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Lab Resource: Single Cell Line

Generation and characterization of human induced pluripotent stem cell line METUi001-A from a 25-year-old male patient with relapsing-remitting multiple sclerosis

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A B S T R A C T

Multiple sclerosis is a chronic disease characterized by inflammation, demyelination, and axonal damage in the central nervous system. Here, we established an induced pluripotent stem cell (iPSC) line METUi001-A from the peripheral blood mononuclear cells of a 25-year-old male individual with clinically diagnosed Relapsing-Remitting Multiple Sclerosis (RRMS) using the integration-free Sendai reprogramming method. We demonstrated that the iPSCs are free of exogenous Sendai reprogramming vectors, have a normal male karyotype, express pluripotency markers, and differentiate into the three germ layers. The iPSC line can serve as a valuable resource to generate cellular model systems to investigate molecular mechanisms underlying RRMS.

1. Resource table

Unique stem cell line identifier	METUi001-A
Alternative name(s) of stem cell line	N/A
Institution	Middle East Technical University, Ankara, Turkey
Contact information of distributor	Erkan Kiris; ekiris@metu.edu.tr
Type of cell line	iPSC
Origin	Human
Additional origin info	Age: 25 Sex: Male Ethnicity if known: N/A
Cell Source	Human Peripheral Blood Mononuclear Cells (PBMCs)
Clonality	Clonal
Associated disease	Relapsing-Remitting Multiple Sclerosis
Gene/locus	N/A
Date archived/stock date	December 2020
Cell line repository/bank	https://hpscreg.eu/cell-line/METUi001-A
Ethical approval	Ethical approval was obtained from the Ethics Board of the University of Health Sciences (Turkey). Approval No: 19/83

2. Resource utility

Multiple sclerosis (MS) is a demyelinating disease without a cure and has a substantial burden on patients and their families. The patient-derived iPSC line METUi001-A represents a valuable resource as these cells can be differentiated to cell types relevant to MS for mechanistic studies and drug screening (see Table 1).

3. Resource details

There is no cure for MS, and currently available therapeutic options mainly focus on modulating the immune system and preventing relapses (Torkildsen et al., 2016). However, to cure the disease, it is necessary to effectively remyelinate the neurons that have lost the myelin sheet, complementary to immunomodulatory therapies (Lubetzki et al., 2020). Therefore, there is a crucial need to better understand remyelination mechanisms in MS. Recent studies suggest that there may be significant differences between rodent models and humans with regard to remyelination processes in MS (Franklin et al., 2020), highlighting the importance of species-specific model systems to study the disease. Additionally, the sex of the model system appears to be crucial as

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epidemiological studies strongly suggest that MS is sex-biased (Gilli et al., 2020). Two-thirds of MS patients are women; however, how sex affects MS initiation or progression remains unknown. Patient-derived iPSCs offer unique approaches to study MS in a species-specific manner, as these cells can be differentiated to obtain a limitless supply of cell types affected by the disease. Such cellular models could be utilized for drug screening, and molecular studies to uncover mechanisms underlying the disease and the sex-specific differences.

Here, we focused on the most common form of MS, Relapsing-Remitting Multiple Sclerosis (RRMS) (Gilli et al., 2020), and established an iPSC line from the peripheral blood mononuclear cells (PBMCs) of a 25-year-old male with clinically diagnosed RRMS, using the Sendai virus-based reprogramming method. The patient was confirmed to have RRMS based on 2017 McDonald criteria, upon thorough neurological examinations and MRI analyses. The blood sample was obtained from the patient, with informed consent, upon confirmation of the disease, but before the patient started taking any disease-modifying medication. The donor represents a patient subject involved in a project that focuses on evaluating sex-specific differences in RRMS at the cellular level. PBMCs were reprogrammed using CytoTune-iPS Sendai Reprogramming Kit according to the manufacturer's instructions. The hiPSCs were cultured on mitotically inactivated (Mitomycin C treated) mouse embryonic fibroblast (MEF) cells as feeder cells and have shown typical human pluripotent stem cell colony morphology Fig. 1**A**). RT-PCR analyses demonstrated the clearance of exogenous Sendai reprogramming vectors at passage 16 (Fig. 1**B**). Pluripotency of the iPSCs was determined by immunocytochemistry using antibodies against the pluripotency markers Oct3/4, Sox2, TRA-1–60, and SSEA-4 (Fig. 1**C**). Real-time quantitative PCR further confirmed the expression of pluripotency genes *POU5F1*, *SOX2*, *NANOG*, relative to corresponding PBMCs, compared to H9 human embryonic stem cells (Fig. 1**D**). The differentiation capacity of the METUi001-A line was determined by embryoid body formation and *in vitro* spontaneous differentiation to the three germ layers. Specifically, immunocytochemistry analyses determined the expression of the endoderm markers AFP and SOX-17, mesoderm markers SMA and Brachyury, and ectoderm markers Pax6 and βIII-tubulin (Fig. 1**E**). METUi001-A line showed a normal diploid 46, XY male karyotype (Fig. 1**F**), and short tandem repeat (STR) analysis authenticated the identity of the line, which is 100% in concordance with parental PBMCs (**Supplementary** Fig. 1). Finally, PCR analysis conducted on the METUi001-A line (p16) showed no trace of mycoplasma (Fig. 1**G**).

