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Stem Cell Research





Lab Resource: Single Cell Line

Production of CSSi013-A (9360) iPSC line from an asymptomatic subject carrying an heterozygous mutation in TDP-43 protein

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ABSTRACT

Amyotrophic Lateral Sclerosis (ALS) is a fatal disease affecting both upper and lower motoneurons. The transactive response DNA binding protein (TARDBP) gene, encoding for TDP-43, is one of the most commonly mutated gene associated with familial cases of ALS (10%). We generated a human induced pluripotent stem cell (hiPSC) line from the fibroblasts of an asymptomatic subject carrying the TARDBP p.G376D mutation. This mutation is very rare and was described in a large Apulian family, in which all ALS affected members are carriers of the mutation. The subject here described is the first identified asymptomatic carrier of the mutation.

1. Resource Table:

https://hpscreg.eu/user/cellline/edit/
CSSi013-A
CSSi013-A cl.D
IRCCS Casa Sollievo della Sofferenza
Jessica ROSATI; j.rosati@css-mendel.it
iPSC
human
Age:34
Sex: Female
Ethnicity if known: Caucasian/Italian
Dermal fibroblasts
Clonal
Amyotrophic lateral sclerosis
TARDBP: $c.1127G > A$
June 2019
https://hpscreg.eu/user/cellline/edit/
CSSi013-A
Comitato etico Palermo 1, 04/19

2. Resource utility

Amyotrophic lateral sclerosis is a very complex disease. The iPSC line was derived from an individual carrying a p.G376D mutation (TARDBP gene) in an asymptomatic stage. This patient-derived iPSC line will be a useful cellular system for modelling those pathogenetic mechanisms that might precede symptoms' onset.

3. Resource details

Amyotrophic lateral sclerosis (ALS) is an incurable neurodegenerative disease characterized by the selective vulnerability and the progressive loss of the motoneurons both in the brain and spinal cord (Zarei et al., 2015). Most of the cases (90%) are considered sporadic (sALS) while in circa 10% of the cases it is possible to individuate a family history (fALS). Several mutated genes have been associated with ALS occurrence, among these, mutations in C9ORF, SOD1, FUS and TARDBP concur to almost half of the genetic cases, in particular mutated TARDBP is present in 2% of fALS and 1% of sALS. The TARDBP gene (transactive response DNA binding protein) encode for the protein TDP-43 that is an ubiquitously expressed and highly conserved nuclear protein involved in

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Fig. 1. Characterization of iPSC line from an asymptomatic subject carrying a heterozygous mutation in TDP-43 protein. (A) DNA sequencing confirmed the presence of the disease-related mutation in the iPSCs line; (B) Morphology of iPSC culture, scale bar = $500 \ \mu\text{m}$; (C) Real-time PCR confirmed the absence of exogenous reprogramming factors; (D) Immunofluorescence staining for TRA1-60 and OCT4, scale bar = $100 \ \mu\text{m}$; (E) qRT-PCR confirmed for the expression of endogenous stemness markers; (F) Histological analysis of iPSC-derived teratoma showed three germ layers formation; (G) Embryoid bodies derived from the iPSC, scale bar = $100 \ \mu\text{m}$; (H) qRT-PCR showed three germ layer expression in the embryoid bodies; (I) Karyotyping analysis of iPSC.

Table 1

Characterization and validation.

