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Published in:
Stem Cell Research

DOI (link to publication from Publisher):
10.1016/j.scr.2019.101657

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Publication date:
2020

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

Link to publication from Aalborg University

Citation for published version (APA):
Chen, M., Maimaitili, M., Buchholdt, S. H., Jensen, U. B., Febbraro, F., & Denham, M. (2020). Generation of eight human induced pluripotent stem cell lines from Parkinson's disease patients carrying familial mutations. Stem Cell Research, 42, [101657]. https://doi.org/10.1016/j.scr.2019.101657
Lab Resource: Multiple Stem Cell Lines

Generation of eight human induced pluripotent stem cell lines from Parkinson's disease patients carrying familial mutations

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ABSTRACT

We generated eight induced pluripotent stem cell (iPSC) lines from Parkinson's disease (PD) patients using non-integrating episomal plasmids. All iPSC lines have a normal karyotype, express pluripotent genes including POU5F1, NANOG, and show alkaline phosphatase activity, as well as the ability to differentiate into all three germ layers. These PD iPSC lines can be used for disease modeling to identify PD mechanisms and for the development or stratification of new drugs.

Resource table

| Unique stem cell lines identifier | DANi-002C DANi-003H DANi-004A DANi-005A DANi-006F DANi-007A DANi-008F DANi-009C |
|----------------------------------|----------------------------------|
| Alternative names of stem cell lines | GBA-002-C3 (DANi-002C) GBA-003-C8 (DANi-003H) PRKN-004-C1 (DANi-004A) LRRK2-GBA-005-C1 (DANi-005A) GBA-006-C6 (DANi-006F) PINK1-007-C1 (DANi-007A) SNCA-008-C6 (DANi-008F) SNCA-009-C3 (DANi-009C) |
| Institution | Danish Research Institute of Translational Neuroscience (DANDRITE), Aarhus, Denmark |
| Contact information of distributor | Mark Denham, mden@dandrite.au.dk |
| Type of cell lines | iPSC |
| Origin | Human |
| Cell Source | DANi002-DANI008: skin fibroblasts. DANi009: lymphoblasts |
| Clonality | Clonal |
| Method of reprogramming | Non-integrating episomal vectors |
| Multiline rationale | Same disease non-isogenic cell lines (Parkinson's disease patients carrying different familial mutation) |
| Gene modification | Yes |
| Type of modification | Hereditary |
| Associated disease | Parkinson's disease |
| Gene/locus | DANi-002C: Gene GBA, Locus 1q22, Mutation NM_001005741.2:c.1448 T>C (NP_000148.2:p.Leu483Pro) GBA-003-C8: Gene GBA, Locus 1q22, Mutation NM_001005741.2:c.1226 A>G (NP_000148.2:p.Asn409Ser) PRKN-004-C1: Gene PRKN, Locus 1q22, Mutation NM_004562.2:c.758 G>A (NP_004553.2:p.Cys253Tyr) DANi-005A: Gene LRRK2, Locus 12q12, Mutation NM_198578.3:c.6055 G>A (NP_940980.3:p.Gly2019Ser) and Gene GBA, Locus 1q22, Mutation NM_001005741.2:c.1226 A>G (NP_000148.2:p.Asn409Ser) DANi-006F: Gene GBA, Locus 1q22, Mutation NM_001005741.2:c.1448 T>C (NP_000148.2:p.Leu483Pro) DANi-007A: Gene PINK1, Locus 1p36.12, Mutation NM_032409.2:c.1366 C>T (NP_115785.1:p.Glu456Ter) DANi-008F: Gene SNCA, Locus 4q22.1, Mutation NM_000345.3:c.157 G>A (p.Ala53Thr) DANi-009C: Gene SNCA, Locus 4q22.1, Mutation duplication |

Method of modification
Name of transgene or resistance system
Inducible/constitutive system
Date archived/stock date
Cell line repository/bank
Ethical approval

