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Lab Resource: Single Cell Line

# Establishment of a novel human iPSC line (YCMi003-A) from a patient with dilated cardiomyopathy carrying genetic variant LMNA p.Asp364His

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A B S T R A C T

Cardiac laminopathy caused by mutations in the LMNA gene are common and highly penetrant with a poor prognosis. We have generated a novel human induced pluripotent stem cell(iPSC) lines YCMi003-A from a patient with dilated cardiomyopathy associated with genetic variant LMNA c.1090G > C; p.Asp364His. We reprogrammed patient-specific peripheral blood mononuclear cells using five episomal vectors Oct4, Sox2, Lin28, L-Myc, and Klf4. The reported iPSC line would be a useful model for in vitro modeling of cardiac laminopathy.

Resource Table:

| Unique stem cell line identifier | YCMi003-A |
|---|---|
| Alternative name(s) of stem cell line | YCMi003-hDCM003-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: 41<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 LMNA gene (NM_005572.3) / c.1090G > C, p.Asp364His |
| Date archived/stock date | April 2021 |
| Cell line repository/bank | https://hpscreg.eu/cell-line/YCMi003-A |
| Ethical approval | Ethical committee: Yonsei University Health System, Severance Hospital, Institutional review board approval number: 4-2020-0112 |

## 1. Resource utility

LMNA is responsible for gross nuclear abnormalities, including cardiac laminopathy. This patient-specific induced pluripotent stem cell (iPSC) line, YCMi003-A, would be a useful cellular model for investigating molecular mechanisms underlying the development of cardiac laminopathies.

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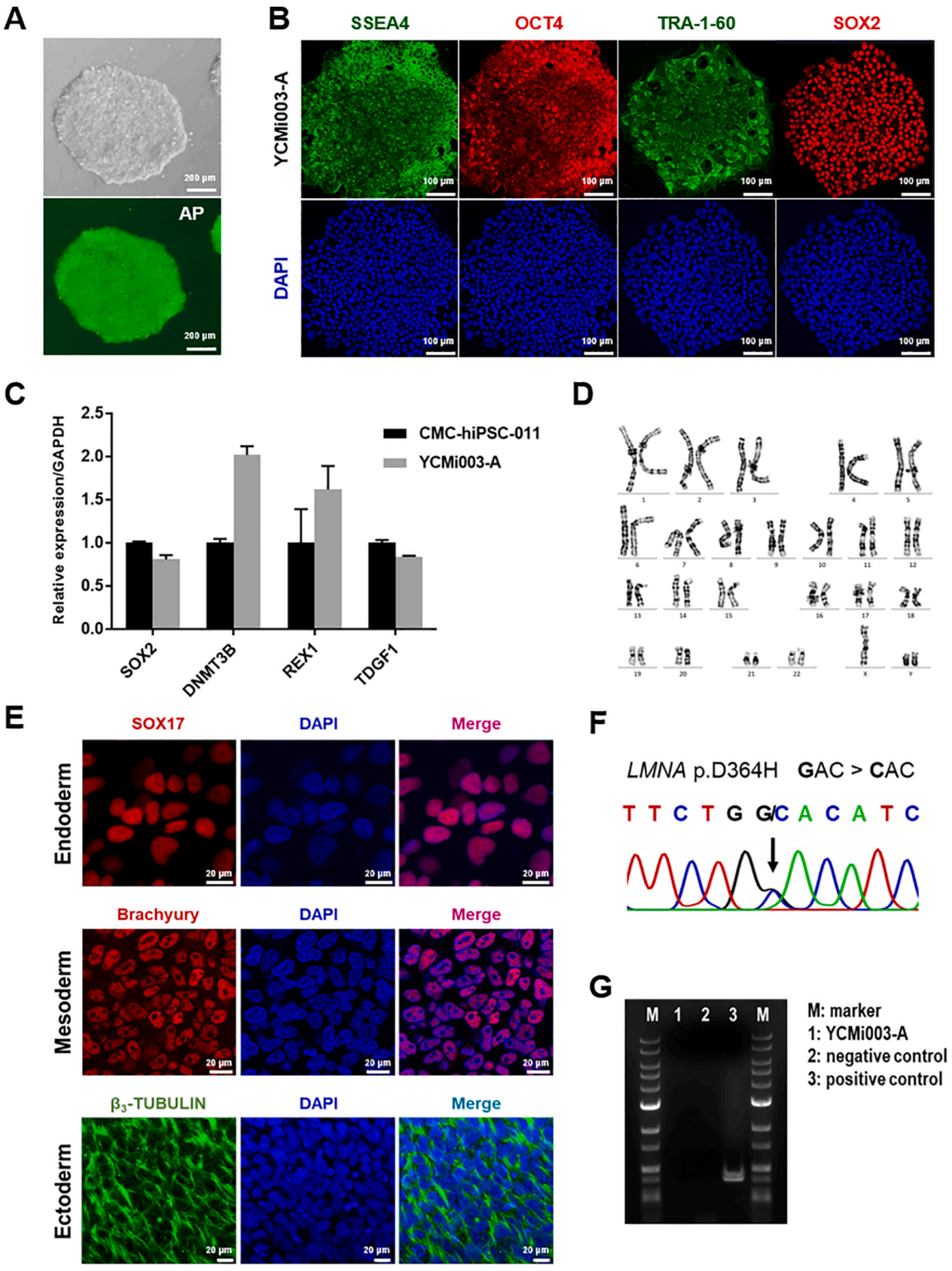


