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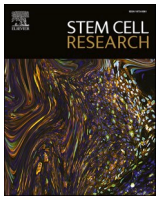

Lab Resource: Single Cell Line

Generation and characterization of a genetic Parkinson's disease-patient derived iPSC line DJ-1-delP (LCSBi008-A)

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A B S T R A C T

Here, we describe an induced pluripotent stem cell (iPSC) line that was derived from fibroblasts obtained from a monogenic Parkinson's disease (PD) patient. The disease was caused by a c.634-636delGCC mutation in the *PARK7* gene leading to p.158P deletion in the protein DJ-1. iPSCs were generated via electroporation using three episomal plasmids encoding human *Oct3/4, Sox2, Klf4, Lin28, L-Myc* combined with a short hairpin RNA for p53. The presence of the c.471_473delGCC mutation in exon 7 of *PARK7* was confirmed by Sanger sequencing. The iPSCs express pluripotency markers, are capable of *in vitro* differentiation into the three germ layers and obtain karyotypic integrity.

1. Resource table

Unique stem cell line identifier	LCSBi008-A
Alternative name(s) of stem cell line	DJ-1-delP delP
Institution	Luxembourg Centre for Systems Biomedicine (LCSB)
Contact information of distributor	Dr. Wim Mandemakers, w.mandemakers@erasmusmc.nl
Type of cell line	Induced pluripotent stem cell line (iPSC)
Origin	Human
Additional origin info	Age: 66 Sex: male Ethnicity: Caucasian
Cell Source	fibroblasts
Clonality	Clonal
Method of reprogramming	electroporation using three episomal plasmids encoding human Oct3/4 (pCXLE-hOCT3/4 (Addgene #27076)), Sox2 and Klf4 (pCXLE-hSK ((Addgene #27078)), Lin28, L-Myc (pCXLE-hUL (Addgene #27080)) combined with a short hairpin RNA for p53
Genetic Modification	Yes
Type of Modification	Autosomal recessive mutation
Associated disease	Parkinson's disease
Gene/locus	*PARK7*
Method of modification	N/A
Name of transgene or resistance	N/A
Inducible/constitutive system	N/A
Date archived/stock date	03. Sep. 2018
Cell line repository/bank	https://hpscreg.eu/cell-line/LCSBi008-A
Ethical approval	Medical Ethical Committee, Erasmus MC Rotterdam, The Netherlands; MEC-2012-001/NL38860.078.11 The cell line can be obtained by third parties using appropriate MTA

2. Resource utility

Parkinson s disease (PD) is the second most common neurodegenerative disease affecting 1–2% of the population that is over 60 years old with its main symptoms being tremor, rigidity and bradykinesia (Tysnes and Storstein, 2017). Most PD cases are sporadic, however, mutations in PD-associated genes (*PARK* genes) can lead to genetic PD (Klein and Westenberger, 2012). The p.158Pdel mutation in the *PARK7* gene encoding the protein DJ-1 leads to autosomal recessively inherited early onset PD (Ramsey and Giasson, 2010). By generating PD-patient iPSC-derived neuronal cell models, the iPSC line will be used to investigate

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Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography	Normal	Fig. 1 panel A
Phenotype	Qualitative analysis: Immunocytochemistry	staining/ expression of pluripotency markers: Oct3/ 4, Nanog, Sox2	Fig. 1 panel D
	Quantitative analysis: RT-qPCR	Transcripts for antigen & cell surface markers	Fig. 1 panel C
Genotype	Karyotype (G-banding) and resolution	46XY, Resolution 450–500	Fig. 1 panel B File available with author
Identity	Array-based karyotyping	performed	File available with author
Mutation analysis (IF APPLICABLE)	Sequencing	homozygous c.634-636delGCC	Fig. 1 panel F
	Southern Blot OR WGS	N/A	N/A
Microbiology and virology	Mycoplasma	Mycoplasma testing by luminescence Negative	Supplementary Fig. 1 B
Differentiation potential	Directed differentiation	Proof of three germ layer formation	Fig. 1 panel E
Donor screening (OPTIONAL)	HIV 1 + 2, Hepatitis B, Hepatitis C	negative	Supplementary file 1
Genotype additional info (OPTIONAL)	Blood group genotyping	N/A	N/A
	HLA tissue typing	N/A	N/A

underlying pathological mechanisms of genetic PD that are caused by the loss of DJ-1 protein due to the p.158Pdel mutation.

