Generation of a gene-corrected isogenic control hiPSC line derived from a familial Alzheimer's disease patient carrying a L150P mutation in presenilin 1

Poon, Anna Fong-Yee; Schmid, Benjamin; Pires, Carlota; Nielsen, Troels Tolstrup; Hjermind, Lena Elisabeth; Nielsen, Jørgen Erik; Holst, Bjørn; Hyttel, Poul; Freude, Kristine

Published in:
Stem Cell Research

DOI:
10.1016/j.scr.2016.09.018

Publication date:
2016

Document Version
Publisher's PDF, also known as Version of record

Stem Cell Research 17 (2016) 466–469

Contents lists available at ScienceDirect

Stem Cell Research

journal homepage: www.elsevier.com/locate/scr

Lab Resource: Stem Cell Line

Generation of a gene-corrected isogenic control hiPSC line derived from a familial Alzheimer's disease patient carrying a L150P mutation in presenilin 1

Anna Poon [a], Benjamin Schmid [b], Carlota Pires [a], Troels T. Nielsen [c], Lena E. Hjermind [c], Jørgen E. Nielsen [c], Bjørn Holst [b], Poul Hyttel [a], Kristine K. Freude [a,*]

[a] Faculty of Health and Medical Sciences, University of Copenhagen, Gronnegaardsvej 7, 1870 Frederiksberg C, Denmark
[b] Bioneer A/S, Kogle Alle 2, 2970 Hørsholm, Denmark
[c] Danish Dementia Research Centre, Rigshospitalet, University of Copenhagen, Blegdamsvej 9, 2100 Copenhagen Ø, Denmark

ARTICLE INFO

Article history:
Received 14 September 2016
Accepted 22 September 2016
Available online 24 September 2016

ABSTRACT

Mutations in the presenilin 1 (PSEN1) gene lead to the most aggressive form of familial Alzheimer's disease (AD). Human induced pluripotent stem cells (hiPSCs) derived from AD patients and subsequently differentiated can be used for disease modeling. We have previously generated a hiPSC line from a familial AD patient carrying a L150P point mutation in PSEN1. Here we used CRISPR/Cas9 gene editing to correct for the single base pair mutation. This gene-corrected line, L150P-GC-hiPSC, serves as an isogenic control to the mutant line for future investigation of mechanisms and cellular phenotypes altered by this specific PSEN1 mutation.

Resource details

A human induced pluripotent stem cell (hiPSC) line was previously generated from a skin biopsy obtained from a 58-year-old male Alzheimer's disease (AD) patient carrying a heterozygous point mutation (c.449C>T) in exon 6 of the presenilin 1 (*PSEN1*) gene (Tubsuwan et al., 2016). The single base pair mutation results in the substitution of leucine (L) to proline (P) at position 150 of the amino acid sequence (i.e., L150P). To generate a patient-specific and integration-free hiPSC line, episomal plasmids carrying gene sequences for *hOCT4*, *hSOX2*, *hKLF4*, *hL-MYC*, *hLIN28* and a short hairpin against TP53 (Okita et al., 2011) were used to reprogram patient's fibroblasts into hiPSCs (Tubsuwan et al., 2016). Here, we used this hiPSC line to further generate an isogenic control line, L150P-GC-hiPSC, by employing the CRISPR/Cas9 system (Ran et al., 2013) to correct the c.449C>T mutation. Genomic sequences manipulated for gene editing are shown in Fig. 1A. Correction of the point mutation (C > T) was validated by sequencing (Fig. 1B). The L150P–GC-hiPSC was characterized to be karyotype normal with 46, XY (Fig. 1C) and expressed common pluripotency markers:

