Unravelling CACNA1A Channelopathies: CRISPR and iPSC Models for Rapid Functional Analysis of Genetic Variants

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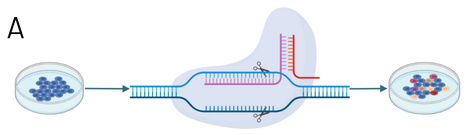
BACKGROUND

- *CACNA1A* encodes the α1A subunit of $Ca_V2.1$ voltage gated calcium channels.[1]
- $Ca_V2.1$ channels are key modulators of fast synaptic transmission in excitatory and inhibitory neurons in the CNS.[1]
- *CACNA1A* related channelopathies are associated with a spectrum of disease including hemiplegic migraine, episodic ataxia, epilepsy, intellectual disability.[1]
- Two patients were identified with VUS in *CACNA1A*:
  - NM_001127221.2:c.4312_4314del; p.(Lys1438del)
  - NM_001127221.2:c.5876C>A; p.(Ala1959Asp)
- Aims: 1. Generate iPSC lines harbouring patient VUSs
  2. Differentiate iPSC to NPCs for future functional analysis.

METHOD



A. Precise CRISPR-Cas9 homology directed repair to generate isogenic matched VUS and WT iPSC lines.[2]

B. iPSC to NPC induction using the STEMdiff SMADi neural induction kit.[2]

C. Neural maturation using the BrainPhys™ hPSC Neuron Kit[3]

RESULTS

CACNA1A structure and location of variants

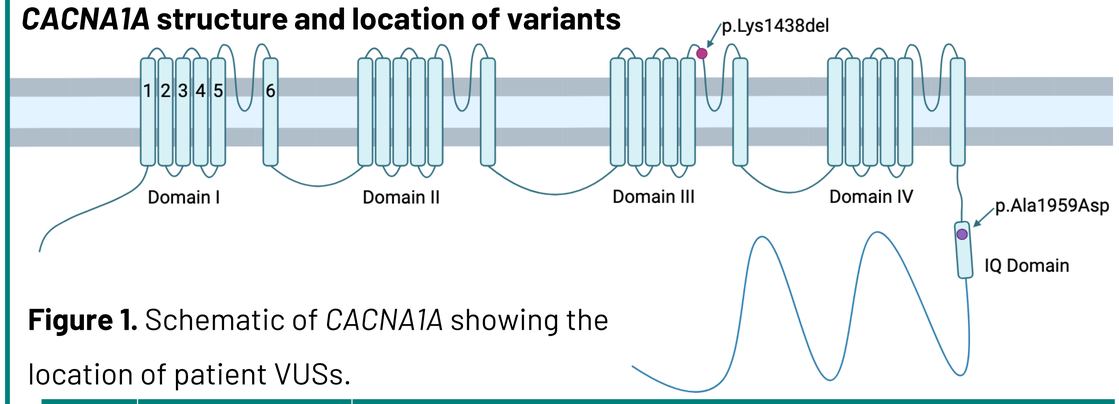
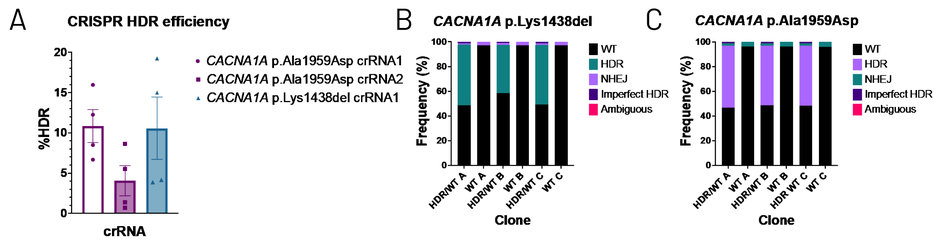


Figure 1. Schematic of *CACNA1A* showing the location of patient VUSs.

