the syndrome.

1.2.2 NEUROLOGICAL EVENTS

Neurological involvement is almost always described individuals affected by Aicardi Goutieres Syndrome.

Encephalopathy with Altered Mental Status (AMS), or variation in the usual state of consciousness and alertness is often described at disease onset. It manifests mainly as a persistent state of sleepiness, or lethargy. Other clinical signs might include movement

difficulty, involuntary grasping, sucking motion of the mouth, and respiratory abnormalities.

Encephalopathy can disrupt the sleeping and feeding abilities of affected children. Specifically, individuals with encephalopathy may experience difficulty reaching deeper phases of sleep. Patients with AGS may develop sleep apnea (both obstructive and/or central). This is defined as a temporary stop of respiration due to either an obstruction of airways or a defective signal coming from the respiratory areas of the brain. Apneas can lead to a state of hypoxia (low oxygenation in the blood), which might cause damage in different organs.

The most widespread and observed neurological sign is the presence of abnormal muscle tone, usually dominated by the absence of postural control (with difficult head and trunk control) associated with hypotonia of the trunk, severe strength deficit, pyramidal and extrapyramidal signs (persistence of archaic reflexes, postures and dystonic movements), and spasticity and stiffness located mostly at the four limbs (Aicardi J et al, 2002).

Severe involvement of the basal ganglia structure can determine occurrence of dystonia, which might become the predominant manifestation of the neurologic phenotype.

A common symptom characteristic of affected subjects is the presence of startle reactions in response to even minimal sensory stimuli.

Characteristic of the disease is the presence of microcephaly. At birth, the child may present normal anthropometric parameters. In conjunction with the active phase of the disease, it's possible to observe a progressive reduction in the head circumference growth rate, which progressively falls below the third percentile of the growth curves (secondary microcephaly). In the forms with neonatal onset, a congenital microcephaly (primary microcephaly) can be observed.

The visual function might be severely affected in AGS children: abnormal eye movements, nystagmus and, generally, impaired visual behavior (fixation anomalies, visual and saccadic tracking) are often described. Due to the involvement of the central nervous system, the affected population can present the so called Cortical Visual Impairment (CVI), characterized by an abnormal visual response to visual stimuli due to injuries along the

optic radiation system. Children with AGS can appear slower in the identification of a moving or complex object due to a central processing deficit.

The evolution of psychomotor development within the syndrome is classically characterized by an acquisition of the key milestones in a typical or slightly delayed fashion during the first months of life. At the onset of the acute phase of the disease, the child might present a loss of previously acquired psychomotor stages which are, in some cases, slowly and only partially regained during the later phases of the disease. In individuals with neonatal onset, the failure to achieve developmental milestones is observed since the very early stages of life.
The majority of affected children are severely impaired both from a motor and intellectual point of view: postural acquisitions are frequently limited to partial control of the head, and expressive language is often absent or severely limited. The presence of a residual modest capacity for environmental participation and understanding is described.
However, there is heterogeneity in clinical severity: less severe phenotypes are described, although rare, with conservation of motor and intellectual skills. Different disease severity are reported within siblings sharing the same genetic abnormality.

Epilepsy is another frequent symptom in subjects suffering from AGS. In the scientific literature its frequency varies between 10-30% and 53-75% of affected individuals (Ramantani G et al, 2014). It is now known that about 50% of children affected by AGS will present epileptic seizures, with a higher incidence in the more compromised and early-onset clinical phenotypes, in which seizures often occur during the first or second month of life. In these subjects, epilepsy correlates with a more severe prognosis, having the risk of progressing towards a status epilepticus or to present drug resistance. In subjects with late-onset AGS or with a less severe phenotype, epilepsy occurs as a symptom of localized brain injury (falling into the category of symptomatic epilepsies) and critical episodes are usually well controlled by appropriate antiepileptic therapy (Ramantani G et al, 2014).
The discrimination of critical episodes from other non-epileptic paroxysmal episodes (startle, paroxysmal dystonia) can present a diagnostic challenge often require further investigation for an appropriate differential diagnosis.

Over time, many patients with AGS will develop autonomic dysfunction, defined as an abnormal function of the autonomic nervous system (ANS). The ANS is a division of the Peripheral Nervous System (PNS) involved in the regulation of non-voluntary muscle tissues and glands, influencing internal organ function. The ANS function is unconscious and it regulates the heart (heart rate control), lungs (respiratory frequency), bowel (peristalsis), bladder (urination) and eyes (pupillary constriction). This system is also involved in blood vessel constriction (particularly when changing from a lying/sitting to a vertical position) and some reflexes such as coughing, swallowing, vomiting. Dysfunction of the ANS can cause extremely variable clinical manifestations. These may include episodes of sweating, fast heart rate, heavy breathing, and temperature changes. This may also affect coloration (alternating red and purplish discoloration) and temperature of the extremities.

1.2.3 EXTRANEUROLOGICAL EVENTS

In Aicardi Goutieres syndrome, extra-neurological involvement is described with high frequency (Fazzi et al, 2013; Cattalini M et al, 2016).

- Integumentary system: it is the system most involved in this syndrome. The presence of chilblains-like skin lesions (chilblains), characterized by areas of inflammation and necrosis localized distally (mainly on fingers and toes), has been reported at least once in life since about 35 % of affects. Sometimes these lesions can be confluent, increasing the risk of ulceration and secondary infection. Skin lesions occur with greater frequency during the cold months, although their persistence during the summer period is documented in numerous individuals, in particular in association with the TREX1 genotype (Tungler V et al, 2012).

- Gastrointestinal system: a variety of gastrointestinal diseases are described in AGS. Gastro-esophageal reflux (GER) is a very common occurrence, often diagnosed

during infancy. It is caused by a reduced functionality of the pylori, a valve which allows communication between the esophagus and the stomach. In the most severe cases the gastric liquid can ascend the esophagus and reach the lungs, increasing dramatically the risk of developing ab-ingestis pulmunitis. Constipation is another common symptom associated with AGS. It is defined as a reduced frequency of evacuation compared to the average population. This might be related the patient's reduced mobility and dysautonomia. More rarely, individuals with AGS can have inflammatory bowel disease. patients with AGS will often show impaired swallowing (dysphagia). Careful monitoring for aspiration risk (ab-ingestis) and management of hypersalivation should be considered to ensure safe feeding.

- Hepatic system: sporadic cases with neonatal onset have been reported in the literature in which hepatomegaly with increased transaminases has been observed, sometimes associated with secondary thrombocytopenia (Rice G et al, 2007).

- Eye system: in the literature occurrence of congenital glaucoma (a form of glaucoma caused by congenital malformations in the corner of the anterior chamber of the eye, with subsequent damage of retina and other ocular structures) is described in individuals with AGS. Congenital glaucoma is a rare occurrence in the general population (incidence <1: 10,000 live births). In the overwhelmingly majority of cases it occurs bilaterally, not always symmetrically. Buphthalmias (swelling of the eyeball due to excessive internal pressure) is typical, often accompanied by other minor symptoms such as profuse lacrimation, photophobia and blepharospasm. Failure to treat this disorder can lead to severe corneal opacity, thinned sclera and cataracts, with severe impact on visual acuity.

- Cardio-vascular system: myocarditis (inflammation of the heart) and pericarditis (inflammation of the membrane surrounding the heart) have been described in AGS individuals. While not particularly common, a severe manifestation of AGS is pulmonary hypertension (PH). This is defined as an increased blood pressure within the arteries in the lungs. Manifestations of PH might include shortness of breath,

increased heart rate, reduced resistance to stress and/or swelling of the extremities. Easier fatigability and reduced appetite are often associated as well. Due to higher pressure levels there, is an increase of the diameter of the arteries and a thickening of the muscular walls of the heart. This can reduce heart contraction efficacy and may result in less blood pumped to the lungs. Another risk of PH is the increased production of blood cloths and an increased possibility of pulmonary embolism (blood cloth with obstructs pulmonary arteries) or stroke. PH is most commonly observed in IFIH1 individuals. Increased stroke/brain hemorrhage rate is observed in AGS children (particularly in those with SAMHD1 gene mutations) due to stenosis (reduction of diameter) of major cerebral vessels and related spurt of new and immature vases, reminiscent of a specific cerebral disorder denominated Moya-Moya Syndrome.

- Hematopoietic system: polygammaglobulinemia, hemolytic anemia and increased levels of autoantibodies have been described in AGS, with a higher frequency in neonatal onset forms.

- Genitourinary system: lupus-like nephritis (inflammation of kidneys on an auto-immune base) has been described in AGS. This can lead to less efficient kidney function, which can lead to reduced excretion of toxic substances. This may require the adjustment of drug prescriptions to avoid high concentration of the drug in the body, which could result in a toxic effect. A nephrological follow-up is suggested. Micropenia has been described in 2 male subjects with mutations in the TREX1 and RNASEH2C.

- Respiratory system: children with AGS may have respiratory complications related to underlying brain injury. The AGS population experiences a higher incidence of pulmunitis and reduced ventilatory capacity. Difficulty in the management of secretions (hypersalivation) and swallow (dysphagia) can play a key role in determining this complication.

- Osteoarticular system: the occurrence of arthropathy with progressive contractures has been described in relation with AGS, more frequently with a SAMHD1 genotype. A complication observed with relatively high frequency is early onset scoliosis (a sideways curvature of the spine), that can rapidly evolve over time, becoming a concern due to limitation of respiratory and digestive functions.

- Endocrine system: patients with AGS may develop thyroid disease such as autoimmune thyroiditis. This is defined as an acute inflammation of the thyroid gland, an organ located at the base of the neck. It produces hormones involved in the regulation of a high number of functions, including determination of basal metabolic rate, nutrients absorption, blood flow and body's temperature regulation, cognitive function.

- Autoimmune thyroiditis can lead to a phase of hyperthyroidism, usually followed by hypothyroidism. More rarely, individuals may develop diabetes mellitus type 1 and diabetes insipidus. Symptoms such as increased urination and recurrent dehydration should be evaluated for these disorders. Due to the increased risk of complications (in particular diabetic coma), specific treatment should be considered if one of these conditions is diagnosed.

1.3 NEURORADIOLOGICAL FINDINGS

Aicardi Goutieres Syndrome is classically characterized by the presence of a neuroradiological triad (Livingston JH et al, 2014, La Piana R et al, 2016), consisting of:
- cerebral calcifications
- white matter signal abnormalities (leukodystrophy)
- brain atrophy

1.3.1 CEREBRAL CALCIFICATIONS

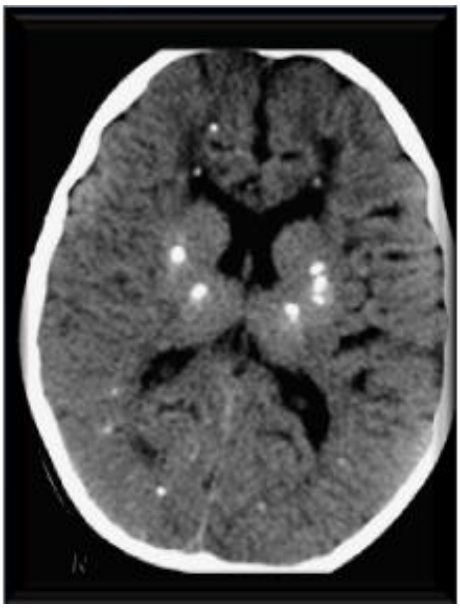


Fig 1. Diffuse cerebral calcifications, mostly localized in basal ganglia area (MRI T2 section)

Cerebral calcifications (deposits of calcium salts) are generally localized bilaterally in the basal ganglia, more often at the level of the pallidus, the putamen, the caudate nuclei, the cerebellar dentate nuclei, and in the frontal and parietal white matter. Less common localizations are however reported in the literature. Calcifications can be punctiform or larger, single or confluent.

It is possible that these findings may be absent at disease onset and become more evident over time. In the majority of cases, however, they are recognizable at the time of diagnosis (Livingston JH et al, 2014).

It is important to underline that in the presence of a clinical picture suggesting AGS, calcifications are almost always a diagnostic sign and must therefore be carefully sought, with appropriate neuroimaging techniques, in all cases of undiagnosed leukoencephalopathy.

Recently, a minority of subjects with genetically confirmed AGS lacking this neuroradiological finding has been observed: therefore, the diagnostic hypothesis of AGS should be taken into consideration in subjects with a suggestive phenotype even in the absence of cerebral calcifications.

1.3.2 LEUKODYSTROPHY

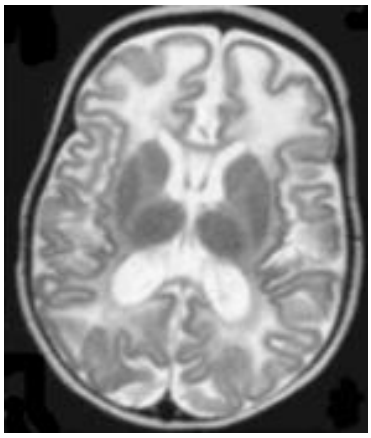


Fig 2. White matter abnormalities configuring leukodystrophy

Leukodystrophy is defined as an alteration of the white matter of the brain, and is a typical element of the syndrome, observed in more than 90% of cases.

Typically, these changes are diffuse, confluent and symmetrical and sometimes show a tendency to cystic degeneration.

They are mainly localized in the periventricular areas and in the deep and subcortical white matter, sometimes diffuse, sometimes with an antero-posterior distribution. These changes appear as hypodensity on brain CT and as hyperintense areas in T2-weighted brain MRI sequences.

1.3.3 CEREBRAL ATROPHY

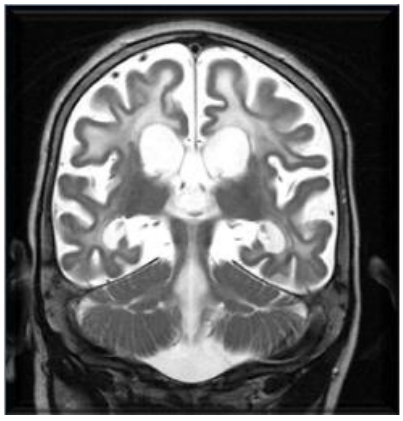


Fig 3. Diffuse cerebral atrophy (MRI T2 section)

Cerebral atrophy is the third neuroradiological element characteristic of the syndrome, evident in over 90% of the cases described in the literature. It can be presented both as a stable finding and with a tendency to progression. The localization of these alterations is prevalent in the frontal and/or temporal lobes, sometimes assuming a characteristic simplified gyral pattern. Frequently, the brain stem, cerebellum, basal ganglia, and corpus callosum may also be atrophic. Atrophy is a consequence of alterations and loss of white matter and does not usually involve the cerebral cortex.

1.3.4 OTHER FINDINGS

In addition to the classic neuroradiological triad, cerebral vasculopathy has been reported in individuals with a diagnosis if AGS. In subjects with mutation in the SAMDH1 gene episodes of stroke may occur due to stenosis of the cerebral arteries (which take on characteristics similar to Moyamoya syndrome, an occlusive vasculopathy with the formation of compensatory collateral circulation) and / or intracerebral aneurysms (Ramesh V et al, 2010; Thiele H et al, 2010, Troedson C et al, 2013)). Other vascular events have been reported in patients with mutations in the TREX1 gene: thrombosis of perforating vessels in one child and anti-phospholipid antibody syndrome and cerebral hemorrhage in another, without previously evidenced vascular abnormalities have been described in the literature.

In subjects with mutation of the ADAR1 gene, a characteristic neuroradiological pattern of bilateral striatal necrosis is observed (Livingston JH et al, 2014), i.e. the presence of a selective, bilateral and symmetrical lesion at the level of the basal ganglia.

1.4 LABORATORY FINDINGS

1.4.1 CEREBRAL SPINAL FLUID ANALYSIS

Cerebral spinal fluid (CSF) analysis has been mandatory for a laboratory diagnosis of AGS for many years (Izzotti A et al, 2008). Historically, the first CSF finding correlated with

the syndrome was the finding of important lymphocytosis, with cellular values usually between 5 and 100 cells/mm^3 (Baechler EC et al, 2000)
Subsequently, an increased concentration of circulating IFNα was demonstrated, with values even much higher than the universally recognized cut-off (equal to 2 IU/ml) (Lebon P et al, 2002).

Both lymphocytosis and CSF IFNα increase are observable at disease onset or during the first year of life. In later stages of the disease these values tend to gradually decrease and a normalization of the values is observed within a few years.
Since 2003, alterations of CSF metabolites have been described, particularly with an increase of the pterins and a reduction in folates (Blau N et al, 2003).
The increase in CSF neopterin values has subsequently been confirmed in many patients with AGS and is currently considered a possible marker of the disease.

1.4.2 INTERFERON STIMULATED GENES (INTERFERON SIGNATURE)

It has recently been shown that, in the peripheral blood of patients with interferonopathies, the transcription level of some genes stimulated by IFNα (interferon-stimulated genes, ISGs) is increased and remains high for a prolonged period of time. This increased expression of interferon-regulated genes is referred to as the "interferon signature".
In 2013 G. Rice used this method in 82 patients affected by AGS, by analyzing 6 ISGs:
- IFI27
- IFI44L
- IFIT1
- ISG15
- RSAD2
- SIGLEC1

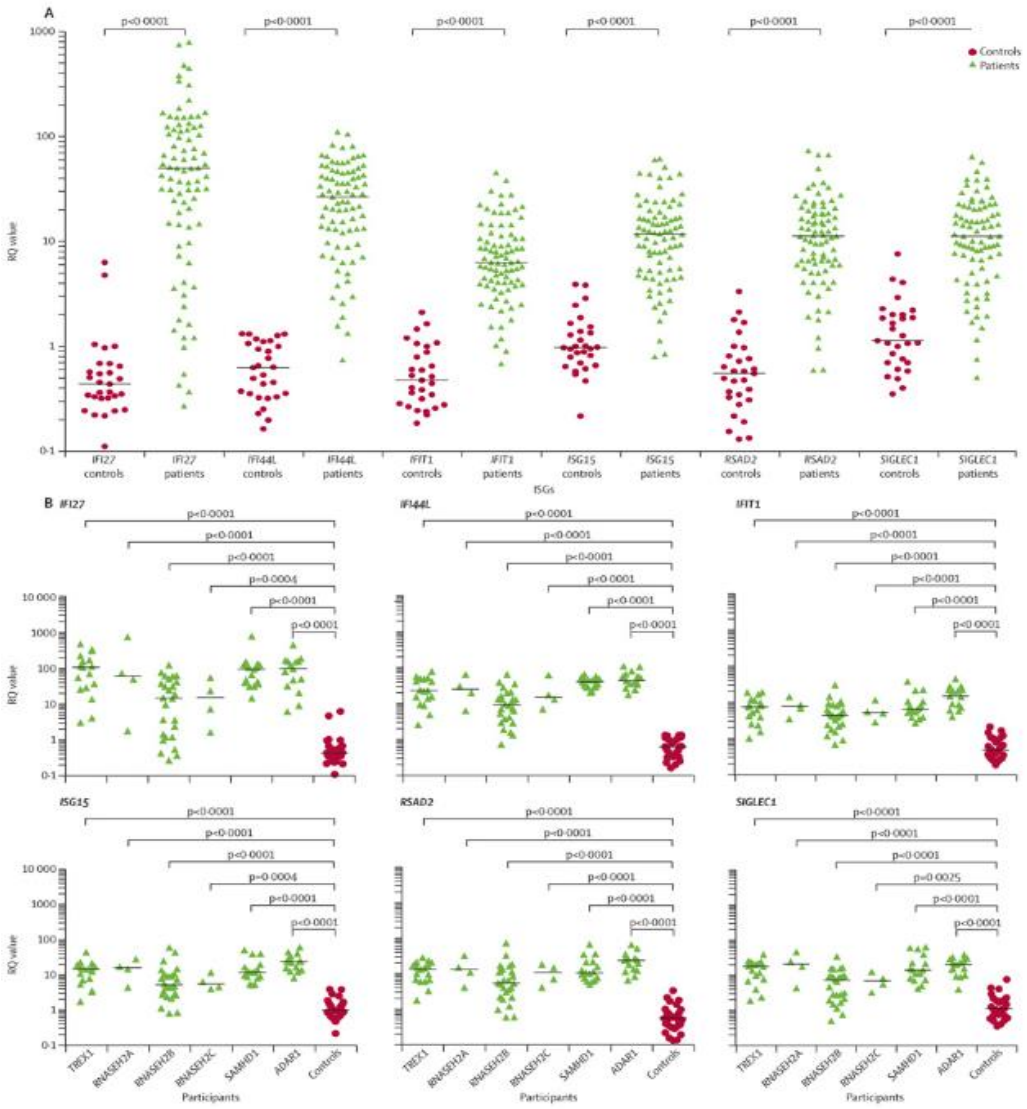


Fig 4. Increased expression of ISGs levels in AGS population, when compared to healthy controls (Rice G et al, 2013)

This method proved to be extremely sensitive in the selection of patients suffering from Aicardi Goutieres Syndrome: 74 out of 82 patients (90% of the sample) showed increased levels of gene transcription of these 6 genes in comparison with a control group of healthy individuals.

The study also demonstrated a correlation between the values of circulating CSF interferon and the increase in transduction of ISGs in the active phase of the disease.

Another characteristic of the interferon signature was the persistence of the abnormal genetic transcription for a longer period of time than what observed for the CSF IFN.

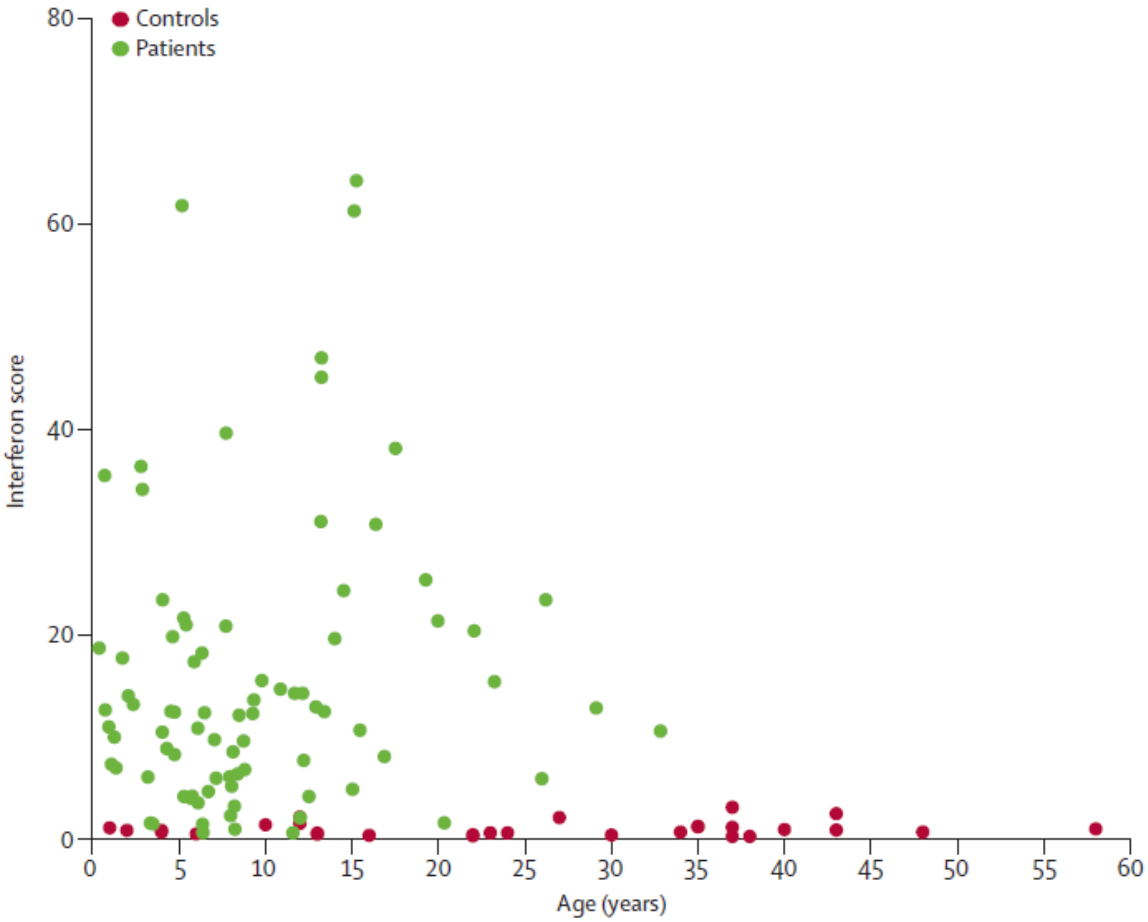


Fig. 5. ISGs levels by age at laboratory analysis (Rice G et al, 2013)

In light of these findings, the interferon signature can therefore be considered a biological marker of disease. Compared to the analysis of CSF factors, the signature also has the considerable advantage of being easily measurable with minimally invasive methods, such as a simple blood sample. As already stated by Rice, it is important to underline that the interferon signature is positive in all subjects with the TREX1, RNASEH2A, RNASEH2C, SAMHD1, ADAR1, IFIH1 genotype and is negative only in 25% of the affected carriers of mutations in the gene RNASEH2B.

1.5 GENETIC ASPECTS

Aicardi-Goutières syndrome is an encephalopathy of genetic etiology, with predominantly autosomal recessive (AR) transmission, although some cases are described in which mutations affecting a single allele have nevertheless generated a pathological phenotype. The picture is caused by mutations in one of the 7 disease genes discovered up to now (TREX1, RNASEH2A, RNASEH2B, RNASEH2C, SAMHD1, ADAR, IFIH1), all involved in the transduction of enzymes involved in the sensing and metabolism of nucleic acids (DNA and RNA). Worldwide, it can be observed that the most expressed genotype is that linked to mutations of the RNASEH2B gene, which alone is associated with the majority AGS cases (36%, Crow Y, 2016). By frequency, therefore, follows the TREX1 (23%), SAMHD1, (13%), RNASEH2C (12%), ADAR1 (7%), RNASEH2A (5%), IFIH1 (3%) genotype.

However, it is important to underline that this data is significantly influenced by the geographical area where the study is carried out (in Italy, for example, there is a lower frequency of subjects carrying mutations in the TREX1 gene), and by the access of extensive genetic testing.

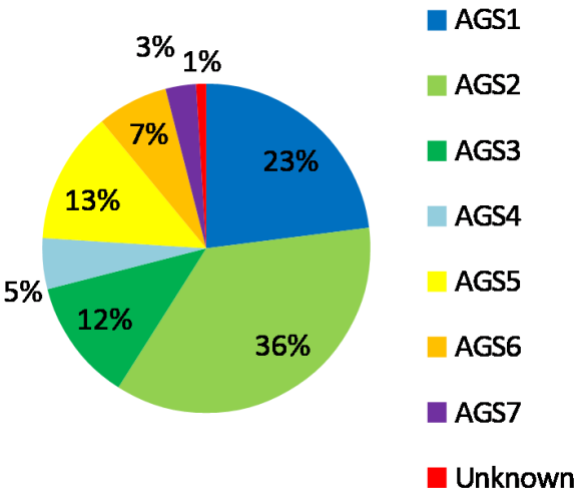


Fig. 6. AGS genotype distribution (Crow Y et al, 2016)

1.5.1 TREX1

Located on chromosome 3.21.31, TREX1 (Three Prime Repair Exonuclease 1) was the first gene discovered in association with Aicardi-Goutieres syndrome (Crow Y et al, 2000; Crow Y et al, 2002; Crow et al, 2006).

Coding for the 3'-5 'DNA exonuclease protein, whose function is of removing non-coding DNA fragments located at the level of the cell cytoplasm. DNA is composed of a coding portion (equal to about 1% of the total DNA) and a coding portion (the remaining 99%). The function of this portion of genetic material is not known to date, although it is known that it is partly composed of genetic residues of primordial viruses, which have integrated into the human genetic code in the course of evolution. These primordial viruses are in fact able to create copies by inserting themselves in different regions of the DNA, with a process similar to what observed during viral infections. Normally cells have defensive mechanisms that block the activity of these elements, eliminating their DNA. One of the proteins involved in this process is the TREX1 protein.

The loss of function of the exonucleases therefore leads to an increase in the concentration of cytoplasmic DNA. In the literature it is reported that 23% of subjects affected by Aicardi Goutieres Syndrome present mutations in this gene. Transmission is autosomal recessive, caused by transmission of mutated alleles from both parents or "de novo" onset. However, some cases of pathology caused by mutations in a single allele (resulting in autosomal dominant transmission) are described. The clinical phenotype is usually severe, characterized by neonatal onset and characterized by evolution in spastic-dystonic tetraparesis associated with severe/profound psychomotor delay. Affected subjects frequently present autoimmune manifestations such as skin lesions (chilblain type or oral aphthous) or thyroid alterations (Olivieri I et al, 2013).

Other pathologies related to the TREX1 gene mutation are known in the literature. Those with the highest incidence are:

- Cerebral Hereditary Angiopathy with vascular Retinopathy and Impaired Organ function caused by TREX1 mutations (CHARIOT): pathology affecting the retina and white matter of the CNS, characterized by reduced visual acuity, lacunar infarcts and late dementia. The symptomatic onset is usually around the age of 40, treatment is only symptomatic. The prognosis is severe, with exitus around 10 years after clinical onset.
- Familiar Chilblain Lupus (FLE): it is a rare form of systemic lupus erythematosus with prevalent cutaneous manifestations localized in the acral regions. Clinical onset occurs in early childhood, with the presence of hypergammaglobulinemia and an increase in the rheumatoid factor titer.

- Cree leukoencephalopathy: it is a rare encephalopathy characterized by psychomotor delay, secondary microcephaly, cerebral atrophy, white matter hyperintensity, cerebral calcifications, lymphocytosis in the CSF, immune system abnormalities.

1.5.2 RNASEH2 COMPLEX

In eukaryotic cells, the RNASEH2 complex is an obligate heterotrimer composed of the catalytic subunit A and the structural subunits B and C. This complex has the ability to cleave single ribonucleotides from a nucleic acid strand. The substrate specificity of this complex gives it a central role in the repair of nucleic acids with ribonucleotide excision, being fundamental both in the removal of mispositioned elements within the DNA and in addition to the R-loop processing. This complex plays a role in maintaining genomic stability (Crow Y et al, 2006; Chon H et al, 2013).

1.5.2.1 RNASEH2A

The RNASEH2A gene is located on chromosome 19p13.13.

In the literature it is reported that 5% of subjects affected by Aicardi Goutieres Syndrome present mutations in this gene. Transmission is autosomal recessive. The clinical phenotype is usually severe, characterized by neonatal onset and characterized by evolution in spastic-dystonic tetraparesis associated with often severe cognitive impact.

1.5.2.2 RNASEH2B

The gene is located at the 13q14.3 level.

It codes for the beta subunit of the H2 ribonuclease complex. The protein is composed of 308 amino acids, with ubiquitous expression. In the literature it is reported that 36% of subjects affected by Aicardi Goutieres Syndrome present mutations in this gene. Transmission is autosomal recessive. The phenotype associated with mutations of the RNASEH2B gene appears to be extremely heterogeneous (Uyur Yalçın E et al, 2016).

1.5.2.3 RNASEH2C

The RNASEH2C gene is located on chromosome 11q13.1. In the literature it is reported that 12% of subjects affected by Aicardi Goutieres Syndrome present mutations affecting this gene (Crow Y et al, 2015). Transmission is autosomal recessive.

The clinical phenotypic is often severe, although also intrafamilial phenotypic variability is reported.

1.5.3 SAMHD1

SAMHD1 is located on chromosome 20q11.23.

The protein is capable of blocking the replication of the HIV virus in dendritic cells, macrophages and monocytes. Specifically, the catalytic activity of the enzyme consists in converting the nucleotides to the corresponding form of nucleosides (e.g. nucleotides without the phosphate group). SAMHD1 therefore prevents the replication of the virus by lowering the level of triphosphate nucleotides, which are necessary for the reverse transcription of the viral cDNA. Furthermore, it has recently been shown that the enzyme also possesses ribonuclease activity, essential in the inhibition of viral replication (Powedd RD et al, 2011).

In the literature it is reported that 13% of subjects affected by Aicardi Goutieres Syndrome present mutations in this gene. Transmission is autosomal recessive.

Phenotypic characteristics of the forms of Aicardi Goutieres related to mutations of the SAMHD1 gene are the presence of cerebral vasculopathy, including both stenosis (with manifestations that moya-moya like) and aneurysms (Dale R et al, 2010), and increased frequency of “chilblain” type skin lesions and glaucoma

Mutations in this gene are also described as causative of Familiar Chilblain Lupus (FLE)

1.5.4 ADAR1

ADAR1 (adenosine deaminase acting on RNA) is located on chromosome 1q21.3 and encodes the homonymous protein. The ADAR family of enzymes has a catalytic function of the pairing reaction of double-stranded RNA (dsRNA), converting an adenosine to inosine by deamination. This reaction interferes with the normal binding of adenosine-uracil nitrogenous bases, which make RNA pairing unstable. In the literature it is reported

that 7% of subjects affected by Aicardi Goutieres Syndrome present mutations affecting this gene (Rice G et al, 2012). Transmission is predominantly autosomal recessive, although cases of subjects with autosomal dominant mutations have been described (usually “de novo”).

Association with an acute presentation of bilateral striatal necrosis is described in the literature. A subgroup of individuals in fact presented signal alterations on MRI, symmetrical and localized in the nuclei of the caudate and putamen base, often associated with subsequent swelling and therefore atrophy. These neuroradiological findings are associated with severe dystonia. The hypothesis of AGS related to ADAR1 gene mutations should be considered in the differential diagnosis of apparently isolated bilateral striatal necrosis associated with variable evolution dystonia (Izzotti A et al, 2013, Rice G et al, 2017).

Mutations of the ADAR1 gene have also been described in association with hereditary symmetric dyschromathosis (DSH1), a disorder characterized by skin hyperpigmentation at the extremities (Li M et al, 2011, Kono M et al, 2016).

1.5.5 IFIH1

The IFIH1 gene is located on chromosome 2q24.2 and encodes the MDA5 protein (Melanoma Differentiation-Associated protein 5), a subunit of the RIG-I-like receptor (the other two components are RIG-I and LGP2). The function of this receptor is the recognition of circulating dsRNA, fundamental for the identification of viruses. Mutations in this gene cause 3% of Aicardi Goutieres syndrome cases. Phenotypic characteristics linked to this genotype is a wide heterogeneity in the level of neurocognitive and motor impairment. Transmission is predominantly autosomal dominant, although numerous cases of mutations arising "de novo" (therefore absent in the parents) have been described.

Mutations in the IFIH1 gene are also causative of Singleton-Merten Syndrome (Bursztejn AC et al, 2015; Buers I et al, 2017), and are also reported in association with a form of immunodeficiency called "MDA5 deficiency".

1.6 PATHOGENESIS

1.6.1 INTERFERON: DEFINITION AND BIOLOGICAL FUNCTIONS

Interferons (IFNs) are a family of proteins produced by both immune system cells (white blood cells) and tissue cells in response to the presence of external agents such as viruses, bacteria and parasites or cancer cells. Interferons belong to the class of glycoproteins known as cytokines.

Their specific function is to inhibit viral replication within infected cells, prevent intercellular viral spread, increase the activity of the cells responsible for the immune defenses, inhibit the growth of cancer cells

Interferons stimulate the production in the cell of some antiviral enzymes, including Protein Kinase R, the RNase-L and the proteins encoded by the Interferon Stimulated Genes (ISGs, defined as genes activity is regulated by the concentration of circulating Interferon).

Human interferons are divided into three main categories:

- Type 1 interferons: they are cytokines that bind to a specific surface membrane receptor known as the interferon-α / β receptor (IFNAR). IFN-α, IFN-β, IFN-ε, IFN-κ and IFN-ω belong to this class.
- Type II interferons (IFN-γ): this molecule, also known as immunity-bound interferon, is activated by a cytokine, Interleukin 10 (IL-12). This interferon is specifically produced by cytotoxic and T-helper T lymphocytes. The binding receptor is called IFNGR
- Type III interferons: the receptor through which the signal is transmitted is a complex structure composed of the IL10R2 receptor and IFNLR1. It is a more recently identified molecule mainly involved in the immune defense in viral and fungal infections.

1.6.2 INTERFERON INDUCTION

Interferon production is regulated in response to bacterial or viral agents. The binding by pattern recognition receptors (PPRs, such as Toll-like membrane receptors or RIG-I or MDA5 cytoplasmic receptors) of microbial molecules (such as viral glycoproteins, viral RNA, bacterial endotoxins) increases the production and release of interferon (Miyake K et al, 2017).

The Toll Like Type 3 receptor (TLR3) is critical in the induction of interferon in response to the presence of viral double-stranded RNA. The binding of this substrate activates the transcription of factors IRF3 and NF- κ B, with consequent initiation of the synthesis of inflammatory proteins.

Other cytokines, such as interleukins 1, 2 and 12, tumor necrosis factor (TNF) and colony-stimulating factor (C-SF) can equally stimulate the production of circulating interferon.

1.6.3 INTERFERON SIGNALING PATHS

By interacting with specific surface receptors, interferons are able to activate STAT complexes (signal transducer and activator of transcription).

STATs are a family of transcription factors that regulate the expression of genes involved in the human immune system. The different STATs are activated by specific types of interferon.

Activation of STAT generates a defined signaling pathway, called the classical Janus-Kinase-STAT (JAK-STAT, kinase bound to surface interferon receptors) pathway. In this signaling pathway the JAKs, activated by the specific interferon-receptor binding, cause a phosphorylation of STAT1 and STAT2.

The result of this process is the formation of a complex called ISGF3 (IFN-stimulated gene factor 3, composed of STAT1, STAT2 and the transcription factor IRF9) which migrates to the cell nucleus. Here this complex binds specific nucleotide sequences called IFN-stimulated response elements (ISREs), located in the promoter sequences of specific genes,

called interferon stimulated genes (ISGs). This link activates the transcription of these genes.

In association with the above, homo or STAT heterodimers form various combinations of STAT1, STAT3, STAT4, STAT5, STAT6 during the IFN signaling pathway. These dimers are able to stimulate gene transcription by binding IFN-activated site (GAS) elements located within promoter genes. Type I interferon can induce the expression of genes with either ISRE or GAS elements, while type II interferon can generate gene induction only in the presence of GAS elements.

Interferons are also capable of activating several other signal cascades. In particular, type I interferon is able to activate the mitogen-activated protein kinase p38 (belonging to the MAP kinase family), in turn able to induce gene transcription. The antiviral and anti-retro proliferative effects specific to IFN type I result from the signaling of this protein.

1.6.4 ROLE OF TYPE I INTERFERON IN AICARDI GOUTIERES SYNDROME: INTERFERONOPATHIES

In 2003 Y. Crow and his collaborators observed an overlap of clinical manifestations in subjects with Aicardi-Goutières Syndrome, Systemic Lupus Erythematosus (SLE) and multiple congenital viral infections (including the HIV virus infection). It was hypothesized that the phenotypic similarity could depend on a common pathogenetic process: the altered regulation of the production of Interferon alpha (IFNα).
The Aicardi-Goutières Syndrome is currently classified within the class of interferonopathies, disorders caused by an excessive production of IFNα. Mutations in TREX1, RNASEH2, SAMHD1, ADAR1 and IFIH1 can in fact cause an alteration of the physiological process of disposal of endogenous nucleic acids (self-DNA and RNA) which therefore accumulate intracellularly (Crow Y et al, 2009).

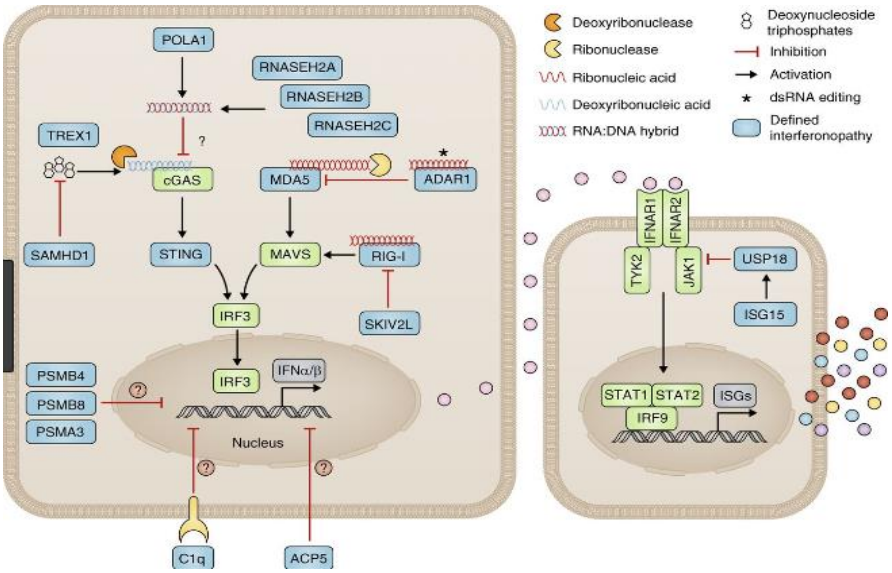


Fig. 7. AGS genes role in signaling and degradation of nucleic acids (Rodero M et al, 2016)

The increased concentration of these components is recognized by the immune system as genetic material of viral origin (non-self), activating the signaling pathway of interferon and determining, as an ultimate effect, an increase in circulating IFNα levels. This increase causes a dysregulated pro-inflammatory state, with the development of tissue damage that causes a release of nucleic acids (Brooks PJ et al, 2008; Hertruch C et al, 2013; Lee-Kirsch et al, 2014).

Therefore, a self-feeding vicious circle is created whereby an increase in circulating nucleic acids generates an immune hyperactivation, with increased production of circulating IFNα. The tissue damage associated with this overproduction further increases the circulating share of nucleic acids, with amplification of the response (Hofer MJ et al, 2013)

An in vitro cellular model has been devised to evaluate the effects of chronic exposure of astrocytes to high concentrations of IFNα (Cuadrado E et al, 2013). A reduction in cellular proliferative capacity has been demonstrated, with a reduction in the vascular components of tissue support in both quantitative (reduced neo-angiogenesis) and qualitative (formation of vessels with immature endothelium and subject to rupture and reactive gliosis). These changes appear long-lasting since, after prolonged exposure to interferon, it is not possible

to convert the model back to the initial cellular condition once the excessive concentration of IFNα has been eliminated.

1.6.5 AICARDI GOUTIERES SYNDROME AND AUTOIMMUNITY

The role of IFNα in the pathogenesis of the disease has long been described and confirmed. This finding justifies the presence of autoimmune symptoms in subjects with AGS.
In particular, the scientific literature has extensively debated the possible overlap between AGS and SLE, an autoimmune disease in which IFNα hypersecretion is recognized as an essential mechanism in the genesis of the disease.
Some works, supporting the hypothesis of an overlap between the two conditions, document lupus-like skin rash, chilblains, intermittent oral ulcers, purpura-like lesions, chronic arthritis and SLE in subjects with AGS; others the absence of associated lupus symptoms.
To date, the most widespread interpretation is that the spectrum of clinical manifestations of AGS may have elements in common with SLE, given the commonality of the pathogenetic mechanism underlying both. The discrepancies between the various studies are likely to be attributed to methodological errors in the recognition of autoimmune manifestations, also due to the lack of universal diagnostic criteria and to the difficulty in interpreting as autoimmune those symptoms present in neurologically compromised children (recurrent canker sores, joint pain, seizures).
In light of the data currently available, it is however believed that the autoimmune-like clinical manifestations (autoimmune-like) should be interpreted as part of the Syndrome. This aspect is further demonstrated by the fact that other clinical pictures belonging to the class of "interferonopathies" have been described in individuals carrying mutations in genes responsible for AGS (TREX1, IFIH1)

1.7 DIAGNOSIS

1.7.1 CARDINAL ELEMENTS FOR DIAGNOSIS AND DIAGNOSTIC PATH

Despite the recent acquisitions in the genetic field, it is important to recognize signs and symptoms of the syndrome using all clinical and instrumental tools available (clinical history, laboratory tests on blood and CSF - in particular the interferon signature - and neuroimaging - new MRI techniques and possibly Brain CT). The diagnostic suspicion of AGS can be subsequently confirmed with genetic testing.

It is also important to underline that some criteria of the syndrome are "age-specific" or rather "evolution-specific", in the sense that their presence / absence takes on a different meaning in the different phases of the disease: for example, at the onset of symptoms, the presence of cerebral calcifications , although very frequent, it is not an indispensable criterion for diagnosis and they must be sought over time, as well as the absence of increased values of CSF INFα years after the onset does not exclude the diagnosis.

Since cerebral calcifications are not easily recognizable on traditional MRI, a neuroradiological examination routinely used in the diagnostic approach, a second aspect to underline is the importance of searching for calcifications through the execution of a brain MRI with gradient echo techniques. 3D gradient echo or CT of the brain, in all cases of undiagnosed early-onset leukoencephalopathy.

Given these premises, the key elements for the diagnosis are:

- The finding of an early onset encephalopathy with a clinical picture characterized by impaired psychomotor development, spasticity and extrapyramidal signs and microcephaly which typically appears during the first (secondary) year of life.
- Appearance of cerebral calcifications localized mainly in the basal ganglia (putamen, pale, thalamus), but sometimes also extended to the white matter
- Changes in the white matter of the brain (leukodystrophy)
- Cerebral atrophy
- Exclusion of pre-perinatal / infections, especially those of the TORCH complex
- Chronic lymphocytosis (> 5 elements / mm3) in the CSF, with no other signs of ongoing infection

- Increase in the level of IFNα in the CSF (> 2 IU / ml)
- Positive Interferon Signature Analysis
- Increase in neopterins and, to a lesser extent, CSF biopterins, sometimes associated with a decrease in CSF folate
- Suggestive systemic symptoms, present in particular in the early stages of the disease (acute phase): presence of irritability, sleep and eating disorders, hyperpyrexia sine causa and appearance of chilblain-like skin lesions in the extremities and auricles;
- Consequential genetic analysis for the search for mutations in the 7 known genes makes it possible to obtain a definitive diagnostic confirmation in the majority of cases.

1.7.2 DIFFERENTIAL DIAGNOSIS

Aicardi Goutieres syndrome is part of the differential diagnosis with numerous neurological and systemic pathologies (Lanzi G et al, 2002, Crow Y et al, 2016), including:

- Congenital infections TORCH: infections by Toxoplasma, Rubella, Cytomegalovirus (CMV) and Herpes Simplex occur with clinical signs and symptoms typical of Aicardi Goutieres syndrome (in particular the presence of microcephaly and cerebral calcifications). However, these findings are atypical with respect to AGS: microcephaly is almost always primary and cerebral calcifications are widespread. These congenital infections also manifest themselves with the association of malformations of different districts. Early differential diagnosis is fundamental both to offer adequate genetic counseling (evaluating any recurrence risks for the family) and to manage the evolution and complications of the affected subject. Due to the relative high incidence of TORCH congenital infections (1.1 / 1000 for Toxoplasmosis, 10.1 / 100000 for syphilis, isolated cases for rubella, 1/100 for CMV, 2.5 / 1000 for Herpes Simplex, US data) this diagnostic hypothesis must be always investigated.

- Congenital HIV and Zika virus infections: Congenital infections by these viral agents can cause phenotypes characterized by primary microcephaly and diffuse brain calcifications.
- Microcephaly-intracranial calcification syndrome (MICS): also known as pseudo-TORCH syndrome or Baraitser-Reardon syndrome, this syndrome has in the past been differentiated from AGS on the basis of findings of congenital microcephaly and the presence of non-neurological anomalies found in such subjects (increased levels of liver enzymes, hepatomegaly, thrombocytopenia). The best definition of the phenotypic spectrum associated with AGS, which provides for the inclusion of such findings.
- Band-like calcification polymicrogyria (BLC-PMG; pseudo-TORCH syndrome): this syndrome presents clinical and neuroradiological signs overlapping with AGS, in particular intracranial calcifications and important psychomotor retardation associated with microcephaly and epilepsy. This condition can be differentiated by the finding of polymicrogyria (never observed or described in the AGS) and by the finding of diallelic mutations in the OCLN gene
- Leukoencephalopathy: at a first analysis MRI findings of fronto-temporal white matter anomalies and cysts, sometimes found in Aicardi Goutieres syndrome, can initially be confused with Alexander's disease, megalencephalic leukoencephalopathy with subcortical cysts or an onset of vanishing white matter disease. White matter hypomyelination in the first years of life can also raise the diagnostic doubt of Pelizeaus-Merzbacher syndrome. In general, Aicardi Goutieres syndrome should always be considered in the differential diagnosis of a leukoencephalopathy of unknown etiology, considering the fact that cerebral calcifications can also occur later than the clinical onset and may not be highlighted by non-specific MRI sequences.
- Classic Cockayne syndrome (CS type 1): it is a leukodystrophy with striatal-cerebellar calcifications, characterized by specific facial features, dwarfism, sensorineural deafness, cataract, retinal dystrophy, skin photosensitivity. Transmission is autosomal recessive.

- Neonatal Onset Lupus Erythematosus: Neuroradiological findings reminiscent of AGS (such as basal ganglia brain calcifications and attenuation of spot white matter signal) have been found in children with neonatal lupus erythematosus. These children had diffuse erythematous skin lesions different from the chilblain-like lesions often found in AGS (Dale RC et al, 2000; Ramantani G et al, 2010). The neurological evolution of these children was normal.
- Cerebro-retinal microangiopathy with calcifications and cysts (CRMCC): pathology caused by diallelic mutations in the CTC1 gene. The neurological involvement of the subjects varies according to the extent of the brain abnormalities, although common signs are focal epilepsy, asymmetric spasticity, ataxia and intellectual disability. An interest in visual-spatial and visual-constructive skills are characteristic. Migraine-like headaches are described. At the ocular level, telangiectasias with frequent retinal hemorrhages are observed. The risk of retinal detachment related to these events is extremely high.
- Labrune syndrome: also called Leukoencephalopathy, brain calcifications, and cysts (LCC), this pathology is characterized by leukoencephalopathy, cerebral calcifications and cysts. The associated phenotype sees the presence of spasticity, dystonia, seizures and psychomotor regression / cognitive decline. The age of onset six clinical signs and symptoms varies from early life to mature adulthood. The disease has an autosomal recessive inheritance with mutations in the SNORD118 gene.
- Mitochondrial encephalopathies including Leigh syndrome and familial mitochondrial encephalopathy with intracerebral calcifications.
- 3-hydroxyisobutyric aciduria: two monozygotic twins, born to unrelated parents, with dysmorphic features of the face, microcephaly, brain cell migration abnormalities, congenital intracranial calcifications have been described in the literature.
- Other metabolic encephalopathies with calcifications to the basal ganglia: disorders of parathyroid hormone metabolism and biotinidase deficiencies enter the differential diagnosis.

1.8 TREATMENTS

1.8.1 SYMPTOMATIC THERAPY AND MANAGEMENT OF AFFECTED SUBJECTS

Aicardi Goutieres syndrome can currently be considered a pathology without specific treatment. Its management is therefore focused on the management of symptoms and their implications on the life of the subject.

Once the diagnosis of Aicardi Goutieres syndrome has been made, it is essential to establish the clinical severity of the phenotype by:

- assessment of the level of psychomotor development, in order to define which areas may be the most compromised, focusing on a targeted and specific intervention
- assessment of the ability to access nutrition, with detailed analysis of sucking and swallowing, in order to exclude or recognize early the presence of any dysphagia (for liquids or solids) or ineffective sucking
- neurophysiological evaluations by performing electroencephalographic recordings, in order to evaluate the cerebral electrical organization and exclude the presence of critical paroxysms, in particular in the presence of paroxysmal episodes of dubious epileptic nature.

The symptomatic treatment of the subject with AGS (to be interpreted in the broadest sense of the term, i.e. including both the pharmacological and rehabilitative aspects), includes:

- antiepileptic drugs (AEDs) for the control of epileptic seizures, if detected
- botulinum toxin and / or muscle relaxant drugs (administered orally or by continuous infusion) for the management of muscle spasticity and dystonia, often extremely disturbing and painful
- pharmacological management of the pain related component of the syndrome, linked both to the presence of skin lesions and to the anomalies of muscle tone in the sense of spasticity or dystonia.

- prevention of complications of postural anomalies (tendon retractions, hip dislocation, scoliosis) through enabling treatments
- respiratory physiotherapy aimed at preventing recurrent lung infections (related to the stagnation of fluids and mucus in the lungs caused by ineffective expectoration)
- management of skin lesions, currently limited to the protection of the extremities from the cold and to the prevention of infections when there is skin ulceration.
- control of diet and feeding methods to ensure adequate caloric intake and avoid the risk of inhaling the food bolus with consequent development of ab ingestis pathology
- the control of constipation, a disorder often reported by the families of affected subjects as extremely stubborn and disturbing, must be treated promptly.

Other possible extra-neurological clinical manifestations of the syndrome (such as glaucoma, type 1 diabetes mellitus or hypothyroidism, scoliosis etc) must be periodically sought and treated early if found, in order to reduce the consequences related to them as much as possible (specifically low vision or blindness and metabolic imbalances).

The team that manages the follow-up of these individuals should therefore be multidisciplinary, including the figure of the child neuropsychiatrist, the pediatrician, the geneticist, the ophthalmologist, the nutritionist, the dietician, the physiotherapist / neuro psychomotor, the physiatrist and the 'orthopedic.

1.8.2 PREVIOUS THERAPEUTIC HYPOTHESIS

Specific experimental treatments for Aicardi Goutieres syndrome are difficult to perform due to the small number of affected subjects and, therefore, the difficulty of having samples with a sufficient number to achieve statistically significant results.

In the few studies conducted in this area, biases frequently found are related to the inclusion, within the same sample, of subjects in different stages of the disease (acute phase, su-acute phase and stabilized phase) and with different genotypes.
The interpretation of the results obtained is also made even more difficult by the absence of tools for assessing the outcome of the disease.
The results of these studies are therefore severely limited in their interpretation and difficult to extend to the AGS population (Crow Y et al, 2013).
In light of this premise, the use of some pharmacological treatments in the context of the syndrome has been considered in the literature:

- In the light of the involvement of the immune system and its activation in the pathogenesis of the disease, a treatment with immunomodulating therapies (prednisone in combination with azathioprine, and isolated intravenous methylprednisolone) has been proposed by some authors. Bias in the selection of the sample and in the drafting of the therapeutic protocols did not allow to achieve results of proven efficacy of the treatment.
- In 2003, Blau and collaborators hypothesized the use of an oral supplementary therapy with folic acid in a subgroup of subjects with folate deficiency at the CSF level. The sample of subjects described in the study showed a modest improvement in general clinical conditions after pharmacological supplementation. However, this figure was not subsequently confirmed in the literature.
- In relation to the treatment of skin lesions typical of the syndrome, treatment with vasodilator or immunosuppressive drugs, described by some authors in the literature, did not show any specific efficacy.

In subjects with the SAMHD1 mutation, the presence of vascular pathology affecting the great vessels has raised questions regarding the management of these subjects. Aneurysmal and occlusive arteriopathies could in fact be deserving of surgical treatment (by surgical clipping of the vessel or revascularization, respectively). Furthermore, the inflammatory element underlying the arteriopathy would suggest the possible use of immunosuppressants in the treatment of these complications. A key element for a considered decision in this sense would be to understand whether this manifestation is related to an active inflammatory process or is the result of a previous and currently dead inflammatory

situation. However, due to the absence of definitive data in this regard, no definite therapeutic strategies can be defined.

1.8.3 NEW THERAPEUTIC PERSPECTIVES

The better understanding of the molecular mechanisms underlying the pathogenesis of AGS has led various authors to hypothesize new therapeutic strategies:

- Biotechnological drugs: considering the possible primary role of interferon in the pathogenesis of AGS, a treatment based on the blocking of IFNα activity has been proposed through the use of selective monoclonal antibodies for various IFNα subtypes and for the IFN type I (alpha and beta). This category includes drugs with inhibitory action on the JAK-mediated signaling pathway (precisely defined JAK-inhibitors). Currently this pharmacological class is mainly used in the treatment of autoimmune neoplastic and inflammatory pathologies, with three specific drugs currently used in a widespread manner. Some pharmacological trials are currently underway with the use of this class in subjects suffering from Aicardi-Goutieres syndrome, one of them being conducted at the Children's Hospital of Philadelphia (CHOP), United States.
- Immunomodulating drugs: based on the role of immune system dysregulation in the pathogenesis of AGS, therapeutic strategies have been hypothesized with substances capable of destroying B lymphocytes (such as the monoclonal antibody rituximab) or inhibiting autoreactive T lymphocytes (such as mycophenolate mofetil).
- Reverse transcriptase inhibitors: considering the role of reverse transcriptase in the accumulation of DNA derived from endogenous RNA fragments (retroelements) normally metabolized by proteins encoded by genes that are mutated in AGS, the use of antiretroviral drugs (inhibitors of reverse transcriptase, i.e. capable of interrupting the replication cycle of both retroviruses and endogenous retroelements). To date, the pharmacodynamic profiles, safety and toxicity of these therapies are well known as they are commonly prescribed to adults and children with HIV1 infection.

The application of these new drugs if carried out in the early stage of the disease could lead to an attenuation of the inflammatory process and therefore of tissue damage; if carried out in subjects at an advanced stage in which the neurological manifestations are already overt, it could lead to an improvement of the clinical manifestations associated with the dysimmune process (e.g. chilblains).

Currently none of the therapeutic proposals mentioned above appears to be of proven efficacy since clinical trials involving the use of these drugs are still ongoing and the results are not yet available.

CHAPTER 2: CHILDREN'S DEVELOPMENT AND HOW TO MEASURE IT

Child development involves the biological, psychological and emotional changes that occur in human beings between birth and the conclusion of adolescence.

Early childhood occurs in the timespan between birth and 6 years of age. During this period a significant number of abilities is acquired (defined as developmental milestones), in 4 main areas:

- Gross motor abilities: such as rolling, crawling, walking and running.
- Fine motor abilities: with the refinement of prehension from a full palmar one (grasping) to a thumb-finger one, allowing better manipulation of objects.
- Communicative and social abilities (cognitive skills): communicative abilities include receptive and expressive language, allowing the child to participate more in the environment in which they live and communicate with others in an improved fashion (social interaction).
- Adaptive behavior abilities: defined as the capability of responding to the requests of the environment.

Within the course of development, the individual human progresses from complete dependency to increased autonomy.

While the process shows a predictable sequence, there is specific uniqueness for every single individual: not everybody progress at the same rate and each stage is affected by the preceding developmental experiences. Multiple factors, including prenatal and perinatal events, in association with genetic factors, can strongly influence the development of a child. Developmental change (in a sense of gain of skills) may occur as a result of genetically-controlled processes known as maturation, as a result of environmental factors and learning, and/or as a combination of the two of them. Parental care also plays a fundamental role in determining the development of a growing child, facilitating learning capabilities and social skills.

Definition of specific periods in childhood is blurred, due to them being a continuum of events. It is usually possible to define 5 stages of development:

- Newborn period (the first 28 days of life)
- Infancy (ages 1-12 months)
- Toddler (ages 1-2 years)
- Preschooler (ages 2-5 years)
- School-ages child (ages 6–13 years)
- Adolescent (ages 14–18/19 years)

2.1 MOTOR SKILL DEVELOPMENT

Motor skills are movements and actions of the muscles. Typically, they are categorized into two categories groups:

- Gross motor skills: skills that require the use of large muscle groups to perform tasks like walking, balancing, and crawling. The skill required is not extensive and therefore are usually associated with continuous tasks. Much of the development of these skills occurs during early childhood. The performance level of gross motor skill typically remains unchanged after periods of non-use. Gross motor skills can be further divided into two subgroups: oculomotor skills, and object-control skills. They can be impaired by occurring events (injuries, cerebrovascular events, neurologic disorders)
- Fine motor skills: skills that requires the use of smaller muscle groups to perform smaller movements with specific sections of the body. These tasks that are precise in nature, and there is a loss of quality of the movement if there is no practice of it. Fine motor skills can become impaired due to occurring events as well (injuries, cerebrovascular events, neurologic disorders)

Motor skills acquisition is representative of maturation of the nervous system (Assaiante C et al, 2005). Motor skills develop in different parts of a body along three principles: cephalocaudal (development from head to foot), proximo-distal, gross to specific. Many factors contribute to the rate that children develop their motor skills. Unless afflicted with a severe disability, children are expected to develop a wide range of basic movement abilities and motor skills.

Development is age-related but is not age dependent. In regard to age, it is seen that typical developments are expected to attain gross motor skills used for postural control and vertical mobility by 5 years of age. The environment in which a child grows strongly influences their motor capabilities (Golding J et al, 2014). A well accepted motor learning theory was proposed by Adams JA in 1971: he described motor learning as a change, resulting from practice, involving improvement of movements accuracy as one's environment changes. Motor learning is considered a relatively permanent skill as the capability to respond appropriately is acquired and retained. There are three stages included in this theory:

- Cognitive phase: considerable cognitive activity is required so to discover a motor gesture that leads to a successful goal. Good strategies are retained and inefficient strategies are discarded.
- Associative phase: the learner has determined the most-effective way to do the task and starts to make subtle adjustments in performance. Improvements are more gradual and movements become more consistent.
- Autonomous phase: once this phase is achieved, the performer can now complete the task without having to pay any attention to performing it.

Multiple Central Nervous System (CNS) structures are involved in the acquisition and refinement of the motor performance, including the primary motor cortex, the supplemental motor area, and the premotor cortex. The supplemental motor area (located anterior to the primary motor cortex), is involved with postural stability and adjustment, while the premotor cortex integrates sensory information from the posterior parietal cortex and is involved with the sensory-guided planning of movement and begins the programming of movement. Some basal ganglia (Globus Pallidus and Putamen) are involved with voluntary motor movement, and motor learning. The cerebellum controls fine motor skills, balance, and coordination.

2.2 COGNITIVE DEVELOPMENT: PIAGET'S THEORY

Cognitive development is defined as the emergence of the ability to consciously cognize, understand, and articulate their understanding in adult terms. Cognitive development is how a person perceives, thinks, and gains understanding of their world through the relations

of genetic and learning factors (Sellers PD et al, 2018). There are four stages to cognitive information development: reasoning, intelligence, language, and memory.

Piaget's theory of cognitive development is a comprehensive theory about the nature and development of human intelligence. It was originated by the Swiss developmental psychologist Jean Piaget (1896–1980). The theory deals with the nature of knowledge itself and how humans gradually come to acquire, construct, and use it. Piaget's theory is mainly known as a developmental stage theory.

In 1919, Piaget was “intrigued by the fact that children of different ages made different kinds of mistakes while solving problems".

Piaget departed from previous beliefs (that saw children as “little adults”) believing that children think and speak in a different way than adults, while showing great cognitive abilities.

In his observations, Piaget defined four different cognitive stages, based on the evolution of cognitive skills and the processes used to jump from one stage to the subsequent one.

To Piaget, cognitive development was a progressive reorganization of mental processes resulting from biological maturation and environmental experience. He believed that children construct an understanding of the world around them, experience discrepancies between what they already know and what they discover in their environment, then adjust their ideas accordingly (Piaget J et al, 1966). Moreover, Piaget claimed that cognitive development is at the center of the human organism, and language is contingent on knowledge and understanding acquired through cognitive development.

In his theory of cognitive development, Jean Piaget proposed that humans progress through four developmental stages: the sensorimotor stage, preoperational stage, concrete operational stage, and formal operational stage.

- Sensorimotor stage: defined as a period that "extends from birth to the acquisition of language". In this stage, infants construct knowledge and understanding of the world by coordinating experiences from physical interactions with objects (explored orally, or through contact with their limbs). Children learn that they are separate from the environment. In this stage, according to Piaget, the development of object permanence is one of the most important accomplishments. Object

permanence is defined as a child's understanding that an object continues to exist even though they cannot see or hear it.

- Preoperational stage: By observing sequences of play, Piaget was able to demonstrate the second stage of his theory, the pre-operational stage. The stage occurs when the child begins to learn to speak multiple words in association (word-phrase) and lasts up until the age of seven. During the pre-operational stage of cognitive development, Piaget noted that children do not yet understand concrete logic and cannot mentally manipulate information. Symbolic and pretended games are observed in this phase. Their observations of symbols exemplify the idea of play with the absence of the actual objects involved. However, the child still present limitation in the ability of envisioning a situation from an external perspective. The pre-operational stage is sparse and logically inadequate in regard to mental operations. The child is able to form stable concepts as well as magical beliefs. The child, however, is still not able to perform operations, which are tasks that the child can do mentally, rather than physically. Thinking in this stage is still egocentric, meaning the child has difficulty seeing the viewpoint of others.
- Concrete operational stage: this stage, occurs between the ages of 7 and 11 years, and is characterized by the appropriate use of logic. During this stage, children start solving problems in a more logical fashion. Abstract, hypothetical thinking is not yet developed, and children can only solve problems that apply to concrete events or objects. Piaget determined that children are able to incorporate inductive reasoning. Inductive reasoning involves drawing inferences from observations in order to make a generalization. In contrast, children struggle with deductive reasoning, which involves using a generalized principle in order to try to predict the outcome of an event. Two other important processes in the concrete operational stage are logic and the elimination of egocentrism (with the consequence of the child being able to mentalize different perspectives and point of views of the same situation).
- Formal operational stage: the final stage of Piaget’s theory spans from ages 12 to adulthood. Intelligence is demonstrated through the logical use of symbols related to abstract concepts. This form of thought includes "assumptions that have no

necessary relation to reality". At this point, the person is capable of hypothetical and deductive reasoning. During this time, people develop the ability to think about abstract concepts. Specifically, children tend to think very concretely and specifically in earlier stages, and begin to consider possible outcomes and consequences of actions. Typical of this phase are also metacognition (the capacity for "thinking about thinking") and dramatic improvement of problem-solving skills. "However, research has shown that not all persons in all cultures reach formal operations, and most people do not use formal operations in all aspects of their lives" (Jeffrey A, 2013)

Despite its success, Piaget's theory has some limitations (recognized by the psychologist himself), the most outstanding being the sharp distinction that is implied between the different phases, within a process that is perceived as a continuum. Nonetheless, Piaget is considered a fundamental author of the cognitive developmental sciences.

2.3 HOW TO EVALUATE THE DEVELOPMENT OF A CHILD

As described before, developmental milestones are changes in specific physical and mental abilities that mark the end of one developmental period and the beginning of another (Dosman CF et al, 2012). Per Piaget's stage theories, milestones indicate a stage transition. Studies of the accomplishment of many developmental tasks have established typical chronological ages associated with developmental milestones. However, there is considerable variation in the achievement of milestones, even between children with developmental trajectories within the typical range. Some milestones are more variable than others, particularly expressive skills.

