Modeling epilepsy-related SCN2A mutation L1342P *with CRISPR/Cas9-edited human-induced pluripotent stem cell-derived cortical spheroids*

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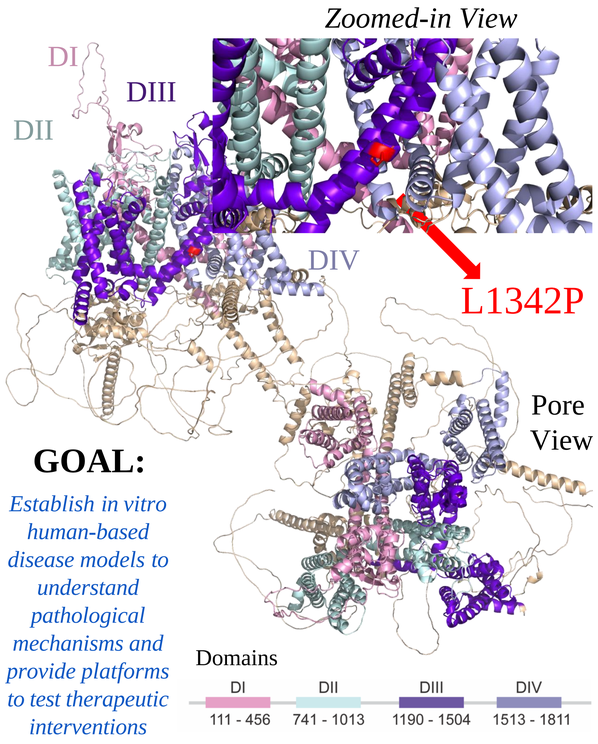
Advancing pharmacogenomics to cure diseases of the nervous system and cancer

Introduction

The SCN2A gene encodes for sodium channel Nav1.2, a protein that mediates action potentials in neurons. SCN2A pathogenic mutations have been associated with **epilepsy**. An example is the **L1342P** mutation, identified in several patients with untreatable seizure episodes **(Que, Olivero-Acosta et al., 2021).**

3D Structure of Sodium Channel Nav1.2



GOAL:

Establish in vitro human-based disease models to understand pathological mechanisms and provide platforms to test therapeutic interventions

Figure 1. *Schematic representation of an Alphafold predicted folding topology of sodium channel Nav1.2. Structures Rendered using PYMOL. Mutated residue L1342P is indicated in red.*

In our recent work, we have demonstrated that hiPSC-derived 2D-neuronal monolayers carrying the CRISPR-Cas9-edited L1342P-mutant channel display a marked hyperexcitability phenotype (Que, Olivero-Acosta et al., 2021). **However, the hyperexcitable L1342P mutation's impact on neurodevelopment remains unknown.** Cortical spheroids (organoids) are *in-vitro* generated 3D cellular aggregates that resemble the features of the human cortex.

In this poster, we describe the generation of the first SCN2A Cortical Spheroid model, aiming to understand the impact of the L1342P mutation on neuron development and further probe at its characteristic hyperexcitability phenotype.

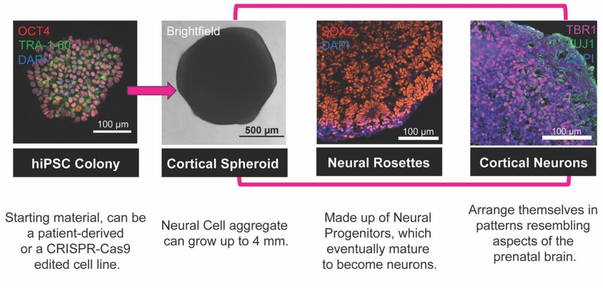


Figure 2. *Representative immunofluorescence and brightfield images of hiPSC-derived cortical spheroids through different maturation stages.*

Methods

CRISPR/Cas 9-edited hiPSCs → Cortical spheroids with epilepsy-related *SCN2A* L1342P mutation → To study **excitatory neuron development** and **characterize disease phenotypes**

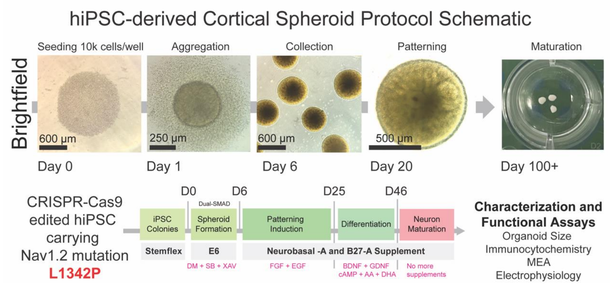


Figure 3. *The procedure used to generate human induced pluripotent stem cell-derived cortical spheroids in the Yang Lab.* Based on **(Sloan et al., 2018).**

Results

L1342P hiPSC lines display high levels of commonly used pluripotency markers indicating undifferentiated status

