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Lab Resource: Multiple Cell Lines

Generation of two human iPSC lines, FINCBi002-A and FINCBi003-A, carrying heteroplasmic macrodeletion of mitochondrial DNA causing Pearson's syndrome

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A B S T R A C T

Pearson marrow pancreas syndrome (PMPS) is a sporadic mitochondrial disease, resulting from the clonal expansion of a mutated mitochondrial DNA (mtDNA) molecule bearing a macro-deletion, and therefore missing essential genetic information. PMPS is characterized by the presence of deleted (Δ) mtDNA that co-exist with the presence of a variable amount of wild-type mtDNA, a condition termed heteroplasmy. All tissues of the affected individual, including the haemopoietic system and the post-mitotic, highly specialized tissues (brain, skeletal muscle, and heart) contain the large-scale mtDNA deletion in variable amount. We generated human induced pluripotent stem cells (hiPSCs) from two PMPS patients, carrying different type of large-scale deletion.

Resource table

Unique stem cell lines identifier	FINCBi002-A FINCBi003-A
Alternative names of stem cell lines	FINCBi002-A: mt8742 (fibroblasts); mt8742 #118 (iPSC) FINCBi003-A: mt8792 (fibroblasts); mt8792 #118 (iPSC)
Institution	Fondazione IRCCS Istituto Neurologico C. Besta
Contact information of distributor	Valeria Tiranti, valeria.tiranti@istituto-besta.it
Type of cell lines	iPSC
Origin	Human
Cell Source	Skin fibroblasts
Clonality	Clonal
Method of reprogramming	Sendai virus
Multiline rationale	Same disease but patients carried a different macro-deletion of mtDNA
Gene modification	NO
Type of modification	Spontaneous mutation
Associated disease	Pearson's syndrome (PMPS)
Gene/locus	Mitochondrial DNA macro-deletion
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	Fondazione IRCCS Istituto Neurologico Carlo Besta, approval number n. 60, date 06/03/2019

1. Resource utility

PMPS is a multi-systemic mitochondrial disorder (Rotig et al., 1990; Broomfield et al., 2015) for which no therapy is available. The generation of iPSC from affected patients gives the unique opportunity to generate *in vitro* differentiated cells and organoids (Lancaster and Knoblich, 2014), which are not accessible *in vivo*, and to try therapeutic interventions aimed at reducing the burden of mutated ΔmtDNA.

2. Resource details

Skin fibroblasts from two male patients, 4 months (mt8742) and 8 years (mt8792) old respectively (Table 1), carrying mtDNA macro-deletion of different extension (Fig. 1 A and B) and heteroplasmic level (Fig. 1C) were used to generate iPS cell colonies by the non-

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Table 1
Summary of lines.

iPSC line names	Abbreviation in figures	Gender	Age	Ethnicity	Genotype of locus	Disease
FINCBi002-A	Mt8742	Male	5 month	Caucasian	m.9449_14550 del	Pearson Syndrome
FINCBi003-A	Mt8792	Male	8 years	Caucasian	m.8469_13460 del	Pearson Syndrome

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). We verified iPSC colonies for loss of Sendai virus expression (Fig. 1D), typical stem cell morphology (Fig. 1 E and F) and we performed colorimetric assay designed to measure Alkaline Phosphatase activity (Supplementary A and B). Clones' fully characterization was done investigating the expression of master regulators of pluripotent stem cells and associated markers, assessed by immunofluorescence for NANOG, TRA1-60 and OCT4 (Fig. 1G–J) and RT-PCR for NANOG, REX1, SOX2 and OCT4 (Fig. 1K), and specific ability to generate Embryoid Bodies (EBs) (Supplementary C and D), composed by cells from all three germ layers. RT-PCR analysis showed the endogenous expression of the three germ layers markers MSX1, BRACHYURY, PAX6, SOX1, FOXA2 and SOX17 (Fig. 1L). We analyzed karyotype by CGH-array (Table 2 and Supplementary E, and F). 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).

