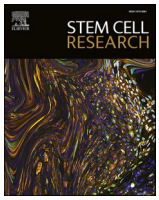
Stem Cell Research 72 (2023) 103208

Contents lists available at ScienceDirect

Stem Cell Research

journal homepage: www.elsevier.com/locate/scr



Lab Resource: Multiple Cell Lines

# Generation and characterization of novel human induced pluripotent stem cell (iPSC) lines originating from five asymptomatic individuals carrying the PLN-R14del pathogenic variant and a non-carrier relative

Valentina Balducci [a,1], Francesco Scardigli [b,1], Magdalena Harakalova [b,c], J. Peter. van Tintelen [d], Pieter A. Doevendans [b,e], Kevin D. Costa [f], Irene C. Turnbull [f], Joost P. G. Sluijter [b,c], Francesca Stillitano [b,c,f,*]

[a] Department of Neurosciences, Psychology, Drugs and Child Health, University of Florence, Florence, Italy
[b] Department of Cardiology, Laboratory of Experimental Cardiology, University Medical Center Utrecht, Utrecht University, Utrecht, The Netherlands
[c] Regenerative Medicine Utrecht, Circulatory Health Research Center, Utrecht University, Utrecht, The Netherlands
[d] Department of Genetics, University Medical Center Utrecht, The Netherlands
[e] Netherlands Heart Institute, Utrecht, The Netherlands
[f] Cardiovascular Research Institute, Icahn School of Medicine at Mount Sinai, New York, NY, USA

A B S T R A C T

The rare genetic alteration PLN-c.(40_42delAGA), leading to the deletion of arginine 14 (p.R14del) in phospholamban, is associated with dilated and arrhythmogenic cardiomyopathies occurring in early-adulthood. However, some carriers remain asymptomatic with normal lifespans. Here, we report human induced pluripotent stem cell (iPSC) lines generated from peripheral blood mononuclear cells (PBMCs) of five PLN-R14del carriers, who were asymptomatic at the time of blood collection, and one non-carrier family member. Each line exhibited typical iPSC morphology, pluripotency markers, and tri-lineage differentiation. These cell lines provide a valuable model to investigate the mechanisms underlying the onset, progression, and patient-specific resistance to PLN-R14del-induced cardiomyopathy.

***Resource Table***

| | |
|---|---|
| Unique stem cell lines identifier | ISMMSi052-A<br>ISMMSi053-A<br>ISMMSi054-A<br>ISMMSi056-A<br>ISMMSi057-A<br>ISMMSi058-A |
| Alternative name(s) of stem cell lines | (ISMMSi052-A) PLN-3CC5<br>(ISMMSi053-A) aPLN-R14del-2AC4<br>(ISMMSi054-A) aPLN-R14del-3AC3<br>(ISMMSi056-A) aPLN-R14del-4BC3<br>(ISMMSi057-A) aPLN-R14del-5CC6<br>(ISMMSi058-A) aPLN-R14del-1AC2 |
| Institution | Cardiovascular Research Institute, Icahn School of Medicine at Mount Sinai, New York, NY |
| Contact information of distributor | Francesca Stillitano, f.stillitano@umcutrecht.nl |
| Type of cell lines | iPSCs |
| Origin | Human |

(*continued on next column*)

(*continued*)

| | |
|---|---|
| Additional origin info required *for human ESC or iPSC* | ISMMSi052-A, Age: 68, Sex: M; ISMMSi053-A, Age: 20, Sex: M ISMMSi054-A, Age: 27, Sex: F; ISMMSi056-A, Age: 28, Sex: M ISMMSi057-A, Age: 39, Sex: M; ISMMSi058-A, Age: 65, Sex: MEthnicity (all) : White. |
| Cell Source | Total PBMCs |
| Clonality | Clonal |
| Method of reprogramming | Sendai Virus reprogramming (OCT4, SOX2, KLF4, c-MYC) |
| Genetic Modification | Yes |
| Type of Genetic Modification | Hereditary |
| Evidence of the reprogramming transgene loss | RT-qPCR |
| Associated disease | Dilated and arrhythmogenic cardiomyopathies |
| Gene/locus | PLN/6q22.31 |
| Date archived/stock date | 11.05.2023 |

