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Lab Resource: Single Cell Line

Establishment of a human induced pluripotent stem cell line (TAUi008-A) derived from a multiple sclerosis patient

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A B S T R A C T

Multiple sclerosis (MS) is a complex autoimmune disease of the central nervous system where the main pathogenetic events include demyelination and axonal degeneration. Here, we generated a human induced pluripotent stem cell (hiPSC) line from peripheral blood mononuclear cells of an MS patient utilizing Sendai virus reprogramming. The produced hiPSC line expressed pluripotency markers, differentiated into three germ layers, showed a normal karyotype and was free of virus vectors, transgenes and mycoplasma. Established hiPSCs are a valuable source for studies of MS disease modeling and drug discovery.

Resource Table

Unique stem cell line identifier	TAUi008-A
Alternative name(s) of stem cell line	TAU2106C1.MS
Institution	Tampere University, Tampere, Finland
Contact information of distributor	Sanna Hagman sanna.hagman@tuni.fi
Type of cell line	iPSC
Origin	Human
Additional origin info required for human ESC or iPSC	Age: 64 Sex: Female Ethnicity: Caucasian
Cell Source	Peripheral blood mononuclear cells
Clonality	Clonal
Method of reprogramming	CytoTune™-iPS 2.0 Sendai Reprogramming Kit
Genetic Modification	NO
Type of Genetic Modification	N/A
Evidence of the reprogramming transgene loss (including genomic copy if applicable)	qPCR
Associated disease	Multiple sclerosis
Gene/locus	N/A
Date archived/stock date	April 2022
Cell line repository/bank	https://hpscreg.eu/cell-line/TAUi008-A
Ethical approval	Ethics Committee, Hospital District of Southwest Finland (approval no. 48/1801/2019)

1. Resource utility

The pathophysiological mechanisms of multiple sclerosis (MS) are not entirely understood. A human induced pluripotent stem cell (hiPSC) line from an MS patient was generated to study the role of neural cells in the pathogenesis of MS disease and for use in drug screening and discovery.

2. Resource details

MS is a chronic autoimmune disease of the central nervous system (CNS) that leads to neuroinflammation, demyelination, astrogliosis and neurodegeneration (Thompson et al., 2018). It is an uncurable disease that affects approximately 2.8 million people globally (Walton et al., 2020). MS disease has complex pathophysiological mechanisms that are still not completely understood, which has limited the development of effective neuroprotective therapies. Both peripheral immune cells and different cell types of the CNS contribute to disease progression. However, knowledge about their exact functions and the mechanisms of neurodegeneration are still incomplete (Thompson et al., 2018).

Animal models have been widely used to study MS, while human-specific disease models are mostly lacking. Rodent cells do not accurately recapitulate human disease due to species-specific differences (Martínez-Larrosa et al., 2020). This leads to low success rates in the translation of preclinical findings to clinical trials; thus, more precise

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human cell-based disease models are required (Martínez-Larrosa et al., 2020). Human MS in vitro disease models are urgently needed to reveal the pathophysiological mechanisms and to discover novel drug targets. In particular, MS patient-derived hiPSC models are advantageous for studying the interactions of different CNS cell types, such as neurons and glial cells, which availability is otherwise extremely limited (Fortune et al., 2022; Martínez-Larrosa et al., 2020).

Here, an MS patient-specific hiPSC line (Table 1) was generated with genome integration-free Sendai virus transduction (CytoTune™-iPS 2.0 Sendai Reprogramming Kit) from peripheral blood mononuclear cells (PBMCs) of an MS patient. The utilized CytoTune™ Sendai vectors KOS, hc-Myc and hKlf4 contained four Yamanaka factors Oct3/4, Sox2, Klf4 and c-Myc. Cells were reprogrammed in a feeder cell-dependent culture and subsequently transferred to feeder-free culture for analysis of flow cytometry, short tandem repeat (STR) and karyotype.

The established hiPSC line showed normal morphology (Fig. 1A), and cells expressed typical pluripotency markers (OCT3/4, SOX2, NANOG and SSEA-4) detected by immunocytochemistry (ICC) (Fig. 1B). The expression of SSEA-4 was analyzed with flow cytometry, and it yielded an expression level of 97.7 % (Fig. 1C). The *in vitro* differentiation capacity of hiPSCs into three germ layers; the endoderm, ectoderm and mesoderm, was confirmed through embryoid body (EB) formation and positive ICC staining of the endodermal marker alpha-fetoprotein (AFP), ectodermal marker orthodenticle homolog 2 (OTX2) and mesodermal marker smooth muscle actin (SMA) (Fig. 1D). The absence of Sendai virus vectors and transgenes (SeV, KOS, Klf4 and cMyc) was confirmed with qPCR at passage 8 by comparing their gene expression level to samples collected after transduction (passage 0) (Fig. 1E). Karyotype analysis showed that the hiPSC line had a normal diploid 46, XX karyotype (Fig. 1F). Mycoplasma analysis was negative (Fig. 1G). The genetic identity of the generated hiPSCs was verified with STR analysis by comparing 24 matched genetic loci io respective DNA isolated from PBMCs of the MS patient.

Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography Bright field	Normal	Fig. 1A
Phenotype	Immunocytochemistry	Positive for pluripotency markers Oct3/4, Nanog, Sox2, SSEA-4	Figure 1B
	Flow cytometry	SSEA-4: 97.7 %	Figure 1C
Genotype	Karyotype (G-banding) and resolution	Normal 46XX, Resolution 300–400	Figure 1F
Identity	Microsatellite PCR (mPCR) OR	N/A	N/A
	STR analysis	24 loci, all matched	Submitted in archive with journal
Mutation analysis (IF APPLICABLE)	Sequencing	N/A	N/A
	Southern Blot OR WGS	N/A	N/A
Microbiology and virology	Mycoplasma	RT-PCR: Negative	Fig. 1G
Differentiation potential	Embryoid body formation	Positive for three germ layer markers: endoderm marker AFP, mesoderm marker SMA and ectoderm marker OTX2	Fig. 1D
Donor screening (OPTIONAL)	HIV, Hepatitis B, Hepatitis C, Covid-19	Negative	N/A
Genotype additional info (OPTIONAL)	Blood group genotyping	N/A	N/A
	HLA tissue typing	N/A	N/A

3. Materials and methods

3.1. Ethics statements

Supportive ethical statements for producing hiPSC lines (approval no. 48/1801/2019) and for culturing hiPSCs (R20159) have been obtained from the Ethics Committee of Hospital District of Southwest Finland and Pirkanmaa Hospital District, respectively. Informed consent was obtained from the patient.

3.2. Reprogramming of PBMCs and culturing of hiPSCs

PBMCs were isolated from whole blood samples using a Ficoll-Paque Plus density gradient (GE Healthcare). Cells were cryopreserved in RPMI 1640 medium (Thermo Fisher Scientific) containing 40 % FBS (Merck) and 10 % DMSO (Sigma). After thawing, PBMCs were cultured in StemPro-34 -medium supplemented with GlutaMAX (both from Thermo Fisher Scientific), 100 ng/ml SCF, 100 ng/ml FLT3-ligand, 20 ng/ml IL-3 and 20 ng/ml IL-6 (all from Peprotech) at + 37 °C under 5 % CO_2. Four days after thawing, Sendai virus transduction was performed using the CytoTune™-iPS 2.0 Sendai Reprogramming Kit (Thermo Fisher Scientific) according to the manufacturer's instructions. Three days after transduction, cells were plated on a feeder layer of mitomycin C inactivated human foreskin fibroblasts (CRL-2429™, ATCC) and cultured in StemPro-34 -medium without cytokines. Seven days later, the cells were cultured in iPSC medium containing KnockOut™-DMEM (Thermo Fisher Scientific), 20 % KnockOut™ Serum Replacement (Thermo Fisher Scientific), 1 % GlutaMAX, 1 % NEAA (Lonza), 0.5 % penicillin–streptomycin (Lonza), 0.2 % 2-mercaptoethanol (Thermo Fisher Scientific) and 8 ng/ml FGF-basic (Peprotech). Two weeks after transduction, formed hiPSC colonies were mechanically picked and cultured on the feeder layer in iPSC medium and mechanically passaged weekly. After passage 10, hiPSCs were transferred to adherent feeder-free cultures on 0.6 μg/cm^2 recombinant human laminin-521 (LN521, Biolamina) coated cell culture plates in Essential 8™ Flex medium (Thermo Fisher Scientific) and passaged enzymatically with TrypLE™ Select Enzyme and Defined Trypsin Inhibitor (both from Thermo Fisher Scientific) twice a week.

3.3. Embryoid body (EB) formation and differentiation capacity

To determine the differentiation capacity of hiPSCs, EBs were generated from hiPSC colonies at passage 10 by cutting colonies mechanically into small pieces and culturing in suspension in Corning™ Costar™ Ultra-Low Attachment plates in iPSC medium without FGF-basic for two weeks. Thereafter, formed EBs were transferred to 0.6 μg/cm2 LN521 coated culture plates and cultured in the adherent culture for two weeks. Spontaneously differentiated cells were stained and imaged as described in Section 3.4.