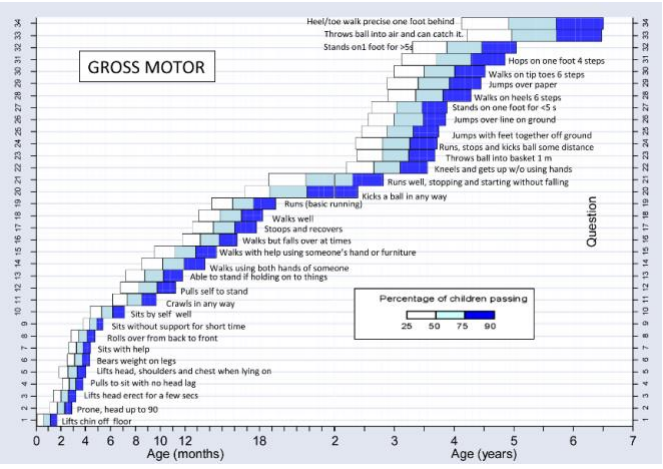
Milestones are categorized into social/emotional, gross and fine motor, language, and cognitive. This activity highlights the role of the interprofessional team in assessing developmental milestones. Understanding and identifying the developmental milestones can help the provider more adeptly recognize delayed development, facilitating earlier interventions and improving outcomes

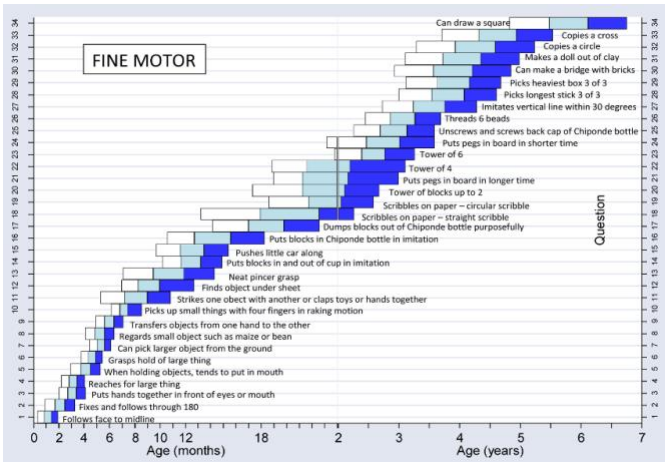
2.3.1 DEVELOPMENTAL SCREENING TOOLS: THE DENVER DEVELOPMENTAL SCREENING TEST II

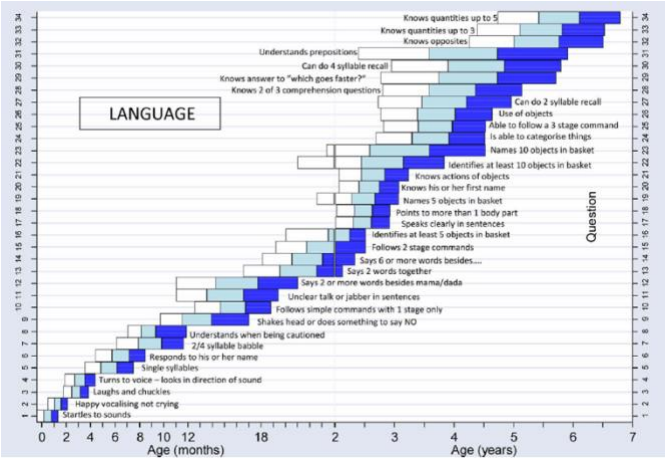
The Denver Developmental Screening Test was conceived and published in 1967 with the function of being a tool capable of identifying children, aged between 0 and 6 years, with psychomotor developmental problems. A revised version, called Denver II, was published in 1992. The tool was created with the aim of being able to ensure early care of the child with developmental problems identified through a screening test. The scale provides for the exploration of 4 domains of the child's psychomotor development: personal and social skills, the area of the child's fine motor skills and adaptive abilities, expressive language and the area of gross motor skills. The test was designed to be administered by medical or healthcare personnel involved in the child's health. The Denver scale II is made up of 125 items, which can be rated as a pass, a fail, or a failed test. Items that are performed by 75-90% of children but failed by the proband of the same age must lead to an alarm in the clinician. Failure to perform tests that are routinely passed by 90% of peers must lead to the presence of a delay. The test can be considered normal if there is no delay in the different domains and no more than an alarm bell.

The evidence is suspicious in cases where the presence of a late domain and one or two domains are found that are interpreted as alarm bells.

The test fails and identifies a probable delay if two or more domains are delayed. For the interpretation of the scale it is possible to refer to the reference scales of the 4 specific domains, shown below.







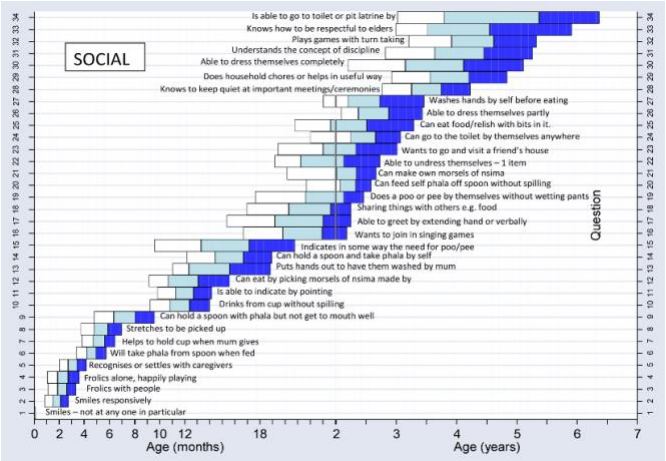


Fig 8. Denver Developmental Scale II: gross motor, fine motor, language and social milestones

2.3.2. CLINICAL CLASSIFICATION OF FUNCTIONAL ABILITIES: THE FUNCTIONAL CLASSIFICATION SYSTEMS

As seen before, multiple factors can influence the acquisition of motor abilities. In the pediatric age, cerebral palsy (CP) is the most common physical disability in childhood (Gorter JW et al, 2004). CP comprises a heterogeneous group of disorders that are the result of a non-progressive disruption or injury that occurred during fetal brain development or within the first two years of life. This disruption can result in spasticity, dystonia, muscle contractures, weakness and difficulty in coordination that ultimately affects the ability to control movements. Resultant activity limitations may affect gross motor movements, fine motor movements, speech and communication, as well as eating and drinking. CP severity is frequently described subjectively in terms of mild, moderate or severe (Graham HK et al, 2005). Imprecise terms such as these tell very little about what a person is able to do functionally. Moreover, when a large range of ability and performance can be encapsulated by the same term (such as moderate spastic quadriplegia CP), providers struggle to accurately interpret communications from one another. In research, this can create subject groups that are very heterogeneous (Paulson A et al, 2017).

Over the last 20 years, clinicians and researchers have created classification systems using a simple ordinal grading system of functional capacity, allowing a more precise discussion between providers. These classification systems use a common language to describe function. They do not describe potential improvements or assess underlying etiology. The most commonly used are: the Communication Function Classification System (CFCS), the Gross Motor Function Classification System (GMFCS), and the Manual Ability Classification System (MACS) and its mini version (Mini-MACS). These measures are standardized, reliable, and complementary to one another (Paulson A et al, 2017).

- **Communication Function Classification System (CFCS)**

The CFCS was developed by Hidecker et al. in 2011 and is a valid measure in cerebral palsy that assesses everyday communication (not optimal communication). The CFCS is a simple, five-point ordinal classification system, and was designed to be analogous and complementary to the GMFCS and MACS. Being an effective communicator requires both sending and receiving information, and the CFCS accordingly assesses both how

information is expressed and how it is received. The CFCS allows all methods of communication (e.g., vocalizations, manual signs, eye gaze, pictures, communication boards, speech generating devices) to be included when assessing an individual's classification. In this way, the CFCS is inclusive and descriptive for individuals who do not vocalize to communicate. The CFCS also considers familiar and unfamiliar communication partners.

Communication Function Classification System (CFCS)	
Score	**Example**
I: Normal Quality and Performance with Complete Sentences	Normal enunciation and ability to use a minimum of 3-word sentences. This level would typically not be able to be attained by a 2-year-old even with typical development.
II: Reduced Quality of Complete Sentences	Reduced quality of spoken language, but individual still attempts to speak in a minimum of 3-word sentences. This could be difficulty with vocabulary, articulation, or grammar; or this could be dysarthria or slowed speech.
III: 2-Word Phrases	Ability to generate unique 2-word phrases. This would not include common phrases such as "Thank You".
IV: Single, Meaningful Words	Ability to generate single words by spoken or sign language. This would not include grunting or pointing. This includes preserved receptive language and the ability to choose between binary options.
V: Complete Loss of Expressive Language	No words (sign or spoken). This would include grunting or pointing. Complete inability to communicate.

Table 1. Communication Function Classification System (CFCS)

- **Gross Motor Function Classification System (GMFCS)**

The GMFCS is the most established and recognized of the functional classification measures in CP. The GMFCS is a simple, five-level, ordinal grading system created to describe the gross motor function of an individual with CP. First described in 1997 by Palisano et al., the GMFCS provides a common language for a practitioner that is meaningful, quick and easy to use. The GMFCS describes self-initiated movement and use of assistive devices (walkers, crutches, canes, wheelchairs) for mobility during an individual's usual activity. Following research to stratify typical motor function for children with CP, the authors concluded that a five-level classification system worked well to discriminate clinically meaningful distinctions in motor function. Motor abilities considered to score the functional level at the GMFCS changes over time, based on the age of the child evaluated with it.

Gross Motor Function Classification System (GMFCS)	
Score	**Example**
Before 2nd Birthday	
Level I: Unassisted walking with normal quality and performance.	Children walk without assistance by 2 years of age. Pull to stand and cruise along furniture as primary means of mobility.
Level II: Independent sitting.	Children walk with assistance by 2 years of age either with one-hand assist or the use of furniture. Children sit without support. May crawl on hands and knees or creep.
Level III: Sitting with support.	Children sit with low back support. Roll or creep forward on their stomachs.
Level IV: Head control.	Children have head support but sit with support. Can roll to supine.
Level V: No head control.	Children are unable to roll. No independent mobility.
Between 2nd and 4th Birthday	
Level I: Unassisted walking with normal quality and performance.	Children walk independently as preferred method of mobility. No need for hand-held device. May need use of leg braces or ankle orthotics.

Level II: Walking Without Support, but with Reduced Quality.	Children are able to sit independently. May cruise along furniture. Crawl on hands and knees with reciprocal pattern.
Level III: Walking with support (i.e. fewer than 5 independent steps).	Children may have a W-posture while floor sitting. Can walk indoors using furniture as assistance. May creep on stomach or crawl on hands and knees.
Level IV: Ambulation through wheelchair or floor mobility (such as crawling).	Children need support for sitting. Only able to move short distances inside by creeping or crawling.
Level V: No independent mobility.	Transported in wheelchair in all settings. No head or neck control. No volitional movement. Children may use power wheelchair with extensive adaptations.
Between 4th and 6th Birthday	
Level I: Unassisted walking with normal quality and performance.	Children walk independently as preferred method of mobility. No need for hand-held device. May need use of leg braces or ankle orthotics. Children climb stairs. Not necessarily running or jumping.
Level II: Walking Without Support, but with Reduced Quality.	Children sit independently in a chair. Minimal walking. Walk up-stairs but are unable to run or jump.
Level III: Walking with support (i.e. fewer than 5 independent steps).	Children need support of a walker, i.e. kaye or posterior walker, for independent mobility. Need wheelchair or transportation for longer distances.
Level IV: Ambulation through wheelchair or floor mobility (such as crawling).	Children use wheelchair or alternative transportation for most daily ambulation. Need support for sitting.
Level V: No independent mobility.	Children are transported in wheelchair in all settings. No head or neck control. No volitional movement. Children may use power wheelchair with extensive adaptations.
Between 6th and 12th Birthday	
Level I: Unassisted walking with normal quality and performance.	Children walk independently as preferred method of mobility in all settings. No need for hand-held device. May need use of leg

	braces or ankle orthotics. Children are able to run and jump.
Level II: Walking Without Support, but with Reduced Quality.	Children walk in most settings. May need some additional means of support. May use walker in school or wheelchair for long distances.
Level III: Walking with support (i.e. fewer than 5 independent steps).	Children need assistance with mobility in most settings, i.e. kaye or posterior walker.
Level IV: Ambulation through wheelchair or floor mobility (such as crawling).	Children use alternative means for mobility in most settings, i.e. wheelchair. Children may crawl at home as primary means of mobility.
Level V: No independent mobility.	Children are transported in wheelchair in all settings. No head or neck control. No volitional movement.
Between 12th and 18th Birthday	
Level I: Unassisted walking with normal quality and performance.	Children walk independently as preferred method of mobility in all settings. No need for hand-held device. May need use of leg braces or ankle orthotics. Children are able to run and jump but coordination may be limited.
Level II: Walking Without Support, but with Reduced Quality.	Children walk in most settings. May need some additional means of support. May use walker in school or wheelchair for long distances.
Level III: Walking with support (i.e. fewer than 5 independent steps).	Children need assistance with mobility in most settings, i.e. kaye or posterior walker.
Level IV: Ambulation through wheelchair or floor mobility (such as crawling).	Children use alternative means for mobility in most settings, i.e. wheelchair. Children may crawl at home as primary means of mobility.
Level V: No independent mobility.	Children are transported in wheelchair in all settings. No head or neck control. No volitional movement.

Table 2. Gross Motor Function Classification System (GMFCS)

- **Manual Ability Classification System (MACS)**

The Manual Ability Classification System (MACS) was developed by Eliasson et al. in 2006, specifically to be an upper extremity analogue of the GMFCS. The MACS is also a simple, five-point ordinal classification system, analogous and complementary to the GMFCS, and was designed for use in children ages 4–18 years. The MACS is a validated measure in cerebral palsy that can be used to classify a child's typical use of both hands and upper limbs; it is not meant to reflect best use or individual hand function.

Manual Ability Classification System (MACS)	
Score	**Example**
I: Handles objects easily and successfully.	Can have limitations in handling very small, heavy, or fragile items that demand detailed fine motor control.
II: Handles objects with reduced quality of speed.	Quality or speed of performance is reduced compared to level I. Often children try to simplify tasks (such as supporting self on a table to perform tasks).
III: Handles objects with difficulties; needs help.	Need help to prepare the activity or need environmental adjustments. Children have decreased independence and require supervision and more time to complete tasks.
IV: Handles a limited selection of easily managed objects in adapted situations.	Need continuous help to complete a task and can at best complete only part of the task.
V: Does not handle objects and has severely limited ability to perform simple actions.	Can only participate in a simple movement (i.e. pushing a button or holding an object).

Table 3. Manual ability Classification System (MACS)

In 2017, Eliasson et al validated an extended version of the MACS was validated to measure the functional motor abilities of younger children (age 1-4 years).

Mini - Manual Ability Classification System (Mini-MACS)	
Score	**Example**
I: Handles objects easily and successfully.	The child may have a slight limitation in performing actions that require precision and coordination between the hands but they can still perform them. The child may need somewhat more adult assistance when handling objects compared to other children of the same age.
II: Handles most objects, but with somewhat reduced quality and/or speed of achievement	Some actions can only be performed and accomplished with some difficulty and after practice. The child may try an alternative approach, such as using only one hand. The child needs adult assistance to handle objects more frequently compared to children at the same age.
III: Handles objects with difficulties; needs help.	Performance is slow, with limited variation and quality. Easily managed objects are handled independently for short periods. The child often needs adult help and support to handle objects.
IV: Handles a limited selection of easily managed objects in simple situations.	The actions are performed slowly, with exertion and/or random precision. The child needs constant adult help and support to handle objects.
V: Does not handle objects and has severely limited ability to perform even simple actions.	At best, the child can push, touch, press, or hold on to a few items, in constant interaction with an adult.

Table 4. Mini-Manual ability Classification System (Mini-MACS)

2.3.3. PROVIDER ADMINISTERED OUTCOME MEASURES FOR THE EVALUATION OF FUNCTIONAL LEVEL

Functional outcomes define results of care focused on the patient's abilities (motor, psychomotor/cognitive, adaptive behavior).

Functional outcomes determined by observing physical/cognitive/psychomotor/adaptive-behavior ability is called performance testing. Performance testing provides a snapshot of the patient's functional level for a specific task. Typically, performance testing will involve timing the performance of a task, counting the completed repetitions of a specific task performed during an allotted amount of time, or observing a sequence of movement patterns and scoring the performance.

When considering a pediatric population, specific attention must be put into the selection of the more appropriate set of assessments included for the evaluation of the functional level. As seen before, the vast majority of motor and cognitive abilities are acquired by pediatric patients before reaching adulthood. Due to that, multiple tests are available to evaluate the same function following the child's growth.

Parameters to consider when choosing the best tool to evaluate a child are:

- the age
- the functional level
- the underlying diagnosis (if determined)

These aspects are incredibly relevant to achieve the scope of the assessment: determine the functional level of an individual, focusing on the identification of dynamic abilities, that might change (in both improvement and worsening sense) over time.

An appropriate outcome measure is a test that is able to avoid floor and ceiling effects, providing a good representation of the abilities of a patient.

Floor effect occurs when responses on a measure, questionnaire or scale cluster at the more negative health state end of the scale. This means that, if the assessment were administered a second time, there would be no room to detect any possible deterioration (or small improvement) in health or functional abilities, even if it had occurred.

On the other side, the ceiling effect occurs when responses on a measure or questionnaire cluster at the more positive health state end of the scale. This means that if the assessment were administered a second time, there would be no room to detect any possible improvements in health or functional abilities, even if they had occurred.

CHAPTER 3: SCOPE OF THE WORK

Aicardi Goutieres syndrome, as discussed above, is a rare monogenic interferonopathy.

Recent scientific advances in the etiopathogenetic field, linked to the ever-greater understanding of the mechanisms linked to the genesis of the disorder, have allowed the possibility to achieving an ever higher diagnostic accuracy.

The progress in the genetic field, linked to the ever-increasing availability of genetic panels including genes linked to AGS, has made it possible to achieve diagnosis even in individuals presenting atypical phenotypic characteristics. The clinical spectrum linked to the syndrome is therefore gradually expanding and widening.

The review of the literature made it possible to observe the absence of scientific works aimed at analyzing the specific psychomotor development of subjects with AGS. In fact, if from a general point of view a seriousness of the classic phenotype of the affected subject has been defined (with psychomotor acquisitions in great delay or lost in a subsequent period), phenotypic differences are described mainly in an anecdotal and unsystematic way.

No comprehensive natural history study focused on developmental trajectories, multi-organ involvement or functional level derived from validated outcome measures is currently available for this disease.

In addition to that, no information about the burden of disease is available in the scientific literature, as well as no description of population performance in standardized functional testing to evaluate motor, cognitive, and adaptive behavior abilities.

In light of the above, the purpose of this work, will be organized in multiple sections:

- Determination of developmental trajectories in an international cohort of individuals with clinical and molecular diagnosis of AGS, in order to better define disease heterogeneity and define existing genotype-phenotype correlations.
- Determination of disease effects on organ functionality, in order to allow monitoring of possible critical disease complications. The focus of this work will be on the hepatic involvement of AGS. This project will fall under the disease natural history umbrella as well.

- Determination of disease perception and burden of disease on caregivers through the use of designed semi-structured interviews designed to explore the topic.
- Determination of existing outcome measures able to describe the functional level of affected individuals. If floor effect will be observed with traditional assessments, a novel specific tool will be designed and validated in the study population
- Application of the existing outcome measures or the novel tool to determine efficacy of experimental treatments

CHAPTER 4: MATERIALS AND METHODS

4.1 DEVELOPMENTAL OUTCOMES OF AICARDI GOUTIÈRES SYNDROME

A total of 100 individuals from 94 families affected by Aicardi Goutieres Syndrome were recruited through the Myelin Disorders Bioregistry Project (MDBP, Institutional Review Board Approved), an arm of the Global Leukodystrophy Initiative Clinical Trial Network (GLIA-CTN).

Aicardi Goutieres syndrome was defined as documented neurologic disability in the context of a change in an Aicardi Goutieres Syndrome–related gene: TREX1, RNASEH2A, RNASEH2B, RNASEH2C, SAMHD1, ADAR1, or IFIH1.

This neurologic disability ranged from isolated spastic paraparesis to profound global developmental delay. Individuals without a known genetic diagnosis or those with insufficient developmental histories were excluded.

Demographic information, including age at last evaluation and mortality, was collected. Date of acquisition of milestones was obtained from parental questionnaires and confirmed with medical record review. Microcephaly was identified in medical records and defined as more than 2 standard deviations from the mean for head circumference at the time of final data col- lection. Presenting information was extracted from concurrent medical records wherever feasible to minimize recall bias.

Standard functional scores, the Gross Motor Function Classification System, the Manual Ability Classification System, and the Communication Function Classification System, were assigned from medical records as previously described, at the last available clinical encounter. These scales are composed of 5 levels, with I representing normal function and V representing complete impairment. The Gross Motor Function Classification System describes motor impairment. 11 Children scored as I are independently ambulatory without limitations, whereas children scored as a V require full assistance including with head control. The Manual Ability Classification System helps to score fine motor skills, 10 with a score of I representing normal dexterity and V representing the requirement for total assistance. The Communication Function Classification System is used to classify communication skills. A Communication Function Classification System score of I

represents effective communication as both sender and receiver, whereas a score of V represents severe impairment even between familiar partners.

Developmental milestones were grouped by genotype and compared to the normative data derived from the Denver Developmental Screening Test II (DDST-II) using the P value log rank test. Skill acquisition was binned by percentage of the cohort who had attained a skill at a given age. In order to compare achievement of milestones by genotype, we created Kaplan-Meier curves for acquisition of developmental milestones. We included indicator variables for each genotype group to evaluate the independent effects of genotype.

Age of acquisition of milestones was also plotted, including the age at which percentiles of the population (p10 for 10%, p25 for 25%, etc) had achieved that specific milestone. These data were grouped by genotype and compared to the normative data derived from the Denver Developmental Screening Test II (DDST-II). The 1992 revision of the DDST, the DDST-II, has been validated across many populations of children with developmental disabilities and brain injuries. We selected specific items from the DDST-II to represent the range of skills observed in the Aicardi Goutieres Syndrome population**.**

4.2 LATE-ONSET AICARDI GOUTIÈRES SYNDROME: A CHARACTERIZATION OF PRESENTING CLINICAL FEATURES

From total of 231 individuals enrolled under the MDBP project or research projects in 3 Italian Centers (Spedali Civili of Brescia, Istituto Mondino of Pavia, Ospedale Buzzi of Milano), we identified 34 individuals from 32 families presenting after the age of twelve months.

Genotype, ages, past medical history, prodromal history, fulminant presenting symptoms, MRI features, and initial differential diagnoses were collected through retrospective chart review. Charts with insufficient details and MRIs obtained greater than 1 year from the initial symptoms were excluded from analysis. Pathology-targeting pharmacologic treatments, such as immunomodulatory agents, and responses to treatment were noted. Age at diagnosis was defined as the date that genetic confirmation was obtained.

Early AGS-related symptomatology was divided into three categories: past medical history, prodrome, and fulminant presentation. Pertinent past medical history was defined as non-specific signs of abnormal growth or development in an otherwise healthy child. This category included isolated or rare inflammatory symptoms. The prodrome was defined as an increased burden of recurrent or persistent symptoms associated with inflammation prior to fulminant presentation. Systemic symptoms were noted and included fevers, fatigue, irritability/colic, myalgias/fatigue and chilblains. Fulminant symptoms were defined as new observable neurologic deficits.

The first magnetic resonance imaging (MRI) results obtained within the first two years of disease were included for review (n=31). Original MR images for 13 individuals were reviewed by a pediatric neuroradiologist, and otherwise the original radiology reports were used. One individual had MR images obtained prior to symptom onset for unrelated reasons.

4.3 HEPATIC INVOLVEMENT IN AICARDI GOUTIÈRES SYNDROME

This study began with the pretreatment data from a cohort of international MDBP children (n: 48) enrolled in therapeutic studies, where a higher than expected incidence of liver enzyme elevation was noted. From this cohort, detailed pretreatment medical histories (n= 48) and banked serum samples (n= 33) were available. Next, we evaluated liver enzyme abnormalities within our expanded cohort (n= 102, inclusive of the detailed cohort above). From this expanded cohort, laboratory values and limited medical histories (e.g., clinical presentation and genotype) were collected. The additional subjects included in the larger cohort were comparable to the detailed cohort by key parameters of age at last evaluation (Wilcoxon rank sum test, p: 0.606), genotype (Pearson chi-squared test, p: 0.241), and presence of microcephaly (Pearson chi- squared test, p: 0.168). Age of onset was different between the two groups (Wilcoxon rank sum test, p: 0.039). With removal of an outlier with disease onset over 200 months, this association no longer reached significance (Wilcoxon rank sum test with removal of outlier, p: 0.059). Clinical manifestations of

liver-disease were described for two individuals across the expanded cohort in the overall cohort.

Results of aspartate-aminotransferase (AST), alanine-aminotransferase (ALT), and gamma-glutamyl transferase (GGT) testing were collected as available from the medical records. Laboratory analyses stated as normal but for which an exact value was not available were scored as normal (0.5-fold increase over normal). Liver enzyme fold increase was calculated based on the upper limit of normal values included in the medical report for each test. AST and ALT fold increase of three times and GGT fold increase of 2.5 times from upper limit of normal were considered meaningful representation of abnormal liver enzyme levels consistent with hepatitis. To minimize the over-representation of repeat laboratory measures in individual patients, the value of greatest elevation was used for visualization if measures were repeated within 1 month, unless individuals were greater than 100 months, in which case the highest value within a 10-month period was used.

Values Banked serum samples were available from 33 individuals in the detailed cohort. These samples were used to measure liver autoantibody levels, which included ANA, ANCA, ASMA, and anti-liver/kidney microsome 1 in the clinical immunology laboratory. A positive value for the autoantibody panel was determined by the presence of one or more elevated autoantibody levels.

Simultaneous liver enzyme testing and IFN signaling gene (ISG) expression scores were available for 52 individuals in the expanded cohort, inclusive of 46 individuals within the de- tailed cohort. To calculate the ISG expression score, extracted RNA (Qiagen PAXgene Blood RNA kit) was quantified using the Nanostring nCounter Digital as previously described. The 18 copy number of mRNA transcripts were measured from type I IFN-inducible genes (IFI27, IFI44L, IFIT1, ISG15, RSAD2, and SIGLEC1) as compared to housekeeping genes (ALAS1, HPRT1, TBP, and TUBB). An ISG score of 1.96 (>98th percentile) was considered positive.

Demographic, clinical, and genetic features were described with descriptive statistics. Analyses were performed using Stata 16.0 statistical software and GraphPad Prism 8. Statistical significance was set at a two-sided p-value of less than 0.05. Bland–Altman analysis was used to evaluate the agreement between age at first available measurement

and age at presentation. This found that as age at onset increased, so did the difference between age at onset and the first laboratory measurement. Thus, it was determined that age at first measurement of liver enzymes could not be used to deter- mine age at onset of hepatitis. In all cases, elevations of ALT, AST, and GGT were considered both for any abnormal values (e.g., fold change >1) as well as for fold changes that were consistent with hepatitis.15 Kaplan–Meier curves were constructed to visualize the acquisition of liver enzyme elevation within groups. The log- rank test was used to compare the survival distributions between the subgroups defined according to microcephaly, disease onset, and genotype (SAMHD1 versus other genotypes combined). We also performed tests for the assumption of proportional hazards based on the Schoenfeld residuals.

Comparisons between liver enzyme abnormalities and ISGs and between liver enzyme abnormalities and age at sample were assessed by fitting longitudinal models. These longitudinal models were fitted using quasi-least squares (QLS) regression and QLS logistic regression. QLS is a computational approach for estimation of intrasubject correlations in the framework of generalized estimating equations. Comparison between ISGs and liver autoantibody abnormalities was performed by Mann–Whitney (Wilcoxon rank sum tests) U test as only single values were available. Comparisons between liver autoantibody abnormalities and AST, ALT, and GGT abnormalities were performed by chi-squared test. Comparisons between subcohorts were performed by Wilcoxon rank sum and chi-squared tests values to existing measures. Significance was defined when $p < 0.05$, adjusted for multiple comparisons with an overall Bonferroni correction. All comparisons maintained significance when an overall Bonferroni correction was applied to define significance between multiple groups.

4.4 DEVELOPMENT OF A NEUROLOGIC SEVERITY SCALE FOR AICARDI GOUTIÈRES SYNDROME

This retrospective natural history study was designed to develop a multimodal AGS scale. Individuals affected by AGS were recruited through the MDBP project.

Individuals with pathogenic or likely pathogenic mutations in the following AGS-related genes were included in this cohort: TREX1, RNASEH2A, RNASEH2B, RNASEH2C, SAMHD1, ADAR1, and IFIH1.

Information, including genetics, neurologic function, and age at each evaluation, was collected from medical records. Milestone acquisition was obtained from medical records and confirmed by parental questionnaires. Data was extracted by a child neurologist.

This cohort was comprised of all individuals with available electronic medical records. A clinical severity assessment was assigned retrospectively to the last available clinical encounter by two child neurologists: “mild”, “moderate”, and “severe” (n = 65). When there was a discrepancy (n = 8), (mild-moderate or severe-moderate), the individual was assigned to the moderate classification.

Because of the age requirements for the application of the functional scales, this cohort was comprised of all individuals with available electronic medical records over the age of 18 months (n = 61/65). Three validated functional scores were assigned retrospectively to the last available clinical encounter as previously described for AGS: Gross Motor Functional Classification Scale (GMFCS), Manual Abilities Functional Classification Scale (MACS), and Communication Function Classification Scale (CFCS) (Eliasson AC et al, 2006; Crow YJ et al, 2015; Palisano RJ et al, 2018; Paulson A et al, 2017; Ramsey D et al, 2017). Each of these scales is composed of 5-levels with ‘I’ as normal function and ‘V’ representing complete impairment. These values were reversed and summed, so that higher numbers re- present greater function. This was designated as the ‘rComposite’.

The AGS scale was developed by a panel of AGS experts by a consensus review process. Each item was reviewed and modified for content validity, clarity, and face validity.

Items were selected from the rates of developmental skill acquisition in the AGS population. Across the AGS population, the most commonly attained skills were smiling, head control, and babbling. As AGS affects all developmental domains, we selected three key develop- mental categories: cognitive development, fine motor development, and gross motor skills to be included in our scale. We removed items that were frequently absent from clinical records (e.g. the differentiation between immature and mature pincer grasp). We focused on the inclusion of items that could be consistently understood and administered.

To address issues around the validation of this outcome measure given the limitations of sample size in a rare disease and familiarity of the research team with this population, pilot testing was performed using blinded clinical encounters. These encounters were selected based on the presence of developmental skill information related to gross motor and cognitive function (n = 140 encounters from 35 individuals). In addition to the removal of all HIPPA identifiers, all identifying information was redacted from these clinical encounters, including gender pronouns and any unique characteristics and histories, by two unblinded members of the study team. These encounters retained only essential developmental and examination information. These blinded encounters were scored by two child neurologists. After scoring a limited set of encounters, the scores were compared between raters, and the definitions for each item were refined.

In the final AGS scale, one point is awarded for the presence of the following skills: normocephaly, smiling, vocalizations, single words, sentences, fine motor skills (pincer grasp or self-feeding), head control, sitting independently, and rolling or crawling, mobile with assistance, and independently mobile. The points are summed to generate the final AGS scale score, which has a range of 0–11.

A subset of blinded encounters was separately scored by two child neurologists and used to calculate the AGS scale agreement and kappa coefficient (total of 24 encounters per rater (92% agreement, kappa coefficient of 0.91; 95% confidence interval 0.81–0.99))

The AGS scale was applied to retrospective clinical encounters (533 clinical encounters from 92 individuals affected by AGS). Each score was assigned and verified by two child neurologists.

A subset of individuals underwent Gross Motor Function Measure- 88 (GMFM-88) assessments by trained therapists (n = 24) (Alotaibi M et al, 2014; A. Lundkvist Josenby A et al, 2009). This cohort includes the first GMFM-88 assessment for all individuals. The GMFM-88 is divided into 5 subdomains: subdomain A (lying and rolling skills), subdomain B (sitting skills), subdomain C (crawling and kneeling skills), subdomain D (standing skills), and subdomain E (walking, running, and jumping skills).

This cohort included the first encounter at which ISG scores were obtained concurrently with clinical evaluations (n = 36). ISG scores (z- scores) were calculated as available from

each clinical visit where blood samples were obtained. The methodology used is the same explained on section 2.2.

Excel 16.23 and Prism 8.0 were used to generate the graphs and statistics. Inter-rater reliability (agreement between two independent providers) of the AGS scale were assessed by kappa statistics and intraclass correlation coefficients (ICC). Mann Whitney test with two tailed p values were used when comparing two independent groups; the Kruskal-Wallis test was used to compare more than two independent groups. Spearman correlations were used to compare the AGS scale

4.5 EXPERIENCES AND HOPE IN CAREGIVERS OF CHILDREN WITH AICARDI GOUTIÈRES SYNDROME

The study participants were recruited from a large US pediatric hospital with a leukodystrophy center. Eligibility included any parent of a child with clinical and molecularly characterized Aicardi Goutières Syndrome (AGS). When multiple caregivers were available, interviews were conducted separately. This AGS cohort represents the breadth of the AGS spectrum by sex, genotype, severity, and age. Additional caregivers were recruited until thematic saturation (absence of new themes observed during interviews administration) was achieved. Severity was assigned using a disease specific AGS scale, which was categorized as mild (score of 8-11), moderate (4-7), and severe (0-3). In total, 45 interviews were conducted with the caregivers of 33 subjects (27 interviews with mothers, 18 with fathers). One caregiver refused interview participation. A copy of the interview is included as Appendix 1.

An expert panel of child neurologists and neonatologists created the interview guide based on clinical experience and the natural history of AGS. The final survey underwent construct and face validity with this group of experts and was reviewed by parent advocates. Study visits were conducted in person. All interview audio files were transcribed verbatim into a text document by a professional medical transcription service. All interview transcripts were uploaded to Atlas.ti v7 qualitative data analysis software for management and coding. Responses to open-ended questions were analyzed using

standard methods of inductive theory-building, and the constant comparative method to develop modified grounded theory about parent perspectives on the impact of AGS on their children. In order to maintain coding consistency and establish validity, analysis progressed in five stages:

(1) analytical note and memo formation to capture thoughts regarding theory,

(2) open coding to identify broad categories/concepts and themes,

(3) prolonged engagement with the data and peer debriefing with study team members to interpret data,

(4) thick description with impartial peers to assign context and meaning to the data, and

(5) axial coding to solidify specific core categories and concepts, and form concrete themes

Using grounded theory methodology, the study team developed analytical memos and logged in the project, and solidified a codebook. Data were examined using open coding leading to overall categories of interest and importance. A team of two coders was used for all interviews. During the open coding phase, the most salient categories and concepts were defined.

Peer debriefing and thick description maintained coding consistency and established validity of those categories and concepts. During the axial coding phase, codes were further refined and themes were generated within each code, as well as explanations for the patterns that emerged. After axial coding was completed, output for the study included several code reports with textual quotations organized by theme. Query tool reports and network views determined the specific quotes used in the paper.

Reliability and validity of the analyses were assessed further through four criteria described in previous research: prolonged engagement with the data, credibility, transferability, and confirmability.

The themes were developed from responses. With the exception of some minor edits to improve clarity, all quotes are provided verbatim.

4.6 RELIABILITY OF THE TELEMEDICINE APPLICATION OF THE GROSS MOTOR FUNCTION MEASURE-88 IN PATIENTS WITH LEUKODYSTROPHY

4.6.1 MATERIALS

The Gross Motor Function Measure (GMFM) and its subsequent revisions, has become the most common functional outcome measure used by rehabilitation specialists to measure gross motor functioning in children with CP and other neurologically based conditions, such as Down syndrome and traumatic brain injury (Krach LE et al, 2005; Boyd RN et al, 2001). The earliest version of the GMFM is known as the GMFM-88. This tool is standardized criterion referenced measurement tools designed to measure gross motor function over time for children with disabilities, ages 5 months to 16 years of age. This tool has been validated to measure change in children with CP and other neurologic disorders such as Down syndrome, traumatic brain injury, and osteogenesis imperfecta. This assessment is able to provide outcome scores that reflect how much of an activity a child can accomplish (function) rather than how well the activity is performed. The scores provide an enhanced understanding of activity outcomes; ultimately leading towards achievement of contextual participation goals specific to the individual child. Organization of test items reflects developmental gross motor milestones. Items on the GMFM-88 are grouped into five dimensions: lying and rolling (17 items), sitting (20 items), crawling and kneeling (14 items), standing (13 items), and walking, running and jumping (24 items). The items are scored on four-point ordinal scales (0 1⁄4 cannot initiate; 1 1⁄4 initiates; 21⁄4partially completes item; 31⁄4completes item independently). Any item that has been omitted or the child is unable, or unwilling to attempt is scored as 0. The child is allowed a maximum of three trials on each item. Percentage scores are calculated within each dimension and averaged to obtain a total score that ranges from 0 to 100. Higher scores indicate better capacity (Alotaibi M et al, 2014)

4.6.2 METHODS

Participants were included if they had completed the GMFM-88 during an in-person visit occurred after January 1st, 2020. The family of two patients refused to participate to this project. All subjects carried a molecularly-confirmed leukodystrophy diagnosis. A total of

21 subjects (age range 1.3 - 52.5 years, 17 of them with a molecular and clinic diagnosis of AGS) were evaluated during sequential in-person and telemedicine encounters. The subject's diagnosis, age at symptom onset, and disease severity were determined by medical records review, as well as all GMFM-88 administrations available before the year 2020.

All in-person GMFM-88 have been administered by physical therapists who had extensive experience using the GMFM-88 and followed the instructions of the GMFM-88 manual. The remote assessments were performed by two trained physical therapists that administered and scored the children live, having the patients' parents assisting their children. One adult subject was evaluated without the assistance of any caregiver. All assessments were performed without the use of orthotics.

The families participating in this study were provided with information prior to the research encounter regarding the necessary environment and technology needed for home evaluations. Sessions were conducted over a secure video conferencing platform (Cisco WebEx). Existing home equipment, including typical home furniture, was selected to be as close as possible to the standard equipment and for subsequent evaluations and logged for consistency, in accordance with the GMFM-88 manual. All 88 items were administered during the virtual encounter, and time needed for the assessment was similar to in-person assessment. Session were recorded with a fixed camera angle.

Composite scores and were calculated and compared between assessments. We assessed inter-rater reliability by administering the GMFM remotely to 10 patients at an average interval of 2 weeks by 2 different therapists. Six of the 10 subjects' telemedicine visits were recorded for reliability assessments. Intra-rater reliability was assessed using these video-recorded assessments by measuring the degree of agreement between two separate scorings of the same video administration.

Stata 16.0 was used for analysis with two-sided tests and a p-value < 0.05 as the criterion for statistical significance. Descriptive and correlative statistics were used to compare the assessments which were done in person to the remote assessments. Descriptive statistics included tabulation of categorical variables and computation of means, standard deviations (SD), medians, ranges (minimum to maximum values), interquartile-ranges (IQR, median to 75th percentile) of continuous variables.

To evaluate agreement, we used the concordance correlation coefficient (ρ_c) that evaluates the degree to which pairs of observations fall on the 45° line through the origin (the line of perfect concordance). The concordance correlation ρ_c is the product of the Pearson correlation and a bias correction factor. McBride (2005) suggested that ρ_c <0.90 indicates poor agreement, while 0.90 - 0.95; 0.95 - 0.99; and > 0.99 suggest moderate, substantial and almost perfect levels of agreement, respectively. We used the bootcor command in Stata 16.0 to obtain ρ_c, with 95% confidence intervals (CI) obtained via bootstrap sampling. Bootstrap resampling does not require an assumption of bivariate normality for the two measures being compared and can also be applied if the samples are not independent. We used the concord command in Stata to obtain a scatter plot of values with the line of perfect concordance.

We also used Bland-Altman analysis to evaluate agreement. We used the agree command in Stata to plot the difference versus average of remote and in-person GMFM-88, with a horizontal line at the average difference and the upper and lower limits of agreement. We also obtained 95% CI for the average difference and upper and lower limits of agreement using the agree command. As suggested in Zagreb acceptable values for the limits of agreement (within which 95% of differences between measurements would be expected to fall) should be established a priori. Bland-Altman analysis assumes normality of the differences. We evaluated the normality assumption of individual variables and paired differences using diagnostic plots (histograms and plots of quantiles of the variable under consideration versus quantiles of the normal distribution [Q-Q plot]) and Shapiro-Wilk and tests for skewness and kurtosis with the agree command.

There were 21 subjects with both in-person and remote assessments of GMFM-88. Of these 21 subjects, 4 (4/21, 19%) had 1 prior in-person assessments; 5 (5/21, 23.8%) had 2 prior in-person assessments; 1 (1/21, 4.8%) had 3 prior in-person assessments; and 11 (11/21, 52.4%) had 4 prior in-person assessments. Remote and in-person assessments were compared by individual level profile plots for each patient. Individual level regression lines of score versus age were fitted to the previous scores on each patient and were used to obtain a predicted value at the age of remote assessment. The agreement between the remote and predicted values from the individual level regressions was then evaluated using

the same approaches that were used to evaluate agreement between the remote and prior value. This analysis was considered to be exploratory and descriptive only.

To evaluate inter-rater reliability, we estimated the intra-class correlation coefficient (ICC) for a two-way mixed effects model. We used the bootcor command to use bootstrap resampling to obtain the 95% CI for the ICC because the distribution of the scores deviated from normality. To evaluate intra-rater reliability, we also estimated the ICC (with 95% CI) using the bootcor command in Stata 16.0.

4.7 JANUS KINASE INHIBITION IN THE AICARDI–GOUTIÈRES SYNDROME

Thirty-five subjects with AGS were treated. Three additional patients without genetically confirmed AGS were enrolled, but excluded from analyses. Four enrolled patients did not complete the initiation period because of infectious risk and disease- related death.

Individuals were dosed with Baricitinib. Dose was increased to control symptoms, according to weight and renal function. In individuals with normal renal function, and a weight of less than 20 kg, a dosing goal of approximately 0.3 mg/kg/day was used, with highest final doses of 0.5 mg/kg/day in some individuals. In individuals with weight between 20-40 kg, a goal of 0.15-0.3 mg/kg/day was used up to 8 mg per day. In individual with a weight of >40 kg, a maximum of 12 mg per day was used. In individuals with altered eGFR, lower dosing was used. In some cases, dosing adjustments were made for hematologic parameters. Participants continued to receive standard of care, including medication and surgical interventions. Children were advised to avoid all live vaccinations while on baricitinib.

Safety was evaluated by the incidence of adverse events, changes in clinical laboratory findings, and physical examination. Based on the FDA-approved prescribing information, participants were monitored for serious infections, thrombosis, and key laboratory measurements of leukocytes, neutrophils, hemoglobin, liver enzymes, creatinine kinase, alkaline phosphatase, creatinine, estimate glomerular filtration rate (eGFR), and lipids. Statistical analysis was performed on the full set of data.

Patient-proxies (parents) completed a daily symptom diary during the run-in and study period. This diary allowed parents to evaluate neurologic disability, crying, sleep, irritability, seizures, fevers, and skin inflammation of the body and extremities. Clinical scores were generated for the intervals before study drug initiation, and then for each visit interval by averaging scores between visits.

All available medical records pre-treatment and on study were retrospectively evaluated to determine neurologic function using quantitation of ten key developmental milestones included in the AGS neurologic scale: mobility (1: head control, 2: sitting, 3: rolling/crawling, 4: supported ambulation, 5: independent ambulation), fine motor control (6: pincer grasp or self-feeding) and social communication (7: smiling, 8: babbling, 9: single words, 10: sentences). AGS developmental information were available from disease onset or a minimum of 12 months prior to treatment initiation and on study.

Baseline neurologic skills were obtained from all 35 subjects. Baseline was collected medical record review from disease onset to study enrollment. Less than 12 months of data was collected from patients with new onset disease. We collected pre-treatment data of a median of 16.5 months (25th to 75th percentile of 7.4 to 41.5) and mean of 36.9 months (standard deviation 53.2; 95% confidence interval 18.7 to 55.2).

Levels of mRNA for 6 IFN-inducible genes: IFI27, IFI44L, IFIT1, ISG15, RSAD2, and SIGLEC1, and 4 housekeeping genes: ALAS1, HPRT1, TBP, and TUBB, were assayed as previously described.5,6 In brief, patient blood samples were collected in PAXgene blood RNA tubes (PreAnalytiX), and RNA was purified using PAXgene blood RNA kits (Qiagen). Copy number of mRNA transcripts was quantified using Nanostring nCounter™ Digital Analyzer using 200ng of sample RNA. The raw copy number of mRNA transcripts of each type I IFN-inducible gene was standardized (stdGene) to the housekeeping genes for each individual. The 6-gene IFN signature was calculated using the sum of the median Z scores of these genes. The IFN signature was positive (IFN high) if ≥1.96 (>98centile) by one tail analysis. The baseline measurement was calculated as the average of all pre-treatment ISG values for each subject (minimum of 3 per subject). On study samples were collected with safety laboratory testing. The average number of ISG scores per patient was 8.8 (minimum = 6, maximum = 12).

Analyses were performed with Stata 16.0. Prism8 was used to construct graphs. Preliminary analyses were descriptive and included frequency tables of categorical variables and summaries (mean, median, 25th to 75th percentile, range) of outcomes, and changes from baseline in each outcome. Graphical displays of the data included histograms and quantile-quantile plots to assess normality; Lowess smoother plots (locally weighted regression of outcome versus day on treatment) to evaluate linearity of change over time; box plots to assess the distribution of outcomes and of change in outcomes versus visit; and individual level profile plots to examine outcomes within individuals over time. McNemar's test for paired dichotomous measurements was used in some cases to assess pre and post treatment change with 95% confidence intervals (CI). In addition, heat maps were used to visually represent gradation in change.

For each outcome, we calculated the intra-subject changes from baseline: immediate (evaluated at the first visit post baseline), at one year, and overall (final measurement). We also obtained the median (25th, 75th percentile) and mean with 95% confidence-intervals (CI).

For each outcome, we also fit longitudinal models. These longitudinal models were fitted using quasi-least squares (QLS) regression, a computational approach for estimation of intra-subject correlations in the framework of generalized estimating equations (GEE). We modelled the intra- subject correlation of measurements using AR(1), Markov, and exchangeable structures, with robust sandwich estimators of covariance. To evaluate temporal changes in outcomes, we included time as a continuous variable as well as an indicator variable for each visit, with baseline as the reference category. Goodness of fit criteria for GEE and QLS8,9 were used to choose between models. The adjusted QLS model included age at symptom onset, age at drug initiation, microcephaly, male gender, and indicator variables for the more severe TREX1 and RNASEH2B genotypes. We then used the lincom procedure in Stata 16 to estimate the average change on therapy based on the estimated regression coefficient.

We fitted models that included all the pre-treatment development skill counts for each subject. When we included the pre-treatment data, we fitted models with indicator variables for each visit (with pre-treatment as the reference category); in addition, we fitted models that allowed us to decompose the longitudinal effects of treatment versus the cross-

sectional effects due to participants starting treatment at different ages. The results for the QLS analysis for AGS are based on the final model for the data set that includes all pre-treatment values for AGS.

CHAPTER 5: RESULTS

5.1 DEVELOPMENTAL OUTCOMES OF AICARDI GOUTIÈRES SYNDROME

One hundred subjects with molecular and clinical diagnosis of AGS were recruited in the 4 centers mentioned in the Methods section.

The age of presentation proved extremely variable when analyzed within the specific genotypes, with the exception of TREX1, which has a consistently early onset compared to the other genotypes (Figure 9).

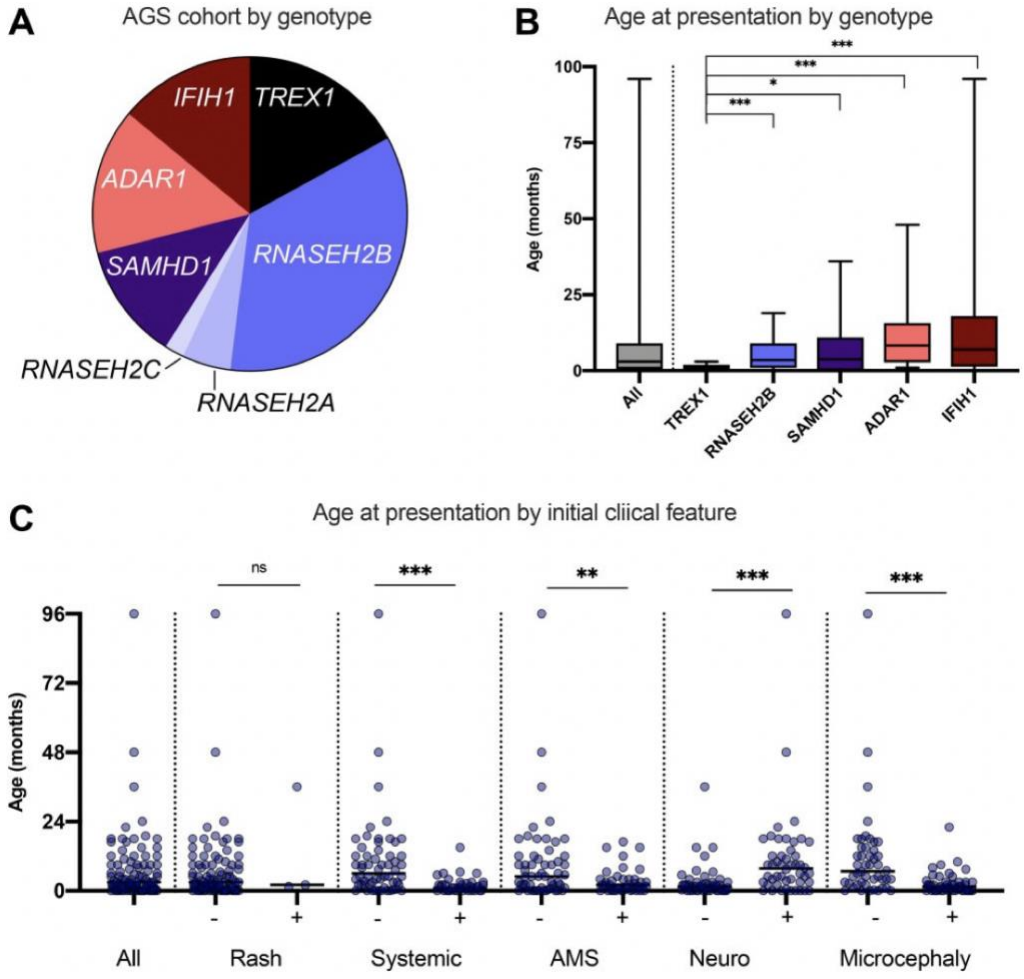


Fig 9. Genetics and features at presentation of the US-Italian Aicardi Goutieres syndrome cohort. (A) Genotypic distribution of individuals with Aicardi Goutieres syndrome across the US and Italian cohorts. (B) The age at presentation in months is shown as sorted by genotype. The error bars represent min-max. Individuals with symptoms present at birth were noted as presenting at 0 months. (C) Features at presentation were classified into nonexclusive categories: neurologic symptoms [Neuro] (eg, seizures, sudden change in tone, or developmental regression), altered

mental status [AMS] (irritability or lethargy), systemic inflammation [Systemic] (eg, feeding intolerance and fevers), rash [Rash], and Microcephaly. Clinical presentation is shown by age. P values were calculated using Mann-Whitney 2-tailed t tests and designated as *s.05, **s.01, and ***s.005.

The most frequent symptoms at onset were altered mental status (AMS), with inclusion of irritability or lethargy, intolerance to nutrition, alterations in the neurological picture (Nwith loss of motor skills, alterations in muscle tone, epileptic seizures). In association with this, many individuals presented with typical manifestations of systemic self-inflammation (rash, fever).

Kaplan-Meier curves were used to determine the percentage of patients who achieved specific skills at certain ages.

Correlation between microcephaly and non-acquisition of psychomotor milestones was found for all psychomotor milestones evaluated ($p < 0.005$, Figure 10).

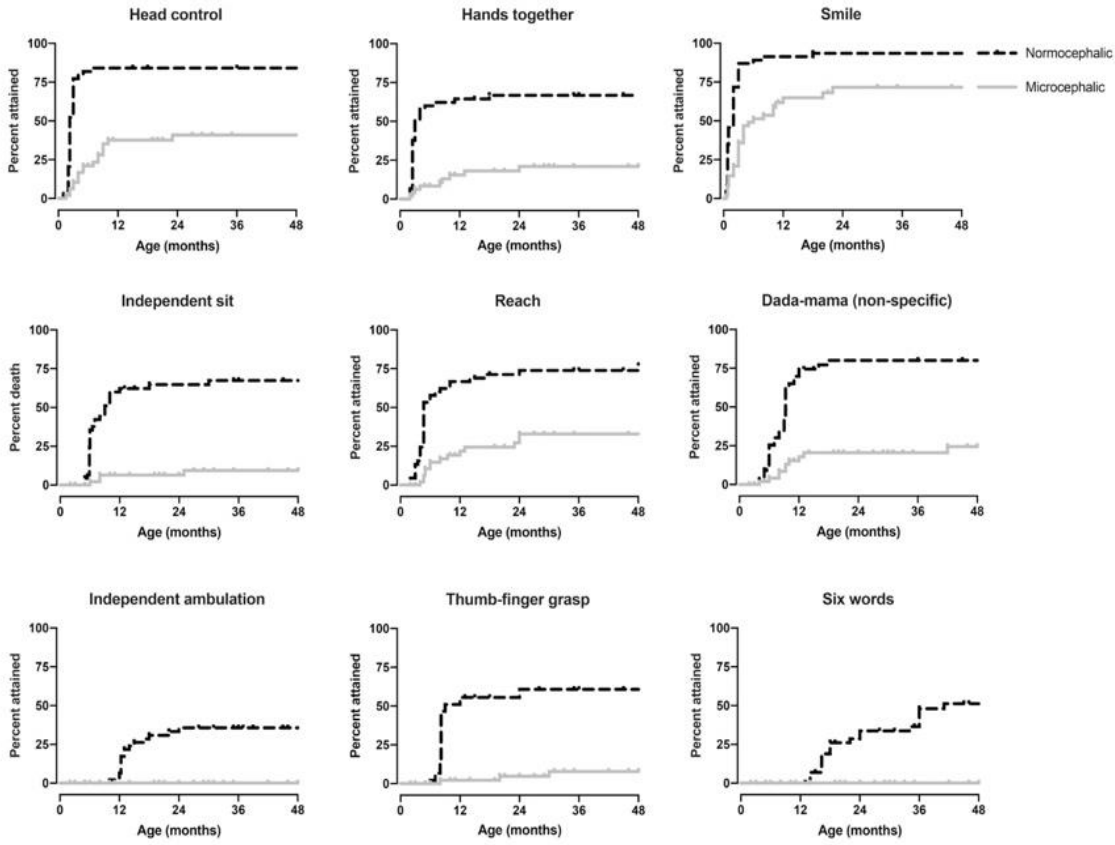


Fig 10. Kaplan Meier curves: normocephaly vs microcephaly

Similarly, an early age of onset or altered mental status as a symptom of onset were associated with a worse disease outcome in terms of the acquisition of motor and cognitive skills ($p < 0.005$).

Due to the limitations related to the genotypic incidence within the cohort, genotypic sub-analyzes were possible in 5 out of 7 genotypes (IFIH1, TREX1, RNASEH2B, SAMHD1 and ADAR). Subjects with mutations in the TREX1 genotype showed greater phenotypic severity while subjects with alterations in the SAMHD1, ADAR and IFIH1 genes showed a greater ability to acquire psychomotor developmental milestones

Evolutionary trajectories were then analyzed for each genotype, by creating tables for the acquisition of psychomotor stages (including the age of acquisition of the same, Figure 11).

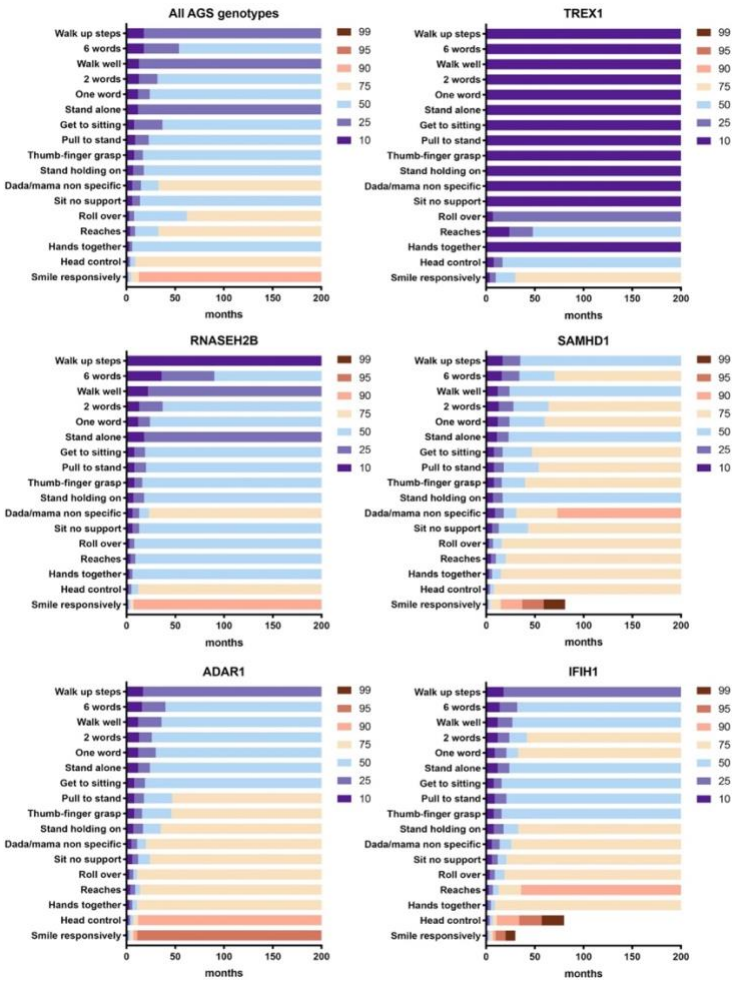


Fig 11: Acquisition of psychomotor milestones in the population affected by AGS, percentiles (including genotype-specific sub-analysis)

5.2 LATE-ONSET AICARDI GOUTIÈRES SYNDROME: A CHARACTERIZATION OF PRESENTING CLINICAL FEATURES

34 subjects belonging to the international cohort met the study inclusion criteria (molecular diagnosis of AGS, disease onset> 12 months of life). All subjects had mutations in RNASEH2B, SAMHD1, ADAR1, or IFIH.

The majority of individuals presented a history of psychomotor retardation or manifestation of systemic symptoms (fever without specific cause, skin lesions like chilblain - chilblain), followed by a prodromal phase characterized by an increase in symptoms typically found in AGS, such as asthenia and irritability. The median age of the acute phase of the disease was 1.33 years, with an extremely wide range (1.00-17.68 years). The majority of subjects presented gross motor skill abnormalities (97%), typically associated with spasticity (78.8%). See figure 12.

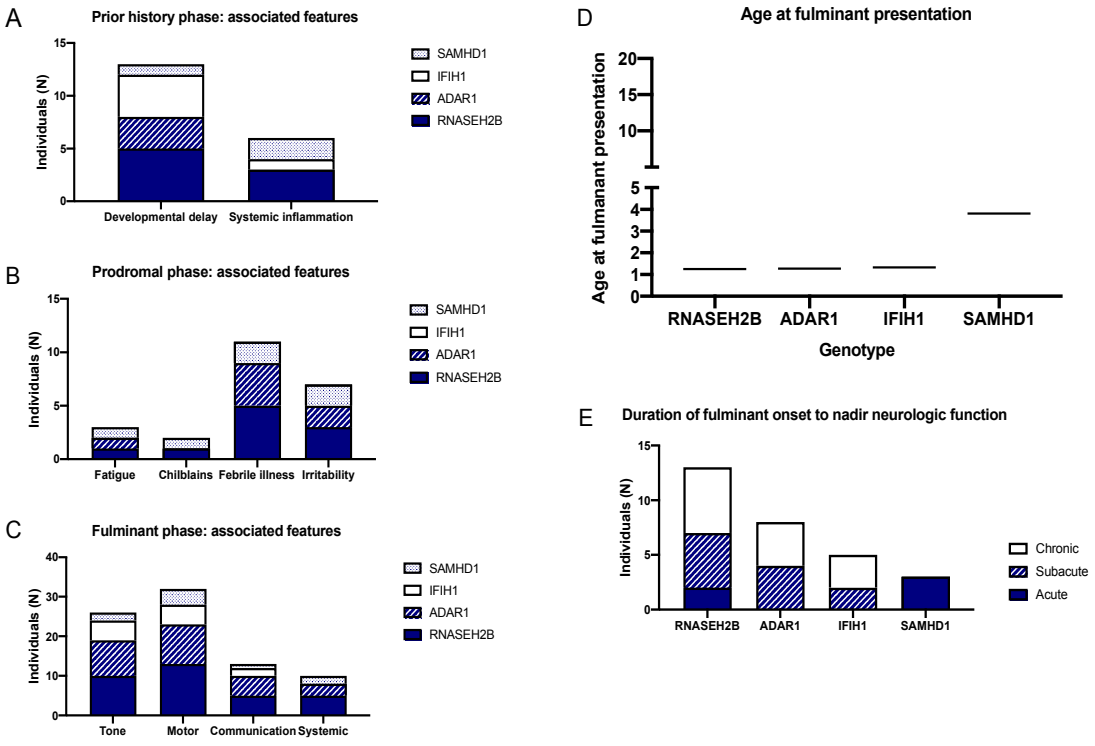


Fig. 12. Disease stages in individuals with late-onset AGS

The most frequent neuroradiological manifestation was leukoencephalopathy (40.0%), while calcifications were less frequent (12.9%).

The most frequent differential diagnosis made at symptomatic onset was that of spastic diplegia or infantile cerebral palsy, acute demyelinating encephalomyelitis (ADEM) or post-viral encephalitis. Other differential diagnoses were made with undefined leukodystrophies or other genetic pathologies.

5.3 HEPATIC INVOLVEMENT IN AICARDI GOUTIÈRES SYNDROME

Following the finding of a high prevalence of liver enzyme level abnormalities (alanine aminostransferase - ALT, aspartate aminostransferase - AST, gamma-glutamyl transferase - GGT) in subjects recruited in clinical trials, a retrospective study was conducted to assess the frequency of these anomalies within the population affected by AGS (in the literature hepatitis is reported as an event associated only with the neonatal form of AGS).

An initial analysis, conducted on medical reports of 48 individuals prior to recruitment in the clinical trial with Baricitinib, revealed the presence of liver enzyme abnormalities in 36 individuals (75%). These anomalies were associated with intercurrent elements (infections, febrile states, therapies with hepatic metabolism) in less than 10% of the episodes. All individuals were vaccinated for hepatitis B (HBV) and were not affected by hepatitis C (HCV).

Following this result, the analysis of these parameters was extended within the entire population with available blood values (102 individuals). 74.5% of the cohort (76/102) had abnormal enzyme levels (74.5%). 29 of these individuals presented alterations that could be compatible with a clinical diagnosis of hepatitis (Common Terminology Criteria for Adverse Events (CTCAE) Version 5.0., 2017, figure 13A-C). There was no correlation between the enzymatic elevation and the age of the subjects at the time of evaluation (Figure 13D).

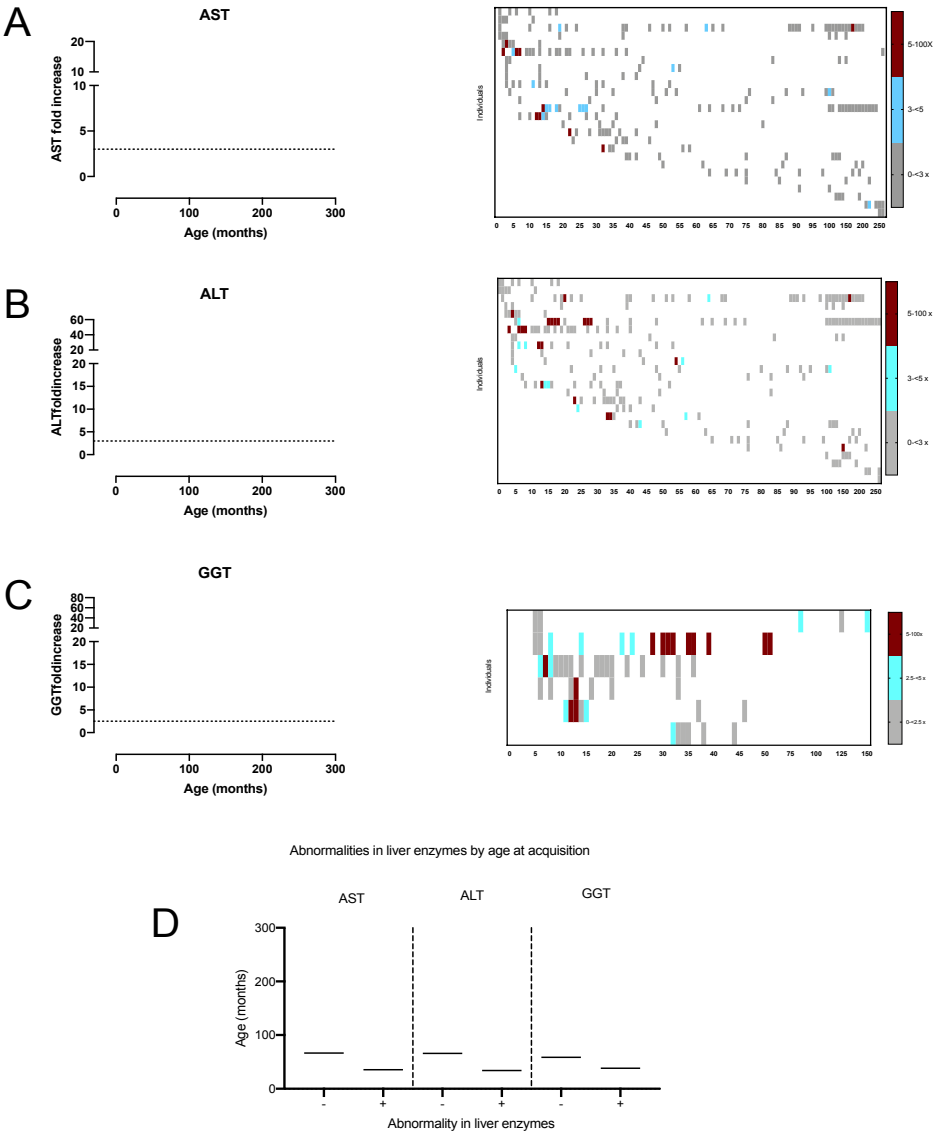


Fig 13. A-C Enzyme Abnormalities in the Cohort. D Absence of correlation between enzymatic elevation and age of the subject at the time of the assessment (QLS logistic regression, AST p = 0.1603, ALT p = 0.3375, GGT = 0.2808)

Enzyme abnormalities compatible with hepatitis are found to be correlated with clinical elements such as microcephaly and a minor age of onset of the disease. The SAMHD1 genotype was protective of the possible occurrence of hepatitis (Figure 14).

