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Lab resource: Stem Cell Line

Generation of an induced pluripotent stem cell line (MHHi018-A) from a patient with Cystic Fibrosis carrying p.Asn1303Lys (N1303K) mutation

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A B S T R A C T

Cystic Fibrosis (CF) is a genetic disease caused by mutations in the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) gene which encodes for a chloride ion channel regulating the balance of salt and water across secretory epithelia. Here we generated an iPSC line from a CF patient homozygous for the p.Asn1303Lys mutation, a Class II folding defect mutation. This iPSC line provides a useful resource for disease modeling and to investigate the pharmacological response to CFTR modulators in iPSC derived epithelia.

Resource table

Unique stem cell line identifier	MHHi018-A
Alternative name(s) of stem cell line	CF19_iPSCK9
Institution	LEBAO, Hannover Medical School, Germany
Contact information of distributor	merkert.sylvia@mh-hannover.de; martin.ulrich@mh-hannover.de
Type of cell line	iPSC
Origin	human
Additional origin info	Age: 25 Sex: male
Cell Source	CD34+ cells from peripheral blood
Clonality	clonal
Method of reprogramming	Sendai Virus, transgene-free
Genetic Modification	no
Type of Modification	n/a
Associated disease	Cystic Fibrosis, p.Asn1303Lys
Gene/locus	CFTR, 7q31.2, c.3909C>*G*
Method of modification	n/a
Name of transgene or resistance	n/a
Inducible/constitutive system	n/a
Date archived/stock date	January 2019
Cell line repository/bank	https://hpscreg.eu/cell-line/MHHi018-A
Ethical approval	The Local Ethics Committee of Erasmus MC Rotterdam approved the study (internal No. NL61623.078.17.V03) and informed consent was obtained from the patient.

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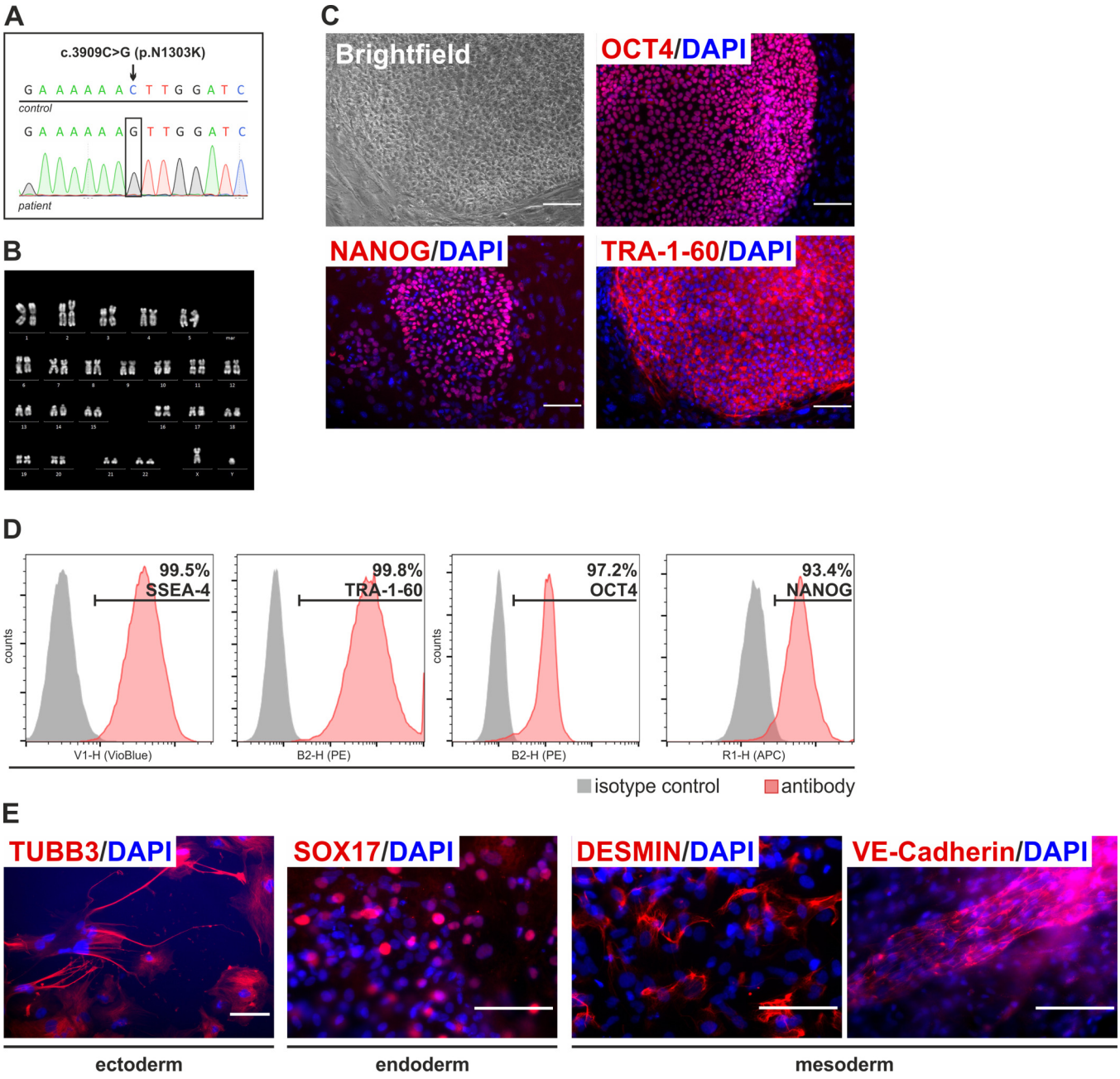

