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# Induced pluripotent stem cell line (LCSBi001-A) derived from a patient with Parkinson's disease carrying the p.D620N mutation in VPS35

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A B S T R A C T

Fibroblasts were obtained from a 76 year-old man diagnosed with Parkinson's disease (PD). The disease is caused by a heterozygous p.D620N mutation in *VPS35*. Induced pluripotent stem cells (iPSCs) were generated using the CytoTune™-iPS 2.0 Sendai Reprogramming Kit (Thermo Fisher Scientific). The presence of the c.1858G > *A* base exchange in exon 15 of *VPS35* was confirmed by Sanger sequencing. The iPSCs are free of genomically integrated reprogramming genes, express pluripotency markers, display *in vitro* differentiation potential to the three germ layers and have karyotypic integrity. Our iPSC line will be useful for studying the impact of the p.D620N mutation in *VPS35 in vitro*.

## Resource table

| | |
|---|---|
| Unique stem cell line identifier | LCSBi001-A |
| Alternative name of stem cell line | *VPS35* Clone 33 |
| Institution | LCSB, University of Luxembourg, Belvaux, Luxembourg |
| Contact information of distributor | Rejko Krüger, rejko.krueger@uni.lu |
| Type of cell lines | Induced pluripotent stem cell line (iPSC) |
| Origin | Human |
| Additional origin info | Age: 76 years old<br>Sex: male<br>Ethnicity: caucasian |
| Cell Source | Dermal fibroblasts |
| Clonality | Clonal |
| Method of reprogramming | Transgene free (Cytotune™-IPS 2.0 Sendai Reprogramming kit) |
| Gene modification | YES |
| Type of modification | Familial mutation |
| Associated disease | Parkinson's disease |
| Gene/locus | Vacuolar protein sorting 35 (*VPS35*)/ chromosome 16q11 |
| Method of modification | N/A |
| Name of transgene or resistance | N/A |
| Inducible/constitutive system | N/A |
| Date archived/stock date | 31/07/2019 |
| Cell line repository/bank | https://hpscreg.eu/cell-line/LCSBi001-A |
| Ethical approval | Ethical approval for the development of and research pertaining to patient-derived cell lines have been given by informed consent for the academic research project (CNER #201,411/05): "Disease modeling of Parkinson's disease using patient-derived fibroblasts and induced pluripotent stem cells" (DiMo-PD). |

### Resource utility

Parkinson's disease (PD) usually occurs sporadically, but in approximately 10% of the cases, a monogenic cause was identified. The *VPS35* p.D620N (PARK17) mutation causes a late-onset autosomal-dominant form of PD (Vilariño-Güell et al., 2011; Zimprich et al., 2011). We aim to explore the molecular mechanisms underlying neurodegeneration in iPSC-derived neurons from one patient carrying p.D620N mutation in *VPS35*.

### Resource details

Dermal fibroblasts were obtained from a 76 year old man heterozygous for the p.D620N mutation in the *VPS35* gene (Bentley et al., 2018). The patient was clinically diagnosed with PD at age 60, displaying typical signs of parkinsonism with rigidity and tremor as predominant symptoms. Reprogramming of the patient fibroblasts was performed by co-expressing the Yamanaka factors OCT3/4, SOX2, KLF4 and cMYC using the integration free CytoTune™-iPS 2.0 Sendai Reprogramming Kit (Thermo Fisher Scientific). Four weeks after

