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Lab Resource: Single Cell Line

Generation of an induced pluripotent stem cell line (UCSCi002-A) from a patient with a variant in *TARDBP* gene associated with familial amyotrophic lateral sclerosis and frontotemporal dementia

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A B S T R A C T

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease that selectively affects motor neurons. In 20% of cases, ALS appears in comorbidity with frontotemporal dementia (FTD). We generated patient-derived-induced Pluripotent Stem Cells (iPSCs), from an ALS/FTD patient. The patient had a familial form of the disease and a missense variant in *TARDBP* gene. We used an established protocol based on Sendai virus to reprogram fibroblasts. We confirmed the stemness and the pluripotency of the iPSC clones, thus generating embryoid bodies. We believe that the iPSC line carrying a *TARDBP* mutation could be a valuable tool to investigate TDP-43 proteinopathy linked to ALS.

Resource Table:

Unique stem cell line identifier	*UCSCi002-A*
Alternative name(s) of stem cell line	*LIF-1989*
Institution	*Università Cattolica del Sacro Cuore- Fondazione Policlinico Universitario A.Gemelli IRCCS*
Contact information of distributor	*Serena Lattante, email: serena.lattante@unicatt.it*
Type of cell line	*iPSC*
Origin	*Human*
Additional origin info required for human ESC or iPSC	*Age:65* *Sex: F* *Ethnicity if known: Caucasian/ Italian*
Cell Source	*Dermal fibroblasts*
Clonality	*Clonal*
Associated disease	*Amyotrophic Lateral Sclerosis with Frontotemporal Dementia*
Gene/locus	*TARDBP c.995G > A*
Unique stem cell line identifier	*UCSCi002-A*
Date archived/stock date	*04/11/2021*
Cell line repository/bank	*https://hpscreg.eu/user/cellline/edit/UCSCi002-A*
Ethical approval	*Ethical Committee "Fondazione Policlinico Universitario Agostino Gemelli IRCCS", Protocol n. 0036530/19 (28/08/2019)*

1. Resource utility

Amyotrophic lateral sclerosis and frontotemporal dementia (ALS/FTD) exist as a disease spectrum as they share not only clinical signs but also pathomechanisms and genetic causes. Generating iPSCs from an ALS/FTD patient with a pathogenic variant in *TARDBP* could help in the investigation of TDP-43 proteinopathy. Table 1.Table 2.

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Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	*Brightfield microscopy*	*Normal*	Figure 1 *panel A*
Phenotype	*Immunocytochemistry*	*Staining of pluripotency markers: Oct4, Sox2, Tra 1–60, SSEA-4*	Figure 1 *panel C*
	RT-qPCR	*Expression of pluripotency markers: OCT4, LIN28, LMYC, SOX2 (qPCR Sybr Green)*	Figure 1 *panel D*
Genotype	*Array-CGH*	*46XX Resolution 8X60K*	*With authors*
Identity	*STR analysis*	*23 sites tested, all matched*	*With authors*
Mutation analysis	*Sequencing*	*Heterozygous, missense variant*	Figure 1 *panel B*
	Southern Blot OR WGS	*N/A*	
Microbiology and virology	*Mycoplasma*	*Mycoplasma tested by NGarde Mycoplasma PCR kit (EuroClone). Negative*	*Supplementary* Figure 1*A*
Differentiation potential	*Embryoid body formation*	*Expression of the three embryonic germ layer markers demonstrated at mRNA level (by Taqman Scorecard) Ectoderm:* DMBX1/OTX3, EN1, LMX1A, NR2F2, PAX3, PAX6, SDC2, TRPM8, WNT1, ZBTB16 Endoderm: AFP, CABP7, CDH20, FOXA1, GATA4, HHEX, HMP19, HNF4A, KLF5, PHOX2B, PRDM1, RXRG, SOX17, SST Mesoderm: ABCA4, ALOX15, BMP10, CDH5, CDX2, ESM1, FCN3, FOXF1, HAND1, HAND2, HEY1, HOPX, IL6ST, ODAM, PDGFRA, PLVAP, RGS4, SNAI2, TBX3, TM4SF1)	Figure 1 *panel E and F*
Donor screening	HIV 1 + 2 Hepatitis B, Hepatitis C	*N/A*	
Genotype additional info (OPTIONAL)	Blood group genotyping	*N/A*	
	HLA tissue typing	*N/A*	

Table 2
Reagents details.

