Stem Cell Research 59 (2022) 102629

Contents lists available at ScienceDirect

Stem Cell Research

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

Lab Resource: Single Cell Line

# Generation of a human induced pluripotent stem cell line YCMi004-A from a patient with dilated cardiomyopathy carrying a protein-truncating mutation of the Titin gene and its differentiation towards cardiomyocytes

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A B S T R A C T

Dilated cardiomyopathy (DCM) is a heart muscle disease that causes heart failure and is the leading cause for heart transplantation. It is a heart muscle disease resulted from a variety of genetics, toxic, metabolic, and infectious causes. One of the most prevalent genetic causes of DCM is a protein-truncating variant in the Titin gene (TTNtv). We have generated a human-induced pluripotent stem cell (hiPSC) line from patients who underwent heart transplantation due to DCM carrying a TTNtv mutation (c.70051C > T, p.Arg23351Ter) at the age of 20. The generated hiPSCs showed normal karyotype (46, XY) and expression of pluripotency markers, and were differentiated towards cardiomyocytes successfully.

## 1. Resource table

| Unique stem cell line identifier | YCMi004-A |
|---|---|
| Alternative name(s) of stem cell line | YCMi004-hDCM004-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: 24<br>Sex: Male<br>Ethnicity: Korean |
| Cell Source | Peripheral blood mononuclear cells (PBMCs) |
| Clonality | Clonal |
| Method of reprogramming | Episomal plasmid vectors, Transgene-free |
| Associated disease | Dilated cardiomyopathy |
| Gene/locus | Heterozygous mutation in TTN gene (NM_001267550.2) / c.70051C > T, p. Arg23351Ter |
| Date archived/stock date | April 2021 |
| Cell line repository/bank | https://hpscreg.eu/cell-line/YCMi004-A |
| Ethical approval | Ethical committee: Yonsei University Health System, Severance Hospital, Institutional review board approval number: 4-2020-0112 |

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https://doi.org/10.1016/j.scr.2021.102629
Received 11 October 2021; Accepted 14 December 2021
Available online 16 December 2021

## 2. Resource utility

A variety of genetic variations has been reported in dilated cardiomyopathy (DCM) patients. Most of mutations occur in sarcomeric genes, including Titin (TTN). Although protein-truncating variants of the Titin gene (TTNtv) are regarded as major genetic mutations in DCM, the relationship between TTNtv and pathomechanism is not yet fully elucidated. Therefore, human-induced pluripotent stem cell (hiPSC) generated from DCM patients with TTNtv could be an important resource for DCM-related research.

## 3. Resource details

The TTN protein is one of the largest proteins in human with a molecular weight of up to 3 million daltons, and the TTNtv comprises approximately 25% of familial DCM causes (Herman et al., 2012). However, there is a limited clinical interpretation of the TTNtv because of its incomplete penetrance (Franaszczyk et al., 2017). Consequently, there have been many attempts to generate hiPSC-CM from TTNtv patients for evaluating pathomechanisms of DCM. Previously, iPSCs from DCM with TTNtv showed that they had sarcomere insufficiency and impaired responses to mechanical stress more in the A-band than in the

