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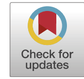

Generation of a human induced pluripotent stem cell (iPSC) line ERPLi004-A from an Alpha-1 antitrypsin deficiency (AATD) patient with SERPINA1 mutation

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ABSTRACT

Alpha-1 antitrypsin deficiency (AATD) is an autosomal disorder that causes liver and lung disease. The risk of developing lung emphysema, chronic obstructive pulmonary disorder and liver cirrhosis is observed in >75 % people affected with a homozygous mutation. Here, we describe the generation of an induced pluripotent stem cell (iPSC) line from peripheral blood mononuclear cells (PBMC) isolated from a AATD patient using non viral and non-integrating episomal vectors. The iPSC line expresses pluripotency markers, generates three germ layers in vitro and retains a normal karyotype (P20) and can provide an ideal tool for disease modelling, drug screening, and personalized medicine.

Resource table

Unique stem cell line identifier	*ERPLi004-A*
Alternative name(s) of stem cell line	*A1AT iPSC C1*
Institution	*Eyestem Research Private Limited, C-CAMP, NCBS-TIFR, Bengaluru, India.*
Contact information of distributor	*Dr. Rajarshi Pal*
Type of cell line	*iPSC*
Origin	*Human*
Additional origin info required for human ESC or iPSC	*Age: 49* *Sex: Male* *Ethnicity if known: Caucasian*
Cell Source	*PBMC*
Clonality	*Clonal*
Method of reprogramming	*Episomal*
Genetic Modification	*No*
Type of Genetic Modification	*Hereditary, Autosomally inherited*
Evidence of the reprogramming transgene loss (including genomic copy if applicable)	*Genomic DNA Semiquantitative PCR for plasmid backbone genes*
Associated disease	*Alpha-1 antitrypsin deficiency (AATD)*
Gene/locus	*NM_001127701.1(SERPINA1): c.1096G>A (p.Glu366Lys - E366K)*
Date archived/stock date	*12 Sep 2024*
Cell line repository/bank	https://hpscreg.eu/cell-line/ERPLi004-A
Ethical approval	*ACE Independent Ethics Committee Protocol number: CEGR 02*

1. Resource utility

The iPSC derived from AATD patient is well characterized, established and would help study the pathophysiology of the disease at molecular level and serve as a prospective drug screening platform. This line with point mutations can potentially be corrected using CRISPR/ CAS9 gene editing technology for autologous cell/ gene therapies.

2. Resource details

The SERPINA1 gene encodes for alpha-1 antitrypsin (AAT) protein, a serine protease inhibitor made in the liver, travels through the blood to protect the lungs and liver from inflammation. It also safeguards the alveolar air sacs from the damage caused by inhaling irritants. During injury/ inflammation, WBCs release neutrophil elastase, a major protease as primary defense mechanism. AAT helps in breakdown of the excess elastase released during injury. When there is inadequate AAT, elastase can target healthy tissues, particularly the liver and the lungs leading to tissue destruction and decline in lung function. AATD is a genetic condition, estimated to be 1/1600–1/5000 in the Western

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Europe and in the USA, mostly diagnosed at a very late stage (Table 1).

