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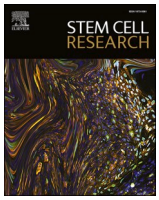

Lab Resource: Single Cell Line

# Generation and characterization of induced pluripotent stem cells from a Parkinson's disease patient carrying the digenic LRRK2 p.G2019S and GBA1 p.N409S mutations

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A B S T R A C T

We describe an induced pluripotent stem cell (iPSC) line that was derived from fibroblasts obtained from a Parkinson's disease (PD) patient carrying the p.G2019S mutation in the *LRRK2* gene and the p.N409S mutation in the *GBA1* gene. iPSCs were generated via Sendai virus transduction of Yamanaka factors. The presence of *GBA1* p.N409S and *LRRK2 p.*G2019S was confirmed by Sanger sequencing. The iPSCs express pluripotency markers, are capable of *in vitro* differentiation into the three germ layers and have a normal karyotype. The newly generated line will be used for *in vitro* PD modeling by investigating the role of each mutation in iPSC-derived dopaminergic neurons.

## 1. Resource Table

| Unique stem cell line identifier | LCSBi013-A |
|---|---|
| Alternative name(s) of stem cell line | GL2 |
| Institution | Luxembourg Centre for Systems Biomedicine (LCSB), University of Luxembourg, Esch-sur-Alzette, Luxembourg |
| Contact information of distributor | Prof. Dr. Rejko Krüger, rejko.krueger@uni.lu |
| Type of cell line | Induced pluripotent stem cell line (iPSC) |
| Origin | Human |
| Additional origin info required for human ESC or iPSC | Age at biopsy: 67 years<br>Sex: Male |
| Cell Source | Dermal fibroblasts |
| Clonality | Clonal |
| Method of reprogramming | Sendai transduction of Yamanaka factors |
| Genetic Modification | YES |
| Type of Genetic Modification | Missense mutation in *LRRK2* (p.G2019S) and GBA1 (p.N409S) genes |
| Evidence of the reprogramming transgene loss (including genomic copy if applicable) | PCR |
| Associated disease | Parkinson's disease (OMIM #168600) |
| Gene/locus | *GBA1*/ chromosome 1q21 (GC01M156443, NM_000157)<br>*LRRK2*/chromosome 12p11 (GC12P040196, NM_198578) |
| Date archived/stock date | 01/06/2023 |
| Cell line repository/bank | https://hpscreg.eu/cell-line/LCSBi013-A |
| Ethical approval | Informed consent was approved by the Ethics Committee of the Liguria Region, Italy (Approval n.8/2015 on 14/09/2015). |

## 2. Resource utility

The p.N409S mutation in the *GBA1* gene (Usenko et al., 2021) and the p.G2019S mutation in the *LRRK2* gene (Pischedda et al., 2021) are genetic risk factors for PD. The iPSC line described here has been established from a PD patient carrying both mutations, and will be used to investigate underlying pathological mechanisms in iPSC-derived

