Stem Cell Research 45 (2020) 101764

Contents lists available at ScienceDirect

Stem Cell Research

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

Lab Resource: Multiple Cell Lines

Generation of two human induced pluripotent stem cell lines, LUMCi020-A and LUMCi021-A, from two patients with Catecholaminergic Polymorphic Ventricular Tachycardia carrying heterozygous mutations in the *RYR2* gene

Viviana Meraviglia[a], Christiaan H. Arendzen[a,b], Christian Freund[a,b], Douwe E. Atsma[c], Christine L. Mummery[a], Milena Bellin[a,*]

[a] Department of Anatomy and Embryology, LUMC, Leiden, the Netherlands
[b] Leiden University Medical Center hiPSC Hotel, Leiden, The Netherlands
[c] Department of Cardiology, LUMC, Leiden, the Netherlands

A B S T R A C T

Catecholaminergic Polymorphic Ventricular Tachycardia (CPVT) is a malignant channelopathy associated with exercise- and stress-induced cardiac sudden death. The autosomal dominant form of CPVT is due to mutations in the ryanodine receptor 2 (*RYR2*) gene. We generated induced pluripotent stem cells (hiPSCs) from skin fibroblasts of two patients carrying the c.12441 G > T and c.14885 A > G *RYR2* missense mutations, respectively, using non-integrating Sendai virus. These lines show the typical morphology of pluripotent cells, express pluripotency markers, display a normal karyotype and differentiate towards the three germ layers *in vitro*. These lines represent a human cellular model to study the molecular basis of CPVT.

Resource Table

Unique stem cell lines identifier	LUMCi020-A LUMCi021-A
Alternative names of stem cell lines	LUMC0159iRYR08 LUMC0160iRYR01
Institution	Leiden University Medical Center, LUMC
Contact information of distributor	Milena Bellin (m.bellin@lumc.nl) Viviana Meraviglia (v.meraviglia@lumc.nl)
Type of cell lines	iPSCs
Origin	Human
Cell Source	Skin Fibroblasts
Clonality	Clonal
Method of reprogramming	Sendai virus
Multiline rationale	Non-isogenic cell lines obtained from patients with mutations in the same gene (*RYR2*)
Gene modification	YES
Type of modification	Spontaneous mutation
Associated disease	Catecholaminergic Polymorphic Ventricular Tachycardia type 1 (OMIM: 604,772)
Gene/locus	*RYR2*/1q43 Heterozygous *RYR2* c.12441 G > T (LUMCi020-A) Heterozygous *RYR2* c.14885 A > G (LUMCi021-A)
Method of modification	N/A
Name of transgene or resistance	N/A
Inducible/constitutive system	N/A
Date archived/stock date	August 2018 (LUMCi020-A) September 2018 (LUMCi021-A)
Cell line repository/bank	N/A
Ethical approval	The generation of the lines was approved by the Leiden University ethics committee under the P 13.080 "Parapluprotocol: hiPSC".

1. Resource utility

The hiPSC lines generated here represent an unlimited source of human cardiac cells *in vitro* and are thus an excellent tool for modeling CPVT, to elucidate the underlying molecular mechanisms of the disease and screen for drugs that may provide treatment.

2. Resource details

Catecholaminergic Polymorphic Ventricular Tachycardia (CPVT) is a rare inherited arrhythmogenic disease characterized by adrenergic stress-induced ventricular tachyarrhythmia, which may lead to syncope or sudden cardiac death in the setting of a structurally normal heart (Imberti et al., 2016). Mutations in ryanodine receptor 2 gene (*RYR2)*

* Corresponding author.
E-mail address: m.bellin@lumc.nl (M. Bellin).

https://doi.org/10.1016/j.scr.2020.101764
Received 28 November 2019; Received in revised form 13 February 2020; Accepted 9 March 2020
Available online 08 April 2020

Table 1
Summary of lines.

iPSC line names	Abbreviation in figures	Gender	Age	Ethnicity	Genotype of locus	Disease
LUMCi020-A	LUMCi020-A	Male	28	Caucasian	Heterozygous *RYR2* c.12441 G>T	Catecholaminergic Polymorphic Ventricular Tachycardia
LUMCi021-A	LUMCi021-A	Male	49	Caucasian	Heterozygous *RYR2* c.14885 A>G	Catecholaminergic Polymorphic Ventricular Tachycardia

Table 2
Characterization and validation.

