Generation of an induced pluripotent stem cell line HHUUKDi013-A (ISRM-AATD-iPSC-3) from a pediatric patient of Alpha-I Antitrypsin Deficiency (AATD)

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Lab Resource: Single Cell Line

Generation of an induced pluripotent stem cell line HHUUKDi013-A (ISRM-AATD-iPSC-3) from a pediatric patient of Alpha-I Antitrypsin Deficiency (AATD)

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A B S T R A C T

Renal progenitor cells were isolated from urine from a male, pediatric AATD-patient, harboring the homologous Pi*ZZ genotype. SIX2-positive urine derived renal progenitor cells (UdRPCs) were reprogrammed to iPSCs by integration-free nucleofection of episomal-based plasmids expressing *OCT4, KLF4*, *c-MYC*, *SOX2*, *NANOG* and *LIN28*. ISRM-AATD-iPSC-3 show pluripotent gene and protein expression, are capable of forming embryoid bodies and carry the parental Pi*ZZ genotype. Global transcriptome analyses revealed a correlation coefficient 0.9091 between the human embryonic stem cell line H9 and ISRM-AATD-iPSC-3.

Resource Table:

Unique stem cell lines identifier	HHUUKDi013-A
Alternative name(s) of stem cell lines	ISRM-AATD-iPSC-3
Institution	Institute for Stem Cell Research and Regenerative Medicine, Medical Faculty, Heinrich-Heine University Düsseldorf
Contact information of distributor	Prof. Dr. James Adjaye James.adjaye@med.uni-duesseldorf.de
Type of cell lines	iPSCs
Origin	Human
Additional origin info required	Age: 11 years Sex: Male Ethnicity: Unknown
Cell Source	Urine derived renal progenitor cells (UdRPCs)
Clonality	Clonal
Method of reprogramming	Episomal expression of *OCT4*, *c-MYC*, *KLF4*, *SOX2*, *NANOG*, *LIN28*
Genetic Modification	Yes
Type of Genetic Modification	Hereditary
Evidence of the reprogramming transgene loss	PCR
Cell culture system	N/A
Unique stem cell lines identifier	HHUUKDi013-A
Associated disease	Alpha-1 Antitrypsin deficiency
Gene/locus	*SERPINA*1rs28929474
Date archived/stock date	06.06.2024
Cell line repository/bank	https://hpscreg.eu/cell-line/HHUUKDi013-A
Ethical approval	Ethical committee of the medical faculty of Heinrich Heine University Düsseldorf, Germany Approval number: 2021-1627_1

1. Resource Utility

Alpha-1 Antitrypsin Deficiency (AATD) is caused by a mutation in the *SERPINA*1rs28929474 locus resulting in misfolded AAT-polymers. AATD liver disease mainly manifests biphasic during early childhood or later adulthood, however the underlying mechanisms remain unclear. Therefore, AATD-iPSCs from pediatric patients may serve as a tool for studying the etiology at the molecular and cellular levels. A summary of the characterization can be found in Table 1.