Fig. 1. Characterization of the MHHi018-A iPSC line.

1. Resource utility

Our established CFTR_p.Asn1303Lys iPSC line offers a patient-specific disease model of Cystic Fibrosis and can be used to investigate the pharmacological response to CFTR modulators in iPSC derived epithelia.

2. Resource details

Cystic Fibrosis (CF) is an autosomal recessive genetic disease caused by ~350 pathogenic variants in the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) gene (https://cftr2.org/ database), encoding a cAMP-regulated chloride channel of secretory epithelia, *e.g.,* in the lungs or the pancreatic ducts (Riordan et al., 1989). p.Asn1303Lys (N1303K) belongs to the most frequent disease-causing mutations in the CFTR gene with an allele frequency of 1.58% of all CF chromosomes varying significantly between countries and ethnic groups (based on https://cftr2.org/ database_ January 2020). Similar to the more prominent CFTR p.Phe508del mutation, p.Asn1303Lys is a Class II mutation, which results in trafficking and gating defects of the chloride channel (Gregory et al., 1991).

In this study the MHHi018-A iPSC line was generated from a 25-year-old male CF patient carrying a homozygous p.Asn1303Lys mutation in the CFTR gene. $CD34^+$ hematopoietic stem cells were isolated from peripheral blood donation and reprogrammed with CytoTune™-iPS 2.0 Sendai Reprogramming Kit for the generation of transgene-free iPSCs. Resulting single cell clones were tested in passage 14 after reprogramming by RT-PCR analysis for the absence of Sendai virus (SeV) and exogenous reprogramming factor transcripts (Suppl. Fig. A). Clone 9 (MHHi018-A) was chosen for further characterization. Sanger sequencing confirmed the homozygous presence of the c.3909C>G mutation in exon 21 of the CFTR gene (Fig. 1A) and a normal human karyotype 46,XY was detected by fluorescence R-banding in passage 24 (Fig. 1B). The clone showed typical pluripotent stem cell like morphology (Fig. 1C) and the expression of the

pluripotency markers OCT4, NANOG, TRA-1-60 and SSEA-4 could be confirmed via fluorescence staining and flow cytometry (Fig. 1C and D). The clone was free from mycoplasma contamination (Suppl. Fig B) and STR analysis of MHHi018-A has been performed. Due to the lack of further patient material, the STR analysis of the parental DNA has not been submitted. Spontaneous embryoid body formation was performed to verify the differentiation potential of MHHi018-A. Immunofluorescence staining against endodermal (SOX17), ectodermal (TUBB3) and mesodermal (DESMIN, VE-Cadherin) markers demonstrated the capacity to differentiate into cells of all three germ layers (Fig. 1E). Scale bars in all microscopic pictures represent 100 µm. All results are summarized in Table 1.

3. Materials and methods

3.1. Reprogramming of $CD34^+$ PBHSCs

10 mL peripheral blood was obtained from the CF patient and peripheral blood mononuclear cells (PBMCs) were collected by Biocoll density gradient centrifugation. Subsequently, $CD34^+$ cells were isolated using the CD34 MicroBead Kit from Miltenyi Biotec (Cat#130-046-702) following the manufacturer's instructions.

