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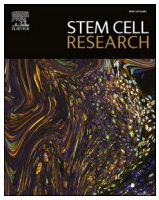

Lab Resource: Single Cell Line

Generation of the induced pluripotent stem cell line UNIBSi017-A from an individual with cardiospondylocarpofacial syndrome and the MAP3K7 c.737-7A > G variant

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A B S T R A C T

TAK1 is a serine threonine kinase that mediates signal transduction induced by TGFβ and bone morphogenetic proteins, and controls a variety of cell functions by modulating the downstream activation of NF-kkB, JNK, and p38. Heterozygous variants in the coding *MAP3K7* gene cause the cardiospondylocarpofacial syndrome, characterized by various abnormalities. Skin fibroblasts derived from a patient carrying the *MAP3K7* c.737-7A>G heterozygous variant were reprogrammed using Sendai viral vector system carrying the Yamanaka factors. The generated induced pluripotent stem cells (iPSC) line retained the original genotype, expressed pluripotency markers, and differentiated into cells of the three germ layers.

1. Resource table

Unique stem cell line identifier	*UNIBSi017-A*
Alternative name(s) of stem cell line	*EDS1331-iPSC*
Institution	*Cell Fate Reprogramming Unit, Dept. Molecular and Translational Medicine, University of Brescia*
Contact information of distributor	*Patrizia Dell'Era, patrizia.dellera@unibs.it*
Type of cell line	*iPSC*
Origin	*human*
Additional origin info required for human ESC or iPSC	*Age: 10* *Sex: Female* *Ethnicity: Caucasian*
Cell Source	*Fibroblasts*
Clonality	*Clonal*
Associated disease	*Cardiospondylocarpofacial syndrome*
Gene/locus	*MAP3K7 gene c.737-7A>G p.(Asn245_Gly246insValVal).*
Date archived/stock date	*June 2022*
Cell line repository/bank	*https://hpscreg.eu/search?q = UNIBSi017-A*
Ethical approval	*The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Institutional Review Board (or Ethics Committee) of at Fondazione IRCCS-Casa Sollievo della Sofferenza (approval no. 2021/13/CE).*

2. Resource utility

Cardiac complications are relevant determinants of quality of life of individuals with variants in *MAP3K7*. The generation of patient iPSC-derived cardiomyocytes will allow to understand the cellular defects that lead to cardiac anomalies in cardiospondylocarpofacial syndrome and evaluate the effectiveness of selected molecules in restoring the cardiac functions.

3. Resource details

Heterozygous variants in the *Mitogen-Activated Protein Kinase Kinase*

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Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography Bright field	Normal	Fig. 1 panel A
Phenotype	Qualitative analysis Immunocytochemistry	Assess staining of pluripotency markers: Oct3/4, Nanog, Sox2, SSEA-3, Tra-1–81.	Fig. 1 panel G
	Quantitative analysis Gene expression	Expression of pluripotency markers OCT4, SOX2, NANOG. Assess % of positive cells for antigen marker: Oct3/4: 95.5%; SOX2: 92.85%; NANOG 98.67%	Fig. 1 panel F
	Immunocytochemistry counting		Fig. 1 panel G
Genotype	Karyotype (QFQ-banding) and resolution	46, XX rob (13;14) (q10;q10) Resolution 450–500	Fig. 1 panel E
Identity	STR analysis	27 loci tested in fibroblasts and iPSC; all matched	Submitted in archive with journal
Mutation analysis	DNA Sequencing	Heterozygous c.737-7A > G	Fig. 1 panel C
	RNA Sequencing	r.736_737insTTGTAG	Fig. 1 panel D
Microbiology and virology	Mycoplasma	Mycoplasma testing by RT-PCR: Negative	Fig. 1 panel B
Differentiation potential	Embryoid body formation	Three germ layers formation	Fig. 1 panel G
List of recommended germ layer markers	Expression of these markers demonstrated at protein (IF) levels	Ectoderm: TUBB3, Mesoderm: TNNT2 Ectoderm: FOXA2	IF with specific antibodies
Donor screening (OPTIONAL)	HIV 1 + 2 Hepatitis B, Hepatitis C	N/A	
Genotype additional info (OPTIONAL)	Blood group genotyping	A Rh+	
	HLA tissue typing	N/A	

Kinase 7 (*MAP3K7*, OMIM#602614) cause the cardiospondylocarpofacial syndrome (CSCFS, OMIM#157800) which is mainly characterized by short stature, dysmorphic facial features, gastrointestinal motility disorders, various cardiac anomalies, and skeletal anomalies. To date, nine individuals with CSCFS from seven families with *MAP3K7* heterozygous variants have been reported and its molecular pathogenesis is only partially understood (Morlino et al., 2018). The *MAP3K7* gene encodes for the TAK1 serine threonine kinase that mediates signal transduction induced by TGFβ and bone morphogenetic proteins, and controls a variety of cell functions by modulating the downstream activation of NF-kB, JNK, and p38 (Wang et al., 2021; Mora et al., 2017).

