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Lab Resource: Single Cell Line

Generation of an induced pluripotent stem cell line (CSS012-A (7672)) carrying the p.G376D heterozygous mutation in the TARDBP protein

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A B S T R A C T

Amyotrophic lateral sclerosis (ALS) is an incurable neurodegenerative condition with phenotypic and genetic heterogeneity. It is characterized by the selective vulnerability and the progressive loss of the neural population. Here, an induced pluripotent stem cell (iPSC) line was generated from dermal fibroblasts of an individual carrying the p.G376D mutation in the TDP-43 protein. Fibroblasts were reprogrammed using non-integrating episomal plasmids. There were no karyotype abnormalities, and iPSCs successfully differentiated into all three germ layers. This cell line may prove useful in the study of the pathogenic mechanisms that underpin ALS syndrome.

1. Resource Table

Please fill in right-hand column of the table below. All information requested in the table is MANDATORY, except where otherwise indicated. Manuscripts with incomplete or incorrect information will be sent back to author.

Unique stem cell line identifier	CSSi012-A (7672) https://hpscreg.eu/cell-line/CSSi012-A
Alternative name(s) of stem cell line	*GAFU cl.A*
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: 38* *Sex: Male* *Ethnicity: Caucasian/Italian*
Cell Source	*Dermal Fibroblasts*
Clonality	*Clonal*
Associated disease	*Amyotrophic lateral sclerosis*
Gene/locus	*TARDBP: c.1127G > A*

(*continued on next column*)

(*continued*)

Unique stem cell line identifier	CSSi012-A (7672) https://hpscreg.eu/cell-line/CSSi012-A
Date archived/stock date	*April 2019*
Cell line repository/bank	*HPSC registry*
Ethical approval	Università Cattolica del Sacro Cuore A.1320/CE/2012

2. Resource utility

Amyotrophic lateral sclerosis is a neurodegenerative condition, and it is recognized as a very complex disease. iPSCs technology may be particularly important to elucidate this disorder, allowing us to deepen our knowledge on the mutated gene's pathological influence on brain development and function (Table 1).

3. Resource details

Amyotrophic lateral sclerosis (ALS) is an incurable neurodegenerative condition characterized by the selective vulnerability and the

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Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography	*Normal*	Fig. 1*A*
Phenotype	*Immunocytochemistry*	*Staining of pluripotency markers: Oct4; Tra1-60.*	Fig. 1*D*
	qRT-PCR	*Expression of pluripotency markers: OCT4, LIN28, L-MYC, KLF4, SOX2*	Fig. 1*E*
Genotype	Karyotype (G-banding) and resolution	46 XY, Resolution 450–500	Fig. 1*I*
Identity	STR analysis	*19 sites tested, all matched*	*With Authors*
Mutation analysis (IF APPLICABLE)	Sequencing	*Heterozygous mutation*	Fig. 1*B*
Microbiology and virology	Mycoplasma	*Mycoplasma tested by N-Garde Mycoplasma PCR kit (EuroClone) is 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. 1*F, G, 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*G*: *qRT-PCR*
Donor screening (OPTIONAL)	HIV 1 + 2 Hepatitis B, Hepatitis C	*N/A*	
Genotype additional info (OPTIONAL)	Blood group genotyping	*N/A*	ì
	HLA tissue typing	*N/A*	