Resource table

Name of Stem Cell construct	L150P-GC-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 induced pluripotent cell line (hiPSCs)
Type of resource	Biological reagent: gene-corrected induced pluripotent stem cells, originally derived from skin fibroblasts of a *PSEN1* L150P heterozygous mutation patient
Sub-type	Induced pluripotent stem cells (iPSCs)
Key transcription factors	*hOCT4*, *hSOX2*, *hKLF4*, *hL-MYC*, *hLIN28*, and shRNA against TP53 (Addgene plasmids 27077, 27078 and 27,080; Okita et al., 2011)
Authentication	Identity and purity of this cell line were confirmed by mutation sequencing, karyotyping and pluripotency markers (Figs. 1A, 1B, 1C and 1D)
Link to related literature (direct URL links and full references)	doi: 10.1016/j.scr.2015.12.015 Tubsuwan A, Pires C, Rasmussen MA, Schmid B, Nielsen JE, Hjermind LE, Hall V, Nielsen TT, Waldemar G, Hyttel P, Clausen C, Kitiyanant N, Freude KK, Holst B. Generation of induced pluripotent stem cells (iPSCs) from an Alzheimer's disease patient carrying a L150P mutation in PSEN-1. Stem Cell Research. 2016; 16: 110–112.
Information in public databases	N/A

* Corresponding author.
E-mail address: kkf@sund.ku.dk (K.K. Freude).

http://dx.doi.org/10.1016/j.scr.2016.09.018

(A) Patient *PSEN1* genomic sequence

actagatggagccagtgtctgcttcacatgttaagtccttctttccatatgttagacattttctttgaagcaattttagagtgtagctgtttttctcaggttaaaaattcttagctagatt
ggtgagttggggaaaagtgacttataagatacgaattgaattaagaaaaagaaaattctgtgttggaggtggtaatgtggttggtgatctccattaacactgacctagggctttt
gtgtttgttttattgtagAATCTATACCCCATTCACAGAAGATACCGAGACTGTGGGCCAGAGAGCCCTGCACTCAAT
TCTGAATGCTGCCATCATGATCAGTGTCATTGTTGTCATGACTATCCTCCCGGTGGTTCTGTATAAATACAG
GTGCTATAAGgtgagcatgagacacagatctttgctttccaccctgttcttcttatggttgggtattcttgtcacagt

(B) ssODN

TCTGAATGCTGCCATCATGATCAGTGTCATTGTTGTCATGACTATCCTACTGGTCGTTCTGTATAAATACAG
GTGCTATAAGgtgagcatgagacacagat

Fig. 1A. CRISPR/Cas9 and ssODN used to correct for the point mutation in L150P-hiPSC. (A) Genomic sequence of *PSEN1* exon 6 containing the point mutation is highlighted in red. The sgRNA recognizes the 23 bp genomic sequence as delineated by the blue box and the triangle indicates the CRISPR cutting site. Forward and reverse primer sequences highlighted in yellow were used for colony screening and sequencing. (B) The ssODN sequence serves as a template for the correction of point mutation from C to T.

OCT4, NANOG, SSEA3, SSEA4, TRA-1-60 and TRA-1-81 (Fig. 1D). This study was approved by the "De Videnskabsetiske Komiteer for Region Hovedstaden" (protocol number H-4-2011-157), Copenhagen, Denmark, and informed consent was obtained from the patient and his family. As part of privacy protection, no personal information of the patient is provided here.

Materials and methods

CRISPR design

The isogenic gene-corrected control L150P-GC-hiPSC was generated using the CRISPR/Cas9 technology in combination with single-stranded oligonucleotides (ssODNs) to guide the site-specific correction of the L150P mutation.

We adopted the protocol published by Ran et al. (2013) and designed the single-guide RNAs (sgRNAs) using the Zhang Lab software (http://crispr.mit.edu/). The sgRNAs was cloned into Cas9 expressing plasmids (pSpCas9(BB)-2 A–Puro (PX459) V2.0; addgene ID 62988). The ssODN contained the T (i.e., wild-type) nucleotide to replace the mutant "C" at the site of mutation. It also contained two additional silent mutations, one up- and one down-stream of the mutation site for positive colony selection with the appropriate restriction enzyme.

