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Lab resource: Stem Cell Line

Generation of a human iPS cell line (CABi003-A) from a patient with age-related macular degeneration carrying the CFH Y402H polymorphism

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A B S T R A C T

Age-related macular degeneration (AMD) is the leading cause of adult blindness in developed countries and is characterized by progressive degeneration of the macula, the central region of the retina. A human induced pluripotent stem cell (hiPSC) line was derived from peripheral blood mononuclear cells (PBMCs) from a patient with a clinical diagnosis of dry AMD carrying the *CFH Y402H* polymorphism. Sendai virus was using for reprogramming and the pluripotent and differentiation capacity of the cells were assessed by immunocytochemistry and RT-PCR.

Resource table

Unique stem cell line identifier	CABi003-A
Alternative name(s) of stem cell line	OF_163/DH05-C16
Institution	Andalusian Molecular Biology and Regenerative Medicine Centre (CABIMER), Seville, Spain.
Contact information of distributor	Francisco Díaz-Corrales; francisco.diaz@cabimer.es
Type of cell line	Induced pluripotent stem cell
Origin	Human
Additional origin info	Age: 81 Sex: Female Ethnicity: Caucasian
Cell Source	Peripheral blood mononuclear cells
Clonality	Clonal
Method of reprogramming	Sendai viral reprogramming
Genetic modification	Yes
Type of modification	Hereditary
Associated disease	Age-related macular degeneration
Gene/locus	*CFH*/p.Y402H polymorphism (rs1061170)
Method of modification	N/A
Name of transgene or resistance	N/A
Inducible/constitutive system	N/A
Date archived/stock date	2018/11/30
Cell line repository/bank	https://hpscreg.eu/user/cellline/edit/CABi003-A
Ethical approval	Cellular Reprograming Board of Andalusia. Ethical Approval number: PR-01-2015

1. Resource utility

hiPSCs from AMD patients allow us the generation of a cellular model to better understand the pathophysiology of the disease and to test new therapeutic strategies.

2. Resource details

PBMCs were collected from 4 mL of peripheral blood sample from 81-year-old woman who was diagnosed with dry AMD carrying a single-nucleotide polymorphism (SNP) in the *complement factor H* gene (*CFH*) caused by a substitution of a thymine (T) for a cytosine (C) in the position 1204 of the exon 9 (c.1204 T > C p.Y402H), this is the most frequent SNP linked to the risk of developing AMD (Toomey et al., 2018). The human reprogramming factors *OCT3/4*, *c-MYC*, *KLF4,* and *SOX2* (Takahashi et al., 2007) were transduced into the PBMCs via the non-integrative Sendai virus according to manufacturer's instructions. After 21–27 days, we obtained the hiPSC line DH05-C16 (registered as