progressive loss of the neural population (1). It affects 1–1.8/100,000 individuals worldwide, and the number of cases is projected to increase as the population ages. Despite the significant advances in the understanding of the molecular and the genetic aspects of ALS, the causes and the mechanisms of the neurodegenerative process typical of this disease are still unknown. Among the numerous defective genes associated with ALS, we focused our attention on the TARDBP gene (transactive response DNA binding protein) (2). Fibroblast cells were isolated from a patient with familial ALS carrying the missense c.1127G > A variant in the TARDBP gene. Skin biopsy was performed six months after disease onset, when the patient was 38 years old. The patient had a spinal onset and a flail arm phenotype. The disease showed an aggressive course, and the patient underwent tracheostomy 15 months after the onset. Death occurred six years after tracheostomy at the age of 44. iPSCs were generated by a non-integrating episomal plasmid-based method through expression of the reprogramming factors oct4, sox2, klf4, l-myc, lin28 and p53 shRNA (3). iPSC colonies were manually picked and expanded in culture over several passages for further characterisation. The established iPSC line showed typical human stem cell-like morphology as judged by brightfield microscopy (Fig. 1A). Sequencing analysis confirmed the presence of the missense TARDBP variant found in the parental fibroblast line (Fig. 1B). Quantitative real-time PCR (qRT-PCR) analysis confirmed that the reprogramming factors were no longer present in iPSCs after ten passages using, as positive control, the fibroblasts after one week from episomal nucleofection (Fig. 1C). iPSC line (pVII) expressed the pluripotency marker OCT4 and the endogenous cell surface marker TRA-1-60, as shown by immunofluorescence staining (Fig. 1D). The expression of endogenous stemness markers lin28, oct4, klf4, sox2, L-myc was detected by qRT-PCR after ten passages, using fibroblast cells as negative control (Fig. 1E). Mechanically splitted iPSC colonies, at 10th passage, spontaneously differentiated into embryoid bodies, representative of the three embryonic germ layers (Fig. 1F), as confirmed through qRT-PCR in which the gene expression was calculated by comparing the embryoid bodies with iPS cells (Fig. 1G). Furthermore, the teratoma assay demonstrated the pluripotent potential of the iPS cells, after XIV passages, to differentiate into endoderm, mesoderm and ectoderm (Fig. 1H), in vivo. Short tandem repeat (STR) analysis confirmed that parental fibroblasts and iPSCs (pXI) were both from the same patient. Chromosomal analysis showed a normal karyotype (46, XY), at 7th passage (Fig. 1I). iPS cell line was negative for Mycoplasma contamination (Supplementary Fig. 1).

4. Materials and methods

4.1. Fibroblast culture and reprogramming

Fibroblasts were cultured in Dulbecco's Modified Eagle Medium High Glucose supplemented with 20% FBS, 2 mM L-Glutamine, 100 U/ml Penicillin-Streptomicin and 1 × Non-Essential Amino Acids (Sigma Aldrich) at 37 °C, 5% CO_2. 1 × 10^5 fibroblasts were nucleofected with 4D-Nucleofector™ X unit (Lonza), FF113 program, using P2 buffer with a mix of the episomal plasmids pCXLE-hUL (Addgene #27080), pCXLE-hSK (Addgene #27078) and pCXLE-hOCT4-shp53 (Addgene #27077). Seven days after nucleofetion, fibroblasts were plated on Matrigel (Corning) and cultured in Nutristem XF medium (Biological Industries). The hiPSC colonies were picked according to their hESC-like colony morphology and expanded under feeder-free conditions. Absence of mycoplasma contamination was verified using N-Garde Mycoplasma PCR kit (EuroClone). After ten passages, the absence of the exogenous reprogramming factors was confirmed by qRT-PCR.

4.2. Embryoid body and teratoma formation assays

The hiPSc were picked and plated in a Petri dish in floating conditions. Nutristem-XF medium was gradually switched with 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, combined with a Matrigel substrate, were injected into the flank of nude mice. After 1 month, teratomas were collected for histological analysis.

4.3. Real-time PCR analysis

Total RNAs were isolated using Trizol reagent (Life Technology) following the manufacturer's recommendations and cDNA synthesized using the High capacity cDNA RT (Life Technology). qPCR analysis was performed in three minimum independent biological experiments. Three germ layers were analyzed through TaqMan primers (Table 2), pluripotency markers through Sybergreen primers (Table 2). The expression ratio of the target genes was calculated by using the 2-ΔΔCt method, considering *β-ACTIN* as the reference gene.