Nucleofection

L150P-hiPSCs grown on matrigel-coated (Corning Bioscience) dishes with E8 medium (Gibco) were detached with Accutase (Gibco). 2×10^6 single cells were transfected with 10 μg of CRISPR/Cas9 plasmid and 100 pmol of ssODN using the Amaxa 4D Nucleofector (program CA137, Lonza) and P3 Primary Cell Kit (Lonza) according to the manufacturer's instructions. Transfected hiPSCs were cultured on matrigel-coated dish with E8 medium supplemented with 1 mM ROCK inhibitor (Sigma). Transfected hiPSCs were then subjected to puromycin selection 4 h after nucleofection and cultured in the presence of puromycin for the next 5 days. Puromycin-resistant colonies were

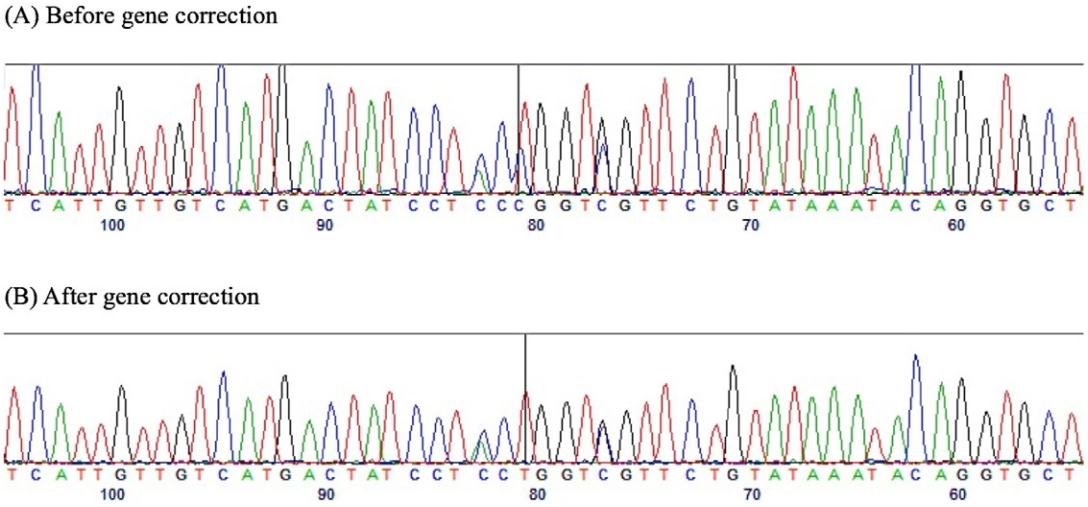

Fig. 1B. Sequencing of *PSEN1* to confirm CRISPR/Cas9 and ssODN-mediated gene correction. (A) Patient *PSEN1* sequence before gene correction. The black vertical line indicates the site of point mutation where the mutant allele with "C" nucleotide (blue) is detected in addition to the normal allele with the "T" nucleotide (red). (B) Sequencing result after gene correction indicating the successful replacement of the "C" with a "T".

