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Generation and characterization of human iPSC lines from two patients with therapy-resistant epilepsy carrying nonsense heterozygous variants in the *SMC1A* gene

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A B S T R A C T

Different *SMC1A* variants contribute to a spectrum of phenotypes. Missense or small in-frame deletions are associated with Cornelia de Lange syndrome (CdLS) while *SMC1A* truncation variants have been detected in subjects with a clinical phenotype different from CdLS, with moderate-to-severe intellectual disability (ID) and pharmaco-resistant epilepsy. We generated two human iPSC lines from two patients with pharmaco-resistant epilepsy carrying nonsense heterozygous c.901C > T (p.E323*) and c.3103C > T (p.R1035*) variants in the *SMC1A* gene. These cell lines will be a valuable resource for *in vitro* disease modeling and drug testing for pharmaco-resistant epilepsy due to *SMC1A* variants.

1. Resource table

Resource table

Unique stem cell lines identifier	ITBi001-A ITBi002-A https://hpscreg.eu/cell-line/ITBi001-A https://hpscreg.eu/cell-line/ITBi002-A
Alternative name(s) of stem cell lines	Alternative name of ITBi001-A: EP1A Alternative name of ITBi002-A: EP2B
Institution	National Research Council
Contact information of distributor	Dr. Antonio Musio antonio.musio@itb.cnr.it
Type of cell lines	iPSC
Origin	Human
Additional origin info required	Age: 6y (EP1A), 7y (EP2B) Sex: Females Ethnicity: Caucasian
Cell Source	Blood- Peripheral Blood Mononuclear Cells
Clonality	Clonal
Method of reprogramming	Transgene free Sendai virus (Cytotune™ iPS 2.0 reprogramming kit)
Genetic Modification	No
Type of Genetic Modification	N/A
Evidence of the reprogramming transgene loss (including genomic copy if applicable)	RT- PCR
Associated disease	Developmental and Epileptic Encephalopathy-85
Gene/locus	*SMC1A*
Date archived/stock date	N/A
Cell line repository/bank	N/A
Ethical approval	National Research Council Ethical Clearance (199894/2023)

2. Resource utility

These patient-specific iPSC lines offer a valuable resource for in *vitro* exploration of *SMC1A*-pharmaco-resistant epilepsy syndrome, disease pathogenesis and therapeutic interventions such as new drug screening

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and gene therapy.

3. Resource details

The *SMC1A* gene encodes a protein that is part of the cohesin complex, which plays a crucial role in proper chromosome segregation, maintenance of genome stability, and regulation of gene expression. Variants in the *SMC1A* gene result in varied phenotypes. Missense variants or small in-frame deletions in this gene are linked to CdLS in both males and females. CdLS is a rare developmental disorder marked by facial dysmorphia, premature aging, limb malformations, multisystemic alterations, and growth and cognitive delays. In contrast, nonsense and frameshift variants, which lead to premature termination of protein synthesis, are associated exclusively with females, intellectual disability (ID) and therapy-resistant epilepsy (Musio, 2020). In order to model ID and *SMC1A*-pharmaco-resistant epilepsy *in vitro*, we generated two iPSC lines from peripheral blood mononuclear cells (PBMCs) obtained from two patients carrying the heterozygous c.901C > T and c.3103C > T variants in *SMC1A* (p.E323* and p.R1035* respectively). PBMCs, isolated from 10 mL of whole blood, were infected with Sendai virus expressing *OCT4*, *SOX-2*, *KLF4*, and *MYC*. After approximately 3–4 weeks, human embryonic stem cell (hESC)-like colonies emerged and were manually picked and transferred to a new Matrigel-coated culture plate for expansion. A clone for each patient (EP1A and EP2B) was selected for karyotyping analysis and further characterization (see Table 1). All clones exhibited a normal karyotype (Fig. 1B and D), and Sanger sequencing confirmed that it carried the same variants (c.901C > T and c.3103 > T) of *SMC1A* as the PBMCs (Fig. 1E and F) and had a short tandem repeat (STR) profile identical to that of its donor cells (Submitted in archive with journal). These observations indicated that clones were reprogrammed from the selected donor cells. They also exhibited hESC-like morphology (Fig. 1A and C) and immunofluorescent staining revealed that all clones expressed *OCT4*, *NANOG, SOX-2* and *TRA 1–60* (Fig. 1G and H and Supplementary Fig. S1A) and could spontaneously differentiate into embryoid bodies (EBs) which contained cells of all three germ layers, evidenced by the markers SOX-17, SMA, and NESTIN (Fig. 1I–L). By passage 12, Sendai virus was undetectable in all clones using RT-PCR, indicating the absence of residual Sendai virus (Supplementary Fig. S1C–G). Additionally, a mycoplasma detection confirmed that all clones were free of mycoplasma contamination (Supplementary Fig. S1B).

Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography (bright field)	Normal	Fig. 1 panel A and C
Phenotype	Qualitative analysis (Immunocytochemistry)	Positive expression of *OCT4, NANOG, SOX-2, TRA1–60* markers	Fig. 1 panel G and H
	Quantitative analysis (Immunocytochemistry counting)	Percentage (%) of cells positive for each marker: *EP1A (OCT4:* 98 % ± 2 %; *SOX-2:* 99 % ± 2 %; *NANOG:* 95 ± 2 %) *EP2B* (*OCT4:* 94 % ± 2 %; *SOX-2:* 96 % ± 1 %; *NANOG:* 98 ± 2 %)	Suppl. Fig. 1 panel A
Genotype	Karyotype (DAPI-banding) and resolution	Both normal karyotype (46, XX) Resolution: 400–450 bands	Fig. 1 panel B and D
Identity	Microsatellite PCR (mPCR) OR	not performed	
	STR analysis	24 sites tested	Submitted in archive with journal
Mutation analysis	Sequencing	Both heterozygous Both nonsense mutations	Fig. 1 panel E and F
	Southern Blot OR WGS	Not performed	
Microbiology and virology	Mycoplasma	Mycoplasma testing by RT-PCR:negative	Suppl. Fig. 1 panel B
Differentiation potential	Spontaneous in vitro differentiation: Embryoid body (photography – bright field) formation followed by immunostaining	Spontaneous differentiation resulted in cells positive for SOX17, SMA and Nestin.	Fig. 1 panel I and L
List of recommended germ layer markers	Spontaneous in vitro differentiation: Embryoid body formation followed by TaqMan™ hPSC Scorecard™ Panel, Fast 96-well (Cat. N. A15876)	Expression of markers (qPCR) of Endoderm, Mesoderm and Ectoderm germ layer confirmed by Scorecard	Fig. 1 panel M
Donor screening (OPTIONAL)	HIV 1 + 2 Hepatitis B, Hepatitis C	N/A	
Genotype additional info (OPTIONAL)	Blood group genotyping	N/A	
	HLA tissue typing	N/A	

4. Materials and Methods

4.1. Reprogramming

PBMCs were reprogrammed to human iPSCs using our previously established protocol (Castelli et al., 2019; Paulis et al., 2020). PBMCs were isolated from 10 mL of peripheral blood using Ficoll and reprogrammed with the CytoTune®-iPS 2.0 Sendai Reprogramming Kit (ThermoFisher Scientific) following the manufacturer's instructions. Briefly, the PBMCs were transduced with non-integrating SeV vectors at a multiplicity of infection (MOI) of 5: 5: 3 (KOS: C-MYC: KLF4). After 24 h, the transduced cells were fed with fresh medium and after three days they were seeded into a 12-well plate covered with mitomycin C-treated mouse embryonic fibroblast feeder cells. On day 7, the medium was changed to iPS medium (DMEM/F-12 GlutaMAX™, KnockOut™ Serum Replacement 20 %, MEM-NEAA 1X, 2- Mercaptoethanol 55uM, bFGF 4 ng/ml) until day 28 post-transduction, at which time colonies were ready to be picked. Individual colonies were manually picked and cultured in Stem Flex medium (Life Technologies) on recombinant vitronectin (VTN-N) (ThermoFisher Scientific)-coated 6-wells. The cells were passaged at 70–80 % confluency using 0.5 mM EDTA. To promote cell survival and recovery after passaging, 5 μM ROCK kinase inhibitor (Y27632, Med Chem Express) was added to Stem Flex medium for 5 h. After 5 h, the medium was replaced with fresh StemFlex medium without ROCK inhibitor. The cells were incubated at 37 °C, 5 % CO_2, and 20 % O_2. After 12 passages, the absence of SeV vectors was confirmed in both iPSC lines by RT-PCR (Supplementary Fig. S1C–G).

Total RNA was extracted from iPSCs at passage 12 using RNeasy Mini Kit (Qiagen). cDNA was synthetized from 1 μg of total RNA using High-Capacity cDNA Reverse Transcription kit (Applied Biosystems). PCR products were separated by electrophoresis on 2 % (w/v) agarose gel.

