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Lab resource: Stem Cell Line

Generation of an induced pluripotent stem cell line (DANi-011A) from a Parkinson's disease patient with a LRRK2 p.G2019S mutation

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ABSTRACT

We generated an induced pluripotent stem cell (iPSC) line from fibroblasts of a clinically diagnosed 70 year old female Parkinson's disease (PD) patient heterozygous for a pathogenic missense variant (p.G2019S; c. 6055 G > A) in the leucine-rich repeat kinase 2 (LRRK2) gene by using non-integrating Sendai viruses. The DANi-011A iPSC line has a normal karyotype and is free from Sendai viruses. The expression of pluripotent markers in the iPSC line was confirmed by immunofluorescent staining, and we confirmed its ability to differentiate into the three germ layers. The DANi-011A iPSC line can be used for modeling PD and as a drug-screening platform.

Resource table:

Unique stem cell line identifier	DANi-011A
Alternative name(s) of stem cell line	LRRK2-011-C1
Institution	Danish Research Institute of Translational Neuroscience (DANDRITE), Aarhus, Denmark
Contact information of distributor	Mark Denham, mden@dandrite.au.dk
Type of cell line	iPSC
Origin	Human
Additional origin info	Age: (age of disease onset) 70 Sex: Female Ethnicity if known: Unknown
Cell Source	Skin fibroblasts
Clonality	Clonal
Method of reprogramming	Sendai Virus with <i>POU5F1</i> , <i>SOX2</i> , <i>KLF4</i> and <i>MYCL</i>
Genetic Modification	Yes
Type of Modification	Hereditary
Associated disease	Parkinson's disease
Gene/locus	Gene <i>LRRK2</i> , Locus 12q12, Mutation NM_198,578.3:c.6055 G > A (NP_940,980.3:p.Gly2019Ser)
Method of modification	Not applicable
Name of transgene or resistance	Not applicable
Inducible/constitutive system	Not applicable

Date archived/stock date	Jan. 14, 2018
Cell line repository/bank	https://hpscereg.eu/cell-line/DANi011-A
Ethical approval	Ethics Committee at the Medical Faculty of Eberhard-Karls University and at the University Hospital Tübingen: 199 / 2011BO1

1. Resource utility

LRRK2 mutations have been identified in both inherited and sporadic cases of Parkinson's disease (PD), and the G2019S point mutation is the most common pathogenic variant of LRRK2 across populations worldwide (Nichols et al., 2005; Paisán-Ruiz et al., 2004; Zimprich et al., 2004). The established induced pluripotent stem cell (iPSC) line, DANi-011A, can be used to study disease mechanisms as well as in a drug-screening platform.

2. Resource details

LRRK2 mutations cause autosomal dominant PD (Paisán-Ruiz et al., 2004; Zimprich et al., 2004); however, the penetrance varies considerably among ethnic populations (Marder et al., 2015; Sierra et al., 2011). The majority of G2019S LRRK2-associated PD patients display comparable clinical symptoms and Lewy body pathology to that of sporadic PD patients (Nichols et al., 2005). In this paper, we report the

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Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology Phenotype	Photography	Normal morphology	Fig. 1 panel A
	Qualitative analysis by immunocytochemistry	Staining/expression of pluripotency markers: Alkaline phosphatase (ALP), POU5F1, TRA1-81, NANOG, SSEA-4	Fig. 1 panel A, C, D
	Quantitative analysis by immunocytochemistry counting	Assess% of positive cells for antigen markers. POU5F1: 98.81% \pm 2.05 S.D, NANOG: 99.51% \pm 0.43 S.D.	Fig. 1 panel B
Genotype Identity	Karyotype (Q-banding) and resolution	46XX, Resolution 450–500	Fig. 1 panel G
	STR analysis	DNA Profiling Performed. 10 genomic markers 100% matched between parental cells and iPSCs	Available with the corresponding author
Mutation analysis (If applicable)	Sequencing	heterozygous	Fig. 1 panel E
Microbiology and virology	Southern Blot OR WGS	N/A	N/A
	Mycoplasma	Mycoplasma testing by RT-PCR: Negative	Supplementary Figure 1
Differentiation potential	Embryoid body formation	Embryoid bodies express endoderm markers: SOX17/FOXA2; mesoderm marker: BRACHYURY; ectoderm marker: PAX6/SOX2.	Fig. 1 panel H

generation of an iPSC line DANI-011A, from a PD patient carrying the LRRK2 p.G2019S (c.6055G>A) variant (Table 1). The fibroblasts were reprogrammed using Sendai virus particles containing transcripts for *POU5F1*, *SOX2*, *KLF4* and *MYCL*. After four weeks, the appearance of iPSC clones was observed. Subsequently, the clone LRRK2-011-C1 (DANI-011A) was picked and expanded on feeders for further characterization. Chromosomal analysis showed a normal female karyotype, 46,XX (Fig. 1G). The LRRK2 p.G2019S (c.6055G>A) variant was confirmed in the patient fibroblasts and the iPSCs (Fig. 1E). The iPSCs expressed the pluripotent markers, POU5F1, NANOG, SSEA4 and TRA-1-81 and were alkaline phosphatase positive (Fig. 1A, C, D). Pluripotency was quantified by counting the percentage of NANOG⁺/DAPI and POU5F1⁺/DAPI cells from three different colonies (Fig. 1B).

