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Generation of human iPSC line from an arrhythmogenic cardiomyopathy patient with a *DSP* protein-truncating variant

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Abstract

Arrhythmogenic cardiomyopathy is an inheritable heart disease characterized by lethal heart rhythms and abnormal contractile function. Mutations in desmoplakin (DSP), a protein linking the cardiac desmosome with intermediate filaments, are associated with arrhythmogenic cardiomyopathy. Here we generated a human induced pluripotent stem cell (hiPSC) line from a patient with a heterozygous protein-truncating variant in *DSP* (c.1386del Leu462Serfs*22). This line has a normal karyotype and expression of pluripotency markers, and can differentiate into all three germ layers. This line is well suited for *in vitro* mechanistic studies of mechanism of *DSP* protein-truncation mutations in the context of arrhythmogenic cardiomyopathy.

1. Resource utility

The patient carrying the DSP (c.1386 Leu462Serfs*22) mutation showed an arrhythmogenic cardiomyopathy phenotype and died of heart failure at age 47. The hiPSC line described here provides a means for generating *in vitro* populations expressing the DSP (c.1386 Leu462Serfs*22) protein-truncating variant, enabling study of disease mechanism and potential drug screening.

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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.

The content is solely the responsibility of the authors and does not necessarily represent the official views of the Department of Veterans Affairs or the United States Government.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scr.2022.102987.

2. Resource details

Arrhythmogenic cardiomyopathy (AC) is an inherited heart disease estimated to affect 0.02 % to 0.1 % of the population with increased risk of sudden cardiac death and heart failure (Corrado et al., 2017). AC typically presents with ventricular arrhythmia and systolic dysfunction, myocyte depletion, and incursion of fibrotic and fatty tissue. Diagnosis relies on a combination of tools including electrocardiogram, echocardiograph, genetic screening, and biopsy.

Genetic variants associated with AC are predominantly clustered in genes associated with the desmosome, a key intracellular structure in cardiac tissue connecting the cytoskeleton and cell surface across cell junctions (Delmar and McKenna, 2010). Desmoplakin (*DSP*) is a critical protein in the desmosome, linking intermediate filaments with intercalated discs to maintain structure during contraction. Mutations in *DSP* are associated with many related diseases including AC, Naxos disease, and Carvajal syndrome; 5–10 % of AC cases exhibit a pathogenic *DSP* variant (Gerull et al., 2019). A conditional mouse knockout model of *DSP* recapitulated the AC phenotype, suggesting *DSP* haploinsufficiency as a possible mechanism (Garcia-Gras et al., 2006). 334 of the reported 488 pathogenic and likely pathogenic *DSP* variants on ClinVar are protein-truncating variants (as of 17 August 2022). However, no patient-derived induced pluripotent stem cell (iPSC) model with a *DSP* protein-truncating variant has been described. A missense mutation iPSC model showed reduced DSP expression but failed to exhibit the characteristic arrhythmia or contractile dysfunction in cardiomyocytes (Ng et al., 2019). As such, a *DSP* protein-truncating variant iPSC model would provide a new tool for studying of disease mechanisms in an *in vitro* context.

We generated an iPSC line from a 47-year old female carrying *DSP* (c.1386del Leu462Serfs*22) protein-truncation variant and exhibiting severe biventricular dysfunction (Table 1). With family consent, peripheral mononuclear blood cells (PBMCs) were reprogrammed into iPSCs using Yamanaka factors Oct3/4, Sox2, Klf4, and c-Myc and Sendaivirus transfection. Transgene and vector removal were induced with four days of culture at 38° C and confirmed via PCR (Fig. 1A). The resulting iPSC clone was confirmed to have the *DSP* protein-truncating variant by Sanger sequencing of the genomic DNA and of the individual alleles via standard subcloning methods (Fig. 1B). Short tandem repeat (STR) analysis verified these hiPSCs genetically matched the donor PBMCs (submitted in the archive with journal). The karyotype of the iPSC clone was assessed as normal at passage 9 (Fig. 1C), and the culture was assessed as free of mycoplasma contamination (Supp Fig. A). The iPSC clone exhibited normal morphology (Fig. 1D, scale 100 μm) and nuclear expression of pluripotency markers Nanog and Oct3/4 at passage 11 (Fig. 1E–F, scale 25 μm). Expression of *NANOG* and *SOX2* was confirmed by RT-qPCR as compared to WTC-11 hiPSCs (Fig. 1F). Trilineage differentiation was confirmed by RT-qPCR detection of expression of markers for ectoderm (*PAX6, OTX2*), mesoderm (*TBX6, MYH6*), and endoderm (*SOX17, FOXA2*). In sum, this *DSP* variant line exhibits all characteristics of pluripotency and is well suited for modelling the effects of *DSP* protein-truncating variants in the context of AC and in other desmosomal diseases.

