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Lab resource: Multiple stem cell lines

Generation of two isogenic iPSC lines with either a heterozygous or a homozygous E280A mutation in the PSEN1 gene

Henriette R. Frederiksen[a], Bjørn Holst[b], Ulrike A. Mau-Holzmann[c], Kristine Freude[a], Benjamin Schmid[b,*]

[a] Stem Cells and Modeling of Neurodegeneration, Department of Veterinary and Animal Sciences, Faculty of Health and Medical Sciences, University of Copenhagen, Grønnegardsvej 7, 1870C, Frederiksberg, Denmark
[b] Bioneer A/S, Kogle Alle 2, 2970 Hørsholm, Denmark
[c] Institute of Medical Genetics and Applied Genomics, Division of Cytogenetics, Calwerstrasse 7, University of Tuebingen, 72076, Germany

A B S T R A C T

Alzheimer's disease (AD) is the most common form of dementia. Mutations in the gene *PSEN1* encoding Presenilin1 are known to cause familial forms of AD with early age of onset. The most common mutation in the *PSEN1* gene is the E280A mutation. iPSCs are an optimal choice for modeling AD, as they can be differentiated *in vitro* into neural cells. Here, we report the generation of two isogenic iPSC lines with either a homozygous or a heterozygous E280A mutation in the *PSEN1* gene. The mutation was introduced into an iPSC line from a healthy individual using the CRISPR-Cas9 technology.

Resource table

Unique stem cell lines identifier	1. *BIONi010-C + homozygous E280A = BIONi010-C-29* 2. *BIONi010-C + heterozygous E280A = BIONi010-C-30*
Alternative names of stem cell lines	1. *BIONi010-C E280 +/+ (homozygous line)* 2. *BIONi010-C E280 +/− (heterozygous line)*
Institution	*Bioneer A/S Hørsholm Denmark and University of Copenhagen (UCPH) Copenhagen Denmark*
Contact information of distributor	*Contact at Bioneer: Benjamin Schmid,bsc@bioneer.dk– Contact at UCPH: Kristine Freude,kkf@sund.ku.dk*
Type of cell lines	*iPSCs*
Origin	*Human*
Cell Source	*Fibroblasts*
Clonality	*Clonal*
Method of reprogramming	*Episomal plasmids (Okita et al., 2011)*
Multiline rationale	*Mutated isogenic clones*
Gene modification	*YES*
Type of modification	*Induced point mutation*
Associated disease	*Alzheimer's disease (AD)*
Gene/locus	*PSEN1/Chr14:73664808*
Method of modification	*CRISPR-Cas9*
Name of transgene or resistance	*N/A*
Inducible/constitutive system	*N/A*
Date archived/stock date	*September 2017*
Cell line repository/bank	1. *BIONi010-C-29:https://hpscreg.eu/cell-line/BIONi010-C-29* 2. *BIONi010-C-30:https://hpscreg.eu/cell-line/BIONi010-C-30*
Ethical approval	*The study was approved by the Ethics Committee of the Capital Region of Denmark (H-4-2011-157), and written informed consent was obtained from the participant before enrolment*

* Corresponding author.
E-mail address: bsc@bioneer.dk (B. Schmid).

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Resource utility

The exact mechanisms leading to Alzheimer's disease (AD) remain still unknown. In order to better understand disease development and underlying cellular pathological mechanisms, we have established two isogenic iPSC lines containing either a heterozygous or a homozygous E280A mutation in the PSEN1 gene, which are known to result in AD.

Resource details

The mutation E280A in the *PSEN1* is one of the most common mutations related to familial forms of Alzheimer's disease (AD). Ample patient based information is available based on studies from a large kindred from the Colombian state of Antioquia. (Sepulveda-Falla et al., 2012). This specific mutation is therefore highly relevant for establishment of an iPSC based disease model, as the cell model is substantially supported by patient phenotype information, which will facilitate validation of the *in vitro* model.

For this study, the E280A mutation was inserted into the human iPSC line BIONi010-C, which had earlier been established from a skin biopsy obtained from a healthy male individual aged 18 (Rasmussen et al., 2014).

