EXPERIMENTAL STUDY

Functional Evaluation of Human Bioengineered Cardiac Tissue Using iPS Cells Derived from a Patient with Lamin Variant Dilated Cardiomyopathy

Koichiro Miura,1,2 MD, Katsuhisa Matsuura,1,2 MD, Yu Yamasaki Itoyama,1 MD, Daisuke Sasaki,1 PhD, Takuma Takada,1,3 MD, Yoshiyuki Furutani,1 PhD, Emiko Hayama,1 PhD, Masamichi Ito,4 MD, Seitaro Nomura,1 MD, Hiroyuki Morita,1 MD, Masashi Toyoda,6,8 MD, Akihiro Umezawa,9 MD, Kenji Onoue,7 MD, Yoshikiko Saito,1 MD, Hiroyuki Aburatani,5 MD, Toshio Nakanishi,3 MD, Nobuhisa Hagiwara,2 MD, Issei Komuro,4 MD and Tatsuya Shimizu,1 MD

Summary

Dilated cardiomyopathy (DCM) is caused by various gene variants and characterized by systolic dysfunction. Lamin variants have been reported to cause a poor prognosis. Medical and device therapies are not sufficient to improve the recovery of DCM with the lamin variants. Recently, induced pluripotent stem (iPS) cells have been used for research on genetic disorders. However, few studies have evaluated the contractile function of cardiac tissue with lamin variants. The aim of this study was to elucidate the function of cardiac cell tissue derived from patients with lamin variant DCM. iPS cells were generated from a patient with lamin A/C (LMNA)-mutant DCM (LMNA p.R225X mutation). After cardiac differentiation and purification, cardiac cell sheets were fabricated through cultivation on a temperature-responsive culture dish were transferred to the surface of the fibrin gel, and the contractile force was measured. The contractile force and maximum contraction velocity, but not the maximum relaxation velocity, were significantly decreased in cardiac cell sheet tissue with the lamin variant. A qRT-PCR analysis revealed that mRNA expression of some contractile proteins, cardiac transcription factors, Ca2+-handling genes, and ion channels were downregulated in cardiac tissue with the lamin variant.

Human iPS-derived bioengineered cardiac tissue with the LMNA p.R225X mutation has the functional properties of systolic dysfunction and may be a promising tissue model for understanding the underlying mechanisms of DCM.

Key words: Cell sheet, Contractile measurement, Tissue engineering

Dilated cardiomyopathy (DCM) is a common cause of heart failure and is characterized by left ventricular dysfunction and wall thinning. Over 50 genes are related to DCM, and 20-50% of DCM cases are considered to include gene variants.11 In a previous study, 65% of DCM cases exhibited gene variants, of which the titin (TTN) (33%) and lamin A/C (LMNA) (11%) variants were the most frequently observed.12 Recent advances in medicine and device therapies have improved the left ventricular dysfunction and prognosis in patients with DCM.13 Left ventricular ejection fraction (LVEF) recovery is observed in approximately 40% of patients with DCM, and the mortality of these patients tended to decrease.14,15 However, patients with the lamin variant of DCM have shown an unrecovered LVEF despite optimal medical therapy, leading to a poor prognosis.16 Therefore, current therapeutic strategies may not be sufficiently effective for patients with lamin variants of DCM, and the development of a disease model for understanding the molecular mechanisms of lamin variant-mediated cardiac dysfunction is necessary.

Recently, tissue engineering technologies have been developed and applied to regenerative therapy and tissue models; an example of which is sheet-based cell tissue engineering. The cell sheet, a scaffold-free bioengineered monolayered tissue, is harvested from a temperature-
responsive culture surface that is coated with a temperature-responsive polymer (poly N-isopropylacrylamide) by lowering the culture temperature. Various types of cell sheets have been fabricated and applied for regenerative medicine in the cornea, heart, esophagus, cartilage, gingiva, ear, and lungs. Cell sheets have also been used in tissue models. Furthermore, induced pluripotent stem (iPS) cell technology enables the generation of human cardiomyocytes. We have developed a human cardiac cell sheet tissue derived from iPS cells and contractile force measurement system to evaluate cardiac tissue function.

