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Lab resource: Stem Cell Line

Generation of the iPSC line IISHDOi007-A from peripheral blood mononuclear cells from a patient with McArdle disease harbouring the mutation c.2392 T > C; p.Trp798Arg

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A B S T R A C T

Peripheral blood mononuclear cells (PBMCs) from a McArdle patient carrying a homozygous mutation in the *PYGM* gene: c.2392 T > C; p.Trp798Arg were used for the generation of the human iPSC line, IISHDOi007-A. For the delivery of the reprogramming factors Oct3/4, Sox2, Klf4, and c-Myc, a non-integrative methodology that implies the use of Sendai virus has been applied.

1. Resource table:

Unique stem cell line identifier	IISHDOi007-A
Alternative name(s) of stem cell line	McA2.7
Institution	Instituto de Investigación Sanitaria Hospital 12 de Octubre, i + 12
Contact information of distributor	Dr. M. Esther Gallardo egallardo.imas12@h12o.es
Type of cell line	iPSC
Origin	Human
Additional origin info	Age: 70 Sex: Female Ethnicity if known: Caucasian
Cell Source	Peripheral blood mononuclear cells (PBMCs)
Clonality	Clonal
Method of reprogramming	Transgene free Sendai virus vectors: Klf4, Oct3/4, Sox2 and cMyc
Genetic Modification	Yes
Type of Modification	Hereditary
Associated disease	McArdle disease
Gene/locus	Gene *PYGM* NM_005609: c.2392 T > C; p.Trp798Arg; Chromosome: 11q13.1
Method of modification	N/A N/A

(*continued on next column*)

(*continued*)

Name of transgene or resistance	
Inducible/constitutive system	N/A
Date archived/stock date	June 2020
Cell line repository/bank	National Bank of Stem Cell Lines, ISCIII, Madrid, Spain
Ethical approval	Patient informed consent was obtained. This study was reviewed and approved by the Institutional Research Ethical Committee of the "Hospital Universitario 12 de Octubre", 18/474.

2. Resource utility

McArdle disease (glycogen storage disease type V) is an autosomal recessive disorder caused by mutations in the *PYGM* gene (Nogales-Gadea et al., 2015). The main characteristic of this disease is exercise intolerance, manifested by myalgia, rhabdomyolysis and even myoglobinuria. Here, we report the generation of the iPSCs line IISHDOi007-A. This line will be very useful for modelling McArdle disease and searching for therapeutic approaches.

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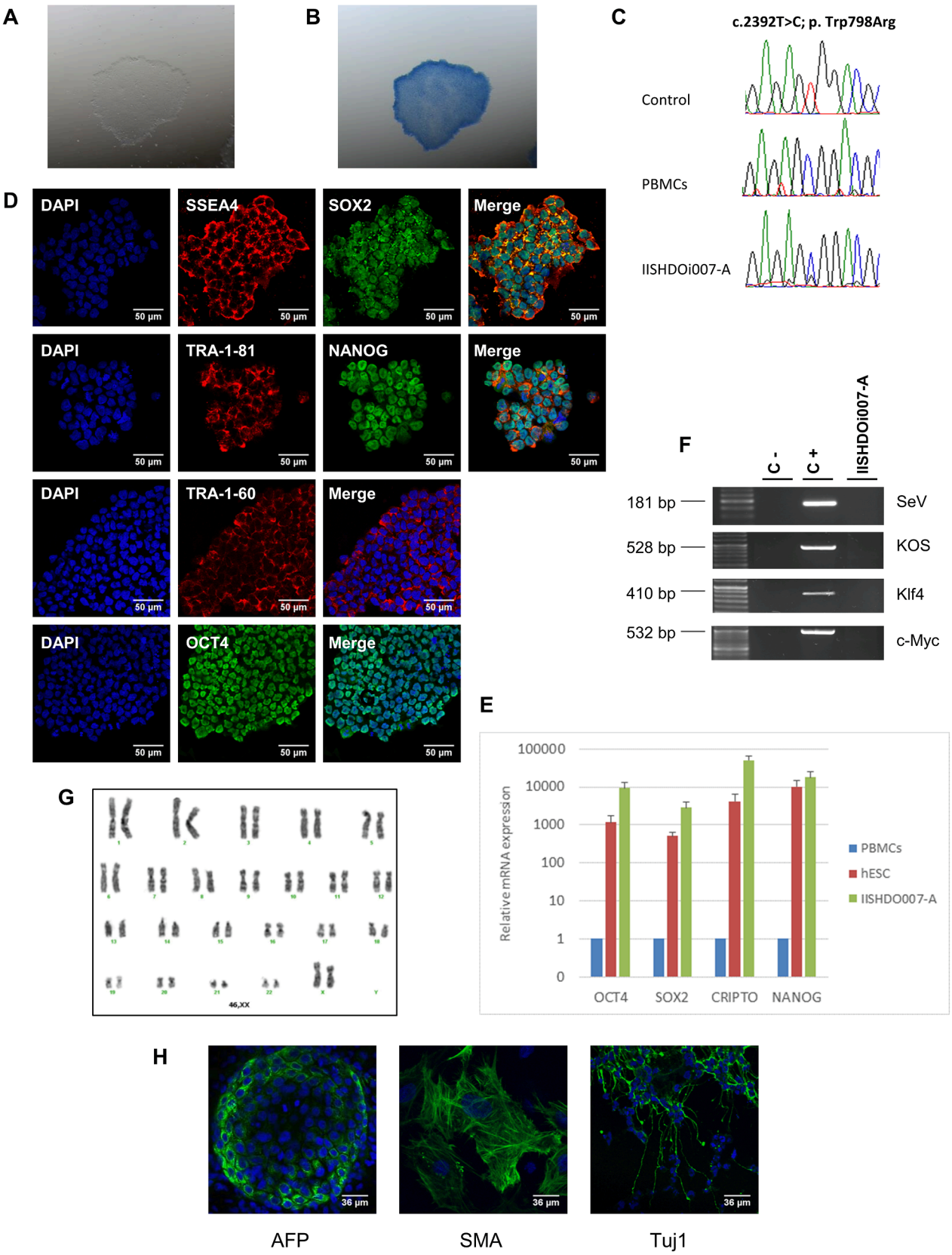

