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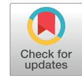
Lab Resource: Stem Cell Line

Generation of a human induced pluripotent stem cell line from a patient with a rare A673T variant in amyloid precursor protein gene that reduces the risk for Alzheimer's disease



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A B S T R A C T

An amyloid precursor protein (APP) A673T mutation was found to be protective against Alzheimer's disease (AD) and cognitive decline in the Icelandic population and to associate with decreased levels of plasma β-amyloid in a Finnish population-based cohort. Human fibroblasts from a Finnish male individual carrying the protective mutation were used to generate integration-free induced pluripotent stem cell (iPSCs) line by Sendai virus technology. The iPSC line retained the mutation and expressed pluripotency markers, had a normal karyotype and differentiated into all three germ layers.

Resource table.

Unique stem cell line identifier	UEFi001-A
Alternative name(s) of stem cell line	MADGIC 4A
Institution	A.I.Virtanen Institute for Molecular Sciences University of Eastern Finland
Contact information of distributor	Jari Kostinaho, jari.koistinaho@uef.fi; jari.koistinaho@helsinki.fi
Type of cell line	iPSC
Origin	Human
Additional origin info	Age: 65 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	NO modification
Type of Modification	NO modification
Associated disease	Protective against Alzheimer's disease
Gene/locus	APP (MIM # 104760) located on the chromosome 21q21.3 genotype c.2017G > A substitution (rs63750847)
Method of modification	NO modification
Name of transgene or resistance	NO transgene or resistence
Inducible/constitutive system	NO inducible
Date archived/stock date	N/A
Cell line repository/bank	N/A
Ethical approval	Northern Savo Hospital district (license no. 123/2016)

Resource utility

Alzheimer's disease (AD) is the leading cause of dementia worldwide. While several mutations in *APP* gene are responsible for familial early-onset AD, APP A673T mutation is associated with reduced risk for

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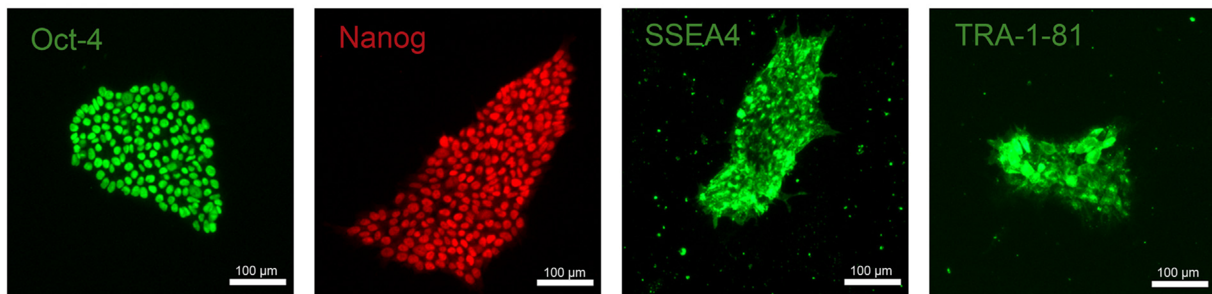

