



## Lab Resource: Multiple Cell Lines

## Generation of induced pluripotent stem cell lines from a sister pair who suffered post-partum or recurrent Spontaneous Coronary Artery Dissections



Monique Bax <sup>a,c</sup>, Keerat Junday <sup>a</sup>, Siiri E Iismaa <sup>a,c</sup>, Xenia Kaidonis <sup>a,c</sup>, David Muller <sup>a,b,c</sup>, Stephanie Hesselson <sup>a</sup>, Robert M Graham <sup>a,b,c,\*</sup>

<sup>a</sup> Victor Chang Cardiac Research Institute, Darlinghurst 2010, Australia

<sup>b</sup> St Vincent's Hospital, Darlinghurst, New South Wales 2010, Australia

<sup>c</sup> University of New South Wales Sydney, Kensington, New South Wales 2052, Australia

## A B S T R A C T

Spontaneous Coronary Artery Dissection (SCAD) results from a bleed within a coronary artery wall that impairs blood flow as it expands. It is the most common cause of myocardial infarction in pregnant women. Here, peripheral blood mononuclear cells from two sisters who had suffered SCADs were reprogrammed using Sendai Virus. Expression of pluripotency markers, capability to differentiate to the three germ layers, and cellular integrity were confirmed. This is the first report of a SCAD family induced pluripotent stem cell (iPSC) cohort, including a sister who suffered post-partum SCAD, and one who suffered from multiple recurrences.

## 1. Resource table

Unique stem cell lines identifier	VCCRIi002-A VCCRIi003-A
Alternative names of stem cell lines	069 (VCCRIi002-A) 096 (VCCRIi003-A)
Institution	Victor Chang Cardiac Research Institute
Contact information of distributor	Robert M Graham, B. Graham@Victorchang.edu.au
Type of cell lines	iPSC
Origin	Human
Additional origin info required	VCCRIi002-A Age: 43 VCCRIi002-A Sex: Female VCCRIi002-A Ethnicity: Caucasian VCCRIi003-A Age: 37 VCCRIi003-A Sex: Female VCCRIi003-A Ethnicity: Caucasian
Cell Source	Peripheral Blood Mononuclear cells (PBMC)
Clonality	Cloned
Method of reprogramming	Sendai Virus
Gene modification	NO
Type of gene modification	N/A
Evidence of the reprogramming transgene loss (including genomic copy if applicable)	q-PCR
Associated disease	Spontaneous Coronary Artery Dissection
Gene/locus	N/A
Date archived/stock date	09.03.2020

(continued on next column)

(continued)

Cell line repository/bank	<a href="https://hpscreg.eu/cell-line/VCCRIi002-A">https://hpscreg.eu/cell-line/VCCRIi002-A</a> <a href="https://hpscreg.eu/cell-line/VCCRIi003-A">https://hpscreg.eu/cell-line/VCCRIi003-A</a>
Ethical approval	St Vincent's Hospital Human Research Ethics Committee (HREC/16/SVH/338); SVH File number: 16/245

## 2. Resource utility

Spontaneous Coronary Artery Dissections (SCAD), the cause of up to one third of myocardial infarctions in women under 50, is now recognized as a familial condition, which likely requires a two-hit (gene and environment) trigger. Here, we report the first iPSC family cohort (sister pair), to investigate the mechanism of SCAD. **Tables 1 – 3.**

## 3. Resource details

SCAD is a member of the dissection vasculopathy family (Bax et al., 2022) that is increasingly recognised as a cause of myocardial infarction in women. It is the primary cause of myocardial infarction in pregnant, and recently pregnant women. Structural failure of the coronary artery results in the formation of an intramural haematoma, which restricts perfusion of the myocardium. The mechanisms underpinning these dissection events are poorly understood, and no specific therapeutic treatments are available to survivors, despite a high risk of recurrence

\* Corresponding author at: Victor Chang Cardiac Research Institute, Darlinghurst 2010, Australia.

E-mail address: [B.Graham@victorchang.edu.au](mailto:B.Graham@victorchang.edu.au) (R.M. Graham).

