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Generation of a human induced pluripotent stem cell line (UEFi003-A) carrying heterozygous A673T variant in amyloid precursor protein associated with a reduced risk of Alzheimer's disease

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A B S T R A C T

A673T mutation in the amyloid precursor protein (APP) is a rare variant associated with a reduced risk of late-onset Alzheimer's disease (AD) and age-related cognitive decline. The A673T mutation decreases beta-amyloid (Aβ) production and aggregation in neuronal cultures *in vitro*. Here we have identified a Finnish non-diseased male individual carrying a heterozygous A673T mutation, obtained a skin biopsy sample from him, and generated an iPSC line using commercially available integration-free Sendai virus-based kit. The established iPSC line retained the mutation, expressed pluripotency markers, had a normal karyotype, and differentiated into all three germ layers *in vitro*.

Resource Table:

Unique stem cell line identifier	UEFi003-A
Alternative name(s) of stem cell line	MADGIC 12B
Institution	A.I.Virtanen Institute for Molecular Sciences University of Eastern Finland
Contact information of distributor	Šárka Lehtonen, sarka.lehtonen@uef.fi; sarka.lehtonen@helsinki.fi
Type of cell line	iPSC
Origin	Human
Additional origin info	Age: 58 Sex: male Ethnicity if known: Finnish
Cell Source	Skin fibroblasts
Clonality	Clonal
Method of reprogramming	Sendai virus delivery of *OCT-3/4, KLF-4, SOX-2 and c-MYC* genes
Genetic Modification	Yes
Type of Modification	Hereditary
Associated disease	Protective against Alzheimer's disease
Gene/locus	APP (MIM # 104760) located on the chromosome 21q21.3 genotype Chr21: 25,897,620 G > A substitution (rs63750847)
Method of modification	NO modification
Name of transgene or resistance	NO transgene or resistance
Inducible/constitutive system	NO inducible
Date archived/stock date	N/A
Cell line repository/bank	Registered in hPSCreg.eu
Ethical approval	Northern Savo Hospital district (license no. 123/2016)

1. Resource utility

Aβ aggregates in the brain are the main hallmark of AD pathology. The A673T mutation in APP reduces the production of Aβ peptides. The generated line could be used to study the effects of a decreased Aβ production on neuronal cell function alone and in co-cultures with glial cells.

2. Resource details

APP is an evolutionarily well-conserved type I transmembrane glycoprotein. Its cleavage can produce several different products, including β-amyloid (Aβ), the accumulation of which has long been considered as the major driving force in AD pathogenesis. The A673T mutation in APP strongly decreases APP cleavage by beta-secretase 1, thus reducing the generation of Aβ peptides and soluble APP-β (Jonsson

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Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography	Visual record of the line: normal	Fig. 1 panel A
Phenotype	Immunocytochemistry	Positive staining of pluripotency markers: Oct4, Nanog, TRA-1-81, SSEA4	Fig. 1 panel B
	RT-qPCR	Expression of Lin28, Nanog, Sox2	Fig. 1 panel C
Genotype	Karyotype (G-banding) and resolution	46 XY Resolution of 400 band level	Fig. 1 panel E
Identity	STR analysis	13 sites tested, all matched with parental fibroblasts cell line	available with author
Mutation analysis	Sequencing	Heterozygous p.A673T in *APP*	Fig. 1 panel F
Microbiology and virology	Mycoplasma	Mycoplasma testing by PCR. Negative.	Fig. 1 panel D
Differentiation potential	Embryoid body formation	Positive staining for smooth muscle actin (SMA), beta-III-tubulin (BIIITub) and α-feto protein (AFP)	Fig. 1 panel G
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

et al., 2012; Maloney et al., 2014). It is also increasingly recognized that APP plays an important role in normal physiological processes (Brothers et al., 2018). APP is upregulated early in the development, and its cleavage products can regulate neurogenesis, neuronal migration, synapse formation and activity (Nicolas and Hassan, 2014). Aβ is also known to have antimicrobial activity (Brothers et al., 2018). We have previously reported the generation of an iPSC line from a 65-year old male expressing a rare Chr21: 25,897,620 G > A variant (rs63750847) in the *APP* gene (Lehtonen et al., 2018). Here we have recruited another 58-year-old healthy (without neurological conditions) Finnish male individual carrying the same mutation and generated a new iPSC line (Table 1).