(*continued on next page*)

* Corresponding author at: Department of Cardiology, Laboratory of Experimental Cardiology, University Medical Center Utrecht, Utrecht University, Utrecht, The Netherlands.
*E-mail address:* f.stillitano@umcutrecht.nl (F. Stillitano).
[1] These authors contributed equally.

https://doi.org/10.1016/j.scr.2023.103208
Received 17 July 2023; Received in revised form 12 September 2023; Accepted 18 September 2023
Available online 21 September 2023

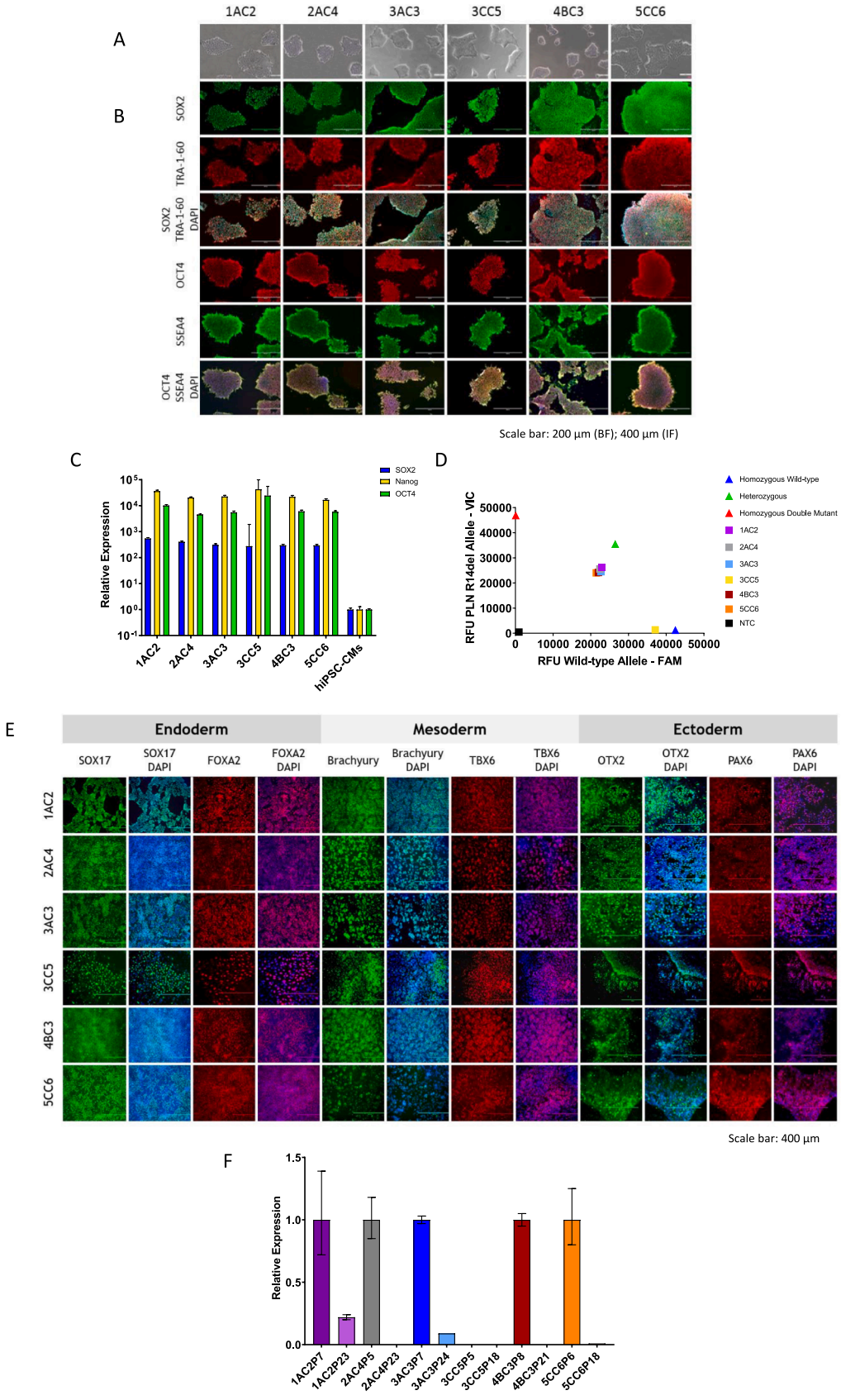



**Fig. 1.** Characterization of five iPSC lines from asymptomatic PLN-R14del carriers and a non-carrier relative.

**Table 1**
Characterization and validation.

| Classification | Test | Result | Data |
|---|---|---|---|
| Morphology | Brightfield imaging | Normal | Fig. 1A |
| Phenotype | Qualitative analysis: immunocytochemistry | Positive for pluripotency markers OCT4, SSEA4, SOX2, TRA-1–60 | Fig. 1B |
| | Quantitative analysis: RT-qPCR | Positive expression of pluripotency genes Sox2, Nanog and Oct4 | Fig. 1C |
| Genotype | Karyotype and resolution | iCS-digital™ PSC test | Fig. S1A |
| Identity | STR analysis | 18 loci analyzed, ruled out cross-contamination | Submitted in archive with journal |
| Mutation analysis (IF APPLICABLE) | TaqMan Genotype Assay | Heterozygous for PLN-R14del:<br>ISMMSi053-A<br>ISMMSi054-A<br>ISMMSi056-A<br>ISMMSi057-A<br>ISMMSi058-A<br>Wild type:<br>ISMMSi052-A | Fig. 1D |
| Microbiology and virology | Mycoplasma | MycoAlert™ Mycoplasma Detection Kit: all negative | Fig. S1B |
