Lab Resource: Multiple Cell Lines

Generation of human induced pluripotent stem cell (iPSC) lines derived from five patients carrying the pathogenic phospholamban-R14del (PLN-R14del) variant and three non-carrier family members

Nishka Mittal [a], Jaydev Dave [a], Magdalena Harakalova [b,c], J. Peter. van Tintelen [d], Folkert W. Asselbergs [b], Pieter A. Doevendans [b,e], Kevin D. Costa [a], Irene C. Turnbull [a], Francesca Stillitano [a,*]

[a] Cardiovascular Research Institute, Icahn School of Medicine at Mount Sinai, New York, NY, USA
[b] Department of Cardiology, University Medical Center Utrecht, Utrecht University, Utrecht, The Netherlands
[c] Regenerative Medicine Utrecht, Utrecht University, Utrecht, The Netherlands
[d] Department of Genetics, University Medical Center Utrecht, Utrecht University, Utrecht, The Netherlands
[e] Netherlands Heart Foundation, Utrecht, The Netherlands

A B S T R A C T

The R14del pathogenic variant in the phospholamban (*PLN*) gene (PLN-R14del), has been identified in families with hereditary cardiomyopathy, including dilated and arrhythmogenic cardiomyopathies. Here we have generated human iPSC lines from five PLN-R14del carriers and three non-carrier family members. Peripheral blood mononuclear cells (PBMC) were obtained from the eight individuals and reprogrammed using Sendai viral vector system carrying the Yamanaka factors. All eight lines show typical iPSC morphology, normal karyotype, high expression of pluripotency markers, and possess the ability to differentiate into all three germ layers. These lines represent valuable resources for studying the pathophysiological mechanisms of PLN-R14del associated cardiomyopathy.

Resource Table

Unique stem cell lines identifier	ISMMSi044-A, ISMMSi045-A, ISMMSi046-A, ISMMSi047-A, ISMMSi048-A, ISMMSi049-A, ISMMSi050-A, ISMMSi051-A
Alternative name(s) of stem cell lines	PLN-R14del-1BC1 (ISMMSi044-A) PLN-R14del-2BC4 (ISMMSi045-A) PLN-R14del-3BC7 (ISMMSi046-A) PLN-R14del-4AC4 (ISMMSi047-A) PLN-R14del-5BC4 (ISMMSi048-A) PLN-1CC1 (ISMMSi049-A) PLN-4CC4 (ISMMSi050-A) PLN-5AC2 (ISMMSi051-A)
Institution	Cardiovascular Research Institute, Icahn School of Medicine at Mount Sinai, New York, NY
Contact information of distributor	Francesca Stillitano, francesca.stillitano@mssm.edu
Type of cell lines	iPSC
Origin	Human
Additional origin info	ISMMSi044-A, Age: 56, Sex: M; ISMMSi045-A, Age: 55, Sex: M; ISMMSi046-A, Age: 56, Sex: F; ISMMSi047-A, Age: 31, Sex: M; ISMMSi048-A, Age: 64, Sex: M; ISMMSi049-A, Age: 62, Sex: M; ISMMSi050-A, Age: 33, Sex: F; ISMMSi051-A, Age:

Resource Table (continued)

	40, Sex: F; Ethnicity (all): Caucasian
Cell Source	Total PBMCs
Clonality	Clonal
Method of reprogramming	Sendai virus reprogramming (OCT4, SOX2, KLF4, and c-MYC)
Genetic Modification	Yes
Type of Genetic Modification	Hereditary
Evidence of the reprogramming transgene loss	RT-/q-PCR
Associated disease	Dilated and arrhythmogenic cardiomyopathies
Gene/locus	PLN/6q22.31
Date archived/stock date	12/20/2021
Cell line repository/bank	https://hpscreg.eu/cell-line/ISMMSi044-A https://hpscreg.eu/cell-line/ISMMSi045-A https://hpscreg.eu/cell-line/ISMMSi046-A https://hpscreg.eu/cell-line/ISMMSi047-A https://hpscreg.eu/cell-line/ISMMSi048-A https://hpscreg.eu/cell-line/ISMMSi049-A https://hpscreg.eu/cell-line/ISMMSi050-A https://hpscreg.eu/cell-line/ISMMSi051-A
Ethical approval	Medical Ethical Committee (TCBio) of University Medical Center (UMC) Utrecht; approval number: 12–387

* Corresponding author.

cellular model to study *PLN*-R14del cardiomyopathy *in vitro*.