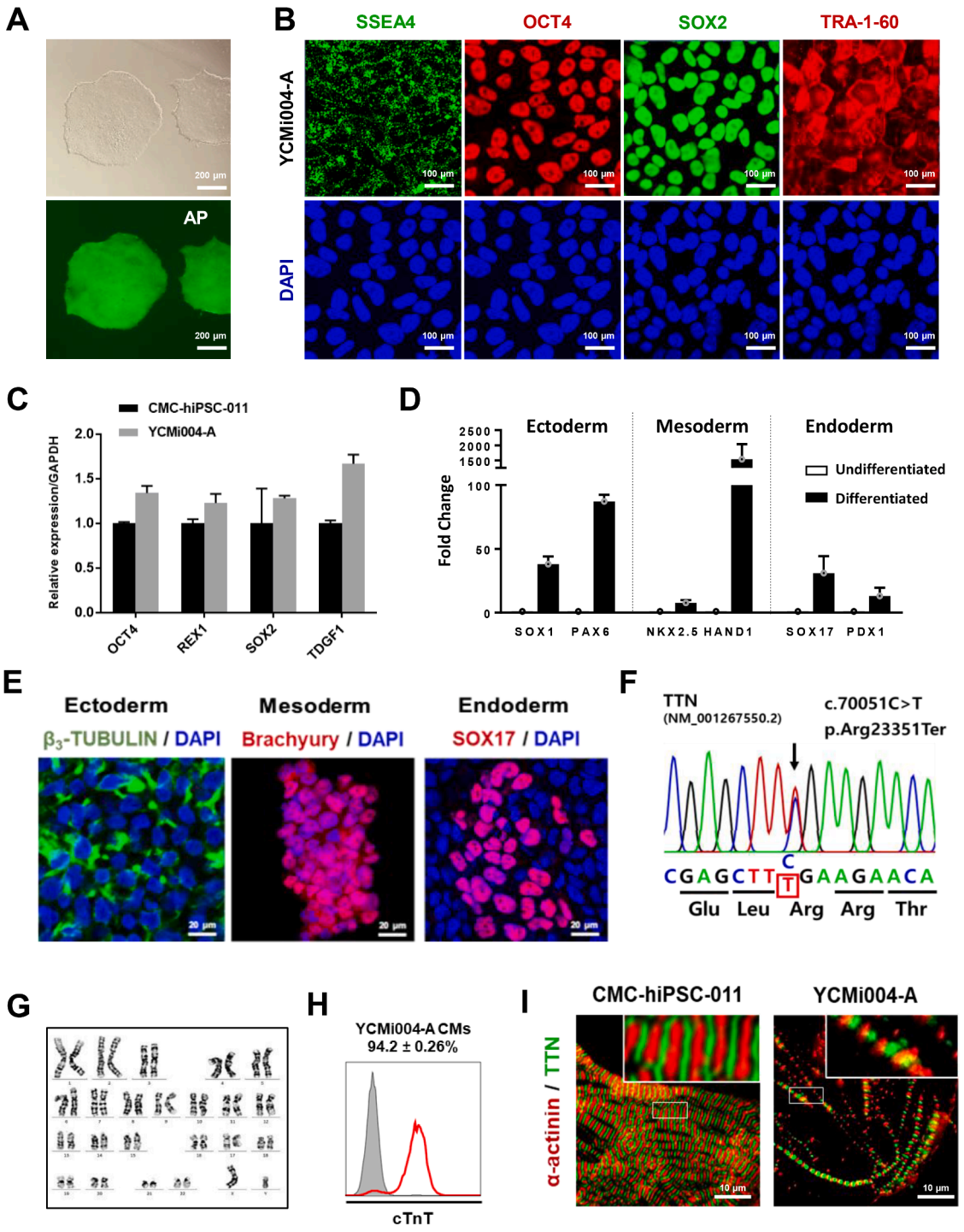


**Fig. 1.**

**Table 1**
Characterization and validation.

| Classification | Test | Result | Data |
|---|---|---|---|
| **Morphology** | Photography Bright field | Normal | Fig. 1 panel A |
| **Phenotype** | **Quantitative analysis** Immunocytochemistry | Positive for pluripotency markers including OCT4, SOX2, SSEA4, and TRA-1–60 | Fig. 1 panel B |
| | **Quantitative analysis** RT-qPCR | Positive for OCT4, REX1, SOX2, and TDGF1 | Fig. 1 panel C |
| **Genotype** | Karyotype (G-banding) and resolution | 46XY, Resolution 450–500 | Fig. 1 panel G |
| **Identity** | Microsatellite PCR (mPCR) OR STR analysis | Not performed 16 loci tested, all matched | NA Submitted in archive with journal |
| **Mutation analysis** | Sequencing | Heterozygous mutation | Fig. 1 panel F |
| | Southern Blot OR WGS | NA | NA |
| **Microbiology and virology** | Mycoplasma | Mycoplasma testing by RT-PCR, Negative | Not shown but available with author |
| **Differentiation potential** | **Directed differentiation** *In vitro* trilineage differentiation Immunocytochemistry | Endoderm: αSOX17 Mesoderm: αBrachyury Ectoderm: αβIII-tubulin | Fig. 1 panel E |
| | **Directed differentiation** *In vitro* trilineage differentiation RT-qPCR | Endoderm: SOX17, PDX1 Mesoderm: NKX2.5, HAND1 Ectoderm: SOX1, PAX6 | Fig. 1 panel D |
| | **Directed differentiation** Cardiomyocyte differentiation Flow cytometry | TNNT2 positive: 94% | Fig. 1 panel H |
| **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, PDX1 Mesoderm: Brachyury, NKX2.5, HAND1 Ectoderm: αβIII-tubulin, SOX1, PAX6 | RT-qPCR with specific primers IF with specific antibodies |
| **Donor screening** | HIV 1 + 2 Hepatitis B, Hepatitis C | Negative | Not shown but available with author |
| **Genotype additional info** | Blood group genotyping HLA tissue typing | Not performed Not performed | NA NA |