30.000 $CD34^+$ cells were transduced with the CytoTune™-iPS 2.0 Sendai Reprogramming Kit (Thermo Fisher; KOS MOI = 5, c-Myc MOI = 5, hKlf4 MOI= 3) for 24 h in StemSpan™ SFEM supplemented with 100 ng/mL SCF, 50 ng/mL TPO, 100 ng/mL FLT3 and 8 µg/mL Polybrene. After 4 days the cells were transferred onto mouse embryonic fibroblasts (MEFs) in knockout-DMEM supplemented with 20% knockout serum replacement, 1 mM L-glutamine, 0.1 mM β-mercaptoethanol, 1% nonessential amino acid stock (all from Thermo Fisher) and 10 ng/mL b-FGF (supplied by the Institute for Technical Chemistry, Leibniz University Hannover, Germany). Cell colonies with appropriate iPSC morphology were picked on day 14 of reprogramming and expanded clonally (see below). After 12 passages, the temperature shift to enhance the loss of reprogramming factors was performed for 6 days at 39 °C. Afterwards, iPSCs were split every 5–6 days using Collagenase IV (Thermo Fisher) in a 1:10 split ratio onto fresh feeder cells and were maintained at 37 °C under 5% CO_2.

3.2. RT-PCR and mutation analysis

Total RNA was isolated from TRIzol®-lysed cells via NucleoSpin® RNA II Kit (Machery-Nagel) followed by c-DNA synthesis via RevertAid™ H Minus First Strand cDNA Synthesis Kit (Thermo Fisher) using random primers according to manufacturer's protocol. RT-PCR was performed with GoTaq® (Promega) polymerase using a Mastercycler ProS (Eppendorf) with following cycling conditions: initial denaturation at 95 °C for 5 min; 30 cycles of 95 °C for 30 s, 30 s annealing at 55 °C and 72 °C for 30 s; final extension at 72 °C for 5 min.

For mutation analysis, genomic DNA was isolated with QIAamp blood Mini Kit (Quiagen). 100 ng DNA was amplified with GoTaq® polymerase using a Mastercycler ProS (Eppendorf). Cycling conditions were as follows: initial denaturation at 95 °C for 2 min; 35 cycles of 95 °C for 30 s, annealing at 59 °C for 1 min, and 72 °C for 1 min; final extension at 72 °C for 5 min. The PCR product was purified and sent for Sanger sequencing to Microsynth Company. Primers are listed in Table 2.

3.3. Flow cytometry

Before flow cytometry analysis, cells were cultured as single-cell monolayers in iPS Brew XF (Miltenyi Biotech) on Geltrex®-coated plates at a seeding density of 3.6×10^4 cells/cm^2. For the intracellular markers OCT4 and NANOG, the cells were detached with Accutase® (Thermo Fisher) and fixed with 1% PFA for 15 min at room temperature (RT). The incubation with direct labeled antibodies was performed in solution B/FIX&PERM® at 4 °C for 20 min. Staining of living cells for the surface markers SSEA-4 and TRA-1-60 was performed in 1% BSA in PBS for 20 min at 4 °C. Cells were analyzed with a MACSQuant Analyzer 10 and FlowJo analysis software. Antibodies are listed in Table 2.

3.4. Immunofluorescence staining

Cells were fixed in 4% paraformaldehyde (20 min, RT) and permeabilized with triton blocking solution (20 min, RT). Afterwards, cells were stained with primary antibodies and corresponding isotype controls in 1% BSA in PBS (1 h, RT), followed by washing and incubation with secondary antibodies (30 min, RT) and DAPI nuclear staining (15 min, RT). Images were acquired with an AxioObserver A1 fluorescence microscope and Axiovision software. Antibodies are listed in Table 2.

3.5. In vitro differentiation

hiPSCs were detached using a cell scraper and transferred into ultra-low attachment plates (Corning) in differentiation medium (80% IMDM supplemented with 20% fetal calf serum, 1 mM L-glutamine, 0.1 mM ß-mercaptoethanol and 1% nonessential amino acid stock). After 7 days,

Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography	normal	Fig. 1 panel C
Phenotype	Qualitative analysis (Immunocytochemistry)	Positive for OCT4, NANOG, TRA-1-60	Fig. 1 panel C
	Quantitative analysis (Flow cytometry)	OCT4 (97.2%), NANOG (93.4%), TRA 1-60 (99.8%), SSEA-4 (99.5%)	Fig. 1 panel D
Genotype	Karyotype (R-banding) and resolution	46XY, Resolution min 300 bands	Fig. 1 panel B
Identity	Microsatellite PCR (mPCR) OR STR analysis	not performed STR profile for 16 specific sites tested	available with author
Mutation analysis	Sequencing	Homozygous for c.3909C>G	Fig. 1 panel A
	Southern Blot or WGS	N/A	
Microbiology and virology	Mycoplasma	Negative	Suppl. Fig. panel B
Differentiation potential	Embryoid body formation	Spontaneous FSC-based *in vitro* differentiation; positive for ectoderm (TUBB3), endoderm (SOX17) and mesoderm (DESMIN, VE-Cadherin)	Fig. 1 panel E
Donor screening	HIV 1 + 2 Hepatitis B, Hepatitis C	Negative	not shown but available with author
Genotype additional info	Blood group genotyping	N/A	
	HLA tissue typing	N/A	

Table 2
Reagents details.

