A Novel Truncating LMNA Mutation in Patients with Cardiac Conduction Disorders and Dilated Cardiomyopathy

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Summary

The cardiac phenotype of laminopathies is characterized by cardiac conduction disorders (CCDs) and dilated cardiomyopathy (DCM). Although laminopathies have been considered monogenic, they exhibit a remarkable degree of clinical variability. This case series aimed to detect the causal mutation and to investigate the causes of clinical variability in a Japanese family with inherited CCD and DCM. Of the five family members investigated, four had either CCD/DCM or CCD alone, while one subject had no cardiovascular disease and acted as a normal control. We performed targeted resequencing of 174 inherited cardiovascular disease-associated genes in this family and pathological mutations were confirmed using Sanger sequencing. The degree of clinical severity and variability were also evaluated using long-term medical records. We discovered a novel heterozygous truncating lamin A/C (LMNA) mutation (c.774delG) in all four subjects with CCD. Because this mutation was predicted to cause a frameshift mutation and premature termination (p.Gln258HisfsTer222) in LMNA, we believe that this LMNA mutation was the causal mutation in this family with CCD and laminopathies. In addition, gender-specific intra-familiar clinical variability was observed in this Japanese family where affected males exhibited an earlier onset of CCD and more severe DCM compared to affected females. Using targeted resequencing, we discovered a novel truncating LMNA mutation associated with CCD and DCM in this family characterized by gender differences in clinical severity in LMNA carriers. Our results suggest that in patients with laminopathy, clinical severity may be the result of multiple factors.

Key words: Lamin A/C, Laminopathy, Targeted resequencing, Gender difference, Cardiovascular disease-associated gene, Truncating mutation

Lamins A and C, encoded by the lamin A/C gene (LMNA), are nuclear intermediate filament proteins that form one of the major structural components of the lamina network, which underlies and mechanially supports the nuclear envelope.1,2 LMNA mutations cause a variety of inherited diseases referred to as laminopathies and include skeletal muscle disease, premature aging, metabolic disorders, and cardiac abnormalities.3,4 The cardiac phenotype of laminopathies is characterized by cardiac conduction disorders (CCD), atrial fibrillation, ventricular arrhythmias, sudden cardiac death, and dilated cardiomyopathy (DCM).3,4

In the past few years, technical advances and cost reductions in next-generation sequencing (NGS) has made comprehensive genetic testing possible for all known genes of cardiovascular disease.5 As well as its versatile applications towards improved detection of genetic changes in patients with cardiovascular disease and exploration of novel genotype-phenotype correlations,6-17 NGS will enable the prediction of clinical outcomes and the development of individualized treatments (personalized medicine) by revealing the interactions of multiple gene mutations in patients with inherited cardiovascular disease.

In the present study, we performed targeted resequencing of 174 inherited cardiovascular disease-associated genes to screen for culprit genes and to investigate the causes of clinical variability in a Japanese family with inherited CCD and DCM. We discovered a novel truncating LMNA mutation associated with CCD and DCM in four affected individuals from a single family,
which exhibited gender differences in clinical severity in LMNA carriers.

Methods

Subjects and clinical evaluation: A Japanese family with inherited CCD and DCM was identified, and five family members were investigated (Figure 1). CCD was characterized by early-onset sick sinus syndrome and/or atrioventricular block (AVB). Three subjects (II-1, II-2, and the proband III-1) had pacemakers until late middle age, and one (III-2) had first-degree AVB at the age of 30 years. DCM was defined according to international criteria, including left ventricular enlargement with systolic dysfunction after the exclusion of other detectable causes of DCM. Three subjects (II-1, II-2, and III-1) were diagnosed with DCM. The father (II-3) of the proband had no cardiovascular disease and was used as a normal control. Clinical evaluation consisted of a medical history, a family history, physical examination, 12-lead electrocardiography (ECG), and transthoracic echocardiography. Clinical evaluations were made by investigators without knowledge of the genetic status of the subjects.

