HHS Public Access

Author manuscript

Stem Cell Res. Author manuscript; available in PMC 2022 August 21.

Published in final edited form as:

Stem Cell Res. 2021 May ; 53: 102279. doi:10.1016/j.scr.2021.102279.

Generation of two heterozygous *MYBPC3* mutation-carrying human iPSC lines, SCVIi001-A and SCVIi002-A, for modeling hypertrophic cardiomyopathy

Lichao Liu[a], **Sushma P. Shenoy**[a], **James W.S. Jahng**[a], **Yu Liu**[a], **Joshua W. Knowles**[a,b], **Yan Zhuge**[a], **Joseph C. Wu**[a,b,c,*]

[a] Stanford Cardiovascular Institute, Stanford University School of Medicine, CA, USA

[b] Division of Cardiovascular Medicine, Depart of Medicine, Stanford University School of Medicine, CA, USA

[c] Department of Radiology, Stanford University School of Medicine, CA, USA

Abstract

Hypertrophic cardiomyopathy (HCM) is an inherited heart disease that can cause sudden cardiac death and heart failure. HCM often arises from mutations in sarcomeric genes, among which the *MYBPC3* is the most frequently mutated. Here we generated two human induced pluripotent stem cell (iPSC) lines from a HCM patient who has a familial history of HCM and his daughter who carries the pathogenic non-coding mutation. All lines show the typical morphology of pluripotent cells, a high expression of pluripotency markers, normal karyotype, and *in vitro* capacity to differentiate into all three germ layers. These lines provide a valuable resource for studying the molecular basis of HCM and drug screening for HCM.

1. Resource utility

The two iPSC lines generated from individuals carrying a pathogenic mutation in *MYBPC3* provide an unlimited source for differentiating iPSC-derived cardiomyocytes (iPSC-CMs) *in vitro*. Thus, they are an excellent tool for modeling HCM to elucidate the underlying molecular mechanisms of the disease and for drug screenings that may provide treatment.

2. Resource details

Hypertrophic cardiomyopathy (HCM) is a heritable disorder of cardiomyocytes that can cause sudden cardiac death (SCD) and heart failure (Marian and Braunwald, 2017). Unfortunately, the efficacy of conventional HCM treatments is variable and unpredictable

* Corresponding author at: 265 Campus Drive, G1120B, Stanford, CA 94305, USA. joewu@stanford.edu (J.C. Wu).

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.2021.102279.

due to vast differences in variant-specific HCM mechanisms (Mosqueira et al., 2019; Wu et al., 2019). *MYBPC3* is one of the most common mutated genes found in HCM patients (Seeger et al., 2019). *MYBPC3* encodes the myosin binding protein C (MyBP-C), a sarcomeric protein that regulates cardiac contractility by modulating myofilament sliding velocity (Seeger et al., 2019). The non-coding variant (c.3330 + 2 T > G) in *MYBPC3* is known to be both pathogenic and prevalent (Morita et al., 2008). Peripheral blood mononuclear cells (PBMCs) were isolated from a 60-year-old male HCM patient with this pathogenic variant in one allele and his 24-year-old daughter who was genotype-positive but phenotype-negative at that time (Table 1). The PBMCs were reprogrammed using the Sendai virus. The iPSC clones displayed typical morphology and normal karyotype (passages 12 and 13, respectively) as assessed by the KaryoStat assay. Immunostaining showed a high expression of pluripotency markers OCT3/4, NANOG, and SOX2. Reverse transcription-quantitative polymerase chain reaction (RT-qPCR) confirmed the comparable expression of OCT3/4 (also named as *POU5F1*) in these two iPSC lines with that of the widely used iPSC line SCVI273, which is 100-fold higher than that in differentiated cardiomyocytes. Additionally, the iPSCs were able to differentiate into derivatives of all three germ layers. The heterozygous c.3330 + 2 T > G mutation was confirmed by Sanger sequencing (Fig. 1 and Table 2). All lines were mycoplasma-negative. The origin of these lines was confirmed by short tandem repeat (STR) analysis showing that the profile of the lines fully matched that of the donors' somatic cells.

