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Generation of an induced pluripotent stem cell line, CSSi011-A (6534), from an Amyotrophic lateral sclerosis patient with heterozygous L145F mutation in SOD1 gene

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A B S T R A C T

Among the known causative genes of familial ALS, *SOD1* mutation is one of the most common. It encodes for the ubiquitous detoxifying copper/zinc binding SOD1 enzyme, whose mutations selectively cause motor neuron death, although the mechanisms are not as yet clear. What is known is that mutant-mediated toxicity is not caused by loss of its detoxifying activity but by a gain-of-function. In order to better understand the pathogenic mechanisms of SOD1 mutation, a human induced pluripotent stem cell (hiPSC) line was generated from the somatic cells of a female patient carrying a missense variation in SOD1 (L145F).

Unique stem cell line identifier	CSSi011-A (6534)
Alternative name(s) of stem cell line	SLA PZ.2 cl1
Institution	Casa Sollievo della Sofferenza – Viale dei Cappuccini, 71013 San Giovanni Rotondo, Foggia, Italy
Contact information of distributor	Jessica ROSATI, j.rosati@css-mendel.it
Type of cell line	iPSC
Origin	human
Additional origin info	Age:48 yrs Sex: female Ethnicity if known: Caucasian/Albanian
Cell Source	Dermal fibroblasts
Clonality	Clonal
Method of reprogramming	Non integrating episomal vectors
Genetic Modification	Yes
Type of Modification	Spontaneous
Associated disease	Amyotrophic lateral sclerosis
Gene/locus	SOD1:c.435G > C
Method of modification	Hereditary
Name of transgene or resistance	N/A
Inducible/constitutive system	N/A
Date archived/stock date	January 2019
Cell line repository/bank	HPSC registry
Ethical approval	Comitato Etico Interaziendale Novara: CE 54/17

1. Resource utility

The iPSC line was derived from an individual carrying the L145F *SOD1* mutation, typical of Mediterranean countries, sharing peculiar clinical features such as lower-limb onset with slow evolution and cognitive involvement, uncommon in *SOD1-ALS* patients. This patient-derived iPSC line will be a useful cellular system for modelling *SOD1* pathogenetic mechanisms (see Table 1).

2. Resource details

Amyotrophic lateral sclerosis (ALS) is a terminal motor neuron disease, characterized by motor neuron degeneration in the primary motor cortex, brainstem and spinal cord, leading to the paralysis of voluntary muscles. The paralysis begins focally and disseminates in a pattern that suggests that degeneration is spreading among contiguous pools of motor neurons. Respiratory failure causes death, which typically occurs within five years of developing this debilitating condition (Pasinelli and Brown, 2006). In about 10% of patients, the disease is

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Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography	*Normal*	Fig. 1A
Phenotype	Immunocytochemistry	***Staining of pluripotency markers: Oct4; Tra-1–60.***	Fig. 1B
	qRT-PCR	***Expression of pluripotency markers: OCT4, LIN28, L-MYC, KLF4, SOX2***	Fig. 1C
Genotype	Karyotype (G-banding) and resolution	***46 XX, Resolution 450–500***	Fig. 1E
Identity	STR analysis	19 sites tested, all matched	With Authors
Mutation analysis (IF APPLICABLE)	Sequencing	*Heterozygous mutation*	Fig. 1I
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. 1F, 1G, 1H
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*	

