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Lab Resource: Stem Cell Line

Derivation of induced pluripotent stem cells from a familial Alzheimer's disease patient carrying the L282F mutation in presenilin 1

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ABSTRACT

Mutations in presenilin 1 (PSEN1) lead to the most aggressive form of familial Alzheimer's disease (AD). Human induced pluripotent stem cells (hiPSCs) derived from AD patients can be differentiated and used for disease modeling. Here, we derived hiPSC from skin fibroblasts obtained from an AD patient carrying a L282F mutation in PSEN1. We transfected skin fibroblasts with episomal iPSC reprogramming vectors targeting human OCT4, SOX2, L-MYC, KLF4, NANOG, LIN28, and short hairpin RNA against TP53. Our hiPSC line, L282F-hiPSC, displayed typical stem cell characteristics with consistent expression of pluripotency genes and the ability to differentiation into the three germ layers.

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Resource table.

Name of Stem Cell construct	L282F-hiPSC
Institution	University of Copenhagen
Person who created resource	Anna Poon, Kristine K Freude
Contact person and email	Kristine K Freude: kkf@sund.ku.dk
Date archived/stock date	August 2016
Origin	Human skin fibroblasts
Type of resource	Biological reagent: induced pluripotent stem cells derived from *PSEN1* L282F heterozygous mutation patient
Sub-type	Induced pluripotent stem cells (iPSCs)
Key transcription factors	Human *OCT4, SOX2, KLF4, L-MYC, LIN28*, and shRNA against TP53 (Addgene plasmids 27077, 27078 and 27080; Okita et al., 2011)
Authentication	The identity and purity of this cell line were confirmed by analysis of plasmid integration, mutation sequencing, karyotyping, pluripotency markers and *in vitro* differentiation into the three germ layers (Fig. 1)
Link to related literature (direct URL links and full references)	N/A
Information in public databases	N/A

Resource details

A human induced pluripotent stem cell (hiPSC) line was generated from skin fibroblasts collected from a familial 64-year-old male Alzheimer's disease (AD) patient carrying a heterozygous L282F mutation in the presenilin 1 (*PSEN1*) gene. The underlying C → T transversion at the first position of codon 282 (Chr14:73198105, Ensembl genome assembly: GRCh38.p7) results in the substitution of leucine (L) to phenylalanine (F) as previously described (Hamaguchi et al., 2009). We confirmed the presence of this L282F mutation in patient fibroblasts through sequencing (Fig. 1A). In addition, we detected a heterozygous variation (Chr14: 73198104 G>A) in our patient (Fig. 1A). Genetic variation at this position does not lead to any changes at the amino acid level and has been previously reported as a silent single nucleotide polymorphism (The 1000 Genomes Project Consortium, 2015).

To derive hiPSC from patient fibroblasts, we adopted previous protocols (Okita et al., 2011; Rasmussen et al., 2014) and electroporated the patient's skin fibroblasts with episomal plasmids expressing human *OCT4, SOX2, L-MYC, KLF4, NANOG, LIN28*, and short hairpin RNA against *TP53*. To demonstrate that our established hiPSC line, referred to as L282F-hiPSC, was free of integrated episomal reprogramming factors, we performed quantitative PCR with primers targeting *OCT4*, *SOX2*, and *LIN28* from the episomal plasmids (Fig. 1B). L282F-hiPSCs was characterized to be karyotypically normal (Fig. 1C), morphologically resembled stem cells, and expressed pluripotency markers including OCT4, NANOG, SSEA4, TRA-1-60, and *TDGF1* (Fig. 1D & E). Pluripotency of L282F-hiPSC was further supported by its ability to differentiate into