3. Resource details

Dermal fibroblasts from a male PD patient (age at biopsy, 66 years) harbouring an autosomal recessive homozygous c.471_473delGCC mutation in *PARK7* were obtained. To generate the presented iPSC line, fibroblasts were reprogrammed by electroporation using three episomal plasmids encoding human *Oct3/4, Sox2, Klf4, Lin28, L-Myc* combined with a short hairpin RNA for p53. The cell line was called DJ-1-delP (see Table 1). We obtained three iPSC clones of the line (data shown only for clone 1) showing the typical morphology of iPSC colonies (Fig. 1 A). The cell line shows a structurally and numerically normal karyotype (46, XY) (Fig. 1 B). qPCR results for fibroblasts, control iPSC GM23338 (Larsen et al. 2020) and DJ-1-delP iPSC in triplicates confirmed that the cells also express Nanog, Oct3/4 and DMNT3B mRNA (Fig. 1 C). The DJ-1-delP iPSC express the stemness marker proteins Sox2, Oct3/4 and Nanog, as validated by immunocytochemistry (Fig. 1 D). DJ-1-delP iPSC are capable of differentiating into the three germ layers mesoderm, ectoderm and endoderm (Fig. 1 E), as shown by *in vitro* differentiation follwed by immunofluorescence staining for the germ layer marker Brachyury and Pax3 (mesoderm), Otx2 and Sox1 (ectoderm) and Sox17 and FOXA2 (endoderm) (Fig. 1 E). PCR followed by agarose gel electrophoresis confirmed that the episomal plasmids disappeared at passage 19 (supplementary Fig. 1A).

The c.471_473delGCC mutation in the *PARK7* gene leading to the deletion of proline 158 of the encoded DJ-1 protein was confirmed by Sanger sequencing in the fibroblasts and the iPSC (Fig. 1 F). The mutation results in stable DJ-1 mRNA (Fig. 1 G), but protein instability of the DJ-1 homodimer leading to a severe loss of DJ-1 protein (Fig. 1 H) (Ramsey and Giasson, 2010).

4. Materials and methods

Fibroblasts derived from the skin biopsy were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 2 mM L-glutamine and 1% penicillin and streptomycin (Pen/Strep). The fibroblasts were reprogrammed into iPSC via electroporation using three episomal plasmids encoding human Oct3/4 (pCXLE-hOCT3/4 (Addgene #27076)), Sox2 and Klf4 (pCXLE-hSK ((Addgene #27078)), Lin28, L-Myc (pCXLE-hUL (Addgene #27080)) combined with a short hairpin RNA for p53. Once colonies had formed, these colonies were picked and plated on a Matrigel™ (Corning)-coated plate. Freshly prepared E8 medium (DMEM F-12 + HEPES, Life Technologies; 1% Pen/Strep, Life Technologies; 1% Insulin-Transferrin-Selenium, Life Technologies; 2 µg/L TGFβ1, Peprotech; 10 µg/L FGF2, Peprotech; 64 mg/L ascorbic acid 2 PM, Sigma-Aldrich; 100 ng/mL Heparin, Sigma-Aldrich; 10% mTesR, StemCell Technologies) was changed each day. The iPSCs were then passaged using EDTA (Life Technologies) once a week at a 1:5 ratio. Fibroblasts and iPSC were cultured at 37 °C under 5% CO2.

5. Mutation analysis

Genomic DNA was purified from fibroblasts passage 9 and iPSC passage 19 using the QIA Blood and Tissue kit (Qiagen). Using the primers listed in Table 2, the exon 7 of the *PARK7* gene was amplified with KOD Hot Start DNA Polymerase (Merck; Annealing temperature 54.8 °C, 40 cycles) on a TProfessional Basic Gradient Thermocycler (Biometra). Sanger sequencing was performed at Eurofins Genomics Germany GmbBH.