I-band (Hinson et al., 2015). In this study, we generated a hiPSC line, YCMi004-A, successfully from a patient carrying a novel A-band TTNtv mutation (c.70051C > T, p.Arg23351Ter), who had an early onset of DCM and underwent heart transplantation at the age of 20 due to being refractory to medical therapy.

Peripheral blood mononuclear cells (PBMCs) were isolated from a 24-year-old male patient with DCM. PBMCs were reprogrammed using the Epi5 Episomal iPSC Reprogramming kit (Thermo Fisher Scientific) containing a set of vectors for five reprogramming factors (Oct4, Sox2, Lin28, Klf4, and L-Myc). After reprogramming, the YCMi004-A exhibited typical hESC-like morphology. The expression of alkaline phosphatase was assessed using the Alkaline Phosphatase Live stain kit (Thermo Fisher Scientific) (Fig. 1A). Immunofluorescence staining showed expressions of typical pluripotent markers, including OCT4, SOX2, SSEA4, and TRA-1-60 (Fig. 1B). Quantitative real-time PCR (qRT-PCR) analyses showed iPSCs expressed endogenous pluripotency genes, including OCT4, REX1, SOX2, and TDGF1 (Fig. 1C). The pluripotency of YCMi004-A was confirmed by in vitro differentiation into three germ layers using the STEMdiff™ Trilineage Differentiation Kit (Stemcell Technologies, 05230). Differentiation to ectoderm was confirmed by βIII-tubulin staining and SOX1, PAX6 qPCR; mesoderm by Brachyury staining and NXK2.5, HAND1 qPCR; endoderm by SOX17 staining and PDX1 qPCR (Fig. 1D, E). DNA sequence analysis revealed the presence of the 70051C > T change in exon 327 of the TTN gene (Fig. 1F). Karyotyping using G-banding showed normal 46, XY karyotype (Fig. 1G). The identity of this iPSC line was also confirmed using short tandem repeat (STR) analysis. Differentiated cardiomyocytes from YCMi004-A expressed cardiac troponin T (cTnT) as a cardiomyocyte-specific marker (Fig. 1H). We confirmed that the sarcomere structure immunostained for α-actin and TTN was disorganized in YCMi004-A compared to the cardiomyocytes differentiated from normal hiPSCs (Fig. 1I). These results are consistent with results reported previously (Hinson et al., 2015).

## 4. Materials and methods

### 4.1. The written informed consent

The written informed consent was obtained from the patient and the study was approved by the Institutional Review Board (IRB) and ethics committee of the Yonsei University Health System (NO. 4-2020-0112).

### 4.2. Reprogramming of human PBMCs

A whole blood sample was obtained from a 24-year-old Korean male patient who had a nonsense mutation in exon 327 of the TTN gene. PBMCs were isolated from a whole blood sample using SepMate™ (StemCell Technologies, 15410) according to the manufacturer's instructions. On day 8, reprograming of PBMCs were induced using the Epi5™ Episomal iPSC Reprogramming Kit (Thermo Fisher Scientific, A15960) with the Neon Electrophoration system according to the manufacturer's instructions. Cells were then plated in a six-well plate coated with Matrigel (hESC-qualifed, Corning, 356278) using ReproTeSR™ medium (Stemcell Technologies, 05920) until cell colonies appeared. The obtained colonies were cultured onto vitronectin coated plates (Truncated VTN-N recombinant human protein, Gibco, A31804) in TeSR™-E8™ medium (Stemcell Technologies, 05990) at 37 °C under a humidified atmosphere of 5% $CO_2$. The cell culture medium was changed daily. Cells were passaged with ReLeSR™ (Stemcell Technologies, 05872) at a ratio of 1:12 every 4–5 days with 10 μM Y-27632 (Tocris, 1254).