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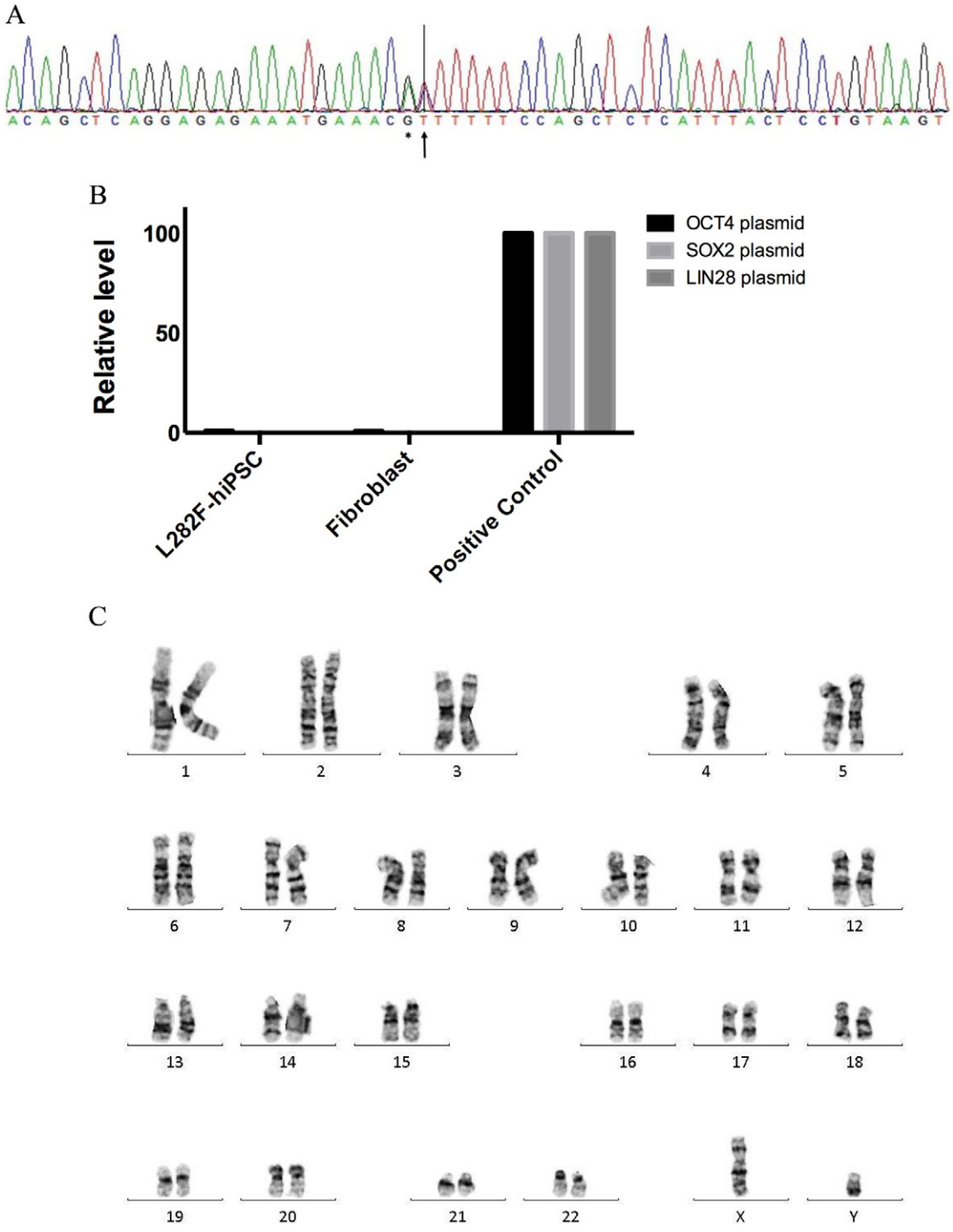

Fig. 1. A Sequencing of *PSEN1* confirmed the heterozygous L282 point mutation at position Chr14:73198105 C>T (arrow) in the AD patient cells. An additional variation at position Chr14:73198104 G>A was also detected (*). B. No integration of reprogramming factors was detected in the L282F-hiPSC. Quantitative PCR using plasmid-specific primers of the episomal reprogramming factors (OCT4, SOX2, and LIN28) was performed on DNA samples from L282F-hiPSCs, non-reprogrammed fibroblasts, and a positive control with presence of all reprogramming factors. All quantitative expression data were normalized to the expression levels of GAPDH. C. Representative normal karyotype of L282F-hiPSC with 46,XY. D. Quantitative RT-PCR analysis of pluripotency in L282F-hiPSC. A panel of pluripotency-associated genes commonly expressed in stem cells was examined (x-axis). The y-axis shows the relative fold change ($2^{-\Delta\Delta Ct}$) in transcript expression of L282F-hiPSC over fibroblasts. All quantitative gene expression data were normalized to the expression levels of GAPDH. E. Immunofluorescence staining of L282F-iPSCs with pluripotency markers: OCT4, NANOG, SSEA3, SSEA4, TRA1-60 and TRA1-81. Scale bars correspond to 100 μm. F. *In vitro* differentiation of L282F-hiPSC derived embryoid bodies on day 21. Cells were stained with smooth muscle actin (SMA), α-fetoprotein (AFP), and β-III tubulin (TUJI). Scale bars correspond to 100 μm.

all three germ layers with positive staining for ectodermal marker β-tubulin III, mesodermal marker smooth muscle actin, and the endodermal marker α-fetoprotein (Fig. 1F).

The study was approved by the Ethics Committee of the Capital Region of Denmark (protocol number H-4-2011-157) and informed consent was obtained from the patient.

