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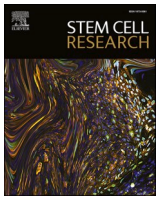

Lab Resource: Multiple Cell Lines

Generation of four induced pluripotent stem cell lines from a family harboring a single nucleotide variant in *SCN5A*

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A B S T R A C T

Patient-derived induced pluripotent stem cells (iPSC) are a valuable approach to model cardiovascular diseases. We nucleofected non-integrating episomal vectors in skin fibroblasts of three family members carrying a single nucleotide variant (SNV) in *SCN5A*, which encodes the cardiac-type sodium channel, and of a related healthy control. The SNV *SCN5A*_c.4573G > A had been previously identified in a Brugada Syndrome patient. The resulting iPS cell lines differentiate into cells of the 3 germ layers, display normal karyotypes and express pluripotency surface markers and genes. Thus, they are a reliable source to study the effect of the identified mutation in a physiologically relevant environment.

1. Resource Table

Unique stem cell lines identifier	IDIBGIi002-A IDIBGIi003-A IDIBGIi004-A IDIBGIi005-A
Alternative name(s) of stem cell lines	Rb20234 Rb20235 Rb20236 Rb20237
Institution	Girona Biomedical Research Institute (IDIBGI)
Contact information of distributor	Elisabet Selga: elisabet.selga@umedicina.cat Fabiana Scornik: fabianasilvia.scornik@udg.edu
Type of cell lines	iPSC
Origin	Human
Additional origin info required	IDIBGIi002-A: - Age: 14 - Sex: Female - Ethnicity if known: Caucasian IDIBGIi003-A: - Age: 11 - Sex: Male - Ethnicity if known: Caucasian IDIBGIi004-A: - Age: 45 - Sex: Female - Ethnicity if known: Caucasian IDIBGIi005-A: - Age: 48 - Sex: Male - Ethnicity if known: Caucasian
Cell Source	Skin fibroblasts
Clonality	Clonal

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(*continued*)

Unique stem cell lines identifier	IDIBGIi002-A IDIBGIi003-A IDIBGIi004-A IDIBGIi005-A
Method of reprogramming	Episomal, transgene-free
Evidence of the reprogramming transgene loss (including genomic copy if applicable)	Copy number-PCR; qRT-PCR
Cell culture system used	Feeder free conditions in TeSR™-E8 or mTeSR™1
Type of Genetic Modification	Spontaneous mutation
Associated disease	Brugada Syndrome
Gene/locus	*SCN5A*_c.4573G > A
Date archived/stock date	2022
Cell line repository/bank	Rb20234: https://hpscreg.eu/cell-line/IDIBGIi002-A Rb20235: https://hpscreg.eu/cell-line/IDIBGIi003-A Rb20236: https://hpscreg.eu/cell-line/IDIBGIi004-A Rb20237: https://hpscreg.eu/cell-line/IDIBGIi005-A Registration ongoing at Spanish National Stem Cell Bank: https://eng.isciii.es/eng.isciii.es/QueHacemos/Servicios/BIOBANCOS/BNLC/Paginas/default.html
Ethical approval	Ethics Committee Of Clinical Research-CMRB. Catalan Authority for Stem Cell Research (Approval number 374 3071) Advisory committee for Human Tissue and Cell Donation and Use, Instituto de Salud Carlos III. Approval number P11/2015

2. Resource utility

We differentiated the generated iPSC into cardiomyocytes (iPS-CM). These iPS-CM allowed us to characterize the effect of the identified SNV *SCN5A*_c.4573G > A on the electrophysiological characteristics of the cardiac sodium current in a tissue and patient-specific background (Martinez-Moreno et al., 2020).