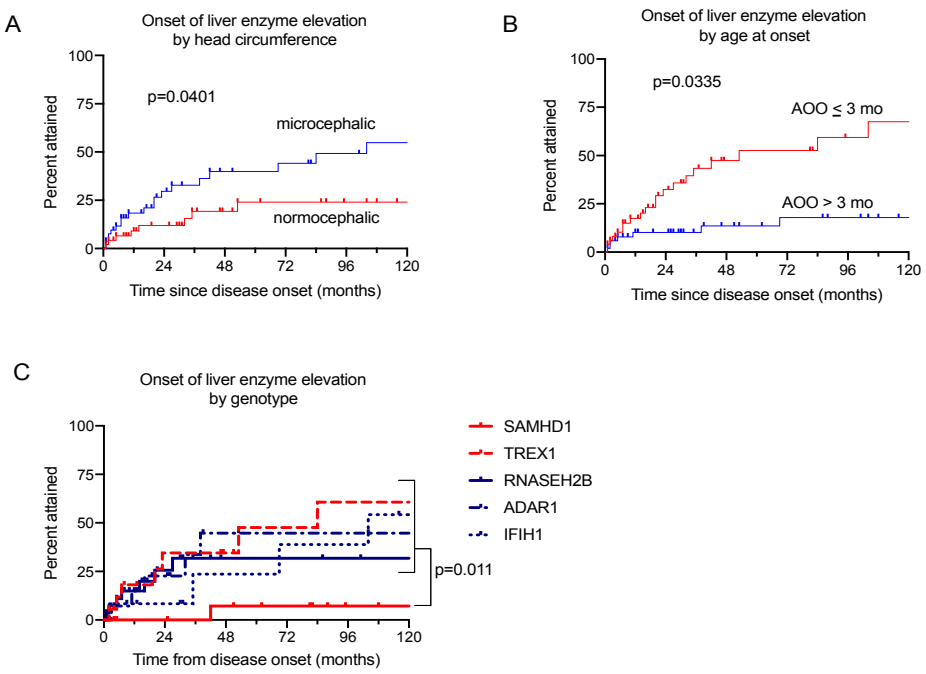


Fig 14. Enzyme abnormalities compatible with hepatitis were associated with the presence of microcephaly (A; log-rank test, p = 0.0401), age of disease onset less than 3 years (B; log-rank test, p = 0.0335), non-SAMHD1 genotypes (C; log-rank test, p = 0.011)

No correlation was found between enzymatic levels and Interferon Stimulated Genes Score (possible biomarker of disease, analysis conducted on 52 individuals) or with autoimmunity panels (analysis conducted on 33 individuals). See figure 15.

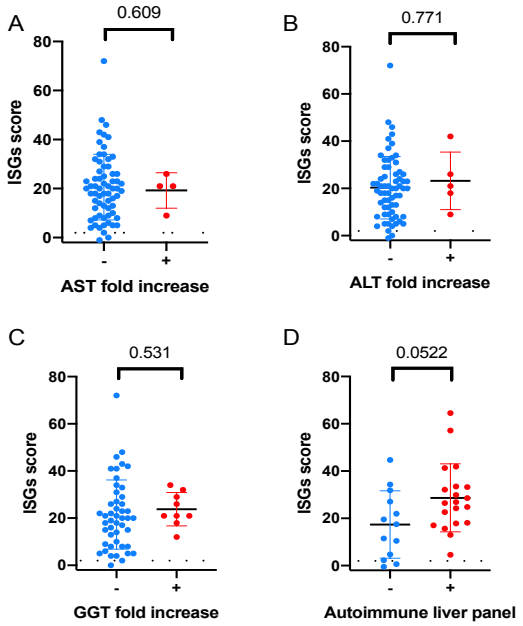


Fig 15. Correlation between ISGs and hepatic autoimmune panels and liver enzymatic levels

5.4 DEVELOPMENT OF A NEUROLOGIC SEVERITY SCALE FOR AICARDI GOUTIÈRES SYNDROME

In light of what emerged from the analysis of the natural history of the disease, the subsequent focus was on the search for measures for the assessment of psychomotor development, cognitive skills and gross motor skills and fine motor skills in this population. At the Children's Hospital of Philadelphia, a compassionate study has been underway since 2017 using a JAK-inhibitor drug, Baricitinib in an AGS population, used for the purpose of evaluating its safety in use. A clinical trial has been underway since 2019, aimed at evaluating the therapeutic efficacy of the drug. My role, within the compassionate study and clinical trial, is to evaluate research and the applicability of the disease outcome assessment tools currently available. Previous neuropsychological assessments conducted by me (Vineland Adaptive Behavior Scale, Mullen Scales of Early Learning, Wechsler Scales based on the reference age), in association with physiotherapy assessments (Gross Motor Function Measure-88) and occupational therapies (Peabody Developmental Motor Scale) showed limits in the capacity to describe the population, due to the important floor effect of the measures used.

In consideration of this, my work has focused on the creation of a specific clinical scale for AGS syndrome, in collaboration with an American pediatric neurologist. The scale takes into consideration aspects of gross motor skills, fine motor skills and cognitive abilities reported in the previous research work aimed at the study of natural history as acquired more frequently in the population. The minimum score obtainable on the scale is 0, the maximum 11. A copy of the scale is shown below (Table 5).

Item	Points	
	0 Absent	1 Present
Normocephaly (< 2 SD from mean)		
Social smile		
Vocalizations (cooing or babbling)		
Single, meaningful words		
Minimum of three-word phrases		
Head control (> 60 seconds)		
Pincer grasp or self-feeding		
Independent sitting (> 2 minutes)		
Rolling or crawling to goal		
Ambulation with assistance (devices or two-hand assist)		
Independent ambulation (no assistive devices)		
Total Score (range 0-11)		

Table 5: AGS Neurologic Scale

The scale was then retrospectively tested in 35 patients (140 de-identified clinical assessments) by the writer and an American pediatric neurologist, with high inter and intra-rater reliability. The scale showed high correlation with a reverse Composite score (opposite of the score obtained on the functional scales GMFCS, MACS, CFCS, figure 16) and with the degree of clinical disease severity (defined as mild - moderate - severe by the writer and by an American pediatric neurologist, figure 17), in light of the absence of specific measures of severity assessment in AGS.

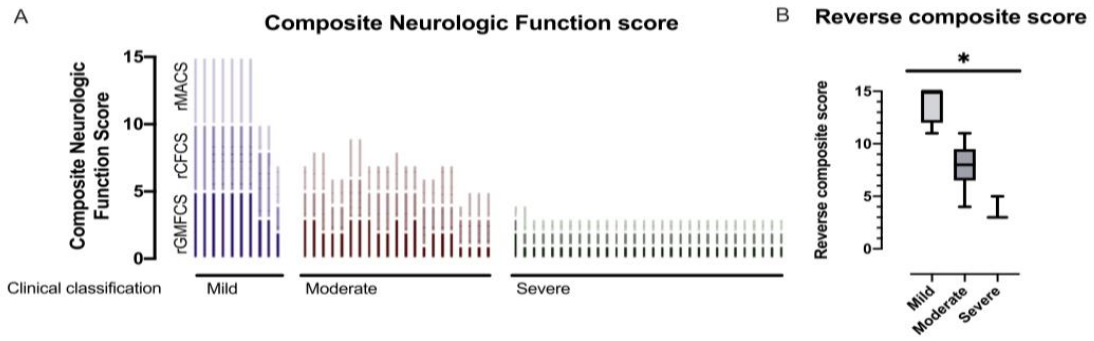


Fig 16: RComposite score and correlation with clinical severity (Kruskal-Wallis test p value<0.0001)

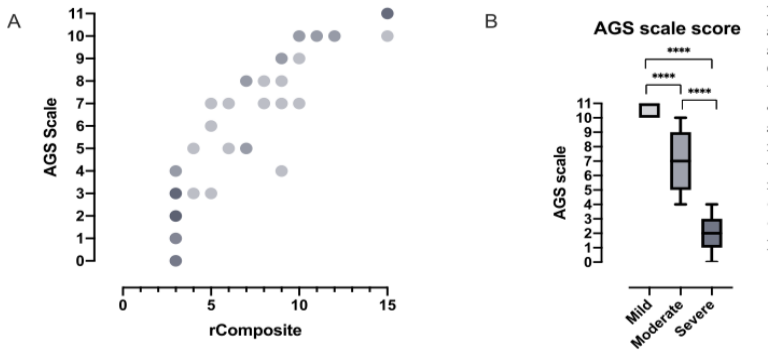


Fig 17: AGS scale and correlation with clinical severity (Kruskal-Wallis test p value <.0001)

The clinical scale was subsequently applied retrospectively to 533 clinical evaluations of 92 patients. Genotype specific correlation and GMFM-88 correlation were found (Figure 18 and 19).

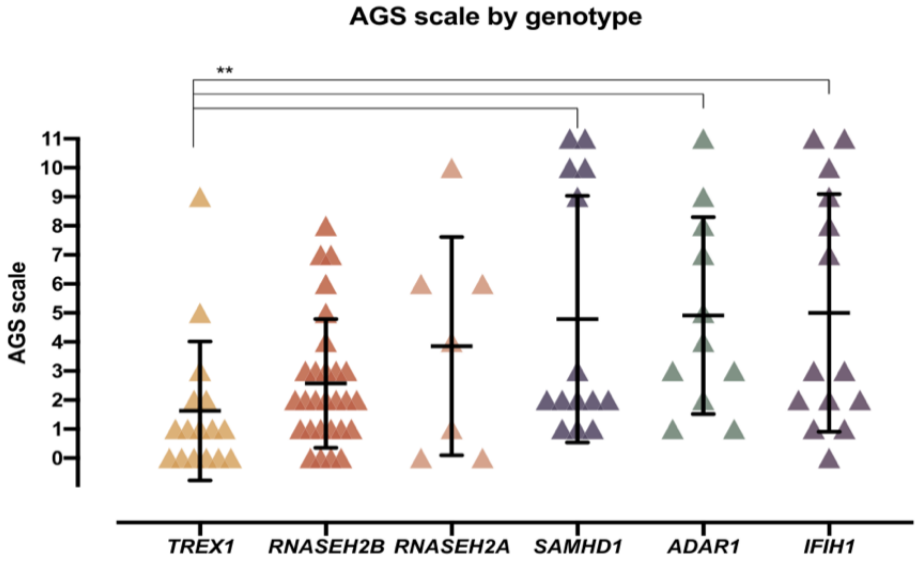


Fig 18: AGS scale and correlation with genotype (Kruskal-Wallis test p value <.0001). The Scala nadir was compared with the different genotypes (Kruskal-Wallis test: TREX1 vs SAMHD1 p value 0.0053; TREX1 vs ADAR1 p value 0.0022; TREX1 vs IFIH1 p value 0.0032)

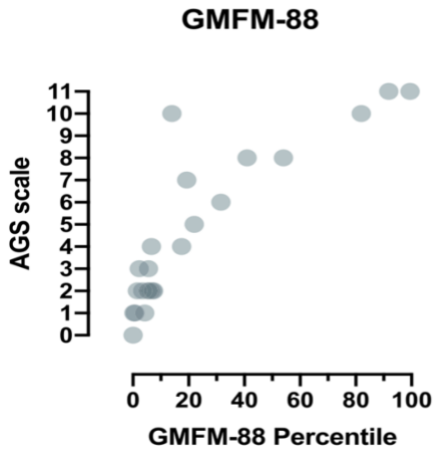


Fig 19: AGS scale and GMFM-88 correlation (available for 24 individuals). The percentage of the GMFM-88 was compared with the retrospective scores of the AGS scale (Spearman r 0.8931, 95% confidence interval 0.76-0.95, 2-tailed p value <.0001).

However, no correlation with the ISGs was found.

The scale has proved useful in its ability to capture longitudinal clinical variations of disease. In our cohort (92 subjects), the majority of individuals showed a stable or progressively worsening score (66 subjects), while a limited subgroup of individuals showed clinical improvement. The results obtained are graphically represented below (figure 20A-B), with subdivision of patients by genotype (figure 20C)

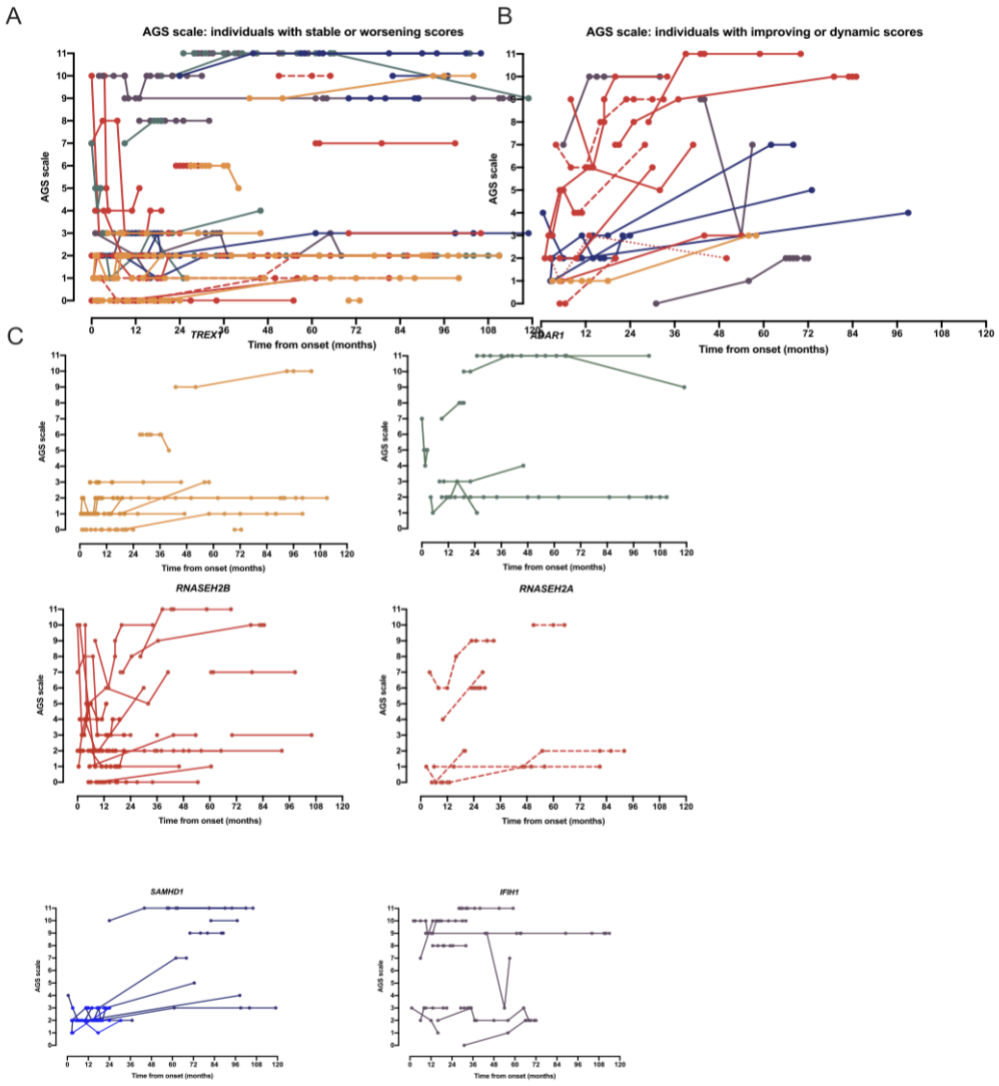


Fig 20: AGS scale scores administered retrospectively in a cohort of 92 individuals. A: individuals with stable or declining scores; B: individuals with improved score; c- subdivision by genotype

Changes in the AGS scale score were more common within the first 6 months of disease onset (Figure 21).

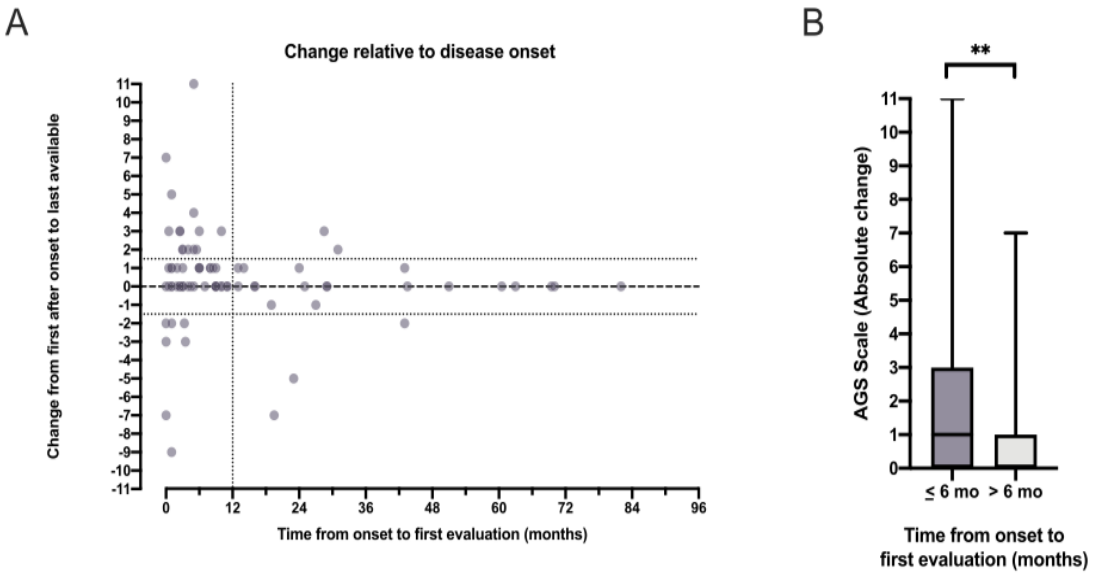


Fig 21. The dynamism of the AGS scale is greater within the first 6 months of disease onset (Mann-Whitney, two-tailed p = .0057)

5.5 EXPERIENCES AND HOPE IN CAREGIVERS OF CHILDREN WITH AICARDI GOUTIÈRES SYNDROME

45 caregivers were interviewed (27 mothers, 18 fathers) of 33 patients. The families represented in this cohort demonstrated a similar genotypic distribution previously observed in the broader AGS population. The median age of the child at the time of interview was 6.08 years (range 1.5-23.08 years). The children with caregivers included in this study represented the spectrum of disease severity seen in AGS (n=12 mild; n=4 moderate; n=17 severe).

Six major themes emerged. In general, we did not find discrepancies in themes by genotype, severity, or time from disease onset. The results are presented in aggregate.

Theme 1: The early symptoms of AGS are stressful to families

Aicardi Goutières Syndrome is a heritable type I interferonopathy characterized by increasing burden of neurologic and systemic symptomatology. Universally, the families described stress associated with the early features of AGS. As described by one participant, AGS can result in the acute onset of severe symptoms: "… he started screaming one night nonstop out of nowhere and before that he never cried [...]. And from that moment on for about three and a half months, he would not eat. He would not sleep. And he screamed 24/7"). Many families noted distress surrounding the early symptoms: "He would just cry and cry, and we couldn't figure out what it was". Other common sources of stress were feeding difficulties, recurrent fevers, skin inflammation, and abnormal sleep patterns. These early parental concerns are consistent with published medical reports.

Theme 2: Families view AGS as globally affecting their child's function

AGS is a systemic disorder associated with severe disability. One caregiver reported that AGS has "impacted every part of her life". Stress regarding motor limitations were reported by caregivers, which was often described as the feature of AGS that had the largest impact. The disassociation between motor and cognitive function was noted as a unique stressor: "[he] wants to do something, but his body just doesn't follow". Families noted the negative impact of AGS on peer interactions and self-esteem. One family described the impact of AGS on their child's self-perception: "a lot of times she sees her restrictions almost more than I do. And so, she won't be eager to participate unless she knows that she will be welcomed in an activity."

Theme 3: AGS is viewed as impacting the entire family

Caregivers described AGS as having major effects on the entire family. Many caregivers noted an emotional burden from AGS, which included feelings of inadequacy and challenges in sharing their children's condition with others. One caregiver reported feels of guilt for "not really doing my part" because of fatigue. Families described the stress on marriages, with both positive and negative effects. Economic burden of AGS on families was reported, with specific concerns related to the costs of treatments and insurance

coverage. Several caregivers reported having to change their employment after the diagnosis of AGS: “I had to stop working. I have to stop working to take care of her”.

Theme 4: Families rely on many resources for support

Caregivers reported a spectrum of overlapping approaches to coping with AGS: faith, reliance on the medical system, self-care, and formal psychotherapy approaches. Several caregivers noted the importance of having a support system that understands AGS: “the problem is people are uncomfortable around him, and they want to help, but they don’t know how to help. So, even though you feel that there is support, it’s still different.”

Theme 5: Hope

Most caregivers described a desire for an improvement in neurologic function. Most families voiced the desire for their children to be able to better communicate, such as “to be able to express when he is hurting or uncomfortable or not feeling well or happy". Caregivers expressed hope for improved motor skills: “it would be different for us if he was mobile”. Even small gains were important to families: “One big thing, miracle […] for him just to move his arm is so huge for me already, for him to just prop himself a little would be huge”. Common goals were improved comfort, happiness, and independence for their children: “I think mainly just him being settled and happy is the first thing to improving his quality of life”.

Theme 6: Diagnostic delay

The diagnostic process was a common theme discussed in most interviews (n=34/45 caregiver interviews from n=28/33 subjects). The interviews from the sub-cohort that discussed the diagnostic process were included in further analyses. The average time elapsed from disease onset to genetic diagnosis of AGS in this cohort was 5.24 years (mean 2.54, SD 5.83; range 0.16-20.79 years) (Figure 21).

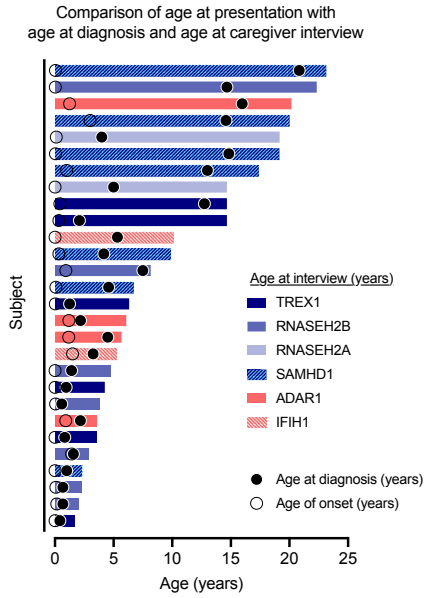


Fig 21. Comparison of age at presentation with age at diagnosis and age at caregiver participation. Genotype is designated within the bar graph.

Most caregivers reported that the period of time from symptom onset to final diagnosis to be one of stress. Parents reported that many times their child was mislabeled with a more common diagnosis, such as cerebral palsy (CP). This was a common source of stress for families: "His local neurologist that we saw at that time said he was too young [for a leukodystrophy], but he told me: possible CP".

Caregivers expressed an overall sense of frustration elicited by the time lost. Often the diagnosis process required families to search for answers: "we have traveled everywhere just to check what's really wrong with our son". There was the perception that time lost in the diagnosis was detrimental because the child was managed without a definitive diagnosis.

Many caregivers discussed their perceptions regarding the barriers to the diagnosis of AGS. A key theme of healthcare provider knowledge of AGS emerged. Another perceived barrier was the lack of care coordination. The lack of access to information about AGS for families was identified as a barrier to diagnosis as well: "The knowledge just isn't there. It isn't easy to find out that that's what it is"). Parents often reported challenges feeling their concerns were heard, both in their private life and in the medical field. This was a major source of stress for the families.

5.6 RELIABILITY OF THE TELEMEDICINE APPLICATION OF THE GROSS MOTOR FUNCTION MEASURE-88 IN PATIENTS WITH LEUKODYSTROPHY

A total of 21 subjects with leukodystrophy were enrolled in this study. Nine of these subjects were male (42.9%). The mean age at the last in-person evaluation prior to the remote evaluation was of 9.6 years (range 1.3-52.5, SD 11.0). The mean age at the telemedicine assessment was 10.1 years (range 1.8-52.8, SD 11.0). The mean difference between age at telemedicine assessment and age at baseline assessment was 0.5 years (range 0.4-0.7, SD 0.1). Of the 21 subjects, 17 individuals had a confirmed diagnosis of AGS (81%), 2 had a diagnosis of POLR3-related leukodystrophy (9.5%), 1 had a diagnosis of Alexander Disease (AxD) (4.7%), and 1 had a diagnosis of TUBB4A-related leukodystrophy (4.7%).

We first evaluated the agreement between GMFM-88 remote administration total score and the closest in-person GMFM-88 total score. Diagnostic plots (Q-Q plot and histogram) and tests for normality suggested that the distribution of GMFM-88 remote and in-person scores deviated from normality; however, the normality assumption was reasonable for the paired differences (GMFM-88 minus remote scores). The mean difference was 0.2 (95% CI = -0.9 to 1.4; lower limit of agreement = -4.8 [95% CI = -6.8 to -2.8]; upper limit of agreement = 5.3 [95% CI = 3.3 to 7.3]; Lin's concordance correlation coefficient = 0.997 [95% CI = 0.993 to 0.998]. (Figure 22A,B).

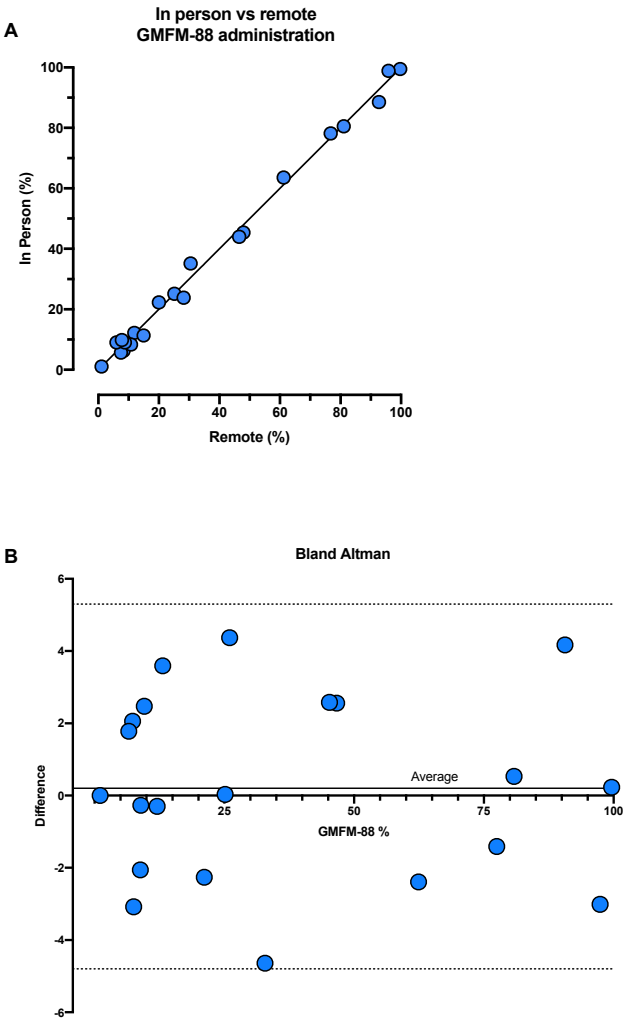


Fig. 22: A. Scatter plot of GMFM-88 in-person and remote administration (total score) with the line of perfect concordance. Lin's concordance correlation ρ_c (with 95% CI) is also provided (ρ_c = 0.997 [95% CI = 0.993 to 0.998]). B. The Bland-Altman plot displays the variance between remote and in-person assessments. The lines represent $\pm$ 5% variation (upper and lower limits of agreement for the paired differences).

We next compared the GMFM-88 remote administration raw score with the predicted values from the individual level regressions using all previous in-person assessments (n=17 individuals had at least two prior assessments, n=12 individuals had 3 or more assessments). Lin's concordance coefficient for GMFM-88 Remote versus predicted scores was 0.994 (95% CI = 0.975 to 0.998). The evaluation of the Bland Altman plot visualized agreement. The average difference (remote - predicted GMFM-88) was 0.3 (95% CI = -

1.5 to 2.0). The lower limit of agreement was -6.5 (95% CI = -9.5 to -3.4) and the upper limit of agreement was 7.0 (95% CI = 3.9 to 10.0). Individual level profile plots of the prior scores that excluded and included the remote assessment on each patient are shown in Figure 23.

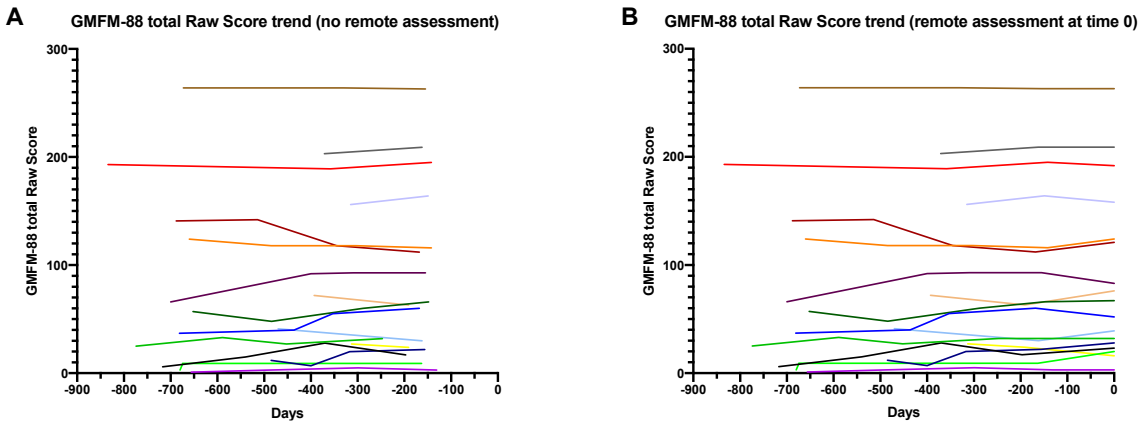


Fig 23: A. Profile Plots Excluding Remote Assessment of GMFM-88 B. Profile Plots Including Remote Assessment of GMFM-88. Time at remote assessment is 0.

Interrater reliability was assessed through remote evaluation of the same subject by two independent raters within a 3-week period (n=20 encounters for n=10 subjects). The estimated ICC (95% CI) was 0.996 (95% CI = 0.964 to 0.999). Agreement between raters was determined using Lin's concordance coefficient and Bland Altman plots (Supplemental Figure 2A-B). Concordance between raters was 0.995 (95% CI = 0.961 to 0.999). The average difference between rater scores was -1.7 (95% CI = -3.9 to 0.5). The lower limit of agreement was -7.8 (95% CI = -11.6 to -4.0) and the upper limit of agreement was 4.3 (95% CI = 0.5 to 8.1).

Intra-rater reliability was assessed by having one clinician score the same encounter twice via a blinded scoring of a video recording of the encounter (n=6 subjects; ICC = 0.999; 95% CI = 0.996 to 1.000). Agreement was determined using Lin's concordance coefficient and Bland Altman plots (Supplemental Figure 3A-B). With respect to agreement, Lin's concordance coefficient for the scores on both raters was 0.999 (95% CI = 0.985 to 1.000). The average difference between second and first scores was 0.2 (95% CI = -1.1 to 1.5).

The lower limit of agreement was -2.3 (95% CI = -4.5 to 0.0) and the upper limit of agreement was 2.7 (95% CI = 0.4 to 5.0).

5.7 JANUS KINASE INHIBITION IN THE AICARDI–GOUTIÈRES SYNDROME

The median age at the onset of symptoms was 6.0 months, and the median age at the initiation of baricitinib was 2.9 years (range, 0.2 to 21.8). The patients participated in the study for a minimum of 12.0 months (range, 11.8 to 43.8). The dose of baricitinib, which ranged from 0.1 to 0.6 mg per kilogram of body weight per day, administered in 2 to 4 dosing increments per day, was determined according to the patient's renal function, age, and symptoms.

JAK inhibitors are associated with infection, anemia, lymphopenia, and thrombosis. Two patients died during the study: one who had been receiving glucocorticoids for a decade, including a 7-year period before the study, had Aicardi–Goutières syndrome–related multisystem organ failure with an opportunistic infection, and the other had Aicardi–Goutières syndrome–related pulmonary hypertension with thrombotic microangiopathy. Clinical variables were measured with laboratory tests and graded according to Common Terminology Criteria for Adverse Events, version 5.0, in which grades range from 1 to 5, with higher grades indicating more severe adverse events. Among these variables, only thrombocytosis increased in severity during the study (mean change in grade from baseline, 0.30; 95% confidence interval [CI], 0.04 to 0.60).

The ISGs indicated a response to treatment (Fig. 24).

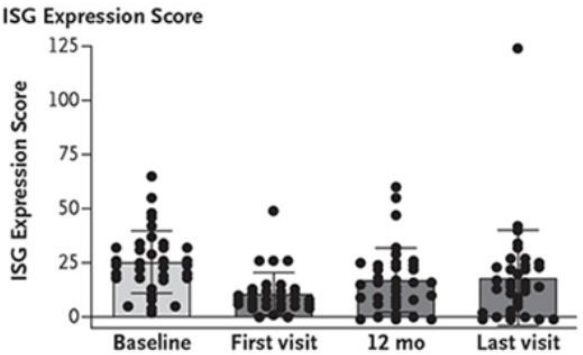


Fig. 24. ISGs response to treatment

The parents of the patients used diaries to record the children's Aicardi–Goutières syndrome–related symptoms, including neurologic disability, crying, sleep disturbances, irritability, seizures, fever, and skin inflammation of the trunk, arms, and legs (see the Supplemental Methods section and Table S5 in the Supplementary Appendix). The diary score included a total of 24 possible points, assigned every day, with daily scores averaged across the time period before each visit (1 month after the initiation of baricitinib and then every 3 months). Scores obtained during the study ranged from 0 to 10.6, with higher scores indicating more severe symptoms. The skin subscore included a total of 6 possible points, with higher scores indicating more severe symptoms. The overall diary scores improved within 1 month after the initiation of therapy (mean change in overall score, −0.7 diary points; 95% CI, −1.2 to −0.2) and were sustained (mean change in overall score, −1.4; 95% CI, −2.2 to −0.6). The percentage of patients with a lower (improved) skin subscore of only 0 or 1 increased from 66% (23 of 35 patients) to 83% (29 of 35 patients) from the baseline visit to the final visit (difference, 17 percentage points; 95% CI, 2 to 32).

We evaluated the patients' developmental histories from disease onset (median age since disease onset, 16.5 months; interquartile range, 7.4 to 41.5) to the clinical data cutoff date. We extracted information on key milestones, including head control, sitting, rolling, assisted and independent ambulation, grasp, smiling, babbling, and the use of single words and word combinations. Before treatment, 26 of the 35 patients had stable or declining neurologic function, and 9 of the 35 patients gained one or two skills after disease onset. During the study, 20 patients met new milestones, and 12 patients gained two to seven new skills. This improvement was noted by 3 months (mean change in the number of milestones, 0.5; 95% CI, 0.2 to 0.9) and persisted (mean change, 1.3; 95% CI, 0.6 to 1.9). One patient lost skills during an acute illness while participating in the study. Children in a higher daily dose category of baricitinib met more milestones (mean increase, 1.3 milestones; 95% CI, 0.6 to 1.9) than those in a lower daily dose category according to weight-based calculations (Fig. 25).

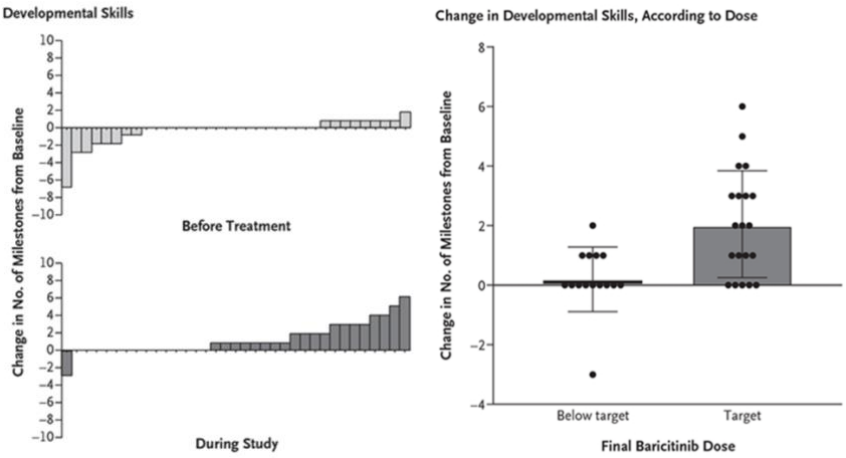


Fig 25: Application of the AGS Neurological Scale as a measure of the outcome of the experimental clinical trial using Baricitinib

The primary risks associated with baricitinib among patients with the Aicardi–Goutières syndrome were thrombocytosis, leukopenia, and infection. Patients who are receiving baricitinib should be monitored closely, especially those with underlying thrombotic risk factors or those who are receiving systemic glucocorticoids or immunosuppressive regimens. Because this study drew from an international population, we anticipate that our findings will be generalizable. The measurement of neurologic improvement is complex, but our data suggest improvement in neurologic function, even in patients with severe and long-standing disease.

CHAPTER 6: DISCUSSION

6.1 NATURAL HISTORY OF AICARDI GOUTIERES SYNDROME

Aicardi Goutieres syndrome is a genetically heterogeneous disorder caused by changes within the nucleic acid sensing machinery, resulting in sustained interferon production.

Although all children described to date with Aicardi Goutieres syndrome demonstrate some degree of neurologic impairment, this project has confirmed that the presentation and degree of disability is highly variable. This study contributes detailed developmental information to collective published works related to the neurologic features of Aicardi Goutieres syndrome (Rice G et al, 2007; Rice G et al, 2014, Rice G et al, 2017, Crow YJ et al, 2015).

In particular, the current work builds on our understanding of overall neurologic outcomes in Aicardi Goutieres syndrome through detailed developmental history analyses. As we move toward therapeutic development in Aicardi Goutieres syndrome and clinical trial design, it is increasingly important to establish a deeper understanding of the neurologic trajectory of Aicardi Goutieres syndrome.

To this end, it has been fundamental to clarify and characterize the features of disease presentation and the overall developmental skill acquisition across the Aicardi Goutieres syndrome genetic spectrum.

As anticipated, we found that children across all genotypes had variable age of onset and presenting symptoms. This underscores one difficulty with early recognition and diagnosis of Aicardi Goutieres syndrome as the presentation can be heterogeneous. Additionally, the ascertainment of presenting features is complicated by parental recall bias, typical of retrospective studies of natural history of rare disorders.

The majority of patients showed delayed development prior to an Aicardi Goutieres syndrome–specific clinical presentation, as already hypothesized in previous studies. We hypothesize that this pre-presentation period is a key window for potential future therapeutic interventions.

Although this study supports genotype as a significant variable affecting neurologic outcomes in Aicardi Goutieres syndrome, significant intra–genetic cohort variability

suggests the importance of other unidentified factors that may influence the age of presentation and overall outcome. We hypothesize that additional confounding variables may include environmental exposures (such as illnesses) and complementary genetic polymorphisms within the interferon pathway. The difficulty in accounting for these variables is a limitation of the studies.

As most individuals with Aicardi Goutieres syndrome are currently identified because of a prominent central nervous system involvement, this study may overestimate severity of neurologic dysfunction of the ultimate population.

Given the variable involvement of other organ systems in Aicardi Goutieres syndrome, it is possible that individuals with the same genotypes, but without central nervous system injury, have not been yet identified.

This underscores the need for a better appreciation of the breadth of disease associated with Aicardi Goutieres syndrome–related genes.

As an example of recruitment bias, we hypothesize that the differences in the developmental skill acquisition in the Italian versus the US cohorts may be due to differences in ascertainment by diagnostics strategies. In Italy, the primary diagnostic method is targeted mutation or gene panel analysis, and the children selected for testing are typically “classic” severe presentations with microcephaly and/or intracranial calcifications. In the United States, an increasing number of individuals are ascertained by whole exome sequencing for more nonspecific symptoms. A more agnostic approach to genetic diagnosis leads to an expansion of phenotypes of Aicardi Goutieres syndrome.

Overall, children with Aicardi Goutieres syndrome reached milestones in the same linear developmental course as neurotypical children. Interestingly, there was a small cohort of children who were able to attain new skills for years beyond the expected age of acquisition.

In our study, we identified 3 distinct cohorts: mild, intermediate, and severe impairment. The mild involvement category included children who were either walking independently or with assistive devices, or had normal speech with some floor mobility. Intermediate category patients have floor mobility through rolling, and some communication skills (includes babbling and language). Severe patients have no mobility, poor head control, and no communication beyond a social smile. These groups clustered by genotype, although

genotype alone was not sufficient to predict the course in an individual patient. Individuals with Aicardi Goutieres syndrome related to SAMHD1, IFIH1, and ADAR mutations achieve a greater number of developmental milestones compared to individual with TREX1-related Aicardi Goutieres syndrome.

TREX1-related Aicardi Goutieres syndrome was statistically distinct from the other genotypes for most milestone acquisitions, and the RNASEH2B was similar to the milder cohort except for early fine motor function (bringing hands together and reaching), rolling over, and the acquisition of 6 words.

From this first study, we have been able to get a deeper understanding of the kinetics of development of children with Aicardi Goutieres syndrome. This study also illustrates potential challenges in clinical trials in the Aicardi Goutieres syndrome population. As the developmental trajectory of Aicardi Goutieres syndrome is heterogeneous, it may be challenging to demonstrate clinical efficacy of any future interventions across a mixed population requiring careful consideration of variables including microcephaly, age of onset, and genotype. Additionally, in individuals with the severe developmental phenotype, traditional measures of motor and cognitive performance may have significant floor effects. Future clinical studies would benefit from the creation of an Aicardi Goutieres syndrome–specific scale, similar to what has been developed in other rare diseases (Ramsey D et al, 2017; Kehrer C et al, 2011). Identifiable variables that may affect long-term outcome, such as age of onset, presenting symptoms, genotype, and microcephaly, should be considered in the design of clinical trials with the selection of appropriate clinical endpoints.

This first natural history study has arisen the need to explore more the spectrum of clinical manifestations of AGS, specifically in regard of uncommon phenotypes. A subsequent study was then conducted to characterize the atypical, later onset cases within our international cohort.

We found that most atypical AGS cases demonstrated distinct periods of clinical symptoms. The prior history phase was characterized by chronic symptoms including global developmental delay or subtle symptoms of systemic inflammation; the prodromal phase was characterized by worsening systemic inflammatory symptoms. Patients

transitioned into a fulminant phase, which was characterized by the loss of neurologic function.

All individuals in our cohort demonstrated a decline in motor skills at presentation. Often the neurologic decline was accompanied by a history of subtle systemic symptoms, including sterile pyrexias, myalgias, and GI intolerance. These features were often different from the classic systemic features noted in the neonatal and infantile forms of the disease, including altered mental status, irritability, seizures, failure to thrive, hepatitis, or blood dyscrasia. In contrast to prior studies characterizing AGS as an acute or subacute process, half of our cohort developed worsening neurologic function over greater than one month.

One of the most common misdiagnoses in the atypical AGS population was ADEM. Of note, none of the individuals within our cohort diagnosed with ADEM had altered mental status (including irritability or confusion) at the time of fulminant presentation, which is part of the diagnostic criteria for ADEM. Another common misdiagnosis was congenital spastic diplegia, despite the fact that disease onset developed after one year of life.

The imaging findings within this atypical AGS cohort were diverse, but most demonstrated evidence of white matter involvement. Classic early-onset AGS findings of calcifications and cerebral atrophy were noted in minority of MR images. We hypothesize that this may be due to the proximity of the imaging to the onset of fulminant symptoms. Only one of our atypical cases was noted to have bilateral striatal necrosis at disease onset, as has been previously described with ADAR1-related AGS. This underscores the need to more broadly consider AGS regardless of the MRI features noted at onset. This study focused on the retrospective information up to the time of fulminant disease onset, and serial, subsequent imaging was not reviewed. Future prospective studies will be better suited to the characterization of the evolution of imaging findings after disease onset.

The individuals in this cohort treated with immunomodulatory therapies demonstrated an incomplete and transient improvement. These immunomodulatory therapies did not result in a sustained improvement in neurologic function or a change in the neurologic trajectory associated with AGS. In the first months after fulminant disease onset, individuals with AGS can demonstrate fluctuating neurologic function. We hypothesize that the improvement attributed to the immunomodulatory therapies may have been due to natural fluctuations in the disease.

This leg of the project presented several limitations. The inclusion criteria may have excluded atypical cases presenting at less than one year of age. As our cohort is also limited by ascertainment bias, we hypothesize that even further diversity in the AGS-spectrum exist as part of an AGS genes-related type I interferonopathy spectrum. Despite these limitations, we were able to demonstrate that later, atypical AGS cases have a pattern of evolution: from early subtle symptoms, to worsening systemic inflammation, and culminating in neurologic decline. This encompasses subtypes previously classified as spastic paraparesis. As such we would recommend consideration of AGS in the differential diagnosis for young children presenting clusters of AGS-associated symptoms of systemic inflammation, particularly when accompanied by a change in neurologic function.

Another element of focus of this project was the exploration of the multisystemic manifestation of the disease. While the impact of AGS on the brain is generally well characterized, the impact on systemic organs is less well understood. The neonatal form of AGS is associated with an inflammatory hepatitis (Crow Y et al, 2008; Ramantani G et al, 2010), but there is limited information about the pathogenesis or incidence of liver abnormalities across the lifespan. Other pediatric liver diseases are associated with the presence of autoantibodies, such as anti-nuclear antibodies (ANA), anti-neutrophil cytoplasmic antibodies (ANCA), and anti-smooth muscle antibody (ASMA). While liver specific auto-antibodies can be causative of disease (such as in autoimmune hepatitis), they may develop as a secondary phenomenon resulting from exposure of hepatocyte antigens (as occurs in Hepatitis C infection and Wilson disease) (Gilman A et al, 2018, Narkewicz MR et al, 2017). The role of antibodies in AGS-related hepatitis is unknown.

Given the association between AGS and liver disease, particularly in the neonatal period, we sought to characterize the natural history of liver involvement in AGS across the lifespan and its relationship to biomarkers of inflammation.

We found that approximately sixty percent to three quarters of individuals with AGS experience elevated liver enzymes. More than one quarter have liver enzyme elevations consistent with hepatitis during their lifetime. As our data is limited by the availability of laboratory measurements, we hypothesize that this is likely an underestimation of the incidence of hepatitis in the AGS population. A younger age at disease onset and the

presence of microcephaly correlated with the early presence of hepatitis, which is consistent with the existing literature. All genotypes were susceptible to liver enzyme elevation, although this was the least pronounced in the SAMHD1 population. We found that enzyme abnormalities may be observed years after disease onset and were not limited to the neonatal period, as previously reported.

While a significant proportion of the population had a transient elevation in liver enzymes, the clinical impact and prognostic significance of this information is uncertain. Within our cohort, there were only rare cases with liver imaging or findings of synthetic liver dysfunction. Only two individuals were noted in their clinical records to have clinical manifestations of liver disease. One limitation of a retrospective study is that the absence of documentation of a specific feature (i.e. the lack of clinical symptoms from hepatitis) is not equivalent to the absence of signs and symptoms. In future investigations, the clinical implications of the common elevations of liver enzymes should be explored with measurements of liver function, such as coagulation laboratory testing, and imaging. Additionally, clinical histories at the time of enzyme measurement were not consistently available. Finally, it is unknown if chronic liver disease may develop in the third or fourth decade of life, since this data was not captured. Additionally, this cohort may be affected by ascertainment bias as it includes only individuals who were diagnosed with AGS (and thus milder forms of the disease may be under-represented), and those individuals whose families choose to enroll in the natural history study. The antibody tested was limited to the individuals from whom samples were available. Despite the incomplete data set due to the structure of a retrospective natural history study, we anticipate that our cohort is representative of AGS. This is because this cohort includes the spectrum of genotypes and neurologic disabilities associated with AGS. When concurrent medical documentation was available, infection or hepatitis-associated medication exposure was identified in approximately 10% of episodes. We hypothesize that the inflammation associated with AGS may drive the recurrent elevation of liver enzymes.

To better characterize the potential pathophysiologic mechanisms underlying the elevations in liver enzymes, we assessed the correlation between liver enzymes, ISG scores and liver autoantibodies in a subset of individuals with available banked samples. No clinically meaningful correlation between liver enzyme levels and ISGs score was

observed. Autoantibodies consistent with autoimmune hepatitis were positive in more than half of our cohort. While these findings are consistent with what described previously in literature, they did not strongly correlate with ISG levels, or liver enzyme elevations. While this study suggests that AGS is associated with recurrent and transient elevations of liver enzymes, the mechanism of these changes is still unknown.

6.2 DISEASE PERCEPTION: A CAREGIVERS' INSIGHT

In general, families of children affected by AGS experience extraordinary stress. Caregivers shared their earliest recollections of how AGS affected their children. Similar to reports in the medical literature (Rice G et al, 2007; Adang L et al, 2020; Crow YJ et al, 2015), caregivers reported an increasing burden of inflammatory symptomatology accompanying severe neurologic decline. The first signs of AGS can be accompanied by extreme irritability and months of continuous crying. This early period of time was noted to be a major source of stress for the child and family. Several families noted the importance of knowledgeable providers as part of their child's medical team. This underscores the need for development of educational resources targeting rare diseases.

After a period of increasing symptomatology, neurodevelopment typically reaches a plateau Families described the profound impact of gross motor and communication impairment. Stress related to motor function was a common theme with caregivers and was noted to be a cause of lower self-esteem and more limited independence. Prior studies have established associations between poor self-image and chronic disability in other populations. This is particularly important in AGS as there can be a significant discrepancy between motor and cognitive function; for example, one subtype of AGS is notable for relatively isolated spastic paraparesis. Of note, several families also described the disconnect between receptive and expressive language. Approaches better measuring receptive language function are needed to capture cognitive abilities in the AGS population. Similarly, many intelligence assessment tools require gross motor function, such as the ability to hold a pencil or point, which can be disproportionately impaired in AGS.

Similar to reports in other contexts (Pelentsov LJ et al, 2015; Cardinali P et al, 2019), caregivers of children with AGS described the emotional impact of the disease. There was a perception of inadequacy and isolation in their daily lives. The life perspective of caregivers changed after the diagnosis of AGS, with a re-evaluation of priorities. Strain on marital relationships was reported in our cohort, as previously seen in parents of children with neurodevelopmental disorders (Hartley SL et al, 2010; Namkung EH et al, 2015). Despite this, some couples reported a positive impact on their relationship and suggested it was a source of strength. Families described the financial impact of the care of affected children on their careers and economic status.

Despite the challenges of AGS, many families described their desires for the future. Increased independence was frequently reported. Caregivers noted the importance of small changes in neurologic function. Common caregiver goals were to improve comfort and happiness. Our data suggest that symptomatic relief, maximizing neurologic function, and providing family support are critical components of the comprehensive care of children with AGS.

Caregivers reported also feelings of anxiety and stress related to diagnostic delay. In this cohort, the most common perceived reasons for delayed diagnosis was the lack of knowledge among health professionals, which led to frustration and lack of trust towards them. This process can be exacerbated by the lack of attention paid to parental concerns prior to diagnosis. Families reported feelings of loneliness, expressing the perception of not being listened to and understood in the first phases of the disease when their worries were downplayed by their social network and healthcare professionals. Trust in medical providers directly correlates to better outcomes of disease and better effectiveness of treatment in general (Birkhäuer, J. et al, 2017). As such, the delay in diagnosis may be a key influence on the long-term relationships with the medical system.

Our findings are consistent with previous studies that described the psychological impact of coping with uncertainty and hopelessness associated with a missing diagnosis, compromising the parental ability to provide care (Dellve L et al, 2006; Knott M et al, 2012). A decrease of parental stress levels is beneficial to their quality of life, the quality of life of the affected child and their siblings (Knott M et al, 2012). The identification and creation of easy to access clinical networks may improve both quality of care and patient

outcomes, facilitating earlier diagnosis and better patient management (Brown BB et al, 2016).

This study has several limitations. The cohort in this study, despite being drawn from a single institution, is geographically diverse and represents the genotypic and neurologic spectrum of disease seen in AGS. As this study is based at a large specialty care center and only includes families who consented to participate research studies, our cohort could be biased to preferentially include families with the greater clinical knowledge of AGS and improved access to medical resources. This may limit its generalizability. Improving access to specialized medical care and research opportunities is an area for future development.

Without prompting, the majority of families addressed importance of diagnosis and its implications on their family's well-being.

6.3 OUTCOME MEASURES IN AGS

6.3.1 VALIDATION OF A NOVEL SCALE

Disease-specific measures have been successful in assessing therapeutic benefit in other rare, neurologic disorders (Augustine EF et al, 2015; Finkel RS et al, 2017; Marshall FJ et al, 2005). Most existing assessment tools are unable to sufficiently evaluate individuals at the lower end of function. In our work, approximately half of the AGS population was severely affected. To address the need for simple assessment tools for neurologic function in this population, we developed an AGS scale to measure key items relevant to development in AGS abilities. This AGS scale was able to assess neurologic function, had excellent interrater reliability, and correlated with overall severity. Important for a rare disease, this scale could be applied retrospectively as it is dependent on key developmental steps often included in medical encounters. The prospective application of this tool is currently underway.

While AGS affects all domains of development, most existing tools focus on a single area: gross motor, fine motor, or cognitive skills. As such, to measure the full impact of AGS on neurologic function, multiple tools may be required. We hypothesize that future

interventions in AGS may result in small, but meaningful improvements, across the modalities. Separate measures may be insensitive to these changes, but a single tool including key neurologic milestones could potentially capture improvements across neurologic domains.

Because gross motor function is impaired in most individuals affected by AGS, we compared performance on the AGS scale to a validated measure of gross motor function, GMFM-88. The AGS Scale and the GMFM-88 demonstrated significant correlation. This underscores the potential utility of the simpler AGS Scale. Of note, half of the values for GMFM-88 were below the 10th percentile, suggestive of a significant floor effect.

We also compared the AGS scale to a biomarker of disease, the ISG score. We found no correlation between this diagnostic biomarker and the AGS scale. This finding is supported by other reports that do not correlate ISG scores with severity (Adang L et al, 2018; Rice G et al, 2020). One confounder of our results is that ISG scores can be dynamic both day-to-day and over the lifetime of a patient. External influences, such as viral infection, would increase the ISG score. Because of these reasons, a single ISG measurement can be difficult to interpret. It is possible that initial ISG scores correlates with severity and the AGS scale.

Given the genetic heterogeneity of AGS, we next characterized performance of the AGS scale across genotypes. In order to minimize the bias from unequal numbers of clinical encounters, we compared nadir AGS scores. We found that individuals with *TREX1*-related AGS demonstrated the lowest scores, which is consistent with clinical experience.

We were able to map the dynamic neurologic function found in AGS by retrospectively applying the scale to historical clinical encounters. We were able to identify several distinct patterns: those with stable disease, those with recurrent relapses, and those with progressive improvement. Future work is needed to identify variables associated with each trajectory, although we hypothesize that genotype and age at disease onset will be influential in future neurologic recovery. The overall rate of recovery after the periods of regression are unknown, although few children are suspected to return to their full neurologic baseline. Importantly, we found that most improvement occurred in the first 6 months after disease onset. This is an important consideration in clinical trial design, as the expected disease trajectory may be dependent on the time from disease onset. Of note, our data are limited by the availability of detailed and complete medical records, including vari- able distance

from disease onset to first available encounter. Future studies will be designed to assess which variables are associated with neurologic improvement, even in the absence of therapeutic intervention.

As this novel tool provides the first opportunity to easily map neurologic function over time, we wished to characterize the dynamic nature of the disease. We found the majority of dynamic AGS scores coincided with when the first post-disease onset encounter was within the first 6 months of disease. This suggests that after an early dynamic period, there is a trend toward neurologic stability in the majority of patients.

Our novel AGS tool allows for the easy assessment of basic neuro- logic function in this unique population. This scale correlates with established measures, microcephaly, and genotype. With the longitudinal and prospective application of this instrument, we can begin to characterize the dynamic neurologic function of this rare disease. Moreover, because this scale does not include AGS-specific parameters, this scale holds promise for use across other severe neurologic disorders with dynamic function.

6.3.2 FEASIBILITY OF REMOTE ADMINISTRATION OF AN IN-PERSON ASSESSMENT: GMFM-88

As described above, widely administered motor assessments such the GMFM-88 suffer some floor effect in the leukodystrophy population. Nonetheless, this test is able to describe quite accurately the gross motor abilities of individuals with a moderate or mild manifestation of the disease.

The field of child neurology has adapted rapidly to the ongoing COVID-19 pandemic by increasing the use of telemedicine encounters to address the needs of patients, and our project has focused on the feasibility of administration of the GMFM-88 using telemedicine, bypassing the need of patients to travel to specialized centers.

Our results demonstrated agreement between the in-person and telemedicine assessments of the GMFM-88 while finding high levels of interrater and intrarater reliability as indicated by ICCs above 0.99. The population studied represents a broad range of function, with scores ranging across the spectrum of GMFM-88 scores so we would predict that this

study may be relevant to most individuals with leukodystrophy. Future research is needed to confirm the reliability of both the in person and remote assessment of the GMFM-88 in a larger sample of subjects with leukodystrophy.

In this investigation, we sought to validate the use of the GMFM-88 via telemedicine more broadly in individuals affected by leukodystrophies, including AGS, POLR3-related leukodystrophy, Alexander Disease (AxD), and TUBB4A-related leukodystrophies. While many individuals affected by leukodystrophies demonstrate a floor effect on outcome measures due to the severity of neurologic dysfunction, the chosen population demonstrates a broad range of gross motor function (Adang L et al, 2020, Gavazzi F et al, 2021). The leukodystrophies selected for this study tend to have a more static course compared to other leukodystrophies (Gavazzi F et al, 2021) which needs to be confirmed by future natural history studies of motor function. This allows for the comparison of outcomes that were obtained asynchronously but it is unknown if our results may have been affected by the time period in between the in-person and remoted assessments.

One key challenge to understanding rare neurologic disorders is recruitment and bias towards including more mobile individuals. Enrollment in natural history studies may be biased against individuals who are have greater disease severity or who live at a greater distance from large academic medical centers. Our results suggest that the GMFM-88 may be a valid tool to assess function remotely which may allow for enrollment of a more diverse population and more frequent assessments in future natural history studies of longitudinal motor function. Future research investigating the remote assessments of other outcome measure of gross motor function as well as assessments of fine motor skills and activities of daily living would allow for more a complete assessment of the functional ability of individuals with leukodystrophy.

While these results are promising, this study has several limitations. Our cohort was limited in number due to rarity of occurrence of leukodystrophies and the ability of families to travel to a central site for in person assessments. However, the inability to recruit larger numbers of participants and to obtain regular study assessments in this population underscores the need to develop alternatives to in-person assessments. Additionally, inclusion in this study required baseline participation at a single centralized site with a high level of expertise with leukodystrophy and the collection of standardized outcome

assessments. The ability to generalize this approach with other remote site facilities will need to be addressed in future studies.

It should be considered that technological limitations, such as unavailability of electronic devices, inconsistent internet connections, or other technical difficulties, which have been reported by Rametta et al (2020) during telehealth encounters with children, may make the remote application of the outcome measures such as the GMFM impossible. The absence of the equipment required to complete the GMFM-88 might limit its application in some home environments. Finally, the use of caregivers to assist in the completion of the GMFM may be difficult for some caregivers, especially for older and more physically dependent children so that alternative assessments which are less physically demanding will need to be developed.

6.4. BARICITINIB MODIFIES AGS DISEASE PROGRESSION: APPLYING THE AGS SCALE IN A CLINICAL TRIAL

Since the identification of the genetic etiology of AGS, there has been intense interest in the application of immunomodulation as disease modifying therapy. Initial efforts focused on use of steroids, intravenous immunoglobulin, cyclophosphamide, and Tocilizumab. More recently, with the development of JAK inhibitors, case reports treating AGS with ruxolitinib and baricitinib have emerged, addressing the impact of this class of molecules on cerebral vasculopathy, skin manifestations, and ISGs. JAK inhibitors have the potential to more directly protect affected individuals from the end organ damage associated with uncontrolled IFN production, though much remains to be understood about the penetration of these agents to key organs, including the blood brain barrier.

This is the first study to assess safety and efficacy of a JAK inhibitor in AGS. Baricitinib was well tolerated in our cohort, with treatment associated laboratory abnormalities limited to mild anemia and moderate thrombocytosis.

Several target organs appeared to have therapeutic improvement with baricitinib administration, particularly thrombocytopenia, skin inflammation, and hepatitis. Chronic thrombocytopenia improved in one individual, and in two individuals, liver enzyme

abnormalities of more than 15-fold upper limit of normal improved on study. Severe skin manifestations by subjective description and symptom diaries also improved, consistent with a prior report.

Neurologic improvement or stabilization is more challenging to measure in AGS. Some individuals in this study had more than 10 years of disease progression before treatment initiation. In addition, the severe neurologic manifestations made objective measurement of neurologic improvement difficult due to floor effects using traditional outcome measures.

To begin to address the limitations of traditional outcome measures, we created a novel AGS scale and applied it to our population.

For the more severely affected individuals, our novel AGS scale showed improvement in a significant subset of individuals, depending on baseline neurologic function. None of these individuals had improvement in this scale in the year before treatment was initiated, although one patient demonstrated dynamic changes over the prior year. Although these evaluations will need to be validated in prospective cohorts, this data is encouraging that some degree of neurologic improvement can be seen even in individuals with longstanding disease.

Additional study of therapeutic efficacy will require longer observation and prospective assignment of the AGS scale.

Overall, this study demonstrates that baricitinib is a safe, well tolerated therapeutic approach in AGS. Treatment-related side effects were limited to those known in this class of medications, including thrombocytosis and anemia. In individuals with significant systemic illness, including thrombocytopenia, hepatitis, and skin inflammation, baricitinib improved disease symptoms. Measurement of neurologic improvement is complex, but early data suggests improvement of neurologic function, even in individuals with severe and long-standing disease. Together, this data suggests that baricitinib is a safe and potentially effective therapy in AGS and warrants further evaluation as a disease modifying treatment.

CHAPTER 7: FUTURE PERSPECTIVE

7.1 DELINEATION OF MORE COMPREHENSIVE OUTCOME MEASURES TO BETTER DETERMINE FUNCTIONAL LEVELS IN A HETEROGENEOUS POPULATION

As described before, the AGS scale has proven to be an effective tool in determining disease severity and clinical dynamicity in a pediatric population with a diagnosis of AGS. That said, the AGS scale presents a number of limitations. While avoiding in some extent the floor effect, this scale suffers a ceiling effect in individuals with a mild phenotype. In this population, clinical improvements may not be reflected by this tool.

It is also to be considered that improvements in aspects like Attention, Memory, and Adaptive Behavior are not represented in this tool.

In order to improve this tool, a future project will focus on the analysis of traditional outcome measures applied in the AGS population, with the goal of identifying specific sections or aspects of the tests that appear to be sensitive and dynamic in the population, through Item Response Theory (IRT statistics).

In order to do so, outcome measures to evaluate gross motor function, fine motor function, cognitive/psychomotor level, and adaptive behavior abilities have been administered on AGS patient.

Outcome measures considered for this project include:

- GMFM-88: to evaluate gross motor abilities.
- Peabody Fine Motor Quotient: to evaluate fine motor abilities.
- Mullen Scales of Early Learning (MSEL): to evaluate psychomotor development in children age 0-5 years and 8 months.
- Leiter International Performance Scale – 3rd edition: to evaluate non-verbal cognitive abilities in individuals age 3-75 years.
- Vineland Adaptive Behavior Scale 3rd edition – parent/caregiver interview: to evaluate adaptive behavior abilities in individuals age 0-90 years.

To date, about 310 encounters in 80 different patients have been performed in relation to this project.

The following administrations have been performed:

- 294 GMFM-88
- 143 Peabody Fine Motor Quotient
- 127 Vineland-3
- 75 MSEL
- 59 Leiter-3

The preliminary results of these assessments are visualized below (Figures 26-30), in association with the AGS scale score at the time of the encounter.

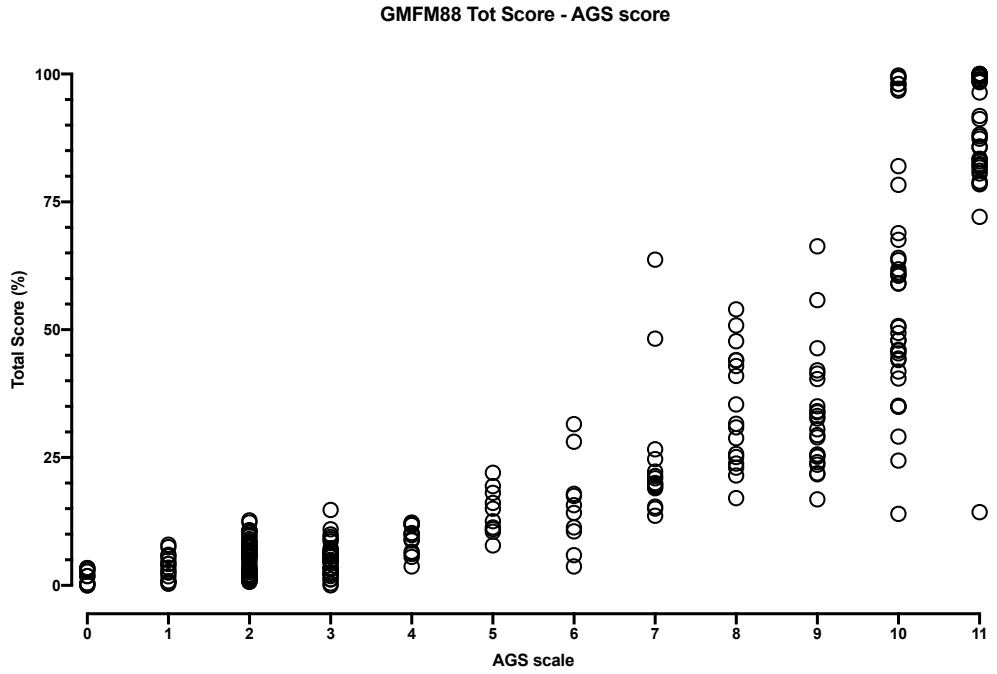


Fig 26. GMFM-88 % distribution in relation with AGS scale score at the time of the encounter.