Classification	Test	Result	Data
Morphology	Brightfield microscopy	Normal morphology	Fig. 1 panel A
Phenotype	Qualitative analysis of immunofluorescence staining	Positive immunostaining of pluripotency markers: SSEA4, OCT3/4, NANOG	Fig. 1 panel B
	Quantitative analysis by RT-qPCR	Fold change for pluripotency genes (RT-qPCR): LUMCi020-A *OCT4* = 7651 ± 487 *SOX2* = 3469 ± 102 *NANOG* = 545 ± 26 LUMCi021-A *OCT4* = 6727 ± 329 *SOX2* = 2714 ± 159 *NANOG* = 648 ± 31	Fig. 1 panel C
Genotype	Whole genome array (KaryoStat™ Assay) Resolution 1-2 Mb	Normal karyotype: 46, XY for all the two iPSC lines	Supplementary Fig. 1C
Identity	Microsatellite PCR (mPCR) OR STR analysis	N/A 24 markers tested with 100% match	N/A Available with the authors
Mutation analysis	Sequencing	Heterozygous *RYR2* c.12441 G>T (LUMCi020-A) Heterozygous *RYR2* c.14885 A>G (LUMCi021-A)	Fig. 1 panel E
	Southern Blot OR WGS	N/A	N/A
Microbiology and virology	Sendai virus	RT-qPCR analysis: Negative	Supplementary Fig. 1A and B
	Mycoplasma	Immunostaining: Negative PCR analysis: Negative	Supplementary File 1
Differentiation potential	Trilineage *in vitro* differentiation by immunofluorescence analysis	Positive immunostaining of the three germ layer markers: ectodermal (β3-tubulin, SOX1, PAX6), mesodermal (Vimentin, αSMA) and endodermal (FOXA2, SOX17)	Fig. 1 panel D
Donor screening (OPTIONAL)	HIV 1 + 2 Hepatitis B, Hepatitis C	PCR analysis: Negative	Supplementary File 1
Genotype additional info (OPTIONAL)	Blood group genotyping	N/A	N/A
	HLA tissue typing	N/A	N/A

are found in approximately 60% of clinically affected patients and cause the autosomal dominant form of CPVT (Cerrone et al., 2009). *RYR2* encodes the cardiac calcium-release channel which, by controlling calcium release from the sarcoplasmic reticulum, is essential for the physiological excitation-contraction coupling in cardiomyocytes. Primary cardiomyocytes from CPVT patients are not easily accessible for research. Given this limited availability, patient-specific hiPSCs could provide a solution by offering an unlimited source of human cardiomyocytes bearing the mutation. We generated hiPSCs from two patients carrying different heterozygous mutations in *RYR2* (Table 1), the novel c.12441 G>T (NM_001035.3; p.R4147S, NP_001026.2) and the previously reported c.14885 A>G (NM_001035.3; p.Y4962C, NP_001026.2, (van der Werf et al. 2011). Fibroblasts cultured from patient skin biopsies were successfully reprogrammed into hiPSCs using replication-defective and persistent Sendai virus (SeV) carrying *OCT3/4, SOX2, KLF4, MYC* (Nishimura et al., 2011). At least three hiPSC clones were generated from each patient; LUMCi020-A and LUMCi021-A clones reported in this work were randomly selected and characterized as reported in Table 2. All hiPSC lines displayed the typical human embryonic-stem cell morphology with high nucleus to cytoplasm ratio (Fig. 1A). Immunofluorescence analysis showed the expression of pluripotency markers OCT3/4, NANOG at the nuclear level and SSEA4 as surface protein (Fig. 1B). In accordance with immunofluorescence results, qPCR analysis revealed the upregulation of endogenous pluripotency genes (*SOX2, OCT3/4, NANOG*) in hiPSCs compared to their parental skin fibroblasts (Fig. 1C). Both hiPSC lines were also negative for SeV after 7 passages *in vitro* as indicated by qPCR (Supplementary Fig. 1A) and immunostaining for SeV (Supplementary Fig. 1B). Trilineage differentiation assay showed the ability of hiPSC lines to differentiate into cells of the three germ layers, as indicated by immunostaining for ectodermal- (TUBB3, SOX1, PAX6), mesodermal- (VIM, αSMA) and endodermal- (FOXA2, SOX17) markers (Fig. 1D). The presence of patient *RYR2* mutations was confirmed by Sanger sequencing (Fig. 1E). The preservation of genomic integrity was determined by whole genome array with a combination of nonpolymorphic (copy number) and polymorphic probes (single nucleotide polymorphism). Using a resolution of 1-2 Mb, no chromosomal aberrations were observed (Supplementary Fig. 1C). Both hiPSC lines were authenticated (100% match) by comparing the short tandem repeat (STR) profiles of the hiPSC clones with the parental skin fibroblasts (Table 2, available with the authors). Finally, hiPSCs were mycoplasma free (Supplementary File 1).