familial (FALS), while the majority of patients are sporadic cases (SALS) (Chia et al., 2018). Mutations in at least 25 genes are implicated in ALS pathogenesis, with *C9orf72 SOD1, FUS* and *TARDBP* representing the most common mutated genes, accounting for 60% of FALS and 10% of SALS cases (Chia et al., 2018). This study concerned a female patient with a family history of ALS, carrying a missense variation in the *SOD1* gene (L145F). The patient presented progressive weakness in the right foot, followed by mild muscle hypotrophy and weakness in the entire right lower limb, also reporting upper right limb weakness. Fibroblasts derived from a skin biopsy were reprogrammed into iPSCs, using three non-integrative episomal vectors containing the reprogramming factors OCT3/4, SOX2, L-MYC, KLF4, LIN28, sh-p53 (Okita et al., 2011). iPSC colonies displayed typical iPSC morphology, with well-defined edges and normal growth behaviour (Fig. 1A). The expanded colonies were stained for the endogenous TRA-1–60, a protein expressed on the surface of iPSCs, and for the homeodomain transcription factor OCT-3/4, demonstrating iPSC pluripotency (Fig. 1B), further confirmed through qRT-PCR (Fig. 1C). We examined the presence of episomal plasmid DNA in this cell line, using primers specific to a common sequence present in all three reprogramming plasmids. We observed that, after 10 passages, the iPSCs were devoid of vector sequences, as shown by RT-qPCR, using the fibroblasts one week from episomal nucleofection as positive controls (Fig. 1D). Genomic stability of the iPSCs was confirmed through karyotype, which provided a normal diploid 46, XX chromosome arrangement without any detectable abnormalities (Fig. 1E). The differentiation potential was demonstrated *in vitro* by the formation of embryoid bodies (EBs) (Fig. 1F) and *in vivo* by teratoma formation (Fig. 1G). The image showed: ectodermal layer represented by primitive epidermal tissue composed by cells characteristically lined up to form palisade-like structures bordered by loose connective layer (typical organization of epithelial tissue), mesodermal layer showed by the presence of loose connective tissue, typically composed by abundant extracellular matrix (pink, eosin stained) with few cellular elements (dark violet, hematoxylin stained) and endodermal layer, represented by a pluri-stratified epithelium organized in villi-like structures characterized by batiprismatic cellular elements. qRT-PCR analysis showed endogenous expression of the three germ layer markers in the embroyoid bodies (Fig. 1H). PCR-based detection tests confirmed the absence of Mycoplasma contamination at this stage (Supplementary Fig. 1). In addition, Short Tandem Repeat (STR) profiling confirmed that these iPSC lines had the same genetic identity as the donor's fibroblasts (data available from the authors). The presence of the heterozygous c.435G > C (NM_000454.4) mutation harboured by parental fibroblasts was confirmed in the generated IPS cell line through Sanger sequencing (Fig. 1I).

3. Materials and methods

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% CO_2. Subsequently, 3×10^5 fibroblasts were nucleofected, using the Nucleofector program named "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. 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 was used as ladder in the running. After ten passages, clearance of the exogenous reprogramming factors was confirmed by qRT-PCR.

4. Embryoid body formation

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 mM β-mercaptoethanol, 1% Pen/Strep the following day. Fourteen days later, the EBs thus obtained were pelletted and RNAs were extracted for qRT-PCR analysis.

5. Teratoma formation

iPSCs derived from six well plates (approximately $6x10^6$ cells) combined with a Matrigel substrate (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.

6. Real-Time PCR analysis

Total RNAs were extracted using Trizol reagent (Life Technology) and cDNA synthesized using the High capacity cDNA RT (LifeTechnology) 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 $2 - \Delta Ct$

