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Lab resource: Stem cell line

Generation of induced pluripotent stem cells (IRMBi001-A) from an Alzheimer's disease patient carrying a G217D mutation in the *PSEN1* gene

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A B S T R A C T

Induced pluripotent stem cells (iPSC) were generated from skin fibroblasts obtained from a 50 year-old patient suffering from Alzheimer's disease and carrying a G217D causal mutation on *presenilin 1* (*PSEN1*). iPSCs were obtained following reprogramming using the integration-free Sendai Virus system which allows expression of the Yamanaka factors. Verification of their pluripotency was achieved by demonstrating the expression of pluripotency markers and their differentiation potential into the three primary germ layers. iPS cells carry the patient G217D mutation and present a normal karyotype. The reported PS1-G217D iPSC line may be used to model and study human AD pathology *in vitro*.

Resource table

Unique stem cell line identifier	IRMBi001-A
Alternative name(s) of stem cell line	AD-PS1 hiPSC
Institution	Institute for Regenerative Medicine and Biotherapy (IRMB), U1183 INSERM, Montpellier, France
Contact information of distributor	Carole Crozet: carole.crozet@inserm.fr
Type of cell line	iPSC
Origin	Human
Additional origin info	Age: 54 y.o. Sex: Male
Cell Source	Fibroblasts
Clonality	Clonal
Method of reprogramming	Sendai Virus Cytotune 2.0 kit (Thermofisher Scientific Inc.), hOCT4, hSOX2,hC-MYC, hKLF4
Genetic Modification	YES
Type of Modification	Spontaneous Mutation
Associated disease	Alzheimer's disease
Gene/locus	PSEN1 c.650G > A p.Gly217Asp exon 7, heterozygous mutation
Method of modification	N/A
Name of transgene or resistance	N/A
Inducible/constitutive system	N/A
Date archived/stock date	30/11/15
Cell line repository/bank	https://hpscreg.eu/cell-line/IRMBi001-A
Ethical approval	DC-2015-2363 régional scientific ethical comittee CPP Sud Med IV Informed consent was obtained from the patient.

Resource utility

The reported PS1-G217D iPSC line may be used as a promising model and to study human Alzheimer's disease pathology *in vitro*.

Resource details

Skin fibroblasts were obtained from a 54 year-old man diagnosed with Alzheimer's disease (AD) and carrying a causal mutation (c.650G > A) in exon 7 of *presenilin 1* (*PS1* or *PSEN1*) gene. Presenilins are part of the γ-secretase complex and are responsible for its catalytic activity. They are thus involved in the proteolytic cleavage of the amyloid precursor protein (APP) into Aβ peptides that are found in