3. Materials and methods

3.1. Ethical statement

This study was approved by the Medical Ethical Committee at the Leiden University Medical Center (P13.080) and informed consent was obtained from both patients.

3.2. Cell culture and reprogramming

Skin fibroblasts were isolated from a skin biopsy and cultured in DMEM/F12 Glutamax supplemented with 10% FBS, 1% penicillin-streptomycin, 10μg/ml Ascorbic acid and 50 μg/ml Gentamicin (only

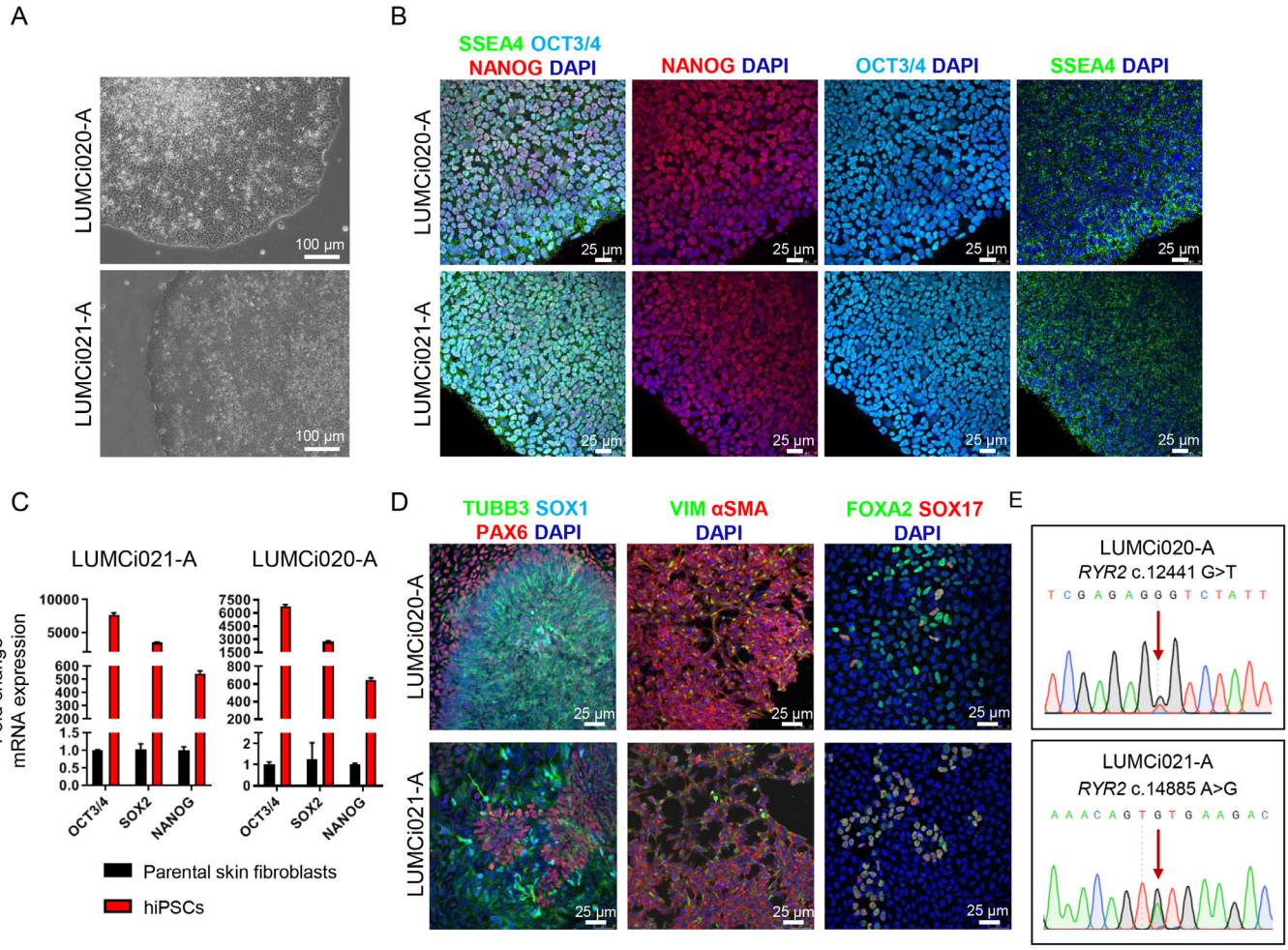

Fig. 1. Generation and characterization of two hiPSC lines LUMCi020-A and LUMCi021-A from two patients with CPVT. (A) Brightfield images showing hiPSC colony morphology. Scale bar: 100 µm. (B) Immunofluorescence analysis for the pluripotency markers SSEA4 (green), OCT3/4 (cyan), and NANOG (red); nuclei stained with DAPI (blue). Scale bar: 25 µm. (C) Gene expression analysis of endogenous pluripotency genes. (D) Immunofluorescence analysis for the three germ layer markers: TUBB3 (green), SOX1 (cyan), and PAX6 (red) for ectoderm; VIM (green) and αSMA (red) for mesoderm; FOXA2 (green) and SOX17 (red) for endoderm; nuclei stained with DAPI (blue). Scale bar: 25 µm. (E) Sanger sequencing results confirming the presence of the heterozygous c.12441G > T mutation (LUMCi020-A) and the heterozygous c.14885A > G mutation (LUMCi021-A) in the RYR2 gene.

after isolation) (all from ThermoFisher Scientific) at 37 °C and 5% CO_2. hiPSCs from skin fibroblasts were generated using Sendai virus and specifically 1 × 10^5 cells were transduced once with 7.5 MOI SeVdp (KOSM302L). One week after transduction, cells