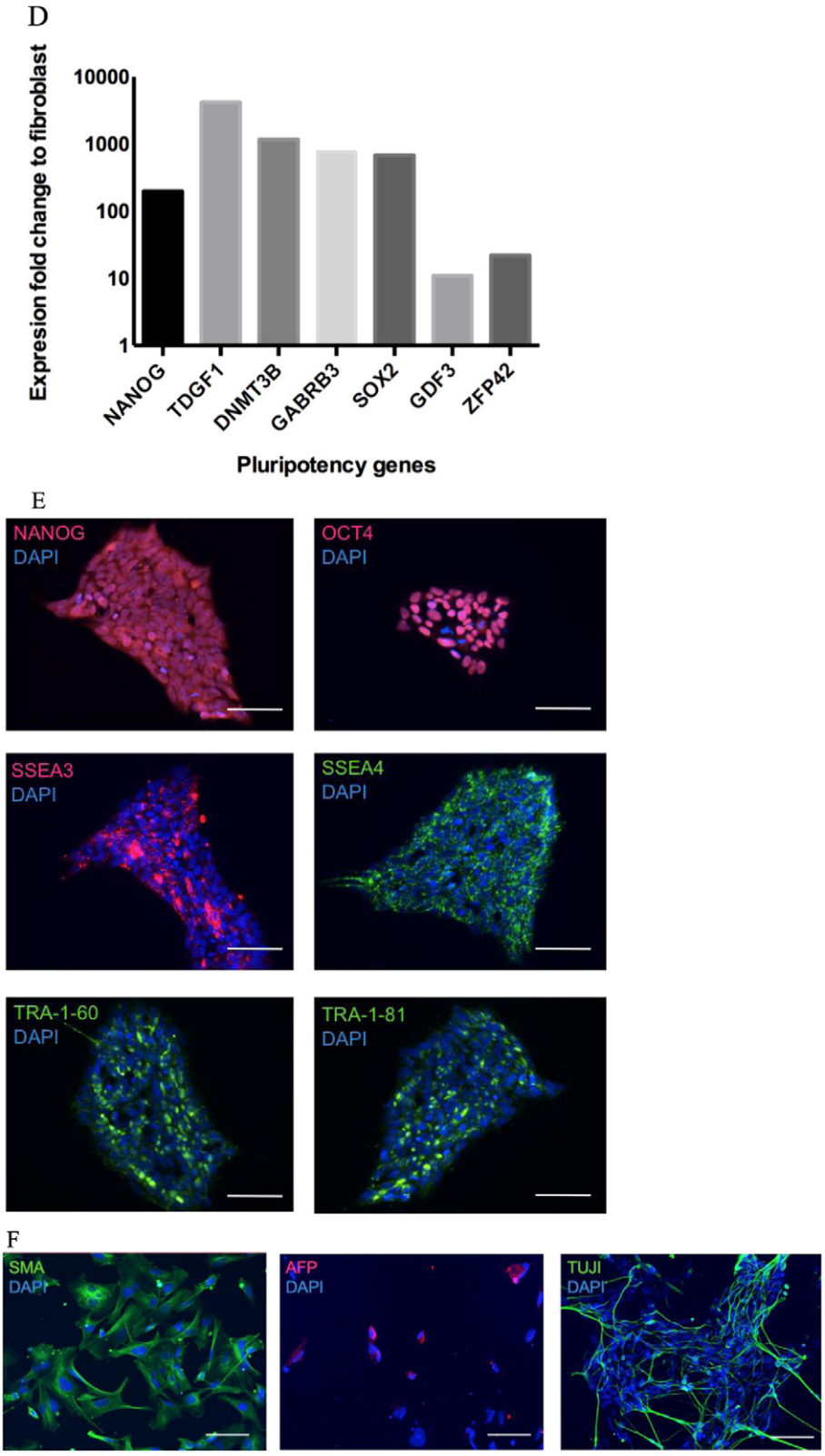

Fig. 1 (*continued*).

Materials and methods

Reprogramming of fibroblasts into hiPSCs

Fibroblasts from a skin biopsy of a 64-year-old man were cultured in DMEM supplemented with 10% fetal bovine serum, 1% Glutamine, and 2 ng/ml of fibroblast growth factor 2. Fibroblasts (1×10^5 cells) were electroporated with 1 μg of episomal plasmids containing gene sequences for human *OCT4*, *SOX2*, *KLF4*, *L-MYC*, *LIN-28* and short hairpin RNA for TP53 (Addgene) using the Neon transfection system (Thermo Fisher Scientific). After electroporation, cells were cultured on Matrigel (BD Biosciences) in Essential 8 medium (E8, Thermo Fisher Scientific).

After four weeks, individual hiPSC colonies were manually picked and transferred to Matrigel-coated dishes in E8 medium. The hiPSC lines were routinely passaged using 0.5 mM EDTA (Sigma) and frozen as stocks in E8 containing 10% DMSO.

