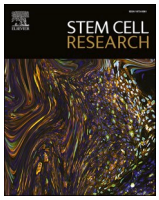
Stem Cell Research 67 (2023) 103048

Contents lists available at ScienceDirect

Stem Cell Research

journal homepage: www.elsevier.com/locate/scr



Lab Resource: Single Cell Line

Human induced pluripotent stem cell line YCMi007-A generated from a dilated cardiomyopathy patient with a heterozygous dominant c.613C > T (p. Arg205Trp) variant of the *TNNT2* gene

Sae-Bom Jeon [a,1], Hyoeun Kim [a,1], Kyeong-Hyeon Chun [b,1], Jaewon Oh [c], Chulan Kwon [d], Hyo-Kyoung Choi [e], Sangwoo Kim [f], Hyoung-Pyo Kim [g], In-Cheol Kim [h], Jung-Yoon Yoo [i], Sahng Wook Park [a,j,*], Seok-Min Kang [c,*], Seung-Hyun Lee [a,d,j,*]

[a] *Department of Biochemistry and Molecular Biology, Yonsei University College of Medicine, 50-1 Yonsei-ro, Seodaemun-gu, Seoul 03722, South Korea*
[b] *Division of Cardiology, Department of Internal Medicine, National Health Insurance Service Ilsan Hospital, Goyang 10444, South Korea*
[c] *Division of Cardiology, Severance Cardiovascular Hospital, Cardiovascular Research Institute, Yonsei University College of Medicine, 50-1 Yonsei-ro, Seodaemun-gu, Seoul 03722, South Korea*
[d] *Division of Cardiology, Department of Medicine, Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA*
[e] *Research Group of Healthcare, Korea Food Research Institute, Wanju-gun, Jeollabuk-do 55365, South Korea*
[f] *Department of Biomedical Systems Informatics and Brain Korea 21 PLUS Project for Medical Science, Yonsei University College of Medicine, 50-1 Yonsei-ro, Seodaemun-gu, Seoul 03722, South Korea*
[g] *Department of Environmental Medical Biology, Institute of Tropical Medicine, Brain Korea 21 PLUS Project for Medical Science, Yonsei Genome Center, Yonsei University College of Medicine, 50-1 Yonsei-ro, Seodaemun-gu, Seoul 03722, Republic of Korea*
[h] *Division of Cardiology, Department of International Medicine, Keimyung University Dongsan Hospital, Keimyung University School of Medicine, Daegu 42601, Republic of Korea*
[i] *Department of Biomedical Laboratory Science, Yonsei University Mirae Campus, Wonju 26493, Republic of Korea*
[j] *Department of Biochemistry and Molecular Biology, Graduate School of Medical Science, Brain Korea 21 Project, Yonsei University College of Medicine, 50-1 Yonsei-ro, Seodaemun-gu, Seoul 03722, South Korea*

A B S T R A C T

Cardiac muscle troponin T protein binds to tropomyosin and regulates the calcium-dependent actin–myosin interaction on thin filaments in cardiomyocytes. Recent genetic studies have revealed that TNNT2 mutations are strongly linked to dilated cardiomyopathy (DCM). In this study, we generated YCMi007-A, a human induced pluripotent stem cell (hiPSC) line from a DCM patient with a p. Arg205Trp mutation in the *TNNT2* gene. The YCMi007-A cells show high expression of pluripotent markers, normal karyotype, and differentiation into three germ layers. Thus, YCMi007-A—an established iPSC—could be useful for the investigation of DCM.

