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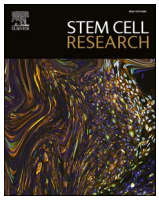

Lab Resource: Multiple Cell Lines

Derivation of two human induced pluripotent stem cell lines carrying a missense mutation in FHL1 (c.377G > A, p.C126Y) linked to familial muscular dystrophy

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A B S T R A C T

FHL1 gene locates in the Xq26 region and encodes for four and half LIM domain protein 1. It plays a crucial role in muscle cells and mutations in *FHL1* are related to muscular dystrophy (MD). Peripheral blood mononuclear cells (PBMCs) were obtained from 2 family patients with MD that carry a pathogenic missense mutation in *FHL1* (c.377G > A, p.C126Y). Induced pluripotent stem cells (iPSCs) were generated by PBMCs reprogramming using the lentiviral-hSTEMCCA-loxP vector, obtaining FHL1-T and FHL1-V iPSCs lines from patients. FHL1 genotype was maintained, and stemness and pluripotency were confirmed in both iPSCs lines.

Unique stem cell lines identifier	INEUi003-A INEUi004-A
Alternative name(s) of stem cell lines	FHL1-T (INEUi003-A) FHL1-V (INEUi004-A)
Institution	Instituto de Neurociencias, Fundación para la Lucha contra las Enfermedades Neurológicas de la Infancia (FLENI).
Contact information of distributor	Dr. Santiago Miriuka. smiriuka@fleni.org.ar / Dra. Lucia N. Moro lmoro@fleni.org.ar
Type of cell lines	iPSC
Origin	Human
Additional origin info required	FHL1-T Age: 17 Sex: Male Ethnicity: white latin FHL1-V Age: 46 Sex:Female Ethnicity: white latin
Cell Source	Peripheral blood mononuclear cells (PBMCs). Total PBMCs.
Clonality	Clonal.
Method of reprogramming	Lentiviral EF1a-hSTEMCCA-loxP vector expressing OCT-4, SOX-2, c-MYC and KLF- 4.

(*continued on next column*)

(*continued*)

Unique stem cell lines identifier	INEUi003-A INEUi004-A
Genetic Modification	YES
Type of Genetic Modification	Hereditary
Evidence of the reprogramming transgene loss (including genomic copy if applicable)	Final Point PCR.
Associated disease	Muscular Dystrophy
Gene/locus	Chromosome X - NC_000023.11 Reference GRCh38.p14 Primary Assembly (c.377G > A, p.C126Y).
Date archived/stock date	23/06/2023
Cell line repository/bank	INEUi003-A: https://hpscreg.eu/user/cellline/edit/INEUi003-A INEUi004-A: https://hpscreg.eu/user/cellline/edit/INEUi004-A
Ethical approval	The study was approved by a local Ethics Committee (Comité de ética en investigaciones biomédicas del Instituto FLENI) (code number: Protocol 018/19). Written informed consents were obtained from the patients.

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1. Resource utility

Generating iPSCs with a specific *FHL1* gene variant from patients with muscular dystrophy serves as a valuable tool for disease modeling and therapy development. FHL1 function in muscle cells is not well understood yet and muscular differentiation of these iPSCs will provide relevant knowledge in both healthy and diseased muscle. Table 1.

2. Resource Details

The generation of pluripotent stem cells derived from somatic cells, called induced pluripotent stem cells (iPSCs), has made it possible to obtain patient-specific stem cells to model diseases and develop personal therapies.