**Fig. 1.**

## 2. Resource details

The LMNA gene encodes lamin A and lamin C, which are principal structural components of nuclear lamina, and is associated with the pathogenesis of various laminopathies, including cardiac laminopathy and dilated cardiomyopathy. Familial cardiomyopathy associated with LMNA mutations accounts for approximately 6%–8% of idiopathic dilated cardiomyopathy cases (Hershberger and Siegfried, 2011), but the prognosis is worse than for other forms of dilated cardiomyopathy (Kayvanpour et al., 2017). Mutations in LMNA are associated with several cellular signaling and gene expression issues in the heart (Lee et al., 2019; Shah et al., 2021). Therefore, it is crucial to generate iPSCs of patients with LMNA genetic mutations to elucidate the disease mechanism of cardiac laminopathy. This study establishes a novel iPSC, YCMi003-A, carrying genetic variant LMNA p.Asp364His with dilated cardiomyopathy. Peripheral blood mononuclear cells (PBMCs) were

isolated from a 41-year-old male patient with familial dilated cardiomyopathy. PBMCs were reprogrammed using Epi5 Episomal iPSC Reprogramming Kit (Thermo Fisher Scientific) containing five reprogramming factors (Oct4, Sox2, Lin28, Klf4, and L-Myc). After reprogramming, the YCMi003-A line showed typical hESC-like morphology. Alkaline phosphatase expression was evaluated using the Alkaline Phosphatase Live stain kit (Thermo Fisher Scientific) (Fig. 1A). Immunofluorescence staining confirmed the expression of essential pluripotent markers, including OCT4, SOX2, SSEA4, and TRA-1–60 (Fig. 1B). Quantitative real-time PCR (qRT-PCR) analysis confirmed iPSCs expressed endogenous pluripotency genes, including SOX2, DNMT3B, REX1, and TDGF1 (Fig. 1C). G band karyotyping analysis showed a 47, XYY in the iPSC line (Fig. 1D). In vitro differentiation into - three germ layers, using the STEMdiff™ Trilineage Differentiation Kit (Stemcell Technologies, 05230), confirmed the pluripotency of YCMi003-A by detection of SOX17 (endoderm), Brachyury (mesoderm), and βIII-tubulin (ectoderm) expression, representing the three germ layers (Fig. 1E). Sanger sequencing also revealed the presence of a c.1090G > C change in exon 6 of the LMNA gene (Fig. 1F). The result of the mycoplasma test using RT-PCR was negative in the YCMi003-A iPSC line (Fig. 1G). The identity of this iPSC line was also confirmed using short tandem repeat (STR) analysis.

## 3. Materials and methods

### *Ethical statement*

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).

### *3.2. Reprogramming of human PBMCs*

A whole blood sample was obtained from a 41-year-old Korean male patient who had a missense mutation (D364H) in exon 6 of the LMNA gene. PBMCs were isolated using SepMate™ (StemCell Technologies, 15410), according to the manufacturer's recommendations. In brief, the fresh blood sample was diluted with an equal volume of PBS and then Lymphoprep™ (StemCell Technologies, 07801) in the SepMate tube. It was centrifuged at 1200 g for 15 min at room temperature (RT), washed twice with 10 ml of PBS, and the cell pellet was resuspended in Erythroid Expansion Medium. The PBMCs were plated into wells of a six-well plate with fresh medium for seven days. The integrating-free Epi5™ Episomal iPSC Reprogramming Kit (Thermo Fisher Scientific, A15960) with the electroporation system (Neon Electroporation) was used for reprogramming, according to the manufacturer's recommendations. The kit contains an optimized mixture of three episomal vectors that deliver five reprogramming factors: Oct4, Sox2, Klf4, L-Myc, and Lin28. Cells were then plated in a six-well plate coated with Corning Matrigel (hESC-qualifed, Corning, 356278). Daily medium changes, using ReproTeSR™ medium, were performed over 25 days. The cells were monitored until iPS cell colonies appeared. The obtained clones were cultured onto vitronectin coated plates (Truncated VTN-N recombinant human protein, Gibco, A31804) in TeSR™-E8™ medium (Stemcell Technologies, 05990) at 37 °C in a 5% $CO_2$ atmosphere. The cell culture medium was changed daily. Cells were passaged with ReLeSR™ (Stemcell Technologies, 05872) at a ratio of 1:10–1:20 every 4–5 days with 10 μM Y-27632 (Tocris, 1254).

### *3.3. Alkaline phosphatase analysis*

YCMi003-A cells were seeded on six-well Matrigel™ coated plates. 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. Plates were analyzed with a fluorescence microscope (OLYMPUS, IX71).