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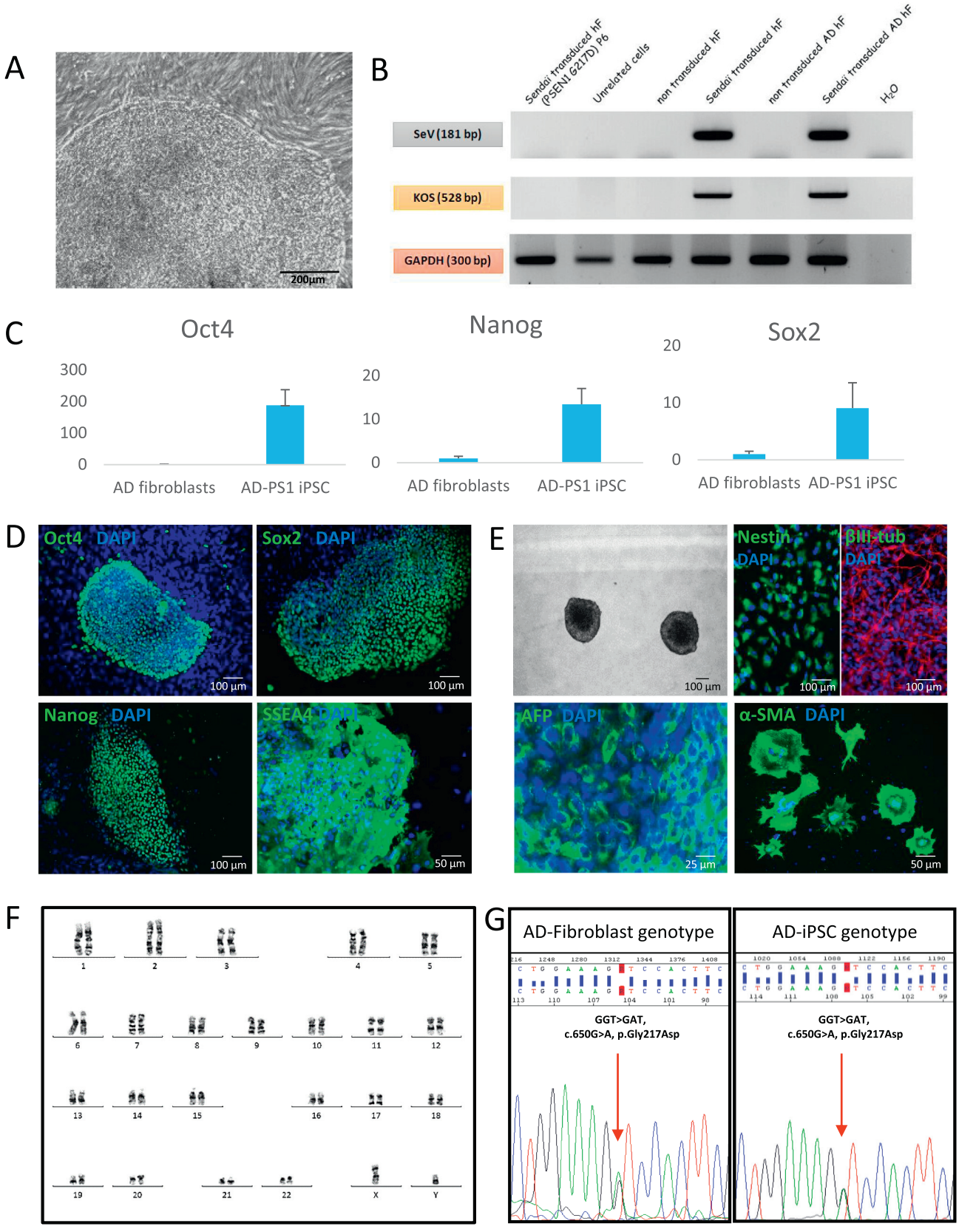

Fig. 1. Characterization and validation of IRMBi001-A.

amyloid plaques in AD. Several mutations in *PS1* are associated with the most severe phenotypes of AD and are considered the most common cause of genetic AD (Lanoiselee et al., 2017). Fibroblasts were reprogrammed into iPSCs (PSEN1G217D iPSC or AD-PS1 iPSC) using the integration-free Sendai Virus (Life Technologies, Invitrogen) carrying the sequences encoding the four Yamanaka factors OCT3/4,

SOX2, KLF4 and c-MYC. Several iPSC colonies were picked for expansion and generation of different iPSC colonies that exhibit normal morphology (Fig. 1, panel A). The absence of Sendaï virus genome from the iPSC, was successfully established by RT-PCR analysis with Sendai Virus and KOS sequence specific primers (Fig. 1, panel B, Table 2). We showed the absence of the transgene and viral sequences in the negative non transduced human fibroblasts (hF) control and in the PSEN1G217D iPSC (collected after 6 passages) compared to positive controls consisting of fibroblasts freshly transduced with the Sendaï virus (Sendaï transduced hF, Fig. 1, panel B). Expression of the endogenous pluripotency genes OCT4, SOX2, NANOG was assessed by RT-qPCR analysis (Fig. 1, panel C) and OCT4, SOX2, NANOG and the embryonic stem cell specific marker SSEA4 by immunofluorescence staining (Fig. 1, panel D). Pluripotency was evaluated following *in vitro* differentiation through the formation of embryoid bodies (Fig. 1, panel E). Expression of nestin and βIII-tubulin ((neuro)ectoderm layer), alpha-feto protein (AFP) (endoderm layer) and the alpha-smooth muscle actin (α-SMA) marker (mesoderm layer) was assessed by immunofluorescence staining more than three months post-differentiation (Fig. 1, panel E). It demonstrates the ability of iPSC to differentiate into the three primary germ layers (Fig. 1, panel E). Clones were found with a normal 46,XY karyotype (Fig. 1, panel F) after 26 passages in culture. Sanger sequencing analysis of AD-iPSCs and AD-fibroblasts showed the presence of a c.650G > A change in exon 7 of *PSEN1* gene, corresponding to a p.Gly217Asp mutation on PSEN1, as illustrated on the DNA electrophoregram (Fig. 1, panel G). Microsatellite analyses were performed on DNA extracted from parental fibroblasts and iPSCs, using the following four short tandem repeats (STR): D1S439, D9S1784, D14S986 and D19S913 (available with authors). The cell lines were also shown to be free of mycoplasma (Supplemental Fig. 1).

