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Generation of two induced pluripotent stem cell lines carrying the phospholamban R14del mutation for modeling ARVD/C

Carlos D. Vera[a,b], **Amit Manhas**[a,b], **Sushma P. Shenoy**[a,b], **Matthew T. Wheeler**[a,b], **Karim Sallam**[a,b], **Joseph C. Wu**[a,b,*]

[a]Stanford Cardiovascular Institute, Stanford University, School of Medicine, United States

[b]Division of Cardiovascular Medicine, Stanford University, School of Medicine, United States

Abstract

The phospholamban (PLN) R14del mutation is associated with arrhythmogenic right ventricular dysplasia (ARVD/C). ARVD/C is a cardiac disease characterized by arrhythmias and structural abnormalities in the right ventricle. Because PLN is a regulator of calcium release, this mutation can have deleterious effects on tissue integrity and contraction. This mutation is a trinucleotide (AGA) deletion that leads to an arginine deletion at position 14 of the PLN structure. Here we show two lines carrying this mutation with typical iPSC morphology, pluripotency, karyotype, ability to differentiate into the three germ layers *in vitro*, and readily availability for studying pathological mechanisms or ARVD/C.

Keywords

Arrhythmogenic right ventricular dysplasia; PLN; Induced pluripotent stem cells

1. Resource utility

Two iPSC lines (Resource Table) have been generated to study the underlying mechanism of arrhythmogenic right ventricular dysplasia/cardiomyopathy (ARVD/C). These lines provide an unlimited and valuable resource to derive cardiomyocytes and other cell types for *in vitro* disease modeling and therapeutics screening.

2. Resource Table:

Unique stem cell lines identifier	SCVIi030-A SCVIi031-A

*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.2022.102834.

Alternative name(s) of stem cell lines	
Institution	Stanford Cardiovascular Institute
Contact information of distributor	Dr. Joseph C. Wu
Type of cell lines	iPSC
Origin	Human
Additional origin info required for human ESC or iPSC	1. SCVIi030-A Age: 54 Sex: Female Ethnicity if known: White 2. SCVIi031-A Age: 27 Sex: Male Ethnicity if known: White
Cell Source	PBMCs
Clonality	Clonal
Associated disease	Arrhythmogenic Right Ventricular Dysplasia/Cardiomyopathy (ARVD/C)
Gene/locus	PLN 6q22.31 SCVIi030-A: Heterozygous PLN (p.Arg14del, c.40_42 AGAdel) SCVIi031-A: Heterozygous PLN (p.Arg14del, c.40_42 AGAdel)
Date archived/stock date	SCVIi030-A: 10/06/2021 SCVIi031-A: 10/06/2021
Cell line repository/bank	SCVIi030-A: https://hpscreg.eu/cell-line/SCVIi030-A SCVIi031-A: https://hpscreg.eu/cell-line/SCVIi031-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)”.

2.1. Resource details

ARVD/C is an inheritable disease with a pathological hallmark of a defective right ventricle and a prevalence ranging from 1:2,000 to 1:5,000, depending on the patient cohort (Corrado et al., 2017). Typically, patients have mutations in desmosomal proteins, which are conventionally present at intercellular junctions and are essential for the tissue integrity (Groeneweg et al., 2014). PLN is a regulator of the sarcoplasmic reticulum Ca^{2+} (SERCA2) pump in cardiac muscle and thus crucial for maintaining Ca^{2+} homeostasis. PLN mutations are associated with dilated cardiomyopathy (DCM) and ARVD/C as several histopathological features overlap in the diagnosis (van der Zwaag et al., 2012). The R14del mutation in PLN has been studied extensively for cardiomyopathy and is present in 13–43% of ARVD/C patients (Groeneweg et al., 2014; Karakikes et al., 2015; van der Zwaag et al., 2012). The consensus mechanism of PLN mutations is irregular Ca^{2+} homeostasis due to disrupted regulation of SERCA2, which leads to disassembly of the desmosomal proteins and loss of myocardial tissue integrity (Groeneweg et al., 2014; van der Zwaag et al., 2012). It is essential to properly understand the disease progression to manage DCM and ARVD/C patients with this mutation. Here we present two resource lines that can enable researchers to study those intricacies and identify potential interventions Table 1.