| Differentiation potential | Directed differentiation | Markers specific for each germ layer (6 in total) were detected by immunocytochemistry | Fig. 1E |
| List of recommended germ layer markers | Immunocytochemistry | Positive expression of germ layer markers:<br>Ectoderm: OTX2, PAX6<br>Endoderm: SOX17, FoxA2<br>Mesoderm: Brachyury, TBX6 | Fig. 1E |
| Donor screening (OPTIONAL) | HIV 1 + 2 Hepatitis B, Hepatitis C | Not performed | N/A |
| Genotype additional info (OPTIONAL) | Blood group genotyping | Not performed | N/A |
| | HLA tissue typing | Not performed | N/A |

(*continued*)

| | |
|---|---|
| Cell line repository/bank | https://hpscreg.eu/cell-line/ISMMSi052-A<br>https://hpscreg.eu/cell-line/ISMMSi053-A<br>https://hpscreg.eu/cell-line/ISMMSi054-A<br>https://hpscreg.eu/cell-line/ISMMSi056-A<br>https://hpscreg.eu/cell-line/ISMMSi057-A<br>https://hpscreg.eu/cell-line/ISMMSi058-A |
| Ethical approval | Medical Ethical Committee (TCBio) of University Medical Center (UMC) Utrecht; approval number 12–387 |

## 1. Resource utility

The PLN-R14 deletion variant is associated with inherited cardiomyopathy, causing heart failure. However, some carriers exhibit resistance and remain asymptomatic with normal lifespans. iPSCs derived from these carriers offer a unique opportunity to explore protective mechanisms and potential therapies that protect PLN-R14del carriers from experiencing the typical disease progression.