**Table 1**  
Summary of lines.

iPSC line names	Abbreviation in figures	Gender	Age	Ethnicity	Genotype of locus	Disease
VCCRII002-A	069; Cell line 1	Female	43	Caucasian	NA	Spontaneous Coronary Artery Dissection
VCCRII003-A	096; Cell line 2	Female	37	Caucasian	NA	Spontaneous Coronary Artery Dissection

**Table 2**  
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photograph	Normal	<a href="#">Fig. 1A</a>
Phenotype	Qualitative analysis: Immunocytochemistry Quantitative analysis: RT-qPCR	Assessed staining/expression of pluripotency markers: Oct4, Nanog, Tra-1–60 and SSEA4 hPSC Scorecard microarray indicates pluripotency expression in range	<a href="#">Fig. 1B &amp; 1C</a>
Genotype	Karyotype (G-banding) and resolution	Normal; 46, XY – 15 cells at resolution 400 bphs	<a href="#">Fig. 1D</a>
Identity	Microsatellite PCR (mPCR) ORSTR analysis	STR Analysis	Supplementary File 2 STR for iPSC Line 069STR for iPSC Line 096
Mutation analysis (IF APPLICABLE)	Sequencing	N/A	N/A
Microbiology and virology	Southern Blot OR WGS	N/A	N/A
Differentiation potential	Sendai Virus EliminationMycoplasma <i>In Vitro</i> Differentiation AND Scorecard AND Directed differentiation	Mycoplasma testing by luminescence. Negative Ectodermal neural rosettes Pax6+, Endodermal progenitors SOX17+, and mesodermal progenitors brachyury +. hPSC Scorecard analysis indicated three germ layer expression in differentiated cells.	Supplementary File 1 Supplementary File 3 <a href="#">Fig. 1E</a> -Ectodermal Fig. 1F – Endodermal Fig. 1G – Mesodermal Fig. 1G – Trilineage Scorecard
Donor screening (OPTIONAL)	HIV 1 + 2 Hepatitis B, Hepatitis C	Negative	Not shown but available from author
Genotype additional info (OPTIONAL)	Blood group genotyping HLA tissue typing	N/A N/A	N/A N/A

(~20%) (Graham et al., 2018). SCAD is believed to occur as a result of both a genetic susceptibility (Tarr et al., 2022) and an environmental cue, however further studies are required to confirm this hypothesis and elucidate the mechanism involved (Graham et al., 2018).

Here, we report the first SCAD family (a sister pair) induced pluripotent stem cells (iPSCs) developed from a patient with post-partum SCAD, and her sister, who has had multiple recurrences. The former, aged 33 years at the time of her SCAD experienced shortness of breath and chest pain less than a month after childbirth (third pregnancy, twins). The other sister, aged 33 years at time of her initial SCAD, experienced two recurrences at 41, and 46 years of age. Both had chest pain and shortness of breath post-SCAD.

Peripheral blood mononuclear cells were reprogrammed to iPSCs using Sendai virus encoding the OSKM factors. Following elimination of

Sendai Virus (Supplementary File 1), clones were selected based on morphology of colonies (Fig. 1A), and immunocytochemical detection of pluripotency factors OCT4, SSEA4 (Fig. 1B) and NANOG, TRA-1–60 (Fig. 1C). Both cell lines expressed a normal karyotype (Supplementary File 2) and both tested negative for mycoplasma (Supplementary File 3).

Expression of pluripotency factors was also assessed via mRNA quantification, using the hPSC Scorecard Assay, which compares to a database of validated stem cells (Fig. 1D). Both lines had high pluripotency factor expression, and low-level expression of markers common to the three germ layers.

Both cell lines were capable of differentiating into the three germ layers: 1. ectodermal lineage, expressing Pax6 in neural rosette formations (Fig. 1E); 2. mesodermal lineage, expressing brachyury in mesenchymal progenitors (Fig. 1F); 3. endodermal lineage, expressing Sox17