DANI-011A was confirmed to be free from Sendai viral particles, which was analyzed by immunostaining (Fig. 1F). The DANI-011A line successfully formed embryoid bodies and neurospheres within which we identified cell types representative of the three germ layers, indicated by immunoreactivity for PAX6/SOX2 (ectoderm), BRACHYURY (mesoderm) and SOX17/FOXA2 (endoderm) (Fig. 1H). The absence of mycoplasma was confirmed by PCR (Supplementary Figure 1). The genetic identity of DANI-011A matched the donor fibroblasts, which was determined by a genetic profile of a set of STR loci.

3. Materials and methods

3.1. Reprogramming and maintenance of human iPSCs

The patient fibroblasts were obtained from the Neuro-Biobank of the University of Tuebingen, Germany (<http://www.hihtuebingen.de/nd/biobank/for-researchers/>) and expanded in RPMI medium supplemented with 1% glutamax, 0.5% pen/strep (both from Thermo Fisher), 10% FCS (Biowest) and FGF2 (10 ng/ml; Peprotech). Reprogramming was performed on 100,000 cells, which were seeded on a 9.6 cm² well (6-well plate, Thermo Fisher) coated with Vitronectin XFTM (STEMCELL Technologies). After cells attached, they were infected with Sendai viral particles, which together contained *POU5F1*, *SOX2*, *KLF4* and *MYCL* (CytoTune-iPS 2.0; Life Technologies), in TeSRTM-E7TM medium (STEMCELL Technologies). The TeSRTM-E7TM media was changed every 3–4 days. When iPSC colonies emerged, media was changed to TeSRTM-E8TM medium (STEMCELL Technologies). After a further 4–7 days, colonies were manually picked and cultured individually on irradiated human foreskin fibroblasts (HFF; ATCC CRL-2097) in KSR media consisting of DMEM/nutrient mixture F-12, supplemented with non-essential amino acids (NEAA) 1%, glutamine 2 mM, β -mercaptoethanol 0.1 mM, 0.5% pen/strep and 20% knockout serum replacement (all from Thermo Fisher). For maintaining pluripotency, KSR media was further supplemented with FGF2 (15 ng/ml; Peprotech) and Activin A

(15 ng/ml; R&D Systems). iPSCs were passaged by mechanical dissection of undifferentiated fragments, which were transferred to freshly prepared HFF every seven days and media was changed every second day. The absence of mycoplasma was confirmed by using the Look Out Mycoplasma PCR Detection Kit (Cat#MP0035, Sigma).

3.2. Pluripotency markers and embryoid body formation assay

Immunocytochemistry analysis was performed by fixing iPSCs in 4% PFA at 4 °C for 10 min. Subsequently, cells were washed 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 were diluted in blocking solution and applied overnight at 4 °C followed by washes in PBT, after which the corresponding secondary antibodies were added for 1 h at RT (antibodies shown in Table 2). Nuclei were counterstained with 4,6-diamidino-2-phenylindole (DAPI; 1 μ g/ml, Sigma). Samples were mounted in PVA-DABCO and visualized under an ApoTome fluorescent microscope (ZEISS), or ZEISS LSM 780 confocal microscope and images captured using the ZEN software (ZEISS). The percentage of positive cells for NANOG and POU5F1 was calculated by counting cells from three different colonies and data expressed as a percentage of NANOG⁺/DAPI and POU5F1⁺/DAPI. Alkaline phosphatase staining was performed following the manufacturer's procedure (Cat#00–0009, Stemgent).

Embryoid bodies (EBs) were generated by culturing fragments of iPSCs in suspension using ultra-low cluster 96-well plates (Corning). For mesoderm and endoderm differentiation, fragments were cultured in KSR media supplemented with 10 ng/ml Activin A, 1.5 μ M CHIR99021 (Cat#04–0004–10, Stemgent) and 40 ng/ml BMP2 (Cat#120–02, Peprotech) for 14 days. For neuroectoderm differentiation, fragments were first cultured for six days on a Vitronectin-coated (STEM CELL Technologies) 35 mm diameter Petri dish (Thermo scientific) in N2B27 medium containing 1:1 mix of Neurobasal medium and DMEM/F-12 medium, supplemented with insulin/transferrin/selenium-A 1%, N2 supplement 1%, B27 Supplement Minus Vitamin A 1%, glucose 0.3%, penicillin 25 U/ml, and streptomycin 25 μ g/ml (all from Life Technologies) supplemented with 10 μ M SB431542 (Tocris) and 0.1 μ M LDN193189 (Stemgent), then each fragment was mechanical dissected into two and transferred to an ultra-low cluster 96-well plate for suspension culture, to form neurospheres, in N2B27 media supplemented with 20 ng/ml FGF2 for one week.