ID	VUS	Phenotype
VUS1	p.Lys1438del	Ataxia, brain atrophy, nystagmus
VUS2	p.Ala1959Asp	Seizures, migraine, episodic ataxia

CRISPR-Cas9 gene editing to introduce *CACNA1A* genetic variants



D

Quality control	Karyotyping qPCR	Off-target site 1	Off-target site 2	Off-target site 3	Off-target site 4	Off-target site 5	Off-target site 6
CACNA1A p.Lys1438del	PASS	PASS	PASS	PASS	PASS	PASS	PASS
CACNA1A p.Ala1959Asp	PASS	PASS	PASS	PASS	PASS	PASS	PASS

Figure 2. A. HDR percentage from polyclonal post-transfection populations for each crRNA designed. B. Amplicon sequencing of selected *CACNA1A* p.Lys1438del clones. C. Amplicon sequencing of selected *CACNA1A* p.Ala1959Asp clones. D. Quality control analysis. Karyotyping qPCR and amplicon sequencing of top six CRISPR off-target sites. Analysis performed on individual clonal populations.

Neural differentiation of *CACNA1A* VUS and WT iPSC lines

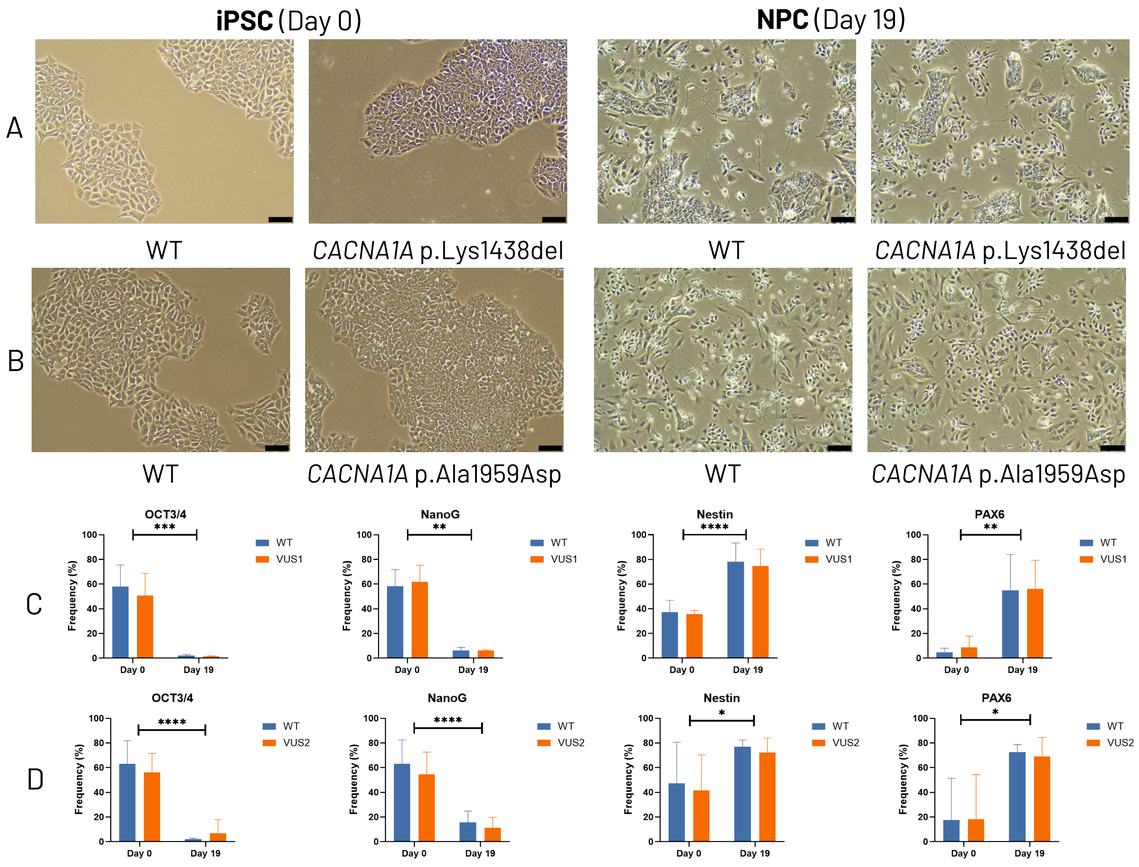


Figure 3. A and B. Cellular morphology of matched VUS and WT cells at days 0 and 19 of neural induction. Scale bar = 100μm. C and D. Percentage of *CACNA1A* p.Lys1438del (C) and *CACNA1A* p.Ala1959Asp (D) cells positive for marker expression at days 0 and 19 of neural induction. Ordinary 2-way ANOVA, $p<0.05$ (*), $p<0.01$ (**), $p<0.001$ (***), $p<0.0001$ (****) day 0 to day 19. No significant difference between WT and VUS.

