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Lab Resource: Single Cell Line

Generation of an induced pluripotent stem cells line, CSSi014-A 9407, carrying the variant c.479C>T in the human iduronate 2-sulfatase (hIDS) gene

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A B S T R A C T

Mucopolysaccharidosis type II (Hunter Syndrome) is a rare X-linked inherited lysosomal storage disorder presenting a wide genetic heterogeneity. It is due to pathogenic variants in the *IDS* gene, causing the deficit of the lysosomal hydrolase iduronate 2-sulfatase, degrading the glycosaminoglycans (GAGs) heparan- and dermatan-sulfate. Based on the presence/absence of neurocognitive signs, commonly two forms are recognized, the severe and the attenuate ones. Here we describe a line of induced pluripotent stem cells, generated from dermal fibroblasts, carrying the mutation c.479C>T, and obtained from a patient showing an attenuated phenotype. The line will be useful to study the disease neuropathogenesis.

Resource Table

Unique stem cell line identifier	https://hpscreg.eu/cell-line/CSSi014-A
Alternative name(s) of stem cell line	MPS II 9991 IPS cl H
Institution	IRCCS Casa Sollievo della Sofferenza
Contact information of distributor	Jessica Rosati; j.rosati@css-mendel.it
Type of cell line	iPSC
Origin	human
Additional origin info required for human ESC or iPSC	Age: 17 years Sex: M Ethnicity if known: Caucasian/Italian
Cell Source	Dermal Fibroblasts
Clonality	Clonal
Method of reprogramming	Non integrating episomal vectors
Genetic Modification	Yes
Type of Genetic Modification	Spontaneous mutation
Evidence of the reprogramming transgene loss (including genomic copy if applicable)	qRT-PCR
Associated disease	Mucopolysaccharidosis type II or Hunter Syndrome
Gene/locus	Iduronate 2-sulfatase (IDS): c. 479C > T
Date archived/stock date	September 2020
Cell line repository/bank	https://hpscreg.eu/cell-line/CSSi014-A
Ethical approval	Original primary fibroblasts were obtained from the ''Cell Line and DNA Biobank from Patients Affected by Genetic Diseases'', member of the Telethon Network of Genetic Biobanks (Istituto Gaslini, Genova, Italy). For the cells use and engineering, the laboratory of Diagnosis and Therapy of Lysosomal Disorders of the University of Padova, has obtained a clearance from the Ethics Committee of the Padova University Hospital.

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Resource Table (*continued*)

1. Resource utility

Many MPS II patients show a neurological involvement that so far

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cannot be explained either by the residual IDS activity or by the genotype. iPSC lines obtained from severe and attenuated patients will hopefully provide the missing model, necessary to study the pathogenesis of brain disease in these patients (Table 1).

2. Resource details

Mucopolysaccharidosis type II (MPS II, Hunter Syndrome) is a rare X-linked inherited lysosomal storage disorder with an incidence ranging from 0.4 to 1.1 per 100.000 live newborns. It is due to pathogenic variants in the IDS gene that cause deficiency of the lysosomal hydrolase iduronate 2-sulfatase activity (D'Avanzo et al., 2020). This determines the progressive intra- and extra-cellular accumulation of the glycosaminoglycans (GAGs) heparan- and dermatan-sulfate, finally leading to a heavy clinical phenotype involving most organ-systems, including the neurological compartment in almost two-thirds of the patients. Indeed, based on the presence/absence of neurocognitive signs the disease forms are classified into severe and attenuated, although a continuum of severity exists (D'Avanzo et al., 2020). The early identification of neuropathic forms through molecular biomarkers of severity would be very helpful for both diagnostic and prognostic purposes; it would also represent an effective tool to both evaluate the response to therapies targeting the CNS manifestations and possibly highlight candidate biomarkers of severity.

Here we describe a line of induced pluripotent stem cells, generated from dermal fibroblasts obtained from a 17 years-old patient. He showed the attenuated form of the disease whose onset was around 6–7 years of age with reduced growth and joint stiffness with movement limitations. The patient carried the missense variant c.479C>T (p.Pro160Leu), still not reported in literature.

