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Lab resource: Stem Cell Line

Derivation of a human DOA iPSC line, IISHDOi006-A, with a mutation in the *ACO2* gene: c.1999G > A; p.Glu667Lys

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A B S T R A C T

Human iPSC line, IISHDOi006-A, was obtained from fibroblasts of a patient with Dominant Optic Atrophy (DOA) carrying a heterozygous mutation in the gene *ACO2:* c.1999G > A; p.Glu667Lys. Reprogramming factors Oct3/4, Sox2, Klf4, and c-Myc were delivered using a non-integrative methodology that involves the use of Sendai virus.

Resource utility

DOA is a genetically heterogeneous mitochondrial blinding disease (Kjer, 1959). We report here a human iPSC line obtained from a patient with DOA harbouring a heterozygous mutation in the *ACO2* gene. This iPSC line will be very useful for modelling this disorder and as a platform for pharmacological screening.

Resource details

The human iPSC line, IISHDOi006-A, has been reprogrammed using Sendai viruses containing the reprogramming factors OCT3/4, SOX2, KLF4 and c-MYC (Takahashi et al., 2007). For this purpose, fibroblasts from a patient with dominant optic atrophy harbouring a heterozygous mutation in the gene *ACO2* (c.1999G > A; p.Glu667Lys) have been obtained from a skin biopsy. IISHDOi006-A iPSC colonies displayed a typical ES-like colony morphology and growth behaviour (Fig. 1A) and they stained positive for alkaline phosphatase activity (Fig. 1B). In addition, the line was confirmed by PCR analysis to be mycoplasma-negative (Supplementary Fig. S1). The 300 bp band represents a mycoplasma-positive sample available in our laboratory (positive control, C+). As a negative control (C-) water instead DNA has been used as a sample for the PCR reaction. Immunofluorescence analysis revealed expression of transcription factors OCT4, NANOG and SOX2, and typical ES cells surface markers SSEA4, TRA-1-60 and TRA-1-81 (Fig. 1C). We also confirmed the clearance of the vectors and the exogenous reprogramming factor genes by RT-PCR after eight culture passages (Fig. 1D). As a positive control (C+), RNA obtained from fibroblasts set aside seven days after transduction with Sendai viruses has been used. As a negative control (C-) we have employed RNA extracted from the original fibroblasts. We have also verified the presence of the mutation in the iPSCs (Fig. 1E). The endogenous expression of the pluripotency associated transcription factors OCT4, SOX2, NANOG and CRIPTO was evaluated by quantitative real time polymerase chain reaction (qPCR) (Fig. 1F). The iPSC line has been adapted to feeder-free culture conditions and a karyotype analysis after more than 18 culture passages has been carried out (Fig. 1G). This analysis displayed a normal karyotype (46, XY). We also confirmed that the line IISHDOi006-A was derived from the patient's fibroblasts by DNA fingerprinting analysis (archived at SCR journal). Finally, we evaluated the capacity of the IISHDOi006-A iPSC line to differentiate into the three germ layers (endoderm, mesoderm and ectoderm) using an *in vitro* embryoid body based assay (Fig. 1H).

Materials and methods

Generation of iPSCs

Fibroblasts from a patient with DOA harbouring the mutation c.1999G > A; p.Glu667Lys in the *ACO2* gene were reprogrammed using

