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Generation of induced pluripotent stem cell lines from peripheral blood mononuclear cells of three drug resistant and three drug responsive epilepsy patients

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A B S T R A C T

Epilepsy is a common neurological disorder characterized by seizures. Unfortunately, 30–40% of all epilepsy patients are resistant to at least two or more anti-seizure medications. Attempts to treat these patients and prevent further seizures necessitates multiple drug trials for the patient. Here we describe the generation and validation of induced pluripotent stem cell (iPSC) lines from peripheral blood mononuclear cells (PBMCs) from 3 drug responsive and 3 drug resistant patients, using a non-integrative Sendai virus vector. These lines can be used to generate 2D and 3D patient-specific human cellular models that will enable personalised drug screening and pharmacogenomic studies.

Resource Table

Unique stem cell lines identifier	*AIBNi001-A* *AIBNi002-A* *AIBNi003-A* *AIBNi004-A* *AIBNi005-A* *AIBNi006-A*
Alternative name(s) of stem cell lines	*AIBNi001-A; GENIE 1* *AIBNi002-A; GENIE 38* *AIBNi003-A; GENIE 68* *AIBNi004-A; GENIE 69* *AIBNi005-A; GENIE 70* *AIBNi006-A; GENIE 74*
Institution	*Australian Institute for Bioengineering and Nanotechnology*
Contact information of distributor	*Professor Ernst J Wolvetang: e. wolvetang@uq.edu.au*
Type of cell lines	*Induced pluripotent stem cells (iPSCs)*
Origin	*Human*
Additional origin info required	***AIBNi001-A*** *Age: 35* *Sex: Female* *Ethnicity if known: N/A* ***AIBNi002-A*** *Age: 48* *Sex: Female* *Ethnicity if known: N/A* ***AIBNi003-A*** *Age: 19* *Sex: Female* *Ethnicity if known: N/A* ***AIBNi004-A*** *Age: 27* *Sex: Female* *Ethnicity if known: N/A* ***AIBNi005-A*** *Age: 31* *Sex: Female* *Ethnicity if known: N/A* ***AIBNi006-A*** *Age: 24* *Sex: Female* *Ethnicity if known: N/A*
Cell Source	*Peripheral blood mononuclear cells*
Clonality	*Clonal*
Method of reprogramming	*Non-integrative Sendai virus delivery of OCT4, SOX2, KLF4 and c-MYC transgenes*
Genetic Modification	*No*
Type of Genetic Modification	*N/A*
Evidence of the reprogramming transgene loss (including genomic copy if applicable)	*PCR*
Associated disease	*Epilepsy*

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(*continued*)

Gene/locus	*N/A*
Date archived/stock date	*20.10.2020*
Cell line repository/bank	*https://hpscreg.eu/cell-line/AIBNi001-A* *https://hpscreg.eu/cell-line/AIBNi002-A* *https://hpscreg.eu/cell-line/AIBNi003-A* *https://hpscreg.eu/cell-line/AIBNi004-A* *https://hpscreg.eu/cell-line/AIBNi005-A* *https://hpscreg.eu/cell-line/AIBNi006-A*
Ethical approval	*Royal Brisbane and Women's Hospital Human Research Ethics Committee HREC/2019/QRBW/54086: Integrating epilepsy genomics into clinical care* *The University of Queensland Human Ethics Research Office 2019002273: Integrating epilepsy genomics into clinical care.*

1. Resource utility

Human induced pluripotent stem cell (hiPSC) lines were generated from 6 epilepsy patients for use as *in vitro* models of drug resistant and drug responsive epilepsy. Recapitulating clinical drug response profiles in these models provides a valuable resource for future drug screening and pharmacogenomic studies.

2. Resource details

Epilepsy is a common and debilitating neurological disorder, affecting around 70 million people globally (Tang et al., 2017). Drug resistant epilepsy occurs in 30–40% of all epilepsy patients and is characterised by a failure to become seizure free after trials of at least 2 or more anti-seizure medications (ASMs) (Kwan et al., 2010). To determine if *in vitro* patient-derived models of epilepsy will demonstrate drug resistant epilepsy profiles, we generated hiPSC lines from 3 drug responsive and 3 drug resistant epilepsy patients, which can be differentiated into 2D neural cultures and 3D organoids. We have since used these *in vitro* models for preliminary investigation into whether responses to particular ASMs in patient-derived models recapitulate clinical responses, with potential utility in future clinical treatment of these patients (Table 1).

