Development of an iPSC-based drug screening platform for DLG4-Related Synaptopathy

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Abstract

DLG4-related synaptopathy (DLG4-RS) is a rare neurodevelopmental disorder characterized by clinical symptoms including developmental delay, intellectual disability, and autism spectrum disorder. Approximately 50% of individuals diagnosed with DLG4-RS experience epilepsy, while approximately 40% experience regression in motor and language skills, highlighting the significant impact on this patient community. Currently, there is no cure for this condition. Our study focuses on accelerating drug discovery efforts for *DLG4*-RS. Here, we describe the generation of cryopreserved iPSC-derived *NGN2*-glutamatergic neurons from both control and from isogenic *DLG4*-RS gene-edited iPSCs with the T654I mutation. In parallel, *NGN2*-glutamatergic neurons were also produced from a patient iPSC line with the T654I *de novo* mutation. The availability of these neurons enabled examination of neuronal electrophysiological properties by microelectrode array analysis, facilitating the identification of *DLG4*-related phenotypes for the development of a drug screening platform, including high-throughput screen (HTS) assays. RNA-seq analysis of cultured NGN2 neurons revealed altered pathways that could serve as potential therapeutic targets. Taken together, the development of patient-derived neurons will be an invaluable resource for identifying therapies for *DLG4*-related synaptopathies.

Experimental Approach

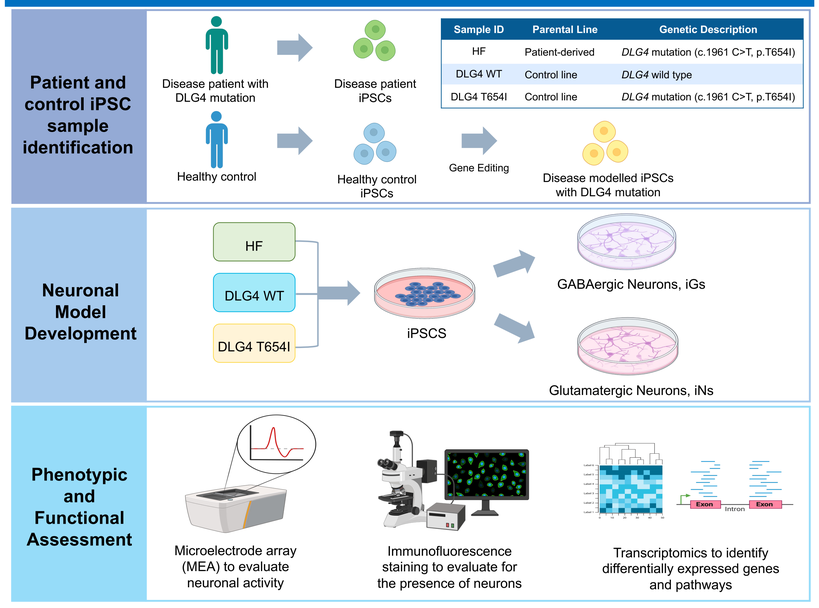

Figure 1. Schematic overview of experimental approach. Induced pluripotent stem cells (iPSCs) were derived from a patient with a DLG4 mutation (T654I), alongside a wild-type control line and an isogenic DLG4 gene-edited line. These iPSCs were obtained from COMBINEDBrain and released to NeuCyte upon request from the Hope for Harvey Foundation (Top Panel). These iPSCs were differentiated into GABAergic (iGs) and Glutamatergic (iNs) neurons using NeuCyte's proprietary platform (Middle Panel). Neuronal activity was evaluated using microelectrode array (MEA), immunocytochemistry was performed to confirm neuronal identity, and RNA sequencing carried out to identify DLG4 T654I gene signatures (Bottom Panel).

iPSCs demonstrate genomic stability

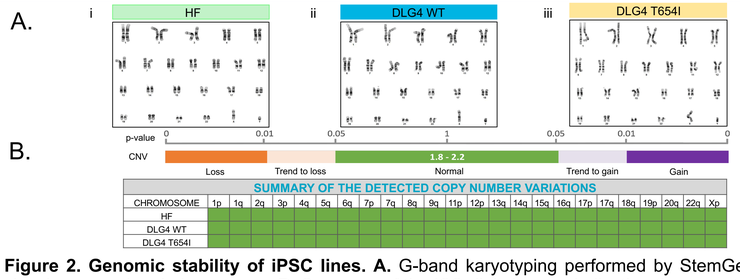

Figure 2. Genomic stability of iPSC lines. A. G-band karyotyping performed by StemGenomics showing normal male karyotypes. **B.** Summary of the iCS-digital assay indicating the expected copy number across 24 genomic regions associated with recurrent iPSC abnormalities.