Two human iPSC lines (SCVIi030-A & SCVIi031-A) were derived from peripheral blood mononuclear cells (PBMCs) of two patients diagnosed with ARVC/D. The lines were derived from a 54-year old female and a 27-year old male (c.40_42 AGAdel), both with family histories of cardiac disease (Resource Table). To reprogram the PBMCs into iPSCs,

we utilized the Sendai virus to deliver the Yamanaka factors. The two iPSC lines had normal morphology. The scale bar = 930 μm (Fig. 1A). The expression of pluripotent markers NANOG, OCT3/4, and SOX2 was verified by immunostaining. The scale bar = 70 μm (Fig. 1B). Both iPSC lines could differentiate into all three germ layer lines. The scale bar = 70 μm (Fig. 1C). Expression levels of NANOG and SOX2 were measured through mRNA and detected by reverse transcription-quantitative polymerase chain reaction (RT-qPCR) (Fig. 1D). For comparison, a healthy control SCVI15 was also measured at both iPSC and cardiomyocyte states to show comparable levels of NANOG and SOX2 for iPSCs, but exhibited low levels for cardiomyocytes (Fig. 1D) (Manhas et al., 2022). Sendai virus was absent in SCVIi030-A and SCVIi031-A at passage ~ 20 but still present at a low passage (P4) in healthy control iPSC culture (Fig. 1E). The PLN R14del mutation was confirmed by Sanger sequencing and was absent in the control SCVI15 (Fig. 1F). Moreover, karyotyping confirmed the biological sex and showed no chromosomal aberrations (Fig. 1G). Mycoplasma testing showed proper culturing of these lines (Supp. Fig. 1A). Finally, a short tandem repeat (STR) analysis confirmed that the iPSCs were derived from their respective PBMC origins.

3. Materials and methods

3.1. Reprogramming

PBMCs were isolated from blood by Percoll density gradient medium (GE Healthcare #17089109), washed with DPBS, and plated in a 24-well plate. The culture medium for the PBMCs consisted of Stem-Pro™−34 medium (Thermo Fisher #14190144) supplemented with 100 ng/mL FLT3 (Thermo Fisher #PHC9414), 20 ng/mL IL-6 (Thermo Fisher #PHC0063), 20 ng/mL EPO (Thermo Fisher #PHC9631), 20 ng/mL IL-3 (Peprotech #200–3), and 100 ng/mL SCF (Peprotech #300–07). To reprogram PBMCs into iPSCs, we used the Sendai virus reprogramming cocktail according to the CytoTune™-iPSC 2.0 Sendai Reprogramming Kit (Thermo Fisher Scientific #A16517). The transduced cells were resuspended and plated in a Matrigel-coated plate using the PBMC culture media. On day-7 after transduction, the medium was switched to StemMACS™ iPS-Brew XF medium (Miltenyi Biotec #130–104–368) until day 10–15 post-transduction, when colonies appeared. Colonies were picked and expanded (Manhas et al., 2022).

3.2. Cell culture

iPSCs were cultured in StemMACS™ iPS-Brew XF medium with supplement at 37 °C and 5% CO_2 until 90% confluency. Cells were further passaged using 10 μM Y-27632, a potent inhibitor of ROCK1 (Selleck Chemicals #S1049). The inhibitor was withdrawn after 24 hr.

3.3. Trilineage differentiation

The ability of iPSCs to differentiate into the three germ layers (ectoderm, endoderm, and mesoderm) was assessed using the STEM-diff™ Trilineage Differentiation Kit (STEMCELL Technologies, #05230) following the manufacturer's instructions.

3.4. Immunofluorescent staining

We performed a qualitative analysis of pluripotency and trilineage differentiation. At room temperature, cells were fixed in 4% paraformaldehyde for 15 min, permeabilized with 50 μg/mL digitonin for 10 min, and blocked for 30 min with 1% BSA plus 5% FBS in PBS. The cells were incubated overnight at 4 °C with primary antibodies diluted in 1% BSA-PBS for staining. The following day, the cells were incubated with secondary antibodies in 1% BSA-PBS for 30 min at room temperature. Nuclei were counter-stained using NucBlue™ from Invitrogen™.

3.5. RT-qPCR

According to the manufacturer's protocol, total RNA was extracted and isolated using the Direct-zol™ RNA Miniprep Kit (ZYMO RESEARCH #3R2061). RT-PCR was performed using the iScript™ cDNA Synthesis Kit (BioRad # 1708891) following the manufacturer's protocol of 5 min at 25 °C, 20 min at 46 °C, and 1 min at 95 °C. Target molecules were amplified using commercial primers (Table 2) and TaqMan™ Gene Expression Assay from Applied Biosystems™.

3.6. Mycoplasma detection

Mycoplasma detection was analyzed using MycoAlert Detection Kit (Lonza #LT07–318) following the manufacturer's protocol.

3.7. Short tandem repeat analysis

Genomic DNA was isolated from iPSCs and PBMCs using DNeasy Blood & Tissue Kit (Qiagen #69504). STR analyses were performed by the Stanford PAN facility using CLA Identifier™ Plus and Identifier™ Direct PCR Amplification Kits (Thermo Fisher #A44661).