In this context, we built up a cellular model that we believe will greatly contribute to elucidate the underlying molecular mechanisms (Table 1). We recruited a previously described CSCFS individual with multiple additional soft connective tissue features and carrying the *MAP3K7* c.737-7A>G heterozygous variant (Morlino et al., 2018). This splicing variant was demonstrated to cause the incorporation of two Valines in the kinase domain of encoded protein TAK1, a mechanism that leads to an impaired binding with TAB1 and loss-of-function (Micale et al., 2020). To analyse the *in vitro* molecular pathogenesis of the TAK1 variant, we successfully generated an induced pluripotent stem cell (iPSC) line, UNIBSi017-A or EDS1331-iPSC, from patient's early passage fibroblasts. iPSC clones were established under feeder-free culture conditions using the CytoTune 2.0 iPS Sendai Reprogramming Kit (Thermo Fisher Scientific) which employs the non-integrating Sendai virus (SeV) to deliver reprogramming factors, OCT4, SOX2, KLF4 and cMYC (Takahashi et al., 2007; Ban et al., 2011). Then, hiPSC colonies were manually picked based on morphology and cultured for further characterization. The absence of SeV in iPSC was confirmed by reverse transcription-PCR (RT-PCR) after 5 passages (Fig. 1B). *MAP3K7* c.737-7A>G variant was confirmed at both DNA and mRNA levels by Sanger sequencing (Fig. 1C; 1D). The EDS1331-iPSCs exhibit the balanced Robertsonian translocation 45, XX,rob(13;14)(q10;q10) (Fig. 1E). The same variant karyotype was previously identified in patient's and her father's peripheral blood and, subsequently, confirmed in patient's fibroblasts. This chromosomal constitution is one of the most common karyotype variations in humans and is regularly considered harmless for the carrier individual, aside for possible effects on reproduction in fertile subjects. The endogenous expression of the pluripotent markers, *OCT4*, *SOX2* and *NANOG* was evaluated by Real Time PCR (Fig. 1F). We also confirmed the protein expression of the pluripotent markers, OCT4, SOX2, NANOG, SSEA-3 and TRA-1–81 by immunofluorescence staining (Fig. 1G). In vitro embryoid bodies formation assay confirmed spontaneous differentiation capacity into the three germ layers as demonstrated by immunocytochemistry analysis of expression of ectodermal Tubulin β3, mesodermal Troponin T and endodermal FoxA2 markers (Fig. 1G).

4. Materials and methods

All the main procedures were already described in Mora et al. (2017). Here we report the specific methodological variations for the cell line EDS1331-iPSC. All the analysis were performed around passage 10th of the cell line.

4.1. Reprogramming of fibroblasts

Primary dermal fibroblasts were established from skin biopsy of a clinically diagnosed 10-year-old girl with CSCFS. Cells were maintained in Dulbecco's-Modified Eagle Medium/Nutrient Mixture F-12 Medium (Thermo Fisher Scientific) supplemented with 20% fetal calf serum and 1% Penicillin/Streptomycin. Fibroblasts were reprogrammed using CytoTune iPS 2.0 Sendai Reprogramming Kit (Thermo Fisher Scientific) following manufacturer's instruction.

4.2. Genotyping

On established patient's iPSC, the presence of the *MAP3K7* (NM_145331.2) pathogenic variant was verified both at gDNA and cDNA level by Sanger sequencing. gDNA was extracted using the DNeasy blood and tissue kit (Qiagen) and PCR amplification was performed with a primer pair encompassing the c.737-7A>G variant in intron 7 of *MAP3K7* (forward primer: 5′-CTGTTCAGCATTGACCAGGA-3′ and reverse primer: 5′-CTCGAAATGTTTCCTCTTCTGA-3′). RT-PCR analysis by standard procedure was achieved by amplification of cDNA covering exons 6–8 of *MAP3K7* (forward primer: 5′-ACCTGAAGTTTTTGAAGGTAGT-3′ and reverse primer: 5′-AGTCATTATTTTCACAATTTCC-3′), as previously described (Morlino et al., 2018). PCR was performed using the GoTaq Mastermix (Promega), according to manufactures' instructions. After enzymatic clean-up of PCR products by ExoSap (Life Technologies), amplicons were sequenced in both orientations using the

