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Lab Resource: Multiple Cell Lines

Generation of two human induced pluripotent stem cell (hiPSC) lines from a long QT syndrome South African founder population

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ABSTRACT

We generated PSMi001-A and PSMi008-A hiPSC lines from two individuals belonging to a South African (SA) founder population in which the malignant KCNQ1-A341V mutation cosegregates with the Long QT Syndrome (LQTS) phenotype. PSMi001-A was derived from an asymptomatic KCNQ1-A341V mutation carrier, whereas PSMi008-A was derived from a healthy non-mutation carrier, heterozygous for the minor variant rs16847548 on the *NOS1AP* gene, associated with QT prolongation in the general population, and with a greater risk for cardiac arrest in the affected members of the SA founder population. The hiPSCs, generated using the Yamanaka's retroviruses, display pluripotent stem cell features and trilineage differentiation potential.

Resource utility

iPSCs and iPSC-CMs can be efficiently used to model LQTS and to test novel pharmacological therapies (Mehta et al., 2018; Gnecchi et al., 2017; Schwartz et al., 2019). PSMi001-A cell line in particular will be useful to elucidate the pathological mechanism underlying the malignant KCNQ1-A341V mutation, while the PSMi008-A line will be of help to elucidate cellular mechanisms by which variants of the *NOS1AP* gene can influence the QT interval prolongation and the arrhythmic risk.

Resource details

Founder effects, by which many individuals share a mutation identical by descent, represent a powerful tool to understand the underlying disease causing mechanisms and to predict the natural history of mutation-associated effects. PSMi001-A and PSMi008-A cell lines were generated from two members of a SA founder population segregating a malignant KCNQ1-A341V mutation causing a severe form of LQTS type 1 (LQT1) (Brink et al., 2005).

LQTS is an autosomal dominant inherited disease which is associated with high risk of sudden cardiac death (SCD) (Brink et al., 2005). 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.

PSMi001-A was derived from the fibroblasts of a 53 years old woman carrying the disease-causing mutation but who never experienced cardiac symptoms. PSMi008-A was derived from a 52 years old man who is wild type (wt) for *KCNQ1* but carries the rs16847548 minor variant on the *NOS1AP* gene in heterozygosis. We have previously de-

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	PSMi001-A		PSMi008-A		
	HDF	hiPSC	HDF	hiPSC	
OCT4	987.54M				
SOX2	Section and				
IANOG			400 ···		
REX1					
GDF3		1			
DPPA2		Acres in		-	
DPPA4		Sec. 10		-	
NODAL					
GAPDH	-	-	-	-	





(caption on next page)

Fig. 1. Characterization of the PSMi001-A and PSMi008-A cell lines. 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 1022 C/T in the *KCNQ1* exon 6 (Ex6) in heterozygosis in the PSMi001-A but not in PSMi008-A parental dermal fibroblasts (HDF) and hiPSC cell line (hiPSC). B. Top: schematic representation of NOS1AP gene upstream region. MAF is the minor allele frequency in the SA founder population. Bottom: DNA sequencing results showing the rs16847548 minor allele in heterozygosis in the PSMi008-A but not PSMi001-A parental dermal fibroblasts (HDF) and hiPSC cell line (hiPSC). C. PH: phase contrast images showing PSMi001-A and PSMi008-A morphology. AP: alkaline phosphatase colorimetric staining. All the other panels: immunofluorescence stainings showing uniform expression of the indicated markers of pluripotency in the PSMi001-A and PSMi008-A. Nuclei were counterstained with Hoechst 33258 (Hoechst, blue). D. RT-PCR analysis showing expression of pluripotency markers in PSMi001-A and PSMi008-A (hiPSC) compared with parental fibroblasts (HDF). E. 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 PSMi001-A and PSMi008-A tipassages 5 and 6 respectively. F. Immunofluorescence staining for markers of the 3 germ layers in iPSC-derived EBs: neuronal class tubulin beta III (Tuj) for ectoderm, smooth muscle actin (SMA) for mesoderm, and alpha Fetoprotein (AFP) for endoderm.

monstrated that this *NOS1AP* variant is significantly associated with increased risk of life-threatening events in LQTS individuals of this SA population (Crotti et al., 2009); therefore we anticipate that the use of this cell line will help us to elucidate the molecular mechanism by which this *NOS1AP* variant worsen the clinical phenotype.