Resource Table:

Unique stem cell line identifier	YCMi007-A
Alternative name(s) of stem cell line	YCMi007-hDCM007-A
Institution	Yonsei University College of Medicine
Contact information of distributor	Seung-Hyun Lee, tiger815@yuhs.ac
Type of cell line	iPSC
Origin	Human
Additional origin info required for human ESC or iPSC	Age: 36 Sex: Male Ethnicity if known: Korean
Cell Source	Peripheral blood mononuclear cells (PBMCs)
Clonality	Clonal
Method of reprogramming	Episomal plasmid vectors, Transgene-free
Genetic Modification	Yes
Type of Genetic Modification	N/A
Evidence of the reprogramming transgene loss (including genomic copy if applicable)	RT-PCR
Associated disease	Dilated cardiomyopathy
Gene/locus	Heterozygous mutation in TNNT2 gene (NM_001001430.2) / c.613C > T, p. Arg205Trp
Date archived/stock date	September 2022
Cell line repository/bank	https://hpscreg.eu/cell-line/YCMi007-A

(*continued on next column*)

(*continued*)

(*continued on next page*)

* Corresponding authors.
E-mail addresses: SWPARK64@yuhs.ac (S.W. Park), smkang@yuhs.ac (S.-M. Kang), tiger815@yuhs.ac (S.-H. Lee).
[1] Contributed equally to this work.

https://doi.org/10.1016/j.scr.2023.103048
Received 21 November 2022; Received in revised form 18 January 2023; Accepted 11 February 2023
Available online 14 February 2023

(*continued*)

Ethical approval	Ethical committee: Yonsei University Health System, Severance Hospital, Institutional review board approval number: 4-2020-0112

1. Resource utility

Cardiac muscle troponin T is a crucial molecule that regulates cardiac muscle contraction and sarcomeric Ca^{2+}. Various *TNNT2* gene

