FUNCTIONAL CHARACTERIZATION OF A NOVEL CANDIDATE GENE FOR CEREBRAL CORTICAL MALFORMATIONS BY USING PATIENT'S IPS DERIVED 3D CELL-BASED MODELS

AUTHORS

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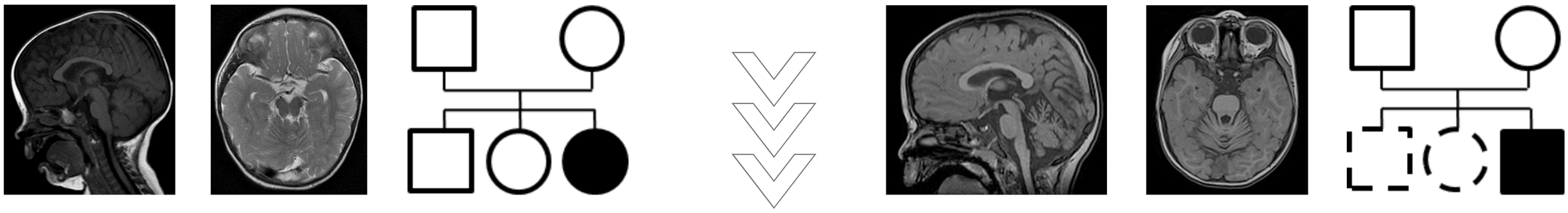
IDENTIFICATION OF A CANDIDATE GENE IN A CEREBRAL MALFORMATION COHORT BY HIGH THROUGHPUT SEQUENCING

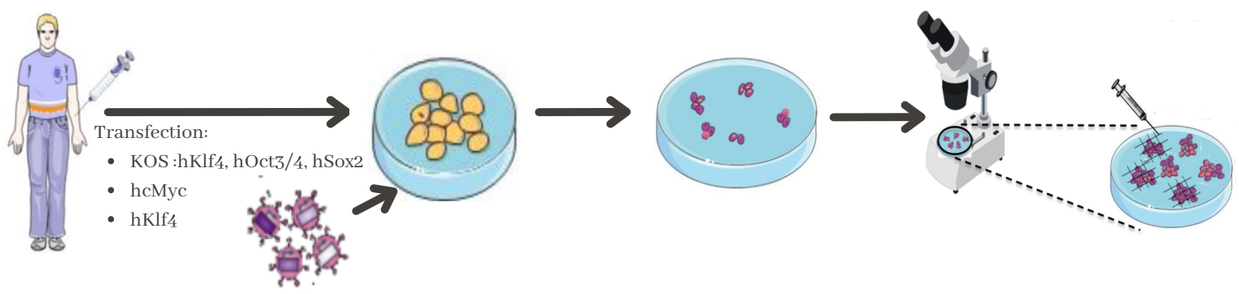
- Case 1: Developmental delay, oculomotor apraxia, vermian hypoplasia, ectopic pituitary gland
- Case 2: Developmental delay, vermian hypoplasia, polymicrogyria



