



Lab Resource: Single Cell Line



Generation of an induced pluripotent stem cell line from a patient with conduction disease and recurrent ventricular fibrillation with a sodium voltage-gated channel alpha subunit 5 (*SCN5A*) gene c.392 + 3A > G splice-site variant

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A B S T R A C T

Variants in the sodium voltage-gated channel alpha subunit 5 gene (*SCN5A*) produce variable cardiac phenotypes including Brugada syndrome, conduction disease and cardiomyopathy. These phenotypes can lead to life-threatening arrhythmias, heart failure, and sudden cardiac death. Novel variants in splice-site regions of *SCN5A* require functional studies to characterise their pathogenicity as they are poorly understood. The generation of an induced pluripotent stem cell line provides a valuable resource to investigate the functional effects of potential splice-disrupting variants in *SCN5A*.

1. Resource table

Unique stem cell line identifier	CIAUi002-C
Alternative name(s) of stem cell line	UBC3 M1
Institution	Centenary Institute of Cancer Medicine and Cell Biology
Contact information of distributor	Christopher Semsarian; c. semsarian@centenary.org.au
Type of cell line	iPSC
Origin	Human
Additional origin info required for human ESC or iPSC	Age: 30Sex: FemaleEthnicity if known: Caucasian
Cell Source	Peripheral blood mononuclear cells
Clonality	Clonal
Method of reprogramming	Episomal vectors; transgene free
Genetic Modification	No
Type of Genetic Modification	N/A
Evidence of the reprogramming	PCR
transgene loss (including genomic copy if applicable)	

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Associated disease	Left bundle branch block, recurrent ventricular fibrillation, conduction disease, non-ischemic cardiomyopathy
Gene/locus	<i>SCN5A</i> NM_000335.5:c.392 + 3A > G
Date archived/stock date	2021
Cell line repository/bank	https://hpscreg.eu/cell-line/CIAUi002-C
Ethical approval	Patient informed consent obtained, and ethics review board-competent authority approval obtained through the Ethics Review Committee, Sydney Local Health DistrictProtocol No: X19-0108 and 2019/ETH00461

2. Resource utility

Patient-derived induced pluripotent stem cells (iPSCs) are a valuable tool to investigate novel variants associated with and resulting in cardiac diseases. Generation of iPSCs provides a resource to elucidate the functional effects of splice-disrupting variants with uncertain clinical

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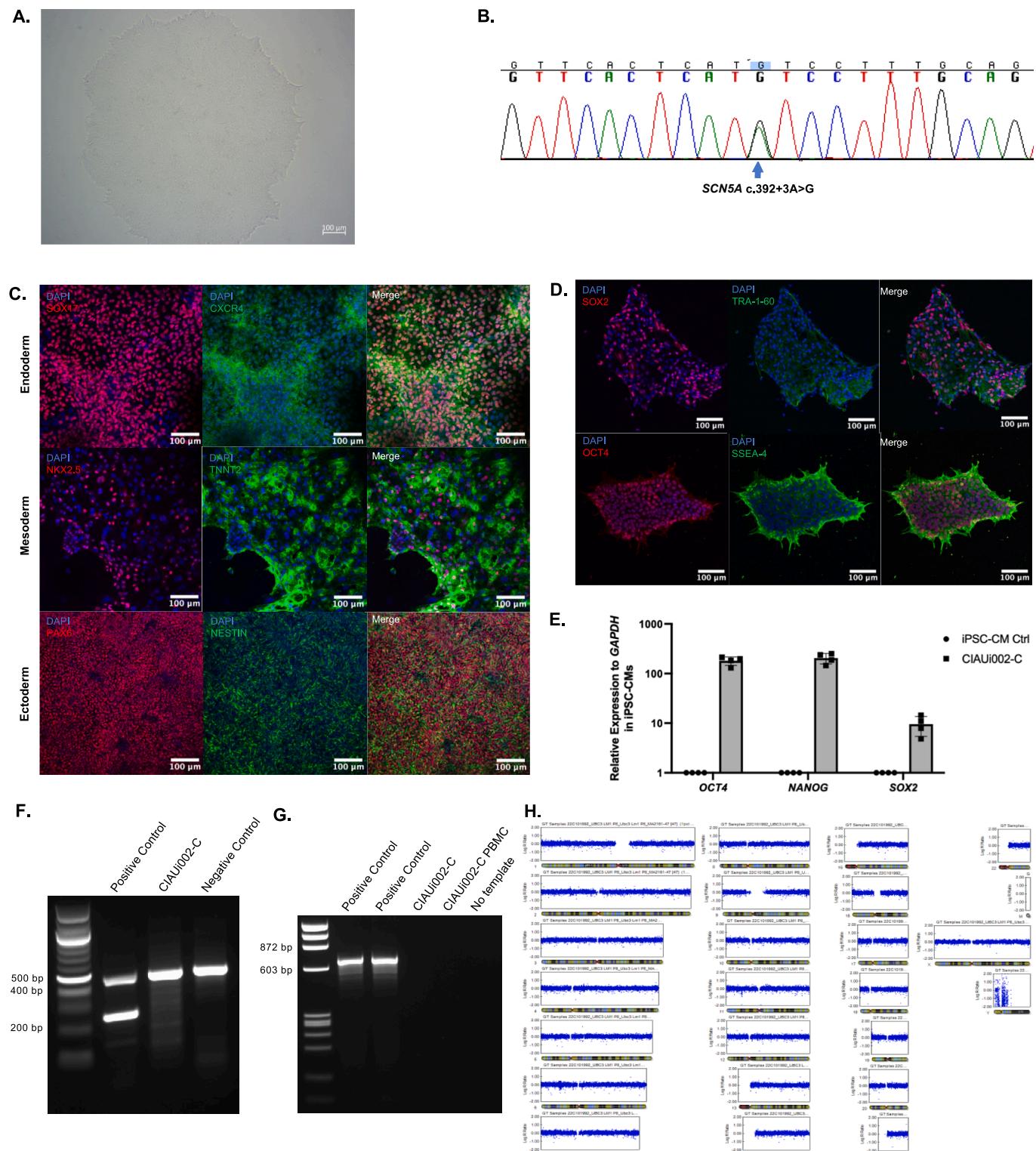


