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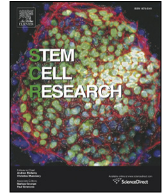

Lab Resource: Stem Cell Line

Generation of an induced pluripotent stem cell line (CSC-41) from a Parkinson's disease patient carrying a p.G2019S mutation in the *LRRK2* gene

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ABSTRACT

The leucine-rich repeat kinase 2 (LRRK2) p.G2019S mutation is the most common genetic cause of Parkinson's disease (PD). An induced pluripotent stem cell (iPSC) line CSC-41 was generated from a 75-year old patient diagnosed with PD caused by a p.G2019S mutation in *LRRK2*. Skin fibroblasts were reprogrammed using a non-integrating Sendai virus-based technology to deliver OCT3/4, SOX2, c-MYC and KLF4 transcription factors. The generated iPSC line exhibits expression of common pluripotency markers, differentiates into the three germ layers and has a normal karyotype. The iPSC line can be used to explore the association between *LRRK2* mutation and PD.

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Resource table

Unique stem cell line identifier	ULUNDi002-A
Alternative name(s) of stem cell line	CSC-41C
Institution	Stem Cell Laboratory for CNS Disease Modeling, Department of Experimental Medical Science, Lund University
Contact information of distributor	Laurent Roybon, Laurent.Roybon@med.lu.se
Type of cell line	iPSC
Origin	Human
Additional origin info	Age of patient: 75 Sex of patient: female Ethnicity: N/A
Cell source	Skin fibroblasts
Clonality	Clonal
Method of reprogramming	Sendai virus-mediated delivery of OCT3/4, SOX2, c-MYC and KLF4
Genetic modification	No modification
Type of modification	No modification
Associated disease	Parkinson's disease
Gene/locus	*LRRK2* (MIM # 607060) located on the chromosome 12q12 Genotype: c.6055G–A transition in exon 41 (p. G2019S substitution)
Method of modification	No modification
Name of transgene or resistance	No transgene or resistance
Inducible/constitutive system	Not inducible
Date archived/stock date	N/A
Cell line repository/bank	N/A
Ethical approval	Parkinson Institute Biobank (part of the Telethon Genetic Biobank Network http://biobanknetwork.telethon.it/): approved by Ethics Committee "Milano Area C" (http://comitatoeticoareac.ospedaleniguarda.it/) on the 26/06/2015, Numero Registro dei pareri: 370-062015. Reprogramming: 202100-3211 (delivered by Swedish work environment Arbetsmiljöverket).

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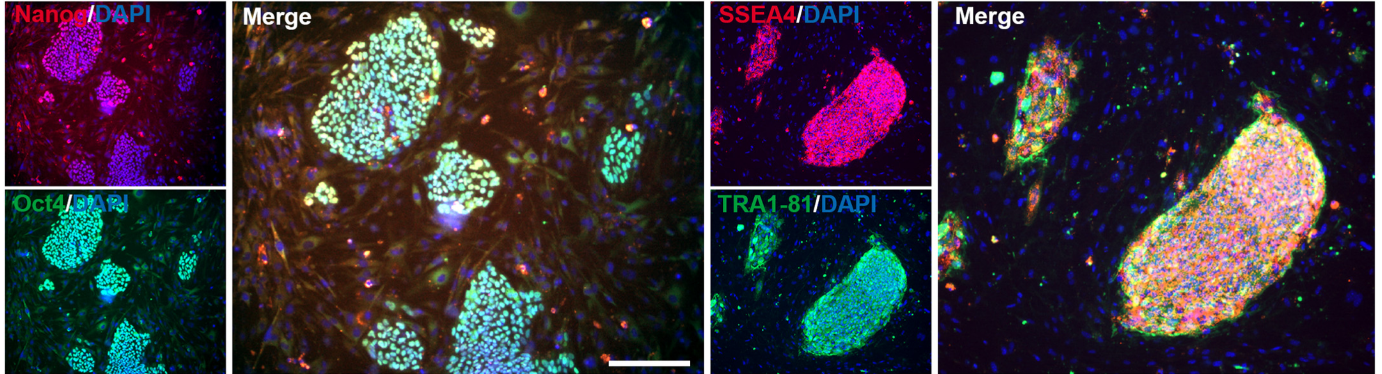