1. Resource utility

PLN-R14del cardiomyopathy is a debilitating inherited cardiac disease that often leads to heart failure. iPSCs generated from patients and their wild-type family members can serve as an important tool for studying the mechanisms underlying the detrimental effects of pathogenic PLN-R14del variants and can ultimately lead to the investigation and development of much needed therapeutic interventions.

2. Resource details

Phospholamban, encoded by the *PLN* gene, is a small protein present in the membrane of the sarcoplasmic reticulum that plays a crucial role in calcium handling within cardiomyocytes. Several pathogenic variants in *PLN* have been described in patients with arrhythmogenic cardiomyopathy (ACM) and dilated cardiomyopathy (DCM). One such variant, a heterozygous deletion of arginine 14 (R14del), has been identified in 12–15% of ACM and DCM patients in the Netherlands and has been shown to lead to increased risk of developing malignant ventricular arrhythmias, contractile dysfunction, and heart failure(Karakikes et al., 2015; Eijgenraam et al., 2020). The prognosis for PLN-R14del cardiomyopathy patients is poor, with symptoms typically emerging from late adolescence onwards. The only treatment options currently available are standard heart failure management, left ventricular assist device (LVAD), and ultimately a heart transplantion. PLN-R14del carriers may exhibit a wide range of phenotypes ranging from asymptomatic to severe cardiomyopathy, with varying degrees of arrhythmia (Karakikes et al., 2015). The development of transgenic mouse models has yielded important insights into the disease (Eijgenraam et al., 2020; Haghighi et al., 2021); however, these models do not recapitulate all aspects of the human phenotype, and clinical translation of such results is not straightforward (Raad et al., 2021). Human induced pluripotent stem cell (iPSC)-derived cardiomyocytes (iPSC-CMs) have been used to model a variety of cardiac diseases in vitro (Oh et al., 2020). Modeling PLN-R14del cardiomyopathy using patient-derived iPSCs provides an opportunity to study the underlying pathophysiology of the disease in the human system to accelerate progress toward finding a cure.

Peripheral blood mononuclear cells (PBMCs) were reprogrammed using the CytoTuneTM-iPS 2.0 Sendai virus reprogramming system. One clone from each line was selected and expanded under feeder-free conditions (37 °C, 5% CO$_2$), with splitting steps every ~4 days by Gentle Cell Dissociation Reagent or ReLeSR (Stem Cell Technologies). All cell lines showed typical iPSC morphology, forming compact colonies with distinct borders and large nuclei (Fig. 1A). The expression of pluripotency markers is indicated by positive immunostaining for OCT4 and SSEA4 (Fig. 1B) and quantitatively assessed by RT-qPCR (Fig. 1D). The expression of pluripotency genes was compared to a reference control of iPSC-CMs derived from a well-validated healthy iPSC line, SKiPS-31.3. All eight lines show the ability to differentiate into the three germ layers as assessed by trilineage differentiation and qPCR (Fig. 1C). PLN-R14 deletion was confirmed in carrier cell lines by TaqMan SNP Genotyping Assay (Fig. 1E). Digital karyotyping revealed normal karyotype without evidence of structural or numerical chromosome aberrations (Fig. S1A). Absence of Sendai-viral transcripts SeV, c-MYC, KLF4, and KOS was confirmed by qPCR (Fig. 1F). Short Tandem Repeat (STR) analysis on 16 genetic loci ruled out cell-line cross-contamination (available from the authors). All cell lines were free of Mycoplasma (Fig. S1B). All investigations were performed around passages 20–25. In summary, we generated five new iPSC lines with a heterozygous PLN-R14 deletion, and three new control iPSC lines from non-carrier family members. All eight of the generated cells have passed the necessary tests as iPSC lines (See Table 1) and are therefore a suitable human

