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Lab resource: Stem Cell Line

Generation of an integration-free iPSC line, ICCSICi005-A, derived from a Parkinson's disease patient carrying the L444P mutation in the *GBA1* gene

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A B S T R A C T

The L444P mutation in the *GBA1* gene which encodes β-glucocerebrosidase-1, is a major risk factor for developing Parkinson's disease (PD) and dementia with Lewy bodies (DLB). We report the generation and characterization of an induced pluripotent stem cell (iPSC) line derived from a female PD patient carrying the L444P/wt mutation. The iPSC line presented a normal morphology, expressed endogenous pluripotency markers, could be differentiated into endodermal, mesodermal and ectodermal cells, was free from Sendai vectors and reprogramming factors, had a normal karyotype and maintained the original *GBA1* genotype. Thus, this iPSC line can serve to establish cellular models of PD.

Resource utility

Generating patient-specific cells bearing a specific gene mutation is a valuable strategy for exploring mechanisms of neurodegeneration and finding potential therapeutic treatments. The cell line reported here, along with our previously reported collection of iPSCs, make a unique set of iPSCs for studying the role of *GBA1* mutations in PD.

Resource details

Among the high number of mutations occurring in the *GBA1* gene, the L444P destabilizes β-glucocerebrosidase-1 structure leading to reduced enzyme activity and protein level. This mutation represents a strong genetic risk factor for developing PD and DLB (García-Sanz et al., 2017; Seto-Salvia et al., 2012; García-Sanz et al., 2018; Migdalska-Richards and Schapira, 2016). In this study, we report the generation and characterization of an iPSC line from a female PD patient carrying the L444P/wt mutation in *GBA1*.

This human iPSC line was generated by reprogramming dermal fibroblasts isolated from a skin biopsy taken from the ventral side of the donor's forearm. Fibroblasts were transduced with non-integrative Sendai viral vectors expressing the transcription factors OCT4, KLF4, SOX2, and c-MYC. Three weeks after transduction, primary colonies were picked and expanded as clonal lines on mouse embryonic fibroblasts (MEFs), and the iPS cell clone ICCSICi005-A was selected for in-depth characterization (Fig. 1; Table 1). This clonal line showed a normal iPSC morphology (flat and compact, with defined borders) by light microscopy observation (Fig. 1A), and expressed the human pluripotency markers NANOG, TRA-1-60, TRA-1-81, SSEA-3 and SSEA-4 as demonstrated by immunofluorescence (Fig. 1B–F) and *OCT4*, *SOX2* and *NANOG* as shown by RT-qPCR (Fig. 1G) This line exhibited a normal karyotype (46, XX; analyzed at passage 7) (Fig. 1H). Next, the iPSC line was differentiated following three distinct protocols that promote the induction of endodermal, mesodermal or ectodermal lineages (see below). The positive immunostaining with specific antibodiesTable 2 against alpha-feto protein (AFP; endoderm), DESMIN (mesoderm) as well as PAX6 and TUJ1 (ectoderm) (Fig. 1I-L, Table 1) confirmed the pluripotency of our iPSC line. Furthermore, the SeV genome and reprogramming transgenes were not detected by RT-PCR (performed in RNA from cells at passage 12), supporting that this line was integration-free (Fig. 1M). Short tandem repeat (STR) analysis of 10 different genomic loci demonstrated that the parental fibroblasts