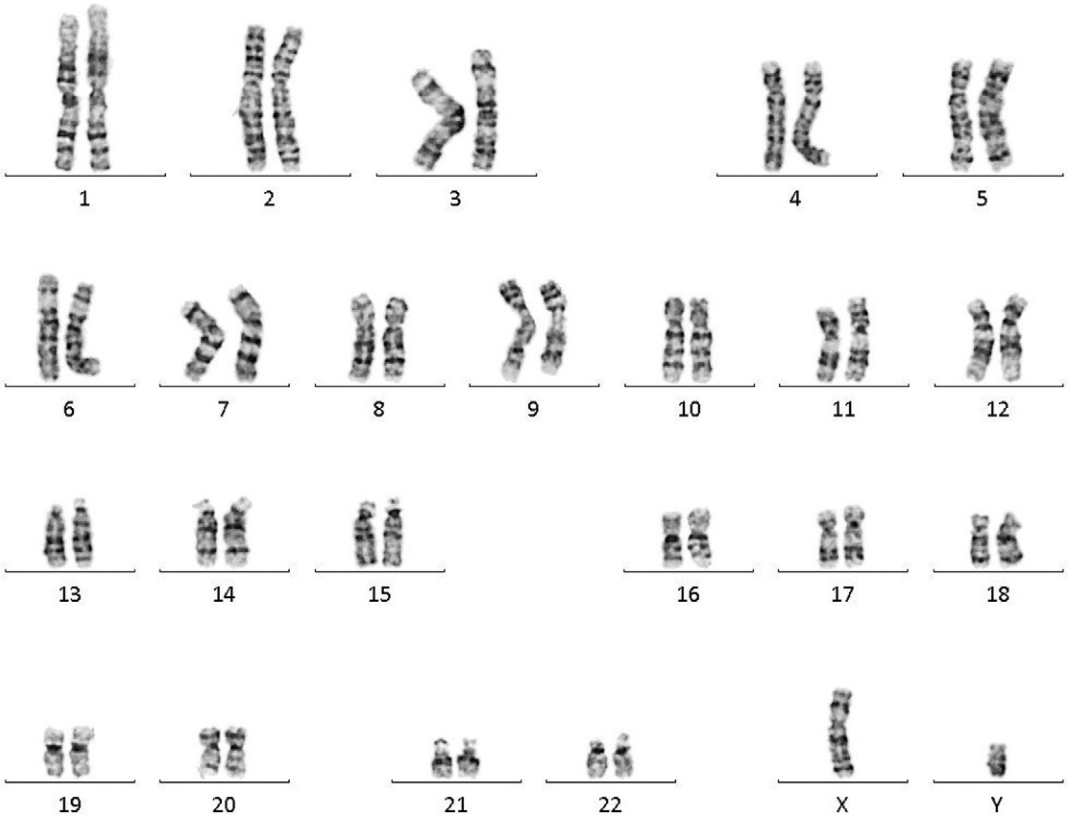

Fig. 1C. Representative normal karyotype of L150-GC-hiPSC with 46, XY.

manually picked into 96 well plates coated with matrigel and expanded in the E8 medium.

Genotyping and restriction assays

DNA for genotyping was extracted using the prepGem™ Kit (ZyGEM, PTI0500) (Qiagen). PCR with primers spanning the site of mutation was performed using TEMPase Hot Start DNA Polymerase (Ampliqon) according to the manufacturer's instructions. PCR products were purified and digested with BseNI (Thermo Scientific) at 65 °C for 20 min. This enzyme only cuts DNA from hiPSC colonies with the corrected allele from the ssODN containing the T nucleotide at the mutation site.

Sanger sequencing

Positive clones were sequenced using the ABI PRISM 310 Genetic Analyzer for the presence of the corrected nucleotide at the site of mutation. Sequences were also inspected for frameshifts and mutations that might arise from the CRISPR-mediated gene editing.

Immunofluorescence staining

L150-GC-hiPSCs were fixed with 4% paraformaldehyde 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 (Leica Microsystems, Wetzlar, Germany).

Karyotyping

L150-GC-hiPSCs were karyotyped using standard cytogenetic procedures. In brief, cells were treated for 45 min with KaryoMAX colcemid (Life Technologies), 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).

Verification and Authentication.

L150-GC-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 and purity of this line was confirmed by sequencing of *PSEN1* (Fig. 1B) and expression of several pluripotency genes (Fig. 1D).

Acknowledgements

We would like to thank Dr. Keisuke Okita and Prof. Shinya Yamanaka for providing the plasmids for reprogramming, Dr. Feng Zhang for providing the plasmid for gene editing and Addgene for their distribution of the plasmids. Furthermore, we would like to thank Ulla Bekker Poulsen for her excellent technical assistance. Our work is supported by the European Union 7th Framework Program (PIAP-GA-2012-324451-STEMMAD), the Innovation Fund Denmark for BrainStem (4108-00008B), and the Programme of Excellence 2016 (Copenhagen as the next leader in precise genetic engineering CDO2016: 2016CDO04210) from the University of Copenhagen.