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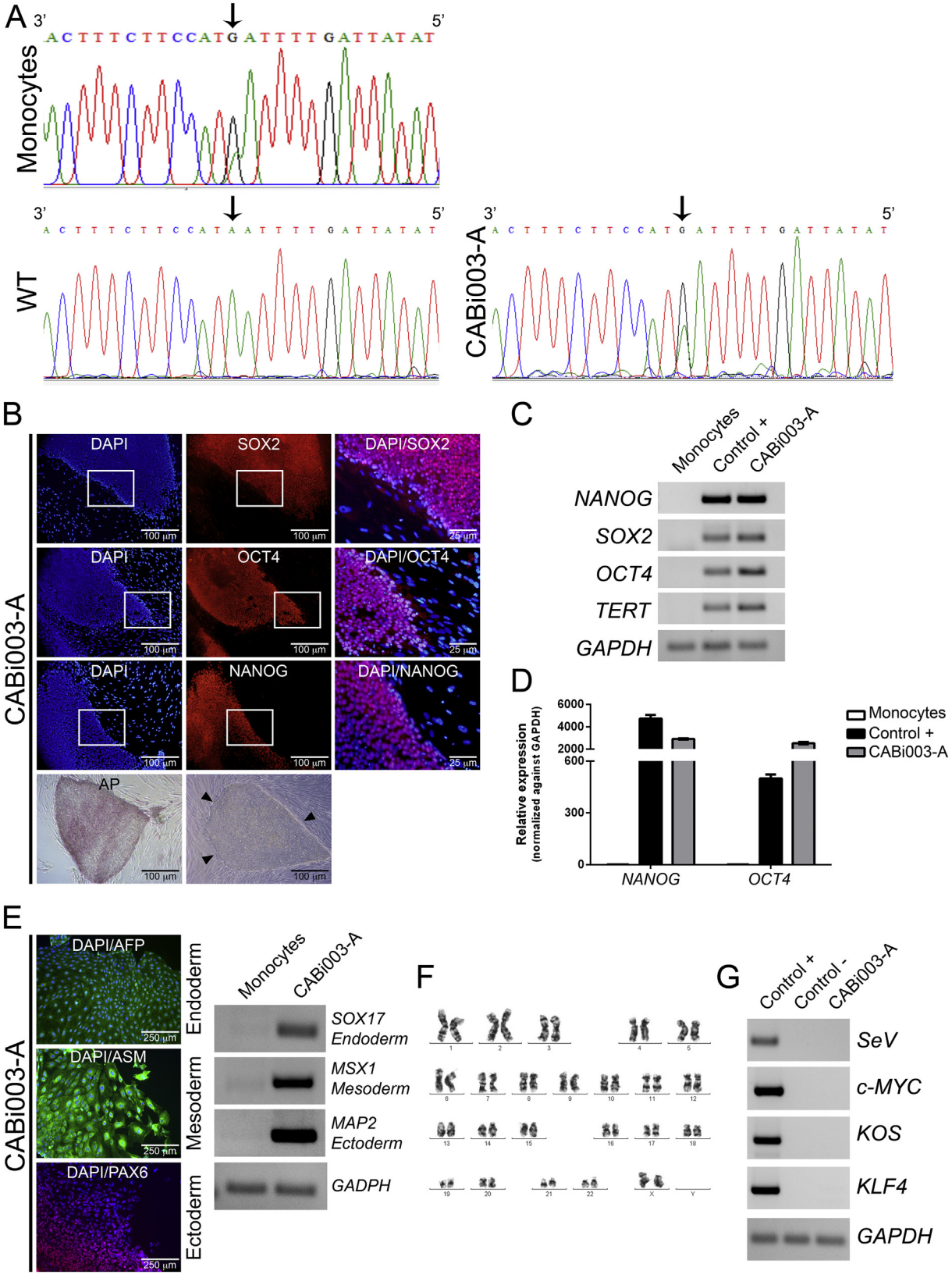

Fig. 1. iPSC characterization.

CABi003-A at www.hPSCreg.com). To genotype the patient's PBMCs (Monocytes) and the CABi003-A cell line, we performed DNA sequencing to confirm the presence of the *CFH* polymorphism (C;T) compared to a wild-type hiPSC line (T;T), the reverse and complement sequences are shown in Fig. 1A. At 27 days, hiPSC showed a typical stem-like growth and morphological features, including high nuclear/cytoplasmic ratio, refractive edges and polygonal shape (Fig. 1B; arrowheads). To evaluate the pluripotency state of CABi003-A cell line,