3. Materials and methods

3.1. Reprogramming

Patient PBMCs were isolated from blood with familial consent. PBMCs were plated at $3x10^5$ cells/well and transformed using CytoTune-iPS 2.0 Sendai Reprogramming Kit (Invitrogen #A16517, Lot #L2170059) with recommended titers at MOI 5:5:3 (Klf4-Oct4-Sox2:c-Myc:Klf4). Cells were spinfected 45 min at 1000 × *g* with virus and 4 μg/mL polybrene. Post-reprogramming, cells were maintained on MEF-feeder cells in StemPro-34 SFM (Gibco #10639) and transitioned to mTeSR+ (STEMCELL Technologies #100-0276) before colony picking. Transgene removal was confirmed via RNA isolation, cDNA generation, and PCR (Table 2).

3.2. Cell culture

iPSCs were cultured in mTeSR + on 80 μg/mL Matrigel (Corning #356231, Lot #1242001) coated plates, 5 % CO_2, 37 °C. Cells were fed every other day and passaged with versene before differentiation was morphologically evident. Media was supplemented with 10 μM ROCK inhibitor (SelleckChem #Y27632) for 24 h post-passage. WTC-11 hiPSC line was a generous gift from the Allen Institute (Coriell #GM25256).

3.3. Trilineage differentiation

Passage 10 iPSCs were seeded in Matrigel-coated 24-well plates at $4x10^5$, $1x10^5$, and $4x10^5$ cells/well for ectoderm, mesoderm, and endoderm differentiations, respectively. Cells were fed daily per manufacturer's protocol (STEMCELL Technologies #5230). Cells were lysed for RNA isolation at day 5 (ectoderm and mesoderm) or 7 (endoderm).

3.4. Immunofluorescence

Passage 11 iPSCs were fixed in 4 % paraformaldehyde 10 min RT. Cells were permeabilized with 0.2 % Triton X-100 in PBS 5 min RT and blocked overnight with 3 % BSA (Sigma #A7030) in PBS. Cells were incubated overnight 4 °C with primary antibody (Table 2) in blocking buffer. Cells were washed and incubated with secondary antibody (Table 2) in PBS2 hours RT. Nuclei were stained with 1:2000 Hoechst 33,342 (Invitrogen H3570) 10 min RT.

3.5. RT-qPCR

RNA from passage 11 iPSCs and passage 10 trilineage differentiated cells was isolated with PureLink RNA minipreps (Invitrogen #12183018A) with PureLink Dnase treatment (Invitrogen #12185010). cDNA was reverse transcription generated using SensiFast cDNA Synthesis Kit (Thomas Scientific #C755H65). qPCR was performed with 10 μL technical triplicate reactions, 40 cycles, with SYBR Select Master Mix (Invitrogen #4472919), 5 ng cDNA, 100 nM forward/reverse primer (Table 2).

3.6. PCR and Sequencing

Genomic DNA was isolated from PBMC passage 1 and iPSC passage 11 using Dneasy Blood and Tissue Kit (Qiagen #69506). *DSP* fragment containing mutation was PCR

amplified using Q5 High-Fidelity DNA Polymerase (New England Biolabs #M0491L), 40 cycles: 95 °C 30 s, 55 °C 30 s, 72 °C 30 s, using Bio Rad T100 Thermocycler. PCR products were run on 2 % agarose gels and extracted using Fermentas Gel Extraction Kit (Invitrogen #K0692). Alleles were cloned into plasmids using CloneJET PCR Cloning Kit (Thermo Scientific #K1321) and Stbl3 chemically competent *E. coli* (Invitrogen #C737303). Sanger sequencing was performed by Eurofins Genomics.

3.7. Karyotyping

G-banded chromosome analysis of twenty metaphase cells at passage 9 was performed by Diagnostic Cytogenetics Incorporated.

3.8. STR analysis

STR analysis was performed by WiCell from 500 ng DNA isolated from PBMC passage 1 and iPSC passage 11.