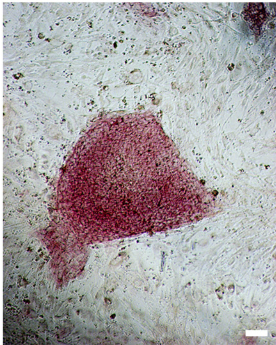

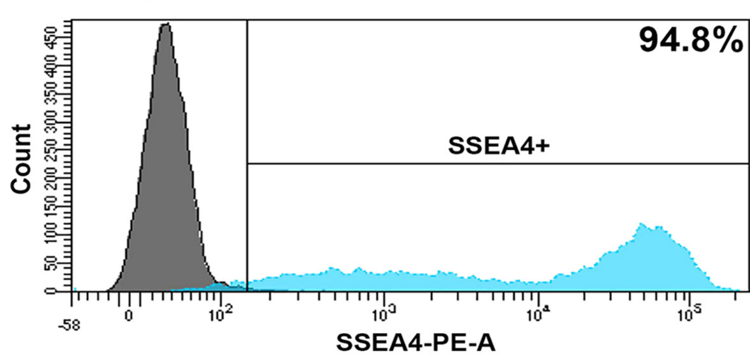

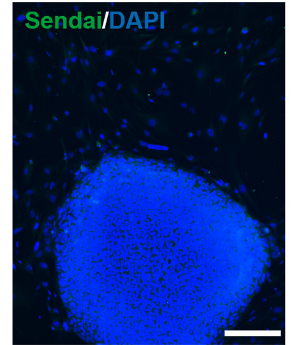

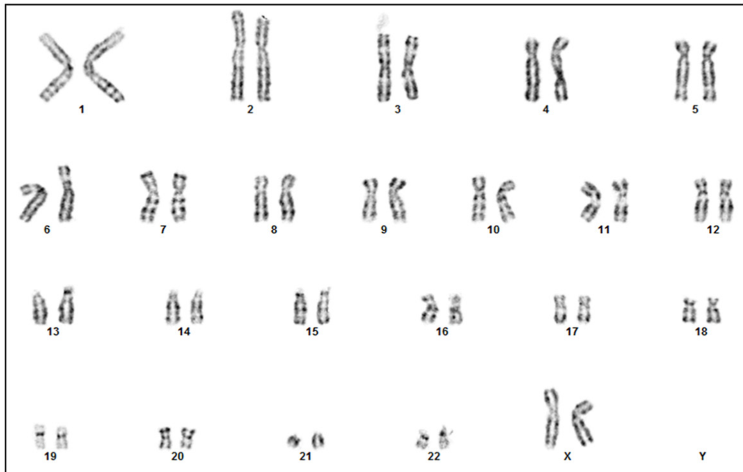

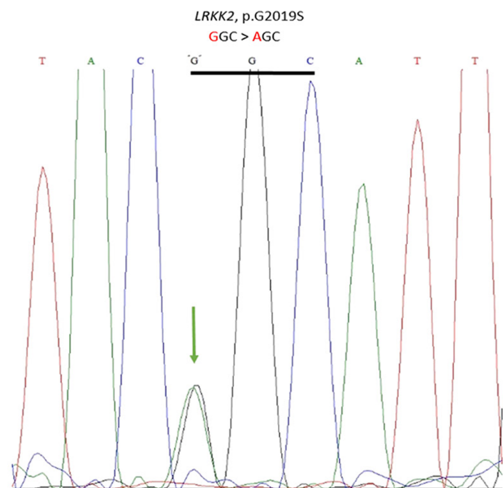

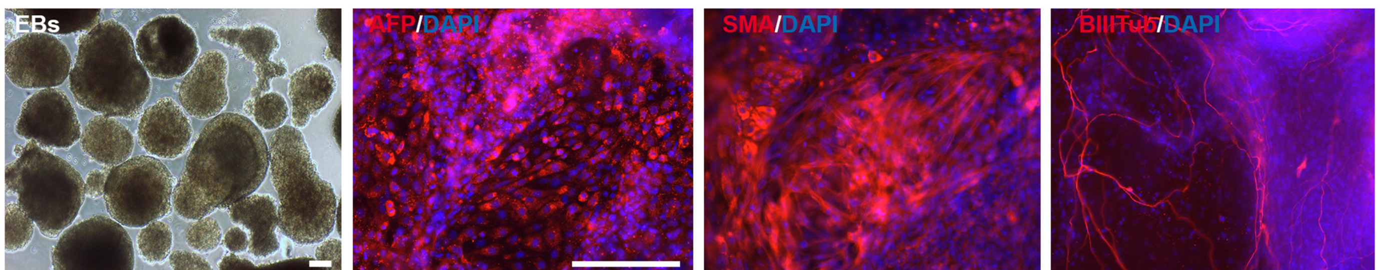

Fig. 1. Characterization of the iPSC line CSC 41C.

Resource utility

The *LRRK2* mutation p.G2019S is the most common genetic cause of Parkinson's disease (PD). The line CSC-41 was generated from a patient with PD carrying a p.G2019S mutation in *LRRK2* encoding for the leucine-rich repeated kinase 2 (LRRK2) protein. This induced pluripotent stem cell (iPSC) line can be used as a model to explore the link between mutant *LRRK2* and PD pathology.

Resource details

Mutations in *LRRK2* result in autosomal-dominant familial PD and have also been identified in sporadic PD cases with no family history of the disease. LRRK2 is encoded by the *LRRK2* gene, and is a protein with GTPase and kinase activity. The most frequent *LRRK2* mutation among PD patients, is a glycine-to-serine substitution at amino acid 2019 (p.G2019S) which causes increased kinase activity and has been reported to have implications in oxidative stress response and neuronal plasticity (Reinhardt et al., 2013). Here we report the generation of an induced pluripotent stem cell line (CSC-41) from a patient with PD carrying a p.G2019S *LRRK2* mutation. CSC-41 iPSC line can be used to better understand the molecular mechanisms underlying p.G2019S *LRRK2* associated PD.

To generate this line, skin fibroblasts collected by punch skin biopsy from a 75-year-old PD patient were reprogrammed using a non-integrating Sendai virus-based technology. Briefly, fibroblasts were seeded (75,000 cells/well) on a 12-well plate, two days before transduction. The