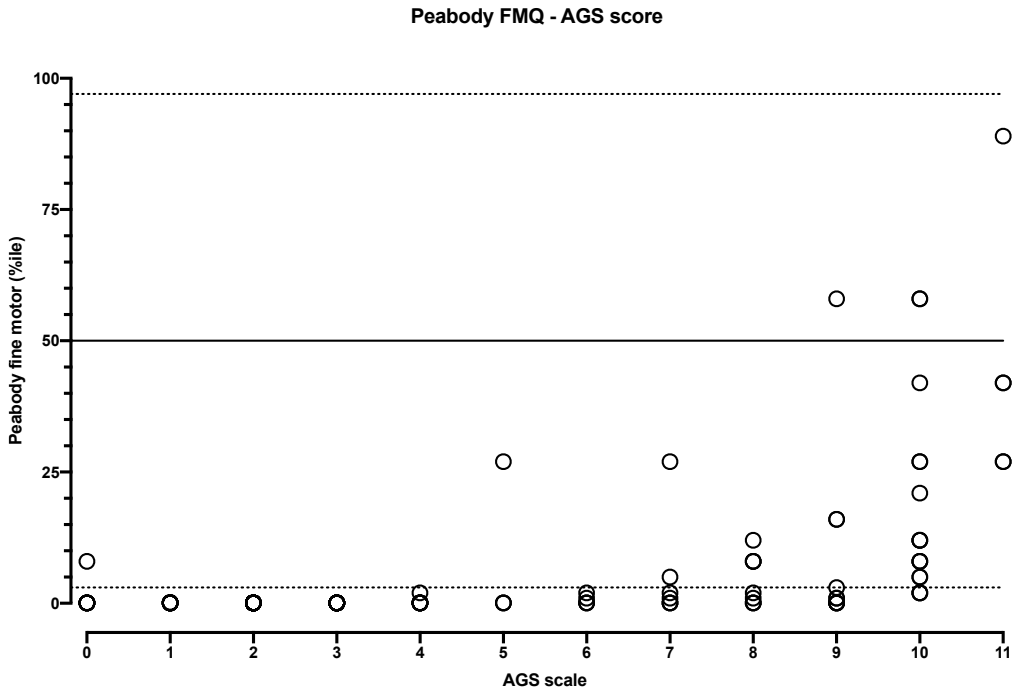


Fig 27. Peabody FMQ %ile distribution in relation with AGS scale score at the time of the encounter

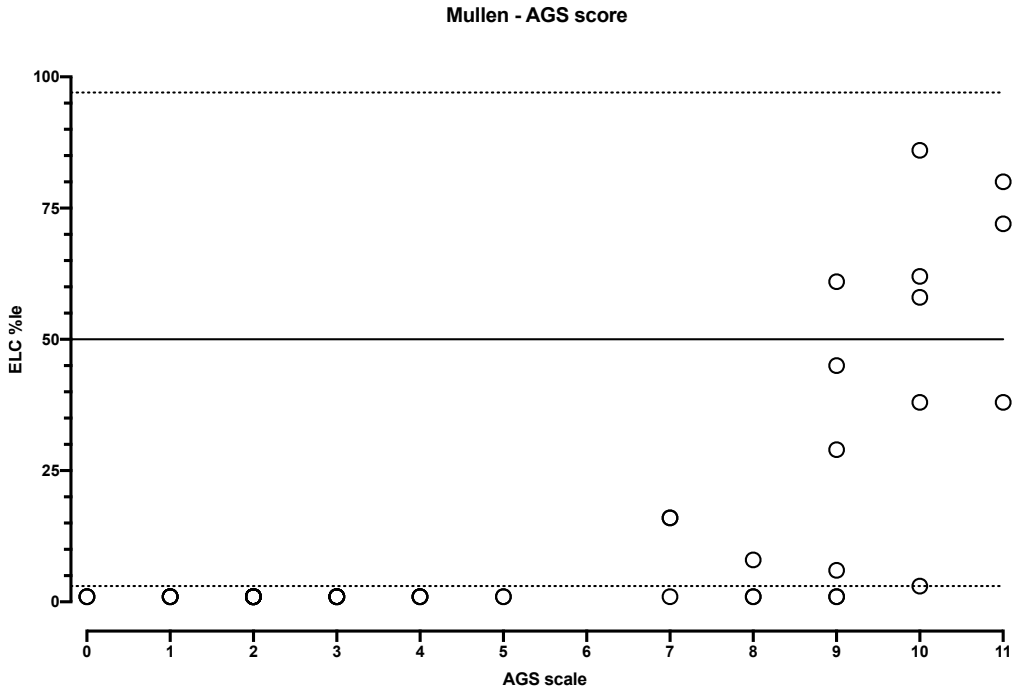


Fig 28. MSEL Early Learning Composite %ile distribution in relation with AGS scale score at the time of the encounter

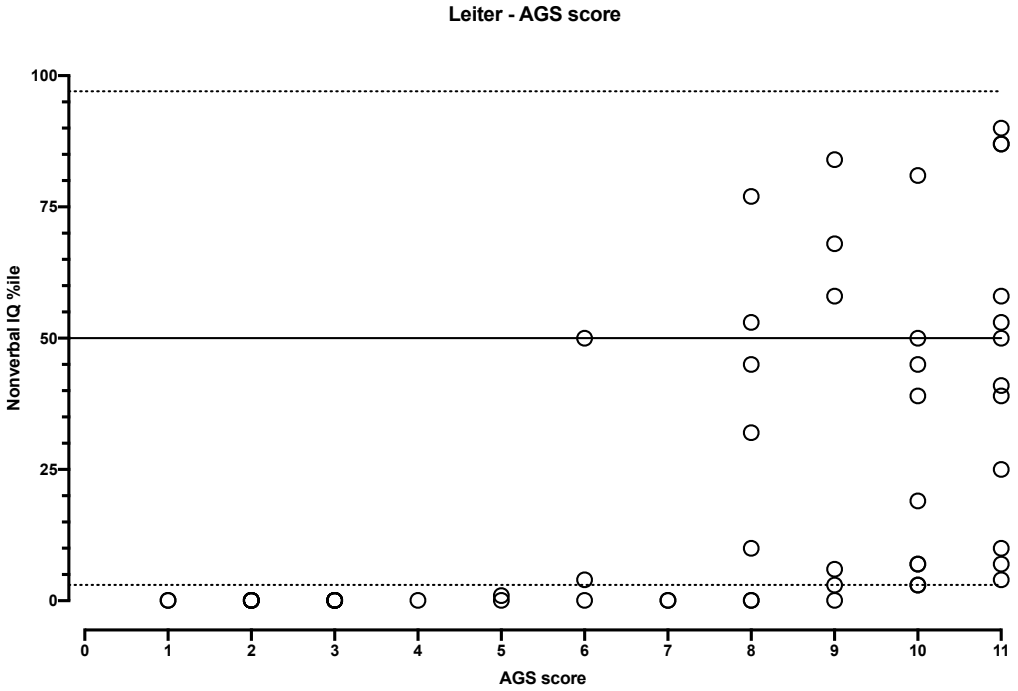


Fig 29. Leiter 3 Non Verbal IQ %ile distribution in relation with AGS scale score at the time of the encounter

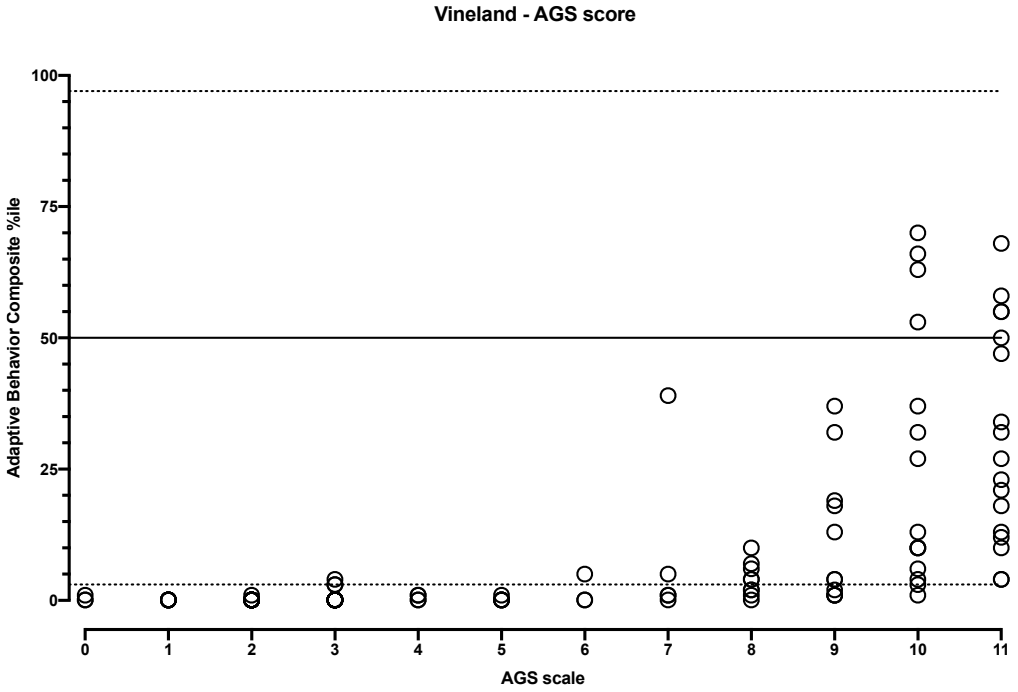


Fig 30. Vineland 3 Adaptive Behavior Composite %ile distribution in relation with AGS scale score at the time of the encounter

A combined visualization of MSEL, Leiter 3 and Vineland 3 performance is included in Figure 31.

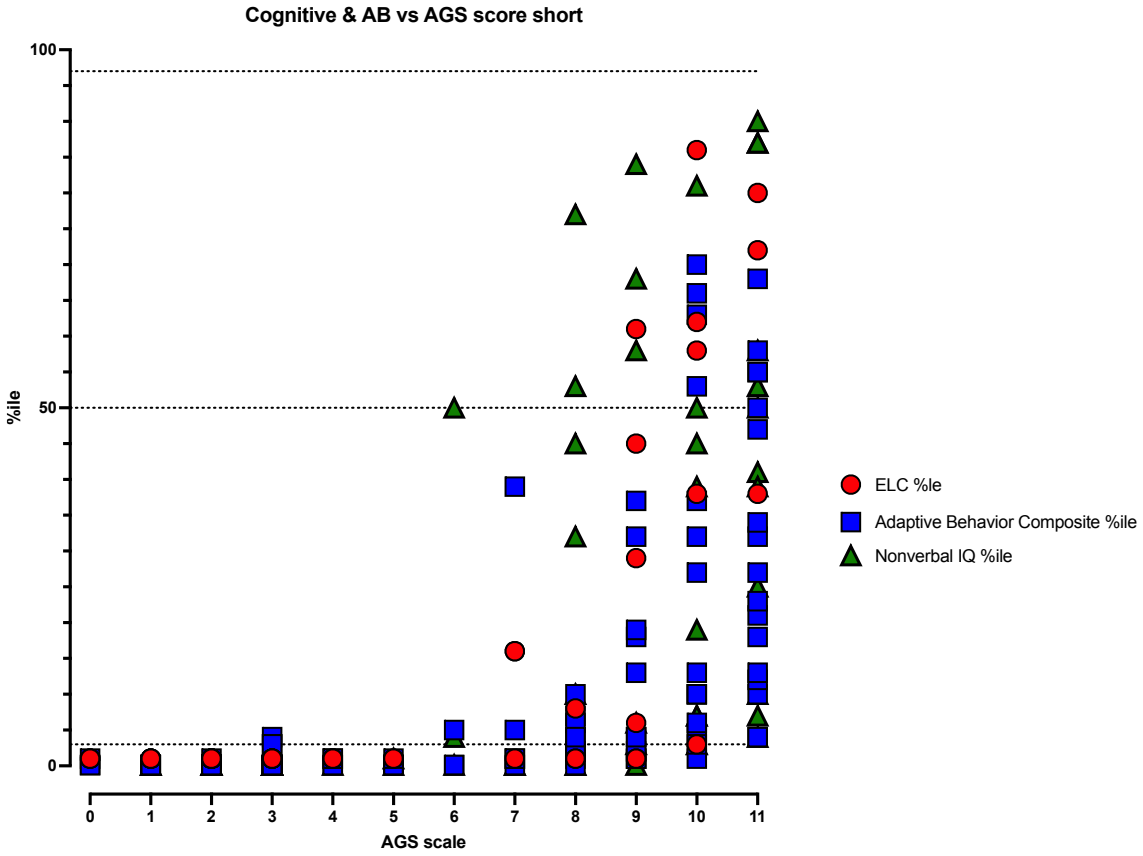


Fig 31. Combined performance of the patients' cohort at MSEL, Leiter 3 and Vineland 3

From a preliminary observation it appears that all measures implemented in the analysis suffer a severe floor effect in individuals with an AGS scale score between 0 and 5/7 (depending on the outcome measure considered). In individuals with a milder impact of disease, these measures appear able to layer different functional levels within populations scored within the sage AGS scale score severity.

IRT analysis will allow us to consider performance of different subsection of the aforementioned assessments, in order to identify the most dynamic items and include them in an expanded AGS score, allowing for more granularity and better separation of functional population, to achieve the identification of appropriate efficacy goals for current and future therapeutic interventions within this disease.

This approach can easily be adopted in other rare disorders, in order to create disease specific severity scales.

7.2 EXPANSION OF THE METODOLOGY TO OTHER RARE LEUKODYSTROPHIES: TUBB4A RELATED LEUKOENCEPHALOPATHY NATURAL HISTORY

The methodological approach used to determine developmental trajectories in AGS can also be expanded to other rare genetic disorders characterized by neurologic impact and heterogeneous spectrum of manifestations. I am now going to present the successful application this methodology in TUBB4A related leukodystrophies.

Mutations in *TUBB4A* are associated with a spectrum of neurologic disorders, including hypomyelination with atrophy of the basal ganglia and cerebellum (H-ABC), isolated hypomyelination, and DYT4/whispering dysphonia (Breuss M et al, 2014; Nogales E et al, 1999; Curiel J et al, 2017). Collectively, these disorders are categorized as *TUBB4A*-related Leukoencephalopathy.

The most common pathologic variant associated with H-ABC in *TUBB4A* is p.Asp249Asn (Vulionovic F et al, 2018). The developmental course of *TUBB4A*-related Leukoencephalopathy can be variable and is only partially explained by genotype alone (Breuss M et al, 2014; Nogales E et al, 1999; Curiel J et al, 2017).

In this project we identified 17 skills from the Denver Developmental Screening Test II (DDST-II) to assess acquisition of developmental milestones in the TUBB4A-related Leukoencephalopathy population, as previously applied in the AGS population (Adang L et al, 2020).

Three sub-cohorts were identified: individuals with the common p.Asp249Asn variant verses all other genotypes with either early (less than 12 months of age) or late onset of presentation. Individuals with the p.Asp249Asn variant or those with non-p.Asp249Asn genotypes with later disease onset attained key milestones, including head control, sitting, and independent walking. Subjects with early onset, non-p.Asp249Asn associated disease were less likely to achieve developmental milestones (figure 31-33).

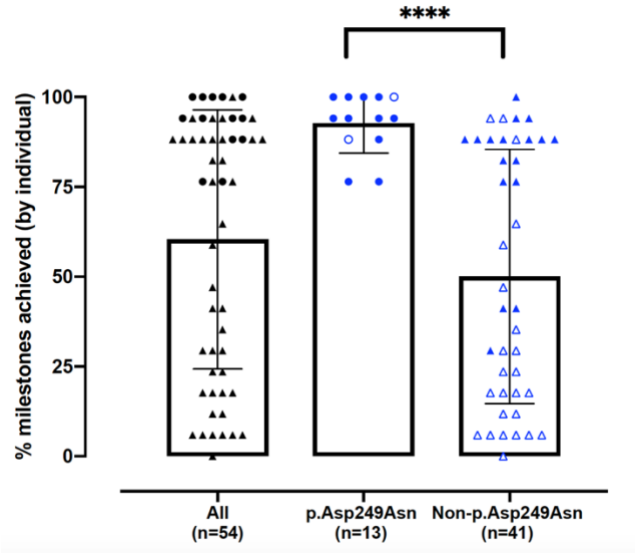


Fig 31. Milestones achieved by genotype. Individuals with p.Asp249Asn variants achieved a higher percentage of selected developmental milestones, when compared to non-p.Asp249Asn patients (Mann-Whitney test, p value <0.0001). Open symbols represent individuals with age of onset <12 months, full symbols represent individuals with age of onset ≥12 months

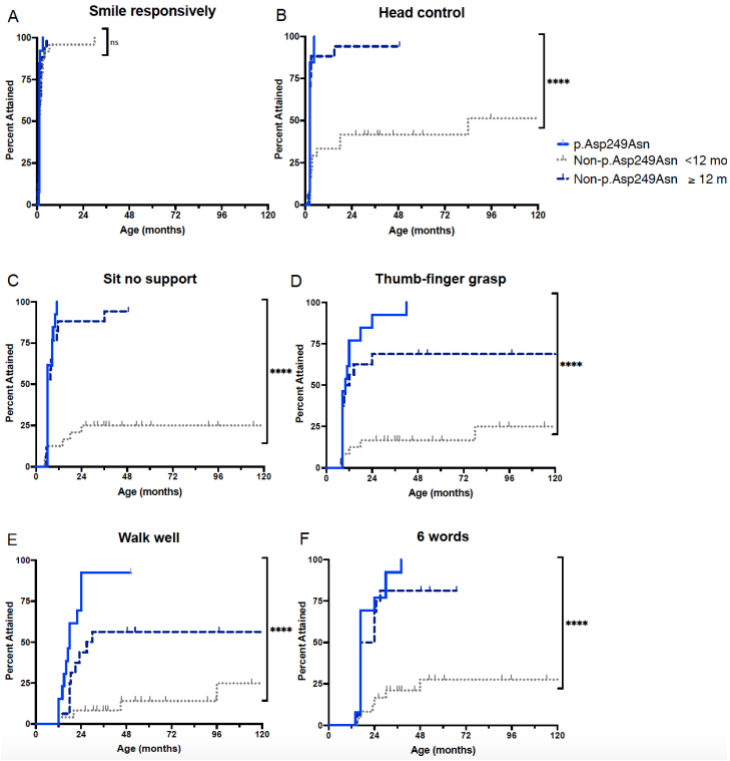


Fig 32. Developmental milestone acquisition by genotype and age of onset. P values were calculated using the log-rank (Mantel-Cox) test comparing genotypes and age of onset (****= p < .0001; ns = not significant).

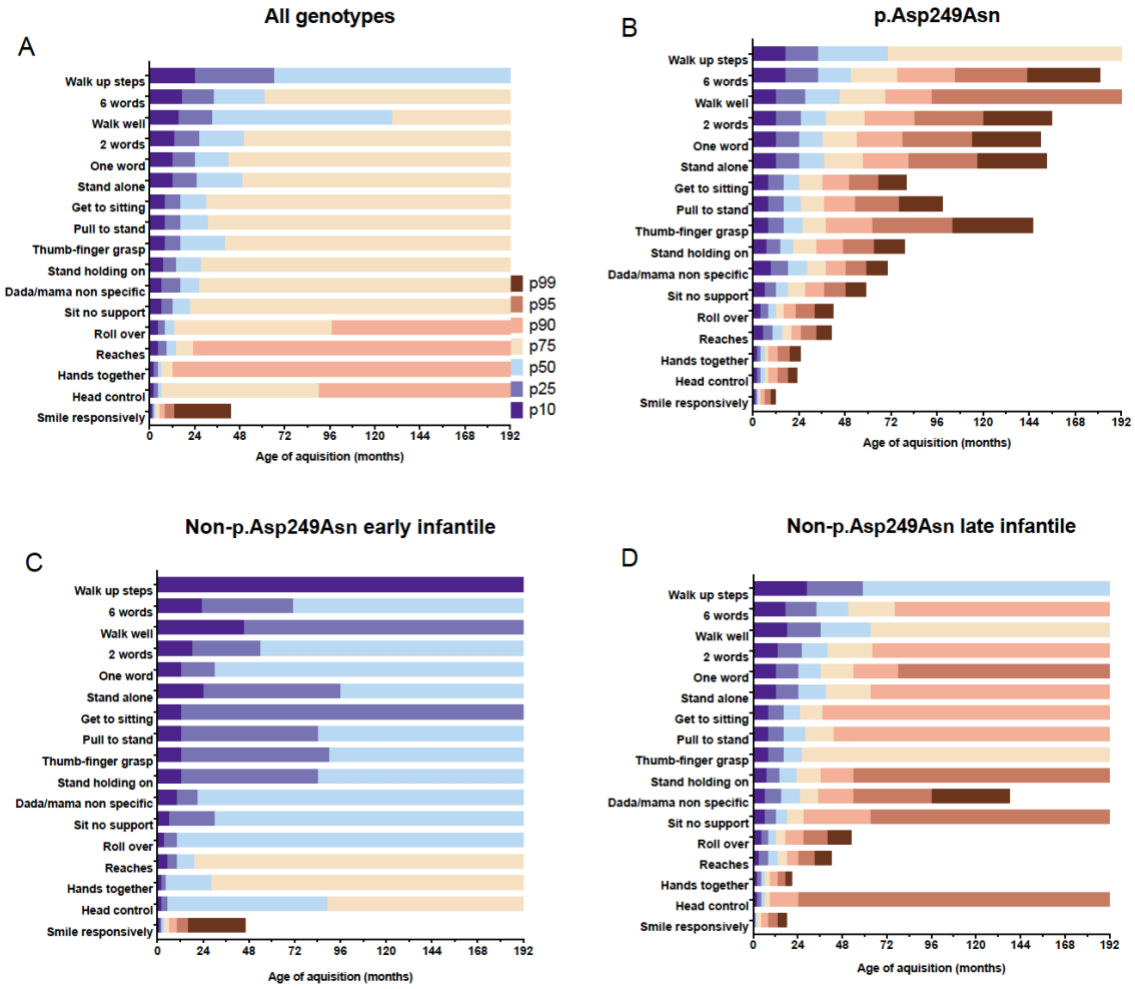


Fig. 33. Developmental acquisition in H-ABC. Developmental skill acquisition plots were created for the overall TUBB4A population based on genotype (p.Asp249Asn variant vs non-p.Asp249Asn variant) and age of disease onset. The designation of 10 indicates that less than 10% of the population has acquired a milestone by the age indicated on the x-axis, whereas the designation of 90 indicates that up to 90% of the population has acquired the skill by the given age. The color-coded heat map indicates the percentage of the population that has attained the milestone at a given age, as shown on the X-axis. The percentiles were estimated using Kaplan-Meier Analysis. A: all genotypes combined. B: p.Asp249Asn related-disease. C: early infantile onset non-p.Asp249Asn related-disease. D: late-infantile onset non-p.Asp249Asn related-disease.

Next, we defined the developmental severity as the percentage of milestones attained by 2 years old. The mild form was defined as attaining at least 75% of key developmental

milestones (Figure 34).

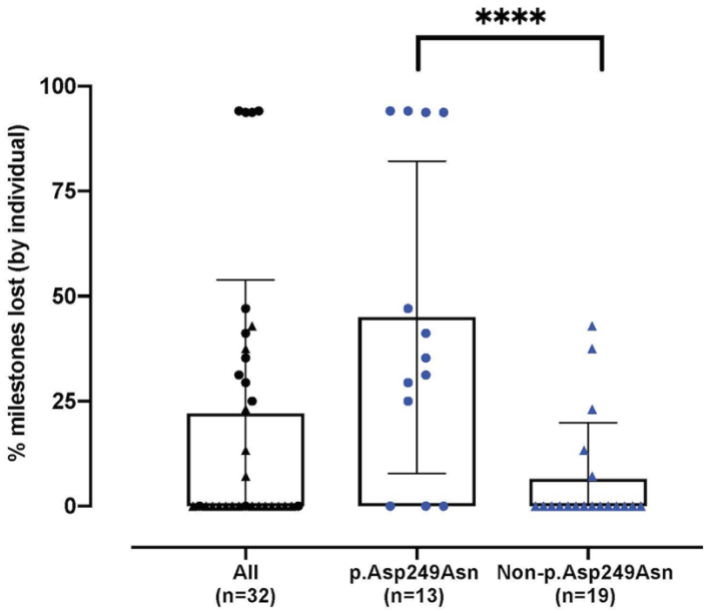


Figure 34. Subsequent milestone loss in individuals achieving 75% of early milestones. Milestones loss at last available evaluation by genotype in individuals who achieved at least 75% of milestones. Individuals with p.Asp249Asn related-disease demonstrate higher loss of developmental milestones, when compared to non-p.Asp249Asn related-disease (Mann-Whitney test, p value<0.001).

Amongst cohort categorized as mild, individuals with p.Asp249Asn variant were more likely to lose acquired abilities when compared to non-p.Asp249Asn individuals.

TUBB4A-related Leukoencephalopathy is a heterogeneous disorder caused by mutations in the beta-tubulin isotype encoded by *TUBB4A*. Although all children described to date with *TUBB4A*-related Leukoencephalopathy demonstrate some degree of neurologic impairment, the magnitude of neurodevelopmental delay can be variable, as can the presence of regression of developmental skills.

Three distinct patterns within childhood onset TUBB4A associated Leukoencephalopathy emerged: the p.Asp249Asn related-disease, characterized by late-infantile onset, with acquisition of early milestones followed by neurologic regression; early infantile (<12 months) onset non-p.Asp249Asn related-disease, characterized by minimal developmental gain; and late infantile (>12 months) onset non-p.Asp249Asn related-disease, characterized by late-infantile onset, with acquisition of early milestones with more moderate neurologic regression. Due to the heterogeneous psychomotor development

observed the late infantile (>12 months) onset non-p.Asp249Asn related-disease cohort, we hypothesize that additional factors may influence neurologic outcome. It will be relevant to future clinical trial planning to better understand factors that may influence outcomes in the late infantile (>12 months) onset non-p.Asp249Asn related-disease cohort. For example, the presence or absence of radiographic features such as cerebellar atrophy or basal ganglia atrophy, may impact long term developmental outcomes. However, in this study, imaging was obtained clinically based on the presence of developmental abnormalities. This ascertainment bias prevented the analysis of these features as predictive of developmental outcomes.

This study explores developmental outcomes in a group of children affected by *TUBB4A*-related Leukoencephalopathy. While this cohort is relatively small (n=54), we anticipate that this international cohort is representative of pediatric onset *TUBB4A*-related Leukoencephalopathy. As the participants were enrolled through a pediatric leukodystrophy research network, it is important to note that there was an ascertainment bias in the identification of cases. This study did not include cases of adult-onset forms of *TUBB4A*-related Leukoencephalopathy and may underrepresent cases without hypomyelination on imaging. All but one individual was noted to have MRI findings consistent with a leukodystrophy. Individuals with adult-onset disease or without hypomyelination are likely underdiagnosed in childhood. We hypothesize that these individuals could have normal or near-normal early development.

In this study, we characterize the association between age of onset, genotype and phenotype in *TUBB4A*-related Leukoencephalopathy. Future identification of variables that may affect long-term outcomes, in addition to age of onset and genotype, will be important in the design of future clinical trials and the selection of appropriate clinical endpoints.

CHAPTER 8: CONCLUSIONS

Aicardi Goutieres Syndrome is a rare monogenic disorder with a wide spectrum of clinical manifestation, identified for the first time about 40 years ago.
Since then, numerous authors have contributed to a better understanding of the disease from both a pathogenic and a clinical point of view.

When this project started to be designed (in 2018), a correlation genotype-phenotype was already available from a neuroradiological and, to some extent, a clinical standpoint.
The choice to focus on the delineation of the developmental trajectories of affected individuals was born from a need to better understand the disease evolution, facilitating early detection of such a kaleidoscopic disorder and, if possible, to predict the evolution of the neurologic status of a child based on their genotype and early clinical manifestations.
The study of the natural history of this disease has then shown correlations between specific mutations and clinical manifestations, and the never reported before ability of affected children to achieve specific developmental milestones in a unique way (in some situations, many years later than expected in typically developing children), configurating genotype specific developmental trajectories. Late onset disease occurrence has been explored in a very extensive cohort of international patients, showing the presence of specific underlying signs and symptoms that should be used as red flags from primary caregivers and pediatricians.

With the existence of experimental and promising disease-specific treatments, it become fundamental to find ways to determine efficacy of treatment. While macroscopic disease manifestations were showing improvements in treated individuals (improvement of skin condition and hematologic analyses), traditional outcome measures assessments were failing to describe accurately improvements in the moderate and severe phenotypes (in the sense of milestones acquisition).
The creation of a disease-specific neurologic severity scale, based on the evolutionary trajectories defined before, has allowed us to demonstrate efficacy of treatment. The tool

has shown that treated children were able to gain motor, communicative and social abilities that the untreated population was not able to achieve.
Semi-structured interviews focused on burden of disease and expectations of the caregivers of these patients have shown that even small improvement of the disease severity of their children were extremely relevant to them.

The determination of critical functional domains in individuals affected by a rare disorder is a multilayered process that requires a deep understanding of the natural evolution of the disease, of its developmental trajectories, and of the real impact of disease on patients and their caregivers. The limited population of affected individuals and the heterogeneity of the phenotype include another degree of complexity, mandating the need of a network of collaboration with multidisciplinary experts that often overcomes continental borders.
The understanding of the rate of progression of a specific disease over time and the determination of the critical functional domains can facilitate the identification of outcome measures to be applied to determine the progression of the functional levels of the individuals examined. Limitations such as travel challenges, geographic distances, or observation of floor and ceiling effects of traditional assessments can be overcame by implementation of alternative ways of administration (for example, using telehealth software), or the creation of more specific and sensitive tools to evaluate the dynamicity of the population.
The ability to assess individuals affected by a disorder with appropriate tools, determining the trajectories, functional levels and occurrence of medical complications can allow to achieve clinical trial readiness: it is therefore possible to evaluate efficacy of an experimental treatment comparing the evolution of an individual with the historical data of the untreated population (which will become the control population).

Future implications of this project include the expansion of the AGS severity scale, to better describe higher functional individuals, by using the Item Response Theory methodology on a series of validated traditional outcome measures that evaluate the main functional aspects of a person. To date, no scientific literature has reported any data on outcome measures applied to this population.

This methodology has proven to be translatable to other rare disorders (such as TUBB4A related leukoencephalopathies), and will facilitate the design of future clinical trials, in order to evaluate the efficacy of determined pharmacologic treatments.

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APPENDIX 1: PARENT/CAREGIVER INTERVIEW GUIDE AICARDI GOUTIERES SYNDROME

Parent/Caregiver Interview Guide
Aicardi-Goutières Syndrome

Interviewer Introduction:

"During this interview, I am going to ask you some questions about your thoughts, opinions and feelings about your child's diagnosis of Aicardi-Goutières Syndrome (AGS). Remember, there are no right or wrong answers. We can also skip any questions that you don't want to answer. If you get tired, we can take a break, and if you don't want to answer any more questions, that's okay, too. If you don't understand a question, or if you need me to repeat anything, just let me know."

Introductory Survey Questions (Rapport Building):

1. *"Talk me about your child's story."*
2. *"How would you describe the impact of AGS on your child?"*
3. *"What is the most important way AGS affects your child?"*
4. *"What is the most important way AGS affects your family?"*

Primary Survey Questions (Details of Diagnosis):

1. *What symptoms did your child have that prompted you to seek medical attention?*
    a. *Free Answer from Parent/Caregiver*
    b. *[PROBES]:*
        i. *Fever*
        ii. *Rash*
        iii. *Irritability or Pain*
        iv. *Fatigue*
        v. *Difficulty Moving*
        vi. *Change in Development*
2. *What other symptoms has your child experienced from AGS? Which of these has the most impact on your child every day? Every month?*
    a. *Free Answer from Parent/Caregiver*
    b. *[PROBES]:*
        i. *Fever*
        ii. *Rash*
        iii. *Irritability or Pain*
        iv. *Fatigue*
        v. *Difficulty Moving*
        vi. *Change in Development*
        vii. *Pulmonary Hypertension*
        viii. *Feeding Intolerance*

3. *How do you feel AGS affect the way your child functions? What does a typical day in your child's life look like?*

    a. *Free Answer from Parent/Caregiver*
    b. *[PROBES]:*
        i. *Does your child go to school?*
        ii. *Can the child assist with activities of daily living, adjusted for functional age of the individual?*

4. *What would be the most meaningful improvement that would impact your child's quality of life?*

    a. *Free Answer from Parent/Caregiver*
    b. *[PROBES]:*
        i. *Rash*
        ii. *Irritability or Pain*
        iii. *Fatigue*
        iv. *Development*
        v. *Head Control*
        vi. *Smiling*
        vii. *Feeding*

5. *How has the way AGS affects your child changed over time?*

    a. *Can you reflect on the improvements? The declines?*
    b. *[PROBES]:*
        i. *Tone*
        ii. *Development*
        iii. *Feeding*
        iv. *Illness*
        v. *Medication Changes*
        vi. *Vaccination*
        vii. *How quickly did things change?*
        viii. *Were these changes transient or permanent?*
        ix. *What do you think may have caused these changes?*

6. *Can you talk a little bit more generally about the experiences you and your family have had with functional testing in clinical settings? These may include developmental tests, IQ tests, or functional assessments by physical and occupational therapists.*

    a. *Free Answer from Parent/Caregiver*
    b. *[PROBES]:*
        i. *Do you feel these tests accurately measure motor and cognitive function?*
        ii. *What do you think these assessments do well?*
        iii. *What assessments do you think are missing?*

7. *Is there anything you would like to add before we conclude the interview?*

APPENDIX 2: PUBLISHED LITERATURE

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Developmental outcomes of Aicardi Goutières Syndrome

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Abstract

Aicardi Goutières Syndrome (AGS) is a monogenic interferonopathy caused by abnormalities in the intracellular nucleic acid sensing machinery (*TRE4X1, RNASEH2A, RNASEH2B, RNASEH2C, SAMHD1, ADAR1,* or *IFIH1*). Most individuals affected by AGS exhibit some degree of neurologic impairment, from spastic paraparesis with relatively preserved cognition to tetraparesis and severe intellectual disability. Because of this heterogeneity, it is important to fully characterize the developmental trajectory in AGS.

To characterize the clinical presentation in AGS, early features were collected from an international cohort of children (n=100) with genetically confirmed AGS. There was a heterogeneous age of onset, with overlapping clusters of presenting symptoms: altered mental status, systemic inflammatory symptoms, and acute neurologic disability. Next, we created genotype-specific developmental milestone acquisition curves. Individuals with microcephaly or *TREX1*-related AGS secondary were the most severely affected, and less likely to reach milestones, including head control, sitting, and nonspecific mama/dada. Individuals affected by *SAMHD1*, *IFIH1*, and *ADAR* attained the most advanced milestones, with 44% achieved verbal communication and 31% independently ambulated. Retrospective function scales (Gross Motor Function Classification System, Manual Ability Classification Scale, and Communication Function Classification System) demonstrated that two-thirds of the AGS population are severely affected.

Our results suggest multifactorial influences on developmental trajectory, including a strong contribution from genotype. Further studies are needed to identify the additional factors that

Information on data in the Myelin Disorders Bioregistry Project can be requested by emailing the corresponding author, Laura Adang (adangl@email.chop.edu).

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influence overall outcomes to better counsel families and to design clinical trials with appropriate clinical endpoints.

Keywords

Leukodystrophy; Developmental disability; Genetics; Neurodevelopment; Pediatric

Introduction

Aicardi Goutières Syndrome (AGS) is a rare genetic disorder of excessive interferon (IFN) production resulting in systemic inflammatory injury (1, 2). Genetic abnormalities in the intracellular nucleic acid sensing machinery (*TREX1, RNASEH2A, RNASEH2B, RNASEH2C, SAMHD1, ADAR1,* and *IFIH1*) trigger an aberrant IFN response, which results in extensive end organ damage (1, 3–5).While all identified individuals affected by AGS exhibit some degree of neurologic impairment, this can range from mild spastic paraparesis with normal intelligence to tetraparesis and severe cognitive disability. The magnitude of this developmental heterogeneity challenges clinical trial development. While there is a known association between genotype and overall clinical severity (6), there is an unmet need for fully understanding the impact of genotype on developmental trajectory. As we transition into the era of therapeutic options, it is important to characterize the impact of genotype on developmental expectations so that appropriate outcomes can be selected (6).

In this study, a retrospective international cohort of 100 individuals affected by AGS was analyzed for age and symptoms at presentation and a detailed developmental history was obtained. Using developmental milestones, we were able to create genotype-specific developmental trajectories. Future studies are needed to characterize additional variables that influence overall clinical course.

Methods

Patient Ascertainment and Enrollment

A total of 100 individuals from 94 families affected by AGS were recruited through the Myelin Disorders Bioregistry Project (MDBP) (IRB approved), an arm of the Global Leukodystrophy Initiative Clinical Trial Network (GLIA-CTN) at 3 institutions (Children's Hospital of Philadelphia, Istituto di Ricerca Clinica C. Mondino, and Spedali Civili of Brescia). AGS was defined as documented neurologic disability in the context of a change in an AGS-related gene: *TREX1, RNASEH2A, RNASEH2B, RNASEH2C, SAMHD1, ADAR1,* or *IFIH1* (Supplemental Table).This neurologic disability ranged from isolated spastic paraparesis to profound global developmental delay. Individuals without a known genetic diagnosis or those with insufficient developmental histories were excluded.

Data Extraction and Collection

Demographic information, including age at last evaluation and mortality, was collected (Table 1; Supplemental Table) (7).Date of acquisition of milestones was obtained from parental questionnaires and confirmed with medical record review. Microcephaly was

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identified in medical records and defined as more than two standard deviations from the mean for head circumference at the time of final data collection. Presenting information was extracted from concurrent medical records wherever feasible to minimize recall bias.

Standard functional scores, the Gross Motor Functional Classification Scale (GMFCS), the Manual Abilities Functional Classification Scale (MACS), and the Communication Function Classification Scale (CFCS), were assigned from medical records as previously described, at the last available clinical encounter (2, 8–11). These scales are composed of 5-levels with 'I' representing normal function and 'V' representing complete impairment. The Gross Motor Function Classification System (GMFCS) describes motor impairment (11). Children scored as 'I' are independently ambulatory without limitations, while children scored as a 'V' require full assistance including with head control. The Manual Ability Classification Scale (MACS) helps to score fine motor skills (10), with a 'I' representing normal dexterity and 'V' representing the requirement for total assistance. The Communication Function Classification System (CFCS) is used to classify communication skills (8). A CFCS score of 'I' represents effective communication as both sender and receiver, while a 'V' score represents severe impairment even between familiar partners.

Statistical analysis

Developmental milestones were grouped by genotype and compared to the normative data derived from the Denver Developmental Screening Test II (DDST-II) using the p-value log rank test (7). Skill acquisition was binned by percentage of the cohort who had attained a skill at a given age. In order to compare achievement of milestones by genotype, we created Kaplan-Meier curves for acquisition of developmental milestones. We included indicator variables for each genotype group to evaluate the independent effects of genotype.

Age of acquisition of milestones was also plotted, including the age at which percentiles of the population (p10 for 10%, p25 for 25%, etc) had achieved that specific milestone. These data were grouped by genotype and compared to the normative data derived from the Denver Developmental Screening Test II (DDST-II). The 1992 revision of the DDST, the DDST-II, has been validated across many populations of children with developmental disabilities and brain injuries (12). We selected specific items from the DDST-II to represent the range of skills observed in the AGS population.

Results

Cohort features and presentation

In total, 100 children were included in our international cohort (Table 1). Genotype, symptoms and age at presentation, and developmental milestone acquisition were extracted by retrospective chart review (Figure 1, Supplemental Table). The median age at final milestone collection was 59 months, with a range of 2 months to 34 years. Five individuals are known to be deceased at the time of last data collection, with a range of age at death of 3 months to 9 years. All deceased individuals were from the *TREX1* and *ADAR1* cohorts. *RNASEH2A*-related AGS (n=5) and *RNASEH2C*-related AGS (n=2) had insufficient numbers for inclusion in comparative analyses (Figure 1A).

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The age at presentation was calculated by genotype (Figure 1B). Within a genotype, there was notable heterogeneity of features and age at presentation. Children with *TREX1*-related AGS presented at a young age (average 1.0 months, range birth to 3 months). Children with *IFIH1*-related AGS had the widest range of age at first clinical symptom: birth to 96 months. Detailed information on presenting symptoms was available for 64 patients. Among all genotypes combined, children with microcephaly had a younger age of onset (mean 2.5 months versus 11.4 months, $p<0.001$).

The most common presenting features were altered mental status (extreme irritability or lethargy; n=41), feeding intolerance (n=40); and neurologic change (loss of gross motor skills and/or spasticity or dystonia, or seizures; n=49). Additionally, some individuals also presented with manifestations of systemic autoinflammation (e.g. fevers, skin involvement, feeding intolerance, hepatitis) (Figure 1C). Clinical onset was more likely to be characterized by acute neurologic decompensation in *ADAR* and *IFIH1* related AGS (p-value = 0.002 compared to other genotypes while *TREX1* and *RNASEH2B* were more likely to present with altered mental status (p-value = 0.0229). Of note, as children with *TREX1*-related disease presented at such a young age, it would be difficult to discern neurologic disability at that age. Systemic symptoms at onset were more likely in TREX1 and RNASEH2B versus other genotypes combined (p-value = 0.03; however, the difference between individual genotypes was not statistically significant (p = 0.083).

Children presenting with altered mental status are characterized by a lower age of onset (3.3 months compared to 9.6 months, p-value=0.011).There was not a statistically significant association between the presence of microcephaly and grouped or individual genotypes (p = 0.422 and 0.09, respectively).

Acquisition of developmental milestones—Kaplan-Meier curves characterized the percentage of children that attained a skill at a given age (Figures 2–5). Developmental milestone acquisition correlated with microcephaly, age at presentation, and presenting symptoms. There was a significant correlation between microcephaly and developmental skill achievement ($p<0.005$) for all assessed milestones (Figure 2).Similarly, earlier age of onset (before 6 and 12 months of age respectively) was associated with significantly worse outcomes regarding developmental milestones acquisition, even when adjusted for genotype in a Cox PH model (log-rank $p<0.005$, Cox PH $p < 0.02$).Children who presented with altered mental status, characterized as either sustained irritability or lethargy, had significantly decreased early milestone acquisition ($p<0.005$ except for babbling, p=0.97).

Given limitations in genotype incidence, we were able to perform sub-analysis for only 5 of the genotypes*: IFIH1, TREX1, RNASEH2B, SAMHD1,* and *ADAR* (Figure 3–5). Responsive smile was the most attained skill across the entire AGS population (Figure 5). *TREX1*-related AGS was the most severely affected, and this cohort was statistically distinct from the other AGS-genotypes for most skills including smiling, bringing hands together, achieving head control, babbling, and sitting without support ($p <0.005$). Children affected by variants in *SAMHD1, ADAR,* and *IFIH1* overall attained the most advanced development. As previously published, individuals with *RNASEH2B*-related AGS demonstrated a milder neurologic course compared to individuals with TREX1 mutations

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(2). The developmental trajectory of *RNASEH2B*-related AGS was compared to the combined cohort of individuals with *SAMHD1*, *IFIH1*, and *ADAR* mutations. These cohorts were distinct in head control ($p = 0.032$), rolling over ($p = 0.044$), standing alone ($p = 0.043$), and walking well ($p = 0.033$), all of which were less likely to be attained in *RNASEH2B*-related AGS.To better assess the developmental trajectory as a whole, the age of milestone acquisition was used to create plots of AGS-specific developmental skill acquisition (Figure 6). These provide a visual demonstration of skill acquisition in our AGS cohorts, for example, fewer than 20% of children with *TREX1*-related AGS achieve rolling over by the age 161 months and fewer than 45% achieve smiling. In *SAMHD1*-related AGS, individuals are more likely to achieve spoken language (6 words) and independent ambulation than in other genotypes.

Functional assessment scales—Finally, we applied retrospective functional assessment scales (GMFCS, MACS and CFCS) to the last available clinical encounter (Figures 7A and 7B).All but one child affected by *TREX1* scored a 'V' on the GMFCS, MACS, and CFCS scales. The other genotypes, *IFIH1, RNASEH2B, SAMHD1,* and *ADAR,* demonstrated significant functional diversity, with both mildly and severely impaired individuals within each genotype.

Conclusions

AGS is a genetically heterogeneous disorder caused by changes within the nucleic acid sensing machinery, resulting in sustained interferon production. While all children described to date with AGS demonstrate some degree of neurologic impairment, we confirm that the presentation and degree of disability is highly variable (Figures 2–7). This study contributes detailed developmental information to collective published works related to the neurologic features of AGS (2, 6, 13–15). In particular, the current work builds upon our understanding of overall neurologic outcomes in AGS through detailed developmental history analyses (6). As we move towards therapeutic development in AGS and clinical trial design, it is increasingly important to establish a deeper understanding of the neurologic trajectory of AGS.

To this end, we characterized the features of presentation and the overall developmental skill acquisition across the AGS genetic spectrum.As anticipated, we found that children across all genotypes had variable age of onset and presenting symptoms (Figure 1). This underscores one difficulty with early recognition and diagnosis of AGS as the presentation can be heterogeneous. Additionally, the ascertainment of presenting features is complicated by parental recall bias. Many children had delayed development prior to an AGS-specific clinical presentation, as previously described (6). We hypothesize that this pre-presentation period is a key window for potential future therapeutic interventions.

While this study supports genotype as a significant variable affecting neurologic outcomes in AGS, significant intra-genetic cohort variability (Figure 7) suggests the importance of other unidentified factors that may influence the age of presentation and overall outcome. We hypothesize that additional confounding variables may include environmental exposures

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(such as illnesses) and complementary genetic polymorphisms within the IFN pathway. The difficulty in accounting for these variables is a limitation of the current study.

As most individuals with AGS are currently identified because of a prominent CNS involvement, this study may overestimate severity of neurologic dysfunction of the ultimate population. Additionally, as part of the Global Leukodystrophy Initiative Clinical Trial Network across 3 child neurology divisions, our population is biased to include children with severe neurologic impairment. Given the variable involvement of other organ systems in AGS, it is possible that individuals with the same genotypes, but without CNS injury, have not been yet identified. For example, no individuals affected by Familial Chilblain Lupus from *TREX1* mutations were included in this cohort. This underscores the need for a better appreciation of the breadth of disease associated with AGS-related genes.

As an example of recruitment bias, we hypothesize that the differences in the developmental skill acquisition in the Italian versus the US cohorts may be due differences in ascertainment by diagnostics strategies. In Italy, the primary diagnostic method is targeted mutation or gene panel analysis, and the children selected for testing are typically “classic” severe presentations with microcephaly and/or intracranial calcifications. In the US, an increasing number of individuals are ascertained by whole exome sequencing for more non-specific symptoms. A more agnostic approach to genetic diagnosis leads to an expansion of phenotypes of AGS.

Overall, children with AGS reached milestones in the same linear developmental course as neurotypical children. Interestingly, there was a small cohort of children who were able to attain new skills for years beyond the expected age of acquisition (Figure 2–5). In our study, we identified 3 distinct cohorts: mild, intermediate, and severe impairment. The mild involvement category included children who were either walking independently or with assistive devices, or had normal speech with some floor mobility. Intermediate category patients have floor mobility through rolling, and some communication skills (includes babbling and language). Severe patients have no mobility, poor head control, and no communication beyond a social smile. These groups clustered by genotype, although genotype alone was not sufficient to predict the course in an individual patient. Individuals with AGS related to *SAMHD1, IFIH1,* and *ADAR* mutations achieve a greater number of developmental milestones compared to individual with *TREX1-related AGS*. *TREX1*-related AGS was statistically distinct from the other genotypes for most milestone acquisitions, and the *RNASEH2B* was similar to the milder cohort except for early fine motor function (bringing hands together and reaching), rolling over, and the acquisition of 6 words. Notably, individuals with *RNASEH2A* and *RNASEH2C* were not able to be included in these analyses due to low rates of enrollment and overall prevalence. This study is also limited in that it was retrospective and thus the results from functional outcome tools were largely unavailable.

From this study, we have a deeper understanding of the kinetics of development of children with AGS. This study also illustrates potential challenges in clinical trials in the AGS population. As the developmental trajectory of AGS is heterogeneous, it may be challenging to demonstrate clinical efficacy of any future interventions across a mixed population

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requiring careful consideration of variables including microcephaly, age of onset and genotype. Additionally, in individuals with the severe developmental phenotype, traditional measures of motor and cognitive performance may have significant floor effects. Future clinical studies may benefit from the creation of an AGS-specific scale, similar to what has been developed in other rare diseases (16, 17). Identifiable variables that may affect long-term outcome, such as age of onset, presenting symptoms, genotype, and microcephaly, should be considered in the design of future clinical trials with the selection of appropriate clinical endpoints. In this study, we began to explore the association between genotype and phenotype in this unique disorder. Future studies are needed to validate our findings and further understand AGS-genotype specific subpopulations.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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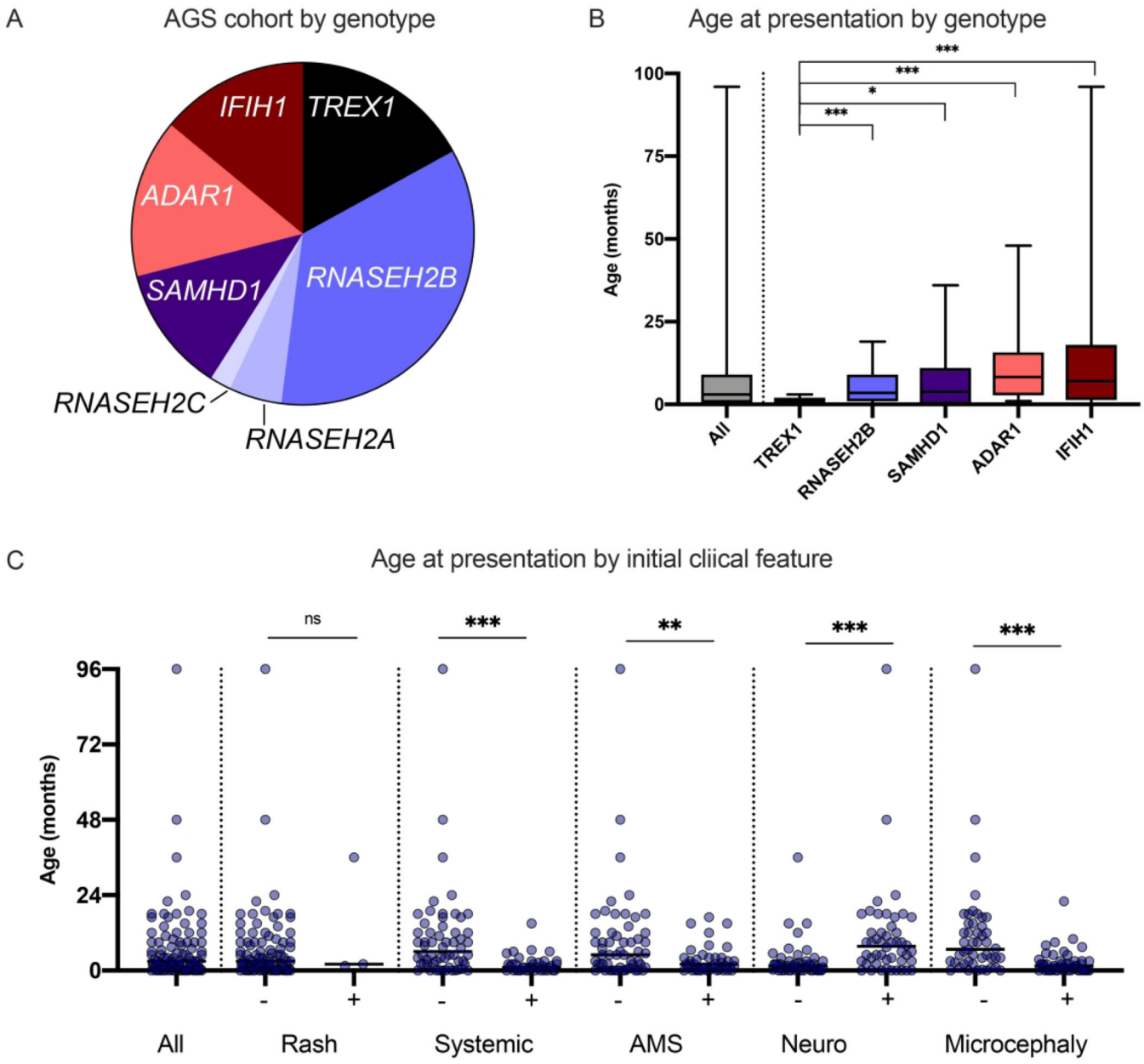

Figure 1.

Genetics and features at presentation of the US-Italian AGS cohort. A. Genotypic distribution of individuals with AGS across the US and Italian cohorts. B. The age at presentation in months is shown as sorted by genotype. The error bars represent min-max. Individuals with symptoms present at birth were noted as presenting at 0 months. C Features at presentation were classified into non-exclusive categories: neurologic symptoms [Neuro] (e.g. seizures, sudden change in tone, or developmental regression), altered mental status [AMS] (irritability or lethargy), systemic inflammation [Systemic] (e.g. feeding intolerance and fevers), rash [Rash], and Microcephaly. Clinical presentation is shown by age. PvalueswerecalculatedusingMann-Whitneytwo-tailedt-testsand designated as * = < 0.05, ** = < 0.01, and *** = < 0.005.

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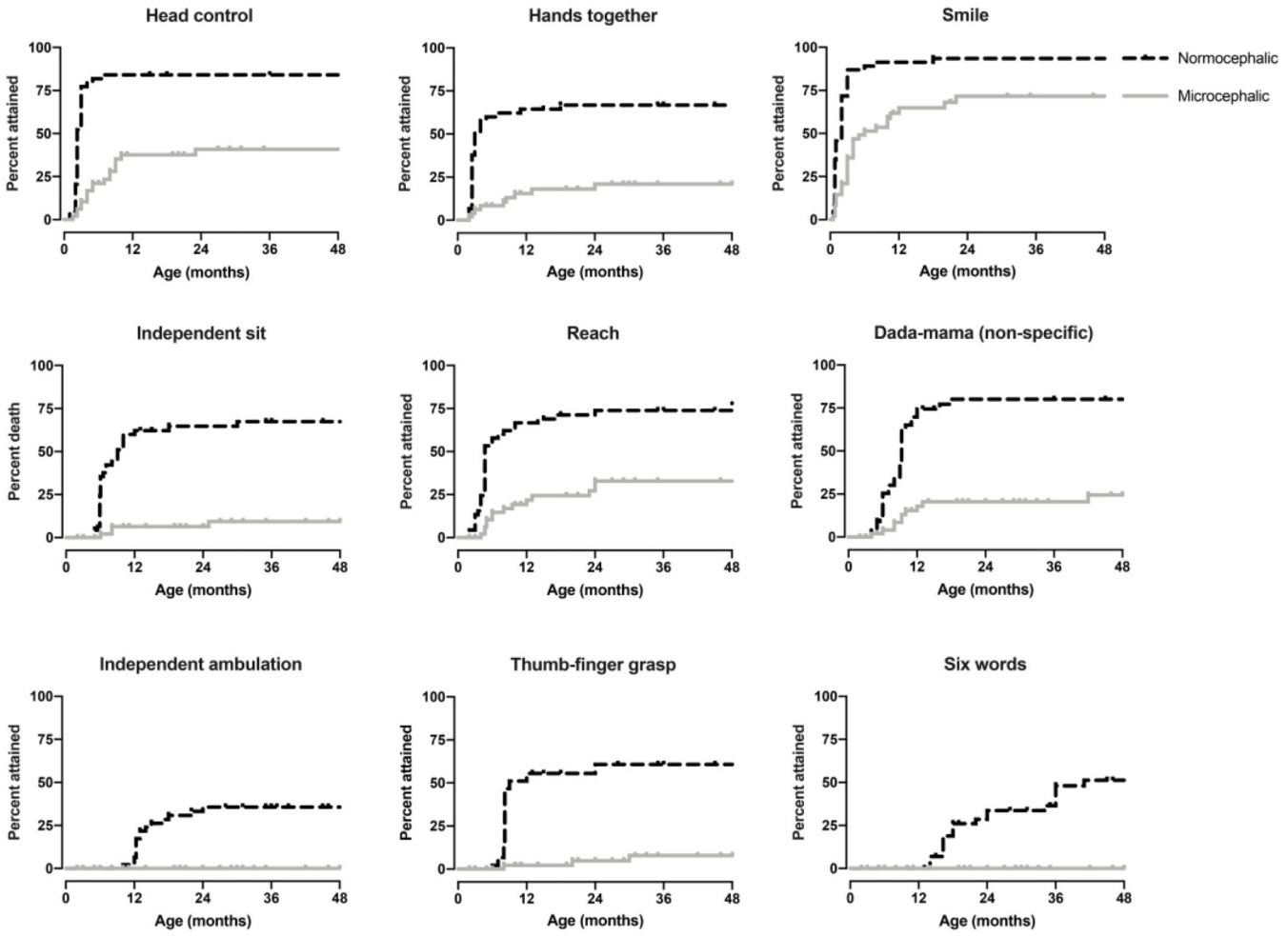

Figure 2.

Developmental milestone acquisition in microcephalic versus nonmicrocephalic individuals. Age at developmental skill acquisition was presented with Kaplan-Meier curves. P-values were calculated using the log-rank (Mantel-Cox) test comparing individuals with microcephaly (>2 standard deviations below the mean head circumference for age) to all other individuals and was <0.005 for all milestones.

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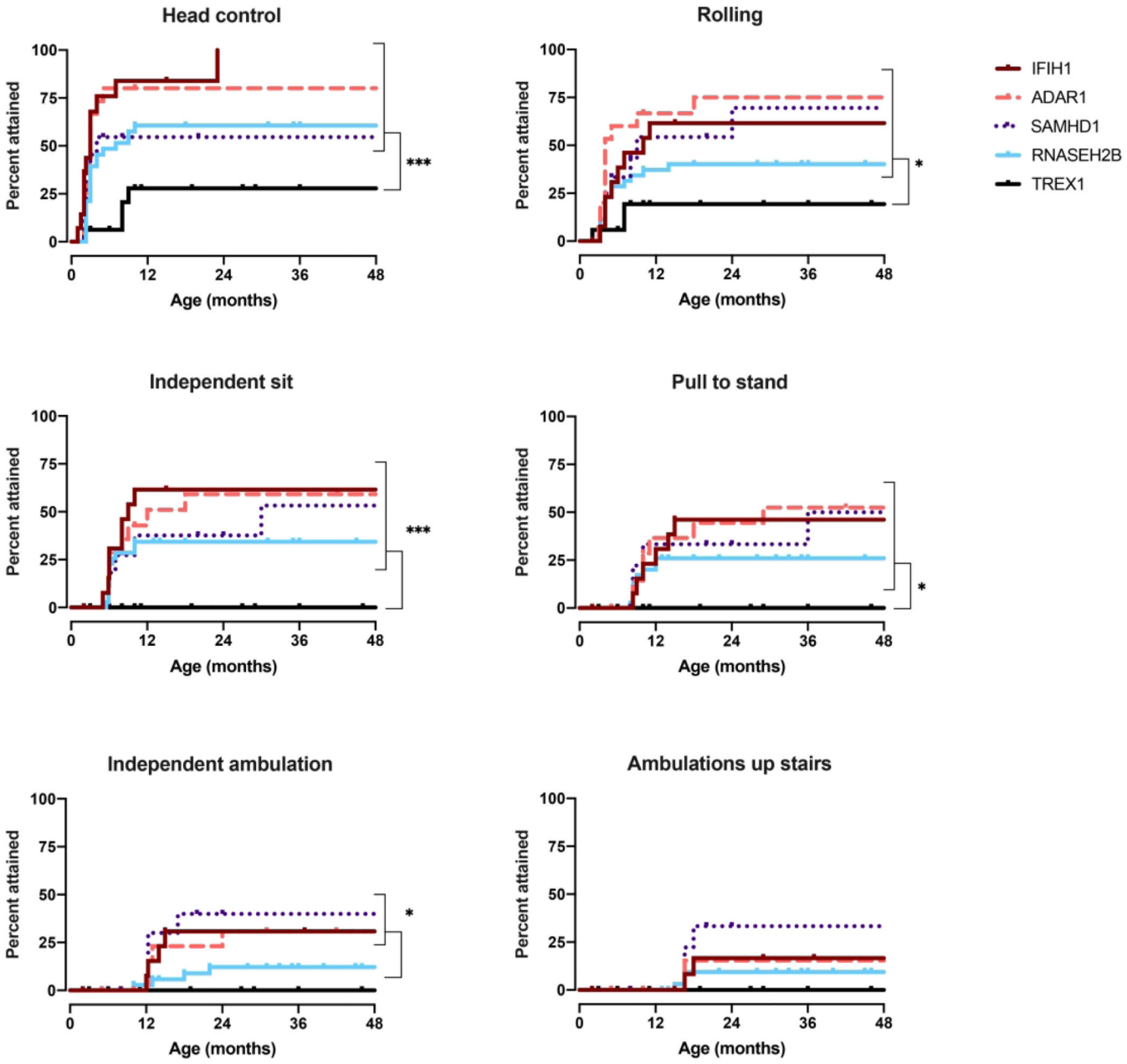

Figure 3.

Gross motor milestone acquisition by genotype. Age at developmental skill acquisition was determined for the 5most prevalent genotypes, IFIH1, TREX1, RNASEH2B, SAMHD1, andADAR, as represented with Kaplan-Meier curves. P-values were calculated using the Log-rank(Mantel-Cox)test comparing genotype sand is designated as * for $p<0.05$, ** for $p < 0.01$, and *** for $p < 0.005$.

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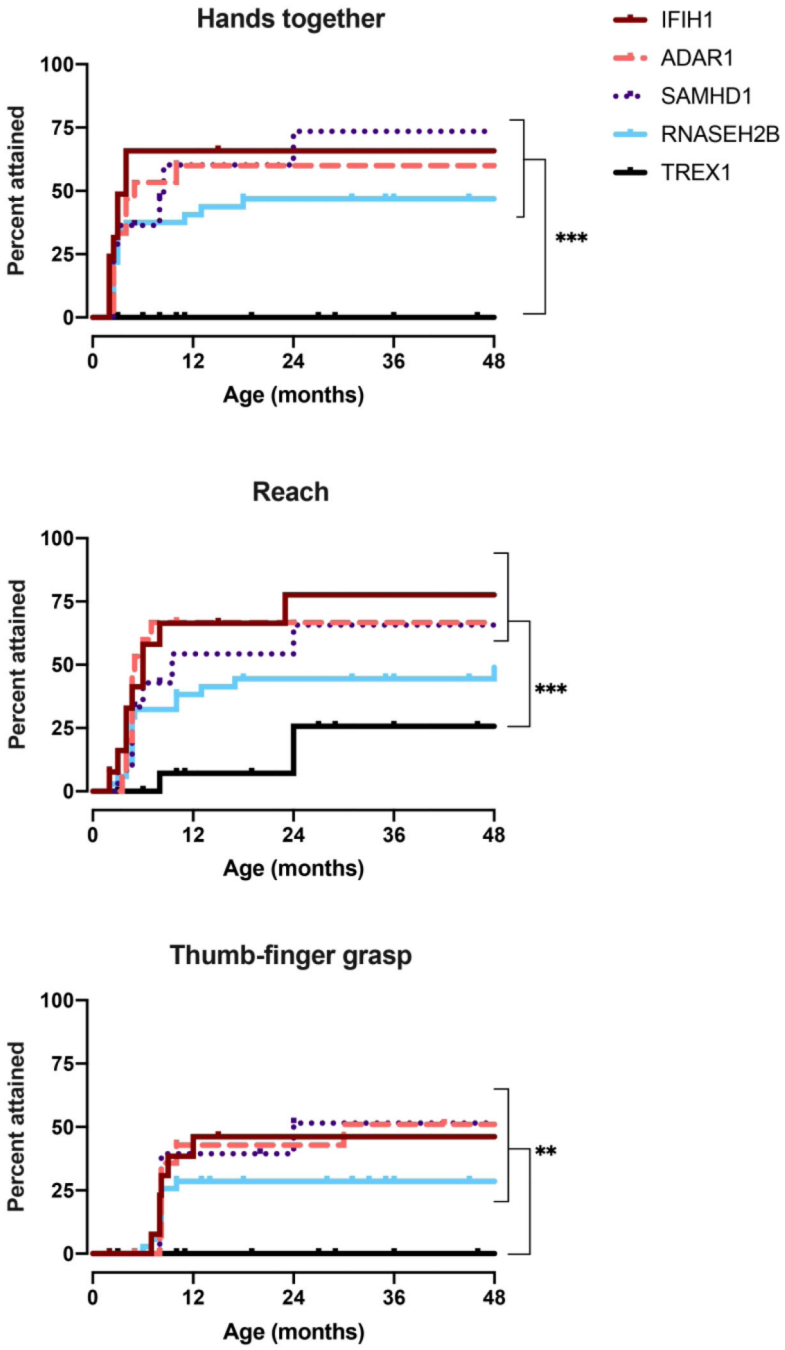

Figure 4.

Fine motor milestone acquisition by genotype. Age at developmental skill acquisition was determined for the 5 most prevalent genotypes, IFIH1, TREX1, RNASEH2B, SAMHD1, andADAR, as presented with Kaplan-Meiercurves. P-values were calculated using the Log-rank(Mantel-Cox)test comparing genotypes and is designated as * for $p<0.05$, ** for $p < 0.01$, and *** for $p < 0.005$.

