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Lab Resource: Single Cell Line

Generation of a human induced pluripotent stem cell line from a familial Alzheimer's disease *PSEN1 T119I* patient

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A B S T R A C T

Human induced pluripotent stem cells (hiPSC) line FLENIi001-A was reprogrammed from dermal fibroblasts using the lentiviral-hSTEMCCA-loxP vector. Fibroblasts were obtained from a skin biopsy of a 72-year-old Caucasian male familial Alzheimer's disease patient carrying the T119I mutation in the *PSEN1* gene. PSEN1 genotype was maintained and stemness and pluripotency confirmed in the FLENIi001-A hiPSC line.

1. Resource table

Unique stem cell line identifier	FLENIi001-A
Alternative name(s) of stem cell line	FFAD1.2 c4
Institution	Fundación para la Lucha contra las Enfermedades Neurológicas de la Infancia (Fleni)
Contact information of distributor	Leonardo Romorini (lromorini@fleni.org.ar)
Type of cell line	Induced pluripotent stem cells (iPSC)
Origin	Human
Additional origin info	Age: 72 Sex: Male Ethnicity: Caucasian
Cell Source	Dermal fibroblasts
Clonality	Clonal
Method of reprogramming	Lentiviral EF1a-hSTEMCCA-loxP vector expressing OCT-4, SOX-2, c-MYC and KLF4
Genetic Modification	Yes
Type of Modification	Congenital mutation
Associated disease	Alzheimer's Disease (AD)
Gene/locus	*PSEN1* (rs1566630791; *c.356C>T; p.T119I*)
Method of modification	N/A N/A
Name of transgene or resistance	
Inducible/constitutive system	N/A
Date archived/stock date	11.07.2020
Cell line repository/bank	No physical repository is available. Cell line has been registered at http://hpsreg.eu with the unique identifier name FLENIi001-A
Ethical approval	The study was approved by local Ethics Committee (Comité de ética en investigaciones biomédicas del Instituto Fleni). Approval number: 0414. Written informed consent was obtained from the patient

2. Resource utility

Familial Alzheimer's disease (fAD) is a neurodegenerative disease characterized by cognitive decline leading to incapacity. The generated iPSC line harbours a newly reported *PSEN1* mutation, making it suitable for *in vitro* studies associated with AD physiopathology (Table 1).

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Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photomicrography	Normal	Fig. 1 panel D
Phenotype	Immunocytochemistry	Positive for pluripotency markers OCT-4, NANOG, TRA1-60, TRA1-81 and SSEA-4	Fig. 1 panel F
	RT-qPCR	cells express OCT-4, SOX-2 and NANOG	Fig. 1 panel E
Genotype	Karyotype (G-banding) and resolution	46, XY, resolution: 440	Fig. 1 panel C
Identity	Microsatellite PCR (mPCR) OR	N/A	N/A
	STR analysis	26 sites tested, matched	Submitted in archive with journal
Mutation analysis (IF APPLICABLE)	Sequencing	Sanger sequencing – heterozygous mutation c.356C>T present in FLENIi001-A	Fig. 1 panel A
	Southern Blot OR WGS	N/A	N/A
Microbiology and virology	Mycoplasma	Mycoplasma testing by PCR (Negative)	Supplementary file 1
Differentiation potential	Embryoid body formation	Expression of α-smooth muscle actin (SMA), α-feto protein (AFP) and βIII-tubulin (TUJ1) were used as a proof of three germ layers formation	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