To generate the hiPSCs, dermal fibroblasts were reprogrammed by retroviral infection of OCT4, SOX2, KLF4 and c-MYC. The obtained clones were maintained on feeders. Both PSMi001-A hiPSC and the parental fibroblasts (HDF) present the disease causing mutation on the *KCNQ1* gene, as proved by DNA sequencing, whereas PSMi008-A HDF and hiPSC are wt (Fig. 1A. *The KCNQ1 coding sequence -CDS- used as a reference is the NCBI sequence NM_000218.2)*. PSMi008-A HDF and hiPSC are heterozygous for the rs16847548 minor allele, whereas PSMi001-A HDF and hiPSC are homozygous for the major allele (Fig. 1B. *MAF = minor allele frequency in the SA founder population*).

Both fibroblasts and the derived PSMi001-A and PSMi008-A hiPSCs show an identical DNA profile at 7 polymorphic loci, as shown by Short tandem Repeat (STR) analysis (available with the authors). Moreover, the DNA karyotyping revealed normal karyotype (46, XX for PSMi001-

A and 46, XY for PSMi008-A) (Suppl Fig. 1A). Both hiPSC lines display embryonic stem cell (ES)-like morphology (Fig. 1C), and uniformly express the human ES surface antigens Tumor Related Antigen-1-60 (TRA-1-60), Stage Specific Embryonic Antigen-3 and -4 (SSEA-3, SSEA-4), and show alkaline phosphatase (AP) activity (Fig. 1C). Likewise, they express the pluripotent markers NANOG, OCT4, SOX2 (Fig. 1C-D), REX1, GDF3, DPPA2, DPPA4 and NODAL (Fig. 1D) at percentages higher than 98% (see immunocytochemistry counting in Suppl Fig. 1B). RT-PCR analysis in Fig. 1E shows no expression of the four viral transgenes (Tg) in naïve fibroblasts (HDF), clear expression of Tg in fibroblasts five days after transduction (OSKM) and silencing of the four Tg in both PSMi001-A and PSM008-A at passage 5 and 6, respectively.

PSMi001-A and PSMi008-A spontaneously form embryoid bodies (EBs) able to differentiate into cells belonging to the three germ layers: endoderm (alpha-fetoprotein - AFP), mesoderm (alpha smooth muscle actin- α SMA) and ectoderm (tubulin beta III - Tuj) (Fig. 1F).

Finally, we excluded the presence of mycoplasma contamination in our PSMi001-A and PSMi008-A lines (Suppl Fig. 1C).

Table 1

Characterization and validation of PSMi001-A and PSMi008-A cell lines.
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Classification	Test	Result	Data
Morphology	Photography	Normal	Fig. 1C
Phenotype	Qualitative analysis	Positive immunostaining for the pluripotency markers OCT4, NANOG, SOX2, TRA-1-60, SSEA-3, SSEA-4	Fig. 1 panel C
		Positive staining for the alkaline phosphatase	Fig. 1 panel C
		Expression of the pluripotency markers OCT3/4, SOX2, NANOG, REX1, GDF3, DPPA2, DPPA4, NODAL, measured by RT-PCR.	Fig. 1 panel D
	Quantitative analysis	Immunocytochemistry counting: 99,2% NANOG ⁺ PSMi001-A cells, 98,6% OCT4 ⁺ PSMi008-A cells.	Suppl Fig. 1 panel B
Genotype	Karyotype (300 G-banding) and	46XX for PSMi001-A and 46XY for PSMi008-A.	Suppl. Fig. 1 panel A
	resolution	Resolution 450–500	
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 authors
Mutation analysis	Sequencing	PSMi001-A is heterozygous for the mutation c. $1022C > T p.A341V$ on the KCNQ1 gene, and homozygous for the rs16847548 major allele T.	Fig. 1 panels A and B
		PSMi008-A is wt for KCNQ1, and heterozygous for the rs16847548 minor allele C.	
Microbiology and virology	Mycoplasma	Mycoplasma testing by RT-PCR. Negative	Suppl. Fig. 1, panel C
Differentiation potential	Embryoid body formation	The EBs expressed neuronal class tubulin beta III (Tuj) (ectoderm), smooth muscle actin (SMA) (mesoderm), and alpha Fetoprotein (AFP) (endoderm).	Fig. 1 panel F
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