This study was performed in accordance with the Helsinki Declaration. The study protocol was approved by the Ethics Committee of the Ehime University School of Medicine (Approval No. 13-1), and written informed consent was obtained from each subject, including their consent for their DNA to be used in genetic analyses.

DNA sample preparation: DNA was extracted from peripheral blood samples of all five subjects using a QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany) in accordance with the manufacturer’s protocol.

Targeted resequencing and data analysis: Targeted resequencing was performed using a TruSight Cardio Sequencing Kit with a MiSeq (Illumina, San Diego, CA, USA), which allowed the enrichment and final analysis of a 174-gene panel including all known genes related to inherited cardiovascular disease. The gene list is shown in Table I. The cumulative target region size was 0.572 Mb including all exons and exon-intron boundaries in the 174 genes comprising the panel.

Library preparation and sequencing was performed according to the manufacturer’s protocol. Briefly, an indexed pooled library was prepared from 50 ng genomic DNA. Targeted regions of the 174 genes were then captured using biotin-labeled TruSight Cardio oligonucleotides (Illumina) and the resulting biotinylated target DNA fragments were purified with streptavidin-coated magnetic beads to obtain enriched libraries. Quantification and validation of the libraries were performed using a Qubit 2.0 Fluorometer system (Life Technologies, Carlsbad, CA, USA) and a 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA). Sequencing of the enriched libraries was performed using a MiSeq system by 150 bp paired-end analysis.

Alignment and variant calling was automatically performed by the on-instrument software, MiSeq Reporter, and variant calling data were exported as VCF files. The variants reported in the VCF files were evaluated and visualized via VariantStudio Variant Analysis Software (Illumina). We filtered the data to remove variants with a minor allele frequency > 1% in the Asian population using the April 2012 phase 1 call set from the 1000 Genomes Project (v3 update), and we used Sorting Intolerant From Tolerant (SIFT; available at http://sift.jcvi.org/) and Polymorphism Phenotyping (PolyPhen-2; available at http://genetics.bwh.harvard.edu/pph2/) to predict mutational changes to protein function.

Sanger sequencing: Mutations identified as pathological
### Table 1. List of Genes Selected to Perform a Targeted Resequencing