Fig. 1. Molecular and functional characterization of the IISHDOi007-A iPSC line.

3. Resource details

The human iPSC line, IISHDOi007-A, has been established using Sendai viruses containing the reprogramming factors Oct3/4, Sox2, Klf4 and c-Myc (Takahashi et al., 2007). For this purpose, peripheral blood mononuclear cells (PBMCs) from a McArdle patient, harbouring a homozygous mutation in the gene *PYGM* (c.2392 T > C; p.Trp798Arg), have been isolated from whole blood. IISHDOi007-A iPSC colonies displayed a typical ES-like colony morphology and growth behavior (Fig. 1A) and they stained positive for alkaline phosphatase activity (Fig. 1B). The line was tested by PCR analysis to be mycoplasma-negative (Supplementary Fig. S1). In addition, the presence of the mutation, c.2392 T > C; p.Trp798Arg, in the iPSCs was confirmed (Fig. 1C). Immunofluorescence analyses revealed expression of the transcription factors OCT4, NANOG and SOX2, and typical ES cells surface markers SSEA4, TRA-1–60 and TRA-1–81 (Fig. 1D). The endogenous expression of the pluripotency associated transcription factors OCT4, SOX2, NANOG and CRIPTO was also evaluated by quantitative real time polymerase chain reaction (qPCR). Total RNA from human embryonic stem cells (hESC) (Celprogen, 36101RNA) has been used as a reference for the gene expression levels of stemness markers (Fig. 1E). Additionally, the clearance of the vectors and the exogenous reprogramming factor genes was confirmed by RT-PCR after eight culture passages (Fig. 1F). We confirmed by DNA fingerprinting analysis that the line, IISHDOi007-A, was derived from the patient's PBMCs (archived at SCR journal). Karyotype analysis after more than 15 culture passages has been carried out, displaying a normal karyotype (46, XX) (Fig. 1G). Finally, we evaluate the capacity of the IISHDOi007-A iPSC line to differentiate into the three germ layers (endoderm, mesoderm and ectoderm) using an *in vitro* embryoid body based assay (Fig. 1H).

Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography	Normal	Fig. 1 panel A
Phenotype	Qualitative analysis: Alkaline phosphatase activity	Positive	Fig. 1 panel B
	Qualitative analysis: Immunocytochemistry	Positive for the pluripotency markers: SSEA4, SOX2, NANOG, TRA-1–81, TRA-1–60 and OCT4	Fig. 1 panel D
	Quantitative analysis: Gene expression (qPCR)	Positive for the pluripotency markers: OCT4, SOX2, CRIPTO and NANOG	Fig. 1 panel E
Genotype	Karyotype (G-banding) and resolution	46XX Resolution 450–500	Fig. 1 panel G
Identity	STR analysis	DNA profiling performed 8 loci, all matched (D2S1338, D7S820, D8S1179, D13S317, D19S433, D21S11, VWA, amelogenin)	Submitted to SCR journal for archiving
Mutation analysis	Sequencing	Confirmation of the mutation: c.2392 T > C; p. Trp798Arg	Fig. 1 panel C
Microbiology and virology	Mycoplasma	Mycoplasma testing by PCR: Negative	Supplementary Fig. S1
Differentiation potential	Embryoid body formation and directed differentiation	Positive for: smooth muscle actin (SMA), β-tubulin (Tuj1) and α-feto protein (AFP).	Fig. 1 panel H
Donor screening	HIV 1 + 2 Hepatitis B, Hepatitis C	N/A	N/A
Genotype additional info	Blood group genotyping	N/A	N/A
	HLA tissue typing	N/A	N/A

