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Lab resource: Stem Cell Line

Generation of one iPSC line (IMEDEAi006-A) from an early-onset familial Alzheimer's Disease (*f*AD) patient carrying the E280A mutation in the PSEN1 gene

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A B S T R A C T

The mutation E280A in PSEN1 (presenilin-1) is the most common cause of early-onset familial Alzheimer's Disease (*f*AD). It presents autosomal dominant inheritance and frequently leads to the manifestation of the disease in relatively young individuals. Here we report the generation of one PSEN1 E280A iPSC line derived from an early-onset patient. OriP/EBNA1-based episomal plasmids containing OCT3/4, SOX2, KLF4, L-MYC, LIN28, BCL-xL and shp53 were used to reprogram oral mucosa fibroblasts. The iPSC line generated has normal karyotype, carry the E280A mutation, is free of plasmid integration, express high levels of pluripotency markers and can differentiate into all three germ layers.

Resource table.

Unique stem cell line identifier	IMEDEAi006-A
Alternative name(s) of stem cell line	iPS 322AD CL7
Institution	IMEDEA - Instituto Mediterráneo de Estudios Avanzados
Contact information of distributor	Daniel Bachiller; d.b@csic.es
Type of cell line	iPSCs
Origin	Human
Additional origin info	Age: 50 years Sex: Female Ethnicity if known: Not Known
Cell Source	Original cell type: primary human Oral Mucosa Fibroblast (hOMF)
Clonality	Clonal cell line
Method of reprogramming	Episomal Plasmids (Klf4, Sox2, Oct4, shp53, L-Myc, Lin28, Bcl-xL)
Genetic Modification	NO
Type of Modification	N/A
Associated disease	Alzheimer's disease 1, familial (AD) (AD; OMIM entry # 104300)
Gene/locus	PSEN1 E280A, Chr14:73664808 A > C
Method of modification	N/A
Name of transgene or resistance	N/A
Inducible/constitutive system	N/A
Date archived/stock date	26/02/2019
Cell line repository/bank	Registered in the Human Pluripotent Stem Cell Registry (https://hpscreg.eu).
Ethical approval	Patient informed consent obtained from the Comité de Bioética de la Universidad de Antioquia (Colombia). Approval number NB-Ci-003

Resource utility

AD is a complex disease whose aetiology is affected by genetic and environmental factors. It has no cure and according to the last World Alzheimer Report, in 2050 the number of people with AD will be above 13 million. A better understanding of AD is necessary, and

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IMEDEAi006-A could be used as a suitable model for studying the disease.

Resource details

Alzheimer's disease is an irreversible, progressive brain disorder associated with aging, which at a time of and increasingly old population is becoming a major concern for public health systems. Determining the genetic bases of AD and their interaction with external and environmental factors would improve the accuracy of disease diagnosis, especially before the appearance of clinical symptoms, as well as increase the possibility of finding an effective treatment. iPS cells carrying AD related mutations constitute an important resource that can be differentiated into neuronal cells and then used to model the disease *in vitro*. The majority of E280A carriers belong to a large kindred in Antioquia, Colombia, the place of origin of the starting cells used to generate the iPSC line IMEDEAi006-A. This is the first time, to our knowledge, that iPSCs from a E280A *f*AD donor with early onset of the disease have been generated, although a recent report has described the introduction by genomic engineering of the E280A mutation into iPS cells from a wild type donor (Frederiksen et al., 2019). This is an interesting approach, but the lack of clinical information concerning the phenotypic effect of the mutation on the whole organism limits the usefulness of the new AD cell lines. IMEDEAi006-A offers the possibility of comparing *in vitro* and *in vivo* data on the aetiology of the disease.

