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Generation of three induced pluripotent stem cell lines, SCVIi003-A, SCVIi004-A, SCVIi005-A, from patients with ARVD/C caused by heterozygous mutations in the PKP2 gene

James W.S. Jahng[a], **Katelyn E. Black**[a], **Lichao Liu**[a], **Hye Ryeong Bae**[a], **Marco Perez**[a,b], **Euan A. Ashley**[a,c], **Karim Sallam**[a,b], **Joseph C. Wu**[a,b,d,*]

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

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

[c]Department of Genetics, Stanford University, School of Medicine, United States

[d]Department of Radiology, Stanford University, School of Medicine, United States

Abstract

Arrhythmogenie right ventricular dysplasia/cardiomyopathy (ARVD/C) is an inherited heart disease which can cause life-threatening ventricular arrhythmias and cardiac dysfunction. The autosomal dominant form of ARVD/C is caused by mutations in the cardiac desmosome, such as those in the plakoglobin plakophilin-2 (PKP2) gene. Here, we generated three human induced pluripotent stem cell (iPSC) lines from the peripheral blood mononuclear cells (PBMCs) of three ARVD/C patients eanying pathogenic variants in their PKP2 genes (c.2065_2070delinsG; c.235C>T; c.1725_1728dup). All lines show the typical morphology of pluripotent stem cells, demonstrate high expression of pluripotent markers, display normal karyotype, and differentiate into all three germ layers in vitro. These lines are valuable resources for studying the pathological mechanisms of ARVD/C caused by PKP2 mutation.

1. Resource utility

The three iPSC lines (Resource Table) generated from different individuals carrying pathogenic mutations in PKP2 provide an accessible, versatile, and adaptable source of cardiomyocytes (iPSC-CMs) or other special cell types to study the pathological mechanism underlying arrhythmogenic right ventricular dysplasia/cardiomyopathy (ARVD/C).

*Corresponding author at: 265 Campus Drive, G1120B, Stanford, CA 94305, United States. 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.2021.102284.

2. Resource details

Arrhythmogenic right ventricular dysplasia/cardiomyopathy (ARVD/C) is an inherited cardiomyopathy affecting approximately 1 in 5000 individuals[1]. Symptoms can include severe cardiac complications such as life-threatening ventricular arrhythmias, right ventricular enlargement, fibrofatty replacement of cardiomyocytes, and progressive decline in ventricular function (James and Calkins, 2013). Pathogenic mutations are often found in five components of the cardiac desmosome including plakoglobin, desmoplakin, desmoglein-2, desmocollin-2, and plakoglobin plakophilin-2 (PKP2) (Awad et al., 2008; Asimaki et al., 2014). The PKP2 protein is highly expressed in cardiomyocytes, where it interacts directly with other desmosome proteins, playing a critical role in normal heart morphogenesis and cardiac junction formation. Approximately 11% to 43% of ARVD/C patients are found to have mutations in PKP2[2].

Here, we report three iPSC lines from three patients carrying pathogenic variants in PKP2 (c.2065_2070delinsG; 235C>T; c.1725_1728dup). The iPSCs were reprogrammed from peripheral blood mononuclear cells (PBMCs) isolated from a 54-year-old male ARVD/C patient (c.2065_2070delinsG), a 34-year-old female ARVD/C patient (235C>T), and a 44-year-old female ARVD/C patient (c.1725_1728dup) (Table 1). The PBMCs were reprogrammed using the Sendai virus. The iPSC clones showed typical morphology and normal karyotype at passage 13, as assessed by the KaryoStat assay (Supplementary Fig 2). Immunofluorescence staining showed high nuclear expression of pluripotency markers SOX2, NANOG, and OCT3/4 (Fig. 1B). The iPSC lines were able to differentiate into derivatives of all three germ layers, indicated by the ectoderm marker, mesoderm marker, and endoderm marker (Fig. 1C). The heterozygous mutations in PKP2 were confirmed by comparing sequencing chromatograms of PCR amplicons SCVIi003-A, SCVIi004-A, and SCVIi005-A with SCVI273 (Kitani et al., 2019; Rhee et al., 2020) (Fig. 1D). All lines were found to be mycoplasma-negative. Short tandem repeat (STR) analysis was performed to match the genetic origin of these lines to those of the donors' somatic cells (Table 2).