4.4. STR analyses

DNAs of fibroblasts and iPSCs were extracted by Dneasy blood and tissue kit (QIAGEN). PCR amplification of 19 distinct STRs (D13S252, D13S305, D13S634, D13S800, D13S628, D18S819, D18S535, D18S978, D18S386, D18S390, D21S11, D21S1437, D21S1409, D21S1442,

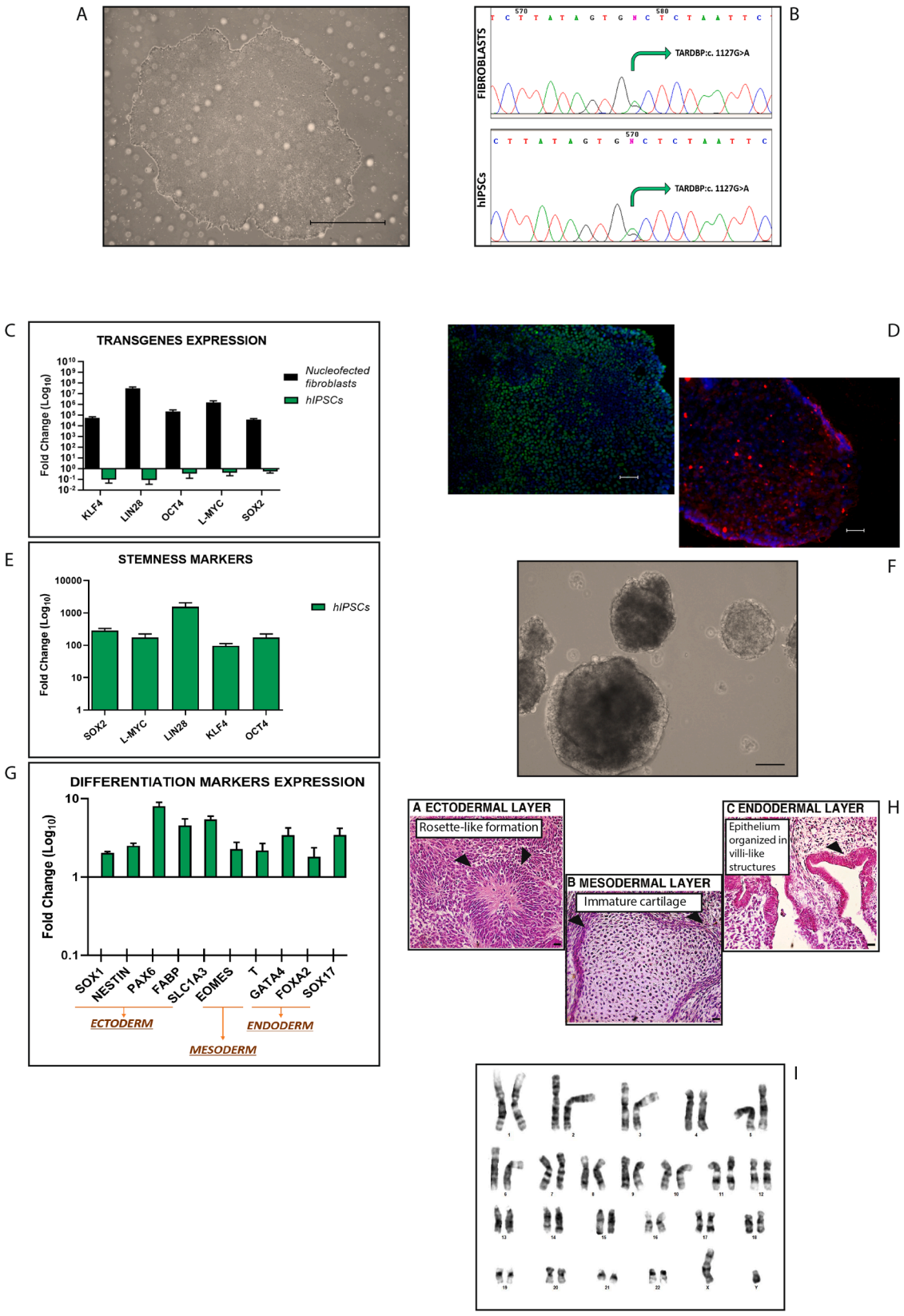

Fig. 1. Characterization of **CSSi012-A (7672)**. A. Phase contrast image of the iPSC line morphology. B. DNA sequencing analysis of (TARDBP): c.1127G>A mutation. Green arrow indicate mutation site. C. Transgene expression analysis through RT-PCR demonstrates the loss of episomal vectors during amplification. D. Representative immunofluorescent picture of IPSCs showing the expression of stem cell markers such as OCT-4 (green) and TRA-1-60 (red), nuclei were stained with Hoechst 33342 (blue). E. Expression analysis of stemness markers in iPSCs with respect to fibroblasts used as reference. F. Phase contrast image of embryoid bodies. G. Expression analysis of differentiation markers in the embryoid bodies. H. Teratoma showing ectodermal, mesodermal and endodermal differentiation. I. Cytogenetic analysis showing a normal karyotype (46, XY).

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; 1:1000e.g. 1:500*	*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*	*Fwd: CAT TCA AAC TGA GGT AAG GG Rev: TAG CGT AAA AGG AGC AAC ATA G*	
	eKLF4	*70–150 bp*	*Fwd: CCA CCT CGC CTT ACA CAT GAA GA Rev: TAG CGT AAA AGG AGC AAC ATA G*	
	eLIN28	*70–150 bp*	*Fwd: AGC CAT ATG GTA GCC TCA TGT CCG C Rev: TAG CGT AAA AGG AGC AAC ATA G*	