3. Resource details

Dermal fibroblasts were isolated from skin biopsies of four members of the same family: 3 carriers of the SNV *SCN5A*_c.4573G > A (named IDIBGIi002-A, IDIBGIi003-A and IDIBGIi004-A), and 1 noncarrier (IDIBGIi005-A). Reprogramming was performed by nucleofection of non-integrating episomal plasmids encoding six human factors (OCT3/4, SOX2, KLF4, LIN28, L-Myc and a p53 knock down shRNA) under feeder-free conditions. A summary of the characterization and validation of the resulting iPSC lines is provided in Table 1. Episomal plasmid copy number was determined in genomic DNA of cells 72 h post-nucleofection by absolute quantitative real time PCR (aqRT-PCR), and showed absence of episomal plasmids (data available upon request). qPCR using specific primers (Table 2) performed in the iPS cell lines once they had been passaged at least 5 times after reprogramming, evidenced the absence of episomal plasmid-derived genes and the presence of endogenous pluripotency markers (Fig. 1A). The obtained iPSC lines (First lane, left of Fig. 1B, 1C, 1D, 1E, scale bar 400 μm), were karyotypically normal (First lane, right of Fig. 1B, 1C, 1D, 1E). Immunocytochemistry analyses with antibodies against endogenous human OCT4, SOX2, NANOG, TRA-1–60, TRA-1–81, SSEA-3 and SSEA-4 (Second lane of Fig. 1B, 1C, 1D, 1E, scale bars 50 μm) and alkaline phosphatase activity (Supplemental Fig. 1A, scale bar 50 μm) confirmed expression of pluripotency markers. Embryoid bodies (EB) were generated and differentiated *in vitro* towards the three germ layers. Immunofluorescence analyses of these cells confirmed their differentiation capacity to definitive endoderm (AFP and FOXA2), mesoderm (ASMA, ASA or GATA4) and ectoderm (TUJ1, GFAP) (Third lane of Fig. 1B, 1C, 1D, 1E, scale bars 50 μm). Short tandem repeat analysis (STR, submitted in archive with journal) of the iPS cell lines matched that of the patient's fibroblasts, thus confirming cell identity. The presence of the SNV in the iPSC from carrier individuals and its absence in the control patient was verified by Sanger sequencing (Fourth lane of Fig. 1B, 1C, 1D, 1E). The sequence of a non-related control iPS cell line (IDIBGIi001-A, https://hpscreg.eu/cell-line/IDIBGIi001-A) is also provided. PCR was used to routinely test samples for absence of mycoplasma contaminations (iPSC only showed amplification of the internal control, as shown in Supplemental Fig. 1B).

Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography Bright field	Normal	Figure 1, first lane left of panel B, C, D and E
Phenotype	Qualitative analysis - Immunocytochemistry	Presence of pluripotency markers: Oct4, Nanog, Sox2, SSEA-3, SSEA-4, Tra 1–60, Tra-1–80.	Figure 1, second lane of panel B, C, D and E
	Quantitative analysis - RT-qPCR	Expression of pluripotency markers Lin28, OCT4, SOX2	Figure 1 panel A
Genotype	Karyotype (G-banding) and resolution	**IDIBGIi002-A and IDIBGIi004-A:** 46XX Resolution 30–500 **IDIBGIi003-A and IDIBGIi005-A:** 46XY Resolution 30–500	Figure 1, first lane right of panel B, C, D and E
Identity	STR analysis	STR Profiling Performed 10 sites tested, all matched	Submitted in archive with journal
Mutation analysis (IF APPLICABLE)	Sequencing	**IDIBGIi002-A, IDIBGIi003-A and IDIBGIi004-A:** Heterozygous *SCN5A*_c.4573G > A **IDIBGIi005-A:** *SCN5A*_c.4573G	Figure 1, fourth lane of panel B, C, D and E
	Southern Blot OR WGS	N/A	
Microbiology and virology	Mycoplasma	Negative testing by PCR	Supplementary Figure 1 panel B
Differentiation potential	Embryoid body formation	Three germ layers formation	Figure 1, third lane of panel B, C, D and E
List of recommended germ layer markers	Expression of markers demonstrated at protein (IF) level	Positive for: Ectoderm: TUJ1, GFAP Endoderm: FOXA2, AFP Mesoderm: ASMA, ASA or GATA4	Figure 1, third lane of panel B, C, D and E
Donor screening (OPTIONAL)	HIV 1 + 2 Hepatitis B, Hepatitis C	N/A	
Genotype additional info (OPTIONAL)	Blood group genotyping	N/A	
	HLA tissue typing	N/A	

Table 2
Reagents details.