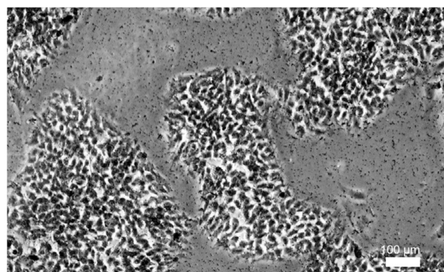

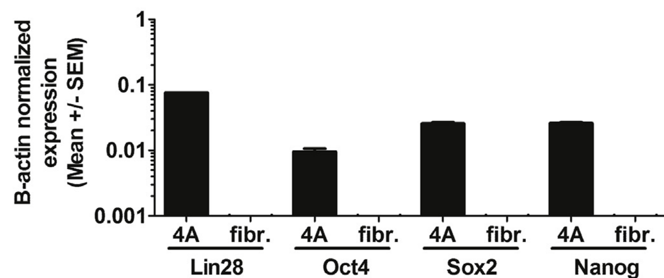

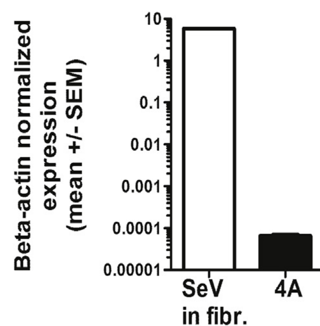

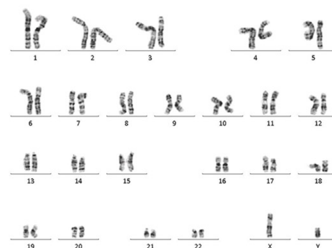

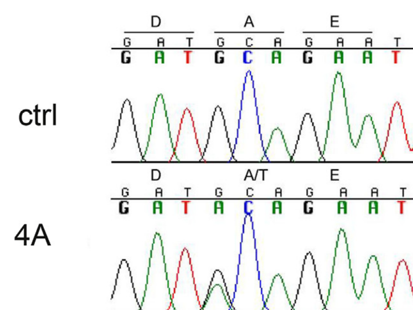

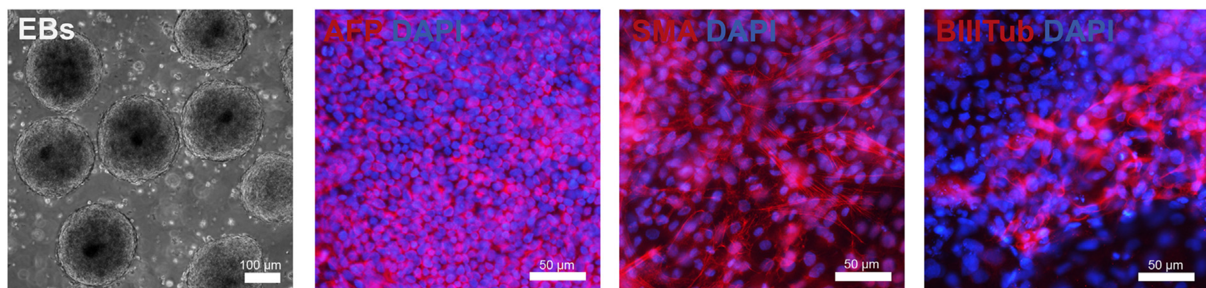

Fig. 1. Characterization of MADGIC 4A iPSC line.

AD. The generated line could be used for *in vitro* modeling to explore the mechanism behind its protective role.

Resource details

The APP is best known for being a precursor molecule for β-amyloid (Aß). The fibrillar form Aß is the primary component of amyloid plaques found in the brain of AD patients. Here, we report the generation of an iPSC line from a 65-year old male expressing a rare c.2017G > A variant in the APP gene. This substitution is next to the aspartyl protease β-site in APP and results in an approximately 40% reduction in the formation of Aß peptides *in vitro* (Jonsson et al. 2012). Moreover, carriers of the APP A673T variant have 28% lower levels of Aß40 and Aß 42 in plasma (Martiskainen et al. 2017).

Fibroblasts were reprogrammed using CytoTune™-iPS 2.0 Sendai Reprogramming Kit. The colonies were manually picked and expanded clonally. Four clones were selected based on morphology for further characterization using methods described previously (Holmqvist et al. 2016). Here, we present the detailed characterization for clone MADGIC 4A. The expression of pluripotency-promoting genes including *OCT4, SOX2, NANOG,* and *LIN28* was confirmed by quantitative real-time PCR (Fig. 1C), and by immunocytochemistry staining for Nanog, Oct-4, TRA-1-81, and SSEA4 (Fig. 1A). The clearance of the Sendai virus was confirmed at passage 11 (Fig. 1D). The alkaline phosphatase

Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography	Visual record of the line: normal	Not shown but available with author
Phenotype	Immunocytochemistry	Positive staining of pluripotency markers: Oct4, Nanog, TRA-1-81, SSEA4	Fig. 1 panel A
	Alkaline Phosphatase activity	Visible activity	Fig. 1 panel B
	RT-qPCR	Expression of Nanog Lin28, Oct4, 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	7 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 luminescence. Negative.	Not shown but available with the author
Differentiation potential	Embryoid body formation	Positive staining for α-feto protein (AFP), smooth muscle actin (SMA) and beta-III-tubulin (BIIITub)	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

Table 2
Reagents details.

Antibodies used for immunocytochemistry

	Antibody	Dilution	Company Cat # and RRID
Pluripotency markers	Mouse anti-Oct4	1:400	EMD Millipore; cat.MAB4401
	Goat anti-Nanog	1:100	R&D Systems; cat. AF1997
	Mouse anti-SSEA4	1:400	EMD Millipore; cat. MAB4304
	Mouse anti-TRA-1-81	1:200	EMD Millipore; cat. MAB4381
Differentiation markers	Mouse anti-SMA	1:300	Sigma-Aldrich; cat. A5228
	Mouse anti-AFP	1:300	Sigma-Aldrich; cat. A8452
	Mouse anti-B -III-tubulin	1:1000	Covance; cat. MMS-435P
Secondary antibodies	Goat anti-mouse Alexa Fluor 488	1:300	Molecular Probes, cat. A11001
	Goat anti-mouse Alexa Fluor 568	1:300	Molecular Probes; cat. A11004
	Donkey anti-goat Alexa Fluor 568	1:300	Molecular Probes; cat. A11057

Primers

	Target	Forward/reverse primer (5′-3′)
Mutation analysis/sequencing	*APP*	Fw 5′-TGGCAAGACAAACAGTAGTGG-3′ Rev. 5′-CTTGCCAACCTCTCAACCAG-3′

Primers	Target	Company
Pluripotency markers (qPCR)	*Nanog*	Thermo Fisher Scientific; cat. Hs02387400_g1
	Lin28	Thermo Fisher Scientific; cat. Hs00702808_s1
	Oct4	Thermo Fisher Scientific; cat. Hs00742896_s1
	Sox2	Thermo Fisher Scientific; cat. Hs01053049_s1
House-keeping genes (qPCR)	*ACTB*	Thermo Fisher Scientific; cat. 4326315E
Sendai virus	SeV	Thermo Fisher Scientific; cat. Mr04269880_mr

staining was positive (Fig. 1B), and chromosomal analysis showed a normal karyotype (Fig. 1E). STR analysis confirmed identical genetic background of the donor fibroblasts and the iPSC clone (data available from the authors). To confirm the presence of the missense mutation, a DNA sample from the iPSC clone was sequenced for the *APP* loci, which confirmed a heterozygous c.2017G > A transition (Fig. 1F). In addition, MADGIC 4A line formed embryoid bodies (EBs) when plated in low adherent plate. Immunocytochemical analyses of the EBs were performed after 14 days of culture and showed a spontaneous differentiation into cell types representative of the three embryonic germ layers, including smooth muscle antibody (SMA)-positive cells (mesoderm), alpha-fetoprotein (AFP)-positive cells (endoderm) and beta-III-tubulin (B-III-TUB)-positive cells (ectoderm) (Fig. 1G). A luminescence-based mycoplasma detection test was negative (data available from the authors).

Materials and methods

Fibroblast culture

Skin biopsy-derived fibroblasts were obtained from a patient recruited by Kuopio University Hospital in Finland, after obtaining informed consent. The fibroblasts were expanded in fibroblast culture media containing Iscove's DMEM media (Thermo Fisher Scientific) with 20% fetal bovine serum, 1% Penicillin-Streptomycin and 1% non-essential amino acids.