Classification Test Result Data Morphology Phenotype Photography munocytochemistry Normal Fig. 18 (1) Morphology Phenotype Photography munocytochemistry Normal Fig. 18 (1) Mutation qRT-PCR Expression of phirpiotency markers OCT4, LIN28, LMVC, LIN28, LMVC, LIN28, LMVC, LIN28, LMVC, Markers Fig. 12 (1) Genotype Karyotype (G- banding) and resolution 65 site strate, dl markers Steendary Fig. 1 Identity Secondary markers Steendary markers Steendary markers Steendary markers Mutation Sequencing Heterozyous markers Fig. 1A markers Stephentary fig. 1G, F, H. Mutation Sequencing Heterozyous markers Fig. 1G, F, H. Fig. 1G, F, H. Microbiology and visology Mycoplasma Supplementary fisting by N. Fig. 1G, F, H. Freentiation potential Embryoid body: formation and markes Fig. 1G, F, H. Fig. 1G, F, H. Freentiation potential Embryoid body: formation and markes Fig. 1G, F, H. Fig. 1F, FIG. F, FIC Fig. 1G, F, H. Fig. 1G, F, H. Fig. 1G, F, H. Fig. 1G, F, H. Freeomended germ layer markers to the markers ho the markers ho the markers ho the markers Fig. 1G, F, H. Fig. 1G, F, H. Fig. 1G, F, H. Fig. 1H: gT.	Characterization ar	nd validation.			Reagents detail	ls.				
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Genotype badding) and resolution 450.500 Fig. 11 Mouse AlexaPluor (A21422) AB.25 AB.25 Identity STR analysis 16 sites tested, all matched Submitted in archive with journal. Primers 525 Forward/Reverse prime bad Mutation Sequencing Heterozygous Fig. 1A Episonal 70 -150 Fwd: CA CA CA CA CA ADPLICABLEJ Microbiology Mycoplasma Mycoplasma Supplementary eKLP4 bp Rev: TAG CGT AAA AGG ATA G analysis (IP APPLICABLEJ Mycoplasma Supplementary eKLP4 bp Rev: TAG CGT AAA AGG ATA G and virology Mycoplasma Mycoplasma Supplementary eKLP4 bp Rev: TAG CGT AAA AGG ATA G ifferentiation Embryoid body Genet expressed Fig. 1G, F, H. 70 -150 Fwd: CAC CT CGC CT ATA G potential formation and in embryoid body: Fig. 1G, F, H. 70 -150 Fwd: CAC ATA GG potential formation and in embryoid body: Genet expression of three Fig. 1G, F, H. 70 -150 Fwd: CC AT AG GT potential formation and in embryoid body: Fig. 1G, F, H. 70 -150 Fwd: CC AC CAC CG CC CC proof of three gern layer Fig. 1G, F, H. 70 -150 Fwd:			markers: OCT4, LIN28, L-MYC, KLF4, SOX2.		Secondary antibodies	anti-Rabbit AlexaFluor 488;anti-	1:1000; 1:1000	Invitrogen (A11034); Invitrogen	RRID: AB_2576217; RRID:	
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Teratoma formation SOX1, NESTIN, eSOX2 bp Fwd: AGC CAT ATG GTA TGT CCG C PAX6, EOMES, T, GATA4, 70 –150 Rev: TAG CGT AAA AGG FOX42, SOX17. bp ATA G Proof of three germ layers proof of three germ layers bp ATA G formation Fig. 1H: qRT- TAC TAC markers has to be NESTIN, PAX6, PCR TAC Rev: TTT GTT TGA CAG GC germ layer demonstrated at FABP, SLC1A3; CAA T markers mRNA Mesoderm: EOMES, T; Endoderm: EOMES, T; Fwd: TTC ACA TGT CCC GOTTO Screening HIV 1 + 2 Hepatitis B, N/A ACC AGA Rev: TTT GTT TGA CAG GC CCC Genotype Blood group N/A Markers GTA CC Genotype Blood group N/A Markers GTA CC	Differentiation potential	Embryoid body formation and	Genes expressed in embryoid body:	Fig. 1 <i>G</i> , <i>F</i> , <i>H</i> .			70 –150	ATA G		
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	Genotype	Blood group	N/A		Markers	OCTA	70 150	GTA CC		
info HLA tissue typing N/A bp Rev: ACC TCA GTT TGA (OPTIONAL) GGG AGA GC	additional info (OPTIONAL)	HLA tissue typing	N/A			UC14	70 –150 bp	Rev: ACC TCA G GGG AGA GC	TT TGA ATG CAT	

