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L. Auboyer, C. Monzo, D. Wallon, A. Rovelet-Lecrux, A. Gabelle, I. Gazagne,  
V. Cacheux, S. Lehmann, C. Crozet

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Lab resource: Stem Cell Line

## Generation of induced pluripotent stem cells (iPSCs) IRMBi002-A from an Alzheimer's disease patient carrying a D694N mutation in the APP gene



Auboyer L.<sup>a</sup>, Monzo C.<sup>a</sup>, Wallon D.<sup>b</sup>, Rovelet-Lecrux A.<sup>c</sup>, Gabelle A.<sup>a,d,e</sup>, Gazagne I.<sup>d</sup>, Cacheux V.<sup>d</sup>, Lehmann S.<sup>a,d,e</sup>, Crozet C.<sup>a,d,\*</sup>

<sup>a</sup> Institute for Regenerative Medicine and Biotherapy (IRMB), Montpellier, France

<sup>b</sup> Normandie Univ, UNIROUEN, Inserm U1245, Rouen University Hospital, Department of Neurology and CNR-MAJ, Normandy Center for Genomic and Personalized Medicine, F 76000 Rouen, France

<sup>c</sup> Normandie Univ, UNIROUEN, Inserm U1245, Rouen University Hospital, Department of Genetics and CNR-MAJ, Normandy Center for Genomic and Personalized Medicine, F 76000 Rouen, France

<sup>d</sup> Hôpital St Eloi, CHU Montpellier, 80 rue augustin Fliche, 30295 Montpellier, France

<sup>e</sup> Université de Montpellier, UM, 163 Rue Auguste Broussonnet, 34090 Montpellier, France

### ABSTRACT

Induced pluripotent stem cells (iPSC) were generated from skin fibroblasts obtained from a 58 year-old woman suffering from Alzheimer's disease and carrying a D694N mutation on Amyloid precursor protein (APP). Fibroblasts were reprogrammed into iPSC using the integration-free Sendai Virus which allows the 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. The cells have the corresponding mutation and present a normal karyotype. The reported APP-D694N iPSC line may be used to model and study human AD pathology *in vitro*.

### Resource table.

Unique stem cell line identifier	IRMBi002-A
Alternative name(s) of stem cell line	AD-APP hiPSC
Institution	Institute for Regenerative Medicine and Biotherapy, IRMB U1183 INSERM, Montpellier
Contact information of distributor	Carole Crozet: <a href="mailto:carole.crozet@inserm.fr">carole.crozet@inserm.fr</a>
Type of cell line	iPSC
Origin	Human
Additional origin info	Age: 58 y.o. Sex: Female Ethnicity: caucasian
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	Hereditary mutation
Associated disease	Alzheimer's disease
Gene/locus	APP c.2080G > A p.Asp694Asn exon 17 Locus 21q21.3 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	The cell line has been archived in hspreg <a href="https://hspreg.eu/cell-line/IRMBi002-A">https://hspreg.eu/cell-line/IRMBi002-A</a>
Ethical approval	DC-2015-2363 régional scientific ethical committee CPP Sud Med IV Informed consent was obtained from the patient.

### Resource utility

The reported APP-D694N iPSC line may be used to model and study human AD pathology *in vitro*.

### Resource details

Skin fibroblasts were obtained from a 58 year-old woman diagnosed with Alzheimer's disease (AD), at the age of 58. This patient carries a causal mutation (c.2080G > A) in exon 17 of Amyloid precursor protein (APP) gene (Grabowski et al., 2001; Van Nostrand et al., 2001; Van

\* Corresponding author at: Neural Stem Cells, MSC and Neurodegenerative Diseases, Institute for Regenerative Medicine and Biotherapy (IRMB), Univ Montpellier, INSERM, Montpellier, France.

E-mail address: [carole.crozet@inserm.fr](mailto:carole.crozet@inserm.fr) (C. Crozet).

