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Generation of two induced pluripotent stem cell lines from dilated cardiomyopathy patients carrying TTN mutations

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Abstract

Dilated cardiomyopathy (DCM) is a common heart disease that can lead to heart failure and sudden cardiac death. Mutations in the *TTN* gene are the most frequent cause of DCM. Here, we generated two human induced pluripotent stem cell (iPSC) lines from the peripheral blood mononuclear cells (PBMCs) of two DCM patients carrying c.94816C>T and c.104188A>G mutations in *TTN*, respectively. The two lines exhibited a normal morphology, full expression of pluripotency markers, a normal karyotype and the ability of trilineage differentiation. The two lines can serve as useful tools for drug screening and mechanism studies on DCM.

Resource Table

Unique stem cell lines identifier	SCVIi068-A SCVIi046-A
Alternative name(s) of stem cell lines	N/A
Institution	Stanford Cardiovascular Institute, Stanford, CA, US
Contact information of distributor	Joseph C. Wu, joewu@stanford.edu
Type of cell lines	iPSC
Origin	Human
Additional origin info required	Age: 59 (SCVIi068-A), 30 (SCVIi046-A) Sex: Female (SCVIi068-A), Female (SCVIi046-A) Ethnicity: White (SCVIi068-A), Pacific Islander (SCVIi046-A)
Cell Source	Blood
Clonality	Clonal
Associated disease	Dilated cardiomyopathy (DCM)

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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.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scr.2022.102941.

Gene/locus	*TTN* c.94816C>T (SCVIi068-A) *TTN* c.104188A>G (SCVIi046-A)
Date archived/stock date	Aug 3rd, 2022
Cell line repository/bank	https://hpscreg.eu/cell-line/SCVIi068-A https://hpscreg.eu/cell-line/SCVIi046-A
Ethical approval	The generation of the lines was approved by the Administrative Panel on Human Subjects Research (IRB) under IRB #29904 "Derivation of Human Induced Pluripotent Stem Cells (Biorepository)"

1. Resource utility

The two iPSC lines were generated from dilated cardiomyopathy (DCM) patients carrying pathogenic mutations in the *TTN* gene. These human induced pluripotent stem cells (iPSCs) provide an unlimited resource for the generation of cardiomyocytes, which represent an essential tool for disease modeling of DCM in vitro and for drug screening identification of therapeutics to mitigate the diseased phenotypes (Chen et al, 2016).

2. Resource details

DCM is a common heart disease that can lead to heart failure and sudden cardiac death. It has been reported that DCM accounts for up to half of heart failure cases, and mutations of the *TTN* gene are the most frequent (20 %–25 %) cause of DCM (Ware and Cook, 2018). Titin, encoded by *TTN*, is the largest protein in the human body and is a substantial component of sarcomere. Titin serves as a molecular spring and is responsible for the passive elasticity of muscle. *TTN* mutation-induced malfunction of titin is highly associated with the development of DCM (Tharp et al., 2019). Thus, cardiomyocytes generated from DCM patient-specific iPSCs can provide essential tools to interrogate the genetic causality of *TTN* mutations in the pathogenesis of DCM (Zhang et al, 2021; Zhao et al, 2021).

Here we generated two iPSC lines, SCVIi068-A and SCVIi046-A, from two DCM patients each carrying different mutations in *TTN*, including a 59-year-old female (SCVIi068-A, c.94816C>T, p.Arg31606X) and a 30-year-old female (SCVIi046-A, c.104188A>G, p.Thr34730Ala). The genetic testing results of the two patients were reviewed by Stanford Center for Inherited Cardiovascular Disease. *TTN* c.94816C>T is a truncating mutation in the A-band of *TTN* previously reported in concert with DCM and atrial fibrillation, and therefore is thought to be pathogenic. *TTN* c.104188A>G is a missense variant in the M-band of *TTN* that is absent in population datasets, and therefore is classified as a variant of uncertain significance. However, given the lack of other high-effect pathogenic variants in this patient, it may contribute to her DCM phenotype. In this report, peripheral blood mononuclear cells (PBMCs) were isolated from the blood samples of the two patients and reprogrammed into iPSCs using Sendai virus carrying reprogramming factors Oct4, Sox2, Klf4, and c-Myc. The two iPSC lines exhibited a typical iPSC morphology (Fig. 1A). Immunofluorescence staining and reverse transcription quantitative polymerase chain reaction (RT-qPCR) were performed to confirm the high expression levels of pluripotency markers Nanog, Oct4 and Sox2 in the two iPSC lines (Fig. 1B and 1C). The presence of *TTN* genetic mutations (c.94816C>T and c.104188A>G) were confirmed by Sanger