Human oral mucosa fibroblasts (hOMF) from 50 years old woman carrying in heterozygosity the PSEN1 E280A mutation were reprogrammed with OriP/EBNA (Epstein-Barr nuclear antigen-1) based episomal plasmids containing OCT3/4, SOX2, KLF4, L-MYC, LIN28, BCL-xL and shp53. The resulting iPSC line showed morphology (Fig. 1A) and growth behavior typical of human Embryonic Stem Cells (hESC), as well as normal female karyotype (46, XX) (Fig. 1E). In addition, after PCR analysis using specific primers (Table 2) that amplify the common element in all plasmids: OriP/EBNA, IMEDEAi006-A was shown to be free of plasmid integration (Fig. 1B). STR analysis (Table 1, data not shown) indicated a 100% match with parental hOMFs. DNA sequencing was used to identify the E280A mutation in PSEN1 (Fig. 1F). Regarding stemness, immunocytochemical analysis demonstrated the expression of the pluripotency-associated markers: SOX2, OCT3/4, TRA-1-60 and NANOG (Fig. 1D), while robust TRA-1-81 and SSEA-4 expression was confirmed by quantitative flow cytometry (Fig. 1C). Finally, in order to probe the differentiation potential of the iPSC line, directed differentiation into all three germ layers was carried out (Fig. 1G). The expression of specific markers for ectoderm, mesoderm and endoderm in the differentiated cells was analyzed by immunocytochemistry (Fig. 1H). The complete characterization of the line was completed determining the absence of mycoplasma in iPSC samples (Supplementary Fig. S1).

In summary, we have generated an iPSC line from an early onset *f*AD, heterozygous patient carrying the E280A mutation in the PSEN1 gene. This new cell line has a great potential for the study and modelling of the disease, as well as a screening tool in pharmacological applications.

Materials and methods

iPSCs derivation and expansion

Human Oral Mucosa Fibroblasts (hOMF) from a *f*AD donor were obtained and cultured in DMEM complete and gelatin-coated plates until passage 6. One million of cells were nucleofected with four OriP/EBNA1-based episomal plasmids: 1 μg of pCXLE-hOCT3/4-shp53, 1 μg of pCXLE-hSK and 1 μg of pCXLE-hUL (Okita et al., 2011) in addition to 1 μg of GBX. After nucleofection, cells were cultured on gelatin-coated plates and DMEM complete (Biowest) supplemented with 2 mM GlutaMAX, 1% NEAA, 50 U/ml Penicillin, 50 mg/ml Streptomycin, 10% FBS (Biowest) for 7 days and then, passed with EDTA-dissociation solution to murine MEF CD1-coated plates and hiPS medium: Knockout DMEM (Invitrogen) supplemented with 20% Knockout Serum Replacement (Invitrogen), 2 mM GlutaMAX, 1% NEAA, 50 U/ml Penicillin, 50 mg/ml Streptomycin, 0,1 m Mercaptoethanol and 8 ng/ml bFGF. After 20 days in culture, iPS colonies were manually isolated and grown on MEF CD1 until passage 3 when cells where change to human HFF1-W3R feeders. Rock Inhibitor Y-27632 was used at 10 μM during the passages. Cells were cultured at 37 °C and 5% CO_2 in a humidified incubator.

Karyotype analysis

iPSCs were passed to Matrigel-coated plates and sent to *Biobanco del Sistema Sanitario Público de Andalucía* (Granada, Spain) for the karyotyping by G-banded metaphases analysis.

Reprogramming plasmid integration analysis

Genomic DNA was isolated from iPSCs on passage 16 and from fibroblast nucleofected with episomal plasmids as a positive control. PCR analysis was used to detect a common element of all the reprogramming plasmids: OriP/EBNA-1. Amplification reactions were carried out with WonderTaq (Euroclone).

STR analysis and sequencing

Genomic DNA from donor cells and iPSC lines were isolated and genotyping for the E280A mutation (PSEN1 exon 8, nucleotide 839) was performed by PCR amplification of the corresponding positions with the primers detailed in Table 3. PCR were performed using EuroTaq (Euroclone). Amplicons were sequenced by SECUGEN S.L (Madrid) using a 96-capillary DNA analyser Abi 3730. STR analysis was performed by the Genomics Core facility at IIBM (Madrid).

