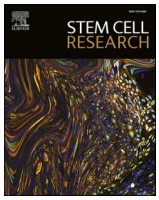
Stem Cell Research 76 (2024) 103367

Contents lists available at ScienceDirect

Stem Cell Research

journal homepage: www.elsevier.com/locate/scr



Lab Resource: Single Cell Line

An iPSC line (FINi003-A) from a male with late-onset developmental and epileptic encephalopathy caused by a heterozygous p.E1211K variant in the *SCN2A* gene encoding the voltage-gated sodium channel $Na_v1.2$

Dmitry A. Ovchinnikov [a], Sharon Jong [a], Claire Cuddy [a], Kelly Dalby [c], Orrin Devinsky [d], Saul Mullen [a,b], Snezana Maljevic [a,*], Steve Petrou [a,c,*]

[a] *The Florey Institute for Neuroscience and Mental Health, University of Melbourne, Melbourne 3010 VIC, Australia*
[b] *Departments of Medicine and Paediatrics, The University of Melbourne, Austin Health and Royal Children's Hospital, Murdoch Children's Research Institute, Melbourne, VIC, Australia*
[c] *Praxis Precision Medicines, Cambridge, MA, USA*
[d] *Department of Neurology, NYU Grossman School of Medicine, New York, NY, USA*

A B S T R A C T

Many developmental and epileptic encephalopathies (DEEs) result from variants in cation channel genes. Using mRNA transfection, we generated and characterised an induced pluripotent stem cell (iPSC) line from the fibroblasts of a male late-onset DEE patient carrying a heterozygous missense variant (E1211K) in $Na_v1.2$ (SCN2A) protein. The iPSC line displays features characteristic of the human iPSCs, colony morphology and expression of pluripotency-associated marker genes, ability to produce derivatives of all three embryonic germ layers, and normal karyotype without SNP array-detectable abnormalities. We anticipate that this iPSC line will aid in the modelling and development of precision therapies for this debilitating condition.

Resource Table:

Unique stem cell line identifier	FINi003-A
Alternative name(s) of stem cell line	FI.SPSM.SCN2A.E1211K.008
Institution	The Florey / University of Melbourne
Contact information of distributor	steven.petrou@florey.edu.au, snezana.maljevic@florey.edu.au
Type of cell line	iPSC
Origin	Human
Additional origin info required for human ESC or iPSC	*Age: 5* *Sex: Male* *Ethnicity if known: Caucasian*
Cell Source	Skin fibroblasts
Clonality	Clonal iPSC line (derivation)
Method of reprogramming	ReproRNA™ kit (StemCellTechnologies. 1 polycistronic mRNA (expressing human KLF4 + OCT4/POU5F1 + SOX2 + GLIS1 + c-Myc, plus puromycin N-acetyltransferase proteins).
Genetic Modification	*Yes*
Type of Genetic Modification	*Hereditary*
Evidence of the reprogramming transgene loss (including genomic copy if applicable)	*RT-PCR*
Associated disease	*Developmental and epileptic encephalopathy (DEE)*
Gene/locus	*SCN2A/Nav1.2*
Date archived/stock date	*17.6.2023*
Cell line repository/bank	https://hpscreg.eu/cell-line/FINi003-A
Ethical approval	The University of Melbourne HREC/16/Austin/472

1. Resource utility

Mutations in the *SCN2A* gene, encoding a neuron-specific type 2 alpha subunit of a voltage-gated sodium channel $Na_v1.2$, are some of the more common causes of DEEs, often constituting *de novo* events (Mason et al., 2019, Shi et al., 2012). The dominant-acting p.E1211K SCN2A variant, confirmed to be present in a heterozygous state in the reported iPSC line, is one of the common mutations associated with DEE, featured in over 40 publications (*e.g.* Berecki et al., 2022, Zeng et al., 2022). The presence of this pathogenic variant in $Na_v1.2$ (located at the highly

* Corresponding authors.
E-mail addresses: snezana.maljevic@florey.edu.au (S. Maljevic), steven.petrou@florey.edu.au (S. Petrou).