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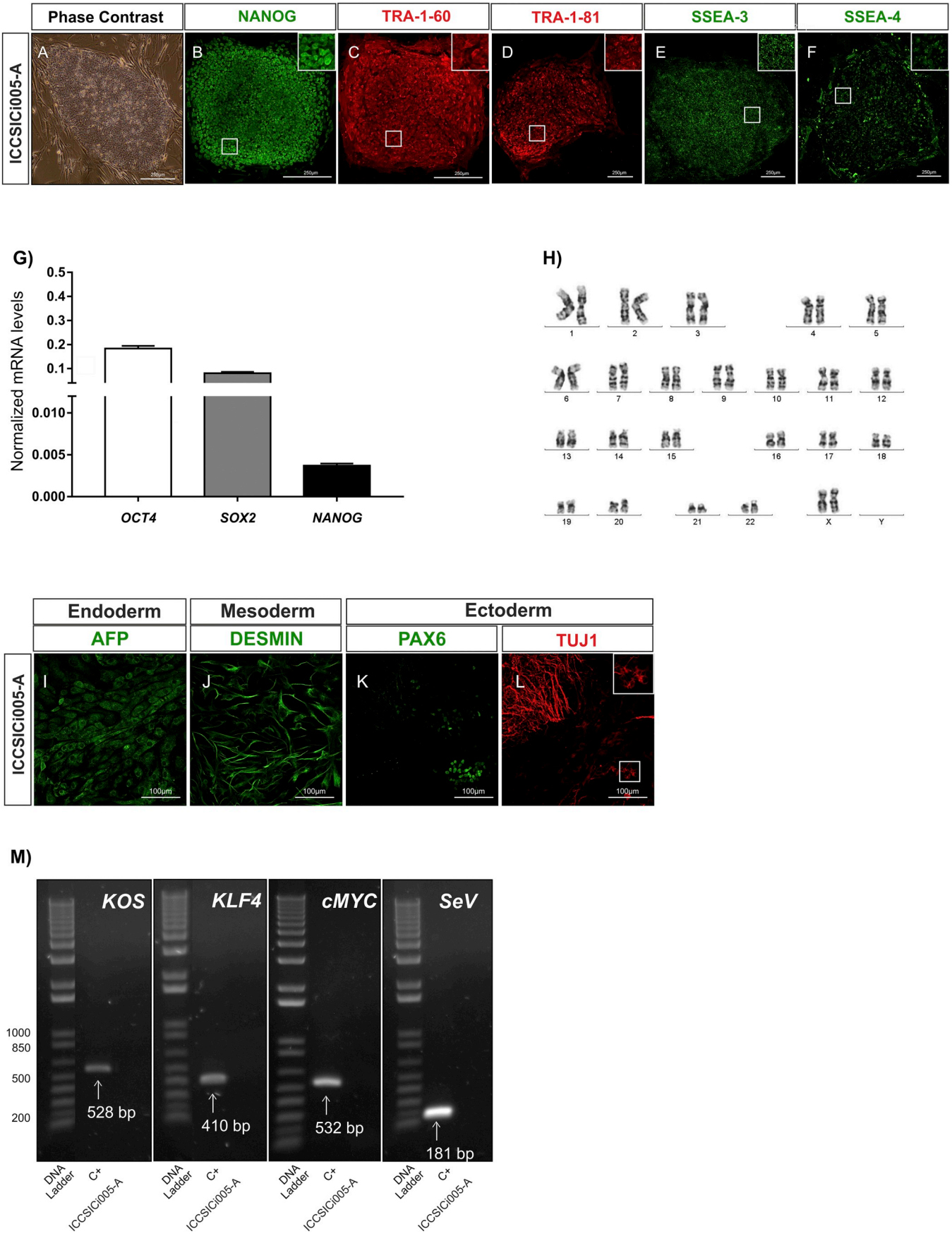

Fig. 1.. Characterization of an iPSC line carrying the GBA1-L444P mutation.

Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography	Visual record of the line: normal iPSC morphology	Fig. 1A
Phenotype	Qualitative analysis Immunocytochemistry	Positive expression of pluripotency markers: NANOG, TRA-1-60, TRA-1-81, SSEA-3 and SSEA-4	Fig. 1B-F
	Quantitative analysis RT-qPCR	The iPSC line expresses *OCT4*, *SOX2* and *NANOG* transcription factors	Fig. 1G
Genotype	Karyotype (G-banding) and resolution	46, XX Resolution 450–500	Fig. 1H
Identity	Microsatellite PCR (mPCR) OR	N/A	N/A
	STR analysis of fibroblasts and iPSC line	10 sites tested, identity verified (100% match)	Submitted in archive with journal
Mutation analysis (IF APPLICABLE)	Sequencing	Heterozygous L444P mutation in *GBA1* (T > C, p.Leu483pro)	Supplementary Fig. 1A
	Southern Blot OR WGS	N/A	N/A
Microbiology and virology	Mycoplasma	Mycoplasma testing by PCR: Negative	Supplementary Fig. 1C
Differentiation potential	Embryoid body formation followed by directed differentiation	Proof of three germ-layers formation by positive immunostaining for AFP, DESMIN, PAX6 and ß-III-TUBULIN	Fig. 1I-L
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

and the ICCSICi005-A iPSC line shared all the tested alleles with a 100% match (archived at SCR journal).