Recently, we confirmed in a human cardiac cell sheet tissue model that the relaxation function is impaired even after the full recovery of systolic function in a hypoxia/reoxygenized condition, which is a common condition in various types of heart disease. Therefore, a human cardiac cell sheet tissue model may be useful for identifying disease-specific functional profiles and for understanding the underlying molecular mechanisms.

In the present study, we generated iPS cells from a patient with the lamin variant of DCM and evaluated the contractile function of iPS cell-derived cardiac cell sheet tissue.

Methods

Establishment of iPS cell lines: This study was performed in line with the principles of the Declaration of Helsinki and approved by the Institutional Review Boards on Human Subjects Research of Tokyo Women’s Medical University, The University of Tokyo, Center for Regenerative Medicine, National Center for Child Health and Development Research Institute, and Nara Medical University. The NI2-1 cell line was established from a patient with familial DCM associated with the LMNA p.R225X mutation at Nara Medical University (Figure 1A). We selected 1 (NI2-1) out of 4 clones from the patient with the LMNA p.R225X mutation, due to the convenience of maintaining iPS cells and efficiency of cardiac differentiation. To establish iPS cells with the lamin variant, a combination of plasmids encoding for OCT3/4, SOX2, KLF4, L-MYC, LIN28, and shRNA for TP53 was induced into peripheral blood mononuclear cells obtained from the patient as previously described. The human iPS cell line 201B7 was purchased from RIKEN (Tsukuba, Japan) as a normal control. A puromycin-resistance gene under the control of the alpha-myosin heavy chain promoter and a neomycin-resistant gene under the control of the Rex-1 promoter were introduced into the iPS cells using the lentiviral vector. The iPS cell line was cultured as previously reported. Phase contrast images of undifferentiated iPS cells were obtained using an inverted microscope (Nikon, Tokyo).

Cardiac differentiation: Cardiac differentiation of iPS cells was induced by slight modification of a previously described procedure. Briefly, iPS cells were harvested from culture dishes and the aggregates were cultured in a stirred bioreactor system (Able, Tokyo). For cardiomyo-
cyte differentiation, a 100 mL vessel was used for the 201 B7 (Wild Type) cell line, and a 30 mL vessel was used for the N12-1 (LMNA p.R225X) cell lines. On day 16, cell aggregates were dissociated with 0.05% trypsin/EDTA (Life Technologies, Carlsbad, CA, USA) and cells were cultured in medium A [defined as Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and penicillin-streptomycin (Sigma-Aldrich, St. Louis, MO, USA)] at 37°C in a humidified atmosphere with 5% CO₂ (Panasonic Healthcare, Tokyo).

**Purification of iPS-derived cardiomyocytes:** Human iPS-derived cardiomyocytes were purified as previously described. On day 21, 1.5 μg/mL puromycin (Sigma-Aldrich) was added for 24 hours. On day 22, the medium was changed, and the cardiomyocytes were incubated for 24 hours in medium A.

**Cardiac cell sheet tissue engineering:** Fibrin gel sheets were prepared as the basis of cardiac cell sheets for contractile force measurements, as previously described and used for cell sheet transfer. The schematic diagram of cardiomyocyte tissue fabrication is shown in Figure 2A. Sterilized silicone frames were attached to the surface of temperature-responsive dishes (UpCell; CellSeed, Tokyo) to restrict the cell culture area to a 12 mm square. The cells were seeded and cultured on temperature-responsive dishes at 1.2 × 10⁶ cells/mL (Figure 2B) and transferred onto the fibrin gel (Figure 2C, D) as previously described.