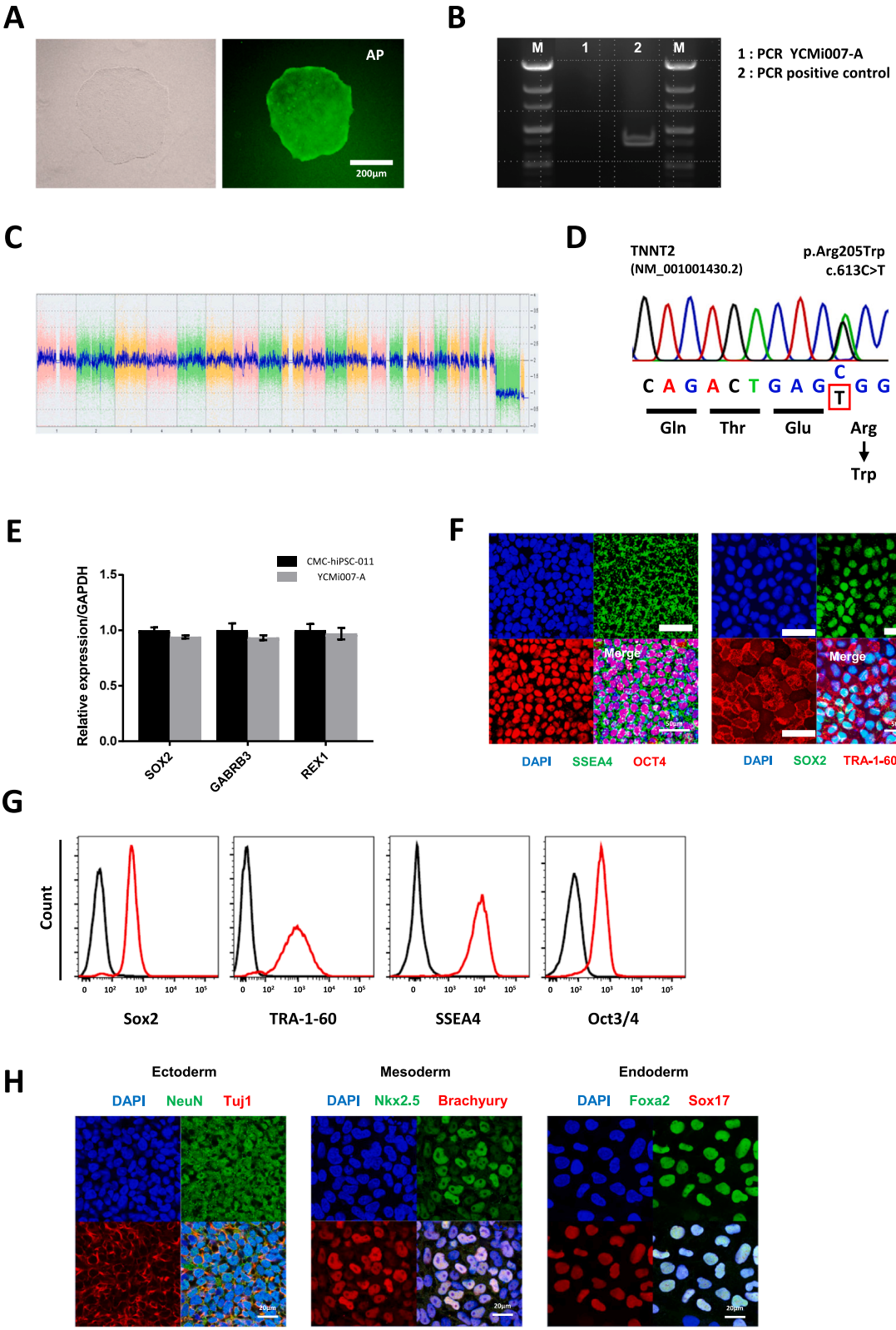


Fig. 1. Characterization of YCMi007-A iPSC cell line.

Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography Bright field	Normal	Fig. 1 panel A
Phenotype	Alkaline Phosphate staining	Positive	Fig. 1 panel A
	Qualitative analysis Immunocytochemistry	Positive for pluripotency markers including SSEA4, OCT4, SOX2, and TRA-1-60	Fig. 1 panel F
	Qualitative analysis RT-qPCR	Positive for SOX2, GABRB3, and REX1	Fig. 1 panel E
	Quantitative analysis Flow cytometry	Oct3/4: 94.1 % TRA 1–60: 95.4 % SSEA-4: 98.1 % SOX2: 97.8 %	Fig. 1 panel G
Genotype	Karyotype (G-banding) and resolution	46, XY Resolution 450–500	Fig. 1 panel C
Identity	Microsatellite PCR (mPCR)	N/A	N/A
	STR analysis	16 loci tested, all matched	Submitted in archive with authors
Mutation analysis (IF APPLICABLE)	Sequencing	Heterozygous mutation	
	Southern Blot OR WGS	N/A	N/A
Microbiology and virology	Mycoplasma	Mycoplasma testing by RT-PCR, negative	Fig. 1 panel B
Differentiation potential	Directed differentiation In vitro trilineage differentiation Immunocytochemistry	Positive expression of three germ layer markers by immunocytochemistry	Fig. 1 panel H
List of recommended germ layer markers	Expression of these markers has to be demonstrated at protein (IF) levels, at least 2 markers need to be shown per germ layer	Endoderm: Sox17, Foxa2 Mesoderm: NKX2.5, Brachyury Ectoderm: NeuN, Tuj1	Fig. 1 panel H
Donor screening (OPTIONAL)	HIV 1 + 2 Hepatitis B, Hepatitis C	Negative	Not shown but available with author
Genotype additional info (OPTIONAL)	Blood group genotyping	N/A	N/A
	HLA tissue typing	N/A	N/A

mutations are associated with DCM. We established an iPSC line from a patient with DCM harboring a heterozygous variant of *TNNT2* (c.613C > T). This cell line could be a useful tool for studying the pathogenic mechanisms underlying DCM.