alkaline phosphatase (Fig. 1B; AP) test and immunostaining for pluripotency markers were performed (Fig. 1B). Qualitative assessment of a panel of pluripotency markers was analyzed by RT-PCR, RNA samples of the PBMCs (Monocytes) were used as negative control and RNA samples of a previously characterized wild-type hiPSC line was used as positive control (Fig. 1C). The expression level of endogenous pluripotency markers *NANOG* and *OCT4* was also quantified by qPCR relative to the PBMCs (Fig. 1D). Moreover, pluripotency was tested by the ability of CABi003-A to generate the three germ layers in vitro: endoderm, mesoderm and ectoderm, as confirmed by immunofluorescent staining of α-fetoprotein (AFP), actin-smooth muscle (ASM) and PAX-6, respectively (Fig. 1E, left) and RT-PCR (Fig. 1E, right). Our results on the karyotype analysis showed that the CABi003-A cell line exhibited a normal and diploid (46, XX) chromosomal content (Fig. 1F) and the genetic fingerprinting proved the genetic identity to parental mononuclear blood cells. After eight cell culture passages, the clearance of Sendai viral vector was checked by absence of gene expression corresponding to the viral capsid (SeV) and the ectopic reprogramming factors (*c-MYC, KOS, KLF4*) compared to iPSCs in passage 1, which was used as positive control for viral expression, RNA samples of the PBMCs (Monocytes) were used as negative control (Fig. 1G). Characterization is summarized in Table 1. Mycoplasma test was negative (Supplementary Table 1).

3. Materials and methods

3.1. PBMCs primary culture

Ficoll-Plaque was used to isolate PBMCs from 4 mL of peripheral blood using Vacutainer® CPT™ tubes (BD Biosciences) and cultured for one week in Expansion Medium (EM; QBSF-60 medium (Quality Biological); 50 µg/mL ascorbic acid (Sigma-Aldrich), 1% Pen/Strep (Gibco), 50 ng/mL SCF (StemCell Technologies), 10 ng/mL IL-3 (StemCell Technologies), 2 U/mL EPO (StemCell Technologies), 40 ng/mL IGF-1 (StemCell Technologies) and 1 µM Dexamethasone (Sigma-Aldrich).

3.2. Reprogramming PBMCs to iPSC

PBMCs were transduced with CytoTune®-iPS 2.0 Sendai Reprogramming Kit (Thermo Fisher Scientific). Briefly, 0.25×10^5 cells were transduced using MOI of 5–5-3 (hKOS, hc-MYC, hKLF4, respectively). After 24 h of incubation cells were collected, centrifuged and seeded in a 24 well plate containing EM. Two days later 0.1×10^5 cells were transferred onto a 6 well plated covered with 0.25×10^6 irradiated human foetal foreskin fibroblasts (ATCC CRL2429) in QBSF-60 medium (Quality Biological), supplemented with 50 µg/mL ascorbic acid (Sigma-Aldrich) and 1% Pen/Strep (Gibco). Seven days post-transduction, culture medium was replaced by iPS medium (KO DMEM (Gibco), 20% KO serum (Gibco), 1% GlutaMAX (Gibco), 1% MEM NEAA (Gibco), 0.23 mM β-mercaptoethanol (Gibco), 1% Pen/Strep (Gibco), 10 ng/mL bFGF (Peprotech)). Individual colonies with stem-like morphology, including CABi003-A (clone 16), were manually isolated and expanded 21 to 27 days post-transduction. hiPSCs were cultured on 6 well plate coated 0.25×10^6 irradiated human foetal foreskin fibroblasts (irHFF) and mantained in iPS medium with change of medium three-times per week. The hiPSCs were mechanically passage once a week with a 1:3 split ratio. All cell culture was performed at 37 °C in humidified atmosphere containing 5% CO_2 and 20% O_2.

3.3. SNP sequencing

Genomic DNA from hiPSCs was isolated using the QIAamp DNA Blood mini kit (Qiagen). Primers for exon 9 of *CFH* gene were used for amplification and directed sequencing was made with complement and reverse primer as described in Table 2.

3.4. Immunocytochemistry

Cells were allowed to grow in glass coverslip and washed in ice-cold PBS before fixation in 4% PFA, for 15 min. Fixed cells were washed twice in PBS and placed in blocking solution (2% donkey serum in 0.2% Triton-X 100/PBS) for 1 h at room temperature. Cells were incubated for 1 h at room temperature with the primary antibody in blocking solution. After incubation, samples were washed 3 times in 0.2% Triton-X100/PBS, and incubated with the secondary antibodies in blocking solution at room temperature for 1 h. After 3 washes, coverslips were mounted with Vestashield mounting medium (Vector H-1200) containing 4,6-diamidino-2-phenylindole (DAPI). Imaging was performed in a fluorescence microscope Leica DM6000 B. Antibodies are listed in Table 2.