4. Materials and methods

4.1. Isolation, expansion and reprogramming of PBMCs

PBMCs were isolated from blood using Histopaque-1077 (Sigma-Aldrich), cultured in StemSpan SFEM II with StemSpan Erythroid Expansion Supplement (Stem Cell Technologies), and transduced with CytoTune 2.0 Sendai Virus Reprogramming Kit (Thermo Scientific), according to manufacturers' protocols. Emerging single colonies were manually passaged from day 21 onwards onto MEF seeded plates in hiPSC medium with 10 μM ROCK inhibitor Y-27632 to enhance viability up until passage 5, and then routinely passaged using 1 U/mL dispase (Gibco) at a ratio of 1:3 every 4–5 days, and incubated at 37C with 5% CO_2. hiPSC medium included Advanced DMEM/F12, 20% KnockOut Serum Replacement, 1% Non-Essential Amino Acids, 1% GlutaMAX, 1% Penicillin-Streptomycin, 55 μM 2-mercaptoethanol, and 4 ng/mL bFGF.

4.2. Karyotype and short tandem repeat (STR) analyses

Karyotype and STR analyses of METUi001-A were both performed at passage 16 by the INTERGEN Genetics and Rare Diseases Diagnosis Research&Application Center. For the karyotype, more than 20 metaphase spreads were analyzed with standard G-banding analyses at a 550–600 band resolution. Purified genomic DNA from iPSCs and the parental PBMCs were utilized for the STR analyses.

4.3. Gene expression analyses

Total RNA was isolated using NucleoSpin RNA Kit (Macherey-Nagel) and converted to cDNA using RevertAid First Strand cDNA Synthesis Kit (Thermo Scientific). The clearance of exogenous Sendai virus genome and transgenes was verified by RT-PCR (T100 Termal Cycler, BIO-RAD), at passage 16 of iPSCs, using the primers listed in Table 2. PCR conditions included initial denaturation at 95 °C for 3 min, followed by 95 °C for 30 s, 55 °C for 30 s, 72 °C for 1 min, and final elongation 72 °C 2 min. Real-time quantitative PCR was utilized to detect endogenous pluripotent genes *POU5F1, SOX2 and NANOG* using Corbett Rotor Gene 6000 system with SsoAdvanced Universal SYBR Green Supermix (BIO-RAD). Data was normalized with PBMC, and analysed by $2^{-\Delta\Delta Ct}$ method, using GAPDH as a reference.

4.4. In vitro spontaneous differentiation

iPSCs (p16) were separated from MEF layers using dispase and

Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography Bright field	Normal Human Pluripotent Stem Cell Morphology	Fig. 1 A
Phenotype	Qualitative analysis (Immunocytochemistry)	Positive for pluripotency markers: OCT4, SOX2, TRA-1–60, SSEA-4	Fig. 1C
	Quantitative analysis (RT-qPCR)	Pluripotency markers *OCT4*, *SOX2* and *NANOG* are expressed endogenously	Fig. 1D
Genotype	Karyotype (G-banding) and resolution	Normal Karyotype, 46 XY, Banding Resolution: 550–600	Fig. 1F
Identity	Microsatellite PCR (mPCR) OR	N/A	N/A
	STR analysis	16 loci analyzed; 100% matching	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 testing by RT-PCR	Negative	Fig. 1G
Differentiation potential	Embryoid body formation and *in vitro* spontaneous differentiation	Expression of markers specific for three germ layers detected by Immunocytochemistry (SOX-17 & AFP for endoderm, Brachyury & SMA for mesoderm, and Pax6 & Beta-III Tubulin for ectoderm)	Fig. 1E
Donor screening (OPTIONAL)	HIV 1 + 2 Hepatitis B, Hepatitis C	N/A	N/A
Genotype additional info (OPTIONAL)	Blood group genotyping	N/A	N/A
	HLA tissue typing	N/A	N/A