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2. Resource details

Alpha-1 Antitrypsin Deficiency (AATD) is a hereditary disease caused by a missense mutation of G > A in the *SERPINA*1rs28929474 locus resulting in Glu342Lys switch (called PI*Z genotype) and misfolding of the AAT protein, causing retention in the hepatocytes (Greene et al., 2016). The disease manifests typically by pulmonary symptoms in adults and or liver cirrhosis in pediatric (<4 years) or adult (>50 years) patients (Strnad et al., 2020; Katzer et al., 2021). Inclusions of AAT-polymers within the hepatocytes are the histological hallmark of the Pi*ZZ liver phenotype, and can lead to chronic inflammation and liver fibrosis (Strnad et al., 2013). Furthermore retention of AAT-polymers results in a reduction of serum AAT and decreased inhibitory effect on proteases, which can cause a pulmonary phenotype due to degradation of lung parenchyma (Strnad et al., 2020). Although there is a relatively high prevalence of AATD within Europe the undiagnosed proportion of patients remains high and further research is necessary to elucidate the underlying molecular mechanisms (Horváth et al., 2019). In addition to our previously published AATD-iPSCs from adults (Ncube et al., 2023), we have now generated an AATD-iPSC line from a pediatric patient (male, 11 years) with compensated liver cirrhosis, carrying the homozygous Pi*ZZ genotype. The cells were nucleofected and episomal-based reprogramming was induced by the ectopic expression of *OCT4*, *KLF4*, *SOX2*, *c-MYC*, *NANOG* and *LIN28* as described in our previous publication (Bohndorf et al., 2017). Reprogrammed cells showed the typical iPSC morphology with a high nucleus/cytoplasm ratio in comparison to the rice-grain shaped UdRPCs (Fig. 1A). Vector dilution of AATD-iPSC-3 (p12) was confirmed (Fig. 1B). Chromosomal analysis from 20 metaphase spreads revealed a normal karyotype (46, XY) (Fig. 1C). The expression of OCT4, SOX2, NANOG, SSEA4, TRA-60 and TRA1-81 was confirmed by immunocytochemistry (Fig. 1D). Reprogramming resulted in 96.8% OCT4+ and 95.9% SSEA4+ cells (p13) (Fig. 1E). Global transcriptome analysis revealed a correlation coefficient of 0.91 between the hESC line H9 and AATD-iPSC-3 (Fig. 1F). Sanger Sequencing confirmed the mis-sense mutation within the locus *SERPINA*1rs28929474 of G to A (Fig. 1G) Immunocytochemistry of spontaneous differentiation to the three germ layers by the embryoid body (EB) assay is shown in Fig. 1H. SRY-box transcription factor-17 (SOX17) and forkhead box A2 (FOXA2) were stained to identify endoderm, alpha smooth muscle actin (aSMA) and Brachyury for mesoderm. Paired box 6 (PAX6) and beta-3-tubulin (βIII-Tubulin) were stained to identify ectoderm (Fig. 1H). Table 2 displays the used reagents.

3. Materials and methods

3.1. Cell culture

SIX2-positive UdRPCs were isolated from urine samples and expanded as previously described (Bohndorf et al., 2017; Rahman et al., 2020). iPSCs were cultivated on Matrigel (Corning) coated wells, using StemMACs iPS Brew (Miltenyi). At 80–90% confluency, iPSCs were detached by incubation with PBS w/o Mg^{2+} and Ca^{2+} and colony-split in a ratio of 1:6.

3.2. Derivation of iPSCs

AATD-UdRPCs were nucleofected with 3 μg of the plasmids pEP4EO2SCK2MEN2L and pEP4EO2SET2K, respectively, as described in our previous publication (Bohndorf et al., 2017; Yu et al., 2011). To activate WNT-signalling 3 μM CHIR99021 was added. TGFβ-, MEK-, and Rock-signaling were inhibited with 0.5 μm A83-01, 0.5 μM PD0325901, 10 μM Y-27632, respectively. After clones attained typical pluripotency-associated morphology they were picked and further cultivated as iPSCs.

3.3. PCR

Vector dilution and endogenous *OCT4* expression was confirmed by PCR using the GoTaq DNA Polymerase kit (Promega). Genomic DNA was isolated from iPSCs using the Tissue DNA Purification Kit (EurX). Supernatants were collected from dense cultures to confirm the absence of mycoplasma contamination. To confirm genetic relationship of AATD-iPSC-3 to the parental AATD-UdRPCs, DNA fingerprinting analysis, short tandem repeats (STR) of 9 distinct loci were amplified by PCR and compared with the parental line.

3.4. Embryoid body (EB) formation

EB formation was performed by seeding single cell iPSCs into a 6 cm petri-dish, coated with anti-adherence-solution (Stem cell technologies) in StemMACS w 10 μM ROCK inhibitor for 24 h. Aggregates were cultivated shaking, in StemMACS w/o ROCK inhibitor for 6 days to form EBs. EBs were plated onto Matrigel coated plates, in DMEM containing 10% FBS, 1% Glutamax and 1% NEEA (all Gibco), for 4 days until immunocytochemistry.

Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	Microphotography Bright field	Normal	Fig. 1 panel A
Phenotype	Immunocytochemistry	Expression of pluripotency associated markers: OCT4, SOX2, NANOG, SSEA-4 TRA-1–60, TRA-1–81,	Fig. 1 panel D
	Flow Cytometry	Assess of 96.8% OCT4 positive cells: Assess of 95.9% SSEA4 positive cells	Fig. 1 panel E
Genotype	Karyotype (G-banding) and resolution	46XY 200–400 Bd	Fig. 1 panel C, Supplementary figure 2
Identity	Microsatellite PCR (mPCR) OR STR analysis	STR analysis by PCR of 6 loci, matched.	Supplementary file 2
Mutation analysis	Sanger sequencing	missense mutation at SERPINA1rs28929474locus, of G to A	Fig. 1 panel G
	Southern Blot OR WGS	Not performed	N/A
Microbiology and virology	Mycoplasma	Mycoplasma testing by PCR: Negative	Supplementary Fig. 1
Differentiation potential	Embryoid body formation	Expression of germ layer specific proteins. Mesoderm: alpha smooth muscle actin (aSMA), Brachyury; Ectoderm: βIII-tubulin, Paired box 6 (PAX6) Endoderm: SRY-box transcription factor 17, Forkhead Box A2 (FOXA2)	Fig. 1 panel H
Donor screening	HIV 1 + 2 Hepatitis B, Hepatitis C	Not performed	N/A
Genotype additional info	Blood group genotyping	Not performed	N/A
	HLA tissue typing	Not performed	N/A