2. Resource details

The cardiac troponin complex, a crucial modulator of cardiac muscle contraction, is composed of three subunits: troponin C (cTnC, Ca^{2+} binding subunit), troponin I (cTnI, actomyosin ATPase inhibitory subunit), and troponin T (cTnT, tropomyosin binding subunit) (Cheng and Regnier, 2016). *TNNT2* encodes the cTnT protein with two functional regions: *N*-terminal region interacting with tropomyosin and C-terminal region integrated into the troponin complex (Jin and Chong, 2010). cTnT protein binds to tropomyosin and regulates the calcium-dependent actin–myosin interaction in the thin filaments (Madan et al., 2020). DCM is a heart muscle disease characterized by the enlargement of one or both chambers and contractile dysfunction. DCM has extensive allelic heterogeneity. Interestingly, both DCM and familial hypertrophic cardiomyopathy, which are regarded as having different pathophysiologic mechanisms, have been linked to pathogenic TNNT2 mutations (Robinson et al., 2007). Therefore, in addition to discovering various *TNNT2* mutations in patients with DCM, intensive studies using patient-specific iPSCs with *TNNT2* mutations are required to elucidate the pathophysiological roles of TNNT2 in DCM. We generated an iPSC line from peripheral blood mononuclear cells (PBMCs) of a male patient with a heterozygous c.613C > T (p. Arg205Trp) mutation in the *TNNT2* gene. PBMCs were reprogrammed into iPSCs using an Epi5 Episomal iPSC Reprogramming Kit (Thermo Fisher Scientific, A15960) containing five reprogramming factors (Oct4, Sox2, Lin28, Klf4, and L-Myc). The loss of reprogramming transgenes in the established iPSC line was confirmed using polymerase chain reaction (PCR) at passage 20 (p20) (data not shown). The YCMi007-A (p20) iPSC line showed typical hESC-like morphology. The expression of the representative pluripotency marker alkaline phosphatase was detected using an Alkaline Phosphatase Live Stain Kit (Thermo Fisher Scientific); the images were captured using fluorescence microscopy (Fig. 1A, scale bar, 200 μm). The absence of mycoplasma contamination was confirmed in YCMi007-A (p20) using PCR and agarose gel electrophoresis (Fig. 1B). Analysis of copy number variation using KaryoStat™ assay (Thermo Fisher Scientific) revealed normal chromosomal integrity of YCMi007-A (p21) (Fig. 1C). Sanger sequencing confirmed the heterozygous c.613C > T mutation in exon 14 of the *TNNT2* gene (Fig. 1D). Short tandem repeat analysis (COSMO GENETECH, Seoul, Korea) based on an algorithm that compares the number of shared alleles between the PBMCs and YCMi007-A (p23) was used to check the identity of YCMi007-A. The transcript levels of endogenous pluripotency markers SOX2, GABRB3, and REX1 in YCMi007-A (p21) were found to be similar to that in the validated hiPSC line CMC-hiPSC-011 using quantitative real-time PCR (qRT-PCR) (Fig. 1E). Immunocytochemistry analysis showed high expression of pluripotency markers SSEA4, OCT4, SOX-2, and TRA-1-60 (Fig. 1F, scale bar, 50 μm). Flow cytometry analysis demonstrated the expression of TRA-1-60 and SSEA4 (surface expression markers) and SOX-2 and OCT3/4 (intracellular expression markers) in YCMi007-A cells (p22). Black lines were used as isotype controls (Fig. 1G). Finally, we tested the differentiation capacity of YCMi007-A cells using the STEMdiff™ Trilineage Differentiation Kit (Stemcell Technologies, 05230). We fostered the differentiation of YCMi007-A cells (p23) into three germ layers. The ectoderm markers (NeuN and Tuj1), mesoderm markers (Nkx2.5 and Brachyury), and endoderm markers (Foxa2 and Sox17) were expressed in the differentiated cells (Fig. 1H). The results of YCMi007-A validation are summarized in Table 1.

3. Materials and methods

3.1. Ethical statement

This study was approved by the Institutional Review Board (IRB) and ethics committee of Yonsei University Health System (Approval No. 4-2020-0112). Written informed consent was obtained prior to patient enrollment.