were replated on Vitronectin coated plates and cultured in TeSR-E7 (STEMCELL Technologies) until the appearance of the first hiPSC colonies. Cells were maintained in TeSR-E8 (STEMCELL Technologies) until hiPSC colonies were ready to be selected. Selected hiPSC colonies were cultured in Essential 8™ Medium on plates coated with Vitronectin Truncated Recombinant Human Protein (all from Gibco) and expanded twice a week using 0.5 mM Ultra Pure EDTA (Invitrogen) with a split ratio of 1:6. Cells were kept at 37 °C, 5% CO_2 in a humidified incubator.

3.3. qPCR

Total RNA from skin fibroblasts and hiPSCs was extracted using NucleoSpin® RNA kit (Macherey-Nagel). Reverse transcription of RNA (1 µg) was performed using iScript™ cDNA Synthesis Kit following manufacturer's instruction. cDNA was amplified on CFX96 Real-Time PCR Detection System using iTaq Universal SYBR Green Supermix (all from BioRad). Primers are shown in Table 3.

3.4. Immunofluorescence staining

For immunostaining, hiPSCs were fixed in PFA 2% for 30 min, permeabilized with 0.1% of Triton X-100 and blocked with 4% normal swine serum (NSS, DAKO) at RT for 1 h. Primary antibodies were incubated overnight at 4 °C, followed by incubation with secondary antibodies for 1 h at RT (Table 3). Nuclei were counterstained with DAPI. All the images have been acquired using Leica TCS SP8 microscope.

3.5. In vitro trilineage differentiation

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

3.6. Karyotype analysis

hiPSCs (2 × 10^6 cells) at passage 15 were analysed using the KaryoStat™ assay (ThermoFisher Scientific).

