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Generation of two induced pluripotent stem cell lines from dilated cardiomyopathy patients caused by heterozygous mutations in the HCN4 gene

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Abstract

Dilated cardiomyopathy (DCM) is a progressive heart muscle disease that can culminate with heart failure and death. Mutations in several genes can cause DCM, including hyperpolarization-activated cyclic nucleotide-gated channel (HCN4), which has a critical function in the autonomic control of the heart rate. Here, we generated two human induced pluripotent stem cell (iPSC) lines generated from two DCM patients carrying variants in the HCN4 gene (c.2587G > T and c.2846G > A). Both lines display normal karyotype, typical morphology of pluripotent stem cells, and differentiate into all three germ layers *in vitro*. These lines are valuable resources for studying the pathological mechanisms of DCM.

Keywords

Dilated cardiomyopathy; iPSC; Stem cells; HCN4

Resource Table.

Unique stem cell lines identifier	**1**	SCVIi063-A
	2	SCVIi064-A
Institution	Stanford Cardiovascular Institute, SCVI	
Contact information of distributor	Dr. Joseph C. Wu (joewu@stanford.edu)	

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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.2022.102951.

Type of cell lines	iPSCs
Origin	Human
Additional origin information	**1** SCVIi063-A; 54-year-old female; Ethnicity: White **2** SCVIi064-A; 44-year-old male; Ethnicity: Other
Cell Source	Blood
Clonality	Clonal
Method of reprogramming	Integration-free Sendai virus expressing human OCT4, SOX2, KFL4, and c-MYC
Evidence of the reprogramming transgene loss	RT-qPCR
Multiline rationale	Non-isogenic cell lines obtained from two patients with heterozygous mutations in HCN4 gene
Gene modification	Yes
Type of modification	Spontaneous mutation
Associated disease	Dilated cardiomyopathy (DCM, OMIM: 115200)
Gene/locus	HCN4 (15q24.1) **1** SCVIi063-A: Heterozygous; HCN4; c.2587G > T **2** SCVIi064-A: Heterozygous; HCN4; c.2846G > A
Method of modification	N/A
Name of transgene or resistance	N/A
Inducible/constitutive system	N/A
Date archived/stock date	1. SCVIi063-A (04/11/2020) 2. SCVIi064-A (11/12/2020)
Cell line repository/bank	1. https://hpscreg.eu/cell-line/SCVIi063-A 2. https://hpscreg.eu/cell-line/SCVIi064-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 generated from different individuals carrying mutation in HCN4 (c.2587G > T and c.2846G > A) provide an accessible, versatile, and adaptable source of cardiomyocytes (iPSC-CMs) or other cell types. These iPSC lines can be used to study the pathological mechanism underlying DCM caused by HCN4 mutation and can serve as a potential tool for drug screening.

2. Resource details

DCM is one of the most common causes of heart failure with a wide spectrum of genetic and nongenetic triggers. The estimated prevalence of DCM in the general population is approximately 1:2800 (Weintraub et al., 2017). Due to its heterogeneity, clinical progression of DCM can be asymptomatic in its early stages. Most patients become symptomatic between 20 and 60 years of age (Mahmaljy et al., 2022). Symptoms can include chamber dilatation, reduced ejection fraction, thromboembolic events, fibrosis or cardiogenic shock (Pooranachandran et al., 2022). Mutations in numerous genes can cause DCM, including the HCN4 gene, a critical regulator of autonomic control of heart rate. HCN4 is highly

expressed in the sinus and atrioventricular node and is essential for the proper function of the developing cardiac conduction system (Stieber et al., 2003). HCN4 has also been shown to promote and stabilize cardiac pacing (Liu et al., 2021).

