Assessing NPC migration and neuronal maturation using a hiPSC-derived model of *SCN3A* encephalopathy

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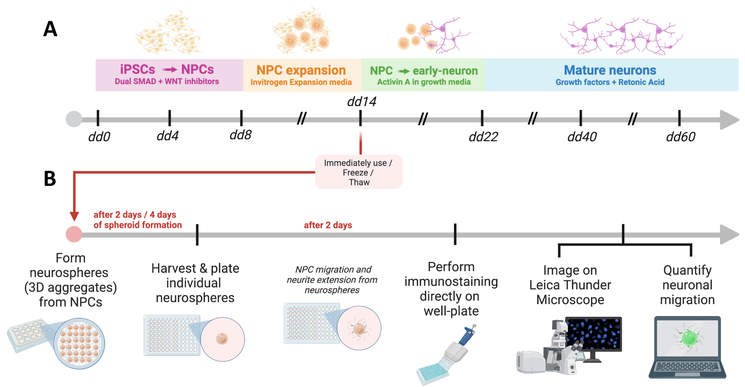
Introduction

- Pathogenic variants in *SCN3A*, a gene encoding the voltage-gated Na+ channel α-subunit Nav1.3, are associated with *SCN3A*-related neurodevelopmental disorders, which include 3 core clinical features: epilepsy, developmental delay/intellectual disability (DD/ID), and/or malformation of cortical development (MCD). Not all patients present with all 3 phenotypes.
- MCD is atypical of a channelopathy yet is surprisingly a key characteristic phenotype in *SCN3A* encephalopathy. Although *SCN3A* is expressed highly in the brain during embryonic development, the mechanism by which mutations in the Nav1.3 channel result in MCD remains unknown.
- In order to address this question, we generated induced pluripotent stem cell (iPSC) lines from a patient with the *SCN3A*-I875T variant–which is associated with all 3 core phenotypes–and its genetically corrected control.
- We directed the differentiation of these isogenic iPSC lines through the NPC stage to cortical neurons in order to model human cortical development *in vitro*.
- To specifically examine early-stage neurodevelopment potentially relevant to *SCN3A* encephalopathy pathogenesis, we developed a NPC neurosphere migration assay to assess NPC migration and maturation.
- **Hypothesis**: The *SCN3A*-I875T variant results in deficits in cell migration and maturation compared to the corrected control.

Methodology

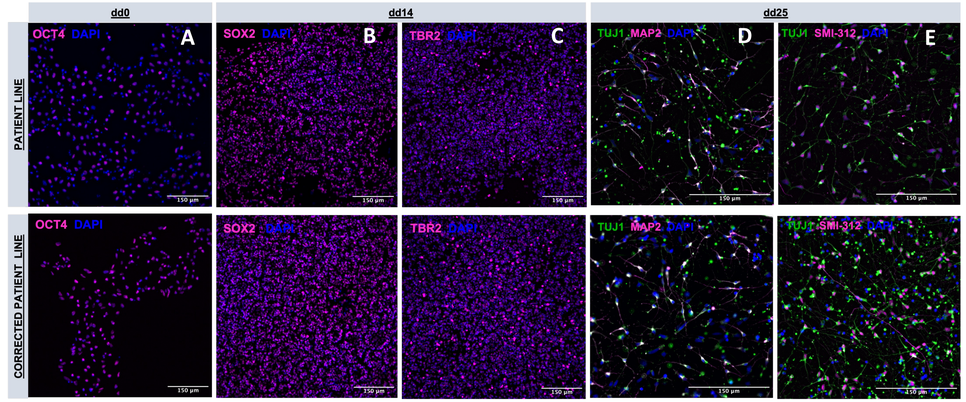
Figure 1. Directing the differentiation of iPSCs to cortical neurons for use in a functional NPC neurosphere migration assay



(**A**) Schematic of the 2D cortical differentiation. Isogenic cell lines containing the *SCN3A*-I875T variant, and its genetically corrected control were directed from iPSCs to NPCs to cortical neurons using a time-sensitive series of developmental signaling molecules. (**B**) Schematic of the NPC neurosphere migration assay in development. At dd14 (day of differentiation), NPCs from each line were plated in a microwell culture plate to form 3D aggregates, or neurospheres with uniform size and shape, for 2 or 4 days. Then, individual neurospheres were plated to separate Matrigel-coated wells of a 96-well plate, and NPC migration was allowed to occur for 2 days. Then, neurospheres were fixed and immunostained using iPSC- and NPC-specific antibodies and images were taken for quantification of cell migration. Total cell migration was calculated as (*total neurosphere area – inner cell mass).*