The point mutation resulting in an amino acid change from glutamic acid (E) to alanine (A) was knocked into the *PSEN1* gene by using the CRISPR-Cas9 system (Jinek et al., 2012). The nucleotide substitution was confirmed by restriction digest (*data not shown*) followed by DNA sequencing. Sequencing analysis confirmed a successful A > C transition causing the E280A mutation on protein level in both a heterozygous and homozygous manner. The two lines are further referred to as BIONi010-C-E280A +/− and BIONi010-C-E280A +/+, respectively (Fig. 1A, Table 1).

Pluripotency of the gene-edited lines was confirmed by immunocytochemistry (ICC) and quantified by flow analysis. Both cell lines show clear expression of OCT4, NANOG, SSEA4, SSEA3, TRA-1-60 and TRA-1-81 (Fig. 1B). The differentiation potential of the iPSCs was confirmed *via* spontaneous differentiation into *endo-* and mesodermal cell types (Fig. 1D). Cells were differentiated to neural progenitor cells and stained for PAX6 and Nestin (Fig. 1D) the cells hereby showed capability of ectodermal differentiation. A karyotype analysis was carried out to confirm chromosomal integrity. The results showed a normal 46, XY karyotype without detectable abnormalities (Supplementary Fig. 1A). Investigation of cells by light microscopy confirmed a normal iPSC morphology throughout the gene editing process (Fig. 1C). Finally, an STR analysis was performed to confirm cell line identity (Available with author) and cells were tested negative for mycoplasma (Supplementary Fig. 1B).

Materials and methods

Gene editing

Cells were grown on Matrigel coated plates in E8 media and incubated at 37 °C and 5% CO_2. At 80% confluency, cells were detached by treating cells with Accutase for 5 min after which 0.8×10^6 cells were nucleofected. Nucleofection was made by using the Lonza nucleofector, P3 solution and the CA167 pulse setting. Nucleofected cells were transferred to Matrigel coated plates and single colonies were picked manually for analysis after 7 days.

Editing was performed using the CRISPR-Cas9 system in combination with an ssODN (single stranded oligo deoxynucleotide) as homologous template containing the E280A mutation. Two silent mutations (changing the DNA sequence but not the protein sequence) were inserted to avoid repeated cutting by the CRISPR-Cas9 and to add a restriction enzyme recognition site for Bsa1. The 20 nt crRNA was designed with the software http://crispr.mit.edu/. crRNA, tracrRNA, ssODN, and Cas9 protein were all ordered from Integrated DNA Technologies (IDT).

Genotyping and restriction enzyme

To screen for positive clones, DNA was extracted using the prepGEM kit (ZyGEM) followed by PCR using the AmpliTaq GOLD DNA polymerase (Thermo Fisher) according to the manufacturer's instructions. 40 cycles consisting of 30 s of denaturation at 94 °C, 30 s of annealing at 60 °C and 60 s of elongation at 72 °C was performed in a T100 thermocycler from Bio-Rad. The primers SURV *PSEN1* FW/RV (IDT) used