3. Resource details

Alzheimer's disease (AD) is a neurodegenerative proteinopathy, which is the main cause of dementia in adults. Prevalence estimates that 5% of AD cases are caused by inherited mutations in genes such as Presenilin-1 (*PSEN1*). Recently, our group reported a novel heterozygous variant in *PSEN1* (c.356C>T; p.T119I) in an Argentine family with early- and late-onset AD (Itzcovich et al., 2020). Dermal fibroblasts were obtained from a skin biopsy of a 72-year-old male AD patient carrying the *PSEN1* T119I mutation. The EF1a-hSTEMCCA-loxP lentiviral vector expressing *OCT-4*, *SOX-2*, *c-MYC* and *KLF4* pluripotency genes was used to generate the iPSC line FLENIi001-A as described previously (Somers et al., 2010). *PSEN1* c.356C>T (NM_000021.4); p.T119I mutation is present in FLENIi001-A iPSCs as well as in the parental fibroblasts (Fig. 1A). Besides, STR profiling confirms a 100% identity match between the parental fibroblasts and FLENIi001-A iPSC cell line. Moreover, transgenes inserted by the STEMCCA lentiviral vector were silenced as no detectable expression was observed by RT-qPCR using specific primers for exogenous expression (Fig. 1B and Table 2). Parental fibroblasts at 6 days post-lentiviral transduction and untransduced fibroblasts were used as positive and negative controls, respectively (Fig. 1B). FLENIi001-A iPSCs (passage 11) exhibited normal karyotype (46, XY) (50 metaphases were studied at a 440-band resolution) (Fig. 1C) and showed typical iPSCs morphological characteristics (formation of compact multicellular colonies with a high nucleus/cytoplasm ratio and distinct colony borders), and high Alkaline Phosphatase (AP) activity (Fig. 1D). Pluripotency was confirmed by quantification of the mRNA expression levels of the pluripotent genes *SOX-2*, *OCT-4*, and *NANOG* by RT-qPCR. Particularly, *SOX-2* was induced >391-fold, *OCT-4* > 341-fold and *NANOG* > 427-fold in comparison to the parental fibroblasts (Fig. 1E). Importantly, control established hiPSCs (Questa et al., 2016) showed similar *SOX-2*, *OCT-4* and *NANOG* mRNA expression levels when compared to parental fibroblasts than FLENIi001-A iPSCs (Fig. 1E). Also, robust expression of stemness-associated markers, such as the nuclear located transcription factors OCT-4 and NANOG and the surface markers SSEA-4 and TRA1-60 was verified by immunofluorescence staining (Fig. 1F). Finally, by *in vitro* spontaneous differentiation (embryoid bodies-based method) we demonstrated that FLENIi001-A iPSCs exhibited pluripotency as they were able to be differentiated into cells from the three germinal layers (mesoderm, endoderm and ectoderm) as shown by immunofluorescence analysis of Smooth muscle actin (SMA), Alpha-fetoprotein (AFP) and βIII tubulin (TUJ1) differentiation markers expression, respectively (Fig. 1G).

4. Material and methods

4.1. Reprogramming and cell culture

Patient fibroblasts carrying *PSEN1 T119I* mutation were cultured in DMEM media containing 10% Fetal bovine serum (FBS). STEMCCA EF1a-hSTEMCCA-loxP lentiviral vector encoding *OCT-4*, *KLF4*, *SOX-2* and *c-MYC* was produced, and fibroblasts infected (MOI = 1) as previously described (Somers et al., 2010). At day 2 post-transduction, media was changed to hESC media (DMEM/F12 + 20% KSR (Gibco), 2 mM non-essential amino acids, 2 mM L-glutamine, 100 U/ml penicillin, 50 μg/ml streptomycin, 0.1 mM 2-mercaptoethanol and 8 ng/ml bFGF). At day 6 post-infection cells were replated on dishes containing irradiated mouse embryonic fibroblasts (iMEFs). Cells were maintained in these conditions until uniform colonies were generated. iPSC colonies were mechanically isolated and expanded on iMEFs. iPSCs were then transferred to Geltrex-coated dishes and cultured in mTeSR1 media (Stemcell Technologies) for further expansion (passaged using Accutase at a split ratio of 1:3 in media containing ROCK inhibitor) and validation. Cells were cultured in a 37 °C, 5% CO_2, 90% humidity incubator.

4.2. Genotyping and STR analysis

Genomic DNA was isolated using the Wizard Genomic DNA Purification kit (Promega). *PSEN1 p.T119I* mutation was screened by PCR amplification and sequenced (exon 5 of *PSEN1*). STR analysis of 26 locations was performed at the Laboratorio de Huellas Digitales Genéticas (Facultad de Farmacia y Bioquímica, UBA, Buenos Aires, Argentina)

4.3. Karyotyping

Chromosomal G-band analyses were performed by Kromos Cytogenetic Laboratory (Buenos Aires, Argentina). 50 metaphases were analysed at 440-band resolution.

4.4. Alkaline phosphatase assay

iPSCs were washed with PBS and subjected to alkaline phosphatase staining following manufacturer's instructions (Sigma).

4.5. RNA isolation and RT-qPCR

RNA was extracted with TRIzol and cDNA was synthesized from 500 ng of total RNA with 15 mM of random hexamers and MMLV reverse transcriptase. For qPCR studies, PCR amplification and analysis were