3.5. RT-PCR and qPCR for detection of viral cleanance and pluripotency markers

Total RNA was isolated from cultured hiPSC cells with Rneasy Mini Kit (Qiagen) and treated with Dnase1 (Qiagen) to removed genomic DNA contamination. 1 µg of total RNA was used as template to obtain cDNA, using QuantiTect Reverse Transcription Kit (Qiagen). Viral clearance and pluripotency markers detection were analyzed using the primers described in Table 2. RT-PCR reaction was performed using MyTaq DNA Polymerase (Bioline GmbH) using Veriti™ 96-Well Thermal

Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography	Visual record of the line: Normal	Fig. 1 panel B
Phenotype	Qualitative analysis: immunocytochemistry' and RT-PCR	Positive for: Oct4, Nanog, SOX2. RT-PCR: NANOG, SOX2, TERT, OCT4	Fig. 1 panel B and C
	Quantitative analysis: qPCR	Positive for: Nanog and OCT4	Fig. 1 panel D
Genotype	Karyotype (G-banding) and resolution	46 XX, Band resolution: 400–550	Fig. 1 panel F
Identity	Microsatellite PCR (mPCR) OR	Not performed	N/A
	STR analysis	10 loci analyzed: all matched with patient's monocytes	Available with the authors.
Mutation analysis (If Applicable)	Sequencing	Heterozygous mutation *CFH* exon 9 c.1204 T > C	Fig. 1 panel A
	Southern Blot OR WGS	N/A	N/A
Microbiology and virology	Mycoplasma	Mycoplasma testing by luminescence: Negative	Supplementary Table 1
Differentiation potential	Embryoid body formation	Immunocytochemistry of AFP (endoderm), ASM (mesoderm) and PAX6 (ectoderm). RT-PCR of SOX17 (endoderm), MSX1 (mesoderm) and MAP2 (ectoderm).	Fig. 1 panel E
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/flow-citometry			
	Antibody	Dilution	Company Cat # and RRID
Pluripotency markers	Rabbit anti-OCT4	1:400	Cell Signaling Technology Cat# 2840, RRID:AB_2167691
	Rabbit anti-NANOG	1:400	Cell Signaling Technology Cat# 4903, RRID:AB_10559205
	Rabbit anti-SOX2	1:400	Cell Signaling Technology Cat# 3579, RRID:AB_2195767
Differentiation markers	Rabbit anti- PAX6	1:100	BioLegend, Cat# PRB-278P RRID:AB_2749901
	Mouse anti-ASM	1:300	Sigma-Aldrich Cat# A5228, RRID:AB_262054
	Mouse anti-AFP	1:20	Sigma-Aldrich Cat# A5228, RRID:AB_262054
Secondary antibodies	Donkey anti-Mouse 488	1:500	Molecular Probes Cat# A-21202, RRID:AB_141607
	Donkey anti-Rabbit 594	1:500	Molecular Probes Cat# A-21207, RRID:AB_141637

