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Lab Resource: Stem Cell Line

Establishment of a human iPSC line (IISHDOi001-A) from a patient with McArdle disease

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ABSTRACT

Human iPSC line IISHDOi001-A was generated from fibroblasts of a patient with McArdle disease harbouring the mutation, c.148C>T; p.Arg50Ter, in the *PYGM* gene. Reprogramming factors Oct3/4, Sox2, Klf4, and c-Myc were delivered using Sendai virus.

Resource table

Unique stem cell line identifier	IISHDOi001-A
Alternative name(s) of stem cell line	MA5622-FiPS4F1
Institution	Instituto de Investigación Sanitaria Hospital 12 de Octubre, i+12
Contact information of distributor	Dr. M. Esther Gallardo egallardo@iib.uam.es
Type of cell line	iPSC
Origin	Human
Additional origin info	Sex: female
Cell source	Human fibroblasts
Method of reprogramming	Sendai virus
Genetic modification	NO
Type of modification	N/A
Associated disease	McArdle disease
Gene/locus	Gene *PYGM*: c.148C>T; p.Arg50Ter
Method of modification	N/A
Name of transgene or resistance	N/A
Inducible/constitutive system	N/A
Date archived/stock date	June 2017
Cell line repository/bank	N/A
Ethical approval	Patient informed consent was obtained. This study was reviewed and approved by the Institutional Ethical Committee of the "Instituto de Investigaciones Biomédicas Alberto Sols", CSIC-UAM, 406 329 1.

Resource utility

McArdle disease is a disorder of carbohydrate metabolism inherited in an autosomal recessive way, associated with mutations in the *PYGM* gene. Patients with this disease experience exercise intolerance including, sometimes, rhabdomyolysis and myoglobinuria. The line IISHDOi001-A will be very useful for modelling this disease and searching for therapeutic approaches.

Resource details

The generation of the human iPSC line, IISHDOi001-A, was performed using non-integrative 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 McArdle disease were employed. These fibroblasts harboured the most prevalent mutation among the Caucasian population, located in the *PYGM* gene (c.148C>T; p.Arg50Ter) (Nogales-Gadea et al., 2016). The presence of this mutation in the iPSCs was confirmed (Fig. 1A). IISHDOi001-A iPSC colonies displayed a typical ES-like colony morphology and growth behavior (Fig. 1B) and they stained positive for alkaline phosphatase activity (Fig. 1C). We confirmed the clearance of the vectors and the exogenous reprogramming factor genes by RT-PCR after twelve culture passages (Fig. 1D). The endogenous expression of the pluripotency associated transcription factors *OCT4*, *SOX2*, *KLF4*, *NANOG*, *CRIPTO* and *REX1* was evaluated by quantitative real time polymerase chain reaction (qPCR) (Fig. 1E). Immunofluorescence analysis revealed expression of transcription factors OCT4, NANOG, SOX2 and surface markers SSEA3,