Materials and methods

Expanded methods are provided in the Supplemental Methods section.

hiPSC generation

Skin fibroblasts were reprogrammed using four retroviruses encoding OCT4, SOX2, KLF4 and c-MYC. Emerging iPSC clones were manually picked, individually placed into a separate cell culture well and expanded on a feeder-layer of mitotically-inactivated mouse

Table	2
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Reagents details.

embryonic fibroblasts (iMEF), and grown in DMEM/F12 supplemented with 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) (Table 1).

Mutation analysis

Genomic DNA was extracted with QIAamp DNA Blood Mini kit (Qiagen), and amplified with Phire Green Hot Start II PCR Master Mix (ThermoFisher) (see Table 2 for primer sequences and product size).

Antibody Dilution Company Cart # and RBD Plarpipetency markers Rabbit and Nanog 1:200 Sterment Cart & 92-002, RRDF: AB 52:096:14 Mouse and tox24 1:500 RED are for 2 = 52:00, RRDF: AB 52:006:11 Mouse and tox24 1:500 RBD Systems Cart # MAB2018, RRDF: AB 52:006:11 Mouse and tox24 1:000 RBD Systems Cart # MAB2018, RRDF: AB 52:006:11 Mouse and tox24 1:000 Millipore Cart # MAB4303, RRDF: AB 52:070 Mouse and toxet stubulin beta III (Tu)) 1:500 Corrace Cart # MAB4303, RRDF: AB 2:31773 Mouse and taphas motion muscle actin (Cast) 1:000 Millipore Cart # SCR039, RRDF: AB 2:31773 Mouse and taphas fetoprotein (AFP) 1:500 ThemoFisher Cart # 11003, RRDF: AB 2:31763 Aleas-Fluore* 594 Goat anti-abbit tgG 1:500 ThemoFisher Cart # 11003, RRDF, AB 2:3166 Aleas-Fluore* 594 Goat anti-mouse tgG 1:500 ThemoFisher Cart # 11003, RRDF, AB 2:3166 Aleas-Fluore* 594 Goat anti-mouse tgG 1:500 ThemoFisher Cart # 11003, RRDF, AB 2:3166 Aleas-Fluore* 594 Goat anti-mouse tgG 1:500 ThemoFisher Cart # 11003, RRDF, AB 2:3176 Primer: Farred Farred Scattarti-ambit tgG 1:500 Aleas-Fluore* 594 Goat anti-mouse tgG 1:500 ThemoFisher Cart # 11003, RRDF, AB 2:3176 Primer: Farred Sca	Antibodies used for immunocytochemistry				
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Alexa-Fluor* 594 Goat anti-rat [gM 1:500 ThermoFisher Cat# A21213, RRD: AB, 1118006 Alexa-Fluor* 488 Goat anti-mouse [gG 1:500 ThermoFisher Cat# A11001, RRD: AB, 2534069 Alexa-Fluor* 546 Goat anti-mouse [gG 1:500 ThermoFisher Cat# A11003, RRD: AB, 2534069 Primers Target Matation analysis/sequencing KCNQ1 Exon 6 Pr: 5- (ggetgaccaetgetcett - 3' 195 bp RCNQ1 Exon 6 Pr: 5- (ggetgaccaetgetcett - 3' 195 bp RCNQ1 Exon 6 Pr: 5- (ggetgaccaetgetcett - 3' 195 bp RCNQ1 Exon 6 Pr: 5- (ggetgaccaetgetcett) 168 bp RCT - 73 273 bp Rev: 5- (aggegacttaactggetce - 3' 579 bp Rev: 5- (aggegacttaactggetce - 3' 168 bp Rev: 5- (aggegacttaactggetce - 3' 273 bp Rev: 5- (aggegacttaactgetce - 3' 273 bp Rev: 5- (aggegactgeact - 3' 273 bp Rev: 5- (aggegact - 3' 273 bp Rev: 5- (aggegact - 3' 273 bp Rev: 5- (aggegact - 3') 273 bp Rev: 5- (aggegact - 3') 274 Rev: 5- (aggegactgeact - 3') 275 Rev: 5- (aggegact	Secondary antibodies	Alexa-Fluor® 488 Goat anti-rabbit IgG	1:500	ThermoFisher Cat# A11008. <i>RRID</i> :AB 143165	
Alexa-Fluor* 488 Goat anti-mouse IgG 1:50 ThermoFisher Cat# A11001, RRD: Ab.2534060 Alexa-Fluor* 546 Goat anti-mouse IgG 1:50 ThermoFisher Cat# A11003, RRD: Ab.2534060 Alexa-Fluor* 546 Goat anti-mouse IgG 1:50 ThermoFisher Cat# A11003, RRD: Ab.2534060 Targeted mutation analysis/sequencing KCNQ1 Exon 6 Provided Teverse primer (5'-3') Targeted mutation analysis/sequencing KCNQ1 Exon 6 Provided Teverse primer (5'-3') Target 55 bp Rev: 5'- tggergaccattgtcccet - 3' T59 bp Rev: 5'- tggergaccattgtccca - 3' T16847548 Provi 5'- aggggaattaaccatgtgcc - 3' T68 bp Rev: 5'- aggggaattaaccatgtgcc - 3' T68 bp Rev: 5'- aggggaattaaccatgtgcc - 3' T68 bp Rev: 5'- cacaaacccatgcataccat - 3' SOX2 Provi 5'- aggagaattaaacctgggaattaa SOX2 Provi 5'- aggagaattaaacctgggaattaa SOX2 Provi 5'- aggagaattaaacctgggaattaa SOX2 Provi 5'- aggagaattaaacctggagaat-3' SOX2 Provi 5'- aggagaattaaagtcgaga-3' SOX2 Provi 5'- aggagaattaaagtcgaga-3' SOX2 Provi 5'- aggagaattaagtcgaga-3' SOX2 Provi 5'- aggaatgaataggagtggggaga-3' SOX2 Provi 5'- aggaatgaatggagtggggaat-3' PDPA4 Provi 5'- ggaatggaatggagtgggagaat-3' DPPA4 Provi 5'- ggaatggaatggatgggaggaat-3' DPPA2 Provi 5'- aggaatggaatggatgggaggaat-3' DPPA2 Provi 5'- aggaatggaatggatggaggat-3' DPPA2 Provi 7'- aggagaaggaatggaggatggagad-3' Sox 2 DDN on pMXs-hOCT3/4 Provi 5'- aggaaggaatggacc-3' SIB bp Provi 7'- aggagaaggaatggacc-3' SIB bp Provi 7'- aggagaaggaatggacc-3' SIB bp Provi 7'- aggagaaggaatggaccagaatggaatgaaggaccag' SIB bp Provi 5'- aggagaaggaatggaccag' SIB bp Provi 5'- aggagaaggaat	obolinally anaboards	Alexa-Fluor® 594 Goat anti-rat IgM	1:500	ThermoFisher Cat# A21213 RRID: AB 11180463	