3.4. Immunocytochemistry

ICC staining of pluripotency (OCT3/4, SOX2, NANOG and SSEA-4) and germ layer markers (AFP, OTX2 and SMA) was performed (Table 2). Pluripotency marker staining was performed for hiPSC colonies at passage 10 that were cultured on top of feeders for one week prior to fixation with 4 % paraformaldehyde in PBS for 15 min. Staining was performed as previously described (Lappalainen et al., 2010), and the primary and secondary antibodies used are listed in Table 2. Stained cells were mounted with ProLong™ Gold Antifade Mountant with DAPI (Thermo Fisher Scientific) and imaged with an Olympus IX51 fluorescence microscope equipped with a Hamamatsu ORCA-Flash4.0 LT +

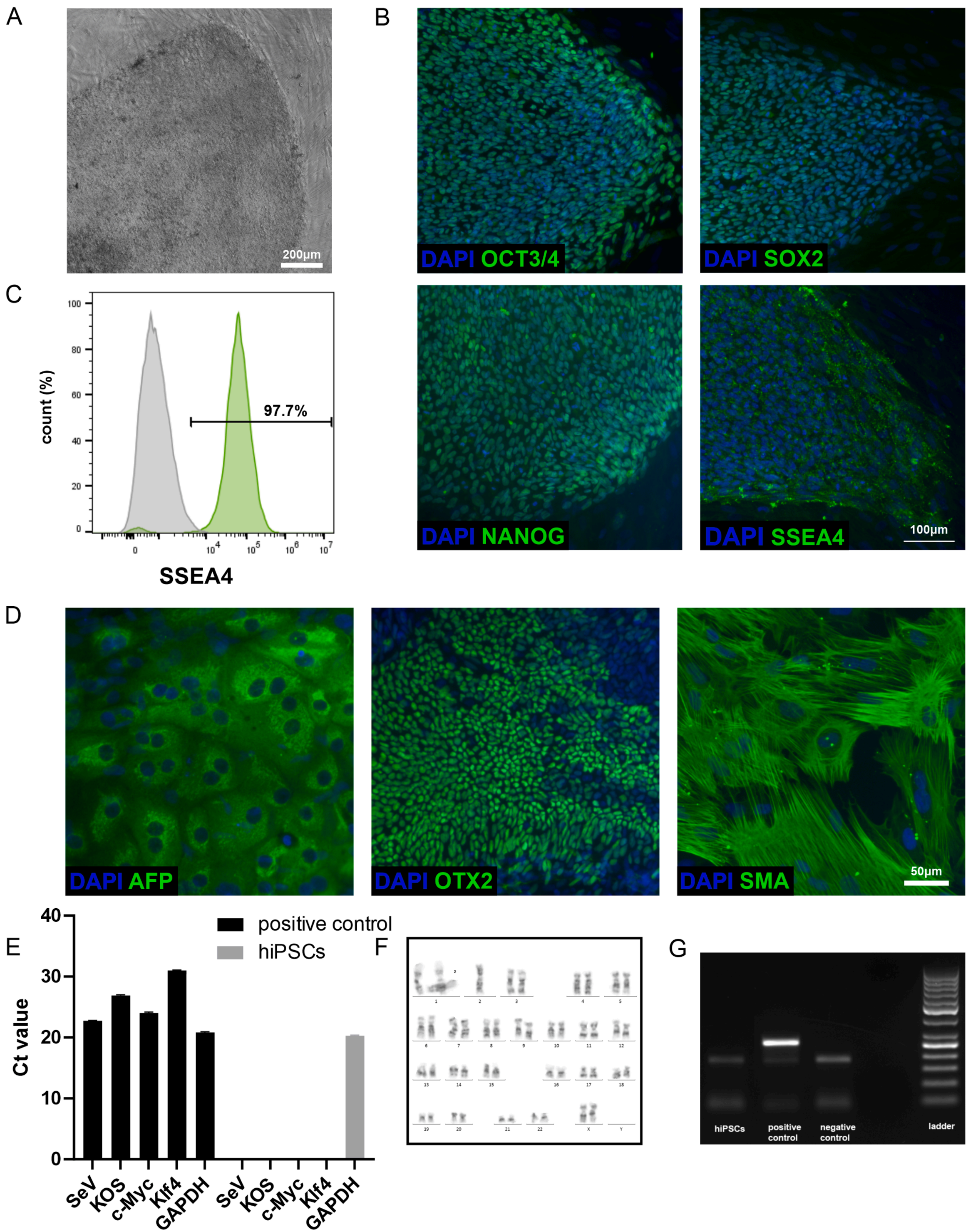

Fig. 1.