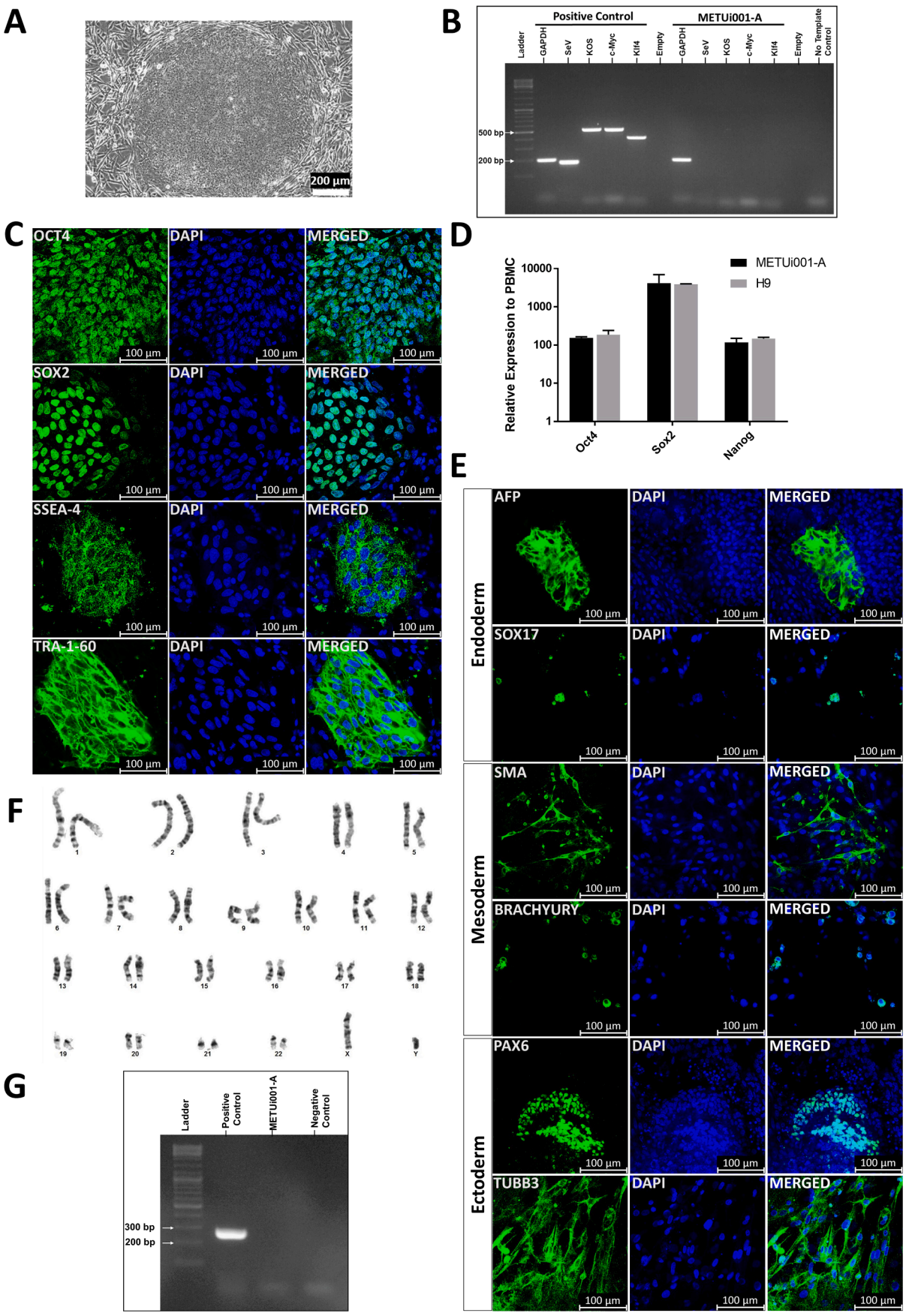
A
200 µm
B
Positive Control
METUi001-A
Ladder
GAPDH
SeV
KOS
c-Myc
Klf4
Empty
GAPDH
SeV
KOS
c-Myc
Klf4
Empty
No Template Control
500 bp
200 bp
C
OCT4
DAPI
MERGED
SOX2
DAPI
MERGED
SSEA-4
DAPI
MERGED
TRA-1-60
DAPI
MERGED
100 µm
D
METUi001-A
H9
Relative Expression to PBMC
10000
1000
100
10
1
Oct4
Sox2
Nanog
E
Endoderm
AFP
DAPI
MERGED
SOX17
DAPI
MERGED
Mesoderm
SMA
DAPI
MERGED
BRACHYURY
DAPI
MERGED
Ectoderm
PAX6
DAPI
MERGED
TUBB3
DAPI
MERGED
100 µm
F
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
X
Y
G
Ladder
Positive Control
METUi001-A
Negative Control
300 bp
200 bp

Fig. 1.