3. Materials and methods

3.1. Reprogramming

Peripheral blood mononuclear cells (PBMCs) were isolated from blood by Percoll separation (GE Healthcare), purified by multiple rounds of washing using DPBS buffer (Thermo Fisher), and plated in a 24-well plate. Then, 1–2 × 10^6 PBMCs were cultured in 1 mL of complete PBMC medium made up of complete StemPro-34 medium (Thermo Fisher) combined with supplements including 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). The medium was replaced every other day until the cell number could remain stable for few days (PBMCs include a heterogeneous population of cells, the medium is targeting a small population). About 2 × 10^5 PBMCs resuspended in 300 μl of complete PBMC medium were infected with a Sendai virus reprogramming cocktail based on the instructions of the CytoTune®-iPSC Sendai Reprogramming Kit (Thermo Fisher). The following day, the cells were washed, resuspended in 1 mL of complete PBMC medium, and plated in one well of a Matrigel-coated 12-well plate. On day 3, the cells were resuspended in 1 mL of StemPro-34 medium (Thermo Fisher) consisting of the StemPro™-34 SFM basal medium supplied with the StemPro™-34 SFM supplement and plated in one well of a Matrigel-coated 12-well plate. The StemPro™-34 medium was replaced every two days. On Day 7, 1 mL of StemMACS medium (Miltenyi Biotec) was added on top of the StemPro™-34 medium and the medium was replaced with fresh StemMACS on Day 8. The medium was replaced every other day with 1 mL of fresh StemMACS until Day 10–15 after Sendai virus infection when colonies appeared and were

ready to be picked up. The picked colonies were expanded when they grew out and were frozen.

3.2. Cell culture

The iPSCs were cultured in StemMACS medium (Miltenyi Biotec), and they were passaged in StemMACS medium combined with 10 μM Y27632 (Selleck Chemicals). Subsequently, the medium was changed every other day with StemMACS medium. All cells were cultured at 37 °C, 5% CO_2, and 85% relative humidity in an incubator.

3.3. Trilineage differentiation

The ability of iPSCs to differentiate into the three germ layers (ectoderm, mesoderm, and endoderm) was assessed using the STEMdiff™ Trillineage Differentiation Kit (STEMCELL Technologies), following the manufacturer's instructions.

3.4. Immunofluorescent staining

Cells were fixed in 4% PFA (EMD Millipore) for 15 min at room temperature, permeabilized with 50 μg/mL digitonin for 10 min, and blocked for 30 min at room temperature with 1% of BSA (Sigma-Aldrich) plus 5% of serum (Thermo Fisher) from host species raised for secondary antibodies. Primary antibodies were incubated with 1% of BSA overnight at 4 °C followed by an incubation with secondary antibodies in 1% of BSA solution for 30 min at room temperature. Nuclei were counterstained with DAPI (Vector Laboratories).

3.5. RT-qPCR

Total RNA from iPSCs and differentiated iPSC-CMs was extracted using the Direct-zol RNA Miniprep Kit (ZYMO RESEARCH). Reverse transcription of RNA was performed using iScript™ cDNA Synthesis Kit (Bio-Rad). cDNA production was amplified on CFX96 Real-Time PCR Detection System using Applied Biosystems TaqMan Fast Advanced Master Mix (Thermo Fisher). Pou5f1 was amplified using *POU5F1 (OCT3/4)* TaqMan™ Gene Expression Assay (Thermo Fisher, Hs00999634). Other primers are shown in Table 3.

3.6. Mycoplasma detection

Mycoplasma was assessed using the MycoAlert Detection Kit (Lonza) following the manufacturer's instructions.

3.7. Short tandem repeat analysis

Genomic DNA was isolated from iPSCs and PBMCs using DNeasy Blood & Tissue Kit (Qiagen) following the manufacturer's instructions. STR-analyses were performed by Stanford PAN Facility, using CLA Identifiler™ Plus and Identifiler™ Direct PCR Amplification Kits (Thermo Fisher).

3.8. Karyotyping

iPSCs at passages 12 and 13 were analysed using KaryoStat™ assay (Thermo Fisher).