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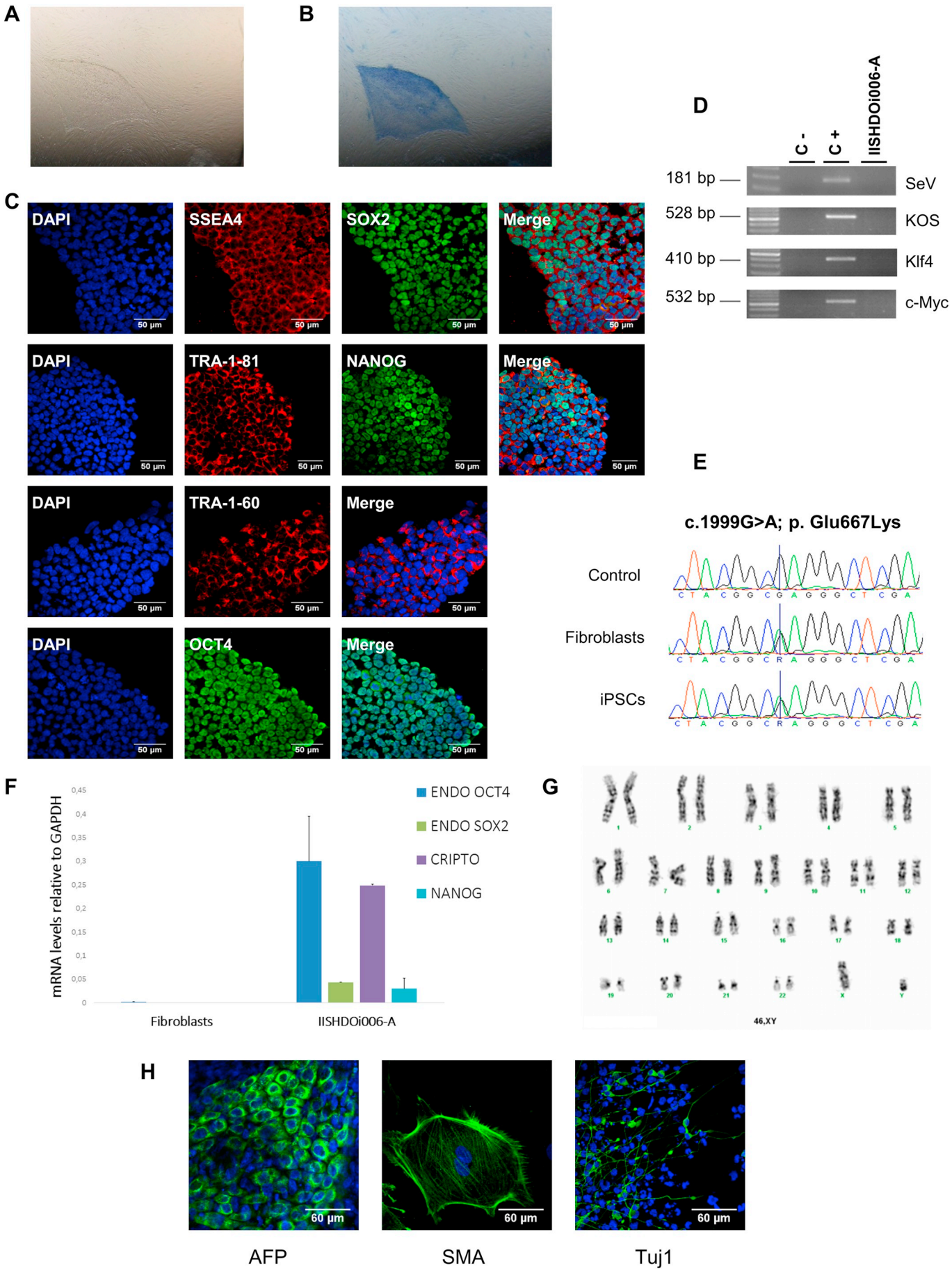
A
B
C
DAPI
SSEA4
SOX2
Merge
TRA-1-81
NANOG
TRA-1-60
OCT4
50 µm
D
C -
C +
IISHDOi006-A
181 bp
528 bp
410 bp
532 bp
SeV
KOS
Klf4
c-Myc
E
c.1999G>A; p. Glu667Lys
Control
Fibroblasts
iPSCs
F
mRNA levels relative to GAPDH
ENDO OCT4
ENDO SOX2
CRIPTO
NANOG
Fibroblasts
IISHDOi006-A
G
46,XY
H
60 µm
AFP
SMA
Tuj1

Fig. 1.

Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography	Normal	Fig. 1 panel A
Phenotype	Qualitative analysis: Alkaline phosphatase activity	Positive	Fig. 1 panel B
	Qualitative analysis: Immunocytochemistry	Positive for the pluripotency markers: SSEA4, SOX2, NANOG, TRA-1-81, TRA-1-60 and OCT4.	Fig. 1 panel C
	Quantitative analysis Gene expression (qPCR)	Positive for the pluripotency markers: *OCT4, SOX2, CRIPTO* and *NANOG*	Fig. 1 panel F
Genotype	Karyotype (G-banding) and resolution	46XY Resolution 450-500	Fig. 1 panel G
Identity	STR analysis	DNA Profiling performed 8 loci, all matched (D2S1338, D7S820, D8S1179, D13S317, D19S433, D21S11, VWA, amelogenin)	Submitted to SCR journal for archiving
Mutation analysis	Sequencing	Confirmation of the mutation: c.1999G > A; p.Glu667Lys	Fig. 1 panel E
Microbiology and virology	Mycoplasma	Mycoplasma testing by PCR: Negative	Supplementary Fig. S1
Differentiation potential	Embryoid body formation and directed differentiation	Positive for: smooth muscle actin (SMA), β-tubulin (Tuj1) and alpha-fetoprotein (AFP)	Fig. 1 panel H
Donor screening	HIV 1 + 2 Hepatitis B, Hepatitis C	N/A	N/A
Genotype additional info	Blood group genotyping	N/A	N/A
	HLA tissue typing	N/A	N/A

Table 2
Reagents details.

Antibodies used for immunocytochemistry/flow-citometry

	Antibody	Dilution	Company Cat # and RRID
Pluripotency Markers	Mouse anti TRA-1-81	1:150	Millipore Cat# MAB4381, RRID: AB_177638
	Mouse anti TRA-1-60	1:150	Millipore Cat# MAB4360, RRID: AB_11211864
	Rabbit anti SOX2	1:100	ThermoFisher Scientific Cat# PA1_16968, RRID: AB_2195781
	Mouse anti SSEA4	1:10	Abcam Cat# ab16287, RRID: AB_778073
	Goat anti NANOG	1:25	R&D Systems Cat# AF1997, RRID: AB_355097
	Mouse anti OCT4	1:100	Santa Cruz Biotechnology Cat# sc_5279, RRID: AB_628051
Differentiation Markers	Mouse anti AFP	1:300	Sigma Aldrich Cat# WH0000174M1, RRID: AB_1839587
	Mouse anti SMA	1:400	Sigma Aldrich Cat# A2547, RRID: AB_476701
	Mouse anti β-Tubulin isotype III	1:300	Sigma Aldrich Cat# T8660, RRID: AB_477590
Secondary antibodies	Cy™2-conjugated AffiniPure Goat anti-Rabbit igG (H + L)	1:50	Jackson Inmunoresearch Labs Cat# 111–225-144, RRID: AB_2338021
	Cy™3-conjugated AffiniPure Goat anti-Mouse IgG, Fcγ Subclass 3 Specific	1:250	Jackson Inmunoresearch Labs Cat# 115–165-209, RRID: AB_2338698
	Cy™2-conjugated AffiniPure Goat anti-Mouse IgG, Fcγ Subclass 2b Specific	1:50	Jackson Inmunoresearch Labs Cat# 115–225-207, RRID: AB_2338749
	Cy™2-conjugated AffiniPure Donkey anti-Goat IgG (H + L)	1:50	Jackson Inmunoresearch Labs Cat# 705–225-147, RRID: AB_2307341
	Goat anti Mouse IgG (H + L), Alexa Fluor 488	1:500	ThermoFisher Scientific Cat# A-11029, RRID: AB_2534088