| Gene   | Gene ID | Gene description                                      | Chromosome |
|--------|---------|-------------------------------------------------------|------------|
| ABCC9  | 10060   | ATP binding cassette subfamily C member 9             | 12p12.1    |
| ABCG5  | 64240   | ATP binding cassette subfamily G member 5             | 2p21       |
| ABCG8  | 64241   | ATP binding cassette subfamily G member 8             | 2p21       |
| ACTA1  | 58      | actin, alpha 1, skeletal muscle                       | 1q42.13    |
| ACTA2  | 59      | actin, alpha 2, smooth muscle, aorta                  | 10q23.3    |
| ACTC1  | 70      | actin, alpha, cardiac muscle 1                        | 15q14      |
| ACTN2  | 88      | actinin alpha 2                                       | 1q42-q43   |
| AKAP9  | 10142   | A-kinase anchoring protein 9                          | 7q21-q22   |
| ALMS1  | 7840    | ALMS1, centrosome and basal body associated protein   | 2p13       |
| ANK2   | 287     | ankyrin 2, neuronal                                   | 4q25-q27   |
| ANKRD1 | 27063   | ankyrin repeat domain 1                               | 10q23.31   |
| APOA4  | 337     | apolipoprotein A4                                     | 11q23      |
| APOA5  | 116519  | apolipoprotein A5                                     | 11q23      |
| APOB   | 338     | apolipoprotein B                                      | 2p24-p23   |
| APOC2  | 344     | apolipoprotein C2                                     | 19q13.2    |
| APOE   | 348     | apolipoprotein E                                      | 19q13.2    |
| BAG3   | 9531    | BCL2 associated athanogene 5                         | 10q25.2-q26.2|
| BRAF   | 673     | B-Raf proto-oncogene, serine/threonine kinase        | 7q34       |
| CACNA1C| 775     | calcium voltage-gated channel subunit alpha1 C        | 12p13.3    |
| CACNA2D1| 781    | calcium voltage-gated channel auxiliary subunit alpha2delta 1 | 7q21-q22 |
| CACNB2 | 1284    | calcium voltage-gated channel auxiliary subunit beta 2| 10p12      |
| CALM1  | 801     | calmodulin 1 (phosphorylase kinase, delta)            | 14q32.11   |
| CALR3  | 125972  | calreticulin 3                                        | 19p13.11   |
| CASQ2  | 845     | calsequastin 2                                        | 1p13.1     |
| CAV3   | 859     | caveolin 3                                            | 3p25       |
| CBL    | 867     | Cbl proto-oncogene                                    | 11q23.3    |
| CBS    | 875     | cystathionine-beta-synthase                           | 21q22.3    |
| CETP   | 1071    | cholesteryl ester transfer protein                    | 16q21      |
| COL3A1 | 1281    | collagen type III alpha 1                             | 2q31       |
| COL5A2 | 1290    | collagen type V alpha 2                               | 9q34.2-q34.3|
| COX15  | 1355    | COX15 cytochrome c oxidase assembly homolog           | 10q24      |
| CREBL3 | 84699   | cAMP responsive element binding protein 3-like 3     | 19p13.3    |
| CRELD1 | 78987   | cysteine rich with EGF like domains 1                 | 3p25.3     |
| CRYAB  | 1410    | crystallin alpha B                                    | 11q23.3    |
| CSRP3  | 8048    | cysteine and glycine rich protein 3                   | 11p15.1    |
| CTF1   | 1489    | cardioprophlin 1                                      | 16p11.2    |
| DES    | 1674    | desmin                                               | 2q35       |
| DMD    | 1756    | dystrophin                                            | Xp21.2     |
| DNAJC19| 131118  | DnaJ heat shock protein family (Hsp40) member C19     | 3q26.33    |
| DOLK   | 22845   | dolichol kinase                                       | 9q34.11    |
| DPP6   | 1804    | dipeptidyl peptidase like 6                           | 7q36.2     |
| DSC2   | 1824    | desmocollin 2                                         | 18q12.1    |
| DSG2   | 1829    | desmoglein 2                                          | 18q12.1    |
| DSP    | 1832    | desmoplakin                                           | 6p24       |
| DTNA   | 1837    | dystrobrevin alpha                                    | 18q12      |
| EEFMP2 | 30008   | EGF containing fibulin-like extracellular matrix protein 2 | 11q13.1 |
| ELN    | 2006    | elastin                                               | 7q11.23    |
| EMD    | 2010    | emerin                                               | Xq28       |
| EYA4   | 2070    | EYA transcriptional coactivator and phosphatase 4     | 6q23       |
| FBNI   | 2200    | fibrillin 1                                           | 15q21.1    |
| FBN2   | 2201    | fibrillin 2                                           | 5q23.3     |
| FHL1   | 2273    | four and a half LIM domains 1                         | Xq26       |
| FHL2   | 2274    | four and a half LIM domains 2                         | 2q12.2     |
| FKRP   | 79147   | fukutin related protein                               | 19q13.32   |