### 4.3. Alkaline phosphatase assay

Passage 12 iPSCs were seeded on a six-well Matrigel™ coated plate. On day 40 after reprogramming, cells were stained using the Alkaline Phosphatase Live stain kit (Thermo Fisher Scientific, A14353). Briefly, an appropriate amount of the stain solution was applied directly on to the iPSCs, incubated for 20 min, and then washed with DMEM/F-12. The expression of alkaline phosphatase was analyzed using a fluorescence microscope (OLYMPUS, IX71).

### 4.4. Immunocytochemistry

Passage 14 iPSCs were fixed with 4% paraformaldehyde for 20 min at room temperature, blocked with 3% bovine serum albumin (LPS solution, 9048-46-8) with 0.3% Triton X (USB®, 9002-93-1) in PBS. After blocking, cells were incubated with primary antibodies against OCT4, SOX2, SSEA4, and Tra-1-60 overnight at 4 °C. Cells were washed twice

**Table 2**
Reagents details.

| | Antibodies used for immunocytochemistry/flow-cytometry | | | |
|---|---|---|---|---|
| | **Antibody** | **Dilution** | **Company Cat #** | **RRID** |
| **Pluripotency Markers** | Rabbit anti-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 |
| | SSEA-4 | 1:100 | Thermo Fisher Scientific Cat# 46-8843-42 | AB_2573850 |
| **Trilineage Differentiation Markers** | Mouse anti-$\beta_3$-TUBULIN | 1:100 | R&D Systems Cat# MAB1195 | AB_357520 |
| | Goat IgG anti-hSOX17 | 1:100 | R&D Systems Cat# AF1924 | AB_355060 |
| | Goat IgG anti-h/mBrachyury | 1:100 | R&D Systems Cat# AF2085 | AB_2200235 |
| **Cardiomyocyte Differentiation Markers** | TNNT2 | 1:100 | abcam Cat# ab10214 | AB_2206574 |
| | TTN | 1:100 | Proteintech Cat# 27867-1-AP | AB_2880998 |
| | α-actinin | 1:100 | Thermo Fisher Scientific Cat# A7811 | AB_476766 |
| **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 ACGC GC ACA T −3′<br>5′- ACC TAC AGC ATG TCC TAC TCG −3′ | |
| | OCT4 | 106 bp | 5′- CCT GAA GCA GAA GAG GAT CAC C −3′<br>5′- AAA GAG GCA GAT GGT CGT TTG G −3′ | |
| | REX1 | 210 bp | 5′- GCA GCC ACG GCC TAT TAA G −3′<br>5′- CCA CCA CGT ACT TGC CAC T −3′ | |
| | TDGF1 | 96 bp | 5′- ACA GCA CAG TAA GGA GCT AAA C −3′<br>5′- CGT CCG TAG AAG GAG GGA GG −3′ | |
| **Trilineage Differentiation Markers (qPCR)** | SOX1 | 287 bp | 5′- CAG TAC AGC CCC ATC TCC AAC −3′<br>5′- GCG GGC AAG TAC ATG CTG A −3′ | |
| | PAX6 | 111 bp | 5′- TGG GCA GGT ATT ACG AGA CTG −3′<br>5′- ACT CCC GCT TAT ACT GGG CTA −3′ | |
| | NKX2.5 | 261 bp | 5′- CCA AGG ACC CTA GAG CCG AA −3′<br>5′- ATA GGC GGG GTA GGC GTT AT −3′ | |
| | HAND1 | 89 bp | 5′- GAG AGC ATT AAC AGC GCA TTC G −3′<br>5′- CGC AGA GTC TTG ATC TTG GAG AG −3′ | |
| | SOX17 | 94 bp | 5′- GTG GAC CGC ACG GAA TTT G −3′<br>5′- GGA GAT TCA CAC CGG AGT CA −3′ | |
| | PDX1 | 98 bp | 5′- CGA TGT GAT TTC GGA CCC TTT −3′<br>5′- GCT TTC TGG GCT TGT TCT CAG −3′ | |
| **House-Keeping Genes (qPCR)** | GAPDH | 197 bp | 5′- GGA GCG AGA TCC CTC CAA AAT −3′<br>5′- GGC TGT TGT CAT ACT TCT CAT GG −3′ | |
| **Mutation sequencing primer** | TTN exon 327 | 982 bp | 5′- ATC CTC CAG GAC CAC CTT −3′<br>5′- CCA GTG ATC TTG CTG CCA −3′ | |