3. Materials and methods

3.1. Reprogramming and cell maintenance

Human donor PBMCs were cultured to expand the erythroblast population for 9–12 days, then reprogrammed using the CytoTuneTM-iPS 2.0 Sendai Reprogramming Kit (Thermo Fisher) containing Sendai virus particles expressing the four Yamanaka factors (OCT4, SOX2, KLF4, and c-MYC). Cells were incubated for 24 h at 37 °C with 5% CO$_2$. On day 3 post-transduction, cells were plated onto mouse embryonic fibroblasts (MEFs) and kept in DMEM/FCS media. From day 8–17, cells were transitioned to hESC media. On day 18–24, individual clones showing typical iPSC morphology were manually selected and transferred to Matrigel-coated plates. iPSCs were passaged every 4–5 days and maintained in mTeSR1 media (STEMCELL Technologies).

3.2. RNA isolation, reverse transcription, and qPCR

Total RNA was extracted using Quick-RNA™ MiniPrep kit (Zymo Research) and transcribed to cDNA using iScript Reverse Transcription Supermix (Bio-Rad). 1 µg RNA, 4 µl iScript RT Supermix, and water were mixed for a 20 µl total reaction volume. qPCR reactions were run in duplicates in a QuantStudio™ 3 Real-Time PCR system (10 min. at 95 °C; 40 cycles of 15 s at 95 °C and 60 s at 60 °C).

3.3. Immunofluorescence staining

iPSCs were fixed with 4% paraformaldehyde for 15 min and washed with phosphate-buffered saline (PBS). After blocking and permeabilization with a buffer containing 2% BSA, 2% FBS, and NP-40 permeating solution (1 h at room temperature), primary antibodies (Table 2) were applied at 4 °C overnight. Secondary antibodies (Table 2) were applied for 45 min at room temperature and nuclei were stained with DAPI. The cells were observed using a Zeiss Axio Observer microscope and analyzed using ZEN blue edition software (Zeiss).

3.4. Karyotyping

Digital karyotyping was performed using the Illumina Human-CoreExome BeadChip and analyzed using GenomeStudio (Illumina). For sample ISMMSi044-A (1BC1), karyotype was performed by G-banding at WiCell with a band resolution of 375–400 in 20 metaphase cells.

3.5. Trilineage differentiation

iPSCs were differentiated into the three germ layers using STEM-diff™ Trilineage Differentiation Kit (STEMCELL Technologies). qPCR was performed using the hPSC Trilineage Differentiation qPCR Array (catalog #07515, STEMCELL Technologies) which includes primers and probes for the detection of 90 genes expressed in either undifferentiated iPSCs or each of the three germ layers. Fold changes in gene expression were determined using the $\Delta\Delta C_t$ method with normalization to the housekeeping gene *18S*.