We used non-integrating episomal vectors containing the reprogramming factors OCT4, SOX2, KLF4, L-MYC, LIN28 and p53-shRNA (Addgene) to reprogram skin fibroblasts into iPSCs. As evidenced by brightfield microscopy, iPSC colonies displayed typical human stem cell-like morphology (Fig. 1**A**). We manually expanded them in culture over several passages for further characterization. Genomic stability was confirmed by chromosomal analysis, which provided a normal karyotype (46, XY), at sixth passage (Fig. 1**B**). Immunofluorescence staining for pluripotency markers such as OCT-4 and TRA-1–60 established the expression of these proteins (passage ninth) (Fig. 1**C**), it was also confirmed through qRT-PCR using fibroblasts as negative control (Fig. 1**D**) and CSSi011-A (6534) hiPSCs as positive control. Additionally, qRT-PCR was also performed to show the absence of episomal plasmid DNA in iPSCs after nine passages, using fibroblasts after one week from nucleofection as positive control and CSSi011-A (6534) as negative control (Fig. 1**E**). The *in vitro* spontaneous differentiation was demonstrated, at tenth passage, through the formation of embryoid bodies (EBs) (Fig. 1**G**), which expressed the markers of three germ layers compared to iPSCs, in qRT-PCR analysis (Fig. 1**F**). Furthermore, the teratoma assay manifested the iPSCs capacity to *in vivo* differentiate into endoderm, mesoderm and ectoderm, after thirteen passages (Fig. 1**H**).

Sanger sequencing confirmed the presence of the missense IDS variant detected in the parental fibroblasts (Fig. 1**I**). In addition, Short Tandem Repeat (STR) profiling confirmed that these iPSC lines, at tenth passage, had the same genetic identity as the donor's fibroblasts. iPSC line resulted negative to Mycoplasma contamination test (Supplementary Fig. 1).

3. Materials and methods

3.1. Fibroblast culture and reprogramming

Fibroblast samples, obtained from the ''Cell Line and DNA Biobank from Patients Affected by Genetic Diseases'' (Filocamo et al., 2014), were cultured in DMEM High Glucose supplemented with 20% FBS, 100 U/ml Penicillin-Streptomycin, 2 mM L-Glutamine, and 1 × Non-Essential Amino Acids (Sigma Aldrich), at 37 °C, 5% CO_2. 1 × 10^5 fibroblasts were nucleofected with 3 μg 1:1:1 mix of the episomal plasmids pCXLE-hUL (Addgene #27080), pCXLE-hSK (Addgene #27078) and pCXLE-hOCT4-shp53 (Addgene #27077), using the Nucleofector program "FF113". Seven days after, nucleofected fibroblasts were plated on Matrigel (Corning) and cultured in Nutristem XF medium (Biological Industries). The hiPSC colonies were picked and expanded under feeder-free conditions. Absence of mycoplasma contamination was verified using N-Garde Mycoplasma PCR kit (EuroClone). At ninth passage, the absence of the exogenous reprogramming factors was confirmed by qRT-PCR.

3.2. Embryoid body and teratoma formation assays

The hiPSCs were manually detached and placed in a 25 mM flask in floating conditions. Nutristem-XF medium was gradually switched to DMEM/F-12, 20% Knock-out serum replacement (Gibco), 0.1 mM β-mercaptoethanol, 1 × NEAA, 50 U/ml Penicillin-Streptomicin, 2 mM l-glutamine in 3 days. Fourteen days later, EBs were collected and RNAs were extracted. For teratoma formation, hiPSCs derived from six well plates were resuspended in Matrigel and injected into immune-deficient mice. After 1 month, teratomas were removed for histological analysis.