The presence of the L444P mutation in *GBA1* was analyzed using Sequenom MassArray technology. Mass spectrum from this iPSC containing the L444P/wt mutation (Supplementary Fig. 1A; Table 1) displayed peaks for C and T at the expected mass (5155 and 5232). These results confirm that the L444P mutation carried by the PD patient was maintained in the corresponding iPSCs. In contrast, the mass spectra for an iPSC line derived from a healthy control carrying no GBA1 mutations shows absence of a peak for the base C (Supplementary Fig. 1B). Additionally, the cells were free from mycoplasma (Supplementary Fig. 1C; Table 1).

Materials and methods

Reprogramming of dermal fibroblasts

Fibroblasts were isolated from a skin biopsy, plated at 10,000 cells/cm^2 and incubated (37 °C, 5% CO2). On day 2, they were transduced using the using the CytoTune™ -iPS 2.0 Sendai Reprogramming Kit (Thermo-Fisher). After seven days, transduced fibroblasts were seeded on MEFs and two-three weeks later, colonies were expanded in hiPSC medium (KnockOut™ DMEM/F12, 100 µM NEAA, 2 mM glutaMAX, 55 µM ß-mercaptoethanol, 100 U/mL Penicillin/100 µl/mL, Streptomycin B, 20% KnockOut serum replacement; Thermo-Fisher).

Embryoid body (EB) formation and three-lineage differentiation

The iPSC colonies were mechanically divided to generate EBs, which on day 5 were plated for initiating differentiation. For ectodermal differentiation, cultures were treated with 250 ng/mL Noggin (Peprotech) and 5 µM A83 (Miltenyi-Biotec). On day 11, the hiPSC medium was changed to Neurobasal (Thermo Fisher) with 2% B27, 2 mM Glutamax, 0.02 mM ascorbic acid (Sigma-Aldrich), 0.05 mM cAMP (Sigma-Aldrich), 20 ng/mL BDNF (Peprotech), 20 ng/mL GDNF (Peprotech) and 1 ng/mL TGFβ-3 (Peprotech). For mesodermal differentiation, 0.01 mM ascorbic acid was added to the hiPSC medium. For endodermal differentiation, no exogenous factors were added to the hiPSC medium. All cultures were fixed on days 16–17.

Immunocytochemistry

Immunofluorescence staining was performed following standard procedures (Nieto-Estévez et al., 2013) using specific primary and secondary antibodies (Table 2). Cells were imaged on a Leica SP-5 confocal microscope.

RT-qPCR

Total RNA was extracted from the hiPSCs and subjected to RT-qPCR following standard procedures (Nieto-Estévez et al., 2013) using specific primers (Table 2). Gene expression levels were determined in triplicate in samples from three experiments and normalized versus *GAPDH* levels.

Sendai virus and transgene detection

Total RNA (500 ng) was used for RT-PCR analysis using the following PCR conditions: 1 min 45 s at 95 °C, 45 s at 95 °C, 45 s at 55–60 °C, 1 min at 72 °C (35 cycles), 5 min at 72 °C (primers listed in Table 2).

Karyotyping

The iPSCs were treated with 10 µg/mL Colcemid (Irvine Scientific; 2 h at 37 °C) and incubated with Trypsin-EDTA (Euroclone) 10 min at 37 °C. Hypotonic treatment was carried out with 0.075 M KCl (30 min at 37 °C) and cells were fixed with methanol / acetic acid (3: 1). Cells were

Table 2
Reagents details.