Fibroblasts were reprogrammed using CytoTune™-iPS 2.0 Sendai Reprogramming Kit. The colonies were manually picked and expanded clonally in feeder-free conditions on Matrigel coating. Here, we present the detailed characterization for clone MADGIC 12B. As expected, MADGIC 12B cells were positive for pluripotency markers OCT-4, NANOG, TRA-1-81 (podocalyxin), and SSEA4 as demonstrated by immunocytochemical staining (Fig. 1B). The cells also strongly upregulated *NANOG*, *SOX2*, and *LIN28* and downregulated Sendai virus (*SeV*) gene expression compared to the parental fibroblasts shown by quantitative real-time PCR (Fig. 1C). Chromosomal analysis showed a normal karyotype (Fig. 1E). STR analysis confirmed the identical genetic background of the donor fibroblasts and the iPSC clone (data available from the authors). PCR amplification of *APP* locus followed by Sanger sequencing confirmed the presence of a heterozygous Chr21: 25,897,620 G > A mutation (Fig. 1F). In addition, MADGIC 12B line formed embryoid bodies (EBs) when plated in the ultra-low adherent plate. Immunocytochemical analysis of the EBs was performed after 28 days of culture. It showed a spontaneous differentiation into cell types representative of the three embryonic germ layers, including smooth muscle antibody (SMA)-positive cells (mesoderm), alpha-feto-protein (AFP)-positive cells (endoderm) and beta-III-tubulin (B-III-TUB)-positive cells (ectoderm) (Fig. 1G). A PCR-based mycoplasma detection test was negative (Fig. 1D).

3. Materials and methods

3.1. Generation of induced pluripotent stem cells

Punch skin biopsy was collected from a healthy male individual recruited by Kuopio University Hospital, Finland, after obtaining informed consent. Skin fibroblasts were expanded as described previously (Lehtonen et al., 2018) and transduced using CytoTune™-iPS 2.0 Sendai Reprogramming Kit (Thermo Fisher Scientific) according to the manufacturer's instructions in fibroblast culture medium containing Iscove's DMEM with 20% fetal bovine serum, 1% penicillin–streptomycin and 1% non-essential amino acids (all from Thermo Fisher Scientific). At day 6 post-transduction, fibroblast culture medium was replaced with Essential 6 Medium (E6, Thermo Fisher Scientific) supplemented with 100 ng/ml basic fibroblast growth factor (bFGF; Peprotech). At three weeks post-transduction, individual reprogrammed iPSC colonies were selected based on morphology and re-plated onto Matrigel (growth factor reduced; Corning; 1:200)-coated 24-well plates. Four iPSC clones were further expanded and grown routinely in feeder-free conditions in Essential 8 Medium (Thermo Fisher Scientific) on Matrigel-coated (1:200) 3.5 cm dishes at 37 °C 5% CO_2. The cells were passaged with 0.5 mM EDTA every 4–5 days.

3.2. Genetic analysis

To confirm identical genetic background of the derived clone and parental fibroblasts, thirteen microsatellite loci (D8S1179, D21S11, D16S538, D2S1338, D18S51, VWA_CHR12, FGA_CHR4, D10S1248, D12S391, D4S385a/b, SE33, CSF1PO and HPTRB; Table 2) were analyzed by conventional PCR using MyTaq DNA polymerase (Bioline). Genomic DNA was isolated from the cells using the NucleoSpin Tissue Mini DNA extraction kit (Macherey-Nagel). PCR products were resolved by running electrophoresis for 1.5 h on a 3% agarose gel in TBE buffer. The presence of the A673T mutation was confirmed by PCR and Sanger sequencing performed at the Institute for Molecular Medicine Finland (FIMM), University of Helsinki (primers listed in Table 2, Rev-primer used for sequencing).

3.3. Embryoid body formation

To prove our new iPSC line's ability to differentiate to all three germ layers spontaneously, we performed an embryoid body (EB) formation assay. IPSCs colonies were detached by using ReLeSR passaging reagent (StemCell Technologies) and cultured in suspension for two weeks in ultra-low adherent 6-well plates (Corning) containing DMEM medium supplemented with 20% Serum replacement and 1% Penicillin-Streptomycin (Thermo Fisher Scientific). Afterwards, EBs were re-plated onto Matrigel-coated 24-well plates and left to differentiate for two more weeks. The scale bar for EBs in suspension is 200 μm.