Blood samples were collected from 3 female drug resistant patients who experienced ongoing seizures despite multiple anti-seizure medications, and 3 female drug responsive patients who were treated with a single anti-seizure medication and had been seizure-free for more than one year. Peripheral blood mononuclear cells (PBMCs) were isolated from the whole blood samples of each patient, expanded in culture, and transduced with a non-integrative Sendai virus (SeV) containing the reprogramming transgenes, OCT4, SOX2, KLF4 and c-MYC. By day 21, cell colonies with the morphology of pluripotent stem cells with prominent nucleoli emerged in each culture. Ten colonies were isolated from each culture, expanded separately, and assessed for the presence of transgene expression by PCR after 5–10 passages (Supplementary Fig. 1C). When the hiPSC colonies of each patient were free of transgene expression, they were subjected to karyotype analysis, revealing a normal female karyotype (46XX), with no G-banding abnormalities (Supplementary Fig. 1A). Short tandem repeat analysis (STR) confirmed that the hiPSC profiles matched those of the donor PBMC. Quantification of immunocytochemical staining of each of the 6 lines, using antibodies for the pluripotency factors OCT4, SOX2, NANOG, TRA-1-60 and TRA-1-81, revealed robust expression in >95% of cells in all cell lines (Fig. 1B). Furthermore, quantitative PCR analysis of cDNA isolated from trilineage differentiations from each cell line revealed the presence of markers for the three germ layers; ectoderm (NR2F2, PAX6), endoderm (SOX17, FOXA2), and mesoderm (RUNX1, HAND1), (Fig. 1C). Table 2 provides additional information about the characterisation and validation of the iPSC lines. Collectively, this data demonstrates the successful generation of pluripotent hiPSC lines from 3 drug resistant and 3 drug responsive epilepsy patients.

3. Materials and methods

3.1. Isolating PBMCs from patient blood samples

Ethical approval was obtained and informed consent was provided to

Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	Light microscopy	Compact flat colonies with a well-defined smooth edge, containing cells with a high nucleus to cytoplasm ratio and prominent nucleoli	Fig. 1, panel A
Phenotype	Qualitative analysis: Immunocytochemistry staining	Positive staining of pluripotency markers: OCT4, SOX2, NANOG, TRA-1-60, TRA-1-81	Fig. 1, panel B and Supplementary Fig. 1, panel B
	Quantitative analysis: Immunocytochemistry counting	Robust endogenous expression of OCT4, NANOG, SOX2, TRA 1-60 and TRA 1-81 in >95% of cells	Fig. 1 panel B
Genotype	Karyotype (G-banding) and resolution	GENIE 1: 46 XX (300bphs) GENIE 38: 46XX (300bphs) GENIE 68: 46XX (400bphs) GENIE 69: 46XX (400bphs) GENIE 70: 46XX (400bphs) GENIE 74: 46XX (300bphs)	Supplementary Fig. 1, panel A
Identity	STR analysis	10 loci tested – matched	Not shown but available with author
Mutation analysis (IF APPLICABLE)	Sequencing	N/A	N/A
	Southern Blot OR WGS	N/A	N/A
Microbiology and virology	Mycoplasma	Mycoplasma testing by Myco Alert Assay: Negative	Not shown but available with author
Differentiation potential	Embryoid body formation	Expression of ectoderm markers (NR2F2, PAX6), endoderm markers (SOX17, FOXA2) and mesoderm markers (RUNX1, HAND1).	Fig. 1, panel C
List of recommended germ layer markers	Germ layer expression validated by qPCR	*Ectoderm:* PAX6, NR2F2 *Endoderm:* SOX17, FOXA2 *Mesoderm:* HAND1, RUNX1	Fig. 1, panel C
Donor screening (OPTIONAL)	HIV 1 + 2 Hepatitis B, Hepatitis C	Negative	Not shown but available with author
Genotype additional info (OPTIONAL)	Blood group genotyping	N/A	N/A
	HLA tissue typing	N/A	N/A