Fig. 1.

significance in the sodium voltage-gated channel alpha subunit 5 gene (*SCN5A*).

3. Resource details

Variants in *SCN5A* produce variable cardiac phenotypes including Brugada syndrome, conduction disease and cardiomyopathy (Veltmann

et al., 2016; McNair et al., 2011). These phenotypes can lead to life-threatening arrhythmias, heart failure, and sudden cardiac death (Wilde et al., 2022). This iPSC line was generated from peripheral blood mononuclear cells (PBMCs) from a 30 year old female with recurrent ventricular fibrillation, conduction disease, and non-ischemic cardiomyopathy. Broad panel genetic testing of 184 genes identified a *SCN5A* c.392 + 3A > G donor splice-site variant which is currently classified as

Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography	Visual record of the line: Normal	Fig. 1A
Phenotype	Bright field Qualitative analysis	Expression of pluripotency markers confirmed by confocal imaging: OCT4, SSEA4, SOX2 and TRA-1-60	Fig. 1D
Genotype	Quantitative analysis RT-qPCR	Expression of OCT4, NANOG and SOX2	Fig. 1E
	Karyotype (G-banding) and resolution	Normal	Fig. 1H
Identity	Microsatellite PCR (mPCR) OR STR analysis	DNA Profiling e.g. Performed/not performed 16 STR sites tested with 100% match with patient blood DNA	Not performed Submitted in archive with journal Fig. 1B
Mutation analysis (IF APPLICABLE)	Sequencing	Heterozygous SCN5A c392 + 3A > G mutation	N/A
	Southern Blot OR WGS	PCR negative	Fig. 1F
Microbiology and virology	Mycoplasma	PCR negative	Fig. 1F
Differentiation potential	Directed differentiation	Formation of three germ layers confirmed by confocal imaging	Fig. 1C
List of recommended germ layer markers	Expression of these markers has to be demonstrated at mRNA (RT PCR) or protein (IF) levels, at least 2 markers need to be shown per germ layer	Endoderm: SOX17, CXCR4 Mesoderm: TNNT2, NKX2.5 Ectoderm: PAX6, NESTIN	Fig. 1C
Donor screening (OPTIONAL)	HIV 1 + 2 Hepatitis B, Hepatitis C	N/A	N/A
Genotype additional info (OPTIONAL)	Blood group genotyping HLA tissue typing	N/A	N/A

a variant of uncertain significance (VUS). This variant segregated to her brother with inducible Brugada syndrome and father who presented with trifascicular block. Variants in splice-site junctions often require RNA-based functional evidence to establish their pathogenicity.

PBMCs from the patient were transfected with reprogramming vectors encoding reprogramming factors Oct4, Sox2, Klf4, L-Myc and Lin28 and expanded in ReproTeSR™. iPSC colonies were selected and expanded in mTeSR™ Plus (Fig. 1A). The heterozygous SCN5A variant was genotyped with Sanger sequencing of DNA isolated from patient PBMCs and iPSCs (Fig. 1B). iPSCs were differentiated into the three germ layers (endoderm, mesoderm and ectoderm) and expression of lineage specific markers confirmed via confocal imaging (Fig. 1C). Pluripotency was confirmed via confocal imaging and RT-qPCR (Fig. 1D and E). Absence of mycoplasma and reprogramming vectors in the iPSCs were confirmed at passage 18 (Fig. 1F and G). A normal female karyotype with no aneuploidies was confirmed (Fig. 1H). Characterisation of the cell line is summarised in Table 1.