3.9. Sanger sequencing

Genomic DNA was isolated from iPSCs using DNeasy Blood & Tissue Kit (Qiagen) following the manufacturer's instructions. *MYBPC3* mutation analysis was performed on a purified PCR product obtained from genomic DNA amplification using Phusion High-Fidelity PCR Kit (Thermo Fisher). The PCR product was purified using QIAquick PCR Purification Kit (Qiagen) and sent for Sanger sequencing. Primers are shown in Table 3.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank Blake Wu and Michelle Bae for proof-reading of our manuscript. This work was supported by National Institutes of Health (NIH) 75N92020D00019, R01 HL126527, and P01 HL141084.

References

Marian AJ, Braunwald E, 2017. Hypertrophic cardiomyopathy: genetics, pathogenesis, clinical manifestations, diagnosis, and therapy. Circ. Res. 121, 749–770. 10.1161/CIRCRESAHA.117.311059. [PubMed: 28912181]

Morita H, Rehm HL, Menesses A, McDonough B, Roberts AE, Kucherlapati R, Towbin JA, Seidman JG, Seidman CE, 2008. Shared genetic causes of cardiac hypertrophy in children and adults. N Engl. J. Med. 358 (18), 1899–1908. 10.1056/NEJMoa075463. [PubMed: 18403758]

Mosqueira D, Smith JGW, Bhagwan JR, Denning C, 2019. Modeling hypertrophic cardiomyopathy: mechanistic insights and pharmacological intervention. Trends Mol. Med. 25, 775–790. 10.1016/j.molmed.2019.06.005. [PubMed: 31324451]

Seeger T, Shrestha R, Lam CK, Chen C, McKeithan WL, Lau E, Wnorowski A, McMullen G, Greenhaw M, Lee J, Oikonomopoulos A, Lee S, Yang H, Mercola M, Wheeler M, Ashley EA, Yang F, Karakikes I, Wu JC, 2019. A premature termination codon mutation in MYBPC3 causes hypertrophic cardiomyopathy via chronic activation of nonsense-mediated decay. Circulation 139 (6), 799–811. 10.1161/CIRCULATIONAHA.118.034624. [PubMed: 30586709]

Wu H, Yang H, Rhee J-W, Zhang JZ, Lam CK, Sallam K, Chang ACY, Ma N, Lee J, Zhang H, Blau HM, Bers DM, Wu JC, 2019. Modelling diastolic dysfunction in induced pluripotent stem cell-derived cardiomyocytes from hypertrophic cardiomyopathy patients. Eur. Heart J. 40 (45), 3685–3695. 10.1093/eurheartj/ehz326. [PubMed: 31219556]