In vitro directed differentiation

In order to perform directed differentiation of IMEDEAi006-A, iPSCs were first cultured on gelatin-coated plates in hiPS medium supplemented with 10 μM Rock Inhibitor and 1% of DMSO. After 75 min cells were passed to a p48 well plate coated with matrigel. Ectoderm differentiation was carried out according to Tchieu et al. (Tchieu et al., 2017), but using 200 ng/ml Noggin (Peprotech) instead of LDN193189. Mesoderm differentiation was attained following Oldershaw et al. (Oldershaw et al., 2008), while the protocol of McCracken et al. (McCracken et al., 2011) was applied, with some minor modifications, for *endoderm* differentiation. After 7 days of culture cells were fixed in PFA 4% for immunocytochemistry.

Immunofluorescence staining

Undifferentiated iPSCs (for pluripotency markers assays) and differentiated cells (for differentiation potential assays) were washed with PBS, fixed for 20 min, washed again with PBS and permeabilized with 0,2% Triton X-100 and 100 mM glycine in PBS for 30 min at room temperature (RT). To block non-specific binding sites, PBS 5% BSA was added an incubated for 60 min at RT. Primary antibodies incubation was done overnight at 4 °C in PBS 2% BSA. After three washes with PBS, cells were incubated with fluorescence-conjugated secondary antibodies in PBS 2% BSA for 60 min at RT and darkness. The antibodies used are described in Table 3. After washing with PBS, cells were stained with DAPI for 5 min at RT in darkness.

