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Generation of two iPS cell lines (HIHDNDi001-A and HIHDNDi001-B) from a Parkinson's disease patient carrying the heterozygous p.A30P mutation in *SNCA*

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A B S T R A C T

Dermal fibroblasts from a patient carrying a heterozygous c.88G > C mutation in the *SNCA* gene that encodes alpha-synuclein were reprogrammed to pluripotency by retroviruses. This pathogenic mutation generates the p.A30P form of the alpha-synuclein protein leading to autosomal dominantly inherited Parkinson's disease (PD). Two clonal iPS cell lines were generated (A30P-3 and A30P-4) and characterised by validating the silencing of viral transgenes, the expression of endogenous pluripotency genes, directed differentiation into three germ layers *in-vitro* and a stable molecular genotype. These iPSC lines will serve as a valuable resource in determining the role of the p.A30P *SNCA* mutation in PD pathogenesis.

1. Resource table

Unique stem cell line identifier	HIHDNDi001-A; HIHDNDi001-B;
Alternative name of stem cell line	A30P-3, SNCA3; A30P-4, SNCA4
Institution	Hertie Institute, Tübingen, Germany
Contact information of distributor	Rejko Krüger, LCSB, University of Luxembourg, Luxembourg. rejko.krueger@uni.lu
Type of cell lines	Induced pluripotent stem cell line (iPSC)
Origin	Human
Additional origin info	Age: 67 years old Sex: male Ethnicity: Caucasian
Cell Source	Dermal fibroblasts
Clonality	Clonal
Method of reprogramming	Retroviral
Multiline rationale	Same diseased iPS clones
Gene modification	Yes
Type of modification	Familial, autosomal dominant
Associated disease	Parkinson's disease
Gene/locus	$SNCA^{A30P}$
Method of modification	N/A
Name of transgene or resistance	N/A
Inducible/constitutive system	N/A
Date archived/stock date	10th February 2020
Cell line repository/bank	https://hpscreg.eu/cell-line/HIHDNDi001-Ahttps://hpscreg.eu/cell-line/HIHDNDi001-B
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 by the National Committee for Ethics in Research (CNER, Luxembourg). (CNER #201411/05): "Disease modelling of Parkinson's disease using patient-derived fibroblasts and induced pluripotent stem cells" (DiMo-PD).

2. Resource utility

PD is a neurodegenerative movement disorder. The p.A30P mutation in alpha-synuclein causes an autosomal dominantly inherited form of PD (PARK1) (Krüger et al., 1998, Krüger et al., 2001). We generated and characterised two iPS cell lines from a patient carrying this p.A30P alpha-synuclein mutation in order to explore the molecular mechanisms underlying neurodegeneration (Table 1).

3. Resource details

Dermal fibroblasts from a 67 year old male carrying the heterozygous p.A30P *SNCA* mutation in alpha-synuclein were donated. The patient had an age at disease onset of 55 years with initial symptoms of rigidity and bradykinesia dominant to the right side, previously referred to as individual IV-5 (Krüger et al., 2001). Reprogramming of the

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Table 1
Characterisation and validation.

Classification	Test	Result	Data
Morphology	Photography	Normal	Fig. 1A
Phenotype	Qualitative analysis Immunocytochemistry	Positive staining of pluripotency markers: OCT4, NANOG, TRA-1–60, SSEA3	Fig. 1A
	Quantitative analysis (RT-qPCR)	Positive expression of pluripotency markers, *OCT4*, *NANOG*, *DNMT3B* and *SOX2*	Fig. 1B
Genotype	Genotyping	HumanOmni2.5 Exome-8 DNA Analysis BeadChip	Fig. 1E
Identity	STR analysis	16 STR loci analysed, all matching to fibroblasts	Available with the authors.
Mutation analysis (IF APPLICABLE)	Sequencing	Heterozygous c.88G > C, p.A30P *SNCA* mutation	Fig. 1.C
	Southern Blot OR WGS	Not done	Not done
Microbiology and virology	Mycoplasma (PlasmoTest™ Invivogen)	Negative	Supplementary Fig. S1
Differentiation potential	Directed differentiation	Expression of ectoderm (OTX2), mesoderm (BRACHYURY) and endoderm (SOX17)	Fig. 1.D
Donor screening (OPTIONAL)	HIV 1 + 2 Hepatitis B, Hepatitis C	HIV 1 & 2, Hepatitis B &C and HTLV1 & 2 were tested. The results were negative.	Available from the authors
Genotype additional info (OPTIONAL)	Blood group genotyping	Not done	Not done
	HLA tissue typing	Not done	Not done

