iPSC-derived 3D Brain organoids cultured in a bioreactor as an *in vitro* model for the study of microcephaly in Aicardi Goutières Syndrome.

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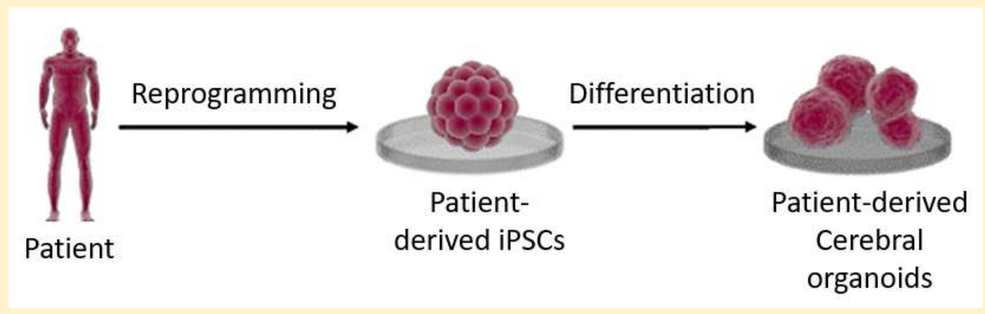
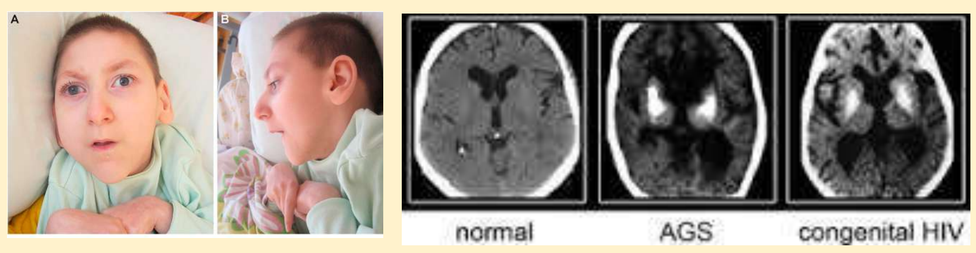
Introduction. The capability to reprogram patient-derived somatic cells to induce Pluripotent Stem Cells (iPSCs) and direct their differentiation towards Neural Stem Cells (NSCs) has provided a renewable source of expandable patient-specific cells to generate a valuable platform for in vitro disease modelling. The 2D monolayer cell culture has certain disadvantages, including the lack of heterogeneous cell-cell and "biomimetic" interactions, which can be partially overcome by the introduction of a 3D organoid culture. The iPSCs potential, combined with the modern 3D culturing technologies, may enable to exploit human brain-like tissues named "brain organoids".



Modified from Chun-Ting Lee et al., 2017.

Neurological genetic disorders can benefit the most from 3D modelling for its capability to generate an organized neuronal and glial network, otherwise only available from post-mortem samples.
We exploited this possibility to create a 3D neural in vitro model of disease to investigate the Aicardi Goutières Syndrome (AGS). AGS is a severe neuro-inflammatory disorder with onset in early infancy. AGS patients exhibit psychomotor retardation, and microcephaly with demyelination and calcification. To date, 9 genes have been identified responsible of the disease.