Alexa-Fluor* 546 Goat anti-mouse lgG1:500ThermoPisher Car# A11003, RRD: AB_141370PrimersTarget of mutation analysis/sequencingTarget (CAR# A11003, RRD: AB_141370)Targeted mutation analysis/sequencingKCNQ1 Exon 6Fw: 5' toggtagaccatgtccctd - 3'1550 bpRev: 5' toggtagaccatgtccctd - 3'Sister (CAR# A11003, RRD: AB_141370)16847548Fw: 5' aggggaacttaaccatgtgtc - 3'Sister (CAR# A11003, RRD: AB_141370)16847548Fw: 5' aggggaacttaaccatgtgtc - 3'Sister (CAR# A11003, RRD: AB_141370)Pluripotency Markers (RT-PCR)OCT4Fw: 5' aggregaccatgtcccatact 3'0C14Fw: 5' caaccatccatcatcataccat 3'Sixter (CAR# A11003, RRD: AB_141370)168 bpRev: 5' caaccatccatcatcatcaccat 3'Sixter (CAR# A11003, RRD: AB_141370)169 bpRev: 5' aggregaccatagtccgtgaat-3'Sixter (CAR# A11003, RRD: AB_141370)160 bpRev: 5' aggregaccatgtcccataccat 3'Sixter (CAR# A11003, RRD: AB_141370)160 bpRev: 5' aggregaccatgtcccataccat 3'Sixter (CAR# A11003, RRD: AB_141370)170 brRev: 5' aggregaccatgtcccataccat 3'Sixter (CAR# A11003, RRD: AB_141370)171 bpRev: 5' aggregaccatgtcccataccat 3'Sixter (CAR# A11003, RRD: AB_141370)172 brRev: 5' aggregaccatgtcccatact 3'Sixter (CAR# A11003, RRD: AB_141370)172 brRev: 5' aggregaccataccataccat 3'Sixter (CAR# A11003, RRD: AB_141370)172 brRev: 5' aggregaccataccataccataccataccataccataccatacc		Alexa-Fluor® 488 Goat anti-mouse IgG	1:500	ThermoFisher Cat# A11001 RRID: AB 2534069	
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579 bpRev.: 5'- agcgcccttatcaccatg - 3'Pluripotency Markers (RT-PCR)OCT4Fw: 5'-gtactcctggccctttc-3'OCT4Fw: 5'-caaaacctggaccacat-3'SOX2Fw: 5'-caaaacctggaccacaca-3'SOX2Fw: 5'-cacacatccatccatccat-3'273 bpRev: 5'-ttcgttggaggct3NANOGFw: 5'-ttcgttggaggct3213 bpRev: 5'-ttggtgaggct3gagt1-3'REX1Fw: 5'-ctggtggaggct3gaggt-3'306 bpRev: 5'-ttggtcgaggct2gaggt-3'631 bpRev: 5'-gtgtcgcaaattaagtccgga-3'631 bpRev: 5'-gtgtcgcaaattaagtccgga-3'644 08 bpRev: 5'-gtgtcgcaaattaagtccgga-3'DPPA4Fw: 5'-gtggtcgcaaggtc3'A0DALFw: 5'-gtggtcgcaaggtc3'234 bpRev: 5'-gtggtcgcaagggccggggtaggtt-3'House-Keeping Genes (RT-PCR)GAPDH642 DNA on pMXs-hOCT3/4Fw: 5'-gtgatcccataggcctcgggagta-3'842 DPASot2 cDNA on pMXs-hOX-2946 bprw: 5'-ggaccccttggcaaggagcc3'842 DPArw: 5'-ggacccctggcaagggccc3'842 DPArw: 5'-gcgcacccctggcaaggccc3'844 CDNA on pMXs-hCMYCFw: 5'-cacaaaccggaaaggagcc-3'842 DPrW: cDNA on pMXs-hCMYC842 DPrW: cDNA on pMXs-hCMYC842 DPrw: 5'-acgaagggcccggaaggcc-3'842 DPrK14 CDNA on pMXs-hCMYC842 DPrw: 5'-acgaagggcccggaagggcc-3'842 DPrw: 5'-acgaaccgggccccggaaagggcc-3'842 DPrw: 5'-acgatcgtggcccggaagggcc-3'842 DPrw: 5'-acgatcgtggcccggaagggcc-3'842 DPrw: 5'-acgatcgtggcccggaaagggcc-3'842		rs16847548	Fw: 5'- agggg	acttaaaccgtgcc -3	
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pMX viral vector Rev: 5'-ccctttttctggagactaaataaa-3'		518 bp			
		pMX viral vector	Rev: 5'-ccctttt	tctggagactaaataaa-3'	

