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Generation of induced pluripotent stem cells (iPSCs) from an Alzheimer's disease patient carrying a L150P mutation in *PSEN-1*

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ABSTRACT

Induced pluripotent stem cells (iPSCs) were generated from skin fibroblasts isolated from a 58-year old male with a L150P mutation in the presenilin 1 (*PSEN-1*) gene, which is responsible for the majority of familial cases of Alzheimer's disease (AD). The iPSCs were established by co-electroporation with episomal plasmids containing *hOCT4*, *hSOX2*, *hL-MYC*, *hKLF4*, *hNANOG*, *hLIN28*, and short hairpin RNA against *TP53*. The iPSCs contained the specific heterozygous mutation c.449C>T, had normal karyotype, expressed the expected pluripotency genes and displayed *in vitro* differentiation potential to the three germ layers. The iPSCs may be useful for studying familial AD pathology *in vitro*.

1. Resource table

Name of stem cell construct	H234 C2
Institution	Bioneer A/S
Person who created resource	Bjørn Holst, Alisa Tubsuwan
Contact person and email	Bjørn Holst, bho@bioneer.dk
Date archived/stock date	December 05, 2014
Origin	Human skin fibroblasts
Type of resource	Biological reagent: induced pluripotent stem cell (iPS); derived from an Alzheimer patient carrying *PSEN-1* L150P mutation
Sub-type	Induced pluripotent stem cells
Key transcription factors	*hOCT4*, *hSOX2*, *hL-MYC*, *hKLF4*, *hNANOG*, *hLIN28*, and shRNA for *TP53* (Addgene plasmids 27,077, 27,078 and 27,080; Okita et al. 2011)
Authentication	Identity and purity of cell line confirmed by integration analysis, sequencing of mutation, pluripotency analysis, karyotyping and *in vitro* differentiation (Fig. 1)
Link to related literature (direct URL links and full references)	N/A
Information in public databases	N/A

2. Resource details

Skin fibroblasts were collected from a 58-year old male with familial Alzheimer's disease (AD) caused by a c.449T>C mutation in the *PSEN-1* gene and electroporated with episomal plasmids of human *OCT4*, *SOX2*, *L-MYC*, *KLF4*, *NANOG*, *LIN28*, and shRNA for *TP53* using a Neon™ electroporation device (Invitrogen). Sequencing analysis of the iPSCs confirmed a heterozygous c.449T>C mutation in *PSEN-1*, which results in lysine being substituted by proline (L150P) (Fig. 1A). The absence of the three reprograming plasmids in the genome was verified by quantitative PCR after 10 passages (Fig. 1B). The iPSCs expressed the pluripotency markers *NANOG*, *OCT4*, *TDGF1*, *DNMT3B*, *GABRB3*, and *GDF3* (International Stem Cell Banking Initiative 2009) in the same range as BION010-A iPSC, a control iPSC line described previously (Rasmussen et al. 2014; Fig. 1C). Expression of the pluripotency markers, *NANOG*, *OCT4*, SSEA4, and TRA-1-60 at the protein level was confirmed by immunofluorescence staining (Fig. 1D). *In vitro* differentiation followed by ICC analysis with the ectodermal marker beta-III-Tubulin (TUJ1), the mesodermal marker smooth muscle actin (SMA) and the endodermal marker alpha-feto protein (AFP) demonstrated the differentiation potential into cells representing all three germ layers (Fig. 1E). In addition, the iPSCs presented a normal karyotype (46, XY) (Fig. 1F).