The *FHL1* gene is located in the Xq26 region, encodes four and a half LIM domain protein 1 and is expressed in skeletal and cardiac muscle, specifically on the myofibrils of the sarcomere and sarcolemma. It has been related to cytoskeletal remodeling, myoblasts differentiation, sarcomere assembly and autophagy regulation (McGrath et al., 2006). Mutations in *FHL1* are related to muscular dystrophy (MD) with a limited life expectancy (Malfatti et al., 2013). The aim of this work was to generate and characterize two induced pluripotent stem cells (iPSCs) lines derived from 2 family patients with MD, mother and son, that carry a pathogenic missense mutation in *FHL1* (c.377G > A, p.C126Y) for *in vitro* disease modeling and personalized therapy development. Since *FHL1* gene locus is on the X chromosome, the mother is heterozygous for the mutation and the son is hemizygous. To generate the iPSCs lines, a blood sample was taken from both patients and peripheral blood mononuclear cells (PBMCs) were isolated and amplified. Cell reprogramming was achieved using the EF1a-hSTEMCCA-loxP lentiviral vector that expresses OCT-4, SOX-2, c-MYC and KLF4 pluripotency genes, as previously described (Somers et al., 2010). After clonal isolation, FHL1-T (XY) and FHL1-V (XX) iPSCs lines were established. *FHL1* c.377G > A mutation was confirmed by Sanger sequencing (Fig. 1A) and short tandem repeat (STR) analysis demonstrated that both iPSCs lines matched those of the donor PBMCs. Silencing of the EF1a-hSTEMCCA-loxP lentiviral transgenes was confirmed by end point PCR using specific primers (Fig. 1D). Transduced human fibroblasts (HF) harvested on day 6 of the reprogramming protocol were used as positive controls. Both FHL1-T and FHL1-V iPSCs lines showed typical iPSCs morphological characteristics (formation of compact multicellular colonies with a high nucleus ratio and distinct colony borders), high alkaline phosphatase activity (Fig. 1C) and normal karyotype [(46XY for FHL1-T and 46XX for FHL1-V (Fig. 1B)]. Moreover, pluripotency was confirmed by RT-qPCR analysis and immunofluorescence staining of OCT-4, SOX2 and NANOG (Fig. 1E), using iPSCs INEUi002-A line as positive control (Questa et al., 2016). Finally, *in vitro* spontaneous differentiation through embryoid bodies-based method proved the pluripotent potential of FHL1-T and FHL1-V iPSCs lines to differentiate into cells derived from the three germ layers as shown by positive expression of Alpha-fetoprotein (AFP, endoderm), and NKX2.5 (mesoderm) and NESTIN (ectoderm) (Fig. 1F). Table 2.

Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography Bright field	Normal	Fig. 1
Phenotype	Qualitative analysis	Expression of pluripotency markers: OCT3/4, NANOG, SOX2.	Fig. 1E
Genotype	Karyotype (G-banding) and resolution	FHL1-T: 46, XY FHL1-V: 46, XXResolution 450–500	Fig. 1B
Identity	Microsatellite PCR (mPCR) OR STR analysis	N/A 27 sites tested, matched	N/A
Mutation analysis (IF APPLICABLE)	Sequencing	Amplified fragment by PCR and Sanger sequencing. FHL1-T: hemizygous FHL1-V: heterozygous	Fig. 1A
	Southern Blot OR WGS	N/A	N/A
Microbiology and virology	Mycoplasma	Mycoplasma testing by PCR: Negative	Supplementary figure
Differentiation potential	Embryoid body formation	Expression of differentiation markers by immunohistochemistry. Endoderm: α-fetoprotein (AFP). Mesoderm: NKX2.5. Ectoderm: NESTIN.	Fig. 1F
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

3. Materials and methods

3.1. Reprogramming and cell culture

PBMCs were isolated from blood using a Ficoll density gradient procedure (HISTOPAQUE®SIGMA). A total of $2x10^6$ cells were cultured in 2 mL expansion media: QBSF-60 Serum-Free Medium (Quality Biological, Cat#: 160–204-101), 100 μg/mL (Gibco), 50 μg/mL ascorbic acid, 50 ng/mL SCF, 10 ng/mL IL-3, 2U/mL EPO, 40 ng/mL IGF-1 and 1 μM Dexamethasone, in 1 well of a 12-well dish at 37 °C, 5 % CO_2. The medium was replaced every 2 days. On day 9, EF1a-hSTEMCCA-loxP lentiviral reprogramming vector, encoding *OCT-4*, *KLF4*, *SOX-2* and *c-MYC* (Somers et al., 2010) was used to transduce cells at MOI = 1.

3.2. iPSCs Cell Culture

iPSCs were maintained on Geltrex (Gibco) coated wells with E8 Flex medium (Gibco). Every three days, cells were passed using PBS 1X (Gibco), Versene (Gibco) and media supplemented with 10uM Y27632 Rock inhibitor (Tocris). Cells were cultured in a 37 °C, 5 % CO2 and 90 % humidity incubator.

3.3. Genotyping, sequencing and STR analysis

Genomic DNA from both iPSCs lines (passage 10) was isolated for PCR amplification of the mutation site. Then, PCR products were purified and Sanger sequenced in Macrogen. STR analysis for 27 locations was performed at the Laboratorio de Huellas Digitales Genéticas (Facultad de Farmacia y Bioquímica, UBA, Buenos Aires, Argentina).

3.4. Karyotyping

Chromosomal G-band analysis of cells at passage 12 (50 metaphases at 450-band resolution) was performed by Laboratorio de Genética Hematológica, Instituto de Medicina Experimental (IMEX-CONICET)/ Academia Nacional de Medicina.

3.5. Alkaline phosphatase assay

iPSCs were washed with PBS and subjected to alkaline phosphatase staining following manufacturer's instructions (Sigma, 86R- 1KT).