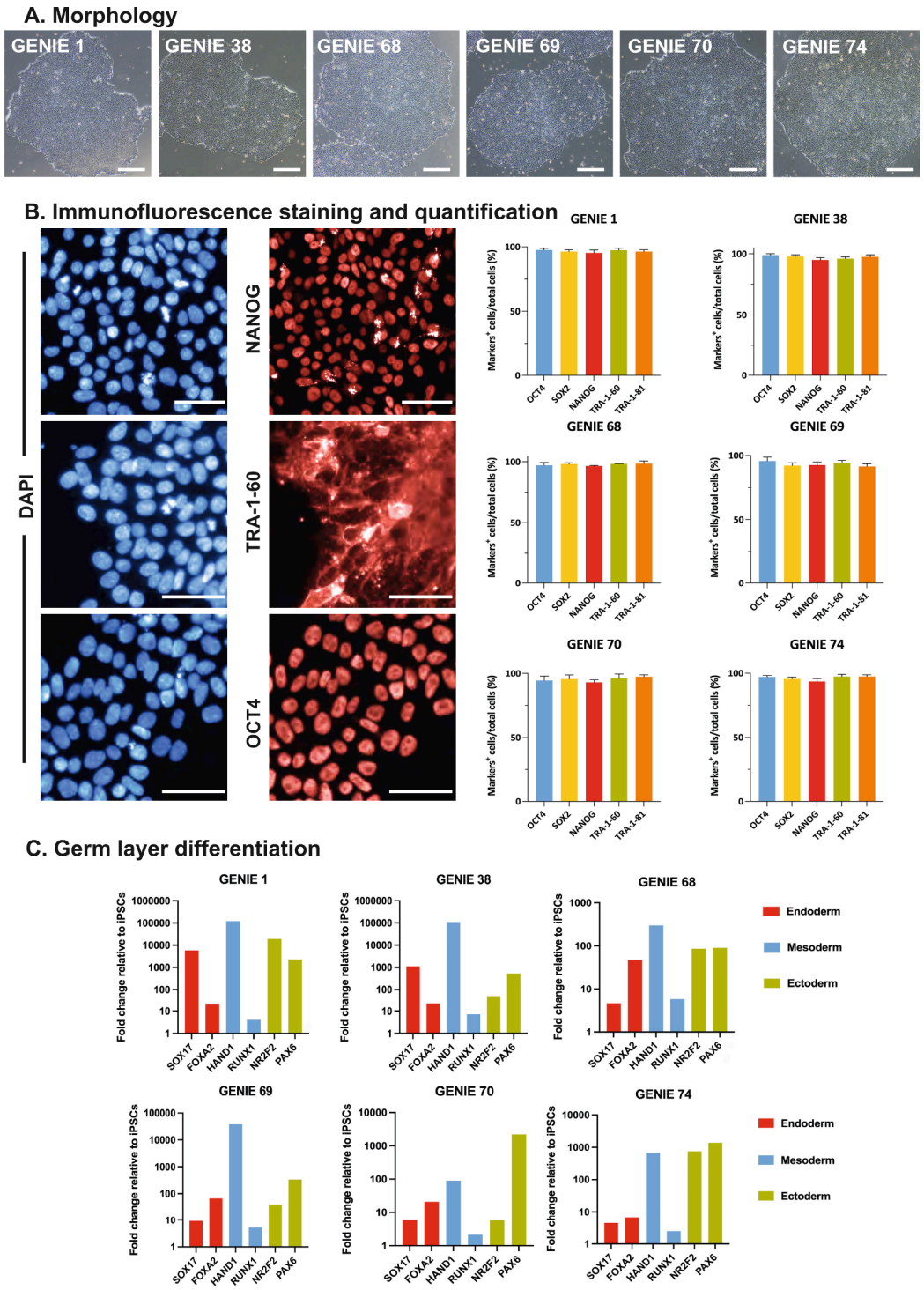

Fig. 1. Characterisation of epilepsy patient-derived iPSCs. (A) Brightfield image of representative iPSC morphology. Scale bar 200 μm. (B) Immunochemistry demonstrated iPSCs were positive for OCT4, NANOG and TRA 1-60, with nuclei counterstained with DAPI. Scale bar 50 μm. Quantification of immunochemistry are shown beside. (C) qPCR analysis following directed differentiations demonstrated the potential for all three germ layers

collect blood samples for the purpose of isolating PBMCs and hiPSC generation. PBMCs were isolated from each whole blood sample using the Leucosep (Greiner) separation technique with centrifugation.