Patient PBMCs were isolated from 20 ml of peripheral blood by density gradient centrifugation using Ficoll and expanded using StemSpan media for 12 days. AATD patient iPSCs were generated through electroporation of PBMCs using episomal plasmids encoding human pluripotent factors – OCT4 along with shRNA against p53, SOX2 and KLF4, L-MYC, and LIN28 (Okita et al., 2011, Konala et al., 2022). Transfected cells were plated on 1 % matrigel coated TC plates in StemSpan medium. Cells were gradually shifted to in ReproTesr medium by day 5. Group of 5–8 cells were noticed at day 7 and it formed a colony by day 14 (Fig. 1A). Cultures were gradually shifted to mTeSR plus medium and by day 20 iPSC colonies were selected by enzymatic dissociation using ReleSR and plated as P1 cells with Rock inhibitor (Y27632) to prevent dissociation associated cell death. In next couple of passages iPSC exhibited classic flattened monolayer morphology, with defined boundaries and increased nuclear to cytoplasmic ratio which are hallmark characteristics of pluripotent cells (Fig. 1A). For subsequent culturing, cells were passaged using ReLeSR at 70–80 % confluency in mTeSR plus medium and all assays reported in this study were performed at P10.The cells stained positive for pluripotent markers such as OCT4, TRA-1–60 by immunofluorescence (Fig. 1B) and showed similar levels of OCT4, LIN28 in Taqman qPCR when compared with established iPSC control line (Baghbaderani et al., 2016) (Fig. 1C). Genomic DNA samples tested for transgene footprints with specific primers against episomal vectors showed absence of episomal vectors in the reprogrammed iPSCs (Fig. 1D). Karyotyping by G-banding was found normal (Fig. 1E). Sanger sequencing analysis confirmed the single nucleotide mutation (p.Glu366Lys, E366K) in iPSC sample (Fig. 1F) as reported by the concerned clinician. In vitro tri-lineage differentiation gave rise to all 3 germ lineages – ectoderm, mesoderm, and endoderm. Cells were grown in suspension for 5 days in 15 % Knockout serum media and attached on matrigel coated dishes and cultured for 10 days. Real time PCR using spontaneously differentiated cells showed the expression of ectoderm (Nestin, PAX6), mesoderm (Brachyury, MSX1), and endoderm (FOXA2, GATA4) markers when compared with iPSCs. Immunostaining against PAX6, SMA and FOXA2 antibodies further validated their tri-lineage differentiation capacity (Fig. 1G) (Table 2.).

3. Materials and methods

The line derivation work was approved by an independent Ethics Committee (EC), Institutional Biosafety Committee (IBSC) and Institutional Committee for Stem Cell Research (IC-SCR). All cell culture work was carried out at 37 °C in humidified atmosphere containing 5 % CO_2.

3.1. Isolation of PBMC

Peripheral blood was collected from patients and PBMCs were isolated using Lymphoprep, Ficoll-based density gradient medium (SCT, 07801). Peripheral blood was mixed with equal volume of density gradient medium and centrifuged at 1000g for 15 min at room temperature. PBMCs at the interphase were carefully transferred to a new 50 ml tube and washed thrice with 2 % FBS media, frozen in CryoStor CS10 (SCT, 100-1061) at 5 million cells per ml, and stored in liquid nitrogen till further use.

3.2. Reprogramming PBMC to iPSC, iPSC culturing

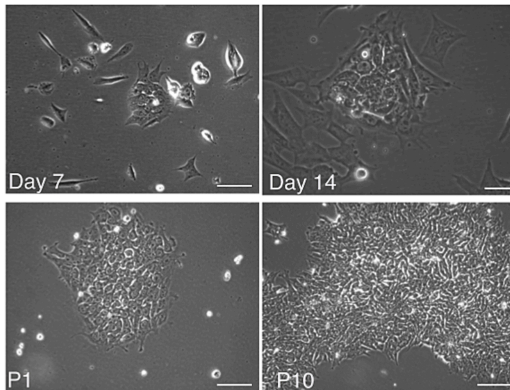
PBMCs were cultured in StemSpan SFEM II medium (SCT, 09605) supplemented with Erythroid Expansion Supplement (SCT, 02692) and transfection was done at day 12. PBMCs were transfected with three episomal plasmids encoding the pluripotency genes – OCT4 along with shRNA against p53, SOX2 and KLF4, L-MYC, and LIN28 (Addgene # 27078, 27080 & 27077) and the electroporation was carried out using the Neon Transfection System (Invitrogen) at 1100 V, 20 ms width, 3 pulses with 0.5–0.8 million cells. Electroporated cells were seeded on 1 % Matrigel (Corning, 354277) coated 35 mm TC dish, maintained in ReproTesr (SCT, 05926) medium for 15–18 days and gradually changed to mTeSR plus medium (SCT, 100–0276) until colony formation was seen. iPSCs were cultured in mTeSR plus medium in plates coated with 1 % Matrigel. Cells were washed with 1X DPBS, added with ReLeSR (SCT, 100-0483) for 1 min, and removed. The plate was incubated at 37 °C for 3–4 min and cells were collected in mTeSR, centrifuged at 200 g for 3 min and used for further experiments. Detailed methodology for iPSC reprogramming is described in Konala et al. (2022).