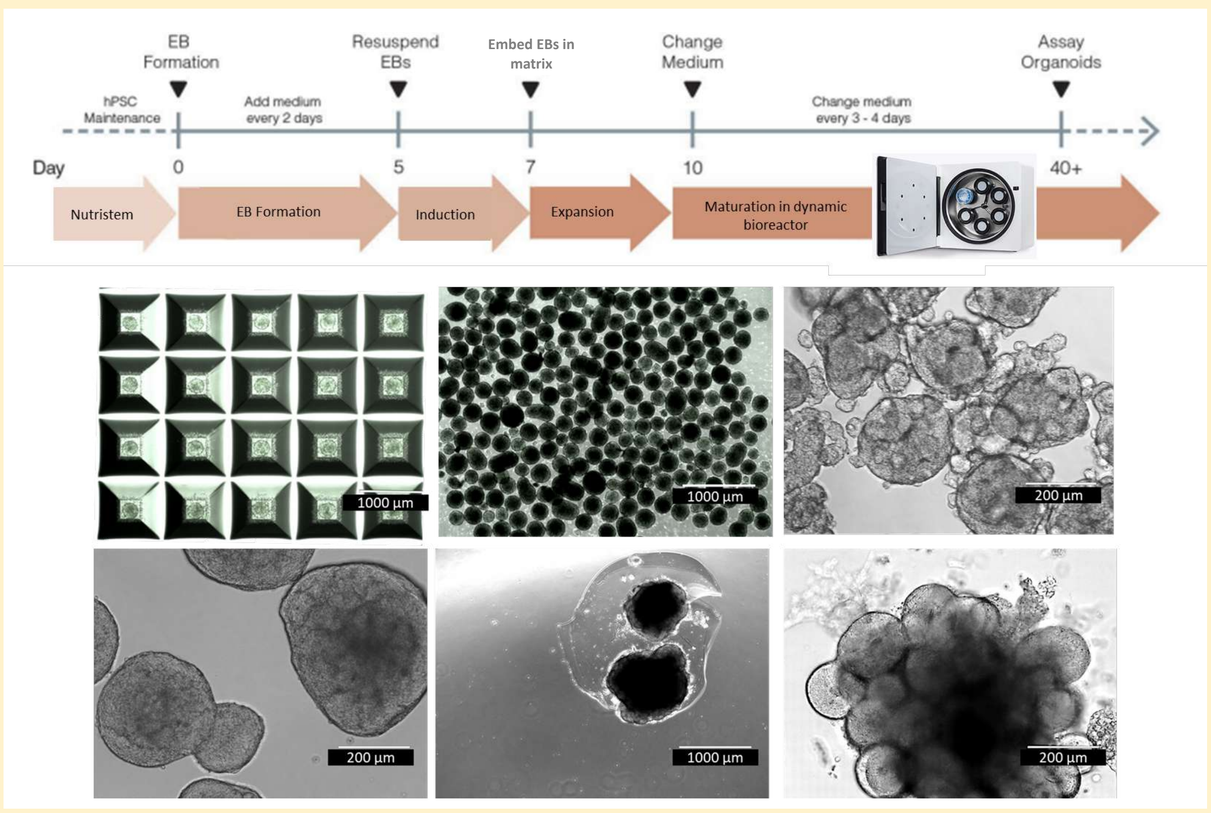


Modified from Fazzi et al., 2014.

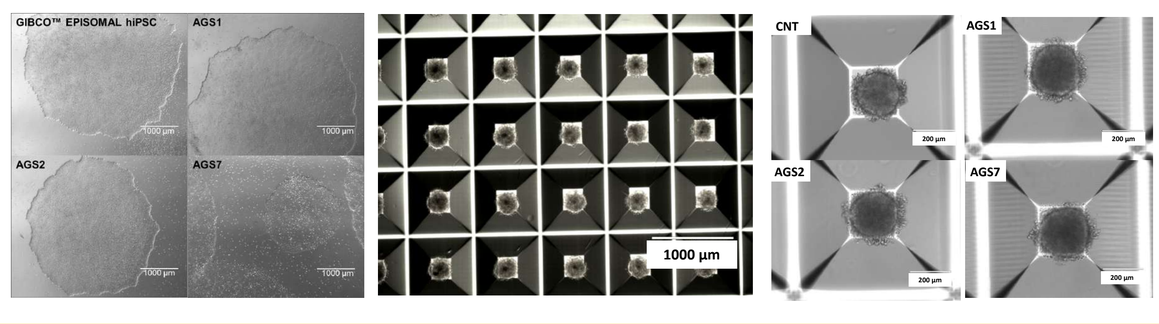
Materials and methods. As one of the features of AGS is the profound microcephaly, we generated iPSC-derived cortical cerebral organoids using a bioreactor (Cel Vivo, ClinoStar system) that let organoids grew in a dynamic suspension as a better *in vitro* model to explore the cytoarchitectural alteration of the disease. We generated iPSC-derived cortical cerebral organoids of 3 patients with AGS, mutated in different genes: *RNaseH2B, IFIH1, and TREX1* and of a commercial iPSC cell line (Gibco Episomal hiPSC).

Patient	Gender	Type of AGS	Gene	Mutation	Protein Effect	iPSCs
PT 1	M	AGS1	*TREX1*	c.[262insAG];[290G>A]	p.[S88fs22X];[R97H]	C12 p.171
PT 2	F	AGS2	*RNAseH2B*	c.[529G>A];[529G>A]	p.[A177T];[A177T]	C3 p.132
PT 3	M	AGS7	*IFIH1*	c.[2471G>A];wt	p.[R824K];wt	C7.4 p.174

