Rescue of neuronal activity in iPSC-derived STXBP1 in vitro disease models using viral vectors

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Overview

QUESTION

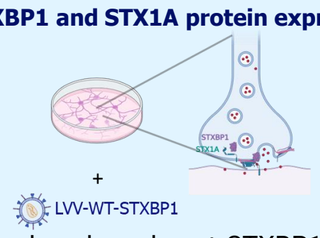
- Do STXBP1 mutant/KO hiPSCs-derived glutamatergic neurons display epilepsy related phenotypes in vitro?
- Can exogenous STXBP1 (over)expression alleviate epilepsy associated phenotypes in vitro?
- Can STXBP1-KO cells be used to assess epilepsy causing STXBP1 variants *in vitro*?

INVESTIGATION

- Evaluate protein expression (STXBP1 and STX1A) and neuronal network activity of STXBP1 mutant and KO$^{-/-}$ hIPSCs-derived glutamatergic neurons.
- Overexpress exogenous STXBP1 using a LVV vector to evaluate the phenotype rescue observed in glutamatergic neurons.
- Evaluate rescue of neuronal network after LVV delivery of 17 patient-associated mutations in STXBP1-KO$^{-/-}$ neurons

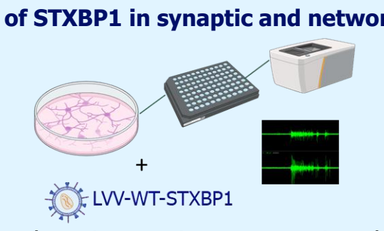
RESULTS

STXBP1 and STX1A protein expression



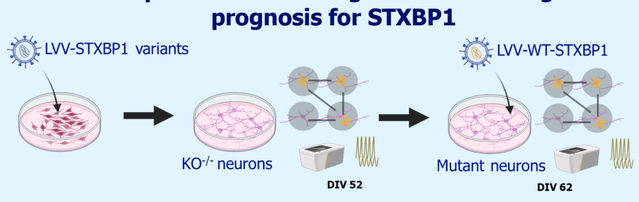
- We confirmed reduced or absent STXBP1 protein, in mutant and KO$^{-/-}$ hIPSC neurons, respectively;
- We observed reduced STX1A protein levels
- Overexpression with LVV-hSTXBP1 increased STXBP1 levels and partially restored STX1A levels.

Critical role of STXBP1 in synaptic and network excitability



- Impaired or absent spontaneous network activity and neuronal synchronization in mutant and KO$^{-/-}$ neurons;
- Overexpression of LVV-hSTXBP1 led to improvements in network connectivity and synchronization.

Human cell-based platform for mutagenesis-screening and treatment-prognosis for STXBP1



- From the 17 mutations screened, 3 exhibited network activity (benign or with no epilepsy history reported in patients), while the remaining have no activity (pathogenic);
- After WT-hSTXBP1 overexpression, network activity can be totally or partially rescued, except in 2 frameshift mutants.

CONCLUSIONS

- Impaired expression of STXBP1 in mutant and KO$^{-/-}$ neurons translates into altered neuronal network activity and synchronization
- Reduced STXBP1 expression in mutant and KO$^{-/-}$ neurons results in lower STX1A levels, showing impaired chaperone function of STXBP1
- Lentiviral delivery of exogenous STXBP1 can restore protein expression, partial chaperone function and ameliorate the neuronal network phenotype in mutant neurons.
- STXBP1-KO$^{-/-}$ neurons can be used as an *in vitro* platform to assess the pathogenicity of STXBP1 patient variants