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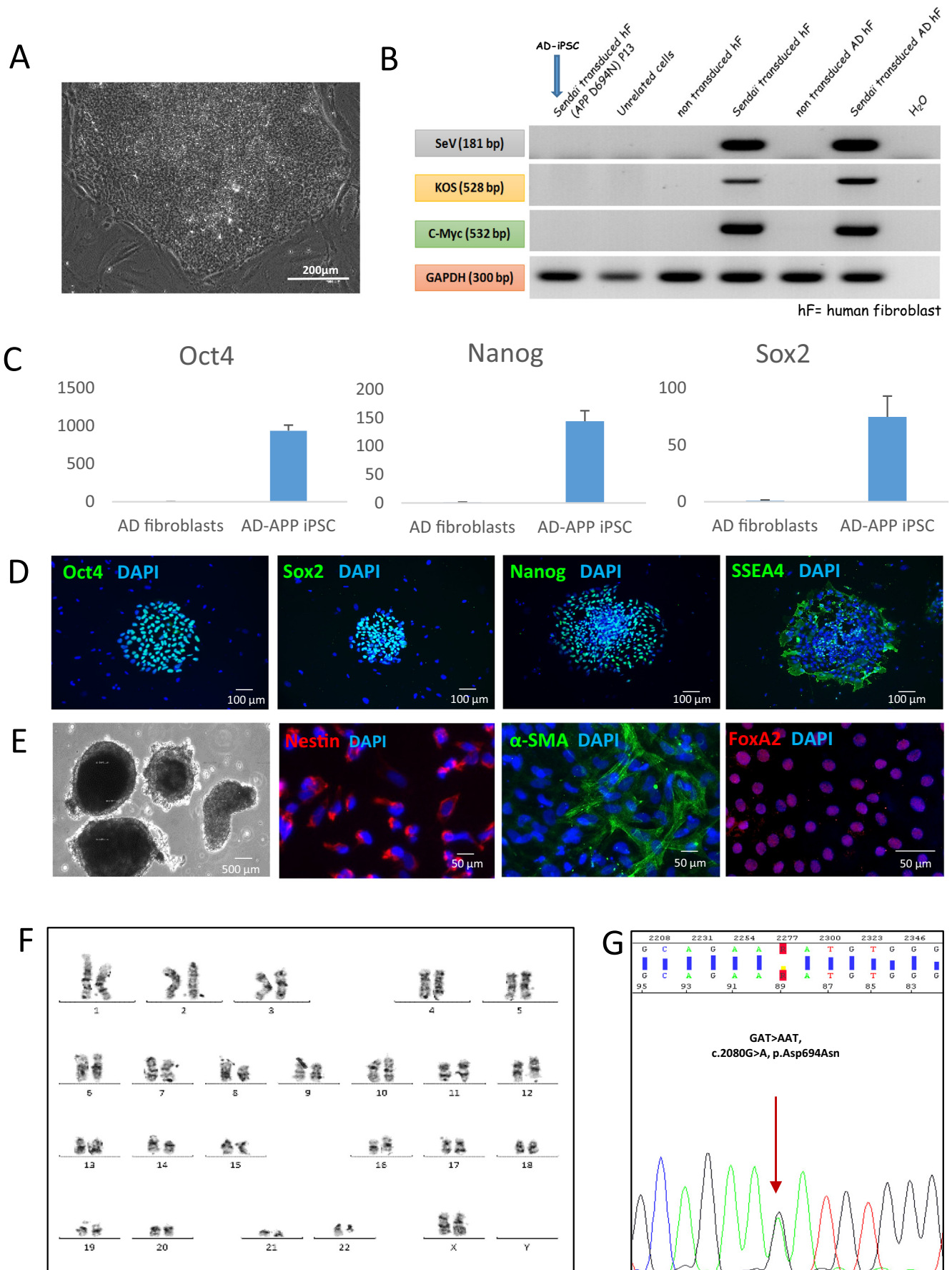


Fig. 1. Characterization of IRMBi002-A iPSC.

**Table 1**  
Characterization and validation.

Classification	Test	Result	Data
<b>Morphology</b>	Photography	Visual record of the line: normal	Fig. 1 panel A
<b>Phenotype</b>	qPCR analysis	Positive for OCT4, NANOG, SOX2	Fig. 1 panel C
	Immunocytochemistry	Positive for OCT4, NANOG, SOX2, SSEA4	Fig. 1 panel D
<b>Genotype</b>	Karyotype (G-banding) and resolution	46XY Resolution: 400	Fig. 1 panel F
<b>Identity</b>	Microsatellite PCR (mPCR)	Not performed	
	STR analysis	4 Matched sites D1S439, D9S1784 D14S986 and D19S913	Not shown but available with author
<b>Mutation analysis</b>	Sequencing	Heterozygous mutation in iPSCs.	Fig. 1 panel G
<b>Microbiology and virology</b>	Mycoplasma	Mycoplasma testing by luminescence (MycoAlert). Negative	Supplemental figure
<b>Differentiation potential</b>	Embryoid body formation	Expression of genes in embryoid bodies: alpha-smooth muscle actin ( $\alpha$ -SMA), $\beta$ III-tubulin and FoxA2.	Fig. 1 panel E
<b>Donor screening (OPTIONAL)</b>	HIV 1 + 2 Hepatitis B, Hepatitis C	Negative	Not shown but available with author
<b>Genotype additional info (OPTIONAL)</b>	Blood group genotyping	NA	NA
	HLA tissue typing	NA	NA