3. Materials and methods

3.1. Reprogramming

Peripheral blood mononuclear cells (PBMCs) were isolated from whole blood with Percoll density gradient medium (GE Healthcare #17089109) and purified with multiple rounds of washing with DPBS (ThermoFisher Scientific #14190144). The PBMCs were cultured in StemPro®-34 SFM medium (ThermoFisher Scientific #10639011) supplemented with 100 ng/mL SCF (Peprotech #300-07), 100 ng/mL FLT3 (ThermoFisher Scientific #PHC9414), 20 ng/mL IL-3 (Peprotech #200-3), 20 ng/mL IL-6 (ThermoFisher Scientific #PHC0063), and 20 ng/mL EPO (ThermoFisher Scientific #PHC9631). iPSC reprograming was performed using the Sendai virus reprogramming cocktail according to the instructions of the CytoTune™-iPSC 2.0 Sendai Reprogramming Kit (ThermoFisher Scientific #A16517). The transduced cells were resuspended and plated in a Matrigel-coated plate. Cells were cultured in StemPro™-34 medium (Thermo Fisher) until day 7 post-transduction. On day 7, the medium was switched to StemMACS™ iPS-Brew XF medium (Miltenyi Biotec #130-104-368) and cultured until day 10–15 post-transduction when colonies appeared and

were ready for clone-picking. The selected colonies were expanded and frozen down until experimental usage.

3.2. Cell culture

The iPSCs were cultured in StemMACS iPS-Brew XF medium. 10 μM of ROCK signalling inhibitor (Y27632, Selleck Chemicals) was added at the time of passaging. One day after passaging, the medium was replaced with StemMACS medium and was changed every other day. All cells were incubated in a humidified incubator at 37 °C with 5% CO_2.

3.3. Trilineage differentiation

The iPSCs were differentiated into three germ layers (ectoderm, mesoderm, and endoderm) to assess their pluripotency. The StemDiff™ Definitive Endoderm Differentiation Kit (STEMCELL™ Technologies #05110) was used to differentiate iPSCs into endoderm, and the Human Pluripotent Stem Cell Functional Identification Kit (R&D Systems #SC027B) was used to differentiate iPSCs into mesoderm and ectoderm.

3.4. Immunofluorescent staining

The iPSCs or iPSC-derivatives were fixed in 10% formalin solution (Sigma-Aldrich #HT501128) for 15 min and permeabilized with 50 μg/mL digitonin (Sigma-Aldrich #D141) for 10 min at room temperature. Cells were blocked for 30 min at room temperature with a blocking solution made of DPBS with 1% Bovine Serum Albumin (BSA, Sigma-Aldrich #A7030) and 5% serum (Donkey Serum, Sigma-Aldrich #D9663; Goat Serum, ThermoFisher Scientific #31873). Cells were incubated overnight at 4 °C with 1% BSA solution containing a 1:200 dilution of primary antibodies. Cells were incubated for 30 min at room temperature with 1% BSA solution containing secondary antibodies at various concentrations (Table 3). Nuclei were counterstained with the Molecular Probes NucBlue Fixed Cell ReadyProbes Reagent (ThermoFisher Scientific #R37606).

3.5. Mycoplasma detection

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

3.6. Short tandem repeat (STR) analysis

Genomic DNA was isolated from iPSCs and PBMCs with the DNeasy Blood & Tissue Kit (Qiagen #69504), and STR analysis was performed with the CLA IdentiFiler™ Direct PCR Amplification Kit (ThermoFisher Scientific #A44661) according to the manufacturer's instructions. Capillary electrophoresis was performed on ABI3130xl by the Stanford Protein Nucleic Acid (PAN) Facility.