4.2. STR analysis

STR analysis was performed by Eurofins Genoma Group Srl using the AmpFlSTR® IdentifilerTM kit (Applied Biosystems). The 24 markers were sequenced on an ABI PRISM 310 Genetic Analyzer and analyzed

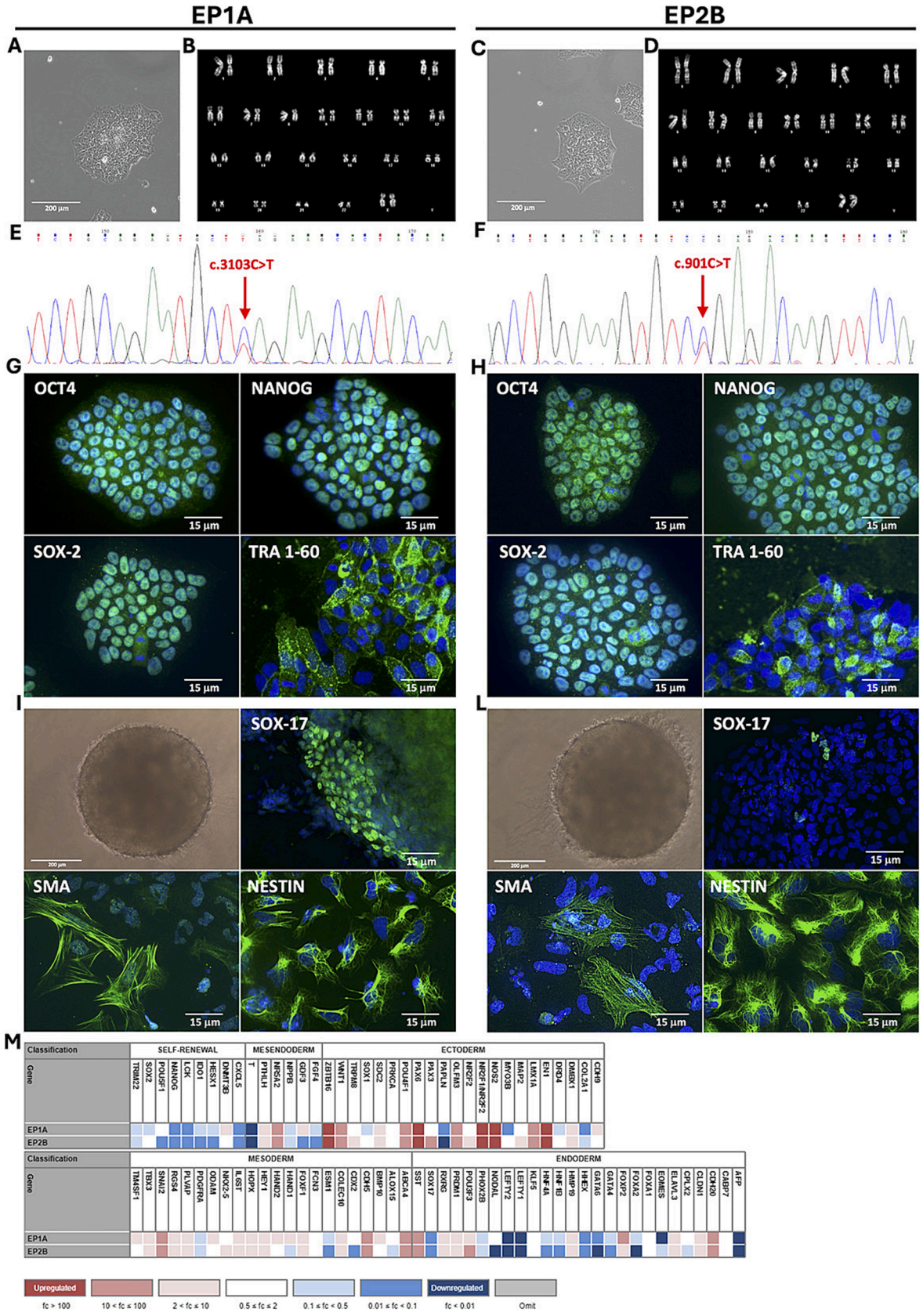

Fig. 1. Characterization of EP1A and EP2B cell lines.

with Genotyper software (Applied Biosystems).

4.3. Karyotyping

iPSCs were treated with 0.1 mg/mL KaryoMAX® Colcemid™ for 2 h at 37 °C. Cells were then subjected to hypotonic treatment with 0.075 M KCl, fixed in methanol-acetic acid (3:1), air-dried and stained with DAPI Vectashield (Vector Laboratories). Chromosome images were captured using an Olympus BX61 microscope with a cooled CCD camera and analyzed with CytoVision software. At least 20 metaphases were examined using DAPI-banding at 400–450 band resolution.

4.4. Immunofluorescence staining

Fixed cells were washed with PBST (PBS + 0.05 % Triton-X) and incubated with primary antibodies for 1 h at room temperature (see Table 2). Cells were subsequently washed and stained with the corresponding secondary antibodies for 1 h at room temperature, washed and then stained by nuclear staining with DAPI Vectashield.

4.5. Immunocytochemistry counting

To quantify the percentage of positive cells for OCT4, SOX-2 and NANOG we used ImageJ software. The percentage of positive cells was determined from 3 different immunofluorescence images for each marker by counting the total number of DAPI-stained nuclei and the number of antibody-stained cells. More than 150 cells were analyzed for each marker.