3.4. Quantitative RT-PCR and immunocytochemistry

RNA was extracted using the RNeasy Mini kit (Qiagen), and quantitative RT-PCR was done using commercially available Taqman probes (Table 2) according to the manufacturer's instructions. For immunocytochemistry, plated iPSCs and EBs were fixed in 4% formaldehyde for 20 min at room temperature and stained with the primary antibodies (Table 2) diluted in 5% normal goat serum in PBS with

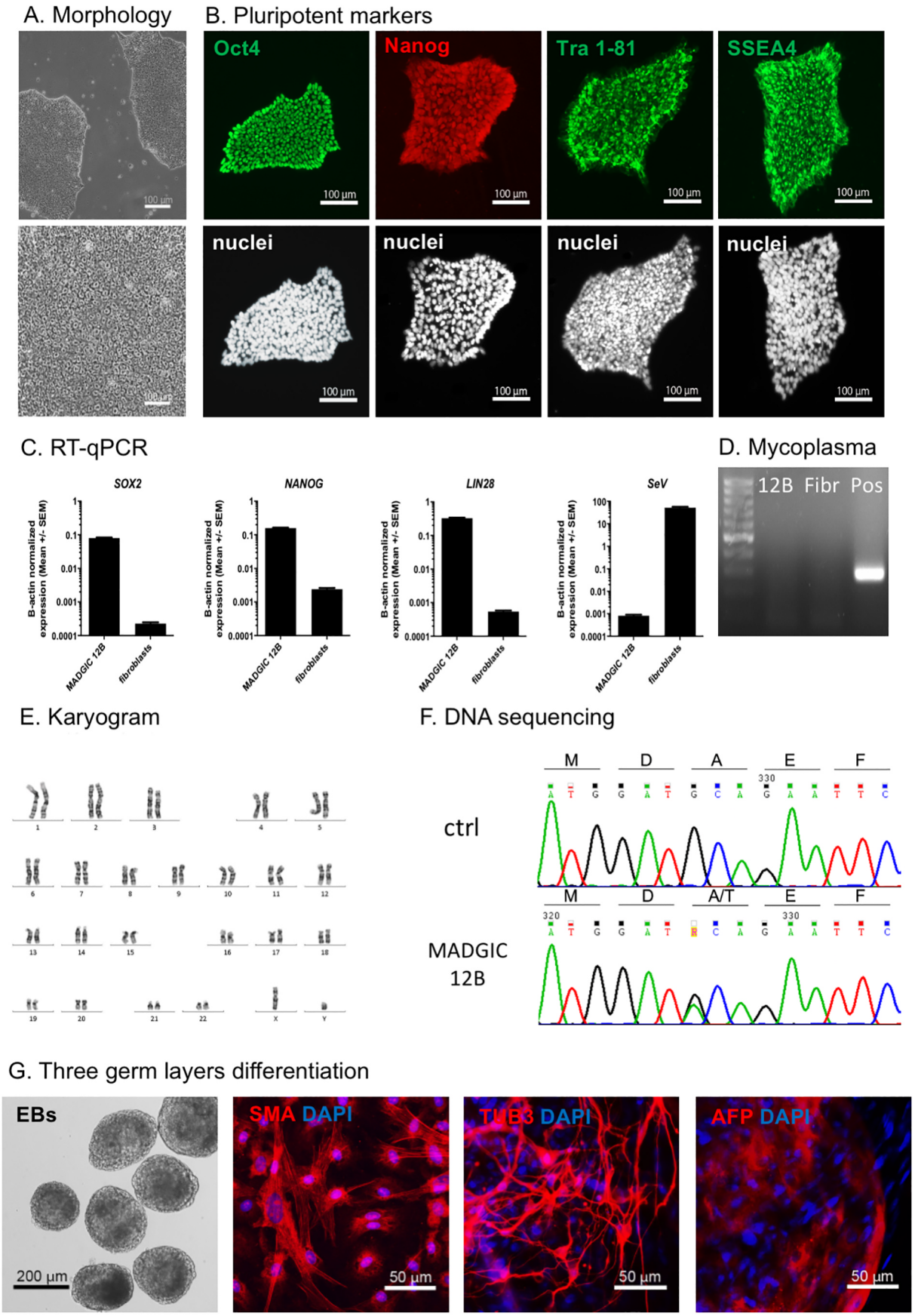
A. Morphology
B. Pluripotent markers
Oct4
Nanog
Tra 1-81
SSEA4
nuclei
100 µm
C. RT-qPCR
SOX2
NANOG
LIN28
SeV
B-actin normalized expression (Mean +/- SEM)
MADGIC 12B
fibroblasts
D. Mycoplasma
12B Fibr Pos
E. Karyogram
F. DNA sequencing
ctrl
MADGIC 12B
M D A E F
M D A/T E F
G. Three germ layers differentiation
EBs
200 µm
SMA DAPI
TUB3 DAPI
AFP DAPI
50 µm

Fig. 1.

Table 2
Reagents details.