Table 2
Reagents details.

	Antibodies used for immunocytochemistry/flow-cytometry			
	Antibody	Dilution	Company Cat #	RRID
Pluripotency Markers (Immunocytochemistry)	SSEA-4	1:100	Thermo Fisher Scientific Cat # 46-8843-42	AB_2573850
	OCT4	1:300	Cell Signaling Technology Cat # 9656	AB_1658242
	SOX2	1:100	Thermo Fisher Scientific Cat # 53-9811-82	AB_2574479
	TRA-1-60	1:100	Thermo Fisher Scientific Cat # 13-8863-82	AB_891594
Pluripotency Markers (Flow cytometry)	SOX2	0.25 μg/test	Thermo Fisher Scientific Cat# 53-9811-82	AB_2574479
	TRA-1-60	0.5 μg/test	Thermo Fisher Scientific Cat # 12-8863-82	AB_891602
	SSEA-4	0.03 μg/test	Thermo Fisher Scientific Cat # 46-8843-42	AB_2573850
	OCT3/4	0.5 μg/test	Thermo Fisher Scientific Cat # 50-5841-80	AB_11218890
Trilineage Differentiation Markers	Rabbit IgG anti-h/m/rNeuN	1:100	Thermo Fisher Scientific Cat # PA5-78639	AB_2736207
	Mouse anti-Neuron-specific beta-III Tubulin (Clone TuJ-1)	1:100	R&D Systems Cat # MAB1195	AB_357520
	Rabbit IgG anti-h/mNkx2.5	1:100	Thermo Fisher Scientific Cat # PA5-49431	AB_2634885
	Goat IgG anti-h/mBrachyury	1:100	R&D Systems Cat # AF2085	AB_2200235
	Goat IgG anti-hSOX17	1:100	R&D Systems Cat # AF1924	AB_355060
	Rabbit IgG anti-h/m/FOXA2	1:100	Thermo Fisher Scientific Cat # PA5-35097	AB_2552407
Secondary antibodies	Alexa® Fluor 488 chicken anti-rabbit IgG	1:500	Thermo Fisher Scientific Cat# A-21441	AB_2535859
	Alexa® Flour 546 goat anti-mouse IgG	1:500	Thermo Fisher Scientific Cat# A-11030	AB_2534089
	Primers			
	Target	**Size of band**	**Forward/Reverse primer (5′-3′)**	
Pluripotency Markers (qPCR)	SOX2	215 bp	5′-TGG ACA GTT ACG CGC ACA T-3′ 5′-ACC TAC AGC ATG TCC TAC TCG-3′	
	GABRB3	153 bp	5′-GAA AAA CCG CAT GAT CCG TCT-3′ 5′-TCC GTG GTG TAG CCA TAG CTT-3′	
	REX1	210 bp	5′-TAG AAT GCG TCA TAA GGG GTG A-3′ 5′-TCT TGC CTG TCA TGT ACT CAG AA-3′	
House-Keeping Genes (qPCR)	GAPDH	197 bp	5′-GGA GCG AGA TCC CTC CAA AAT-3′ 5′-GGC TGT TGT CAT ACT TCT CAT GG-3′	
Mutation sequencing primer	TNNT2	1345 bp	5′-GGA GGA GGG ATA TGT GAA-3′ 5′-GGG AGG TCC AGT AAG AAA-3′	
Episomal Plasmids (PCR)	EBNA-1	666 bp	5′-ATC GTC AAA GCT GCA CAC AG-3′ 5′-CCC AGG AGT CCC AGT AGT CA-3′	
	Orip	544 bp	5′-TTC CAC GAG GGT AGT GAA CC-3′ 5′-TCG GGG GTG TTA GAG ACA AC-3′	