patient fibroblasts was performed using the following retroviral vectors: OCT4 (Addgene #13366), SOX2 (Addgene #13367), KLF4 (Addgene #13370), C-MYC (Addgene #13375). iPSC clones were picked and colonies displayed a typical stem-cell-like round morphology, with immunocytochemistry staining of: OCT4, NANOG, SOX2 and TRA-1-60 confirming the pluripotency at the protein level (Fig. 1A). The stem cells demonstrated expression of endogenous pluripotency genes *OCT4*, *NANOG, DMNT3B* and *SOX2* using quantitative RT-qPCR, which was upregulated compared to the patient-derived fibroblasts, and in the range of a previously characterised iPS line (Schöndorf et al., 2014) (Fig. 1B). Sanger sequencing confirmed the presence of the c.88G > C mutation in the patient lines (Fig. 1C). We demonstrate the *in-vitro* pluripotency ability of the iPSCs to differentiate into all three germ layers (Fig. 1D). Furthermore, the chromosomal integrity was ascertained and confirmed using copy number variants to assess the genotype (Fig. 1E).

4. Materials and methods

4.1. Reprogramming of dermal fibroblasts

Dermal fibroblasts of the patient with the heterozygous p.A30P mutation in *SNCA* were donated with informed consent via a 4 mm^2 biopsy performed at the University Hospital Tübingen. The fibroblasts were cultured in fibroblast media: (Dulbecco's Modified Eagle Media (DMEM) 4.5 g/L Glucose, 10% FCS, 50 μM β-Mercaptoethanol, 1% Penicillin/Streptomycin). For the retroviral transfection, 25 000 cells were seeded in a well of a 6well plate. The cells were transfected twice in 24 h with a total retroviral load of 9 μg. Additionally, 2.25 μg of VSV-G (Addgene #8454), 0.25 μg of Gag-Pol (Addgene #8449), 4 μg/mL Protamine sulphate (Sigma) and 30 μL of Fugene-6 were added to this transfection mix. After 24 h the media was changed to iPS media (Knock-out DMEM, 20% Knock-out serum replacement (KOSR), 50 μM β-Mercaptoethanol, 1% Non-essential amino acids 1% Penicillin/Streptomycin) containing 5 ng/mL FGF-2 (Peprotech), 1 nM Valproic Acid (Sigma), SB-421543 (Sigma) and PD-173074 (Selleckchem). The cells were placed in an incubator at 37 °C; 5% CO_2 with the media changed daily. Colonies would form after 7 days and were passaged to a plate containing inactivated mouse embryonic feeders (MEFs), iPS colonies were expanded by passaging and cryopreserved.

4.2. Cell culture conditions

IPSCs were routinely cultured in 6-well plates (Nunc, 140675). These were coated with high concentration growth factor reduced Matrigel® (1:100; Corning, 354263) according to the manufacturer instructions. The iPSCs were maintained in homemade E8: (DMEM/F12 + HEPES)(Life Technologies; 31330038), Insulin-Transferrin-Selenium (ITS) (1%; Life Technologies, 41400045), Penicillin-Streptomycin (1%; Life Technologies, 15140), L-Ascorbic acid 2-phosphate sesquimagnesium salt hydrate ((AA2PM); 64 μg/mL; Sigma, A8960), bFGF (10 ng/mL; Peprotech, 100-18B), TGF-β1 (2 ng/mL; Peprotech, 100–21), Heparin (100 ng/mL; Sigma, H3149). The iPSCs were maintained as colonies and enzymatically passaged using Dispase® (5U/mL; CellSystems, LS02104) with a split ratio of 1:5. The cells were maintained in an incubator at 37 °C; 5% CO_2 with the media changed daily.

