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Lab resource: Stem Cell Line

Generation of a human iPSC line, FINCBi001-A, carrying a homoplasmic m.G3460A mutation in MT-ND1 associated with Leber's Hereditary optic Neuropathy (LHON)

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A B S T R A C T

Leber's Hereditary Optic Neuropathy (LHON) is a maternally inherited disorder caused by homoplasmic mutations of mitochondrial DNA (mtDNA). LHON is characterized by the selective degeneration of the retinal ganglion cells (RGC). Almost all LHON maternal lineages are homoplasmic mutant (100% mtDNA copies are mutant) for one of three frequent mtDNA mutations now found in over 90% of patients worldwide (m.11778G > A/MT-ND4, m.3460G > A/MT-ND1, m.14484 T > C/MT-ND6). Human induced pluripotent stem cells (hiPSCs) were generated from a patient carrying the homoplasmic m.3460G > A/MT-ND1 mutation using the Sendai virus non-integrating virus.

1. Resource table

Unique stem cell line identifier	FINCBi001-A
Alternative name(s) of stem cell line	F56L cl33
Institution	Fondazione IRCCS Istituto Neurologico C. Besta
Contact information of distributor	Valeria Tiranti, valeria.tiranti@istituto-besta.it
Type of cell line	iPSC
Origin	Human
Additional origin info	Age: 21 Sex: Female Ethnicity if known: Caucasian/Italian
Cell Source	Skin fibroblasts
Clonality	Clonal
Method of reprogramming	Sendai Virus
Genetic Modification	NO
Type of Modification	N/A
Associated disease	LHON
Gene/locus	m.3460G > A/MT-ND1
Method of modification	N/A
Name of transgene or resistance	N/A
Inducible/constitutive system	N/A
Date archived/stock date	13/05/2019
Cell line repository/bank	N/A
Ethical approval	Fondazione IRCCS Istituto Neurologico Carlo Besta, approval number 43-2013, date 26/6/2013

2. Resource utility

LHON disease is a mitochondrial disorder affecting RGC. The generation of iPSC from affected patients gives the unique opportunity to generate in vitro retinal neuronal cells, which are not accessible in vivo.

3. Resource details

LHON is characterized by the selective degeneration of retinal ganglion cells (RGCs), the terminal retinal neurons projecting to the brain their optic nerve-forming axons, which leads to permanent blindness (Carelli et al., 2017; Man, 2002). Despite the causative mutations are present in all tissues, yet only a specific cell type is affected.