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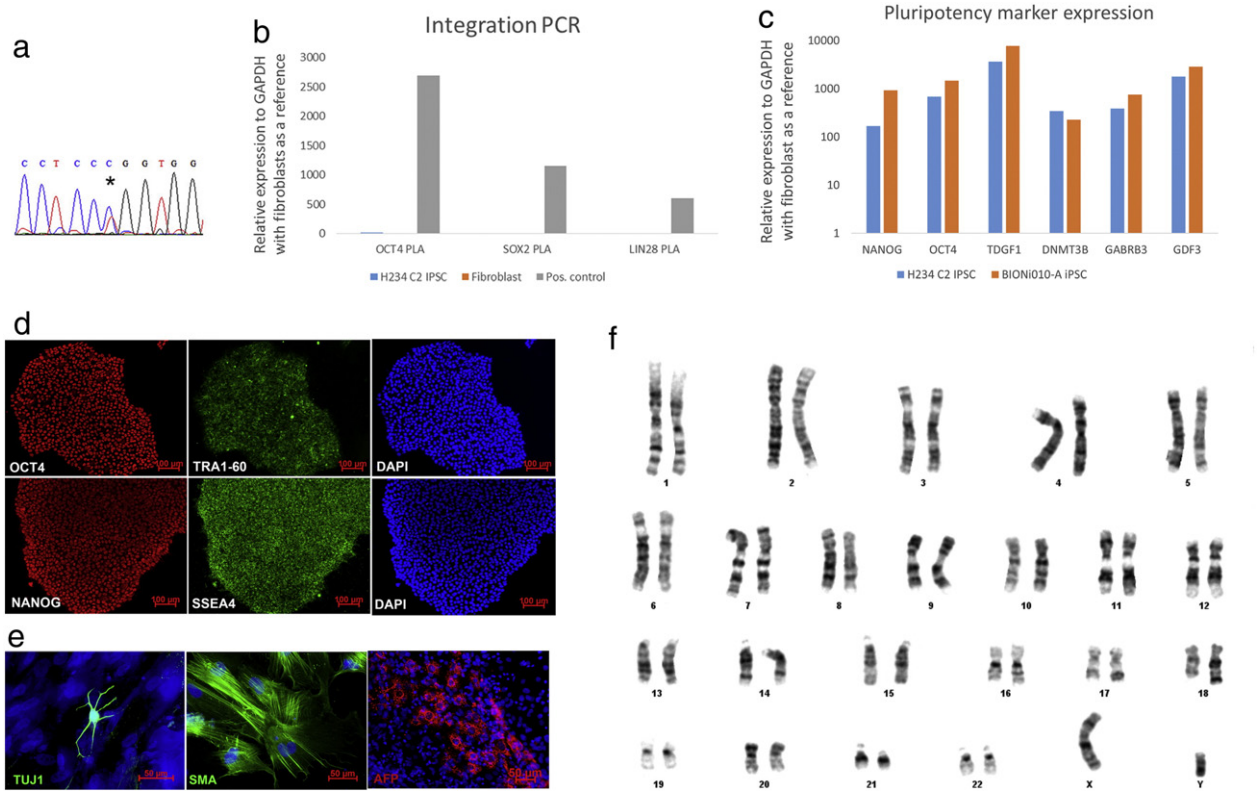

Fig. 1. Characterization of iPSCs generated from an Alzheimer's disease patient with L150P mutation in the *PSEN-1* gene. (A) Sequencing results of mutation in iPSCs confirmed a heterozygous c.449T>C mutation in *PSEN-1*. *Marks the mutation site. (B) Quantitative PCR for reprograming plasmids in H234 C2 iPSC, fibroblasts, and positive control. Data is shown as the fold change ($2^{-\Delta\Delta Ct}$) with fibroblasts and GAPDH as internal controls. (C) Quantitative PCR analysis of pluripotency-associated genes in H234 C2 iPSC and BION010-A iPSC. Data is shown as the fold change ($2^{-\Delta\Delta Ct}$) with fibroblasts and GAPDH as internal controls. (D) Immunofluorescence staining for the pluripotency markers *OCT4* (red), TRA-1-60 (green), *NANOG* (red) and SSEA4 (green). Scale bar correspond to 100 μm. (E) *In vitro* differentiation potential into the three germ layers demonstrated by ICC analyses of adhered EBs using TUJ1 (ectoderm marker; green), SMA (mesoderm marker; green) and AFP (endoderm marker; red). Scale bar correspond to 50 μm. (F) Representative karyotype of H234 C2 iPSC (46, XY).

3. Materials and methods

The study was approved by the regional scientific ethical committee in the Capital Region of Denmark (RH), (protocol number H-4-2011-157), Copenhagen, Denmark and written informed consent was obtained in all cases.

3.1. Cell culture and reprogramming

Skin fibroblasts from a 58-year old male were obtained from a skin biopsy and cultured in DMEM (Life Technologies) supplemented with 10% fetal bovine serum (FBS, Life Technologies), 1% Glutamine (Life Technologies) and 2 ng/ml of fibroblast growth factor 2 (FGF2). Fibroblasts (1×10^5) were electroporated with 1 μg of three episomal plasmids containing gene sequences for *hOCT4*, *hSOX2*, *hKLF4*, *hL-MYC*, *hLIN-28* and short hairpin RNA for *TP53* (*shP53*) (Addgene), using a Neon™ device (Invitrogen) as previously described (Okita et al. 2011). After electroporation, cells were cultured on ESC grade Matrigel (BD Biosciences) in mTeSR1 medium (Stem Cell Technologies, Vancouver, BC, Canada). After four weeks, individual iPSC colonies were manually picked and transferred to Matrigel-coated dishes in mTeSR1 medium. The iPSC lines were routinely passaged using 0.5 mM EDTA (Sigma) and frozen in liquid nitrogen in mTeSR1 containing 10% DMSO and thawed in the presence of 10 μM of Rho-kinase inhibitor (Y-27632, Sigma).

3.2. Sequencing analysis

Genotyping of the *PSEN-1* mutation was performed by PCR amplification of the *PSEN-1* gene using a primer pair covering the mutation in exon 5 (Table 1). 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.