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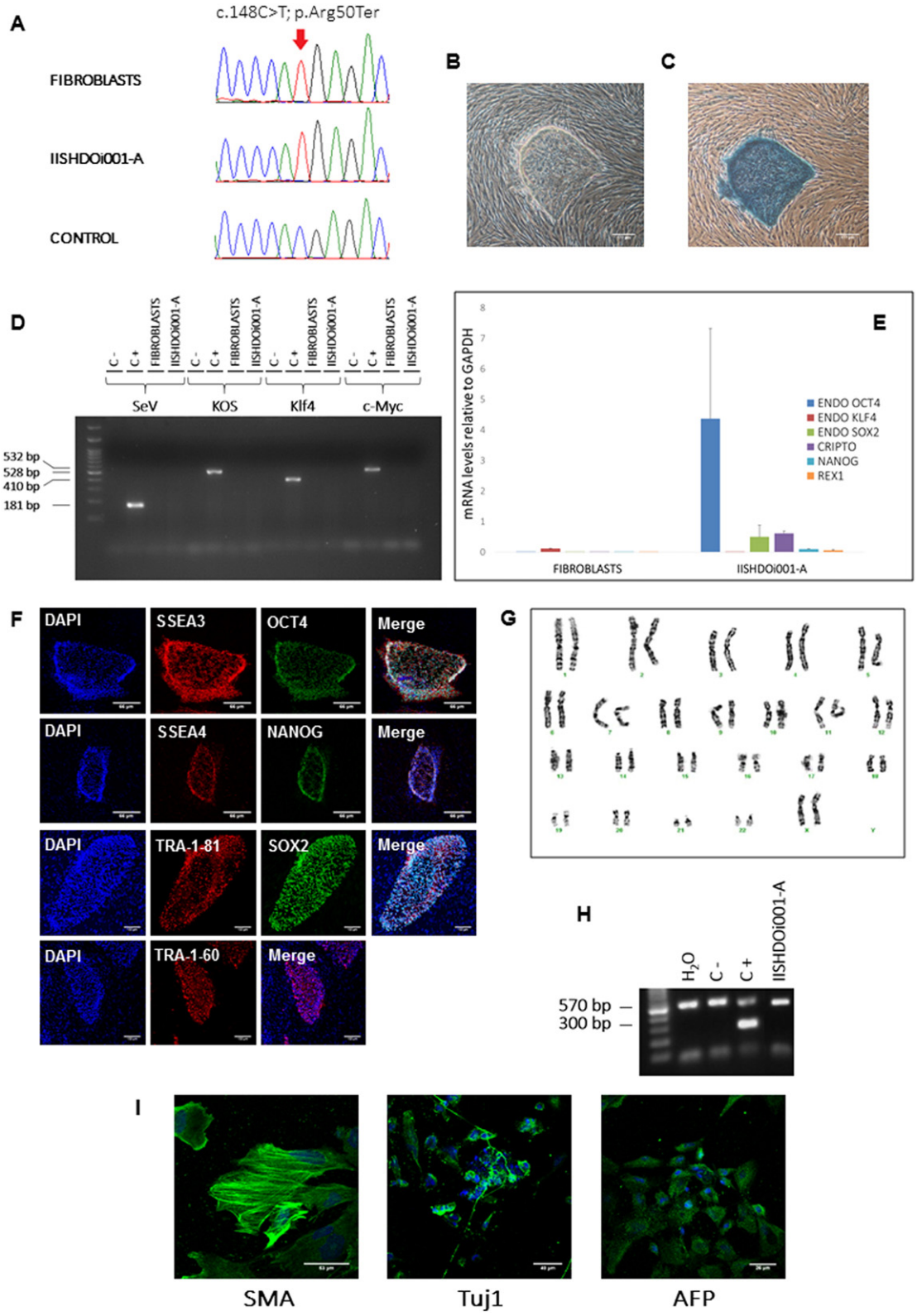

Fig. 1. Molecular and functional characterization of the IISHDOi001-A iPSC line.

SSEA4, TRA-1-60 and TRA-1-81 characteristics of pluripotent ES cells (Fig. 1F). The iPSC line has been adapted to feeder-free culture conditions and displays a normal karyotype (46, XX) after more than twenty culture passages (Fig. 1G). We also confirmed by DNA fingerprinting analysis that the line IISHDOi001-A was derived from the patient's fibroblasts. In addition, the line was confirmed by PCR

analysis to be mycoplasma-negative (Fig. 1H). Finally, the capacity of the generated iPSC line to differentiate into the three germ layers (endoderm, mesoderm and ectoderm) was tested *in vitro* using an embryoid body based assay (Fig. 1I).

Materials and methods

Reprogramming of McArdle fibroblasts into iPSCs

Human McArdle fibroblasts harbouring the mutation p.Arg50Ter in the *PYGM* gene were reprogrammed using the CytoTune-iPS 2.0 Sendai reprogramming kit following the instructions of the manufacturer. IISHDOi001-A was maintained and expanded both on feeder and feeder-free layers as described in Galera et al., 2016.

Phosphatase alkaline analysis

The iPSC line IISHDOi001-A was seeded on a feeder layer plate. After one week, direct phosphatase alkaline activity was determined using the phosphatase alkaline blue membrane substrate solution kit (Sigma, AB0300) (Table 1).

