Lab resource: Stem Cell Line

Generation of the human induced pluripotent stem cell (hiPSC) line PSMi004-A from a carrier of the KCNQ1-R594Q mutation

Manuela Mura[a], Yee-Ki Lee[b,c,l], Federica Pisano[a,d], Monia Ginevrino[e,f], Marina Boni[g], Federica Calabrò[a], Lia Crotti[h,i,j], Enza Maria Valente[e,f], Peter J. Schwartz[h], Hung-Fat Tse[b,c,l], Massimiliano Gnecchi[a,d,k,*]

[a] Coronary Care Unit, Laboratory of Experimental Cardiology for Cell and Molecular Therapy, Fondazione IRCCS Policlinico San Matteo, Pavia, Italy
[b] Department of Medicine, The University of Hong Kong, Hong Kong Special Administrative Region
[c] Hong Kong-Guangdong Joint Laboratory on Stem Cell and Regenerative Medicine, The University of Hong Kong, Hong Kong Special Administrative Region
[d] Department of Molecular Medicine, Unit of Cardiology, Università degli Studi di Pavia, Pavia, Italy
[e] Department of Molecular Medicine, Unit of Genetics, Università degli Studi di Pavia, Pavia, Italy
[f] Neurogenetics Unit, Fondazione IRCCS Santa Lucia, Rome, Italy
[g] Laboratory of Oncohaematological Cytogenetic and Molecular Diagnostics, Division of Haematology, Fondazione IRCCS Policlinico San Matteo, Pavia, Italy
[h] Istituto Auxologico Italiano, IRCCS, Center for Cardiac Arrhythmias of Genetic Origin, Laboratory of Cardiovascular Genetics, Milan, Italy
[i] Istituto Auxologico Italiano, IRCCS, Department of Cardiovascular, Neural and Metabolic Sciences, Ospedale San Luca, Milan, Italy
[j] Department of Medicine and Surgery, Università Milano-Bicocca, Milan, Italy
[k] Department of Medicine, University of Cape Town, Cape Town, South Africa
[l] Guangzhou Institutes of Biomedicine and Health, China

A B S T R A C T

We generated human induced pluripotent stem cells (hiPSCs) from dermal fibroblasts of a male carrier of the heterozygous mutation c.1781 G > A p.R594Q on the *KCNQ1* gene. hiPSCs, generated using four retroviruses each encoding for OCT4, SOX2, KLF4 and cMYC, display pluripotent stem cell characteristics, and can be differentiated into spontaneously beating cardiomyocytes (hiPSC-CMs).

Resource table

Unique stem cell line identifier	PSMi004-A
Alternative name of stem cell line	HDF28-LQT1-iPS
Institution	Fondazione IRCCS Policlinico San Matteo, Pavia, Italy
Contact information of distributor	Massimiliano Gnecchi, m.gnecchi@unipv.it
Type of cell line	hiPSC
Origin	human
Additional origin info	Age: 40
	Gender: male
	Ethnicity: Caucasian
Cell source	Dermal fibroblasts
Clonality	Clonal
Method of reprogramming	Retroviruses encoding for the human cDNA of OCT4, SOX2, cMYC, KLF4
Genetic modification	Yes
Type of modification	Inherited mutation
Associated disease	Long QT Syndrome type 1 (OMIM #192500)
Gene/locus	1781 G > A mutation on *KCNQ1*, 11p15.5-p15.4 (NM_000218.2)
Method of modification	N/A
Name of transgene or resistance	N/A
Inducible/Constitutive system	N/A
Date archived/stock date	Jan 7, 2013

* Corresponding author at: Università di Pavia, Fondazione IRCCS Policlinico S. Matteo, Pavia, Italy.
  E-mail address: m.gnecchi@unipv.it (M. Gnecchi).

Cell line repository/bank	https://hpscreg.eu/cell-line/PSMi004-A
Ethical approval	The study has been approved by the Ethics Committee of our Institution, Fondazione IRCCS Policlinico San Matteo, on the 29th of October 2010, protocol number 20100004354, proceeding P-20100003369.
	We obtained patient written informed consent for both skin biopsy procedure and conservation of biological samples.

Resource utility

It has been proven that iPSCs and iPSC-CMs can be efficiently used to model LQTS and test targeted therapies (Mehta et al., 2018; Gnecchi et al., 2017; Mura et al., 2017; Rocchetti et al., 2017). The PSMi004-A cell line will be of help to: 1) modelling of LQTS type 1; 2) targeted drug testing.