	eL-MYC	*70–150 bp*	*Fwd: GGC TGA GAA GAG GAT GGC TAC Rev: TTT GTT TGA CAG GAG CGA CAA T*	
	eSOX2	*70–150 bp*	*Fwd: TTC ACA TGT CCC AGC ACT ACC AGA Rev: TTT GTT TGA CAG GAG CGA CAA T*	
Pluripotency Markers	*OCT4*	*70–150 bp*	*Fwd: CCC CAG GGC CCC ATT TTG GTA CC Rev: ACC TCA GTT TGA ATG CAT GGG AGA GC*	
	LIN28	*70–150 bp*	*Fwd: CCC CAG GGC CCC ATT TTG GTA CC Rev: ACC TCA GTT TGA ATG CAT GGG AGA GC*	
	L-MYC	*70–150 bp*	*Fwd: GCG AAC CCA AGA CCC AGG CCT GCT CC Rev: CAG GGG GTC TGC TCG CAC CGT GAT G*	
	SOX2	*70–150 bp*	*Fwd: TTC ACA TGT CCC AGC ACT ACC AGA Rev: TCA CAT GTG TGA GAG GGG CAG TGT GC*	
	β-ACTIN			

Table 2 (*continued*)

Antibodies used for immunocytochemistry/flow-cytometry				
	Antibody	Dilution	Company Cat #	RRID
House-Keeping Genes		*70-150bp500bp*	*Fwd: GGC ATC CTC ACC CTG AAG TA Rev: 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*	

D21S1435, D21S1446, DXS6803, HPRT, DXS1187) 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).

4.5. Sequencing

Genomic DNA was extracted from 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.

4.6. Karyotype analysis

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

4.7. Immunofluorescence staining

Cells were fixed using 4% paraformaldehyde for 20 min 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. Next, primary antibodies 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.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Jessica Rosati reports financial support was provided by Italian Ministry of Health.

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.2021.102356.