3.2. Reprogramming of human PBMCs

Peripheral whole blood samples were collected from a 36-year-old Korean male patient with a missense mutation (R205W) in exon 14 of the *TNNT2* gene. PBMCs were isolated from the samples using SepMateTM (StemCell Technologies, 15410) according to the manufacturer's protocol. Reprogramming was performed using the Epi5™ Episomal iPSCs Reprogramming Kit (Thermo Fisher Scientific, A15960) using electroporation (Neon transfection system), based on the manufacturer's recommendations. The cells were then maintained under feeder-free conditions on ReproTeSR™ medium (Stemcell Technologies, 05920) in a Matrigel™ (hESC-qualified, Corning, 356278)-coated six-well plate. The medium was changed daily for 20 days until iPSC colonies appeared. The iPSC colonies were maintained on TeSR™-E8™ medium (Stemcell Technologies, 05990) in vitronectin (truncated VTN-N recombinant human protein, Gibco, A31804)-coated plates in a CO_2 incubator at 37 °C and 5 % CO_2. The medium was refreshed daily until 80–90 % cell confluence was achieved The cells were dissociated using ReLeSR™ (Stemcell Technologies, 05872) at a 1:10 to 1:20 ratio and passaged with TeSR™-E8™ medium supplemented with a ROCK inhibitor (Y-27632, Tocris, 1254).

3.3. Alkaline phosphatase assay

The presence of alkaline phosphatase activity was confirmed using an Alkaline Phosphatase Live Stain Kit (Thermo Fisher Scientific, A14353). The cells were treated with the substrate according to the manufacturer's instructions, and alkaline phosphatase expression was analyzed using a fluorescence microscope (OLYMPUS, IX71).

3.4. Genotype analysis

A Genomic DNA Purification Kit (Thermo Fisher Scientific, K0512) was used to extract the genomic DNA. A KaryoStat™ assay (Thermo

Fisher Scientific, 905403) was performed to confirm the karyotype of YCMi007-A cells at passage 18 using the Cytoscan HT-CMA 96 array, which identifies copy number variants and single nucleotide polymorphisms throughout the genome. A total of 100 ng of gDNA was used to prepare the Cytoscan HT-CMA 96 array for KaryoStat.

3.5. DNA sequence analysis of the mutation site

Genomic DNA was extracted from YCMi007-A cells using the G-spin™ Genomic DNA Extraction Kit (iNtRON Biotechnology, 17121) according to the manufacturer's protocol and amplified using PCR. Information regarding the designed primers is presented in Table 2. The heterozygous c.613C > T mutation in exon 14 of *TNNT2* was identified through DNA sequencing.

3.6. Mycoplasma screening

Mycoplasma was detected in iPSC cultures using the TaKaRa PCR Mycoplasma Detection Set (Takara, 6601) according to the manufacturer's protocol. The presence of positive control band and the correct size band confirmed the presence of mycoplasma.

3.7. Flow cytometry

The cells were washed with Dulbecco's phosphate-buffered saline (DPBS), and a single-cell suspension was generated using the Gentle Cell Dissociation Reagent (Stemcell Technologies, 07174). The cells were counted, permeabilized, and fixed using a Fixation/Permeabilization solution kit (BD Bioscience, 554714) for 15 min. The iPSC cells were stained with pluripotency markers Sox2 (Alexa 488, 0.25 μg/test), TRA-1–60 (PE, 0.5 μg/test), SSEA4 (PerCP-eFluor710, 0.03 μg/test), and OCT3/4 (eFluor 660, 0.5 μg/test). The stained cells were observed using the LSRII flow cytometer (BD Bioscience) and analyzed using FLOWJO v10.0.7 software (Tree Star, Inc.).

3.8. Quantitative RT-PCR

Total RNA was extracted from the iPSCs at passage 16 using the Ribospin™ total RNA purification kit (GeneAll Biotechnology, 314–150), followed by reverse transcription (RT) using PrimeScript™ Reverse Transcriptase (Takara, 2680A) according to the manufacturer's instructions. qPCR was performed using a QuantStudio™ 3 Real-Time PCR system (Applied Biosystems™, A28567) with the FastStart Universal SYBR® Green Master Mix (Roche Applied Science). *GAPDH* was used as the endogenous control gene to standardize and validate human iPSC (CMC-hiPSC-011).