Resource details

Long QT Syndrome (LQTS) is an autosomal dominant inherited disease characterized by the prolongation of cardiac repolarization, which is quantified as the duration of the QT interval on the surface electrocardiogram (ECG). The repolarization defect predisposes to "Torsades de Pointes" (TdP), a type of ventricular tachycardia often causing syncope or sudden cardiac death (SCD) (Schwartz et al., 2012). LQT1 is the most common LQTS sub-type, accounting for ~40–50% of all LQTS cases. It is caused by mutations in the KCNQ1 gene, encoding for the α-subunit of the voltage-dependent potassium channel responsible for the delayed rectifier potassium current (I_{Ks}), one of the repolarization currents in the heart (Schwartz et al., 2012).

The PSMi004-A cell line was generated by reprogramming dermal fibroblasts isolated from a skin biopsy of a 40 years old man, father of a patient affected by LQTS type 1. The man carries the heterozygous mutation c.1781 G > A p.R594Q leading to the substitution of the arginine in position 594 with a glutamine in the KCNQ1 channel. His QTc (QT corrected for heart rate) has always been only slightly prolonged (~470 ms) and he never experienced cardiac symptoms even in the absence of pharmacological therapy.

Fibroblasts were reprogrammed by retroviral infection of human OCT4, SOX2, KLF4 and c-MYC. The obtained hiPSCs were maintained on feeders, retaining embryonic stem cell (ES) -like morphology (Fig. 1C) and pluripotent features up to passage 50. Both patient's fibroblasts and hiPSCs present the disease causing mutation on the *KCNQ1* gene, as proven by DNA sequencing (Fig. 1A. *The KCNQ1 coding sequence -CDS- used as a reference is the NCBI sequence NM_000218.2*), and an identical DNA profile at seven polymorphic loci, as shown by Short tandem Repeat (STR) analysis (available with the authors). Moreover, the DNA karyotyping, performed at passage 8, revealed normal male karyotype (46, XY) (Fig. 1B). PSMi004-A uniformly expresses the human ES surface antigens Tumor Related Antigen-1-60 and − 1-81 (TRA-1-60 and TRA-1-81) (Fig. 1C-D), Stage Specific Embryonic Antigen-3 and -4 (SSEA-3, SSEA-4), and shows alkaline phosphatase (AP) activity (Fig. 1C). Likewise, it expresses the pluripotent markers NANOG, OCT4, SOX2 (Fig. 1C-E), REX1, GDF3, ESG1, DPPA2, DPPA4 and NODAL (Fig. 1E), and shows OCT4 promoter demethylation (Fig. 1F, *open circles indicate unmethylated CpG dinucleotides, while closed circles indicate methylated CpGs*). RT-PCR analysis in Fig. 1G shows no expression of the four viral transgenes (Tg) in naïve fibroblasts (HDF), clear expression of Tg OCT4, SOX2, KLF4 and cMYC in fibroblasts five days after transduction (OSKM) and silencing of the four Tg in PSMi004-A at passage 6.

As expected, PSMi004-A spontaneously forms embryoid bodies (EBs) able to differentiate into cells belonging to the three germ layers: endoderm, mesoderm and ectoderm (Fig. 1H). Most importantly, we have successfully differentiated this LQT1 cell line also into spontaneous beating cells expressing typical cardiac proteins such as sarcomeric proteins alpha-sarcomeric actinin (α-SA) and troponin T (TnT) (Fig. 1I, *the insets show areas of cross-striation*). We also verified the absence of mycoplasma contamination in our PSMi004-A line (Fig. 1J).

Materials and methods

hiPSC generation

The detailed protocol is reported in the Supplemental Methods section.