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Background

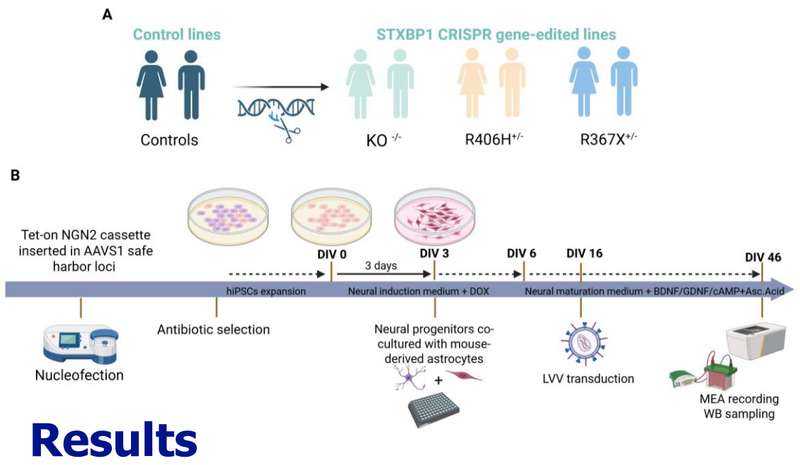
STXBP1 (Syntaxin-binding-protein-1), also known as MUNC-18-1, facilitates synaptic vesicle docking and fusion for synaptic transmission. Mutations in the STXBP1 gene are linked to neurodevelopmental disorders in children, with over 85% experiencing seizures and severe intellectual disability[1, 2, 3]. This study used gene-edited human-induced pluripotent stem cells (hiPSCs) to model STXBP1 pathophysiology and phenotype rescue after wild-type (WT) STXBP1 gene expression.

Objective

In this study we developed and characterized a hiPSCs-derived model of STXBP1 haploinsufficiency.

Methods

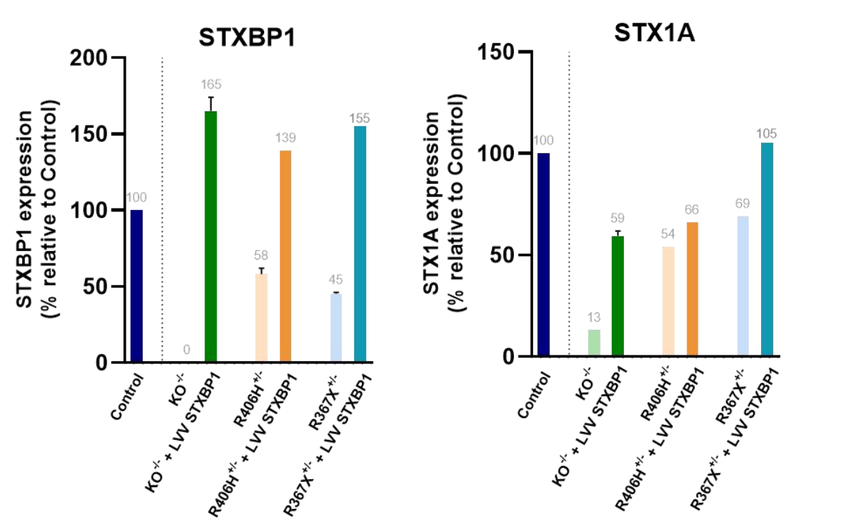
- hiPSCs were CRISPR/Cas9-edited to obtain STXBP1-KO$^{-/-}$ clones and two heterozygous (HET) missense (R406$^{+/-}$) and nonsense mutations (R367X$^{+/-}$). Two healthy lines from different genetic backgrounds have been used for gene edition and are used as isogenic controls - **A**
- The NGN2 transcription factor was introduced to AAVS1 locus by flipase – ligase system to differentiate iPSCs into enriched glutamatergic neuronal population – **B**
- Glutamatergic neurons were then transduced between at day *In vitro* (DIV) 16 with Lentiviral vectors (LVVs) containing wild-type (WT) human STXBP1 – (LVV-STXBP1) - **B**
- STXBP1 protein expression was assessed by Western Blot (WB), as well as Syntaxin-1 (STX1A) protein, which an interaction partner of STXBP1 protein - **B**
- The functional impact of STXBP1 was evaluated by recording extracellular spontaneous neuronal activity using a microelectrode array (MEA) assay - **B**