with PBS, then, were incubated with 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 a secondary antibody for 3 h at RT. Hoechst 33342 (Thermo Fisher Scientific, 62249) was used to counterstain nuclei of cells for 10 min at RT. Slides were analyzed with a confocal microscope (LSM710, Zeiss) using the ZEN software. YCMi004-A derived cardiomyocytes was determined by immunostaining to express TTN, a mutated gene. Immunostaining was performed under the same conditions as confirming iPSCs pluripotency markers. Sarcomeric localization was confirmed by coimmunostaining for α-actinin. All antibodies and reaction conditions are listed in Table 2.

### 4.5. Quantitative RT-PCR

Total RNA was extracted using a Ribospin™ total RNA purification kit (GeneAll Biotechnology, 314-150) from iPSC at passage 14. Reverse transcription was performed with PrimeScript™ Reverse Transcriptase (Takara, 2680A), according to the manufacturer's recommendations. The gene amplification was performed by a QuantStudio™ 3 Real-Time PCR system (Applied Biosystems™, A28567) using FastStart Universal SYBR® Green Master (Roche Applied Science).

### 4.6. DNA sequence analysis of the mutation site

Genomic DNA was extracted from YCMi004-A using the G-spin™ Genomic DNA Extraction Kit (iNtRON Biotechnology, 17121), according to the manufacturer's instructions. After PCR amplification of exon 327 of the TTN gene, specific primers were prepared, and the mutation was confirmed by DNA sequencing. DNA sequences of primers are listed in Table 2.

### 4.7. Karyotyping

Passage 14 iPSCs were treated with KaryoMAX Colcemid (Thermo Fisher Scientific) for 76 min at 37 °C and then dissociated using Gentle Cell Dissociation Reagent (Stemcell Technologies, 07174). Dissociated cells were washed with 3 ml of PBS and incubated with 0.1 M KCl solution for 30 min at 37 °C. Cells were fixed with 4% paraformaldehyde for 5 min. The karyotype of iPSC was analyzed using Ikaros (MetaSystems, Neon 1.2.7) software at a band resolution of 450–500 nm.

### 4.8. In vitro trilineage differentiation

Directed in vitro trilineage differentiation was carried out using the STEMdiff™ Trilineage Differentiation Kit (Stemcell Technologies,

05230), according to manufacturer's instructions. Briefly, dissociated iPSCs were seeded onto a Matrigel coated 12-well plate, and cultured in lineage-specific medium with daily replacement by day five for mesoderm and endoderm, or by day seven for ectoderm differentiation. To assess trilineage differentiation, RT-PCR for lineage-specific markers and immunocytochemistry were performed as described above (Table 1).

### 4.9. Flow cytometry

Passage 12 iPSCs were differentiated into high purity populations of cardiomyocytes as measured by flow cytometry after staining for cardiac troponin T (cTnT). For immunofluorescence staining, cells were fixed and permeabilized with Fixation/Permeabilization solution kit (BD Bioscience) for 15 min. YCMi004-A derived cardiomyocytes were incubated with the rabbit anti-cTnT antibody (Invitrogen, 1:500 dilution) at 4 °C overnight, then incubated with a secondary antibody tagged with Alexa Flour for 1 h at room temperature. Nuclei were stained with DAPI in mounting medium. An irrelevant isotype-match antibody was used as a negative control. Stained cells were analyzed using LSR II flow cytometer (BD Bioscience) and FLOWJO v10.0.7 software (Tree Star, Inc.).

## 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 has supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIT) (No. NRF-2021R1I1A1A01060135, 2019R1C1C1002334, 2018R1D1A1B07049956). This study also was supported by Faculty research grant of Yonsei University College of Medicine (6-2021-0058), and a grant from the Korea Food Research Institute funded by the Ministry of Science, ICT & Future Planning (E0210400). Human stem cell line, CMC-hiPSC-011 was provided by National Stem Cell Bank of Korea (Korea National Institute of Health), originally provided from Catholic University.

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