"DE NOVO" MISSENSE HETEROZYGOUS MUTATIONS IN THE SAME CANDIDATE GENE

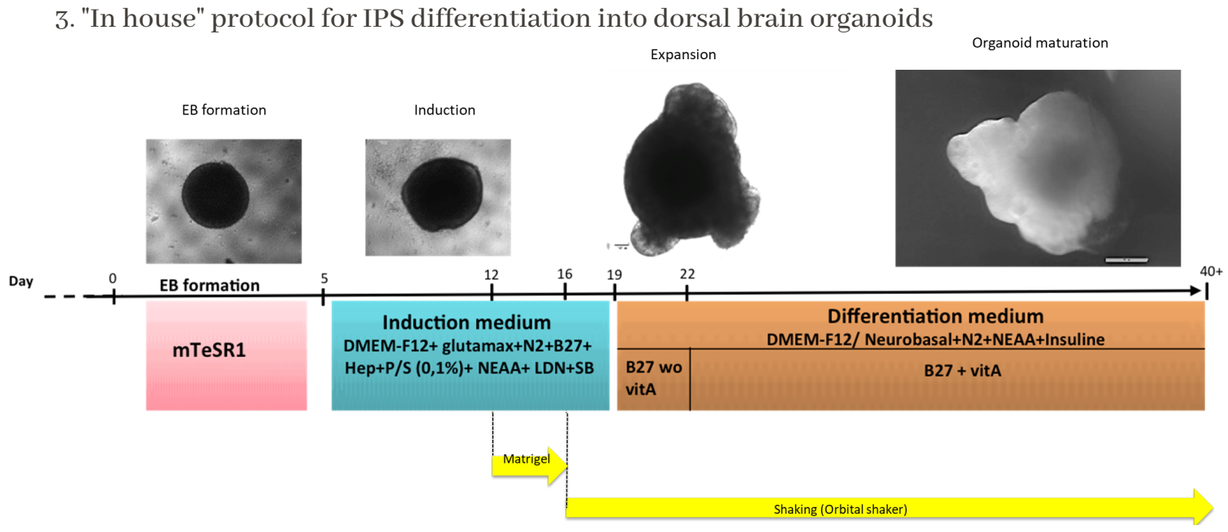
DEVELOPMENT OF AN "IN VITRO" MODEL: DORSAL BRAIN ORGANOIDS

1. Patient cells reprogramming: CytoTune-iPS 2.0 Sendai Reprogramming Kit

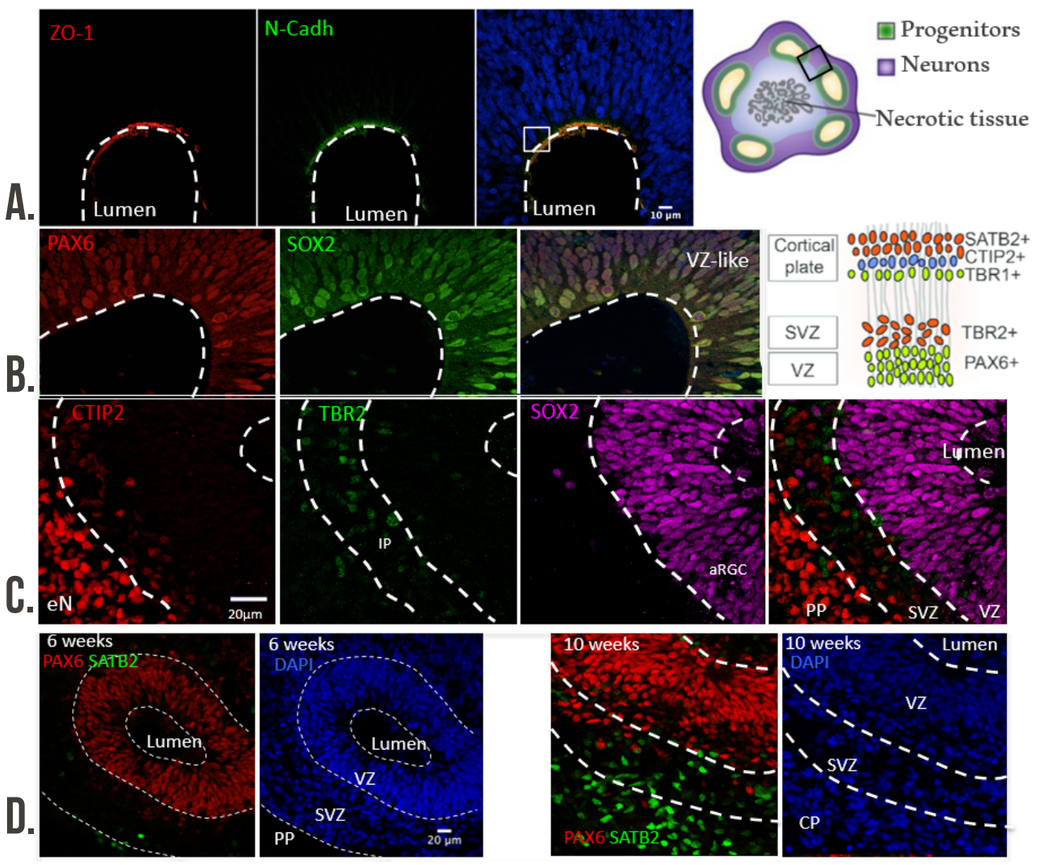


2. CRSIPR/Cas9 genome editing: isogenic controls & mutated iPS cells lines

3. "In house" protocol for IPS differentiation into dorsal brain organoids



VERIFICATION OF THE DORSAL BRAIN ORGANOID MODEL



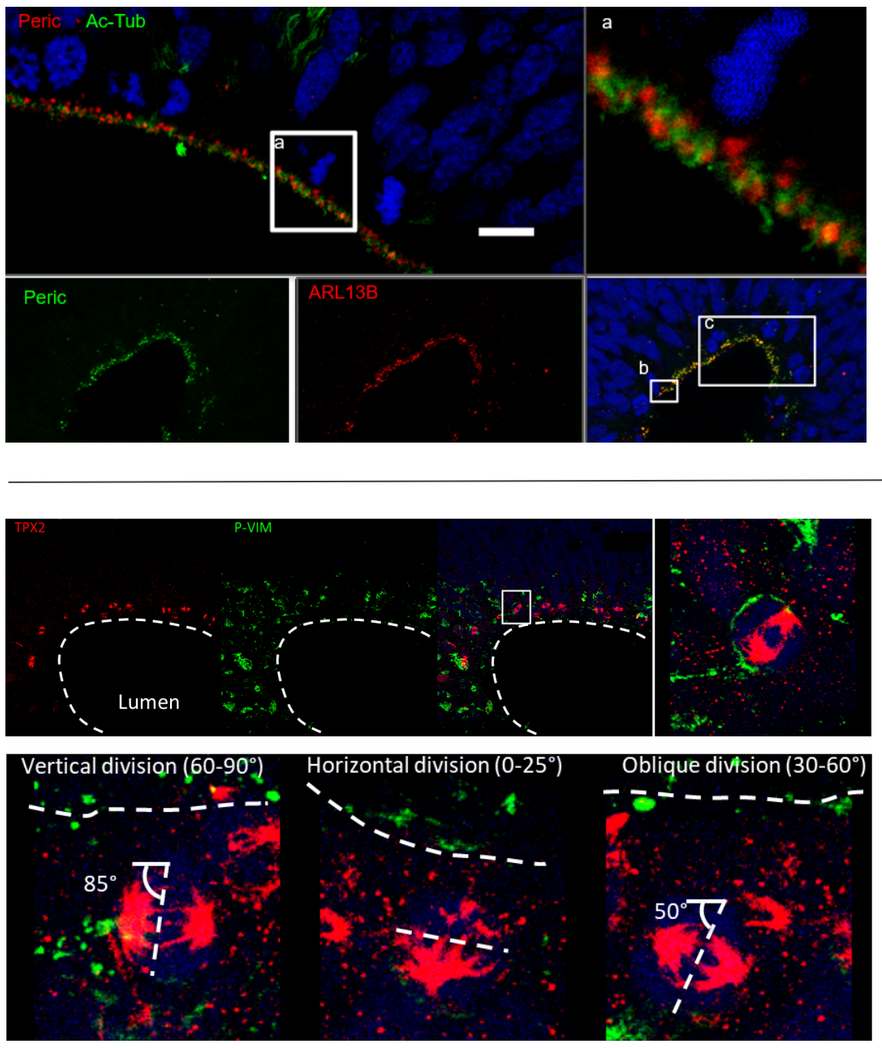
CYTOARCHITECTURE OF 3D CEREBRAL CORTEX–LIKE STRUCTURES

A. Normal apicobasal polarity: accumulation of N-cadherin and zona occludens protein 1 near the lumen. From the center to the periphery

B. Apical progenitors located in the ventricular zone (VZ)-like region co-express Sox2 and Pax6.

C. Subventricular zone (SVZ)-like region populated by intermediate progenitors (IP, TBR2 staining). Preplate (PP)-like region contains early deep layer neurons expressing CTIP2.

D. At the early stage of differentiation, only few late born upper layer neurons are present (stained in green with SATB2 Ab) whereas they are more abundant after 10 weeks according to what happens in vivo.



PRIMARY CILIA AT THE APICAL SURFACE OF APICAL PROGENITORS