CytoTune™-iPS 2.0 Sendai Reprogramming Kit was then used to deliver the four reprogramming factors (OCT3/4, SOX2, c-MYC and KLF4). At day 7 post-transduction, the cells were re-seeded onto irradiated mouse embryonic fibroblasts (MEF)-feeder layer and expanded until colonies presented an embryonic stem cell-like morphology. At day 28, 12 colonies were picked and expanded as individual clones for 7 days. Three clones (CSC-41C, CSC-41I, CSC-41K) were further selected, based on their morphology, for expansion and karyotype analysis. All clones were characterized using the methods we previously described (Holmqvist et al., 2016). Here, we present the characterization of clone CSC-41C.

Briefly, the generated clone, CSC-41C, expressed the common nuclear and cell surface pluripotency markers, OCT4/NANOG and TRA1-81/SSEA4 (Fig. 1A), and exhibited alkaline phosphatase (ALP) activity (Fig. 1B). According to flow cytometry analysis, >94% of the iPSCs were positive for SSEA4 (Fig. 1C; non-stained iPSCs are shown in grey). Additional immunocytochemistry analysis revealed elimination of the Sendai virus at passage 11 (Fig. 1D). Fig. 1E depicts a normal female karyogram of the CSC-41C clone. The identity of the generated iPSC line was confirmed by DNA fingerprint, showing genetic correspondence to parental fibroblasts. The mutation p.G2019S was confirmed by DNA sequencing (Fig. 1F). Embryoid bodies (EBs) generated from CSC-41C iPSCs differentiate into the three-germ layer, *in vitro*. Differentiated cells express endodermal marker alpha-fetoprotein (AFP), mesodermal marker smooth muscle actin (SMA), and ectodermal marker beta-III-tubulin (BIIITub) (Fig. 1G). During the generation of the iPSC clones, plasmocin was used to prevent Mycoplasma contamination.