**Table 3**  
Reagents details.

Antibodies used for immunocytochemistry/flow-cytometry			
	Antibody	Dilution	Company Cat # and RRID
Pluripotency Markers	Rabbit anti-OCT4 Mouse anti-SSEA4 Rabbit anti-Nanog Mouse anti-TRA-1–60	1:400 1:500 1:20001:1000	Cell Signaling Technology Cat. No. 2840, RRID: AB_2167691 Cell Signaling Technology Cat. No. 4755, RRID: AB_1264259 Cell Signaling Technology Cat. No. 4903, RRID: AB_10559205 Cell Signaling Technology Cat. No. 4746, RRID: AB_211905
Differentiation Markers	Rabbit anti-PAX6 Rabbit anti Sox17 Rabbit anti Brachyury	1:100 1:1001:50	Invitrogen Cat. No. 42–6600, RRID: AB_2533534 Invitrogen Cat. No. 703063, RRID: AB_2784556 Invitrogen Cat. No. PA5-82078, RRID: AB_2784556
Secondary Antibodies	Alexa 594 Goat Anti-Rabbit Alexa 488 Goat Anti-MouseAlexa 488 Goat Anti-Rabbit	1:500 1:5001:500	Invitrogen Cat. No. A11012, RRID: AB_2534079 Invitrogen Cat. No. A11029, RRID: AB_2534088 Invitrogen Cat. No. A11034, RRID: AB_2534088
Primers	Target	Forward/Reverse primer (5'-3') GGATCACTAGGTGATATCGAGC/ACCAGACAAGAGTTAACGAGATATGTATC	
Sendai Virus Primer	SeV, 181 bp	TGAAGGTCGGAGTCAACGGA/CCAATTGATGACAAGCTCCCG	
Reference Gene	GAPDH, 203 bp		