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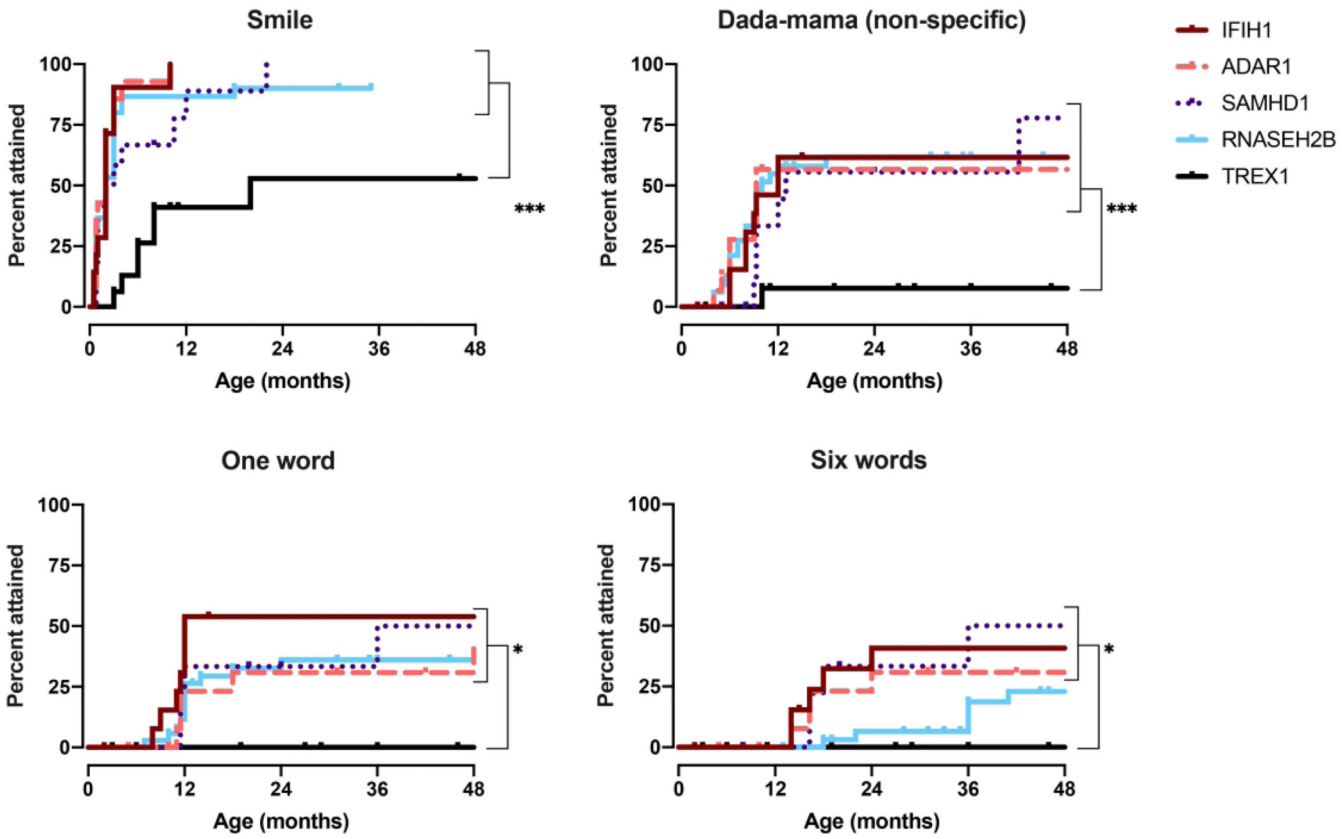

Figure 5.

Social and language milestone acquisition by genotype. Age at developmental skillacquisitionwasdeterminedforthe5mostprevalentgenotypes, IFIH1,TREX1, RNASEH2B,SAMHD1, andADAR, as presented with Kaplan-Meiercurves. P-values were calculated using the Log-rank(Mantel-Cox)test comparing genotypes and is designated as * for $p<0.05$, ** for $p < 0.01$, and *** for $p < 0.005$.

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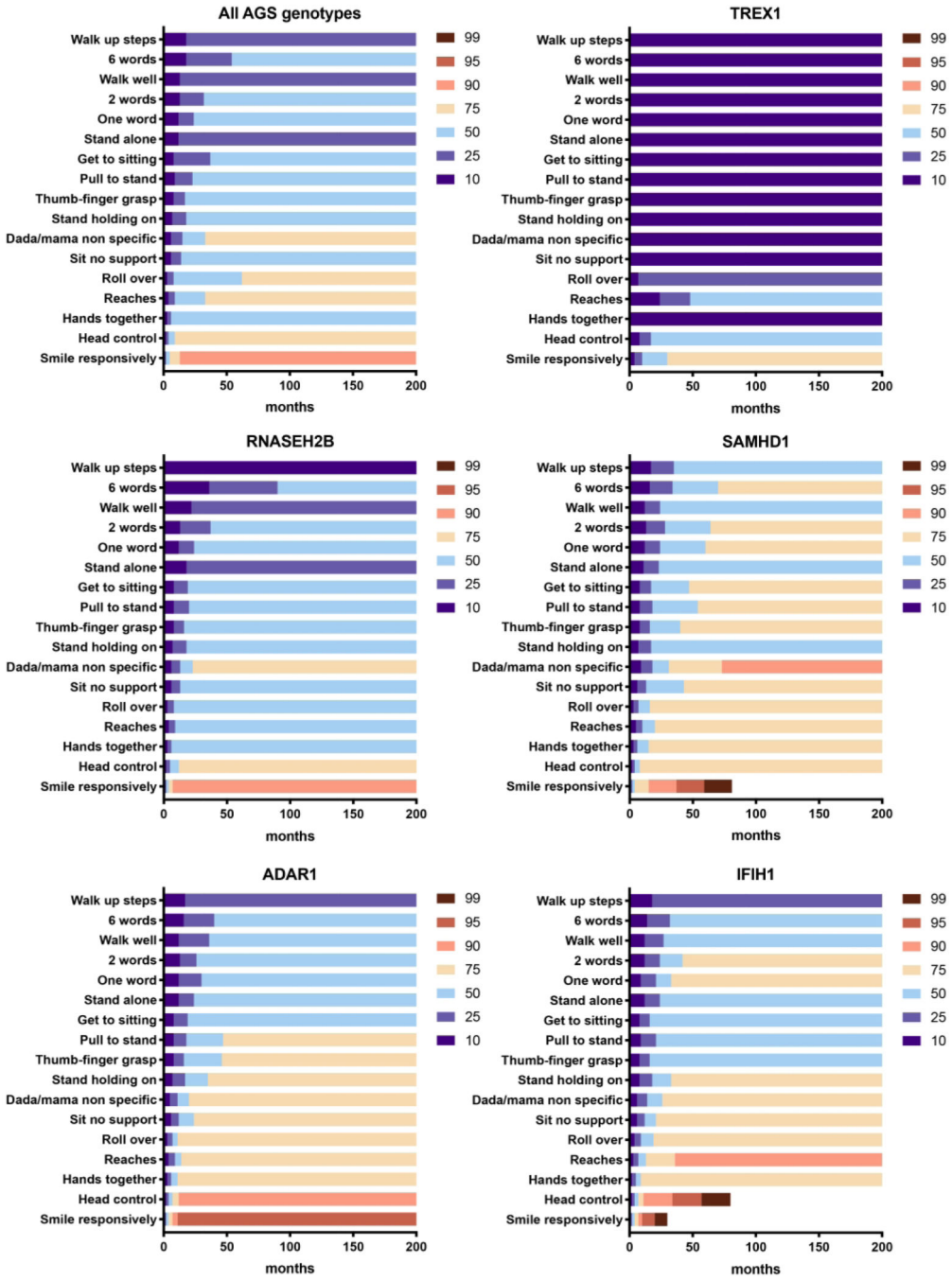

Figure 6.

Developmental skill acquisition plots were created for the overall AGS population and for the 5 most common genotypes, TREX1, RNASEH2B, SAMHD1, ADAR, and IFIH1. The designation of '10' represents less than 10% of the population has acquired a milestone by the age indicated on the x-axis, while the designation of '90' represents that up to 90% of the population has acquired the skill by the given age. The color-coded heat map indicates the percentage of the population who has attained the milestone at a given age, as shown on the x-axis.

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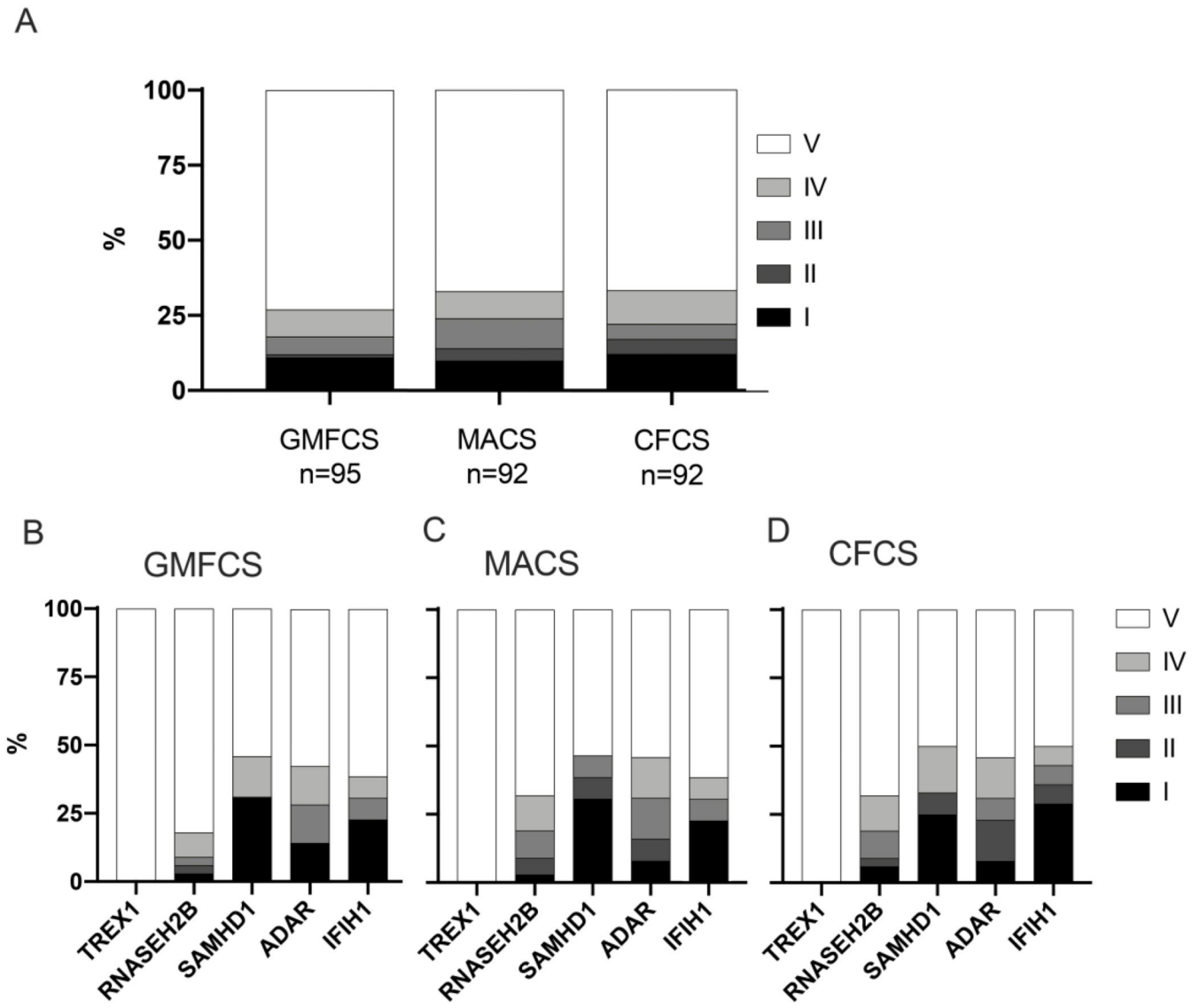

Figure 7.

A.Overall performance by retrospective functional assessment scales in the AGS population. The Gross Motor Function Classification System (GMFCS) describes motor impairment ('I' = independently ambulatory without limitations, 'V' = full assistance including with head control). The Manual Ability Classification Scale (MACS) helps to score fine motor skills ('I' = normal dexterity, 'V' = requirement for total assistance with manual tasks). The Communication Function Classification System (CFCS) is used to classify communication skills ('I' = effective communication as both sender and receiver, 'V' = severe impairment even between familiar partners).B-D. Performance by retrospective functional assessment scales in the AGS population by genotype.

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Table 1.

Demographic Information.

	Total N (% of all individuals)	Age at clinical presentation in months	Systemic symptoms at presentation % of genotype	Altered mental status at presentation % of genotype	Neurologic symptoms at presentation % of genotype	Microcephaly N (%)
All	100	6.9	40	41	49	51 (51)
TREX1	17 (17)	1	37.5	56.3	43.8	13 (81.3)
RNASEH2B	35 (35)	5.4	55.9	52.9	38.2	15 (42.8)
RNASEH2A	5 (5)	2.7	40.0	0.0	60.0	2 (40.0)
RNASEH2C	2 (2)	0.5	100.0	100.0	0.0	2 (100.0)
SAMHD1	12 (12)	7	41.7	50	41.7	7 (58.3)
ADAR	15 (15)	11.5	28.6	21.4	71.4	7 (46.7)
IFIH1	14 (14)	14.6	14.3	21.4	78.6	5 (35.7)

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Late-onset Aicardi Goutières syndrome: a characterization of presenting clinical features

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Abstract

Introduction: Aicardi Goutières Syndrome (AGS) is a genetic interferonopathy characterized by early onset of severe neurologic injury with intracranial calcifications, leukoencephalopathy, and systemic inflammation. Increasingly, a spectrum of neurologic dysfunction and presentation beyond the infantile period is being recognized in AGS. The aim of this study was to characterize late-infantile and juvenile onset AGS.

Methods: We conducted a multi-institution, retrospective review of individuals with AGS who presented over 1 year old, including medical history, imaging characteristics and suspected diagnoses at presentation.

Results: Thirty-four individuals were identified, all with pathogenic variants in *RNASEH2B*, *SAMHD1*, *ADAR1,* or *IFIH1*. Most individuals had a history of developmental delay and/or systemic symptoms, such as sterile pyrexias and chilblains, followed by a prodromal period associated with increasing symptoms. This was followed by an abrupt onset of neurologic decline (fulminant phase), with a median onset at 1.33 years (range 1.00–17.68 years). Most individuals

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Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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presented with a change in gross motor skills (97.0%), typically with increased tone (78.8%). Leukodystrophy was the most common MRI finding (40.0%). Calcifications were less common (12.9%).

Conclusions: This is the first study to characterize presentation of late-infantile and juvenile onset AGS and its phenotypic spectrum. Late-onset AGS can present insidiously and lacks classic clinical and neuroimaging findings. Signs of early systemic dysfunction prior to fulminant disease onset and loss of motor symptoms were common. We strongly recommend genetic testing when there is concern for sustained inflammation of unknown origins or changes in motor skills in children more than one year of age.

Keywords

Aicardi Goutières Syndrome; leukodystrophy; RNASEH2B; ADAR1; IFIH1; SAMHD1; type I interferonopathy; ADEM; spastic diplegia

Introduction

Aicardi Goutières Syndrome (AGS) is a rare genetic interferonopathy that results in heterogeneous neurologic injury. The neonatal (likely in utero) form is a mimic of congenital viral (TORCH) infections and is commonly linked with the *TREX1* genotype [1–5]. Early-infantile AGS typically presents weeks to months after birth, and is typically characterized by an acute encephalitic phase, followed by stabilization of neurologic function [4]. As the recognition of AGS has improved, a spectrum of neurologic dysfunction has been described beyond the early infantile period [1, 3, 6, 7]. Late-onset AGS, or 'atypical' AGS, poses a particular diagnostic challenge because it appears to be the most heterogeneous [8].

AGS is associated with by systemic auto-inflammation secondary to pathologic upregulation of the type I interferon-mediated immune system [9, 10]. There are currently 7 genes linked to AGS: *TREX1*, *RNASEH2A*, *RNASEH2B*, *RNASEH2C*, *SAMHD1*, *ADAR1*, and *IFIH1*. These genes are involved in intracellular nucleic acid metabolism. *TREX1, RNASEH2A/B/C,* and *SAMHD1* regulate nucleic acid maintenance and repair [11–15]. Pathogenic variants in these genes result in an accumulation of endogenous retro-elements, which triggers RNA/DNA sensing pathways and IFN activation [16–18]. *ADAR1* and *IFIH1* are part of a nucleic acid sensing pathway [19–21]. Regardless of the specific mechanism, all forms of AGS result in elevated interferon levels in the blood and cerebrospinal fluid, as measured indirectly by interferon signaling gene expression scores [4, 10, 22, 23]. Despite the common mechanistic pathway, outcomes and age at onset can be variable [1, 24, 25].

Neuroimaging plays a critical role in facilitating the diagnosis of AGS, although the incidence of individual features differs by the subpopulation studied. Brain calcifications, cerebral atrophy, and leukodystrophy are the most common findings [26]. Some gene-specific patterns have been described: *SAMHD1*-related intracerebral large vessel disease [7, 27], *ADAR1*-related bilateral striatal necrosis [9, 22], and *RNASH2B*-associated porencephalic cysts, cerebellar hypoplasia, and globus pallidus iron accumulation [28]. Notably, neuroimaging findings can change, and even improve, over time [29].

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Later onset AGS is poorly understood, having been primarily described in case reports. Some individuals with *ADAR1*-, *IFIH1*- and *RNASEH2B*-related disease present with non-syndromic spastic paraplegia [4, 30]. Other cases of *ADAR1*-related AGS manifest with severe, isolated dystonia [4, 9]. One individual with *IFIH1*-associated AGS presented with posturing, rigidity, and exaggerated startle [3]. These atypical cases can have variable clinical courses after presentation. Some AGS individuals do not experience developmental or cognitive regression with disease onset [3, 4]. These reports reveal the diversity of AGS, and more research needed to characterize the full phenotypic spectrum.

The aim of this study was to identify common misdiagnoses of late-onset AGS and to characterize presenting signs and symptoms within a retrospective natural history study. Our aim was to provide clinicians with information to appropriately consider AGS during initial evaluation of children with neurologic regression. We hypothesized that there are particular clinical or neuroradiographic characteristics that providers can use to appropriately evaluate individuals for AGS.

Methods

Subject Ascertainment and Enrollment

Individuals were identified through the Myelin Disorders Bioregistry Project, an arm of the Global Leukodystrophy Initiative Clinical Trial Network (GLIA-CTN) from several institutions (Children's Hospital of Philadelphia, Spedali Civili of Brescia, and Istituto di Ricerca Clinica C. Mondino). The study was approved by the Institutional Review Board. AGS was defined as a combination of neurologic disability with a pathogenic/likely pathogenic variant in an AGS-related gene. Individuals without a known genetic diagnosis or with insufficient clinical diagnostic information were excluded. Individuals diagnosed with spastic paraparesis and other subforms of AGS were included. From total of 231 individuals, we identified 34 individuals from 32 families presenting after the age of twelve months.

Retrospective history collection

Genotype, ages, prodromal history, fulminant presenting symptoms, MRI features, and initial differential diagnoses were collected through retrospective chart review. Charts with insufficient details and MRIs obtained greater than 1 year from the initial symptoms were excluded from analysis. Pathology-targeting pharmacologic treatments, such as immunomodulatory agents, and responses to treatment were noted. Age at diagnosis was defined as the date that genetic confirmation was obtained.

Early AGS-related symptomatology was divided into three categories: early phase, prodrome, and fulminant presentation. The early phase was defined as non-specific signs of abnormal growth or development in an otherwise healthy child. This category included isolated or rare inflammatory symptoms. The prodrome was defined as an increased burden of recurrent or persistent symptoms associated with inflammation prior to fulminant presentation. Systemic symptoms were noted and included fevers, fatigue, irritability/colic, myalgias/fatigue and chilblains. Fulminant symptoms were defined as new observable neurologic deficits.

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The first magnetic resonance imaging (MRI) results obtained within the first two years of disease were included for review (n=31). Original MR images for 13 individuals were reviewed by a pediatric neuroradiologist, and otherwise the original radiology reports were used. One individual had MR images obtained prior to symptom onset for unrelated reasons.

Statistical analysis

Analyses were performed in GraphPad Prism 8. Demographic, clinical and genetic features were described with descriptive statistics.

Results

Cohort Features

A total of 34 individuals (17 males) were identified from our international cohort. This included individuals with variants in *RNASEH2B* (n=14), *SAMHD1* (n=5), *ADAR1* (n=10), and *IFIH1* (n=5) (Table 1). Twenty individuals were diagnosed by targeted genetic panel (58.8%) and fourteen by exome sequencing (41.2%).

Clinical Presentation of Atypical AGS

Based on clinical experience, early AGS-related symptomatology was divided into three categories: early phase, prodrome, and fulminant presentation (Figure 1). The majority of individuals with atypical AGS had a history of AGS-related complications prior to the onset of disease (n=20 of 29 with available histories, 69.0%%), beginning between birth and 4.76 years of age (median 0.81 years). Common features during this early phase included developmental delay (n=13/29, 44.8%), often accompanied by systemic signs or symptoms of inflammation (n=6/29, 20.7%), including sterile pyrexia, unexplained hypothermia, chronic pain, persistent irritability, or chilblains (Figure 2A).

After the early phase, most individuals with atypical AGS demonstrated increasing features of inflammation during a “prodromal” period (n=18/25 individuals with available clinical information, 72.0%) (Figure 2B). The median age of onset of prodromal symptoms was 1.25 years (range 0.83–17.00 years). Systemic prodromal features included febrile illness (n=11/25, which included infection-related episodes in two cases), irritability (n=7), fatigue (n=3), chilblains (n=2), and myositis (n=1).

Following a period of worsening prodromal symptoms, 33 of 34 individuals with atypical AGS experienced a fulminant period of neurologic decline with a sufficiently detailed course for analysis. The median age of fulminant disease onset was 1.33 years (range 1.00–17.68 years) (Figure 2D). At the time of disease onset, most individuals (n=32 of 33, 97.0%) presented with changes in gross motor function, accompanied by a change in tone in 26 individuals (78.8%). Twenty-one individuals were noted to have new onset of spasticity, eight had dystonia, including three individuals affected by both spasticity and dystonia. Thirteen individuals (39.4%) presented with a change in communication skills, including loss of vocabulary (n=8), decreased speech production (n=9), or dysarthria (n=5). Cranial nerve deficits (n=2) and ischemic strokes (n=2, both associated with Moya Moya vasculopathy) were also observed. Ten individuals (30.3%) presented with systemic features,

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including sterile pyrexia (n=4), feeding intolerance (n=1), and myalgias (n=2). Three patterns of clinical decline during the fulminant period were identified: acute (nadir within 1 week, n=5), subacute (1–4 weeks to reach nadir, n=11), and chronic (nadir reached over 4 weeks after fulminant onset, n=13) (Figure 2E). The remainder of patterns of decline were unknown.

To evaluate the time to diagnosis in later-onset, atypical AGS, we collected information on the time from disease onset to medical evaluation. The median time from fulminant symptom onset to first medical encounter was 4 days (range 0 – 197 days). The median time from fulminant symptom onset to first MRI was 33 days (range 0 days - 2 years) and the time to AGS diagnosis was 1.25 years (median, range 0 – 20 years).

Next, we evaluated the process of diagnosis in atypical AGS cases. The most common (non-mutually exclusive) diagnoses included congenital spastic diplegia or cerebral palsy (n=4), acute demyelinating encephalomyelitis (ADEM) (n=4), and post-viral encephalitis (n=4). Other considerations included non-specified leukodystrophy (n=9), metabolic/mitochondrial disease (n=6), non-specified genetic syndrome (n=2), and behavioral disturbance (n=1). Two individuals had initial symptoms that were attributed to orthopedic injuries (transverse tibial fracture and hyperextension deformity of the knee).

The results from early cerebral imaging was available for review in the majority of cases (n=31), with images available for 13 cases (Figure 3). These images were obtained a median of 0.1 years after the onset of fulminant symptoms (range 0.0–2.0 years). White matter abnormalities (n=16, 51.6%) were the most commonly noted radiographic feature within our atypical AGS cohort. This included delayed myelination (*ADAR1*, n=2; *IFIH1*, n=3; *RNASEH2B,* n=3), large multifocal lesions (n=8), and small focal abnormalities (Figure 3E, n=5). Of note, the imaging of one individual demonstrated multiple large lesions as well as a small focal parietal lesion. The localization of white matter injury was variable, but was predominantly supratentorial involving the periventricular region and deep matter. One individual demonstrated near-confluence of the white matter changes (Figure 3D).

The common features of neonatal AGS were largely absent on these initial images. Only 4 individuals (12.9%) had evidence of calcifications (Figure 3A–B), and no individuals were noted to have cystic lesions. As assessed by T2 hyperintensity, involvement of the thalamus (*ADAR1*, n=1; Figure 3F) and basal ganglia (*ADAR1*, n=7; *RNASH2B*, n=1, *SAMHD1*, n=2) was noted in a subset of individuals. Nine individuals (29.0%) were noted to have mild ventriculomegaly (Figure 3C). Three individuals with *SAMHD1*-related disease were noted to have vascular involvement: two with Moya Moya syndrome with infarcts and another individual with MCA-ICA stenosis with possible calcification of the vessels. The most common radiographic diagnoses from the original reports were leukodystrophy (n=14 of 25 available reports, 56.0%), metabolic disorders (n=7, 28.0%), and ADEM or encephalitis not otherwise specified (n=2 each, 8.0%).

Four individuals with presumed neuroinflammatory disease received immunomodulatory therapy within one month of their fulminant presentation: intravenous (IV) steroids followed by enteral steroids (n=2), IV steroids with intravenous immunoglobulin (IVIG) (n=1), and

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oral steroids alone (n=1). While all 4 individuals showed partial improvement, none of these individuals had a resolution of symptoms with these immunomodulatory approaches.

Discussion

Aicardi Goutières Syndrome is a genetic autoinflammatory leukoencephalopathy with significant phenotypic variability [1, 4, 25, 31]. The goal of our study was to characterize the atypical, later onset cases within our international cohort. Our results suggest that the signs and symptoms AGS often predate fulminant neurologic decline. This has important implications related to the timing of future clinical interventions and underscores the need for early screening platforms, such as newborn screening [2]. The first 'early' phase was characterized by chronic symptoms including global developmental delay or subtle symptoms of systemic inflammation; the prodromal phase was characterized by worsening systemic inflammatory symptoms.

The fulminant neurologic regression was characterized by the loss of motor skills often accompanied by a change in language function and a history of subtle systemic symptoms, including sterile pyrexias, myalgias, and GI intolerance. These features were often different from the classic systemic features noted in the neonatal and infantile forms of the disease, which include altered mental status, irritability, seizures, failure to thrive, hepatitis, or blood dyscrasia [32]. In contrast to prior studies characterizing AGS as an acute or subacute process [4, 32], half of our cohort developed worsening neurologic function over a period of time longer than a month.

One of the most common misdiagnoses in the atypical AGS population was ADEM. Of note, none of the individuals within our cohort diagnosed with ADEM had altered mental status (including irritability or confusion) at the time of fulminant presentation, which is part of the diagnostic criteria for ADEM. Another common misdiagnosis was congenital spastic diplegia, despite the fact that disease onset developed after one year of life.

The imaging findings within this atypical AGS cohort were diverse, but most demonstrated evidence of white matter involvement. Classic early-onset AGS findings of calcifications and cerebral atrophy were noted in minority of MR images. We hypothesize that this may be due to the proximity of the imaging to the onset of fulminant symptoms. Only one of our atypical cases was noted to have bilateral striatal necrosis at disease onset, as has been previously described with *ADAR1*-related AGS [9]. This underscores the need to more broadly consider AGS regardless of the MRI features noted at onset. This study focused on the retrospective information up to the time of fulminant disease onset, and serial, subsequent imaging was not reviewed. Future prospective studies will be better suited to the characterization of the evolution of imaging findings after disease onset.

The individuals in this cohort treated with immunomodulatory therapies demonstrated an incomplete and transient improvement. These immunomodulatory therapies did not result in a sustained improvement in neurologic function or a change in the neurologic trajectory associated with AGS. In the first months after fulminant disease onset, individuals with AGS can demonstrate fluctuating neurologic function [25]. We hypothesize that the improvement

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attributed to the immunomodulatory therapies may have been due to natural fluctuations in the disease.

Our study has several limitations. The inclusion criteria may have excluded atypical cases presenting at less than one year of age. As our cohort is also limited by ascertainment bias, we hypothesize that even further diversity in the AGS-spectrum exist as part of an AGS genes-related type I interferonopathy spectrum. Our study is also limited by the available clinical information from provider notes. For example, typically only MR images were obtained in the early phase of disease, while CT images, which are more sensitive to calcifications, were not available in the majority of subjects. Additionally, early diagnostic testing results, including cerebrospinal fluid cell counts and markers of inflammation were not consistently available across the cohort. Despite these limitations, we were able to demonstrate that later, atypical AGS cases have a pattern of evolution: from early subtle symptoms, to worsening systemic inflammation, and culminate in neurologic decline. This encompasses subtypes previously classified as spastic paraparesis. As such we would recommend consideration of AGS in the differential diagnosis for young children presenting clusters of AGS-associated symptoms of systemic inflammation, particularly when accompanied by a change in neurologic function.

This is the first study to characterize late-onset AGS and underscores the broad phenotypic and radiographic spectrum found in this disorder. Early recognition is critical, particularly as new, targeted therapeutic options are developed. We encourage practitioners to consider AGS in their broader list of potential diagnoses and within their comprehensive diagnostic evaluation.

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LA: Serves on the MLD Foundation Medical/Scientific Advisory Board and the CureMLD Scientific Advisory Board. She is a consultant to Orchard Pharmaceutical.

DT: Serves on the ULF Medical/Scientific Advisory Board Provided scientific advisory services to Ionis.

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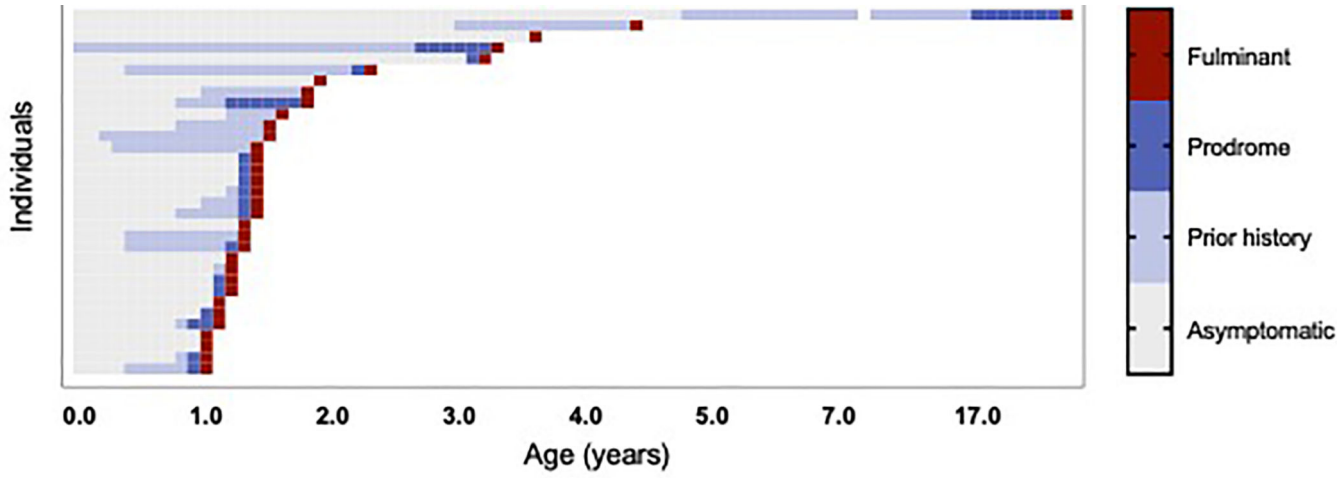

Figure 1.

Progression of disease in atypical AGS cases is characterized by 3 phases: early phase, prodrome, and fulminant presentation. Each individual (n=33 with sufficient data) is represented by a row, with each phase of the disease color coded with age in years on the x-axis. Normal development with no systemic signs or symptoms of inflammation represented by grey boxes, early phase is represented by light blue; prodromal phase is represented by blue; the onset of fulminant symptoms is represented by a red box.

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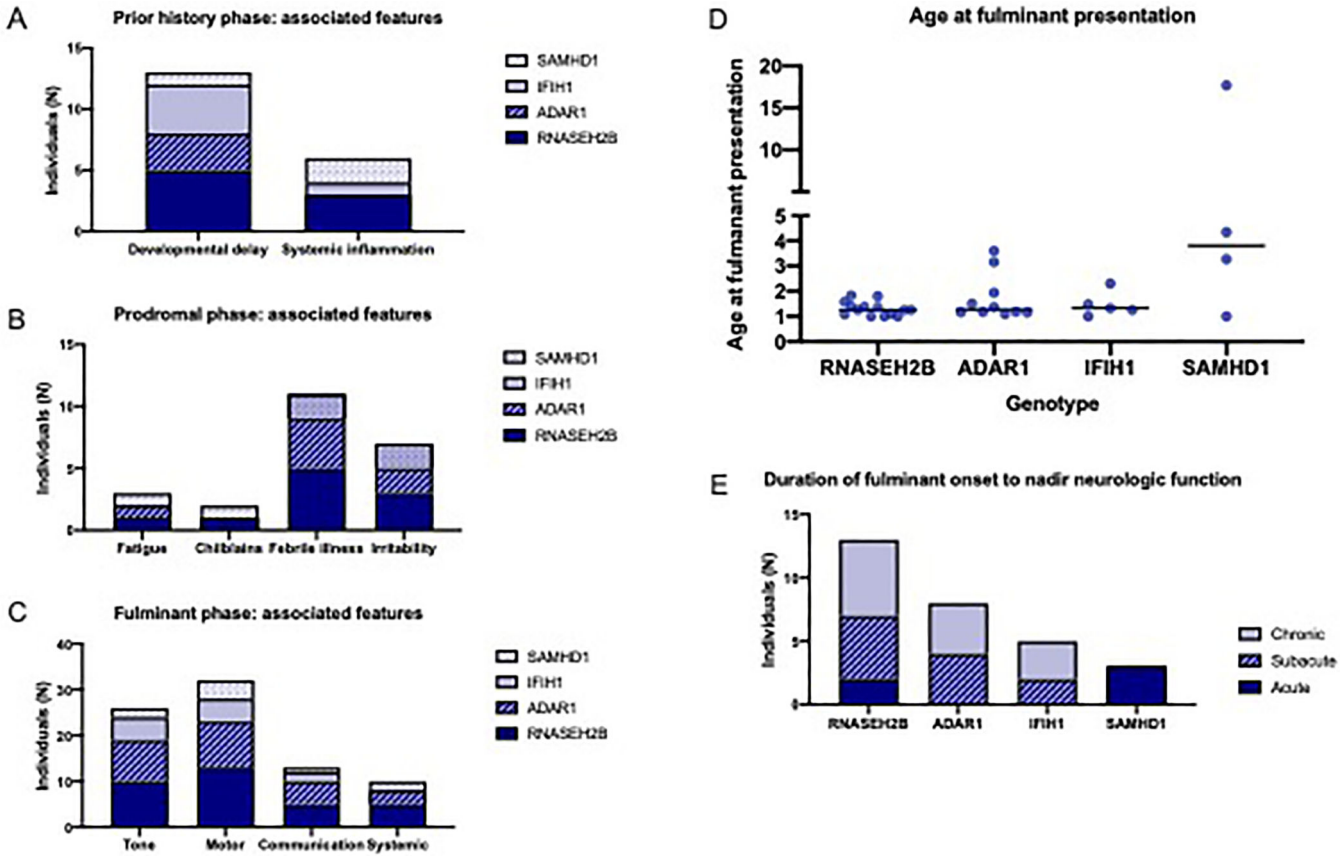

Figure 2.

Common features of the phases of atypical AGS by genotype. The most common clinical features during each phase were noted by genotype in non-exclusive categories. (A) In the early phase, developmental delay and systemic inflammation were most commonly noted; (B) in the prodrome phase, fatigue, irritability, sterile pyrexias (febrile illnesses), and chilblains were noted; while in the fulminant phase (C), abnormalities in tone, motor skills, communication, and systemic signs or symptoms were most common. (D) The age at fulminant presentation is presented by genotypic cohort. (E) The time to neurologic nadir (maximum symptoms) was categorized as acute (within a week), subacute (1 week to 1 month), or chronic (more than 1 month).

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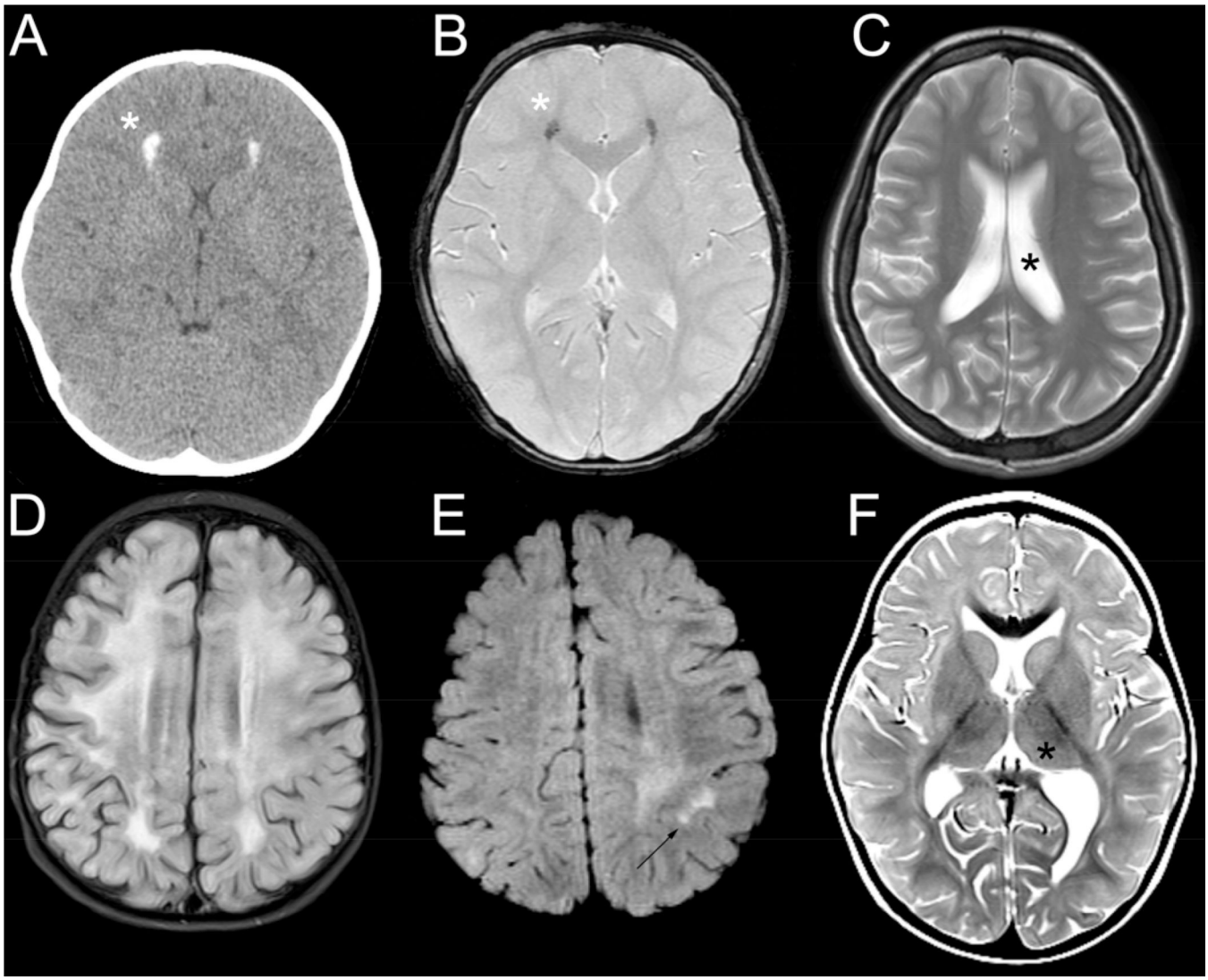

Figure 3.

Imaging features found in atypical AGS. Imaging findings include calcifications (*) as visualized by head CT (A) and GRE (B) as demonstrated in the same individual, mild ventriculomegaly on T2 imaging (*, C), diffuse white matter changes as demonstrated by T2 FLAIR hyperintensity (D), patchy white matter hyperintensities (T2 FLAIR, arrow, E), and basal ganglia and thalami involvement on T2 (*).

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Table 1:

Atypical AGS cohort genotypes

Genotype	Nucleotide #1	Protein #1	Inheritance	Nucleotide #2	Protein #2	Inheritance	Method of diagnosis
ADAR1	c.3019G<A	p.Gly1007Arg	de novo				ES
	c.3019G<A	p.Gly1007Arg	de novo				ES
	c.982C<T	p.Arg328*	UK	c.577C<G	p.Pro193Ala	paternal	ES
	c.577C<G	p.Pro193Ala	maternal	c.3020-3C<G		paternal	ES
	c.3019G<A	p.Gly1007Arg	UK				Targeted/panel
	c.3443+1G<C		maternal	c.577C<G	p.Pro193Ala	paternal	ES
	c.3019G<C	p.Gly1007Arg	UK				Targeted/panel
	c.577C<G	p.Pro193Ala	maternal	c.1076_1080del		paternal	Targeted/panel
	c.3019G<A	p.Gly1007Arg	de novo				Targeted/panel
	c.3463C<T	p.Arg1155Trp	maternal	c.577C<G	p.Pro193Ala	paternal	ES
IFIH1	c.2336G<A	p.Arg779His	paternal				Targeted/panel
	c.2336G<A	p.Arg779His	paternal				ES
	c.1165G<A	p.Gly389Arg	de novo				ES
	c.992C<G	p.Thr331Arg	de novo				Targeted/panel
	c.2335C<T	p.Arg779Cys	UK				Targeted/panel
RNASEH2B	c.529G<A	p.Ala177Thr	maternal	c.529G<A	p.Ala177Thr	paternal	ES
	c.529G<A	p.Ala177Thr	UK	c.132T<A	p.Cys44*	paternal	Targeted/panel
	c.554T<G	p.Val185Gly		c.554T<G	p.Val185Gly		Targeted/panel
	c.529G<A	p.Ala177Thr	UK	c.529G<A	p.Ala177Thr	UK	ES
	c.526G<A	p.Ala177Thr	maternal	c.526G<A	p.Ala177Thr	paternal	ES
	c.529G<A	p.Ala177Thr	UK	c.529G<A	p.Ala177Thr	UK	ES
	c.529G<A	p.Ala177Thr	maternal	c.510+1G<A		paternal	ES
	c.529G<A	p.Ala177Thr	maternal	c.510+1G<A		paternal	ES
	c.529G<A	p.Ala177Thr	UK	c.529G<A	p.Ala177Thr	UK	Targeted/panel
	c.529G<A	p.Ala177Thr	UK	c.529G<A	p.Ala177Thr	UK	Targeted/panel
	c.529G<A	p.Ala177Thr	maternal	c.529G<A	p.Ala177Thr	paternal	Targeted/panel
	c.529G<A	p.Ala177Thr	maternal	c.529G<A	p.Ala177Thr	paternal	Targeted/panel
	c.253C<G	p.Leu85Val	maternal	c.65-13G<Ar.65-11_65-1ins	p.Glu22Valfs*5	paternal	Targeted/panel
	c.529G<A	p.Ala177Thr	UK	c.529G<A	p.Ala177Thr	UK	Targeted/panel
SAMHD1	Exon 1 deletion		UK	Exon 1 deletion		UK	Targeted/panel
	Exon 1 deletion		maternal	Exon 1 deletion		paternal	Targeted/panel
	c.490C<T	p.Arg164*	UK	Deletion (Exons 10–13)		UK	Targeted/panel
	Exon 1 deletion	maternal	Exon 1 deletion		paternal		Targeted/panel
	Exon 1 deletion		UK	Exon 1 deletion		UK	Targeted/panel

UK: unknown; ES: exome sequencing

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Genetic and phenotypic spectrum associated with IFIH1 gain-of-function

A full list of authors and affiliations appears at the end of the article.

Abstract

IFIH1 gain-of-function has been reported as a cause of a type I interferonopathy encompassing a spectrum of autoinflammatory phenotypes including Aicardi–Goutières syndrome and Singleton Merten syndrome. Ascertaining patients through a European and North American collaboration, we set out to describe the molecular, clinical and interferon status of a cohort of individuals with pathogenic heterozygous mutations in *IFIH1*. We identified 74 individuals from 51 families segregating a total of 27 likely pathogenic mutations in *IFIH1*. Ten adult individuals, 13.5% of all mutation carriers, were clinically asymptomatic (with seven of these aged over 50 years). All mutations were associated with enhanced type I interferon signaling, including six variants (22%) which were predicted as benign according to multiple in silico pathogenicity programs. The identified mutations cluster close to the ATP binding region of the protein. These data confirm variable expression and nonpenetrance as important characteristics of the *IFIH1* genotype, a consistent association with enhanced type I interferon signaling, and a common mutational mechanism involving increased RNA binding affinity or decreased efficiency of ATP hydrolysis and filament disassembly rate.

Keywords

Aicardi–Goutières syndrome; IFIH1; MDA5; Singleton Merten syndrome; Type I interferonopathy

1 | INTRODUCTION

In 2014, heterozygous gain-of-function mutations in *IFIH1* were reported to cause a spectrum of neuroimmune phenotypes including classical Aicardi–Goutières syndrome (AGS; Oda et al., 2014; Rice et al., 2014). *IFIH1* encodes interferon-induced helicase C domain-containing protein 1 (IFHI1; also known as melanoma differentiation associated

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CONFLICT OF INTERESTS

Y. J. Crow has undertaken consultancy work with Biogen on behalf of the University of Edinburgh.

DATA AVAILABILITY STATEMENT

Data available on request due to privacy/ethical restrictions. Identified variants submitted to ClinVar (Submission ID: SUB6667166; Organization ID: 507341).

Gillian I. Rice and Sehoon Park equally contributed to this study.

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gene 5 protein: MDA5) which senses viral double-stranded (ds) RNA in the cytosol, leading to the induction of a type I interferon-mediated antiviral response. Consequent to Mendelian determined gain-of-function, it is suggested that IFIH1 inappropriately senses self-derived nucleic acid as viral, leading to an autoinflammatory state classified as a type I interferonopathy (Ahmad et al., 2018; Crow & Manel, 2015). In 2015, a p.Arg822Gln substitution in IFIH1 was shown to cause Singleton Merten syndrome (SMS), an autosomal dominant trait variably characterized by a deforming arthropathy, abnormal tooth development and cardiac valve calcification, again in association with enhanced type I interferon signaling (Rutsch et al., 2015). Although it was initially considered that SMS was a distinct, mutation-specific disorder, subsequent reports indicate that SMS and the neuroinflammatory phenotypes seen in the context of IFIH1 gain-of-function constitute part of the same disease spectrum (Buers, Rice, Crow, & Rutsch, 2017; Bursztejn et al., 2015).

Type I interferonopathy associated *IFIH1* mutations are either absent from control databases, or only present at very low frequency. However, we have noted previously that in silico algorithms are not always reliable in differentiating *IFIH1* disease-causing variants from benign polymorphisms (Ruaud et al., 2018). Such difficulty in assigning molecular pathogenicity is compounded by marked variability in disease expression, sometimes even within the same family, and the observation of complete non-penetrance in certain pedigrees (Rice et al., 2014). Given this background, we considered it important to provide an update of our experience of sequencing individuals for pathogenic *IFIH1* mutations associated with a type I interferonopathy state. In total, we describe molecular and clinical data relating to 74 individuals from 51 families, identifying 27 likely pathogenic mutations that cluster close to the ATP binding region of the protein. Our data confirm variable expression and nonpenetrance as important characteristics of these mutant genotypes, and the consistent association with enhanced type I interferon signaling as assessed by interferon-stimulated gene (ISG) expression, referred to as the interferon score.

2 | MATERIALS AND METHODS

2.1 | Subjects

Patients were ascertained through direct contact and/or collaborating physicians across clinical research laboratories in the UK and France (Crow), the USA (Vanderver), and Italy (Orcesi). The study was approved by the Leeds (East) Research Ethics Committee (10/H1307/132), the Comite de Protection des Personnes (ID-RCB/EUDRACT: 2014-A01017-40), IRB study protocol (Myelin Disorders Bioregistry Project: IRB# 14-011236) and the local ethics committee of the IRCCS Mondino Foundation, Pavia, Italy (3549/2009 of 30/9/2009 and 11/12/2009; n.20170035275 of 23/10/2017). Amino acid substitutions were considered as pathogenic mutations when they were seen in the context of a neuroimmune/autoinflammatory state (including AGS, a spastic-dystonic syndrome, nonsyndromic spastic paraparesis or SMS), and when two or more of the following applied: observation of the same variant in an unrelated family; de novo occurrence; documented increase in ISG expression; in vitro data consistent with IFIH1 gain-of-function.

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2.2 | Mutational analysis

Mutations were identified on a variety of next-generation sequencing platforms. Where Sanger sequencing was undertaken, primers were designed to amplify the coding exons of *IFH1*, with mutation annotation based on the reference cDNA sequence NM_022168.2. Variants were assessed using the in silico programs SIFT (http://sift.jcvi.org), Polyphen2 (http://genetics.bwh.harvard.edu/pph2/), and CADD (https://cadd.gs.washington.edu), summarized in VarCards (http://varcards.biols.ac.cn/). Population allele frequencies were obtained from the gnomAD database (http://gnomad.broadinstitute.org).

2.3 | Protein modeling

Molecular graphics figures were generated with PyMOL (Schrödinger) using the PDB coordinates (4GL2).

2.4 | Interferon score

Interferon scores were calculated on the basis of the expression of ISGs according to previously published protocols. In brief, this involved either a quantitative reverse transcription-polymerase chain reaction (qPCR) analysis using TaqMan probes (Crow laboratory: Rice et al., 2013), or testing on a Nanostring platform (Vanderver laboratory: Adang et al., 2018+). In the former, the relative abundance of *IFI27* (Hs01086370_m1), *IFI44L* (Hs00199115_m1), *IFIT1* (Hs00356631_g1), *ISG15* (Hs00192713_m1), *RSAD2* (Hs01057264_m1), and *SIGLEC1* (Hs00988063_m1) transcripts was normalized to the expression levels of *HPRT1* (Hs03929096_g1) and *18S* (Hs999999001_s1). The median fold change of the six genes, compared to the median of 29 previously collected healthy controls, was then used to create an interferon score for each individual, with an abnormal interferon score being defined as greater than +2 standard deviations above the mean of the control group that is 2.466. Alternatively, the copy number of mRNA transcripts of the six ISGs listed above, and four housekeeping genes (*ALAS1, HPRT1, TBP*, and *TUBB*), was quantified using a Nanostring nCounter™ Digital Analyzer. The raw copy number of mRNA transcripts of each ISG was standardized using the geometric mean of the four housekeeping genes for each individual, and the six-gene interferon signature for each individual calculated using the median of the Z scores, with the result considered positive if ≥1.96 (>98th centile; one tail analysis).

2.5 | Interferon reporter assay

The pFLAG-CMV4 plasmid encoding *IFIH1* has been described elsewhere (Rice et al., 2014). Indicated mutations were introduced using Phusion HiFi DNA polymerase. HEK 293T cells (ATCC) were maintained in 48-well plates in DMEM (Cellgro) supplemented with 10% fetal bovine serum and 1% L-glutamine. At 80% confluence, cells were cotransfected with pFLAGCMV4 plasmids encoding wild-type or mutant *IFIH1* (5 ng, unless indicated otherwise), interferon β (*IFNb*) promoter-driven firefly luciferase reporter plasmid (100 ng), and a constitutively expressed Renilla luciferase reporter plasmid (pRL-TK, 10 ng), by using Lipofectamine 2000 (Life Technologies) according to the manufacturer's protocol. The medium was changed 6 hr after transfection, and cells were subsequently incubated for 18 hr with or without stimulation with poly(I-C) (500 ng;

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InvivoGen) using Lipofectamine 2000. Cells were lysed with Passive Lysis Buffer (Promega), and *IFNb* promoter activity was measured using a Dual-Luciferase Reporter Assay (Promega) and a Synergy 2 plate reader (BioTek). Firefly luciferase activity was normalized to *Renilla* luciferase activity Each experiment was performed in triplicate and data are presented as mean ± standard mean of error. Statistical significance was determined by two-tailed, unpaired Student's *t*-test with *, **, and *** indicating *p* values <.05, <.01, and <.001, respectively. Expression levels of individual constructs were tested by western blot analysis.

3 | RESULTS

3.1 | Molecular data

We collected data on 74 individuals from 51 families, identifying 27 distinct mutations in total (Figure 1; Table 1). Fourteen mutations were recorded in a single proband, seven in more than one individual belonging to a single-family, and six in more than one family. Of these six recurrent mutations, the p.Arg720Gln, p.Arg779Cys, and p.Arg779His substitutions were observed most frequently (6, 8, and 10 times, respectively). Twenty-two mutations were recorded to have occurred de novo in at least one individual, whilst four mutations were only ascertained in familial cases demonstrating autosomal dominant transmission (two mutations, p.Ala489Thr and p.Gly495Arg, were transmitted from a father in whom the mutation arose de novo). Three mutations, p.Thr331Arg, p.Arg779Cys, and p.Arg779His, was documented to have occurred both de novo, in association with severe, AGS-like, neurological disease, and in families with transmission across two or more generations.

For six putative mutations (p.Gly389Arg; p.Asn449Lys; p.Ile583Val; p.Ile803Phe; p.Asp848Glu; p.Ile956Val), in silico predictions using both SIFT and Poyphen2 suggested that the substitutions were benign, with relatively poor evolutionary conservation (Figure S1). However, all of these variants were novel (i.e., not recorded in gnomAD), and assays of interferon signaling (ISG expression and in vitro testing) indicate that they represent pathogenic mutations conferring gain-of-function (Table S1; Figure S2). Of note, four of these variants were seen in the context of a spastic paraparesis phenotype with no or minimal cognitive impairment. Clinical nonpenetrance was observed in three of these families (the other three variants arising in the proband de novo).

3.2 | Clinical phenotype

Consistent with previous data, we observed a spectrum of phenotypes in our cohort, encompassing classical AGS, less easily defined rapid neuroregression, a spastic-dystonic syndrome, spastic paraparesis, SMS, and clinical nonpenetrance (Figure 2; Table 2; Table S2). A single individual, AGS2222, experienced neonatal hepatitis and then developed chronic fibrotic liver disease in the absence of any other clinical features (note that this same variant was seen in another proband, AGS735, presenting with neuroregression at age 1 year). Unequivocal episodes of rapid neuroregression were noted in at least 20 patients, in seven of whom an acute loss of skills occurred after the age of 1 year on a background of completely normal development. Recognition/onset of symptoms was frequently later in

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patients with a spastic paraparesis phenotype, with one patient experiencing the development of lower limb spasticity beginning at 13 years of age (AGS531_P4). Six symptomatic patients were recorded to have died. Five of these individuals demonstrated a severe AGS phenotype with features obvious at, or soon after, birth that is indicating prenatal onset. One further deceased patient presented with neuroregression at age 15 months, and died suddenly of a cardiorespiratory arrest at 16 years of age, with pulmonary hypertension documented on postmortem examination. Ten individuals were reported as asymptomatic mutation carriers, across five mutations (p.Gly389Arg, p.Arg779Cys, p.Arg779His p.Asp848Glu, and p.Ile956Val), with seven aged over 50 years.

3.3 | Interferon status

Where tested, all mutations (i.e., 26 of 27) were associated with increased expression of ISGs in peripheral blood (Table 1). Samples were unavailable for the single patient carrying the p.Glu773Gln substitution. This variant is not recorded in gnomAD, occurring de novo in the context of a phenotype compatible with IFIH1 upregulation, and conferring a gain-of-function in our in vitro assay (Figure S2). Considering all (51) mutation-positive individuals tested for ISG expression in the Crow laboratory (given that a direct comparison of results across laboratories is not possible), 109 of 117 values were positive (Table S3; Figure S3). Only one clinically symptomatic patient (AGS2154_1) demonstrated a negative interferon signature (on two of three occasions tested). The phenotype, in this case, was unusual; a child with white matter disease confined to the right cerebral hemisphere on MRI and no abnormal neurological signs on examination, having presented at age 8 years with headaches. We leave open the possibility that these two normal results, and three normal results from his mother, might be due to technical artifact, given that the samples had been stored for many months before testing. Sixteen samples from seven clinically nonpenetrant subjects exhibited an upregulation of interferon signaling, with two asymptomatic mutation carriers demonstrating normal interferon signatures (each tested on three occasions).

3.4 | Modeling of IFIH1 gain-of-function mutations

Modeling of the 27 mutations described here showed that most residues cluster near the ATP binding site within the helicase domain (Figure 3). Three mutations, p.Ileu583Val, p.Ileu956Val, and p.Leu979Trp were the only residues not situated in the cluster (colored cyan; only p.Ileu583Val and p.Leu979Val are shown since residue p.Ileu956 is disordered in the crystal structure). Within this main cluster, residues can be further categorized into three groups: those at the ATP binding pocket (magenta spheres), those in the double stranded RNA (dsRNA) binding surface (colored blue) and those not directly involved in either ATP or RNA binding (colored green). Three published mutations (p.Leu372phe; p.Ala452Thr; p.Glu813Asp; Table S4) not ascertained in our cohort are also located within the main cluster (colored orange), further supporting the importance of this region in the regulation of IFIH1 signaling activity.

4 | DISCUSSION

Here we present data on 74 individuals, 41 previously unreported, from 51 families, with a putative gain-of-function mutation in IFIH1. Consistent with previous descriptions, we

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observed a spectrum of phenotypes, encompassing AGS, spastic-dystonia, spastic paraparesis, SMS and clinical nonpenetrance. Phenotypic variability was common, both in the context of familial inheritance and mutations seen recurrently across families so that no obvious genotype–phenotype correlations could be ascertained.

Acute regression was noted in almost one-third of symptomatic mutation carriers, occurring after the age of 1 year in seven patients demonstrating completely normal development to that time. Beyond acute regression, a slower onset of disease, and subsequent progression, was seen in patients demonstrating a spastic paraparesis phenotype. Together with the observation of clinical nonpenetrance (10:13.5% of 74 mutation-positive individuals in our series), with seven individuals identified to be apparently disease-free beyond the age of 50 years, these data suggest the importance of additive genetic factors and/or environmental triggers in determining phenotypic status. Although we did not formally record neuroimaging features in our cohort, white matter disease and intracranial calcification were observed frequently. Such imaging characteristics can be seen in the absence of overt neurological signs (see Bursztejn et al., 2015 and de Carvalho et al., 2017). Conversely, significant neurological disease, most typically spastic paraparesis, can occur in the context of normal brain and spinal imaging (e.g., the father in family AGS524).

Clinically manifest extraneurological illness was uncommon in our series, but there appears to be a real association between IFIH1 gain-of-function and lupus-like illness, autoimmune hepatitis, and hypothyroidism. Furthermore, psoriatic-like skin disease is a well-recognized feature of the SMS phenotype. As recently described (Adang et al., 2018), two patients included here were diagnosed with pulmonary hypertension, a feature which was not searched for in most patients and may be under-recognized.

We observed a strong association of mutation status with an enhanced expression of ISGs, with 109 of 117 samples from 51 patients being positive in the experience of one laboratory. A similar conclusion can be drawn from in vitro testing. As such, upregulated interferon signaling represents a reliable biomarker of IFIH1 gain-of-function, and can serve as an indicator of variant pathogenicity where doubt exists as to the significance of a molecular lesion. This is important given that we show here that in silico algorithms do not always accurately predict pathogenicity (involving 22% of the mutations that we recorded). Where tested, clinical nonpenetrance was also associated with a persistent upregulation of interferon signaling, with only two of nine such individuals nonpenetrant on ISG testing in blood. Whether these individuals demonstrate fluctuations in ISG expression is not known at this time.

Despite documented clinical nonpenetrance in some cases, all putative IFIH1 gain-of-function substitutions are rare, with only two of the 30 discrete mutations described here and in previous reports recorded in gnomAD. Furthermore, all ascertained type I interferonopathy associated mutations are missense variants, likely conferring increased sensitivity to a self-derived nucleic acid. Although premature termination mutations in the helicase domain are seen in control populations as common polymorphisms, none has been associated with a type I interferonopathy phenotype, further supporting the role of nucleic acid binding by the helicase domain in disease pathogenesis. Substitutions of the arginine

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residues at positions 720 and 779 were seen in six and 19 probands, respectively, in our series. Given the focus of our laboratories on pediatric neurological disease, our data are likely to subject to ascertainment bias. Indeed, although only observed once by us, the p.Arg822Gln mutation has been reported in an additional five pedigrees demonstrating a classical SMS phenotype (Pettersson et al., 2017; Rutsch et al., 2015).

IFIH1 is a member of the retinoic acid-inducible gene I (RIG-I) receptor family (del Toro Duany, Wu, & Hur, 2015). Recognition of cytoplasmic viral dsRNA by IFIH1 induces filament assembly along the dsRNA axis, with the helicase domains and C terminal domain responsible for RNA recognition. Filament formation then induces oligomerization of the tandem CARD domains (2CARD) of IFIH1, leading to the interaction with mitochondrial MAVS and subsequent induction of interferon and other proinflammatory cytokines. IFIH1 filament stability is intrinsically regulated by ATP hydrolysis, which is stimulated upon dsRNA binding. Mutations that impair ATP hydrolysis generally increase filament stability and, often, but not always, confer gain-of-function signaling activity. The clustering of mutations that we ascertained, and of a further three unique published mutations, near the ATP binding region likely highlights common mechanisms, perhaps increasing RNA binding affinity or decreasing the efficiency of ATP hydrolysis and the rate of filament disassembly.

Summarizing, IFIH1 gain-of-function is associated with a spectrum of phenotypes, occurring due to de novo mutations or transmitted as an autosomal dominant trait. Testing for an interferon signature in blood represents a useful biomarker in this context, which can aid in the interpretation of identified sequence variants.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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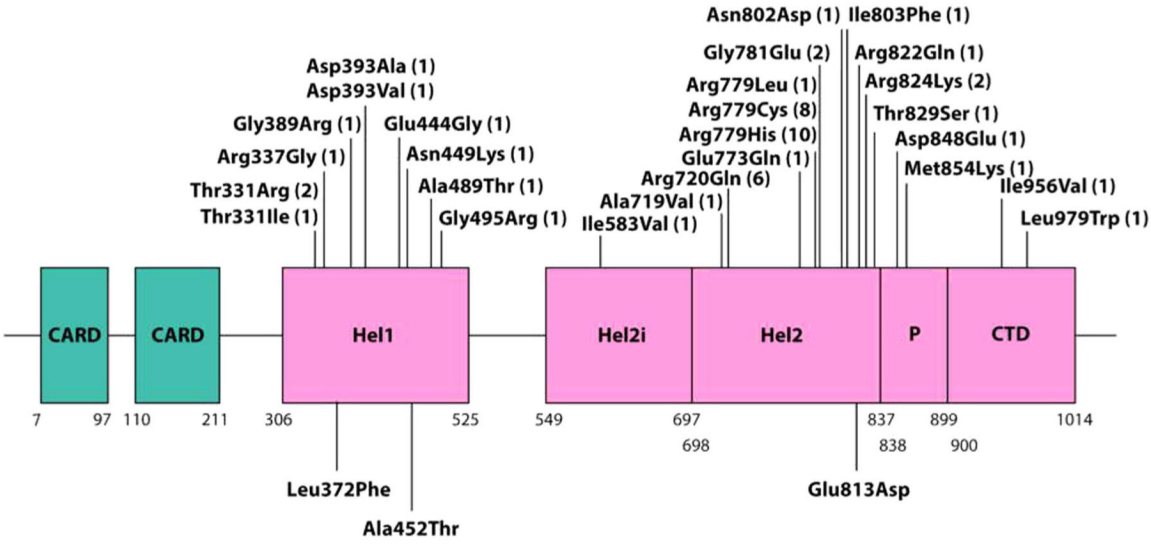

FIGURE 1.

Schematic showing the positions of protein domains and their amino acid boundaries within the 1,025-residue IFIH1 protein. The 27 mutations ascertained in the present study are annotated, with the numbers in brackets indicating the number of families in which each mutation was observed. Three previously published mutations (p.Leu372Phe; p.Ala452Thr; p.Glu813Asp), not ascertained in our series, are also denoted (below the cartoon). CARD, caspase activation recruitment domain; Hel, helicase domain, where Hel1 and Hel2 are the two conserved core helicase domains and Hel2i is an insertion domain that is conserved in the RIG-I-like helicase family; P, pincer or bridge region connecting Hel2 to the C-terminal domain (CTD) involved in binding double stranded RNA

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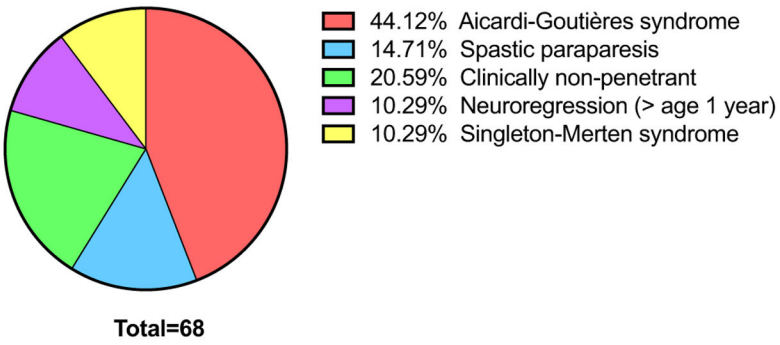

FIGURE 2.