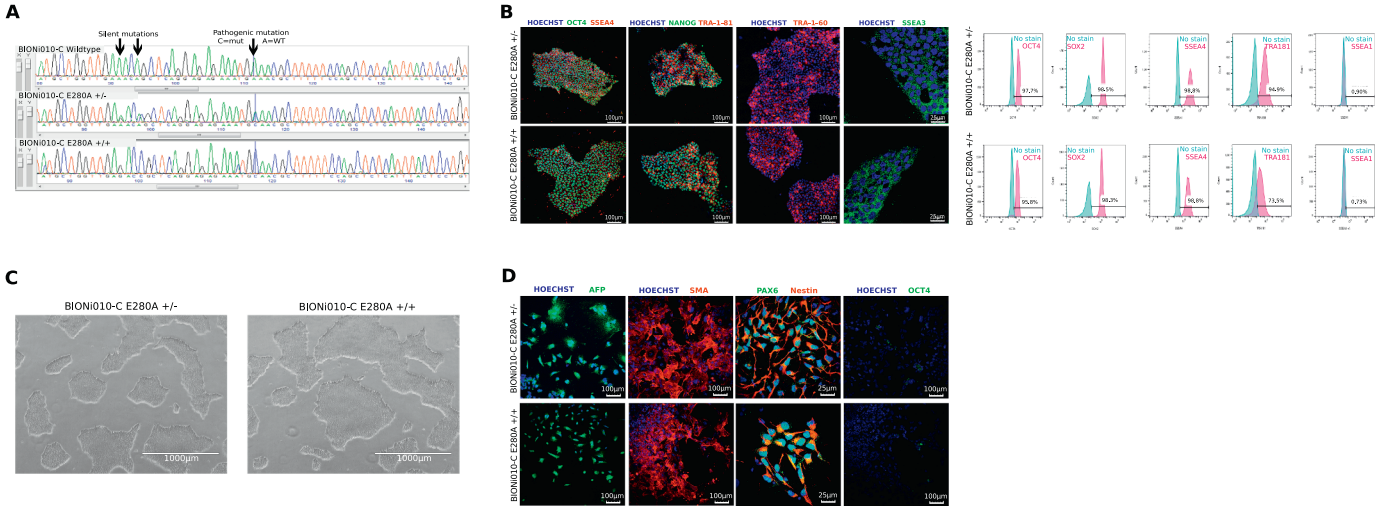

Fig. 1. A) Sequencing analysis of the PSEN1 gene revealed a heterozygous and a homozygous insertion of the pathogenic E280A mutation depicted by the arrow in the middle of the sequencing analysis. In addition, two silent mutations were inserted to block the CRISPR cutting site (two arrows at the left side). B) *Left:* Immunofluorescence microscope analysis of the stem cell markers OCT4, SSEA4, NANOG, TRA-1-81, TRA-1-60 and SSEA3. *Right:* Flow cytometry analysis of the stem cell markers OCT4, SOX2, SSEA4, and TRA-1-81 quantified on unstained cells. SSEA1 was used as marker for differentiated cells. C) Bright-field image of the two gene-edited iPS cell lines. E) Immunofluorescence microscope analysis of endo-, meso-, and ectodermal cell types with the respective markers AFP, SMA and PAX6. Cells were stained with the pluripotency marker OCT4 as a negative control.

Table 1
Summary of lines.

iPSC line names	Abbreviation in figures	Gender	Age	Ethnicity	Genotype of locus	Disease
BIONi010-C-29	*BIONi010-C E280A +/+*	*Male*	*18*	*African*	*CC*	*N/A*
BIONi010-C-30	*BIONi010-C E280A +/−*	*Male*	*18*	*African*	*AC*	*N/A*

for PCR, were designed to cover the mutation at locus Chr14:73664808 and produces a 400 bp product (Table 3). The screening was carried out by restriction digest using Bsa1 for 1 h at 37 °C (New England Biolabs).

Sequencing

Positive clones were sequenced by Sanger sequencing using primer SURV *PSEN1* seq (Table 3).

Karyotyping and quality control

Quality control was performed after the lines had been banked and are listed in Table 2. The general morphology of the cells was investigated daily by light microscopy. When cells were 70–85% confluent, karyotyping was initiated by treating cells with Colcemid (Gibco) for 1.5 h after which they were detached with Accutase for 5 min. Next, cells were incubated with 0.075 M KCl for 30 min at 37^{0}C and afterwards fixed with a mixture of 25% acidic acid and 75% methanol. Fixed cells were placed at -20 °C overnight and then shipped for G-band karyotyping at the Institute of Medical Genetics and Applied Genomics, University of Tuebingen. For each cell line 15 metaphases where analysed. BIONi010-C E280A +/− was sent for karyotyping at passage 35 and BIONi010-C E280A +/+ at passage 38. STR analysis was performed by extracting DNA (Qiagen) and subsequently analyzing the DNA using the AmpFLSTR Identifier PCR Amplification kit according to manufacturer's instructions (Applied Biosystems). Cells were tested for microbiological contamination by growing 500 µL of the supernatant in LB medium for 2 days at 37^{0}C and tested for mycoplasma using the PCR mycoplasma test kit (PromoKine).