Table 2

multiple key cellular processes, including transcription, pre-mRNA processing, splicing and translation (Baralle and Romano, 2021). In this work we describe the generation and characterization of a induced pluripotent stem cell line (hiPSCs) derived from a fALS patient carrying the missense c.1127G > A variant in the TARDBP gene. Skin biopsy was performed when the subject was 34 years old and clinically healthy. In this study, we reprogrammed dermal fibroblasts into iPSCs, using three non-integrative episomal vectors containing the reprogramming factors OCT 3/4, SOX2, L-MYC, KLF4, LIN28, Sh-P53 (Okita et al., 2011). DNA sequencing confirmed the presence of the disease-related mutation in the iPSCs line (Fig. 1A). iPSCs showed typical human stem cell-like morphology and they were manually picked and expanded for further characterization (Fig. 1B). The silencing of exogeneous reprogramming factors was confirmed through quantitative real-time PCR (qRT-PCR) in iPSCs after ten passages using, as positive control, the nucleofected fibroblasts and as negative control a already hiPSC published line named CSSi011-A (6534) (D'Anzi et al., 2020) (Fig. 1C). hiPSC colonies (passage 12) expressed the pluripotency transcription factor OCT4 and the cell surface antigen TRA-1-60, as shown by immunofluorescence staining (Fig. 1D). After ten passages, the expression of endogenous stemness markers LIN28, OCT4, KLF4, SOX2, L-MYC was detected,

	CAG TGT GC
	Fwd: GGC ATC CTC ACC CTG AAG
	TA
70 –150	
bp	Rev: GGG GTG TTG AAG GTC TCA
	AA
	Hs01057642_s1
	Hs04187831_g1
	Hs00240871_m1
	Hs00610080_m1
	Hs00172872_m1

(continued on next page)

LIN28

L-MYC

SOX2

β-ACTIN

SOX1

NESTIN

PAX6

T EOMES

House-Keeping

Differentation

markers

Genes

70 -150

70 –150

70 –150

bp

bp

bp

GTA CC

GGG AGA GC

 $CCT \; GCT \; CC$

CGT GAT G

ACC AGA

Rev: ACC TCA GTT TGA ATG CAT

Fwd: GCG AAC CCA AGA CCC AGG

Rev: CAG GGG GTC TGC TCG CAC

Fwd: TTC ACA TGT CCC AGC ACT

Rev: TCA CAT GTG TGA GAG GGG

Table 2 (continued)

Antib	Antibodies used for immunocytochemistry/flow-cytometry				
Antib	ody Dilution	n Company Cat RRID #			
GATA	4	Hs00171403_m1			
FOXA	2	Hs00232764_m1			
SOX12	7	Hs00751752_s1			
β-ΑСΤ	IN	Hs 99999903_m1			

RRID Requirement for antibodies: use https://antibodyregistry.org/ to retrieve RRID for antibodies and include ID in table as shown in examples.

through qRT-PCR, using parental fibroblast cells as negative control (Fig. 1E). The hiPSC lines (passage 13) could differentiate into all three germ layers through the formation of teratoma, providing evidence for their in vivo differentiation potential (Fig. 1F), and through the formation of embryoid bodies (Fig. 1G), providing evidence for their in vitro differentiation potential Through qRT-PCR, the gene expression of the embryoid bodies was evaluated by comparing them with iPS cells (Fig. 1H). Cytogenetic analysis confirmed a normal female karyotype (46, XX), at 5th passage (Fig. 1I). Short tandem repeat (STR) analysis confirmed that parental fibroblasts and iPSCs (passage 15) were both from the same patient. iPS cell line was negative for Mycoplasma contamination (Supplementary Fig. 1).