https://doi.org/10.1016/j.scr.2024.103367
Received 26 December 2023; Received in revised form 6 February 2024; Accepted 25 February 2024
Available online 28 February 2024

evolutionarily conserved beginning of domain III) results in a substantial alteration of the voltage dependence of the channel's activity. Specifically, it reduces the threshold of depolarization required for the channel's activation due to a substantial hyperpolarizing shift in its voltage dependence curve (Ogiwara et al., 2009). At the normal resting potential levels, most mutant SCN2A channels are inactive, reflecting the complex physiological effects of this variant in cortical neurons. Our iPSC line will be a valuable resource to model this complex SCN2A mutation-caused epileptic phenotypes in 2D and 3D human iPS cell-derived cortical neuronal systems.

2. Resource Details

We report a reprogramming transgene-free iPSC line carrying a recurrent and well-characterised pathogenic dominant-acting mutation in SCN2A/Na_v1.2 protein, p.E1211K (Fig. 1A, glutamate to lysine transition, OMIM Allelic Variant: 182390.0009); introducing changes in the voltage-dependent channel activation and inactivation (Ogiwara et al., 2009). This variant, in one of the patient's *SCN2A* alleles, was confirmed by direct PCR from genomic DNA of fibroblasts and iPSCs using *SCN2A*-specific primers, followed by Sanger sequencing (Fig. 1B).

Primary fibroblasts from a skin biopsy of a male patient with late-onset *SCN2A*-DEE (patient 3 in Howell et al., 2015) were reprogrammed using lipofection with a polycistronic mRNA (ReproRNA, ThermoFisher Scientific) expressing reprogramming factors that are transiently maintained, and loss of which occurs in these reprogramming experiments after passage eight (Ovchinnikov et al., 2023). Clonal iPSC lines were manually isolated, maintained in feeder-free conditions (in the media of mTeSR family, StemCellTechnologies), and displayed characteristic human primer iPSC morphology (phase-contrast image Fig. 1C). We assessed the pluripotency-associated marker expression (members of the PluriNet gene regulatory network) at the protein and mRNA (Fig. 1D) levels. High-resolution karyotyping via evaluation of the genomic integrity using a high-density SNP array (Illumina Global Screening Array 24, v.2.0), and standardised BAF (B-allele frequency) plots across all 24 chromosomes (22 autosomes + X + Y) provide clear evidence of the absence of any detectable loss of heterozygosity in this iPSC line (Fig. 1F). To further validate the pluripotency potential of this iPSC line, a directed differentiation into three embryonic germ layers (mesoderm, ectoderm and endoderm) was performed (Fig. 1G), using validated differentiation-promoting factor containing commercial media (TriLineage diff kit, StemCellTechnologies). Expression of the pluripotency and lineage-specific markers was evaluated using specific and reliable Taqman qPCR assays (relying on internal probe hybridisation in combination with qPCR, see Materials and Methods and Tabel 2 for more detail).

3. Materials and Methods

3.1. Reprogramming of fibroblasts into iPSCs using transient mRNA transfection

Cryopreserved fibroblasts were cultured in DMEM/Glutamax medium supplemented with 15 % normal bovine serum, 1xNEAA and 0.2xPenicillin/Streptomycin mix. Before transfection, 100,000 cells were plated onto a well of a 6-well TC plate coated with hESC-qualified Matrigel® (Corning) to facilitate manual colony isolation for line establishment (Ovchinnikov, 2021). Transfection and cell culture were performed as per the ReproRNA manual, except for using mTeSRplus medium to maintain emerging iPSC colonies rather than mTeSR1 after day 28 average-sized colonies. We found good morphology and manually established clonal iPSC cell lines.

To confirm the loss of mRNA, RT-PCR was carried out from total RNA extracted from iPSC lines at passage 8 (Pavan et al., 2023). An RNA sample of the recently transfected mRNA (day 3 post-transfection) fibroblasts served as a positive control.