Overview of phenotypes observed in the *IFIH1*-mutation-positive cohort. Classification of 68 of 74 individuals according to phenotype. For clarity, six individuals displaying characteristics difficult to classify were omitted from this analysis

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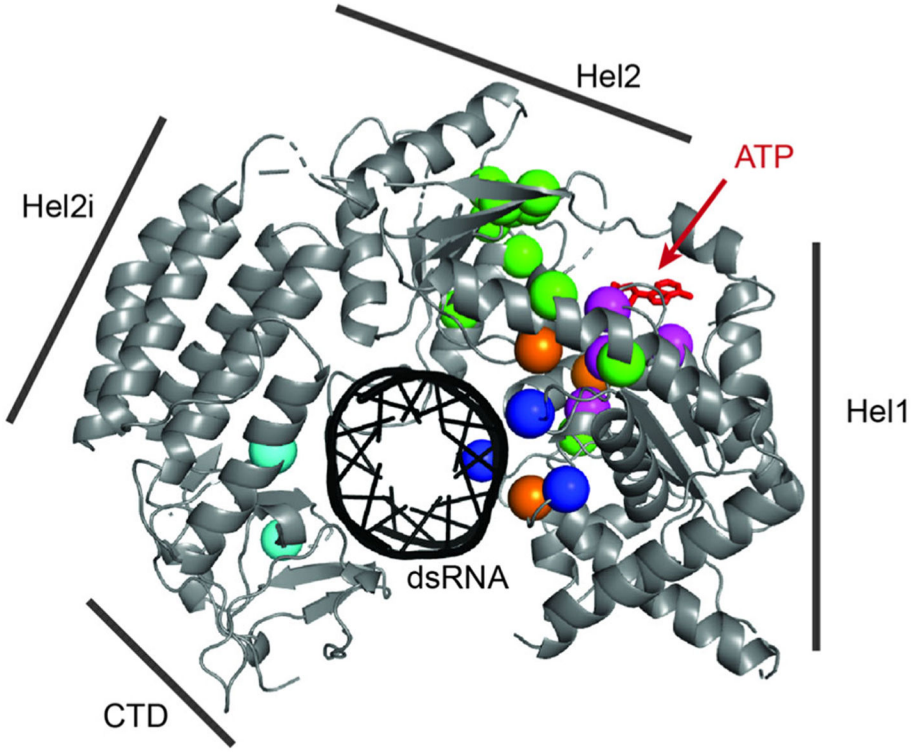

FIGURE 3.

Mutation mapping. Structure of human IFIH1 (4GL2) in complex with double stranded RNA (dsRNA; blue stick model in the center). Only the RNA binding domain (helicase domain and C-terminal domain, CTD) are included in the crystal structure. Note that the helicase domain consists of Hel1, Hel2i, and Hel2. Mutations are indicated by spheres using the following color code: residues in the ATP binding pocket (magenta), residues in the dsRNA binding surface (blue), residues within the main cluster but not directly involved in RNA binding or ATP binding (green), residues outside the main cluster (cyan), and residues previously reported by others but not in our cohort (orange). We considered all 27 mutations reported here plus three previously published mutations (p.Leu372Phe; p.Ala452Thr; p.Glu813Asp) not ascertained in our series. Residues p.Arg822, p.Arg824, and p.Ile956 are not shown because they are disordered in the crystal structure, but are expected to be located in the ATP binding (p.Arg822 and p.Arg824) and RNA binding (p.Ile956) pockets

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TABLE 1

Details of individual *IFIH1* mutations identified in the families included in the present data set

cDNA change	Protein change	Families (de novo inheritance; or, number of symptomatic and non-penetrant individuals where familial)	Associated phenotypes ('/' within family)(';' between families)	Upregulation of interferon signalling	Assessment by interferon reporter assay	gnomAD	SIFT	Polyphen2	CADD score	Var-cards
c.992C>G	p.Thr331Arg	AGS674 (de novo); AGS1972 (2;0)	AGS-SMS; SMS	Yes	Yes (de Carvalho et al., 2017)	Novel	Deleterious 0	Probably damaging 1.000	29.7	22:23
c.992C>T	p.Thr331Ile	AGS1938 (3;0)	SMS	Yes	Yes (de Carvalho et al., 2017)	Novel	Deleterious 0	Probably damaging 1.000	31	22:23
c.1009A>G	p.Arg337Gly	AGS237 (de novo)	NR	Yes	Yes (Rice et al., 2014)	Novel	Tolerated 0.12	Probably damaging 1.000	26.8	17:23
c.1165G>A	p.Gly389Arg	AGS848 (2;1)	AGS/SP/CNP	Yes	Yes (this paper)	Novel	Tolerated 0.88	Benign 0.108	5.325	01:23
c.1178A>T	p.Asp393Val	AGS626 (de novo)	NR	Yes	Yes (Rice et al., 2014)	Novel	Deleterious 0.01	Probably damaging 0.998	28.6	16:23
c.1178A>C	p.Asp393Ala	AGS2586 (de novo)	AGS	Yes	No	Novel	Deleterious 0.03	Possibly damaging 0.913	24.8	12:23
c.1331A>G	p.Glu444Gly	AGS2669 (de novo)	AGS	Yes	Yes (this paper)	Novel	Deleterious 0	Probably damaging 1	31	23.23
c.1347C>G	p.Asn449Lys	AGS1001 (de novo)	SP	Yes	Yes (this paper)	Novel	Tolerated 0.64	Benign 0.163	13.91	03:23
c.1465G>A	p.Ala489Thr	AGS755 (3;0)[a]	CLL/AGS-SMS/SMS	Yes	Yes (Bursztejn et al., 2015)	Novel	Deleterious 0	Probably damaging 1.000	32	21:23
c.1483G>A	p.Gly495Arg	AGS524 (2;0)[a]	SP-LLD/SP	Yes	Yes (Rice et al., 2014)	Novel	Deleterious 0.01	Probably damaging 0.982	23.3	14:23
c.1747A>G	p.Ile583Val	AGS2369 (de novo)	AGS	Yes	Yes (this paper)	Novel	Tolerated 0.48	Benign 0.00	0.573	5.23
c.2156C>T	p.Ala719Val	Hm_1 (de novo)	AGS	Yes	No	Novel	Tolerated 0.07	Possibly damaging 0.949	27.1	09:23
c.2159G>A	p.Arg720Gln	AGS102 (de novo); AGS647 (de novo); AGS1504 (de novo); AGS2422 (NPDT); AGS2548 (de novo); LD_0982.0 (de novo)	AGS; SP	Yes	Yes (Rice et al., 2014)	Novel	Deleterious 0	Probably damaging 0.992	34	17:23
c.2317G>C	p.Glu773Gln	AGS2399 (de novo)	NR	NA	Yes (this paper)	Novel	Tolerated 0.27	Possibly damaging 0.743	24.8	13:23
c.2335C>T	p.Arg779Cys	AGS376 (NPDT); AGS723 (NPDT); AGS1004 (de novo); AGS1156 (de novo);	AGS-LLD; SP-ICC; NR; unilateral white matter	Yes	Yes (Rice et al., 2014)	Novel	Deleterious 0.01	Probably damaging 1.000	34	21:23

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cDNA change	Protein change	Families (de novo inheritance; or, number of symptomatic and non-penetrant individuals where familial)	Associated phenotypes ('/' within family)(';' between families)	Upregulation of interferon signalling	Assessment by interferon reporter assay	gnomAD	SIFT	Polyphen2	CADD score	Var-cards
		AGS2154 (1;1); AGS2180 (de novo); AGS2507 (de novo); LD_1030.0 (de novo)	disease/CNP; AGS							
c.2336G>A	p.Arg779His	AGS163 (de novo); AGS259 (3;2); AGS1351 (de novo); AGS1509 (de novo); AGS2177 (1;2); Berg_1 (de novo); Orc_0098 (de novo); LD_1199.0 (de novo); LD_1381 (3;1); LD_1585.0 (de novo)	AGS; CNP; NR; SP	Yes	Yes (Rice et al., 2014)	1/244230	Tolerated 0.05	Probably damaging 0.994	28.9	19:23
c.2336G>T	p.Arg779Leu	LD_1067.0 (de novo)	AGS	Yes	No	Novel	Tolerated 0.06	Probably damaging 1.000	35	21:23
c.2342G>A	p.Gly781Glu	LD_0940.0 (de novo); LD_0943.0 (de novo)	NR; SP	Yes	No	Novel	Deleterious 0	Probably damaging 1.000	32	19:23
c.2404A>G	p.Asn802Asp	AGS2662 (de novo)	NR	Yes	No	Novel	Tolerated 0.22	Probably damaging 1.000	28.1	18:23
c.2407A>T	p.Ile803Phe	LD_1488.0 (de novo)	AGS	Yes	Yes (this paper)	Novel	Tolerated 0.24	Benign 0.043	11.8	04:23
c.2465G>A	p.Arg822Gln	AGS1514 (de novo)	SD-ICC	Yes	Yes (Rutsch et al., 2015)	6/244096	Deleterious 0	Probably damaging 1.000	35	23:23
c.2471G>A	p.Arg824Lys	AGS735 (de novo); AGS2222 (de novo)	NR; Isolated liver disease	Yes	No	Novel	Deleterious 0	Probably damaging 1.000	34	22:23
c.2486C>G	p.Thr829Ser	AGS1290 (2 siblings and NPDT)	AGS	Yes	No	Novel	Tolerated 0.73	Possibly damaging 0.512	16.61	12:23
c.2544T>G	p.Asp848Glu	AGS531 (3;2)	SP-ICC/CNP	Yes	Yes (Ruaud et al., 2018)	Novel	Tolerated 0.4	Benign 0.004	10.08	02:23
c.2561T>A	p.Met854Lys	AGS2081 (de novo)	AGS/SMS	Yes	No	Novel	Deleterious 0	Probably damaging 1.000	31	18:23
c.2866A>G	p.Ile956Val	AGS1430 (2;1)	SP-ICC/CNP	Yes	Yes (this paper)	Novel	Tolerated 0.77	Benign 0.004	3.576	06:23
c.2936T>G	p.Leu979Trp	LD_1346.0 (de novo)	AGS	Yes	Yes (this paper)	Novel	Deleterious 0.01	Probably damaging 1.000	26.6	16:23

Note: IFIH1 mutation annotation based on the reference complementary DNA sequence NM_022168.2.

Abbreviations: AGS, Aicardi–Goutières syndrome; CLL, Chilblain-like lesions; CNP, clinical nonpenetrance; ICC, Intracranial calcification; LLD, Lupus-like disease; NPDT, no parental DNA testing; NR, neuro-regression; SD, spastic dystonia; SP, spastic paraparesis; SMS, Singleton Merten syndrome.

[a]This mutation was shown to have been paternally inherited by the proband and to have occurred de novo in the proband's father.

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TABLE 2

Molecular and clinical data by family

Family	Individual	Sex	cDNA	Protein	Inheritance (number of mutation-positive individuals)	Previously reported (reference)	Clinical phenotype	Status at last contact (age in years)
AGS102	P1	M	c.2159G>A	p.Arg720Gln	De novo	Rice et al. (2014)	AGS	Deceased (2)
AGS163	P1	M	c.2336G>A	p.Arg779His	De novo	Rice et al. (2014)	AGS	Alive (13)
AGS237 (LD_0762)	P1	M	c.1009A>G	p.Arg337Gly	De novo	Rice et al. 2014; Adang et al., 2018	Neuroregression and SD starting at age 15 months	Deceased (16)
AGS259	P1	M	c.2336G>A	p.Arg779His	Familial (3)	Rice et al. (2014)	AGS	Alive (13)
	P2 (father of P1)	M					Clinically nonpenetrant	Alive (54)
	P3 (mother of P2)	F					Clinically nonpenetrant	Deceased (84)
AGS376	P1	M	c.2335C>T	p.Arg779Cys	No parental testing	Rice et al. (2014)	AGS with LLD	Deceased (3)
AGS524	P1	F	c.1483G>A	p.Gly495Arg	Familial (2)(shown to have occurred de novo in P2)	Rice et al. (2014); Hacohen et al. 2015; Crow et al. 2015; McLellan et al. 2018	SP with LLD and AQP4 + TM	Alive (10)
	P2 (father of P1)	M					Pure SP	Alive (39)
AGS531	P1	F	c.2544T>G	p.Asp848Glu	Familial (5)	Ruaud et al. (2018)	SP with ICC	Alive (13)
	P2 (brother of P1)	M					Clinically nonpenetrant	Alive (13)
	P3 (father of P1 and P2)	M					SP with ICC	Alive (40)
	P4 (brother of P3)	M					SP with ICC	Alive (38)
	P5 (father of P3 and P4)	M					Clinically non-penetrant	Alive (66)
AGS626	P1	M	c.1178A>T	p.Asp393Val	De novo	Rice et al. (2014)	Neuroregression and SD starting at 13 months	Alive (13)
AGS647	P1	M	c.2159G>A	p.Arg720Gln	De novo	Rice et al. (2014)	AGS	Alive (2)
AGS674	P1	M	c.992C>G	p.Thr331Arg	De novo	Unreported	SP-SMS overlap	Alive (14)
AGS723	P1	F	c.2335C>T	p.Arg779Cys	Mother negative; no paternal DNA	Unreported	SP with ICC	Alive (19)
AGS735	P1	M	c.2471G>A	p.Arg824Lys	De novo	Galli et al. 2018	Neuroregression and SD starting at 12 months	Alive (19)
AGS755	P1	M	c.1465G>A	p.Ala489Thr	Familial (3)	Bursztejn et al. (2015)	CLL	Alive (4)
	P2 (brother of P1)	M					AGS-SMS overlap	Alive (3)
	P3 (father of P1 and P2)	M					SMS-like	Alive (41)
AGS848	P1	M	c.1165G>A	p.Gly389Arg	Familial (3)	Unreported	AGS	Alive (8)

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Family	Individual	Sex	cDNA	Protein	Inheritance (number of mutation-positive individuals)	Previously reported (reference)	Clinical phenotype	Status at last contact (age in years)
	P2 (father of P1)	M					SP	Alive (42)
	P3 (maternal grandmother of P2)	F					Clinically nonpenetrant	Alive (84)
AGS1001	P1	M	c.1347C>G	p.Asn449Lys	De novo	Unreported	SP	Alive (19)
AGS1004	P1	F	c.2335C>T	p.Arg779Cys	De novo	Unreported	AGS (neuroregression with onset at age 8 months)	Alive (8)
AGS1156	P1	M	c.2335C>T	p.Arg779Cys	De novo	Kothur et al. 2018	AGS (neuroregression with onset at age 8 months)	Alive (5)
AGS1290	P1	M	c.2486C>G	p.Thr829Ser	2 affected (no parental DNA)	Unreported	AGS	Alive (6)
	P2 (brother of P1)	M					AGS	Alive (4)
AGS1351	P1	F	c.2336G>A	p.Arg779His	De novo	Unreported	AGS	Deceased (2)
AGS1430	P1	M	c.2866A>G	p.Ile956Val	Familial (3)	Unreported	SP with ICC with onset at age 6 years	Alive (14)
	P2 (father of P1)	M					SP with onset at age 2 years	Alive (50)
	P3 (father of P2)	M					Clinically non-penetrant	Alive (72)
AGS1504 (LD_1175)	P1	F	c.2159G>A	p.Arg720Gln	De novo	Unreported	AGS	Alive (10)
AGS1509	P1	M	c.2336G>A	p.Arg779His	De novo	Unreported	AGS	Alive (8)
AGS1514	P1	M	c.2465G>A	p.Arg822Gln	De novo	Buers et al. (2017)	SD with ICC	Alive (6)
AGS1938	P1	F	c.992C>T	p.Thr331Ile	Familial (3)	de Carvalho et al. (2017)	SMS	Alive (18)
	P2 (mother of P1)	F					SMS	Alive (45)
	P3 (sister of P2)	F					SMS	Alive (27)
AGS1972	P1	F	c.992C>G	p.Thr331Arg	Familial (2)	de Carvalho et al. (2017)	SMS	Alive (9)
	P2 (father of P1)	M					SMS	Alive (47)
AGS2081	P1	M	c.2561T>A	p.Met854Lys	De novo	Unreported	SP-SMS overlap	Alive (12)
AGS2154 (LD_1240)	P1	M	c.2335C>T	p.Arg779Cys	Familial (2)	Unreported	Unilateral white matter disease with normal development	Alive (13)
	P2 (mother of P1)	F					Clinically nonpenetrant	Alive (40)
AGS2177	P1	M	c.2336G>A	p.Arg779His	Familial (3)		Neuroregression and SD starting at age 12 months	Alive (29)
	P2 (mother of P1)	F					Clinically nonpenetrant	Alive (62)

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Family	Individual	Sex	cDNA	Protein	Inheritance (number of mutation-positive individuals)	Previously reported (reference)	Clinical phenotype	Status at last contact (age in years)
	P3 (sister of P1)	F					Clinically nonpenetrant	Alive (33)
AGS2180	P1	F	c.2335C>T	p.Arg779Cys	De novo	Unreported	AGS	Alive (4)
AGS2222	P1	M	c.2471G>A	p.Arg824Lys	De novo	Unreported	Isolated liver disease	Alive (9)
AGS2369	P1	M	c.1747A>G	p.Ile583Val	De novo	Unreported	AGS	Alive (10)
AGS2399	P1	M	c.2317G>C	p.Glu773Gln	De novo	Unreported	Neuroregression and SD starting at age 16 months	Alive (8)
AGS2422	P1	F	c.2159G>A	p.Arg720Gln	No parental testing	Unreported	SP	Alive (38)
AGS2507	P1	F	c.2335C>T	p.Arg779Cys	De novo	Unreported	AGS	Alive (1)
AGS2548	P1	M	c.2159G>A	p.Arg720Gln	De novo	Unreported	AGS	Alive (3)
AGS2586	P1	M	c.1178A>C	p.Asp393Ala	De novo	Unreported	AGS-like with frank regression at age 21 months	Alive (3)
AGS2662 (LD_1640)	P1	F	c.2404A>G	p.Asn802Asp	De novo	Unreported	Neuroregression and SD starting at age 11 months	Alive (1)
AGS2669	P1	M	c.1331A>G	p.Glu444Gly	De novo	Unreported	AGS	Deceased (0.5)
Hm_1	P1	F	c.2156C>T	p.Ala719Val	De novo	Unreported	AGS	Alive (2)
Berg_1	P1	F	c.2336G>A	p.Arg779His	De novo	Unreported	Neuroregression and SD starting at age 9 months	Alive (7)
Orc_0098	P1	M	c.2336G>A	p.Arg779His	De novo	Unreported	AGS	Alive (4)
LD_0940.0	P1	M	c.2342G>A	p.Gly781Glu	De novo	Unreported	Neuroregression and SD starting at age 15 months	Alive (5)
LD_0943.0	P1	F	c.2342G>A	p.Gly781Glu	De novo	Unreported	SP	Alive (14)
LD_0982.0	P1	M	c.2159G>A	p.Arg720Gln	De novo	Adang et al. (2018); Case 2	AGS	Alive (9)
LD_1030.0	P1	F	c.2335C>T	p.Arg779Cys	De novo	Unreported	AGS	Alive (5)
LD_1067.0	P1	M	c.2336G>T	p.Arg779Leu	De novo	Unreported	AGS	Alive (8)
LD_1199.0	P1	F	c.2336G>A	p.Arg779His	De novo	Unreported	AGS	Alive (4)
LD_1346.0	P1	M	c.2936T>G	p.Leu979Trp	De novo	Adang et al. (2018); Case 3	AGS	Deceased (0.4)
LD_1381 (Hart)	P1	F	c.2336G>A	p.Arg779His	Familial (4)	Unreported	SP	Alive (4)
	P2 (brother of P1)	M					SP	Alive (3)
	P3 (father of P1 and P2)	M					SP	Alive (32)
	P4 (father of P3)	M					Clinically nonpenetrant	Alive (68)
LD_1488.0	P1	F	c.2407A>T	p.Ile803Phe	De novo	Unreported	AGS	Alive (2)

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Family	Individual	Sex	cDNA	Protein	Inheritance (number of mutation-positive individuals)	Previously reported (reference)	Clinical phenotype	Status at last contact (age in years)
LD_1585.0	P1	F	c.2336G>A	p.Arg779His	De novo	Unreported	AGS	Alive (5)

Note: IFIH1 mutation annotation based on the reference complementary DNA sequence NM_022168.2.

Abbreviations: AGS, Aicardi–Goutières syndrome; CLL, Chilblain-like lesions; F, Female; ICC, intracranial calcification; LLD, Lupus-like disease; M, Male; SD, spastic dystonia; SP, spastic paraparesis; SMS: Singleton Merten syndrome; TM, transverse myelitis

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Hepatic Involvement in Aicardi-Goutières Syndrome

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Abstract

Aicardi-Goutières syndrome (AGS) is a monogenic type-I interferonopathy that results in neurologic injury. The systemic impact of sustained interferon activation is less well characterized. Liver inflammation is known to be associated with the neonatal form of AGS, but the incidence of AGS-related hepatitis across lifespan is unknown.

We compared natural history data including liver enzyme levels with markers of inflammation, (liver-specific autoantibodies and interferon signaling gene expression [ISG] scores). Liver enzymes were classified as normal or elevated by the fold increase over the upper limit of normal (ULN). The highest increases were designated as hepatitis, defined as aspartate-aminotransferase or alanine-aminotransferase threefold ULN, or gamma-glutamyl transferase 2.5-fold ULN. A larger cohort was used to further characterize the longitudinal incidence of liver abnormalities and the association with age and genotype.

Across the AGS cohort ($n = 102$), elevated liver enzymes were identified in 76 individuals (74.5%) with abnormalities at a level consistent with hepatitis in 29 individuals (28.4%). SAMHD1 mutations were less likely to be associated with hepatitis (log-rank test; $p = 0.011$). Hepatitis was associated with early-onset disease and microcephaly (log-rank test; microcephaly $p = 0.0401$, age onset $p = 0.0355$). While most subjects ($n = 20/33$) were found to have liver-specific autoantibodies, there was

Keywords

- Aicardi-Goutières syndrome
- liver
- ISGs

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no association between the presence of autoantibodies or ISG scores with hepatitis-level enzyme elevations.
In conclusion, all genotypes of AGS are associated with transient elevations of liver enzymes and the presence of liver-associated autoantibodies. This adds to our growing understanding of the systemic pathology AGS.

Introduction

Aicardi-Goutières syndrome (AGS) is a rare genetic disorder characterized by dysregulation of the nucleic acid metabolism and sensing pathways. This results in a chronic activation of type I interferons (IFN)[1] and leads to extensive multisystem organ injury.[2–7] Children with AGS have systemic autoinflammation mediated by IFN and, in some cases, by autoantibodies.[8]

While the impact of AGS on the brain is well characterized, the impact on systemic organs is less well understood. The neonatal form of AGS is associated with an inflammatory hepatitis,[8,9] but there is limited information about the pathogenesis or incidence of liver abnormalities across the lifespan. Other pediatric liver diseases are associated with the presence of autoantibodies, such as antinuclear antibodies (ANA), antineutrophil cytoplasmic antibodies (ANCA), and antismooth muscle antibody (ASMA).[10] While liver-specific autoantibodies can be causative of disease (such as in autoimmune hepatitis), they may develop as a secondary phenomenon resulting from exposure of hepatocyte antigens (as occurs in hepatitis C infection and Wilson disease).[11–13] The role of antibodies in AGS-related hepatitis is unknown.

Given the association between AGS and liver disease, particularly in the neonatal period, we sought to characterize the natural history of liver involvement in AGS across the lifespan and its relationship to biomarkers of inflammation.

Methods

Natural History Cohorts

An international cohort of individuals was recruited through the Institutional Review Board-approved Myelin Disorders Bioregistry Project at the Children's Hospital of Philadelphia. This study began with the pretreatment data from a cohort of children ($n = 48$) enrolled in our therapeutic studies (NCT01724580 and NCT03921554), where a higher than expected incidence of liver enzyme elevation was noted.[14] From this cohort, detailed pretreatment medical histories ($n = 48$) and banked serum samples ($n = 33$) were available. Next, we evaluated liver enzyme abnormalities within our expanded cohort ($n = 102$, inclusive of the detailed cohort above). From this expanded cohort, laboratory values and limited medical histories (e.g., clinical presentation and genotype) were collected. The additional subjects included in the larger cohort were comparable to the detailed cohort by key parameters of age at last evaluation (Wilcoxon rank sum test, $p = 0.606$), genotype (Pearson chi-squared test, $p = 0.241$), and presence of microcephaly (Pearson chi-squared test, $p = 0.168$). Age of onset was different between the two groups (Wilcoxon rank sum test, $p = 0.039$). With removal of an outlier with disease onset over 200 months, this association no longer reached significance (Wilcoxon rank sum test with removal of outlier, $p = 0.059$). Clinical manifestations of liver-disease were described for two individuals across the expanded cohort in the overall cohort. A summary of the cohort demographic is included in ►**Table 1**.

Clinical Laboratory Values

Results of aspartate-aminotransferase (AST), alanine-aminotransferase (ALT), and gamma-glutamyl transferase (GGT) testing were collected as available from the medical records. Laboratory analyses stated as normal but for which an exact value was not available were scored as normal (0.5-fold increase over normal). Liver enzyme fold increase was calculated based on the upper limit of normal values included in the medical report for each test. AST and ALT fold increase of three times and GGT fold increase of 2.5 times from upper limit of normal were considered meaningful representation of abnormal liver enzyme levels consistent with hepatitis.[15] To minimize the over-representation of repeat laboratory measures in individual patients, the value of greatest elevation was used for visualization if measures were repeated within 1 month, unless individuals were greater than 100 months, in which case the highest value within a 10-month period was used.

Research Laboratory Values

Banked serum samples were available from 33 individuals in the detailed cohort. These samples were used to measure liver autoantibody levels, which included ANA, ANCA, ASMA, and antiliver/kidney microsome 1 in the clinical immunology laboratory. A positive value for the autoantibody panel was determined by the presence of one or more elevated autoantibody levels.

Simultaneous liver enzyme testing and IFN signaling gene (ISG) expression scores were available for 52 individuals in the expanded cohort, inclusive of 46 individuals within the detailed cohort. To calculate the ISG expression score, extracted RNA (Qiagen PAXgene Blood RNA kit) was quantified using the Nanostring nCounter Digital as previously described.[16,17] The copy number of mRNA transcripts were measured from type I IFN-inducible genes (IFI27, IFI44L, IFIT1, ISG15, RSAD2, and SIGLEC1) as compared to housekeeping genes (ALAS1, HPRT1, TBP, and TUBB). An ISG score of ≥ 1.96 (>98th percentile) was considered positive.[18]

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Table 1 Demographics

Variable		Pretreatment detailed cohort ($n = 48$)	Expanded cohort[a] ($n = 102$)	*p*-Value[b]
		n (%)	*n* (%)	
Sex	Male	27 (56.25)	58 (56.86)	0.906
	Female	21 (43.75)	44 (43.14)	
Genotype	*TREX1*	6 (12.5)	18 (17.65)	0.241
	RNASEH2B	13 (27.08)	29 (28.43)	
	RNASEH2A	1 (2.08)	6 (5.88)	
	RNASEH2C	1 (2.08)	2 (1.96)	
	SAMHD1	9 (18.75)	19 (18.63)	
	ADAR1	9 (18.75)	16 (15.5)	
	IFIH1	9 (18.75)	12 (11.76)	
HC	Microcephaly	22 (45.83)	53 (53%)	0.168
	Normocephaly	26 (54.17)	47 (47%)	
Age of onset (months)	Mean	11.34 (SD: 29.41)	8.78 (SD: 23.52)	0.039
	Median	4 (range: 0–204)	3.5 (range: 0–204)	
Age at last evaluation (months)	Mean	74.10 (SD: 74.72)	73.5 (SD: 74.07)	0.606
	Median	37.0 (range: 2.0–266)	45 (range: 2–266)	

Abbreviations: HC, HC: head circumference; SD, standard deviation.
[a]Inclusive of the detailed cohort.
[b]p-Values were obtained using the chi-squared test for categorical variables and the Wilcoxon rank sum test for age of onset and age of evaluation.

Statistical Analysis

Demographic, clinical, and genetic features were described with descriptive statistics. Analyses were performed using Stata 16.0 statistical software and GraphPad Prism 8. Statistical significance was set at a two-sided *p*-value of less than 0.05. Bland–Altman analysis was used to evaluate the agreement between age at first available measurement and age at presentation. This found that as age at onset increased, so did the difference between age at onset and the first laboratory measurement. Thus, it was determined that age at first measurement of liver enzymes could not be used to determine age at onset of hepatitis. In all cases, elevations of ALT, AST, and GGT were considered both for any abnormal values (e.g., fold change >1) as well as for fold changes that were consistent with hepatitis.[15]

Kaplan–Meier curves were constructed to visualize the acquisition of liver enzyme elevation within groups. The log-rank test was used to compare the survival distributions between the subgroups defined according to microcephaly, disease onset, and genotype (*SAMHD1* versus other genotypes combined). We also performed tests for the assumption of proportional hazards based on the Schoenfeld residuals.

Comparisons between liver enzyme abnormalities and ISGs and between liver enzyme abnormalities and age at sample were assessed by fitting longitudinal models. These longitudinal models were fitted using quasi-least squares (QLS) regression and QLS logistic regression. QLS is a computational approach for estimation of intrasubject correlations in the framework of generalized estimating equations.[19,20] Comparison between ISGs and liver autoantibody abnormalities was performed by Mann–Whitney (Wilcoxon rank sum tests) U test as only single values were available. Comparisons between liver autoantibody abnormalities and AST, ALT, and GGT abnormalities were performed by chi-squared test. Comparisons between subcohorts were performed by Wilcoxon rank sum and chi-squared tests.

Results

This study began with the pretreatment data from a cohort of children ($n = 48$) enrolled in our therapeutic studies, where a higher than expected incidence of liver enzyme elevation was noted at baseline. We collected all available pretreatment laboratory values from this cohort. We found 64 transient episodes of liver enzyme elevation throughout the lifespan. Most of these episodes were independent of events typically associated with hepatitis. Infections or fevers were noted in six episodes (9.4%); six episodes were noted during antiepileptic drug exposure associated with liver enzyme elevation (valproic acid and phenobarbital). One of these six events was associated with simultaneous infection and antiepileptic drug exposure. None of these events were concomitant with infections such as Epstein–Barr virus, cytomegalovirus, or viral hepatitis. All individuals had documentation of serologies consistent with hepatitis B vaccination and lack of seroconversion for hepatitis C ($n = 48$).

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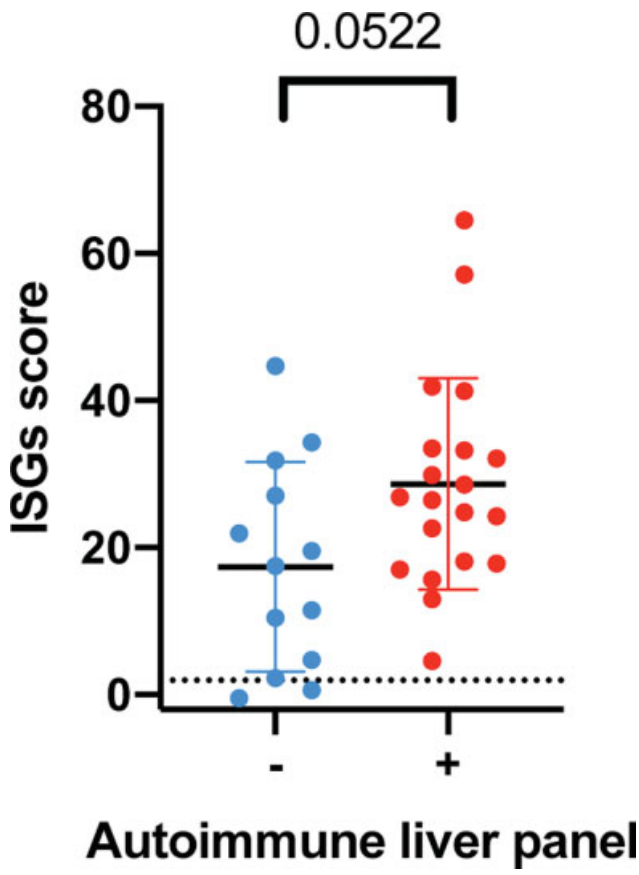

Fig. 1 Interferon signaling gene expression (ISG) scores in paired samples for autoimmune liver panel results ($p = 0.0522$, Mann–Whitney U test).

As AGS has been associated with autoantibody production targeting other organs, liver-specific autoantibodies were measured in banked samples with paired liver enzymes and ISG scores ($n = 33$) (►**Fig. 1**). Within this cohort, 20 individuals were found to have liver autoantibodies (60.61%), which did not correlate with the degree of elevation in ISG scores ($p = 0.0522$, Mann–Whitney U test). None of the individuals with liver-specific antibodies had clinical reports of jaundice, right upper quadrant pain, vomiting, or laboratory evidence of synthetic liver dysfunction. There was no clinically meaningful correlation between liver autoantibodies and AST, ALT, or GGT fold increases within the range of hepatitis (chi-squared test, AST, $p = 0.401$, ALT, $p = 0.227$, GGT $= 0.794$) or when any increase in liver enzymes was considered (chi-squared test, AST, $p = 0.837$, ALT, $p = 0.946$, GGT $= 0.547$).

Next, we assessed a second cohort to include all individuals in our natural history cohort with historical liver enzymes measurements. We combined this cohort with our detailed cohort to assess a combined cohort. Most subjects experienced an episodes of liver enzyme elevation (74.5%; $n = 76/102$), with 29 individuals (28%) meeting the definition of hepatitis ($n = 29/102$) (►**Fig. 2A–C**). There was no association between the fold change in liver enzyme elevation (AST, ALT, or GGT) and age at sample measurement (QLS logistic regression, AST, $p = 0.1603$, ALT, $p = 0.3375$, GGT $= 0.2808$) (►**Fig. 2D**).

Because of the known association between hepatitis and neonatal disease in AGS, we hypothesized that variables associated with the neonatal presentation of AGS would be associated with an earlier onset of hepatitis. We found that microcephaly and early age at onset (within the first 3 months of life) were associated with earlier abnormal liver enzymes levels within the range of hepatitis (microcephaly: log-rank [Mantel-Cox] test, $p = 0.0401$, age onset $p = 0.0335$, ►**Fig. 3A–B**). Children with *SAMHD1*-related AGS had a lower acquisition of liver enzyme elevation within the range of hepatitis (log-rank test, $p = 0.011$) compared to other genotypes (►**Fig. 3C**). Only early age of onset (within the first 3 months of life) was associated with abnormal liver enzyme levels when a simple fold change >1 was considered (log-rank test, $p < 0.001$).

From the expanded cohort, simultaneously obtained liver enzyme measurements and ISG scores were available from 52 individuals (n of paired values $= 71$) (►**Fig. 3**). There was no correlation between ISG score and liver enzyme elevations (►**Fig. 4 A–C**).

Discussion

AGS is a genetic interferonopathy of childhood with extensive multisystemic organ involvement. Despite our growing understanding of AGS, the extent of hepatic involvement in AGS is currently unknown. Liver involvement is known to be associated with the early onset form of AGS (neonatal presentation) and with *TREX1*-related disease,[8,9] but the broader penetration of liver involvement in AGS affected individuals remains unknown.

We found that approximately sixty percent to three quarters of individuals with AGS experience elevated liver enzymes. More than one quarter have liver enzyme elevations consistent with hepatitis during their lifetime. As our data is limited by the availability of laboratory measurements, we hypothesize that this is likely an underestimation of the incidence of hepatitis in the AGS population. A younger age at disease onset and the presence of microcephaly correlated with the early presence of hepatitis, which is consistent with the existing literature. All genotypes were susceptible to liver enzyme elevation, although this was the least pronounced in the *SAMHD1* population. We found that enzyme abnormalities may be observed years after disease onset and were not limited to the neonatal period, as previously reported.

While a significant proportion of the population had a transient elevation in liver enzymes, the clinical impact and prognostic significance of this information is uncertain. Within our cohort, there were only rare cases with liver imaging or findings of synthetic liver dysfunction. Only two individuals were noted in their clinical records to have clinical manifestations of liver disease. One limitation of a retrospective study is that the absence of documentation of a specific feature (i.e., the lack of clinical symptoms from hepatitis) is not equivalent to the absence of signs and symptoms. In future investigations, the clinical implications of the common elevations of liver enzymes should be

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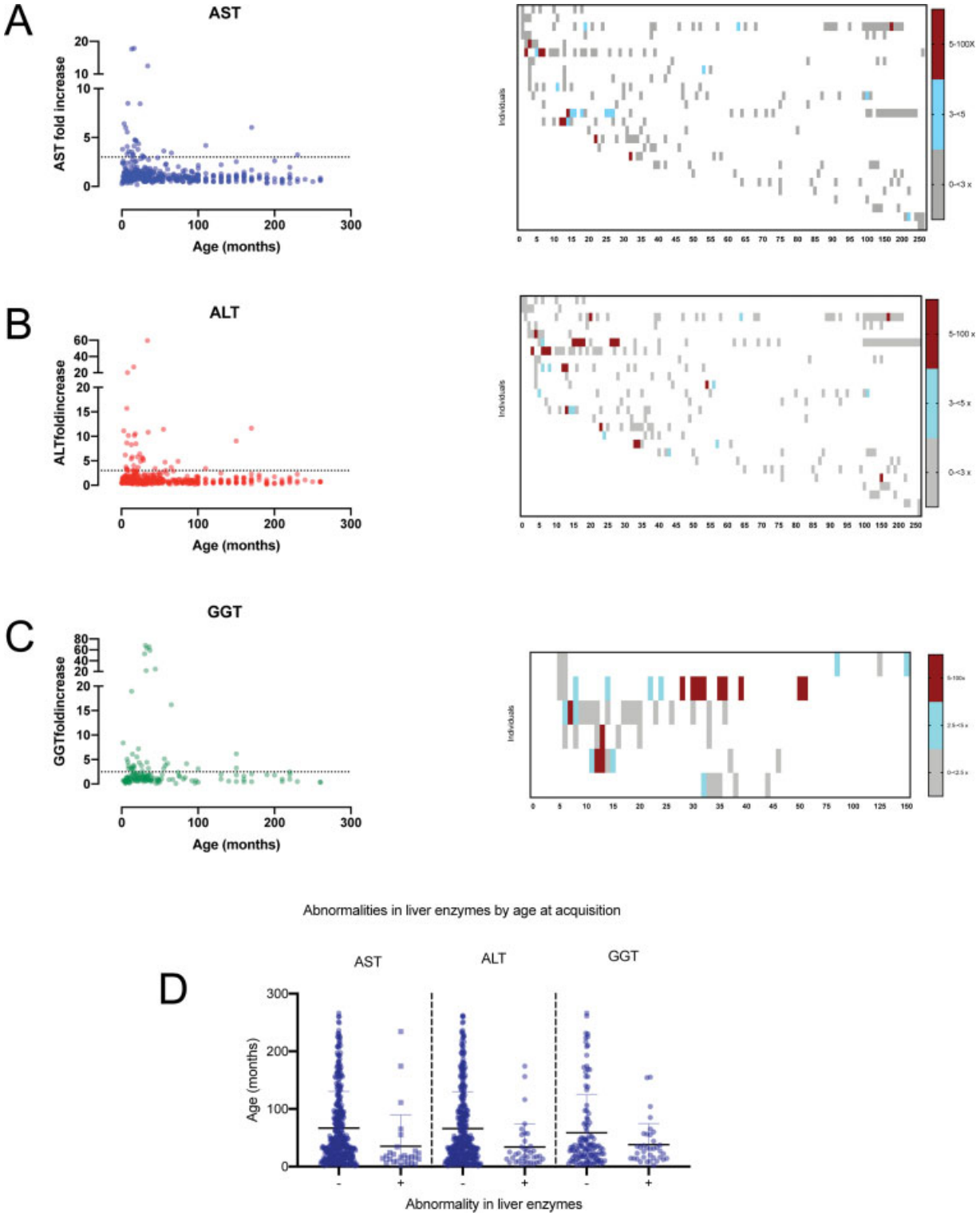

Fig. 2 Serial measurements of liver enzymes (AST, ALT, and GGT) in the AGS cohort. In the left panels for A–C, the fold increase of each liver enzyme for each individual within a time period was compared to age at measurement for AST (**A**), ALT (**B**), and GGT (**C**). The maximal fold increase is shown for each month up to 100 months, and then 10 month increments afterward. In the right panels for A–C, heatmaps represent the sequential laboratory measurements for all individuals with 5 or more measurements (AST, $n = 27$, ALT, $n = 27$, GGT, $n = 6$). (**D**) The age at laboratory acquisition was compared between liver enzyme normal and abnormal values meeting criteria of hepatitis (QLS logistic regression, AST, $p = 0.1603$, ALT, $p = 0.3375$, GGT $= 0.2808$). ALT, alanine-aminotransferase; AST, aspartate-aminotransferase; GGT, gamma-glutamyl transferase; QLS, quasi-least squares.

explored with measurements of liver function, such as coagulation laboratory testing, and imaging. Additionally, clinical histories at the time of enzyme measurement were not consistently available. Finally, it is unknown if chronic liver disease may develop in the third or fourth decade of life, since this data was not captured. Additionally, this cohort

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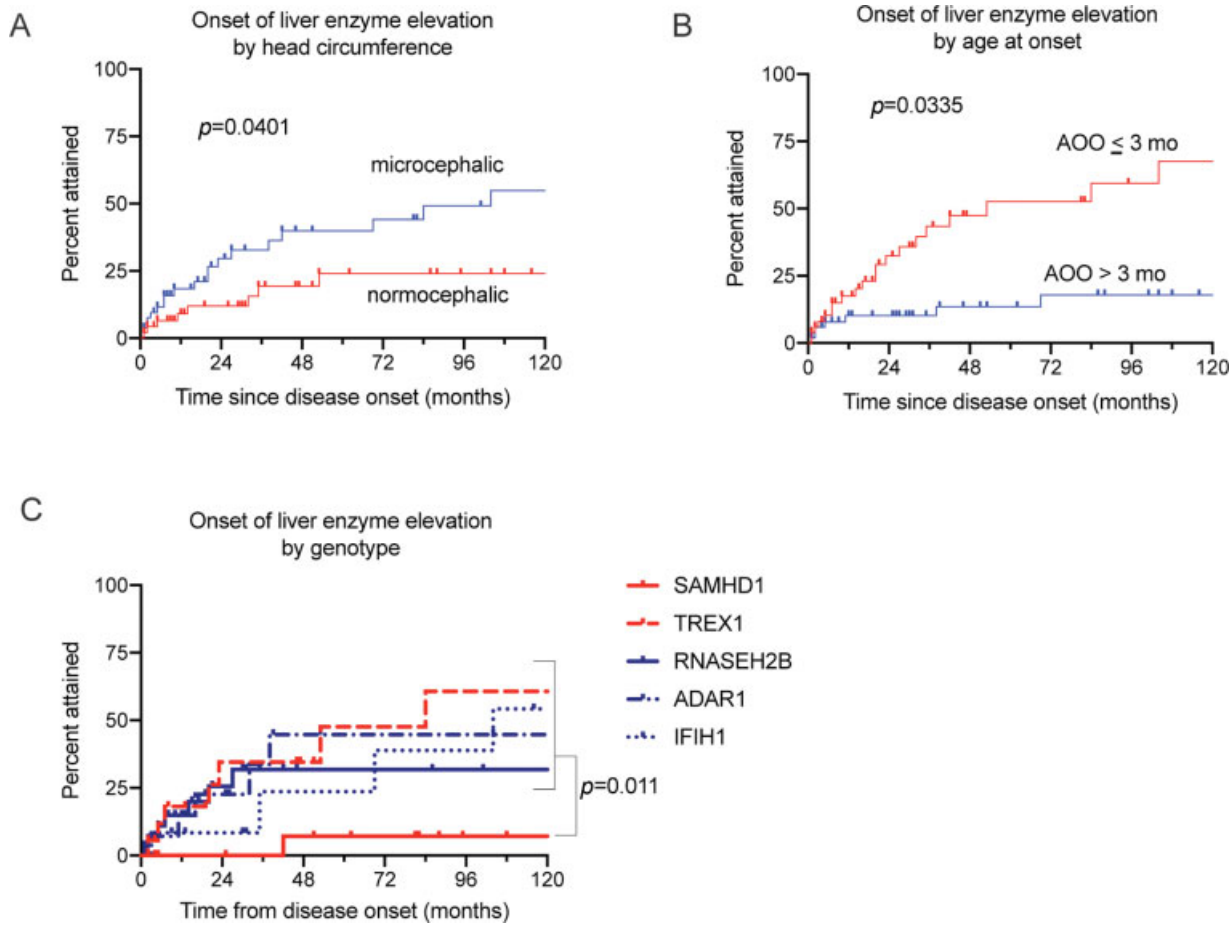

Fig. 3 Acquisition of liver enzyme elevation in the AGS population. Acquisition of liver enzyme elevation was associated with microcephaly (A; log-rank test, $p = 0.0401$), disease onset within 3 months of life (B; log-rank test, $p = 0.0335$), and non-*SAMHD1* genotype (C; log-rank test, $p = 0.011$). ALT, alanine-aminotransferase; AST, aspartate-aminotransferase; GGT, gamma-glutamyl transferase.

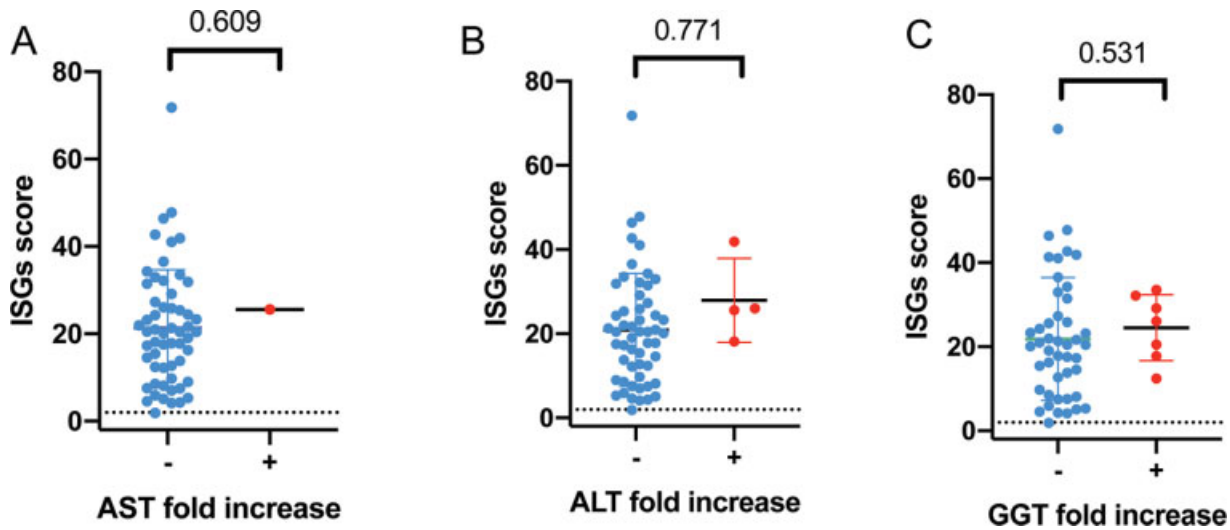

Fig. 4 Interferon signaling gene expression (ISG) scores in paired samples for AST fold increase (**A**), ALT fold increase (**B**), and GGT fold increase (**C**). The QLS model *p*-values are provided for the correlations **A**, **B**, and **C**. ALT, alanine-aminotransferase; AST, aspartate-aminotransferase; GGT, gamma-glutamyl transferase; QLS, quasi-least squares.

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may be affected by ascertainment bias as it includes only individuals who were diagnosed with AGS (and thus milder forms of the disease may be under-represented), and those individuals whose families choose to enroll in the natural history study. The antibody tested was limited to the individuals from whom samples were available. Despite the incomplete data set due to the structure of a retrospective natural history study, we anticipate that our cohort is representative of AGS. This is because this cohort includes the spectrum of genotypes and neurologic disabilities associated with AGS. When concurrent medical documentation was available, infection or hepatitis-associated medication exposure was identified in approximately 10% of episodes. We hypothesize that the inflammation associated with AGS may drive the recurrent elevation of liver enzymes.

To better characterize the potential pathophysiologic mechanisms underlying the elevations in liver enzymes, we assessed the correlation between liver enzymes, ISG scores, and liver autoantibodies in a subset of individuals with available banked samples. No clinically meaningful correlation between liver enzyme levels and ISGs score was observed. Autoantibodies consistent with autoimmune hepatitis were positive in more than half of our cohort. While these findings are consistent with what described previously in literature,[7] they did not strongly correlate with ISG levels, or liver enzyme elevations. While this study suggests that AGS is associated with recurrent and transient elevations of liver enzymes, the mechanism of these changes is unknown.

In this work, we characterized liver enzyme elevation within the AGS population, which we found were present throughout the lifespan of AGS and in all genotypes. Clinicians caring for infants and children with AGS should consider assessment of liver injury and function; similarly, clinicians evaluating children with neurologic disease and elevated liver enzymes should consider the diagnosis of AGS.

Conflict of Interest

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The epileptology of Aicardi-Goutières syndrome: electro-clinical-radiological findings

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ABSTRACT

Objective: Although epileptic seizures occur in approximately a quarter of patients with Aicardi-Goutières syndrome (AGS), their phenotypic and electrophysiological characterization remains elusive. The aim of our study was to characterize epilepsy phenotypes and electroencephalographic (EEG) patterns in AGS and look for possible correlations with clinical, genetic and neuroradiological features.

Methods: We selected patients with an established AGS diagnosis followed at three Italian reference centers. Medical records, EEGs and MRI/CT findings were reviewed. EEGs were independently and blindly reviewed by three board-certified pediatric epileptologists. Chi square and Fisher's exact tests were used to test associations between epilepsy and EEG feature categories and clinical, radiological and genetic variables.

Results: Twenty-seven patients were enrolled. We reviewed 63 EEGs and at least one brain MRI scan per patient. Epilepsy, mainly in the form of epileptic spasms and focal seizures, was present in 37 % of the cohort; mean age at epilepsy onset was 9.5 months (range 1–36). The presence of epilepsy was associated with calcification severity ($p = 0.016$) and startle reactions ($p = 0.05$). Organization of EEG electrical activity appeared to be disrupted or markedly disrupted in 73 % of cases. Severe EEG disorganization correlated with microcephaly ($p < 0.001$) and highly abnormal MRI T2-weighted signal intensity in white matter ($p = 0.022$).

Physiological organization of the EEG was found to be better preserved during sleep (87 %) than wakefulness (38 %). Focal slow activity was recorded in more than one third of cases. Fast activity, either diffuse or with frontal location, was more frequent in the awake state (78 %) than in sleep (50 %). Interictal epileptiform discharges (IEDs) were present in 33 % of awake and 45 % of sleep recordings. IEDs during sleep were associated with a higher risk of a epileptic seizures ($p = 0.008$).

Significance: The hallmarks of EEG recordings in AGS were found to be: disruption of electrical organization, the presence of focal slow and fast activity, and the presence of IEDs, both in patients with and in those without epilepsy. The associations between epilepsy and calcification and between EEG pattern and the finding of a highly abnormal white matter T2 signal intensity suggest a common anatomical correlate. However, the complex anatomical-electroclinical basis of AGS-related epilepsy still requires further elucidation.

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1. Introduction

Aicardi-Goutières syndrome (AGS) is a rare hereditary leukodystrophy, named after Jean Aicardi and Françoise Goutières, who first described it in 1984 [1]. To date, causative mutations have been identified in seven genes: TREX1, RNASEH2B, RNASEH2C, RNASEH2A, ADAR1, SAMHD1, IFIH1 [2]. The disease pathophysiology is based on a "fundamental link between nucleic acid metabolism, innate immune sensors and type I interferon induction" [3].

AGS patients with the classic phenotype typically present with a subacute onset during the first year of life. This is characterized by marked irritability, sleep disturbances, feeding difficulties, recurrent sterile pyrexias, progressive loss of previously acquired psychomotor skills, pyramidal and extrapyramidal signs, and slowing of head growth. This initial "encephalitic-like" phase usually lasts some months, and in most cases evolves into spastic-dystonic tetraplegia, typically accompanied by excessive startle reactions and epileptic seizures [4]. The presence of cerebrospinal fluid lymphocytosis and raised levels of interferon-alpha, without evidence of infection, is an important diagnostic feature [2,3]. At the end of the encephalitic-like phase, the active disease seems to "switch off", and there is no clear further progression over time [4,5]. However, signs of abnormal interferon activity (i.e. recurrent chilblains) may persist [2,3].

The classic neuroradiological findings in AGS include cerebral calcification, white matter abnormalities, and brain atrophy [6].

Epileptic seizures have been reported in AGS from the very first description of the syndrome. The incidence of epilepsy in affected patients varies widely in the literature, ranging from 24 % to 75 % of cases [7–13], and remains a matter of debate.

Moreover, although epileptic seizures occur in at least 24 % of patients, their phenotypic and electrophysiological characterization remains elusive.

Ramantani et al., in 2014, [11] made the first (and currently only) attempt to provide a comprehensive evaluation of seizure and EEG features in patients diagnosed with AGS. However, no specific interictal or ictal EEG pattern that might help to distinguish AGS emerged from their study, nor did they identify any discernible correlation between epilepsy, genotype, and neuroimaging findings.

The seizure semiology in AGS includes epileptic spasms, focal tonic and myoclonic, focal to generalized, and generalized tonic-clonic epileptic seizures [8,9,11,14,15]. Electroencephalographic (EEG) tracings characteristically show diffuse slowing of background activity, poor organization of electrical activity, and occasional regional slow-wave activity in interictal recordings 11]. However, in the majority of AGS subjects, EEG has been found to show diffuse slowing without focal or epileptic abnormalities [7,15,16].

This study was conducted to assess epileptic phenotypes, EEG patterns, and their correlations with clinical, genetic, and neuroradiological features in a cohort of AGS patients.

2. Methods

2.1. Protocol approvals and patient consent

The study was approved by our institutional ethics committee (approval n. 3549/2009 of 30/Sep/2009 and 11/Dec/2009, and approval n. 20170035275 of 23/Oct/2017). Informed consent was obtained from all parents or legal guardians of participants.

2.2. Recruitment and inclusion criteria

This is a multicenter study involving AGS patients longitudinally followed at three centers —Mondino Foundation (Pavia), Vittore Buzzi Children's Hospital (Milan), and Spedali Civili (Brescia) — according to a common protocol.

In collaboration with the International Aicardi-Goutières Syndrome Association (IAGSA), we retrospectively evaluated 125 AGS patients followed at the three reference centers from 2000 to 2018. The diagnosis was based on clinical, laboratory, and neuroimaging criteria for AGS [4,5,17], as described by Tonduti and colleagues in 2018 17].

From this cohort, we enrolled patients who met the following inclusion criteria:

- Available clinical records
- At least one digital scalp video-EEG recording
- MRI examination performed within a week of the video-EEG recording.

2.3. Clinical phenotype

We retrospectively collected demographic, genetic, and clinical data as detailed in Table 1.

Symptom onset in relation to age was defined according to the Livingston classification [5]. Data were collected on each clinical diagnostic criterion (i.e., irritability, feeding difficulties, sleeping difficulties, unexplained fevers, chilblains, startle reactions, pyramidal signs, extrapyramidal signs, microcephaly, etc.).

In addition, for the purpose of our study, we evaluated three criteria clinically related to epileptic manifestations:

Global functional impairment. This was recently demonstrated to be a good indicator of global clinical severity [18]. We used a score obtained by summing the scores of the following scales: the Gross Motor Function Classification System [19], the Manual Ability Classification System [20], and the Communication Function Classification System [21]. The resulting composite functional severity score ranged from three (fully preserved motor and communicative function) to 15 (extremely severe impairment of motor and communicative function). A score $\geq$ 12 denoted severe impairment, a score between 6 and 12 moderate impairment, and a score $<$ 6 mild impairment.

- Microcephaly (defined as a head circumference more than two standard deviations below the mean) for age and sex and the related neuroradiological finding of cerebral atrophy. This criterion was taken as an indicator of the severity of cortical neuronal loss and as a factor affecting long-term clinical outcome [20]
- Startle reactions. These are a frequent finding in epileptic encephalopathy patients, which suggests a possible common pathophysiological basis [22].

2.4. Seizures and epilepsy

We reviewed the patients' hospital charts to collect data on their epilepsy history (age at seizure onset and offset, seizure frequency, history of status epilepticus) and use of anti-epileptic drugs (AEDs) (Table 1), as well as details of seizure semiology and of video-EEG recordings. These data were collected and classified according to the 2017 International League Against Epilepsy (ILAE) classification and terminology [23]. In particular, since the seizures were classified mainly retrospectively, on the basis of anamnesis and home videos, the data were then re-evaluated by expert epileptologists, who assigned definitive classifications. We classified seizure semiology as follows: seizures with generalized manifestations (of unknown origin), focal seizures, or spasms (of unknown origin).

2.5. EEG recording, selection, and blinding

Digital scalp video-EEG recordings in wakefulness and during daytime sleep were obtained using a preformed cap with electrodes placed according to the international 10–20 system. A reduced array of electrodes was used for children who had a head circumference of less than 40 cm. All EEGs were performed during the afternoon and lasted a minimum of 1:10 h. Sleep deprivation of at least 50 % of usual sleep time

was required in all patients prior to obtaining daytime sleep recordings.

All identifying information, except for age, was removed before exporting EEG data from digital archives. An alpha-numeric code identified each EEG. Digital videos were available for all subjects.

EEGs were independently and blindly reviewed by two board-certified pediatric electroencephalographers (LG, MV). Any disagreements relating to major rating categories were resolved by a third board-certified pediatric electroencephalographer (V.D.G.).

2.6. EEG rating

EEGs were rated according to the following categories: awake background activity, non-epileptiform interictal abnormalities in wakefulness and sleep, interictal epileptiform discharges (IEDs) in wakefulness and sleep, and ictal recording (including seizure semiology and ictal EEG characteristics). Each major category had sub-descriptors detailing topographical distribution, morphology of EEG patterns and discharges, and correlation with clinical events.

All terminology used was based on standard usage in clinical practice and research. Indeed, we used terms adapted from the standardized computer-based organized reporting of EEG (SCORE) tool [24] and the revised glossary of terms most commonly used by clinical electroencephalographers, proposed by Kane in 2017 [25].

2.7. Neuroimaging

Neuroradiological imaging (MRI and CT) data were reviewed and analyzed only when performed within a week of the video-EEG acquisition.

Two pediatric neurologists with expertise in white matter disorders and AGS imaging analysis (RLP and DT) performed the qualitative imaging review to evaluate the presence, site, and severity of cerebral atrophy, white matter abnormalities, and calcification, according to the approach described in detail by La Piana and colleagues [6].

The presence, site, and severity of calcification were assessed using head CT images. When CT images were not available, calcification was often seen on MRI as areas of high T1-weighted/low T2-weighted signal intensity or low signal intensity on gradient-echo imaging or susceptibility-weighted imaging. Calcification was defined as severe when it involved multiple locations beyond the classical ones — i.e. the lentiform nuclei, deep white matter, thalami — and had multiple and variable patterns, as previously described [26].

MRI images were also reviewed to assess the presence of cortical malformations.

2.8. Statistical analysis

Demographic, clinical, neuroradiological, genetic, and EEG features were analyzed.

Age at disease onset and at epilepsy onset and offset, as well as ages at the time of EEG recordings, were described as mean, range, and/or standard deviation.

Chi-square and Fisher's exact tests were used to test associations between epilepsy and EEG feature categories, and the clinical and neuroradiological variables described above. A p-value ≤ 0.05 was considered statistically significant. We also reported results with a p-value ≤ 0.1, since we cannot exclude that the p-values obtained were dependent on the small sample size. Only results with p-values ≤ 0.05 or ≤ 0.1 are reported in the text.

3. Results

3.1. Population (Table 1)

Our sample included 27 patients: 11 females (41 %) and 16 males (59 %). The EEG recordings in these patients were performed at a mean age of 5.6 years (range 1−21 years; standard deviation 4.7).

- Neurological, seizure and epilepsy features (Table 1)

The onset of AGS occurred within the first year of life in 96 % of the patients (26/27 patients) — in the neonatal period in 33 % (9/27), and between 2 and 12 months of age in 63 % (17/27) —, while in one individual (4%), it occurred at 15 months of age.

Twenty-two patients in our cohort (81 %) showed severe functional impairment, as measured by the composite functional severity score. Microcephaly was observed in 24 of the 27 (89 %), and startle reactions in 19/27 (70 %).

Epilepsy was present in 37 % of the sample (10/27). The mean age at seizure onset was 9.5 months (range 1–36 months): 40 % of the affected patients (4/10) experienced their first seizure in the first three months of life, 40 % (4/10) at between four and 12 months of age, and 20 % (2/10) after the age of 1 year. A younger age at disease onset was more frequent in the patients who showed epilepsy: 100 % of the patients with epilepsy (10/10) had a disease onset before six months of age, versus 59 % (10/17) of those without (p-value: 0.0538). Notably, startle reactions were also present in all the patients with epilepsy (100 % vs 52 %, p-value: 0.050).

Moderate-severe calcification was more frequent in patients with than without epilepsy, being detected in 9/10 and 9/15 respectively (90 % versus 60 %, p-value: 0.016).

With regard to the use of AEDs, mono-therapy effectively controlled seizures in five patients (50 %), whereas polytherapy was necessary in the other five (50 %).

At the time of the study, three patients (mean age 3.6 years; range 3–5) were still affected by seizures, despite ongoing polytherapy, while epilepsy offset and AED discontinuation (in childhood, at a mean age of 7 years; range 2–12) was reported in four. One patient became seizure free in adulthood; another was still affected by seizures at the time of death, which occurred at the age of 4 years, while no information about possible seizure offset was available in one patient.

The following seizure semiology was observed in these 10 AGS patients with epilepsy: seizures with generalized manifestations in two patients, isolated focal motor seizures in two, and epileptic spasms alone in two. A further two children presented initially with epileptic spasms, and subsequently with focal motor seizures; another showed epileptic spasms, followed by seizures with generalized manifestations. Finally, one patient presented with focal motor seizures and seizures with generalized manifestations.

3.2. Neuroradiological findings (Table 2; Fig. 1a-b)

We reviewed neuroradiological imaging data (MRI and CT) from 25 patients (92.6 % of the total cohort).

Brain calcification was observed in 92 % of these patients (23/25): calcification was mild in 44 % (10/23), moderate in 39 % (9/23), severe in 17 % (4/23).

Cerebral atrophy was found in 88 % of the reviewed patients (22/25) and classed as mild in 18 % (4/22), moderate in 59 % (13/22), and severe in 23 % (5/22). Of the 22 patients with cerebral atrophy, 15 had microcephaly, whereas no patient without microcephaly had cerebral atrophy (68 % versus 0%, p-value: 0.0291). Ventricular enlargement was observed in 60 % of the reviewed patients (15/25).

White matter abnormalities were present in all but one patient (96 %; 24/25): these were diffuse in 33 % of cases (8/24), while they showed periventricular predominance in 17 % (4/24) and frontotemporal predominance in 50 % (12/24).

Highly abnormal signal intensity on MRI was observed in 60 % of cases (15/25).

White matter rarefaction located in the frontotemporal poles was observed in 12 % of patients (3/25).

The cerebral cortex was normal in all the subjects. Of note, two

Table 1
Overview of clinical, genetic and epileptological findings in our cohort.

ID	Sex	Genotype	Age at AGS onset	Neurological phenotype	Disease severity score	Head circumference	Presence of startle reactions	Epilepsy	Age at seizure onset	Seizure Type	Seizure frequency	Age at seizure offset	Anti-epilpetic Drugs	Hisotry of febrile seizures	History of status epilepticus
Pt 1	male	AGS5	neonatal	spastic dystonic quadriplegia	>12	<< 3rd p	yes	yes	7 months	epileptic spasms	daily	NA	DZP, VPA	no	no
Pt 2	female	AGS2	2 months	spastic dystonic quadriplegia	>12	<< 3rd p	no	no						no	no
Pt 3	female	AGS2	neonatal	spastic dystonic quadriplegia	>12	< 3rd p	yes	yes	6 months	focal motor	less than monthly	ongoing at 3 years	VPA	no	no
Pt 4	male	AGS3	6 months	spastic dystonic quadriplegia	>12	< 3rd p	yes	no						no	no
Pt 5	female	AGS4	birth	spastic dystonic quadriplegia	>12	<< 3rd p	yes	yes	3 years	generalized tonic-clonic	occasional	10 years	NA	yes	no
Pt 6	male	AGS2	2 months	spastic dystonic quadriplegia	>12	< 3rd p	yes	no						no	no
Pt 7	male	AGS4	birth	spastic dystonic quadriplegia	>12	< 3rd p	yes	yes	3 months	generalized clonic, generalized myoclonic	daily	exitus at 4 years	PHB	no	yes
Pt 8	male	AGS2	neonatal	right hemiplegia	<6	25–50th p	no	no						no	no
Pt 9	male	AGS2	neonatal	spastic dystonic quadriplegia	>12	<< 3rd p	yes	yes	2 months	epileptic spasms, focal motor	daily	ongoing at 5 years	VGB, ACTH, TPM	no	no
Pt 10	female	AGS5	2 months	spastic dystonic quadriplegia	>12	<3rd p	yes	no						no	no
Pt 11	male	AGS2	4 months	spastic dystonic quadriplegia	>12	<3rd p	yes	no						no	no
Pt 12	male	AGS2	2 months	spastic dystonic quadriplegia	>12	<3rd p	yes	yes	6 weeks	generalized tonic-clonic, focal motor	NA	12 years	OXC	no	yes
Pt 13	female	AGS2	2 months	spastic dystonic quadriplegia	>12	<3rd p	yes	yes	3 months	epileptic spasms, generalized tonic	NA	27 years	NZP, HC, CZP, LTG	no	no
Pt 14	female	UNK	4 months	spastic dystonic quadriplegia	>12	<3rd p	yes	yes	5 months	epileptic spasms, focal motor	daily	2 years	VGV, CZP, TPM, RFN	no	no
Pt 15	male	AGS1	birth	spastic dystonic quadriplegia	>12	< 3rd p	yes	no						no	no
Pt 16	female	AGS2	3 months	dyplegia plus left arm	>12	3rd-10th p	yes	no						no	no
Pt 17	male	AGS2	2 months	spastic dystonic quadriplegia	>12	< 3rd p	yes	yes	2 years	focal motor	NA	4 years	VPA	yes	no

(*continued on next page*)

Table 1 (*continued*)

ID	Sex	Genotype	Age at AGS onset	Neurological phenotype	Disease severity score	Head circumference	Presence of startle reactions	Epilepsy	Age at seizure onset	Seizure Type	Seizure frequency	Age at seizure offset	Anti-epilpetic Drugs	Hisotry of febrile seizures	History of status epilepticus
Pt 18	female	AGS2	15 months	spastic dyplegia	<6	75−90th p	no	no						no	no
Pt 19	female	AGS2	9 months	spastic dystonic quadriplegia	>12	< 3rd p	no	no						no	no
Pt 20	male	AGS2	neonatal	spastic dyplegia	<6	< 3rd p	no	no						no	no
Pt 21	male	AGS2	11 months	spastic dystonic quadriplegia	>12	<3rd p	yes	no						no	no
Pt 22	male	AGS2	6 months	spastic dystonic quadriplegia	>12	< 3rd p	yes	no						no	no
Pt 23	male	AGS6	7 months	spastic dystonic quadriplegia	>12	<< 3rd p	no	no						no	no
Pt 24	male	AGS5	4 months	spastic dystonic quadriplegia	>12	<3rd p	yes	yes	8 months	epileptic spasms	daily	ongoing at 4 years	HC, VGB, LTG	no	no
Pt 25	female	UNK	1 month	spastic dystonic quadriplegia	<6	< 3rd p	yes	no						no	no
Pt 26	male	AGS2	11 months	spastic diplegia	>6 < 12	97th p	no	no						no	no
Pt 27	female	AGS2	5 months	axial hypotonia	>12	< 3rd p	no	no						no	no

Abbreviations: Ptpatient; ppercentile; NAdata not available; DZPdiazepam, VPA, valproic acid; PHBphenobarbital; VGBvigabatrin; ACTHadrenocorticotropic hormone; TPMtopiramate; OXCox-carbamazepine; NZPnitrazepam; HChydrocortisone; CZPclonazepam, LTG, lamotrigine; RFNrufinamide.