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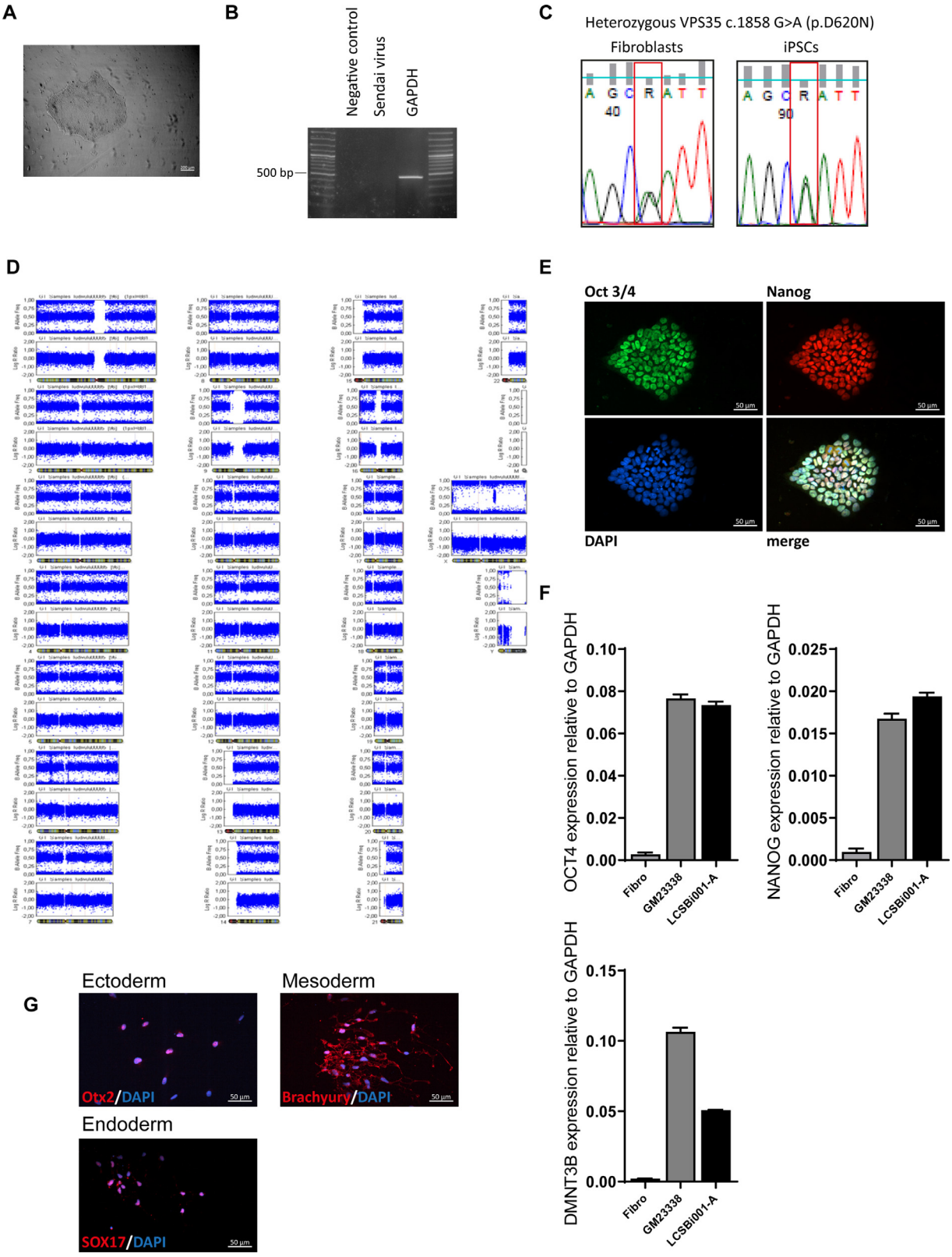


**Fig. 1.**

transduction, we successfully generated an iPS cell line with normal colony morphology (Fig. 1A) (Table 1). Clones were picked and integration analysis with primers against Sendai virus backbone was performed at passage 8. The selected clones were free of integrated viral DNA into their genome (Fig. 1B). Sanger sequencing confirmed the presence of a heterozygous c.1858G > *A* substitution in exon 15 of the *VPS35* gene corresponding to the p.D620N mutation (Fig. 1C). Using SNP-based karyotyping, no chromosomal aberrations were identified in the selected iPSC clone (Fig. 1D). Nevertheless, this technique doesn't allow for detection of balanced translocation. Identity analysis was

**Table 1**
Characterization and validation.

| Classification | Test | Result | Data |
|---|---|---|---|
| Morphology | Photography | Normal | Fig. 1A |
| Phenotype | Qualitative analysis Immunocytochemistry | Positive for pluripotency markers: OCT3/4 and NANOG | Fig. 1E |
| | Quantitative analysis RT-qPCR | Positive for pluripotency markers: OCT4, NANOG and DNMT3B | Fig. 1F |
| Genotype | Genotyping | HumanOmni2.5 Exome-8 DNA Analysis BeadChip | Fig. 1D |
| Identity | SNP analysis | DNA Profiling: matched | Available with the authors |
| Mutation analysis | Sequencing | Heterozygous *VPS35* p.D620N mutation | Fig. 1C |
| Microbiology and virology | Mycoplasma (PlasmoTest™ Invivogen) | Negative | Supplementary Fig. S1 |
| Differentiation potential | Directed differentiation | Positive for specific markers of ectodermal (OTX2), mesodermal (Brachyury) and endodermal (SOX17) lineage | Fig. 1G |
| 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 |

performed to confirm that the iPSC line originates from the parental fibroblasts.