3.8. Karyotyping

The whole-genome array to detect chromosomal abnormalities was performed at passage 12 with KaryoStat™ (Thermo Fisher Scientific) on 2×10^6 cells.

3.9. Sequencing

PCR primers were designed to amplify the region of interest in the PLN sequence (Table 2). iPSC genomic DNA was isolated using DNeasy Blood & Tissue Kit (Qiagen #69504) and served as the PCR template, with NEB Phusion High Fidelity PCR Kit (Thermo Fisher) being used to amplify the template. The PCR reaction was performed under the following conditions: 98 °C for 30 min, 98 °C for 10 s, 68 °C for 15 s, and 72 °C for 1 min for 35 cycles. Sanger sequencing was performed on ABI3130xl by the Stanford PAN Facility. The WT and mutant alleles were parsed out using the web-based Poly Peak Parser tool from http://yosttools.genetics.utah.edu/PolyPeakParser/.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

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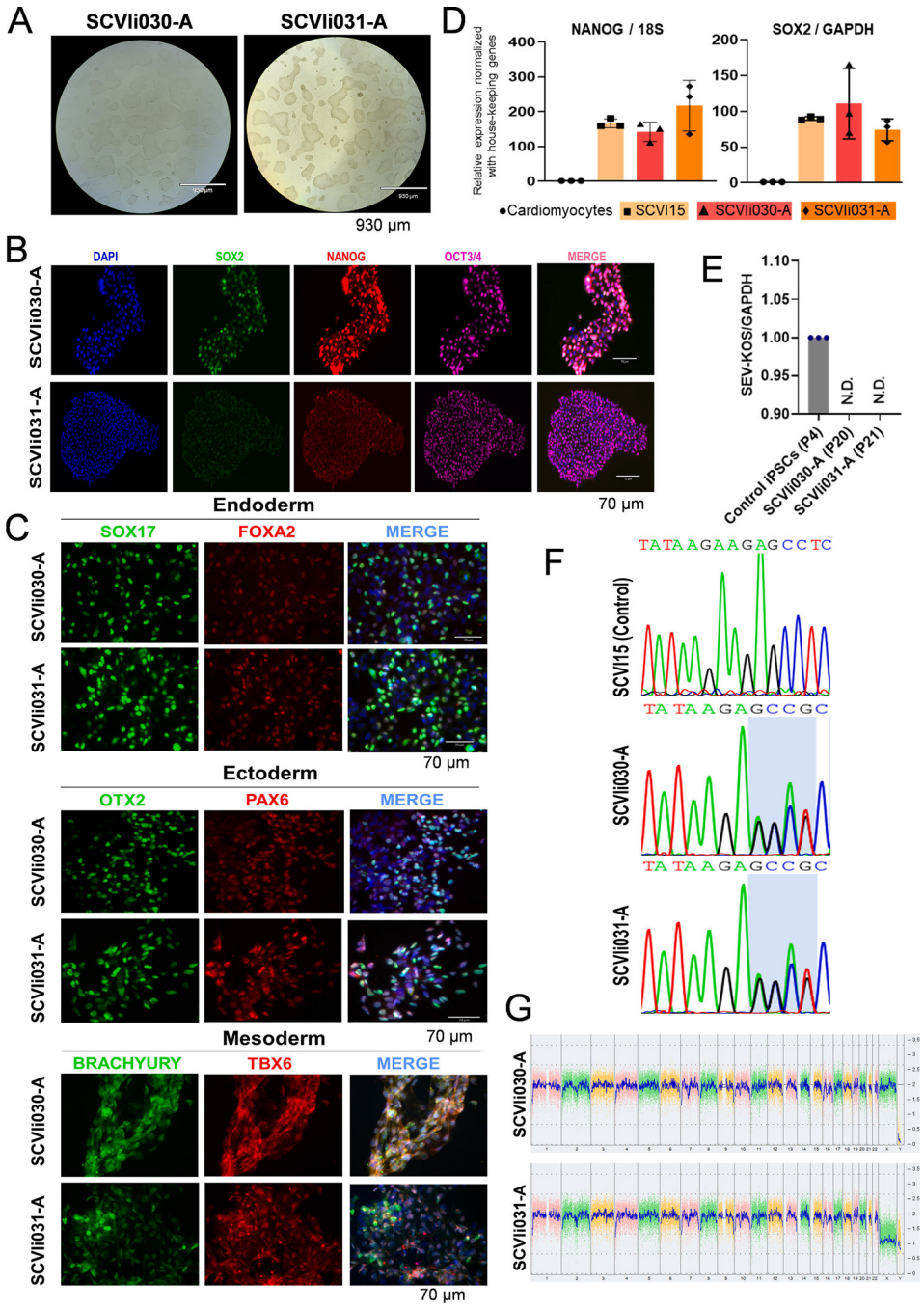

Fig. 1.