Generation of induced pluripotent stem cells

For reprogramming, 150,000 cells were plated on a 6-well plate and maintained in fibroblast culture media for 2 days. The cells at 90% confluency were transduced using CytoTune™-iPS 2.0 Sendai Reprogramming Kit (Thermo Fisher Scientific) with three separate vectors carrying genes h*OCT-3/4, hKLF-4, hSOX-2 and hc-MYC* to induce pluripotency. The media was changed 24 h after transduction and then daily. At day 6, fibroblast culture medium was replaced with Essential 6 Medium (E6, Thermo Fisher Scientific) supplemented with 100 ng/ml basic fibroblast growth factor (bFGF). On the next day, the cells are re-plated onto 6-well matrigel-coated plates with a density of 60,000 cells/well. Between days 17–28, the individual colonies were picked to 24-well matrigel-coated plate containing Essential 8 Medium (E8, Thermo Fisher Scientific) and passaged with 0.5 mM EDTA weekly. One week later, four colonies were selected and expanded on 6-well plates with daily media changes (Table 1).

Characterization of pluripotency by RT-qPCR and immunocytochemistry

The total RNA from the iPSCs was extracted using RNeasy Mini kit (Qiagen), and synthesis of cDNA was carried out using Maxima reverse transcriptase enzyme approach (Thermo Fisher Scientific). Maxima Probe qPCR Master Mix (Thermo Fisher Scientific) and commercially available Taqman probes (Table 2) were used in qPCR measurement by StepOne Plus machine. For immunocytochemistry, plated iPSCs were fixed in 4% paraformaldehyde for 20 min at room temperature (RT), permeabilized with 0.2% Triton X-100 (in case of Nanog and Oct-4), blocked in 5% normal goat serum at RT for 1 h and finally incubated with the primary antibodies (Table 2) overnight at 4 °C. On the following day, the secondary antibodies (1:300 dilution) were added for 1 h at RT. Images were taken using a Zeiss AXIO microscope. Scale bars are 100 μm.

Alkaline phosphatase activity

Plated iPSCs were stained with Nitro blue tetrazolium chloride/5-Bromo-4-chloro-3-indolyl phosphate, toluidine salt) (Roche) at a dilution of 1:50 in buffer containing 100 mM Tris-HCl pH 8.2–8.5, 100 mM NaCl, 50 mM MgCl2 and incubated for 20 min in the dark before imaging.

Embryoid body formation

Pluripotency was confirmed by EB-based differentiation. Small undifferentiated iPSCs colonies were lifted up by scalpel to ultra low-adherent dishes (Corning) and cultured in DMEM media (Thermo Fisher Scientific) supplemented with 20% serum replacement and 1% Penicillin-Streptomycin. Afterwards, EBs were plated onto Matrigel-coated 24-well plates and left to differentiate for two weeks. Scale bar for EBs is 100 μm. Scale bars for differentiated cells are 50 μm.

Karyotype analysis

Karyotyping was performed using Giemsa (G-banding) staining, after arresting the cells in the metaphase by 200 ng/ml *N*-desacetyl-*N*-methyl colchicine. The analyses were performed at the Yhtyneet Medix laboratoriot, Finland (http://www.yml.fi/).

Genetic analysis

Genomic DNA was isolated from the parental fibroblasts and the iPSC clones with geneJET genomic DNA purification kit (Thermo Fisher Scientific). To verify the genetic identity of the clones seven microsatellite loci were analyzed by PCR and gel electrophoresis (primer sequences available upon request). Further, the presence of the mutation was confirmed by PCR and Sanger sequencing (Primers listed in Table 2, Fw-primer used for sequencing.)

Mycoplasma testing

The absence of mycoplasma contamination was confirmed using the MycoAlert™ Mycoplasma Detection Kit (Lonza).

Acknowledgment

We thank Laila Kaskela and Eila Korhonen for their outstanding technical assistance.

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