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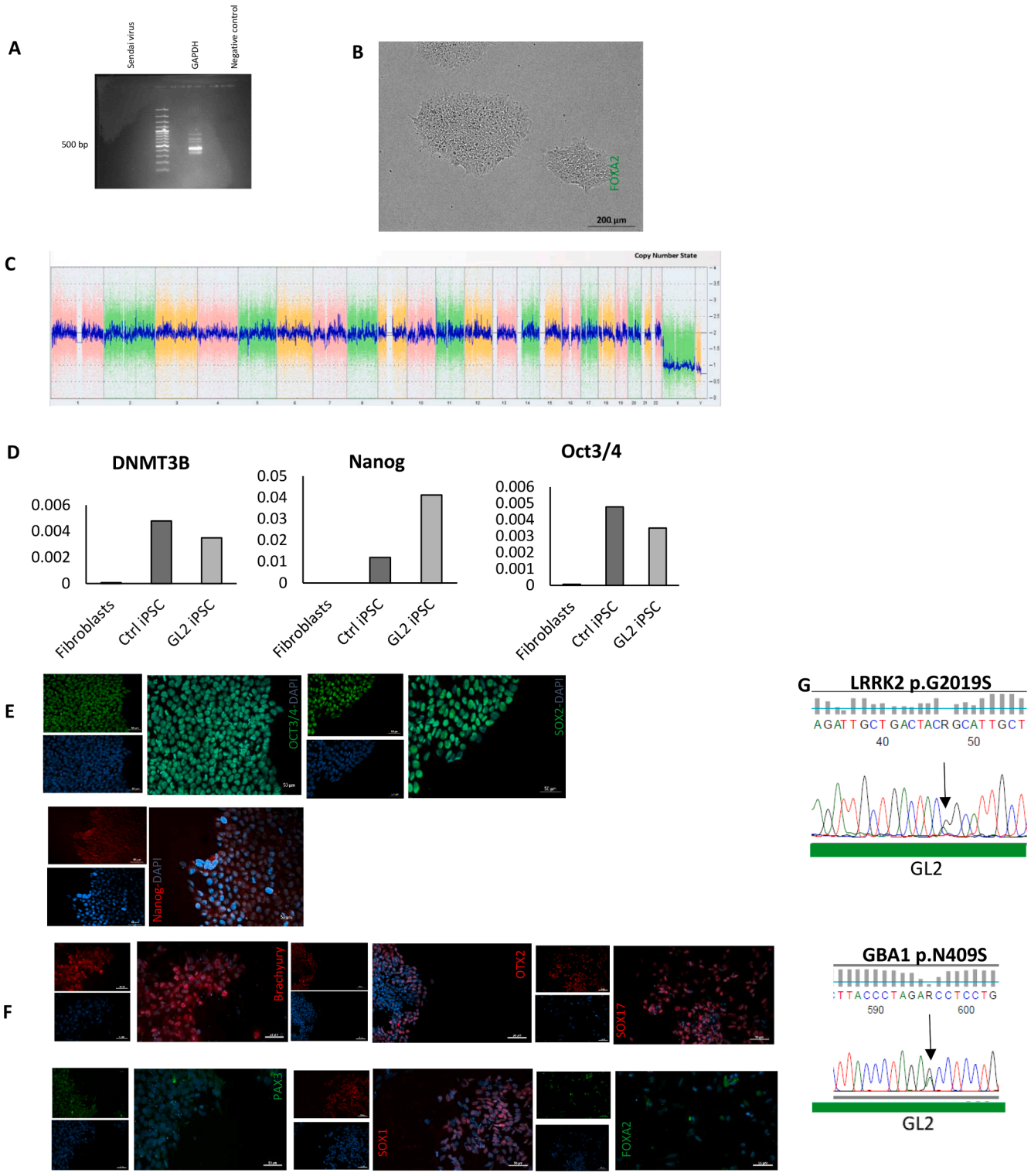


**Fig. 1.**

neuronal models.

## 3. Resource details

To generate the presented iPSC line (aka: GL2), dermal fibroblasts from a PD patient carrying the digenic *LRRK2* p.G2019S and *GBA1* p.N409S mutations were obtained by the "Cell Line and DNA Biobank from Patients Affected by Genetic Diseases" and the "Parkinson Institute Biobank", member of the Telethon Network of Genetic Biobanks (project no. GTB18001). The fibroblasts were reprogrammed using Sendai virus transduction of human OCT4, SOX2, KLF4 and c-MYC Yamanaka factors. PCR analysis using primers against Sendai virus backbone confirmed that the selected clone was free of integrated viral DNA into the genome (Fig. 1 A). GL2 iPSCs displayed a typical stem cell morphology (Fig. 1 B), a normal karyotype (46, XY) (Fig. 1C), and genetic identity with the corresponding fibroblasts (Supplementary Fig. 1).