**Contractile force measurement system:** The contractile force measurement device was composed of a load cell (LVS-10GA; Kyowa Electronic Instruments, Tokyo) and a culture bath made of acrylic plates (Figure 2E). The contractile force measurement was conducted as previously described.

**Flow cytometry:** At day 23, cells were fixed with 4% paraformaldehyde for 15 minutes. The fixed cells were then preserved at 4°C in PBS. The cells were labeled with anti-cardiac troponin T rabbit polyclonal antibody (Abcam, Cambridge, UK) in PBS containing 5% FBS. As isotype controls, the cells were labeled with rabbit polyclonal IgG (Abcam). The cells were then labeled with FITC-conjugated anti-rabbit antibody (Jackson Immuno Research, PA, USA) and analyzed using a Gallios flow cytometer (Beckman Coulter, Brea, CA, USA) and Kaluza analysis software (Beckman Coulter). In this study, 50-75% of cardiac troponin T-positive samples were used for contractile force measurement and qRT-PCR analysis.

**Quantitative reverse transcription polymerase chain reaction (qRT-PCR):** After the contractile measurement, the cardiac tissue was preserved with Buffer RLT (QIAGEN, Hilden, Germany) at -80°C. RNA extraction was performed using the RNeasy Micro Kit (QIAGEN) according to the manufacturer’s protocol. cDNA was synthesized using a high-capacity cDNA reverse transcription kit (Thermo Fisher Scientific, Rockford, IL, USA) with random hexamer primers. Real-time PCR analysis of each sample was performed using the Applied Biosystems ViiA™ 7 RT-PCR system (Thermo Fisher Scientific). TaqMan gene expression assays for real-time PCR (Thermo Fisher Scientific) are listed in the Table. The average copy number of gene transcripts was normalized to that of glyceraldehyde-3-phosphate dehydrogenase for each sample. The data were analyzed using the ΔΔCT method. All statistical analyses were performed by comparing the 2^ΔΔCT values between groups and the results were plotted as fold change ± standard deviation (2^ΔΔCT).

**RT-PCR analysis of undifferentiated markers:** PCR analysis of undifferentiated markers such as OCT3/4,
region. The cells were lysed in the extraction buffer and incubated at 95°C for 10 minutes. A portion of the lysate was used for PCR using Terra PCR Direct Polymerase (Takara). The reaction protocol was as follows: 98°C for 2 minutes (98°C for 10 seconds, 60°C for 15 seconds, 68°C for 20 seconds) × 35, then 4°C. The sequence of the primers was as follows: Forward, GCTGCGTAGTGCCGTC ATGGAG; Reverse, ATACTGCTCACCTGGTCCT. The PCR product was purified using the Wizard® SV Gel and PCR Clean-Up System (Promega, Tokyo), confirmed to be a single band by agarose gel electrophoresis, and submitted to sequencing reactions (FASMAC, Atsugi, Japan).

Karyotype analysis: Karyotypic analysis was also conducted out to Chromosome Science Labo Inc. (Sapporo, Japan). Metaphase spreads were prepared from cells treated with 100 ng/mL of Colcemid for 6 hours. The cells were fixed with methanol: glacial acetic acid (2:5) 3 times, and dropped onto glass slides. The chromosome numbers of 50 cells were analyzed and 20 cells were randomly chosen for karyotype analysis using the Q banded method.

Statistical analysis: Data are presented as the mean ± standard deviation. Statistical comparisons between the 2 groups were performed using the unpaired Student’s t-test. Statistical significance was set at P < 0.05.

**Results**

Generating iPS cell lines and confirming the pluripotency of the cells: NI2-1 cells formed flat-shaped colonies consistent with iPS cells (Figure 1C). When we carried out immunostaining of undifferentiated markers to confirm their pluripotency, almost all cells expressed OCT3/4 and NANOG (Figure 1C). The RT-PCR analysis revealed that NI2-1 cells also expressed mRNA of OCT3/4, NANOG and TERT (Figure 1D), suggesting that the generated cells were compatible as iPS cells. Sanger sequencing revealed that the NI2-1 iPS cells have the p. R225X LMNA mutation (Figure 1E). The karyotype analysis showed that most cells (17/20) of NI2-1 clones at passage 19 had an intact karyotype (Figure 1F).