sequencing (Fig. 1D). Neither reprogramming nor long-term maintenance compromised the karyotype integrity of the two iPSC lines (Fig. 1E). Furthermore, the two iPSC lines demonstrated full potential to differentiate into three lineages, namely endoderm, mesoderm and ectoderm (Fig. 1F). Although Sendai virus genome was detectable at early passages of iPSCs, it was negligible at passages 25–30 (Fig. 1G). Both iPSC lines were mycoplasma-negative (Supplementary Data 1). Short tandem repeats (STR) analysis confirmed the origin identicalness between the two iPSC lines and their corresponding PBMCs (data archived) (See Table 1).

3. Materials and methods

3.1. Reprogramming

PBMCs were isolated and collected from the whole peripheral blood of patients by gradient, followed by DPBS buffer (Thermo Fisher) purification. Then the PBMCs were cultured in complete StemPro-34 medium (Thermo Fisher) supplemented with 100 ng/mL SCF (Peprotech), 100 ng/mL FLT3 (Thermo Fisher), 20 ng/mL IL-3 (Peprotech), 20 ng/mL IL-6 (Thermo Fisher), and 20 ng/mL EPO (Thermo Fisher). When the enlarged PBMCs were observed, 2×10^5 PBMCs were collected and replated. Next, PBMCs were reprogrammed to iPSCs by the CytoTune®-iPSC Sendai Reprogramming Kit (Thermo Fisher) according to the manufacturer's instructions. The transduced cells were harvested and replated in a new well of a Matrigel (Corning)-coated plate 20 hr posttransduction. The cells were cultured in supplement StemPro-34 medium (Thermo Fisher) for 7 days. Then the StemPro-34 medium was changed to StemMACS™ iPS-Brew XF medium (Miltenyi Biotechnology) to promote the growth of iPSCs. The StemMACS™ iPS-Brew XF medium was refreshed every other day until day 10–15 post-transduction when colonies were ready to be picked. Picked colonies were further expanded and frozen down for future experimental use.

3.2. Cell culture

iPSCs were cultured in Matrigel-coated plates with StemMACS™ iPS-Brew XF medium in a humidified incubator at 37 °C with 5 % CO_2. When the confluency reached around 80 %, the cells were passaged with StemMACS™ iPS-Brew XF medium and 10 μM ROCK inhibitor (Y-27632, Selleck Chemicals) at a ratio of 1:6 to 1:12. Fresh StemMACS™ iPS-Brew XF medium was changed every other day, until cells were ready for replating at approximately 80 % confluency.

3.3. Immunofluorescence staining

iPSCs at passages 15–20 were fixed in a 24-well plate with 4 % paraformaldehyde (EMD Millipore) for 15 min at room temperature (RT), then permeabilized with 0.3 % Triton X-100 (Sigma) for 10 min at RT. After that, the cells were incubated with a blocking buffer (3 % bovine serum albumin, BSA, Sigma) for another 30 min at RT. Next, the cells were incubated with primary antibodies overnight at 4 °C, followed by an incubation with fluorescent dye-conjugated secondary antibodies for 60 min at RT. Cell nuclei were counterstained with Hoechst 33342 (Thermo Fisher) for 5 min at RT. Fluorescent images were captured under a fluorescence microscope. The antibody information and dilution ratios are listed in Table 2.

3.4. Trilineage differentiation potential assay

According to the manufacturer's instructions, the STEMdiff trilineage differentiation kit (Stemcell Technologies) was used to functionally validate the potential of the two iPSC lines to differentiate toward the three germ layers when iPSCs were at passages 15–20.

