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Lab Resource: Multiple Cell Lines

Human induced pluripotent stem cells generated from epilepsy patients for use as *in vitro* models for drug screening

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A B S T R A C T

In this paper, we describe the generation and validation of human induced pluripotent stem cell (hiPSC) lines from peripheral blood mononuclear cells (PBMCs) from 6 epilepsy patients using a non-integrative Sendai virus vector. These human cellular models will enable patient-specific drug screening to improve outcomes for individuals with this disorder.

1. Resource Table:

Unique stem cell lines identifier	*AIBNi007-A* *AIBNi008-A* *AIBNi009-A* *AIBNi0010-A* *AIBNi0011-A* *AIBNi0012-A*
Alternative name(s) of stem cell lines	*AIBNi007-A; GENIE 11* *AIBNi008-A; GENIE 29* *AIBNi009-A; GENIE 49* *AIBNi0010-A; GENIE 75* *AIBNi0011-A; GENIE 81* *AIBNi0012-A; GENIE 116*
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	***AIBNi007-A*** *Age: 6* *Sex: Female* *Ethnicity if known: N/A* ***AIBNi008-A*** *Age: 25* *Sex: Female* *Ethnicity if known: N/A* ***AIBNi009-A*** *Age:29* *Sex: Male* *Ethnicity if known: N/A* ***AIBNi0010-A*** *Age:17* *Sex: Female* *Ethnicity if known: N/A* ***AIBNi0011-A*** *Age: 56* *Sex: Female* *Ethnicity if known: N/A* ***AIBNi0012-A*** *Age: 35* *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*
Gene/locus	*N/A*
Date archived/stock date	*7.12.2020*
Cell line repository/bank	https://hpscreg.eu/cell-line/AIBNi007-A https://hpscreg.eu/cell-line/A

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(*continued*)

	IBNi008-A https://hpscreg.eu/cell-line/A IBNi009-A https://hpscreg.eu/cell-line/A IBNi010-A https://hpscreg.eu/cell-line/A IBNi011-A https://hpscreg.eu/cell-line/A IBNi012-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.*

2. Resource utility

For the purposes of creating patient-specific *in vitro* models, human induced pluripotent stem cell lines (hiPSCs) were generated from 6 epilepsy patients (see Table 1). Preliminary data gathered from brain organoid models derived from our earlier hiPSC work indicates that these lines may prove to be a valuable resource for drug screening and pharmacogenomic studies.

3. Resource details

There are over 65 million people around the world currently living with epilepsy, of which more than one third are resistant to two or more anti-seizure medications (ASMs) (Kwan et al., 2010, Tang et al., 2017). Frustratingly, ASM selection remains a largely trial and error process. To address this, hiPSC lines were generated from 6 patients with this disorder. We intend to differentiate each patient cell line into 2D and 3D neuronal models in order to investigate whether responses to drug regimes *in vitro* translate to known clinical responses. We anticipate these patient-specific models will have clinical relevance and will assist with the selection of medication for patients.

Peripheral blood mononuclear cells (PBMCs) were isolated from blood samples donated by both male and female patients. These PBMCs were then expanded and reprogrammed using a Sendai virus containing the transgenes c-MYC, SOX2, OCT4 and KLF4. Cell colonies with the morphology of iPSCs emerged after 18 days of culturing and were subsequently picked and expanded (Figure 1A). Endpoint PCR confirmed the loss of reprogramming transgene expression after 5–10 passages (Supplementary Figure 1B). Karyotype analysis revealed a normal female and male karyotype (46XX, 46XY) without G-banding abnormalities. Short tandem repeat analysis verified the hiPSC profiles matched those of the original patient donor PBMCs. Robust expression of the pluripotency markers, TRA-1–60, TRA-1–81, NANOG, SOX2 and OCT4 was confirmed by quantification of immunofluorescence staining for all patient cell lines (Figure 1B and Supplementary Figure 1A). Likewise, directed differentiation of each patient hiPSC line into endoderm (FOXA2, SOX17), ectoderm (NR2F2, PAX6) and mesoderm (PDGFRA, HAND1) resulted in the upregulation of all cell lineage specific markers, as indicated by the quantitative PCR analysis shown in Figure 1C. Additional information regarding the characterisation and validation of the hiPSC lines can be seen in Table 2.

4. Materials and methods

4.1. Isolating PBMCs from patient blood samples

PBMCs were isolated from each whole blood sample using Leucosep (Greiner) separation technique with centrifugation.