	Antibodies used for immunocytochemistry			
	Antibody	**Dilution**	**Company Cat #**	**RRID**
Pluripotency Markers	*Rabbit anti-OCT4*	*1:200*	*Thermo Fisher Scientific Cat# A24867*	*RRID: AB_2650999*
	Mouse anti-SSEA4	*1:100*	*Thermo Fisher Scientific Cat# A24866*	*RRID: AB_2651001*
	Rat anti-SOX2	*1:100*	*Thermo Fisher Scientific Cat# A24759*	*RRID: AB_2651000*
	Mouse anti-TRA-1–60	*1:100*	*Thermo Fisher Scientific Cat# A24868*	*RRID: AB_2651002*
Secondary antibodies	*Alexa Fluor™ 555 donkey anti-rabbit*	*1:250*	*Thermo Fisher Scientific Cat# A24869*	*RRID: AB_2651006*
	Alexa Fluor™ 488 goat anti-mouse IgG3	*1:250*	*Thermo Fisher Scientific Cat# A24877*	*RRID: AB_2651008*
	Alexa Fluor™ 488 donkey anti-rat	*1:250*	*Thermo Fisher Scientific Cat# A24876*	*RRID: AB_2651007*
	Alexa Fluor™ 555 goat anti-mouse IgM	*1:250*	*Thermo Fisher Scientific Cat# A24871*	*RRID: AB_2651009*
	Primers			
	Target	**Size of band (bp)**	**Forward/Reverse primer (5′-3′)**	
Sendai virus vector (RT-PCR)	*Sendai virus*	*181*	*GGA TCA CTA GGT GAT ATC GAG C/ ACC AGA CAA GAG TTT AAG AGA TAT GTA TC*	
Pluripotency Markers (qPCR)	*LIN28*	*143*	*CCC CAG GGC CCC ATT TTG GTA CC/ ACC TCA GTT TGA*	
	OCT4	*143*	*ATG CAT GGG AGA GC CCC CAG GGC CCC ATT TTG*	
	L-MYC	*143*	*GTA CC/ ACC TCA GTT TGA ATG CAT GGG AGA GC*	
	SOX2	*80*	*GCG AAC CCA AGA CCC AGG CCT GCT CC/ CAG GGG GTC TGC TCG CAC CGT GAT G TTC ACA TGT CCC AGC ACT ACC AGA/TCA CAT GTG TGA GAG GGG CAG TGT GC*	
House-Keeping Genes (qPCR)	*GAPDH*	*207*	*GGC TGG GGC TCA TTT GCA/ GTC ATG AGT CCT TCC ACG ATA CC*	
Targeted mutation analysis	*TARDBP-Ex6*	*773*	*TGC TTA TTT TTC CTC TGG CT/ CTC CAC ACT GAA CAA ACC AA*	

2. Resource details

Amyotrophic lateral sclerosis (ALS) is a devastating neuromuscular disorder caused by the degeneration of upper and lower motor neurons in the cerebral cortex, the brainstem and the spinal cord. Mutations in *TARDBP* (TAR DNA-binding protein) gene occur in about 3% of patients with familial ALS and in 1.5% of patients with sporadic disease. About 50% of ALS cases show concomitant signs of cognitive decline, with 20% having Frontotemporal dementia (FTD), characterized by degeneration of the frontal lobe. Furthermore, aggregates of TDP-43, the protein encoded by *TARDBP* gene, are present in neurons of ALS and FTD patients with and without genetic variants (Neumann et al. 2006). Because of the important role that TDP-43 protein plays in neurodegeneration, studying iPSCs from mutated patients may be helpful to investigate ALS pathogenic mechanisms. To obtain iPSCs, we used fibroblasts from a patient with the missense variant c.995G > A p.Ser332Asn, previously described in association with ALS (Corrado et al. 2009). The patient was first admitted to NEMO Clinical Centre in Rome at age 64 years when she had a bulbar onset of the disease. She presented a familial form of ALS/FTD. Skin biopsy was performed one year after the disease onset and fibroblasts were cultured according to standard protocols. iPSCs were obtained using Sendai virus expressing four reprogramming factors (Klf-