DANi-002C: Oct.22, 2014 DANi-003H: Dec.3, 2014 DANi-004A: Mar.23, 2015 DANi-005A: Jan.6, 2016 DANi-006F: Mar.19, 2015 DANi-007A: Mar.19, 2015 DANi-008F: Mar.19, 2015 DANi-009C: Jul.9, 2015
DANi-002C: Oct.22, 2014 DANi-003H: Dec.3, 2014 DANi-004A: Mar.23, 2015 DANi-005A: Jan.6, 2016 DANi-006F: Mar.19, 2015 DANi-007A: Mar.19, 2015 DANi-008F: Mar.19, 2015 DANi-009C: Jul.9, 2015
https://hpscreg.eu/user/cellline/edit/DANi002-C
https://hpscreg.eu/user/cellline/edit/DANi003-H
https://hpscreg.eu/user/cellline/edit/DANi004-A
https://hpscreg.eu/user/cellline/edit/DANi005-A
https://hpscreg.eu/user/cellline/edit/DANi006-F
https://hpscreg.eu/user/cellline/edit/DANi007-A
https://hpscreg.eu/user/cellline/edit/DANi008-F
https://hpscreg.eu/user/cellline/edit/DANi009-C

Ethics Committee of the Institute Giannina Gaslini: 334358c/fg
Ethics Committee at the Medical Faculty

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https://doi.org/10.1016/j.scr.2019.101657
Received 22 August 2019; Received in revised form 6 November 2019; Accepted 8 November 2019
Available online 13 November 2019
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1. Resource utility

A bank of Parkinson’s disease (PD) iPSC lines from a broad range of familial PD patients can be used to study early disease mechanisms and those involved in its progression, which may be relevant for sporadic cases, and provide a platform for the development or stratification of new drugs.

1.1. Resource details

Parkinson disease is the second most common neurodegenerative disorder, which affects a broad segment of the aging population in our society. The majority of PD cases are sporadic; however, more than 10% of cases are hereditary (Martí et al., 2003). Hereditary cases, where a genetic defect is present, provide the opportunity to investigate PD related mechanisms that may also be relevant for sporadic cases. Induced pluripotent stem cells (iPSCs) offer new opportunities to use these patient cells and generate specific cell type to model PD in vitro in a human context (Soldner et al., 2011). Establishing a bank of PD iPSC lines from a broad range of familial PD patients will enable the analysis of patient-specific neurons from various familial PD genetic backgrounds, which can potentially uncover disease relevant mechanisms and help accelerate the development of new drugs.

In this paper, we report the generation of eight iPSC lines from PD patients. Seven reprogrammed from fibroblasts with the following familial mutations: DANi-002C heterozygous for GBA c.1448 T>C (p.Leu483Pro, previously annotated as Leu444Pro; Tsuji et al., 1987), DANi-003H for heterozygous GBA c.1226 A>G (p.Asn409Ser, previously annotated as Asn370Ser; Tsuji et al., 1988), DANi-004A homozygous for PRKN c.758 G>A (p.Cys253Tyr), DANi-005A digenic affected and heterozygous for both LRRK2 6.605 G>A (p.Gly2019Ser) and GBA c.1226 A>G (p.Asn409Ser), DANi-006F heterozygous for GBA c.1448 T>C (p.Leu483Pro), DANi-007A homozygous for PINK1 c.1366C>T (p.Gln456Ter), and DANi-008F, heterozygous for SNCA c.157G>A (p.Ala53Thr), and one iPSC line DANi-009C reprogrammed from a lymphoblast line derived from a PD patient with a duplication of SNCA (Table 1).

The fibroblasts and lymphoblasts were reprogrammed by transfection with POU5F1, SOX2, KL4F, MYCL1 and LIN28 using non-integrating episomal vectors. After 3–4 weeks, we observed cell morphological changes. Subsequently, iPSCs clones were picked and cultured on feeders for expansion and further characterization. Chromosomal analysis from all iPSCs showed normal karyotypes 46, XX or 46, XY (Supplementary Fig. 1A) and the familial mutations in PD-iPSCs were confirmed (Supplementary Fig. 2). All iPSCs were alkaline phosphatase positive (Fig. 1A) and expressed the pluripotent markers, POU5F1, and NANOG (Fig. 1B, C). Quantitative assessment of pluripotency was determined by counting the percentage of POU5F1+/DAPI and NANOG+/DAPI cells from three different colonies for each cell line (Supplementary Figure 1B and Supplementary Table 1).