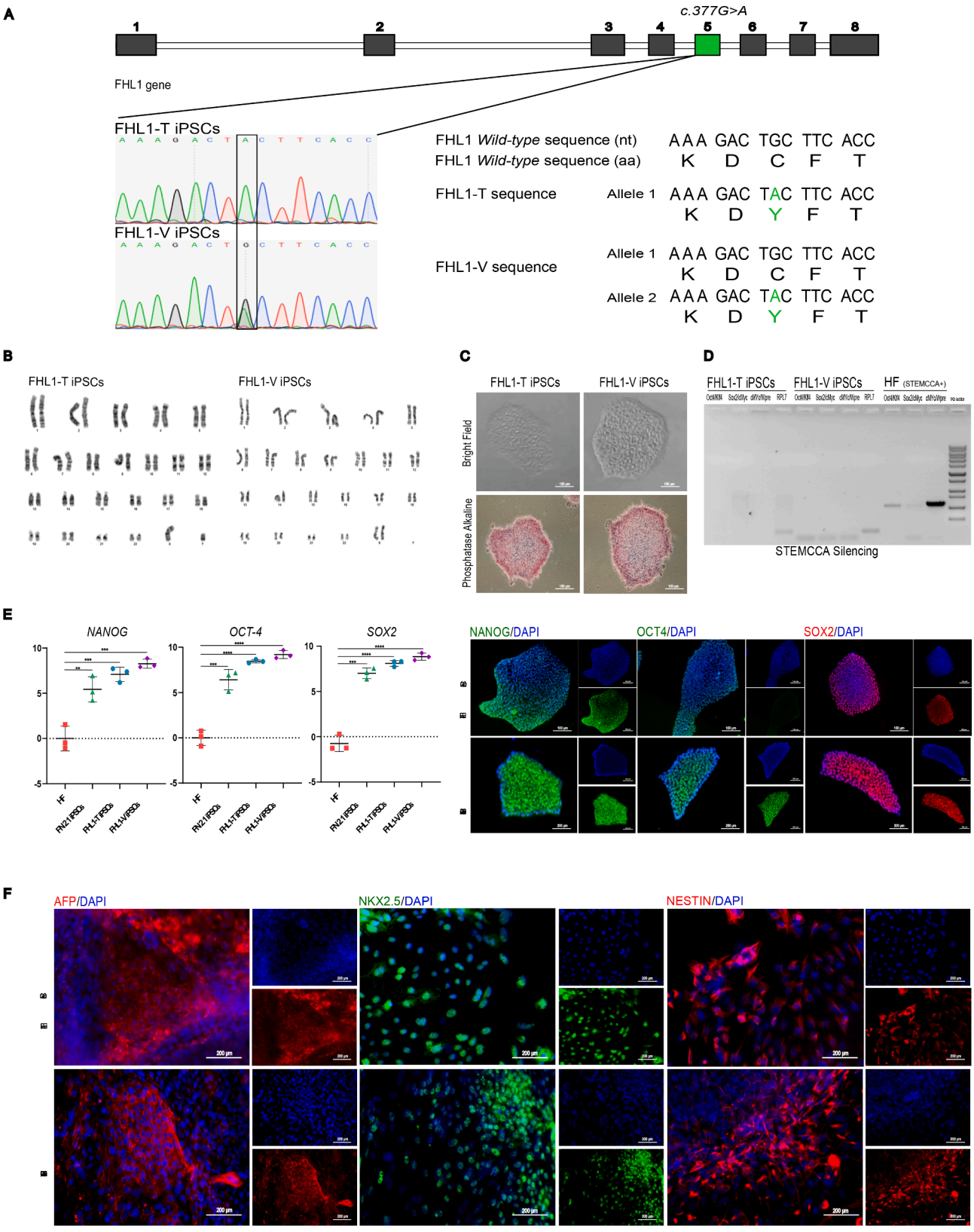

Fig. 1. Characterization of FHL1-T and FHL1-V iPSCs lines.

3.6. RNA isolation and RT-qPCR

RNA isolation and purification was performed using TRIzol (ThermoFisher-Scientific). Then, cDNA was synthesized from 500 ng of total RNA with 15 mM of random hexamers using MMLV reverse transcriptase (Promega), following manufacturer's instructions. cDNA amplification and analysis were done using the FastStart Universal SYBR Green Master Mix (Roche) and StepOnePlus Real Time PCR. After that, LinRegPCR software was used for mRNA values analysis, normalizing the gene values against two housekeeping genes, GAPDH and RPL7

Table 2
Reagents details.