6. RNA and protein status analysis by PCR and Western blotting

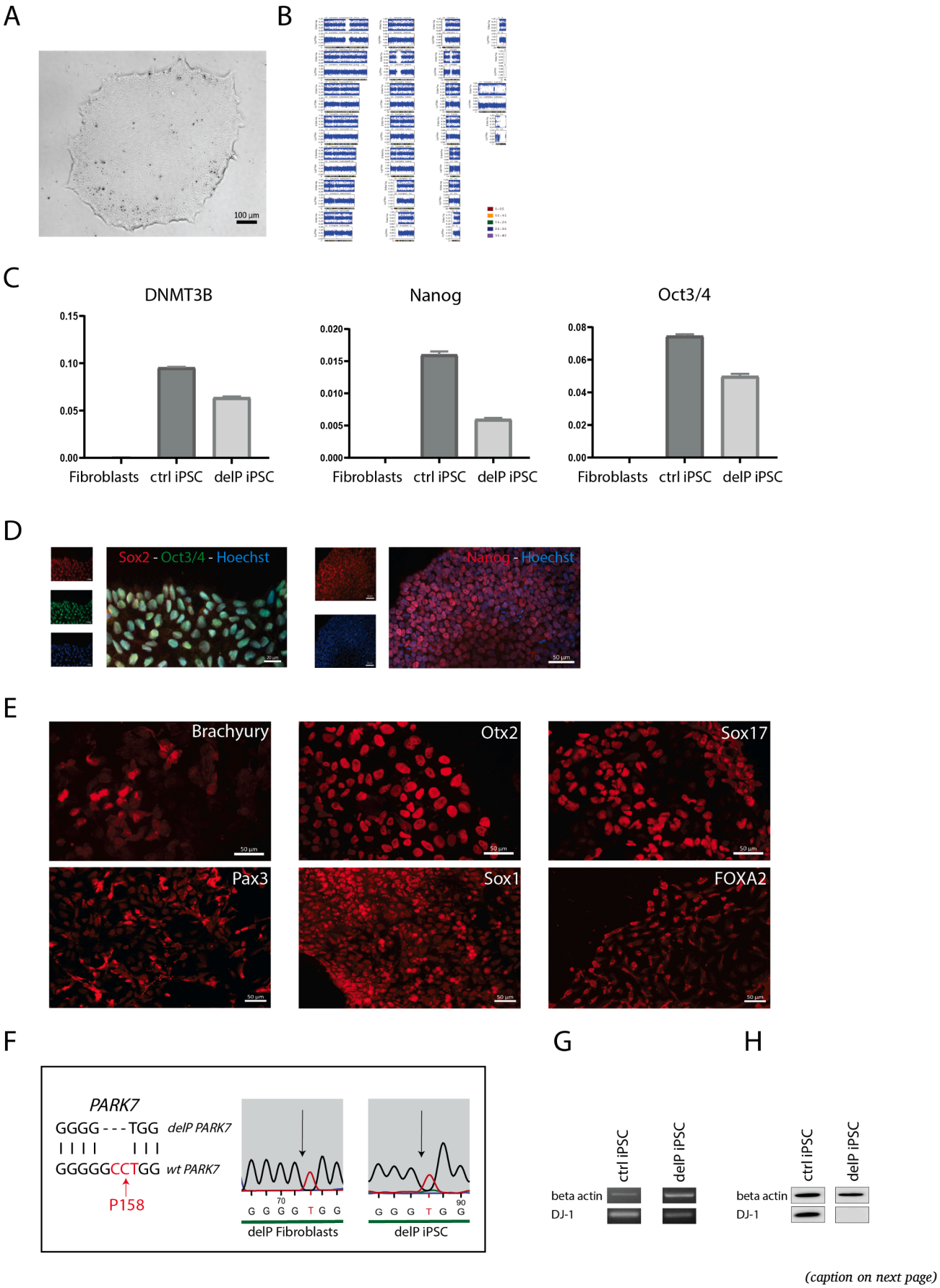
RNA and protein levels of *PARK7*/DJ-1 were evaluated by PCR and Western blotting at passage 12 using the primers and antibodies listed in Table 2 following standard protocols.