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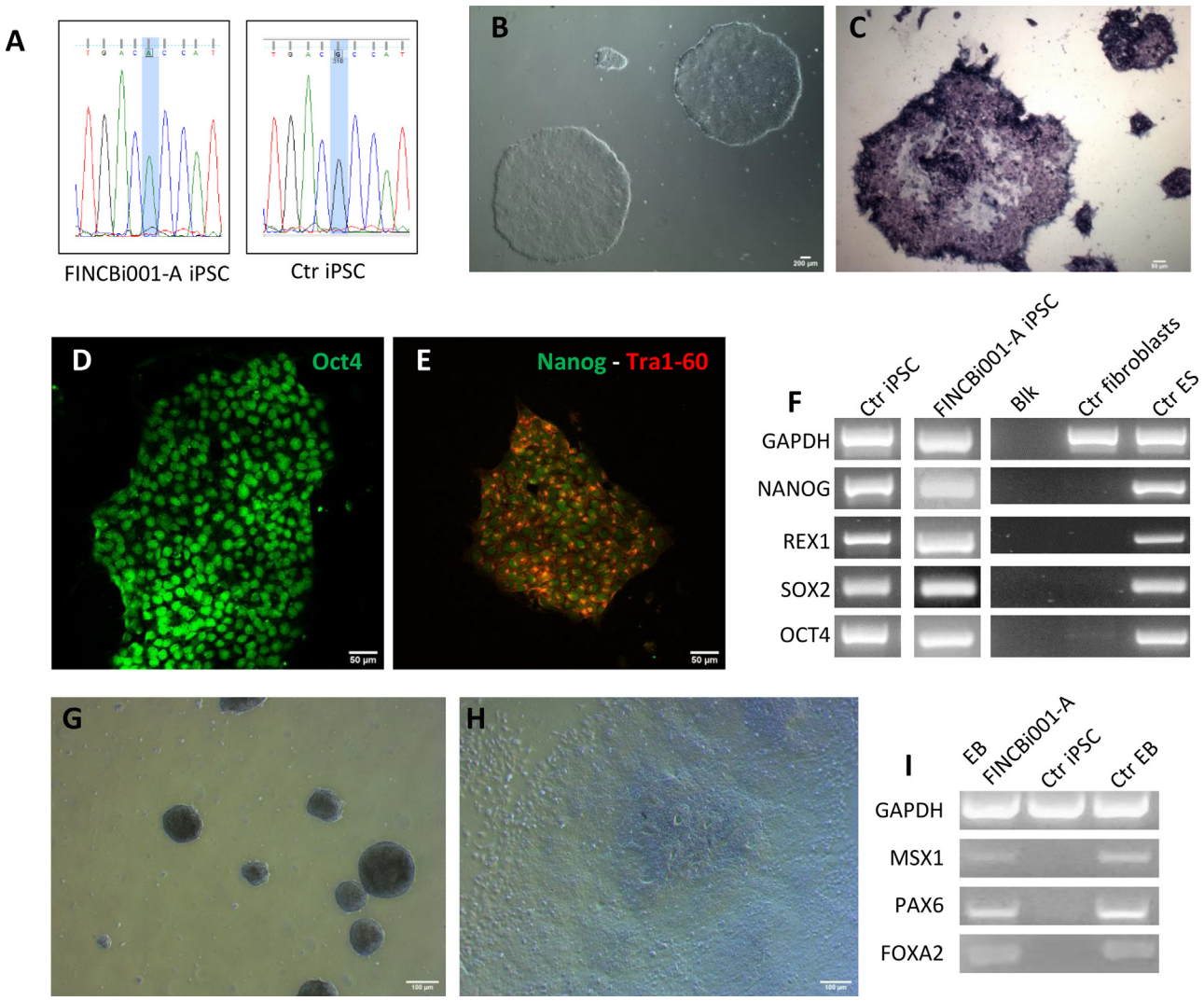

Fig. 1.

Our deep understanding of the pathogenic mechanism is thus hampered by the lack of appropriate models, still preventing the development of effective therapies.

Skin fibroblasts from a 21 year old female patient carrying the m.3460G > A in the mitochondrial gene MT-ND1 (Fig. 1A) were used to generate iPS cell colonies by the non-integrating Sendai Virus System-mediated (CytoTuneTM-iPS 2.0 Sendai Reprogramming Kit from Life Technologies) introduction of the four key factors of Yamanaka (*OCT3/4*, *SOX2*, *c-MYC* and *KLF4*) (Takahashi et al., 2007). Colonies were controlled for typical stem cell morphology (Fig. 1B) and a colorimetric assay designed to measure Alkaline Phosphatase activity was also performed (Fig. 1C). Clones' fully characterization was done investigating the expression of master regulators of pluripotent stem cells and associated markers, assessed by immunofluorescence for Oct4, Nanog and Tra1-60 (Fig. 1D, E) and RT-PCR for *NANOG*, *REX1*, *SOX2* and *OCT4* (Fig. 1F), and specific ability to generate Embryoid Bodies (EBs), composed by cells from all three germ layers (Fig. 1G, H). RT-PCR analysis showed the endogenous expression of the three germ layers markers MSX1, PAX6 and FOXA2 (Fig. 1I). Karyotype analysis was performed by CGH-array (Table 1). PCR-based detection tests confirmed the absence of Mycoplasma contamination at this stage. In addition, microsatellite PCR profiling confirmed that these iPSC lines had the same genetic identity with respect to the donor's fibroblasts (Data available with the authors).