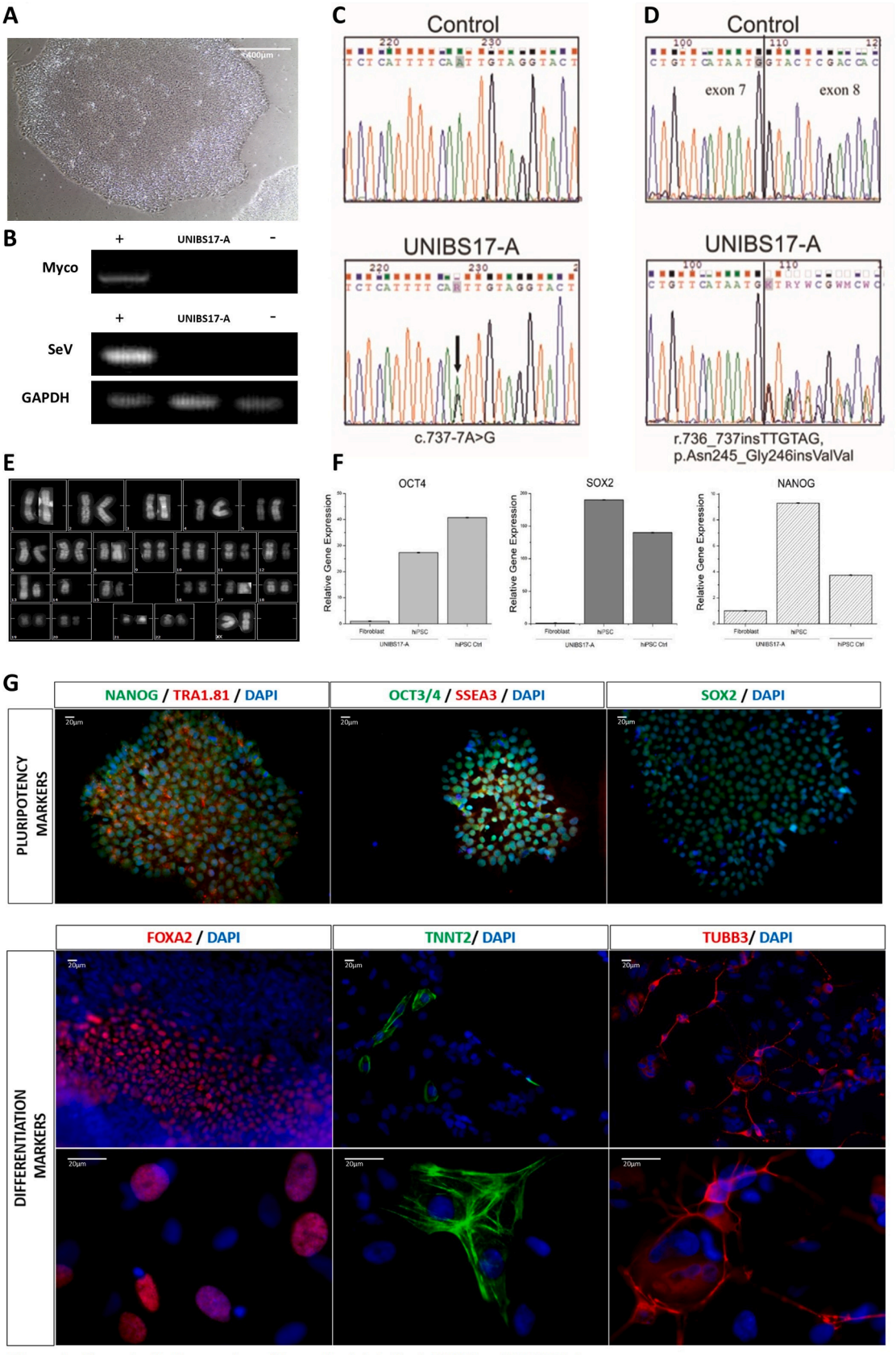

Figure 1: Characterization and quality control data for hiPSC line UNIBS17-A

Fig. 1.

Table 2
Reagents details.