4. Materials and methods

4.1. Cells culture and reprogramming

Fibroblasts derived from skin biopsy were cultured in DMEM high glucose, 20% FBS, 2mML-glutamine and 1% penicillin-streptomycin(all reagents from Sigma Aldrich) at 37 °C, 5% CO2. Subsequently, 3×10^5 fibroblasts were nucleofected at passage 4, using the Nucleofector program "FF113", with 3 µg 1:1:1 mix of the episomal plasmids pCXLE-hUL (Addgene #27080), pCXLEhSK (Addgene #27078) and pCXLE-hOCT4shp53 (Addgene #27077). On day 7, the nucleofected fibroblasts were plated on Matrigel (1:100) (BD Biosciences) and cultured in NutristemXF medium (Biological Industries). The hiPSC colonies were picked and expanded under feeder-free conditions and passaged through manual picking in NutristemXF medium, cells were collected after passage XIII for all characterizations. Absence of mycoplasma contamination was verified using N-Garde Mycoplasma PCR kit (EuroClone). For amplification, the kit provides a reaction mixture containing all ingredients necessary for PCR, including the positive control. 1 Kb plus was used as ladder in the running. After ten passages, clearance of the exogenous reprogramming factors was confirmed by qRT-PCR Table 1.

4.2. Embryoid body formation and teratoma formation assay

Mechanically detached iPSCs were plated in Petri dishes in NutristemXF medium, which was substituted with differentiation medium: DMEM/F12, 20% KOSR (Gibco), 0,1mM NEAA, 0.1 mMβ-mercaptoethanol, 1% Pen/Strep the following day. Fourteen days later, the EBs were collected and RNAs were extracted for qRT-PCR analysis. iPSCs derived from six well plates (approximately $6x10^6$ cells), combined with a Matrigel matrix (Corning, Inc., USA), were injected into the right flank of nude mice. After 1 month, tumors were collected for histological analysis to check their in vivo differentiation capacity into derivatives of all three germ layers.

4.3. Immunofluorescence staining

Cells at passage XIII were fixed with 4% paraformaldehyde for 20' at room temperature and blocked in PBS containing 20% Normal Goat Serum. 0.1% Triton X-100 was used for 30 min for only OCT4 staining. Primary antibodies against OCT4 and TRA1-60, diluted in 5% BSA, were incubated O/N at 4 $^\circ$ C. After washing, Alexa-Fluor-conjugated

secondary antibodies were added for 1 h at room temperature. Cellular nuclei were stained with Hoechst. Microphotographs were taken using a Nikon C2 fluorescence microscope.

4.4. Real-Time PCR analysis

Total RNAs were extracted using Trizol reagent (Life Technology) and cDNAs were synthesized using the High capacity cDNA-RT (Life-Technology) following manufacturer's recommendations. qPCR analysis was performed in three minimum independent biological experiments with TaqMan primers (Table 2) for three germ layers (Thermo Fischer Scientific) and SyBr green primers (Table 2) for stemness markers according to the manufacturer's protocol. The expression ratio of the target genes was calculated by using the $2^{-\Delta Ct}$ method, considering Actin as reference gene.

4.5. STR analyses

DNAs of fibroblasts and iPSCs were extracted by Dneasy blood and tissue kit (QIAGEN). PCR amplification of 16 distinct STRs (D3S1358, TH01, D21S11, D18S51, D10S1248, D1S1656, D2S1338, D16S539, D22S1045, vWA, D8S1179, FGA, D2S441, D12S391, D19S433, SE33) was carried out using the PowerPlex® ESX 17 Fast System (Promega), PCR products were separated on an ABI Prism 3130 DNA sequencer and analyzed by GeneMapper IDX v3.2 (Applied Biosystems).

4.6. Sequencing

Genomic DNA was extracted from both iPSCs and fibroblasts using ReliaPrepTM Blood gDNA Miniprep System. TARDBP exon 6 was amplified by PCR using the following primers: Forward: 5-GACTGAAA-TATCACTGCTGCTGTT-3, Reverse: 5'-GATCCCCAACCAATTGCTGC-3'. The amplicon was sequenced by BigDye terminator v.3.1 Cycle Sequencing kit on ABI 3130XL Genetic Analyzer.

4.7. Karyotype analysis

iPSCs, at passage 8, were cultured with Nutristem XF medium in T25 flasks for 2–3 days. Karyotype analysis of metaphase chromosomes was performed using G-banding, in house. Fifteen metaphases were counted and three karyograms analyzed.

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.2022.102835.

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