Here, we report two iPSC lines from two patients carrying pathogenic variants in the HCN4 gene (c.2587G > T and c.2846G > A). The iPSCs were reprogrammed from peripheral blood mononuclear cells (PBMCs) isolated from a 54-year-old female DCM patient (c.2587G > T) and a 44-year-old male DCM patient (c.2846G > A) (Table 1). The PBMC samples from both patients were reprogrammed using the Sendai virus containing the Yamanaka factors (Oct3/4, Sox2, Klf4, and c-Myc). The iPSC clones displayed typical morphology (Fig. 1A, scale bar = 330 μm) and the Sendai virus retained in these cells up to passage 9 and was lost by passage 17 without any virus removal procedure (Fig. 1B). Both lines have normal karyotype as assessed by the KaryoStat assay (Supplementary Fig. 1A) and demonstrated high nuclear expression of pluripotency markers SOX2, NANOG, and OCT3/4 as shown by immunofluorescence staining (Fig. 1C, scale bar = 330 μm). Furthermore, the mRNA levels of pluripotency markers (SOX2 and NANOG) showed similar levels compared to a previously characterized positive control line (Chen et al., 2022). We tested the differentiation of the two lines into derivatives of all three germ layers; the expression of ectoderm markers (OTX2 and PAX6), mesoderm markers (BRACHY and TBX6), and endoderm marker (SOX17 and FOXA2) was assessed by immunohistochemistry staining (Fig. 1D, scale bar = 330 μm). The heterozygous mutations in HCN4 were confirmed by comparing sequencing chromatograms of PCR amplicons SCVIi063-A and SCVIi064-A to control line (Fig. 1D). All lines tested mycoplasma negative (Supplementary Fig. 1B). The origins of these lines were confirmed by short tandem repeat (STR) analysis, which showed that the profiles of the lines fully matched those of the donors' somatic cells.

3. Materials and methods

3.1. Reprogramming

PBMCs were isolated from whole blood samples using Percoll density gradient medium (17089109, GE Healthcare). Cells were purified with multiple rounds of DPBS wash (14190144, ThermoFisher Scientific). Cells were cultured in complete PBMC medium composed of StemPro®−34 SFM medium (10639011, ThermoFisher Scientific) containing 100 ng/mL SCF (300–07, Peprotech), 100 ng/mL FLT3 (PHC9414, ThermoFisher Scientific), 20 ng/mL IL-3 (200–3, Peprotech), 20 ng/mL IL-6 (PHC0063, ThermoFisher Scientific), and 20 ng/mL EPO (PHC9631, ThermoFisher Scientific). The medium was refreshed daily until the cell count remained stable for a few days. PBMC transduction was performed using the Sendai virus reprogramming cocktail according to the manufacturer's instructions (CytoTune™-iPSC 2.0 Sendai Reprogramming Kit, A16517, ThermoFisher Scientific). The transduced cells were resuspended and plated in a Matrigel-coated plate (356231, Corning) and cultured in StemPro™−34 medium (Thermo Fisher). Media was replaced daily until day 7 post transduction. On Day 7, the medium was switched to StemMACS™ iPS-Brew XF medium (130–104–368, Miltenyi Biotec) and was replaced every other day until Day 10–15 post transduction until the colonies appeared. Selected colonies were expanded and frozen down until experimental usage.

3.2. Cell culture

iPSCs were cultured in StemMACS iPS-Brew XF medium (130–107–086, Miltenyi Biotec) with StemMACS iPS-Brew XF supplement (130–107–087, Miltenyi Biotec). 5 μM of ROCK signalling inhibitor (Y27632, S1049, Selleck Chemicals) was added at the time of passaging and the medium was replaced with fresh medium the next day. Culture medium was refreshed every other day. Cells were cultured in a humidified incubator at 37 °C with 5 % CO_2.

3.3. Trilineage differentiation

iPSCs were differentiated into ectoderm according to the manufacturer's instructions (StemDiff™ Definitive Endoderm Differentiation Kit, 05110, STEMCELL™ Technologies). Ectoderm was induced using Human Pluripotent Stem Cell Functional Identification Kit (R&D Systems #SC027B) according to the manufacturer's instructions. Mesoderm differentiation was induced by RPMI media supplemented with B27 supplement minus Insulin (Gibco, #11875–085 and #A18956–01) with 6 μM CHIR (Selleck Chemicals, #S2924) for 48 h.

3.4. Immunofluorescent staining

Cells were washed with DPBS and fixed in 10 % formalin solution (HT501128, Sigma-Aldrich) for 15 min followed by permeabilization with 50 μg/mL Digitonin (D141, Sigma-Aldrich) for 10 min at RT. Cells were blocked in blocking solution (1 % Bovine Serum Albumin (A7030, Sigma-Aldrich), 5 % Donkey Serum (D9663, Sigma-Aldrich) and 5 % Goat Serum (31873, ThermoFisher Scientific) in DPBS) for 30 min at RT. Cells were then incubated with 1 % BSA solution containing primary antibodies at various dilutions (Table 2) overnight at 4 °C followed by 1 % BSA solution containing secondary antibodies at various dilutions (Table 2) for 30 min at RT. Nuclei were counterstained with the Molecular Probes NucBlue Fixed Cell ReadyProbes Reagent (R37606, ThermoFisher Scientific). Cells were imaged using the ECHO Revolve microscope.

3.5. Mycoplasma detection

Mycoplasma contamination was assessed monthly with the MycoAlert™ Detection Kit (LT07–318, Lonza) according to the manufacturer's instructions.