4.2. Reprogramming the PBMCs to iPSCs

PBMCs were expanded in StemSpan SFEM II medium supplemented with Erythroid Expansion Supplement (Stemcell Technologies) for 7 days. CytoTune-iPS 2.0 Sendai Reprogramming Kit (Thermo Fisher) was used to reprogramme PBMCs, and cells were maintained in ReproTeSR with media replaced daily until colonies formation. Colonies were initially passaged manually and subsequently with 0.5 mM EDTA at a split ratio of 1:20 – 1:50. Colonies were plated on hESC qualified

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	Figure 1, panel A
Phenotype	Qualitative analysis: Immunocytochemistry staining	Positive staining of pluripotency markers: OCT4, SOX2, NANOG, TRA-1–60, TRA-1–81	Figure 1, panel B and supplementary figure 1, panel A
	Quantitative analysis: Immunocytochemistry counting	Robust endogenous expression of OCT4, NANOG, SOX2, TRA 1–60 and TRA 1–81 in > 95% of cells	Figure 1, panel B
Genotype	Karyotype (G-banding) and resolution	GENIE 11: 46 XX (500bphs) GENIE 29: 46XX (500bphs) GENIE 49: 46XY (500bphs) GENIE 75: 46XX (500bphs) GENIE 81: 46XX (500bphs) GENIE 116: 46XX (500bphs)	Not shown but available with author
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 (PDGFRA, HAND1).	Figure 1, panel C
List of recommended germ layer markers	Germ layer expression validated by qPCR	*Ectoderm:* PAX6,NR2F2 *Endoderm:* SOX17, FOXA2 *Mesoderm:* HAND1, PDGFRA	Figure 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