Immunocytochemical (ICC) analyses showed the presence of the pluripotency markers OCT3/4 and NANOG, at the protein level (Fig. 1E). RT-qPCR confirmed that transcription of the endogenous pluripotency genes *NANOG, OCT4* and *DNMT3B* was in the range of a previously characterized iPSC line (GM23338, Coriell Institute) and upregulated compared to fibroblasts (Fig. 1F). *In vitro* differentiation using the Human Pluripotent Stem Cell Functional Identification Kit (R&D Systems) followed by ICC analyses with the mesodermal marker Brachyury, the endodermal marker SOX17 and the ectodermal marker OTX2 demonstrated the differentiation potential into all three germ layers (Fig. 1G). The iPSC cultures are free of mycoplasma contamination (Fig. S1).

## 1. Materials and methods

### 1.1. Reprogramming of dermal fibroblasts

Dermal fibroblasts carrying the heterozygous p.D620N mutation in *VPS35* were collected at the Griffith Institute (Queensland, Australia) after informed consent of the patient. Fibroblasts derived from the skin biopsy were cultured in fibroblasts medium composed of Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 2 mM L-glutamine and 1% penicillin and streptomycin (Pen/Strep) (all Life Technologies). The fibroblasts were transduced using the CytoTune-iPS 2.0 Sendai Reprogramming Kit (Thermo Fisher Scientific) with a multiplicity of infection (MOI) (KOS MOI = 5, hc-Myc MOI = 5, and hKlf4 MOI = 3). Seven days post-transduction, cells were passaged under feeder-free conditions in 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 acid ascorbic, Sigma-Aldrich; 100 ng/mL Heparin, Sigma-Aldrich; 10% mTesR, StemCell Technologies) was changed every other day, supplemented with 100 µM sodium butyrate (Sigma-Aldrich). After four weeks, iPSC colonies formed and were manually passaged into a new Matrigel-coated dish and cultured in E8 medium. The iPSC lines were then enzymatically passaged using Dispase (CellSystems) once a week at a 1:5 ratio. At passage 8, iPSCs were harvested for analysis and cryopreserved in liquid nitrogen. Fibroblasts and iPSC were cultured at 37 °C under 5% $CO_2$.

### 1.2. RT-PCR

Total RNA was purified from cells using Trizol/chloroform (Life Technologies). Transcriptor High Fidelity cDNA Synthesis Kit (Roche) was used to synthesize cDNA. The transgene-free status was carried out using the SeV primer (Table 2) by amplification with the GoTaq G2 Flexi (Promega; Annealing temperature 58 °C, 30 cycles) on a TProfessional Basic Gradient Thermocycler (Biometra). The negative control used was sterile $H_2O$. Quantification of pluripotency markers by multiplex qPCR was performed using the LightCycler® 480 Probes Master kit (Roche) and hydrolysis probes mentioned in Table 2 and run on the LightCycler 480 (Roche). Total RNA purified from fibroblasts was used as a negative control.

### 1.3. Immunofluorescence staining

iPSCs plated on Matrigel-coated coverslips were fixed with 4% paraformaldehyde in PBS for 15 min, permeabilized and blocked in 0.4% Triton-X 100 (Carl Roth), 10% goat serum (Vector Labs) and 2% bovine serum albumin (Sigma-Aldrich) in PBS for 1 h. Then, permeabilized samples were stained with primary antibodies in incubation buffer (0.1% Triton-X, 1% goat serum and 0.2% bovine serum albumin in PBS) overnight at 4 °C, washed three times with PBS, and incubated for 2 h at room temperature with secondary antibodies in incubation buffer. The expression of pluripotency markers OCT3/4 and NANOG were visualized using antibodies listed in Table 2 together with DAPI nuclear stain. Images were acquired using the Zeiss Axio Observer spinning disk confocal microscope (Carl Zeiss Microimaging GmH).