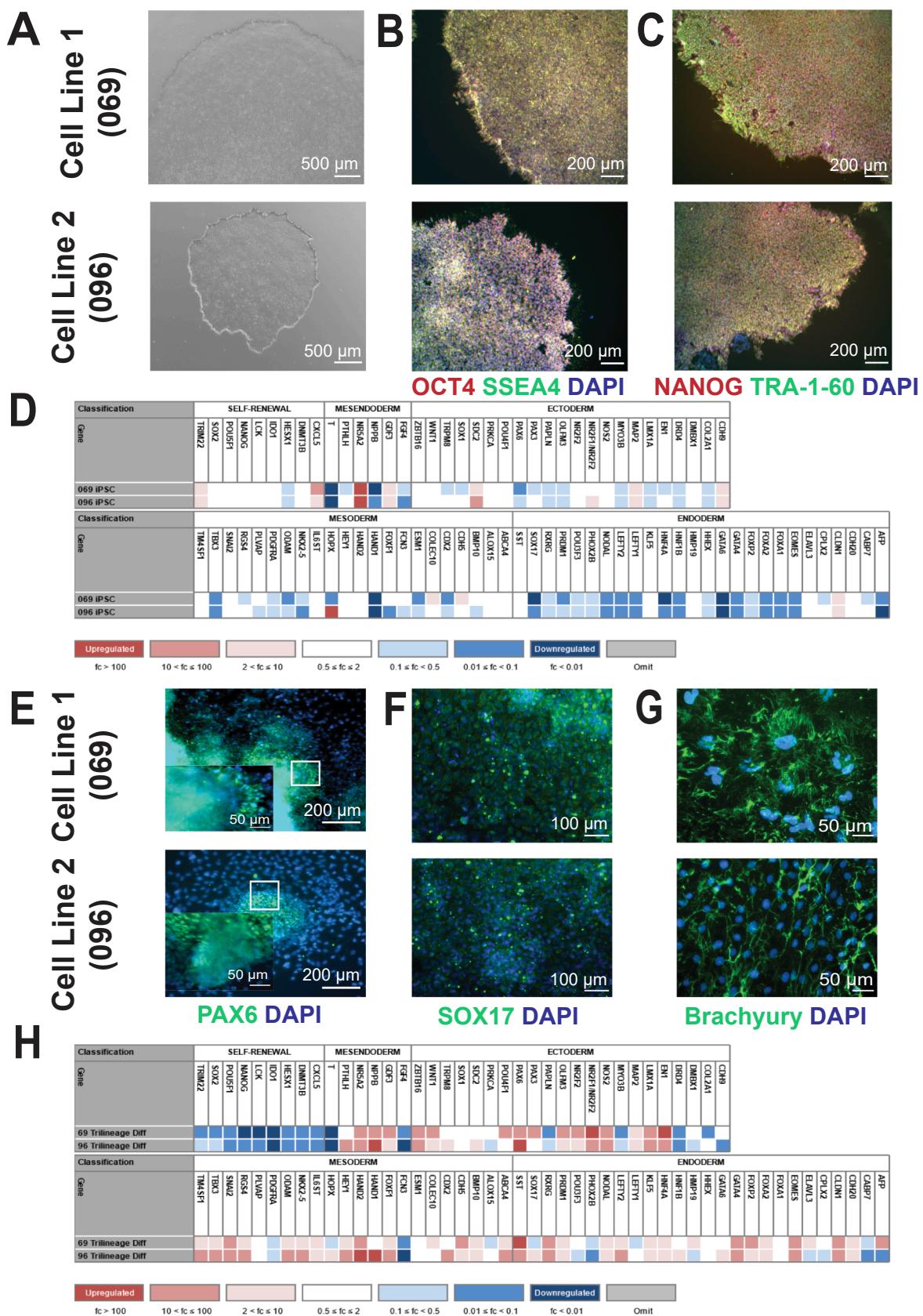


Fig. 1.

in endodermal progenitors (Fig. 1G). Differentiation into the three germ layers was confirmed by hPSC Scorecard Assay assessment of lineage-specific markers and comparison to the Scorecard database (Fig. 1H).

Owing to the complexity and predominantly polygenic nature of SCAD, whole genome sequencing has yet to elucidate a clear single SCAD-causing candidate gene in this family. We are therefore able to use these iPSCs to investigate if a multigenic cause underpins the condition. These novel iPSC lines represent a critically-needed model to dissect the molecular mechanism that underlies SCAD, and to potentially develop suitable treatments to reduce the risk of recurrence.

#### 4. Materials and methods

##### 4.1. Ethics statement

iPSC generation and use were approved by the St Vincent's Hospital Human Research Ethics Committee (HREC/16/SVH/338); SVH File number:16/245. All studies were performed according to guidelines following obtaining informed consent.

##### 4.2. Reprogramming of peripheral blood mononuclear cells and maintenance of induced pluripotent stem cells

PBMCs from two sisters who survived SCADs (aged 43 and 37 at collection), were collected at local pathology centers. Cells were separated using Ficoll centrifugation, and maintained using a StemSpan erythroid progenitor media as directed (Stem Cell Technologies). PBMCs ( $1 \times 10^5$ ) were transduced with Cytotunes 2.0 Sendai Virus (ThermoFisher Scientific). Following a 24 h incubation, cells were centrifuged and a total media change was performed; cells were plated in a single well of a 12-well tissue culture plate for 4 days, then plated on Matrigel-coated plates (diluted according to batch). ReproTeSR was introduced with 50:50 media ratio, and then daily media changes performed. Newly formed iPSC colonies were manually picked, based on iPSC morphology from day 15. All tissue culture was carried out at 37°C, 5% CO<sub>2</sub>, 20% O<sub>2</sub>.

##### 4.3. Confirmation of Sendai virus removal and subsequent clonal expansion

Clones were passaged with ReLeSR for 12 passages. Sendai virus removal was confirmed as previously described (Mishra et al., 2019). Clones were expanded and maintained on Matrigel with mTeSR plus, and passaged with ReLeSR (clumps), splitting 1:40 weekly. Cells at p20-p24 were used for further characterization unless specified.

##### 4.4. Immunocytochemistry

Cells were fixed with 4% paraformaldehyde for 10 min at room temperature (RT), and kept for up to 72 h at 4°C. Cells were blocked (10% goat serum 1 h, RT), and permeabilized with 0.05% v/v Triton-X if required. Primary antibodies were incubated at 4°C overnight. Cells were washed and incubated with corresponding secondary antibodies (1 h, RT). Following wash steps, nuclear DAPI stain was performed (1:50,000 in PBS) for 7 min. All images were taken using an EVOS Cell Imaging System, and assessed using FIJI software.