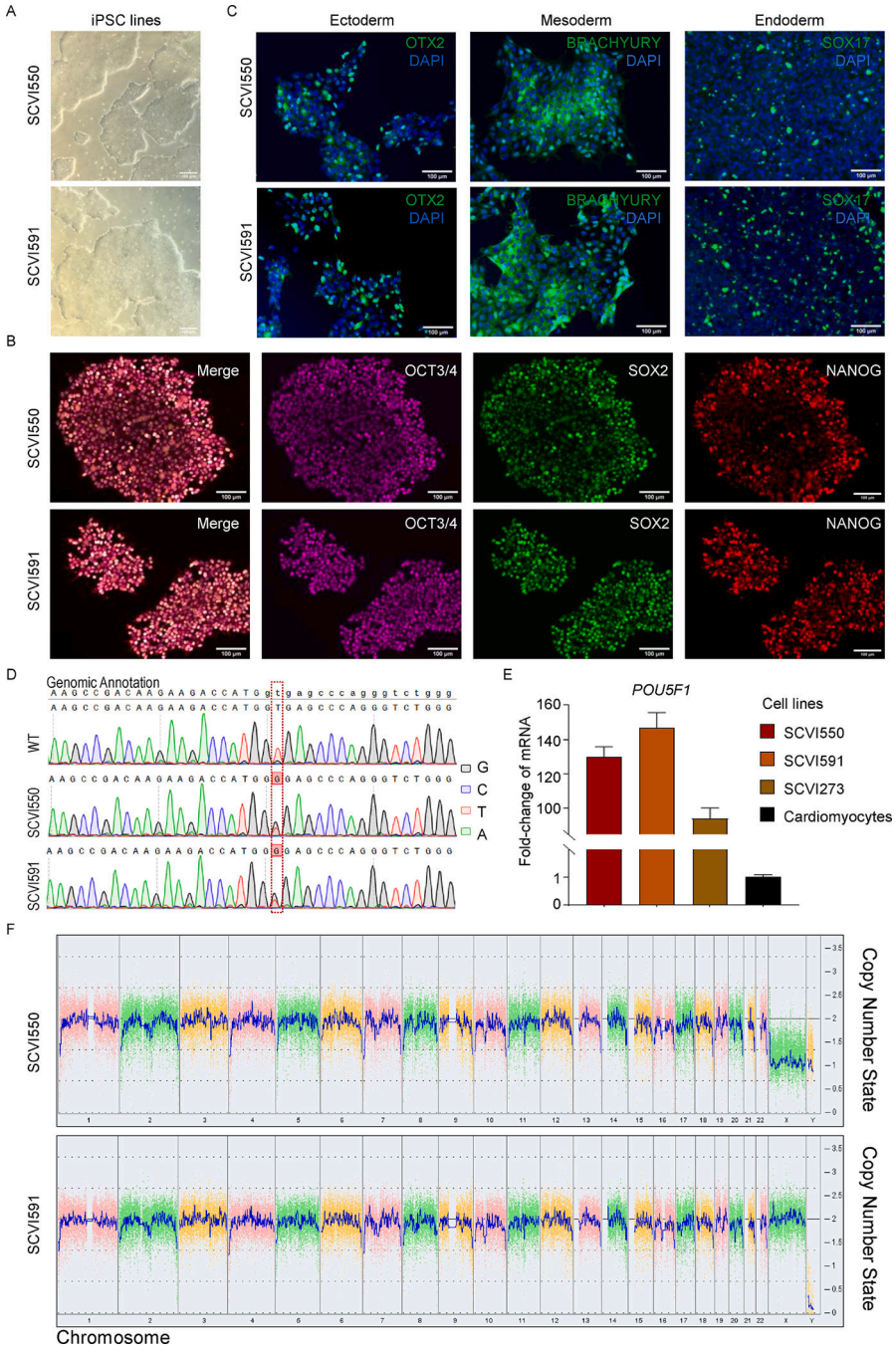

Fig. 1.

Characterization of iPSC lines SCVIi001-A and SCVIi002-A. A. Morphology of the iPSC colonies. B. Immunofluorescent staining for pluripotency markers OCT3/4, SOX2, and NANOG. C. Immunofluorescent staining for ectoderm, mesoderm, and endoderm markers after differentiation. D. Sanger sequencing results. E. Relative expression of pluripotency marker POU5F1 (OCT3/4) in iPSCs and cardiomyocytes. F. Results of KaryoStat assay.

Table 1

Summary of lines.

iPSC line names	Abbreviation in figures	Gender	Age	Ethnicity	Genotype of locus	Clinical disease
SCVIi001-A	SCVI550	Male	60	Not Hispanic or Latino	c.3330 + 2 T > G heterozygous	Hypertrophic cardiomyopathy
SCVIi002-A	SCVI591	Female	24	Not Hispanic or Latino	c.3330 + 2 T > G heterozygous	–