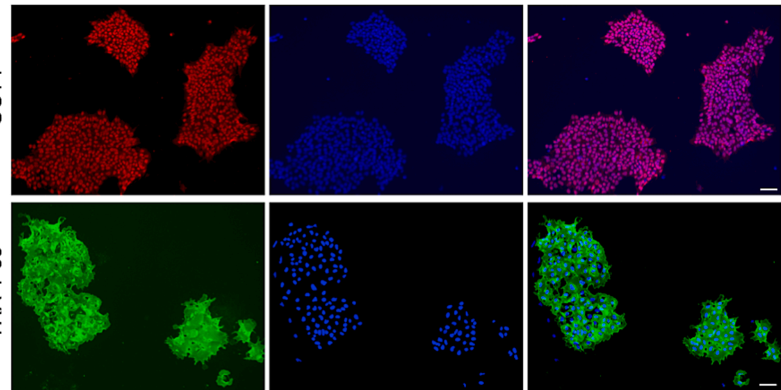
3.3. Immunofluorescence

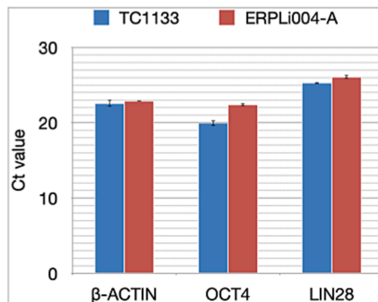
Cultures were fixed with 4 % paraformaldehyde for 10 min, permeabilized in 0.1 % Triton-X-100 for 5 min, and blocked using 4 % fetal bovine serum (FBS) for 45–60 min at room temperature. Primary antibodies were diluted in DPBS and incubated at 4 °C overnight. Secondary

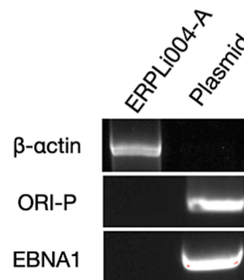
Table 1
Characterization and validation.

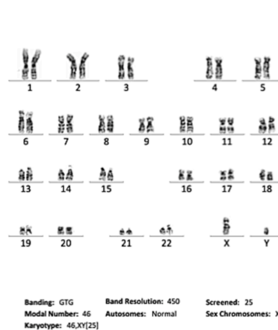
Classification	Test	Result	Data
Morphology	Bright field microscopy	*Visual record of the line: Normal*	Fig. 1 *panel A*
Phenotype	Qualitative analysis *a. Immunocytochemistry*	*Expression of pluripotency markers: OCT4, TRA-1–60 by IF.*	Fig. 1 *panel B*
	Quantitative analysis *a. RT-qPCR*	*Expression of pluripotency markers: OCT4, TRA-1–60,* *expression of pluripotency genes against control iPSC (Baghbaderani et al, 2016).*	Fig. 1 *panel C*
	Qualitative analysis *a. sq-PCR*	*Clearance of vector/ transgene against episomal plasmid as positive control: Ori and EBNA*	Fig. 1 *panel D*
Genotype	Karyotype (G-banding) and resolution	*46XY, Band resolution: 450*	Fig. 1 *panel E*
Identity	Microsatellite PCR (mPCR) OR	*NA*	NA
	STR analysis	*16 Loci tested, 100 % match*	Data available with authors
Mutation analysis (IF APPLICABLE)	Sequencing	*Homozygous*	Fig. 1 *panel F*
	Southern Blot OR WGS	*NA*	*NA*
Microbiology and virology	Mycoplasma	*Mycoplasma testing by RT-PCR*	Supplementary Fig. S1
Differentiation potential	e.g. Embryoid body formation OR Teratoma formation OR Scorecard OR Directed differentiation	*Gene expression in embryoid bodies by Real time PCR – Nestin, Pax6 (ectoderm), MSX1, Brachyury (mesoderm) and GATA4, FOXA2 (endoderm) and Immunofluorescence – Pax6 (ectoderm), SMA (mesoderm) and FoxA2 (endoderm)*	Fig. 1 panel G
Donor screening (OPTIONAL)	HIV 1 + 2 Hepatitis B, Hepatitis C	*Negative*	Data available with authors
Genotype additional info (OPTIONAL)	Blood group genotyping	*NA*	NA
	HLA tissue typing	*NA*	NA