	Antibodies used for immunocytochemistry/flow-cytometry			
	Antibody	**Dilution**	**Company Cat #**	**RRID**
Pluripotency markers	Mouse anti-OCT4	1:2	Santa Cruz, sc-5279	AB_628051
	Goat anti-NANOG	1:5	R&D Systems, AF1997	AB_355097
	Rabbit anti-SOX2	1:100	ABR, PA1-16968	AB_2195781
	Rat anti-SSEA3	1:1	Hybridoma Bank, MC-631	AB_528476
	Mouse anti-SSEA4	1:1	Hybridoma Bank, MC-813–70	AB_528477
	Mouse anti-TRA-1–60	1:100	Millipore, MAB4360	AB_2119183
	Mouse anti-TRA-1–81	1:100	Millipore, MAB4381	AB_177638
Differentiation Markers	Mouse anti-TUJ1	1:40	Covance, MMS-435P	AB_2313773
	Rabbit anti-GFAP	1:1000	Dako, Z0334	AB_10013382
	Mouse anti-ASA	1:400	Sigma, A2172	AB_476695
	Rabbit anti-GATA4	1:25	Santa Cruz sc-9053	AB_2247396
	Rabbit anti-AFP	1:200	Agilent, A0008	AB_2650473
	Goat anti-FOXA2	1:50	R6D Systems, AF2400	AB_2294104
Secondary antibodies	AF488 Goat anti-Mouse	1:200	Jackson, 115–546-071	AB_2338865
	Cy3 Goat anti-Rat	1:200	Jackson, 112–165-020	AB_2338243
	AF488 Donkey anti-Rabbit	1:200	Jackson, 711–545-152	AB_2313584
	DyLight649 Goat anti-Mouse	1:200	Jackson, 115–495-075	AB_2338809
	AF488 Donkey anti-Goat	1:200	Jackson, 705–545-147	AB_2336933
	Cy3 Donkey anti-Mouse	1:200	Jackson, 715–165-140	AB_2340812
	Cy3 Donkey anti-Goat	1:200	Jackson, 705–165-147	AB_2340812
	AF488 Donkey anti-Mouse	1:200	Jackson, 715–545-151	AB_2307351
	Cy3 Donkey anti-Guinea pig	1:100	Jackson, 706–165-148	AB_2341099
	AF488 Goat anti-Mouse	1:200	Jackson, 115–546-071	AB_2338865
	Cy3 Goat anti-Mouse	1:200	Jackson, 115–165-075	AB_2338689
	Primers			
	Target	**Size of band**	**Forward/Reverse primer (5′-3′)**	
Episomal plasmids (aqRT-PCR)	EBNA-1		TGGAAACCAGGGAGGCAAAT/GTCAAGGAGGTTCCAACCCG	
Episomal plasmids (qPCR)	pCXLE-Oct3/4 (plasmid)		CATTCAAACTGAGGTAAGGG/TAGCGTAAAAGGAGCAACATAG	
	pCXLE-SOX2 (plasmid)		TTCACATGTCCCAGCACTACCAGA/TTTGTTTGACAGGAGCGACAAT	
	pCXLE-KLF4 (plasmid)		CCACCTCGCCTTACACATGAAGA/TAGCGTAAAAGGAGCAACATAG	
	pCXLE-LIN28 (plasmid)		AGCCATATGGTAGCCTCATGTCCGC/TAGCGTAAAAGGAGCAACATAG	
	pCXLE-L-Myc (plasmid)		GGCTGAGAAGAGGATGGCTAC/TTTGTTTGACAGGAGCGACAAT	
Endogenous pluripotency genes (qPCR)	endogenous Oct3/4 (cds)		CCCCAGGGCCCCATTTTGGTACC/ACCTCAGTTTGAATGCATGGGAGAGC	
	endogenous SOX2 (cds)		TTCACATGTCCCAGCACTACCAGA/TCACATGTGTGAGAGGGGCAGTGTGC	
	endogenous LIN28 (cds)		AGCCATATGGTAGCCTCATGTCCGC/TCAATTCTGTGCCTCCGGGAGCAGGGTAGG	
	endogenous L-Myc (cds)		GCGAACCCAAGACCCAGGCCTGCTCC/CAGGGGGTCTGCTCGCACCGTGATG	
Controls (qPCR)	EBNA-1		ATCAGGGCCAAGACATAGAGATG/GCCAATGCAACTTGGACGTT	
House-Keeping Gene (qPCR)	GAPDH		GCACCGTCAAGGCTGAGAAC/AGGGATCTCGCTCCTGGAA	
Targeted mutation analysis/sequencing	SCN5A		TTTTCCTCTGCACTCTCTGTG/AAAGAAGCTAGGGTTGTACATG	

