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Establishment of induced pluripotent stem cells IRMBi005-A from a patient with sporadic Alzheimer's disease

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ABSTRACT

Alzheimer's disease (AD) is a progressive neurological disorder and the most common form of dementia worldwide. Sporadic Alzheimer's disease (sAD) cases are the main forms, over 95% of AD cases, but still poorly understood. Thereby there is a crucial need to develop *in vitro* models for studying this multifactorial disorder. Here, we report the reprogramming of skin fibroblasts from a 57-years-old male donor. The new generated iPSC cell line has a normal karyotype and, is pluripotent since it demonstrates the ability to differentiate *in vitro* into the three germ layers. This iPSC line will be used to understand pathological mechanisms of sAD.

1. Resource Table

Unique stem cell line identifier	IRMBi005-A
Alternative name(s) of stem cell line	SAD-hiPSC
Institution	INM, Univ Montpellier, INSERM, Montpellier, France
Contact information of distributor	Carole CROZET carole.crozet@inserm.fr
Type of cell line	iPSC
Origin	Human
Additional origin info required for human ESC or iPSC	Age: 57 years' old Sex: Male <i>Ethnicity if known: white</i>
Cell Source	Fibroblasts
Clonality	Clonal
Method of reprogramming	Sendai virus Cytotune 2.0 kit (ThermoFisher Scientific Inc.), hOCT4, hSOX2, hC-MYC, hKLF4
Genetic Modification	NO
Type of Genetic Modification	N/A
Evidence of the reprogramming	Sendai virus and c-Myc PCR
transgene loss (including genomic copy if applicable)	
Associated disease	Alzheimer's disease
Gene/locus	No Associated mutation
Date archived/stock date	07/26/2023

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(continued)

Unique stem cell line identifier	IRMBi005-A
Cell line repository/bank	https://hpscereg.eu/cell-line/1RMBi005-A
Ethical approval	DC-2015-2363 regional scientific ethical committee CPP Sud Med IV Informed consent was obtained from the patient.

2. Resource utility

The reported cell line may be useful to model and investigate the pathogenesis of sporadic Alzheimer's disease (sAD), the most common and complex form of the disease. It also constitutes a promising tool for drug screening.

3. Resource details

This paper describes the generation of an iPSC cell line from a patient with sporadic AD. Skin fibroblasts were obtained from a 57-year-old man diagnosed with sporadic Alzheimer's disease at 48. Fibroblasts were reprogrammed into iPSC with integration-free Sendai virus carrying gene sequences of the four Yamanaka factors OCT3/4, SOX2, KLF4

