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Published in:
Stem Cell Research

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

Publication date:
2020

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

Stem Cell Research 42 (2020) 101657

Contents lists available at ScienceDirect

Stem Cell Research

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

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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A B S T R A C T

We generated eight induced pluripotent stem cell (iPSC) lines from Parkinson's disease (PD) patients with different familial mutations 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) DANi-003H: Gene *GBA*, Locus 1q22, Mutation NM_001005741.2:c.1226 A > G (NP_000148.2:p.Asn409Ser) DANi-004A: Gene *PRKN*, Locus 6q26, 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.Gln456Ter) 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	Not applicable
Name of transgene or resistance	Not applicable
Inducible/constitutive system	Not applicable
Date archived/stock date	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
Cell line repository/bank	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
Ethical approval	Ethics Committee of the Institute Giannina Gaslini: 3343DSc/fg And 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

of Eberhard-Karls University and at the University Hospital Tübingen: 199 / 2011BO1

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 (Marti et al., 2003). Hereditary cases, where a high penetrant pathogenic variant has been identified, provide the opportunity to investigate PD related mechanism 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 di-genic affected and heterozygous for both *LRRK2* c.6055 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), 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, KLF4, MYCL* 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 FGF2 (10 ng/ml; Peprotech). For reprogramming, 100,000 fibroblast cells were seeded on 9.6 cm^2 (6-well plate, Cat # 140685, ThermoFisher) pre-coated with Vitronectin XF™ (STEMCELL Technologies) and transfected with P3 primary cell 4D-Nucleofector™ X kit L (cat#V4XP-3012, Lonza) with a Lonza 4-D Nucleofector (program: EN-150); using episomal vectors (1 μg each vector) pCXLE-hOCT3/4-shp53-F, pCXLE-hSK and pCXLE-hUL (Addgene plasmid numbers: 27077, 27078, 27080) that together contained the following genes *POU5F1, SOX2, KLF4, MYCL, 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 iPSCs 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%, glutamine 2 mM, penicillin 25 U/ml, streptomycin 25 U/ml and knockout serum replacement 20% (all from Life Technologies), which was further supplemented with FGF2 (15 ng/ml; Peprotech) and Activin A (15 ng/ml; R&D systems). All cells were cultured at 37 °C and 5% CO_2. 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#MP0035, 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 PBT (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

Table 1
Summary of lines.

iPSC line names	Abbreviation in figures	Gender	Age at collection	Ethnicity	Genotype of locus	Disease
DANi-002C (GBA-002-C3)		Male	50	unknown	Heterozygous *GBA* c.1448 T > C (p.L483P)	Parkinson's disease
DANi-003H (GBA-003-C8)		Male	56	unknown	Heterozygous *GBA* c.1226 A > G (p.N409S)	Parkinson's disease
DANi-004A (PRKN-003-C1)		Female	28	unknown	Homozygous *PRKN* c.758 G > A (p.C253Y)	Parkinson's disease
DANi-005A (LRRK2-GBA-005-C1)		Male	66	unknown	Heterozygous *LRRK2* c.6055 G > A (p.G2019S) and Heterozygous *GBA* c.1226 A > G (p.N409S)	Parkinson's disease
DANi-006F (GBA-006-C6)		Female	39	unknown	Heterozygous *GBA* c.1448 T > C (p.L483P)	Parkinson's disease
DANi-007A (PINK1-007-C1)		Male	59	unknown	Homozygous *PINK1* c.1366 C > T (p.Q456X)	Parkinson's disease
DANi-008F (SNCA-008-C6)		Male	45	unknown	Heterozygous *SNCA* c.157 G > A (p.A53T)	Parkinson's disease
DANi-009C (SNCA-009-C3)		Female	45	unknown	*SNCA* duplication	Parkinson's disease