Materials and methods

Fibroblast culture

Dermal fibroblasts were collected by punch skin biopsy from a patient diagnosed with PD, after obtaining informed consent. The fibroblasts were maintained in fibroblast growth medium, composed of DMEM media (ThermoFisher Scientific) with 10% fetal bovine serum and 1% Penicillin-Streptomycin and passaged with 0.05% trypsin.

iPSC generation and expansion

For reprogramming, 75,000 cells were seeded on a 12-well plate and maintained in fibroblast growth medium. Two days after (day 0), the cells were transduced using the three vector preparations (MOI = 5, 5, 3) included in the CytoTune™-iPS 2.0 Sendai Reprogramming Kit (ThermoFisher Scientific). On the following day and on every other day, the medium was replaced with fresh fibroblast growth medium. At day 7, the cells were re-seeded onto irradiated mouse embryonic fibroblasts (MEF) feeder cells with fibroblast growth medium. On the day after and until colony picking, the cells were cultured in WiCell medium composed of advanced DMEM/F12 (ThermoFisher Scientific), 20% Knock-Out Serum Replacement (v/v, ThermoFisher Scientific), 2 mM L-glutamine (Thermo Fisher Scientific), 1% non-essential amino acids (NEAA, v/v, Millipore) and 0.1 mM β-mercaptoethanol (Sigma-Aldrich), supplemented with 20 ng/ml FGF2 (ThermoFisher Scientific). On day 28, individual colonies were picked and re-seeded on a 24-well plate containing fresh MEFs. One week after, three clones were selected and further expanded on 6-well plates. The cells were passaged once a week and seeded on the appropriate cell culture surface for characterization assays at the indicated passage numbers (Table 1).

Immunocytochemistry

The iPSC cultures were fixed with 4% paraformaldehyde for 15 min at room temperature (RT), permeabilized and blocked for 1 h at RT with PBS containing 10% donkey serum and 0.1% TritonX-10 (Sigma)

Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography	Visual record of the line: Normal	Not shown but available with author
Phenotype	Immunocytochemistry	Positive staining for pluripotency markers: OCT4, NANOG, TRA1–81 and SSEA4	Fig. 1 panel A
	Alkaline phosphatase activity	Visible activity	Fig. 1 panel B
	Flow cytometry	98.7% SSEA4	Fig. 1 panel C
Genotype	Karyotype (G-banding) and resolution	46,XX, (300–400 bands resolution in average)	Fig. 1 panel E
Identity	STR analysis	10 sites analyzed, all matched with parent fibroblast cell line	Available with author
Mutation analysis (IF APPLICABLE)	Sequencing	Heterozygous p.G2019S mutation in *LRRK2*	Fig. 1 panel F
Microbiology and virology	Mycoplasma	Mycoplasma testing by RT-PCR. Negative.	Not shown but available with author
Differentiation potential	Embryoid body formation	Positive staining for smooth muscle actin, beta-III-tubulin and alpha-fetoprotein after spontaneous differentiation of embryoid bodies	Fig. 1 panel G
Donor screening (OPTIONAL)	HIV 1 + 2 Hepatitis B, Hepatitis C	N/A	N/A
Genotype additional info (OPTIONAL)	Blood group genotyping	N/A	N/A
	HLA tissue typing	N/A	N/A

Table 2
Reagents details.