Table 3
Reagents details.

Antibodies used for immunocytochemistry Antibody		Dilution	Company Cat # and RRID
Pluripotency markers (Immunocytochemistry)	Mouse IgG2b anti-OCT3/4	1:100	Santa Cruz Biotechnology Cat# sc-5279 RRID:AB_628051
Pluripotency markers (Immunocytochemistry)	Mouse IgG1 anti-NANOG	1:150	Santa Cruz Biotechnology Cat# sc-293121 RRID:AB_2665475
Pluripotency markers (Immunocytochemistry)	Mouse IgG3 anti-SSEA4	1:30	BioLegend Cat# 330402, RRID:AB_1089208
Differentiation markers (Immunocytochemistry)	Mouse IgG2a anti-β3-tubulin	1:4000	Covance Cat# MMS-435P, RRID:AB_2313773
Differentiation markers (Immunocytochemistry)	Anti-SOX1	1:100	R&D Systems Cat# AF3369, RRID:AB_2239879
Differentiation markers (Immunocytochemistry)	Anti-PAX6	1:200	Cell Signaling Technologies Cat# D3A9V, RRID:AB_2797599
Differentiation markers (Immunocytochemistry)	Anti-Vimentin	1:50	Sigma-Aldrich Cat# V6630, RRID:AB_477627
Differentiation markers (Immunocytochemistry)	Anti-αSMA	1:3000	Sigma-Aldrich Cat# A2547, RRID:AB_476701
Differentiation markers (Immunocytochemistry)	Anti-FOXA2	1:100	Millipore Cat# 07-633, RRID:AB_390153
Differentiation markers (Immunocytochemistry)	Anti-SOX17	1:100	R&D Systems Cat# AF1924, RRID: AB_355060
Sendai virus	Anti-SeV	1:600	Kindly provided by N. Nakanishi
Secondary antibodies (Immunocytochemistry)	Donkey anti-Mouse IgG (H + L) Highly cross-Adsorbed Secondary, Alexa Fluor 488	1:250	Thermo Fisher Scientific Cat# A-21202, RRID: AB_141607
Secondary antibodies (Immunocytochemistry)	Donkey anti-Goat Mouse IgG (H + L) Highly cross-Adsorbed Secondary, Alexa Fluor 647	1:250	Thermo Fisher Scientific Cat# A-32849, RRID AB_2762840
Secondary antibodies (Immunocytochemistry)	Donkey anti-Rabbit Mouse IgG (H + L) Highly cross-Adsorbed Secondary, Alexa Fluor 555	1:250	Thermo Fisher Scientific Cat# A-31572, RRID: AB_162543
Secondary antibodies (Immunocytochemistry)	Goat anti-Mouse IgG1 Cross-Adsorbed Secondary Antibody, Alexa 568	1:250	Thermo Fisher Scientific Cat# A-21124, RRID:AB_2535766
Secondary antibodies (Immunocytochemistry)	Goat anti-Mouse IgG2a Cross-Adsorbed Secondary Antibody, Alexa 647	1:250	Thermo Fisher Scientific Cat# A-21241, RRID: AB_2535810

Primers	Target	Forward/Reverse primer (5′−3′)
Pluripotency Markers (RT-qPCR)	*SOX2*	GGGAAATGGGAGGGGTGCAAAAGAGG/ TTGCGTGAGTGTGGATGGGATTGGTG
Pluripotency Markers (RT-qPCR)	*OCT4*	GACAGGGGGAGGGGAGGAGCTAGG/ CTTCCCTCCAACCAGTTGCCCCAAAC
Pluripotency Markers (RT-qPCR)	*NANOG*	TGCAAGAACTCTCCAACATCCT/ATTGCTATTCTTCGGCCAGTT
House-Keeping Gene (RT-qPCR)	*RPL37A*	GTGGTTCCTGCATGAAGACAGTG/TTCTGATGGCGGACTTTACCG
Mutation analysis (PCR/sequencing)	*RYR2* (c.12441G > T)	CAGATGGTGGATATGCTTGTG/GCTTCTTCAGGCTCTTCAGAC
Mutation analysis (PCR/sequencing)	*RYR2* (c.14885A > G)	CTTATGTTGCTCAGGCTGGT/GAAATAAAGAGGCTGGGTGAG
Sendai virus vector (RT-qPCR)	*SeV*	GCAGCTCTAACGTTGTCAAA/CCTGGAGCAAATTCACCATGA

