



Lab Resource: Multiple Cell Lines

Generation of iPSC lines (ICHi001-A, IChi002-A, IChi003-A, IChi004-A) from four patients carrying Titin truncating variants associated with dilated cardiomyopathy

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A B S T R A C T

Inherited dilated cardiomyopathy (iDCM) is a disease of the heart muscle, characterized by left ventricle enlargement, systolic dysfunction and arrhythmias. iDCM represents a common cause of heart failure and the most frequent cause of heart transplantation. Among the causative genes, *TTN*, encoding the sarcomeric protein Titin, represents the most prevalent (about 25 % of cases). The heterogeneous clinical manifestations and variable response to therapy represent a major challenge in patients' clinical management. To deepen the knowledge of this disease, we generated and fully characterized induced Pluripotent Stem Cell lines from 4 iDCM patients carrying 4 different truncating variants of *TTN* gene.

Resource Table

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Unique stem cell lines identifier	ICHi001-A ICHi002-A ICHi003-A ICHi004-A	TTNtv-002 age 56 TTNtv-003 age 53 TTNtv-004 age 41 Sex: TTNtv-001 male TTNtv-002 female TTNtv-003 male TTNtv-004 male Ethnicity: Caucasian
Alternative names of stem cell lines	TTNtv-001 TTNtv-002 TTNtv-003 TTNtv-004	Cell Source
Institution	IRCCS Humanitas Research Hospital	Clonality
Contact information of distributor	Elisa Di Pasquale Elisa.dipasquale@humanitasresearch.it	Method of reprogramming
Type of cell lines	iPSC	CytoTune™-iPS 2.0 Sendai Reprogramming Kit – mediated delivery of Klf4, Oct3/4, Sox2 and c-Myc factors
Origin	Human	YES
Additional origin info required for human ESC or iPSC	Age: TTNtv-001 age 51	Genetic Modification

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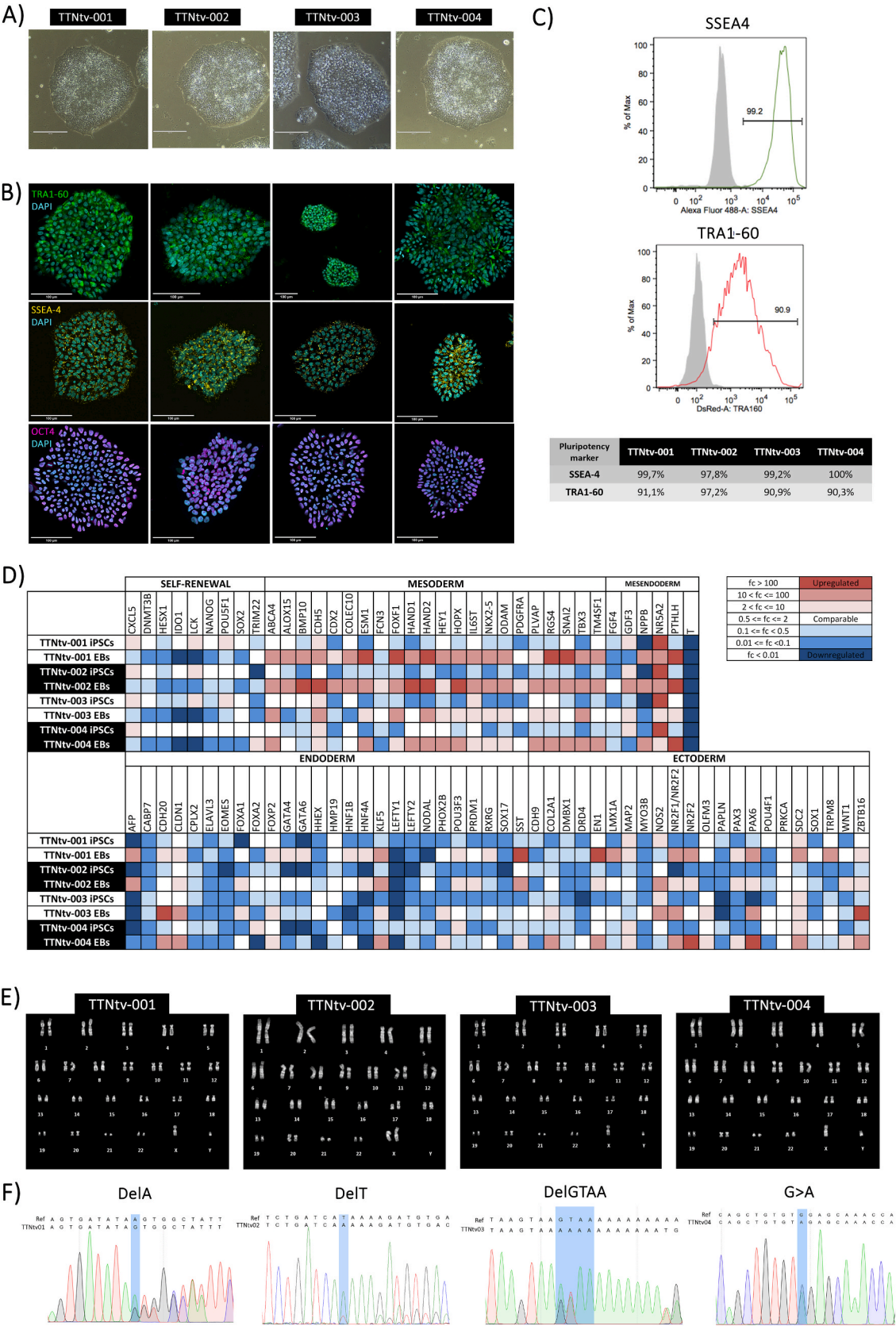


Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography Bright field	Normal	Fig. 1 panel A
Phenotype	Qualitative analysis Immunocytochemistry	Colonies are positive for Oct4, Tra 1–60, SSEA-4,	Fig. 1 panel B
	Quantitative analysis Flow cytometry	More than 90 % of cells are positive for cell surface markers Tra 1–60 and SSEA-4	Fig. 1 panel C
Genotype	Karyotype (G-banding) and resolution	TTNtv-001 46XY TTNtv-002 46XX TTNtv-003 46XY TTNtv-004 46XY 350/400	Fig. 1 panel E
Identity	STR analysis	16 sites tested, all matched between iPSC and T cells	Available with the authors
Mutation analysis (IF APPLICABLE)	Sequencing	All mutations are heterozygous and cause frameshift except for TTNtv04 which is a nonsense nucleotide substitution introducing a STOP codon	Fig. 1 panel F
Microbiology and virology	Mycoplasma	Mycoplasma testing by PCR, negative for all lines	Supplementary Fig. 1 panel D
Differentiation potential	Embryoid body formation: Scorecard and immunofluoresce for specific markers	Embryoid bodies of all lines express markers of the three germ layers	Fig. 1 panel D and Supplementary Fig. 1 panel B, Supplementary Fig. 2 panel A and B
Donor screening	HIV 1 + 2 Hepatitis B, Hepatitis C	NA	
Genotype additional info	Blood group genotyping HLA tissue typing	NA NA	

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Type of Genetic Modification	Spontaneous mutation
Evidence of the reprogramming transgene loss (including genomic copy if applicable)	PCR
Associated disease	Dilated Cardiomyopathy (DCM)
Gene/locus	TTN gene (NM_003319.4) TTNtv-001 c.61800delA TTNtv-002 c.37382delT TTNtv-003 c.57847 + 4delGTAA TTNtv-004 c.65681G > A
Date archived/stock date	20.12.2024
Cell line repository/bank	Lines have been registered in the hPSCreg database https://hpscereg.eu/cell-line/ICHi001-A

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Ethical approval	https://hpscereg.eu/cell-line/ICHi002-A https://hpscereg.eu/cell-line/ICHi003-A http://hpscereg.eu/cell-line/ICHi004-A
	Lines generation was approved by the review boards of two clinical entities: IRCCS Humanitas Research Hospital (ID:1215) and ASUGI CERU (43_2009 Em. 02 dd. 22/06/2022).

Resource utility

iPSC lines were obtained from patients with inherited Dilated Cardiomyopathy (iDCM) carrying *TTN* truncating mutations and displaying different response to Optimal Medical Therapy (OMT); cardiac models developed from these iPSC lines might be useful to discover novel molecular mechanisms underlying *TTN*-related iDCM pathogenesis and to test response to therapies.

Resource Details

Mutations of *TTN* gene are a major cause of iDCM (Herman et al., 2012); more specifically, *TTN* truncating variants (TTNtv) have been associated to impaired sarcomere integrity and cardiac dysfunction. iPSC-derived cardiac models have proven valuable for studying *TTN*-related iDCM, providing hints on disease mechanisms (Fomin et al., 2021; Schick et al., 2018); however significant variability persists in patients' symptoms, age of onset, prognosis, and response to conventional therapies. Thus, development of additional patient-specific models is key for further enhancing current knowledge on disease pathogenesis, potentially contributing to improve patients' prognostic prediction and to develop personalized therapeutic strategies. Here, we generated four iPSC lines from iDCM patients, carrying distinct TTNtv mapping in the A-band region of Titin protein.