Immunocytochemistry

Expression of pluripotency markers was investigated using a Leica DMRB-fluorescence microscope (Leica Microsystems Wetzlar, Germany). Cells were fixed in 4% PFA for 20 min at RT and labeled according to standard ICC procedures by permeabilizing fixed cells with 2% Triton X-100 in PBS for 20 min at RT followed by 30 min of blocking with 3% BSA. All antibodies were diluted in 3% BSA (OCT4, NANOG, SSEA4, SSEA3, Tra-1-60 and Tra-1-81) and incubated overnight at 4 °C. The primary antibodies (Table 3) were visualized with Alexa488 or Alexa647 as secondary antibodies diluted 1:200 (Life Technologies). Secondary antibodies were incubated in darkness for one hour at RT and and all samples were stained with Hoechst bisbenzimide 33,258.

Differentiation potential

For analysis of differentiation potential, embryoid bodies were formed for 7 days in E8 media after which, cells were spontaneously differentiated in fibroblast media for 14 days on Matrigel coated plates. Cells were afterwards immunocytochemically stained for smooth muscle actin (mesoderm), alpha feto protein (endoderm). Fluorescent images showing the expression of the two markers were acquired on a Leica DMRB-fluorescence microscope. iPSCs were also differentiated into neural progenitor cells (ectoderm), by forming embryoid bodies for 7 days in neural induction media and plating the embryoid bodies on Matrigel coated plates for another 7 days in neural induction media. Neural induction media consisted of Neurobasal media (Thermo Fisher), DMEM-F12 (Gibco), B-27 (Thermo Fisher), N-2 (Thermo Fisher), SB431542 (SMS), LDN193189 (Sigma), Glutamax (Thermo Fisher), Pen/Strep (Sigma). Cells were immunocytochemically stained for PAX6 and Nestin and images acquired on a Leica DMRB-fluorescence microscope.

Flow cytometry

Flow cytometry was used to quantify pluripotency markers expressed by the cell lines using the Staining Buffer Set from Invitrogen. Cells were detached with Accutase for 5–10 min and 0.2×10^5 cells were fixed in 0.5 mL of the fixation/permeabilizationbuffer according to the guidelines. After 30 min incubation at RT, the cells were washed three times with permeabilization buffer and then incubated for 45 min with 100 µL of the diluted antibody. Cells were washed additionally three times and afterwards run at a calibrated BD Accuri C6 flow cytometer (analyze 50.000 cells at high speed in 150 µL buffer). The analysis was done using the FlowJo software.

Table 2
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography	*Visual record of the lines: normal*	Fig. 1*panel C*
Phenotype	Qualitative analysis by immunocytochemistry	Positive for OCT4, NANOG, SSEA4, SSEA3, Tra-1-60 and Tra-1-81	Fig. 1*panel B*
	Quantitative analysis by flow cytometry	BIONi010-C-E280A +/− positive for: *Oct3/4:95.8%Sox2:98.3%* *SSEA4:98.8%Tra181:73,5%* *SSEA1(control):0.7%* BIONi010-C-E280A +/+ positive for: *Oct3/4:97.7%Sox2:98.5%* *SSEA4:98.8%Tra181:94.9%* *SSEA1(control):0.9%*	Fig. 1*panel B*
Genotype	Karyotype (G-banding),	*All 46, XY, Resolution 300–400 bp*	*Supplementary Fig. 1 panel A*
Identity	STR analysis	*DNA profiling performed* *16 sites tested, all match*	*Available with the authors*
Mutation analysis	Sequencing	*Successful integration of both heterozygous and homozygous mutation A > C.*	Fig. 1*panel A*
Microbiology and virology	Mycoplasma	*Mycoplasma testing by PCR Mycoplasma Test Kit was negative*	*Supplementary Fig. 1 panel B*
Differentiation potential	Spontaneous differentiation	*Positive for: AFP and SMA. Negative for OCT4. Positive for PAX6 and Nestin after 10 days of neural induction*	Fig. 1*panel D*

Table 3
Reagents details.