3.5. RT-q PCR

Total RNA was extracted by miRNeasy Micro Kit (Qiagen) according to the manufacturer's instructions. RT-qPCR was performed by iScript™ Reverse Transcription Supermix (Bio-rad) according to the manufacturer's instructions. iPSCs at passages 15–20 were used for the detection of pluripotency markers Sox2 and Nanog. iPSCs at passages 25–30 were used to determine the presence of Sendai virus genome.

3.6. Karyotyping

A total of 2×10^6 iPSCs of these two lines were collected when they were at passages 11–15. The cells were analyzed using the KaryoStat™ assay (Thermo Fisher).

3.7. Short tandem repeat (STR) analysis

Genomic DNAs of iPSCs at passages 15–20 and PBMCs from the two patients were isolated by the DNeasy Blood & Tissue Kit (Qiagen) following the manufacturer's instructions. STR analysis was performed using a CLA IdentiFiler™ and a Direct PCR Amplification Kit (Thermo Fisher). Capillary electrophoresis was carried out on ABI3130l by the Stanford PAN facility.

3.8. Mycoplasma detection

The spent cell culture medium of each iPSC line was collected for mycoplasma detection when the cells (passage 11–15) were maintained for at least 3 days with a confluency >60 % after replating. Mycoplasma detection was performed by a MycoAlert™ Detection Kit (Lonza) according to the manufacturer's instructions.

3.9. DNA sequencing

Genomic DNA was extracted from iPSCs using the DNeasy Blood & Tissue Kit (Qiagen) according to the manufacturer's instructions. The primers were designed to include the *TTN* mutation site of each iPSC line (primers are listed in Table 2). Then the PCR was performed using a Phusion High-Fidelity PCR Kit (Thermo Fisher). The purified PCR products were subjected to Sanger sequencing.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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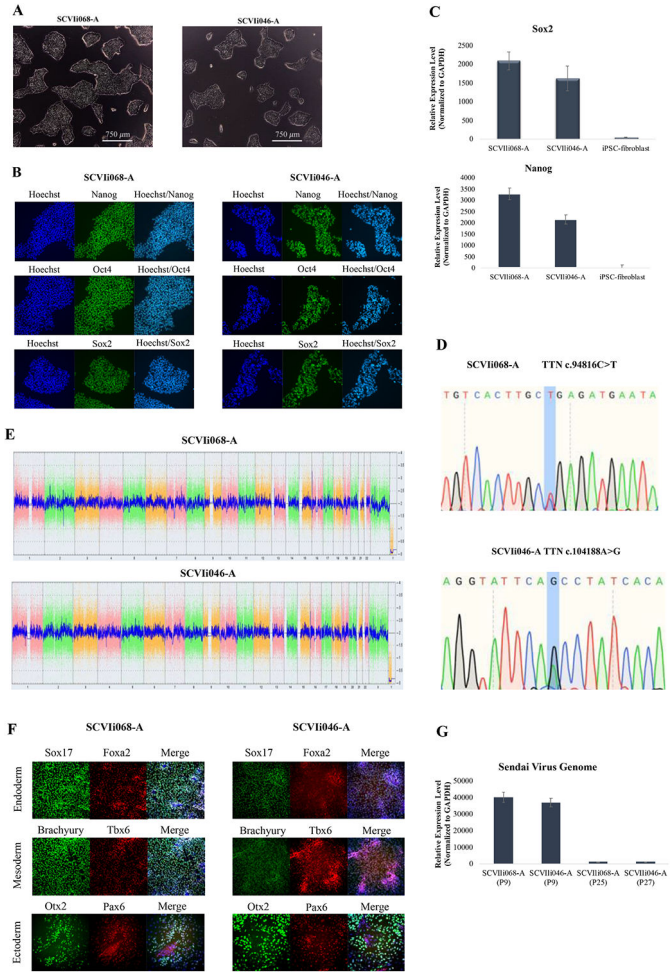

Fig. 1.

Characterization of two iPSC lines derived from DCM patients carrying *TTN* mutations.