The resulting amplicons were purified and sequenced (Lightrun service - GATC Biotech AG – Germany).

STR analysis

STR analysis was carried out using PowerPlex[®] CS7 System kit (Promega), 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 $10 \,\mu$ g/ml demecolcine solution (Sigma Aldrich) for 3 h. Karyotyping was performed using 300 G-banding chromosome analysis.

Immunocytochemistry

hiPSCs and their derivatives were grown on glass coverslips, 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 at RT with the primary antibody (Table 2) diluted in blocking solution for 1 h, washed three times, and incubated for 1 h at RT with an appropriate secondary antibody (Table 2). Finally, the cells were stained with 1 μ g/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).

Immunocytochemistry counting

NANOG⁺ and OCT4⁺ cells were counted using the AxioVision 6.0 software (Zeiss GmbH, Gottingen, Germany). The total number of cells in each field was quantified by counting the nuclei stained with Hoechst 33258.

AP assay

AP was detected by using the Alkaline Phosphatase Staining kit II (00–0055 Stemgent).

RT-PCR

Total RNA was purified using TRIzol (ThermoFisher Scientific). cDNA was synthesized using the Superscript IV Reverse Transcriptase (ThermoFisher). RT-PCR was performed with the Phire Green Hot Start II PCR Master Mix (Thermo Scientific) and primers in Table 2.

EB formation

hiPSCs were enzimatically detached and grown for 7 days in nonadherent conditions in a modified iPS medium deprived of bFGF and containing 20% FBS instead of KO-SR. Forming EBs were then transferred to gelatin-coated dishes to allow differentiation in adhesion in the same medium for additional 7 days. Finally, the cells were processed for immunostaining of the three germ layers as described above.

Mycoplasma test

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

Key resources table

Unique stem cell lines identifier	PSMi001-A
	PSMi008-A
Alternative names of stem cell lines	SA6.27-iPS
	SA2.3-iPS
Institution	Fondazione IRCCS Policlinico San Matteo,
	Pavia, Italy
Contact information of distributor	Massimiliano Gnecchi, m.gnecchi@unipv.it
Type of cell lines	hiPSC
Origin	human
Cell source	Dermal fibroblasts
Clonality	Clonal
Method of reprogramming	Retroviruses encoding for the human cDNA of
	OCT4, SOX2, cMYC, KLF4
Multiline rationale	Control and disease pair
Genetic modification	Yes
Type of modification	PSMi001-A: congenital mutation
, i	PSMi008-A:congenital single nucleotide poly-
	morphism (SNP)
Associated disease	Long QT Syndrome type 1 (OMIM #192500)
Gene/locus	PSMi001-A: 1022C > T mutation on KCNO1
	(NM 000218.2), 11p15.5-p15.4
	PSMi008-A: rs16847548 variant
	(CM000663.2;g.162065484T > C)
Method of modification	N/A
Name of transgene or resistance	N/A
Inducible/Constitutive system	N/A
Date archived/stock date	PSMi001-A: 30/12/2015
Bate arean ea, stock auto	PSMi008-A: 25/2/2016
Cell line repository/bank	https://hpscreg.eu/cell-line/PSMi001-A
den nile repository/ bank	https://hpscreg.eu/cell-line/PSMi008-A
Ethical approval	The study has been approved by the Ethics
Ethical approval	Committee of the University of Stellenbosch
	South Africa on the 4 March 2013 protocol
	number N13/01/002
	We obtained natient written informed consent
	for both skin bionsy procedure and conserva-
	tion of biological samples
	tion of biological samples.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scr.2019.101510.

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