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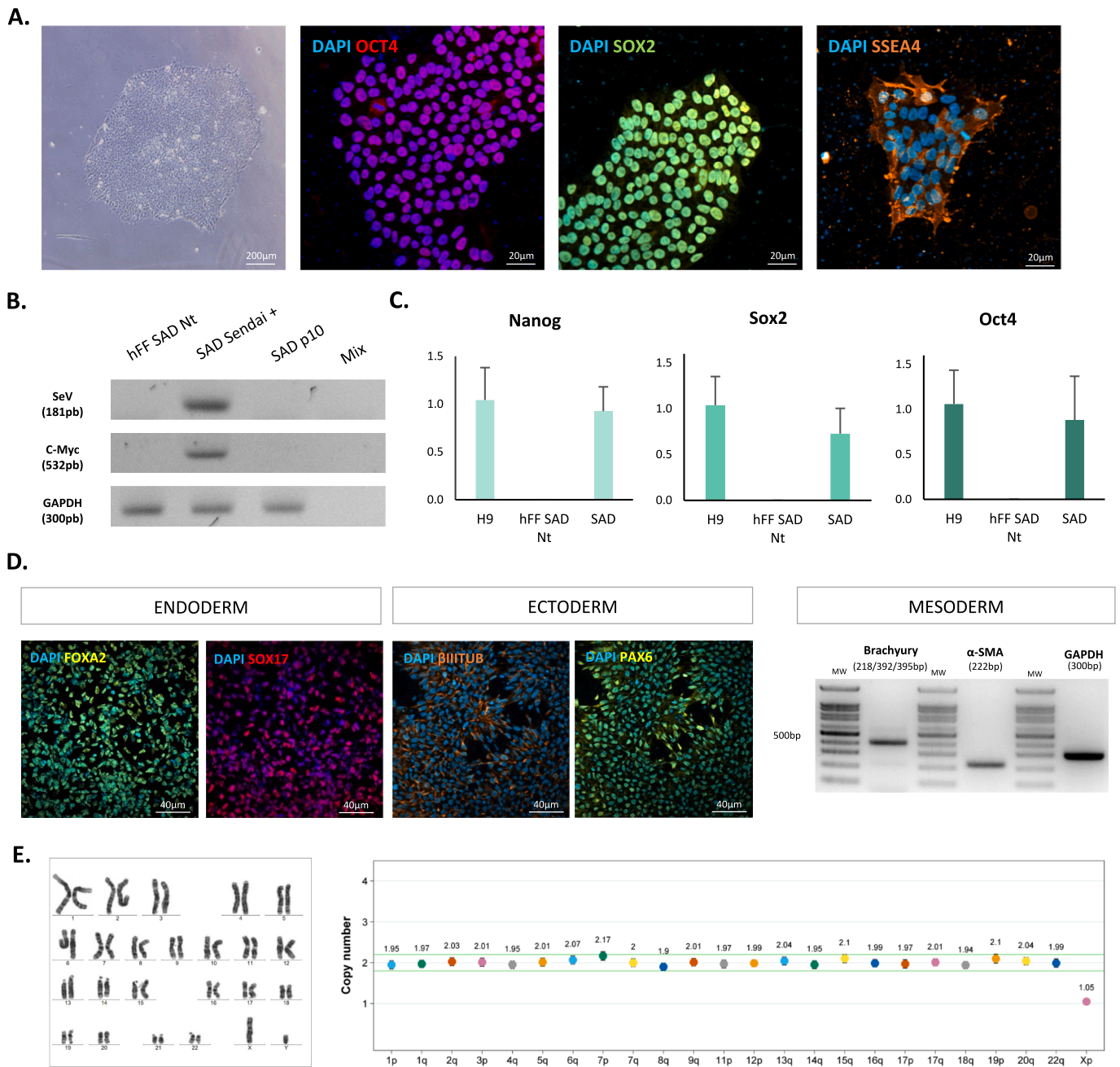


Fig. 1.

and c-MYC. Several iPSC colonies with normal morphology (Fig. 1A) were picked for expansion. The disappearance of Sendai virus was successfully established by RT-PCR analysis with Sendai virus specific primers in a selected iPS clone after 10 passages. The transgenes and viral sequences were also absent in the negative controls, non-transduced human healthy fibroblasts (hFF SAD Nt) and clone of interest, but present in fibroblasts freshly transduced with the Sendai virus (SAD Sendai +) (Fig. 1B). Expression of endogenous pluripotency markers OCT4, SOX2, NANOG was confirmed by qPCR analysis (Fig. 1C) and immunofluorescent staining expression of the embryonic stem cell specific marker SSEA4 (Fig. 1A) and the nuclear localization of the two pluripotency factors OCT4 and SOX2 (Fig. 1A). Pluripotency of the clone was assessed through *in vitro* directed differentiation of iPS colonies to each germ layers, followed by immunofluorescence of ectodermal and endodermal markers, respectively PAX6 and Nestin and FOXA2 and SOX17, or RT-PCR of mesodermal makers, Brachyury and α -smooth

muscle actin (Fig. 1D). The SAD clone presents a normal karyotype 46, XY after 13 passages and no evidence of recurrent genomic abnormalities was detected (Fig. 1E) (Assou et al., 2020). The short tandem repeat (STR) profile established the 100% identity of the iPSC line to parental cells. The SAD clone was also shown to be mycoplasma free (Supplementary Data).

4. Materials and methods

4.1. Generation of iPSC

After amplification in DMEM (Gibco) supplemented with 10% FBS (Lonza), 1% NEAA, 0.1 mM β -mercaptoethanol, 1 mM glutamine and 1% penicillin/streptomycin (Gibco), fibroblasts were reprogrammed using the Cytotune-iPSC 2.0 Sendai Reprogramming kit (Life Technologies) (KOS MOI = 5, hc-Myc MOI = 5, hklf4 MOI = 3). The reprogramming

Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology Phenotype	Photography Bright field	Normal	Fig. 1A
	Qualitative analysis (Immunocytochemistry)	Expression of pluripotency markers: Oct3/4, SOX2, SSEA-4	Fig. 1A
Genotype	Quantitative analysis (RT-qPCR)	Expression of Oct3/4, SOX2, NANOG	Fig. 1C
	Karyotype (G-banding) and resolution Recurrent genomic abnormalities	46XY, Resolution 400 No recurrent genetic abnormalities detected	Fig. 1E
Identity	Microsatellite PCR (mPCR) OR STR analysis	Not performed 4 matched sites	NA Available with authors
Mutation analysis (IF APPLICABLE)	Sequencing	NA	NA
	Southern Blot OR WGS	NA	NA
Microbiology and virology	Mycoplasma	Mycoplasma testing by luminescence. Negative	Supplementary data
Differentiation potential	In vitro directed differentiation	Expression of PAX6, β III-tubulin, SOX17 and FOXA2 by immunocytochemistry. Expression of Brachyury and α SMA by RT-PCR.	Fig. 1D
List of recommended germ layer markers	mRNA (RT-PCR) and protein (IF) levels, at least 2 markers need to be shown per germ layer	Ectoderm: PAX6, TUBB3/TUJ1 Endoderm: SOX17, FOXA2, Mesoderm: BRACHYURY, α SMA	Fig. 1D
Donor screening (OPTIONAL)	HIV 1 + 2 Hepatitis B, Hepatitis C	NA	NA
Genotype additional info (OPTIONAL)	Blood group genotyping	NA	NA
	HLA tissue typing	NA	NA

was performed on mytomicin-inactivated mouse embryonic fibroblasts from CF1 mouse (Charles River) on 0.1% Gelatin coated dishes and maintained, in KODMEM/F-12 with 20% knock-out serum, 1% NEAA, 0.1 mM β -mercaptoethanol, 1 mM glutamax, 1% penicillin/streptomycin and 5 ng/mL bFGF (Miltenyi Biotech). Several iPSC clones were picked up and expanded for the generation of different iPSC lines among which the present SAD line was selected.

4.2. Pluripotent stem cells culture

iPSC were maintained on feeder layers for several passages before adaptation to feeder-free culture condition, on 0.1% Geltrex (Gibco, Fisher Scientific) coated dishes and maintained in mTeSR Plus medium (Stemcell Technologies). Colonies were chemically passaged (1:10) 2 times a week as aggregates with enzymatic-free reagent ReLeSR (Stemcell Technologies). Cells were cultivated in a humidified incubator at 37 °C, 5% CO₂. The iPSC lines were frozen in liquid nitrogen in Cryosrot and 10 μ M of Rock Inhibitor (Stemcell Technologies). See [Table 1](#).

4.3. PCR analysis

Total RNA was extracted from fibroblasts and the SAD clone with RNAeasy micro kit (Qiagen) and cDNA obtained using the Superscript III system (Invitrogen). Extinction of the expression of transgenes and expression of mesodermal markers was performed by conventional PCR with virus specific primers ([Table 2](#)). Expression of endogenous pluripotency genes was confirmed by quantitative PCR analysis using Syber-Green I Master kit (Roche Diagnostic) using H9 human embryonic stem cell mRNA as reference and positive control.

4.4. In vitro differentiation

iPS(p14) cells were differentiated into the three embryonic germ layers, mesoderm, ectoderm and endoderm with the StemMACS Trilineage Differentiation kit (Miltenyi) in 7 days, according to the supplier's recommendations.

4.5. Immunofluorescence staining

iPSC(p14) colonies or EB were fixed on coverlips in 4% PFA (15 min) and washed 3 times with DPBS (Gibco). Cells were permeabilized with 0.5% Triton X-100 (3x10min for nuclear staining, Sigma-Aldrich,). Saturation of non-specific binding sites was performed with 0.2% Bovine Serum Albumin (MP BIOMEDICALS) in DPBS (1 h, RT). Cells were incubated overnight with primary antibodies at 4 °C. Secondary antibodies were applied at RT for 1 h. Primary antibodies and secondary antibodies and their respective dilutions in 0.2% BSA-DPBS solution are listed in [Table 2](#). Nuclei were labelled with DAPI in DPBS. After two washes in DPBS and water, coverslips were mounted with Fluorsave (Millipore). Samples were imaged with Upright Zeiss Apotome.