3. Materials and methods

3.1. Reprogramming in iPSC

We generated iPS cells from skin fibroblasts using the CytoTuneTM-iPS 2.0 Sendai Reprogramming Kit (Life Technologies).

3.2. 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). We passaged colonies every 3 days. To obtain embryoid bodies (EBs), 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. We changed medium without Y27632 every other day. We plated EBs on 1:100 Cultrex® Stem cell for 4–8 days to obtain rosette-like formations. We performed RNA extraction from iPSC and EBs pellets.

3.3. Immunofluorescence

We fixed IPSC using cold EtOH for 15′ at −20 °C and performed antigen retrieval by incubating fixed colonies in 1 mM EDTA solution for 15′ at 65 °C. Cells were permeabilized in 0.5% Triton for 10′ at room temperature (RT) and then washed. After 1 h of blocking solution (10% NGS + 1% BSA) at RT, cells were incubated at 4 °C overnight with primary antibodies (Table 3) in 3% NGS + 1% BSA, followed by incubation with secondary antibody (Table 3) and observation with a Leica TCS SP8 confocal microscope.

3.4. Rt-PCR

We performed RNA extraction with the RNeasy Mini Kit (Quiagen) followed by retrotranscription with the GoTaq®2-Step RT-qPCR System (Promega) using primers in Table 3.

3.5. Alkaline phosphatase Staining

We fixed IPSC 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.

3.6. CGH

We performed array Comparative Genomic Hybridization (CGH) on DNA extracted from cultured iPS cells (about $1x10^6$ 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). 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).

3.7. qPCR

We evaluated mtDNA deletion heteroplasmy by real-time PCR-based quantification (ABI7000 Real-Time PCR System) using specific mtDNA probes (amplicons nt 888–927 (12S), 12857–12872 (deletion)) and a standard, single-copy autosomal gene (RNaseP).

3.8. Sequencing

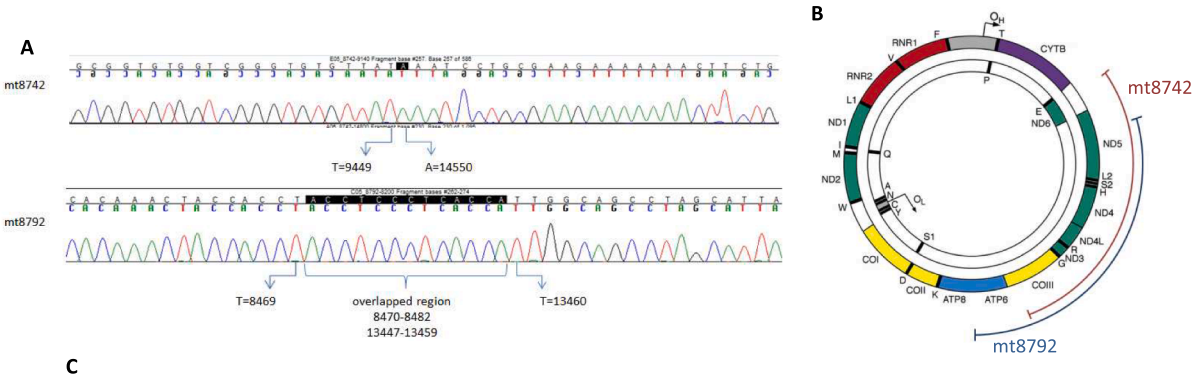
We diluted PCR products to a final concentration of 0.2 ng/μl and processed according to Nextera XT DNA Library Prep protocol (Illumina). Indexed DNA libraries were pooled together with equal molar ratios and sequenced on MiSeq platform with a v3 Illumina Flowcell (600 cycles) and a paired-end read chemistry.

3.9. Mycoplasma

We used MycoAlert Mycoplasma Detection Kit (Lonza).