3.2. Immunofluorescent detection of pluripotency markers

Cultures of pluripotent iPSC, after confirmation of the complete loss of the reprogramming factors-expressing mRNA (at passage 10), were fixed with 4 % PFA in 1xPBS (10 min at RT), rinsed and incubated overnight with primary antibodies diluted in 0.1 M PBS containing 5 % normal bovine serum and 0.3 % Triton X-100 (Amresco, USA). Primary antibodies and dilutions were: rabbit anti-NANOG (1:200; Cell Signalling Technology Cat# 4755), mouse anti-SSEA-4 (1:200; Cell Signalling Technology Cat# 4903). Secondary antibodies generated in donkey were applied for 2 h at room temperature at a dilution of 1:1000 for fluorescent detection using DyLight 549-, or 488-conjugated goat anti-mouse, anti-rabbit (Invitrogen Cat #s A11032, A11008). Cultures were counterstained for 10 min with DAPI in PBS (1:5000; Sigma-Aldrich) and imaged using a compound fluorescent microscope.

3.3. Evaluation of the genomic integrity of iPSC Clones Using An SNP Array

To confirm the absence of loss or gain of a substantial amount of genetic material from the genome of published iPSC lines, we utilised hybridisation of genomic DNA from early-passage cultures (p8) to an SNP array (Illumina Global Screening Array-24, GSA v. 2.0). Genomic DNA was isolated using a tissue genomic DNA extraction kit as per manufacturer's protocol (Qiagen, GmbH), and 2 μg were submitted for the QC and subsequent array hybridisation and bioinformatic analysis to the Australian Genome Research Facility (Melbourne). Equally scaled across the whole genome logR and BAF plots were generated and analysed for the absence of whole chromosomal loss or gain, intra-chromosomal deletions or insertions (best judged by the BAF plot only containing 0, 0.5 and 1.0 frequencies always anticipated for intact autosomes Chr.1–22 with the expected resolution of < 1 MB.

3.4. Quantification of gene mRNA expression levels using qPCR

Extraction of total RNA was performed using the PureLink RNA Mini Kit (ThermoFisher) according to the manufacturer's instructions. Total RNA yield was measured using NanoDrop™ OneC Microvolume UV–Vis Spectrophotometer (ThermoFisher). Coupled synthesis of the first cDNA strand and quantification of each gene's mRNA level was performed using TaqMan RNA-to-Ct 1-Step Kit (Applied Biosystems) per manufacturer's instructions. An input of 500 ng of total RNA from passage 15 iPSCs was used for RT-qPCR. The expression of pluripotent stem cell markers (*NANOG, OCT4, KLF4, SOX2) w*as analysed using ThermoFisher Scientific TaqMan assays listed in Table 2 and QuantStudio 7 Pro (ThermoFisher Scientific). Experiments were conducted in triplicates to obtain average relative levels of each transcript abundance, expressed as normalized for the expression levels of an endogenous reference gene, *GAPDH* (Fig. 1).

3.5. Directed trilineage differentiation of hPSCs

Cultures of enzymatically-passaged iPSCs were differentiated into (ectoderm, endoderm and mesoderm in monolayer culture using the STEMdiff Trilineage Differentiation Kit (StemCell Technologies). Expression of mRNA of each germ layer differentiation marker genes after differentiation was assessed by RT-qPCR using lineage-specific targeted markers (Table 2). Differentiated cultures (from passage 17 iPSCs) were harvested on day 5 (endoderm and mesoderm) or day 7 (ectoderm) for total RNA extraction using PureLink RNA Mini Kit (Invitrogen/ThermoFisher Scientific). After measuring the total RNA yield using NanoDrop™ OneC Microvolume UV–Vis Spectrophotometer (ThermoFisher), 0.5 μg of total RNA was used for gene expression analysis. Reverse transcription of RNA and qPCR was performed simultaneously using the TaqMan RNA-to-Ct 1-Step Kit (Applied Biosystems) and QuantStudio 7 Pro (ThermoFisher Scientific, all steps were