3.6. Short tandem repeat (STR) analysis

Genomic DNA was isolated according to the manufacturer's instructions (69504, Qiagen). STR analysis was performed with the CLA IdentiFiler™ Direct PCR Amplification Kit (A44661, ThermoFisher Scientific) according to the manufacturer's instructions. Fragment analysis was performed on ABI3130xl by the Stanford Protein Nucleic Acid (PAN) Facility.

3.7. Karyotyping

The iPSCs (2×10^6 cells) at passage 12–13 were analyzed using the KaryoStat™ assay (ThermoFisher Scientific).

3.8. Sequencing

HCN4 gene region containing the mutation was amplified using the KOD One PCR Master Mix (KMM-101, DiagnoCine) from genomic DNA. The primers used in the PCR reaction are listed in Table 2. The PCR reaction was performed using the following conditions: 94 °C 1 min; 94 °C 30 s; 62 °C 15 s; 68 °C 1 min for 35 cycles; and 68 °C 5 min. Sanger sequencing was performed by Azenta.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Data availability

Data will be made available on request.

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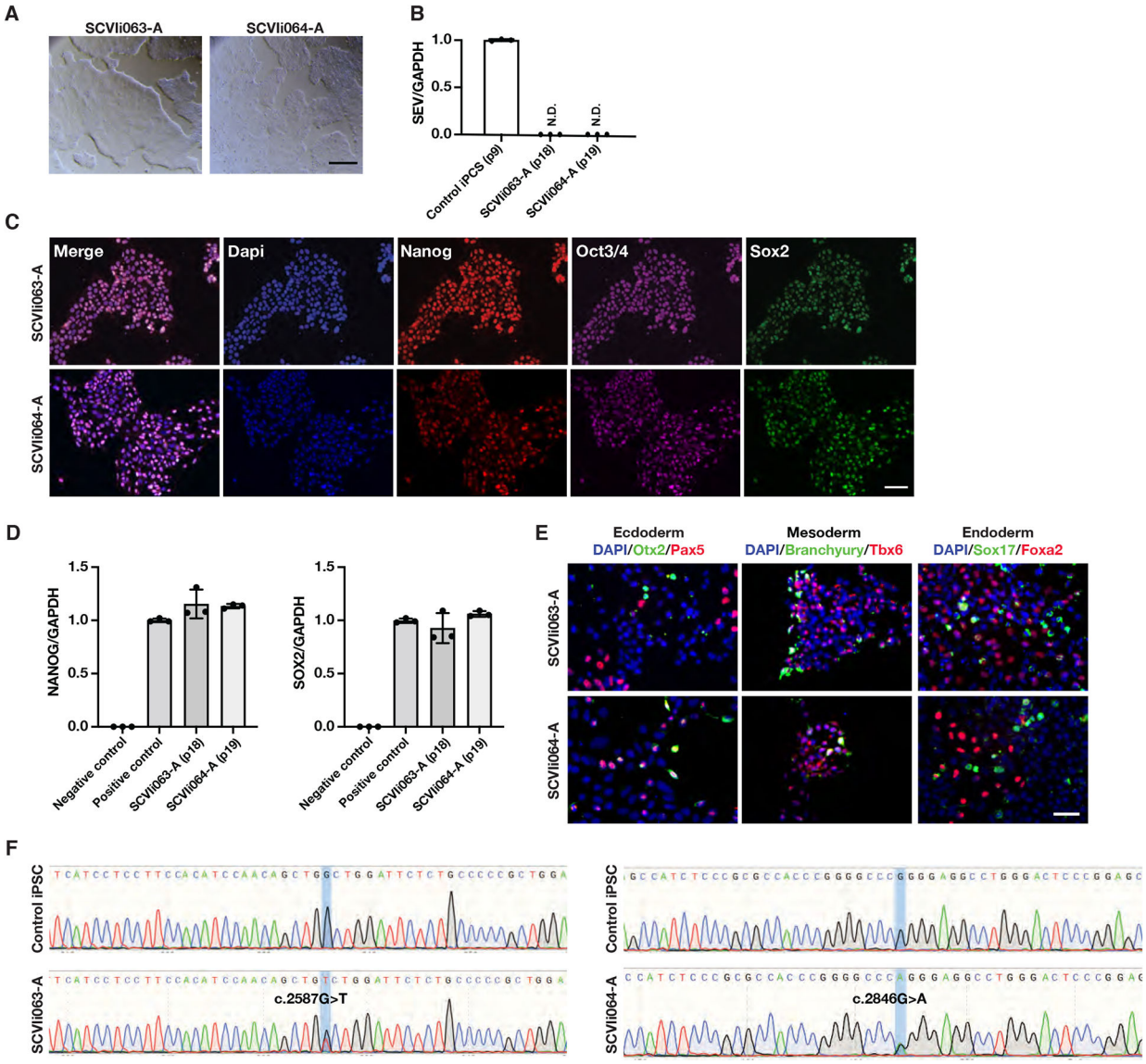

Fig. 1.