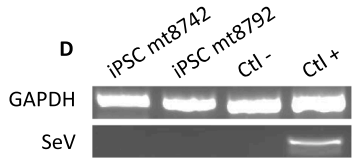
Declaration of Competing Interest

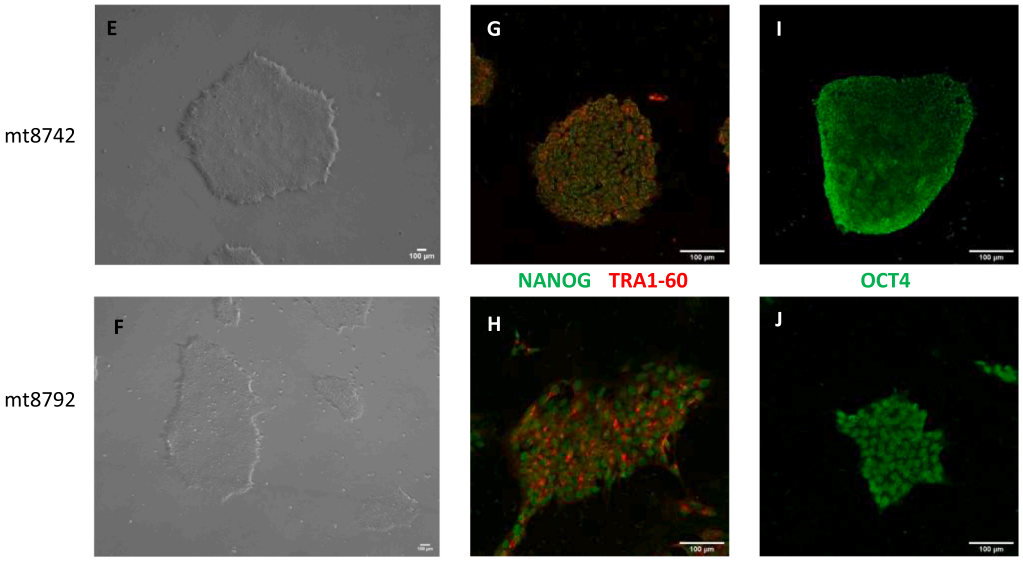
The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

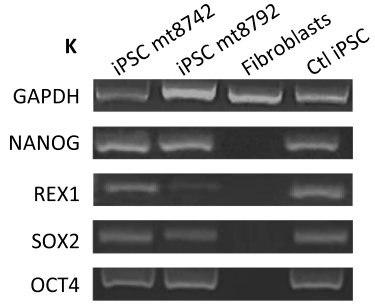


C

qPCR (% mtDNA deleted)	Fibroblasts	iPSC
mt8742	50%	80%
mt8792	10%	30%







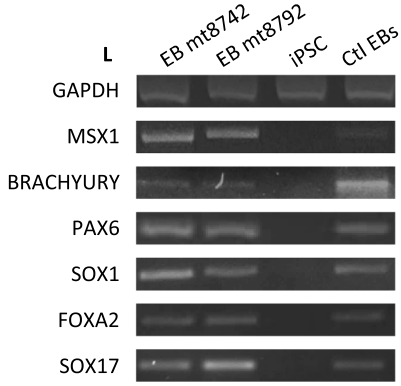


Fig. 1. A-B) Sanger sequences and schematic representation of mtDNA deletions of mt8742 and mt8792 lines. C) heteroplasmic level of mtDNA deletions in fibroblasts and iPSC. D) Sendai virus expression by RT-PCR. E-F) typical morphology of iPSC colonies in brightfield. G to K) expression of master regulators of pluripotent stem cells and associated markers, assessed by immunofluorescence for NANOG, TRA1-60 (G-H) and OCT4 (I-J); and RT-PCR for NANOG, REX1, SOX2 and OCT4 (K). L) endogenous expression of the three germ layers markers MSX1, BRACHYURY, PAX6, SOX1, FOXA2 and SOX17 by RT-PCR.