4. Materials and methods

4.1. Reprogramming of fibroblasts

Fibroblasts were cultured in DMEM supplemented with 10% HyClone FBS and 1% penicillin–streptomycin at 37 °C and 5% CO_2. 0.5x10^6 fibroblasts were reprogrammed at passage 2 by nucleofection (Amaxa NHDF Nucleofector Kit and Nucleofector 2b (Lonza), U023 protocol) with Addgene episomal plasmids #27077, #27078, #27080. Seven days later, cells were seeded onto Matrigel-coated dishes in TeSR-E8 medium (Stemcell Technologies). Approximately 20 days after nucleofection, iPS colonies were manually picked and passaged for expansion.

4.2. iPS cell culture and passaging

Culture media was gradually transitioned from TeSR-E8 to mTeSR1 medium (Stemcell Technologies), which was needed to successfully differentiate the iPS cells into cardiomyocytes. Cells were maintained on Matrigel-coated plates at 37 °C and 5% CO_2 in feeder-free conditions with daily media changes. Passaging was performed using pre-warmed 0.5 mmol/L EDTA (Life Technologies), and iPS cells were plated as single cells.

4.3. PCR and qPCR

A first screening to assess reprogramming plasmids copy number was performed 72 h post-nucleofection. Genomic DNA was extracted and aqRT-PCR for EBNA1 was performed as previously described (Martinez-Moreno, 2021) using the primers listed in Table 2 and fibroblasts as control. qPCR to assess mRNA expression of endogenous and exogenous pluripotency genes was performed in iPSCs once these cell lines had been passaged at least 5 times after reprogramming. mRNA was isolated and reverse transcribed as described (Martínez-Moreno et al., 2022) with primers listed in Table 2. Ct values were analyzed with the $2-\Delta Ct$ method and expressed as % with respect to GAPDH (mean ± SD) for each cell line. Previously established and published iPS cell lines ESi044-B (https://hpscreg.eu/cell-line/ESi044-B) and ESi045-B (https://hpscreg.eu/cell-line/ESi045-B) were used as control.

4.4. Karyotype determination

iPSCs at passage 15 (IDIBGIi002-A), 13 (IDIBGIi003-A), 12 (IDIBGIi004-A) and 19 (IDIBGIi005-A) were treated as described (Martínez-Moreno et al., 2022). Genomic integrity was evaluated by G-banded metaphase karyotype analysis of 20 metaphase spreads at Hospital Sant Joan de Déu, Barcelona, following standard procedures.

4.5. Alkaline phosphatase (AP) staining and immunocytochemistry for pluripotency

To detect AP activity, iPSCs were treated as reported in (Martínez-Moreno et al., 2022). Immunocytochemistry was performed at passage 11 (IDIBGIi002-A and IDIBGIi004-A), 16 (IDIBGIi003-A) and 12 (IDIBGIi005-A), with antibodies against pluripotency factors (Nanog, OCT4, SOX2, TRA-1–81, TRA-1–60, SSEA3 and SSEA4) as previously described