3.3. Real-time PCR analysis

Total RNAs were isolated using Trizol reagent (Life Technology)

Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography Bright field	Normal	Fig. 1A
Phenotype	Qualitative analysis: immunocytochemistry	Staining of pluripotency markers: Oct4; Tra1-60.	Fig. 1C
	Quantitative analysis: RT-qPCR	Expression of pluripotency markers: OCT4, LIN28, L-MYC, KLF4, SOX2	Fig. 1D
Genotype	Karyotype (G-banding) and resolution	46 XY, Resolution 450–500	Fig. 1B
Identity	STR analysis	All the 19 sites tested matched	*e.g. submitted in archive with journal*
Mutation analysis	Sequencing	*IDS*: c.479C>T in hemyzygosis	Fig. 1I
Microbiology and virology	Mycoplasma	Mycoplasma tested by N-Garde Mycoplasma PCR kit (EuroClone). Negative	Supplementary Fig. 1
Differentiation potential	Embryoid body formation and teratoma formation	Genes expressed in embryoid bodies: SOX1, NESTIN, PAX6, EOMES, T, GATA4, FOXA2, SOX17 Proof of three germ layers formation	Fig. 1F, G + **Figure H**
List of recommended germ layer markers	Expression of these markers was demonstrated at mRNA (RT PCR) levels	Ectoderm: SOX1, NESTIN, PAX6, FABP, SLC1A3; Mesoderm: EOMES, T; Endoderm: GATA4, FOXA2, SOX17.	Fig. 1G
Donor screening	HIV 1 + 2 Hepatitis B, Hepatitis C	*Not applicable*	
Genotype additional info	Blood group genotyping	*Not applicable*	
	HLA tissue typing	*Not applicable*	