Table 2
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography	Normal	Fig. 1E and F
Phenotype	Alkaline Phosphatase Staining	STAINING resulted positive	Supplementary A and B
	Immunocytochemistry	Staining of pluripotency markers NANOG; TRA1-60 and OCT4	Fig. 1G–J
	RT-PCR	Loss of Sendai virus expression (SeV); expression of pluripotency markers NANOG; REX1; SOX2; OCT4	Fig. 1D and K
Genotype	CGH array	arr(1–22)x2, (X,Y)x1, normal male	Supplementary file E and F
Identity	Microsatellite PCR (mPCR)	6 loci analyzed, all matching	Data available with Author
Mutation analysis (IF APPLICABLE)	Sequencing	Heteroplasmic mtDNA deletion	Fig. 1A–C
	Southern Blot OR WGS	N/A	
Microbiology and virology	Mycoplasma	Mycoplasma testing by MycoAlert was Negative	
Differentiation potential	e.g. Embryoid body formation	Expression of three germlayers markers MSX1 and BRACHYURY (mesoderm), PAX6 and SOX1 (ectoderm), FOXA2 and SOX17 (endoderm)	Fig. 1L and Supplementary C and D
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 3
Reagents details.

Antibodies used for immunocytochemistry/flow-cytometry

	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′)
Virus loss expression	Sendai Virus (SeV)	GGATCACTAGGTGATATCGAGC/ACCAGACAAGAGTTTAAGAGATATGTATC
Pluripotency Markers	NANOG	CATGGATCTGCTTATTCAGGAC/GGTTCAGGATGTTGGAGAGTT
	REX1	CAGATCCTAAACAGCTCGCAGAAT/GCGTACGCAAATTAAAGTCCAGA
	SOX2	GAATGCCTTCATGGTGTGGTC/GCTTAGCCTCGTCGATGAAC
	OCT4	ATCCTCGGACCTGGCTAAGC/TCTCCAGCTTCACGGCACCA
House-Keeping Genes	GAPDH	GTGTGAACCATGAGAAGTATGACAAC/CTTCACCACCTTCTTGATGTCATC
Targeted mutation analysis	Deletion primers (qPCR)	GCAGCCATTCAAGCAATCCTA/AGGCGAGGATGAAACCGATAT
	Deletion probe (qPCR)	ACAACCGTATCGGCG
	12S primers (qPCR)	CCCCAGGGTTGGTCAATTT/CTATTGACTTGGGTTAATCGTGTG
	12S probe (qPCR)	TGCCAGCCACCGC
	Whole mtDNA sequence	CCGCACAAGAGTGCTACTCTCCTC/GATATTGATTTCACGGAGGATGGTG
Differentiation Markers (mesoderm)	MSX1	CGAGAGGACCCCGTGGATGCAGAG/GGCGGCCATCTTCAGCTTCTCCAG
	BRACHYURY	GCCCTCTCCCTCCCCTCCACGCACAG/CGGCGCCGTTGCTCACAGACCACAGG
Differentiation Markers (ectoderm)	PAX6	ACCCATTATCCAGATGTGTTTGCCCGAG/ATGGTGAAGCTGGGCATAGGCGGCAG
	SOX1	AGATGCACAACTCGGAGATC/GCCAGCGAGTACTTGTCCTT
Differentiation Markers (endoderm)	FOXA2	GGAGCGGTGAAGATGGAA/TACGTGTTCATGCCGTTCAT
	SOX17	CTCTGCCTCCTCCACGAA/CAGAATCCAGACCTGCACAA
Short Tandem Repeats Markers	ApoB (Chr 2)	ATGGAAACGGAGAAATTATG/CCTTCTCACTTGGCAAATAC
	D10S1214 (Chr 10)	ATTGCCCCAAAACTTTTTTG/TTGAAGACCAGTCTGGGAAG
	D11S533 (Chr 11)	GCCTAGTCCCTGGGTGTGGTC/GGGGGTCTGGGAACATGTCCCC
	D17S1290 (Chr 17)	GCAACAGAGCAAGACTGTC/GGAAACAGTTAAATGGCCAA
	D19S894 (Chr 19)	TTACTTGGCCCCAGGAAGC/GTTAAGCCATAAACATGGAATGACC
	D21S2055 (Chr 21)	AACAGAACCAATAGGCTATCTATC/TACAGTAAATCACTTGGTAGGAGA

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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.2020.102151.

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