4. Materials and methods

4.1. Generation and culture of iPSC

PBMCs were reprogrammed using the Erythroid Progenitor Reprogramming Kit according to manufacturer's protocol (STEMCELL Technologies, Vancouver, CAN). Expanded PBMCs (1×10^6) were transfected with Epi5 reprogramming vectors from the Epi5™ Episomal iPSC Reprogramming Kit (Thermo Fisher, MA, USA) according to

Table 2
Reagents details.

	Antibodies used for immunocytochemistry			
	Antibody	Dilution	Company Cat #	RRID
Pluripotency Marker	Rabbit anti-OCT4	1:400	Cell Signaling Technology Cat# 2750	RRID: AB_823583
	Mouse anti-SSEA-4	1:500	Cell Signaling Technology Cat# 4755	RRID: AB_1264259
	Rabbit anti-SOX2	1:400	Cell Signaling Technology Cat# 3579	RRID: AB_2195767
	Mouse anti-TRA-1-60	1:500	Cell Signaling Technology Cat# 4746	RRID: AB_2119059
Endoderm Marker	Rabbit anti-SOX17	1:1000	Cell Signaling Technology Cat# 81778S	RRID: AB_2650582
	Mouse anti-CXCR4	1:100	STEMCELL TechnologiesCat# 60,089	RRID: AB_2936358
	Rabbit anti-NKX2.5	1:1000	Thermo Fisher Scientific Cat# A25974	RRID: AB_2936358
	Mouse anti-TNNT2	1:100	Thermo Fisher Scientific Cat# MA5-12960	RRID: AB_11000742
Mesoderm Marker	Rabbit anti-PAX6	1:40	Thermo Fisher Scientific Cat# 42-6600	RRID: AB_2533534
	Mouse anti-NESTIN	1:100	STEMCELL Technologies Cat# AB_2650581	RRID: AB_2650581
	Alexa Fluor 594 Goat anti-rabbit IgG	1:250	Thermo Fisher Scientific Cat# A11012	RRID: AB_141359
	Alexa Fluor 488 Goat anti-mouse IgG	1:250	Thermo Fisher Scientific Cat# A11001	RRID: AB_2534069
Secondary Antibody	Primers Target	Size of band (bp)	Forward/Reverse primer (5'-3')	
	Epi5 Plasmids EBNA-1	666	TTCCACGAGGGTAGTGAAC/ TCGGGGTGTTAGAGACAAC	
	House-keeping gene (qPCR)	GAPDH	131	GTCTCCTCTGACTTCAACAGCG/ ACCACCTGTTGCTGAGCCAA
	Pluripotency marker (qPCR)	OCT4	121	TCGAGAACGAGTGAGAGGC/ CACACTCGGACCACATCCTTC
		SOX2	146	TACAGCATGATGCAGGACCA/ CGGTTCATGTTAGGCTGCGA
		NANOG	138	GATTGTTGGGCCTGAAGAAA/ TTGGGACTGGTGGAAAGAAC
Variant sequencing	SCN5A	290	AAGGGCTCTGAGCCAAA/ ACTCTAAACTAGAATGGAGGT	
STR analysis	D7	226	TGTCTAGTTAGAACGAACTAACG/ CTGAGGTATCAAACACTCAGAGG	

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Table 2 (continued)

Antibodies used for immunocytochemistry			
Antibody	Dilution	Company Cat #	RRID
D16	157	GATCCCAAGCTTCTCTT/ACGTTTGTGTGCATCTGT	
TH01	246	CAGCTGCCCTAGTCAGCAC/GCTTCCGAGTGCAAGGTACA	
D8	181	TTTTGTATTCTATGTACATTGCG/CGTAGCTATAATTAGTTCTTTCA	
TPOX	232	ACTGGCACAGAACAGGCACTTAGG/GGAGGAACGTGGACCACAGGT	
D19	206	CCTGGCAACAGAATAAGAT/TAGGTTTTAAGGAACAGGGTGG	
D3	131	ACTGCAGTCCAATCTGGGT/ATGAAATCAACAGAGGCTTG	
FGA	196	GCCCCATAGTTTGTAACCTCA/TGATTTGCTGTAATTGCCAGC	
D6S1377	160	TGACATTAGGAGGCACTGG/TTAACTTGTCTGGCTGTTGGAT	
D5S407	111	TGGTTAGAGAATTGCC/CCTGTGATTTGTTGTCATTGGAAGT	
D11S1344	292	AGTGAGCCCTGAACCTCTGC/CCACAGCGCTGGCTTGATAC	
D7S517	253	TGGAGAACCCATGTGAGT/AGCTGTAATAGTTGCTGGTTGAG	
D5	149	GGTGATTTCTCTTGGTATCC/AGGCCACAGTTACAAACATTGATCT	
D14S972	208	GTAAACGCCATAACAGGCCAG/TGACTGCCTCATGATTTC	
D9S157	145	AGCAAGGCCAGCACATT/C TGGGGATGCCAGATAACTATATC	
D19S605	117	TCCACCCAAGTCTCTG/ GCATGTGTGTCCGT	