3.7. Karyotyping

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

3.8. Sequencing

PKP2 mutation analysis was performed on PCR amplicons obtained by the Phusion High-Fidelity PCR Kit (Thermo Fisher) with the primers listed in Table 3. The PCR reaction was performed using the following conditions: 94 °C 1 min/94 °C 30 s; 60 °C 15 s; 72 °C 1 min for 35 cycles/72 °C 5 min. Sanger sequencing was performed on ABI3130xl by the Stanford PAN Facility.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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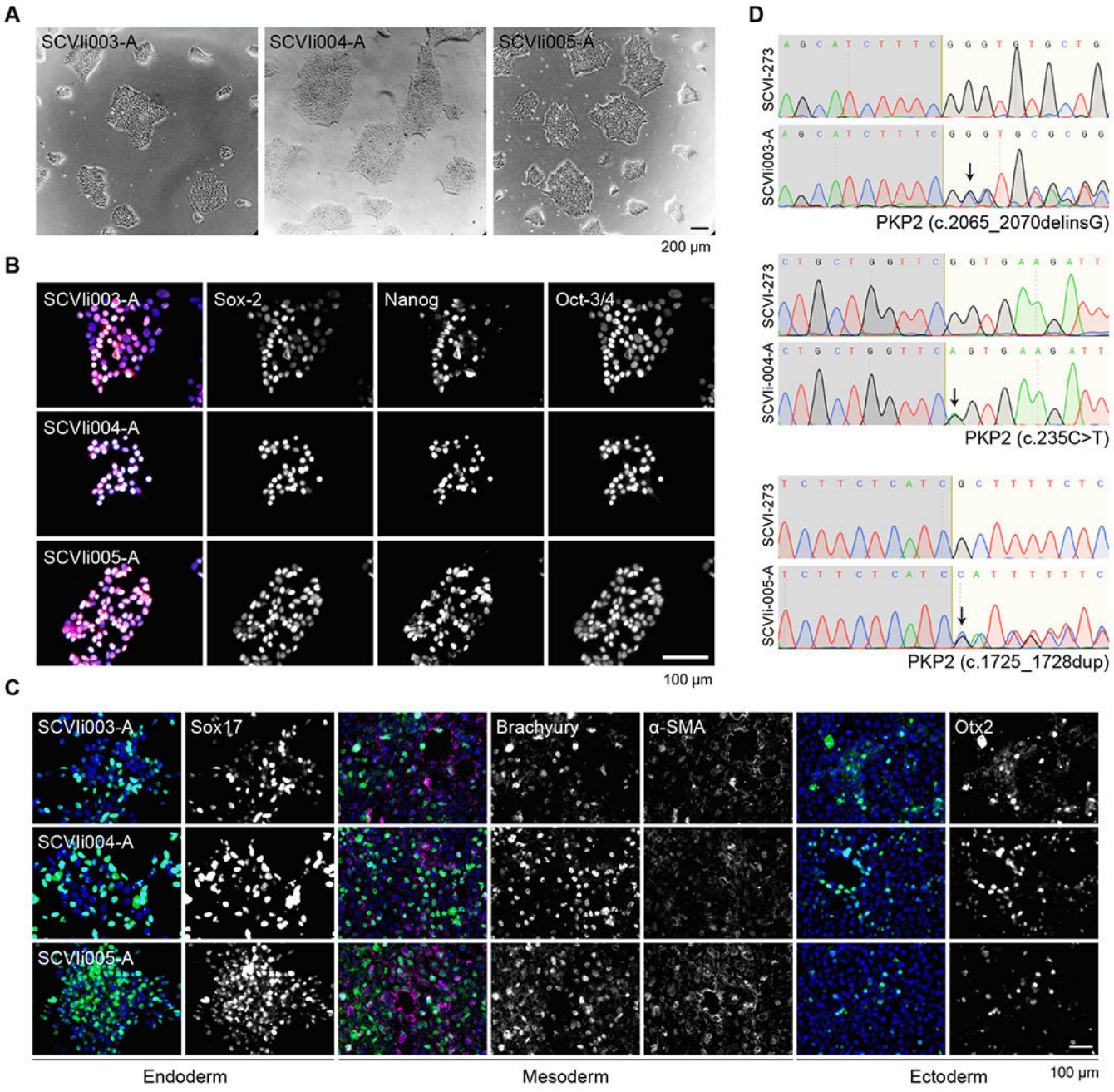

Fig. 1.

Table 1

Summary of lines.

iPSC line names	Abbreviation in figures	Gender	Age	Ethnicity	Genotype of locus	Disease
SCVIi003-A	SCVI-385	Male	54	White	c.2065_2070delinsG Heterozygous	ARVD/C
SCVIi004-A	SCVI-410	Female	34	White	c.235C>T Heterozygous	ARVD/C
SCVIi005-A	SCVI-740	Female	44	East Asian	c.1725_1728dup Heterozygous	ARVD/C