iPSC-derived iNs and iGs display functional maturation

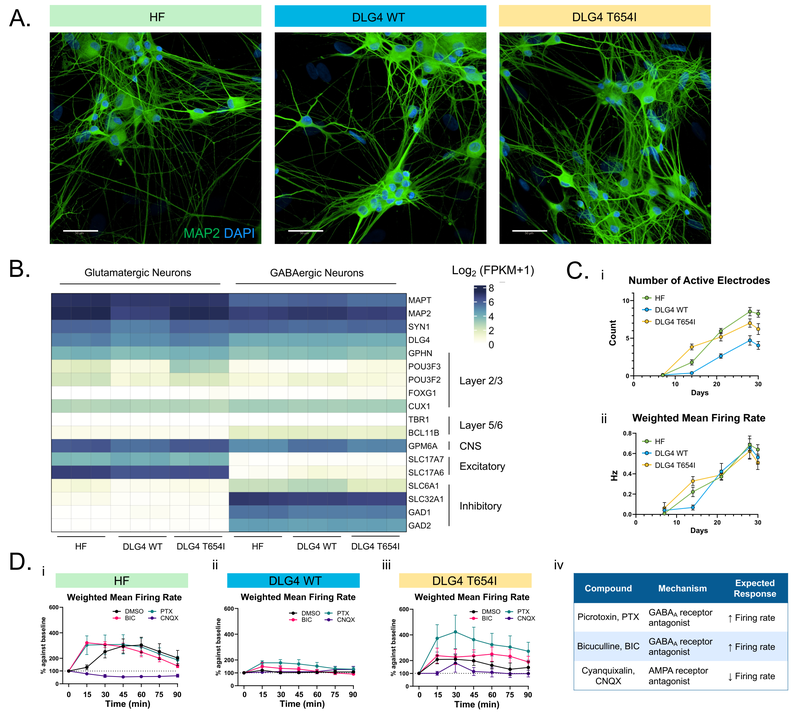

Figure 3. Generation and characterization of glutamatergic and GABAergic neurons from iPSCs. A. Representative immunofluorescent images of iPSC-derived neurons at Day 28, stained for a neuronal marker, MAP2. Cell nuclei were stained with DAPI. Scale bar, 50 µM. **B.** Heatmap showing iPSC-derived glutamatergic and GABAergic neurons expressing canonical neuronal gene signatures. **C.** Line graphs showing the **(i)** number of active electrodes and **(ii)** weighted mean firing rate of neurons as they mature over 30 days in culture. **D.** Line graphs showing weighted mean firing rates of neurons from HF **(i)**, DLG4 WT **(ii)**, and DLG4 T654I **(iii)** in response to picrotoxin, bicuculline, and cyanquixalin, over a 90-minute treatment period.

Effect of T654I mutation on splicing and mRNA stability

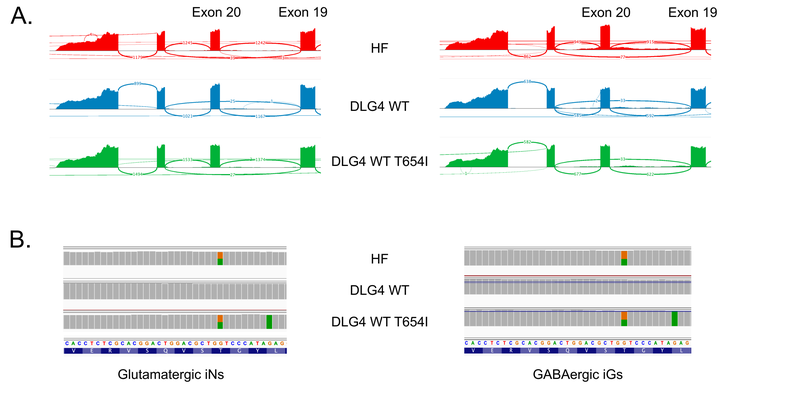

Figure 4. RNA-seq analysis of DLG4 expression in Glutamatergic iNs and GABAergic iGs. A. Sashimi plots generated from the RNA-seq transcriptomic data show that the c.1961 C>T located in exon 19 (hg38) does not affect splicing between exon 19 and exon 20 junction and therefore does not lead to differential isoform expression. **B.** Mapped reads from the RNA-seq analysis of Glutamatergic iNs or GABAergic iGs indicate that c.1961 C>T leads to a decreased ratio of wild-type to T654I mRNA in Glutamatergic iNs (51% wild-type, 49% T654I) and GABAergic iGs (55% wild-type, 45% T654I).