EBs and neurospheres were fixed in 4% PFA for 20 mins at 4 °C, washed in PBS and placed in a 30% sucrose solution overnight before being embedded in Tissue-Tek OCT compound (Labtek). For immunostaining, EBs and neurospheres were cut at 10 μ m on a cryostat and antibodies applied following the protocol stated above.

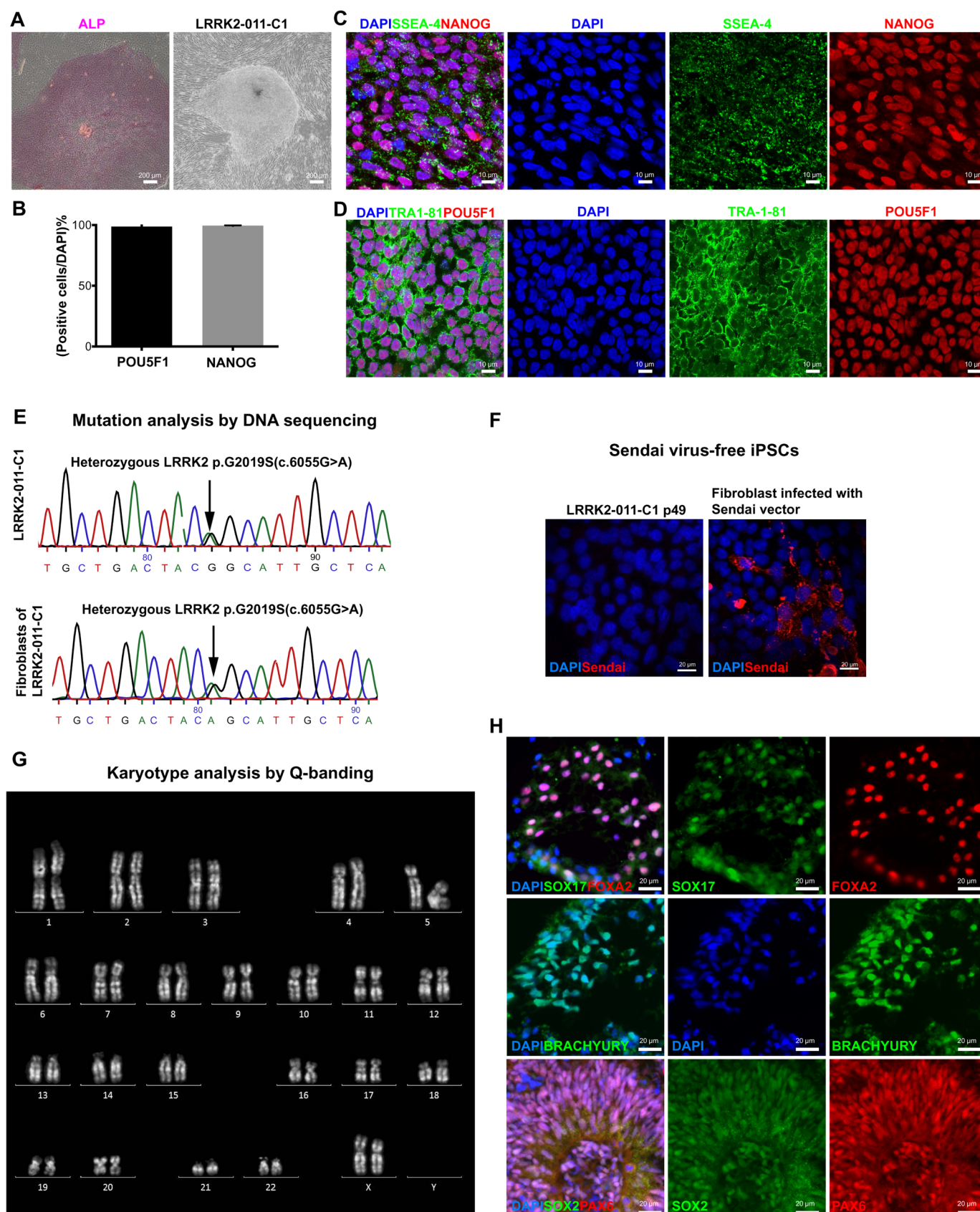


Fig. 1. Characterization of the DANI-011A iPSC line generated from a Parkinson's disease patient with a LRRK2 p.G2019S mutation.