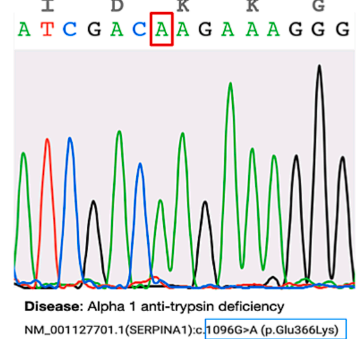












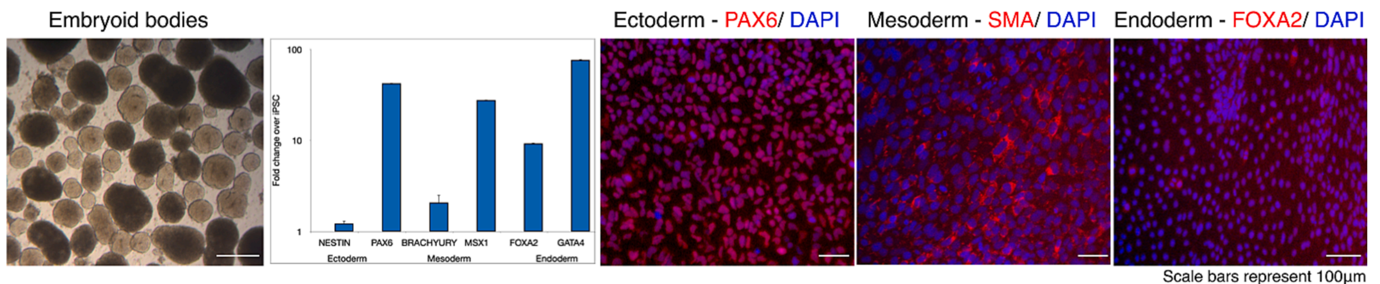


Fig. 1. .

antibodies were incubated for 60 min at room temperature. The nucleus was stained with DAPI for 10 min at room temperature and cells were imaged under EVOS M5000 microscope.

3.4. Semi-quantitative and Real time PCR

For Genomic DNA isolation, cells were lysed in ATL buffer for 20 mins at 56 °C and AL buffer for 15 mins at 70 °C. The lysate was added to the column with equal volumes of ethanol, washed and eluted as per manufacturer's instructions (Qiagen, 51304). 200 ng of the gDNA was used to run the PCR reaction against specific primers for plasmid backbone- ORI-P, EBNA1, Sanger sequencing- SERPINA1. PCR cycle parameters include Denaturation: 94 °C for 30 s, Primer annealing: 60 °C for 45 s, Extension: 72 °C for 45 s for 40 cycles (Applied Biosystems, 2720). PCR products were examined on 1 % agarose gel for transgene absence. Sequencing was performed with SERPINA1 PCR products spanning mutation location using a Sanger Sequencer (ABI-3730).