4.6. EB Formation

iPSCs were treated with 2 mg/mL Dispase (Sigma Aldrich) at 37 °C for 2–3 min, then seeded into a ultra-low attachment plate with Essential 6 medium (Thermo Fisher Scientific) supplemented with 5 μM Y-27632 ROCK inhibitor (Selleckem). After 1 week in suspension, EBs were transferred to vitronectin-coated dishes and cultured in Essential 6 medium for 1 more week. Cells were then collected for the TaqMan hiPSC Scorecard™ assay or fixed with 4 % paraformaldehyde, stained with antibodies against germline markers, and analyzed by immunofluorescence.

4.7. TaqMan hiPSC Scorecard™ assay

Total RNA was extracted from collected EB pellets using RNeasy Mini Kit (Qiagen). cDNA was synthetized from 1 μg of total RNA using High-Capacity cDNA Reverse Transcription kit (Applied Biosystems). qPCR was performed with 384-well TaqMan hPSC Scorecard Panel plates and run on an Applied Biosystems Viia7 instrument.

4.8. Mycoplasma detection

Cell cultures were tested for *Mycoplasma spp*. using PCR with Mycoplasma rDNA-specific GPO1 and MGSO primers (Pruckler et al., 1995). PCR was performed on genomic DNA, with mock (negative) and positive controls. Products were analyzed on a 1.0 % (w/v) agarose gel. All described analyses have been conducted between passages 15–20, after confirming the clearance of the Sendai reprogramming factors.

CRediT authorship contribution statement

Marianna Paulis: Writing – review & editing, Methodology, Investigation, Formal analysis, Data curation. **Maddalena Di Nardo:** Methodology, Investigation. **Lucia Susani:** Methodology, Investigation. **Angela La Grua:** Methodology, Investigation. **Antonio Musio:** Writing – review & editing, Writing – original draft, Validation, Funding acquisition, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial

Table 2
Reagents details.

	Antibodies used for immunocytochemistry/flow-cytometry			
	Antibody	Dilution	Company Cat #	RRID
Pluripotency Markers	Mouse-Anti-TRA-1-60	1:200	(Abcam, Cat# ab16288)	RRID:AB_778563
	Rabbit-Anti-OCT4	1:200	(Abcam Cat# ab18976)	RRID:AB_444714
	Mouse-Anti-Nanog	1:200	(Abcam, Cat# ab62734)	RRID:AB_956161
	Rabbit-Anti-Sox-2	1:200	(Abcam, Cat# ab97959)	RRID:AB_2341193
Ectoderm Marker	Rabbit-Anti-Nestin	1.200	(Abcam, Cat# ab22035)	RRID:AB_446723
Endoderm Marker	Mouse- anti-SOX17	1:50	(Invitrogen, Cat# MA524885)	RRID:AB_2725396
Mesoderm Marker	Mouse anti-smooth muscle actin	1:100	(Abcam, Cat# ab5694)	RRID:AB_2223021
Secondary antibodies	Goat anti-Mouse IgG (H + L), Alexa Fluor™ Plus 488	1:2000	(Invitrogen, Cat# A32723)	RRID:AB_2633275
	Goat anti-Rabbit IgG (H + L), Alexa Fluor™ Plus 488	1:2000	(Invitrogen, Cat# A32731)	RRID:AB_2633280
	Primers			
	Target	Size of band	Forward/Reverse primer (5′-3′)	
House-Keeping Genes (RT-PCR)	GAPDH	115 bp	F:GGTCTCCTCTGACTTCAACA R: AGCCAAATTCGTTGTCATAC	
Reprogramming vectors (RT-PCR)	SeV	181 bp	F:GGATCACTAGGTGATATCGAGC R: ACCAGACAAGAGTTTAAGAGATATGTATC	
	KOS	528 bp	F: ATGCACCGCTACGACGTGAGCGC R: ACCTTGACAATCCTGATGTGG	
	C-MYC	532 bp	F: TAACTGACTAGCAGGCTTGTCG R: CCACATACAGTCCTGGATGATGATG	
	KLF4	410 bp	F: TTCCTGCATGCCAGAGGAGCCC R: AATGTATCGAAGGTGCTCAA	
Mycoplasma detection	GPO-1	700 bp	F: ACTCCTACGGGAGGCAGCAGTA	
	MGSO		R: GCACCATCTGTCACTCTGTTAACCTC	
Targeted mutation sequencing	SMC1A	299bp	EP1A	
		643bp	F: CCACACTCAGTCAGTCATCT R: TGGCATACCCTTAGCCTCTT	
			EP2B	
			F: CTCCTTTGGGTGAAAAGCCT	
			R: GGATTTGGGATGCTCAACCT	

interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scr.2025.103752.

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