Table 2
Reagents details.

Antibodies used for immunocytochemistry/flow-citometry		Dilution	Company Cat # and RRID
	Antibody		
Pluripotency markers	mouse anti-OCT3/4(C-10)	1:100	Santa Cruz Biotechnology Cat# sc-5279, RRID:AB_628,051
Pluripotency markers	mouse anti-NANOG	1:100	eBioscience Cat# 14-5768-82, RRID:AB_467,572
Pluripotency markers	mouse anti-TRA-1-81	1:200	Millipore Cat# MAB4381, RRID:AB_177,638
Pluripotency markers	mouse anti-SSEA4	1:40	DSHB Cat# MC-813-70, RRID:AB_528,477
Differentiation markers	goat anti-SOX17	1:200	R&D Systems Cat# AF1924, RRID:AB_355,060
Differentiation markers	rabbit anti-FOXA2	1:500	Cell signaling Technology Cat#8186, RRID: AB_10,891,055
Differentiation markers	goat anti-BRACHYURY	1:100	R&D Systems Cat# AF2085, RRID:AB_2,200,235
Differentiation markers	mouse anti-PAX6	1:40	DSHB Cat# PAX6, RRID: AB_528,427
Differentiation markers	goat anti-SOX2	1:100	R&D Systems Cat# AF2018, RRID:AB_355,110
Sendai virus free test	rabbit anti-Sendai virus	1:500	MBL International Cat# PD029, RRID:AB_10,597,564
Secondary antibodies for IF	Goat anti-mouse IgG2b Alexa 568	1:1000	ThermoFisher Scientific Cat# A21144, RRID: AB_2,535,780
Secondary antibodies for IF	Goat anti-mouse IgG1 Alexa 488	1:1000	ThermoFisher Scientific Cat# A21121, RRID:AB_141,514
Secondary antibodies for IF	Donkey anti-mouse IgM Alexa 488	1:200	Jackson ImmunoResearch Cat# 715-545-020, RRID:AB_2,340,844
Secondary antibodies for IF	Goat anti-mouse IgG3 Alexa 594	1:200	Jackson ImmunoResearch Cat# 115-585-209, RRID: AB_2,338,889
Secondary antibodies for IF	Donkey anti-goat IgG (H + L) Alexa 488	1:1000	ThermoFisher Scientific Cat# A-11,055, RRID: AB_2,534,102
Secondary antibodies for IF	Donkey anti-rabbit IgG (H + L) Alexa 568	1:1000	ThermoFisher Scientific Cat# A11057, RRID: AB_2,534,104
Secondary antibodies for IF	Donkey anti-mouse IgG (H + L) Alexa 568	1:1000	ThermoFisher Scientific Cat# A10037, RRID: AB_2,534,013

Primers		Forward/Reverse primer (5'–3')
	Target	
Targeted mutation analysis	LRRK2 G2019S Product size 518	GGCAGATACCTCCACTCAGC/ TTGATTGCTCACAAGTGC
Targeted mutation sequencing	LRRK2 G2019S	GGCAGATACCTCCACTCAGC

3.3. Genomic analysis

Karyotype analysis was performed on DANI-011A passage 46 by Q-banded metaphase spreads that were prepared according to standard protocol at an accredited clinical laboratory. Ten metaphases were counted, and two further analyzed in accordance with clinical standards. Briefly, fresh medium was added and supplemented with colcemid at 0.1 µg/ml and incubated at 37 °C for 60–120 min. Single cells were harvested by trypsinization (0.025% W/V in Hanks buffered saline) at 37 °C, after which the trypsinization was inactivated by adding serum-containing medium. Cells were centrifuged and resuspended in a hypotonic KCl 0.56% and incubated at 37 °C for 30 min in a water bath. After which, cells were centrifuged and resuspended in fixation buffer (1 part acetic acid and 3 parts methanol). The cells were again spun down by centrifugation and resuspended in fixative. This step was repeated once. The resuspended cells were added dropwise to glass slides, dried, stained by quinacrine and mounted for microscopy.

The G2019S variant in the patient fibroblasts and iPSCs was validated by standard PCR. Briefly, genomic DNA was collected and purified using the GeneJet Genomic DNA purification kit (Cat #K0721, ThermoFisher Scientific). The PCR amplification was performed in a 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 purified 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 2. The DANI-011A line was tested for Sendai virus particles by staining iPSC colonies with an anti-Sendai virus antibody (Table 2). Cell line identity was performed by the Department of Molecular Medicine (MOMA) at Aarhus University Hospital with the GenePrint® 10 system.

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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.2020.101781](https://doi.org/10.1016/j.scr.2020.101781).

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