Skin fibroblasts were reprogrammed using the four retroviruses pMXs-hOCT3/4 (Addgene #17217), pMXs-hSOX-2 (Addgene #17218), pMXs-hcMYC (Addgene #17220) and pMXs-hKLF4 (Addgene #17219), that were packaged in the 293T cell line (Clontech), using the packaging vector pCL-Eco (Addgene #12371). Emerging iPSC clones were manually picked, individually placed into a separate cell culture well and expanded on a feeder-layer of mitotically-inactivated mouse embryonic fibroblasts (iMEF), in DMEM/F12 (Gibco), 20% Knockout Serum Replacement (KO-SR), 2 mM L-glutamine, 50 U/ml penicillin, 50 U/ml streptomycin, 1% Non-Essential Amino Acids (NEAA), 0.1 mM beta-mercaptoethanol, 10 ng/ml basic Fibroblast Growth Factor (bFGF) (all purchased from Gibco), in a humidified incubator, at 37 °C, 5% CO_2 (Table 1). Passaging was performed every 5–7 days, at 1:4–1:6 split ratio, using a dissociation buffer composed by 1 mg/ml collagenase IV (Invitrogen), 0,25% trypsin (Gibco), 20%KO-SR (Gibco), 1 mM $CaCl_2$ all diluted in PBS 1 ×.

Mutation analysis

Genomic DNA was extracted from hiPSCs and their parental fibroblasts with QIAamp DNA Blood Mini kit (Qiagen). *KCNQ1* exon 15 was amplified with the GoTaq G2 DNA polymerase (Promega), primers in Table 2, and Mastercycler EPGradient S (Eppendorf). Cycle parameters were: 3 min at 95 °C, (30 s at 95 °C, 30 s at 56 °C, 1 min at 72 °C) × 35 times, 10 min at 72 °C. The resulting amplicon, whose size is indicated in Table 2, was purified and sequenced (Lightrun service - GATC Biotech AG – Germany).

STR analysis

STR analysis was carried out using PowerPlex® CS7 System (Promega) kit, following the manufacturer's protocol. Fragments were run on a 3130xl capillary sequencer (Applied Biosystems). Genotypes were assigned using GeneMarker software (SoftGenetics).

Karyotyping

hiPSCs were blocked at metaphase by exposure to 10 µg/ml demecolcine solution (Sigma Aldrich) for 3 h, and then lysed with a hypotonic solution (0.075 M KCl), and fixed with fresh Carnoy's Fixative (3,1 ratio of methanol:glacial acetic acid). Karyotyping was performed using the Giemsa trypsin G-banding (GTG-banding) technique. Whenever possible, we screened at least 20 metaphases, and 6 of them fully karyotyped. Chromosome identification and karyotype description were made in accordance with the International System for Chromosome Nomenclature (ISCN, 2016).

Immunocytochemistry

hiPSCs were grown on feeders, on glass coverslips coated with 0,1% porcine gelatin (Sigma Aldrich), and then fixed for 15 min in 4% paraformaldehyde (Affymetrix USB), permeabilized with 0.1% Triton X-100 (Sigma Aldrich) for 5 min, and blocked in 1% bovine