DLG4 T654I alters expression of DLG4-interacting proteins

A. i

Comparisons	Glutamatergic Neurons		GABAergic Neurons	
	Number of Downregulated Genes	Number of Upregulated Genes	Number of Downregulated Genes	Number of Upregulated Genes
HF vs. DLG4 WT	254	503	401	311
DLG4 T654I vs. DLG4 WT	337	152	128	177

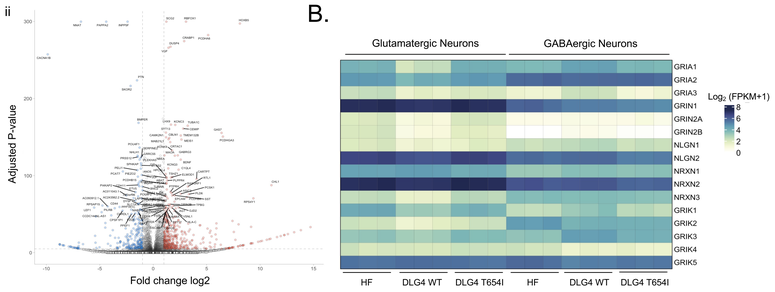

Figure 5. RNA-seq analysis of Glutamatergic iNs and GABAergic iGs show that T654I leads to increased expression of genes encoding DLG4-interacting proteins. A. i. Table summarizing differentially-expressed genes (DEGs) between T654I mutation and wild-type neurons (FDR<1x10^{-5},Two-fold change). **ii.** Representative volcano plot comparing HF vs WT DLG4 Glutamatergic iNs **B.** Heatmap demonstrating increased expression of genes encoding DLG4 interacting proteins including ionotropic AMPA (GRIAs), NMDA (GRINs), and Kainate (GRIKs) glutamate receptors and neuroligins (NLGNs) that are all implicated in seizures.

T654I mutation neurons exhibit a seizurogenic phenotype

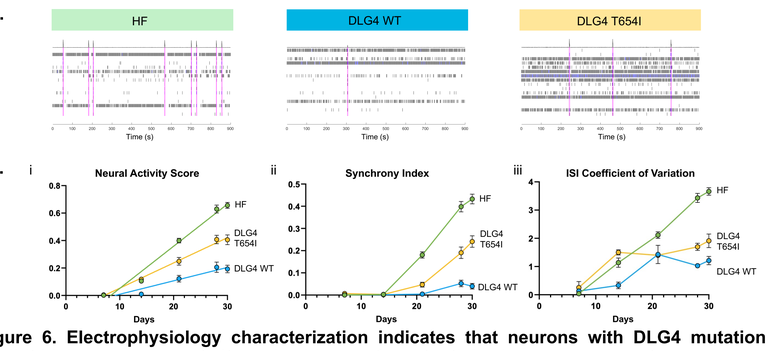

Figure 6. Electrophysiology characterization indicates that neurons with DLG4 mutation exhibit higher neuronal activity and evidence of a potential seizurogenic phenotype. **A.** Representative raster plots showing changes in neuronal firing patterns, highlighting the differences in activity among the different lines over time. **B.** Line graph showing **(i)** neural activity score, which quantifies the overall level of neuronal firing; **(ii)** synchrony index, reflecting the degree of coordinated firing among neurons; and **(iii)** ISI (Inter-Spike Interval) Coefficient of Variation, which measures the variability in the intervals between successive action potentials or neuronal firing. These metrics are compared for neurons derived from HF, DLG4 WT and DLG4 T654I tracked over 30 days in culture.

Conclusions and Future Directions

Generation of patient-derived neurons from DLG4-related synaptopathy identified altered ionotropic glutamate receptor expression in both glutamatergic and GABAergic neurons. This change contributes to an Excitatory/Inhibitory (E/I) imbalance that can lead to seizures, a clinical manifestation of the DLG4 T654I mutation. Electrophysiological studies further revealed seizurogenic phenotypes in T654I neurons. Future studies will employ drug screenings targeting these receptors to identify compounds that can reverse the seizures for these rare disease patients.