Primers

	Target	Forward/Reverse primer (5′-3′)
Pluripotency Markers (qPCR)	Endo *OCT4*	GGGTTTTTGGGATTAAGTTCTTCA / GCCCCCACCCTTTGTGTT
	Endo *SOX2*	CAAAAATGGCCATGCAGGTT / AGTTGGGATCGAACAAAAGCTATT
	CRIPTO	CGGAACTGTGAGCACGATGT / GGGCAGCCAGGTGTCATG
	NANOG	ACAACTGGCCGAAGAATAGCA / GGTTCCCAGTCGGGTTCAC
House-Keeping Genes (qPCR)	*GAPDH*	GCACCGTCAAGGCTGAGAAC / AGGGATCTCGCTCCTGGAA
Targeted mutation analysis/sequencing	*ACO2*	CAGACAGGTGAGGACGGT / GGCAGGGAGAGTGACCTTG
Virus silencing	SeV	GGATCACTAGGTGATATCGAGC / ACCAGACAAGAGTTTAAGAGATATGTATC
	KOS	ATGCACCGCTACGACGTGAGCGC / ACCTTGACAATCCTGATGTGG
	Klf-4	TTCCTGCATGCCAGAGGAGCCC / AATGTATCGAAGGTGCTCAA
	c-Myc	TAACTGACTAGCAGGCTTGTCG / TCCACATACAGTCCTGGATGATGATG
STR analysis	Amelogenin	[6-FAM] CCCTGGGCTCTGTAAAGAATAGTG / ATCAGAGCTTAAACTGGGAAGCTG
	D2S1338	[6-FAM] CCAGTGGATTTGGAAACAGA / ACCTAGCATGGTACCTGCAG
	D7S820	[6-FAM] TGTCATAGTTTAGAACGAACTAACG / CTGAGGTATCAAAAACTCAGAGG
	D8S1179	[6-FAM] TTTTTGTATTTCATGTGTACATTCG / CGTAGCTATAATTAGTTCATTTTCA
	D13S317	[6-FAM] ACAGAAGTCTGGGATGTGGA / GCCCAAAAAGACAGACAGAA
	D19S433	[6-FAM] CCTGGGCAACAGAATAAGAT / TAGGTTTTTAAGGAACAGGTGG
	D21S11	[6-FAM] GTGAGTCAATTCCCCAAG / GTTGTATTAGTCAATGTTCTCC
	VWA	[6-FAM] CCCTAGTGGATGATAAGAATAATC / GGACAGATGATAAATACATAGGATGGATGG
Mycoplasma detection	*GPO-3 /MGSO*	GGGAGCAAACAGGATTAGATACCCT / TGCACCATCTGTCACTCTGTTAACCTC

the CytoTune-iPS 2.0 Sendai reprogramming kit following the instructions of the manufacturer. IISHDOi006-A line was maintained and expanded both on feeder and feeder-free conditions as described in Galera et al., 2016.

Phosphatase alkaline analysis

Direct phosphatase alkaline activity was analyzed on IISHDOi006-A iPSCs seeded on feeder plates using the phosphatase alkaline blue

membrane substrate solution kit (Sigma, AB0300) (Table 1).

Mutation analysis

To confirm the mutation, a PCR was carried out with total DNA, from fibroblasts and iPSCs, extracted using a commercial kit (primers shown in Table 2). The PCR conditions were as follows: 95 °C for 7 min, 35 cycles at 95 °C for 30 s, at 62 °C for 1 min, and at 72 °C for 20 s, and a final extension at 72 °C of 6 min (Applied Biosystems Verity Thermal Cycler). Amplicons were sequenced in an ABI 3730 analyzer (Applied Biosystems).

qPCR analysis

The expression of the endogenous pluripotency associated genes *OCT4*, *SOX2*, *CRIPTO* and *NANOG* (Table 1) was quantified by qPCR. Total mRNA was isolated using TRI Reagent® and 1 µg was used to synthesize cDNA using the Thermo Scientific RevertAid RT Kit. The qPCR was carried out with GoTaq® qPCR Master Mix (Promega), and analyzed using an Applied Biosystems™ 7500 Fast Real-Time PCR System (primers listed in Table 2). All the expression values were normalized to the *GAPDH* gene. Plots are representative of at least three independent experiments.