Primers		
	Target size (bp)	Forward/Reverse primer (5′-3′)
Episomal Plasmids (RT-PCR)	SeV plasmid/181 bp	GGATCACTAGGTGATATCGAGC/ACCAGACAAGAGTTTAAGAGATATGTATC
Episomal Plasmids (RT-PCR)	KOS plasmid/528 bp	ATGCACCGCTACGACGTGAGCGC/ACCTTGACAATCCTGATGTGG
Episomal Plasmids (RT-PCR)	KLF4 plasmid/410 bp	TTCCTGCATGCCAGAGGAGCCC/AATGTATCGAAGGTGCTCAA
Episomal Plasmids (RT-PCR)	c-MYC plasmid/532 bp	TAACTGACTAGCAGGCTTGTCG/TCCACATACAGTCCTGGATGATGATG
Pluripotency Markers (RT-PCR)	NANOG/260 bp	CCAAATTCTCCTGCCAGTGAC/CACGTGGTTTCCAAACAAGAAA
Pluripotency Markers (RT-PCR)	OCT4/165 bp	AAGCCCTCATTTCACCAGG/CTTGGAAGCTTAGCCAGGTC
Pluripotency Markers (RT-PCR)	SOX2/181 bp	TCACATGTCCCAGCACTACC/CCCATTTCCCTCGTTTTTCT
Pluripotency Markers (RT-PCR)	TERT/259 bp	GCGTTTGGTGGATGATTTCT/GGCATAGCTGGAGTAGTCGC
Differentiation Potential (RT-PCR)	MAP2/225 bp	GCACGCCTGCAGCTTGCATC/TCTCCACCACCCCGTACGCA
Differentiation Potential (RT-PCR)	MSX1/350 bp	CGAGAGGACCCCGTGGATGCAGAG/GGCGGCCATCTTCAGCTTCTCCAG
Differentiation Potential (RT-PCR)	SOX17/182 bp	CGCTTTCATGGTGTGGGCTAAGGACG/TAGTTGGGGTGGTCCTGCATGTGCTG
House-Keeping Genes (RT-PCR)	GAPDH/86 bp	TGCACCACCAACTGCTTAGC/GGCATGGACTGTGGTCATGAG
Genotyping	CFH exon 9/126 bp	GAAAATGTTATTTTCCTTATTTGGAAAATGG/GACACGGATGCATCTGGGA

Cycler 9902 (Applied Biosystems) with the program: 35 cycles of 94 °C for 15 s, 58 °C for 45 s and 72 °C for 45 s (for GADPH, 30 cycles). PCR products were analyzed on 2% agarose gels (Pronadisa). For qPCR a CFX96 Realtime PCR detection system (BioRad) was used with a program: 95 °C for 1 min followed by 40 cycles of 95 °C for 15 s and 60 °C for 1 min. Triplicate reactions were prepared with TaqMan Gene expression Master Mix and TaqMan probes: NANOG (Hs02387400g1), OCT4 (Hs01654807s1) and GADPH (Hs02786624g1), all for Applied Biosystems.

3.6. Three lineage differentiation

In vitro differentiation was performed by embryoid body (EB) formation to generated the three germ layers (endoderm, mesoderm and ectoderm). The hiPSCs were separated manually from feeder cells and cultured in non-adherent conditions in iPS medium without bFGF for the following 7 days. Then, the EBs were seeded on glass coverslips and plates treated with 0,1% gelatin for 2 h/RT and cultured between 7 and 9 days with EBs medium (DMEM/F12 (Thermo Scientific), 10% FBS (Gibco), 1% GlutaMAX (Gibco), 1% MEM NEAA (Gibco) and 1% Pen/Strep (Gibco)). The EBs were analyzed by immunofluorescence and RT-PCR.

3.7. Karyotype analysis

Metaphase arrest was performed as usually and 30 metaphase were counted and genome integrity of the hiPS cells was analyzed by G-banding at 400–550 band resolution in Biobanco de Sistema Sanitario Publico, Granada, Spain.

3.8. Fingerprinting

Genomic DNA from PBMC's and hiPS cells were extracted using QIAamp DNA Blood mini kit (Qiagen). STR analysis was performed by Biobanco de Sistema Sanitario Publico, Granada, Spain using the GenePrint 10 System (Promega) to check STRs for AMEL, CSF1PO, D13S317, D16S539, D21S11, D5S818, D7S820, TH01, TPOX and vWA.

3.9. Mycoplasma detection

The presence of mycoplasma was tested regularly by luminescence using the MycoAlert™ PLUS Mycoplasma Detection Kit (Lonza) (Supplementary Table 1).

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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.2019.101473.

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