Table 1

Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography brightfield	Visual record of the line: normal	Fig. 1 panel A
Phenotype	Qualitative analysis Immunocytochemistry Quantitative analysis RT-qPCR	Positive expression of pluripotency markers: Oct3/4, Nanog, Sox2 Nanog and Sox2 are highly expressed	Fig. 1 panel B Fig. 1 panel C
Genotype	Whole genome array (KaryoStat™ Assay) Resolution 1–2 Mb	Normal karyotype: 46, XX	Fig. 1 panel E
Identity	Microsatellite PCR (mPCR) or STR analysis	N/A 16 loci tested, all matched	N/A Submitted in archive with journal
Mutation analysis (IF APPLICABLE)	Sequencing Southern blot or WGS	Heterozygous Heterozygous N/A	Fig. 1 panel D N/A
Microbiology and virology	Mycoplasma	Mycoplasma testing by luminescence. Negative	Supplementary Fig. 1
Differentiation potential	Directed differentiation	Positive expression of three germ layer markers by immunocytochemistry	Fig. 1 panel F
List of recommended germ layer markers	Expression of these markers has to be demonstrated at mRNA (RT-PCR) or protein (IF) levels, at least 2 markers need to be shown per germ layer	Ectoderm: Pax6, Otx2 Endoderm: Soxl7, Foxa2 Mesoderm: Brachyury, Tbx6	Fig. 1 panel F
Donor screening (OPTIONAL)	HIV 1+2 Hepatitis B, Hepatitis C	N/A	N/A
Genotype additional info (OPTIONAL)	Blood group genotyping HLA tissue typing	N/A N/A	N/A N/A

Table 2

Reagents details.

	Antibodies used for immunocytochemistry/flow-cytometry			
	Antibody	**Dilution**	**Company Cat #**	**RRID**
Pluripotency marker	Rabbit Anti-Nanog	1:200	Proteintech Cat# 142951-1-AP	RRID: AB_1607719
Pluripotency marker	Mouse IgG2bκ Anti-Oct-3/4	1:200	Santa Cruz Biotechnology Cat# sc-5279	RRID: AB_628051
Pluripotency marker	Mouse IgG1κ Anti-Sox2	1:200	Santa Cruz Biotechnology Cat# sc-365823	RRID: AB_10842165
Ectoderm marker	Goat Anti-Otx2	1:200	R&D Systems Cat# 963273	RRID: AB_2157172
Ectoderm marker	Rabbit Anti-Pax6	1:100	Thermo Fisher Scientific Cat# 42-6600	RRID: AB_2533534
Endoderm marker	Goat Anti-Sox17	1:200	R&D Systems Cat# 963121	RRID: AB_355060
Endoderm marker	Rabbit Anti-Foxa2	1:250	Thermo Fisher Scientific Cat# 701698	RRID: AB_2576439
Mesoderm marker	Goat Anti-Brachyury	1:200	R&D Systems Cat# 963427	RRID: AB_2200235
Mesoderm marker	Rabbit Anti-Tbx6	1:200	Thermo Fisher Scientific Cat# PA5-35102	RRID: AB_2552412
Secondary antibody	Alexa Fluor 488 Goat Anti-Mouse (H + L)	1:500	Thermo Fisher Scientific Cat# A-32723	RRID: AB_2633275
Secondary antibody	Alexa Fluor 488 Goat Anti-Rabbit (H + L)	1:500	Thermo Fisher Scientific Cat# A-32731	RRID: AB_2633280
Secondary antibody	Alexa Fluor 594 Donkey Anti-Goat (H + L)	1:500	Thermo Fisher Scientific Cat# A-11058	RRID: AB_2534105
	Primers			
	Target	**Size of band**	**Forward/Reverse primer (5′-3′)**	
Sendai virus plasmids (qPCR)	Sendai virus genome	181 bp	Mr04269880_mr	
Pluripotency marker (qPCR)	Sox2	258 bp	Hs04234836_s1	
Pluripotency marker (qPCR)	Nanog	327 bp	Hs02387400_g1	
House-keeping gene (qPCR)	GAPDH	91 bp	Hs02758991_g1	
Genotyping	TTN c.94816C>T Heterozygous	345 bp	Forward: 5′-TATGATGGAGGCAGCAAGGTTGT-3′ Reverse: 5′-GCTTTGGGTGGAGCTGTCAGTAG-3′	
Genotyping	TTN c.104188A>G Heterozygous	756 bp	Forward: 5′-TGATGCCTCTTCCACGCATT-3′ Reverse: 5′-GCTCAGACACTGGCCTCATT-3′	