For RNA isolation, cells were lysed in TRIZOL (Sigma, T9424), added to the column with equal volumes of ethanol, washed and eluted as per manufacturer's instructions (Qiagen, 74104). 1 ug of total RNA was converted to cDNA using the Verso cDNA synthesis kit (ThermoFisher Scientific, AB1453B); For real-time PCR, SYBR green or TaqMan gene expression assay kit was used in triplicates in Quantstudio 3 (Applied Biosystems), normalized to β-actin and relative expressions were calculated by 2^-ddCt method.

3.5. Karyotyping

GTG-banding analysis was performed by Neurberg Anand Diagnostic Laboratory, Bengaluru (https://www.anandlab.com). Metaphase was arrested in the fixed cells using KaryoMAX Colcemid Solution (Gibco, 15212012) overnight at 37 °C and chromoseome spreads were analyzed using routine procedures.

3.6. Short Tandem Repeat (STR) typing

STR analysis was performed at Medgenome, Bengaluru (https://diagnostics.medgenome.com) using PowerPlex® 16 System (Promega, DC6531) following manufacturer's instructions.

Table 2
Reagents details *RRID Requirement for antibodies*: use http://antibodyregistry.org/ to retrieve RRID for antibodies and include ID in table as shown in examples.

	Antibodies used for immunocytochemistry			
	Antibody	**Dilution**	**Company Cat #**	**RRID**
Pluripotency Markers	*Rabbit anti-OCT4*	*1:500*	*Abcam*	*Cat# ab18976, RRID: AB_444714*
	Mouse anti-Tra-1–60	*1:300*	*Abcam*	*Cat# ab16288, RRID: AB_778563*
Differentiation Markers	*Rabbit anti-PAX6*	*1:300*	*Abcam*	*Cat# ab195045, RRID: AB_2750924*
	Mouse anti-Actin, smooth muscle, clone ASM-1	*1:200*	*Merck*	*Cat# CBL171, RRID: AB_2223166*
	Mouse anti-FOXA2	*1:300*	*Abcam*	*Cat# ab60721, RRID: AB_941632*
Secondary antibodies	*Goat Anti-Mouse IgG H&L (Alexa Fluor®594)*	*1:700*	*Abcam*	*Cat# ab150116, RRID: AB_2650601*
	Goat anti rabbit IgG H&L [Alexa Fluor 488]	*1:800*	*Abcam*	*Cat# ab150077, RRID: AB_2630356*
	Donkey Anti-Mouse IgG H&L (Alexa Fluor®488)	*1:800*	*Abcam*	*Cat# ab150105, RRID: AB_2732856*

	Primers used for PCR		
	Target	**Size of band / Amplicon length**	**Forward/Reverse primer (5′-3′)**
Episomal Plasmids (PCR)	*ORI P*	*524 bp*	*TTCCACGAGGGTAGTGAACC / TCGGGGGTGTTAGAGACAAC*
	EBNA1	*665 bp*	*ATCGTCAAAGCTGCACACAG/ CCCAGGAGTCCCAGTAGTCA*
Pluripotency Markers (qPCR – Taqman probe)	*OCT4/ POU5F1P3*	*64 bp*	*Hs03005111_g1*
	LIN28	*130 bp*	*Hs01013729_m1*
House-Keeping Genes (qPCR – Taqman probe)	*β-actin*	*171 bp*	*Hs01013729_m1*
Targeted mutation analysis/ sequencing	SERPINA1	*525 bp*	*GGAAGATGGACAGAGGGGAG/ CAGGCAAAGGGAGACTCAGA*

CRediT authorship contribution statement

Harshini Surendran: Writing – original draft, Visualization, Validation, Methodology, Investigation, Data curation. **Rajani Battu:** Writing – review & editing, Validation, Investigation, Data curation, Conceptualization. **Renjitha Gopurappilly:** Writing – review & editing, Visualization, Resources, Project administration, Methodology. **Chethala N. Vishnuprasad:** Writing – review & editing, Project administration, Formal analysis, Conceptualization. **Rajarshi Pal:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Rajarshi Pal reports was provided by Eyestem Research Pvt Ltd. If there are other authors, they 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.2025.103664.

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