4. Materials and methods

4.1. Isolation of PBMCs

A peripheral blood sample was collected from a McArdle patient harbouring the homozygous mutation c.2392 T > C; p.Trp798Arg in the *PYGM* gene. PBMCs were isolated from the whole blood by a density gradient centrifugation method using Lymphoprep™ (StemCell, 07851). PBMCs were cultured for 4 days in StemSpam™ SFEM II medium (StemCell, 09605) supplemented with SCF (C-Kit ligand) (StemCell, 78062.1), Flt-3 Ligand (StemCell, 78009.1), IL-3 (StemCell, 78040.1) and IL-6 (Thermofisher, PHC 0064).

4.2. Generation of iPSCs

PBMCs were reprogrammed in feeder-free conditions using the CytoTune-iPS 2.0 Sendai reprogramming kit following the manufacturer's guide. IISHDOi007-A was maintained and expanded using mTeSR Plus medium (StemCell, 05825) in a 5% CO2 incubator at 37 °C. Cells were passaged at a confluence of 80% employing ReLeSR™ (StemCell, 05873).

4.3. Phosphatase alkaline analysis

Direct phosphatase alkaline activity was determined using the phosphatase alkaline membrane substrate solution kit (Sigma, AB0300), Table 1.

4.4. Mutation analysis

Total DNA was extracted using a commercial kit (Ilustra™ blood genomicPrep Mini Spin Kit, GE Healthcare, 28–9042-65). Subsequently, a PCR was performed to confirm the mutation (primers shown in Table 2) with the following conditions: 95 °C for 2 min, 35 cycles at 95 °C for 30 s, at 69 °C for 1 min, and at 72 °C for 15 s, and a final extension at 72 °C of 5 min (Applied Biosystems™ Verity Thermal Cycler). Amplicons were sequenced in an ABI 3730 analyzer (Applied Biosystems).

4.5. qPCR analysis

A qPCR analysis was performed to quantify the expression of the pluripotency associated genes *OCT4, SOX2, CRIPTO* and *NANOG*. Total mRNA was isolated using TRI Reagent® and cDNA was synthetized using the Thermo Scientific RevertAid RT Kit. The qPCR was carried out with GoTaq® qPCR Master Mix (Promega), and analysed using an Applied Biosystems™ 7500 Fast Real-Time PCR System (primers listed in Table 2). All the expression values were normalized to the *GAPDH* gene. Plots are representative of at least four independent experiments.

4.6. Karyotype analysis

iPSCs with more than 15 passages were treated with 10 μg/ml of Colcemid (Gibco) for 90 min at 37 °C. Afterwards they were trypsinized, treated with hypotonic solution KCl 0.075 M and fixed with Carnoy's fixative. Twenty metaphases were G banded using Wright staining and analyzed.

Table 2
Reagents details.

Antibodies used for immunocytochemistry/flow-citometry			
	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	ThermoFisher Scientific Cat# PA1_16968, RRID: AB_2195781
	Mouse anti SSEA4	1:10	Abcam Cat# ab16287, RRID: AB_778073
	Goat anti NANOG	1:25	R&D Systems Cat# AF1997, RRID: AB_355097
	Mouse anti OCT4	1:100	Santa Cruz Biotechnology Cat# sc_5279, RRID: AB_628051
Differentiation Markers	Mouse anti α-feto protein (AFP)	1:300	Sigma Aldrich Cat# WH0000174M1, RRID: AB_1839587
	Mouse anti smooth muscle actin (SMA)	1:400	Sigma Aldrich Cat# A2547, RRID: AB_476701
	Mouse anti β-Tubulin isotype III (Tuj1)	1:300	Sigma Aldrich Cat# T8660, RRID: AB_477590
Secondary antibodies	CyTM2-conjugated AffiniPure Goat anti-Rabbit igG (H + L)	1:50	Jackson Inmunoresearch Labs Cat# 111–225-144, RRID: AB_2338021
	CyTM3-conjugated AffiniPure Goat anti-Mouse IgG, Fc□ Subclass 3 Specific	1:250	Jackson Inmunoresearch Labs Cat# 115–165-209, RRID: AB_2338698
	CyTM2-conjugated AffiniPure Goat anti-Mouse IgG, Fc□ Subclass 2b Specific	1:50	Jackson Inmunoresearch Labs Cat# 115–225-207, RRID: AB_2338749
	CyTM2-conjugated AffiniPure Donkey anti-Goat IgG (H + L)	1:50	Jackson Inmunoresearch Labs Cat# 705–225-147, RRID: AB_2307341
	Goat anti Mouse IgG (H + L), Alexa Fluor 488	1:500	ThermoFisher Scientific Cat# A-11029, RRID: AB_2534088