Table 2
Reagents details.

	Antibodies used for immunocytochemistry/flow-cytometry			
	Antibody	Dilution	Company Cat #	RRID
Pluripotency Markers	Goat anti-OCT3/4	1:200	R&D Systems Cat#AF1759	RRID: AB_354975
	Mouse anti-SSEA4	1:200	R&D Systems Cat#MAB1435	RRID: AB_357704
	Mouse anti-SOX2	1:200	R&D Systems Cat#MAB2018	RRID: AB_358009
	Goat anti-Nanog	1:200	R&D Systems Cat#AF1997	RRID: AB_355097
	PE mouse anti-SSEA4	1:25	BD Pharmingen Cat#560128	RRID: AB_1645533
Differentiation Markers	Mouse anti-SMA	1:200	R&D Systems Cat#MAB1420	RRID: AB_262054
	Mouse anti-AFP	1:200	R&D Systems Cat#MAB1369	RRID: AB_2258005
	Goat anti-OTX2	1:200	R&D Systems Cat#AF1979	RRID: AB_2157172
Secondary antibodies	Alexa Fluor 488 donkey anti-mouse IgG	1:400	Thermo Fisher Scientific Cat#A21202	RRID: AB_141607
	Alexa Fluor A568 donkey anti-mouse IgG	1:400	Thermo Fisher Scientific Cat#A10037	RRID: AB_2534013
	Alexa Fluor A488 donkey anti-goat IgG	1:400	Thermo Fisher Scientific Cat#A11055	RRID: AB_2534102
	Alexa Fluor A568 donkey anti-goat IgG	1:400	Thermo Fisher Scientific Cat#A11057	RRID: AB_2534104
	Alexa Fluor A568 goat anti-Mouse IgM	1:400	Thermo Fisher Scientific Cat#A21043	RRID: AB_1500924
	Primers			
	Target	Size of band	Forward/Reverse primer (5′-3′)	
Sendai virus vectors (qPCR)	SeV		Assay ID: Mr04269880_mr	
	KOS		Assay ID: Mr04421257_mr	
	Klf4		Assay ID: Mr04421256_mr	
	cMyc		Assay ID: Mr04269876_mr	
House-Keeping Genes (qPCR)	GAPDH		Assay ID: Hs99999905_m1	

sCMOS camera (type C11440-42U30).

3.5. Flow cytometry

hiPSCs (total passages 20, feeder-free passages 9) were enzymatically dissociated into single cells and washed twice with staining buffer (0.5 % BSA and 1 mM EDTA in PBS). Thereafter, the cells were stained with PE mouse anti-SSEA-4 (Table 2) in staining buffer for 20 min on ice and were washed twice with staining buffer. Flow cytometry analysis was performed using CytoFLEX S (Beckman Coulter). Unstained negative controls were treated similarly to stained samples and used for gating. The data were analyzed using FlowJo™ v10 software (BD Bioscience).

3.6. Sendai virus and transgene detection with qPCR

The gene expression of the Sendai virus vectors and transgenes was investigated in hiPSCs at passage 0 (positive control) and passage 8 by qPCR. RNA was isolated from samples with a NucleoSpin® RNA kit (Macherey-Nagel) and reverse transcribed into cDNA with a High-Capacity cDNA Reverse Transcription kit (Thermo Fisher Scientific). Gene expression analysis of SeV (Mr04269880_mr), KOS (Mr04421257_mr), Klf4 (Mr04421256_mr) and cMyc (Mr04269876_mr) was performed with pre-validated TaqMan™ assays (Table 2) using Applied Biosystems QuantStudio 12 K Flex System (Thermo Fisher Scientific). GAPDH was used as an endogenous control. The absence of Sendai vectors was confirmed when gene expression levels were nondetectable.

3.7. Karyotyping

G banding karyotype analysis was performed for feeder-free hiPSCs (total passages 21, feeder-free passages 10) by Fimlab Laboratoriot Oy, Tampere, Finland. The resolution was 300–400 bands per haploid chromosome set.

3.8. STR analysis

DNA was isolated from PBMCs obtained from the MS patient and the feeder-free hiPSC line using QIAamp DNA Mini Kit (Qiagen). The identity of PBMCs and hiPSCs was analyzed with the GenePrint 24 system (Promega) by comparing 24 loci. Genotyping was performed by the Institute for Molecular Medicine Finland FIMM Technology Centre, University of Helsinki, Finland.

3.9. Mycoplasma test

hiPSC line (total passages 16, feeder-free passages 5) was tested for mycoplasma using the Venor GeM Classic Mycoplasma Detection Kit (Minerva Biolab).

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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