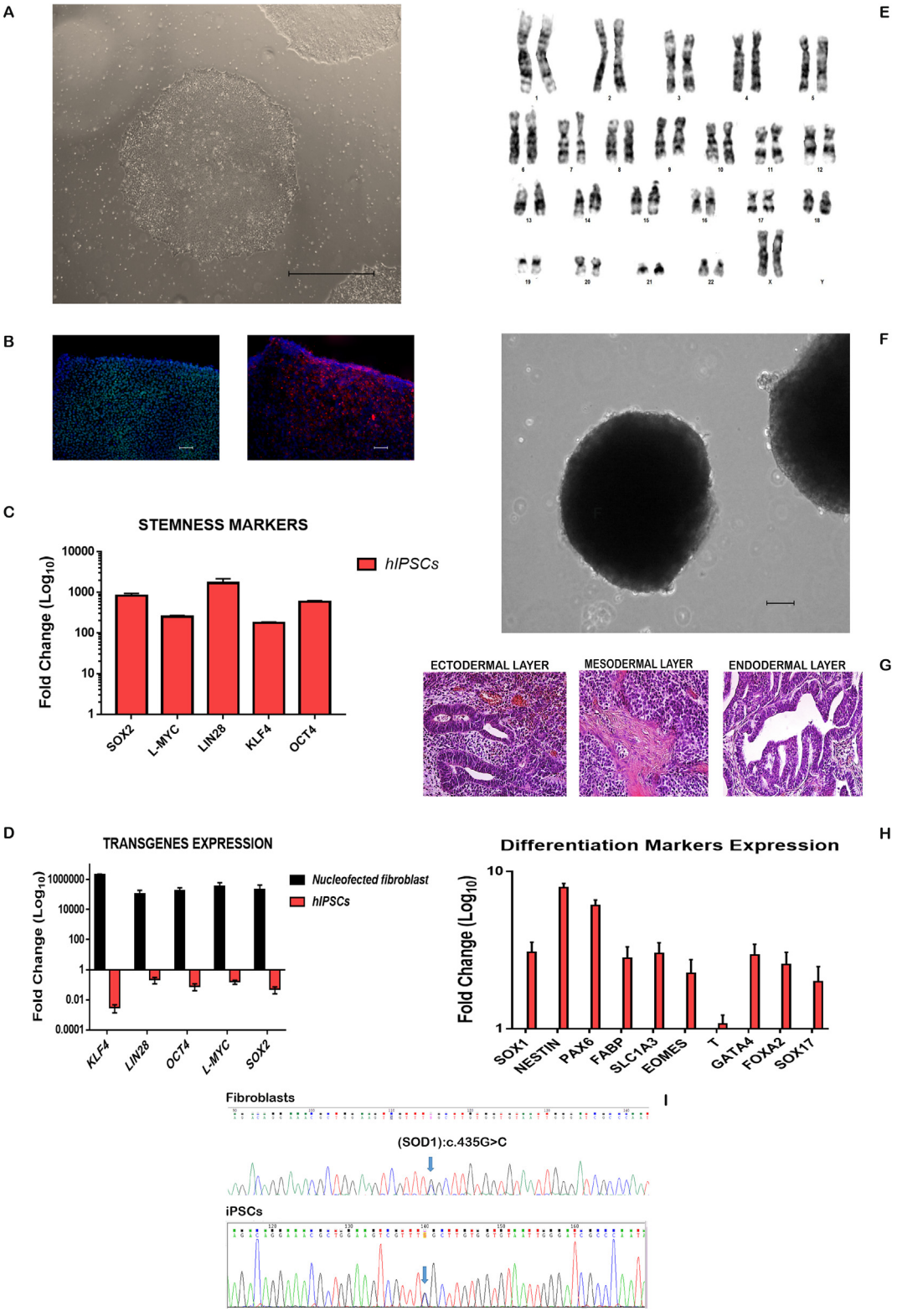

Fig. 1. Characterization of CSSi011-A (6534) A. Phase contrast image of the iPSC morphology. B. Representative immunofluorescent images of iPSCs marked for stem cell markers, OCT4 (green) and TRA-1-60 (red). Nucleus is labelled with Hoechst 33342 (blue). C. Expression analysis of stemness markers D. qRT-PCR analysis of transgene expression showing the loss of episomal vectors during iPSC amplification. E. Cytogenetic analysis showing a normal karyotype (46, XX). F. Phase contrast image of embryoid bodies. G. Teratoma showing a normal ectodermal, mesodermal and endodermal differentiation. H. Expression analysis of differentiation markers. I. DNA sequencing analysis of (SOD1):c.435G > C mutation. Blue arrows indicate mutation site.

Table 2
Reagents details. RRID Requirement for antibodies: use http://antibodyregistry.org/ to retrieve RRID for antibodies and include ID in table as shown in examples.