Primary cilia formation at the apical surface of apical progenitors (AP) of the VZ stained with pericentrin Ab (basal body) and ARL13B or acetylated alpha tubulin (axoneme).

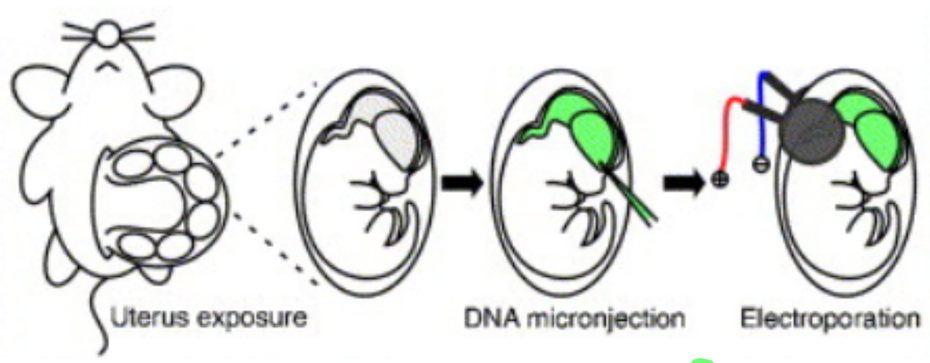
INTERKINETIC NUCLEAR MIGRATION AND SYMMETRIC/ASYMMETRIC MITOTIC DIVISIONS

Alignment of mitotic nuclei at the apical surface of the VZ showing the characteristic interkinetic nuclear migration of AP (P-Vim and TPX2 Ab). Measurement of the angle of the mitotic plane defining the division mode of neocortical progenitors which regulation is crucial in determining the size of the neocortex.

CONCLUSIONS AND PERSPECTIVES

DORSAL FOREBRAIN ORGANOIDS

Dorsal forebrain 3D cell-based model recapitulates the cytoarchitecture of developing human cortex. We are now comparing control and patients organoids to demonstrate the involvement of our candidate gene mutations in humain CNS anomalies, particularly in cortical dysplasia.
We are also assaying live imaging to test cortical growth and neuron migration.



"IN VIVO" MODEL: IN UTERO ELECTROPORATION

Finally, electroporation of WT and mutant constructs in brain of mice embryos will help us to see neuronal migration abnormalities as one of the patient has polymicrogyria.

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