| FKTN   | 2218    | fukutin                                               | 9q31.2     |
| FXN    | 2395    | frataxin                                              | 9q21.11    |
| GAA    | 2548    | glucosidase alpha, acid                              | 17q25.2-q25.3|
| GATAD1 | 57798   | GATA zinc finger domain containing 1                  | 7q21-q22   |
| GCCKR  | 2646    | glucokinase (hexokinase 4) regulator                  | 2p23       |
| GJA5   | 2702    | gap junction protein alpha 5                         | 1q21.1     |
| GLA    | 2717    | galactosidase alpha                                   | Xq22       |
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| Gene   | Gene ID     | Gene description                                                                 | Chromosome |
|--------|-------------|-----------------------------------------------------------------------------------|------------|
| GP1D1L | 23174       | glycerol-3-phosphate dehydrogenase 1-like                                        | 3p22.3     |
| GPHBP1 | 338328      | glycosylphosphatidylinositol anchored high density lipoprotein binding protein 1 | 8q24.3     |
| HADHA  | 3030        | hydroxyacyl-CoA dehydrogenase/G-3-ketoacyl-CoA thiolase/enoyl-CoA hydratase        | 2p23       |
|         |             | (trifunctional protein), alpha subunit                                            |            |
| HCN4   | 10021       | hyperpolarization activated cyclic nucleotide gated potassium channel 4           | 15q24.1    |
| HFE    | 3077        | hemochromatosis                                                                   | 6p21.3     |
| HRAS   | 3265        | Harvey rat sarcoma viral oncogene homolog                                         | 11p15.5    |
| HSPB8  | 26353       | heat shock protein family B (small) member 8                                      | 12q24.23   |
| ILK    | 3611        | integrin linked kinase                                                             | 11p15.4    |
| JAG1   | 182         | jagged 1                                                                          | 20q12.1-p11.23 |
| JPH2   | 57158       | junctophilin 2                                                                     | 20q13.12   |
| JUP    | 3728        | junction plakoglobin                                                               | 17q21      |
| KCNA5  | 3741        | potassium voltage-gated channel subfamily A member 5                              | 12p13      |
| KCND3  | 3752        | potassium voltage-gated channel subfamily D member 3                              | 1p13.3     |
| KCNE1  | 3753        | potassium voltage-gated channel subfamily E regulatory subunit 1                  | 21q22.12   |
| KCNE2  | 9992        | potassium voltage-gated channel subfamily E regulatory subunit 2                  | 21q22.12   |
| KCNE3  | 10008       | potassium voltage-gated channel subfamily E regulatory subunit 3                  | 11q13.4    |
| KCNH2  | 3757        | potassium voltage-gated channel subfamily H member 2                              | 7q36.1     |
| KCNJ2  | 3759        | potassium voltage-gated channel subfamily J member 2                              | 17q24.3    |
| KCNJ5  | 3762        | potassium voltage-gated channel subfamily J member 5                              | 11q24      |
| KCNJ8  | 3764        | potassium voltage-gated channel subfamily J member 8                              | 12p11.23   |
| KCNQ1  | 3784        | potassium voltage-gated channel subfamily Q member 1                              | 11p15.5    |
| KLF10  | 7071        | Kruppel-like factor 10                                                             | 8q22.2     |
| KRAS   | 3845        | Kirsten rat sarcoma viral oncogene homolog                                        | 12p12.1    |
| LAMA2  | 3908        | laminin subunit alpha 2                                                            | 6q22-q23   |
| LAMA4  | 3910        | laminin subunit alpha 4                                                            | 6q21       |
| LAMF2  | 3920        | lysosomal associated membrane protein 2                                            | Xq24       |
| LDB3   | 11155       | LIM domain binding 3                                                               | 10q22.3-q23.2 |
| LDLR   | 3949        | low density lipoprotein receptor                                                   | 19p13.2    |
| LDLRAP1| 26119       | low density lipoprotein receptor adaptor protein 1                                | 1p36.11    |
| LMF1   | 64788       | lipase maturation factor 1                                                         | 16p13.3    |
| LMNA   | 4000        | lamin A/C                                                                         | 1q22       |
| LPL    | 4023        | lipoprotein lipase                                                                 | 8p22       |