##### 4.5. Trilineage differentiation

Mesodermal differentiation was initiated by treating single cell iPSCs seeded on Matrigel with mesodermal media (DMEM/F12, Insulin-Transferrin-Selenium, MEM non-essential and Glutamax) supplemented with 50 ng/mL BMP4 and 20 ng/mL FGF2 (Life Technologies). Alternate day media changes were performed for six days. Media was

supplemented with 50 ng/mL VEGF for two days before harvesting for immunocytochemistry or RNA. Ectodermal and endodermal differentiation performed as previously described (Bax et al., 2019).

##### 4.6. Scorecard quantification

Quantification of pluripotency and trilineage expression were performed via Scorecard analysis. iPSCs, and trilineage differentiated cells were harvested in Trizol. RNA (1 µg) was analyzed by TaqMan Scorecard (ThermoFisher Scientific) for each line and each cell type as per the manufacturer's instructions. Expression levels were compared to the hPSC Scorecard analysis software database.

##### 4.7. Karyotyping, mycoplasma and Short Tandem Repeat (STR) verification

Karyotyping was carried out by Monash Health Services. Metaphase spreads (N = 15 cells) were reviewed at a 400 bphs resolution. Monthly mycoplasma testing was performed via Mycoplasma Detection Kit (Lonza Biosciences) as per manufacturer's instruction. STR analysis was performed from cell pellet by the Garvan Molecular Genetics Facility.

#### Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Robert M Graham reports financial support was provided by Australian National Health & Medical Research Council. Robert M Graham reports financial support was provided by Cardiovascular Society of Australia and New Zealand. Robert M Graham reports financial support was provided by New South Wales Government.

#### Data availability

Data will be made available on request.

#### Acknowledgements

We would like to acknowledge our cell donors, without whom this work would not have been possible, the SCADdadle Inc. group for their continued support, and the multiple pathology centers' staff who kindly provided their services free of charge. This work was partially funded by an Australian National Health & Medical Research Council Grant; a Cardiovascular Society of Australia and New Zealand grant and a NSW Health Senior Clinician Cardiovascular Grant (RG193092) to RMG, and completed at the Innovation Centre within the Victor Chang Cardiac Research Institute, established with New South Wales Government funding.

#### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scr.2023.103238>.

#### References

- Bax, M., Balez, R., Muñoz, S.S., Do-Ha, D., Stevens, C.H., Berg, T., Cabral-da-Silva, M.C., et al., 2019. Generation and Characterization of a Human Induced Pluripotent Stem Cell Line UOWi005-A from Dermal Fibroblasts Derived from a CCNF Familial Amyotrophic Lateral Sclerosis Patient Using mRNA Reprogramming. *Stem Cell Research* 40 (October), 101530. <https://doi.org/10.1016/j.scr.2019.101530>.
- Bax, M., Romanov, V., Junday, K., Giannoulatou, E., Martinac, B., Kovacic, J.C., Liu, R., Iismaa, S.E., Graham, R.M., 2022. Arterial Dissections: Common Features and New Perspectives. *Frontiers in Cardiovascular Medicine* 9 (December), 1055862. <https://doi.org/10.3389/fcvm.2022.1055862>.

Graham, R.M., McGrath-Cadell, L., Muller, D.W.M., Holloway, C.J., 2018. The Mystery and Enigma of Spontaneous Coronary Artery Dissection. *Heart, Lung and Circulation* 27 (4), 401–405. [https://doi.org/10.1016/S1443-9506\(18\)30060-X](https://doi.org/10.1016/S1443-9506(18)30060-X).

Mishra, K., Junday, K., Wong, C.M.Y., Chan, A.Y., Hesselson, S., Muller, D.W., Iismaa, S.E., Mehta, A., Graham, R.M., 2019. Generation of VCCRI001-A, a Human Induced Pluripotent Stem Cell Line, from a Patient with Spontaneous Coronary Artery

Dissection. *Stem Cell Research* 41 (December), 101584. <https://doi.org/10.1016/j.scr.2019.101584>.

Tarr, Ingrid, Stephanie Hesselson, Siiri E. Iismaa, Emma Rath, Steven Monger, Michael Troup, Ketan Mishra, et al. 2022. "Exploring the Genetic Architecture of Spontaneous Coronary Artery Dissection Using Whole-Genome Sequencing." *Circulation: Genomic and Precision Medicine*. <https://doi.org/10.1161/CIRCGEN.121.003527>.