These patients suffer from non-ischemic iDCM presenting with severe heart failure in adult age. The left ventricle at baseline was severely dilated, with ejection fraction below 35%. All were subjected to genetic testing revealing distinct heterozygous truncating variants of *TTN* gene, classified as pathogenic or likely-pathogenic according to ACMG guidelines (Richards et al., 2015). These patients were treated following guideline OMT including ace-inhibitors/sacubitril valsartan, beta-blockers, mineralcorticoid receptor antagonists and SGLT2-inhibitors. During clinical follow up, two of them (TTNtv-001 and TTNtv-003) presented left ventricular reverse remodelling leading to normalization of LV geometry and function, whereas TTNtv-002 and -004 didn't show significant improvement in LV function.

iPSC lines were generated from activated T cells from total PBMCs using the Cytotune – iPSC 2.0 Sendai Reprogramming Kit for transduction of the reprogramming factors (cMYC, OCT4, Klf4, Sox2). iPSC colonies from all four reprogrammed iPSC lines showed the typical embryonic stem cell-like morphology, with a round and compact appearance, cells with large nuclei, scant cytoplasm and prominent nucleoli (Fig. 1A). Generated iPSC lines were tested for pluripotency as follows: the totality of the analysed colonies were positive for alkaline phosphatase staining (Supplementary Fig. 1A) and expressed the pluripotency markers TRA1-60, SSEA4 and OCT4, assessed via immunofluorescence (Fig. 1B). FACS analysis confirmed more than 90 % of cells from each line expressed the surface markers TRA1-60 and SSEA4 (Fig. 1C). Moreover, gene expression analysis by ScoreCard assay in both undifferentiated iPSC lines and derived embryoid bodies showed an enriched expression of pluripotency genes in all undifferentiated iPSC lines, while markers specific for the three germ layers (endoderm, mesoderm and ectoderm) were induced in the samples from differentiated embryoid bodies (Fig. 1D, Supplementary Fig. 1B, Supplementary Fig. 2A). Detection of expression of tubulin beta III (ectoderm) and Sox17 (endoderm) and differentiation of contracting cardiomyocytes (mesoderm) further demonstrated the developmental competence of the generated lines and their ability to differentiated into three germ layers

Table 2

Reagent Details.

Antibodies used for immunocytochemistry/flow-cytometry				
	Antibody	Dilution	Company Cat #	RRID
Pluripotency Markers	Mouse anti-OCT4	1:500	Millipore, Cat. No. MAB4401	RRID: AB_2167852
Pluripotency Markers	Mouse anti-TRA1-60	1:200	Abcam, Cat. No. Ab16288	RRID: AB_778563
Pluripotency Markers	Mouse anti-SSEA-4	1:100	Stemcell Technology, Cat. No. 60062	RRID: AB_2721031
Endodermal Marker	Mouse anti-SOX17	1:50	Invitrogen, MA5-24885	RRID: AB_2725396
Ectodermal Marker	Mouse anti-TUBIII	1:500	Promega, G7121	RRID: AB_430874
Secondary antibody	Goat anti-Mouse	1:500	ThermoFisher Scientific, Cat No. A32723	RRID: AB_2866489
FACS staining	PE mouse anti-TRA1-60	1:10	BD Biosciences, Cat. No. 560193	RRID: AB_1645539
FACS staining	FITC mouse anti-SSEA-4	1:10	BD Biosciences, Cat. No. 560126	RRID: AB_1645491
Primers				
	Target	Size of band	Forward/Reverse primer (5'-3')	
Sendai virus (PCR)	Sendai Virus	181 bp	GGATCACTAGGTGATATCGAGC/ACCAGACAAGAGTTTAAGAGATATGTATC	
Targeted mutation analysis for sequencing (PCR)	TTNtv-001 mutation	639 bp	TGTGTGGTCTATGGTGTCTGAAC/AGCGTTTACAGCACAACTCTG	
Targeted mutation analysis for sequencing (PCR)	TTNtv-002 mutation	682 bp	TGCACCACTATAGCTCTCCA/CCCGGAAGATGTACTCTCTGG	
Targeted mutation analysis for sequencing (PCR)	TTNtv-003 mutation	1005 bp	CATTGAAGGCAACAGCTTGA/GGAAATAGAGGTTTGTGTGAGGCA	
Targeted mutation analysis for sequencing (PCR)	TTNtv-004 mutation	744 bp	ATGTGTAAGCCGACTTAGG/TGGTCTGGCATTTCATAGG	
Mycoplasma detection (PCR)	Mycoplasma	700 bp	ACTCTACGGGAGGCAGCAGTA/TGCACCATCTGTCTACTCTGTAAACCTC	

derivatives (Supplementary Fig. 2B and Videos). In term of genome integrity, all iPSC lines showed a normal karyotype (either 46,XY or 46,XX, depending on the specific line) (Fig. 1E). Moreover, short tandem repeat analysis confirmed cell identity proving that the parental T cells and the respective iPSC lines belonged to the same patient; presence of the specific mutations was confirmed by direct sequencing (Fig. 1F). Finally, all cell lines were assessed for the absence of the reprogramming vectors via PCR with specific primers (Supplementary Fig. 1C) and tested free from mycoplasma contamination (Supplementary Fig. 1D).