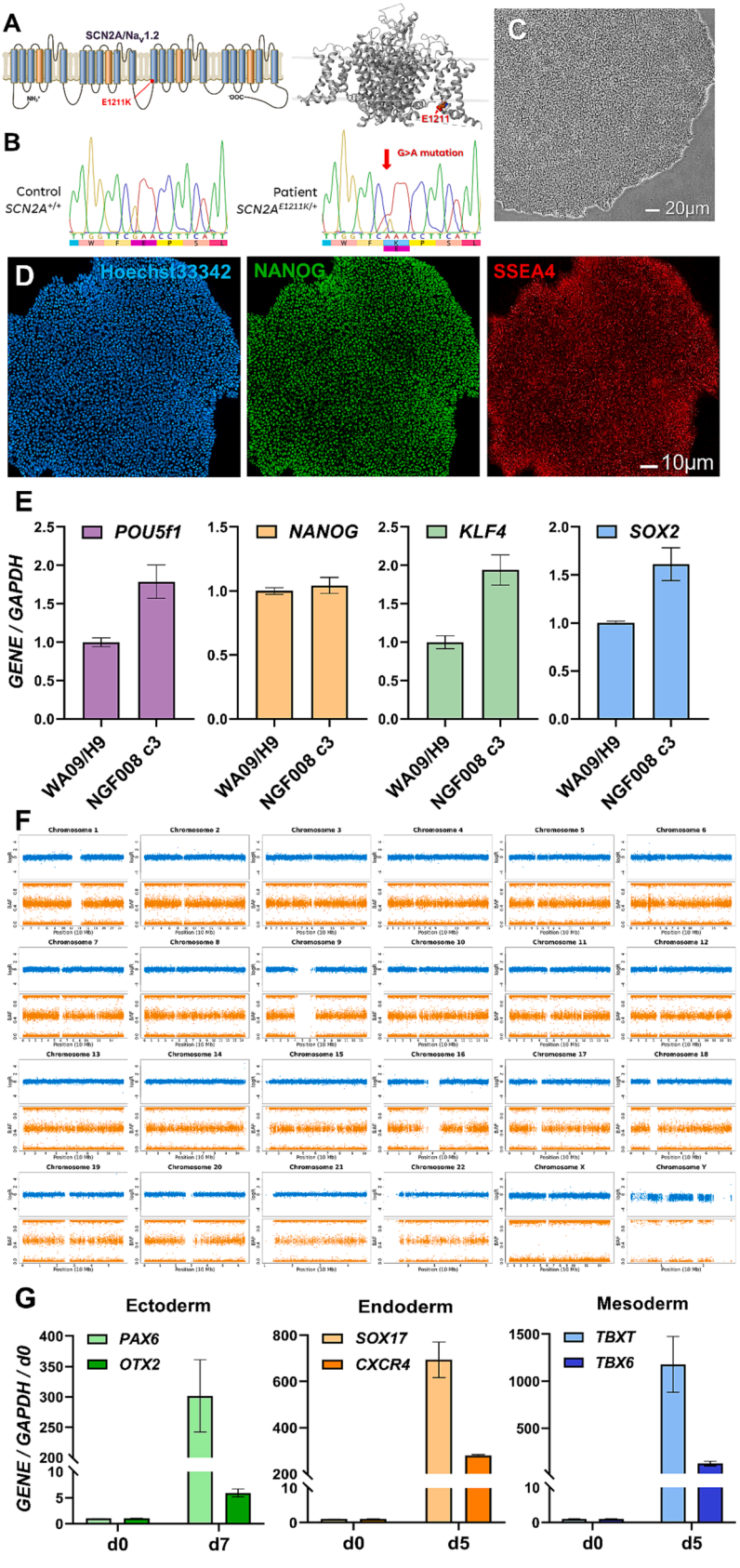

A
SCN2A/Na$_v$1.2
E1211K
E1211
B
Control
SCN2A+/+
Patient
SCN2AE1211K/+
G>A mutation
C
20μm
D
Hoechst33342
NANOG
SSEA4
10μm
E
GENE / GAPDH
POU5f1
NANOG
KLF4
SOX2
WA09/H9
NGF008 c3
F
Chromosome 1
Chromosome 2
Chromosome 3
Chromosome 4
Chromosome 5
Chromosome 6
Chromosome 7
Chromosome 8
Chromosome 9
Chromosome 10
Chromosome 11
Chromosome 12
Chromosome 13
Chromosome 14
Chromosome 15
Chromosome 16
Chromosome 17
Chromosome 18
Chromosome 19
Chromosome 20
Chromosome 21
Chromosome 22
Chromosome X
Chromosome Y
G
Ectoderm
Endoderm
Mesoderm
GENE / GAPDH / d0
PAX6
OTX2
SOX17
CXCR4
TBXT
TBX6
d0
d7
d5

Fig. 1.

Table 2
Reagents details.

	Antibodies used for immunocytochemistry/flow-cytometry			
	Antibody	**Dilution**	**Company Cat #**	**RRID**
Pluripotency markers	Mouse mAb *anti-SSEA4*	1:200	Cell Signaling Technology Cat# 4903	RRID: AB_10559205
	Rabbit mAb *anti-NANOG*	1:200	Cell Signaling Technology Cat# 4755	RRID: AB_1264259
Secondary antibodies	Alexa Fluor 594 goat anti-mouse IgG (H + L)	1:1000	Invitrogen Cat# A11032	RRID: AB_141637
	Alexa Fluor 488 goat anti-rabbit IgG (H + L)	1:1000	Invitrogen Cat# A11008	RRID: AB_143165
Primers				
	Target	**Amplicon Length**	**Forward/Reverse primer (5′-3′)**	
Pluripotency Markers (qPCR)	*NANOG*, Hs02387400_g1 (FAM) Cat# 4331182*	109	**All predesigned Taqman assays are proprietary ThermoFisherScientific assays, hence we are unable to provide primers sequence.*	
	KLF4, Hs00358836_m1 (FAM) Cat# 4331182*	110		
	SOX2, Hs01053049_s1 (FAM) Cat# 4331182*	91		
	POU5f1, Hs00999632_g1 (FAM) Cat# 4331182*	77		