4.6. Karyotype and recurrent genomic abnormalities testing

G-banding karyotyping and iCS-digital PCS test (detection of recurrent genetic abnormalities) were performed by StemGenomics at passage p13.

4.7. STR analysis

Microsatellite analysis was performed on DNA extracted from parental fibroblasts and iPSCs at p13, using the following four short tandem repeats (STR): D1S439, D9S1784, D14S986 and D19S913.

4.8. Mycoplasma testing

Absence of mycoplasma contamination was assessed on cell culture supernatants of iPS cells (p13) using the MycoAlert® Mycoplasma Detection assay (Lonza).

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.

Table 2
Reagents details.

	Antibodies used for immunocytochemistry/flow-cytometry			
	Antibody	Dilution	Company Cat #	RRID
Pluripotency Markers	Mouse anti-OCT3-4 (C-10)	1:500	Santa Cruz Biotechnology Cat# sc-5279	RRID: AB_628051
	Rabbit anti-SOX2	1:100	Bethyl Cat#301-739A	RRID: AB_1211354
	Mouse anti-SSEA4 (MC-813-70)	1:500	Thermo Fisher Scientific Cat# MA1-021	RRID: AB_2536687
Differentiation Markers	Mouse anti-Beta-Tubulin III, Clone TUJ1	1:200	STEMCELL Technologies Cat#60052	RRID:AB 2,313,773
	Rabbit anti-PAX6	1:20	Thermo Fisher Scientific Cat# PA5-25970	RRID: AB_2543470
	Rabbit anti-HNF3beta/FOXA2	1:75	Millipore Cat# 07-633	RRID: AB_390153
Secondary antibodies	Mouse anti-SOX17	1:75	R&D Systems Cat# MAB1924	RRID: AB_2195646
	Donkey Anti-Mouse IgG, Alexa Fluor 555-conjugated	1:1000	Molecular Probes Cat# A21424	RRID: AB_141780
	Goat anti-Rabbit IgG, Alexa Fluor 488-conjugated	1:1000	Molecular Probes Cat# A-11008	RRID: AB_143165
	Donkey anti-Rabbit IgG, Alexa Fluor 488-conjugated	1:1000	Molecular Probes Cat# A21206	RRID: AB_2535792
	Donkey anti-Goat IgG, Alexa Fluor 555-conjugated	1:1000	Thermo Fisher Scientific Cat# A21432	RRID: AB_2535853
Primers Target	Donkey anti-Mouse IgG, Alexa Fluor 488-conjugated	1:1000	Molecular Probes Cat# A21202	RRID: AB_141607
	SeV	181 bp	Forward: GGA TCA CTA GGT GAT ATC GAG C Reverse: ACC AGA CAA GAG TTT AAG AGA TAT GTA TC	
	c-Myc	532 bp	Forward: TAA CTG ACT AGC AGG CTT GTC G Reverse: TCC ACA TAC AGT CCT GGA TGA TGA TG	
	GAPDH	300 bp	Forward: CTG GCG TCT TCA CCA CCA TGG Reverse: CAT CAC GCC ACA GTT TCC CGG	
Mesodermal markers (PCR)	BRACHYURY/TBXT	218/392/395 bp	Forward: ACC CAG TTC ATA GCG GTG AC Reverse: CAA TTG TCA TGG GAT TGC AG	
	αSMA	222 bp	Forward: TTC AAT GTC CCA GCC ATG TA Reverse: GAA GGA ATA GCC ACG CTC AG	
Pluripotency Markers (qPCR)	OCT4	150 bp	Forward: TGT ACT CCT CGG TCC CTT TC Reverse: TCC AGG TTT TCT TTC CCT AGC	
	SOX2	144 bp	Forward: ATG CAC CGC TAC GAC GTG A Reverse: CTT TTG CAC CCC TCC CAT TTC	
	NANOG	354 bp	Forward: TGC CTC ACA CGG AGA CTG TC	

Table 2 (continued)

Antibodies used for immunocytochemistry/flow-cytometry				
Antibody	Dilution	Company Cat #	RRID	
House-Keeping Genes (qPCR)	GAPDH	NA	Reverse: TGC TAT TCT TCG GCC AGT TG	Forward: CGC TCT CTG CTC CTC CTG TT
			Reverse: CCA TGG TGT CTG AGC GAT GT	

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scr.2023.103216>.

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