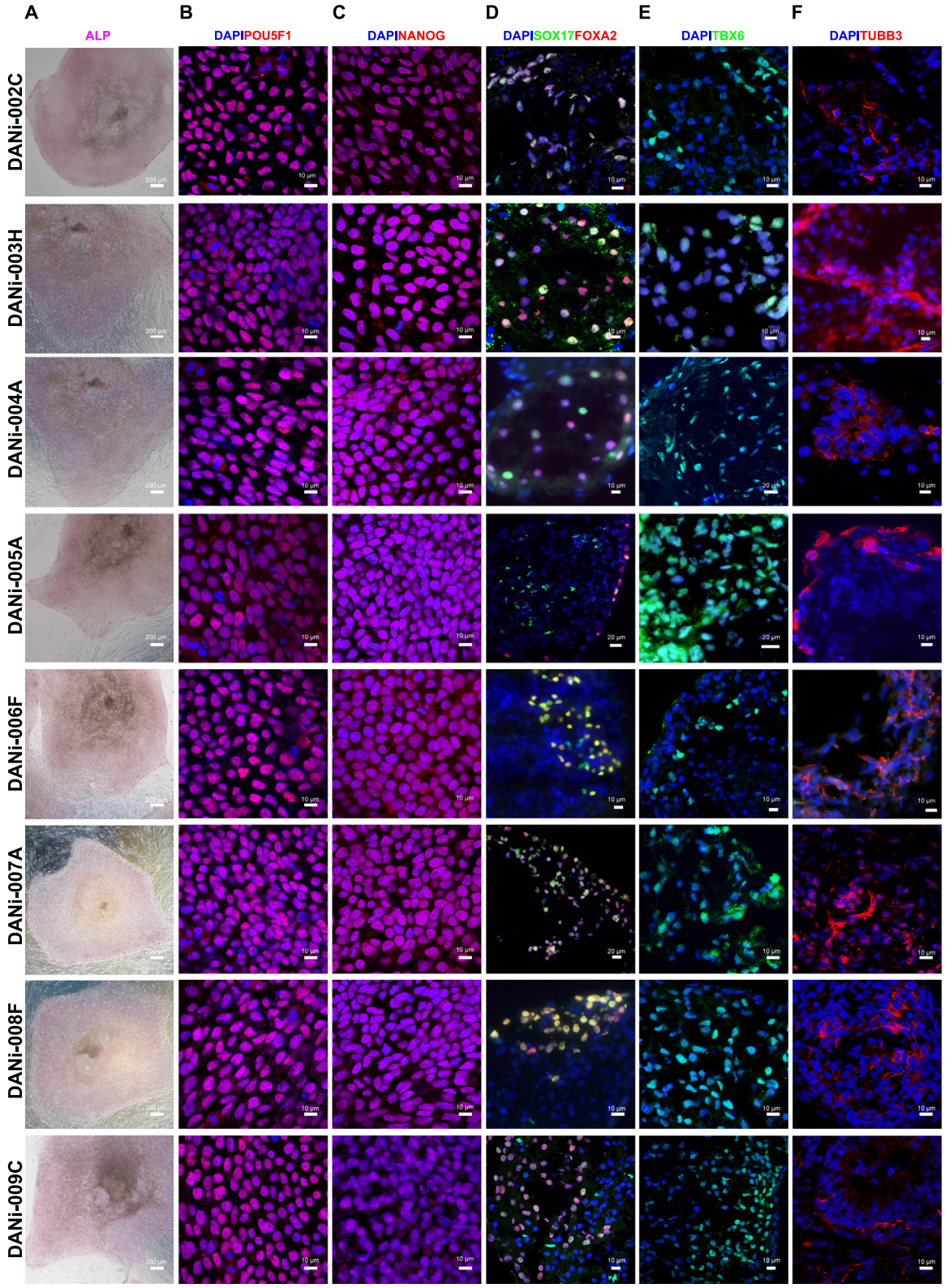

Fig. 1. Characterization of eight human iPSC lines generated from Parkinson's disease patients carrying familial mutations.