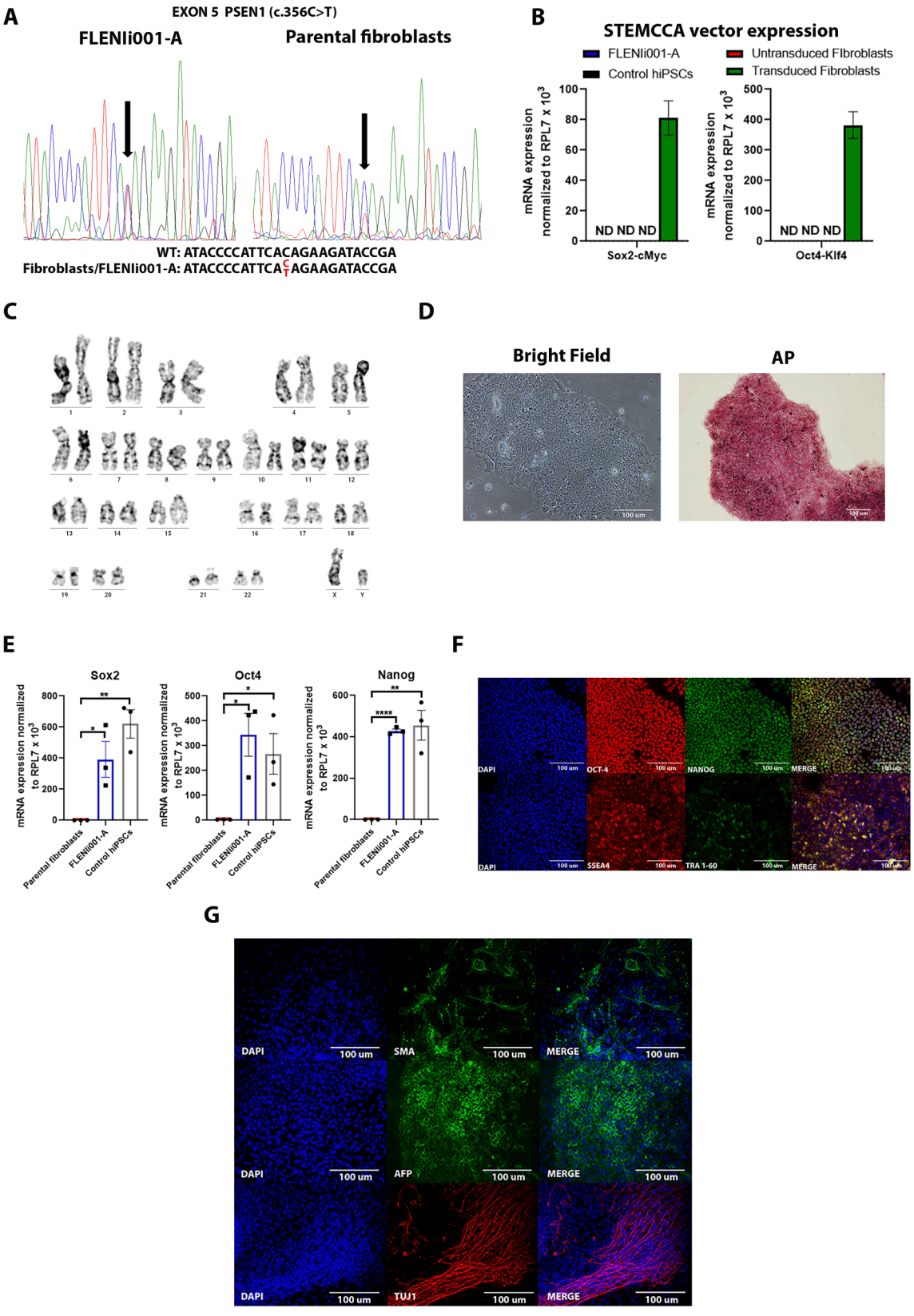

Fig. 1.

performed with StepOnePlus Real Time PCR System (PE Applied Biosystems). The FastStart Universal SYBR Green Master Mix (Roche) was used for all reactions. Transgenes expression was assessed by using primers that bridge over two of the four transgenes on the STEMCCA lentiviral vector sequence. This design allows amplification of only cDNA generated from the exogenous mRNA expression of these genes. Values were analysed using LinRegPCR and normalized against a housekeeping (RPL7).

Table 2
Reagents details.