Materials and methods

iPSC generation

iPSCs were generated from PBMCs isolated from total blood with Ficoll separation methods (Lympholyte, CL5020), activated to T cell with a 5-days treatment with CD28 and CD3 (BD Bioscience, #555336 and #555726). Reprogramming of 5×10^5 cells was achieved by 18-hours transduction with CytoTune™-iPS 2.0 Sendai Reprogramming Kit (ThermoFisher Scientific, A16517) with Polybrene 4 ng/ml (Sigma-Aldrich, H9268). Medium used for T cells activation and transduction was RPMI (Euroclone, ECB9006) 10 % FBS, 1 % Pen/Strep, 1 % Glutamine. Cells were plated onto a layer of Mouse Embryonic Fibroblasts, where iPSC colonies appeared after 7–12 days, when they were picked and cultured as separate clones for further characterization.

Cells were maintained under 5 % CO₂ in Stem Flex medium (Gibco, A3349401) on Vitronectin (Gibco, A14700) and passaged at ~70 % confluency, using 0.5 mM EDTA (Invitrogen, #15575-038) and 5 μM Rock inhibitor Y-27632 (Selleck Chemicals, S1049). All characterization assays were performed with iPSCs between passage 10–20.

Alkaline Phosphatase

Staining was performed with Alkaline Phosphatase Kit (Sigma-Aldrich, 86R).

Immunofluorescence

iPSCs plated onto vitronectin-coated glass coverslips were fixed with 4 % paraformaldehyde. Blocking and permeabilization was performed with PBS 0.1 % Triton X-100 1 % BSA for 10 min at room temperature. Cells were then incubated 1 h with primary antibodies. Nuclei were stained with DAPI. Images were acquired on an Olympus FV1000 confocal laser-scanning microscope and analyzed using ImageJ Fiji software.

FACS

iPSCs were dissociated with Tryple (Gibco, #12605036), washed with PBS and stained with Live/Dead Zombie Aqua (1:800 Biolegend, Cat. No. 77143) and FACS antibodies diluted in FACS buffer (PBS 2 %

FBS) for 15 min. Cells were then fixed in 1 % paraformaldehyde. Detection was performed with BD LSRFortessa and data analysed with FlowJo.

Karyotype

Chromosome counts and karyotype analyses were done on 15 to 20 metaphases stained with Vectashield mounting medium with DAPI for Q-banding. Images were captured using an Olympus BX61 Research Microscope with a cooled CCD camera and analyzed with Applied Imaging Software CytoVision.

Genotyping

Genomic DNA was extracted using QuickExtract DNA Extraction Solution (Lucigen, QE09050) and amplification of the mutation-specific *TTN* locus performed via GoTaq G2 Flexi DNA Polymerase (Promega, M7801). Sanger sequencing was performed with the 3500Dx Genetic Analyzer Applied Biosystem.

Embryoid Bodies generation and Scorecard analysis

Embryoid Bodies (EBs) were generated by detaching iPSC colonies with Dispase (1 mg/mL) and plating them into ultra-low attachment plates (ThermoFisher Scientific, #174932). After 4–7 days in suspension, EBs are attached to a Vitronectin-coated plate and let differentiate until 14 days. Spontaneously differentiated cells were then collected in TRizol (ThermoFisher Scientific, #18596-018) for RNA extraction, performed with Direct-zol RNA MiniPrep (Zymo Research, R2052). cDNA was obtained with High-capacity cDNA Reverse Transcription Kit with RNase Inhibitor (Life Technologies, #4374966) and loaded on the TaqMan hPSC Scorecard Kit 384w (Life Technologies, A15872).

Short Tandem Repeat analysis

Genomic DNA from parental T cells and iPSC lines extracted with QuickExtract DNA Extraction Solution (Lucigen, QE09050) were analyzed with Cell Line Authentication Service from Eurofins Genomics and performed by using Applied Biosystems AmpFLSTR Identifier Plus PCR Amplification Kit with 16 markers.

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 material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scr.2025.103719>.

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