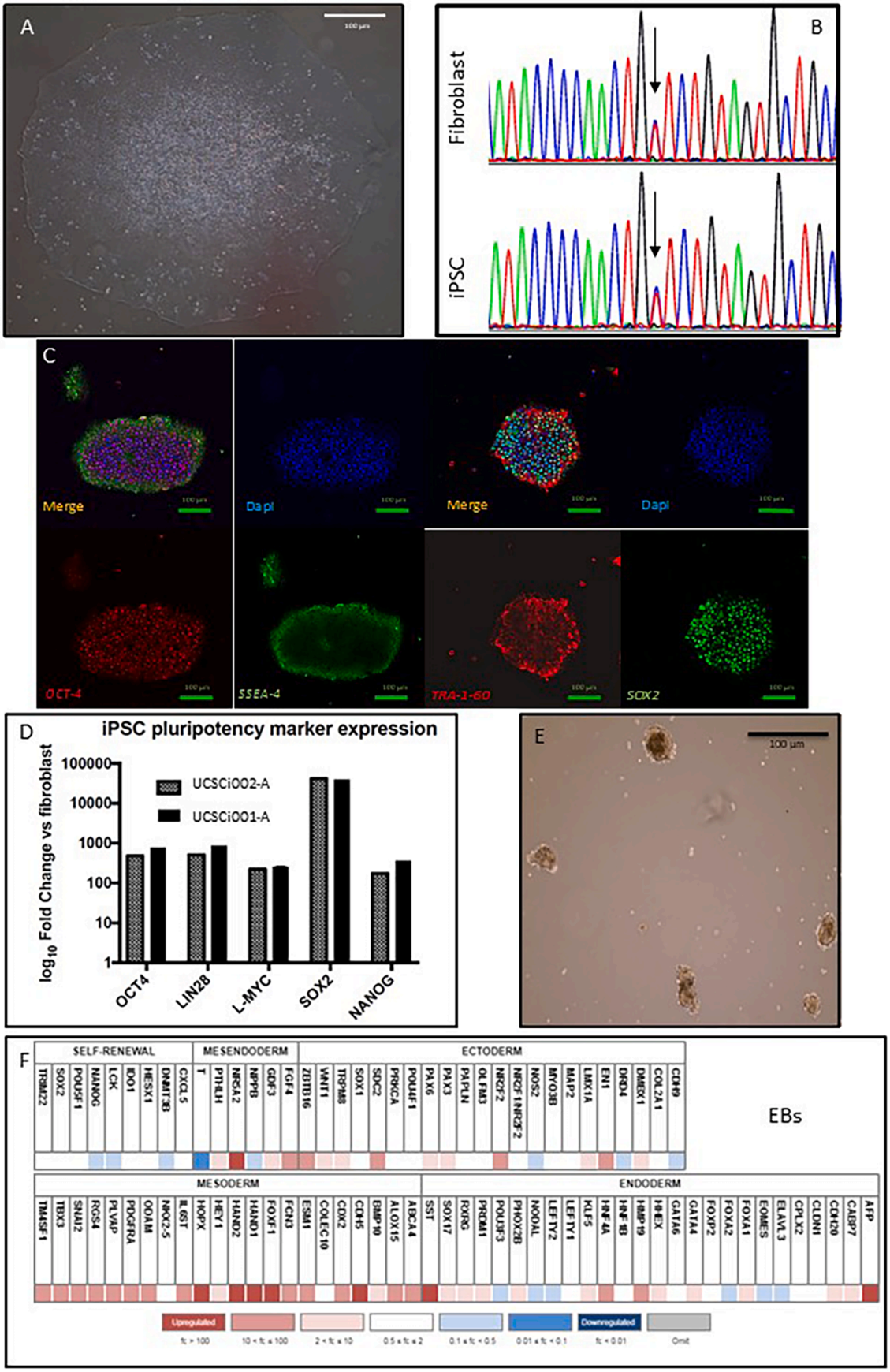
A
B
Fibroblast
iPSC
C
Merge
Dapi
Merge
Dapi
OCT-4
SSEA-4
TRA-1-60
SOX2
D
iPSC pluripotency marker expression
log10 Fold Change vs fibroblast
100000
10000
1000
100
10
1
UCSCi002-A
UCSCi001-A
OCT4
LIN28
L-MYC
SOX2
NANOG
E
100 µm
F
SELF-RENEWAL
MESENDODERM
ECTODERM
EBs
MESODERM
ENDODERM
Upregulated
Downregulated
fc > 100
10 < fc ≤ 100
2 < fc ≤ 10
0.5 ≤ fc ≤ 2
0.1 ≤ fc < 0.5
0.01 ≤ fc < 0.1
fc < 0.01
Omit

Fig. 1. .

4, c-Myc, Oct-4 and Sox-2). iPSC colonies displayed typical stem cell morphology (Fig.1A). Sanger Sequencing was used to check the presence of the *TARDBP* p.Ser332Asn variant, previously identified in DNA from blood and from fibroblasts, in iPSCs at passage 14 (Figure 1B). The pluripotency of iPSCs was confirmed by evaluating the expression of the pluripotency markers Oct4, Sox2, Tra1-60, SSEA-4, detected by immunofluorescence staining at P20 (Fig. 1C), as well as by evaluating the expression of OCT4, SOX2, LIN28, L-MYC and NANOG, quantified by qPCR at P15(Fig. 1D). Mechanically splitted iPSC colonies, at P23, spontaneously differentiated into embryoid bodies (EBs), when allowed to grow without matrix (Fig. 1E). Their differentiation potential was demonstrated by the expression of the three embryonic germ layer markers, detected by TaqMan hPSC Scorecard panels (ThermoFisher Scientific), containing 85 gene specific primer and probe sets (Fig. 1F). At passage 14, DNA was extracted and analyzed by array-comparative genomic hybridization (CGH) and Short Tandem Repeat (STR) profiling. Array-CGH detected a normal molecular karyotype (46,XX) and STRs confirmed the identity between iPSCs and parental fibroblasts. Furthermore, the absence of Mycoplasma contamination and Sendai virus was confirmed at passage 15, as shown by PCR and RT-PCR (Supplementary Figures 1A and 1B).

