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Lab Resource: Single Cell Line

Generation of an induced pluripotent stem cell line (UCSCi001-A) from a patient with early-onset amyotrophic lateral sclerosis carrying a *FUS* variant

Francesco Martello [a,b,1], Serena Lattante [a,b,*,1], Paolo Niccolò Doronzio [a,b], Amelia Conte [c], Giulia Bisogni [c], Daniela Orteschi [b], Filomena Pirozzi [d], Mario Sabatelli [c,e], Marcella Zollino [a,b], Giuseppe Marangi [a,b]

[a] *Section of Genomic Medicine, Department of Life Sciences and Public Health, Università Cattolica del Sacro Cuore, Rome, Italy*
[b] *Unit of Medical Genetics, Department of Laboratory and Infectious Disease Sciences, Fondazione Policlinico Universitario A. Gemelli IRCCS, Rome, Italy*
[c] *Adult NEMO Clinical Center, Unit of Neurology, Department of Aging, Neurological, Orthopedic and Head-Neck Sciences, Fondazione Policlinico Universitario A. Gemelli IRCCS, Rome, Italy*
[d] *Seattle Children's Research Institute, Center for Integrative Brain Research, Seattle, WA, USA*
[e] *Section of Neurology, Department of Neuroscience, Faculty of Medicine and Surgery, Università Cattolica del Sacro Cuore, Rome, Italy*

A R T I C L E I N F O

Amyotrophic lateral sclerosis, FUS, Sendai virus

A B S T R A C T

Amyotrophic lateral sclerosis (ALS) is a devastating neurodegenerative disease affecting upper and lower motor neurons. We generated patient-derived-induced Pluripotent Stem Cells (iPSCs), from an ALS patient affected by an early-onset and aggressive form of the disease, carrying a missense pathogenic variant in *FUS* gene. We reprogrammed somatic cells using an established Sendai virus protocol and we obtained clones of iPSC. We confirmed their stemness and further generated embryoid bodies, showing their potential of differentiating in all three germ layers. This iPSC line, carrying a pathogenic *FUS* variant, is a valuable tool to deeply investigate pathogenic mechanisms leading to ALS.

1. Resource table

Unique stem cell line identifier	UCSCi001-A
Alternative name(s) of stem cell line	SII-1802 cl. R
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: 31 Sex: M Ethnicity if known: Caucasian/ Italian
Cell Source	Dermal fibroblasts
Clonality	Clonal
Associated disease	Amyotrophic Lateral Sclerosis
Gene/locus	*FUS* c.1553G > T
Date archived/stock date	28/06/2021
Cell line repository/bank	https://hpscreg.eu/cell-line/UCSCi001-A
Ethical approval	Ethical Committee "Fondazione Policlinico Universitario Agostino Gemelli IRCCS", Protocol n. 0036530/19 (28/08/2019)

2. Resource utility

Mutations in the *FUS* gene are very rare and are associated with an early-onset form of amyotrophic lateral sclerosis. The generation of iPSCs carrying *FUS* variants and their differentiation into motor neurons may provide the possibility to investigate the cell type selectively affected from the disease.

* Corresponding author at: Fondazione Policlinico Universitario A. Gemelli IRCCS, Largo F. Vito 1, 00168 Roma, Italia.
E-mail address: serena.lattante@unicatt.it (S. Lattante).

[1] These authors contributed equally to the work.