Antibodies used for immunocytochemistry/flow-citometry			
	Antibody	Dilution	Company Cat # and RRID
Pluripotency Markers	*Rabbit anti-OCT4 Mouse anti-TRA-1-60*	*1:100 1:100*	*Life technologies (A13998) RRID: AB_2534182 Life technologies (411000) RRID: AB_2533494*
Secondary antibodies	*anti-Rabbit AlexaFluor 488 anti-Mouse AlexaFluor 555*	*1:10000 1:10000*	*Invitrogen (A11034) RRID: AB_2576217 Invitrogen (A21422) RRID: AB_2535844*

SyBr green Primers used for qPCR	Target	Forward/Reverse sequence (5′-3′)	*(411000)*
Episomal genes	*eOCT4*	*Fwd: CAT TCA AAC TGA GGT AAG GG Rev: TAG CGT AAA AGG AGC AAC ATA G*	
	eKLF4	*Fwd: CCA CCT CGC CTT ACA CAT GAA GA Rev: TAG CGT AAA AGG AGC AAC ATA G*	
	eLIN28	*Fwd: AGC CAT ATG GTA GCC TCA TGT CCG C Rev: TAG CGT AAA AGG AGC AAC ATA G*	
	eL-MYC	*Fwd: GGC TGA GAA GAG GAT GGC TAC Rev: TTT GTT TGA CAG GAG CGA CAA T*	
	eSOX2	*Fwd: TTC ACA TGT CCC AGC ACT ACC AGA Rev: TTT GTT TGA CAG GAG CGA CAA T*	
Pluripotency markers	*OCT4*	*Fwd: CCC CAG GGC CCC ATT TTG GTA CC Rev: ACC TCA GTT TGA ATG CAT GGG AGA GC*	
	LIN28	*Fwd: CCC CAG GGC CCC ATT TTG GTA CC Rev: ACC TCA GTT TGA ATG CAT GGG AGA GC*	
	L-MYC	*Fwd: GCG AAC CCA AGA CCC AGG CCT GCT CC Rev: CAG GGG GTC TGC TCG CAC CGT GAT G*	
	SOX2	*Fwd: TTC ACA TGT CCC AGC ACT ACC AGA Rev: TCA CAT GTG TGA GAG GGG CAG TGT GC*	
House-Keeping Gene	*β-ACTIN*	*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*

method, considering 18S as reference gene.

7. Sequencing

Genomic DNA was extracted from iPSC and parental fibroblasts using *ReliaPrep*™ Blood gDNA Miniprep System. *SOD1* exon 5 was amplified by PCR using the following primers: Forward: 5′-TTGTTGGGA GGAGGTAGTG-3′, Reverse: 5′-AAAGCAACTCTGAAAAAGT-3′. The amplicon was sequenced by BigDye terminator v.3.1 Cycle Sequencing kit on ABI 3130XL Genetic Analyzer.

8. Immunofluorescence staining

Cells were fixed using 4% paraformaldehyde and stained. The cells were incubated with Blocking Buffer (PBS containing 20% Normal Goat Serum, 0.1% Triton X-100) for 30 min at room temperature. Next, primary antibodies, listed in Table 2, diluted in blocking buffer were added and incubated O/N at 4 °C. After extensive washing, Alexa Fluor 594- and/or Alexa Fluor 488-conjugated secondary antibodies were added 1 h at room temperature. Cellular nuclei were counterstained with DAPI. Microphotograps were taken using a Nikon C2 fluorescence microscope and NIS Elements 1.49 software.

9. STR analysis

Fibroblasts and iPSCs DNA was 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, D21S1435, D21S1446, DXS6803, XHPRT, 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).

10. Karyotyping

iPSCs were cultured in chamber slides (Thermo Fisher Scientific) coated with Matrigel (1:100) in NutristemXF medium for 2–3 days. Cells were blocked in metaphase by adding 0.1 μg/ml of COLCEMID solution (Thermo Fisher Scientific) to culture medium for 60 min at 37 °C and by adding hypotonic KCl solution (30 mM) in 10%FBS at 37 °C for 6 min. Cells were fixed in a cold fresh-made 3:1 ethanol:acetic acid solution. Karyotype analysis was carried out on GTG-banded metaphases. Fifteen metaphases were counted and three karyotypes analyzed. Only clonal aberrations were considered, following ISCN recommendations.

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.2020.101924.

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