serum albumin (BSA, Sigma Aldrich) for 1 h at room temperature (RT). Then they were incubated for 1 h at RT with the primary antibody (Table 2) diluted in blocking solution, washed three times, and incubated for 1 h at RT with an appropriate secondary antibody (Table 2). Finally, the cells were stained with 1 μg/ml of Hoechst 33258 (Sigma Aldrich). Images were acquired using the Carl Zeiss fluorescence microscope Observer.Z1 equipped with the Apotome system and AxioVision 6.0 software (Zeiss GmbH, Gottingen, Germany).
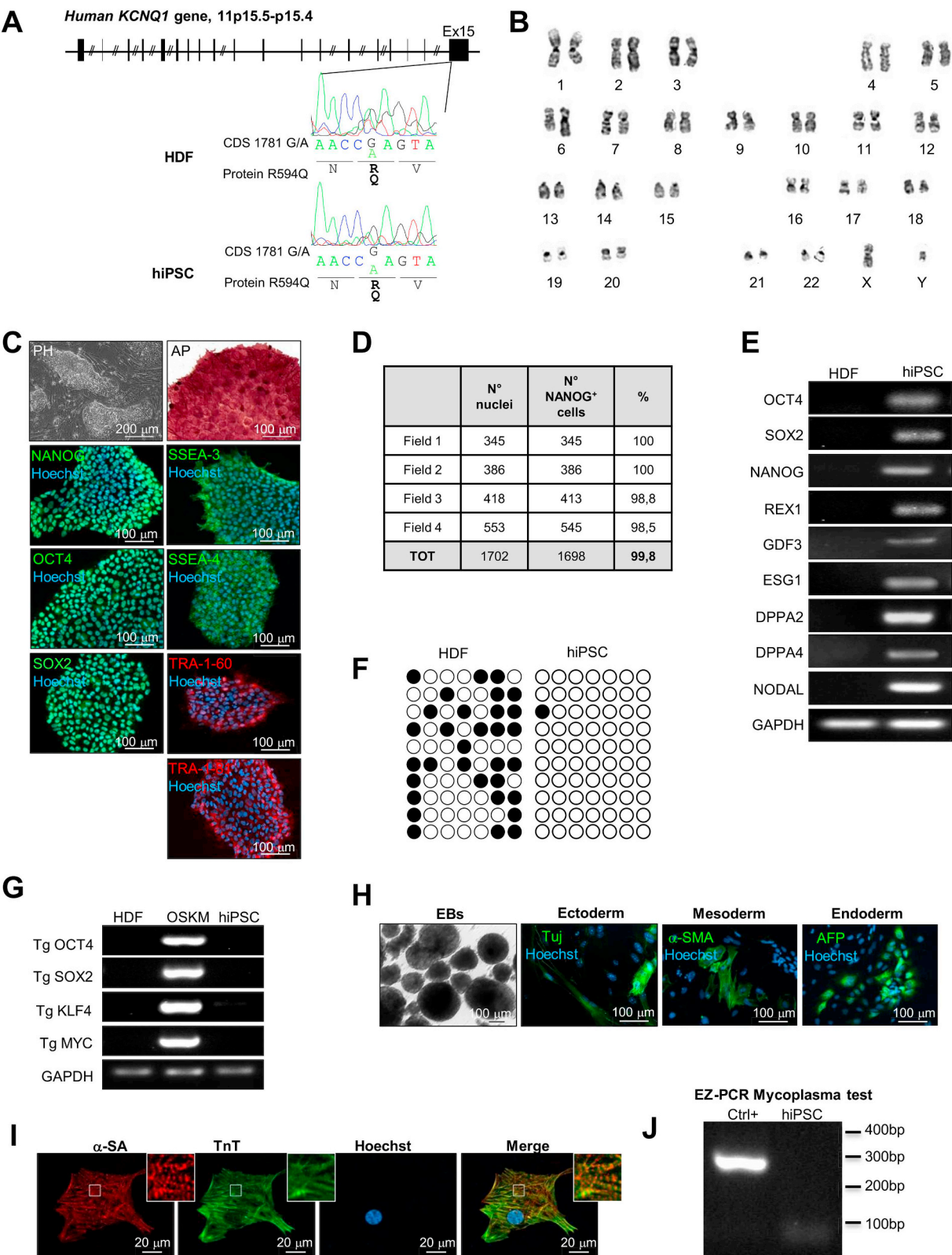
(caption on next page)

Fig. 1. **Characterization of the PSMi004-A cell line**. **A.** Top: schematic representation of *KCNQ1* gene (exons are vertical lines/boxes). The *KCNQ1* coding sequence (CDS) used as a reference is the NCBI sequence NM_000218.2. Bottom: DNA sequencing results showing the mutation 1781 G/A in the *KCNQ1* gene in heterozygosis in both patient-derived dermal fibroblasts (HDF) and PSMi004-A cell line (hiPSC). **B.** Karyotype analysis of PSMi004-A (300 G-bandings) showing normal male karyotype (46, XY). **C.** Top left: phase contrast image showing PSMi004-A morphology (PH). Top right: alkaline phosphatase colorimetric staining (AP). Bottom panels: immunofluorescence stainings showing uniform expression of pluripotency markers in the PSMi004-A. Nuclei were counterstained with Hoechst 33258 (Hoechst, blue). **D.** Immunocytochemistry counting of Nanog$^+$ cells. The total number of cells in each of the four fields analyzed was quantified by counting the nuclei stained with Hoechst 33258. **E.** RT-PCR analysis showing expression of the indicated markers of pluripotency in PSMi004-A (hiPSC) compared with its parental fibroblasts (HDF). **F.** OCT4 promoter methylation analysis with bisulfite sequencing in patient's dermal fibroblasts (HDF) and in the derived hiPSCs. Open circles indicate unmethylated CpG dinucleotides, while closed circles indicate methylated CpGs. **G.** RT-PCR analysis showing no expression of the four viral transgenes (Tg) in naïve fibroblasts (HDF), expression of Tg OCT4, SOX2, KLF4 and cMYC five days after transduction (OSKM) and silencing of the four Tg in PSMi004-A at passage 6. **H.** Far left panel: floating embryoid bodies (EBs) formed after 7 days of PSMi004-A culture in suspension. Panels on the right: immunofluorescence staining for markers of the 3 germ layers in iPSC-derived EBs: neuronal class tubulin beta III (Tuj) for ectoderm, smooth muscle actin (α-SMA) for mesoderm, and alpha Fetoprotein (AFP) for endoderm. **I.** Co-immunofluorescence staining for the alpha-sarcomeric actinin (α-SA, red) and troponin T (TnT, green) in cardiomyocytes differentiated from the PSMi004-A. Nuclei were counterstained with Hoechst 33258 (Hoechst, blue). Magnifications show areas of cross-striations. **J.** EZ-PCR test showing the absence of mycoplasma contamination in PSMi004-A. Ctrl + is the positive PCR control provided by the kit.