Table 2
Overview of neuroradiological findings in our cohort.

PATIENT	leukoencephalopathy presence	symmetrical distribution	highly abnormal signal intensity	predominance of white matter involvement	atrophy severity	ventricular enlargement	cerebral cortex	calcification presence
Pt 1	present	yes	present	antero-posterior gradient	severe	present	normal	severe
Pt 2	present	yes	absent	antero-posterior gradient	moderate	absent	normal	mild
Pt 3	present	yes	present	antero-posterior gradient	severe	absent	normal	severe
Pt 4	present	yes	present	antero-posterior gradient	moderate	present	normal	mild
Pt 5	present		present	antero-posterior gradient	moderate	present	normal	severe
Pt 6	present	yes	present	antero-posterior gradient	mild	present	normal	moderate
Pt 7	present	yes	present	periventricular	moderate	present	normal	severe
Pt 8	present	yes	absent	diffuse	absent	absent	normal	moderate
Pt 9	present	yes	present	antero-posterior gradient	moderate	present	abnormal with calcification	moderate
Pt 10	NA	NA	NA	NA	NA	NA	NA	NA
Pt 11	NA	NA	NA	NA	NA	NA	NA	NA
Pt 12	present	yes	present	antero-posterior gradient	moderate	present	normal	moderate
Pt 13	present	yes	absent	antero-posterior gradient	moderate	absent	normal	moderate
Pt 14	present	yes	absent	diffuse	mild	present	abnormal, thin	mild
Pt 15	present	yes	present	antero-posterior gradient	severe	absent	normal	moderate
Pt 16	present	yes	absent	periventricular	absent	absent	normal	mild
Pt 17	present	yes	present	antero-posterior gradient	severe	present	normal	mild
Pt 18	present	yes	absent	periventricular	moderate	absent	normal	mild
Pt 19	present	yes	present	antero-posterior gradient	mild	absent	normal	moderate
Pt 20	present	yes	absent	antero-posterior gradient	moderate	present	normal	mild
Pt 21	present	yes	present	periventricular	moderate	present	normal	absent
Pt 22	present	yes	present	diffuse	severe	present	normal	moderate
Pt 23	present	yes	present	periventricular	moderate	present	normal	moderate
Pt 24	present	yes	present	diffuse	moderate	present	abnormal, thin	mild
Pt 25	present	yes	absent	periventricular	moderate	present	normal	mild
Pt 26	absent		absent		absent	absent	normal	absent
Pt 27	present	yes	absent	diffuse	mild	absent	normal	mild

Abbreviations: NA, data not available.

patients presented specific cortical atrophy, and one showed cortical calcification.

3.3. EEG features (Table 3; Fig. 2)

We reviewed 63 EEGs from 27 patients. Two EEG recordings were of suboptimal quality and could be evaluated only for background activity. We had at least one evaluable EEG recording per patient, and in 55 % of the cohort (15/27 cases) more than one. On average, 2.3 recordings were evaluated per patient (range 1–7, standard deviation 1.6), with a mean of five years (standard deviation 4.4) separating the first and last EEG.

Background activity analysis showed age-appropriate dominant posterior activity in four individuals (15 % of the cohort), a slower dominant posterior rhythm in 11 (41 %), and diffuse delta activity in 12 (44 %). A significant association was found between slowing of EEG background activity and highly abnormal signal intensity on MRI (p-value: 0.028). Normal organization of electrical activity on EEG was found in four individuals (15 % of the cohort), while three (12 %) showed discrete organization; conversely, disorganized and markedly disorganized electrical activity was found in 16 (58 %) and 4 (15 %) cases, respectively. These 20 cases with disorganized or markedly disorganized electrical activity on awake EEG accounted for the majority (83 %) of the 24 patients with microcephaly, while the other microcephalic AGS patients showed normal or discrete organization (p-value <0.0001). Moreover, disorganized electrical activity on awake EEG was also more frequent in the patients with versus without highly abnormal signal intensity on MRI (70 % versus 21 %, p-value: 0.022). The patients with an early disease onset (<6 months of age) were more likely to show marked disorganization of awake EEG activity compared with the late-onset patients (17/20, 80 % versus 57 %, p-value: 0.075). Marked EEG awake disorganization was also found to be more frequent in the patients with greater disease severity (score $\geq$12) than in those with lower disease severity scores (16/22, 77 % versus 40 %, p-value: 0.059). Interhemispheric asymmetry was present in 15 % of the cohort (4/27). Normal sleep waves were detectable in 50 % of the available sleep EEG recordings (11/ 22).

Focal slow activity during wake or sleep EEG was seen in 33 % of the cohort (9/27). Specifically, focal slow activity in awake recordings was found 15 % of the cases (4/27), whereas it was present in 32 % of the available sleep EEG recordings (7/22 recordings). In the awake recordings, it was frontal in two cases, central in one, and occipital in the other, whereas in the seven sleep recordings, it was centrally located in four recordings, and located in frontal, temporal, and occipital regions in one recording each.

Fast electrical activity was present in 78 % of the cohort (21/27) and in 50 % of sleep recordings (11/22). In the awake recordings, it was generalized in 13 of cases (62 %), involved frontal regions in six (28 %), and was observed in posterior regions in two (10 %), whereas in the sleep recordings, it was generalized in six (55 %) and located in the frontal lobes in the other five (45 %). The use of drugs potentially able to influence electrical activity speed was excluded in all individuals.

Interictal epileptiform discharges (represented by spikes/polyspikes, sharp/polysharp waves, spike and slow-wave complexes, and sharp and slow-wave complexes) were present in 33 % (9/27) of patients. They were generalized in 22 % (2/9), located in the temporal lobes in 66 % (6/9), and observed in the frontal lobes in 11 % (1/9). IEDs were present during sleep in 45 % of available recordings (10/22), showing a generalized distribution in 30 % and a focal distribution in 70 % (being detected in central areas in 30 % and in temporal areas in 40 %). IEDs during sleep were more frequent in patients with epileptic seizures (71 %) than in those without (33 %) (p-value 0.008).

No hypsarrhythmia pattern was recorded in any recording.

Ictal EEGs were recorded in two cases. In one, the event manifested itself clinically as a cluster of spasms with corresponding electrical activity compatible with diffuse slow-wave complexes with fast activity. The other event presented as a focal motor seizure associated with electrical activity compatible with focal epileptiform discharge located

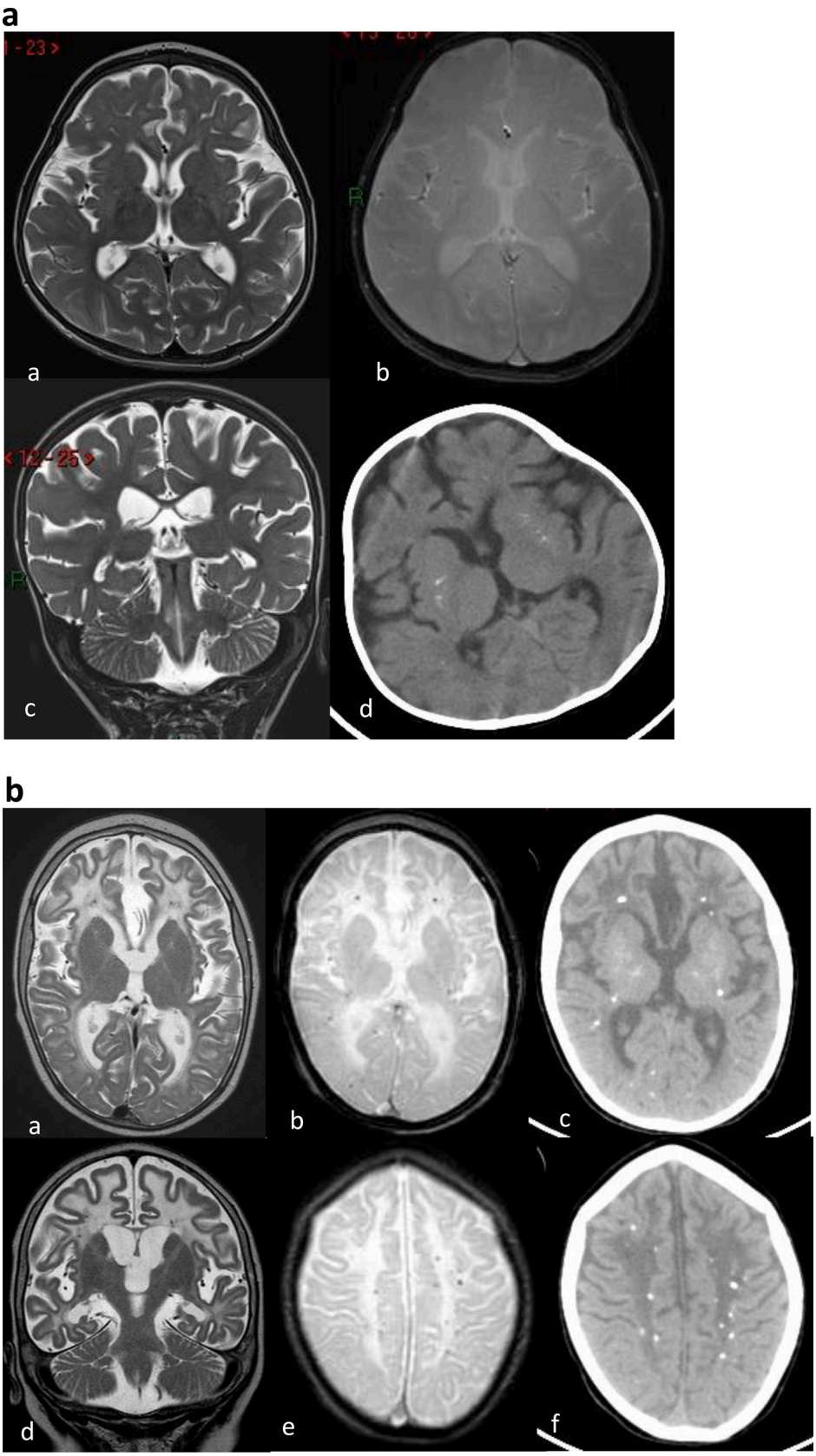

Fig. 1. a. Patient #20 (mild course). Axial and coronal T2 TSE (a,c) and T2 GE (b) MRI sequences and axial CT (d) of the same patient #20, showing mild cerebral atrophy with no white matter abnormalities (a,c) and the presence of concomitant bilateral basal ganglia calcifications (b,d). b. Patient #9 (severe course).
Axial T2 TSE (a,d) and T2 GE (b,e) MRI images and axial CT (c,f) of the same patient #9. The figure shows diffuse white matter abnormalities, detectable as highly abnormal signal intensity on T2-weighted images, associated with global enlargement of the ventricular system and mild cortical atrophy (a,b,d,e). CT images (c,f) show diffuse scattered calcification evident in both basal ganglia, in the thalami, in the hemispheric white matter bilaterally and the cortical-juxtacortical junction mainly on the fronto-parietal left side (f); the same calcification is are evident as multiple focal hypointensities on T2 GE images (b,e)

Table 3
Overview of electroencephalographic findings in wake and sleep in our cohort.

PATIENT	BACKROUND ACTIVITY		INTERICTAL WAKE						ICTAL (CLINICAL)	ICTAL (EEG)	DROWSINESS AND SLEEP							INVOLUNTARY MOVEMENTS
	FREQUENCY	ORGANIZATION	FOCAL SLOW ACTIVITY	FAST ACTIVITY	EPILEPTIFORM DISCHARGES						FOCAL SLOW ACTIVITY	FAST ACTIVITY	EPILEPTIFORM DISCHARGES				physiological sleep waves	
					SPIKE WAVE / POLYSPIKES	SHARP / POLYSHARPS	SPIKE AND SLOW-WAVE COMPLEX	SHARP AND SLOW-WAVE COMPLEX					SPIKE WAVE / POLYSPIKES	SHARP / POLYSHARPS	SPIKE AND SLOW-WAVE COMPLEX	SHARP AND SLOW-WAVE COMPLEX		
Pt 1	slowing posterior rythhm	disorganized	FCP, bilateral	FCP, bilateral	FCP, bilateral	FCP, bilateral	generalized	FCP, bilateral	spasms	generalized	FCP, bilateral	FCP, bilateral	FCP, bilateral	absent	FCP, bilateral	absent	present	present
Pt 2	diffuse delta activity	markedly disorganized	absent	generalized	absent	absent	absent	absent	absent	absent	absent	absent	absent	absent	absent	absent	absent	present
Pt 3	slowing posterior rythhm	disorganized	absent	F, bilateral	absent	absent	absent	absent	absent	absent	PO, bilateral	F, bilateral	O, bilateral	absent	absent	absent	present	absent
Pt 4	diffuse delta activity	markedly disorganized	FP, bilateral	generalized	absent	absent	absent	generalized	absent	absent	absent	absent	absent	absent	absent	generalized	absent	present
Pt 5	slowing posterior rythhm	disorganized	absent	generalized	absent	absent	absent	absent	absent	absent	NA	NA	NA	NA	NA	NA	NA	present
Pt 6	diffuse delta activity	disorganized	absent	F, right	absent	absent	absent	absent	absent	absent	absent	F, bilateral	absent	absent	generalized	absent	absent	present
Pt 7	slowing posterior rythhm	disorganized	absent	absent	absent	T, bilateral	absent	absent	absent	absent	absent	absent	absent	T, bilateral	absent	absent	absent	present
Pt 8	adequate for age	normal	absent	FC, bilateral	absent	absent	absent	absent	absent	absent	absent	absent	absent	absent	absent	absent	absent	absent
Pt 9	diffuse delta activity	markedly disorganized	absent	PT, bilateral	absent	T, left	absent	absent	focal seizure	FT, bilateral	absent	PT, bilateral	absent	absent	absent	absent	absent	present
Pt 10	diffuse delta activity	disorganized	absent	absent	absent	absent	absent	absent	absent	absent	CT, right	absent	absent	absent	absent	absent	present	absent
Pt 11	slowing posterior rythhm	discrete organization	absent	generalized	absent	absent	absent	absent	absent	absent	CP, right	generalized	absent	absent	absent	absent	present	absent
Pt 12	diffuse delta activity	markedly disorganized	absent	generalized	absent	absent	absent	absent	absent	absent	absent	absent	absent	FCT, bilateral	absent	absent	absent	present
Pt 13	adequate for age	normal	absent	absent	absent	PT, bilateral	absent	absent	absent	absent	NA	NA	NA	NA	NA	NA	NA	absent
Pt 14	diffuse delta activity	disorganized	absent	FC, bilateral	absent	absent	absent	absent	absent	absent	NA	NA	NA	NA	NA	NA	NA	absent
Pt 15	diffuse delta activity	disorganized	absent	FT, bilateral	absent	absent	absent	absent	absent	absent	absent	absent	absent	absent	absent	absent	present	absent
Pt 16	slowing posterior rythhm	discrete organization	absent	TPO, bilateral	absent	absent	absent	absent	absent	absent	absent	absent	absent	absent	absent	absent	present	absent
Pt 17	slowing posterior rythhm	disorganized	absent	generalized	absent	O, bilateral	absent	absent	absent	absent	absent	absent	absent	absent	absent	absent	present	absent
Pt 18	adequate for age	normal	absent	absent	absent	absent	absent	absent	absent	absent	CTO, bilateral	absent	absent	absent	generalized	absent	present	absent

(*continued on next page*)

Table 3 (*continued*)

PATIENT	BACKROUND ACTIVITY		INTERICTAL WAKE						ICTAL (CLINICAL)	ICTAL (EEG)	DROWSINESS AND SLEEP							INVOLUNTARY MOVEMENTS
	FREQUENCY	ORGANIZATION	FOCAL SLOW ACTIVITY	FAST ACTIVITY	EPILEPTIFORM DISCHARGES						FOCAL SLOW ACTIVITY	FAST ACTIVITY	EPILEPTIFORM DISCHARGES				physiological sleep waves	
					SPIKE WAVE / POLYSPIKES	SHARP / POLYSHARPS	SPIKE AND SLOW-WAVE COMPLEX	SHARP AND SLOW-WAVE COMPLEX					SPIKE WAVE / POLYSPIKES	SHARP / POLYSHARPS	SPIKE AND SLOW-WAVE COMPLEX	SHARP AND SLOW-WAVE COMPLEX		
Pt 19	diffuse delta activity	disorganized	absent	generalized	absent	FCT, right	absent	absent	absent	absent	absent	FT, bilateral	absent	absent	FCT, bilateral	absent	absent	present
Pt 20	slowing posterior rythhm	disorganized	absent	generalized	absent	absent	absent	absent	absent	absent	CT, right	generalized	absent	absent	absent	absent	present	absent
Pt 21	slowing posterior rythhm	discrete organization	T, bilateral	generalized	absent	absent	absent	absent	absent	absent	T, bilateral	a	absent	absent	absent	absent	present	absent
Pt 22	slowing posterior rythhm	disorganized	absent	absent	absent	absent	absent	absent	absent	absent	NA	NA	NA	NA	NA	NA	NA	absent
Pt 23	diffuse delta activity	disorganized	absent	generalized	absent	absent	absent	TO, bilateral	absent	absent	absent	generalized	absent	absent	absent	TO, bilateral	absent	absent
Pt 24	diffuse delta activity	disorganized	absent	generalized	absent	absent	absent	TO, bilateral	absent	absent	absent	generalized	absent	absent	absent	TO, bilateral	absent	absent
Pt 25	diffuse delta activity	disorganized	absent	generalized	absent	absent	absent	absent	absent	absent	absent	generalized	absent	absent	absent	absent	absent	present
Pt 26	adequate for age	normal	absent	F, bilateral	absent	absent	absent	absent	absent	absent	absent	F, bilateral	absent	absent	absent	absent	present	absent
Pt 27	slowing posterior rythhm	disorganized	C, bilateral	absent	absent	absent	absent	absent	absent	absent	NA	NA	NA	NA	NA	NA	NA	absent

Abbreviations: Pt, patient; F, frontal; C, central; P, parietal; T temporal; O, occipital; NA, data not available.

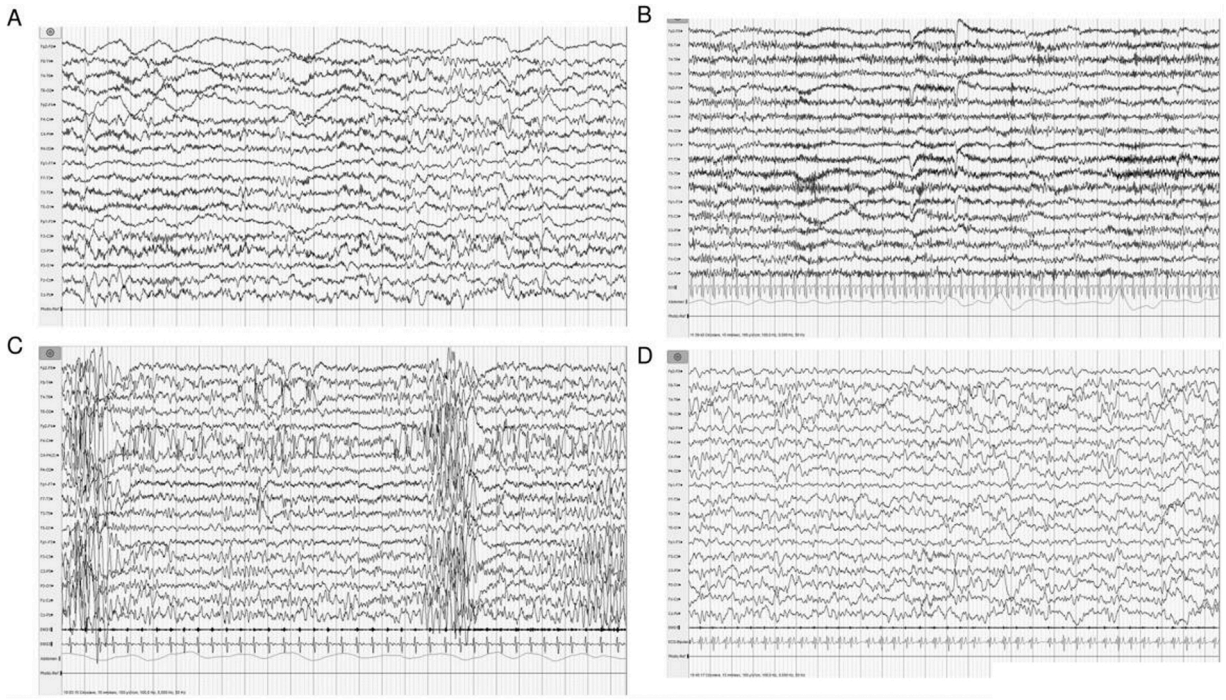

Fig. 2. A. focal fast activity in the fronto-central region in an awake recording; B. diffuse fast activity in an awake recording; C. generalized interictal epileptiform discharges, prevalently in fronto-temporo-parietal regions; D. focal slow activity in temporo-posterior regions in a sleep recording.

in the frontotemporal regions bilaterally.

The video-EEG recordings captured startle reactions, provoked by noise or spontaneous, but these were never associated with EEG modifications (Fig. 3).

4. Discussion

Our study provides a detailed description of EEG features and their relationships with clinical and neuroradiological data in AGS. Only one previous study sought to furnish a comprehensive evaluation of seizure and EEG features in patients with AGS [11]. However, Ramantani et al. did not investigate the presence of specific interictal or ictal EEG patterns in AGS patients, and, due to their small sample size, they did not perform any correlation analysis between epilepsy, genotype, and neuroimaging findings.

The clinical and radiological features and genotypes of our sample of AGS patients confirm what has already been reported in more extensive series [1,2,6,9,13,17,26–28].

Epilepsy was found in approximately 40 % of our cohort, a rate similar to that reported by Crow [2], but lower than that found by Ramantani et al. [11]. This difference might be due to a possible selection bias in Ramantani's cohort, which was recruited in an epilepsy centre; our patients, on the other hand, were followed at third-level institutes (all three are Italian and international referral centers for AGS).

In 80 % of our sample, epilepsy onset occurred within the first year of life and its presence was statistically significantly correlated with an early disease onset.

Since this was a retrospective study, the seizure semiology had to be established on the basis of anamnestic dataoccasionally supported by patients' home videos. It consisted of epileptic spasms and focal motor seizures in equal measure and, to a slightly less extent, seizures with generalized manifestations.

Although our study did not set out to assess the efficacy of AEDs in AGS patients, our data suggest that in at least 50 % of cases epilepsy is easily controlled with the first AED administered, and that more than 50 % of these patients become seizure free before adolescence.

While acknowledging the limitations due to the small size of our sample, we consider it useful to underline that vigabatrin was the drug of choice for spasms.

In 70 % of patients, we observed startle reactions, and these were statistically significantly correlated with the presence of epilepsy. Startle reactions are a physiological motor response to sudden sensitive stimuli; in some pathological conditions they can be provoked by even mild stimuli and are not susceptible to habituation effects. The bulbopontine reticular formation is the anatomical generator of the startle reaction [22]. The precise pathophysiology of the exaggerated startle reaction observed in AGS is still not clear. However, its higher incidence in AGS patients with epilepsy may point to the presence, in AGS, of a neuronal dysfunction involving both cortical and reticular neurons and/or their interconnection.

Our analysis of EEG activity revealed that physiological EEG organization was better preserved in sleep than in awake recordings. Electrical activity showed moderate to marked disorganization in 70 % of our subjects, and unsurprisingly this finding was more frequent in patients with a higher disease severity score. EEG organization did not differ between patients with and without epilepsy, an observation that reflects the fact that AGS is not an epileptic encephalopathy in the strict sense, rather an encephalopathy that can be associated with epileptic seizures. Severe EEG disorganization was statistically significantly correlated with microcephaly, which in turn was found to be directly associated with an increased likelihood of cerebral atrophy. Therefore, we can infer that marked EEG disorganization correlates with atrophy. As reported in previous papers [6,26], atrophy in AGS is due to white matter loss rather than involvement of the primary cortex, which suggests that brain white matter may play a role in EEG organization. This hypothesis is further supported by the finding of a correlation between highly abnormal white matter T2-weighted signal intensity on MRI and moderate-severe EEG disorganization.

A peculiar low-amplitude fast activity at about 12−16 Hz, previously described in just one AGS patient [1], was a frequent finding in our cohort's awake EEGs. EEG fast rhythms are known to be a typical finding

Fig. 3. A. startle reaction, provoked by noise, with no EEG modifications; B. epileptic spasms with symmetrical limb flexion, preceded by generalized slow wave with superimposed fast activity.

in lissencephaly [29], and have also been described as non-specific finding in other brain malformations [30,31]. We did not observe any cortical malformations in our cohort. These observations, taken together with other reports in non-malformative genetic syndromes [32], suggest that increased fast activity may reflect the presence of cortical and subcortical abnormalities or dysfunctional circuitries caused by microscopic cortical disruption. Future in-depth studies are needed to identify a possible pathogenic mechanism underlying this pattern.

Focal slow activity was present in more than one third of patients, more frequently in sleep recordings, as previously described by Ramantani et al. [11].

Interictal epileptiform discharges were present in one third of wake EEG recordings and almost half of sleep recordings. We observed polyspikes, sharp waves, and sharp slow waves. In our sample, IEDs more often showed a focal than a generalized location. Topographically, focal IEDs were more commonly seen in central and temporal regions, similarly to what was reported by Ramantani et al. [11]. As expected, IEDs in sleep recordings occurred more frequently in patients with a diagnosis of epilepsy.

In 62 % of the cases with focal EEG activity, this activity corresponded to a localized neuroradiological characteristic (such as a particular pattern of predominance of white matter involvement, calcification or cystic degeneration), while in the remaining patients, focal EEG abnormalities did not correlate with focal neuroradiological findings. This complex anatomical-electroclinical picture needs to be further explored in future studies.

Overall, the pathogenesis of epileptic seizures and EEG abnormalities in AGS requires further eludication. With the exception of “astrocytopathies” [33,34], epilepsy is rarely documented in the early stages of leukodystrophies. Astrocytes have many essential functions, including brain ion and water homeostasis, and their dysfunction can trigger seizures [35,36]. Moreover, astrocytes are key players in neuroinflammation, which plays a central role in the pathogenesis of AGS [37]. Both innate and adaptive immunity are involved in seizure genesis. The authors of a fairly recent study [38] comparing epileptic patients with healthy subjects suggested that postictal and interictal inflammatory cytokines (IL-6, IL-1 beta, IL-10, IL-17A, TNF-α, and IFN-γ) are elevated in the plasma of active epilepsy patients. In most of our

patients, epilepsy appeared early in the disease course, specifically in the "encephalitic phase" that is related to high interferon levels. Thus, interferon may play a role in both disease and epilepsy onset in AGS. Should further studies confirm this hypothesis, it would have important therapeutic implications for the management of patients with interferonopathies.

Of note, in our cohort, seizure burden was directly correlated with calcification severity, a finding that could indicate a causal relationship between the two parameters, or point to a common pathogenic mechanism. In recent decades, there has been a growing interest in the role of cerebral calcification in the pathogenesis of epileptic seizures [39–41] and in disorders with a primary extraneurological phenotype associated with seizures [42,43].

In conclusion, this is the first study conducted to provide a detailed description of EEG and epileptic seizure features in AGS and to explore their possible correlations with clinical and neuroradiological data. The hallmarks of EEG recordings in AGS were found to be: disruption of electrical organization, the presence of focal slow and fast activity, and the presence of IEDs, both in patients with and in those without epilepsy.

The mechanism underlying the development of epilepsy in AGS remains to be clarified. Taken together, various aspects — the severity of the cerebral abnormalities, the presence of cortical calcification, the possible roles of astrocytic dysfunction and of neuroinflammation, and the hypothetical involvement of neurons and neuronal circuits of the cerebral cortex and reticular formation — support the argument for a multifactorial basis.

Our study was conducted in a limited number of patients. Further studies in larger samples will be needed to confirm our results.

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Ethical publication statement

We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

Declaration of Competing Interest

Dr. De Giorgis received a consultancy fee from Nutricia Gmbh. Dr. Varesio received a consultancy fee from the Italian GLUT1 association (a no-profit organization). Prof. Veggiotti received financial support (speaker fee, travel expenses) from Nutricia GmbH, Dr. Schär AG/SPA, Eisai. None of the other authors has conflict of interest to disclosure.

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Appendix A

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Development of a neurologic severity scale for Aicardi Goutières Syndrome

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Abstract

Background and purpose: Aicardi Goutières Syndrome (AGS) is a severe, autoinflammatory leukodystrophy characterized by global neurologic dysfunction. Our goal was to create an easy-to-apply scale relevant to the unique developmental challenges associated with AGS.

Methods: All individuals were recruited through our natural history study. Individuals were classified by AGS severity as mild, moderate, or severe, and clinical encounters were assigned a composite score for neurologic function calculated from the sum of three functional classification scales. Through expert consensus, we identified 11 key items to reflect the severity of AGS across gross motor, fine motor, and cognitive skills to create the AGS Scale. There was strong interrater reliability. The AGS scale was applied across available medical records to evaluate neurologic function over time. The AGS scale was compared to performance on a standard measure of gross motor function (Gross Motor Function Measure-88, GMFM-88) and a putative diagnostic biomarker of disease, the interferon signaling gene expression score (ISG).

Results: The AGS scale score correlated with severity classifications and the composite neurologic function scores. When retrospectively applied across our natural history study, the majority of individuals demonstrated an initial decline in function followed by stable scores.

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Appendix A. Supplementary data

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Within the first 6 months of disease, the AGS score was the most dynamic. The AGS scale correlated with performance by the GMFM-88, but did not correlate with ISG levels.

Conclusions: This study demonstrates the utility of the AGS scale as a multimodal tool for the assessment of neurologic function in AGS. The AGS scale correlates with clinical severity and with a more labor-intensive tool, GMFM-88. This study underscores the limitations of the ISG score as a marker of disease severity. With the AGS scale, we found that AGS neurologic severity is the most dynamic early in disease. This novel AGS scale is a promising tool to longitudinally follow neurologic function in this unique population.

Keywords

Leukodystrophy; Interferonopathy; Neurodegenerative; Genetic; Outcome measure

1. Introduction

Aicardi Goutières Syndrome (AGS) is a rare genetic disorder of excessive interferon (IFN) production resulting in severe neurologic disability [7]. AGS results from pathogenic changes in genes associated within the nucleic acid sensing machinery *(TREX1, RNASEH2A, RNASEH2B, RNASEH2C, SAMHD1, ADAR1*, and *IFIH1*) [2,7,11,20]. Almost all individuals affected by AGS exhibit severe neurologic dysfunction with impairments in gross motor, fine motor, and cognitive domains ([1]).

Existing outcome measures have significant limitations in the AGS population. Floor effects make it difficult to distinguish among skill levels in the lower register. Additionally, children with AGS can demonstrate an atypical sequence of development, creating challenges with sequential scoring (e.g. standing before sitting), and insensitivity to delayed skill acquisition (e.g. new skill of babbling at age 5) [1]. Traditional outcome measures are labor-intensive and fatiguing in fragile populations, and often multiple tools are required to assess the multimodal disability found in AGS. As such, there is a lack of existing tools to easily measure the full neurologic disability found in AGS.

Disease-specific assessment tools have been beneficial in demonstrating change in other rare, neurologic disorders [5,10,13–15,19]. The lack of validated measures to study AGS has limited the evaluation of neurologic function in this rare disease. As targeted therapeutics are developed for AGS, it becomes increasingly important to be able to measure neurologic function in this unique population. To this end, we developed an AGS-specific neurologic scale. Our goal was to create a simple to apply tool, capable of measuring neurologic function longitudinally for use in future natural history studies and as a potential outcome measure in future clinical trials.

2. Methodology

2.1 Study design

This is a retrospective natural history study designed to develop a multimodal AGS scale. Individuals affected by AGS were recruited through the Myelin Disorders Bioregistry Project (MDBP) (NCT03047369; IRB approved), which is part of the Global

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Leukodystrophy Initiative Clinical Trial Network (GLIA-CTN). Individuals were included across three participating institutions: Children's Hospital of Philadelphia, Istituto di Ricerca Clinica C. Mondino, and Spedali Civili of Brescia.

2.2 Subjects

Individuals with pathogenic or likely pathogenic mutations in the following AGS-related genes were included in this cohort: *TREX1, RNASEH2A, RNASEH2B, RNASEH2C, SAMHD1, ADAR1*, and *IFIH1*. Individuals without a known genetic diagnosis or insufficient developmental records were excluded. The cohorts for the development, validation, and clinical cohorts are described in Supplemental Fig. 1.

2.3 Data collection

Information, including genetics, neurologic function, and age at each evaluation, was collected from medical records. Milestone acquisition was obtained from medical records and confirmed by parental questionnaires. Data was extracted by a child neurologist.

2.4 Definition of AGS severity classes

This cohort was comprised of all individuals with available electronic medical records. A clinical severity assessment was assigned retrospectively to the last available clinical encounter by two child neurologists: "mild", "moderate", and "severe" (n = 65). When there was a discrepancy (n = 8), (mild-moderate or severe-moderate), the individual was assigned to the moderate classification.

2.5 Composite neurologic function score (rComposite)

Because of the age requirements for the application of the functional scales, this cohort was comprised of all individuals with available electronic medical records over the age of 18 months (n = 61/65). Three validated functional scores were assigned retrospectively to the last available clinical encounter as previously described for AGS: Gross Motor Functional Classification Scale (GMFCS), Manual Abilities Functional Classification Scale (MACS), and Communication Function Classification Scale (CFCS) [8,9,16,18,22]. Each of these scales is composed of 5-levels with 'I' as normal function and 'V' representing complete impairment. These values were reversed and summed, so that higher numbers represent greater function. This was designated as the 'rComposite'.

2.6 Development of the AGS Scale

The AGS scale was developed by a panel of AGS experts by a consensus review process [6]. Each item was reviewed and modified for content validity, clarity, and face validity.

Items were selected from the rates of developmental skill acquisition in the AGS population [1]. Across the AGS population, the most commonly attained skills were smiling, head control, and babbling. As AGS affects all developmental domains, we selected three key developmental categories: cognitive development, fine motor development, and gross motor skills to be included in our scale. We removed items that were frequently absent from clinical records (e.g. the differentiation between immature and mature pincer grasp). We focused on the inclusion of items that could be consistently understood and administered [6].

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To address issues around the validation of this outcome measure given the limitations of sample size in a rare disease and familiarity of the research team with this population, pilot testing was performed using blinded clinical encounters. These encounters were selected based on the presence of developmental skill information related to gross motor and cognitive function (n = 140 encounters from 35 individuals). In addition to the removal of all HIPPA identifiers, all identifying information was redacted from these clinical encounters, including gender pronouns and any unique characteristics and histories, by two unblinded members of the study team. These encounters retained only essential developmental and examination information. These blinded encounters were scored by two child neurologists. After scoring a limited set of encounters, the scores were compared between raters, and the definitions for each item were refined.

In the final AGS scale (Fig. 1), one point is awarded for the presence of the following skills: normocephaly, smiling, vocalizations, single words, sentences, fine motor skills (pincer grasp or self-feeding), head control, sitting independently, and rolling or crawling, mobile with assistance, and independently mobile. The points are summed to generate the final AGS scale score, which has a range of 0–11.

A subset of blinded encounters were separately scored by two child neurologists and used to calculate the AGS scale agreement and kappa coefficient (total of 24 encounters per rater (92% agreement, kappa coefficient of 0.91; 95% confidence interval 0.81–0.99))

2.7 Application of the AGS scale

The AGS scale was applied to retrospective clinical encounters (533 clinical encounters from 92 individuals affected by AGS). Each score was assigned and verified by two child neurologists.

2.8 Gross motor function measure-88

A subset of individuals underwent Gross Motor Function Measure-88 (GMFM-88) assessments by trained therapists (n = 24) [3,12]. This cohort includes the first GMFM-88 assessment for all individuals. The GMFM-88 is divided into 5 subdomains: subdomain A (lying and rolling skills), subdomain B (sitting skills), subdomain C (crawling and kneeling skills), subdomain D (standing skills), and subdomain E (walking, running, and jumping skills).

2.9 Interferon signaling gene expression scores

IFN-signaling gene (ISG) scores are a diagnostic biomarker of AGS. This cohort included the first encounter at which ISG scores were obtained concurrently with clinical evaluations (n = 36). ISG scores (z-scores) were calculated as available from each clinical visit where blood samples were obtained. These scores were derived from the mRNA measurement of 6 IFN-inducible genes (*IFI27, IFI44L, IFIT1, ISG15, RSAD2*, and *SIGLEC1*) and 4 housekeeping genes (*ALAS1, HPRT1, TBP*, and *TUBB*) as previously described [4]. Briefly, patient blood samples were collected in PAXgene blood RNA tubes (PreAnalytiX), and RNA was purified using PAXgene blood RNA kits (Qiagen). RNA quality was assessed by RNA TapeStation (Agilent) and the RIN number of all of the samples was above 6.0. The

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concentration of RNA was quantified by Qubit High Sensitivity RNA assay (Thermo), and 200 ng RNA was used for each sample in the nCounter™ Digital Analyzer.

2.10. Statistical analysis

Excel 16.23 and Prism 8.0 were used to generate the graphs and statistics. Inter-rater reliability (agreement between two independent providers) of the AGS scale were assessed by kappa statistics and intraclass correlation coefficients (ICC). Mann Whitney test with two tailed *p* values were used when comparing two independent groups; the Kruskal-Wallis test was used to compare more than two independent groups. Spearman correlations were used to compare the AGS scale values to existing measures. Significance was defined when P < .05, adjusted for multiple comparisons with an overall Bonferroni correction. All comparisons maintained significance when an overall Bonferroni correction was applied to define significance between multiple groups.

3. Results

3.1. Neurologic severity in AGS

There is no validated tool to objectively measure neurologic severity in AGS. As AGS is a multimodal neurologic disorder, the sum of three neurologic severity scales that address gross motor (GMFCS), communication (CFCS), and fine motor (MACS) has been previously used in this population as a surrogate marker for global neurologic function. In this study, these three retrospective scales were applied to the most recent clinical encounters for individuals older than 18 months (n = 61). This value was calculated as a reverse composite score (rComposite) where larger numbers represent higher abilities.

To further address this need to categorize neurologic severity in AGS, two independent child neurologists assessed the neurologic severity of AGS (n = 65 individuals) as mild, moderate, and severe (Fig. 2). There was agreement in the categorizations in 87% of the encounters (n = 57 of 65). There was a significant difference between the rComposite scores between the mild (n = 10, mean 14.00 ± 1.63 standard deviation [SD], range 11–15), moderate (n = 21, mean 7.91 ± 1.95 SD, range 4–11), and severe (n = 30, mean 3.10 ± 0.40 SD, range 3–5) categories (Kruskal-Wallis test *p* value < .0001 for all comparisons). (Fig. 2B).

3.2. AGS scale development and validation

To address the need for a simple, multimodal scale to assess neurologic function in AGS, the AGS Scale was developed. This scale is a simple tool where one point is awarded in the presence of key developmental steps and for head circumference (Fig. 1). Microcephaly was included because it can be acquired in AGS and is strongly correlated with neurologic impairment [17]. The AGS scale was applied retrospectively to the rComposite encounters (n = 61). There was a The AGS scale was applied retrospectively to the rComposite encounters (n = 61). There was a significant correlation between the multimodal AGS Scale and the composite scale derived from the GMCSF, CSCS, and MACS (Spearman r 0.9273, 95% confidence interval 0.8795–0.9565, 2 tailed *p* value < .0001) (Fig. 3A).

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The AGS scale was also compared between the clinical categories (n = 65). There was a significant difference between the AGS scale scores between the mild (n = 11, mean 10.55 ± 0.52 standard deviation [SD], range 10–11), moderate (n = 23, mean 7.13 ± 1.89 SD, range 4–10), and severe (n = 31, mean 2.00 ± 1.16 SD, range 0–4) categories (Kruskal-Wallis test *p* value < .0001) (Fig. 3B).

AGS is a unique genetic disorder as it is caused by pathogenic changes in 7 distinct genes within a common pathway. We sought to correlate the lowest level of neurologic function (the nadir score of the AGS scale) with genotype to explore genotypic differences. This nadir score was compared between the 6 most common genotypes (n = 90) (Fig. 4). We excluded the *RNASEH2C*, the rarest genotype, from comparative analyses because of small sample size. As previously described [1], each genotype had a heterogeneous distribution of severity. There was a significant difference between the nadir score identified within the cases associated with *TREX1* genotypes and the genotypes for *SAMHD1, ADAR1*, and *IFIH1* (Kruskal-Wall test: *TREX1* vs *SAMHD1 p* value 0.0053; *TREX1* vs *ADAR1* p value 0.0022; *TREX1* vs *IFIH1 p* value 0.0032).

Formal testing of gross motor function was available in a subset of individuals (n = 24) (Fig. 5). GMFM-88 is an intensive gross motor test administered by a qualified physical therapist. We compared the GMFM-88 percentiles to our simple AGS scale, noting that the latter also encompasses fine motor and cognitive function as well. There was a significant correlation between the two measures (Spearman r 0.8931, 95% confidence interval 0.76–0.95, 2-tailed p value < .0001).

There is no validated biomarker for AGS disease, although ISG scores are often used a diagnostic biomarker. The relevance of this biomarker to clinical status and outcomes is unknown. To begin to understand the relationship between interferon-related activity and neurologic status, we compared concurrent AGS Scale results to ISG score (n = 36) (Fig. 6). There was no correlation between ISG score and performance by the AGS scale.

3.3. Longitudinal application of the AGS scale

Clinical records were scored at approximately 6-month intervals to assess for longitudinal performance and to evaluate neurologic trajectory over the first 10 years of disease (Fig. 7). The average age at clinical presentation was 8.2 months (range 0–120 months). The average age at the first AGS scale application was 28.1 months (range 0–186 months). The average age at last scale application was 83.0 months (range 2–305 months).

Change in the AGS scale was defined as improvement or decline by 2 or more points over the course of the available medical records. The majority of individuals had stable or declining neurologic function during the period of available medical records (n = 66; Fig. 7A). Neurologic function improved or was dynamic over the course of disease in 19 individuals (Fig. 7B). The longitudinal course by AGS scale by genotype is presented in Fig. 7C for the 6 most common genotypes.

Finally, the absolute change in AGS scores between the first and last evaluations was compared to the duration between symptom onset and first evaluation across the cohort for

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whom 2 evaluations within the first 10 years were available (n = 80; Fig. 8A). As was previously hypothesized [11], we found that the AGS scale was the most dynamic when the first available encounter was within the first 6 months of disease (Fig. 8B).

4. Conclusions

Our detailed understanding of the neurologic course of AGS has been limited by the rarity of the disease and the lack of validated assessment tools. Disease-specific measures have been successful in assessing therapeutic benefit in other rare, neurologic disorders [5,10,13–15,19]. Most existing assessment tools are unable to sufficiently evaluate individuals at the lower end of function. In this study, approximately half of the AGS population was severely affected (Fig. 2). To address the need for simple assessment tools for neurologic function in this population, we developed an AGS scale to measure key items relevant to development in AGS abilities. This AGS scale was able to assess neurologic function, had excellent interrater reliability, and correlated with overall severity. Important for a rare disease, this scale could be applied retrospectively as it is dependent on key developmental steps often included in medical encounters. The prospective application of this tool is currently underway.

While AGS affects all domains of development, most existing tools focus on a single area: gross motor, fine motor, or cognitive skills. As such, to measure the full impact of AGS on neurologic function, multiple tools may be required. We hypothesize that future interventions in AGS may result in small, but meaningful improvements, across the modalities. Separate measures may be insensitive to these changes, but a single tool including key neurologic milestones could potentially capture improvements across neurologic domains.

Because gross motor function is impaired in most individuals affected by AGS, we compared performance on the AGS scale to a validated measure of gross motor function, GMFM-88. The AGS Scale and the GMFM-88 demonstrated significant correlation. This underscores the potential utility of the simpler AGS Scale. Of note, half of the values for GMFM-88 were below the 10th percentile, suggestive of a significant floor effect (Fig. 3A).

We also compared the AGS scale to a biomarker of disease, the ISG score. We found no correlation between this diagnostic biomarker and the AGS scale. This finding is supported by other reports that do not correlate ISG scores with severity [2,21]. One confounder of our results is that ISG scores can be dynamic both day-to-day and over the lifetime of a patient. External influences, such as viral infection, would increase the ISG score. Because of these reasons, a single ISG measurement can be difficult to interpret. It is possible that initial ISG scores correlates with severity and the AGS scale.

Given the genetic heterogeneity of AGS, we next characterized performance of the AGS scale across genotypes. In order to minimize the bias from unequal numbers of clinical encounters, we compared nadir AGS scores. We found that individuals with *TREX1*-related AGS demonstrated the lowest scores, which is consistent with clinical experience.

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We were able to map the dynamic neurologic function found in AGS by retrospectively applying the scale to historical clinical encounters. We were able to identify several distinct patterns: those with stable disease, those with recurrent relapses, and those with progressive improvement. Future work is needed to identify variables associated with each trajectory, although we hypothesize that genotype and age at disease onset will be influential in future neurologic recovery. The overall rate of recovery after the periods of regression are unknown, although few children are suspected to return to their full neurologic baseline. Importantly, we found that most improvement occurred in the first 6 months after disease onset. This is an important consideration in clinical trial design, as the expected disease trajectory may be dependent on the time from disease onset. Of note, our data are limited by the availability of detailed and complete medical records, including variable distance from disease onset to first available encounter. Future studies will be designed to assess which variables are associated with neurologic improvement, even in the absence of therapeutic intervention.

As this novel tool provides the first opportunity to easily map neurologic function over time, we wished to characterize the dynamic nature of the disease (Fig. 8). We found the majority of dynamic AGS scores coincided with when the first post-disease onset encounter was within the first 6 months of disease (Fig. 8B). This suggests that after an early dynamic period, there is a trend toward neurologic stability in the majority of patients.

Our novel AGS tool allows for the easy assessment of basic neurologic function in this unique population. This scale correlates with established measures, microcephaly, and genotype. With the longitudinal and prospective application of this instrument, we can begin to characterize the dynamic neurologic function of this rare disease. Moreover, because this scale does not include AGS-specific parameters, this scale holds promise for use across other severe neurologic disorders with dynamic function.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Item	Points	
	0 Absent	1 Present
Normocephaly (< 2 SD from mean)		
Social smile		
Vocalizations (cooing or babbling)		
Single, meaningful words		
Minimum of three-word phrases		
Head control (> 60 seconds)		
Pincer grasp or self-feeding		
Independent sitting (> 2 minutes)		
Rolling or crawling to goal		
Ambulation with assistance (devices or two-hand assist)		
Independent ambulation (no assistive devices)		
Total Score (range 0-11)		

Fig. 1.
AGS Scale. © 2020, The Children's Hospital of Philadelphia. All Rights Reserved.

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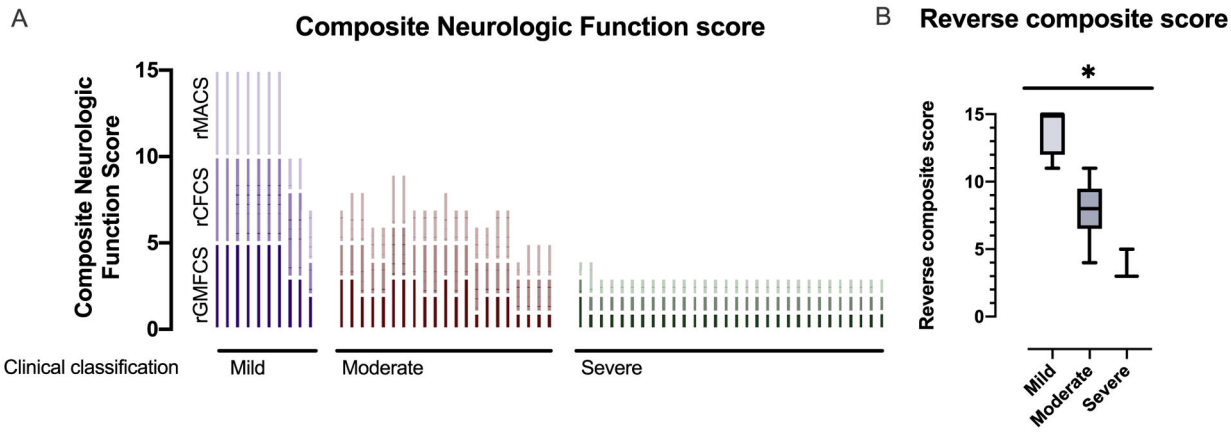

Fig. 2.

Neurologic severity in AGS. (A) Three classification scales (GMFCS, MACS, and CFCS) were applied to the most recent available clinical encounter retrospectively in 61 children with molecularly confirmed AGS and are presented as reverse composite (rComposite) scores. Individuals are clustered by severity classifications. Higher rComposite scores indicate less impairment. (B) Clinical severity was assigned to each clinical encounter as mild-moderate-severe. The rComposite scores were compared across the severity classifications: mild (n = 10, mean 14.00 ± 1.63 standard deviation [SD], range 11–15), moderate (n = 21, mean 7.91 ± 1.95 SD, range 4–11), and severe (n = 30, mean 3.10 ± 0.40 SD, range 3–5) categories (Kruskal-Wallis test p value < .0001 for all comparisons).

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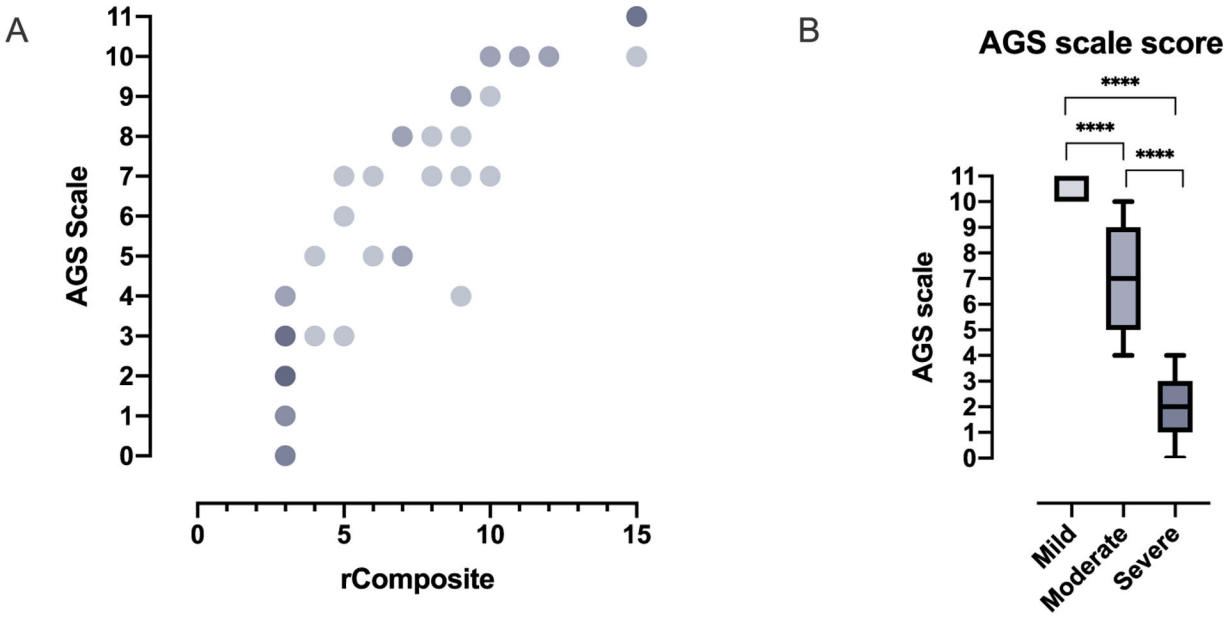

Fig. 3.

AGS Scale scores correlate with neurologic severity. (A) The AGS scale was compared to disease severity using the rComposite scores (Spearman r 0.9273, 95% confidence interval 0.8795–0.9565, 2 tailed *p* value < .0001). Overlapping values are indicated by increasing color density. (B) The AGS score was compared to clinical severity assessments: mild (n = 11, mean 10.55 ± 0.52 standard deviation [SD], range 10–11), moderate (n = 23, mean 7.13 ± 1.89 SD, range 4–10), and severe (n = 31, mean 2.00 ± 1.16 SD, range 0–4) (Kruskal-Wallis test *p* value < .0001 for all comparisons).

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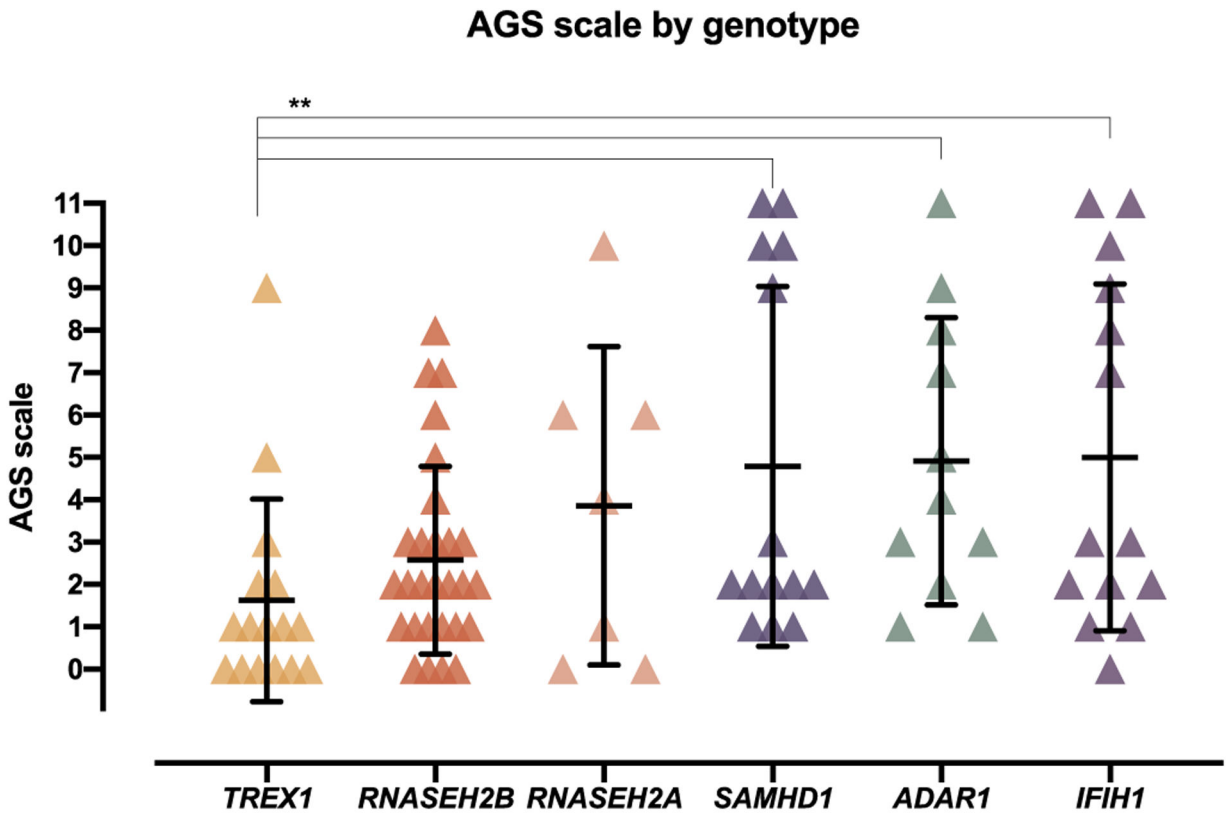

Fig. 4.

Comparison of the AGS Scale to genotype. The nadir AGS Scale was compared across genotypes (n = 90; mean with SD bars; Kruskal-Wall test: *TREX1* vs *SAMHD1* p value 0.0053; *TREX1* vs *ADAR1* p value 0.0022; *TREX1* vs *IFIH1* p value 0.0032).

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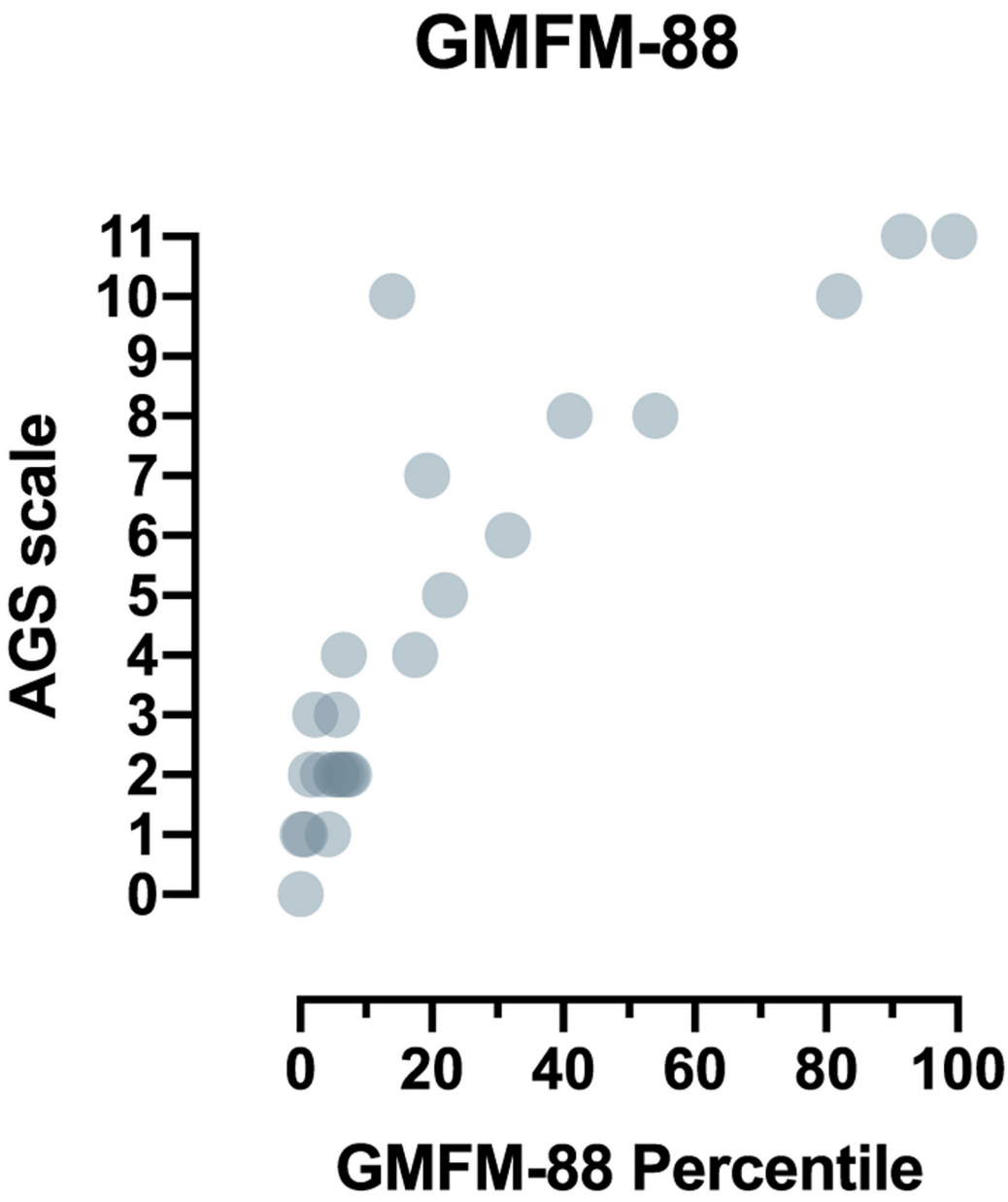

Fig. 5.

The correlation of the AGS scale to a standardized tool for gross motor function, GMFM-88. Formal testing of gross motor function was available in a subset of individuals (n = 24). The GMFM-88 overall percentile was compared to retrospective assignment of the AGS scale (Spearman r 0.8931, 95% confidence interval 0.76–0.95, 2-tailed p value < .0001).

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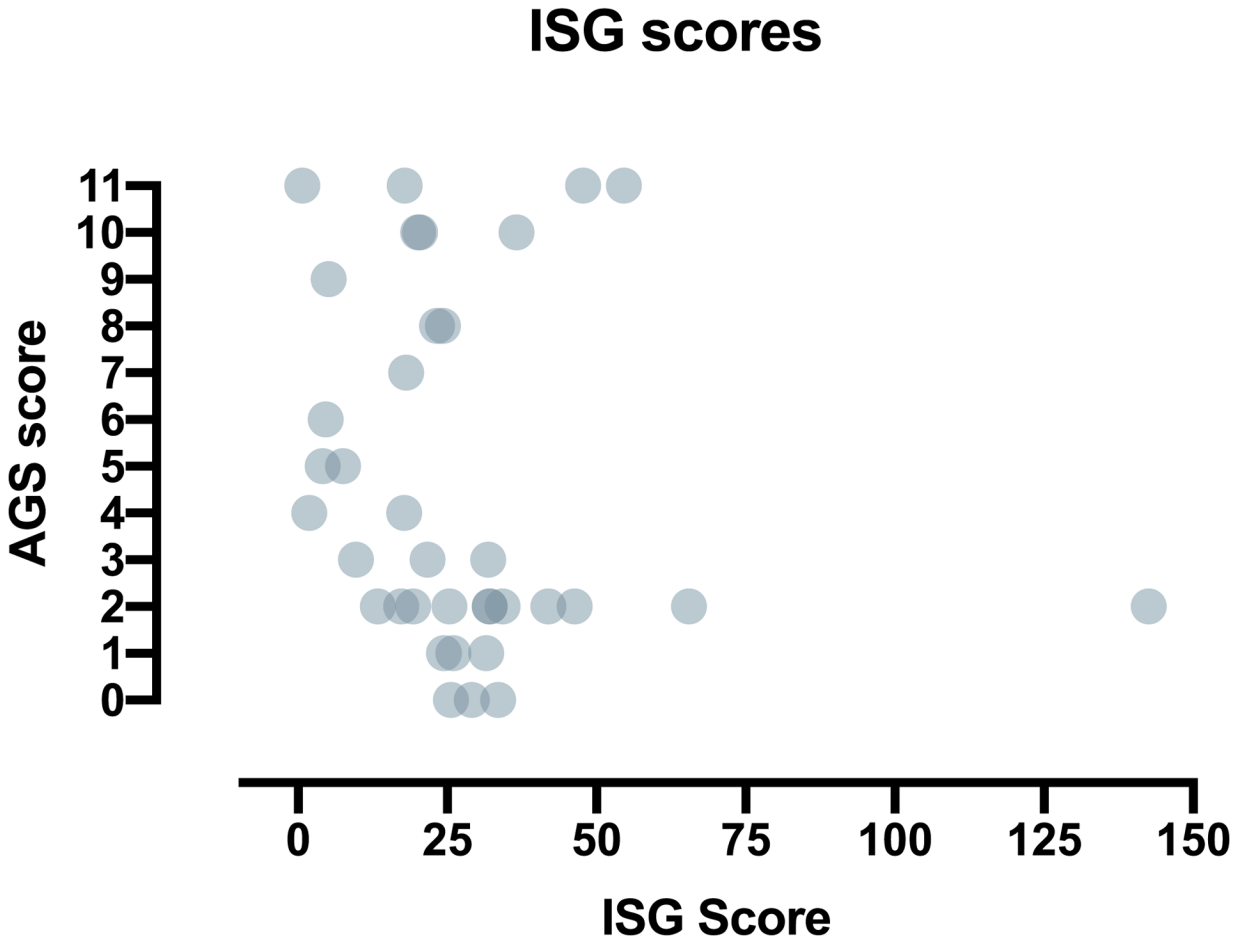

Fig. 6.

Correlation of the AGS scale to a potential AGS biomarker. The AGS Scale was retrospectively applied to clinical encounters at which ISG measurements were obtained (n = 36). There was no correlation between ISG score and performance by the AGS scale.