MUTATION DOES NOT ALTER PLURIPOTENCY

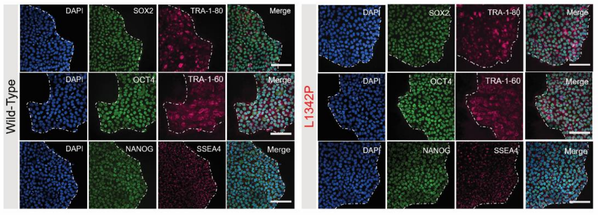


Figure 4. *Representative images of pluripotent colonies. Markers include DAPI (nuclei),* SRY-Box Transcription Factor 2 (*SOX2*)*, Tra-1-80, OCT4, Tra-1-60, NANOG and SSEA4. Scale bar set to 100 µm.*

L1342P variant seems to enhance synapse formation in 120-day-old spheroids

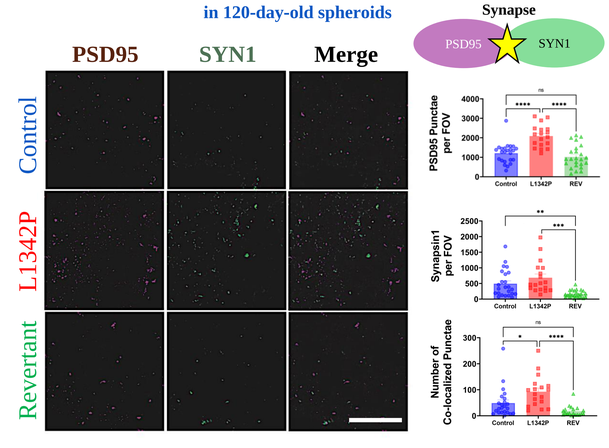


Figure 5. *Preliminary data shows the cortical spheroids carrying the L1342P-SCN2A Mutation display increased synapse formation at Day 120. Markers include Postsynaptic density protein-95 (PSD95) in magenta, Synapsin1 (green). Analysis performed using Zen Blue. Magnification 63X. Each dot represents one field of view. At least 2 organoids per genotype. One-Way ANOVA. Scale bar set to 50 µm.*

Results

hiPSC-derived cortical spheroids display active calcium transients

L1342P MUTANT NEURONS DISPLAY INCREASED NUMBER OF CALCIUM EVENTS

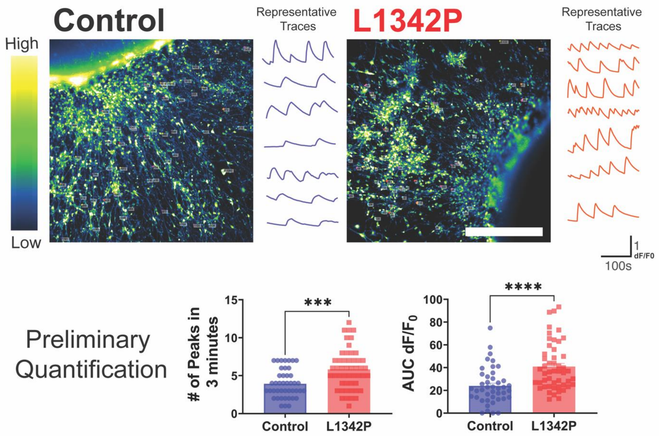


Figure 6. *Mature cortical spheroid derived neurons carrying the L1342P-variant display active calcium transients, increased peak frequency and area under the curve. Pseudocolored fields of views containing neurons loaded with Fluo-4. Each dot represents an active neuron. Data are reported as mean ± error (SEM). Scale bar is set to 500 µm. Data analyzed by Student's t test; *p < 0.05.*

hiPSC-derived cortical spheroids are electrophysiologicaly active

L1342P MUTANT NEURONS DISPLAY INCREASED NUMBER OF ACTION POTENTIALS

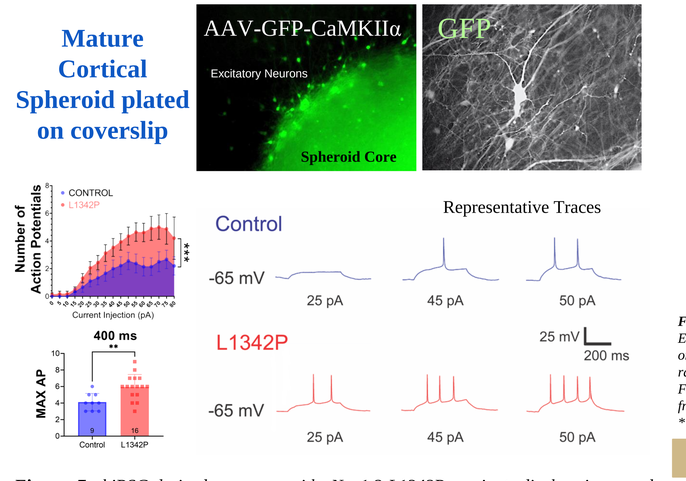


Figure 7. *hiPSC-derived neurons with Nav1.2-L1342P variant display increased excitability. The L1342P variant enhances the repetitive firing of hiPSC-derived neurons. Plot showing AP number per epoch in response to graded inputs from 0- to 80-pA current injection (400-ms duration). Representative sustained AP firings from hiPSC-derived Nav1.2-L1342P (red) cortical neurons or isogenic control (blue). Data were collected from two differentiated batches, with two clones used for each genotype. Data analyzed by repeated-measures two-way ANOVA and Student's t test; *p < 0.05.*