Table 2

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: Oct4, Nanog, Sox2	Fig. 1 panel B
	Quantitative analysis: RT-qPCR	Fold-change for OCT4: SCVIi001-A: 130 ± 6 SCVIi002-A: 146 ± 9	Fig. 1 panel E
Genotype	Whole genome array (KaryoStat™ Assay) Resolution 1–2 Mb	Normal karyotype: 46, XY for SCVIi001-A; 46, XX for SCVIi002-A	Fig. 1 panel F
Identity	Microsatellite PCR	N/A	N/A
	(mPCR) or STR analysis	16 loci tested match well	Available with the authors
Mutation analysis (IF APPLICABLE)	Sequencing	Heterozygous *MYBPC3* c.3330 + 2 T > G for the two iPSC lines	Fig. 1 panel D
	Southern blot or WGS	N/A	N/A
Microbiology and virology	Mycoplasma	Luminescence: Negative	Supplementary Table 1
Differentiation potential	Trilineage *in vitro* differentiation by immunofluorescence analysis	Positive staining of three germ layer markers: ectodermal (OTX2), mesoderm (BRACHYURY), endoderm (SOX17)	Fig. 1 panel C
Donor screening (OPTIONAL)	HIV 1 + 2 Hepatitis B, Hepatitis C	Not performed	Not performed
Genotype additional info (OPTIONAL)	Blood group genotyping	Not performed	Not performed
	HLA tissue typing	Not performed	Not performed

Table 3

Reagents details.

Antibodies used for immunocytochemistry/flow-cytometry			
	Antibody	**Dilution**	**Company Cat # and RRID**
Pluripotency Marker	Mouse IgG_{2b} κ Anti-OCT-3/4	1:200	Santa Cruz Biotechnology Cat# sc-5279, RRID: AB_628051
Pluripotency Marker	Rabbit Anti-NANOG	1:200	Proteintech Cat# 142951-1-AP, RRID:AB_1607719
Pluripotency Marker	Mouse IgG_1 κ Anti-SOX 2	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
Endoderm Marker	Goat Anti-SOX17	1:200	R&D Systems Cat# 963121, RRID: AB_355060
Mesoderm Marker	Goat Anti-BRACHYURY	1:200	R&D Systems Cat# 963427, RRID: AB_2200235
Secondary Antibody	Alexa Fluor 488 Goat Anti-Mouse IgG_1	1:1000	Thermo Fisher Scientific #A-21121 RRID: AB_2535764
Secondary Antibody	Alexa Fluor 647 Goat Anti-Mouse IgG_{2b}	1:250	Thermo Fisher Scientific #A-21242 RRID: AB_2535811
Secondary Antibody	Alexa Fluor 555 Goat Anti-Rabbit IgG (H + L)	1:500	Thermo Fisher Scientific #A-21428 RRID: AB_141784
Secondary Antibody	Alexa Fluor 488 Donkey Anti-Goat IgG (H + L)	1:1000	Thermo Fisher Scientific #A-11055 RRID: AB_2534102

Primers		
	Target	**Forward/Reverse primer (5′-3′)**
Pluripotency Markers (qPCR)	OCT4	Hs00999634_gH
House-keeping genes	18S	F: AGAAACGGCTACCACATCCA R: CCCTCCAATGGATCCTCGTT
Genotyping	MYBPC3	F: CCACAGCTCACATTTTCCAGTCCAC R: TAATGCTCCAAGACGGTGAACCACT

Resource Table

Unique stem cell lines identifier	SCVIi001-A SCVIi002-A
Alternative names of stem cell lines	SCVI550 SCVI591
Institution	Stanford Cardiovascular Institute
Contact information of distributor	Dr. Joseph C. Wu; joewu@stanford.edu
Type of cell lines	iPSC
Origin	Human
Cell Source	Blood
Clonality	Clonal
Method of reprogramming	Integration-free Sendai virus expressing human OCT4, SOX2, KLF4, and c-MYC
Multiline rationale	Non-isogenic cell lines obtained from one patient and one healthy relative with the same mutation in *MYBPC3*
Gene modification	Yes
Type of modification	Spontaneous mutation
Associated disease	Hypertrophic cardiomyopathy
Gene/locus	*MYBPC3*/chr11 (p11.2) Heterozygous *MYBPC3* c.3330 + 2 T > G
Method of modification	N/A
Name of transgene or resistance	N/A
Inducible/constitutive system	N/A
Date archived/stock date	SCVIi001-A: 4/26/2016 SCVIi002-A: 8/16/2016
Cell line repository/bank	 https://hpscreg.eu/cell-line/SCVIi001-A https://hpscreg.eu/cell-line/SCVIi002-A
Ethical approval	The generation of the lines was approved by the Administrative Panel on Human Subjects in Medical Research, Stanford University under IRB #29904 for working with human subjects