Cardiac tissue of the lamin variant exhibits impaired contractile force: Next, we fabricated the cardiac tissue and measured the contractile force as shown in Figure 2 A-E. Since the purity of cardiomyocytes in tissue might affect its contractile properties and certain extent levels of non-cardiomyocytes such as fibroblasts are indispensable for fabricating cardiac cell sheet, we used differentiated cells with a cardiomyocyte purity of 50-75% for cell sheet tissue fabrication. As shown in Figures 3A and B, the purity of cardiomyocytes was identical between the groups [201B7 (Wild Type), 59.1 ± 5.5% (n = 3); NI2-1 (LMNA p.R225X), 55.9 ± 4.9% (n = 3)]. After initiating the contractile force measurement, the contractile force tended to increase gradually and was stabilized at day 37 (day 5 on the measurement system, data not shown). Therefore, we compared the contractile properties of the cardiac cell sheet tissue at day 37. The representative cardiac force traces are shown in Figure 4A. The cardiac parameters were defined as follows: the contractile force was the maximum height of the curve; the beating rate

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**Table.** TaqMan Gene Expression Assays for Real-Time PCR

| Taqman assay ID | Gene       |
|-----------------|------------|
| Hs01011425_m1   | MYH6       |
| Hs0110632_m1    | MYH7       |
| Hs00166405_m1   | MYL2       |
| Hs01085598_g1   | MYL7       |
| Hs00943911_m1   | TNNT2      |
| Hs00231763_m1   | NNX2-5     |
| Hs00171403_m1   | GATA4      |
| Hs00231149_m1   | MEF2C      |
| Hs00361155_m1   | TBX5       |
| Hs00232018_m1   | GATA6      |
| Hs01105751_m1   | WT1        |
| Hs00975492_m1   | HCN4       |
| Hs00267499_s1   | KCNE1      |
| Hs04234270_g1   | KCNH2      |
| Hs00923522_m1   | KCNQ1      |
| Hs01062258_m1   | SLC8A1     |
| Hs00748445_s1   | GJA1       |
| Hs00979198_m1   | GJA5       |
| Hs00271416_s1   | GIC1       |
| Hs00164004_m1   | COL1A1     |
| Hs00383230_g1   | NPPA       |
| Hs00173590_m1   | NPPB       |
| Hs00167681_m1   | CACNA1C    |
| Hs00181461_m1   | RYR2       |
| Hs00544877_m1   | ATP2A2     |
| Hs01848144_s1   | PLN        |
represented the beating time per min; the time of systole was the time taken from 20% of the maximum height of the peak, and the time of relaxation was the time taken from the peak to 20% of the peak; the contraction velocity was the maximum +dF/dt during systole, and the relaxation velocity was the maximum -dF/dt during relaxation (Figure 4B).

Although the systolic functions, including contractile force and contraction velocity, of the cardiac cell sheet tissue with the lamin variant were significantly impaired [contractile force: 201B7 (Wild Type), 1.10 ± 0.15 mN \((n = 4)\); NI2-1 (LMNA p.R225X), 0.56 ± 0.18 mN \((n = 7)\), \(P = 0.001\); contraction velocity: 201B7, 6.6 ± 1.2 mN/second \((n = 4)\); NI2-1, 3.7 ± 1.0 mN/second \((n = 7)\), \(P = 0.004\)], relaxation function such as relaxation velocity was identical between the groups [201B7, -3.0 ± 0.9 mN/second \((n = 4)\); NI2-1, -2.9 ± 1.0 mN/second \((n = 7)\), \(P = 0.95\)] (Figure 4C). Beating rate and time of systole were also identical between the groups [beating rate: 201B7, 30.5 ± 6.7 bpm \((n = 4)\); NI2-1, 38.6 ± 19.3 bpm \((n = 7)\) \((P = 0.48)\); time of systole: 201B7, 0.25 ± 0.13 seconds \((n = 4)\); NI2-1, 0.26 ± 0.03 seconds \((n = 7)\) \((P = 0.61)\)]. Time of relaxation was significantly decreased in NI2-1 compared to the control (201B7, 0.34 ± 0.03 seconds \((n = 4)\); NI2-1, 0.24 ± 0.03 seconds \((n = 7)\), \(P = 0.001\)). These findings suggest that systolic dysfunction, but not relaxation dysfunction, might be a functional property of cardiac cell sheet-tissue with the lamin variant. During the measurement period, no apparent arrhythmia was observed in either sample.