Characterization of two iPSC lines from ARVC patients carrying PLN R14del mutation.

Table 1

Characterization and validation.

Classification	Test	Result	Data
Morphology	Bright field 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 B*
	Quantitative analysis *RT-qPCR*	mRNA expression of NANOG and SOX2 that is absent in differentiated cardiomyocytes	Fig. 1 *panel D*
Genotype	Whole genome array (KaryoStat™ Assay) Resolution 1–2 Mb	*Normal karyotype,* *SCVIi030-A: 46XX* *SCVIi031-A: 46XY*	Fig. 1 *panel G*
Identity	STR analysis	*16 loci tested, 100% matching identity*	*Submitted in archive with journal*
Mutation analysis	Sequencing	Heterozygous (c.40_42 AGAdel) for both iPSC lines	Fig. 1 *panel F*
	Southern blot or WGS	*N/A*	*N/A*
Microbiology and virology	Mycoplasma	*Luminescence: negative*	Supplementary Fig. 1A
Differentiation potential	Directed differentiation	*Positive IF staining of three germ layer markers.*	Fig. 1 *panel C*
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, OTX1/2; Endoderm: SOX17, FOXA2; Mesoderm: BRACHYURY, TBX6	Fig. 1 *Panel C*
Donor screening	HIV 1 + 2, Hepatitis B, Hepatitis C	N/A	N/A
Genotype additional info	Blood group genotyping	N/A	N/A
	HLA tissue typing	N/A	N/A

Table 2

Reagents details.

	Antibodies used for immunocytochemistry			
	Antibody	**Dilution**	**Company Cat #**	**RRID**
Pluripotency marker	Mouse IgG_{2b} κ Anti OCT-3/4	1:200	Santa Cruz Biotechnology Cat# sc-5279	AB_628051
	Rabbit Anti-NANOG	1:200	Proteintech Cat# 142951–1-AP	AB_1607719
	Mouse IgG_1 κ Anti-SOX2	1:200	Santa Cruz Biotechnology Cat# sc-365823	AB_10842165
Ectoderm marker	Goat Anti-OTX2	1:200	R&D Systems Cat# 963,273	AB_2157172
	Rabbit Anti-PAX6	1:200	Thermo Fisher Cat# 42-6600	AB_2533534
Endoderm marker	Goat Anti-SOX17	1:200	R&D Systems Cat# 963,121	AB_355060
	Rabbit Anti-FOXA2	1:200	Thermo Fisher Cat# 701,698	AB_2576439
Mesoderm marker	Goat Anti-BRACHYURY	1:200	R&D Systems Cat#963427	AB_2200235
	Rabbit Anti-TBX6	1:200	Thermo Fisher Cat# PA5-35102	AB_2552412
Secondary Antibody	Alexa Fluor 488 Goat, Anti-mouse IgG1	1:200	Thermo Fisher Cat# A-21121	AB_2535764
	Alexa Fluor 488 Donkey, Anti-goat IgG (H + L)	1:200	Thermo Fisher Cat# A-11055	AB_2534102
	Alexa Fluor 647 Goat, Anti-mouse IgG2b	1:200	Thermo Fisher Cat# A-21242	AB_2535811
	Alexa Fluor 555 Goat, Anti-rabbit IgG (H + L)	1:200	Thermo Fisher Cat# A21428	AB_141784
	Alexa Fluor 555 Donkey, Anti-rabbit IgG (H + L)	1:200	Thermo Fisher Cat# A-31572	AB_162543
	Primers Target	Size of band	Forward/Reverse primer (5′-3′)	
Sendai virus plasmids (qPCR)	Sendai virus genome	181 bp	Mr04269880_mr	
Pluripotency marker (qPCR)	NANOG	327 bp	Hs02367400_g1	
	SOX2	256 bp	Hs04234836_g1	
House-keeping gene (qPCR)	GAPDH	471 bp	Hs02786624_g1	

	Antibodies used for immunocytochemistry			
	Antibody	**Dilution**	**Company Cat #**	**RRID**
	18S	207 bp	Hs03003631_g1	
Genotyping For PLN mutation	PLN (c.40_42 AGAdel)	350 bp	*FWD:* TACATTCCAGGCTACCTAAAAGAAG *REV:* TTTCCTGTCTGCATGGGATGAC	