3.7. Sequencing

Genomic DNA used for *RYR2* sequencing was isolated using High Pure PCR Template Preparation Kit (Roche), following manufacturer's instructions. *RYR2* mutation analysis was performed on a PCR product obtained by genomic DNA amplification using Platinum™ Taq DNA Polymerase High Fidelity (Invitrogen), following manufacturer's instructions (primers are listed in Table 3). The PCR reaction was performed using the following conditions: 94 °C 1 min/94 °C 30 s; 58 °C 30 s; 68 °C 1 min for 35 cycles/68 °C 5 min; 12 °C ∞. The amplification product was purified using QIAquick PCR Purification Kit (Qiagen). Then, *RYR2* mutation was confirmed on hiPSCs on the purified PCR product by Sanger sequencing (primers are shown in Table 3).

3.8. STR analysis

Cell identity was assessed on genomic DNA from both hiPSCs and corresponding skin fibroblasts by Promega PowerPlex Fusion System 5C autosomal STR kit (Westen et al., 2014).

3.9. Mycoplasma test

The absence of human pathogens, including mycoplasma contamination was evaluated by PCR analysis performed by IDEXX BioAnalytics (Supplementary File 1).

Declaration of Competing Interest

Christine L. Mummery is co-founder of Pluriomics (now Ncardia) bv.

Acknowledgements

We thank N. Nakanishi (National Institute of Advanced Industrial Science and Technology, Japan) for providing SeV and SeV antibody, T. de Korte and P. Voskamp (Ncardia) for human pathogen analysis and P. de Knjiff (Dept. of Human Genetics, LUMC) for STR analysis. This study was supported by Health~Holland TKI-LSH PPP-allowance (LSHM17013-H007).

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.scr.2020.101764.

References

Cerrone, M., Napolitano, C., Priori, S.G., 2009. 'Catecholaminergic polymorphic ventricular tachycardia: a paradigm to understand mechanisms of arrhythmias associated to impaired Ca(2+) regulation'. Heart Rhythm 6, 1652–1659.
Imberti, J.F., Underwood, K., Mazzanti, A., Priori, S.G., 2016. 'Clinical challenges in

catecholaminergic polymorphic ventricular tachycardia'. Heart Lung Circ. 25, 777–783.
Nishimura, K., Sano, M., Ohtaka, M., Furuta, B., Umemura, Y., Nakajima, Y., Ikehara, Y., Kobayashi, T., Segawa, H., Takayasu, S., Sato, H., Motomura, K., Uchida, E., Kanayasu-Toyoda, T., Asashima, M., Nakauchi, H., Yamaguchi, T., Nakanishi, M., 2011. 'Development of defective and persistent Sendai virus vector: a unique gene delivery/expression system ideal for cell reprogramming'. J. Biol. Chem. 286, 4760–4771.
van der Werf, C., Kannankeril, P.J., Sacher, F., Krahn, A.D., Viskin, S., Leenhardt, A., Shimizu, W., Sumitomo, N., Fish, F.A., Bhuiyan, Z.A., Willems, A.R., van der Veen, M.J., Watanabe, H., Laborderie, J., Haissaguerre, M., Knollmann, B.C., Wilde, A.A., 2011. 'Flecainide therapy reduces exercise-induced ventricular arrhythmias in patients with catecholaminergic polymorphic ventricular tachycardia'. J. Am. Coll. Cardiol. 57, 2244–2254.
Westen, A.A., Kraaijenbrink, T., Robles de Medina, E.A., Harteveld, J., Willemse, P., Zuniga, S.B., van der Gaag, K.J., Weiler, N.E., Warnaar, J., Kayser, M., Sijen, T., de Knijff, P., 2014. 'Comparing six commercial autosomal STR kits in a large Dutch population sample'. Forensic Sci. Int. Genet. 10, 55–63.