Mutation analysis

Total DNA from patient's fibroblasts and iPSCs was extracted using a standard phenol-chloroform protocol. Subsequently, a PCR was carried out with the primers listed in Table 2. Following PCR amplification, direct sequencing of amplicons was performed in an ABI 3730 sequencer (Applied Biosystems).

qPCR analysis

Total mRNA was isolated using TRIZOL and 1 μg was used to synthesize cDNA using the QuantiTect RT cDNA synthesis kit. One microliter of the reaction was used to quantify by qPCR the expression of the endogenous pluripotency associated genes (*OCT4*, *SOX2*, *KLF4*, *NANOG*, *CRIPTO* and *REX1*). Primers are listed in Table 2 (Aasen et al., 2008). All the expression values were normalized to the *GAPDH* gene. Plots are representative of at least three independent experiments.

Karyotype analysis

Karyotype analyses were carried out using cells with more than twenty culture passages. Briefly, cells were treated with 10 μg/mL of Colcemid (Gibco) for 90 min at 37 °C, trypsinized, treated with hypotonic solution KCl 0.075 M, and fixed with Carnoy's fixative. Cells were then dropped on a microscope glass slide and dried. Metaphase cells were G banded using Wright staining. At least 20 metaphases were karyotyped.

Immunofluorescence analysis

Cells were grown on 0.1% gelatin-coated 35 mm culture plates (81,156, Ibidi), 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. Secondary antibodies for 2 h at RT. Nuclei were stained with DAPI (Sigma, 28718-90-3). All the antibodies are listed in Table 2.

In vitro differentiation assay

The *in vitro* pluripotency capacity of the line IISHDOi001-A was tested by spontaneous embryoid body differentiation. The protocol we have used has been described in detail by Galera et al., 2016.

Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography	Normal	Fig. 1 panel B
Phenotype	Immunocytochemistry	Positive for the pluripotency markers: SSEA3, SSEA4, TRA-1-81, TRA-1-60, OCT4, NANOG, SOX2	Fig. 1 panel F
	Flow cytometry	N/A	
	Gene expression (qPCR)	Positive for the pluripotency markers *OCT4*, *KLF4*, *SOX2*, *CRIPTO*, *NANOG*, *REX1*	Fig. 1 panel E
	Alkaline phosphatase activity	Positive	Fig. 1 panel C
Genotype	Karyotype (G-banding) and resolution	46, XX Resolution 450–500	Fig. 1 panel G
Identity	Microsatellite PCR (mPCR)	N/A	
	STR analysis	8 loci, all matched (D2S1338, D7S820, D8S1179, D13S317, D19S433, D21S11, VWA, amelogenin)	Submitted in archive with journal
Mutation analysis (IF APPLICABLE)	Sequencing	Confirmation of the mutation: *PYGM* c.148C>T; p.Arg50Ter	Fig. 1 panel A
	Southern Blot OR WGS	N/A	
Microbiology and virology	Mycoplasma	Negative	Fig. 1 panel H
	Sendai virus silencing	Virus silenced	Fig. 1 panel D
Differentiation potential	Embryoid body formation and directed differentiation	Positive for: smooth muscle actin (SMA), β-tubulin (Tuj1) and alpha-fetoprotein (AFP)	Fig. 1 panel 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-cytometry			
	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	Thermo Fisher Scientific Cat# PA1-16968, RRID: AB_2195781
	Mouse anti-SSEA4	1:10	Millipore Cat# MAB4304, RRID: AB_177629
	Rat anti-SSEA3	1:20	Abcam Cat# ab16286, RRID: AB_882700
	Goat anti-NANOG	1:25	R and D Systems Cat# sc-5279, RRID: AB_628051
	Mouse anti-OCT4	1:100	Santa Cruz Biotechnology Cat# sc-5279, RRID: AB_628051
Differentiation markers	Mouse anti-β tubulin isotype III	1:300	Sigma-Aldrich Cat# T8660, RRID: AB_528427
	Mouse anti- AFP	1:300	Sigma-Aldrich Cat# WH0000174M1, RRID: AB_1839587
	Mouse anti- SMA	1:400	Sigma-Aldrich Cat# A2547, RRID: AB_476701
Secondary antibodies	Cy™2-conjugated AffiniPure Donkey Anti-Goat IgG (H + L)	1:50	Jackson ImmunoResearch Labs Cat# 705-225-147, RRID: AB_2307341
	Cy™2-conjugated AffiniPure Goat Anti-Mouse IgG, Fcγ Subclass 2b specific	1:50	Jackson ImmunoResearch Labs Cat# 115-225-207, RRID: AB_2338749
	Cy™2-conjugated AffiniPure Goat Anti-Rabbit IgG (H + L)	1:50	Jackson ImmunoResearch Labs Cat# 111-225-144, RRID: AB_2338021
	Cy™3-conjugated AffiniPure Goat Anti-Rat IgM, μ chain specific	1:250	Jackson ImmunoResearch Labs Cat# 112-165-075, RRID: AB_2338249
	Cy™3-conjugated AffiniPure Goat Anti-Mouse IgG, Fcγ Subclass 3 specific	1:250	Jackson ImmunoResearch Labs Cat# 115-165-209, RRID: AB_2338698
	Cy™3-conjugated AffiniPure Donkey Anti-Mouse IgM, μ chain specific	1:250	Jackson ImmunoResearch Labs Cat# 715-165-020, RRID: AB_2340811
	Goat anti-mouse IgG (H + L), Alexa Fluor 488	1:500	Thermo Fisher Scientific Cat# A-11029, RRID: AB_2534088
Primers			
	Target		Forward/Reverse primer (5′-3′)
Pluripotency markers (qPCR)	*Endo-KLF4*		AGCCTAAATGATGGTGCTTGGT/TTGAAAACTTTGGCTTCCTTGTT
	Endo-OCT4		GGGTTTTTGGGATTAAGTTCTTCA/GCCCCCACCCTTTGTGTT
	Endo-SOX2		CAAAAATGGCCATGCAGGTT/AGTTGGGATCGAACAAAAGCTATT
	REX1		CCTGCAGGCGGAAATAGAAC/GCACACATAGCCATCACATAAGG
	CRIPTO		CGGAACTGTGAGCACGATGT/GGGCAGCCAGGTGTCATG
	NANOG		ACAACTGGCCGAAGAATAGCA/GGTTCCCAGTCGGGTTCAC
House-Keeping Genes (qPCR)	*GAPDH*		GCACCGTCAAGGCTGAGAAC/AGGGATCTCGCTCCTGGAA
Targeted mutation analysis/sequencing	*PYGM*		CAAATCAGTGTGCGTGGCCT/CCTTCTCATAGTAGTGCTGC
Virus silencing	SeV		GGATCACTAGGTGATATCGAGC/ACCAGACAAGAGTTTAAGAGATATGTATC
	KOS		ATGCACCGCTACGACGTGAGCGC/ACCTTGACAATCCTGATGTGG
	Klf4		TTCCTGCATGCCAGAGGAGCCC/AATGTATCGAAGGTGCTCAA
	c-Myc		TAACTGACTAGCAGGCTTGTCG/TCCACATACAGTCCTGGATGATGATG
STR analysis	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
	Amelogenin		[6-FAM] CCCTGGGCTCTGTAAAGAATAGTG/ATCAGAGCTTAAACTGGGAAGCTG
Mycoplasma detection	MGSO		TGCACCATCTGTCACTCTGTTAACCTC/GAGGTTAACAGAGTGACAGATGGTGCA
	GPO-3		GGGAGCAAACAGGATTAGATACCCT/AGGGTATCTAATCCTGTTTGCTCCC

DNA fingerprinting analysis

For DNA fingerprinting analysis the 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

Mycoplasma detection was performed by PCR analysis using 1 mL of the cell culture supernatant (3 days culture at 90% confluence). Primers used are specified in Table 2. The 300 bp band represents that the sample is positive for mycoplasma (positive control, C+). The band at 570 bp is an internal control to discard the inhibition of the polymerase.

Author disclosure statement

There are no competing financial interests in this study.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.scr.2017.07.020.

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