Antibodies used for immunocytochemistry/flow-citometry			
	Antibody	Dilution	Company Cat # and RRID
Pluripotency Markers	Rabbit anti-NANOG	1:250	Stemgent Cat#09–0020 RRID:AB_2298294
	Rat anti-SSEA-3	1:100	Stemgent Cat#09–0014 RRID:AB_1512168
	Mouse anti-SSEA-4	1:100	Hybridoma Bank Cat#MC-813-70 RRID:AB_528477
	Mouse anti-TRA-1-60	1:200	Stemgent Cat#09–0010 RRID:AB_1512170
	Mouse anti-TRA-1-81	1:200	Stemgent Cat#09–0011 RRID:AB_1512171
Differentiation Markers	Mouse anti-AFP	1:100	R&D Systems Cat#MAB1368 RRID:AB_357658
	Rabbit anti-DESMIN	1:250	Lab Vision Cat#RB-9014-P0 RRID:AB_149768
	Rabbit anti-PAX6	1:300	Covance Cat#PRB-278P RRID:AB_291612
	Mouse anti-β-III TUBULIN (TUJ1)	1:1000	Covance Cat#MMS-435P RRID:AB_2313773
Secondary antibodies	Alexa Fluor 488 Goat Anti-Rabbit IgG	1:750	Thermo Fisher Scientific Cat# A-11008, RRID:AB_143165
	Alexa Fluor 488 Donkey Anti-Mouse IgG	1:750	Thermo Fisher Scientific Cat# A-21202, RRID:AB_141607
	Alexa Fluor488 Goat Anti-Rat IgM	1:500	Thermo Fisher Scientific Cat# A-21212, RRID:AB_2535798
	Alexa Fluor 594 Donkey	1:750	Thermo Fisher Scientific Cat# A-21203, RRID:AB_2535789
	Anti-Mouse IgG	1:400	Jackson ImmunoResearch Labs Cat# 115–076-075, RRID:AB_2338588
	Texas Red Goat Anti-Mouse IgM		

Primers		
	Target	Forward/Reverse primer (5′-3′)
Sendai Viral Vectors (PCR)	*SeV*	GGATCACTAGGTGATATCGAGC/ ACCAGACAAGAGTTTAAGAGATATGTATC
	KOS	ATGCACCGCTACGACGTGAGCGC/ ACCTTGACAATCCTGATGTGG
	KLF4	TTCCTGCATGCCAGAGGAGCCC/ AATGTATCGAAGGTGCTCAA
	c-MYC	TAACTGACTAGCAGGCTTGTCG/ TCCACATACAGTCCTGGATGATGATG
Pluripotency Markers (qPCR)	*NANOG*	TCTGCTGAGATGCCTCACACGG/ CACACAGCTGGGTGGAAGAGAAC
	OCT4	CCCCTGTCTCTGTCACCACT/ TGAATGAAGAACTTAATCCCAAA
	SOX2	ACACTGCCCCTCTCACACAT/ TCCCATTTCCCTCGTTTTT
House-Keeping Genes (qPCR)	*GADPH*	AACCATGAGAAGTATGACAACAGCC/ TGAGTCCTTCCACGATACCAAAGT

dropped on a microscope slide and metaphases were G-banded with Wright stain. At least 10 to 20 metaphases were counted and structurally evaluated (450 to 500 bands per haploid number).

Genotyping

GBA1 genotype was determined in DNA extracted from human fibroblasts and hiPSCs using Sequenom MassArray IPLEX technology performed at the CEGEN-ISCIII (Santiago de Compostela, Spain). This technology couples single-base extension after a PCR using ddNTPs with a MALDI-TOF mass spectrometry analysis. For a specific allele, differences in mass will depend on a single base (the studied one). Accordingly, it is a powerful tool for genotyping single nucleotide polymorphisms (SNPs) with great accuracy. Since it uses multiplex and mass array formats it allows the analysis of several mutations in a single reaction well; thus, minimizing DNA amount, cost and time for processing. Moreover, multiple samples can be processed at the same time.