Results

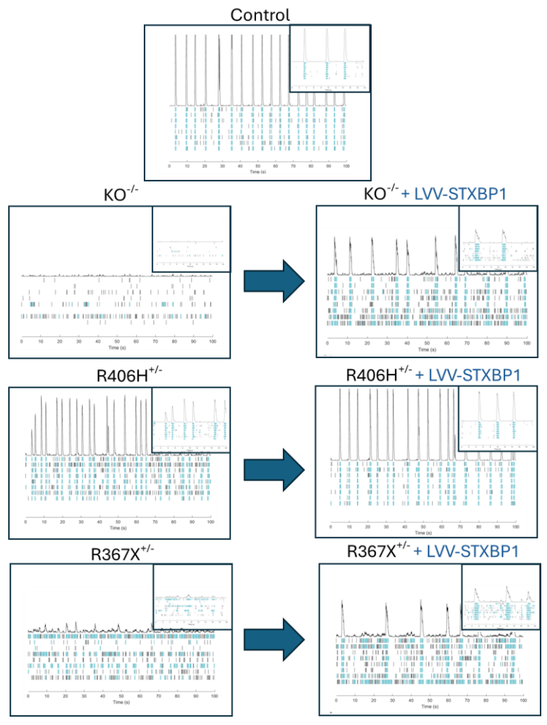
1. Mutant hiPSCs-neurons have reduced endogenous STXBP1 and STX1A levels and which can be rescued by LVV mediated overexpression of WT STXBP1

- STXBP1 and STX1A representative WB and respective quantification (DIV46)

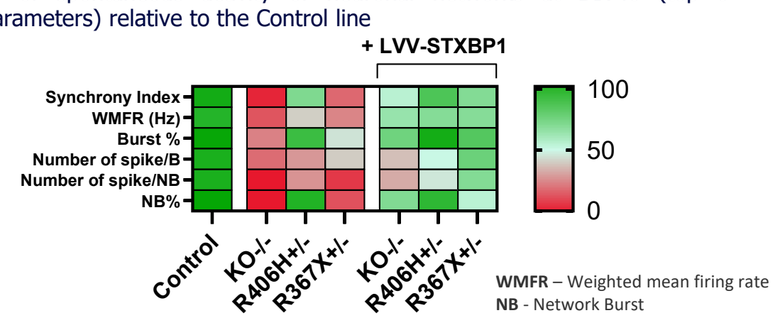


2. Impaired spontaneous neuronal network activity in STXBP1 mutant and KO$^{-/-}$ neurons is ameliorated after exogenous SXTBP1 (over)expression

- Representative spike raster plots (100s and 20s) from a MEA recording at DIV46 before and after exogenous STXBP1 overexpression.

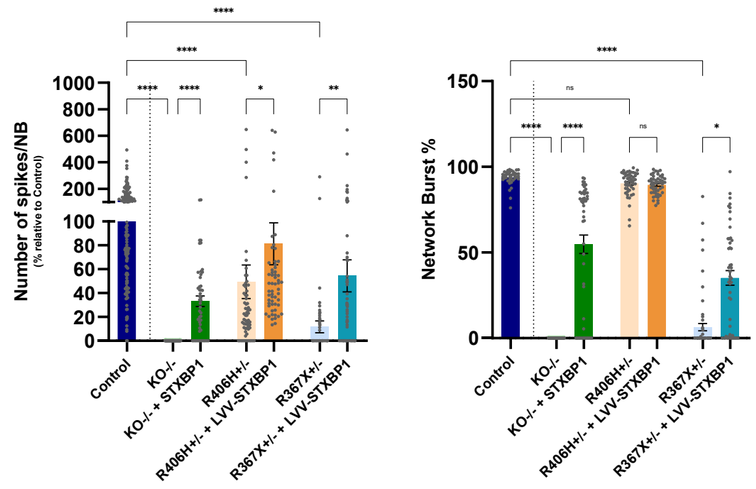


- % of spontaneous activity of neuronal networks at DIV46 (top 6 parameters) relative to the Control line