3.3. Analysis of reprogramming plasmid integration

Genomic DNA was isolated from parental fibroblasts, iPSCs, and fibroblasts electroporated with episomal plasmids as a positive control using DNeasy Blood and Tissue Kit (Qiagen). Quantitative PCR was carried out using primers specific to the episomal plasmids (Okita et al. 2011). The data was analyzed using the $2^{-\Delta\Delta Ct}$ method relative to fibroblasts and GAPDH was used as an internal control.

3.4. Quantitative PCR analysis of pluripotency markers

Total RNA was purified from iPSCs, fibroblasts and the iPSC line BIONi010-A (Rasmussen et al. 2014) using RNeasy Mini Kit (Qiagen) and converted to cDNA using High-Capacity cDNA Reverse Transcription Kit (Thermo Scientific) according to the manufacturer's instructions. qPCR analysis was performed using TaqMan probes obtained from Applied Biosystems (Table 1) according to the manufacturer's instructions. qPCR assays were carried out in duplicate or triplicate with the StepOnePlus™ system (Thermo Scientific). The relative fold-changes in expression were calculated using the $2^{-\Delta\Delta Ct}$ method relative to *GAPDH* with fibroblasts as a reference (set to 1).

3.5. In vitro differentiation by embryoid body (EB) formation

Embryoid body (EB) formation was performed by transferring Dispase-treated clumps of iPSCs onto ultra-low attachment plates (Corning, Corning, NY, USA) in mTeSR1. After 2 days of culture, the

Table 1
Primers and TaqMan probes used for sequencing and qRT-PCR.

Assays	Amplicons	Sequence (5′ to 3′) or TaqMan gene expression assay number	Modification
Sequencing analysis	*PSEN-1*	GTGAGTTGGGGAAAAGTGAC	–
	gene	TCCACAGTGAGGAGGAAGAA	–
Pluripotency analysis	*TDGF1*	HS02339497_g1	5′Fam3′NFQ-MGB
	GABRB3	HS00241459_m1	5′Fam3′NFQ-MGB
	NANOG	HS02387400_g1	5′Fam3′NFQ-MGB
	GDF3	HS00220998_m1	5′Fam3′NFQ-MGB
	OCT4	HS0999632_g1	5′Fam3′NFQ-MGB
	DNMT3B	HS00171876_m1	5′Fam3′NFQ-MGB
	GAPDH	Hs03929097_g1	5′Fam3′NFQ-MGB

medium was changed to DMEM/F12 containing 20% knockout serum replacement (Life Technologies, Carlsbad, CA, USA), 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 0.1% gelatin-coated culture dishes and cultured in DMEM supplemented with 10% FBS, 2 mM L-gultamine and 1% pen/strep for up to three weeks. The cells were fixed for 15 min in 4% paraformaldehyde (PFA) for ICC analysis with the antibodies TUJI, SMA and AFP.

3.6. Immunofluorescence staining

Cells grown on cover slips were fixed at room temperature with 4% PFA in PBS for 10 min and then permeabilized with 0.1% Triton X-100 in PBS for 15 min. Non-specific binding was blocked with 1% BSA for 30 min. The cells were then incubated with primary antibodies overnight at 4 °C. To assess the expression of pluripotent markers, immunofluorescence staining was performed with primary antibodies against *OCT4* (1:500, 09–0023, Stemgent), *NANOG* (1:1000, 500-P236, Peprotech), SSEA4 (1:400, 330302, BioLegend), TRA-1-60 (1:400, 330602, BioLegend). In addition, primary antibodies against β-III tubulin (TUJ1; 1:3000, T8660, Sigma Aldrich), smooth muscle actin (SMA;1:1000, M0851, DAKO), and α-fetoprotein (AFP;1:1000, A0008, DAKO) were used to confirm the differentiation potential of H234 iPSC into all three germ layers. After three PBS washes, the cells were incubated with fluorescence-conjugated secondary antibodies (AlexaFluor 488: goat anti-mouse, and Cyanine 3: goat anti-rabbit) for 60 min, and mounted on glass slides with mounting medium containing DAPI (Life technologies).

3.7. Karyotype analysis

iPSCs and fibroblasts were treated for 45 min with KaryoMAX colcemid (Life Technologies) and karyotyping was performed on G-banded metaphase chromosomes using standard cytogenetic procedures.

4. Verification and authentication

Karyotyping was performed at the Cell Guidance Systems, England. At least 10 metaphases were analyzed per sample with an approximate resolution of 550 to 600 bands per haploid genome. The results showed a normal 46, XY karyotype, free of any discernible abnormalities (Fig. 1F). iPSC line identity and purity were furthermore confirmed by sequencing of the *PSEN-1* gene (Fig. 1A) and ICC with pluripotency markers (Fig. 1C).

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