**qRT-PCR analysis:** We performed qRT-PCR analysis to clarify the underlying mechanisms of impaired systolic function in cardiac cell sheet tissue with the lamin variant. As shown in Figure 5, the cardiac gene expressions of contractile proteins including MYL2, MYL7, MYH6, MYH7, TNNT2, transcription factors including NNX2.5, GATA4, GATA6, MEF2C, ion channels such as HCN4, KCNE1, KCN2, KCNQ1, SLC8A1, gap junction proteins such as GJA1, GJA5, GJC1 and Ca\(^{2+}\) handling proteins including ATP2A2, RYR2 were downregulated in cardiac cell sheet tissue with the lamin variant. On the other hand, the expression of certain cardiac genes for transcription factors including TBX5 and WT1, and Ca\(^{2+}\) handling proteins including CACNA1C and PLN, were not different in cardiac tissue between the cell lines 201B7 and NI2-1. Therefore, downregulated gene expression of contractile proteins, transcription factors, ion channels, and Ca\(^{2+}\) handling proteins might be responsible for the impaired systolic function of cardiac cell sheet tissue with the lamin variants.
Figure 4. Contractile force measurement. A: Schematic illustration of contractile measurement parameters. B: Representative trace of contractile force and maximum contraction and relaxation velocity of cardiac cell sheet tissue; C: Contractile force, beating rate, systolic and relaxation time, maximum contraction velocity, and maximum relaxation velocity of the 201B7 and NI2-1 cell lines.

Figure 5. qRT-PCR analysis of gene expression. Y axis indicates relative gene expression of target genes compared with glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene. Results are shown for the 201B7 (n = 3–4) and NI2-1 (n = 4–10) cell lines. qRT-PCR indicates quantitative reverse transcription polymerase chain reaction.
Discussion

In the present study, we generated iPS cells from a patient with the lamin variant and succeeded in identifying impaired systolic function as the functional property of cardiac cell sheet tissue. We also observed the downregulation of mRNA expression associated with some contractile proteins, cardiac transcription factors, ion channels, and Ca\(^{2+}\) handling proteins.

We have identified that contractile force along with contraction velocity was impaired in cardiac cell sheet tissue with the lamin variant. Further, the systolic dysfunction in cardiac tissue with the lamin variant is comparable to the clinical profile of patients with DCM. The clinical phenotypes of patients with lamin variants of DCM have been reported to be distinct between the ages of 30 and 40. However, in the present study, impaired contractility was observed in freshly differentiated cardiomyocytes, which are considered fetal-like cardiomyocytes. Various types of compensatory mechanisms, including the Frank-Starling law and neurohormonal factor activation in the living body, may delay the typical onset even in cardiomyocytes with systolic dysfunction. Further, the inadequate inborn or responsive cell proliferation capacity of cardiomyocytes has been reported to contribute to the development of DCM with the lamin mutation. Therefore, intensive observation and management might be necessary in patients with the lamin variant of DCM. Diastolic function is mediated by various factors such as Ca\(^{2+}\) overload and fibrosis and becomes increasingly evident in DCM with disease progression and age.