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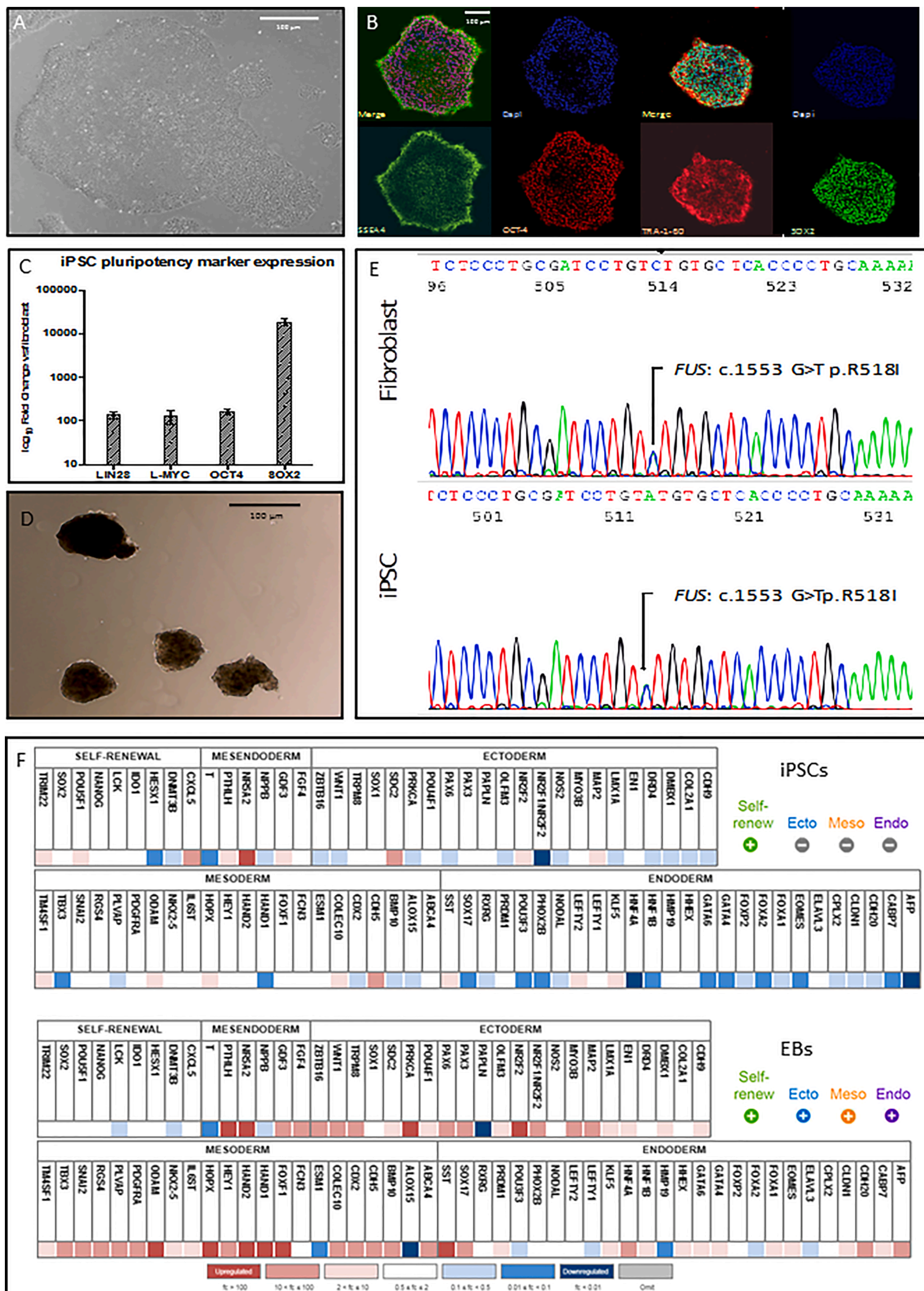

Fig. 1.

3. Resource details

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disorder, affecting upper and lower motor neurons in the cerebral cortex, the brainstem and the spinal cord. Genetic defects have been identified in about 65% of familial cases (FALS) and in about 12% of sporadic patients (SALS). Mutations in the *FUS/TLS* gene are responsible for about 5% of FALS and for less than 1% of SALS(Taylor et al., 2016) (Taylor et al., 2016). FUS protein is mainly localized in the nucleus and plays a role in RNA transcription, splicing and export to the cytoplasm. Variants found in ALS patients are predominantly clustered in the C-terminal region, encoded by exon 15, and have been often associated

Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	Brightfield microscopy	Normal	Fig. 1 panel A
Phenotype	Immunocytochemistry	Staining of pluripotency markers: Oct4, Sox2, Tra 1–60, SSEA-4.	Fig. 1 panel B
	RT-qPCR	Expression of pluripotency markers: OCT4, LIN28, LMYC, SOX2 (qPCR Sybr Green)- CXCL5, LCK, NANOG, POU5F1, SOX2, TRIM22 (TaqMan Scorecard)	Fig. 1 panel C and F
Genotype	Array-CGH	46XY Resolution 8X60K	With authors
	STR analysis	10 sites tested, all matched	With authors
Mutation analysis (IF APPLICABLE)	Sequencing	Heterozygous, missense variant	Fig. 1 panel E
	Southern Blot OR WGS	N.A.	
Microbiology and virology	Mycoplasma [mandatory]	Mycoplasma tested by NGarde Mycoplasma PCR kit (EuroClone). Negative	Supplementary Fig. 1B
Differentiation potential	Embryoid body formation and Taqman Scorecard	Describe expression of genes in embryoid bodies: e.g. muscle actin, β-tubulin and α-feto protein. OR proof of three germlayers formation	Fig. 1 panel D and F
List of recommended germ layer markers	Expression of these markers has to be demonstrated at mRNA level (Taqman Scorecard)	Ectoderm: CDH9, DMBX1, EN1, LMX1A, MAP2, MYO3B, NR2F1/NR2F2, NRF2, OLFM3, PAX3, PAX6, POU4F1, PRKCA, SDC2, TRPM8, WNT1, ZBTB16 Endoderm: AFP, CABP7, CDH20, CLDN1, EOMES, FOXA1, GATA4, GATA6, HHEX, HNF1B, HNF4A, KLF5, PRDM1, SOX17, SST,Mesoderm: ABCA4, BMP10, CDH5, CDX2, COLEC10, FOXF1, HAND1, HAND2, HEY1, HOPX, IL6ST, NKX2-5, ODAM, PDGFRA, PLVAP, RGS4, SNAI2, TBX3, TM4SF1	Fig. 1 panel F
Donor screening	HIV 1 + 2	N/A	With authors
	Hepatitis B, Hepatitis C	Negative	
Genotype additional info (OPTIONAL)	Blood group genotyping	N/A	
	HLA tissue typing	N/A	

with a juvenile onset of the disease and a very aggressive course (Hübers et al., 2015) (Hübers et al., 2015). To further investigate the pathomechanisms of *FUS* mutations, we generated iPSCs from a patient carrying a *FUS* variant. The patient developed first clinical symptoms when he was 30-years old. He had a spinal onset with involvement of upper limbs. The progression of the disease was very rapid and the patient underwent tracheostomy 5 months after the onset, due to respiratory muscles failure. When the patient was first admitted to NEMO Clinical Centre in Rome, he presented with tetraplegia with impairment of oculomotor muscles, resulting in a locked-in syndrome. Sanger sequencing of ALS-associated genes revealed the pathogenic missense variant c.1553G > T (p.Arg518Ile) in *FUS* (NM_004960.3). Skin biopsy was performed 16 months after the onset (11 months after tracheostomy). Fibroblasts were reprogrammed using Sendai virus harbouring four reprogramming factors: Klf-4, c-Myc, Oct-4 and Sox-2. iPSC colonies, with typical stem cell morphology Fig. 1A were manually picked and expanded over several passages. Sendai virus, present in the fibroblasts after the transfection, was not detected after passage 15, as shown by PCR Figure Supplementary Fig. 1(Supplementary Fig. 1A). iPSCs expressed the pluripotency markers Oct4, Sox2, Tra1-60, SSEA-4, as confirmed by the immunofluorescence staining performed at passage 15 Fig. 1B. The expression of the pluripotency genes (OCT4, SOX2, LIN28, and FOXD3) was demonstrated by qPCR in comparison to parental fibroblasts Fig. 1C. The expression of the endogenous stemness markers was detected by Real Time-PCR after 15 passages, using hPSC Scorecard Analysis Fig. 1F. Array-comparative genomic hybridization (CGH), performed at passage 15, revealed a normal molecular karyotype (46, XY), indicating that no unbalanced chromosomal anomalies were acquired during consecutive passaging. In fact, array-CGH does not detect balanced translocations, inversions, or any other balanced alterations in chromosome structure, low-grade mosaicism or polyploidy. The identity between parental fibroblasts and iPSCs was further confirmed by short tandem repeat (STR) analysis. Furthermore, the generated iPSCs were negative for mycoplasma contamination Supplementary Fig. 1B. iPSC colonies spontaneously differentiated into embryoid bodies (EBs), when allowed to grow in low attachment vessels without matrix, thus demonstrating their differentiation potential Fig. 1D. The expression of the three embryonic germ layer markers was confirmed by qRT-PCR Fig. 1F. The presence of the *FUS* p.Arg518Ile variant, first identified in DNA from blood and from fibroblasts, was confirmed by Sanger sequencing in iPSCs Fig. 1E (Table 1).