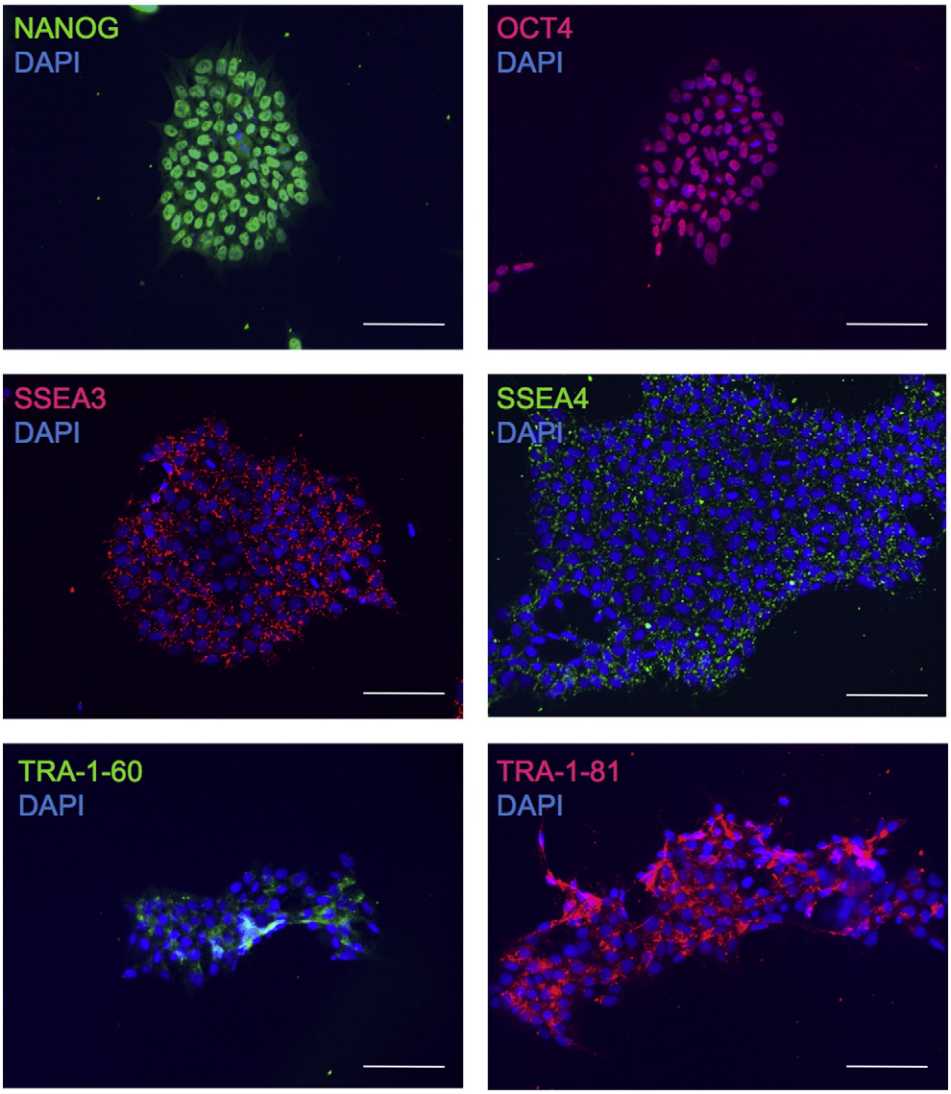

Fig. 1D. Immunofluorescence staining of L150P-GC-iPSCs with pluripotency markers: OCT4, NANOG, SSEA3, SSEA4, TRA1–60 and TRA1–81. Scale bars correspond to 100 μm.

Table 1
Primary antibodies used for immunocytochemistry.

Antibody and host species	Dilution	Company and catalog number
rabbit anti-NANOG	1:50	Peprotech, 500-P236
goat anti-OCT4	1:100	Santa Cruz, sc-8628
rat anti-SSEA3	1:100	Biolegend, 330302
mouse anti-SSEA4	1:100	Biolegend, 330402
mouse anti-Tra-1-60	1:200	Biolegend, 330602
mouse anti-Tra-1-81	1:200	Biolegend, 330702

References

Okita, K., Matsumura, Y., Sato, Y., Okada, A., Morizane, A., Okamoto, S., Hong, H., Nakagawa, M., Tanabe, K., Tezuka, K., Shibata, T., Kunisada, T., Takahashi, M., Takahashi, J., Saji, H., Yamanaka, S., 2011. A more efficient method to generate integration-free human iPS cells. Nat. Methods 8, 409–412.

Ran, F.A., Hsu, P.D., Wright, J., Agarwala, V., Scott, D.A., Zhang, F., 2013. Genome engineering using the CRISPR\-Cas9 system. Nat. Protoc. 8, 2281–2308.

Tubsuwan, A., Pires, C., MA, R., Schmid, B., JE, N., LE, H., Hall, V., TT, N., Waldemar, G., Hyttel, P., Clausen, C., Kitiyanant, N., KK, F., Holst, B., 2016. Generation of induced pluripotent stem cells (iPSCs) from an Alzheimer's disease patient carrying a L150P mutation in PSEN-1. Stem Cell Res. 16, 110–112.