Antibodies used for immunocytochemistry/flow-cytometry				
	Antibody	Dilution	Company Cat #	RRID
Pluripotency Markers	Mouse anti-OCT4	1:50	Thermo Fisher Scientific Cat#MA1-104	RRID:AB_2536771
	Rabbit anti-SOX2	1:200	Cell Signaling Technology Cat#3579	RRID:AB_2195767
	Mouse anti-NANOG	1:100	Santa Cruz Cat#sc-293121	RRID:AB_2665475
Differentiation Markers	Rabbit anti-NKX2.5	1:200	Thermo Fisher Scientific Cat# PA5-49431	RRID:AB_2634885
	Mouse anti-NESTIN	*1:200*	Millipore Cat#MAB5326	RRID:AB_2251134
	Mouse anti-AFP	*1:50*	Santa Cruz Biotechnology Cat# SC-166325	RRID:AB_2305278
Secondary antibodies	Alexa Fluor 488 goat anti rabbit IgG (H + L)	1:400	Invitrogen Cat#A11034	RRID:AB_2576217
	Alexa Fluor 594 goat anti mouse IgG (H + L)	1:400	Invitrogen Cat# A11032	RRID:AB_2534091
	Alexa Fluor 594 donkey anti rabbit IgG (H + L)	1:500	Thermo Fisher Scientific Cat# A-21207	RRID:AB_141637
	Alexa Fluor 488 donkey anti mouse IgG (H + L)	1:500	Thermo Fisher Scientific Cat# A-21202	RRID:AB_141607
Primers				
	Target	**Size of band**	**Forward/Reverse primer (5′-3′)**	
Pluripotency Markers (qPCR)	NANOG	120 bp	AAAGGATCTTCACCTATGCC/GAAGGAAGAGGAGAGACAGT	
	OCT4	128 bp	CTGGGTTGATCCTCGGACCT/CACAGAACTCATACGGCGGG	
	SOX2	110 bp	AGCATGGAGAAAACCCGGTACGC/CGTGAGTGTGGATGGGATTGGTGT	
House-Keeping Genes (qPCR)	RPL7	138 bp	AATGGCGAGGATGGCAAG/TGACGAAGGCGAAGAAGC	
	GAPDH	98 bp	ACAGCCTCAAGATCATCAG/GAGTCCTTCCACGATACC	
Genotyping	FHL1	472 bp	TTGGAGGTGTGAGGCCAGTA/ACTGACAGCCTGACTTGGCT	
STEMCCA expression	STEMCCA - OCT4/KLF4	561 bp	CAACGAGAGGATTTTGAGGC/ATCGTTGAACTCCTCGGTCTCTCT	
	STEMCCA - SOX2/CMYC	550 bp	TTGGCTCCATGGGTTCGGTG/AAGGGTGTGACCGCAACGTAGG	
	CMYC/WPRE	580 bp	GGAACTCTTGTGCGTAAGTCGATAG/GGAGGC GGCCCAAAGGGAGATCCG	
Mycoplasma	*Mycoplasma sp.*	500 bp	ACACCATGGGAGYTGGTAAT/CTTCWTCGACTTYCAGACCCAAGGCAT	

[One-Way ANOVA, followed by Dunnett's - test. (**p < 0,01; ***p < 0,001; ****p < 0,0001)]. Cells were analyzed at passages 13–15-17.

3.7. In vitro differentiation

Cells were detached with Dispase (Gibco) and transferred to non-adherent Petri dishes in DMEM/F12+ (Gibco), 20 % knock-out serum replacement (KSR, Gibc), 1x GlutaMAX (Gibco™, #35050061), 1x non-essential amino acids (Sigma, #M7145), 0.1 μM 2-mecaptoethanol (KSR-EBs medium), to induce formation of embryoid bodies (EBs). On day 4, EBs were plated onto 0.1 % gelatin coated 24-well plates and cultured for additional 17 days in KSR-EBs medium supplemented with 20 % Fetal Bovine Serum (Gibco).

3.8. Immunofluorescence staining

iPSCs at passage 11 and EBs were fixed with 4 % paraformaldehyde (Sigma) during 15 min, permeabilized and blocked with PBS –0.1 % Triton X-100 and 3 % Normal Goat Serum, and incubated with primary antibodies overnight at 4 °C. Secondary antibodies were incubated for 1 h at room temperature. Cells were counterstained with DAPI and examined under an Evos XL Core inverted microscope.

CRediT authorship contribution statement

Federico Zabalegui: Writing – original draft, Methodology, Investigation. **Guadalupe Amin:** Methodology. **Carolina Belli:** Methodology.

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.2024.103307.

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