4. Materials and methods

4.1. Reprogramming in iPSC

Skin fibroblasts were collected to generate iPS cell colonies using the CytoTuneTM-iPS 2.0 Sendai Reprogramming Kit (Life Technologies).

4.2. Cell culture

IPSC were growth on 1:100 Cultrex® Stem cell qualified Reduced Growth factor basement membrane matrix (Trevigen) and fed with the Essential 8™ Flex medium (Gibco). Colonies were passaged every 3 days using 0,5mM EDTA-Na2 solution, adding 10uM Y27632 (DBA) in the new medium. Then medium was changed without Y27632. In order to obtain embryoid bodies (EBs), when iPSC reached 70–80% confluence, little clumps of colonies were cultivated in suspension in Essential 8 medium plus 1X N2 Supplement (Life Technologies), 5 ng/ml Noggin Recombinant Protein (Tebu Bio), 10 μM SB431542 (Sigma) and Y27632 for 4–8 days. Medium without Y27632 was changed every other day. EBs were then plated on 1:100 Cultrex® Stem cell for 4–8 days to obtain rosette-like formations. Pellets of iPSC and EBs were used to perform RNA extraction.

Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography	Normal	Fig. 1B
Phenotype	Alkaline phosphatase Staining	Staining resulted positive	Fig. 1C
	Immunocytochemistry	Staining of pluripotency markers OCT4; Tra-1–60; Nanog	Fig. 1D, E
	RT-PCR	Expression of pluripotency markers OCT4, SOX2, REX1, NANOG	Fig. 1F
Genotype	CGH array	arr(1–22,X)x2, normal female	Supplementary file
Identity	Microsatellite PCR (mPCR)	6 loci analyzed, all matching	Data available with the Authors
Mutation analysis (IF APPLICABLE)	Sequencing	Homoplasmic mtDNA mutation	Fig. 1A
	Southern Blot OR WGS	N/A	
Microbiology and virology	Mycoplasma	Mycoplasma testing by MycoAlert was Negative	
Differentiation potential	Embryoid body formation and characterization by RT-PCR	Expression of three germ layers markers PAX6 (ectoderm), MSX1 (mesoderm), FOXA2 (endoderm)	Fig. 1G–I
Donor screening (OPTIONAL)	HIV 1 + 2 Hepatitis B, Hepatitis C	N/A	
Genotype additional info (OPTIONAL)	Blood group genotyping	N/A	
	HLA tissue typing	N/A	

Table 2
Reagents details.

Antibodies used for immunocytochemistry/flow-citometry			
	Antibody	Dilution	Company Cat # and RRID
Pluripotency Markers	Rabbit anti-NANOG	1:100	ReproCELL Incorporated Cat# RCAB004P-F, RRID:AB_1560380
	Mouse anti-TRA1-60	1:200	Abcam Cat# ab16288, RRID:AB_778563
	Rabbit anti-OCT4	1:200	Abcam Cat# ab19857, RRID:AB_445175
Secondary antibodies	Goat anti-Mouse IgG Alexa Fluor 546	1:1000	Thermo Fisher Scientific Cat# A-11030, RRID:AB_2534089
	Goat anti-Rabbit IgG Alexa Fluor 488	1:1000	Thermo Fisher Scientific Cat# A-11034, RRID:AB_2576217
Primers			
	Target	Forward/Reverse primer (5′–3′)	
Pluripotency Markers	NANOG	CATGGATCTGCTTATTCAGGAC/GGTTCAGGATGTTGGAGAGTT	
	REX1	CAGATCCTAAACAGCTCGCAGAAT/GCGTACGCAAATTAAAGTCCAGA	
	SOX2	GAATGCCTTCATGGTGTGGTC/GCTTAGCCTCGTCGATGAAC	
	OCT4	ATCCTCGGACCTGGCTAAGC/TCTCCAGCTTCACGGCACCA	
House-Keeping Genes	GAPDH	GTGTGAACCATGAGAAGTATGACAAC/CTTCACCACCTTCTTGATGTCATC	
Targeted mutation analysis	m.3460G > A	CGAAAGGACAAGAGAAATAA/GAGGTTGACCAGGGGGTTGGGTAT	
Differentiation Markers (ectoderm)	PAX6	ACCCATTATCCAGATGTGTTTGCCCGAG/ATGGTGAAGCTGGGCATAGGCGGCAG	
Differentiation Markers (mesoderm)	MSX1	CGAGAGGACCCCGTGGATGCAGAG/GGCGGCCATCTTCAGCTTCTCCAG	
Differentiation Markers (endoderm)	FOXA2	GGAGCGGTGAAGATGGAA/TACGTGTTCATGCCGTTCAT	
Short Tandem Repeats Markers	ApoB (Chr 2)	ATGGAAACGGAGAAATTATG/CCTTCTCACTTGGCAAATAC	
	D10S1214 (Chr 10)	ATTGCCCCAAAACTTTTTG/TTGAAGACCAGTCTGGGAAG	
	D11S533 (Chr 11)	GCCTAGTCCCTGGGTGTGGTC/GGGGGTCTGGGAACATGTCCCC	
	D17S1290 (Chr 17)	GCAACAGAGCAAGACTGTC/GGAAACAGTTAAATGGCCAA	
	D19S894 (Chr 19)	TTACTTGGCCCCAGGAAGC/GTTAAGCCATAAACATGGAATGACC	
	D21S2055 (Chr 21)	AACAGAACCAATAGGCTATCTATC/TACAGTAAATCACTTGGTAGGAGA	