3.9. Mycoplasma detection

Supernatant media from iPSCs at passage 10 was tested for mycoplasma per manufacturer's protocol with MycoAlert Mycoplasma Detection Kit (Lonza #LT07-118) and MycoAlert Assay Control Set (Lonza #LT07-518).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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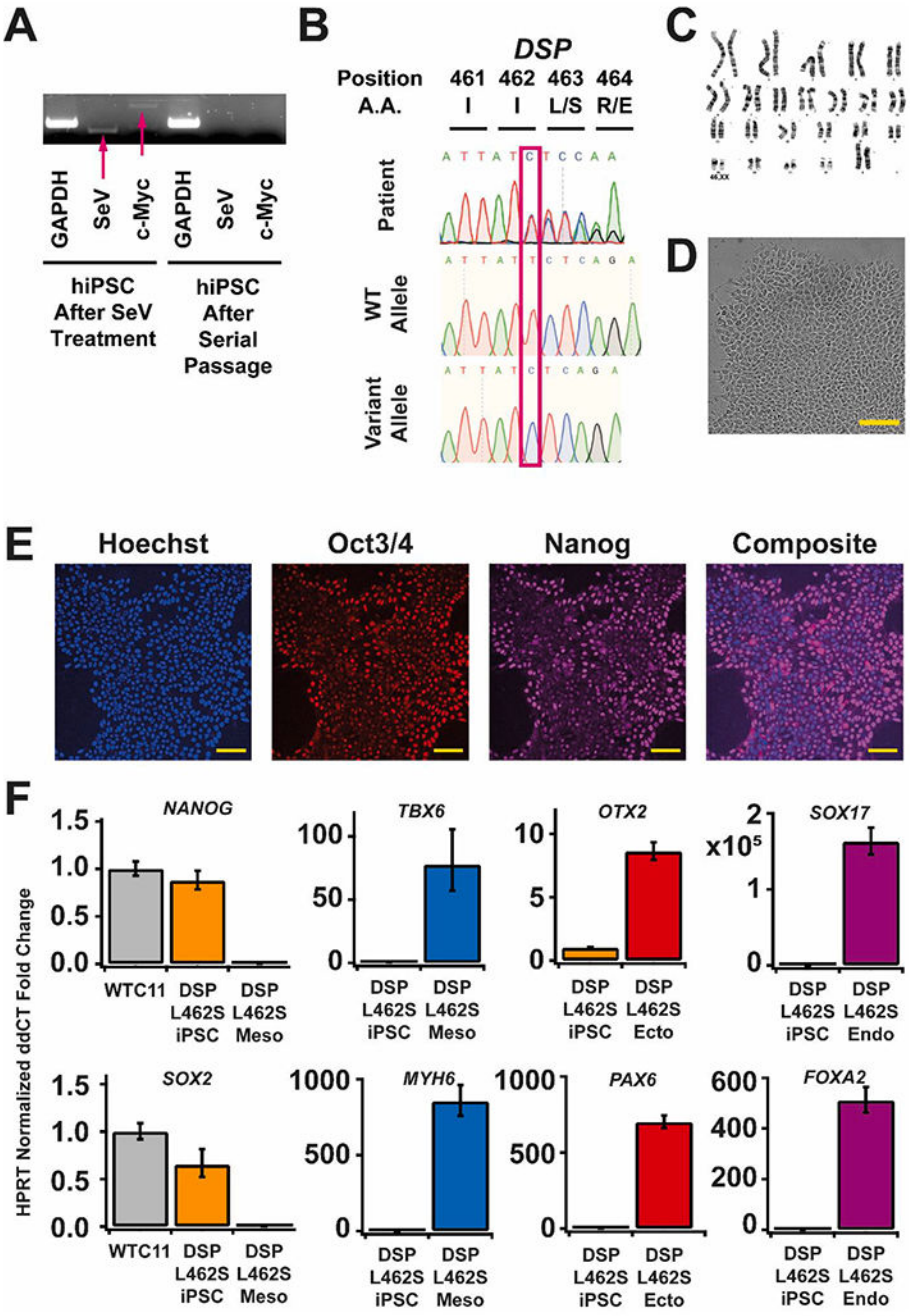

Table 1

Characterization and validation.

classification	Test	Result	Data
Morphology	Photography Bright field	Normal	Fig. 1D
	Quantitative analysis (RT-qPCR)	mRNA expression of *NANOG* and *SOX2*	Fig. 1G
Genotype	Karyotype (G-banding) and resolution [mandatory]	Normal Karyotype 46XX Resolution 5 Mb	Fig. 1C
Identity	Microsatellite PCR (mPCR) OR	N/A	N/A
	STR analysis [mandatory]	Matched 27 allelic polymorphisms across 15 STR loci	Available with the authors
Mutation analysis (IF APPLICABLE)	Sequencing	Heterozygous	Fig. 1B
	Southern Blot OR WGS	N/A	N/A
Microbiology and virology	Mycoplasma	Luminescence:Negative	Supplementary
Donor screening (OPTIONAL)	HIV 1 + 2 Hepatitis B, Hepatitis C	*N/A*	N/A
Genotype additional info (OPTIONAL)	Blood group genotyping	*N/A*	N/A
	HLA tissue typing	*N/A*	N/A

Table 2

Reagents details.