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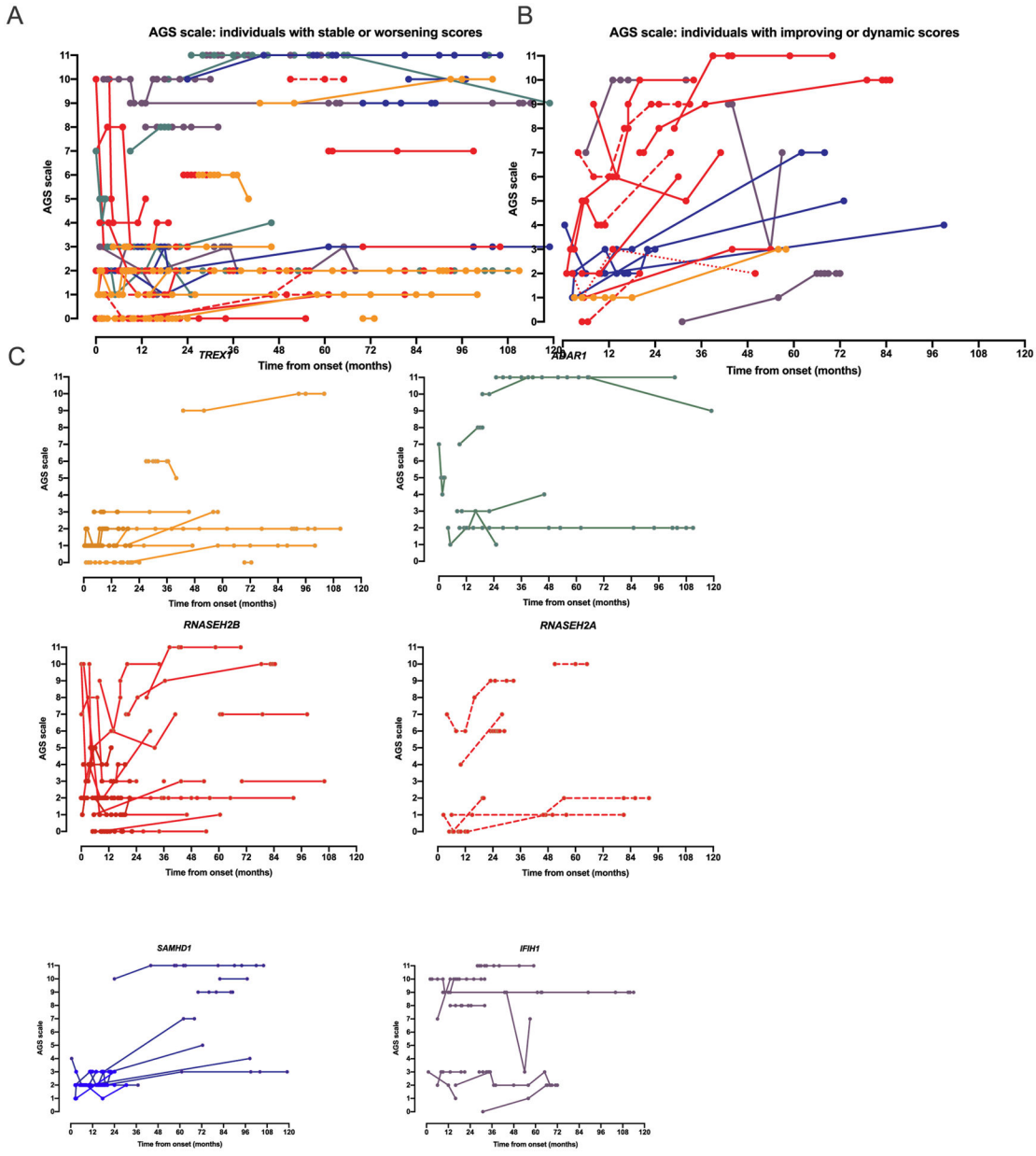

Fig. 7.

The AGS scale captures longitudinal change in individuals affected by AGS. All plots represent time from disease onset (n = 92). A. The majority of individuals across all genotypes had stable or worsening AGS scale scores (n = 66). B. A subset of individuals had improvement in their AGS scale score (≥ 2 points increase from baseline at any time point) or were dynamic (both increasing and decreasing by ≥2 points). C. Genotype specific plots are provided for the 6 genotypes with more than 2 individuals.

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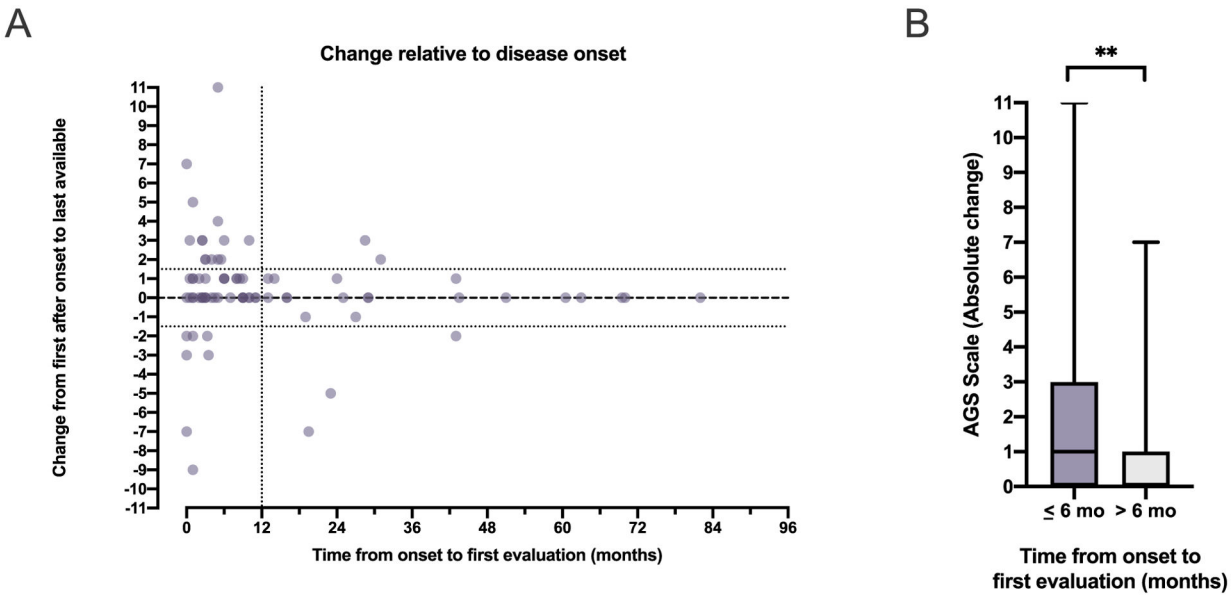

Fig. 8.

AGS scale change correlates with proximity to disease onset. (A) The change in AGS scale between the first and last evaluations was compared to the duration between symptom onset and first evaluation in the 80 individuals for whom 2 evaluations within the first 10 years of disease were available. (B) Dynamic AGS scores (absolute change between the first and last evaluation) were more likely when the first evaluation occurred within the first 6 months of disease (Mann Whitney, two-tailed p = .0057).

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Original Article

Reliability of the Telemedicine Application of the Gross Motor Function Measure-88 in Patients With Leukodystrophy

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ABSTRACT

Background: Leukodystrophies are a rare class of disorders characterized by severe neuromotor disability. There is a strong need for research regarding the functional status of people with leukodystrophy which is limited by the need for in-person assessments of mobility. The purpose of this study is to assess the reliability of the Gross Motor Function Measure-88 (GMFM-88) using telemedicine compared with standard in-person assessments in patients with leukodystrophy.

Methods: A total of 21 subjects with a diagnosis of leukodystrophy (age range = 1.79-52.82 years) were evaluated by in-person and by telemedicine evaluations with the GMFM-88 by physical therapists. Inter-rater reliability was assessed through evaluation of the same subject by two independent raters within a three-week period (n = 10 encounters), and intrarater reliability was assessed through blinded rescoring of video-recorded assessments after a one-week time interval (n = 6 encounters).

Results: Remote assessments were performed by caregivers in all 21 subjects using resources found in the home with remote guidance. There was agreement between all paired in-person and remote measurements (Lin's concordance correlation $\geq$0.995). The Bland-Altman analysis indicated that the paired differences were within ±5%. Intrarater and inter-rater reliability demonstrated an intraclass correlation coefficient of >0.90.

Conclusions: These results support that remote application of the GMFM-88 is a feasible and reliable approach to assess individuals with leukodystrophy. Telemedicine application of outcome measures may be of particular value in rare diseases and those with severe neurologic disability that impacts the ability to travel.

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Introduction

Leukodystrophies are a broad class of genetic disorders that affect the myelin of the brain which often result in severe neurologic disability in early childhood. Children with leukodystrophies are noted to have a low health-related quality of life[1-4] and prolonged hospitalizations that cost more than $59 million per year.[2] There is a need to lessen the burden of disease through improved symptomatic care and targeted therapeutics.[5-15] One barrier to the development of novel therapies for leukodystrophy is the lack of comprehensive natural history studies describing the functional abilities of patients with the disorder. Standardized assessments of motor function will likely be a key outcome in future natural history studies and future clinical trials.[16] Motor outcomes that are valid and reliable are critical for monitoring the change in neurologic function over time.

Because of the rarity of leukodystrophy, enrollment in natural history studies via traditional in-person assessments may require travel to a specialty medical center, time off work for caregivers, and transportation of medically fragile individuals which may limit enrollment.[17,18] The ability to capture motor function in a more natural environment without the fatigue or risks of travel may prove to be a powerful approach to more accurate and frequent outcome assessments. One potentially useful tool to assess motor function that may avoid these challenges is the use of remote assessments via telemedicine. Remote assessments would allow for a broader and more diverse population to participate in research, including people who live in long-term care facilities and isolated rural areas,[19] but few outcome assessments are validated for use with telemedicine approaches.

The field of child neurology has adapted rapidly to the ongoing COVID-19 pandemic by increasing the use of telemedicine encounters to address the needs of patients.[20] However, motor assessments via telemedicine has been mostly limited to adults with neurological conditions which largely focus on measures of upper extremity function[21] or walking.[22] Other approaches to assess motor function using telemedicine require specialized equipment[23] which limits its broad use in clinical practice. Camden and Silva report that therapists value telehealth evaluations while noting that use of norm-referenced and criterion-referenced assessments in telehealth have not been implemented owing to a lack of information on how to complete these tests remotely.

One commonly used outcome measure that may be adaptable to the telehealth environment is the Gross Motor Function Measure-88 (GMFM-88), which was originally developed to assess children with cerebral palsy.[24] The GMFM-88 is divided into 5 subdomains: subdomain A (lying and rolling skills), subdomain B (sitting skills), subdomain C (crawling and kneeling skills), subdomain D (standing skills), and subdomain E (walking, running, and jumping skills). The GMFM-88 requires equipment that is easily available in clinical practice and no special technology so that its use in telehealth may be promising. In addition, the use of the GMFM-88 has been recently reported in patients with leukodystrophy.[8,25,26] In our previous work, we reported a significant correlation between the GMFM-88 and a disease-specific scale for children with Aicardi-Goutières syndrome (AGS).[25] However, the reliability of the GMFM-88 has not been reported in patients with leukodystrophy and the assessment of the GMFM-88 via telemedicine has not been described to date. In this study, we sought to assess the reliability of the GMFM-88 using telemedicine compared with standard in-person assessments in patients with leukodystrophy.

Methods

All subjects were recruited from the institutional review board–approved protocol, Myelin Database and Biorepository Project (MDBP), at the Children's Hospital of Philadelphia. Participants were included if they had completed the GMFM-88 during an in-person visit occurred after January 1st, 2020. The family of two patients refused to participate in this project. All subjects underwent a molecularly confirmed leukodystrophy diagnosis. A total of 21 subjects (age range = 1.3-52.5 years) were evaluated during sequential in-person and telemedicine encounters. The subject's diagnosis, age at symptom onset, and disease severity were determined by medical records review, as well as all GMFM-88 administrations available before the year 2020.

All in-person GMFM-88 asessments have been administered by physical therapists who had extensive experience using the GMFM-88 and followed the instructions of the GMFM-88 manual.[27] The remote assessments were performed by two trained physical therapists that administered and scored the children live, having the patients' parents assisting their children. One adult subject was evaluated without the assistance of any caregiver. All assessments were performed without the use of orthotics.

The families participating in this study were provided with information before the research encounter regarding the necessary environment and technology needed for home evaluations (Supplemental Table 1). Sessions were conducted over a secure video conferencing platform (Cisco WebEx). Existing home equipment, including typical home furniture, was selected to be as close as possible to the standard equipment and for subsequent evaluations and logged for consistency, in accordance with the GMFM-88 manual.[27] All 88 items were administered during the virtual encounter, and time needed for the assessment was similar to in-person assessment. Sessions were recorded with a fixed camera angle.

Composite scores were calculated and compared between assessments. We assessed inter-rater reliability by administering the GMFM remotely to 10 patients of the original cohort at an average interval of two weeks by two different therapists. Six of the 10 subjects' telemedicine visits were recorded for reliability assessments. Intrarater reliability was assessed using these video-recorded assessments by measuring the degree of agreement between two separate scorings of the same video administration after a one-week time interval (range = 6-8 days).

Statistics

Stata 16[28] was used for analysis with two-sided tests and a *P*-value < 0.05 as the criterion for statistical significance. Descriptive and correlative statistics were used to compare the assessments which were carried out in person with the remote assessments. Descriptive statistics included tabulation of categorical variables and computation of means, standard deviations (SDs), medians, ranges (minimum to maximum values), and interquartile ranges (IQRs, median to 75th percentile) of continuous variables.

To evaluate agreement, we used the concordance correlation coefficient (ρ_c)[29,30] that evaluates the degree to which pairs of observations fall on the 45° line through the origin (the line of perfect concordance). The concordance correlation ρ_c is the product of the Pearson correlation and a bias correction factor. McBride[30] suggested that ρ_c <0.90 indicates poor agreement, while 0.90-0.95, 0.95-0.99, and >0.99 suggest moderate, substantial, and almost perfect levels of agreement, respectively. We used the bootcor command in Stata 16.0 to obtain ρ_c, with 95% confidence intervals (CIs) obtained via bootstrap sampling. Bootstrap resampling does not require an assumption of bivariate normality for the two

measures being compared and can also be applied if the samples are not independent. We used the concord command in Stata to obtain a scatterplot of values with the line of perfect concordance.

We also used the Bland-Altman analysis to evaluate agreement. We used the agree command in Stata to plot the difference versus average of remote and in-person GMFM-88, with a horizontal line at the average difference and the upper and lower limits of agreement. We also obtained 95% CI for the average difference and upper and lower limits of agreement using the agree command. As suggested in the study by Zagreb,[31] acceptable values for the limits of agreement (within which 95% of differences between measurements would be expected to fall) should be established a priori. The Bland-Altman analysis assumes normality of the differences. We evaluated the normality assumption of individual variables and paired differences using diagnostic plots (histograms and plots of quantiles of the variable under consideration versus quantiles of the normal distribution [Q-Q plot]) and Shapiro-Wilk and tests for skewness and kurtosis with the agree command.

There were 21 subjects with both in-person and remote assessments of the GMFM-88. Of these 21 subjects, four (4/21, 19%) had one prior in-person assessment; five (5/21, 23.8%) had two prior in-person assessments; one (1/21, 4.8%) had three prior in-person assessments; and 11 (11/21, 52.4%) had four prior in-person assessments. Remote and in-person assessments were compared by individual-level profile plots for each patient. Individual-level regression lines of score versus age were fitted to the previous scores on each patient and were used to obtain a predicted value at the age of remote assessment. The agreement between the remote and predicted values from the individual-level regressions was then evaluated using the same approaches that were used to evaluate agreement between the remote and prior value. This analysis was considered to be exploratory and descriptive only.

To evaluate inter-rater reliability, we estimated the intraclass correlation coefficient (ICC) for a two-way mixed-effects model. We used the bootcor command to use bootstrap resampling to obtain the 95% CI for the ICC because the distribution of the scores deviated from normality. To evaluate intrarater reliability, we also estimated the ICC (with 95% CI) using the bootcor command in Stata, version 16.0.

Results

Demographics

A total of 21 subjects with leukodystrophy were enrolled in this study. Nine of these subjects were male (42.9%). The mean age at the last in-person evaluation before the remote evaluation was of 9.6 years (range = 1.3-52.5, SD = 11.0). The mean age at the telemedicine assessment was 10.1 years (range = 1.8-52.8, SD = 11.0). The mean difference between age at telemedicine assessment and age at baseline assessment was 0.5 years (range = 0.4-0.7, SD = 0.1). Of the 21 subjects, 17 individuals had a confirmed diagnosis of AGS (81%), 2 had a diagnosis of POLR3-related leukodystrophy (9.5%), 1 had a diagnosis of Alexander disease (AxD) (4.7%), and 1 had a diagnosis of TUBB4A-related leukodystrophy (4.7%).

GMFM-88 evaluations

We first evaluated the agreement between GMFM-88 remote administration total score and the closest in-person GMFM-88 total score. Diagnostic plots (Q-Q plot and histogram) and tests for normality suggested that the distribution of GMFM-88 remote and in-person scores deviated from normality; however, the normality assumption was reasonable for the paired differences (GMFM-88 minus remote scores). The mean difference was 0.2 (95% CI = −0.9 to 1.4; lower limit of agreement = −4.8 [95% CI = −6.8 to −2.8]; upper limit of agreement = 5.3 [95% CI = 3.3 to 7.3]; Lin's concordance correlation coefficient = 0.997 [95% CI = 0.993 to 0.998]) (Fig 1).

We next compared the GMFM-88 remote administration raw score with the predicted values from the individual-level regressions using all previous in-person assessments (n = 17 individuals had at least two prior assessments, n = 12 individuals had three or more assessments). Lin's concordance coefficient for GMFM-88 Remote versus predicted scores was 0.994 (95% CI = 0.975 to 0.998) (Supplemental Figure 1). The evaluation of the Bland-Altman plot visualized agreement (Supplemental Figure 1). The average difference (remote minus predicted GMFM-88) was 0.3 (95% CI = −1.5 to 2.0). The lower limit of agreement was −6.5 (95% CI = −9.5 to −3.4), and the upper limit of agreement was 7.0 (95% CI = 3.9 to 10.0). Individual-level profile plots of the prior scores that excluded and included the remote assessment on each patient are shown in Fig 2.

Inter-rater reliability was assessed through remote evaluation of the same subject by two independent raters within a 3-week period (n = 20 encounters for n = 10 subjects). The estimated ICC (95% CI) was 0.996 (95% CI = 0.964 to 0.999). Agreement between raters was determined using Lin's concordance coefficient and Bland-Altman plots (Supplemental Figure 2). Concordance between raters was 0.995 (95% CI = 0.961 to 0.999). The average difference between rater scores was −1.7 (95% CI = −3.9 to 0.5). The lower limit of agreement was −7.8 (95% CI = −11.6 to −4.0), and the upper limit of agreement was 4.3 (95% CI = 0.5 to 8.1).

Intrarater reliability was assessed by having one clinician score the same encounter twice via a blinded scoring of a video recording of the encounter (n = 6 subjects; ICC = 0.999; 95% CI = 0.996 to 1.000). Agreement was determined using Lin's concordance coefficient and Bland-Altman plots (Supplemental Figure 3). With respect to agreement, Lin's concordance coefficient for the scores on both raters was 0.999 (95% CI = 0.985 to 1.000). The average difference between the second and first scores was 0.2 (95% CI = −1.1 to 1.5). The lower limit of agreement was −2.3 (95% CI = −4.5 to 0.0), and the upper limit of agreement was 2.7 (95% CI = 0.4 to 5.0).

Discussion

Our results demonstrated agreement between the in-person and telemedicine assessments of the GMFM-88 while finding high levels of inter-rater and intrarater reliability as indicated by ICCs above 0.99. The population studied represents a broad range of function, with scores ranging across the spectrum of GMFM-88 scores so we would predict that this study may be relevant to most individuals with leukodystrophy. Future research is needed to confirm the reliability of both the in-person and remote assessment of the GMFM-88 in a larger sample of subjects with leukodystrophy.

In this investigation, we sought to validate the use of the GMFM-88 via telemedicine more broadly in individuals affected by leukodystrophies, including AGS, POLR3-related leukodystrophy, AxD, and TUBB4A-related leukodystrophies. While many individuals affected by leukodystrophies demonstrate a floor effect on outcome measures owing to the severity of neurologic dysfunction, the chosen population demonstrates a broad range of gross motor function.[32,33] The leukodystrophies selected for this study tend to have a more static course than other leukodystrophies[25,33-35] which needs to be confirmed by future natural history studies of motor function. This allows for the comparison of outcomes that were obtained asynchronously, but it is unknown if our results may have

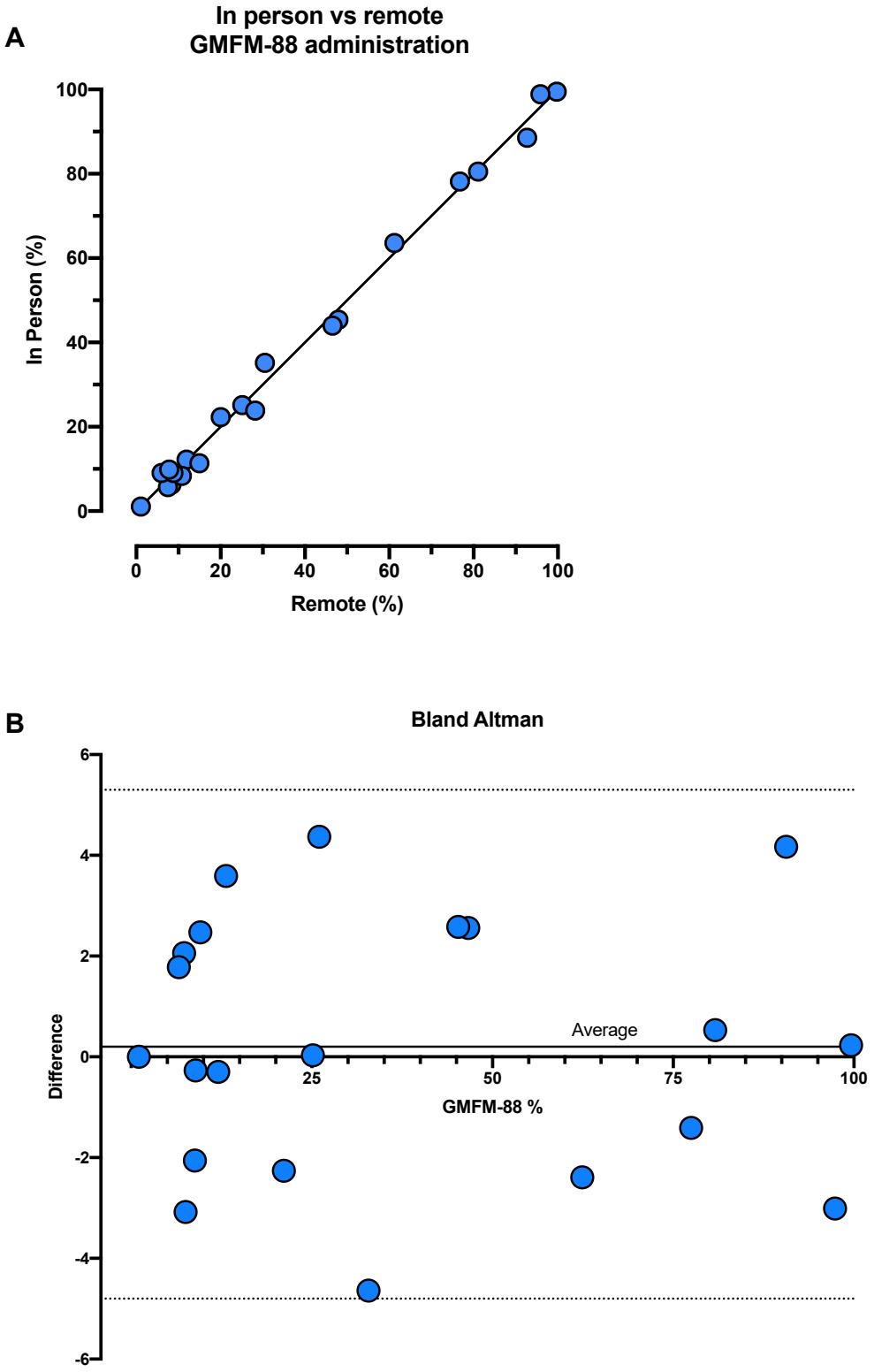

FIGURE 1. (A) Scatterplot of GMFM-88 in-person and remote administration (total score) with the line of perfect concordance. Lin's concordance correlation ρ_c (with 95% CI) is also provided ($\rho_c = 0.997$ [95% CI = 0.993 to 0.998]). (B) The Bland-Altman plot displays the variance between remote and in-person assessments. The lines represent ±5% variation (upper and lower limits of agreement for the paired differences). CI, confidence interval; GMFM-88, Gross Motor Function Measure-88. The color version of this figure is available in the online edition.

been affected by the time period in between the in-person and remote assessments.

One key challenge to understanding rare neurologic disorders is recruitment and bias toward including more mobile individuals.[32] Enrollment in natural history studies may be biased against individuals who have greater disease severity or who live at a greater distance from large academic medical centers. Our results suggest that the GMFM-88 may be a valid tool to assess function remotely

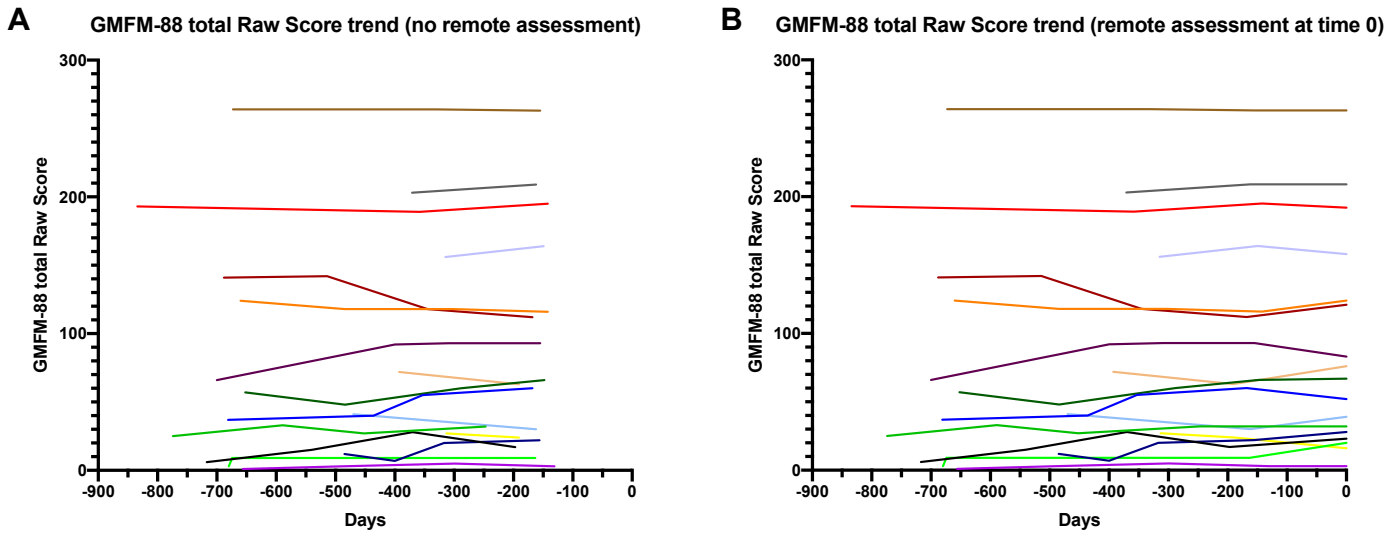

FIGURE 2. (A) Profile plots excluding remote assessment of the GMFM-88. (B) Profile plots including remote assessment of the GMFM-88. Time at remote assessment is 0. GMFM-88, Gross Motor Function Measure-88. The color version of this figure is available in the online edition.

which may allow for enrollment of a more diverse population and more frequent assessments in future natural history studies of longitudinal motor function. Future research investigating the remote assessments of other outcome measure of gross motor function as well as assessments of fine motor skills and activities of daily living would allow for more complete assessment of the functional ability of individuals with leukodystrophy.

Although these results are promising, this study has several limitations. Our cohort was limited in number owing to rarity of occurrence of leukodystrophies and the ability of families to travel to a central site for in-person assessments. However, the inability to recruit larger numbers of participants and to obtain regular study assessments in this population underscores the need to develop alternatives to in-person assessments. Additionally, inclusion in this study required baseline participation at a single centralized site with a high level of expertise with leukodystrophy and the collection of standardized outcome assessments. The ability to generalize this approach with other remote site facilities will need to be addressed in future studies. It should be considered that technological limitations, such as unavailability of electronic devices, inconsistent internet connections, or other technical difficulties, which have been reported by Rametta et al. during telehealth encounters with children, may make the remote application of the outcomes measures such as the GMFM impossible. The absence of the equipment required to complete the GMFM-88 might limit its application in some home environments. Finally, the use of caregivers to assist in the completion of the GMFM may be difficult for some caregivers, especially for older and more physically dependent children, so that alternative assessments which are less physically demanding will need to be developed.

In summary, we validated the use of remote GMFM-88 application using comparison with baseline in-person data. As future therapeutic trials for the leukodystrophies become available, the remote GMFM-88 may serve as a valuable tool to supplement in-person traditional outcome evaluations.

Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.pediatrneurol.2021.09.012.

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Janus Kinase Inhibition in the Aicardi–Goutières Syndrome

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TO THE EDITOR: The Aicardi–Goutières syndrome is a genetic interferonopathy that is associated with severe disability and death.[1] Most children with this syndrome are unable to walk or talk and have multisystemic complications, including skin inflammation (Fig. 1A). Janus kinase (JAK) inhibitors may be effective in blocking interferon activation in patients with the Aicardi–Goutières syndrome.[2–4]

We conducted an open-label study of a single-center, expanded-access program (ClinicalTrials.gov numbers, NCT01724580 and NCT03047369; see the protocol, available with the full text of this letter at NEJM.org) involving 35 patients with genetically confirmed Aicardi–Goutières syndrome. The patients received baricitinib, an oral JAK1 and JAK2 inhibitor. Eli Lilly provided the medication for the study and provided support for safety monitoring.

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The median age at the onset of symptoms was 6.0 months, and the median age at the initiation of baricitinib was 2.9 years (range, 0.2 to 21.8). The patients participated in the study for a minimum of 12.0 months (range, 11.8 to 43.8). The dose of baricitinib, which ranged from 0.1 to 0.6 mg per kilogram of body weight per day, administered in 2 to 4 dosing increments per day, was determined according to the patient's renal function, age, and symptoms.

JAK inhibitors are associated with infection, anemia, lymphopenia, and thrombosis. Two patients died during the study: one who had been receiving glucocorticoids for a decade, including a 7-year period before the study, had Aicardi–Goutières syndrome–related multisystem organ failure with an opportunistic infection, and the other had Aicardi–Goutières syndrome–related pulmonary hypertension with thrombotic microangiopathy.[5] Clinical variables were measured with laboratory tests and graded according to Common Terminology Criteria for Adverse Events, version 5.0, in which grades range from 1 to 5, with higher grades indicating more severe adverse events. Among these variables, only thrombocytosis increased in severity during the study (mean change in grade from baseline, 0.30; 95% confidence interval [CI], 0.04 to 0.60).

A biomarker for interferon signaling, the interferon-signaling gene-expression score, indicated a response to treatment (Fig. 1B). This score is calculated from the expression of six interferon-signaling genes normalized to housekeeping genes. The six-gene interferon signature is the sum of the median z scores of these genes. The interferon signature is positive (i.e., type 1 interferon signaling is high) if it is at least 1.96 (>98th percentile) on a one-tailed test (see the Supplemental Methods section in the Supplementary Appendix, available at NEJM.org).

The parents of the patients used diaries to record the children's Aicardi–Goutières syndrome–related symptoms, including neurologic disability, crying, sleep disturbances, irritability, seizures, fever, and skin inflammation of the trunk, arms, and legs (see the Supplemental Methods section and Table S5 in the Supplementary Appendix). The diary score included a total of 24 possible points, assigned every day, with daily scores averaged across the time period before each visit (1 month after the initiation of baricitinib and then every 3 months). Scores obtained during the study ranged from 0 to 10.6, with higher scores indicating more severe symptoms. The skin subscore included a total of 6 possible points, with higher scores indicating more severe symptoms. The overall diary scores improved within 1 month after the initiation of therapy (mean change in overall score, −0.7 diary points; 95% CI, −1.2 to −0.2) and were sustained (mean change in overall score, −1.4; 95% CI, −2.2 to −0.6) (Fig. 1A and 1C). The percentage of patients with a lower (improved) skin subscore of only 0 or 1 increased from 66% (23 of 35 patients) to 83% (29 of 35 patients) from the baseline visit to the final visit (difference, 17 percentage points; 95% CI, 2 to 32).

We evaluated the patients' developmental histories from disease onset (median age since disease onset, 16.5 months; interquartile range, 7.4 to 41.5) to the clinical data cutoff date (Fig. 1D). We extracted information on key milestones, including head control, sitting, rolling, assisted and independent ambulation, grasp, smiling, babbling, and the use of single words and word combinations. Before treatment, 26 of the 35 patients had stable or

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declining neurologic function, and 9 of the 35 patients gained one or two skills after disease onset. During the study, 20 patients met new milestones, and 12 patients gained two to seven new skills. This improvement was noted by 3 months (mean change in the number of milestones, 0.5; 95% CI, 0.2 to 0.9) and persisted (mean change, 1.3; 95% CI, 0.6 to 1.9). One patient lost skills during an acute illness while participating in the study. Children in a higher daily dose category of baricitinib met more milestones (mean increase, 1.3 milestones; 95% CI, 0.6 to 1.9) than those in a lower daily dose category according to weight-based calculations (Fig. 1E).

The primary risks associated with baricitinib among patients with the Aicardi–Goutières syndrome were thrombocytosis, leukopenia, and infection (see Section 4.1.2 in the Supplementary Appendix). Patients who are receiving baricitinib should be monitored closely, especially those with underlying thrombotic risk factors or those who are receiving systemic glucocorticoids or immunosuppressive regimens. Because this study drew from an international population, we anticipate that our findings will be generalizable. The measurement of neurologic improvement is complex, but our data suggest improvement in neurologic function, even in patients with severe and long-standing disease.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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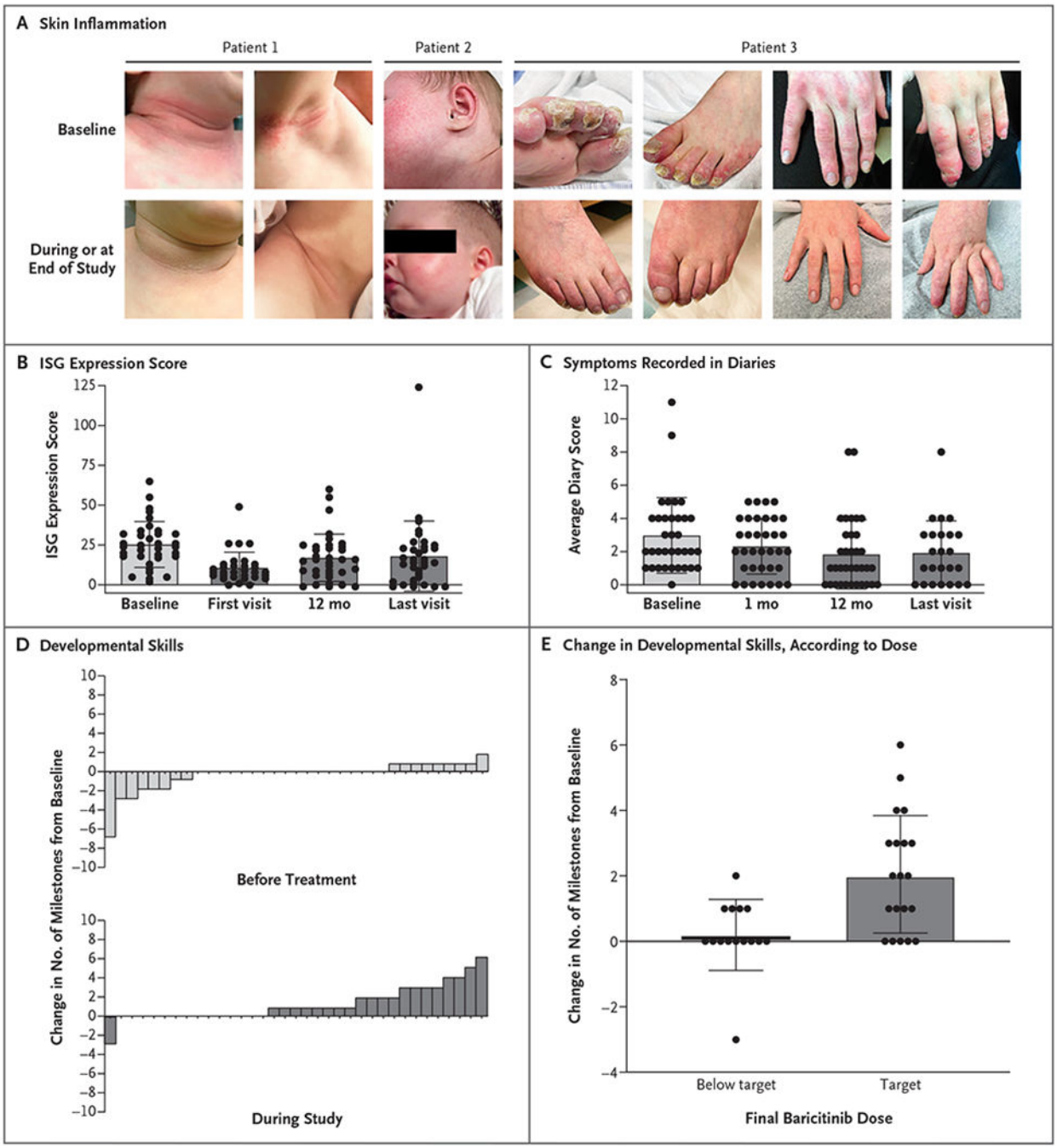

Figure 1. Changes in Symptoms, Interferon Gene Expression, and Developmental Skills in Children with the Aicardi–Goutières Syndrome.

As shown in Panel A, a decrease in symptoms during the study was indicated by reduced skin inflammation in the neck, face, toes, and hands of the patients. In Patient 1, the findings shown are from 3 days after the initiation of treatment; in Patient 2, from 1 month after the initiation of treatment; and in Patient 3, from the 18-month visit (the clinical cutoff date for data analysis). As shown in Panel B, the interferon-signaling gene (ISG) expression scores decreased during treatment. This score is calculated from the expression of six interferon-signaling genes normalized to housekeeping genes. The six-gene interferon signature is the

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sum of the median z scores of these genes. The interferon signature is positive (i.e., type 1 interferon signaling is high) if it is at least 1.96 (>98th percentile) on a one-tailed test (see Section 3.1.6 in the Supplementary Appendix). As shown in Panel C, symptom scores recorded in diaries by the patients' parents decreased during treatment. Recorded symptoms were neurologic disability, crying, sleep disturbances, irritability, seizures, fever, and skin inflammation of the trunk, arms, and legs (see the Supplemental Methods section and Table S5 in the Supplementary Appendix). As shown in Panel D, developmental milestones in the individual patients were met during treatment. Each bar represents an individual patient. As shown in Panel E, the gain of new milestones correlated with the dose of baricitinib. Target doses varied according to weight-based norms (see the Supplementary Methods section in the Supplementary Appendix). In Panels B, C, and E, each circle represents an individual patient. Horizontal lines indicate means, and I bars standard deviations.

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Acquisition of Developmental Milestones in Hypomyelination With Atrophy of the Basal Ganglia and Cerebellum and Other TUBB4A-Related Leukoencephalopathy

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Abstract

Mutations in *TUBB4A* are associated with a spectrum of neurologic disorders categorized as *TUBB4A*-related leukoencephalopathy. Affected children can present with global developmental delay or normal early development, followed by a variable loss of skills over time. Further research is needed to characterize the factors associated with the divergent developmental trajectories in this rare monogenic disorder because this phenotypic spectrum is not fully explained by genotype alone.

To characterize early psychomotor features, developmental milestones and age of disease onset were collected from medical records (n=54 individuals). Three subcohorts were identified: individuals with the common p.Asp249Asn variant vs all other genotypes with either early (<12 months of age) or late onset of presentation. Individuals with the p.Asp249Asn variant or those with non-p.Asp249Asn genotypes with later disease onset attained key milestones, including head control, sitting, and independent walking. Subjects with early-onset, non-p.Asp249Asn–associated disease were less likely to achieve developmental milestones. Next, we defined the developmental severity as the percentage of milestones attained by age 2 years. The mild form was defined as attaining at least 75% of key developmental milestones. Among cohort categorized as mild, individuals with p.Asp249Asn variant were more likely to lose acquired abilities when compared with non-p.Asp249Asn individuals.

Our results suggest multiple influences on developmental trajectory, including a strong contribution from genotype and age of onset. Further studies are needed to identify additional factors that influence overall outcomes to better counsel families and to design clinical trials with appropriate clinical endpoints.

Keywords

developmental delay, genetics, leukodystrophy, neurodevelopment, pediatric

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Mutations in *TUBB4A* are associated with a spectrum of neurologic disorders, including hypomyelination with atrophy of the basal ganglia and cerebellum (H-ABC), isolated hypomyelination, and DYT4/whispering dysphonia.[1-8] Collectively, these disorders are categorized as *TUBB4A*-related leukoencephalopathy.

Microtubules are cytoskeletal complexes composed of heterodimers of alpha and beta tubulins.[1,2] One beta tubulin, *TUBB4A* (MIM 602662), plays an integral role in neuronal migration and axonal projections and is highly expressed in neurons and oligodendrocytes within the basal ganglia and cerebellum.[1] Pathogenic changes in *TUBB4A* result in a spectrum of clinical disorders ranging from isolated dystonia to severe hypomyelination with atrophy of the basal ganglia and cerebellum (H-ABC).[5-8,10-15] The most common pathologic variant

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Table 1. Demographic Table.

	Cohort	D249N	Non-D249N
Number	54	13 (24.07)	41 (75.92)
Average age at onset, mo (range)	20.33 (0-180)	25.77 (8.04-54.48)	18.61 (0-180)
Sex (n = 52)			
Male	32 (61.5)	9 (69.2)	23 (59)
Female	20 (38.5)	4 (30.8)	16 (41)
Average age at last follow-up, mo (range)	139.44 (30-552.84)	149.76 (54.96-261)	136.17 (30-552.84)

associated with H-ABC in *TUBB4A* is p.Asp249Asn.[2,17] The developmental course of *TUBB4A*-related leukoencephalopathy can be variable and is only partially explained by genotype alone.[1-9,16] Children can demonstrate mild or profound global developmental delay, or normal early development, followed by a variable loss of skills over time.

In this study, a retrospective international cohort of 54 individuals affected by *TUBB4A*-related leukoencephalopathy was analyzed for age of onset and genotype, and a detailed developmental history was obtained. Using age of acquisition of developmental milestones, we were able to evaluate the influence of genotype and age of onset on developmental trajectories. Future studies are needed to characterize additional variables that influence overall clinical course.

Methods

Patient Ascertainment and Enrollment

Fifty-four individuals with clinical and molecular confirmation of pediatric-onset *TUBB4A*-related leukoencephalopathy (defined as disease onset occurred before 18 years of age) were recruited through the Myelin Disorders Bioregistry Project (institutional review board approved), an arm of the Global Leukodystrophy Initiative Clinical Trial Network (GLIA-CTN) at the Children's Hospital of Philadelphia (Supplemental Table S1). Individuals without a known genetic diagnosis and those with insufficient developmental histories (available up to less than 24 months of age) were excluded.

Data Extraction and Collection

Demographic information was collected for all individuals (Table 1), including genotype. A report of neuroimaging was available for all the individuals (Supplemental Table S1), and presence of leukodystrophy/hypomyelination, cerebellar atrophy, and basal ganglia atrophy was documented.

We identified 17 skills from the Denver Developmental Screening Test II (DDST-II) to assess acquisition of developmental milestones in the *TUBB4A*-related leukoencephalopathy population (Supplemental Table S2), as previously applied in the leukodystrophy population.[18] The age at acquisition and loss of milestones was obtained from historical medical records. Information was extracted from medical records synchronous with the time of acquisition and loss of a specific milestone when feasible to minimize recall bias. Ages were confirmed by telephone parental interview conducted by a child neurologist. In order to reduce the bias from missing data related to "delayed" early milestones, if a particular milestone was described as "delayed acquisition," the date of acquisition was defined as the 50th percentile in typically developing individuals + 3 SD (based on DDST-II). We compared the average percentage of milestones achieved at 24 months of age between 3 clinically relevant thresholds for age of onset (6, 9, and 12 months). Age of disease onset was defined as the youngest age in which a disease-specific manifestation was noted. Neuroradiologic reports and disease-specific medical histories were collected for the cohort.

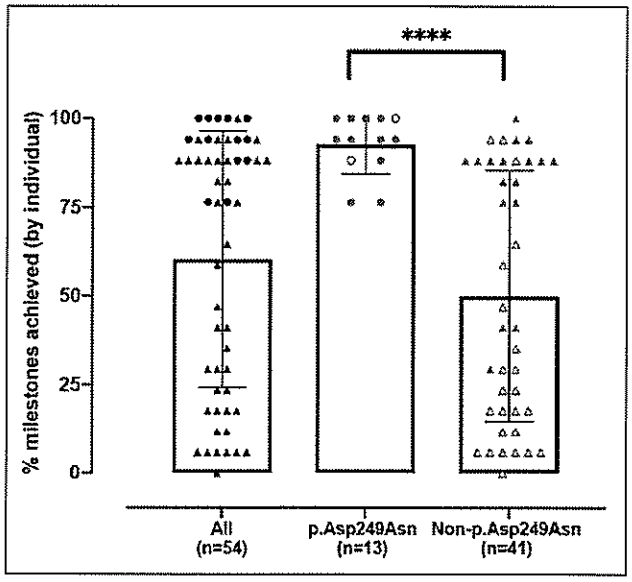

Figure 1. Milestones achieved by genotype. Individuals with p.Asp249Asn variants achieved a higher percentage of selected developmental milestones, when compared to non-p.Asp249Asn patients (Mann-Whitney test, *P* value <.0001). Open symbols represent individuals with age of onset <12 months, full symbols represent individuals with age of onset ≥12 months.

Statistical Approach

Analyses were performed using Stata 16, SAS 9.4, and PRISM graphical software. In order to determine the ideal age of onset cut off that best described discrete cohorts, we fit a linear regression model of the percentage of milestones achieved at 24 months. The presence or absences of the common genotype p.Asp249Asn was further used to define cohorts. Kaplan-Meier curves and the Mann-Whitney test were used to compare the percentage of milestones achieved by 24 months between the cohorts. Log rank testing was also used to compare subsequent loss of milestones between groups. We also estimated the 25th, 50th, 75th, 90th percentiles of the age of acquisition for each developmental milestone as the age at

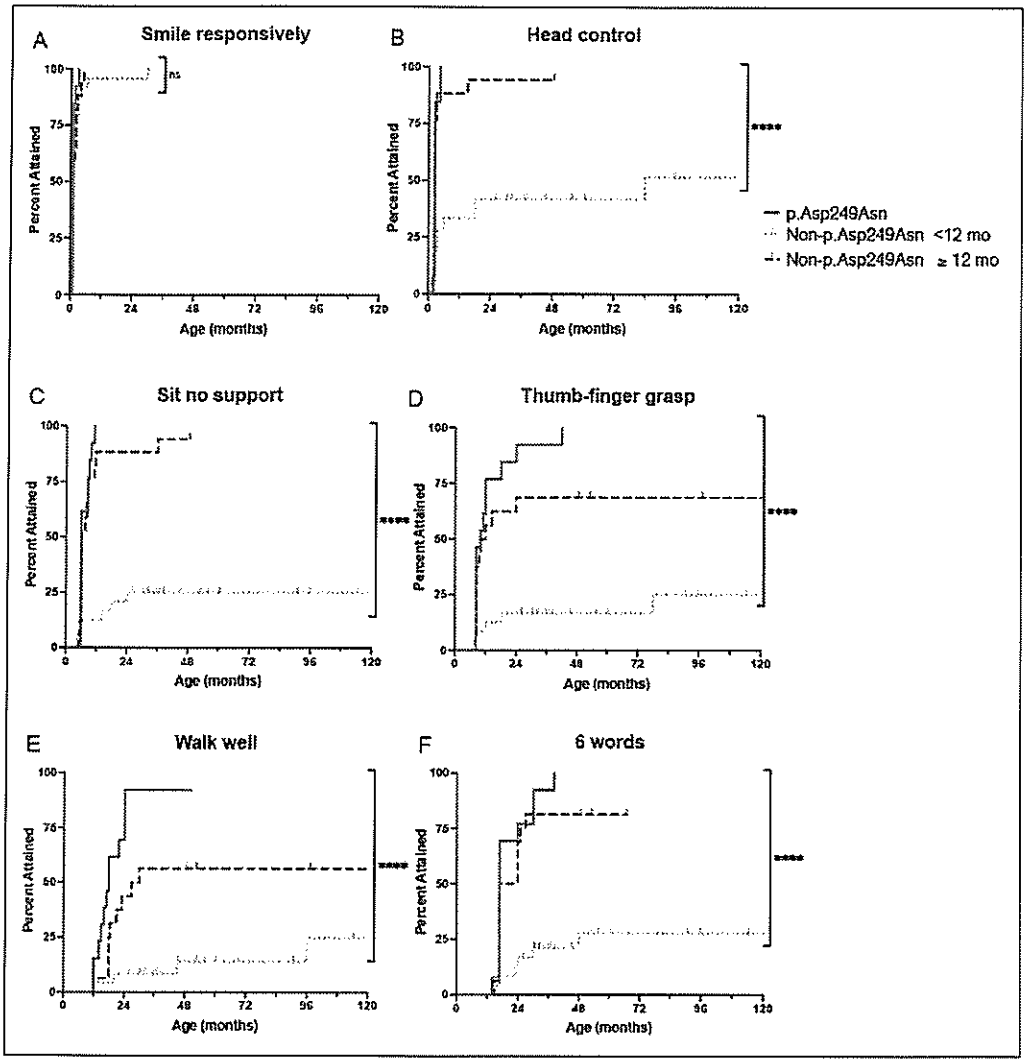

Figure 2. Developmental milestone acquisition by genotype and age of onset. *P* values were calculated using the log-rank (Mantel-Cox) test comparing genotypes and age of onset (****$P < .0001$; ns, not significant).

which the estimated Kaplan-Meier survival curve crossed 0.25, 0.50, 0.75, and 0.90, respectively. Two-sided tests of hypotheses were applied, with a P value <.05 as the criterion for statistical significance. We also performed a descriptive analysis of acquisition and loss of milestones by constructing a stacked bar chart that plotted the number of children in each of 3 categories (milestone not yet acquired, milestone acquired, milestone lost) by age in months.

Results

Demographics and Neuroimaging Findings

Fifty-four individuals with clinical and molecular confirmation of pediatric-onset *TUBB4A*-related leukoencephalopathy (defined as disease onset occurred before 18 years of age) were included in this analysis. Genotype in 13 individuals identified the common p.Asp249Asn pathogenic variant, whereas the remainder of the individuals has less common variants. A report of neuroimaging was available for all the individuals (Supplemental Table 1), obtained at variable ages relative to disease presentation. Of those, 53 had evidence of white matter changes consistent with leukodystrophy/hypomyelination (98.1% of the cohort), 41 with cerebellar atrophy (75.9% of the cohort), and 27 with basal ganglia atrophy, with predominant putaminal involvement (50% of the cohort). One subject (subject 40) met the overall study inclusion criteria but was noted to have basal ganglia atrophy on MRI, without evident white matter changes.

Determination of Age at Onset Threshold

Earlier age of onset is often associated with disease severity.[19] To determine a more precise threshold for "early onset," we compared the average percentage of milestones achieved at 24 months of age between 3 clinically relevant thresholds for

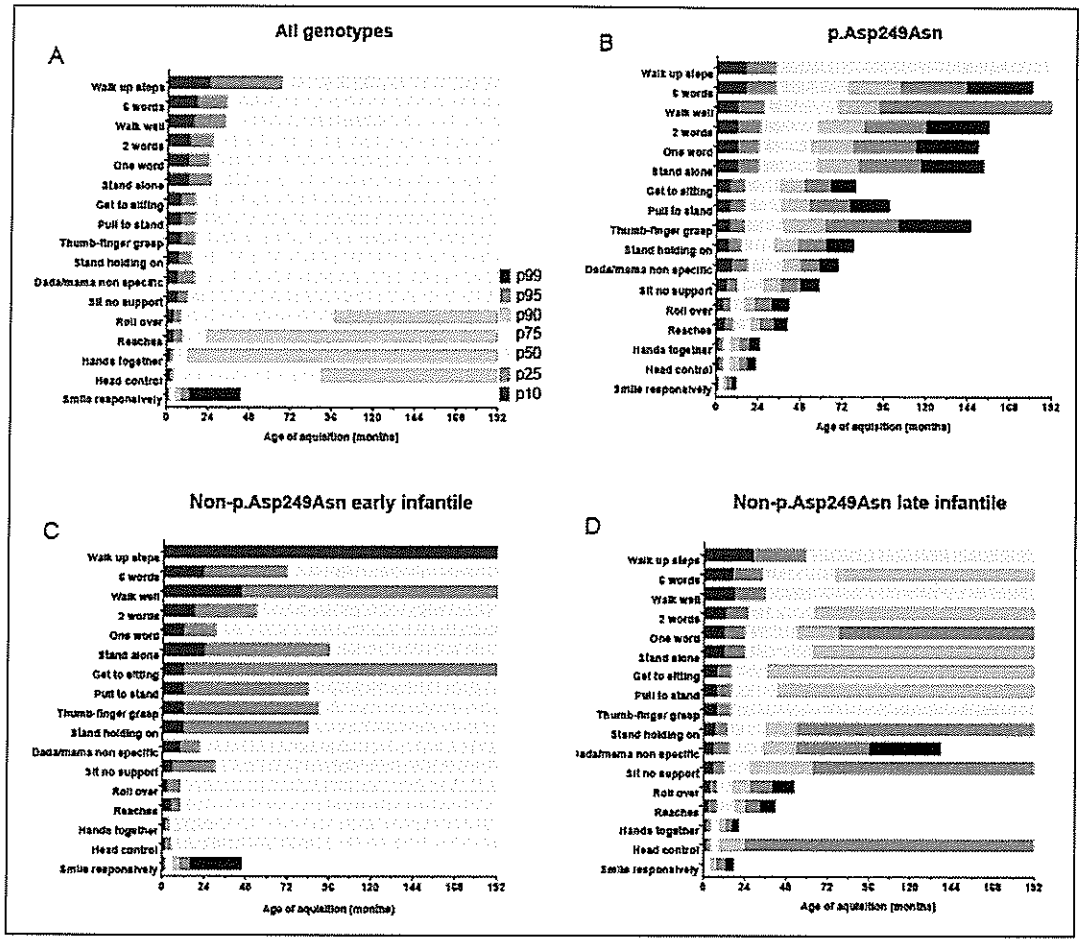

Figure 3. Developmental acquisition in H-ABC. Developmental skill acquisition plots were created for the overall TUBB4A population based on genotype (p.ASP249ASN variant vs non-p.Asp249Asn variant) and age of disease onset. The designation of 10 indicates that less than 10% of the population has acquired a milestone by the age indicated on the *x*-axis, whereas the designation of 90 indicates that up to 90% of the population has acquired the skill by the given age. The color-coded heat map indicates the percentage of the population that has attained the milestone at a given age, as shown on the *x*-axis. The percentiles were estimated using Kaplan-Meier analysis. (A) All genotypes combined; (B) p.Asp249Asn-related disease; (C) early infantile onset non–p.Asp249Asn related disease; (D) late-infantile onset non–p.Asp249Asn related disease.

age of onset (6, 9, and 12 months). Twenty-four months was selected because most early milestones are attained by this age. We fit a linear regression model of the percentage of milestones achieved at 24 months vs an indicator variable (having age of onset $\geq$ the threshold). We estimated the effect-size for each threshold as the regression coefficient for the indicator variable divided by the root mean square error for the regression model. The 12-month age at onset threshold had the highest effect size (1.5) and R-squared value (0.4).

Determination of Cohorts

The enrolled cohort was divided by genotype: p.Asp249Asn variant vs all other genotypes. The remaining non-p.Asp249Asn variants included 25 discrete variants occurring between one and 6 individuals each. The low numbers of each non-p.Asp249Asn genotype precluded any additional genotype-phenotype correlations. We further divided individuals by age of onset: <12 vs $\geq$12 months. Most (11/13; 85%) children with p.Asp249Asn-related disease had an onset $\geq$12 months of age. The 2 children with early onset demonstrated a developmental trajectory similar to the other subjects with the p.Asp249Asn variant. We thus created 3 cohorts: p.Asp249Asn-related disease, early infantile (<12 months) onset non–p.Asp249Asn related disease, and late infantile (>12 months) onset non–p.Asp249Asn related disease. Cohort groups are visually presented in Supplemental Figure S1.

Developmental Heat Maps and Developmental Function in Cohort Groups

We compared developmental achievements between cohorts by ranking percentiles estimated by the Kaplan-Meier curves with heat maps. These heat maps display the ages at which a

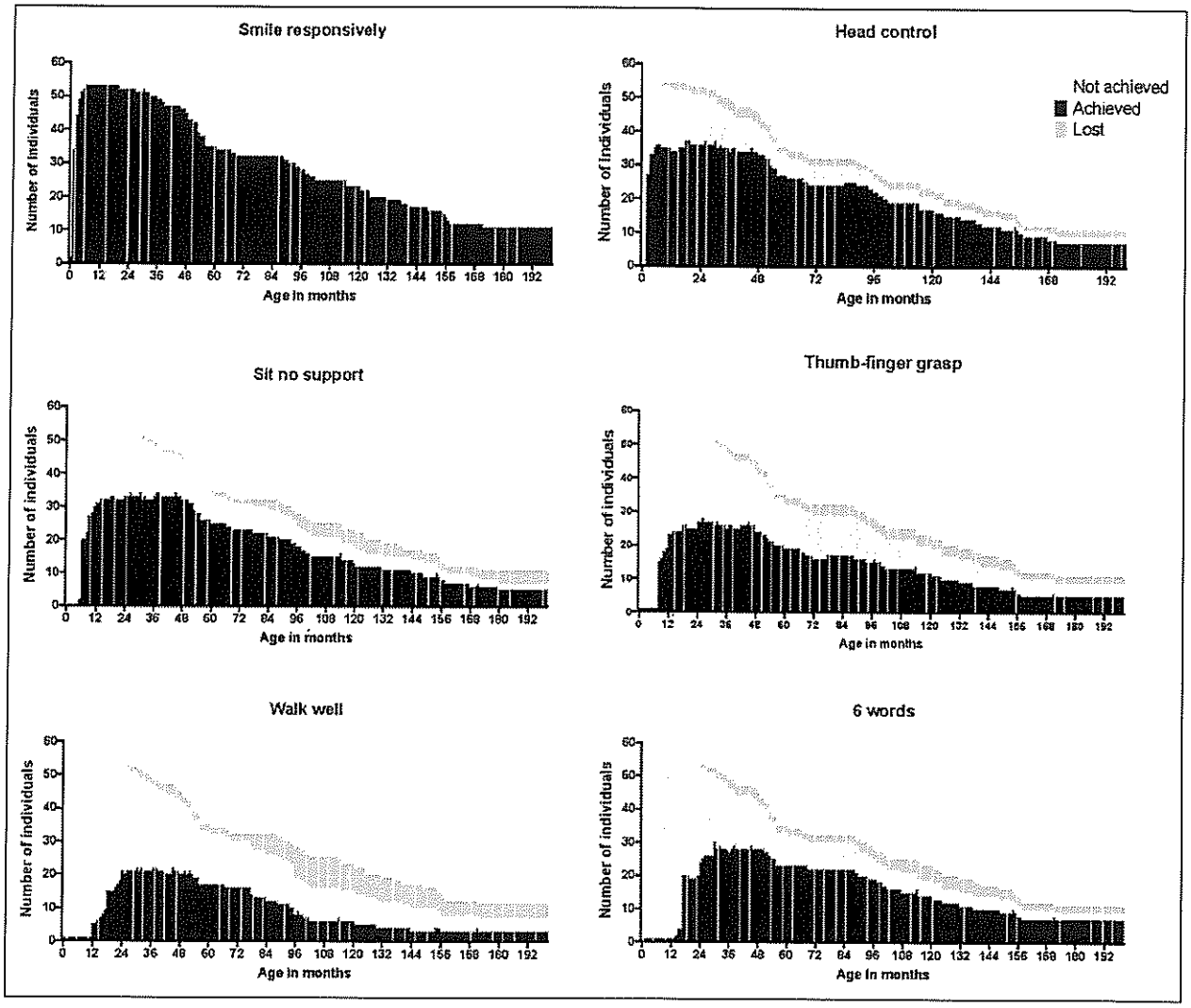

Figure 4. Milestone acquisition over time. Bar charts representing achievement and loss of specific developmental milestones over time. The *y*-axis represents the number of individuals included in the cohort at those specific ages.

given percentiles of the cohort achieved a milestone (p10 for 10%, p25 for 25% etc.).[18] Additionally, the milestone status for each individual was divided into the categories of: not yet achieved, achieved, and lost. These milestone categories were assigned to each individual at 1-month intervals to tract the transition between each category.

The p.Asp249Asn-related disease was correlated with a higher percentage of developmental skills achievement by age 24 months (Mann-Whitney test, P value <.0001) (Figure 1), followed by late infantile (>12 months) onset non–p.Asp249Asn related disease. Early infantile (<12 months) onset non–p.Asp249Asn related disease was associated with a more severe developmental trajectory, for all assessed milestones (log-rank test, P value < .0001), except smiling responsively, which was attained universally across the cohorts (Figure 2). p.Asp249Asn-related disease was also associated with the earliest gain of milestones. Age of milestone acquisition was used to create plots of developmental skill acquisition by genotype and age of onset (Figure 3). Across all 3 cohorts, we characterized developmental outcomes as mild if individuals attained at least 75% of key developmental milestones. Individuals with the p.Asp249Asn-related disease, or those with late infantile onset non–p.Asp249Asn related disease were more likely to be characterized as mild. Subjects with early infantile non–p.Asp249Asn related disease were more likely to be characterized as severe.

The evolution of gain and loss of skills was tracked for each individual over time (Figure 4). In addition to having delayed acquisition of milestones, many individuals lost skills over time. The loss of previously acquired specific milestones (roll over, get to sitting, pull to stand, stand alone, and one word) was greater in individuals with p.Asp249Asn-related disease (log-rank test: roll over $P < .05$, get to sitting $P < .005$, pull to stand $P < .005$, stands alone $P < .05$, one word $P < .05$).

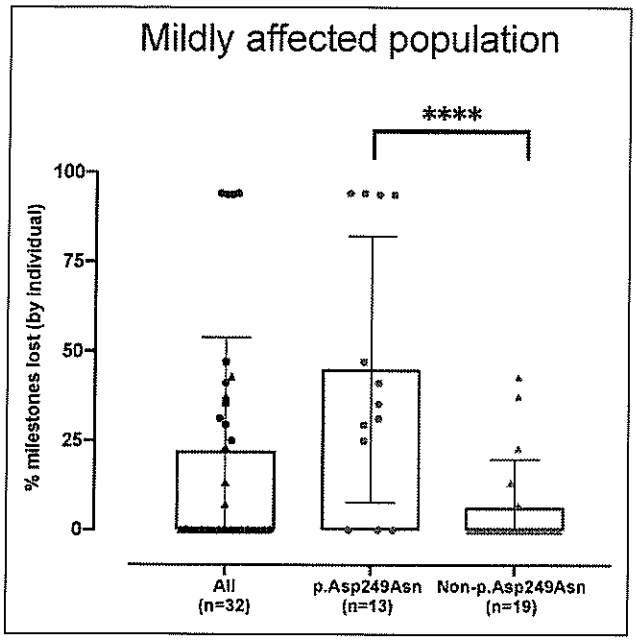

Figure 5. Subsequent milestone loss in individuals achieving 75% of early milestones. Milestones loss at last available evaluation by genotype in individuals who achieved at least 75% of milestones. Individuals with p.Asp249Asn-related disease demonstrate higher loss of developmental milestones, when compared to non–p.Asp249Asn related disease (Mann-Whitney test, *P* value <.001).

Additionally, when the percentage of all milestones lost at last evaluation was compared between individuals having achieved at least 75% of all milestones (mildly affected), individuals with p.Asp249Asn variant were more likely to have lost milestones (Mann-Whitney test, $P < .001$) (Figure 5).

Discussion

TUBB4A-related leukoencephalopathy is a heterogeneous disorder caused by mutations in the beta-tubulin isotype encoded by *TUBB4A*. Although all children described to date with *TUBB4A*-related leukoencephalopathy demonstrate some degree of neurologic impairment, the magnitude of neurodevelopmental delay can be variable, as can the presence of regression of developmental skills.

Three distinct patterns within childhood-onset TUBB4A-associated leukoencephalopathy emerged: the p.Asp249Asn-related disease, characterized by late infantile onset, with acquisition of early milestones followed by neurologic regression; early infantile (<12 months) onset non–p.Asp249Asn related disease, characterized by minimal developmental gain; and late infantile (>12 months) onset non–p.Asp249Asn related disease, characterized by late infantile onset, with acquisition of early milestones with more moderate neurologic regression. Because of the heterogeneous psychomotor development observed in the late infantile (>12 months) onset non–p.Asp249Asn related disease cohort, we hypothesize that additional factors may influence neurologic outcome. It will be relevant to future clinical trial planning to better understand factors that may influence outcomes in the late infantile (>12 months) onset non–p.Asp249Asn related disease cohort. For example, the presence or absence of radiographic features such as cerebellar atrophy or basal ganglia atrophy, may impact long term developmental outcomes. However, in this study, imaging was obtained clinically based on the presence of developmental abnormalities. This ascertainment bias prevented the analysis of these features as predictive of developmental outcomes.

This study explores developmental outcomes in a group of children affected by *TUBB4A*-related leukoencephalopathy. Although this cohort is relatively small (n = 54), we anticipate that this international cohort is representative of pediatric-onset *TUBB4A*-related leukoencephalopathy. As the participants were enrolled through a pediatric leukodystrophy research network, it is important to note that there was an ascertainment bias in the identification of cases. This study did not include cases of adult-onset forms of *TUBB4A*-related leukoencephalopathy and may underrepresent cases without hypomyelination on imaging. All but one individual was noted to have MRI findings consistent with a leukodystrophy. Individuals with adult-onset disease or without hypomyelination are likely underdiagnosed in childhood. We hypothesize that these individuals could have normal or near-normal early development.

In this study, we characterize the association between age of onset, genotype, and phenotype in *TUBB4A*-related leukoencephalopathy. Future identification of variables that may affect long-term outcomes, in addition to age of onset and genotype, will be important in the design of future clinical trials and the selection of appropriate clinical endpoints.

Author Note

Information on data in the Myelin Disorders Bioregistry Project can be requested by emailing the corresponding author.

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Author Contributions

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FFG, BC, JS, CA, LA, AV, CW, drafted the article or revised it critically for important intellectual content.

FG, BC, JS, CA, LA, AV, CW, approved the version to be published.

FG, BC, JS, CA, LA, AV, CW, each author should have participated sufficiently in the work to take public responsibility for appropriate portions of the content.

Declaration of Conflicting Interests

The authors declared the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article: FG: no conflicts of interest to disclose. BAC: no conflicts of interest to disclose. LA: consultant to Orchard Therapeutics and Takeda Pharmaceuticals. AV: research support from Gilead Sciences Inc, Orchard Therapeutics, Eli Lily and Company, Biogen, Homology, Takeda, Ionis and Illumina Inc. Justine Shults: research support from Eli Lily and Company, Homology, and Takeda. CAA: no conflicts of interest to disclose.

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Supplemental Material

Supplemental material for this article is available online.

Ethical Approval

The Myelin Disorders Bioregistry Project was approved by the Institutional Review Board at the Children's Hospital of Philadelphia.

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