Nostrand et al., 2002). Several mutations on APP, the precursor of the amyloid- $\beta$  peptide (A $\beta$ ), are known to be responsible for familial cases of AD mainly since they promote the increase of the A $\beta$  peptide aggregation in the brain of the patients. Fibroblasts were reprogrammed into iPSCs using integration-free Sendai Virus carrying gene sequences of 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 disappearance of Sendai virus from AD-iPSC (Fig. 1B, first panel Sendai transduced hF (APPD694N) P13) collected after 13 passages was successfully established by RT-PCR analysis with Sendai Virus (SeV), C-MYC and KOS sequence specific primers (Fig. 1, panel B, Table 1). The transgenes and viral sequences were also absent in the negative non transduced healthy human fibroblast (hF) and AD human fibroblast controls (Fig. 1B, panel 3 and 5 respectively) but present in positive controls consisting of healthy and AD fibroblasts freshly transduced (6 days post-transduction) with the Sendai virus (Sendai transduced hF, Fig. 1B, panel 4 and 6 respectively). Expression of endogenous pluripotency markers OCT4, SOX2, NANOG was verified by qPCR analysis (Fig. 1, panel C) and OCT4, SOX2, NANOG and 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) and immunofluorescence analysis of the beta-III-tubulin ectodermal marker ( $\beta$ III-tubulin), the Fox2A endodermal marker and the alpha-smooth muscle actin mesodermal marker ( $\alpha$ -SMA), more than three months post-differentiation. The results demonstrates the ability of iPSC to differentiate into the three primary germ layers (Fig. 1, panel E). Clones were found with a normal 46,XX karyotype (Fig. 1 panel F) after 23 passages in culture. Sanger sequencing analysis of iPSCs confirmed the presence of the c.2080G > A change in exon 17 of APP gene corresponding to a p.Asp694Asn mutation on APP (also called 'IOWA' mutation) (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 woman carrying the APPD694N 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, 0,1 mM  $\beta$ -mercaptoethanol, 1 mM glutamine 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 » (Life Technologies, Thermo Fisher Scientific) following exactly the manufacturer's instruction using MOI of 5–5–3 (KOS MOI = 5, hc-Myc MOI = 5, hklf4 MOI = 3).

### Pluripotent stem cells culture

IRMBi002-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  $\beta$ -mercaptoethanol (Gibco, Life Technologies, Thermo Fisher Scientific), 1 mM glutamine (Gibco, Life Technologies, Thermo Fisher Scientific), 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<sub>2</sub> and 5% O<sub>2</sub> (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 13) 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 expression of transgenes was assessed using Sendai-virus-specific primers (Table2) and conventional PCR. The PCR mix was composed of cDNA (300 ng), the forward and reverse primers (see Table 2), 0.3  $\mu$ l at 10  $\mu$ M 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 LC480 device (Roche Diagnostics) using Syber-Green I Master Kit (Roche Diagnostics). The relative quantification was calculated using the 2<sup>- $\Delta\Delta$ Ct</sup> method with fibroblasts as controls and GAPDH as housekeeping gene (Primers are listed in table2).

**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 $\alpha$ -SMA	1/200	Lab Vision Cat# MS-113-P0, RRID:AB_64001
	Goat anti-FoxA2	1/50	R and D Systems Cat# AF2400, RRID:AB_2294104
	Mouse anti- $\beta$ III-TUBULIN	1/1000	Covance Research Products Inc. Cat# MMS-435P, RRID:AB_2313773
	Rabbit anti-GFAP	1/500	Dako Cat# Z0334, RRID:AB_10013382
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	
	c-MYC product size 532 bp	Forward: TAA CTG ACT AGC AGG CTT GTC G* Reverse: TCC ACA TAC AGT CCT GGA TGA TGA TG	
	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: TGTACTCCTCGGTCCTTC Reverse: TCCAGGTTTTCTTTCCCTIAGC	
	SOX2	Forward: ATGCACCGCTAGCAGCTGA Reverse: CTTTTGCACCCCTCCCATTTTC	
	NANOG	Forward: TGCCTCACACGGAGACTGTC Reverse: TGCTATTCTTCGGCCAGTTG	
House-Keeping Gene (qPCR)	GAPDH	Forward: CGCTCTCTGCTCCTCTGTT Reverse: CCATGGTGTCTGAGCGATGT	
Targeted mutation analysis/sequencing	APP c.2080G > A exon 17 Locus 21q21.3	Primers available with authors	

### Immunofluorescence staining

Cells were fixed on coverslips with 4% paraformaldehyde (15 min) and washed 3 times in Dulbecco phosphate-buffered saline (D-PBS; Gibco, Life Technologies, 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, 0.2% BSA (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 22 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 one day. Cells were then fixed for 15 min in 4% paraformaldehyde for immunofluorescence analysis with anti nestin (neuroectoderm), FOXA2 (endoderm) and  $\alpha$ -SMA (mesoderm) antibodies (Fig. 1, panel E).

### Karyotyping

Following 23 passages in culture, iPSCs were treated with colcemid (0,1  $\mu$ g/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, XX), without any discernable abnormalities (Fig. 1, panel F).

### Verification and authentication

DNA was extracted from fibroblasts and from iPSCs (after 14 passages) 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 APP variant c.2080G > A was 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 mycoplasma free cultures.

#### **Appendix A. Supplementary data**

Supplementary data to this article can be found online at <https://>

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