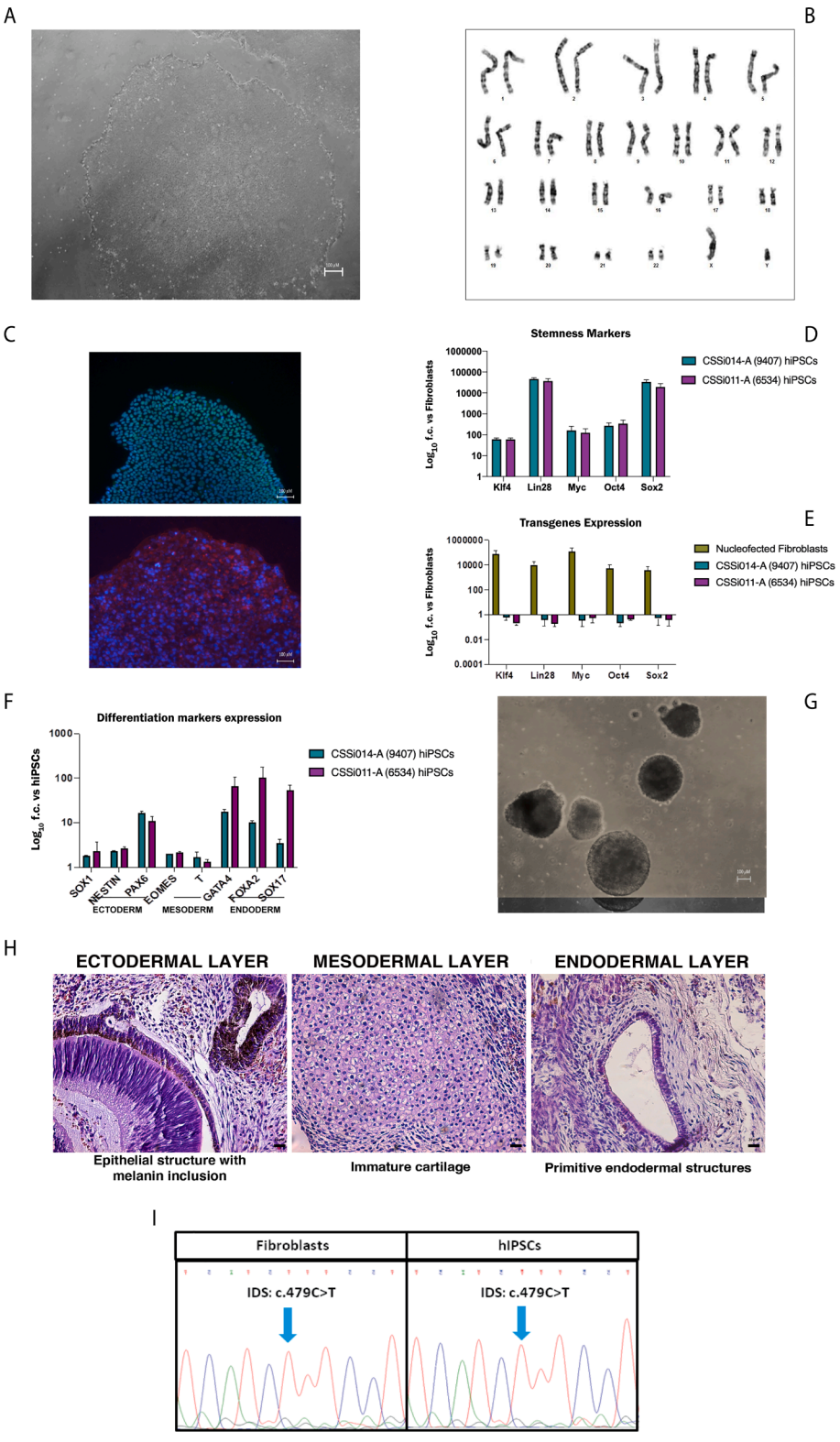

Fig. 1.

following the manufacturer's instructions and cDNA synthesized using the High-capacity cDNA RT (Life Technologies). Pluripotency markers were analyzed through SyBr green primers (Table 2), three germ layers were analyzed through TaqMan primers (Table 2). The expression ratio of target genes was calculated by using the 2-ΔΔCt method, considering β-ACTIN as the reference gene. qPCR analysis was performed in three independent biological experiments. An already published reference line is shown for comparison of the results (D'Anzi et al., 2020).

3.4. STR analysis

DNA of fibroblasts and iPSCs was extracted by Dneasy blood and tissue kit (QIAGEN). PCR amplification of 17 distinct STRs (AMEL, D3S1358, TH01, D21S11, D18S51, D10S1248, D1S1656, D2S1338,

Table 2
Reagents details.

Antibodies used for immunocytochemistry/flow-cytometry				
	Antibody	**Dilution**	**Company Cat #**	**RRID**
Pluripotency Markers 1:100	Rabbit antiOCT4; Mouse antiTRA-1–60 Life Technologies (A13998); Life Technologies (411000)	1:100; RRID: AB 2534182; RRID: AB_2533494.		
Secondary antibodies	anti-Rabbit AlexaFluor 488; antiMouse AlexaFluor 555	1:1000; 1:1000	Invitrogen (A11034); Invitrogen (A21422)	RRID: AB_2576217; RRID: AB_2535844
	Primers			
SyBr green Primers used for qPCR	**Target**	**Size of band**	**Forward/Reverse primer (5′-3′)**	
Episomal genes	eOCT4	70–150 bp	Fw: CAT TCA AAC TGA GGT AAG GG/ Rv: TAG CGT AAA AGG AGC AAC ATA G	
	eKLF4	70–150 bp	Fw: CCA CCT CGC CTT ACA CAT GAA GA/ Rv: TAG CGT AAA AGG AGC AAC ATA G	
	eLIN28	70–150 bp	Fw: AGC CAT ATG GTA GCC TCA TGT CCG C/ Rv: TAG CGT AAA AGG AGC AAC ATA G	
	eL-MYC	70–150 bp	Fw: GGC TGA GAA GAG GAT GGC TAC/ Rv: TTT GTT TGA CAG GAG CGA CAA T	
	eSOX2	70–150 bp	Fw: TTC ACA TGT CCC AGC ACT ACC AGA/ Rv: TTT GTT TGA CAG GAG CGA CAA T	
Pluripotency Markers	OCT4	70–150 bp	Fw: CCC CAG GGC CCC ATT TTG GTA CC/ Rv: ACC TCA GTT TGA ATG CAT GGG AGA GC	
	LIN28	70–150 bp	Fw: CCC CAG GGC CCC ATT TTG GTA CC/ Rv: ACC TCA GTT TGA ATG CAT GGG AGA GC	
	L-MYC	70–150 bp	Fw: GCG AAC CCA AGA CCC AGG CCT GCT CC/Rv: CAG GGG GTC TGC TCG CAC CGT GAT	
	SOX2	70–150 bp	Fw: TTC ACA TGT CCC AGC ACT ACC AGA/ Rv: TCA CAT GTG TGA GAG GGG CAG TGT GC	
House-Keeping Genes (qPCR)	β-ACTIN	70–150 bp	Fw: GGC ATC CTC ACC CTG AAG TA/ Rv: GGG GTG TTG AAG GTC TCA AA	
TaqMan primers used for qPCR	**Target**		**Probe**	
Differentation markers	SOX1		Hs01057642_s1	
	NESTIN		Hs04187831_g1	
	PAX6		Hs00240871_m1	
	T		Hs00610080_m1	
	EOMES		Hs00172872_m1	
	GATA4		Hs00171403_m1	
	FOXA2		Hs00232764_m1	
	SOX17		Hs00751752_s1	
	β-ACTIN		Hs 99999903_m1	
Targeted mutation analysis/sequencing	*IDS* (exon 4)		Fw: ACCAGCTTCACAGAACATGC/ Rv: AATTATGGGGAGTGGGGTGT	