## 2. Resource details

Phospholamban (PLN) is a small membrane protein that plays a critical role in regulating calcium handling within cardiomyocytes by interacting with the calcium-ATPase (SERCA) in the sarcoplasmic reticulum. Mutations in the PLN gene have been associated with the development of arrhythmogenic cardiomyopathy (ACM), dilated cardiomyopathy (DCM) and heart failure (van der Zwaag et al. 2012). The PLN-R14del has been identified as increasing the risk of malignant ventricular arrhythmias, contractile dysfunction, and heart failure (Haghighi et al. 2006). PLN-R14del disease typically manifests between late adolescence and the fourth decade of life (van Rijsingen et al. 2014). However, extensive epidemiological studies have revealed that many individuals carrying the PLN-R14del pathogenic variant remain asymptomatic, and little is known regarding the triggers that could influence the clinical course of the disease in an individual carrier (Hof et al 2019). Here, we present six resource lines that, together with our recently published iPSCs from symptomatic PLN-R14del carriers and their non-carrier family members (Mittal et al., 2022), can facilitate research into disease progression versus inherent protection, and the identification of potential interventions. Peripheral Blood Mononuclear Cells (PBMCs) of the donors were reprogrammed into iPSCs using the non-integrating Sendai virus (CytoTuneTM-iPS 2.0 reprogramming system), containing the four Yamanaka factors (OCT4, SOX2, KLF4 and c-MYC). From each line, a single clone was selected and expanded under feeder-free conditions (37 °C, 5% $CO_2$), with splitting steps approximately every 4 days by 0.5 mM EDTA or ReLeSR (Stem Cell Technologies). All lines displayed a typical iPSC morphology, with compact colonies and defined edges (Fig. 1A). The expression of pluripotency markers was confirmed by positive immunostaining for SOX2, TRA-1–60, OCT4 and SSEA4 (Fig. 1B). Additionally, the expression of SOX2, NANOG and OCT4 was quantitatively assessed using RT-qPCR and comparing the results to a reference control consisting of iPSC-derived cardiomyocytes (iPSC-CMs) generated from a well-validated healthy iPSC line (SKiPS-31.3) (Fig. 1C). The PLN-R14del pathogenic variant was verified in all cell lines by allelic discrimination using TaqMan™ Genotyping Assay. Cell lines 1AC2, 2AC4, 3AC3, 4BC3 and 5CC6 correspond to mutation carriers, while cell line 3CC5 represents a non-carrier related to the 3AC3 individual. Positive controls included wild-type (blue triangle), mutant heterozygous (green triangle) and mutant homozygous (red triangle) PLN plasmids (Fig. 1D). The ability to differentiate into all three germ layers (ectoderm, endoderm, and mesoderm) was assessed by positive immunocytochemistry for multiple germ layer-specific markers (Fig. 1E). Furthermore, the presence/absence of Sendai-viral transcript (SeV) was confirmed by RT-qPCR (Fig. 1F) in low passage (P5-7) and high passage (P18-24) cell lines. Digital karyotyping revealed no evidence of chromosomal aberrations for all the cell lines, as shown in the table reporting a summary of the depicted copy number variations (CNV) (Fig. S1A). Additionally, Short Tandem Repeat (STR) analysis on 18 genetic loci ruled out cell-line cross-contamination (submitted in archive with journal). The B/A ratios of all cell lines were less than 1.2, indicating that they were free from mycoplasma contamination (Fig. S1B). In summary, each of the six cell lines has successfully undergone the required assessments confirming