Flow cytometry analysis of pluripotency marker

Cells were detached with Tryple and incubated 45 min and 4 °C with

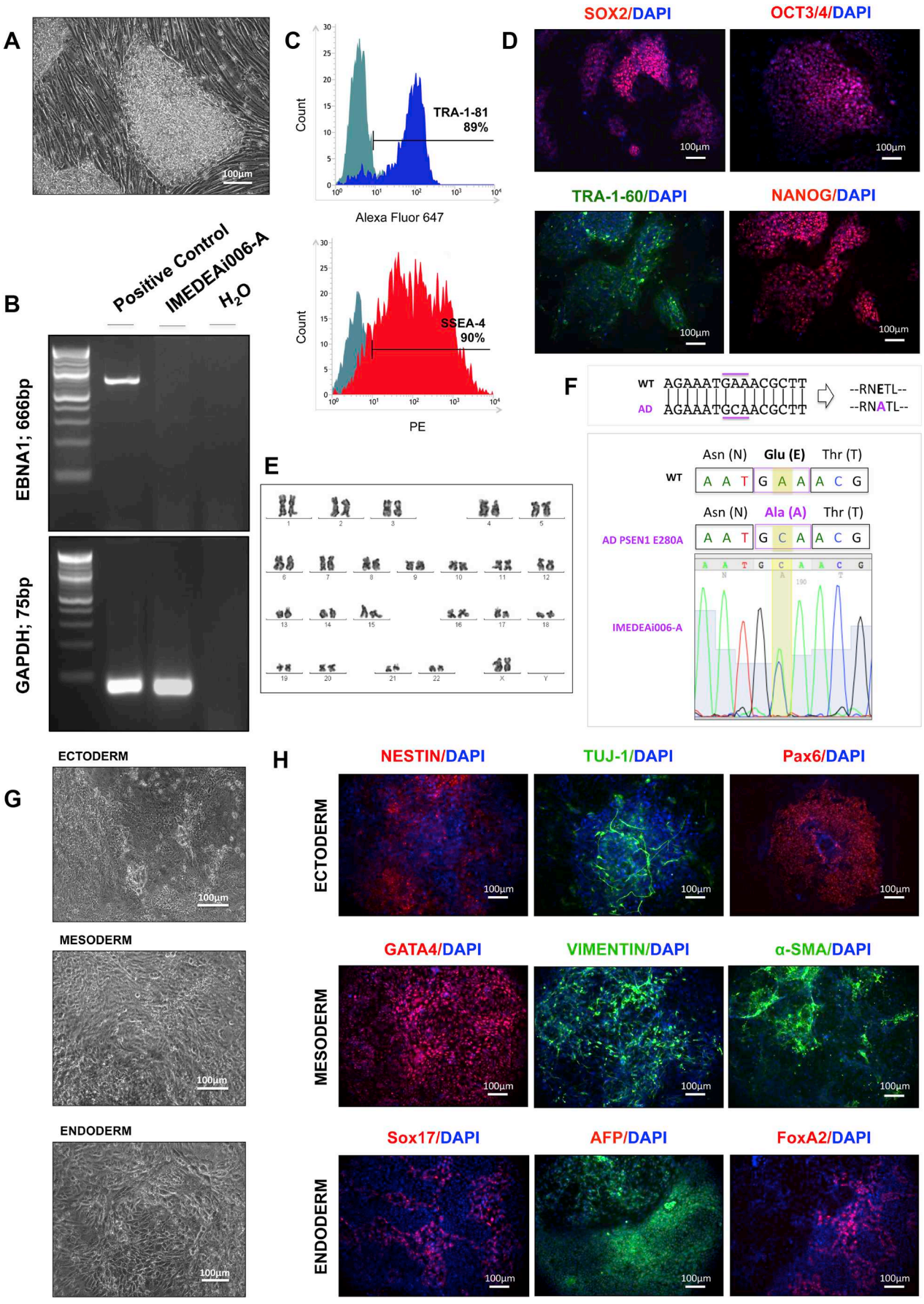

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Fig. 1. Characterization of the novel fAD-iPSC line IMEDEAi006-A. (A) Phase contrast image of the iPSC line at passage 17 on a feeder-coated plate (scale bar = 100 m). (B) PCR analysis demonstrating the absence of episomal plasmid integration in the iPSC line. (C) Flow cytometry analysis of TRA-1-81 (up) and SSEA-4 (down) expression. (D) Expression of SOX2, OCT3/4, TRA-1-60 and NANOG, assessed by immunofluorescence assay, confirmed the pluripotent phenotype of the line (scale bars = 100 m). (E) G band analysis demonstrates a normal female karyotype. (F) Sequence of the genomic PSEN1 E280A region. (G) Phase contrast images of the iPSC line after 7 days of directed differentiation into ectoderm, mesoderm and endoderm (scale bars = 100 m). (H) Immunocytochemistry images of differentiation specific marker expression. Nuclei were counterstained with DAPI (scale bars = 100 m)

Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography	Normal appearance of packed iPS colonies. Scale bars: 100 µm	Fig. 1 panel A
Phenotype	Qualitative analysis by Immunocytochemistry	All cell lines expressed the pluripotency markers: SOX2, OCT3/4, TRA-1-60 and NANOG.	Fig. 1 panel D
	Quantitative analysis by Flow Cytometry	All cell lines expressed high levels of the pluripotency marker sTRA-1-81 and SSEA-4	Fig. 1. Panel C
Genotype	Karyotype (G-banding) and resolution	IMEDEAi006-A: 46, XX Resolution 450-500	Fig. 1. Panel E
Identity	Microsatellite PCR analysis	Not performed	
	STR analysis	The STR profiles of cell lines matched 100% with that of the parental fibroblast cells (10 loci analyzed)	Submitted in archive with journal
Mutation analysis	Sequencing	Heterozygous: Glu280Ala (E280A).	Fig. 1. Panel F
Microbiology and virology	Mycoplasma	Mycoplasma testing by PCR using MycoSPY Kit (Biontex).	Submitted in archive with journal
Differentiation potential	Directed differentiation	All cell lines differentiated into the three germ layers including ectoderm (Nestin, TUJ-1 and Pax6), mesoderm (GATA-4, Vimentin and α-SMA) and endoderm (Sox17, AFP and FoxA2).	Fig. 1. Panel G and H
Donor screening	NA	NA	NA
Genotype additional info	NA	NA	NA

Table 2
Reagent details.