3. Materials and methods

3.1. Fibroblasts culture

Skin biopsy was performed at the distal leg of the patient by using a 4-mm punch. After dissection, small pieces were cultured in BIOAMF-2 complete medium (Biological Industries), as previously described (Sabatelli et al., 2015).

3.2. Generation of iPSCs

CytoTune™-iPS 2.0 Sendai Reprogramming Kit (Invitrogen) was used to transfect patients' fibroblasts at P3, after mixing components at MOI = 5:5:3 (KOS:c-Myc:Klf4), according to the manufacturer's protocol. Cells were incubated at 37C°C, 5% CO_2. After seven days, cells were dissociated using TrypLE™ Select reagent (ThermoFisher) and plated on Geltrex (Gibco) coated plates in StemFlex medium (Gibco). When they reached ~ 85% confluency, cells were dissociated using Versene solution (Gibco) and cultured in Stemflex medium (Gibco) and RevitaCell supplement (Gibco). The N-Garde Mycoplasma PCR kit (EuroClone) was used to verify the absence of mycoplasma contamination.

3.3. Embryoid body formation

iPSCs colonies were detached using Versene (Gibco) and plated in a 60 mm Petri dish in Complete KnockOut™ Serum Replacement EB medium (Gibco), containing Knockout DMEM/F12, GlutaMAX –I, KnockOut Serum and Non-Essential Amino Acids (Gibco). At days 15, embryoid bodies were collected.

3.4. Real-Time PCR analysis

Total RNA was isolated using E.Z.N.A. Total RNA kit (Omega Biotek), following the manufacturer's recommendations. The Multiskan GO (Thermo Scientific) was used to quantify the RNA and to evaluate its quality, by determining the ratio of absorbance readings at 260 nm and 280 nm (A260/A280). Agarose gel electrophoresis was used to check the integrity of the RNA. cDNA was obtained using the High capacity cDNA reverse transcription kit (ThermoFisher). To detect pluripotency markers, SYBR-green quantitative real- time PCR analysis was performed using SYBR Green on a 7900 system (Applied Biosystems). The expression was quantified using the ΔΔCt method, comparing iPSCs and parental fibroblasts and the iPSC line UCSCi002-A and considering GAPDH as reference gene. TaqMan hPSC Scorecard panels (ThermoFisher Scientific) were used to predict the differentiation potential of EBs, using a Viia7 instrument (Applied Biosystems), following manufacturers' instructions.

3.5. Genetic analysis (sequencing and STR analyses)

Genomic DNA was extracted from iPSCs and fibroblasts using QIAamp DNA mini kit (Qiagen). Mutational analysis of exon 6 of *TARDBP* gene was performed using BigDye terminator v.3.1 Cycle Sequencing kit (Life Technologies). The Investigator 24plex QS Kit (Qiagen) was used for multiplex PCR in a Veriti 96 Weell Thermal Cycler (ThermoFisher Scientific) to simultaneously amplify 22 polymorphic STR markers (TH01, D3S1358, vWA, D21S11, TPOX, DYS391, D1S1656, D12S391, SE33, D10S1248, D22S1045, D19S433, D8S1179, D2S1338, D2S441, D18S51, FGA, D16S539, CSF1PO, D13S317, D5S818, D7S820) along with the gender-specific marker Amelogenin. PCR products were run on an ABI 3130 Genetic Analyzer (Applied Biosystems) and analysed using Sequencing Analyses version 6 and Gene Mapper version 4.0, respectively.

3.6. Array-CGH

Array-CGH analysis was performed on DNA samples derived from fibroblasts and iPSCs, by using the commercial Agilent 8 × 60 K kit (Agilent Technologies, Santa Clara, CA, USA), following manufacturer's instructions.

3.7. Immunofluorescence analysis

Pluripotent Stem Cell 4-Marker Immunocytochemistry Kit (Invitrogen) was used to test four key markers of human pluripotent stem cells: OCT4, SOX2, SSEA4, and TRA-1–60. Cells were first washed with DPBS, fixed with Fixative Solution (for 15 min at room temperature) and incubated in Permeabilization Solution (for 15 min) followed by incubation in Blocking Solution (for 30 min). Primary antibodies were incubated for 3 h at 4 °C and secondary antibodies for 1 h. Nuclei were counterstained with NucBlue Fixed Cell Stain (DAPI) for 5 min.

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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.2022.102825.

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