All the iPSC lines reported in this paper were confirmed to be free from random integration of the reprogramming plasmids, which were analyzed by qPCR (Supplementary Fig. 1C). All iPSC lines successfully formed embryoid bodies and at day 14 cultures contained cell types representative of the three germ layers, indicated by positive staining for SOX17/FOXA2 (endoderm), TBX6 (mesoderm), and TUBB3 (ectoderm) (Fig. 1D–F). In addition, the absence of mycoplasma for all the lines was confirmed by PCR (Supplementary Figure 1D). Cell line identities were confirmed to match the original donors by a genetic profile of a set of STR loci on each cell line (Table 2).

2. Materials and methods

2.1. Reprogramming patients fibroblasts to iPSCs

Patient fibroblasts and lymphoblasts from Hertie biobank or Gaslini biobank were expanded in RPMI media supplemented with 1% glutamax, pen/strep 10,000 µg/ml (all from Life Technologies), 10% FCS (Biowest) and GFF2 (10 ng/ml; Peprotech). For reprogramming, 100,000 fibroblast cells were seeded on 9.6 cm² (6-well plate, Cat # 140685, ThermoFisher) pre-coated with Vitronectin XF™ (STEMCELL Technologies) and transfected with P3 primary cell 4D-Nucleofector™ X kit I (cat#V4X-3012, Lonza) with a Lonza 4-D Nucleofector program (program: EN-150); using episomal vectors (1 µg each vector) pCXLE-hOCT3/4- shp53-F, pCXLE-hSK and pCXLE-hUI (Addgene plasmid numbers: 27077, 27078, 27080) that together contained the following genes POU5F1, SOX2, KL4F, MYCL1, LIN28 and shRNA against TP53, in TeSR™-E7™ medium (STEMCELL Technologies). The medium was changed every 3–4 days, and after 3–4 weeks without passaging, iPSC colonies were isolated and expanded as individual clones.

The iPSC clones were cultured on irradiated human foreskin fibroblasts (HFF; ATCC CRL-2097) in KSR media consisting of DMEM/ nutrient mixture F-12, supplemented with β-mercaptoethanol 0.1 mM, non-essential amino acids (NEAA) 1%, glucose 2.0 mM, penicillin 25 U/ml, streptomycin 25 U/ml and knockout serum replacement 20% (all from Life Technologies), which was further supplemented with GFF2 (15 ng/ml; Peprotech) and Activin A (15 ng/ml; R&D systems). All cells were cultured at 37°C and 5% CO₂. Colonies were mechanically dissected every seven days and transferred to freshly prepared HFF. Mycoplasma detection was performed by using LookOut Mycoplasma PCR Detection Kit (Cat#M0035, Sigma) according to manufacturer’s instructions.

2.1.1. Pluripotency markers and embryoid body formation assay

iPSCs analyzed by immunocytochemistry were first fixed in 4% PFA at 4°C for 10 min and washed briefly in PBS and blocked for 1 h at room temperature (RT) with 5% donkey serum in PBS, with 0.25% triton-X solution. Primary antibodies diluted in blocking solution were applied at 4°C overnight followed by washes in PBT, after which the corresponding secondary antibodies were applied for 1 h at RT.
(antibodies shown in Table 3). Nuclei were counterstained with 4,6-diamidino-2-phenylindole (DAPI; 1 µg/ml, Sigma). Positive cells for POU5F1 and NANOG staining was counting from three different colonies and data expressed as a percentage of POU5F1⁺/DAPI and NANOG⁺/DAPI.

Alkaline phosphatase staining was performed following manufacturer's procedure (Cat#00-0009, Stemgent).

Embryoid bodies (EBs) were generated from iPSCs by culturing fragments in ultra-low cluster 96-well plate (Cat#3474, Corning) in suspension and cultured in KSR media supplemented with 1.5 µM CHIR99021 (Cat#04-0004-10, Stemgent), 40 ng/ml BMP2 (Cat#120-02, Peprotech), and 10 ng/ml Activin A. At day 14, EBs were collected,

Fig. 1. Characterization of eight human iPSC lines generated from Parkinson's disease patients carrying familial mutations.
Table 2
Characterization and validation.