Antibodies used for immunocytochemistry			
	Antibody	**Dilution**	**Company Cat # and RRID**
Pluripotency Markers	Mouse anti-Oct4	1:400 1:100	EMD Millipore; cat.MAB4401; RRID:AB_2167852
	Goat anti-Nanog	1:200 1:400	R&D Systems; cat. AF1997; RRID:AB_355097
	Mouse anti-TRA-1-81		EMD Millipore; cat. MAB4381; RRID:AB_177638
	Mouse anti-SSEA4		EMD Millipore; cat. MAB4304; RRID:AB_177629
Differentiation Markers	Mouse anti-SMA	1:300 1:1000	Sigma-Aldrich; cat. A5228; RRID:AB_262054
	Mouse anti-B -III-tubulin	1:300	Covance; cat. MMS-435P; RRID:AB_2313773
	Mouse anti-AFP		Sigma-Aldrich; cat. A8452; RRID:AB_258392
Secondary antibodies	Goat anti-mouse Alexa Fluor 488	1:300 1:300	Molecular Probes; cat. A11001; RRID:AB_2534069
	Goat anti-mouse Alexa Fluor 568	1:300	Molecular Probes; cat. A11004; RRID:AB_2534072
	Donkey anti-goat Alexa Fluor 568		Molecular Probes; cat. A11057; RRID:AB_142581
Primers			
	Target	**Forward/Reverse primer (5′-3′)**	
Mutation analysis/ sequencing	*APP*	TGGCAAGACAAACAGTAGTGG/ CTTGCCAACCTCTCAACCAG (453 bp)	
STR analysis	*D8S1179*	GTATCGTATCCCATTGCGTG/ CGCCTTTGCCTGAGTTTTG (197–249 bp)	
	D21S11	TGTGAGTCAATTCCCCAAGTG/ CACTGAGAAGGGAGAAACACTG (286–344 bp)	
	D16S538	GTTCCCATTTTTATATGGGAGC/ TTTACGTTTGTGTGTGCATCTG (165–213 bp)	
	D2S1338	GAAGCCAGTGGATTTGGAAAC/ TCCTACCAGAATGCCAGTCC (206–274 bp)	
	D18S51	CATGCCACTGCACTTCACTC/ AAGGTGGACATGTTGGCTTC (169–256 bp)	
	VWA_CHR12	CCCTAGTGGATGATAAGAATAATCAGTATG/ GGACAGATGATAAATACATAGGATGGATGG (122–182 bp)	
	FGA_CHR4	TGCCCCATAGGTTTTGAAC/ CTTTGCGCTTCAGGACTTC (266–422 bp)	
	D10S1248	GGAATAAGTGCAGTGCTTGG/ ACCAATCTGGTCACAAACAT (227–271 bp)	
	D12S391	AACAGGATCAATGGATGCAT/ TGGCTTTTAGACCTGGACTG (209–253 bp)	
	DYS385 a/b	AGCATGGGTGACAGAGCTA/ TGGGATGCTAGGTAAAGCTG (352–436 bp)	
	SE33	AATCTGGGCGACAAGAGTGA/ ACATCTCCCCTACCGCTATA (197–343 bp)	
	CSF1PO	AACCTGAGTCTGCCAAGGACTAGC/ TTCCACACACCACTGGCCATCTTC (287–331 bp)	
	HPRTB	ATGCCACAGATAATACACATCCCC/ CTCTCCAGAATAGTTAGATGTAGG (259–303 bp)	
Mycoplasma testing	*MGSO_GPO3*	TGCACCATCTGTCACTCTGTTAACCTC/ GGGAGCAAACAGGATTAGATACCCT (271 bp)	
Primers	**Target**	**Company**	
Pluripotency Markers (qPCR)	*Nanog*	Thermo Fisher Scientific; cat. Hs02387400_g1	
	Lin28	Thermo Fisher Scientific; cat. Hs00702808_s1	
	Sox2	Thermo Fisher Scientific; cat. Hs01053049_s1	
Housekeeping Genes (qPCR)	*ACTB*	Thermo Fisher Scientific; cat. 4326315E	
Sendai virus	SeV	Thermo Fisher Scientific; cat. Mr04269880_mr	

the exception of Nanog antibody, which was diluted in 0.1% bovine serum albumin in PBS, at 4 °C overnight. For the staining of nuclear markers, cells were permeabilized with 0.2% Triton X-100. The following day, the secondary antibodies (1:300 dilution, Table 2) were added for 1 h at room temperature. Images were taken using a Zeiss AXIO microscope. Scale bars are 100 μm for iPSCs and 50 μm for plated EBs.

3.5. Karyotype analysis

Karyotyping was performed using Giemsa (G-banding) staining as described (Lehtonen et al., 2018). Twenty metaphases were analyzed. The analyses were performed at the Yhtyneet Medix laboratoriot,

Finland (http://www.yml.fi/).

3.6. Mycoplasma testing

The absence of mycoplasma contamination was confirmed by PCR reaction targeting mycoplasma-specific 16S ribosomal RNA (primer sequence listed in Table 2) followed by gel electrophoresis.

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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