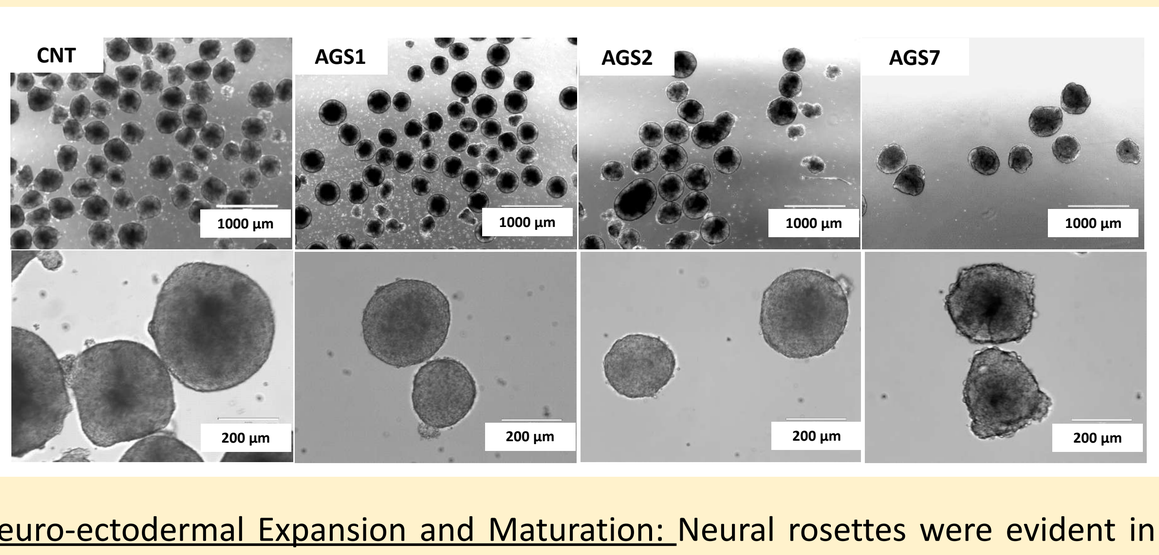
Mini-brains were generated from optimizing the protocol described by Lancaster et al. in 2014, through the use of commercial media enriched with growth factors and supplements (STEMdiff™ Cerebral Organoid Kit, Stem Cell) to improve the experimental standardization and reproducibility.
The workflow consists in iPSC-derived embryoid bodies generation, neuroectodermal induction, matrix embedding for the neuroepithelium expansion, and cerebral organoids maturation.



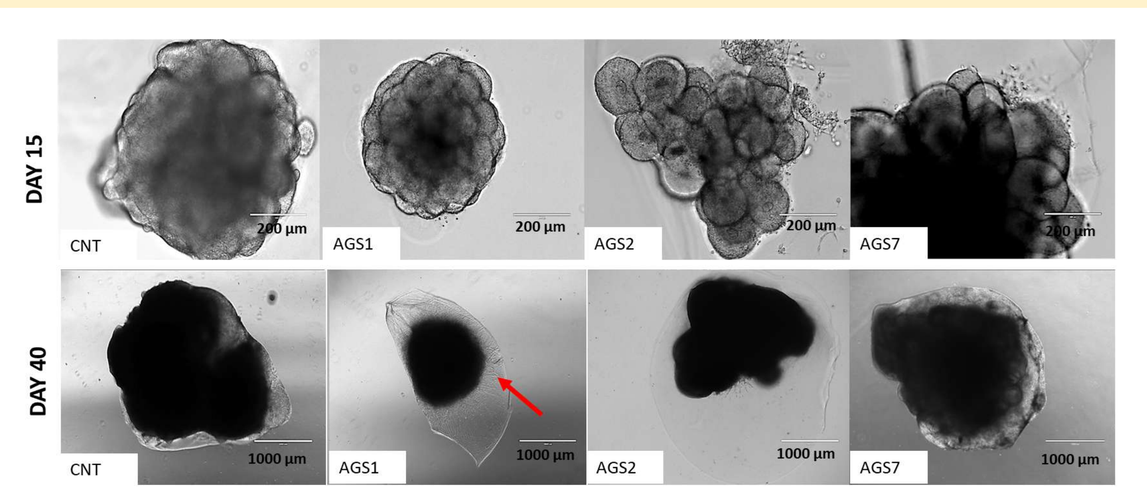
Results. <u>iPSC-derived Embryoid bodies (EBs)</u>: iPSCs colonies were dissociated to single-cell and cultured in Microwell Culture Plates in order to obtain a scalable production of EBs similar in shape and size (6000 cells/EB). iPSC-derived AGS EBs showed no differences in terms of number, size and structure compared to the control.



<u>Neuro-ectodermal Induction:</u> On day 6 EBs reached a diameter of > 200 µm. AGS and control EBs appeared homogeneous in size and round shape, delimited by a well-defined edge (ectoderm) surrounding a compact area, darker in the center



<u>Neuro-ectodermal Expansion and Maturation:</u> Neural rosettes were evident in all samples and resembled the folding structure of the cerebral ventricles. CNT and AGS7 organoids appeared similar in terms of size and rounded shape. AGS1 organoids had an extremely regular structure but a smaller size. AGS2 organoids showed a smaller and irregular shape. We observed a progressive growth in the organoids' size which tend to colonize the entire available area of matrix, becoming increasingly dense and dark under optical microscope vision.



Conclusion. The description of AGS iPSCs-derived brain organoids was documented only for TREX1 mutated-samples. We thus expanded the study cohort to investigate the pathogenic contributions and interaction between neurons and glia also in other AGS-derived brain organoids characterized by specific genetic mutations.