Materials and methods

Generation of iPSC

Fibroblasts were obtained from a skin biopsy of a man carrying the PS1G217D causal mutation (Table 1). Fibroblasts were amplified in DMEM (Gibco, Life-Technologies, Thermo Fisher Scientific), 10% fetal bovine serum (Biowhittaker, Lonza), 1% non-essential-amino-acids (Gibco, Life Technologies, Thermo Fisher Scientific), 0,1 mM β-mercaptoethanol (Gibco, Life Technologies, Thermo Fisher Scientific), 1 mM glutamine (Gibco, Life Technologies, Thermo Fisher Scientific) and 1% penicillin/streptomycin (Gibco, Life Technologies, Thermo Fisher Scientific). Fibroblasts were reprogrammed using the non-integrating Sendai Virus («CytoTune™-iPS 2.0 Sendai Reprogramming Kit» LifeTechnologies, Thermo Fisher Scientific) following the manufacturer's instruction using MOI of 5–5-3 (KOS MOI = 5, hc-Myc MOI = 5, hklf4 MOI = 3).

Pluripotent stem cells culture

IRMBi001-A iPSC colonies were cultured on feeder (Mouse embryonic (E12.5) fibroblast (MEF) from CF1 mice, Charles River). They were mechanically passaged every 4–5 days into clumps, with a split ratio of 1:5, on mitomycin-inactivated MEF on 0.1% Gelatin (Sigma Aldrich) coated dishes (Corning, Dutscher) and maintained in KODMEM/F12 (Gibco, Life Technologies, Thermo Fisher Scientific), 20% KOSR (Gibco, Life Technologies, Thermo Fisher Scientific), 1% non-essential-amino-acids (Gibco, Life Technologies, Thermo Fisher Scientific), 0,1 mM β-mercaptoethanol (Gibco, Life Technologies, Thermo Fisher Scientific), 1 mM glutamine, 1% penicillin/streptomycin (Gibco, Life Technologies, Thermo Fisher Scientific), and 5 ng/mL bFGF (Miltenyi Biotec). The cells were cultivated in a humidified incubator at 37 °C, in hypoxic conditions 5% CO_2 and 5% O_2 (New Brunswick™ Innova, Laboservices). The iPSC lines were frozen in liquid nitrogen in iPSC medium containing 10% DMSO (Miltenyi Biotec).

PCR analysis

Total RNA was extracted from fibroblasts, and the iPSC (passage 6) using RNeasy micro kit (Qiagen). cDNA were obtained following reverse transcription using the Superscript III First-strand synthesis system (Invitrogen, Thermo Fisher Scientific) on a Master Cycle gradient device (Eppendorf). Disappearance of the Sendaï virus genome and transgene sequences was assessed using Sendai-virus-specific primers (Table 2) and conventional PCR. The PCR mix was composed of cDNA (300 ng), the forward and reverse primers (see Table 2), 0.3 µl at 10 µM for each, dNTPs (10 nM) GoTaq buffer and GoTaq DNA polymerase (Promega). The PCR was performed on a Master Cycle gradient device (Eppendorf): 5 min of DNA denaturation at 95 °C, 40 cycles (95 °C during 60s, 55 °C for 60s and 72 °C for 20s), final elongation for 5 min at 72 °C. Products were analysed on 2% agarose gel, ran at 130 Velectric field. Finally the picture was acquired using Ebox Vilber System.

For the expression of the endogenous genes of pluripotency, qPCR analysis was performed using the Light Cycler 480 device (Roche Diagnostics) using SyberGreen I Master Kit (Roche Diagnostics). The relative quantification was calculated using the $2^{-\Delta\Delta Ct}$ method with fibroblasts as controls and GAPDH as housekeeping gene (Primers are listed in Table 2).

Immunofluorescence staining

Cells were fixed on coverslips with 4% paraformaldehyde (15 min)

Table 1
Characterization and validation.