Antibodies used for immunocytochemistry			
	Antibody	Dilution	Company Cat # and RRID
Pluripotency markers	Mouse anti-OCT4	1:200	Millipore Cat# MAB4401, RRID:AB_2167852
	PE-conjugated mouse anti-human Nanog	1:200	BD Biosciences Cat# 560483, RRID:AB_1645522
	Mouse anti-TRA-1-81	1:200	Thermo Fisher Scientific Cat# 41-1100, RRID:AB_2533495
	PE-conjugated mouse anti-SSEA4	1:200	Thermo Fisher Scientific Cat# A14766, RRID:AB_2534281
Sendai	Chicken anti-Sendai virus	1:1000	Abcam Cat# ab33988, RRID:AB_777877
Differentiation markers	Mouse anti-AFP	1:200	Sigma-Aldrich Cat# A8452, RRID:AB_258392
	Mouse anti-SMA	1:200	Sigma-Aldrich Cat# A2547, RRID:AB_476701
	Mouse anti-beta-III tubulin	1:200	Sigma-Aldrich Cat# T8660, RRID:AB_477590
Secondary antibodies	Donkey anti-mouse Alexa Fluor® 488	1:400	Molecular Probes Cat# A-21202, RRID:AB_141607
	Donkey anti-chicken Alexa Fluor® 488	1:400	Jackson ImmunoResearch Labs Cat# 703-545-155, RRID:AB_2340375
	Donkey anti-mouse Alexa Fluor® 555	1:400	Thermo Fisher Scientific Cat# A-31570, RRID:AB_2536180

Primers		
	Target	Forward/reverse primer (5′-3′)
Mutation sequencing	*LRRK2*	TTTTGATGCTTGACATAGTGGAC/CACATCTGAGGTCAGTGGTTATC

and incubated overnight at +4 °C with the primary antibodies (Table 2) diluted in the blocking buffer. The secondary antibodies were thereafter added for 1 h at RT in the dark, followed by nuclei counterstain with DAPI (1:10,000 (Life Technologies)) and image acquisition on inverted epifluorescence microscope LRI - Olympus IX-73. Scale bars are 200 μm.

Alkaline phosphatase activity

Alkaline phosphatase staining was performed using Alkaline Phosphatase Staining Kit (Stemgent, MA).

In vitro differentiation by embryoid body (EB) formation

Human iPSCs were grown for 2 weeks as embryoid bodies (EBs) in low-attachment 24-well plates (Corning) in WiCell medium supplemented with 20 ng/ml FGF2. The EBs were then seeded on a 0.1% gelatin-coated 96-well plate (Greiner Bio-One) in DMEM media containing 10% fetal bovine serum and 1% Penicillin-Streptomycin for subsequent spontaneous differentiation, with media changes every 2–3 days. After 2 weeks, the cells were fixed and stained for three germ-layer markers as described in the Table 2.

Karyotype analysis

The G-banding analysis was performed after 14 passages at 300–400 band resolution in average, at the Department of Clinical Genetics and Pathology in Lund.

Mutation sequencing

Genomic DNA from fibroblasts and iPSCs was extracted using conventional lysis buffer composed of 100 mM Tris (pH 8.0), 200 mM NaCl, 5 mM EDTA and 0.2% SDS in distilled autoclaved water supplemented with 1.5 mg/ml Proteinase K. The mutation, p.G2019S in *LRRK2*, was confirmed by direct DNA sequencing (Macrogen Europe, Amsterdam, The Netherlands). Primers used for amplification and directed sequencing of *LRRK2* around the mutation sites are listed in the Table 2.

DNA fingerprinting

Genomic DNA from fibroblasts and iPSCs was isolated as described above and fingerprinting analyses were outsourced to the IdentiCell STR profiling service (Department of Molecular Medicine, Aarhus University Hospital, Skejby, Denmark).

Mycoplasma detection

Absence of mycoplasma contamination was confirmed by the real-time PCR method at GATC Biotech AG (European Genome and Diagnostics Centre, Konstanz, Germany).

Acknowledgements

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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.2018.01.022.

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