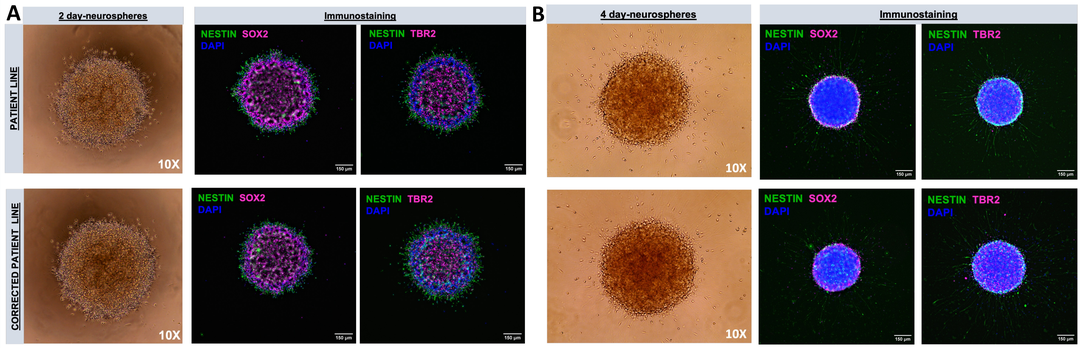
Results

Figure 2. Both lines produce healthy NPCs and differentiated neurons with expected marker expression



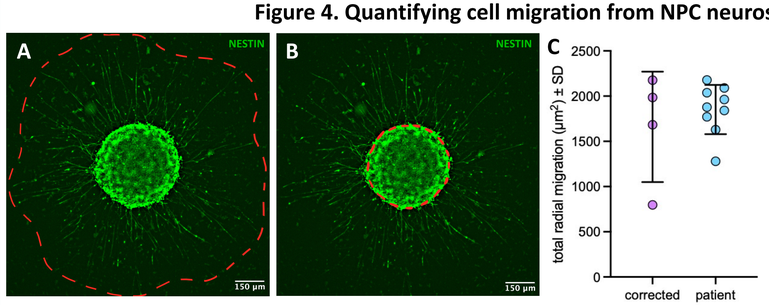
(**A**) Cells at dd0 express OCT4, a pluripotency marker, suggesting both lines produced good quality iPSCs to begin the 2D cortical differentiation. (**B-C**) Cells at dd14 express NPC markers: SOX2, an iPSC and NPC marker, and TBR2, a forebrain progenitor marker, suggesting both lines have progressed towards a neural cell fate. (**D-E**) Cells at dd25 express a combination of: TUJ1, an axonal and dendritic marker, MAP2, a dendritic marker, and SMI-312, an axonal marker, suggesting both lines have successfully differentiated into neurons and show typical neuronal morphology.

Figure 3. 4-day neurospheres show NPC migration and neurite extension



(**A-B**) Neurospheres from both lines express TBR2, NESTIN (filament protein) and SOX2 (transcription factor). Neurospheres allowed to aggregate for 2 days followed by plating on Matrigel-coated wells show minimal NPC migration, but notable rosette organization (**A**), whereas neurospheres allowed to aggregate for 4 days before plating show robust and quantifiable neurite extension (**B**).

Figure 4. Quantifying cell migration from NPC neurospheres



(**A-B**) Representative trace of the total neurosphere area (**A**) and inner cell mass (**B**) based on NESTIN expression.
(**C**) Preliminary quantification of cell migration, where *total radial migration = total neurosphere area – inner cell mass.* Values are shown for 4-day neurospheres, n = 9 patient line, 4 corrected line.

Discussion

- We successfully implemented a 2D differentiation protocol and generated NPCs and cortical neurons using a human-iPSC line from a *SCN3A* encephalopathy patient with the *SCN3A*-I875T variant and a CRISPR/Cas9-corrected control line, which can now be confidently used in functional assays.
- We developed a NPC neurosphere migration assay to evaluate NPC migration and neurite extension in the patient and corrected lines to investigate the mechanistic underpinnings of cortical malformation–a peculiar, yet key associated phenotype–in *SCN3A* encephalopathy.
- NPCs allowed to form spheroids for 4 days produce neurospheres with visible migration compared to NPCs only allowed to form spheroids for 2 days. Immunocytochemistry shows the latter expresses rosette-type NPC organization; perhaps the influence of apico-basal polarity during the mid-NPC stage may provide an explanation for lack of migration. This strongly suggests the period of spheroid formation is pertinent for migration to occur, which informs further development of the neurosphere assay.

Implications / Future Directions

- We will repeat the NPC migration assay to allow neurospheres to form for 4 days before the plating stage, which will allow us to determine whether a significant phenotypic difference in migration exists in the SCN3A-I875T patient versus corrected lines.
- Future directions include performing this assay on a range of lines with different pathogenic variants in *SCN3A*, from patients presenting epilepsy without brain malformation, brain malformation only, and epilepsy and brain malformation.

References

1. Topol, A., Tran, N.N., Brennand, K.J. A Guide to Generating and Using hiPSC Derived NPCs for the Study of Neurological Diseases. J. Vis. Exp. (96), e52495, doi:10.3791/52495 (2015).

2. Zaman, T., et al. (2020), *SCN3A*-Related Neurodevelopmental Disorder: A Spectrum of Epilepsy and Brain Malformation. Ann Neurol, 88: 348-362. https://doi.org/10.1002/ana.25809

3. Zaman, T., et al. (2018), Mutations in *SCN3A* cause early infantile epileptic encephalopathy. Ann Neurol., 83: 703-717. https://doi.org/10.1002/ana.25188

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