**Table 1**
Characterization and validation.

| Classification | Test | Result | Data |
|---|---|---|---|
| Morphology | Photography | Typical iPSC morphology | Fig. 1 panel B |
| Phenotype | Qualitative analysis: Immunocytochemistry | Robust nuclear staining of the pluripotency markers Oct3/4, Sox2, and Nanog | Fig. 1 panel E |
| | Quantitative analysis: RT-qPCR | mRNA expression of the stemness markers *Nanog*, *Oct3/4* and *DMNT3B* | Fig. 1 panel D |
| Genotype | SNP array (KaryoStat + ) Resolution: > 2 Mb for chromosomal gains; > 1 Mb for chromosomal losses; ~5 Mb for telomere ends and centromeres | arr(1–22)x2, (XY)x1 *No aneuploidies detected* | Fig. 1 panel C |
| Identity | Correlation analysis of 150 k SNPs across the genome | Identical genotype between patient's fibroblasts and newly generated iPSCs | Supplementary Fig. 1 |
| Mutation analysis (IF APPLICABLE) | Sequencing | Heterozygous, *LRRK2 p. G2019S, GBA1 p.N409S* | Fig. 1 panel G |
| | Southern Blot OR WGS | *Not performed* | *N/A* |
| Microbiology and virology | Mycoplasma detection (colorimetric assay) | Negative | Supplementary Fig. 2 |
| Differentiation potential | Directed differentiation | Proof of three germ layers formation | Fig. 1 panel F |
| Donor screening (OPTIONAL) | HIV 1 + 2 Hepatitis B, Hepatitis C | Not performed | N/A |
| Genotype additional info (OPTIONAL) | Blood group genotyping | Not performed | N/A |
| | HLA tissue typing | Not performed | N/A |

RT-qPCR assays demonstrated that, different from fibroblasts, GL2 iPSCs express the pluripotency markers *Nanog*, *Oct3/4* and *DMNT3B*, to a similar extent as in a previously characterized control line 17608/6, referred to as C1-1 in the publication (Schöndorf et al., 2014) (Fig. 1D). Expression of the stemness markers Oct3/4, Sox2 and Nanog was also confirmed at protein level by immunocytochemistry (Fig. 1E). *In vitro* differentiation of GL2 iPSCs, followed by immunofluorescence staining of mesoderm (Brachyury and Pax3), ectoderm (Otx2 and Sox1) and endoderm (Sox17 and FOXA2) markers (Fig. 1F), confirmed their ability to differentiate into the three germ layers. The presence of *LRRK2* p. G2019S and *GBA1* p.N409S mutations in the newly-generated GL2 line was confirmed by Sanger sequencing (Fig. 1G). Finally, we excluded any contamination of GL2 iPSCs by mycoplasma (Supplementary Fig. 2) (Table 1).

## 4. Materials and methods

### 4.1. Fibroblast cell culture and reprogramming

PD patient-derived fibroblasts 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). 150.000 fibroblasts were reprogrammed into iPSCs using the CytoTune iPS 2.0 Sendai Reprogramming Kit, following the manufacturer's instructions (Thermo Fisher Scientific). Undifferentiated iPSC colonies were grown on a geltrex-coated plate in mTESR medium (mTeSR™1) and identified by morphology using bright-field microscopy (Fig. 1B). The undifferentiated iPSC colonies were picked manually and re-plated on Geltrex-coated wells containing mTESR medium (mTeSR™1). Feedings were performed every day, and iPSCs passaged (1:3) once a week using EDTA 0.5 mM in PBS (Life Technologies). Both fibroblasts and iPSCs were maintained at 37 °C under 5 % CO2 and humidified atmosphere.

### 4.2. RT-qPCR

Total RNA was extracted from the already characterized control fibroblasts, and control iPSCs and GL2 iPSCs 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 (Table 2, Fig. 1D). cDNA from GL2 fibroblasts was used as a negative control.

### 4.3. Loss of reprogramming vector

To analyze the transgene-free status, genomic DNA was isolated using the DNeasy Blood and Tissue Kit (Qiagen), followed by PCR analysis using the SeV primers (Table 2). Amplification was performed using the GoTaq G2 Flexi (Promega; Annealing temperature 58 °C, 30 cycles) on a TProfessional Basic Gradient Thermocycler (Biometra). The negative control used was sterile $H_2O$.

### 4.4. Immunofluorescence

iPSCs were plated on Geltrex-coated coverslips and fixed at passage 13 with 4 % paraformaldehyde in PBS for 15 min. Cells were blocked and permeabilized 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), diluted in PBS containing 0.1 % Triton-X, 1 % goat serum and 0.2 % bovine serum albumin, were incubated overnight at 4 °C. Next day, coverslips were washed three times with PBS and then incubated for 2 h at room temperature with secondary antibodies (Table 2). Nuclei were stained with Hoechst. Images were acquired using a Zeiss spinning disk confocal microscope (Carl Zeiss Microimaging GmbBH). Scale bar: 50 μm (Fig. 1E).