Classification	*Test*	*Result*	*Data*
Morphology	Photography	Normal	Fig. 1 panel A
Phenotype	qPCR analysis	Positive for OCT4, NANOG, SOX2	Fig. 1 panel C
	Immunocytochemisty	Positive for OCT4, NANOG, SOX2, SSEA4	Fig. 1 panel D
Genotype	Karyotype (G-banding) and resolution	46XY Resolution: 400	Fig. 1 panel F
Identity	Microsatellite PCR (mPCR)	Not performed	
	STR analysis	4 Matched sites D1S439, D9S1784 D14S986 and D19S913	Not shown but available with author
Mutation analysis	Sequencing	Heterozygous mutation in fibroblasts and iPSCs.	Fig. 1 panel G
Microbiology and virology	Mycoplasma	Mycoplasma testing by luminescence (MycoAlert Kit, Lonza). Negative	Supplementary Figure
Differentiation potential	Embryoid body formation	Expression of genes in embryoid bodies: alpha-smooth muscle actin (a-SMA), βIII-tubulin and α-feto protein (AFP).	Fig. 1 panel E
Donor screening (OPTIONAL)	HIV 1 + 2, Hepatitis B, Hepatitis C	Negative	Not shown but available with author
Genotype additional info (OPTIONAL)	Blood group genotyping	NA	NA
	HLA tissue typing	NA	NA

Table 2
Reagents details.

Antibodies used for immunocytochemistry/flow-cytometry			
	Antibody	*Dilution*	*Company Cat # and RRID*
Pluripotency markers	Mouse anti-OCT4	1/500	Santa Cruz Biotechnology Cat# sc-5279, RRID:AB_628051
	Rabbit anti-SOX2	1/200	Bethyl Cat# A301-739A, RRID:AB_1211354
	Goat anti-NANOG	1/100	R and D Systems Cat# AF1997, RRID:AB_355097
	Mouse anti-SSEA4	1/100	Millipore Cat# MAB4304, RRID:AB_177629
Differentiation markers	Mouse anti-actin α-SMA	1/200	Lab Vision Cat# MS-113-P0, RRID:AB_64001
	Rabbit anti-AFP	1/1000	Lab Vision Cat# RB-9064-P0, RRID:AB_177884
	Mouse anti-βIII-TUBULIN	1/1000	Covance Research Products Inc. Cat# MMS-435P, RRID:AB_2313773
	Rabbit anti-NESTIN	1/500	Millipore Cat# ABD69, RRID:AB_2744681
Secondary antibodies	A555 Goat Anti-Mouse IgG	1/7000	Molecular Probes Cat# A21424, RRID:AB_141780
	A488 Donkey Anti-Mouse IgG	1/7000	Molecular Probes Cat# A21202, RRID:AB_141607
	A488 Donkey Anti-Rabbit IgG	1/7000	Molecular Probes Cat# A21206, RRID:AB_141708

Primers		
	Target	*Forward/Reverse primer (5′-3′)*
Transgenes from SeV vector (PCR)	SeV product size 181 bp	Forward: GGA TCA CTA GGT GAT ATC GAG C Reverse: ACC AGA CAA GAG TTT AAG AGA TAT GTA TC
	KOS product size 528 bp	Forward: ATG CAC CGC TAC GAC GTG AGC GC Reverse: ACC TTG ACA ATC CTG ATG TGG
	GAPDH product size 300 bp	Forward: CTG GCG TCT TCA CCA CCA TGG Reverse: CAT CAC GCC ACA GTT TCC CGG
Pluripotency markers (qPCR)	OCT4	Forward: TGTACTCCTCGGTCCCTTTC Reverse: TCCAGGTTTTCTTTCCCTAGC
	SOX2	Forward: ATGCACCGCTACGACGTGA Reverse: CTTTTGCACCCCTCCCATTTC
	NANOG	Forward: TGCCTCACACGGAGACTGTC Reverse: TGCTATTCTTCGGCCAGTTG
House-keeping gene (qPCR)	GAPDH	Forward: CGCTCTCTGCTCCTCCTGTT Reverse: CCATGGTGTCTGAGCGATGT

and washed 3 times in Dulbecco phosphate-buffered saline (D-PBS; Gibco, Life Technology, Thermo Fisher Scientific). After permeabilization with 0,5% Triton X-100 (Sigma Aldrich) during 30 min for nuclear stainings at room temperature (RT), non-specific binding sites were blocked with 0.2% BSA (MP BIOMEDICALS) in D-PBS during 1 h at RT. Cells were then incubated with primary antibodies diluted in D-PBS (Gibco Lifetechnology, Thermo Fisher Scientific) overnight at 4 °C. Primary antibodies are listed in Table 2. The Alexa-488 or − 555 secondary antibodies (Molecular Probe, Thermo Fisher Scientific) were used at a dilution of 1:7000 at RT for 1 h. The coverslips were then mounted in DAPI mounting medium (Life Technologies, Thermo Fisher Scientific). Pictures were captured with Axovision using a Zeiss Axiovert 200 M microscope.