4.3. RT-qPCR

Total RNA was extracted and purified from the fibroblasts and iPS cells using the RNeasy Mini Kit (Qiagen) according to manufacturer instructions. Transcriptor High Fidelity cDNA Synthesis Kit (Roche) was used to synthesize cDNA. Pluripotency markers were quantified by Multiplex qPCR using the LightCycler® 480 Probes Master kit (Roche) run on the LightCycler® 480 (Roche). Hydrolysis probes, used previously (Larsen et al., 2020) are shown in Table 2 with gene expression normalised to *ACTB*. For a positive control, previously published iPS control line 17608/6 was used, this was referred to as C1-2 in the publication (Schöndorf et al., 2014). Total RNA purified from the patient-derived fibroblasts were used as a negative control with the fold increase in iPS gene expression normalised to the patient-derived fibroblasts.

4.4. Immunocytochemistry

iPS cells were fixed on Geltrex™-coated glass coverslips in 4% paraformaldehyde (Sigma) for 15 mins at room temperature, blocked and permeabilised also at room temperature for 1 h (PBS + 10% goat serum (Vectorlabs), 2% BSA (Sigma), 0.4% Triton-X (Sigma), and placed overnight at 4 °C in antibody staining solution (primary antibody (Table 2), 0.1% Triton-X, 1% goat serum, 0.2% BSA). Cells were washed in PBS and incubated in staining solution containing the secondary antibody (Table 2). Hoechst 33,342 (Invitrogen) was used for 15 mins as a nuclear stain before mounting the coverslip on a frosted microscope slide containing a droplet of Vectashield® mounting medium (Vectorlabs). Images were acquired using the Zeiss Spinning Disk confocal microscope (Carl Zeiss Microimaging GmBH).

4.5. In-vitro differentiation

iPSCs were plated onto Geltrex™-coated glass coverslips. Directed *in-vitro* differentiation to the three germ layers was performed using the Human Pluripotent Stem Cell Functional Identification Kit (R&D