4.3. Immunofluorescence

IPSC were fixed using cold EtOH for 15′ at −20 °C. Antigen retrieval was performed by incubating fixed colonies in 1 mM EDTA solution for 15′ at 65 °C. Cells were washed three times with PBS + 1% BSA and then permeabilized in PBS + 0,5% Triton for 10′ at room temperature (RT) and washed again. After 1 h of blocking solution (PBS + 10% NGS + 1% BSA) at RT, cells were incubated at 4 °C overnight with primary antibodies (Table 2) in PBS + 3% NGS + 1% BSA. Cells were then washed and incubated for 1 h at RT with secondary antibodies (Table 2), washed again and then observed with a Leica TCS SP8 confocal microscope.

4.4. Rt-PCR

RNA extraction was performed with the RNeasy Mini Kit (Quiagen) and retrotranscription with the GoTaq®2-Step RT-qPCR System (Promega). RT-PCR was conducted using primers in Table 2.

4.5. Alkaline phosphatase staining

IPSC were fixed with 2% PFA for 10′ at RT and washed tree times. Cells were then incubated with the Sigmafast™ BCIP®/NBT substrate (Sigma) for 20′ at RT and visualized with a phase-contrast microscope.

4.6. CGH

Deletion and duplication of nuclear DNA were verified by array Comparative Genomic Hybridization (CGH) DNA was extracted from cultured iPS cells (about 1 × 106 cells) using DNA extraction kit (Gentra kit, Qiagen, Hilden, Ge). Array CGH analyses were performed using Cytosure oligo ISCA60K platform: array design was performed by Oxford Gene Technology (OGT, Begbroke, Oxfordshire, UK) and manufactured by Agilent Technologies (Santa Clara, CA, USA). The DNA test was hybridized with sex-matched DNA from pooled controls (reference DNA, Promega, Madison, Wisconsin, USA), according to the manufacturer's protocol. Data were analyzed using Cytosure Interpret software (OGT). Clinical interpretation of array CGH results are based on published literature and public databases (ENSEMBL, USBC,

Database for Genetic Variants, DECIPHER, the Italian database of Troina) following Cytogenetic European and International Guidelines (Hastings et al., 2012; Mascarello et al., 2011). Genomic coordinates are based on the February 2009 Human Genome Build (GRCh37/hg19).

4.7. Sequencing

Sanger sequencing was performed using a 3130 Abi Prism sequencer.

4.8. Mycoplasma

MycoAlert mycoplasma detection Kit (Lonza) was used.

Declaration of Competing Interest

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.

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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.2020.101939.

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