In vitro differentiation

Embryoid-bodies formation was performed following 8 passages by transferring iPSC clumps to low-adhesion dishes (Falcon) in iPSC medium without bFGF. The medium was exchanged every two days during 30 days. After this period, the embryoid-bodies were mechanically dissociated and plated on 1% geltrex-coated dishes and cultured for up to two months in the same medium. Cells were then fixed for 15 min in 4% paraformaldehyde for immunofluorescence analysis with anti-NESTIN (neurectoderm), AFP (endoderm) and SMA (mesoderm) antibodies (Fig. 1, panel E). As our final goal for future studies is to analyse neural cells, we also plated out some EB in a medium containing KODMEM/F12, 1 mM L-glutamine, and 1% penicillin/streptomycin supplemented with 1%B27 (all from Gibco, Life Technologies, Thermo Fisher Scientific) during 10 additional days. Cells were then fixed for 15 min in 4% paraformaldehyde for immunofluorescence analysis with anti βIII-TUBULIN (neuronal lineage).

Karyotyping

Following 26 passages in culture, iPSCs were treated with colcemid (0,1 µg/ml, Sigma Aldrich) in KODMEM medium for 2 h at 37 °C and cells were harvested in cold fixative (75% methanol (Sigma Aldrich), 25% acetic acid (Sigma Aldrich)). Karyotyping was performed on G-banded metaphase chromosomes by the Cytogenetic platform at Hôpital St Eloi (Montpellier, France). At least 18 metaphases were analysed per sample. Picture acquisition was performed using the Metacyte Metafer System and chromosome analysis using the IKAROS software. The karyotype was found normal (46, XY), without any discernable abnormalities (Fig. 1 panel F).

Verification and authentication

DNA was extracted from fibroblasts and from iPSCs using the DNA blood and Tissue kit (Qiagen). RNA was extracted from fibroblasts and iPSCs using the RNeasy mini kit (Qiagen) and reverse transcription was performed using the Verso cDNA kit (Thermo Fisher Scientific).

STR analyses were performed on DNA extracted from fibroblasts and iPSCs, using the following markers: D1S439, D9S1784, D14S986 and D19S913. The PSEN1 c.650G > A variant p.Gly217Asp and the APP variant p.Asp694Asn were validated on DNA and cDNA from RNA extracted from iPSCs and fibroblasts by Sanger sequencing (primers available upon request). Sanger sequences and microsatellite profiles were run on a 3500 Genetic Analyzer (Applied Biosystems). Sequences (Fig. 1, panel G) and microsatellite profiles were analysed using the Sequencing Analysis Software v6.0 and the GeneMapper Software 5 (Applied Biosystems), respectively. STR results are available with the authors.

Mycoplasma testing

Absence of mycoplasma contamination was assessed on cell culture supernatants of iPS cells, using bioluminescence MycoAlert® Detection Kit (Lonza) according to manufacturer's instructions. The ratio Lecture B/Lecture A should be < 0.9 in mycoplasm free cultures.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scr.2018.101381.

References

Lanoiselee, H.M., Nicolas, G., Wallon, D., Rovelet-Lecrux, A., Lacour, M., Rousseau, S., Richard, A.C., Pasquier, F., Rollin-Sillaire, A., Martinaud, O., Quillard-Muraine, M., de la Sayette, V., Boutoleau-Bretonniere, C., Etcharry-Bouyx, F., Chauvire, V., Sarazin, M., le Ber, I., Epelbaum, S., Jonveaux, T., Rouaud, O., Ceccaldi, M., Felician, O., Godefroy, O., Formaglio, M., Croisile, B., Auriacombe, S., Chamard, L., Vincent, J.L., Sauvee, M., Marelli-Tosi, C., Gabelle, A., Ozsancak, C., Pariente, J., Paquet, C., Hannequin, D., Campion, D., 2017. C.N.R.M.A.J.p. collaborators of the, APP, PSEN1, and PSEN2 mutations in early-onset Alzheimer disease: a genetic screening study of familial and sporadic cases. PLoS Med. 14, e1002270.