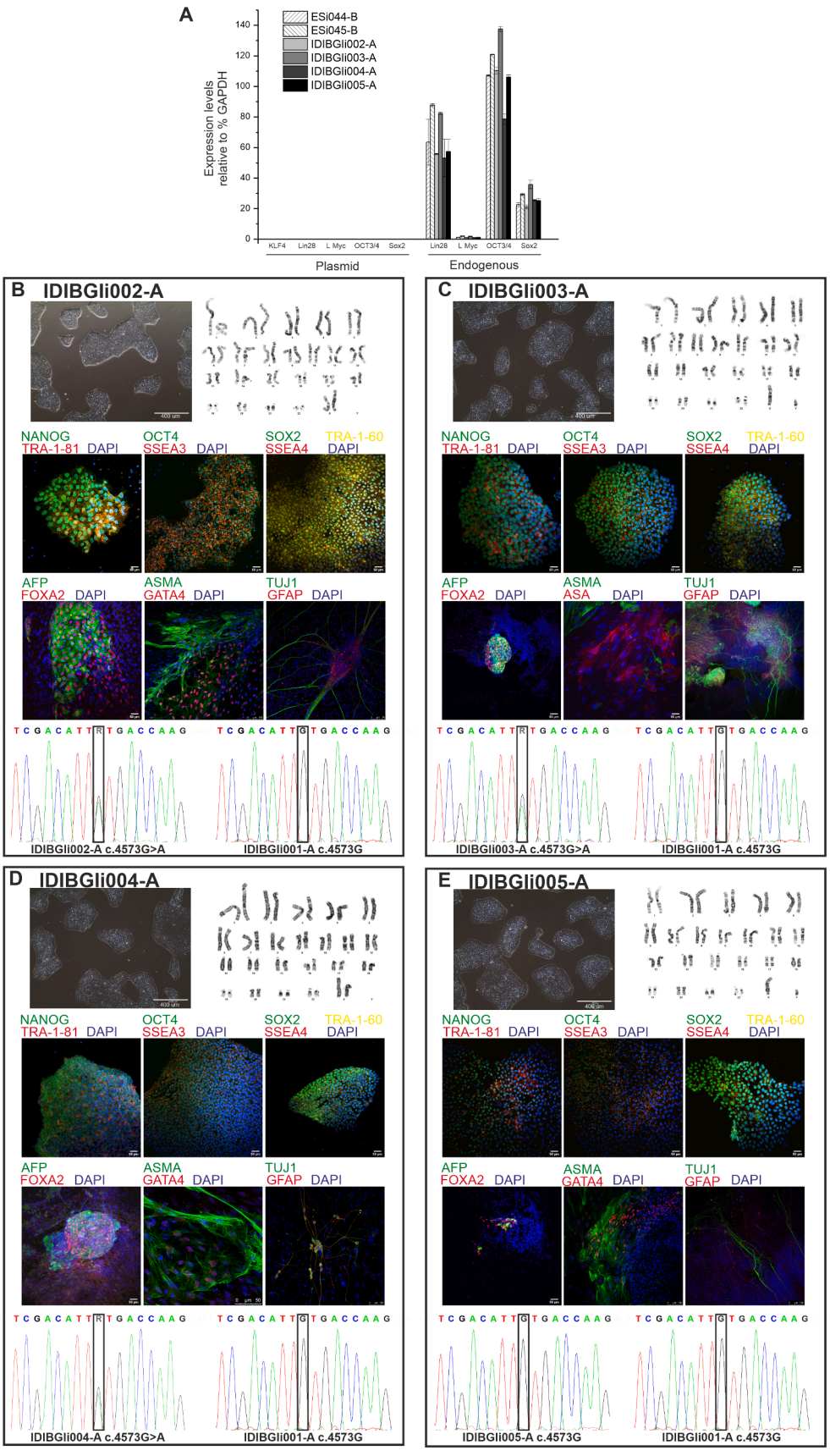

Fig. 1. Characterization of the IDIBGIi002-A, IDIBGIi003-A, IDIBGIi004-A and IDIBGIi005-A iPSC lines.

(Kuebler et al., 2017). Primary and secondary antibodies used are listed in Table 2. Confocal images were taken using a Leica TSC SPE/SP5 microscope.

4.6. Embryoid body formation and immunocytochemistry for differentiation

In vitro differentiation was promoted by embryoid bodies (EB) formation. iPSC colonies (passages 13–14) were prepared as described elsewhere (Martínez-Moreno et al., 2022) and analysed by immunocytochemistry with specific antibodies (Table 2) against endodermal markers AFP and FOXA2, ectodermal marker TUJ1 and mesodermal markers ASMA, ASA or GATA4 as previously described (Kuebler et al., 2017). Confocal images were taken as above.

4.7. Authentication and mycoplasma testing

To confirm line identity, genomic DNA was obtained from fibroblasts and from iPSCs and used for STR analysis. Genomic DNA from iPSC was extracted with the NucleoSpin Tissue kit (Macherey-Nagel), and was used to confirm the presence or the absence of the SNV by Sanger sequencing. Samples were routinely tested for absence of mycoplasma contaminations by PCR.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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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.2022.102847.

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