**Table 2**
Reagents details.

| | Antibodies used for immunocytochemistry/flow-cytometry | | | |
|---|---|---|---|---|
| | **Antibody** | **Dilution** | **Company Cat #** | **RRID** |
| Pluripotency Markers | Rabbit anti-OCT4 | 1:200 | Thermo Fisher Scientific, Cat# A24867 | AB_2650999 |
| | Rat anti-SOX2 | 1:100 | Thermo Fisher Scientific, Cat# A24759 | AB_2651000 |
| | Mouse anti-SSEA4 | 1:100 | Thermo Fisher Scientific, Cat# A24866 | AB_2651001 |
| | Mouse anti-TRA-1–60 | 1:100 | Thermo Fisher Scientific, Cat# A24868 | AB_2651002 |
| Differentiation Markers | Goat anti-FOXA2 | 1:50 | R&D Systems, Cat# AF2400-SP | AB_2294104 |
| | Goat anti-SOX17 | 1:50 | R&D Systems, Cat# AF1924-SP | AB_355060 |
| | Goat anti-Brachyury | 1:100 | R&D Systems, Cat# AF2085 | AB_2200235 |
| | Goat anti-TBX6 | 1:100 | R&D Systems, Cat# AF4744 | AB_2200834 |
| | Goat anti-OTX2 | 1:100 | R&D Systems, Cat# AF1979 | AB_2157172 |
| | Rabbit anti-PAX-6 | 1:100 | Thermo Fisher Scientific, Cat# 42–6600 | AB_2533534 |
| Secondary Antibodies | Donkey anti-rabbit 555 | 1:250 | Thermo Fisher Scientific, Cat# A24869 | AB_2651006 |
| | Donkey anti-rat 488 | 1:250 | Thermo Fisher Scientific, Cat# A24876 | AB_2651007 |
| | Goat anti-mouse IgG3 488 | 1:250 | Thermo Fisher Scientific, Cat# A24877 | AB_2651008 |
| | Goat anti-mouse IgM 555 | 1:250 | Thermo Fisher Scientific, Cat# A24871 | AB_2651009 |
| | Donkey anti-goat 488 | 1:250 | Thermo Fisher Scientific, Cat# A11055 | AB_2534102 |
| | **Primers** | | | |
| | **Target** | **Size of band** | **Forward/Reverse primer (5′-3′)** | |
| Pluripotency Markers (qRT-PCR) | *OCT4* | 163 bp | GAGAACCGAGTGAGAGGCAACC/ CATAGTCGCTGCTTGATCGCTTG | |
| | *NANOG* | 116 bp | TGCAAGAACTCTCCAACATCCT/ ATTGCTATTCTTCGGCCAGTT | |
| | *SOX2* | 215 bp | CGAGTAGGACATGCTGTAGGT/ TGGACAGTTACGCGCACAT | |
| Reference Gene | *18S* | 159 bp | ACCCGTTGAACCCCATTCGTG/ GCCTCACTAAACCATCCAATCGG | |
| Sendai virus detection (qPCR) | SeV | N/A | Assay ID: Mr04269880_mr | |
| SNP Genotyping Assay | PLN | 72 bp | GGAGAAAGTCCAATACCTCACTCG/ CGTGCTTGTTGAGGCATTTCA WT-Probe: FAM-CAGCTATAAGAAGAGCCTCA Mutant-Probe: VIC-CAGCTATAAGAAGAGCCTCA | |

their status as iPSCs (Table 1).

## 3. Materials and methods

### 3.1. Reprogramming and cell culture

Peripheral blood mononuclear cells (PBMCs) were reprogrammed as previously described (Mittal et al., 2022). Briefly, PBMCs were isolated from human donors and cultured to expand the erythroblast population for 9–12 days. The cells were then reprogrammed using CytoTune™ iPS 2.0 Sendai Reprogramming Kit (Thermo Fisher Scientific) containing Sendai virus particles expressing the four Yamanaka factors (OCT4, SOX2, KLF4, c-MYC). Three days after 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 to 24, individual clones showing typical iPSC morphology were manually selected and transferred to 6-well plates coated with 1X Matrigel (Matrigel hESC-Qualified Matrix, Corning). iPSCs were passaged every 4–5 days and maintained in Essential 8 (E8) medium (Thermo Fisher Scientific) at 37 °C, 5% $CO_2$ and 5% $O_2$. iPSCs underwent characterization upon reaching passages 18 to 24.

### 3.2. Immunofluorescent staining

iPSCs were fixed at room temperature with 4% paraformaldehyde for 15 min and washed with phosphate-buffered saline (PBS). After blocking and permeabilization for one hour with 2% BSA, 2% FBS, and 0.05% Triton X-100 solution, primary antibodies (Table 2) were applied at 4 °C overnight. Secondary antibodies (Table 2) were applied for 1 h at room temperature and nuclei were stained with DAPI (NucBlue™ Fixed Cell Stain, DAPI nuclear DNA stain, Thermo Fisher Scientific). The cells were observed using EVOS™ FL Digital Inverted Fluorescence Microscope (Invitrogen) and analyzed using ImageJ software.

### 3.3. RNA isolation, reverse transcription, and qPCR

Total RNA was extracted using RNeasy Plus Mini Kit (Qiagen) and reverse transcribed to cDNA using qScript™ cDNA Synthesis Kits (QuantaBio). qPCR was performed on CFX96 Real-Time PCR Detection System (BioRad) using commercial primers and TaqMan Gene expression Assay (Table 2).

### 3.4. Karyotyping

Genomic DNA was extracted using Blood & Cell Culture DNA Kit (Qiagen). Digital karyotyping was performed using the iCS-digital™ PSC test (Stem Genomics).