| Classification       | Test                                      | Result                                      | Data                                      |
|----------------------|-------------------------------------------|---------------------------------------------|-------------------------------------------|
| Morphology           | Photography                               | Normal morphology                           | Fig. 1 panel A                             |
| Phenotype            | Qualitative analysis by Immunocytochemistry | Positive staining/expression of pluripotency markers: Alkaline phosphatase (ALP), POU5F1, NANOG | Fig. 1 panel A, B, C                      |
| Genotype             | Karyotype (Q-banding) and resolution       | Asses% of positive cells for antigen markers. POU5F1: all above 97%, NANOG: all above 96%. | Supplementary Fig. 1 panel B, Table 1, Supplementary Fig. 1 panel A |
| Identity             | STR analysis                              | DNA Profiling Performed                     | Available with the authors                |
| Mutation analysis    | Sequencing                                | Heterozygous: DANi002, DANi003, DANi005, DANi006, DANi008, DANi009. | Supplementary Fig. 2                      |
| Microbiology and virology | Mycoplasma                          | Mycoplasma testing by PCR: Negative          | N/A                                       |
| Differentiation potential | Embryoid body formation                | Embryoid bodies formation expressing endoderm markers: SOX17/FOXA2; mesoderm marker: TBX6; ectoderm marker: TUBB3. | Supplementary Fig. 1D, Fig. 1 panel D-F.  |

Table 3
Reagents details.

Antibodies used for immunocytochemistry

| Antibody Description | Antibody | Dilution | Company Cat # | RRID |
|----------------------|----------|----------|---------------|------|
| Pluripotency Markers | Mouse anti-OCT3/4(C-10) | 1:100 | Santa Cruz Biotechnology Cat# sc-5279, RRID:AB_628051 |
| Pluripotency Markers | Mouse anti-NANOG | 1:100 | eBioScience Cat# 14-5768-82, RRID:AB_467572 |
| Differentiation Markers | Goat anti-SOX17 | 1:200 | R&D Systems Cat# AF1924, RRID:AB_355060 |
| Differentiation Markers | Rabbit anti-FOX2A | 1:500 | Cell signaling Technology Cat#B816; RRID:AB_10891055 |
| Differentiation Markers | Goat anti-TBX6 | 1:100 | R&D Systems Cat# AF4744 RRID:AB_2200834 |
| Secondary antibodies for IF | Mouse anti-TUBB3 | 1:1000 | Millipore Cat# MAB1637, RRID:AB_2210524 |
| Secondary antibodies for IF | Goat anti-mouse IgG2B Alexa 568 | 1:1000 | ThermoFisher Scientific Cat# A21144, RRID:AB_2535780 |
| Secondary antibodies for IF | Goat anti-mouse IgG1 Alexa 488 | 1:1000 | ThermoFisher Scientific Cat# A21121, RRID:AB_141514 |
| Secondary antibodies for IF | Donkey anti-mouse IgM Alexa 488 | 1:200 | Jackson ImmunoResearch Cat# 715-545-020, RRID:AB_2340844 |
| Secondary antibodies for IF | Donkey anti-goat IgG (H+L) Alexa 488 | 1:1000 | ThermoFisher Scientific Cat# A-11055, AB_2534102 |
| Secondary antibodies for IF | Donkey anti-rabbit IgG (H+L) Alexa 568 | 1:1000 | ThermoFisher Scientific Cat# A11057, AB_2534104 |
| Secondary antibodies for IF | Donkey anti-mouse IgG (H+L) Alexa 568 | 1:1000 | ThermoFisher Scientific Cat# A10037, AB_2534013 |

Primers

| Target | Forward/Reverse primer (5′−3′) |
|--------|--------------------------------|---|
| Episomal Plasmids (qPCR) | Plasmid DNA Product size 95 | AGTGCCTAGAGAAGGTTCA |
| Episomal Plasmids Template control (qPCR) | Albumin Product size 73 | TTTGCAGGTCGATGAGGAAGA |
| Targeted mutation analysis | GBA N499S Product size 497 | ATCCATCGGGTAAAGGCACCC |
| Targeted mutation sequencing primer | GBA N499S | ATCATCAAGGCTGAACCC |
| Targeted mutation sequencing | GBA L483P Product size 1445 | CTGGTCTGCAATTTGGCA |
| Targeted mutation sequencing | GBA L483P | CTGAGTGTCCTGCGAGCC |
| Targeted mutation analysis | SNCA A53T Product size 486 | TGGACGTTCCAAAAGCCAG |
| Targeted mutation analysis | SNCA A53T | CTGTCGAAACAGTGCTT |
| Targeted mutation analysis | RINK1 Q456X Product size 430 | AGATCTGGTGATGCTTGG |
| Targeted mutation sequencing | RINK1 Q456X | GTGTTCTGCAGTGGAGTC |
| Targeted mutation analysis | PRKN C253Y Product size 239 | TGCTGCTGCTGCTGAGG |
| Targeted mutation analysis | PRKN C253Y | TGCTGCTGCTGCTGAGG |
| Targeted mutation analysis | LRRK2 G2019S Product size 518 | GGGGAGTACCTGCAGTATGG |
| Targeted mutation analysis | LRRK2 G2019S | GTGTTCTGCAGTGGAGTC |
| Targeted mutation analysis (qPCR) | SNCA (duplication) Product size 73 | GGAACATTCACCATCCGTG |
| Targeted mutation analysis (qPCR) | SNCA (duplication) | GGAACATTCACCATCCGTG |