Sequencing analysis

Genotyping of *PSEN1* mutation was performed by PCR amplification using a primer pair spanning the L282F mutation in exon 8 of *PSEN1* (forward primer: 5′-ccaccagttcacctgccattt-3′ and reverse primer: 5′-tggaactcctgcagatctctt-3′). PCR was performed using Taq DNA polymerase (Takara) according to the manufacturer's instructions. Amplicons were sequenced and electropherograms were analyzed with an ABI PRISM310 Genetic Analyzer.

Karyotyping

L282F-hiPSCs were karyotyped using standard cytogenetic procedures. In brief, cells were treated for 45 min with KaryoMAX colcemid (Thermo Fisher Scientific), harvested in fresh fixative consisted of 25% acetic acid and 75% methanol, and subjected for G-band karyotyping at the Institute of Medical Genetics and Applied Genomics (University of Tübingen, Tübingen, Germany).

Reprogramming factors integration assay

Genomic DNA was isolated from passage 10 L282F-hiPSCs, non-reprogrammed fibroblasts as negative control, and fibroblasts collected 48 h after electroporation with episomal plasmids as a positive control. Quantitative PCR was carried out using primers specific to the episomal plasmids (Okita et al., 2011). All quantitative expression data were normalized to the expression levels of GAPDH.

Quantitative reverse transcription PCR analysis of pluripotency genes

Total RNA was purified from L282F-hiPSCs and fibroblasts using RNeasy Mini Kit (Qiagen) and converted to cDNA using High-Capacity cDNA Reverse Transcription Kit (Thermo Fisher Scientific). Real-time quantitative reverse transcription PCR (qRT-PCR) was performed using the StepOnePlus system (Thermo Fisher Scientific). Applied Biosystems' TaqMan assays was used to measure the expression of specific pluripotency genes including *NANOG* (assay no.: HS02387400_g1), *TDGF1* (assay no.: HS02339497_g1), *GABRB3* (assay no.: HS00241459_m1), *GDF3* (assay no.: HS00220998_m1), *DNMT3B* (assay no.: HS00171876_m1), and *GAPDH* (assay no.: Hs03929097_g1). All quantitative gene expression data were normalized to the expression levels of GAPDH. The relative fold changes ($2^{-\Delta\Delta Ct}$) in transcript expression of L282F-hiPSC over fibroblasts were calculated.

Immunofluorescence staining

L282F-hiPSCs were fixed with 4% paraformaldehyde (PFA) in PBS for 15 min, blocked, and incubated with primary antibodies listed in Table 1 overnight at 4 °C. The next day, cells were incubated with the appropriate secondary antibodies and counterstained with DAPI. Images were acquired using the Leica DMRB-fluorescence microscope.

In vitro differentiation by embryoid body (EB) formation

Embryoid body (EB) formation was performed by transferring Dispase-treated clumps of hiPSCs onto ultra-low attachment plates (Corning) in E8. After 2 days of culture, the medium was changed to DMEM/F12 containing 20% knockout serum replacement (Thermo Fisher Scientific), 1 × nonessential amino acid, 2 mM L-glutamine, 0.1 mM 2-mercaptoethanol, and 1% pen/strep. After 7 days, the EBs were plated on Matrigel-coated culture dishes and cultured in DMEM supplemented with 10% FBS, 2 mM L-glutamine and 1% pen/strep for up to three weeks. The cells were fixed for 15 min in 4% PFA for immunofluorescence staining with the antibodies TUJI, SMA and AFP (Table 1).

Table 1
A list of primary antibodies used for immunofluorescence staining.

	Antibody and host species	Dilution	Company and catalog number
Pluripotency	Rabbit anti-NANOG	1:50	Peprotech, 500-P236
	Goat anti-OCT4	1:100	Santa Cruz, sc-8628
	Rat anti-SSEA3	1:100	Biolegend, 330,302
	Mouse anti-SSEA4	1:100	Biolegend, 330,402
	Mouse anti-Tra-1-60	1:200	Biolegend, 330,602
	Mouse anti-Tra-1-81	1:200	Biolegend, 330,702
In vitro differentiation	Rabbit anti-α-1-fetoprotein (AFP)	1:100	DAKO, A0008
	Mouse anti-Smooth muscle Actin (SMA) 1:500	1:100	DAKO, M0851
	Mouse anti-β-III tubulin (TUJI)	1:1000	Sigma-Aldrich, T8660

Verification and authentication

L282F-hiPSC karyotyping was performed by the Institute of Medical Genetics and Applied Genomics, University of Tübingen (Tübingen, Germany). A minimum of 20 metaphases were analyzed. The results showed a normal 46, XY karyotype without any detectable abnormalities (Fig. 1C). The identity of this line was confirmed by sequencing of *PSEN1* (Fig. 1A), integration assay (Fig. 1B), and expression of several pluripotency genes as well as genes expressed in the three germ layers following *in vitro* differentiation (Fig. 1D–F).

Acknowledgments

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