Table 1

Characterization and validation of PSMi004-A cell line.

Classification	Test	Result	Data
Morphology	Photography	Normal	Fig. 1 panel C
Phenotype	Qualitative analysis	Positive immunostaining for the pluripotency markers OCT4, NANOG, SOX2, TRA-1-60, TRA-1-81, SSEA-3, SSEA-4	Fig. 1 panel C
		Positive staining for the alkaline phosphatase	Fig. 1 panel C
		RT-PCR: expression of the pluripotency markers OCT3/4, SOX2, NANOG, REX1, GDF3, ESG1, DPPA2, DPPA4, NODAL	Fig. 1 panel E
	Quantitative analysis	Immunocytochemistry counting: 99,8% NANOG$^+$ cells	Fig. 1 panel D
Genotype	Karyotype (300 G-banding) and resolution	46XY, Resolution 450–500	Fig. 1 panel B
Identity	Microsatellite PCR (mPCR)	Not performed	Not available
	STR analysis	7 sites tested for iPSC, all sites matched with donor HDF STR profile	Available with the authors
Mutation analysis	Sequencing	Heterozygous for the mutation c.1781 G > A p.R594Q on the KCNQ1 gene	Fig. 1 panel A
Microbiology and virology	Mycoplasma	Mycoplasma testing by RT-PCR. Negative	Fig. 1 panel J
Differentiation potential	Embryoid body formation	The EBs expressed neuronal class tubulin beta III (Tuj) (ectoderm), alpha smooth muscle actin (α-SMA) (mesoderm), and alpha Fetoprotein (AFP) (endoderm).	Fig. 1 panel H
	Differentiation into cardiomyocytes	The iPSC-derived cardiomyocytes expressed the cardiac sarcomeric proteins alpha-sarcomeric actinin (α-SA) and troponin T (TnT)	Fig. 1 panel I
Donor screening	HIV 1 + 2 Hepatitis B, Hepatitis C	Not performed	Not available
Genotype additional info	Blood group genotyping	Not performed	Not available
	HLA tissue typing	Not performed	Not available

Immunocytochemistry counting

Nanog$^+$ cells were counted using the AxioVision 6.0 software (Zeiss GmbH, Gottingen, Germany). More than 1000 cells were counted in 4 fields.

AP assay

AP was detected by using the Alkaline Phosphatase Staining kit (00–0009 Stemgent).

RT-PCR

Total RNA was purified using TRIzol (ThermoFisher) and quantified using Nanodrop ND-1000 spectrophotometer (Celbio). 500 ng of purified RNA was reverse transcribed into cDNA using the Superscript II Reverse Transcriptase (ThermoFisher), following manufacturer's instructions. 1 μl of the RT reaction was amplyfied by PCR with the GoTaq G2 DNA polymerase (Promega) and primers in Table 2. Cycle parameters were: 3 min at 95 °C, (30 s at 95 °C, 30 s at 56 °C, 1 min at 72 °C) × 25–35 times, 10 min at 72 °C. Expected product sizes for each PCR reaction are indicated in Table 2. As a thermocycler for both reverse transcription and PCR, we used the Mastercycler EPGradient S (Eppendorf).