Antibodies used for immunocytochemistry/flow-cytometry

	Antibody	Dilution	Company Cat # and RRID
Pluripotency Marker	*Goat anti-OCT4*	*1:200*	Santa Cruz Cat: sc-8628 RRID:AB_653551
Pluripotency Marker	*NANOG*	*1:50*	Peprotech Cat: 500-P236 RRID:AB_1268805
Pluripotency Marker	*Rat anti-SSEA3*	*1:100*	Biolegend Cat: 330302 RRID: AB_1236554
Pluripotency Marker	*Mouse anti-SSEA4*	*1:100*	Biolegend Cat: 330402 RRID: AB_1089208
Pluripotency Marker	*Mouse anti-TRA-1-60*	*1.200*	Biolegend Cat: 330602 RRID: AB_1186144
Pluripotency Marker	*Mouse anti-TRA-1-81*	*1:200*	Biolegend Cat: 330702 RRID: AB_1089240
Pluripotency Marker	*OCT4 PE*	*1:25*	BD Pharmigen Cat: 560186 RRID:AB_1645331
Pluripotency Marker	*SOX2 AF647*	*1:50*	BD Pharmigen Cat: 560294 RRID:AB_1645324
Pluripotency Marker	*TRA-1-81 AF647*	*1:25*	BD Pharmigen Cat: 500793 RRID:AB_1645449
Pluripotency Marker	*SSEA4 PE*	*1:25*	BD Pharmigen Cat: 560128 RRID:AB_1645533
Differentiation Markers	*SSEA1 PE*	*1:25*	BD Pharmigen Cat: 560142 RRID:AB_1645246
Differentiation Markers	*Rabbit anti-Alpha-1-Fetoprotein*	*1:200*	*DAKO Cat: A0008 RRID:*AB_2650473
Differentiation Markers	*Mouse anti-Smooth Muscle Actin*	*1:200*	*DAKO Cat: M0851* RRID:AB_2313736
Differentiation Markers	Rabbit anti-PAX6	1:100	Convanca Cat# PRB-278P RRID: AB_2565003
Differentiation Markers	Mouse anti-Nestin	1:1000	Millipore Cat# MAB5326 RRID: AB_11211837
Secondary antibodies	Donkey Anti-Mouse IgG Alexa fluor 488	1:200	Life technologies Cat#A21202 RRID: AB_141607
Secondary antibodies	Donkey Anti-Mouse IgG Alexa fluor 647	1:200	Invitrogen Cat# A31571 RRID: AB_162542
Secondary antibodies	Donkey Anti-Rat IgG Alexa fluor 594	1:200	Invitrogen Cat# A21209 RRID: AB_2535795
Secondary antibodies	Donkey Anti-Goat IgG Alexa fluor 488	1:200	Invitrogen Cat# A11055 RRID: AB_2534102
Secondary antibodies	Donkey Anti-Rabbit IgG Alexa fluor 488	1:200	Life technologies Cat# A21206 RRID: AB_2535792

sgRNA, ssODN and primers

	Target	Forward/Reverse primer (5′-3′)
Cr-RNA	*PSEN1*	ATGCTGGTTGAAACAGCTCA
ssODN	*PSEN1*	GATTTAGTGGCTGTTTTGTGTCCGAAAGGTCCACTTCGTATG CTGGTTGAGACCGCTCAGGAGAGAAATGCAACGCTTTTTCCAG CTCTCATTTACTCCT
SURV PSEN1 FW/RV (PCR mutation analysis)	*PSEN1*	CCACCAGTTCACCTGCCATTT/AAGAGATCTGCAGGAGTTCCA
SURV *PSEN1* seq *(Sequencing)*	*PSEN1*	CACCCATTTACAAGTTTAGC

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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.2019.101403.

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