INDUCED PLURIPOTENT STEM CELLS-DERIVED NEURONS AS DISEASE MODELS TO INVESTIGATE THE MOLECULAR PATHOGENESIS AND PHARMACOLOGICAL RESPONSES IN GENETICALLY-DETERMINED EPILEPTIC CHANNELOPATHIES

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Introduction: The lack of suitable patient-derived biological material is a major limitation when investigating the molecular pathogenesis and pharmacological responses in neurological diseases. Induced Pluripotent Stem Cells (iPSC)-derived neurons overcome such limitation and provide suitable alternatives to the use of heterologous expression systems or animal models when addressing neurological diseases such as epileptic channelopathies (Tindball and Parent, 2016; Simkin and Kiskinis, 2018). In the present work we describe the generation of (iPSC)-derived neurons from an 8years-old proband with neonatal-onset seizures and non-syndromic intellectual disability carrying a homozygous mutation in KCNQ3, a gene encoding for neuronal voltage-gated potassium channel subunits exerting inhibitory control over cell excitability (Soldovieri et al., 2011). The KCNQ3frameshift mutation (c.1599dup; p.Phe534Ile Fs*15) truncates a C-terminal domain where critical regions controlling subunit assembly, biophysical properties, and subcellular localization have been identified.

Materials and methods: Fibroblasts from the proband and one healthy control brother who carried wild-type KCNQ3alleles, were obtained from punch skin biopsies. Virus-free hiPSCs from fibroblasts were obtained using a non-integrating, episomal-based reprogramming system, under feeder-free conditions. The reprogramming factors (Oct4, Sox2, Klf4, L-Myc, and Lin28) were delivered by electroporation. From iPSCs, neural precursor cells (NPCs) were obtained upon cell differentiation using Neuronal Induction medium; once NPCs were obtained, mature neurons were obtained in Neuronal Differentiation Medium (Verpelli et al., 2013). Immunofluorescence and RT-PCR were used to molecularly characterize cell subtypes; G-banding served to assess the chromosomal morphology and integrity. Patch clamp recordings were used to evaluate the electrophysiological properties of iPSCs derived neurons.

Results: Two independent clones of iPSCs, from each individual were generated; these clones displayed a normal karyotype and typical morphological features such as growth in tightly packed colonies with defined borders and a high nucleus to cytoplasm ratio. Expression of the major stemness markers SSEA4and Nanog via immunofluorescence, and Klf4and Oct4via RT-PCR confirmed the acquisition of staminal properties. Transcripts encoding for all five members of the KCNQ subfamily were also detected by RT-PCR. iPSCs were differentiated into NPCs and mature neurons, as revealed by the expression of the relative markers Nestin/Pax6and NeuN, respectively. In these (iPSC)-derived neurons, electrophysiological recordings revealed a large density of voltage-gated sodium and potassium currents, confirming the acquisition of a differentiated neuronal phenotype. Functional, morphological and molecular characterization is ongoing to identify pathogenetically-relevant differences between proband and control cells.

Conclusions: Our preliminary data indicate the possibility to generate iPSCs from fibroblasts of a patient carrying an epileptogenic mutation in KCNQ3, and their differentiation into mature neurons. This newly-available resource might serve as a powerful research platform to characterize molecular pathogenesis and pharmacological responses in genetically-determined epilepsies.