### 3.5. Genotyping

The presence or absence of the heterozygous PLN-R14del pathogenic variant was evaluated using a custom TaqMan™ Genotyping Assay (Thermo Fisher Scientific), with primers and probes specific for the human wild-type and mutant PLN sequence. PCR reaction was performed in duplicates per the following protocol: 3 min at 95 °C; 40 cycles of 10 sec at 95 °C and 30 sec at 60 °C.

### 3.6. Trilineage Differentiation

iPSCs were differentiated into the three germ layers using STEMdiff™ Trilineage Differentiation Kit (Stem Cell Technologies). The differentiated cells were fixed on day 5 (endoderm and mesoderm) and on day 7 (ectoderm) for immunofluorescent staining.

### 3.7. STR analysis

Short Tandem Repeats analysis (STR) was performed using the FTA Sample Collection Kit for Human Cell Authentication Service (ATCC®). Seventeen STR loci plus Amelogenin were amplified using the Promega's PowerPlex® 18D system. STR for parental lines was not conducted due to the limited number of available PBMCs, which were all used for reprogramming.

### 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

We thank the UNRAVEL Research Data Platform team at the University Medical Centre Utrecht (UMCU) and the Dutch PLN Foundation for their assistance with individual identification and PBMC collection. We also acknowledge the Stem Cell Engineering CoRE Facility at the Icahn School of Medicine at Mount Sinai, for their valuable assistance in cell reprogramming. This work was supported by the Project EVICARE (No. 725229) of the European Research Council (ERC), ZonMw PSIDER-Heart grant (no 10250022110004), NWO TTP Harvey (2021/TTW/01038252) (to J.P.G.S), and the Leducq Foundation (18CVD01) (to P.A.D., K.D.C., P.v.T., M.H., F.S.).

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scr.2023.103208.

## References

Haghighi, K., Kolokathis, F., Gramolini, A.O., Waggoner, J.R., Pater, L., Lynch, R.A., Fan, G.C., Tsiapras, D., Parekh, R.R., Dorn II, G.W., MacLennan, D.H., Kremastinos, D.T., Kranias, E.G., 2006. A mutation in the human phospholamban gene, deleting arginine 14, results in lethal, hereditary cardiomyopathy. PNAS 103 (5), 1388–1393.

Hof, I.E., van der Heijden, J.F., Kranias, E.G., Sanoudou, D., de Boer, R.A., van Tintelen, J.P., van der Zwaag, P.A., Doevendans, P.A., 2019. Prevalence and cardiac phenotype of patients with a phospholamban mutation. Neth Heart J. 27, 64–69.

Mittal, N., Dave, J., Harakalova, M., van Tintelen, J.P., Asselbergs, F.W., Doevendans, P. A., Costa, K.D., Turnbull, I.C., Stillitano, F., 2022. 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. Stem Cell Res. 60, 102737.

van der Zwaag, P.A., van Rijsingen, I.A., Asimaki, A., Jongbloed, J.D., van Veldhuisen, D. J., Wiesfeld, A.C., Cox, M.G., van Lochem, L.T., de Boer, R.A., Hofstra, R.M., Christiaans, I., van Spaendonck-Zwarts, K.Y., Lekanne dit Deprez, R.H., Judge, D.P., Calkins, H., Suurmeijer, A.J., Hauer, R.N., Saffitz, J.E., Wilde, A.A., van den Berg, M. P., van Tintelen, J.P., 2012. Phospholamban R14del mutation in patients diagnosed with dilated cardiomyopathy or arrhythmogenic right ventricular cardiomyopathy: evidence supporting the concept of arrhythmogenic cardiomyopathy. J Heart Fail. 14 (11), 1199–1207.

van Rijsingen, I.A., van der Zwaag, P.A., Groeneweg, J.A., Nannenberg, E.A., Jongbloed, J.D., Zwinderman, A.H., Pinto, Y.M., Dit Deprez, R.H., Post, J.G., Tan, H. L., de Boer, R.A., Hauer, R.N., Christiaans, I., van den Berg, M.P., van Tintelen, J.P., Wilde, A.A., 2014. Outcome in phospholamban R14del carriers: results of a large multicentre cohort study. Circ. Cardiovasc. Genet. 7 (4), 455–465.