

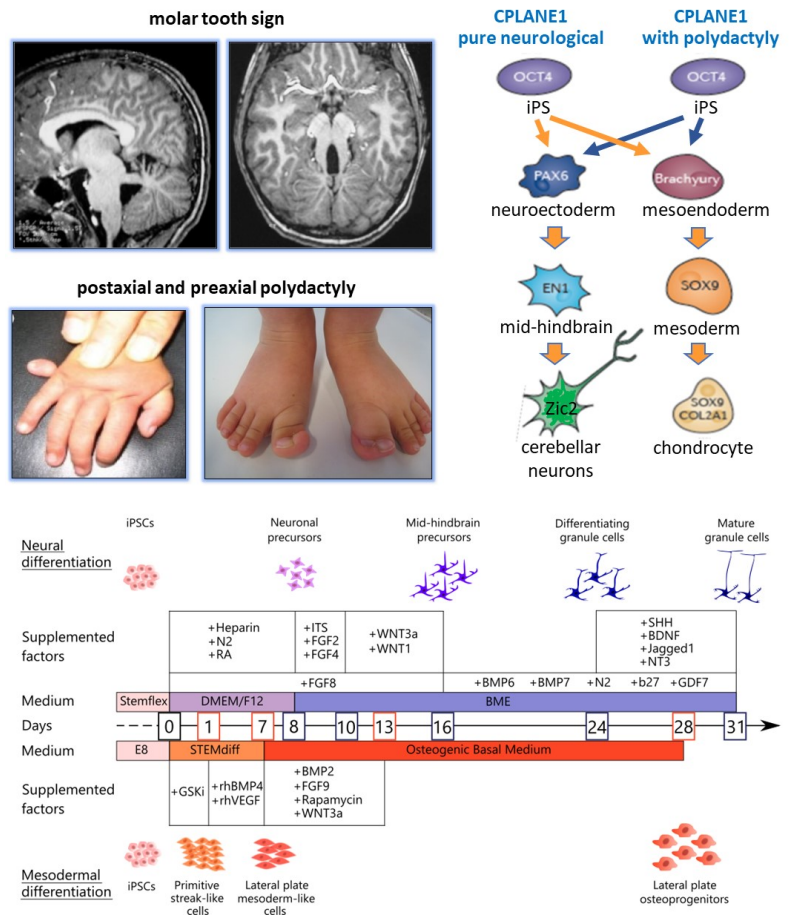
**Project title:** Explaining clinical variability in Joubert syndrome making use of patient-derived cell models

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**Background:** Joubert syndrome (JS) is a clinically heterogeneous inherited neurodevelopmental disorder characterized by a unique mid-hindbrain malformation, so called “molar tooth sign”. Over 40 causative genes are known, all encoding proteins implicated in the formation or functioning of the primary cilium, a ubiquitous organelle playing several key functions in development and adult life. Notably, besides the typical neurological manifestations and “molar tooth sign”, patients with JS variably experience the involvement of other organs, mainly the retina, kidneys, liver and skeleton. Despite some gene-phenotype correlates, there is wide clinical variability, and variants in the same gene can give rise to different phenotypes, a phenomenon which still remains unexplained.

**Aims:** We hypothesize that the variable organ involvement seen in JS patients with mutations in the same gene may relate at least in part on tissue-specific modulation of the expression of the mutant gene and related pathways.



This project, funded by Telethon, will take advantage of patients-derived iPSCs to explore this specific hypothesis. As pilot phenotype, we have chosen polydactyly, a developmental trait frequently observed in JS. We chose polydactyly as: 1) it develops during embryonic development; 2) it occurs in ~15% JS patients regardless of the mutated gene; 3) it is common in patients mutated in *CPLANE1*, with no genotype-phenotype correlations; 4) a protocol is available to differentiate iPSCs into lateral plate mesoderm (from which limb buds originate). If successful, this approach can be easily translated also to other tissues variably affected in JS and for which differentiation protocols are available.

**Experimental Plan:** The PhD student will use patient-derived iPSCs differentiated towards distinct lineages, to recapitulate phenotypic variability at cellular level, and detect transcriptional differences which may explain it. We have selected 4 *CPLANE1* patients with/without polydactyly (n=2 each), with iPSCs already available and fully characterized. iPSCs will be differentiated towards cerebellar lineage and towards osteogenic progenitors from lateral plate mesodermal (LPM) lineage, giving rise to limb buds. Developmental markers and transcriptional profiles will be compared, to detect shared or distinct cellular phenotypes and deregulated expression pathways in the two lineages and between patients with/without polydactyly. Analysis of cilia formation and morphology will be performed.

**Techniques:** Cell lines and iPSC cultures, differentiation protocols, immunofluorescence and confocal microscopy, western blot, RNAseq and related data analysis, qRT-PCR, assessment of cilia formation and morphology.