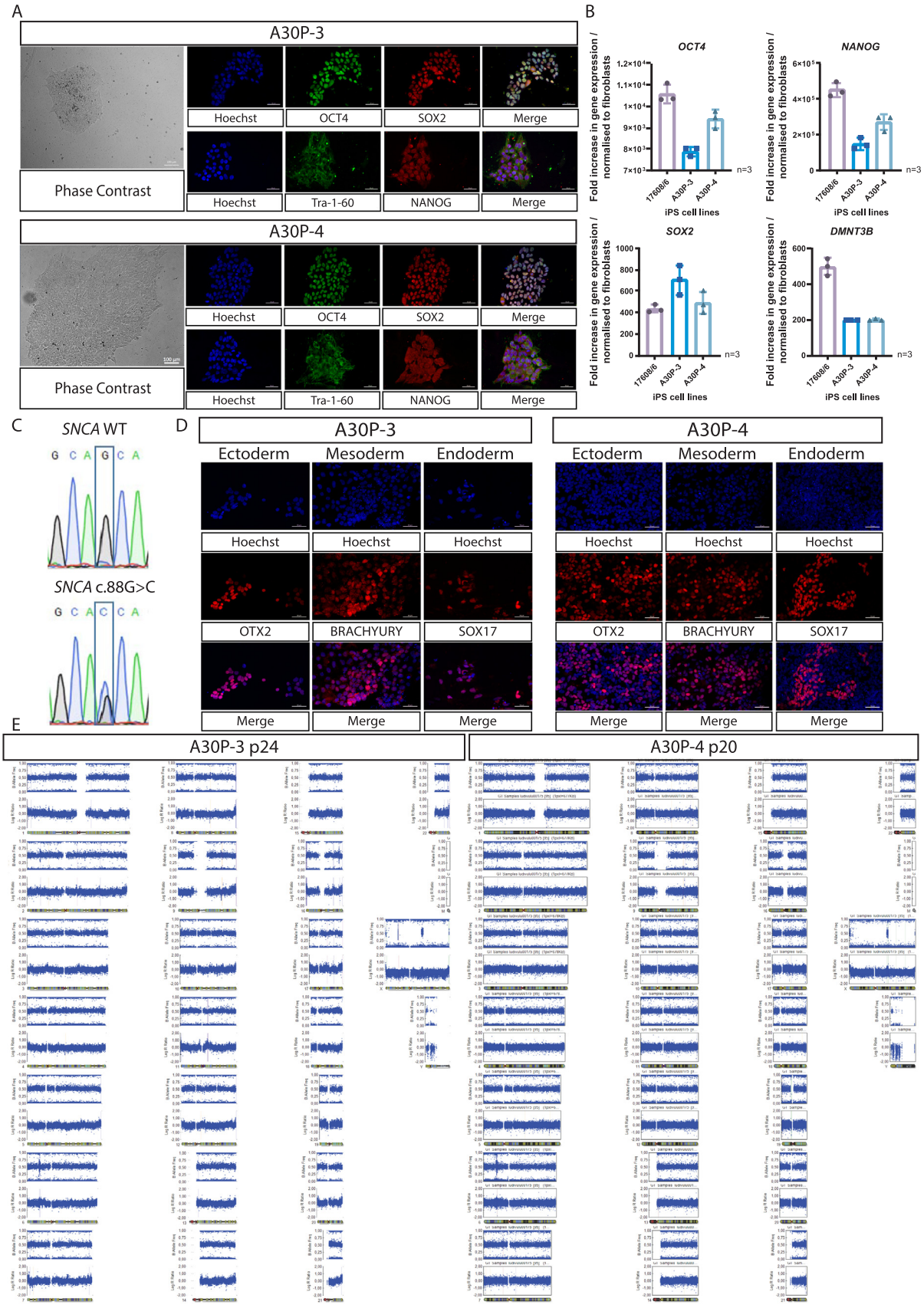

Fig. 1. Characterization of two iPS cell lines derived from a familial Parkinson's disease patient harbouring the p.A30P *SNCA* mutation in alpha synuclein.

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_628051
Pluripotency Marker	Rabbit anti-NANOG	1:500	Abcam, Cat #: ab21624; RRID: AB_446437
Pluripotency Marker	Goat anti-SOX2 (Y-17)	1:200	Santa Cruz, Cat #: sc-17320 ; RRID : AB_2286684
Pluripotency marker	Mouse anti-TRA-1-60	1:300	Abcam Cat #: ab 16288; RRID: AB_778563
Ectoderm	Goat anti-OTX2	1:1000	R&D Systems, Cat # 963273; RRID:AB_2157172
Endoderm	Goat anti-SOX17	1:1000	R&D Systems, Cat # 963121; RRID:AB_355060
Mesoderm	Goat anti-BRACHYURY	1:1000	R&D Systems, Cat # 963427; RRID:AB_2200235
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-Rabbit IgG (H + L)	1:1000	Invitrogen, Cat #: A11036; RRID: AB_143011
Secondary antibody	Alexa Fluor 647 Donkey anti-Goat IgG (H + L)	1:1000	Invitrogen, Cat #: A21447; RRID: AB_2535864
Primers			
	Target	**Forward/Reverse primer (5′-3′)**	
Sequencing	*SNCA*	CCCCGAAAGTTCTCATTCAA / TCCACCTTTTTGACAAGCAA	
Hs03023880_g1	*ACTB*	N/A	
Hs00999632_g1	*OCT4*	N/A	
Hs02387400_g1	*NANOG*	N/A	
Hs00171876_m1	*DMNT3B*	N/A	
Hs01053049_s1	*SOX2*	N/A	

Systems) according to manufacturer instructions. Cells were fixed, stained as described in the kit and imaged as above.

4.6. Chromosomal analysis

Molecular karyotyping and identity analysis was performed at Life& Brain GmbH using HumanOmni2.5 Exome-8 DNA Analysis BeadChip.

4.7. Mutational analysis

Genomic DNA was extracted and purified from the cell lines using the QIA Blood and Tissue Kit (Qiagen) according to manufacturer instructions. Exon 2 of SNCA was amplified by PCR with Sanger sequencing performed by Eurofins Genomics GmbH.

4.8. Mycoplasma methodology

For the exclusion of mycoplasma contamination, the PlasmoTest™ Mycoplasma Detection Kit was used. The assay is a cell-based colorimetric kit based upon the activation of Toll-Like Receptor 2 (TLR2). A positive control was provided with the kit.

4.9. STR analysis

Short Tandem Repeat (STR) analysis was performed by CLS GmbH with 16 loci analysed.

Declaration of Competing Interests

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.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scr.2020.101951.

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