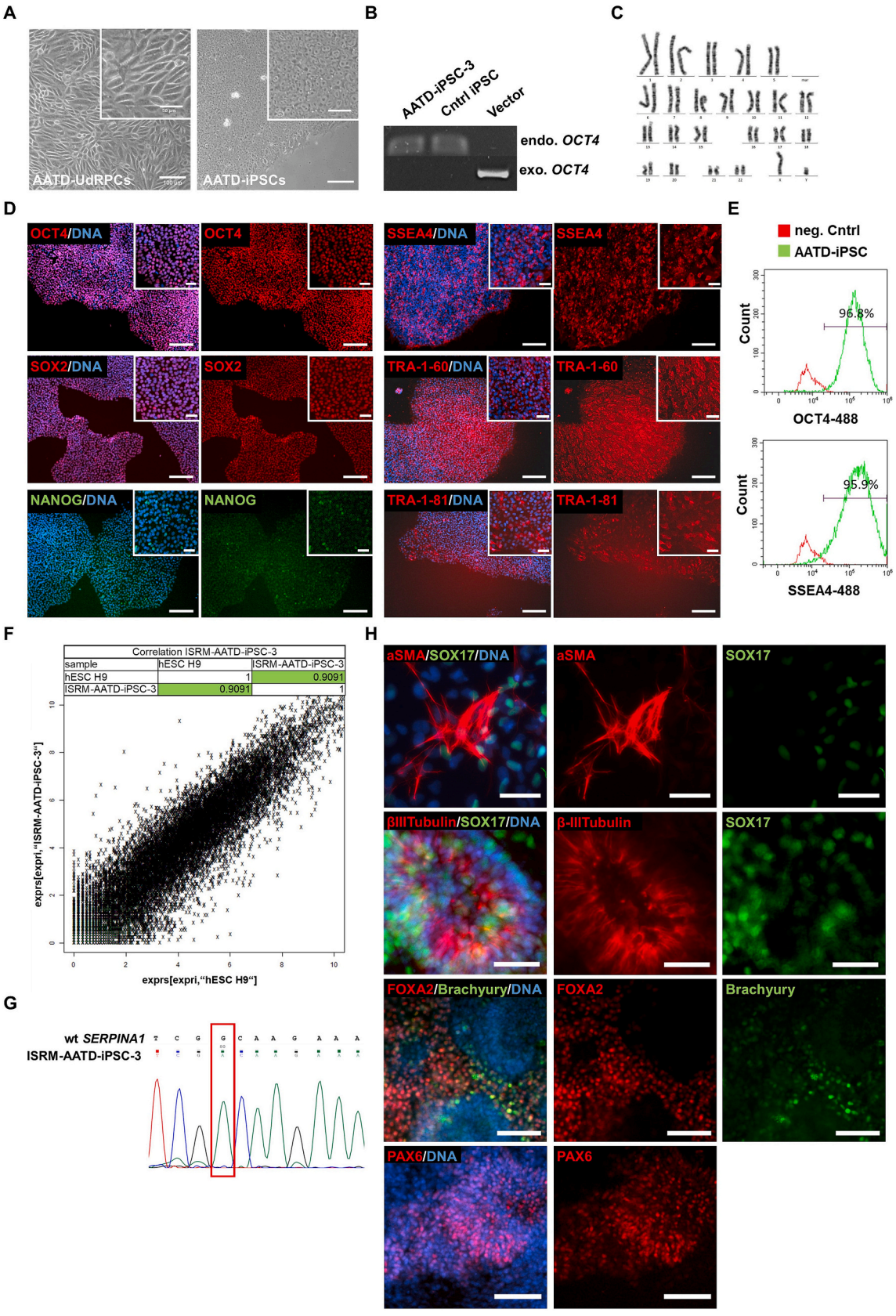

Fig. 1. Characterization of iPSC-line HHUUKDi013-A / ISRM-AATD-iPSC-3.

Table 2
Reagents details.

	Antibodies used for immunocytochemistry/flow-cytometry			
	Antibody	**Dilution**	**Company Cat #**	**RRID**
Flow Cytometry	Rabbit anti-OCT4	1:50	Cell Signaling Technologies #2840S	AB_2167691
Flow Cytometry	Mouse anti-SSEA4	1:50	Cell Signaling Technology	AB_1264259
Pluripotency Marker	Rabbit anti-OCT4	1:400	Cell Signaling Technologies #2840S	AB_2167691
Pluripotency Marker	Rabbit anti-SOX2	1:400	Cell Signaling Technologies #3579S	AB_2195767
Pluripotency Marker	Rabbit anti-NANOG	1:200	Cell Signaling Technologies #4903S	AB_10559205
Pluripotency Marker	Mouse anti-SSEA4	1:1000	Cell Signaling Technology	AB_1264259
Pluripotency Marker	Mouse anti-Tra-1-60	1:1000	Cell Signaling Technologies #4746S	AB_2119059
Pluripotency Marker	Mouse anti-Tra-1-81	1:1000	Cell Signaling Technologies #4745S	AB_2119060
Differentiation Marker	anti-aSMA	1:1000	Dako; # M0851	AB_2223500
Differentiation Marker	Mouse anti- SOX17	1:50	R and D Systems Cat# AF1924	AB_355060
Differentiation Marker	anti-BIII Tubulin	1:250	Cell Signaling Technologies #4466S	AB_1904176
Differentiation Marker	anti-PAX6	1:200	Synaptic Systems # 153 011	AB_887758
Secondary Antibody	anti-mouse- Alexa555	1:500	Thermo Fisher Scientific Cat# A10521	AB_2534030
Secondary Antibody	anti-rabbit- Alexa488	1:500	Thermo Fisher Scientific Cat# A27034	AB_2536097
Secondary Antibody	anti-rabbit- Alexa647	1:500	Thermo Fisher Scientific Cat# A-31573	AB_2536183
Nuclear Co– Staining	Hoechst33258	1:5000	Thermo Fisher Scientific Cat# H3569	AB_2651133
	Primers			
	Target	**Size of band**	**Forward/Reverse primer (5′-3′)**	
Endogenous OCT4	OCT4	113 bp	AGTTTGTGCCAGGGTTTTTG ACTTCACCTTCCCTCCAACC	
Episomal Plasmids (exo)	OCT4	657 bp	AGTGAGAGGCAACCTGGAGA AGGAACTGCTTCCTTCACGA	
Mycoplasma PCR	mycoplasma-specific 16S rRNA gene	265 – 278 bp	GGGAGCAAACAGGATTAGATACCCT TGCACCATCTGTCACTCTGTTAACCTC	
PiZ genotyping by Sanger Sequencing	SERPINA1	N/A	AGCGCTTCCTGGGAGGTGT	
STR-PCR	D16S539	N/A	GGGGGTCTAAGAGCTTGTAAAAAG GTTTGTGTGTGCATCTGTAAGCATGTATC	
	D13S317	N/A	ACAGAAGTCTGGGATGTGGAGGA GGCAGCCCAAAAAGACAGA	
	D7S820	N/A	ATGTTGGTCAGGCTGACTATG GATTCCACATTTATCCTCATTGAC	
	TH01	N/A	ATTCAAAGGGTATCTGGGCTCTGG GTGGGCTGAAAAGCTCCCGATTAT	
	vWA	N/A	CTAGTGGATGATAAGAATAATCAGTAT GTG GGACAGATGATAAATACATAGGATGGATGG	
	Amel	N/A	ACCTCATCCTGGGCACCCTGGTT AGGCTTGAGGCCAACCATCAG	
	CSF1PO	N/A	AACCTGAGTCTGCCAAGGACTAGC TTCCACACACCACTGGCCATCTTC	
	TPOX	N/A	ACTGGCACAGAACAGGCACTTAGG GGAGGAACTGGGAACCACACAGGTTA	
	D5S818	N/A	GGTGATTTTCCTCTTTGGTATCC AGCCACAGTTTACAACATTTGTATCT	