7. RT-qPCR

Total RNA was extracted from DJ-1-delP fibroblasts passage 16, control iPSC passage 24 and DJ-1-delP iPSCs passage 9 using the RNeasy Mini Kit (Qiagen). Transcriptor High Fidelity cDNA Synthesis Kit (Roche) was used to synthesize cDNA. Quantification of pluripotency markers by multiplex qPCR was performed using the LightCycler® 480 Probes Master kit (Roche) and hydrolysis probes detecting NANOG-FAM (Hs02387400_g1, Thermo Fisher Scientific), OCT4-FAM (Hs00999632_g1, Thermo Fisher Scientific) and DNMT3B (Hs00171876_m1, Thermo Fisher Scientific). ACTB (Hs03023880_g1, Thermo Fisher Scientific) was used as a housekeeping gene. cDNA from DJ-1-delP fibroblasts was used as a negative control.

8. Immunofluorescence staining

Expression of stemness markers was analysed via immunocytochemistry. iPSCs were plated on Matrigel-coated coverslips and were fixed at passage 26 with 4% paraformaldehyde in PBS for 15 min. Cells were permeabilized and blocked for 1 h in PBS supplemented with 0.4% Triton-X 100 (Carl Roth), 10% goat serum (Vector Labs) and 2% bovine serum albumin (Sigma-Aldrich). Primary antibodies (Table 2) in antibody buffer (0.1% Triton-X, 1% goat serum and 0.2% bovine serum albumin in PBS) were added for overnight incubation at 4 °C. Cells were washed three times with PBS, incubated for 2 h at room temperature with secondary antibodies in antibody buffer. Nuclei were stained with Hoechst. Images were acquired using a Zeiss spinning disk confocal microscope (Carl Zeiss Microimaging GmbBH).



(caption on next page)

Fig. 1. A: Brightfield image of DJ-1-delP iPSC colony. iPSC show the typical iPSC morphology. B: The karyotype of DJ-1-delP iPSC has no abnormalities. C: Gene expression analysis of pluripotency markers by qPCR shows the expression of DNMT3B, Nanog and Oct3/4 in control and DJ-1-delP iPSC and no expression of the markers in control fibroblasts. D: Expression of stemness markers by immunocytochemistry. DJ-1-delP iPSCs express the stemness marker Sox2, Oct3/4 and Nanog. E: DJ-1-delP iPSCs are able to differentiate into the three germ layers, as assessed by three germ layer differentiation and subsequent immunocytochemistry analysis of the markers for Brachyury and Pax3 (mesoderm), Otx2 and Sox1 (ectoderm) and Sox17 and FOXA2 (endoderm). F: The c.471_473delGCC mutation in the PARK7 gene leading to the deletion of proline 158 of the encoded DJ-1 protein was confirmed by Sanger sequencing in the DJ-1-delP fibroblasts and iPSC. G: DJ-1-delP iPSC have normal DJ-1 mRNA levels, as assessed by RT-PCR. H: The mutation in the DJ-1-delP iPSC leads to loss of DJ-1 protein, as seen by Western blotting.

9. In vitro differentiation

The ability of the iPSC to differentiate into the three germ layers was tested at passage 18 using the manufacturer's differentiation protocol of the Human Pluripotent Stem Cell Functional Identification Kit (R&D Systems). Expression of the mesodermal markers Brachyury and Pax3, the ectodermal markers Otx2 and Sox1 and the endodermal markers Sox17 and FOXA2 was confirmed after differentiation by immunocytochemistry. Images were acquired using a Zeiss spinning disk confocal microscope (Carl Zeiss Microimaging GmBH).

10. Karyotyping and identity analysis

Molecular karyotyping and identity analysis of fibroblasts passage 9 and iPSC passage 6 was performed at Life&Brain GmbH (Bonn) using the HumanOmni2.5 Exome-8 DNA Analysis BeadChip. This method does not detect translocations or inversions, alterations in chromosome structure, mosaicism or polyploidy.

11. Mycoplasma test

iPSCs were tested for Mycoplasma contamination at passage 9 by

Table 2
Reagents details.