	Antibodies used for immunocytochemistry/flow-cytometry			
	Antibody	**Dilution**	**Company Cat #**	**RRID**
Pluripotency Markers	Mouse anti-OCT3/4	1:30	Santa Cruz Cat# sc-5279	RRID: AB_628051
	Rabbit anti-NANOG	1:100	Proteintech Cat# 14295-1-AP	RRID: AB_1607719
Secondary Antibodies	Alexa Fluor Plus 647 Goat anti-Mouse IgG	1:1000	Invitrogen Cat# A32728	RRID: AB_2633277
	Alexa Fluor Plus 647 Goat anti-Rabbit IgG	1:1000	Invitrogen Cat# A32733	RRID: AB_2633282
	Primers			
	Target	**Size of band**	**Forward/Reverse primer (5′-3′)**	
Sendai Virus (PCR)	Sendai Virus Genome	181 bp	GGATCACTAGGTGATATCGAGC/ACCAGACAAGAGTTTAAGAGATATGTATC	
Transgene (PCR)	*c-Myc*	532 bp	TAACTGACTAGCAGGCTTGTCG/TCCACATACAGTCCTGGATGATGATG	
House-Keeping Gene (PCR)	*GAPDH*	238 bp	GAGTCAACGGATTTGGTCGT/TTGATTTTGGAGGGATCTCG	
Pluripotency Markers (qPCR)	*NANOG*	237 bp	AAGGTCCCGGTCAAGAAACAG/CTTCTGCGTCACACCATTGC	
	SOX2	155 bp	GCCGAGTGGAAACTTTTGTCG/GGCAGCGTGTACTTATCCTTCT	
Ectoderm Markers (qPCR)	*PAX6*	111 bp	TGGGCAGGTATTACGAGACTG/ACTCCCGCTTATACTGGGCTA	
	OTX2	179 bp	CAAAGTGAGACCTGCCAAAAAGA/TGGACAAGGGATCTGACAGTG	
Endoderm Markers (qPCR)	*SOX17*	94 bp	GTGGACCGCACGGAATTTG/GGAGATTCACACCGGAGTCA	
	FOXA2	83 bp	GGAGCAGCTACTATGCAGAGC/CGTGTTCATGCCGTTCATCC	
Mesoderm Markers (qPCR)	*TBX6*	139 bp	CATCCACGAGAATTGTACCCG/AGCAATCCAGTTTAGGGGTGT	
	MYH6	187 bp	CAAGTTGGAAGACGAGTGCT/ATGGGCCTCTTGTAGAGCTT	
House-Keeping Gene (qPCR)	*HPRT*	94 bp	TGACACTGGCAAAACAATGCA/GGTCCTTTTCACCAGCAAGCT	
DSP Amplification for Sequencing (PCR)	*DSP*	1406 bp	GGGGCTCCAGGACTCCATCAG/GTCCTTCAGGATACACTCATCCCCC	
DSP Sequencing (Sanger)	*DSP*		GCGTCAGGTGCAGAACTTGG	

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Unique stem cell line identifier	**ISCRMi001-A**
Alternative name(s) of stem cell line	3-0062; *DSP* Leu462Serfs*22
Institution	University of Washington Department of Medicine
Contact information of distributor	Kai-Chun Yang, kcyang@uw.edu
Type of cell line	iPSC
Origin	Human
Additional origin info required for human ESC or iPSC	Age: 47 Sex: Female Ethnicity if known: White
Cell Source	Peripheral blood mononuclear cells
Clonality	Clonal
Method of reprogramming	Integration-free Sendai virus expressing human *OCT4, SOX2, KLF4, c-MYC*
Genetic Modification	N/A
Type of Genetic Modification	N/A
Evidence of the reprogramming transgene loss (including genomic copy if applicable)	PCR
Associated disease	Arrhythmogenic cardiomyopathy
Gene/locus	GRCh38 6: 7,568,555NM_004415.4 (*DSP*):c.1386 (3′ rule) (p. Leu462Serfs*22)
Date archived/stock date	Aliquots frozen 11 Aug 2022 Currently under application for archival at EBiSC
Cell line repository/bank	https://hpscreg.eu/cell-line/ISCRMi001-A
Ethical approval	IRB Consent STUDY00002544