fixed in 4% PFA for 20 min at 4 °C and then washed briefly in PBS, kept in 30% sucrose overnight and embedded in Tissue-Tek OCT compound (Labtek). Sections were cut at 10 µm on a cryostat and used for immunostaining (Table 3). After immunostaining, slides were mounted in PVA-DABCO for viewing under a fluorescent microscope (ZEISS ApoTome, and images captured using the ZEISS software. Confocal microscopy was performed using a ZEISS LSM 780 Confocal Microscope (Fig. 1).

2.2. Genomic analysis

Karyotype analysis was performed on Q-banded metaphase spreads that were prepared according to standard protocol at a clinical accredited laboratory. Ten metaphases were counted and two analysed according to clinical standards. Briefly, growth medium was renewed and colcemide was added to the cultures at 0.1 µg/ml and incubated at 37 °C for 60–120 min depending on the donor. The PD iPSC cells were harvested by trypsinization (0.025% W/V in Hanks buffered saline) at 37 °C. The trypsinization was stopped by adding serum-containing medium. Cells were collected by centrifugation and then incubated in 37 °C for 5 min. The resuspended cells were added dropwise to slide glasses, dried, stained with quinacrine and mounted for fluorescence microscopy.

Genomic DNA were collected and purified using GeneJet Genomic DNA purification kit (Cat #K0721, ThermoFisher Scientific). Familial
mutations for each of the PD iPSC lines and their parental cell lines were validated by either standard PCR or qPCR. The standard PCR amplification was done with Thermo Scientific™ Arktik™ Thermal Cycler with the following program: initial denaturation at 94 °C for 30 s; 35 cycles of (94 °C for 30 s, 60 °C for 30 s, 68 °C for 30 s); final extension at 68 °C for 5 min and hold at 15 °C. PCR products were extracted and cleaned with DNA Clean and concentrator kit (Cat#D4005, Zymo Research) and then samples were prepared and sent to Eurofins Genomics for Sanger sequencing using primers in Table 3. qPCR were done with 7500 Fast Real-Time PCR system (Applied Biosystems) using Taqman Universal Master Mix II no UNG (Cat#444040, ThermoFisher Scientific) to confirm the mutations of SNCA duplication of the DANi-009C and the original parental lymphoblasts. iPSC clones were tested for random integration of episomal plasmids by qPCR with a GoTaq® qPCR System kit (Cat#A6001, Promega) for EBNA/OriP sequences using primers in Table 3 and those positive for plasmid integration were excluded. Cell line identity was performed by the Department of Molecular Medicine (MOMA) at Aarhus University Hospital with the GenePrint® 10 system.

Acknowledgments

This study was supported by Lundbeckfonden grant no. DANDRITE-R248-2016-2518. MC is supported by a postdoctoral fellowship from the Lundbeckfonden grant no. R209-2015-3100. MD is a partner of BrainStem—Stem Cell Center of Excellence in Neurology, funded by Innovation Fund Denmark. For providing us with patient samples, we would like to thank the Neuro-Biobank of the University of Tuebingen, Germany (http://www.hihtuebingen.de/nds/biobank-for-researchers/). This biobank is supported by the Hertie Institute and the DZNE. We would also like to thank the ‘Cell Line and DNA Biobank from Patients affected by Genetic Diseases’ (Istituto G. Gaslini) and the “Parkinson Institute Biobank” (Milan, http://www.parkinsonbiobank.com/), members of the Telethon Network of Genetic Biobanks (project no. GTB12001), funded by Telethon Italy.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.scr.2019.101657.

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