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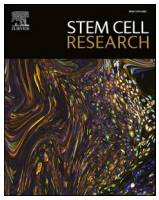

Lab Resource: Single Cell Line

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

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A B S T R A C T

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:

Unique stem cell line identifier	https://hpscreg.eu/user/cellline/edit/CSSi013-A
Alternative name(s) of stem cell line	*CSSi013-A cl.D*
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:34* *Sex: Female* *Ethnicity if known: Caucasian/Italian*
Cell Source	*Dermal fibroblasts*
Clonality	*Clonal*
Associated disease	*Amyotrophic lateral sclerosis*
Gene/locus	TARDBP: c.1127G > A
Date archived/stock date	*June 2019*
Cell line repository/bank	https://hpscreg.eu/user/cellline/edit/CSSi013-A
Ethical approval	*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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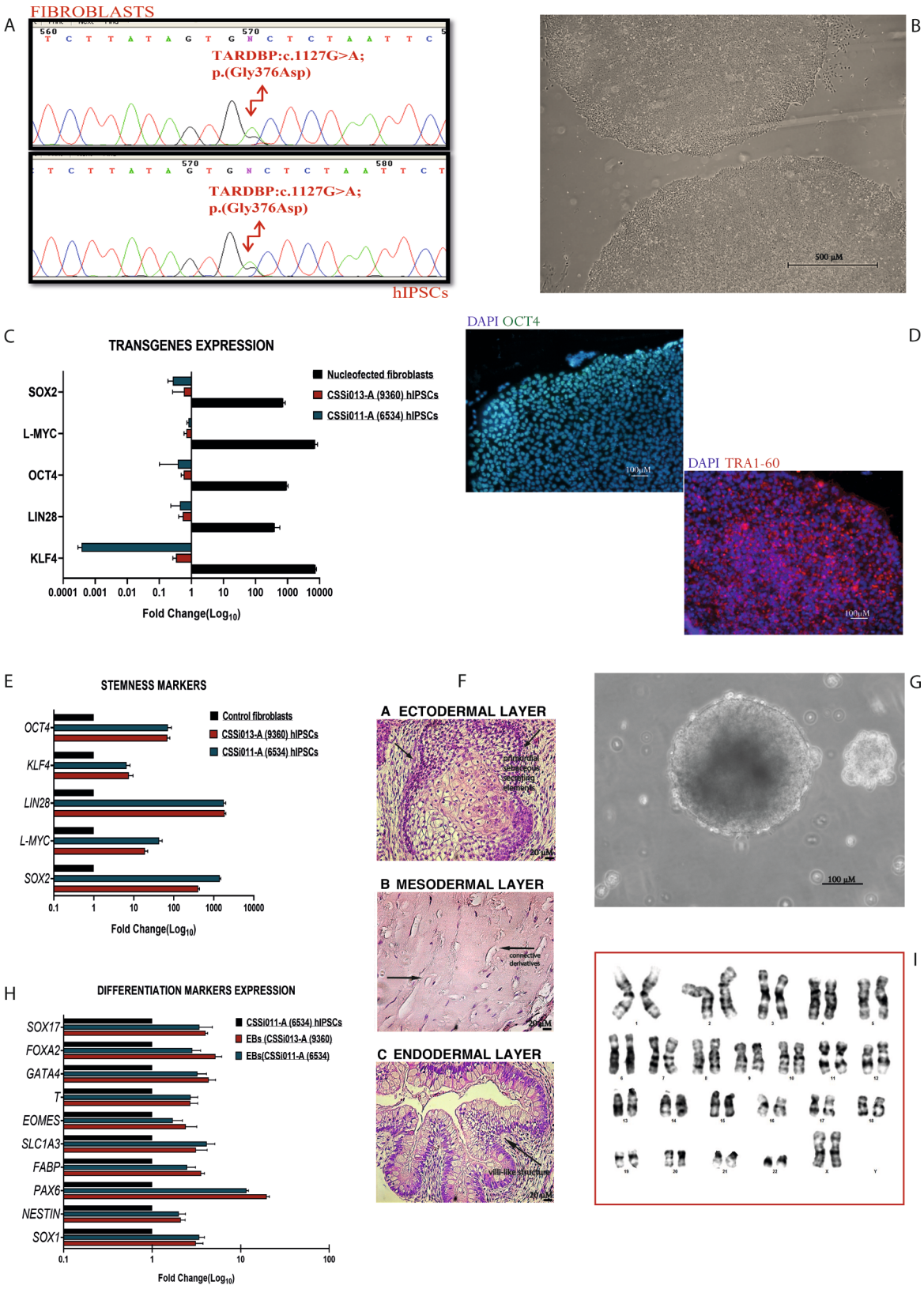

Fig. 1. Characterization of iPSC line from an asymptomatic subject carrying a heterozygous mutation in TDP-43 protein. (A) DNA sequencing confirmed the presence of the disease-related mutation in the iPSCs line; (B) Morphology of iPSC culture, scale bar = 500 μm; (C) Real-time PCR confirmed the absence of exogenous reprogramming factors; (D) Immunofluorescence staining for TRA1-60 and OCT4, scale bar = 100 μm; (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 μm; (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	Photography	*Normal*	Fig. 1*B*
Phenotype	*Immunocytochemistry*	*Staining pluripotency markers: Oct4, Tra 1–60.*	Fig. 1*D*
	qRT-PCR	*Expression of pluripotency markers: OCT4, LIN28, L-MYC, KLF4, SOX2.*	Fig. 1*E*
Genotype	Karyotype (G-banding) and resolution	*46XX, Resolution 450–500*	Fig. 1*I*
Identity	STR analysis	*16 sites tested, all matched.*	*Submitted in archive with journal.*
Mutation analysis (IF APPLICABLE)	Sequencing	*Heterozygous mutation*	Fig. 1*A*
Microbiology and virology	Mycoplasma	*Mycoplasma testing by N-Garde Mycoplasma PCR kit (EuroClone) is Negative.*	*Supplementary* Fig. 1
Differentiation potential	Embryoid body formation and Teratoma formation	*Genes expressed in embryoid body: SOX1, NESTIN, PAX6, EOMES, T, GATA4, FOXA2, SOX17. Proof of three germ layers formation*	Fig. 1*G, F, H.*
List of recommended germ layer markers	**Expression of these markers has to be demonstrated at mRNA**	*Ectoderm: SOX1, NESTIN, PAX6, FABP, SLC1A3; Mesoderm: EOMES, T; Endoderm: GATA4, FOXA2, SOX17.*	Fig. 1*H: qRT-PCR*
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*	

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,

Table 2
Reagents details.

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

(*continued on next page*)

Table 2 (*continued*)

Antibodies used for immunocytochemistry/flow-cytometry			
Antibody	**Dilution**	**Company Cat #**	**RRID**
GATA4		*Hs00171403_m1*	
FOXA2		*Hs00232764_m1*	
SOX17		*Hs00751752_s1*	
β-ACTIN		*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-hOCT4-shp53 (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 °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 ReliaPrep™ Blood gDNA Miniprep System. TARDBP exon 6 was amplified by PCR using the following primers: Forward: 5-GACTGAAATATCACTGCTGCTGTT-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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