(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,

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
	Quantitative analysis by Immunocytochemistry counting	Assess% of positive cells for antigen markers. POU5F1: all above 97%, NANOG: all above 96%.	Supplementary Fig. 1 panel B, Supplementary Table 1
Genotype	Karyotype (Q-banding) and resolution	46 XY, 46 XX. Resolution 450–500	Supplementary Fig. 1 panel A
Identity	STR analysis	DNA Profiling Performed	
		10 genomic markers 100% matched between parental cells and respective iPSCs	Available with the authors
Mutation analysis (IF APPLICABLE)	Sequencing	Heterozygous: DANi002, DANi003, DANi005, DANi006, DANi008, DANi009.Homozygous: DANi004, DANi007.	Supplementary Fig. 2
	Southern Blot OR WGS	N/A	N/A
Microbiology and virology	Mycoplasma	Mycoplasma testing by PCR: Negative	Supplementary Fig. 1D
Differentiation potential	Embryoid body formation	Embryoid bodies formation expressing endoderm markers: SOX17/FOXA2; mesoderm marker: TBX6; ectoderm marker: TUBB3.	Fig. 1 panel D–F.

Table 3
Reagents details.

Antibodies used for immunocytochemistry			
	Antibody	Dilution	Company Cat # and 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-FOXA2	1:500	Cell signaling Technology Cat#8186; RRID: AB_10891055
Differentiation Markers	Goat anti-TBX6	1:100	R&D Systems Cat# AF4744 RRID:AB_2200834
Differentiation Markers	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	Goat anti-mouse IgG3 Alexa 594	1:200	Jackson ImmunoResearch Cat# 115-585-209, RRID:AB_AB_2338889
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	AGGTCCCTCGAAGAGGTTCA/ TTCCAACGCGAGAAGGTGTT	
Episomal Plasmids Template control (qPCR)	Albumin Product size 73	TTTGCAGATGTCAGTGAAAGAGA/ TGGGGAGGCTATAGAAAATAAGG	
Targeted mutation analysis	*GBA* N409S Product size 497	ATCATCACGGTAAGCCACCC/ CGACAAAGTTACGCACCCAA	
Targeted mutation sequencing primer	*GBA* N409S	ATCATCACGGTAAGCCACCC	
Targeted mutation analysis	*GBA* L483P Product size 1445	TTGGGTGCGTAACTTTGTCG / CTCACGCTCCCAAGACTGG	
Targeted mutation sequencing	*GBA* L483P	CTGAGAGTGTGATCCTGCCAA	
Targeted mutation analysis	*SNCA A53T* Product size 486	TGTAGGCTCCAAAACCAAGG/ CTGTCCAAGGGTGTTTCCTG	
Targeted mutation sequencing	*SNCA* A53T	ATGTTCTTAGAATGCTCAGTGATTG	
Targeted mutation analysis	*PINK1* Q456X Product size 430	GAGTTCAGATTAGCCCATGG/ ATCTGTCACTGTGGCTCTGG	
Targeted mutation sequencing	*PINK1* Q456X	GAGTTCAGATTAGCCCATGG	
Targeted mutation analysis	*PRKN* C253Y Product size 239	TGCCTTTCCACACTGACAGGTACT/ TCTGTTCTTCATTAGCATTAGAGA	
Targeted mutation sequencing	*PRKN* C253Y	TGCCTTTCCACACTGACAGGTACT	
Targeted mutation analysis	*LRRK2* G2019S Product size 518	GGCAGATACCTCCACTCAGC/ TTGATTTGCCTCACAAGTGC	
Targeted mutation sequencing	*LRRK2* G2019S	GGCAGATACCTCCACTCAGC	
Targeted mutation analysis (qPCR)	*SNCA* (duplication) Product size 73	GGAACATTAACCCTACACTCGG/ GGAACCAGTGCATACCAAAAC	
Targeted mutation analysis (qPCR)	*SNCA* (duplication)	Probe [6FAM]TCCCTGAAGCAACACTGCCAGAA[BHQ1]	

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 Apo-Tome), and images captured using the ZEN 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 colcimide 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 hypotonic KCl 0.56% at 37 °C for 30 min in a water bath. Cells were collected by centrifugation and resuspended in fixation buffer (1 part glacial acetic acid and 3 parts methanol). The cells were spun down by centrifugation and resuspended in fixative. This step was repeated once. The resuspended cells were added dropwise to slide glasses, dried, stained by 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/nd/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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