Table 2
Reagent details.

Antibodies used for immunocytochemistry				
	Antibody	Dilution	Company Cat #	RRID
Pluripotency Markers	Mouse anti-Oct-3/4	1:100	Santa Cruz Biotechnology Cat# sc-5279	RRID: AB_628051
	Mouse anti-Sox-2	1:100	Santa Cruz Biotechnology Cat# sc-365823	RRID: AB_10842165
	Mouse anti-TRA-1–60	1:100	Santa Cruz Biotechnology Cat# sc-21705	RRID: AB_628385
	Mouse anti-SSEA-4	1:100	DSHB Cat# MC-813-70 (SSEA-4)	RRID: AB_528477
Differentiation Markers	Mouse anti-AFP	1:100	Santa Cruz Biotechnology Cat# sc-8399	RRID: AB_626665
	Mouse anti-SMA	1:100	Santa Cruz Biotechnology Cat# sc-53142	RRID: AB_2273670
	Mouse anti-Pax6	1:100	DSHB Cat# pax6	RRID: AB_528427
	Mouse anti-Beta-III Tubulin	1:500	R and D Systems Cat# BAM1195	RRID: AB_356859
	Mouse anti-Sox-17	1:100	Santa Cruz Biotechnology Cat# sc-130295	RRID: AB_2286667
	Mouse anti-Brachyury	1:100	Santa Cruz Biotechnology Cat# sc-166962	RRID: AB_10610035
Secondary antibodies	Goat anti-Mouse IgG (H + L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor 488	1:200	Thermo Fisher Scientific Cat# A-11029	RRID: AB_2534088

Primers			
	Target	Size of band	Forward/Reverse primer (5′-3′)
SeV specific primers (RT-PCR)	SeV	181 bp	GGATCACTAGGTGATATCGAGC/ ACCAGACAAGAGTTTAAGAGATATGTATC
SeV specific primers (RT-PCR)	KOS	528 bp	ATGCACCGCTACGACGTGAGCGC/ ACCTTGACAATCCTGATGTGG
SeV specific primers (RT-PCR)	Klf4	410 bp	TTCCTGCATGCCAGAGGAGCCC/ AATGTATCGAAGGTGCTCAA
SeV specific primers (RT-PCR)	c-Myc	532 bp	TAACTGACTAGCAGGCTTGTCG/ TCCACATACAGTCCTGGATGATGATG
House-Keeping gene (RT-qPCR)	GAPDH	197 bp	GGAGCGAGATCCCTCCAAAAT/ GGCTGTTGTCATACTTCTCATGG
Pluripotency marker primers (RT-qPCR)	OCT4	63 bp	GGGTTTTTGGGATTAAGTTCTTCA/ GCCCCCACCCTTTGTGTT
Pluripotency marker primers (RT-qPCR)	SOX2	63 bp	CAAAAATGGCCATGCAGGTT/ AGTTGGGATCGAACAAAAGCTATT
Pluripotency marker primers (RT-qPCR)	NANOG	190 bp	CCTGAAGACGTGTGAAGATGAG/ GCTGATTAGGCTCCAACCATA
Mycoplasma detection (PCR)	MGSO & GPO3	270 bp	TGCACCATCTGTCACTCTGTTAACCTC/ GGGAGCAAACAGGTTAGATACCCT

seeded onto low-attachment dishes with hiPSC medium without bFGF for embryoid body (EB) formation. EBs were then incubated for three weeks with medium change every 2–3 days and then seeded on 0.1% gelatin-coated 4-well chamber slides with the same media for seven additional days.

4.5. Immunocytochemistry

The iPSCs (p16) were cultured on 0.1% gelatin-coated, MEF including 4-well chamber slides for the pluripotency analyses. iPSCs with MEFs and differentiated EBs, which were also grown on chamber slides (as described in section 4.2.), were fixed with 4% PFA, permeabilized with 0.3% Triton-X-100, and then blocked with 3% BSA. Primary and secondary antibodies (Table 2) were diluted in 0.3% BSA and incubated overnight at 4 °C, and 2 h at room temperature, respectively. ProLong Gold Antifade with DAPI was utilized for mounting. Confocal imaging was performed using a Leica SP8 Microscope (Bilkent University).

4.6. Mycoplasma detection

Mycoplasma detection was performed by PCR using primers (MGSO & GPO3, Table 2) targeting 16S rRNA of a wide range of *Mycoplasma* species, as previously described (Young et al., 2010).

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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