Preliminary immunohistochemistry of iPSC derived NPCs and mature neurons

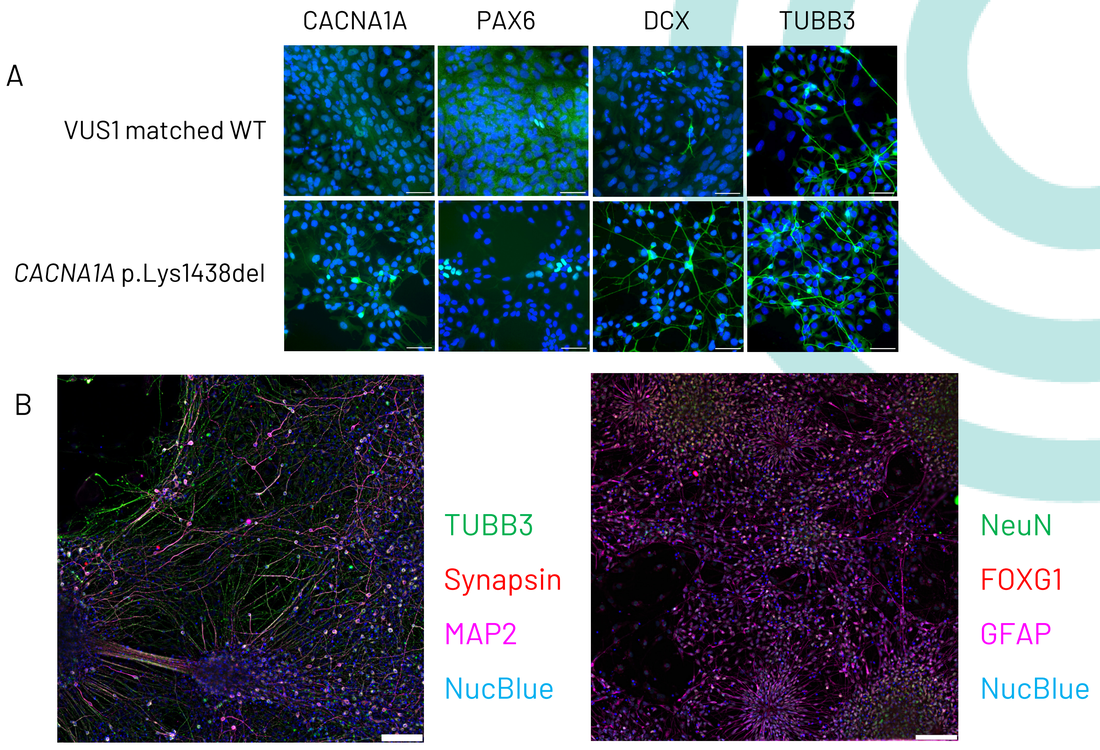


Figure 5. A. Expression of CACNA1A, PAX6, DCX, and TUBB3 in *CACNA1A* p.Lys1438del VUS and WT NPCs at day 19 of neural induction. B. Expression of TUBB3, Synapsin, MAP2, NeuN, FOXG1, and GFAP in WT neurons at 21 days of maturation. Scale bar = 50μm.

CONCLUSION

- Matched WT and VUS iPSC lines were established for each patient VUS.
- iPSC lines were differentiated to NPCs. Preliminary immunofluorescence indicates limited expression of *CACNA1A*.
- Preliminary immunofluorescence indicates successful maturation of neurons via BrainPhys™ hPSC Neuron Kit.
- Matched VUS and WT NPCs to be matured via BrainPhys™ hPSC neuron kit ahead of functional assessment of variant effect by calcium flux imaging and functional transcriptomic profiling.

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References: 1. Indelicato & Boesch (2021) *Frontiers in Neurology* 12:6399994 **2.** Shaw et al. (2024) *Molecular Autism* 15(1):42 **3.** van den Hurk et al. (2018) *Front. Mol. Neurosci.* 11:261