Antibodies used for immunocytochemistry/flow-cytometry			
	Antibody	Dilution	Company Cat # and RRID
Pluripotency Markers	mouse anti-OCT4 (IgG2b)	1:100	Santa Cruz Biotechnology Cat# sc-5729, RRID:AB_628051
	mouse anti-NANOG (IgG1)	1:500	Abcam Cat# ab62734, RRID:AB_956161
	mouse anti-TRA-1-60 (IgM)	1:100	Abcam Cat# ab16288, RRID:AB_778563
	RE anti-OCT4_PE	1:25	Miltenyi Biotec Cat# 130-105-554, RRID:AB_2653085
	RE anti-NANOG_APC	1:25	Miltenyi Biotec Cat# 130-105-049, RRID:AB_2652991
	RE anti-TRA-1-60_PE	1:25	Miltenyi Biotec Cat# 130-100-347, RRID:AB_2654227
	RE anti-SSEA4_VioBlue	1:25	Miltenyi Biotec Cat# 130-098-366, RRID:AB_2653521
Differentiation Markers	mouse anti-DESMIN (IgG1)	1:20	Progen Cat# 10519, RRID:AB_1541098
	goat anti-SOX17 (IgG)	1:200	R&D Systems Cat# AF1924, RRIP:AB_355060
	mouse anti-TUBB3 (IgG2a)	1:400	Millipore Cat# 05-559, RRID:AB_309804
	rabbit anti-VE-Cadherin (IgG)	1:500	Abcam Cat# ab33168, RRIP:AB_870662
Secondary antibodies	Cy™3-AffiniPure donkey anti-mouse IgG	1:200	Jackson ImmunoResearch Labs Cat# 715-165-150, RRID:AB_2340813
	Cy™3-AffiniPure donkey anti-goat IgG	1:200	Jackson ImmunoResearch Labs Cat# 705–165-147, RRID:AB_2307351
	Cy™3-AffiniPure donkey anti-rabbit IgG	1:200	Jackson ImmunoResearch Labs Cat# 711-165-152, RRID:AB_2307443
	Cy™3-AffiniPure Donkey anti-Mouse IgM	1:200	Jackson ImmunoResearch Labs Cat# 715-165-020, RRID:AB_2340811

Primers	Target	Forward/Reverse primer (5′ − 3′)
Genotyping	CFTR (620 bp)	1981_gggactccaaatattgctgtagt 1982_cttgtcgcgccaggtatttt
Absence of reprogramming factors	SeV (181 bp)	fwd_GGATCACTAGGTGATATCGAGC
	RT-PCR	rev_ACCAGACAAGAGTTTAAGAGATATGTATC
	KOS (528 bp)	fwd_ATGCACCGCTACGACGTGAGCGC
	RT-PCR	rev_ACCTTGACAATCCTGATGTGG
	Kfl4 (410 bp)	fwd_TTCCTGCATGCCAGAGGAGCCC
	RT-PCR	rev_AATGTATCGAAGGTGCTCAA
	c-Myc (532 bp)	fwd_TTACTGACTAGCAGGCTTGTCG
	RT-PCR	rev_TCCACATACAGTCCTGGATGATG

formed embryoid bodies were plated onto gelatin-coated cell culture plates in differentiation medium for another 14 days before PFA fixation and immunofluorescence staining.

3.6. Karyotyping

Adherent cells were incubated at 37 °C with 75 mM potassium chloride for 20 min and for 30 minutes with colcemide. After trypsination, metaphases were prepared according to standard procedures. Fluorescence R-banding using chromomycin A3 and methyl green was performed and metaphases were captured using a Zeiss Imager.Z2 microscope and Ikaros software (MetaSystems). At least 14 metaphase spreads were analysed at a minimum level of 300 bands.

3.7. STR analysis

Cell pellets were provided to Microsynth and profiling was performed using highly-polymorphic short tandem repeat loci (STRs). STR loci were amplified using the PowerPlex® 16 HS System (Promega). Fragment analysis was done on an ABI3730xl (Life Technologies) and the resulting data were analyzed with GeneMarker HID software (Softgenetics). Tested loci: D3S1358, TH01, D21S11, D18S51, Penta_E, D5S818, D13S317, D7S820, D16S539, CSF1PO, Penta_D, AMEL, vWA, D8S1179, TPOX, FGA

3.8. Mycoplasma test

Exclusion of mycoplasma contamination was confirmed by MycoAlert™ mycoplasma detection kit (Lonza), according to manufactures' instructions. The test exploits the activity of mycoplasmal enzymes by biochemical luminescence.

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.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.scr.2020.101744.

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