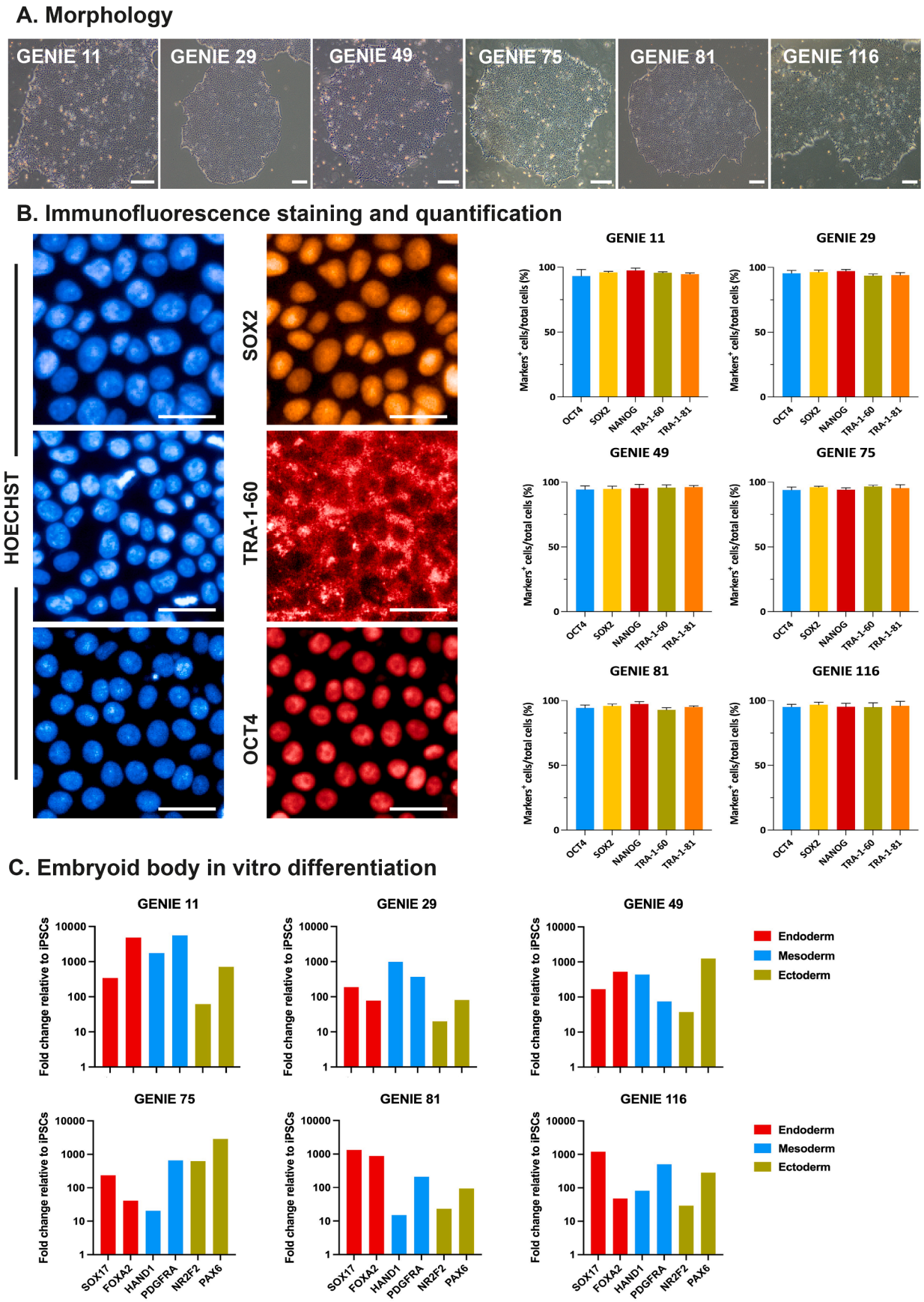

Fig. 1. Characterisation of epilepsy patient-derived iPSCs. (A) Representative image of iPSC morphology using brightfield microscopy. Scale bar 200 μm. (B) Immunochemistry and subsequent quantification of images revealed iPSCs were positive for OCT4, NANOG and TRA 1–60. Nuclei were counterstained with Hoechst. Scale bar 50 μm. (C) qPCR analysis validated the potential for all three germ layers.

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# 23,064	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)	PDGFRA (mesoderm)	92 bp	GTCTTCTCACAGGGCTGAG/ TGAATTCAGCTGCACAACC	
	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	

Matrigel (Corning) at a batch specific concentration. Colonies were maintained in mTeSR Plus (Stemcell Technologies) at 37 °C in a 5% CO_2 incubator.

4.3. Immunofluorescence staining

Immunocytochemistry was performed as previously described (Shaker et al., 2020). Briefly, hiPSCs (passage 7 onward) were fixed with 4% paraformaldehyde for 10 min at 4 °C followed by blocking and permeablisation for 1hr with 3% Bovine Serum Albumin and 0.1% TritonX-100 in 1xPBS. Primary and secondary antibodies and dilutions are recorded in Table 2. Primary antibodies were incubated overnight at 4 °C, followed by 3 × washes with 1xPBS. Secondary antibodies were incubated at room temperature for 1hr with 1ug/mL Hoechst. Cells were imaged with PerkinElmer Operetta CLS High-Content Analysis System and quantified with Harmony High-Content Imaging and Analysis Software.

4.4. Endpoint PCR (PCR)

Nucleospin RNA extraction kit (Macherey-Nagel) was used. Positive controls were harvested the day after transduction. BioRad iScript cDNA Synthesis kit was used for cDNA synthesis as described before (Lee et al., 2020). OneTaq 2X Master Mix and GoTaq Green Master Mix were used to carry out PCRs. Primers are listed in Table 2.

4.5. Germ layer directed differentiation

Endoderm differentiations were directed using STEMDiff Definitive Endoderm Kit (Stemcell Technologies). Ectoderm differentiations were maintained in media consisting of DMEM/F12 (Gibco), 0.5X B-27, 1X GlutaMax, 0.5X NEAAs, 55uM B-mercaptoethanol with dual SMAD inhibitors (10 μM SB431542 and 0.1 μM LDN-193189). For mesoderm differentiations, cells were cultured in the ectoderm differentiation media with 3uM CHIR. Differentiations were performed over 3–5 days.

4.6. Quantitative PCR (qPCR)

RNA was extracted from samples and cDNA synthesised as mentioned above. PowerUp SYBR Green Master Mix (Thermo Fisher) was used for qPCR and carried out with BioRad CXF Maestro Real Time System, in triplicate. Results were quantified using 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.

4.7. Karyotyping

Patient hiPSC lines (passage 15 onward) were karyotyped by Virtus Diagnostics (Brisbane), at a resolution of 500 bands. 10 metaphase spreads were analysed for each line.

4.8. Short tandem repeat (STR) analysis

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

4.9. Mycoplasma testing

Cell culture medium was routinely collected from each cell line for mycoplasma analysis using 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.2022.102673.

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