D16S539, D22S1045, vWA, D8S1179, FGA, D2S441, D12S391, D19S433, SE33) was carried out using the QST*Rplusv2 kit (Elucigene Diagnostics), PCR products were separated on an ABI Prism 3130 DNA sequencer and analyzed by GeneMapper version 4.0 (Applied Biosystems).

3.5. Sequencing

Genomic DNA was extracted from fibroblasts and iPSC using QIAamp DNA Blood Mini Kit (Qiagen). IDS exon 4 was PCR-amplified by using the following primers: Forward 5′-ACCAGCTTCACAGAACATGC-3′, Reverse: 5′-AATTATGGGGAGTGGGGTGT-3′. The purified amplicon was sequenced by BigDye terminator v.3.1 Cycle Sequencing kit on ABI 3100 Genetic Analyzer (Applied Biosystems).

3.6. Karyotype analysis

iPSCs were cultured in Nutristem XF medium for 2–3 days. Karyotyping of metaphase chromosomes was performed using G banding. Fifteen metaphases were counted and three karyograms analyzed.

3.7. Immunofluorescence staining

Cells were fixed using 4% paraformaldehyde for 20 min at room temperature and blocked in PBS with 20% Normal Goat Serum. 0.1% Triton X-100 was used for 30 min for only OCT4 staining. Primary antibodies diluted in 5% BSA were incubated O/N at 4 °C. After washing, Alexa Fluor 594- and/or Alexa Fluor 488-conjugated secondary antibodies in PBS 1x were added 1 h at room temperature. Nuclei were counterstained with Hoechst. Microphotographs were taken with a Nikon C2 fluorescence microscope.

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.

Data availability

No data was used for the research described in the article.

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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.2022.102846.

References

D'Avanzo, F., Rigon, L., Zanetti, A., Tomanin, R., 2020. Mucopolysaccharidosis type II: one hundred years of research, diagnosis, and treatment. Int J Mol Sci. 21 (4), 1258. https://doi.org/10.3390/ijms21041258.

Filocamo, M., Mazzotti, R., Corsolini, F., Stroppiano, M., Stroppiana, G., Grossi, S., Lualdi, S., Tappino, B., Lanza, F., Galotto, S., Biancheri, R., 2014. Cell line and DNA biobank from patients affected by genetic diseases. Open J. Bioresour. 1, e2.

D'Anzi, A., Altieri, F., Perciballi, E., Ferrari, D., Bernardini, L., Goldoni, M., Mazzini, L., De Marchi, F., Di Pierro, A., D'Alfonso, S., Gelati, M., Vescovi, A.L., Rosati, J., 2020. Generation of an induced pluripotent stem cell line, CSSi011-A (6534), from an Amyotrophic lateral sclerosis patient with heterozygous L145F mutation in SOD1 gene. Stem Cell Res. 25 (47), 101924 https://doi.org/10.1016/j.scr.2020.101924. PMID: 32739880.