4. Materials and methods

4.1. Fibroblasts culture

A 4-mm punch skin biopsy at the distal leg was performed on the patient. The biopsy was further dissected into small pieces and cultured in BIOAMF-2 complete medium (Biological Industries), as previously described (Sabatelli et al., 2015).

4.2. Generation of iPSCs

Fibroblasts at P3 were used for transduction. CytoTune™-iPS 2.0 Sendai Reprogramming Kit (Invitrogen) was used. Components were mixed based on the cell number at MOI = 5:5:3 (KOS:c-Myc:Klf4), according to the manufacturer's protocol. Cells were incubated for 24 h at 37C°C, 5% CO_2. Seven days after transduction, cells were dissociated using TrypLE™ Select reagent (ThermoFisher) and plated on Geltrex (Gibco) coated plates. iPSCs colonies were grown in StemFlex medium (Gibco). Cells were dissociated and expanded when they reached ~85% confluency using Versene solution (Gibco), Stemflex medium (Gibco) and RevitaCell supplement (Gibco). Absence of mycoplasma contamination was verified using N-Garde Mycoplasma PCR kit (EuroClone).

4.3. 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). After 15 days, embryoid bodies were collected and RNA was extracted.

4.4. Real-Time PCR analysis

Total RNA was isolated using E.Z.N.A. Total RNA kit (Omega Biotek), following the manufacturer's recommendations, and measured using Multiskan GO (Thermo Scientific). RNA was converted in cDNA using the High capacity cDNA reverse transcription kit (ThermoFisher). Quantitative real- time PCR analysis was performed to detect pluripotency markers using SYBR Green on a 7900 system (Applied Biosystems) (see Table 2 for oligonucleotides sequences). The expression was quantified using the ΔΔCt method, considering GAPDH as reference gene, comparing iPSCs and parental fibroblasts. TaqMan hPSC Scorecard panels (ThermoFisher Scientific), containing a collection of gene

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 ATG CAT GGG AGA GC	
	OCT4	143	CCC CAG GGC CCC ATT TTG GTA CC/ ACC TCA GTT TGA ATG CAT GGG AGA GC	
	L-MYC	143	GCG AAC CCA AGA CCC AGG CCT GCT CC/ CAG GGG GTC TGC TCG CAC CGT GAT G	
	SOX2	80	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	FUS-Ex15	412	TCGCTGGGTTAGGTAGGAGG/ TATTCCAGTTCCTGCTGGGC	

specific primer and probe sets, have been used to confirm iPSCs pluripotency and to predict the differentiation potential of EBs, following manufacturers'instructions, using a Viia7 instrument (Applied Biosystems).

4.5. *Genetic analysis (sequencing and STR analyses)*

Genomic DNA was extracted from iPSCs and fibroblasts using QIAamp DNA mini kit (Qiagen). Exon 15 of *FUS* was amplified using PCR Master Mix (Promega) and sequenced using BigDye terminator v.3.1 Cycle Sequencing kit (Life Technologies) (see Table 2 for sequencing primers). Ten distinct STRs (D1S1656, D3S1358, D5S818, D7S796, D8S1179, D10S1214, BAT-25, BAT-26, NR-21, NR-24) were amplified by PCR. Sequences and fluorescent PCR products were run on an ABI 3130XL Genetic Analyzer (Applied Biosystems) and analysed using Sequencing Analyses version 6 and Gene Mapper version 4.0, respectively.

4.6. *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.

4.7. *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) (see Table 2 for antibodies)

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Giuseppe Marangi reports financial support was provided by Italian Ministry of Health. Mario Sabatelli reports financial support was provided by ICOMM (Insieme COntro le Malattie del Motoneurone) ONLUS.

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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.2021.102461.

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