3.5. Immunofluorescence

Cells were fixed with 4% PFA for 10 min at RT, and permeabilized for 10 min with 0.5% Triton-x-100 (Sigma). For extracellular staining the permeabilization step was skipped. Cells were blocked for 1 h at RT with 3% BSA and incubated with respective primary antibodies overnight at 4 °C. Fluorophore-coupled antibodies against the host IgG were incubated for 1 h at RT in the dark and DNA was counterstained with 4 μM Hoechst33342. Fluorescence microscopy was performed using the LSM700 (Zeiss) microscope and ZEN2012 (blue edition) software, version 6.1.7601.

3.6. Flow cytometry

Cells were detached to single cells, centrifuged and fixed in 4% PFA for 10 min at RT. After 3x wash with PBS-/-, cells were permeabilized for 10 min with 0.5 % Triton-x-100. For extracellular staining this step was skipped. Cells were blocked in 0.5% BSA and 2 mM EDTA for 1 h at RT and primary antibodies were incubated overnight, at 4 °C. Fluorophore-coupled antibodies against the host IgG were incubated for 1 h at RT in the dark. Cells were washed and resuspended in blocking buffer. Fluorescence was measured using the CytoFLEX S Flow Cytometer (Beckman Coulter) and analyzed with the CytExpert software version 2.6.0.105 (Beckman Coulter).

3.7. Karyotype analysis

Karyotype analysis was performed and evaluated at the Institute of Human Genetics and Anthropology, Heinrich-Heine-University, Düsseldorf.

3.8. Sanger sequencing and global transcriptome analysis

Sanger sequencing and 3'RNA-Seq were performed at the core facility BMFZ-GTL of Heinrich-Heine-University Düsseldorf.

CRediT authorship contribution statement

Christiane Loerch: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Rabea Hokamp:** Writing – review & editing, Visualization, Methodology, Investigation, Formal analysis, Data curation. **Wasco Wruck:** Writing – review & editing, Methodology, Investigation, Formal analysis, Data curation. **David Katzer:** Writing – review & editing, Writing – original draft, Resources, Project administration, Investigation. **Alexander Weigert:** Writing – review & editing,

Resources. **Alexander Machui:** Writing – review & editing, Resources, Methodology, Investigation. **Nina Graffmann:** Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization. **Rainer Ganschow:** Writing – review & editing, Supervision, Resources, Project administration, Conceptualization. **James Adjaye:** Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Christiane Loerch reports financial support was provided by Else Kroner-Fresenius Foundation. Nina Graffmann reports financial support was provided by Else Kroner-Fresenius Foundation. 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.

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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.2025.103762.

Data availability

Data will be made available on request.

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