### 1.4. In vitro differentiation

The iPSC were plated on Matrigel-coated coverslips four days prior to the *in-vitro* differentiation. The ability of the iPSC to differentiate into cell types of the three germ layers was tested using the manufacturer's differentiation protocol (Human Pluripotent Stem Cell Functional Identification Kit, R&D Systems). We confirmed it by ICC using the ectodermal marker OTX2, the mesodermal marker Brachyury and the endodermal marker SOX17. Images were acquired using the Zeiss Axio Observer spinning disk confocal microscope (Carl Zeiss Microimaging GmBH).

### 1.5. Chromosomal analysis

Molecular karyotyping and identity analysis were performed on iPSC at passage 8 by Life&Brain GmBH (Bonn) using HumanOmni2.5 Exome-8 DNA Analysis BeadChip.

### 1.6. Mutation analysis

Genomic DNA was purified from LCSBi001-A iPSC using the QIA Blood and Tissue kit (Qiagen). The exon 15 of *VPS35* was amplified by PCR (Table 2) with the GoTaq G2 Flexi (Promega; Annealing temperature 60 °C, 30 cycles) on a TProfessional Basic Gradient Thermocycler (Biometra). Sanger sequencing was carried out at Eurofins Genomics Germany GmbH.

**Table 2**
Reagents details.

| Antibodies used for immunocytochemistry | | | |
|---|---|---|---|
| | Antibody | Dilution | Company Cat # and RRID |
| Pluripotency Marker | Mouse anti-OCT3/4 | 1:1000 | Santa Cruz, Cat #: sc-5279; RRID: AB_628,051 |
| Pluripotency Marker | Rabbit anti-Nanog | 1:500 | Abcam, Cat #: ab21624; RRID: AB_446,437 |
| Ectoderm Marker | Goat Anti- OTX2 | 1:500 | Human Pluripotent Stem Cell Functional Identification Kit (SC027B, R&D Systems) |
| Mesoderm Marker | Goat Anti-Brachyury | 1:500 | Human Pluripotent Stem Cell Functional Identification Kit (SC027B, R&D Systems) |
| Endoderm Marker | Goat Anti-SOX17 | 1:500 | Human Pluripotent Stem Cell Functional Identification Kit (SC027B, R&D Systems) |
| Secondary antibody | Alexa Fluor 488 Goat anti-Mouse IgG (*H + L*) | 1:1000 | Invitrogen, Cat #: A11029; RRID: AB_138,404 |
| Secondary antibody | Alexa Fluor 568 Goat anti-Rabbit IgG (*H + L*) | 1:1000 | Invitrogen, Cat #: A11036; RRID: AB_143,011 |
| Secondary antibody | Alexa Fluor 647 Donkey α-Goat IgG (*H + L*) | 1:1000 | Invitrogen, Cat #: A21447; RRID: AB_141,844 |
| Primers | Target | Forward/Reverse primer (5′−3′) | |
| Sendai virus detection | SeV plasmid (181 bp) | GGATCACTAGGTGATATCGAGC/ACC AGACAAGAGTTTAAGAGATATGTATC | |
| House-Keeping Gene | *GAPDH* (447 bp) | CAGGGCTGCTTTTAACTC/AAGTTGTCATGGATGACCTTG | |
| *VPS35* exon 15 | *VPS35* | AAATGGATATCCTGGAACAAG/ CAAATCTCCTAAGAGTAGGAAGGG | |
| Hs02758991_g1 | GAPDH | N/A | |
| Hs02387400_g1 | NANOG | N/A | |
| Hs00999632_g1 | OCT4 | N/A | |
| Hs00171876_m1 | DNMT3B | N/A | |

### *1.7. Mycoplasma test*

iPSCs were tested for Mycoplasma contamination by using a colorimetric mycoplasma detection kit (Plasmotest, Invivogen). Briefly, cell supernatant was collected and heat at 100 °C for 15 min then placed on Mycoplasma Sensor cells overnight. Detection of blue/purple wells by eye was indicating a contamination.

## Conflict of interest and authorship conformation form

We hereby confirm that:

- ○ All authors have participated in (a) conception and design, or analysis and interpretation of the data; (b) drafting the article or revising it critically for important intellectual content; and (c) approval of the final version.
- ○ This manuscript has not been submitted to, nor is under review at, another journal or other publishing venue.
- ○ The authors have no affiliation with any organization with a direct or indirect financial interest in the subject matter discussed in the manuscript

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## Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.scr.2020.101776.

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