Antibodies used for immunocytochemistry

	Antibody	Dilution	Company Cat # and RRID
Pluripotency Markers	Mouse anti-OCT4	1:50	Santa Cruz Biotech; sc-5279; RRID:AB_628051
	Rabbit anti-NANOG	1:200	Cell Signaling; D73G4; RRID:AB_10559205
	Mouse anti-SOX2	1:100	R and D Systems; MAB2018; RRID:AB_177629
	Mouse anti-TRA-1-60	1:100	Millipore; MAB4360; RRID:AB_2119183
Differentiation Markers	Rabbit anti-Nestin	1:500	Sigma-Aldrich; N5413; RRID:AB_1841032
	Rabbit anti-TUJ-1	1:500	Covance; MRB-435P-100; RRID:AB_663339
	Mouse anti-Pax6	1:100	DSHB pax6, RRID:AB_528427
	Mouse anti-GATA-4	1:300	Santa Cruz Biotech; sc-25310; RRID:AB_627667
	Mouse anti-Vimentin	1:100	Abcam; ab80667 RRID:AB_1603290
	Mouse anti-α-SMA	1:200	Sigma-Aldrich; A2547 RRID:AB_476701
	Goat anti-SOX-17	1:100	R&D Systems; AF1924; RRID:AB_355060
	Rabbit anti-AFP	1:200	Dako; A0008; RRID:AB_2650473
	Rabbit anti-FoxA2	1:400	Cell Signaling; 8186 RRID:AB_10891055
Secondary antibodies	Alexa Fluor 555 Donkey Anti-Rabbit IgG	1:500	Invitrogen; A-31572; RRID:AB_162543
	Alexa Fluor 555 Donkey Anti-Mouse IgG	1:500	Invitrogen; A-315700 RRID:AB_2536180
	Alexa Fluor 488 Donkey Anti-Mouse IgG	1:500	Invitrogen; A-21202; RRID:AB_141607
	Alexa Fluor 488 Donkey Anti-Goat IgG	1:500	Invitrogen; A-21208, RRID:AB_141709
	Alexa Fluor 488 Donkey Anti-Rabbit IgG	1:500	Invitrogen; A-21206 RRID:AB_141708

Antibodies used for flow cytometry

	Antibody	Vol. per test	Company Cat # and RRID
Pluripotency Markers	Mouse Anti TRA-1-81, APC	20 µl	BD Biosciences; 560793; RRID:AB_10550550
	Mouse Anti SSEA-4, PE	20 µl	BD Biosciences; 560128 RRID:AB_1645533
	Mouse IgM, k Isotype Control, APC	1 µl	BD Biosciences; 560806; RRID:AB_2034030
	Mouse IgG3, k Isotype Control PE	1 µl	BD Biosciences; 559926 RRID:AB_10050453

Primers

	Target	Forward/Reverse primer (5′-3′)
Episomal Plasmids Integration PCR	EBNA	ATCGTCAAAGCTGCACACAG/CCCAGGAGTCCCAGTAGTCA
House-Keeping Genes (PCR)	GAPDH	GCACCGTCAAGGCTGAGAAC/ AGGGATCTCGCTCCTGGAA
Sequencing	GLU280ALA (E280A)	GGTCCACTTCGTATGCTGGT/AGGAGTTCCAGGAATGCTGTG

conjugated antibodies (Table 3) against cells surface markers TRA-1-81 and SSEA-4. The corresponding isotype antibodies were used as controls. Influx Cell Sorter and BD 1.0.0.650 Software were used for the cytometry analysis.

Mycoplasma detection

Mycoplasma testing was carried out using the MycoSPY Mycoplasma PCR detection kit (Biontex), which detects a wide range of bacteria from the *Mollicutes* class. An Internal Control template was included to discard the presence of PCR inhibitors and rule out false positive results.

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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.2019.101440.

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