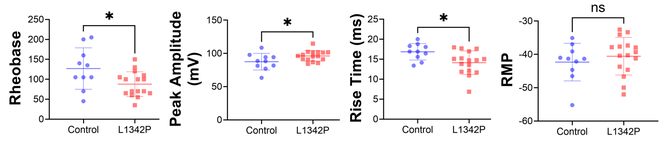


Figure 8. *The L1342P variant increases the intrinsic excitability of hiPSC-derived neurons. Data analyzed by Student's t test; *p < 0.05.*

L1342P leads to increased electrical firing in cortical spheroids

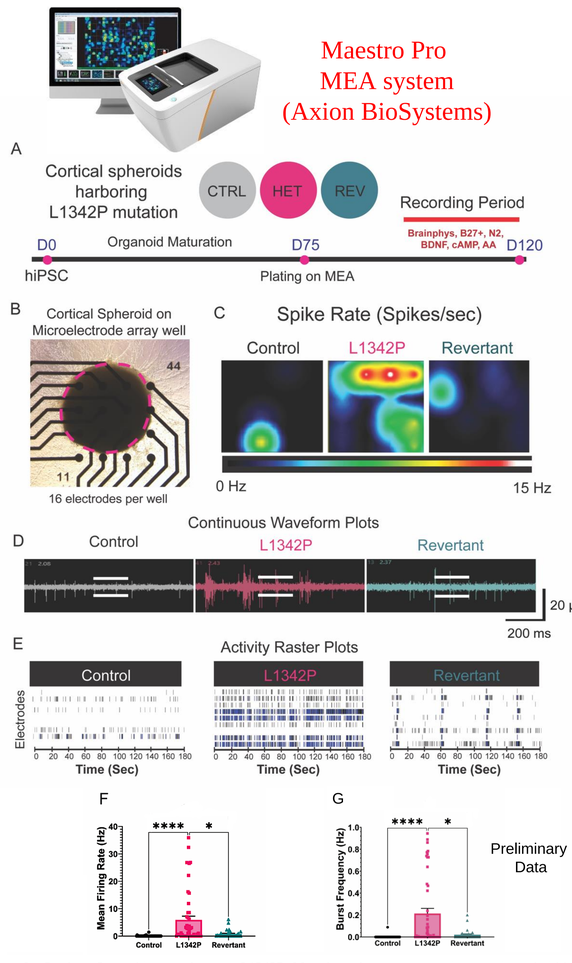


Figure 9. *Cortical Spheroids carrying the L1342P Mutation display enhanced network excitability. (A) Experimental Design. (B) Description of organoids used in study and representative image of organoid plated on a 16-electrode MEA well. (C) Activity Heat-maps (D) Representative raw spikes (E) Representative spike raster plots Bursting events are depicted by a cluster of ticks in blue. (F) Mean Firing Rate (G) Burst Frequency. Each dot represents an active electrode. Data are reported as mean ± error (SEM). Data pooled from Control: n = 48, L1342P: n = 48, Revertant: n = 32. Kruskal-Wallis test was performed with * p < 0.05; ** p < 0.01; ****p < 0.001*

Conclusions

- L1342P Mutation displays characteristic hyperexcitability phenotype.

References

- Que, Z., Olivero-Acosta, M. I., Zhang, J., Eaton, M., Tukker, A. M., Chen, X., Wu, J., Xie, J., Xiao, T., Wettschurack, K., Yunis, L., Shafer, J. M., Schaber, J. A., Rochet, J. C., Bowman, A. B., Yuan, C., Huang, Z., Hu, C. D., Trader, D. J., … Yang, Y. (2021). Hyperexcitability and Pharmacological Responsiveness of Cortical Neurons Derived from Human iPSCs Carrying Epilepsy-Associated Sodium Channel Nav1.2-L1342P Genetic Variant. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *41*(49). https://doi.org/10.1523/JNEUROSCI.0564-21.2021
- Sloan, S. A., Andersen, J., Paşca, A. M., Birey, F., & Pașca, S. P. (2018). Generation and assembly of human brain region–specific three-dimensional cultures. *Nature Protocols*, *13*(9). https://doi.org/10.1038/s41596-018-0032-7

Acknowledgements

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