Antibodies used for immunocytochemistry/flow-cytometry			
	Antibody	Dilution	Company Cat # and RRID
Pluripotency Markers	mouse anti-OCT-4 IgG	1:200	Santa Cruz Biotechnology Cat# sc-5279, RRID: AB_628051
Pluripotency Markers	rabbit anti-NANOG IgG	1:400	Cell Signaling Technology Cat#4903, RRID: AB_10559205
Pluripotency Markers	mouse anti-SSEA4 IgG	1:200	Santa Cruz Biotechnology Cat# sc-21704, RRID: AB_628289
Pluripotency Markers	mouse anti-TRA1-60 IgM	1:200	Santa Cruz Biotechnology Cat# sc-21705, RRID: AB_628385
Pluripotency Markers	mouse anti-TRA1-81 IgM	1:200	Santa Cruz Biotechnology Cat# sc-21706, RRID: AB_628386
Differentiation Markers	mouse anti-AFP IgG	1:200	Santa Cruz Biotechnology Cat# sc-166325, RRID: AB_2305278
Differentiation Markers	mouse anti-SMA IgG	1:400	Invitrogen Cat# PA5-87638, RRID: AB_2804309
Differentiation Markers	mouse anti-TUJ1 IgG	1:400	Covance Cat# MMS-435P, RRID: AB_2313773
Secondary antibodies	Goat anti-Mouse IgG Alexa Fluor 594	1:400	Thermo Fisher Scientific Cat# A-11005, RRID: AB_2534073
Secondary antibodies	Goat anti-Mouse IgG Alexa Fluor 488	1:400	Thermo Fisher Scientific Cat# A-11001, RRID: AB_2534069
Secondary antibodies	Goat anti-Mouse IgM Alexa Fluor 488	1:400	Thermo Fisher Scientific Cat# A-21042, RRID: AB_2535711
Secondary antibodies	Goat anti-Rabbit IgG Alexa Fluor 488	1:400	Thermo Fisher Scientific Cat# A-11008, RRID: AB_143165

Primers		
	Target	Forward/Reverse primer (5′–3′)
Exogenous factors (RT-PCR)	STEMCCA plasmid OCT-4/ KLF4	CAACGAGAGGATTTTGAGGC/ ATCGTTGAACTCCTCGGTCTCTCT
Exogenous factors (RT-PCR)	STEMCCA plasmid SOX-2/ c-MYC	TTGGCTCCATGGGTTCGGTG/ AAGGGTGTGACCGCAACGTAGG
Pluripotency Markers (qPCR)	OCT-4	CTGGGTTGATCCTCGGACCT/ CACAGAACTCATACGGCGGG
Pluripotency Markers (qPCR)	SOX-2	AGCATGGAGAAAACCCGGTACGC/ CGTGAGTGTGGATGGGATTGGTGT
Pluripotency Markers (qPCR)	NANOG	AAGAATCTTCACCTATGCC/ GAAGGAAGAGGAGAGACAGT
House-Keeping Genes (qPCR)	RPL7	AATGGCGAGGATGGCAAG/ TGACGAAGGCGAAGAAGC
Sequencing	PSEN1	GTGGTAATGTGGTTGGTGAT/ CCCAACCATAAGAAGAACAG

4.6. In vitro differentiation

Cells were dispersed with Dispase and transferred to non-adherent Petri dishes in hESC media (without bFGF) to induce formation of embryoid bodies (EBs). On day 4 media was changed to DMEM/F12 supplemented with 20% FBS, 2 mM L-glutamine, 100 U/ml penicillin and 50 μg/ml streptomycin. EBs incubated in suspension for 4 days were then plated onto 0.1% gelatin coated 24-well plates and cultured for additional 17 days.

4.7. Immunofluorescence staining

Cells fixed with 4% formaldehyde and permeabilized with 0.1% Triton X-100 were analysed for *in situ* immunofluorescence. Fluorescent secondary Alexa Fluor antibodies were used to localize the antigen/ primary antibody complexes. Cells were counterstained with DAPI and examined under a NIKON Eclipse TE2000-S inverted microscope.

4.8. Statistical analysis

All results are expressed as mean ± SEM. Two-tailed Student's *t*-test were used to detect significant differences (*$p < 0.05$; **$p < 0.01$; ****$p < 0.0001$) as indicated.

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.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scr.2021.102325.

References

Itzcovich, T., Chrem-Méndez, P., Vázquez, S., Barbieri-Kennedy, M., Niikado, M., Martinetto, H., Allegri, R., Sevlever, G., Surace, E.I., 2020. A novel mutation in PSEN1 (p.T119I) in an Argentine family with early- and late-onset Alzheimer's disease. Neurobiol. Aging 85, 155.e9–155.e12.

Questa, M., Romorini, L., Blüguermann, C., Solari, C.M., Neiman, G., Luzzani, C., Scassa, M.É., Sevlever, G.E., Guberman, A.S., Miriuka, S.G., 2016. Generation of iPSC line iPSC-FH2.1 in hypoxic conditions from human foreskin fibroblasts. Stem Cell Res. 16 (2), 300–303.

Somers, A., Jean, J.C., Sommer, C.A., Omari, A., Ford, C.C., Mills, J.A., Ying, L., Sommer, A.G., Jean, J.M., Smith, B.W., Lafyatis, R., Demierre, M.F., Weiss, D.J., French, D.L., Gadue, P., Murphy, G.J., Mostoslavsky, G., Kotton, D.N., 2010. Generation of transgene-free lung disease-specific human iPS cells using a single excisable lentiviral stem cell cassette. Stem Cells 28 (10), 1728–1740.