3.2. Reprogramming the PBMCs to iPSCs

Patient PBMCs were expanded in StemSpan SFEM II medium supplemented with the Erythroid Expansion Supplement (Stemcell Technologies) for 7 days. The expanded cells were next transduced with a non-integrating Sendai virus carrying the 4 transgenes, OCT4, SOX2, KLF4 and c-MYC, and reprogrammed using the CytoTune-iPS 2.0 Sendai Reprogramming Kit (Thermo Fisher), according to the manufacturer's instructions (5:5:3 Klf: KOS: c-Myc transgene ratios). Cells were maintained daily in ReproTeSR until colony formation. Colonies were manually picked and medium was transitioned to mTeSR Plus (Stemcell Technologies). Subsequent passages were performed using 0.5 mM EDTA at a split ratio of 1:5–1:8 approximately every 5 days. Clumps were plated without ROCK inhibitor on hESC qualified Matrigel

Table 2
Reagents details.

	Antibodies used for immunocytochemistry/flow-cytometry			
	Antibody	Dilution	Company Cat #	RRID
Pluripotency Markers	Mouse Anti OCT4 IgG	1:100	Millipore Cat# MAB4419	RRID:AB_1977399
	Rabbit Anti SOX2 IgG	1:400	Cell Signaling Technology Cat# 23064	RRID:AB_2714146
	Mouse Anti NANOG IgG	1:2000	Cell Signaling Technology Cat# 4893	RRID:AB_10548762
	Mouse Anti Tra-1-60 IgG	1:200	Millipore Cat# MAB4360	RRID:AB_2119183
	Mouse Anti Tra-1-81 IgG	1:100	Millipore Cat# MAB4381	RRID:AB_177638
Secondary Antibodies	Goat Anti Mouse IgG H + L Alexa Fluor 647	1:500	Thermo Fisher Scientific Cat# A-21235	RRID:AB_2535804
	Goat Anti Mouse IgG H + L Alexa Fluor 488	1:500	Thermo Fisher Scientific Cat# A-11029	RRID:AB_2534088
	Goat Anti Rabbit IgG H + L Alexa Fluor 647	1:500	Thermo Fisher Scientific Cat# A-21245	RRID:AB_2535813
	Donkey Anti Mouse IgG H + L Alexa Fluor 568	1:500	Thermo Fisher Scientific Cat# A-10037	RRID:AB_2534013
	Primers			
	Target	Size of band	Forward/Reverse primer (5′-3′)	
Viral vector (PCR)	Sendai virus genome	181 bp	GGATCACTAGGTGATATCGAGC/ACCAGACAAGAGTTTAAGAGATATGTATC	
Vector transgenes (PCR)	KOS	528 bp	ATGCACCGCTACGACGTGAGCGC/ACCTTGACAATCCTGATGTGG	
	KLF-4	410 bp	TTCCTGCATGCCAGAGGAGCCC/AATGTATCGAAGGTGCTCAA	
	c-MYC	532 bp	TAACTGACTAGCAGGCTTGTCG/TCCACATACAGTCCTGGATGATGATG	
Differentiation primers (qPCR)	RUNX1 (mesoderm)	76 bp	TGAGCTGAGAAATGCTACCGC/ACTTCGACCGACAAACCTGAG	
	HAND1 (mesoderm)	170 bp	CCATGCTCCACGAACCCTTC/CCTGGCGTCAGGACCATAG	
	NR2F2 (ectoderm)	151 bp	TCATGGGTATCGAGAACATTTGC/TTCAACACAAACAGCTCGCTC	
	PAX6 (ectoderm)	320 bp	ACACACTTGAGCCATCACCA/ TTCCACGGGGCTCGAATATG	
	SOX17 (endoderm)	94 bp	GTGGACCGCACGGAATTTG/GGAGATTCACACCGGAGTCA	
	FOXA2 (endoderm)	83 bp	GGAGCAGCTACTATGCAGAGC/CGTGTTCATGCCGTTCATCC	

(Corning) prepared at the specific concentration detailed on the batch sheet. Cells were maintained in mTeSR Plus at 37 °C in a 5% CO_2 incubator.