Table 2

Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography	Normal	Fig. 1A
Phenotype	Qualitative analysis	Positive for Oct4, Nanog, Sox2	Fig. 1B
	Quantitative analysis	SCVIi003 (Oct4-99%; Nanog-80.1 ± 8.1%; Sox2-99%) SCVIi004 (Oct4-99%; Nanog-82.6 ± 6.4%; Sox2-99%) SCVIi005 (Oct4-99%; Nanog-80.1 ± 3.4%; Sox2-99%)	Supplementary Fig. 1
Genotype	Whole genome array (KaryoStat™ Assay) Resolution 1–2 Mb	Normal karyotype: 46, XY for SCVIi003-A and XX for SCVIi004-A and SCVIi005-A	Supplementary Fig. 2
Identity	Microsatellite PCR (mPCR) OR STR analysis	N/A 24 markers tested with 100% match	N/A Not shown but available with author
Mutation analysis	Sequencing	Heterozygous Heterozygous Heterozygous	Fig. 1D
	Southern Blot or WGS	N/A	N/A
Microbiology and virology	Mycoplasma	Negative	Supplementary Fig. 3
Differentiation potential	Trilineage in vitro differentiation by immunofluorescence analysis	Positive staining of three germ layer markers: ectodermal (OTX2), mesoderm (BRACHYURY), endoderm (SOX17)	Fig. 1C
Donor screening	HIV 1 + 2 Hepatitis B, Hepatitis C	N/A	N/A
Genotype additional info	Blood group genotyping	N/A	Not shown but available with author
	HLA tissue typing	N/A	Not shown but available with author

Table 3:

Reagents details.

Antibodies used for immunocytochemistry/flow-cytometry			
	Antibody	**Dilution**	**Company Cat # and RRID**
Pluri potency Marker	Mouse $IgG_{2b}\kappa$ Anti-OCT-3/4	1:200	Santa Cruz Biotechnology Cat# sc-5279, RRID: AB_628051
Pluri potency Marker	Rabbit Anti-NANOG	1:200	Proteintech Cat# 142951-1-AP, RRID: AB_1607719
Pluripotency Marker	Mouse $IgG_1\kappa$ Anti-SOX 2	1:200	Santa Cruz Biotechnology Cat# sc-365823, RRID: AB_10842165
Ectoderm Marker	Goat Anti-OTX 2	1:200	R&D Systems Cat# 963273, RRID: AB_2157172
Endoderm Marker	Goat Anti-SOX 17	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
Mesoderm Marker	Mouse Anti-Smooth Muscle Actin	1:200	R&D Systems Cat# MAB1420, RRID: AB_262054
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′)**	
Genotyping	PKP2 (c.2065_2070delinsG)	F: AGGCCGGTTTATCACCTACT R: TCTTCATCAACCTCTGGTAATC	
Genotyping	PKP2 (c.235C>T)	F: TGACGAGAAGTTTGCCCCTT R: ACGTTTAATACTTGTTCTTGGCCT	
Genotyping	PKP2 (c.1725_1728dup)	F: AACCTAAAACCAAGCGGCTAT R: TCCAAACACCTGGAAGAAGCA	

Resource Table

Unique stem cell lines identifier	1. SCVIi003-A 2. SCVIi004-A 3. SCVIi005-A
Alternative names of stem cell lines	1. SCVIi003-A: SCVI-385, SCVI385 2. SCVIi004-A: SCVI-410, SCVI410 3. SCVIi005-A: SCVI-740, SCVI740
Institution	Stanford Cardiovascular Institute, SCVI
Contact information of distributor	Dr. Joseph C. Wu (joewu@stanford.edu)
Type of cell lines	iPSCs
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 three patients with heterozygous mutation in PKP2
Gene modification	Yes
Type of modification	Spontaneous mutation
Associated disease	Arrhythmogenie right ventricular dysplasia/cardiomyopathy (OMIM: 107970)
Gene/locus	PKP2 (12p11.21) SCVIi003-A: Heterozygous; PKP2; c.2065_2070delinsG SCVIi004-A: Heterozygous; PKP2; c.235C>T SCVIi005-A: Heterozygous; PKP2; c.1725_1728dup
Method of modification	N/A
Name of transgene or resistance	N/A
Inducible/constitutive system	N/A
Date archived/stock date	1. SCVIi003-A (04-27-2017) 2. SCVIi004-A (05-17-2017) 3. SCVIi005-A (05-30-2017)
Cell line repository/bank	https://hpscreg.eu/cell-line/SCVIi003-A https://hpscreg.eu/cell-line/SCVIi004-A https://hpscreg.eu/cell-line/SCVIi005-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)”.