Mycoplasma detection

Mycoplasma testing in the culture medium was performed using Mycoplasma Detection Kit (SoutherBiotech).

Short tandem repeat (STR) analysis

DNAs from parental fibroblasts and the iPSC line were analyzed at the Genomics Unit of CNIO-ISCIII (Madrid, Spain). Nine STR loci plus the Amelogenin (AMEL) locus for gender determination were PCR amplified (GenePrint 10 kit; Promega). Each DNA was subsequently analyzed using an ABI Prism 3730xl Genetic Analyzer and the Osiris v2.6 software (NIH).

Key resource table

Unique stem cell line identifier	ICCSICi005-A
Alternative name(s) of stem cell line	IC-PD5-F-iPS-4F-1
Institution	Instituto Cajal- CSIC and CIBERNED, Madrid, Spain
Contact information of distributor	Carlos Vicario, cvicario@cajal.csic.es
Type of cell line	iPSC
Origin	Human
Additional origin info	Age: 76 Sex: Female Ethnicity: Caucasian
Cell Source	Dermal fibroblasts
Clonality	Clonal
Method of reprogramming	Non-integrative (Sendai viral vectors) expressing hOCT4, hKLF4, hSOX2 and hc-MYC
Genetic Modification	Yes
Type of Modification	Hereditary
Associated disease	Parkinson's disease
Gene/locus	*GBA1* / 1q22. L444P/wt mutation (exon 10)
Method of modification	N/A
Name of transgene or resistance	N/A
Inducible/constitutive system	N/A
Date archived/stock date	11/04/2019
Cell line repository/bank	IC-PD5-F-iPS-4F-1 Banco Nacional de Líneas Celulares (BNLC, Spanish Stem Cell Bank): http://www.isciii.es/ISCIII/es/contenidos/fd-el-instituto/fd-organizacion/fd-estructura-directiva/fd-subdireccion-general-investigacion-terapia-celular-medicina-regenerativa/fd-centros-unidades/fd-banco-nacional-lineas-celulares/fd-lineas-celulares-disponibles/lineas-de-celulas-iPS.shtml
Ethical approval	The study was approved by the Commission of Guarantees for the Donation and Utilization of Human Cells and Tissues, Instituto de Salud Carlos III (ISCIII) (approval numbers 3212701 and 4103331). Informed consent was obtained from the patient.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scr.2019.101578.

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Declaration of Competing Interest

The authors declare no conflict of interest.

References

García-Sanz, P., Orgaz, L., Bueno-Gil, G., Espadas, I., Rodríguez-Traver, E., Kulisevsky, J., ... Moratalla, R., 2017. N370S-GBA1 mutation causes lysosomal cholesterol accumulation in Parkinson's disease. Mov. Disord. 32 (10), 1409–1422. https://doi.org/10.1002/mds.27119.

García-Sanz, P., Orgaz, L., Fuentes, J.M., Vicario, C., Moratalla, R., 2018. Cholesterol and multilamellar bodies: Lysosomal dysfunction in GBA-Parkinson disease. Autophagy 14 (4), 717–718. https://doi.org/10.1080/15548627.2018.1427396.

Migdalska-Richards, A., Schapira, A.H., 2016. The relationship between glucocerebrosidase mutations and Parkinson disease. J. Neurochem. 139 Suppl 1, 77–90. https://doi.org/10.1111/jnc.13385.

Seto-Salvia, N., Pagonabarraga, J., Houlden, H., Pascual-Sedano, B., Dols-Icardo, O., Tucci, A., ... Clarimon, J., 2012. Glucocerebrosidase mutations confer a greater risk of dementia during Parkinson's disease course. Mov. Disord. 27 (3), 393–399. https://doi.org/10.1002/mds.24045.

Nieto-Estévez, V., Pignatelli, J., Araúzo-Bravo, M.J., Hurtado-Chong, A., Vicario-Abejón, C., 2013. A global transcriptome analysis reveals molecular hallmarks of neural stem cell death, survival, and differentiation in response to partial FGF-2 and EGF deprivation. PLos One 8 (1), e53594.