3.3. Immunofluorescence staining

The hiPSCs (passage 3 onward) were fixed with 4% paraformaldehyde (PFA) for 10 min at 4 °C before blocking and permeablisation for 1 h with 3% Bovine Serum Albumin (BSA) and 0.1% TritonX-100 in PBS at room temperature (RT). Each well was then stained with the primary antibodies listed in Table 2 at the dilution indicated and incubated overnight at 4 °C. Following 3x PBS washes, the secondary antibodies listed in Table 2 were added the next day at the indicated dilution and left for 1hr at RT. Cell nuclei were next stained with 1ug/ml DAPI or Hoechst for 5 min after removing the secondary antibodies. Cells were imaged with the PerkinElmer Operetta CLS High-Content Analysis System and quantified with Harmony High-Content Imaging and Analysis Software.

3.4. Endpoint PCR (PCR)

To confirm loss of transgene expression, RNA was extracted from cell samples (passage 5 onward) using the Nucleospin RNA extraction kit (Macherey-Nagel). Positive controls were harvested 18–21 days after transduction. To synthesise cDNA, the BioRad iScript cDNA Synthesis kit was used according to manufacturer's instructions. OneTaq 2X Master Mix and the GoTaq Green Master Mix were used to carry out the PCRs. The primers used for the PCRs are listed in Table 2.

3.5. Germ layer directed differentiation

hiPSC cells were maintained in lineage specific medium for 5 days. Endoderm differentiations were cultured using the STEMDiff Definitive Endoderm Kit (Stemcell Technologies) for 5 days. Mesoderm and Ectoderm differentiations were maintained in media consisting of DMEM/F12 (Gibco), 0.5X B-27 (Gibco), 1X GlutaMax (Gibco), 0.5X NEAA (Gibco), 50uM B-mercaptoethanol (Sigma Aldrich). Dual SMAD inhibition (10 μM SB431542 and 0.1 μM LDN-193189) was used for the ectoderm differentiations, whilst 3uM CHIR was used for the mesoderm differentiations. Fresh medium was added daily following for 5 days. Differentiations were harvested for RNA and analysed by qPCR.

3.6. Quantitative PCR (qPCR)

RNA was extracted from cell samples using Nucleospin RNA extraction kit (Macherey-Nagel). cDNA was synthesised using BioRad iScript cDNA Synthesis kit. PowerUp SYBR Green Master Mix (Thermo Fisher) was used for the qPCR. The qPCR was carried out with the BioRad CXF96 Real Time System and results quantified using the double delta CT analysis. Cycle conditions were: initial denaturation (95 °C for 2 min), denaturation (95 °C for 15 s), annealing (55–60 °C for 15 s), extension (72 °C for 1 min), for 40 cycles. Primers are listed in Table 2. Each qPCR was performed in triplicate.

3.7. Karyotyping

Patient hiPSC lines (passage 9 onward) were karyotyped by Sullivan Nicolaides Pathology (Bowen Hills, Queensland), at a resolution of between 300 and 400 bands. 15 metaphase spreads were analysed for each line.

3.8. Short tandem repeat (STR) analysis

DNA from patient hiPSCs and the corresponding PBMC lines were extracted using the DNeasy Blood and Tissue Kit (Qiagen). DNA samples were analysed using the GenePrint-10 assay by the Australian Genomic Research Facility (AGRF) in Melbourne.

3.9. Mycoplasma testing

Cell culture medium was routinely collected from each cell line for mycoplasma analysis using the MycoAlert Assay.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scr.2021.102564.

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