WMFR – Weighted mean firing rate
NB - Network Burst

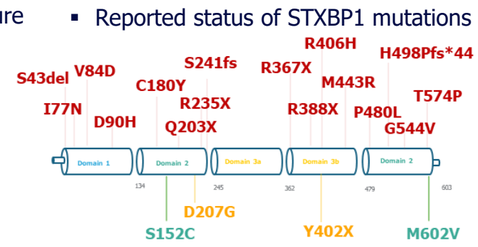
- Network Burst parameters at DIV46, before and after exogenous STXBP1 overexpression



3. In vitro platform for investigating epilepsy-related variants and predicting pathogenicity for both existing and novel variants

- STXBP1 protein structure

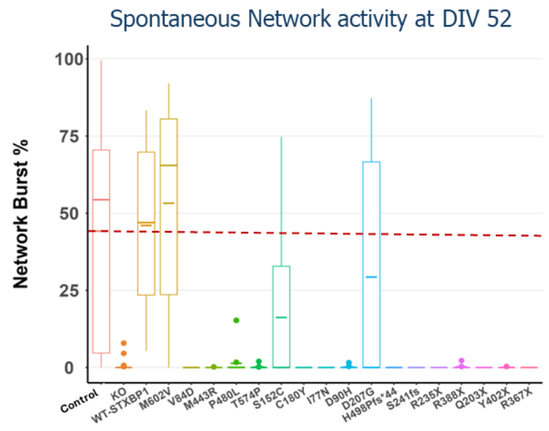
- Reported status of STXBP1 mutations



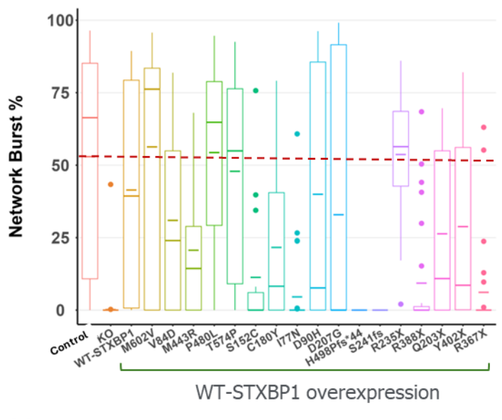
- Benign/likely benign
- Pathogenic/Likely pathogenic
- Likely pathogenic, but no history of epilepsy

*STXBP1 variant pathogenicity information was collected from published sources

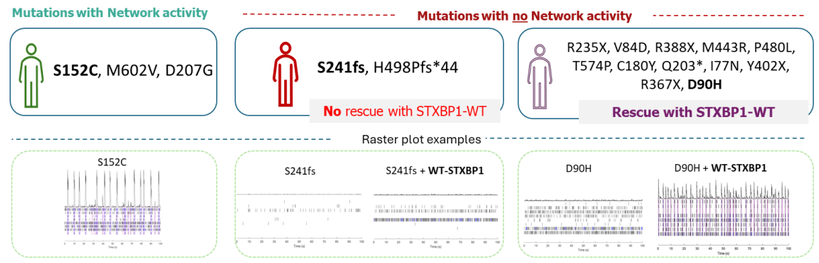
Spontaneous Network activity at DIV 52



Spontaneous Network activity at DIV62
(10 days post-transduction with WT-STXBP1)



Summary table



Conclusions

We confirmed that STXBP1 gene-edited hiPSCs-derived glutamatergic neurons are a suitable model recapitulating the pathophysiology of STXBP1 *in vitro*. Moreover, the data demonstrates the potential of hiPSCs-derived model to be used to hypothesize a likely genotype/phenotype correlation and to explore novel approaches for disease modifying therapies.

References

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