House-Keeping Gene (qPCR)	*GAPDH*, Hs02786624_g1 (VIC) Cat# 4331182*	157		
Trilineage differentiation markers (qPCR)	*PAX6*, Hs00240871_m1 (FAM) Cat# 4331182*	76		
	OTX2, Hs00222238_m1 (FAM) Cat# 4331182*	72		
	SOX17, Hs00751752_s1 (FAM) Cat# 4331182*	149		
	CXCR4, Hs00976734_m1 (FAM) Cat# 4331182*	79		
	TBX6, Hs00365539_m1 (FAM) Cat# 4331182*	75		
	BRACHYURY/TBXT, Hs00610080_m1 (FAM) Cat# 4331182*	132		
Targeted mutation analysis/ sequencing	F CAAAGGGAAACTCTGGTGGAR GTGAGTGAGTGGAGTTCGC	519 bps		

carried out according to the manufacturer's instructions). Expression levels of targeted genes in triplicates were normalized for each replicate to the housekeeping gene (*GAPDH*). The lineage marker gene expression levels of differentiated iPSCs were compared to undifferentiated iPSCs and expressed as a fold increase in mRNA levels compared to day 0 equivalent iPSC cultures.

3.6. Confirmation of the pathogenic variant status

To confirm the status of the *SCN2A* pathogenic variant in generated iPSCs, amplification from genomic DNA and RNA has been performed using specific primer sets designed using Primer-BLAST (NLM NCBI) to generate 300—700 bps target gene-specific products, with the mutation site at least 70 bps away from its end. The specificity of genomic DNA amplification was also validated using the UCSC Genome Browser In-silico PCR tool. PCR using primers located within the last exon 27 of the *SCN2A* gene using 1x GoTaq® Green Master Mix (Promega, M712) as per the manufacturer's instructions, PCR fragment separated on 1 % TAE agarose gel, extracted and Sanger sequencing with one of the primers, performed by AGRF (Melbourne).

Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography Bright field	Visual record of the line: normal	Fig. 1 *Panel C*
Phenotype	Qualitative analysis (Immunohistochemistry)	Assess expression of pluripotency markers: NANOG, OCT4	Fig. 1 *Panel D* NANOG, SSEA4
	Quantitative analysis (RT-qPCR)	*Assess the pluripotency genes mRNA expression NANOG, OCT4, KLF4, SOX2*	Fig. 1 *Panel E*
Genotype	Karyotype (G-banding) and resolution	*E.g. 46XX, Resolution 450–500*	Fig. 1 *panel F*
Identity	Microsatellite PCR (mPCR) OR	*DNA Profiling e.g. Performed/not performed*	*e.g.* supplementary file *2*
	STR analysis	STR (CellID) using 10 markers	Supplemental STR PDF
Mutation analysis (IF APPLICABLE)	Sequencing	Sanger sequencing of genomic DNA PCR	*e.g.* Fig. 1 *panel D* Fig. 1 panels B
	Southern Blot OR WGS	*e.g. number of insertions in the genome, off-target effects*	*e.g. sequencing deposited at DATABASE: recordID*
Microbiology and virology	Mycoplasma	Mycoplasma testing by luminescence. Negative	*e.g.* Fig S1/supplementary
Differentiation potential	*e.g.* Embryoid body formation OR Teratoma formation OR Scorecard OR Directed differentiation	Three germ layers formation/ marker expression, Directed differentiation	*e.g* Fig. 1 *panel D and E* Fig. 1 Panel G
List of recommended germ layer markers	Expression of these markers has to be demonstrated at mRNA (RT PCR) or protein (IF) levels, at least 2 markers need to be shown per germ layer	Ectoderm: *PAX6, OTX2* Endoderm: *SOX17, CXCR4* Mesoderm: *BRACHYURY/TBXT, TBX6*	qRT-PCR for lineage-specific and reference genes results shown in Fig. 1 panel G (see Methods)
Donor screening (OPTIONAL)	*HIV 1 + 2 Hepatitis B, Hepatitis C*	*Negative/Positive*	*e.g. not shown but available with author*
Genotype additional info (OPTIONAL)	*Blood group genotyping*	*DNA analysis e.g. AA*	*e.g. not shown but available with author*
	HLA tissue typing	*HLA typed Class I and Class II*	*e.g. not shown but available with author*

3.7. Verification of the iPSC line origin using the short tandem repeat PCR fingerprinting

To confirm that the iPSC line was derived from the fibroblast population from this patient in the EpiliPSC bank (Florey Institute, University of Melbourne), genomic DNA extracted from primary fibroblasts and described in this manuscript iPSC clonal line at passage 14 (1 μg/sample) was submitted to the Australian Genome Research Facility (Melbourne) for a short tandem repeat (STR) "fingerprinting" analysis by a PCR using 10 sets of primers for short tandem repeat-carrying polymorphic loci spread across the human genome (GenePrint-10, Table 1). This human cell line identification system provides an informative genetic profile ("fingerprint") with a random coincidental match probability of less than 1 in 2.92×10^9 (https://www.agrf.org.au/genetic-id). An identical STR fingerprint profile was confirmed for all 10 markers from the genomic DNA from the primary fibroblast culture and the iPSC clone (see STR data file/table).

CRediT authorship contribution statement

Dmitry A. Ovchinnikov: Conceptualization, Funding acquisition, Data curation, Writing – original draft, Supervision. **Sharon Jong:** Investigation, Writing – review & editing. **Claire Cuddy:** Investigation, Validation. **Kelly Dalby:** Resources. **Orrin Devinsky:** Resources. **Saul Mullen:** Resources, Project administration, Software. **Snezana Maljevic:** Conceptualization, Funding acquisition, Resources. **Steve Petrou:** Conceptualization, Funding acquisition, Resources, Supervision.