### *3.4. Quantitative RT-PCR*

Total RNA was extracted using a Ribospin™ total RNA purification kit (GeneAll Biotechnology, 314–150) from iPSCs at passage 12. 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). The expression of an endogenous control gene (GAPDH) and pluripotency-related genes (SOX2, DNMT3B, REX1, and TDGF1) were amplified using the primers listed in Table 2. Validated human iPSC (CMC-hiPSC-011) was used as a positive control.

**Table 1**
Characterization and validation.

| Classification | Test | Result | Data |
|---|---|---|---|
| Morphology | Photography Bright field | Normal | Fig. 1 panel A |
| Phenotype | Qualitative analysis Immunocytochemistry | Positive for pluripotency markers including OCT4, SOX2, SSEA4, and TRA-1–60 | Fig. 1 panel B |
| | Quantitative analysis RT-qPCR | Positive for SOX2, DNMT3B, REX1, TDGF1 | Fig. 1 panel C |
| Genotype | Karyotype (G-banding) and resolution | 46XYY, Resolution 450–500 | Fig. 1 panel D |
| Identity | Microsatellite PCR (mPCR) OR | Not Performed | NA |
| | STR analysis | 16 loci tested, all matched | 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 | Fig. 1 panel G |
| Differentiation potential | Directed differentiation | Endoderm: αSOX17 Mesoderm: αBrachyury Ectoderm: αβIII-tubulin | Fig. 1 panel E |
| 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: αSOX17Mesoderm: αBrachyury Ectoderm: αβIII-tubulin | IF with specific antibodies |
| Donor screening (OPTIONAL) | HIV 1 + 2 Hepatitis B, Hepatitis C | Negative | Not shown but available with author |
| Genotype additional info (OPTIONAL) | Blood group genotyping | Not performed | NA |
| | HLA tissue typing | Not performed | NA |

**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 |
| 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′ | |
| | DNMT3B | 199 bp | 5′- CCC AGC TGT TAC CTT ACC ATC G −3′<br>5′- GGT CCC CTA TTC CAA ACT CCT −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′ | |
| 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 | LMNA exon 6 – 7 | 524 bp | 5′- TGC TGA GAG GAA CAG CAA −3′<br>5′- CAA ACT TGC CCT CCT CAT −3′ | |

### 3.5. Immunocytochemistry

Passage 12 iPSCs were fixed using 4% paraformaldehyde for 20 min, blocked with 3% bovine serum albumin (LPS solution, 9048–46-8) with 0.3% Triton X (USB®, 9002–93-1), and incubated overnight at 4 °C with primary antibodies for OCT4, SOX2, SSEA4, and Tra-1–60. Then, 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 33,342 (Thermo Fisher Scientific, 62249) was used for counterstaining the nuclei of cells for 10 min at RT. Slides were analyzed with a confocal microscope (LSM710, Zeiss) using ZEN software. All antibody information is listed in Table 2.

### 3.6. Sequencing of the mutation site

Genomic DNA was extracted from YCMi003-A using the G-spin™ Genomic DNA Extraction Kit (iNtRON Biotechnology, 17121), following the manufacturer's instructions. After PCR amplification of exon 6–7 of the LMNA gene, specific primers were prepared and the mutation site was identified through Sanger Sequencing. The specific primers are listed in Table 2.

### 3.7. Karyotyping

Passage 12 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). Single-dissociated iPSCs were washed in 3 ml PBS and incubated at 37 °C in 0.1 M hypotonic KCL solution for 30 min. They were then fixed with FIXATION buffer for 5 min. The karyotype of iPSCs was analyzed using Ikaros (MetaSystems, Neon 1.2.7) software with a 450–500 band resolution.

### 3.8. In vitro trilineage differentiation

Directed in vitro trilineage differentiation was achieved using the STEMdiff™ Trilineage Differentiation Kit (Stemcell Technologies, 05230), according to manufacturer's instructions. In brief, single-dissociated iPSCs were seeded onto a Matrigel coated 12-well plate. Cells were cultured in lineage-specific medium with daily replacement until day five, for mesodermal (200,000 cells/well) and endodermal (800,000 cells/well), and until day seven for ectodermal (800,000 cells/well) differentiation. To assess trilineage differentiation, RT-PCR of lineage-specific markers and immunofluorescence assays were performed (Table 1).

### 3.9. STR analysis

An STR analysis was performed on the iPSCs and the parental PBMCs, with detection of 16 loci (D8S1179, D21S11, D7S820, CSF1PO, D3S1358, TH01, D13S317, D16S539, D2S1338, D19S433, vWA, TPOX, D18S51, AMEL, D5S818). In brief, PCR was used to amplify the STR loci. The PCR products were analyzed with Gene Mapper Software 5 (Applied Biosystems, 5.0) software.

### 3.10. Mycoplasma screening

Mycoplasma was detected using TaKaRa PCR Mycoplasma Detection Set (Takara, 6601), according to the manufacturer's recommendations. In brief, the PCR product (8 μl) was loaded onto 1% agarose gel for electrophoresis. The correct size band indicates the presence of mycoplasma species in the cell culture.

## 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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