Antibodies used for immunocytochemistry/flow-cytometry			
	Antibody	Dilution	Company Cat # and RRID
Pluripotency Markers	Goat anti SOX2 (Y-17)	1:250	Santa Cruz, Cat #: sc-17320; RRID: AB_2286684
Pluripotency Markers	Mouse anti Oct3/4	1:1000	Santa Cruz, Cat #: sc-5279; RRID: AB_628051
Pluripotency Markers	Rabbit anti Nanog	1:1000	Abcam, Cat #: ab21624; RRID: AB_446437
Pluripotency Markers	Mouse anti Pax3	1:1000	DSHB AB_528426
Pluripotency Markers	Goat anti Sox1	1:1000	R & D Systems, Cat #: AF3369, RRID: AB_2239879
Pluripotency Markers	Mouse anti FOXA2	1:1000	Santa Cruz, Cat #: sc-101060, RRID: AB_1124660
DJ-1	Rabbit anti DJ-1 (D29E5)XP	1:1500	cell signaling, Cat #: 5933; RRID: AB_11179085
β-Actin	mouse anti β-Actin (8H10D10)	1:20.000	cell signaling, Cat #: 3700S; RRID: AB_2242334
Secondary antibody	Alexa Fluor 488 Goat anti Mouse IgG (H + L)	1:1000	Invitrogen, Cat #: A11029; RRID: AB_138404
Secondary antibody	Alexa Fluor 568 Goat anti Mouse IgG (H + L)	1:1000	Invitrogen, Cat #: A-11031, RRID: AB_144696
Secondary antibody	Alexa Fluor 568 Goat anti Rabbit IgG (H + L)	1:1000	Invitrogen, Cat #: A11036; RRID: AB_143011
Secondary antibody	Alexa Fluor 568 Donkey anti Goat IgG (H + L)	1:1000	Invitrogen, Cat #: A-11057, RRID: AB_142581
Secondary antibody	Alexa Fluor 647 Donkey anti Goat IgG (H + L)	1:1000	Invitrogen, Cat #: A-21447, RRID: AB_2535864
Secondary antibody	Goat anti Rabbit IgG (H + L) Secondary Antibody, HRP, 0.5 mg GTXRB IgG F AB'2 HRP X ADS	1:5000	Invitrogen, Cat #: A24537; RRID AB_2536005
Secondary antibody	Goat anti Mouse IgG (H + L) Secondary Antibody, HRP 0.5 mg GTXMU IgG F AB'2 HRP X ADS	1:10.000	Invitrogen, Cat #: A24524; RRID AB_2535993

Primers		
	Target	Forward/Reverse primer (5′-3′)
Targeted mutation analysis	*PARK7* gene, exon 7 1815 bp	CTGAAGGAGCAAGGAACTGGA
		GGAATGCTGGGTGCTATTACCT
Sequencing	*PARK7* gene, exon 7, locus of DJ-1 mutation in DJ-1-delP line	GCCCATTAGGATGTCACCTTT
	213 bp	GCAGTTCGCTGCTCTAGTCTT
RNA status	*PARK7*, whole transcript	atatatggccATGGCTTCCAAAAGAGC
	595 bp	cccccagatctCTAGTCTTTAAGAACAAG
	Beta actin	CTGGAACGGTGAAGGTGACA
	140 bp	AAGGGACTTCCTGTAACAATGCA
Plasmid specific primers (PCR)	*OriP*	TCGGGGGTGTTAGAGACAAC
		TTCCACGAGGGTAGTGAACC
Plasmid specific primers (PCR)	*EBNA1*	ATCGTCAAAGCTGCACACAG
		CCCAGGAGTCCCAGTAGTCA

using a colorimetric mycoplasma detection kit (PlasmoTest™, Invivogen).

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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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.2022.102792.

References

Klein, C., Westenberger, A., 2012. Genetics of Parkinson's disease. Cold Spring Harb. Perspect. Med. 663–697 https://doi.org/10.1016/B978-044452809-4/50169-1.

Larsen, S.B., et al., 2020. Induced pluripotent stem cell line (LCSBi001-A) derived from a patient with Parkinson's disease carrying the p. D620N mutation in VPS35. Stem Cell Res. 45, 101776.

Ramsey, C.P., Giasson, B.I., 2010. L10p and P158DEL DJ-1 mutations cause protein instability, aggregation, and dimerization impairments. J. Neurosci. Res. 88, 3111–3124. https://doi.org/10.1002/jnr.22477.

Tysnes, O.B., Storstein, A., 2017. Epidemiology of Parkinson's disease. J. Neural Transm. 124, 901–905. https://doi.org/10.1007/s00702-017-1686-y.