Karyotype analysis

Cells with more than twenty culture passages were treated with 10 µg/mL of Colcemid (Gibco) for 90 min at 37 °C. Subsequently, they were trypsinized, treated with hypotonic solution KCl 0.075 M and fixed with Carnoy's fixative. Metaphase cells were G banded using Wright staining. At least 20 metaphases were karyotyped.

Immunofluorescence analysis

Cells were fixed with 4% paraformaldehyde for 30 min at RT and permeabilized using TBS+ (0.1% Triton X-100 in Tris-buffered saline, TBS) for 45 min. Then, the cells were incubated in TBS++ (3% donkey serum, 0.3% Triton X-100 in TBS) for 2 h at RT. Primary antibodies were applied overnight at 4 °C and secondary antibodies for 2 h at RT (shown in Table 2). Nuclei were stained with DAPI (Sigma, 28718-90-3).

In vitro differentiation assay

The *in vitro* pluripotency capacity of the line IISHDOi006-A was tested by a spontaneous embryoid body differentiation. The protocol used has been described in detail by Galera et al., 2016.

DNA fingerprinting analysis

Markers D13S317, D7S820, VWA, D8S1179, D21S11, D19S433, D2S1338 and amelogenin for sex determination have been amplified by PCR and analyzed by ABI PRISM 3100 Genetic analyzer and Peak Scanner v3.5 (Applied Biosystems), (Table 2).

Mycoplasma detection

A PCR analysis using 1 mL of the cell culture supernatant (90% confluence) was carried out with the primers specified in Table 2. After initial denaturation at 95 °C for 2 min, 35 cycles of amplification at 95 °C for 30 s, at 55 °C for 30 s, and at 72 °C for 1 min, and a final extension at 72 °C of 3 min were performed in an Applied Biosystems Verity Thermal Cycler (Applied Biosystems).

Key resources table

Unique stem cell line identifier	IISHDOi006-A
Alternative name(s) of stem cell line	DOA2259-FiPS4F-8
Institution	Instituto de Investigación Sanitaria Hospital 12 de Octubre, i + 12
Contact information of distributor	Dr. M. Esther Gallardo egallardo.imas12@h12o.es
Type of cell line	iPSC
Origin	Human
Additional origin info	Age: N/A Sex: Male Ethnicity if known: N/A
Cell source	Skin Fibroblasts
Clonality	Clonal
Method of reprogramming	Transgene free (Sendai Virus)
Genetic modification	Yes
Type of modification	Hereditary
Associated disease	Dominant Optic Atrophy
Gene/locus	Gene *ACO2* NM_001098: c.1999G > A; p.Glu667Lys; Chromosome: 22q13.2
Method of modification	N/A
Name of transgene or resistance	N/A
Inducible/constitutive system	N/A
Date archived/stock date	July 2019
Cell line repository/bank	N/A
Ethical approval	Patient informed consent was obtained. This study was reviewed and approved by the Institutional Research Ethical Committee of the "Fundación Jiménez Díaz", 03/14; 404327 1.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scr.2019.101566.

Declaration of Competing Interest

There are no conflicts of interest in this study.

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STR analysis

Marker	Fibroblasts		IISHDOi006-A	
D2S1338	164	168	164	168
D7S820	205	209	205	209
D8S1179	180	184	180	184
D13S317	191	194	191	194
D19S433	195	206	195	206
D21S11	222	226	222	226
VWA	142	147	142	147
Amelogenin	X	Y	X	Y

Mycoplasma testing by PCR analysis