3.9. Immunocytochemistry

The pluripotency of YCMi007-A cells in a Matrigel™-coated slide chamber was detected through immunocytochemistry. The cells were fixed with 4 % paraformaldehyde at room temperature for 20 min and blocked with 3 % bovine serum albumin (LPS solution, 9048–46-8) and 0.3 % Triton X (USB®, 9002-93-1) in PBS. After overnight incubation at 4 °C with the respective primary antibodies (OCT4, SOX2, SSEA4, and TRA-1–60), the cells were washed twice with PBS, and incubated for 3 h at room temperature with either diluted Alexa® Fluor 488 chicken anti-rabbit IgG (1:500, Thermo Fisher Scientific, A21441) or Alexa® Flour 546 goat anti-mouse IgG (1:500, Thermo Fisher Scientific, A11030) as the secondary antibody. The nuclei were stained with Hoechst 33,342 (Thermo Fisher Scientific, 62249). The slides were viewed using a confocal microscope (LSM710, Zeiss) and analyzed using ZEN software. The antibodies used are listed in Table 2.

3.10. In vitro trilineage differentiation

The differentiation potential of the three iPSC germ layers was verified using a STEMdiff™ Trilineage Differentiation Kit (Stemcell Technologies, 05230). IPSCs were cultured in Matrigel™-coated 12-well plates in lineage-specific media for mesoderm, endoderm, and ectoderm according the manufacturer's instructions.

3.11. STR analysis

STR analysis was performed on the generated iPSCs and parental PBMCs, and 16 loci were detected (D8S1179, D21S11, D7S820, CSF1PO, D3S1358, TH01, D13S317, D16S539, D2S1338, D19S433, vWA, TPOX, D18S51, AMEL, and D5S818). The results were analyzed using ANALYZER software.

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.

Acknowledgements

This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIT) (No. NRF-2022R1H1A2003919, 2021R1I1A1A01042945, 2021R1I1A1A01049625, 2022M3A9B6017424). This study also was supported by a grant from the Korea Food Research Institute funded by the Ministry of Science, ICT & Future Planning (E0210400). This research was supported by a grant of the Korea Health Technology R&D Project through the Korea Health Industry Development Institute (KHIDI), funded by the Ministry of Health & Welfare, Republic of Korea (HI22C0198). The human stem cell line, CMC-hiPSC-011 was provided by the National Stem Cell Bank of Korea (Korea National Institute of Health), originally provided by Catholic University.

References

Cheng, Y., Regnier, M., 2016. Cardiac troponin structure-function and the influence of hypertrophic cardiomyopathy associated mutations on modulation of contractility. Arch. Biochem. Biophys. 601, 11–21.

Jin, J.P., Chong, S.M., 2010. Localization of the two tropomyosin-binding sites of troponin T. Arch. Biochem. Biophys. 500 (2), 144–150.

Madan, A., Viswanathan, M.C., Woulfe, K.C., Schmidt, W., Sidor, A., Liu, T., Nguyen, T. H., Trinh, B., Wilson, C., Madathil, S., Vogler, G., O'Rourke, B., Biesiadecki, B.J., Tobacman, L.S., Cammarato, A., 2020. TNNT2 mutations in the tropomyosin binding region of TNT1 disrupt its role in contractile inhibition and stimulate cardiac dysfunction. Proc. Natl. Acad. Sci. USA 117 (31), 18822–18831.

Robinson, P., Griffiths, P.J., Watkins, H., Redwood, C.S., 2007. Dilated and hypertrophic cardiomyopathy mutations in troponin and alpha-tropomyosin have opposing effects on the calcium affinity of cardiac thin filaments. Circ. Res. 101 (12), 1266–1273.