OCT4 promoter demethylation analysis

Genomic DNA were treated with the EZ DNA methylation kit (Zymo Research, Orange, CA, USA). The promoter region of the human OCT4 gene was amplified with biotinylated primers (Table 2) using Amplitaq gold 360 (Applied Biosystems). PCR products were sequenced (sequencing primer sequence in Table 2) and Pyrosequencing PSQ96 HS System (Biotage, Uppsala, Sweden). The methylation status of each locus was analyzed using PyroQ-CpG software (Qiagen).

EB formation

hiPSCs were detached using our dissociation buffer (see "hiPSC generation" paragraph), and grown for 7 days in suspension in a modified iPS medium deprived of bFGF and containing 20% FBS instead of KO-SR. Forming EBs were then transferred to gelatin-coated glass coverslips to allow differentiation in adhesion for additional 7 days in the same medium. Finally the EBs were immunostained for the three germ layers.

Cardiac differentiation

Cardiac differentiation was induced using the PSC Cardiomyocyte Differentiation Kit (ThermoFisher).

Table 2
Reagents details.

Antibodies used for immunocytochemistry

	Antibody	Dilution	Company Cat # and RRID
Pluripotency Markers	Rabbit anti NANOG	1:200	Stemgent *Cat#* 09–0020, *RRID:* AB_2298294
	Mouse anti OCT3/4 (C-10)	1:500	SCBT *Cat#* sc-5279, *RRID:* AB_628051
	Mouse anti SOX2	1:500	R&D Systems *Cat#* MAB2018, *RRID:* AB_358009
	Mouse anti TRA-1-60	1:100	Stemgent *Cat#* 09–0010, *RRID:* AB_1512170
	Mouse anti TRA-1-81	1:100	Stemgent *Cat#* 09–0011, *RRID:* AB_1512171
	Rat anti SSEA-3	1:100	Millipore *Cat#* MAB4303, *RRID:* AB_177628
	Mouse anti SSEA-4	1:100	Stemgent *Cat#* 09–0006, *RRID:* AB_1512169
Differentiation Markers (EBs)	Mouse anti neuronal class Tubulin beta III (Tuj)	1:500	Covance *Cat#* MMS-435P, *RRID:* AB_2313773
	Mouse anti alpha Smooth Muscle Actin (α-SMA)	1:1000	Millipore *Cat#* CBL171, *RRID:* AB_2223166
	Mouse anti alpha-fetoprotein (AFP)	1:500	Millipore *Cat#* SCR030, *RRID:* AB_597591
Cardiac Markers	Mouse anti cardiac Troponin T (cTnT)[a]	1:250	ThermoFisher *Cat#* MA5–12960, *RRID:* AB_11000742
	Mouse anti alpha Sarcomeric Actinin (α-SA)[a]	1:800	Sigma Aldrich *Cat#* A7811, *RRID:* AB_476766
Secondary antibodies	Alexa-Fluor® 488 Goat anti-rabbit IgG	1:500	ThermoFisher Cat# A11008, *RRID:* AB_143165
	Alexa-Fluor® 488 Goat anti-rat IgM	1:500	ThermoFisher Cat# A21212, *RRID:*AB_11180047
	Alexa-Fluor® 488 Goat anti-mouse IgG	1:500	ThermoFisher Cat# A11001, *RRID:* AB_2534069
	Alexa-Fluor® 546 Goat anti-mouse IgG	1:500	ThermoFisher Cat# A11003, *RRID:* AB_141370