Declaration of competing interest

Steven Petrou is an equity holder of RogCon, Inc. and Praxis Precision Medicine, Inc., Cambridge, MA, USA. Orrin Devinsky has equity and/or compensation from the following companies: Hitch Biosciences, Tevard Biosciences, Regel Biosciences, Script Biosciences, Actio Biosciences, Empatica, SilverSpike, Ajna Biosciences, and California Cannabis Enterprises (CCE). He has received consulting fees or equity options from Emotiv, Zogenix, Ultragenyx, and GeneMedicine. He holds patents for the use of cannabidiol in treating neurological disorders but these are owned by GW Pharmaceuticals and he has waived any financial interests. He holds other patents in molecular biology. He is the managing partner of the PhiFund Ventures. Kelley Dalby is an equity holder of RogCon, Inc. and Praxis Precision Medicines, Inc., Cambridge, MA, USA. Dmitry A. Ovchinnikov is a Lab Resource editor at the Stem Cell Research (Elsevier, B.V.). Other authors declare no relevant interests/relationships.

Acknowledgements

We are grateful to the patient and family for participating in our research. We thank the Australian Genomics Research Facility (Melbourne) for assistance with Sanger sequencing and high-resolution karyotyping using SNP array (hybridisation and data analysis). We also thank StemCore (UQ AIBN, Brisbane) for assistance in the generation of this iPSC line. This study was supported by RogCon, Inc., a National Health and Medical Research Council (NHMRC) program grant (10915693) to S.P., Medical Research Future Fund (MRFF) Accelerated Research Stem Cell grant to D.O., S.P. and S.M., and MRFF Genomic Health Futures Mission Project Grant to S.M. and S.P.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scr.2024.103367.

References

Berecki, G., Howell, K.B., Heighway, J., Olivier, N., Rodda, J., et al., 2022. Functional correlates of clinical phenotype and severity in recurrent SCN2A variants. Commun Biol 5, 515.

Howell, K.B., McMahon, J.M., Carvill, G.L., Tambunan, D., Mackay, M.T., et al., 2015. SCN2A encephalopathy: a major cause of epilepsy of infancy with migrating focal seizures. Neurology 85, 958–966.

Mason ER, Wu F, Patel RR, Xiao Y, Cannon SC, Cummins TR. 2019. Resurgent and Gating Pore Currents Induced by De Novo SCN2A Epilepsy Mutations. *eNeuro* 6.

Ogiwara, I., Ito, K., Sawaishi, Y., Osaka, H., Mazaki, E., et al., 2009. De novo mutations of voltage-gated sodium channel alphaII gene SCN2A in intractable epilepsies. Neurology 73, 1046–1053.

Ovchinnikov, D.A., 2021. Reprogramming fibroblasts and mononuclear blood cells into iPSCs using Sendai virus. Protocols.io. https://doi.org/10.17504/protocols.io.bypqpvmw.

Ovchinnikov, D.A., Jong, S., Cuddy, C., Scheffer, I.E., Maljevic, S., Petrou, S., 2023. Generation of an iPSC line (FINi001-a) from a girl with developmental and epileptic encephalopathy due to a heterozygous gain-of-function p. R1882Q variant in the voltage-gated sodium channel Na(v)1.2 protein encoded by the SCN2A gene. Stem Cell Res 71, 103179.

Pavan, C., Jin, J., Jong, S., Strbenac, D., Davis, R.L., Sue, C.M., Johnston, J., Lynch, T., Halliday, G., Kirik, D., Parish, C.L., Thompson, L.H., Ovchinnikov, D.A., 2023. Generation of the iPSC line FINi002-a from a male parkinson's disease patient carrying compound heterozygous mutations in the PRKN gene. Stem Cell Res 73, 103211.

Shi, X., Yasumoto, S., Kurahashi, H., Nakagawa, E., Fukasawa, T., et al., 2012. Clinical spectrum of SCN2A mutations. Brain Dev 34, 541–545.

Zeng, Q., Yang, Y., Duan, J., Niu, X., Chen, Y., et al., 2022. SCN2A-related epilepsy: the phenotypic Spectrum. Treatment and Prognosis. *Front Mol Neurosci* 15, 809951.