	Antibodies used for immunocytochemistry			
	Antibody	**Dilution**	**Company Cat #**	**RRID**
Pluripotency Markers	Mouse anti-OCT3/4 (C-10)	1:60	Santa Cruz, Cat#sc5279	RIDD: AB_628052
	Mouse anti-SOX2 (Clone 245610)	1:200	R&D, Cat#MAB2018 Everest Biotech, Cat#EB06860	RIDD: AB_358009
	Goat anti-NANOG	1:200	Millipore, Cat#MAB4381 DSHB, Cat#MC-631	RIDD: AB_2150379
	Mouse anti-Tra-1–81	1:200		RIDD: AB_177638
	Rat anti-SSEA3	1:100		RIDD: AB_528406
Differentiation Markers	Rabbit anti-FOXA2	1:400	Thermo Fisher Scientific, Cat#720061 Abcam; Cat#ab8295	RIDD: AB_2576441
	Mouse anti-TNNT2 [1C11]	1:400	Sigma-Aldrich, Cat# T8660	RIDD: AB_306445
	Mouse anti-TUBB3	1:500		RIDD: AB_477590
Secondary antibodies	Donkey anti-Mouse IgG 488	1:800	Thermo Fisher Scientific, Cat#A-21202 Thermo Fisher Scientific, Cat#A-21203	RIDD: AB_141607
	Donkey anti-Mouse IgG 594	1:600	Thermo Fisher Scientific, Cat#A-21207 Invitrogen, Cat#A11055	RIDD: AB_141633
	Donkey anti- Rabbit IgG 594		Invitrogen, Cat#A21213	
	Donkey anti-goat IgG 488	1:600		RIDD: AB_141637
	Goat anti-rat IgM 594	1:600		RIDD: AB_2534102
		1:600		RIDD: AB_2535799
	Primers			
	Target	**Size of band**	**Forward/Reverse primer (5′-3′)**	
Sendai viral vector (PCR)	SeV Genome sequence	180 bp	GGATCACTAGGTGATATCGAGC/ ACCAGACAAGAGTTTAAGAGATATGTATC	
Pluripotency Markers (qPCR)	OCT4	63 bp	GGGTTTTTGGGATTAAGTTCTTCA/ GCCCCCACCCTTTGTGTT	
	SOX2	63 bp	CAAAAATGGCCATGCAGGTT/ AGTTGGGATCGAACAAAAGCTATT	
	NANOG	174 bp	AGGAAGACAAGGTCCCGGTCAA/ TCTGGAACCAGGTCTTCACCTGT	
House-Keeping Genes (qPCR)	GAPDH	170 bp	GAAGGTCGGAGTCAACGGATT/ TGACGGTGCCATGGAATTTG CACTCTTCCAGCCTTCCTTC/	
	ACTB	176 bp	AGTGATCTCCTTCTGCATCCT	
Mycoplasma (PCR)	Mycoplasma 16S rRNA	717 bp	TGCACCATCTGTCACTCTGTTAACCTC/ ACTCCTACGGGAGGCAGCAGTA	
Genotyping				
Targeted mutation analysis/sequencing	MAP3K7 (c.737-7A > G)	560 bp	CTGTTCAGCATTGACCAGGA/ CTCGAAATGTTTCCTCTTCTGA CTGTTCAGCATTGACCAGGA/ AGTCATTATTTTCACAATTTCC	
	MAP3K7 (mRNA)	265 bp		

BigDye® Terminator Cycle Sequencing kit v.3.1 (Life Technologies) and the Performa DTR Ultra 96-Well Plates (EdgeBio) for PCR clean-up, followed by capillary electrophoresis on the ABI3130XL Genetic analyser (Life Technologies). Sequences were analysed with the Alamut Visual Plus ver.1.7 software (Sophia Genetics).

4.3. Molecular analysis

The expression of endogenous pluripotency-related genes *OCT4*, *SOX2*, and *NANOG* of EDS1331-iPSC were confirmed by quantitative PCR (qPCR). Thermal cycling protocol was performed on the ViiA7™ system (Thermo Fisher Scientific) using the following cycling condition: pre-denaturation at 95 °C for 10 min followed by 40 cycles of denaturation at 95 °C for 15 sec, annealing and extension at 60 °C for 1 min. The amplification program was then followed by melting cycle of 95 °C for 15 sec, 60 °C for 1 min, and 95 °C for 15 sec, with a ramp rate of 0.05 °C per second. An iPSC line previously reprogrammed in our lab was used as positive control. The expression ratio of the target genes in EDS1331 fibroblasts and in the EDS1331-iPSC line was calculated by the 2-ΔCt method, using *ACTB* as reference. Each individual determination was repeated in triplicate. Primers used are listed in Table 2.

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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