Primers

	Target	Forward/Reverse primer (5′-3′)
Targeted mutation analysis/sequencing	*KCNQ1* Exon 15	Fw: 5′-ctacctccccccagccctac-3′
	317 bp	Rev.: 5′-caactcccaagaggggcc-3′
Pluripotency Markers (RT-PCR)	OCT4	Fw: 5′-gtactcctcggtccctttcc-3′
	168 bp	Rev.: 5′-caaaaaccctggcacaaact-3′
	SOX2	Fw: 5′-acaccaatcccatccacact-3′
	273 bp	Rev.: 5′-tttttcgtcgcttggagact-3′
	NANOG	Fw: 5′-ttccttcctccatggatctg-3′
	213 bp	Rev.: 5′-tctgctggaggctgaggtat-3′
	REX1	Fw: 5′-cagatcctaaacagctcgcagaat-3′
	306 bp	Rev.: 5′-gcgtacgcaaattaaagtccaga-3′
	GDF3	Fw: 5′-cttatgctacgtaaaggagctggg-3′
	631 bp	Rev.: 5′-gtgccaacccaggtcccggaagtt-3′
	ESG1	Fw: 5′-atatcccgccgtgggtgaaagttc-3′
	243 bp	Rev.: 5′-actcagccatggactggagcatcc-3′
	DPPA4	Fw: 5′-ggagccgcctgccctggaaaattc-3′
	408 bp	Rev.: 5′-tttttcctgatattctattcccat-3′
	DPPA2	Fw: 5′-ccgtccccgcaatctccttccatc-3′
	606 bp	Rev.: 5′-atgatgccaacatggctcccggtg-3′
	NODAL	Fw: 5′-gggcaagaggcaccgtcgacatca-3′
	234 bp	Rev.:5′-gggactcggtggggctggtaacgtttc-3′
House-Keeping Genes (RT-PCR)	GAPDH	Fw 5′-catgttccaatatgattccaccc-3′
	112 bp	Rev. 5′-gggatctcgctcctggaagat-3′
Retroviral transgenes	OCT4 cDNA on pMXs-hOCT3/4	Fw: 5′-ccccagggcccccattttggtacc-3′
	339 bp	
	SOX2 cDNA on pMXs-hSOX-2	Fw: 5′-ggcacccctggcatggctcttggctc-3′
	496 bp	
	cMYC cDNA on pMXs-hcMYC	Fw: 5′-caacaaccgaaaatgcaccagccccag-3′
	542 bp	
	KLF4 cDNA on pMXs-hKLF4	Fw: 5′-acgatcgtggcccccggaaaaggacc-3′
	518 bp	
	pMX viral vector	Rev: 5′-cccttttttctggagactaaataaa-3′
OCT4 promoter demethylation analysis/Bisulfite sequencing	OCT4 promoter	Fw: 5′-gaggttggagtagaaggattgttttggttt-3′
	467 bp	Rev.: 5′-cccccctaacccatcacctccaccacctaa-3′
	Sequencing primer	Fw: 5′-agagaggggttgagtagtttt-3′

[a] To perform the co-staining with these two antibodies, we used the Zenon Tricolor Mouse IgG labeling Kit (Molecular Probes).

Mycoplasma test

For the detection of mycoplasma in cell culture we used the EZ-PCR Mycoplasma Test Kit (Biological Industries).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scr.2019.101431.

References

Gnecchi, M., Stefanello, M., Mura, M., 2017. Induced pluripotent stem cell technology: toward the future of cardiac arrhythmias. Int. J. Cardiol. 237, 49–52.

ISCN, 2016. In: Mcgowan-Jordan, J., Simons, A., Schmid, M. (Eds.), An international system for human cytogenomics nomenclature 2016. S. Karger, Basel.

Mehta, A., Ramachandra, C.J.A., Singh, P., Chitre, A., Lua, C.H., Mura, M., Crotti, L.,

Wong, P., Schwartz, P.J., Gnecchi, M., Shim, W., 2018. Identification of a targeted and testable antiarrhythmic therapy for long-QT syndrome type 2 using a patient-specific cellular model. Eur. Heart J. 39, 1446–1455.

Mura, M., Mehta, A., Ramachandra, C.J., Zappatore, R., Pisano, F., Ciuffreda, M.C., Barbaccia, V., Crotti, L., Schwartz, P.J., Shim, W., Gnecchi, M., 2017. The KCNH2-IVS9-28A/G mutation causes aberrant isoform expression and hERG trafficking defect in cardiomyocytes derived from patients affected by long QT syndrome type 2. Int. J. Cardiol. 240, 367–371.

Rocchetti, M., Sala, L., Dreizehnter, L., Crotti, L., Sinnecker, D., Mura, M., Pane, L.S., Altomare, C., Torre, E., Mostacciuolo, G., Severi, S., Porta, A., De Ferrari, G.M., George Jr., A.L., Schwartz, P.J., Gnecchi, M., Moretti, A., Zaza, A., 2017. Elucidating arrhythmogenic mechanisms of long-QT syndrome CALM1-F142L mutation in patient-specific induced pluripotent stem cell-derived cardiomyocytes. Cardiovasc. Res. 113, 531–541.

Schwartz, P.J., Crotti, L., Insolia, R., 2012. Long-QT syndrome: from genetics to management. Circ. Arrhythm. Electrophysiol. 5, 868–877.