3.6. STR analysis

Genomic DNA was extracted using PureLink™ Genomic DNA Mini Kit (Thermo Fisher). STR analysis for 16 loci was performed at WiCell using the Promega PowerPlex 16HS system. STR for parental lines was not conducted due to the limited number of avilable PBMCs, which were all used for reprogramming.
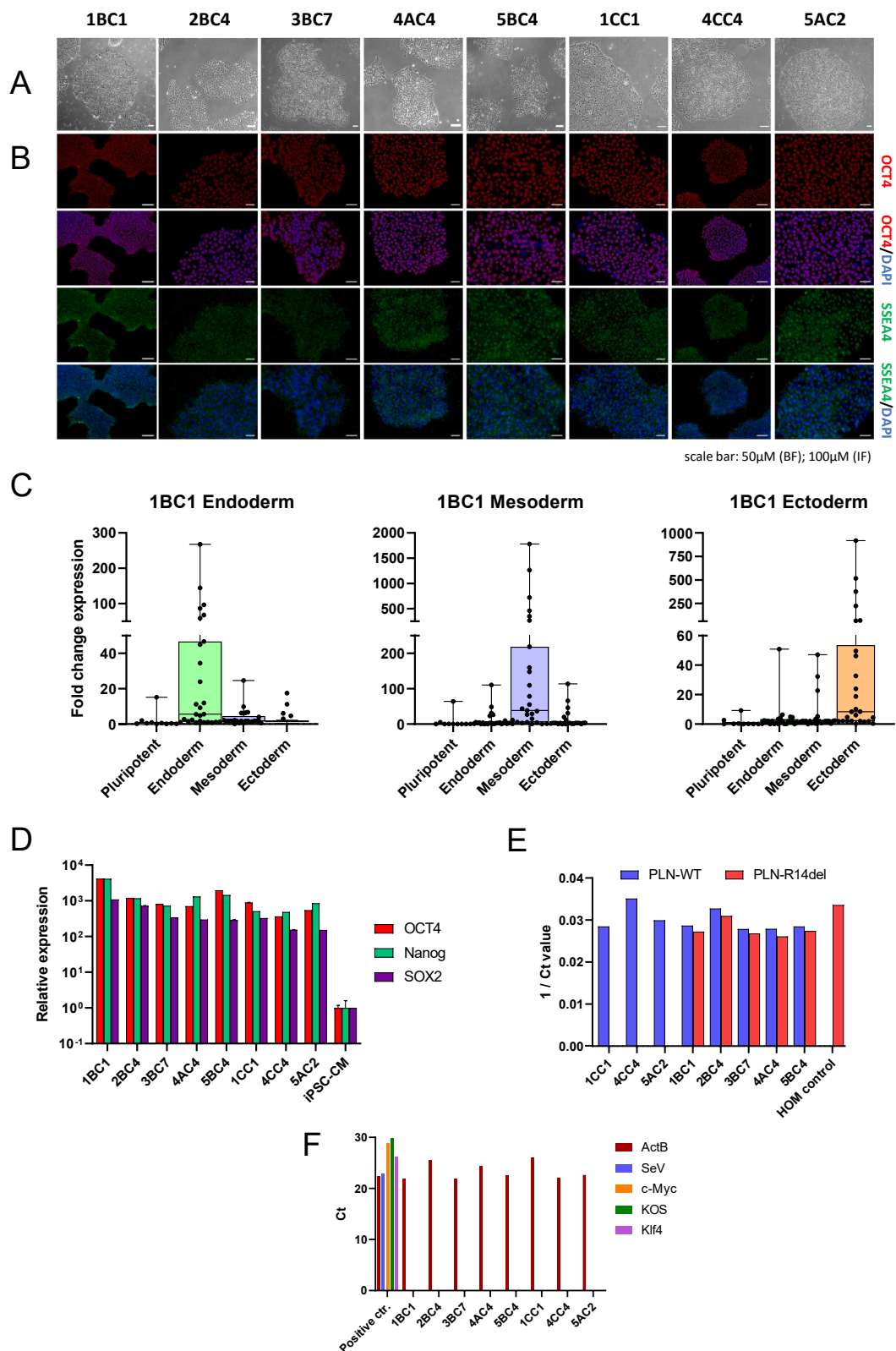
Fig. 1. Characterization of iPSC lines. (A) Brightfield microscopy showing iPSC colony morphology. (B) Immunostainings of pluripotency markers OCT4 (red) and SSEA4 (green). (C) Trilineage differentiation potential of 1BC1 cell line. Box and whisker plots showing fold expression change relative to iPSCs, calculated using 18S rRNA as a housekeeping gene. Data for other lines available from the authors. (D) Relative expression of pluripotency markers OCT4, NANOG, and SOX2 compared to iPSC-derived cardiomyocytes, as measured by qPCR. (E) The genotypes of three non-carrier and five carrier lines were verified by qPCR. (F) Absence of Sendai-viral transcripts SeV, c-MYC, KLF4, and KOS confirmed by qPCR.

Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	Brightfield imaging	Normal	Fig. 1A
Phenotype	Qualitative analysis: immunocytochemistry	Positive for pluripotency markers Oct4 and SSEA-4	Fig. 1B
	Quantitative analysis: RT-qPCR	Expression of pluripotency genes was assessed by qPCR	Fig. 1D
Genotype	Karyotype and resolution	ISMMSi044-A: 46,XY (375–400 resolution) All other lines: Illumina HumanCoreExome BeadChip digital karyotyping	Fig. S1A; available from the authors
Identity	STR analysis	16 loci analyzed, ruled out cross-contamination	Available from the authors
Mutation analysis (IF APPLICABLE)	TaqMan Genotype Assay	ISMMSi044-A, ISMMSi045-A, ISMMSi046-A, ISMMSi047-A, ISMMSi048-A: Heterozygous for PLN-R14del ISMMSi049-A, ISMMSi050-A, ISMMSi051-A: Wild-type	Fig. 1E
Microbiology and virology	Mycoplasma	MycoAlert™ Mycoplasma Detection Kit: all negative	Fig. S1B
Differentiation potential	Directed differentiation	Markers specific for each germ layer (96 total genes) were detected by qRT-PCR and fold expression from iPSC was calculated	Fig. 1C
List of recommended germ layer markers	Trilineage differentiation qPCR array	This qPCR array contains validated primers and probes for detection of 90 genes whose expression is correlated with undifferentiated iPSCs or their derivatives undergoing the early stages of differentiation, as well as six endogenous (housekeeping) control genes.	Fig. 1C
Donor screening (OPTIONAL)	HIV 1 + 2 Hepatitis B, Hepatitis C	Not performed	N/A
Genotype additional	Blood group genotyping	Not performed	N/A

Table 1 (*continued*)

Classification	Test	Result	Data
info (OPTIONAL)	HLA tissue typing	Not performed	N/A

Table 2
Reagents details.

	Antibodies used for immunocytochemistry/flow-cytometry			
	Antibody	Dilution	Company Cat #	RRID
Pluripotency Markers	Rabbit anti-OCT4	1:100	Abcam, Cat# ab19857	AB_445175
	Mouse anti-SSEA4	1:100	Thermo Fisher, Cat# MA1-021	AB_2536687
Secondary antibodies	Goat anti-Rabbit IgG, Alexa Fluor 555	1:1000	Thermo Fisher, Cat# A-21428	AB_2535849
	Goat anti-Mouse IgG, Alexa Fluor 488	1:1000	Thermo Fisher, Cat# A-11001	AB_2534069

	Primers		
	Target	**Size of band**	**Forward/Reverse primer (5′-3′)**
Pluripotency markers (qRT-PCR)	OCT4	106 bp	CCTGAAGCAGAAGAGGATCACC/ AAAGCGGCAGATGGTCGTTTGG
	NANOG	115 bp	CTCCAACATCCTGAACCTCAGC/ CGTCACACCATTGCTATTCTTCG
	SOX2	109 bp	GCTCGCAGACCTACATGAAC/ GCCTCGGACTTGACCACAG
Reference gene	18S	159 bp	ACCCGTTGAACCCCATTCGTG/ GCCTCACTAAACCATCCAATCGG
Sendai virus detection (qPCR)	SeV c-MYC KOS KLF4 ACTB (positive control)	N/A	Assay ID: Mr04269880_mr Assay ID: Mr04269876_mr Assay ID: Mr04421257_mr Assay ID: Mr04421256_mr Assay ID: Hs01060665_g1
SNP Genotyping Assay	hPLN	72 bp	GGAGAAAGTCCAATACCTCACTCG/ CGTGCTTGTTGAGGCATTTCA WT-Probe: FAM-CAGCTATAAGAAGAGCCTCA Mutant-Probe: VIC-CAGCTATAAGAAGAGCCTCA

3.7. Genotyping

Genotyping was performed using a Custom TaqMan™ SNP Genotyping Assay (Thermo Fisher), including primers and probes specific to the human wild-type and mutant PLN coding sequences (Table 2). The PCR reaction was run in duplicates per the following protocol: 30 s at 60 °C [pre-read]; 2 min. at 50 °C, 10 min. at 95 °C, followed by 40 cycles of 15 s at 95 °C and 60 s at 60 °C [amplification]; 30 s at 60 °C [post-read]. Data were analyzed by QuantStudio Design and Analysis software.

3.8. Mycoplasma detection

Mycoplasma contamination was evaluated using MycoAlert™ Mycoplasma Detection Kit (Lonza).

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.

Data availability

Data will be made available on request.

Acknowledgments

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scr.2022.102737.

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