manufacturer's protocol. Transfection was performed with the CD34+ Nucleofector Kit with the Nucleofector I (Lonza Technologies, Basel, CHE) on program U-014 and plated onto Matrigel® hESC-Qualified Matrix (Corning, New York, USA) coated plates. After appearance of single reprogrammed iPSC colonies, cells were passaged as aggregates with Gentle Cell Dissociation Reagent (STEMCELL Technologies) and cultured in mTeSR™ Plus (STEMCELL Technologies). Cells were cultured in a humidified incubator at 37 °C with 5% CO₂.

4.2. Confirmation of heterozygous *SCN5A* c.392 + 3A > G variant

Genomic iPSC DNA from CIAUi002-C and a control cell line were isolated using GenElute™ Blood Genomic DNA kit (Sigma-Aldrich, MO, USA). *SCN5A* was amplified in DNA using primers described in Table 2. The samples were Sanger sequenced at Macrogen (Seoul, KOR) and the resulting chromatograms were analysed using Sequencher® v5.4.6 to verify the presence of the heterozygous *SCN5A* c.392 + 3A > G variant.

4.3. Immunocytochemistry

iPSCs were differentiated at passage 20 into endoderm and ectoderm lineages using the STEMdiff™ Trilineage Differentiation Kit and mesoderm using the STEMdiff™ Ventricular Cardiomyocyte Differentiation Kit (Stem Cell Technologies) following manufacturer's instructions. Cells were stained for lineage specific and pluripotency markers (Table 2), at passages 18–19, as previously described (Holliday et al., 2018) and imaged on the Leica SP8 confocal microscope (Leica, Wetzlar, DE).

4.4. Reverse Transcription-Quantitative PCR (RT-qPCR) of pluripotency markers

Expression of pluripotency markers (*OCT4*, *NANOG* and *SOX2*) in iPSCs were compared with iPSC-derived cardiomyocytes (iPSC-CMs) using RT-qPCR. RNA was isolated from iPSCs (passage 19, 20–23, n = 4)

using Trizol™ Reagent (Thermo Fisher Scientific) and reverse transcribed into cDNA using SuperScript™ III Reverse Transcriptase kit (Thermo Fisher Scientific) according to manufacturer's protocol. qPCR was performed on the Stratagene Mx3005P qPCR system (Agilent Technologies, CA, USA) using 500 ng of cDNA and Fast SYBR™ Green Master Mix (Thermo Fisher Scientific) following manufacturer's protocol. cDNA primer sequences are shown in Table 2. Ct values were normalised to *GAPDH* expression and relative fold change was compared against expression in iPSC-CMs.

4.5. Testing for absence of episomal vectors and mycoplasma

DNA from iPSC at passage 18 was amplified for the presence of Epi5 vector DNA using EBNA-1 primers (Table 2) and analysed using gel electrophoresis. The presence of mycoplasma was tested at passage 18 using the LookOut™ Mycoplasma Kit (Sigma-Aldrich, St Louis, USA) according to manufacturer's protocol.

4.6. STR analysis

Short tandem repeat (STR) sites (n = 16) across the genome were amplified in DNA from patient PBMCs and iPSCs using primer sequences shown in Table 2. Fragments were separated using Applied Biosystems 3730xl platform and analysed using Peak Scanner Software v1.0 (Thermo Fisher Scientific).

4.7. Karyotype analysis

Karyotype analysis was performed at the Victorian Clinical Genetics Services (VCGS, VIC, AUS) using genomic DNA isolated from iPSC. The Infinium Global Screening Array-24 v1.0 (Illumina, SA, USA) was used at 0.50 Mb resolution.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Christopher Semsarian reports financial support was provided by National Health and Medical Research Council. Richard D. Bagnall reports financial support was provided by New South Wales Ministry of Health. Emma S. Singer reports financial support was provided by Australian Government Department of Education.

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