The intact relaxation function of cardiac cell sheet tissue with the lamin variant in the present study may also be compatible with the early phase of DCM. According to the qRT-PCR analysis, mRNA expression levels of contractile proteins, ATP2A2, RYR2, transcriptional factors, and ion channels were downregulated in cardiac cell sheet tissue with the lamin variant. Although the downregulation of contractile protein gene expression directly affects systolic function in cardiac tissues, we cannot exclude the possibility that this phenomenon might be the result of the impaired contraction of cardiac tissue with the lamin variant. In the present study, cardiomyocyte purity was identical between the groups. However, since the mRNA expression levels of cardiac transcription factors, including NKX2.5, GATA4, GATA6, and MEC2C, were downregulated in cardiac tissue with the lamin variant, cardiomyocytes might be more immature, which can result in impaired systolic function.

It is well known that the NKX2.5,\(^{20}\) GATA4,\(^{20}\) and MEF2C\(^{30}\) genes are critical for heart development, and variants in these transcription factors cause congenital heart diseases including atrial septal defect and ventricular septal defect. It has been reported that iPS cell-derived cardiomyocytes from subjects with a heterozygous GATA4-G296S missense mutation have impaired contractility, calcium handling, and metabolic activity.\(^{25}\) The GATA4 binding site has also been reported to be located within the 5’ flanking sequence of the human cardiac alpha-myosin heavy chain encoding gene.\(^{26}\) Since MYH6 was downregulated in cardiac tissue with the lamin variant, insufficient expression levels of GATA4 might impair systolic function through the downregulation of MYH6, NKX2.5 and MEF2C genes have been reported to upregulate each other’s expression in the process of cardiac differentiation in P19 cells,\(^{27}\) and these transcription factors have been reported to interact cooperatively in heart development.\(^{28}\) Although it remains unclear how NKX2.5 and MEF2C expression levels are downregulated in patients with the lamin variant, lower expression levels of these genes might affect cardiomyocyte differentiation and contractile function. ATP2A2 codes for SERCA2a, which acts as a subtype of SERCA expressed in the heart. As SERCA2a mediates Ca\(^{2+}\) reuptake into the sarcoplasmic reticulum in cardiomyocytes, the downregulation of ATP2A2 in cardiac tissue with the lamin variant might cause Ca\(^{2+}\) reuptake dysfunction. SERCA2a expression levels have been reported to be reduced in failing hearts, and the amelioration of SERCA2a expression using adeno-associated virus leads to the attenuation of reduced cardiac contractility and heart failure.\(^{29}\) Therefore, the downregulation of ATP2A2 may be a cause of contractile impairment in cardiac tissue with the lamin variant. Although cardiac cell sheet tissue with the lamin variant showed low expression levels of KCNE1, KCNH2, KCNQ1, which regulate the repolarization of cardiomyocytes, an apparent tendency of arrhythmia was not observed. iPS cells have been used for research on DCM with lamin variants. Lee, et al. generated iPS cells from patients with DCM who carry a frameshift of LMNA, leading to the early termination of translation (348-349 insG; K117fs).\(^{30}\) Further, they showed that cardiomyocytes have impaired Ca\(^{2+}\) intensity, and that aberrant calcium homeostasis led to arrhythmia through the activation of the PDGF signaling pathway.\(^{30}\) Bertero, et al. reported that iPS-derived cardiomyocytes with the LMNA p.R225X mutation showed increased Ca\(^{2+}\) intensity and increased contractility.\(^{31}\) In contrast, cardiac cell sheet tissue with the LMNA p.R225X mutation showed impaired systolic function in the present study. Differences in contractile force measurement strategy and cellular components in cardiac tissue might explain this discrepancy. They evaluated the contractile function by calculating the change of motion in the imaging data,\(^{31}\) while the contractile force measurement system of the present study enabled a direct evaluation of the contractile force. The cardiomyocyte purity in a previous study was over 95%.\(^{33}\) Since certain levels of fibroblasts are necessary for fabricating cardiac cell sheets,\(^{31}\) we used cardiomyocytes whose purity was 50-75% in the present study. We previously reported that almost all non-cardiomyocytes after cardiac differentiation of human iPS cells were fibroblast-like cells,\(^{32}\) and that the gene expression profile of iPS cell-derived fibroblast-like cells after cardiac differentiation was similar to that of human atrium and ventricle-derived fibroblasts.\(^{32}\) Recently, Lachaize, et al. reported that cardiac fibroblasts from neonatal rat hearts with a missense mutation in the lamin A/C gene (LMNA D192G mutation) showed cytoskeleton disorganization, decreased elasticity, and altered cell-cell adhesion properties.\(^{33}\) The analysis of the profile of non-cardiomyocytes with the LMNA p.R225X mutation will be necessary to understand the differences in non-cardiomyocytes between 201B7 and N12-1. However, con-
sistent with the evidence that the native heart contains various types of cells, including cardiomyocytes and non-cardiomyocytes such as fibroblasts, the co-existence of non-cardiomyocytes in cardiac tissue might be similar to that in the native heart, and the systolic dysfunction observed in the present study might be more compatible with the phenotype of DCM with the lamin variant.