Characterization of the two DCM lines carrying heterozygous HCN4 gene mutation.

Table 1

Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography	Visual record of the line: normal	Fig. 1 panel A
Phenotype	Qualitative analysis: Immunofluorescence staining	Positive expression of pluripotency markers: Oct3/4, Nanog, Sox2	Fig. 1 panel C
	Quantitative analysis: RT-qPCR	*NANOG* and *SOX2* expressed in the iPSC lines and absent in differentiated CMs	Fig. 1 panel D
Genotype	Whole genome array (KaryoStat™ Assay) Resolution 1–2 Mb	Normal karyotype: SCVIi063-A 46, XX SCVIi064-A 46, XY	Supplementary Fig. 1 panel A
Identity	STR analysis	16 loci tested match well	Submitted in archive with authors
Microbiology and virology	Mycoplasma	Mycoplasma testing by luminescence: Negative	Supplementary Fig. 1 panel B
Differentiation potential	Directed differentiation	Positive IF staining of three germ layer markers	Fig. 1 panel E
Germ layer markers	Expression of these markers demonstrated at mRNA (RT-PCR) and protein (IF) levels	Ectoderm: Pax6, Otx2 Endoderm: Sox17, Foxa2 Mesoderm: Brachyury, Tbx6	Fig. 1 panel E
Mutation analysis	Sequencing	Heterozygous	Fig. 1 panel F

Table 2

Reagent details.

Antibodies used for immunocytochemistry				
	Antibody	**Dilution**	**Company Cat #**	**RRID**
Pluripotency marker	Rabbit anti-Nanog	1:200	Proteintech Cat# 142951–1-AP	AB_1607719
Pluripotency marker	Mouse IgG2bκ anti-Oct-3/4	1:200	Santa Cruz Biotechnology Cat# sc-5279	AB_628051
Pluripotency marker	Mouse IgG1κ anti-Sox2	1:200	Santa Cruz Biotechnology Cat# sc-365823	AB_10842165
Ectoderm marker	Goat anti-Otx2	1:200	R&D Systems Cat# 963273	AB_2157172
Ectoderm marker	Rabbit anti-Pax6	1:100	Thermo Fisher Scientific Cat# 42–6600	AB_2533534
Endoderm marker	Goat anti-Sox17	1:200	R&D Systems Cat# 963121	AB_355060
Endoderm marker	Rabbit anti-Foxa2	1:250	Thermo Fisher Scientific Cat# 701698	AB_2576439
Mesoderm marker	Goat anti-Brachyury	1:200	R&D Systems Cat# 963427	AB_2200235
Mesoderm marker	Rabbit anti-Tbx6	1:200	Thermo Fisher Scientific Cat# PA5–35102	AB_2552412
Secondary antibody	Alexa Fluor 488 Goat anti-Mouse IgG1	1:1000	Thermo Fisher Scientific Cat# A-21121	AB_2535764
Secondary antibody	Alexa Fluor 488 DonkeyAnti-Goat IgG (H + L)	1:1000	Thermo Fisher Scientific Cat# A-11055	AB_2534102
Secondary antibody	Alexa Fluor 555 Goat anti-Rabbit IgG (H + L)	1:500	Thermo Fisher Scientific Cat# A-21428	AB_141784
Secondary antibody	Alexa Fluor 647 Goat anti-Mouse IgG2b	1:250	Thermo Fisher Scientific Cat# A-21242	AB_2535811

Primers			
	Target	**Size of band**	**Forward/Reverse primer (5′ −3′)**
Sendai virus plasmids (qPCR)	Sendai virus	181	Mr04269880_mr
Genotyping	HCN4; c.2587G > T and c.2846G >A	843	Forward: 5′GCTGATCCAGGCACCACTGCAG 3′ Reverse: 5′GCAGGTGGCAGGAGCAAGGATC3′
House-keeping gene (qPCR)	*GAPDH*	471	Hs02786624_g1
Pluripotency marker (qPCR)	*SOX2*	258	Hs04234836_s1
Pluripotency marker (qPCR)	*NANOG*	327	Hs02387400_g1