Primers		
	Target	Forward/Reverse primer (5′-3′)
Pluripotency Markers (qPCR)	Endo *OCT4*	GGGTTTTTGGGATTAAGTTCTTCA / GCCCCCACCCTTTGTGTT
	Endo *SOX2*	CAAAAATGGCCATGCAGGTT / AGTTGGGATCGAACAAAAGCTATT
	CRIPTO	CGGAACTGTGAGCACGATGT / GGGCAGCCAGGTGTCATG
	NANOG	ACAACTGGCCGAAGAATAGCA / GGTTCCCAGTCGGGTTCAC
House-Keeping Genes (qPCR)	*GAPDH*	GCACCGTCAAGGCTGAGAAC / AGGGATCTCGCTCCTGGAA
Targeted mutation analysis/sequencing	*PYGM*	TGCCAGGAGAAAGTCAGCGCC / CTGGCGGGAAGGCTCCACAC
Virus silencing	SeV	GGATCACTAGGTGATATCGAGC / ACCAGACAAGAGTTTAAGAGATATGTATC
	KOS	ATGCACCGCTACGACGTGAGCGC / ACCTTGACAATCCTGATGTGG
	Klf-4	TTCCTGCATGCCAGAGGAGCCC / AATGTATCGAAGGTGCTCAA
	c-Myc	TAACTGACTAGCAGGCTTGTCG / TCCACATACAGTCCTGGATGATGATG
STR analysis	Amelogenin	[6-FAM] CCCTGGGCTCTGTAAAGAATAGTG / ATCAGAGCTTAAACTGGGAAGCTG
	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
Mycoplasma detection	*GPO-3 /MGSO*	GGGAGCAAACAGGATTAGATACCCT / TGCACCATCTGTCACTCTGTTAACCTC

4.7. Immunofluorescence analysis

Cells were fixed using 4% paraformaldehyde (30 min at RT). Subsequently, a permeabilization step with 0.1% Triton X-10 in Tris-buffered saline (TBS) was performed. Then, cells were incubated with TBS++ (3% donkey serum, 0.3% Triton X-100 in TBS). Primary antibodies were applied overnight at 4 °C and secondary antibodies for 2 h at RT (Table 2). Nuclei were stained with DAPI (Sigma, 28718–90-3). Images were obtained with a confocal microscope (Espectral LSM510 META ConfoCor 3, ZEISS) and processed with Zen 2009 software (ZEISS).

4.8. In vitro differentiation assay

The *in vitro* pluripotency capacity of the line IISHDOi007-A was evaluated by a spontaneous embryoid body differentiation assay. The protocol used has been detailed by Galera et al., 2016.

4.9. DNA fingerprinting analysis

A PCR amplification of the markers D13S317, D7S820, VWA, D8S1179, D21S11, D19S433, D2S1338 and amelogenin for sex determination (Table 2), was carried out. Amplicons were analyzed in the ABI PRISM 3100 Genetic analyzer and by Peak Scanner v3.5 (Applied Biosystems).

4.10. Mycoplasma detection

When cultures reached 90% of confluence, 1 ml of the cell culture supernatant was extracted to perform a PCR in an Applied BiosystemsTM Verity Thermal Cycler, using the primers described in Table 2 and the following conditions: An initial denaturation at 95 °C for 2 min, 35 cycles of amplification at 95 °C for 30 s, at 55 °C for 1 min, and at 72 °C for 1 min, and a final extension at 72 °C of 5 min.

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.2020.102108.

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STR analysis

	PBMCs		IISHDOi007-A	
Amelogenin	X	X	X	X
D2S1338	180	192	180	192
D7S820	214	218	214	218
D8S1179	172	180	172	180
D13S317	182	190	182	190
D19S433	199	204	199	204
D21S11	227	233	227	233
VWA	141	154	141	154