It has been reported that cardiomyocyte apoptosis contributes to the pathogenesis in DCM with the lamin variant. Since cardiomyocytes with lamin variants may be more fragile or susceptible to contraction in an in vitro stringent environment compared to normal cardiomyocytes, we cannot exclude the possibility that loss of cardiomyocytes in the experimental period affected the decreased contractile force in the cardiac tissue with the LMNA p.R225X mutation. Analysis of the number of cardiomyocytes in cardiac tissue will be necessary to understand the precise mechanisms of impaired contractility of cardiac tissue with the LMNA p.R225X mutation.

In conclusion, we have showed that systolic dysfunction provides a phenotype for cardiac tissue with the LMNA p.R225X mutation. Bioengineered cardiac tissue may provide a novel tool for understanding the molecular mechanisms of DCM with the lamin variant.

Limitations: In this study, we used 201B7 as a healthy control since an isogenic control of the corrected LMNA mutation was not available. We observed a small amount of cells with abnormal karyotypes and cannot exclude the possibility that a small amount of cells with abnormal karyotype affects the function of cardiac tissue from N12-1.

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Disclosure
Conflicts of interest: Tatsuya Shimizu is a shareholder of CellSeed Inc. Tokyo Women’s Medical University received a research fund from CellSeed Inc. for practical application of cell sheet tissue engineering. Katsuhisa Matsuura and Tatsuya Shimizu are the inventors of bioreactor systems. The other authors declare no conflicts of interest.

Ethics approval: This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Institutional Review Boards on Human Subjects Research of Tokyo Women’s Medical University, The University of Tokyo, Center for Regenerative Medicine, National Center for Child Health and Development Research Institute, and Nara Medical University.

Consent: Informed consent was obtained from all individual participants included in the study.

Data availability: Data are available from the corresponding author upon reasonable request.

Authors’ contribution: Koichiro Miura designed and performed the experiments, analyzed the data, and wrote the manuscript. Katsuhisa Matsuura conceived and supervised the project, designed the experiments, and edited the manuscript. Yu Yamasaki Itoyama, Daitsuke Sasaki, Takuma Takada, Nobuhisa Hagiwara, and Tatsuya Shimizu analyzed the data. Yoshiyuki Furutani, Emiko Hayama, Yoshio Nakanishi, Masamichi Ito, Settaro Nomura, Hiro-yuki Morita, Masashi Toyoda, Akihiro Umezawa, Kenji Onoue, Yoshihiko Saito, Hiroyuki Aburatani, and Isssei Komuro generated iPS cells from patients. Nobuhisa Hagiwara, Isssei Komuro, and Tatsuya Shimizu supervised the project. All authors read and approved the final manuscript.

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