### 4.5. Three-germ layer differentiation

The iPSC's ability to differentiate into the three germ layers was verified at passage 14, using the Human Pluripotent Stem Cell Functional Identification Kit (R&D Systems) and following the manufacturer's instructions. A Zeiss spinning disk confocal microscope (Carl Zeiss Microimaging GmBH) was used for image acquisition. Scale bar: 50 μm (Fig. 1F).

### 4.6. Karyotyping and identity analysis

Molecular karyotyping of GL2 iPSCs was performed at passage 12, using a CytoScan HT-CMA 96F array for Karyostat+ (Thermo Fisher Scientific, Madison, WI, USA) (Fig. 1C). Genetic identity between patient-derived fibroblasts and iPSCs was assessed through correlation analysis of 1.1 million SNPs between samples (Cell ID assay, Thermo Fisher Scientific) (Supplementary Fig. 1).

**Table 2**
Reagents details.

| | Antibodies used for immunocytochemistry/flow-cytometry | | | |
|---|---|---|---|---|
| | Antibody | Dilution | Company Cat # | RRID |
| Pluripotency Markers | Mouse anti Oct3/4 | 1:1000 | Santa Cruz, Cat #: sc-5279 | RRID: AB_628051 |
| Pluripotency Markers | Goat anti SOX2 (Y-17) | 1:250 | Santa Cruz, Cat #: sc-17320 | RRID: AB_2286684 |
| Pluripotency Markers | Rabbit anti Nanog | 1:1000 | Abcam, Cat #: ab21624 | RRID: AB_446437 |
| Differentiation Markers | Goat anti Sox1 | 1:1000 | R&D Systems, Cat #: AF3369, | RRID: AB_2239879 |
| Differentiation Markers | Mouse anti FOXA2 | 1:1000 | Santa Cruz, Cat #: sc-101060, | RRID: AB_1124660 |
| Differentiation Markers | Mouse anti PAX3 | 1:1000 | DSHB AB_528426 | DSHB AB_528426 |
| 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 |

| | **Primers** | | |
|---|---|---|---|
| | **Target** | **Size of band** | **Forward/Reverse primer (5′-3′)** |
| Targeted mutation analysis | *LRRK2* gene, exon 41 | 129 bp | AGACCTGAAACCCACAATG/GGTGTGCCCTCTGATGTTTT |
| Targeted mutation analysis | *GBA1* gene, exon 9 | 1.6 kb | TGTGTGCAAGGTCCAGGATCAG/ACCACCTAGAGGGGAAAGTG |
| Sequencing | *LRRK2* gene, exon 41 | 1210 bp | AGACCTGAAACCCACAATG |
| Sequencing | *GBA1* gene, exon 9 | 1090 bp | TGTGTGCAAGGTCCAGGATCAG |
| Sendai Virus Detection | SeV plasmid | 181 bp | GGATCACTAGGTGATATCGAGC/ACCAGACAAGAGTTTAAGAGATATGTATC |
| Housekeeping gene | *GAPDH* | 447 bp | CAGGGCTGCTTTTAACTC/AAGTTGTCATGGATGACCTTG |

### 4.7. Mycoplasma test

iPSCs were tested for mycoplasma contamination at passage 5 by using a colorimetric mycoplasma detection kit (InvivoGen) (Supplementary Fig. 2).

### 4.8. Sanger sequencing

Genomic DNA was purified from GL2 iPSC using the QIA Blood and Tissue kit (Qiagen). Using the primers listed in Table 2, the exon 41 of the *LRRK2* gene and the exon 9 of the *GBA1* gene was amplified by PCR and Sanger sequenced at Microsynth AG.

## 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.

## Acknowledgements

The Cell Line and DNA Biobank from Patients affected by Genetic Diseases (Instituto 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, provided us with specimens. Work of RK is supported by the Fonds National de Recherche (FNR) within the following projects: National Centre for Excellence in Research on Parkinson’s disease (NCER-PD) (FNR; NCER13/BM/11264123), PEARL (/P13/6682797), MotaSYN (12719684), MAMaSyn (INTER/LEIR/18/12719318) and MiRisk (C17/BM/ 11676395). Work of GA is supported by the FNR, grant number C21/BM/15850547/PINK1-DiaPDs.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scr.2023.103212.

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