| LTPB2  | 4053        | latent transforming growth factor beta binding protein 2                          | 14q24      |
| MAP2K1 | 5604        | mitogen-activated protein kinase kinase 1                                          | 15q22.1-q22.33 |
| MAP2K2 | 5605        | mitogen-activated protein kinase kinase 2                                          | 19p13.3    |
| MIB1   | 57534       | mindbomb E3 ubiquitin protein ligase 1                                             | 18q11.2    |
| MURC   | 347273      | muscle related coiled-coil protein                                                 | 9q31.1     |
| MYBPC3 | 4607        | myosin binding protein C, cardiac                                                 | 11p11.2    |
| MYH11  | 4629        | myosin, heavy chain 11, smooth muscle                                             | 16p13.11   |
| MYH6   | 4624        | myosin, heavy chain 6, cardiac muscle, alpha                                     | 14q12      |
| MYH7   | 4625        | myosin, heavy chain 7, cardiac muscle, beta                                      | 14q12      |
| MYL2   | 4633        | myosin light chain 2                                                               | 12q24.11   |
| MYL3   | 4634        | myosin light chain 3                                                               | 3p21.3-p21.2 |
| MYLK   | 4638        | myosin light chain kinase                                                          | 3q21       |
| MYLK2  | 85366       | myosin light chain kinase 2                                                        | 20q13.31   |
| MYO6   | 4646        | myosin VI                                                                         | 6q13       |
| MYOZ2  | 51778       | myozin 2                                                                          | 4q26-q27   |
| MYPN   | 84665       | myopalladin                                                                       | 10q21.3    |
| NEXN   | 91624       | nexilin F-actin binding protein                                                    | 1p31.1     |
| NKX2-5 | 1482        | NK2 homeobox 5                                                                    | 5q34       |
| NODAL  | 4838        | nodal growth differentiation factor                                                | 10q22.1    |
| NOTCH1 | 4851        | notch 1                                                                           | 9q34.3     |
| NPPA   | 4878        | natriuretic peptide A                                                              | 1p36.21    |
| NRAS   | 4893        | neuroblastoma RAS viral oncogene homolog                                          | 1p13.2     |
| PCSK9  | 255738      | proprotein convertase subtilisin/kexin type 9                                     | 1p32.3     |
| PDLIM3 | 27295       | PDZ and LIM domain 3                                                               | 4q35       |
| PKP2   | 5318        | plakophilin 2                                                                      | 12p11      |
| PLN    | 5350        | phospholamban                                                                     | 6q22.1     |
| PRDM16 | 63976       | PR domain 16                                                                       | 1p36.32    |
| PRKAG2 | 51422       | protein kinase AMP-activated non-catalytic subunit gamma 2                         | 7q36.1     |
| PRKAR1A| 5573        | protein kinase cAMP-dependent type I regulatory subunit alpha                      | 17q24.2    |
were validated using Sanger sequencing with the BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) following the standard protocol.

**Results**

**Clinical characteristics and severity of cardiovascular disease:** The clinical characteristics and phenotypes of all subjects are summarized in Table II. The proband (III-1) of this family was diagnosed with sick sinus syndrome and first-degree AVB at the age of 30 years. He has a strong family history of CCD; his grandfather, mother, and uncle all had pacemakers. Implantation of a pacemaker was recommended by his family physician that he originally rejected; however, he experienced syncope due to third-degree AVB at the age of 33 years (Figure 2A), and so a pacemaker was implanted. At the time, transasto-

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| Gene    | Gene ID | Gene description                      | Chromosome |
|---------|---------|---------------------------------------|------------|
| PTPN11  | 5781    | protein tyrosine phosphatase, non-receptor type 11 | 12q24      |
| RARF    | 5894    | Raf-1 proto-oncogene, serine/threonine kinase | 3p25       |
| RANGRF  | 29098   | RAN guanine nucleotide release factor   | 17p1       |
| RBM20   | 282969  | RNA binding motif protein 20           | 10q25.2    |
| RYR1    | 6261    | ryanodine receptor 1                   | 19q13.2    |
| RYR2    | 6262    | ryanodine receptor 2                   | 1q43       |
| SALL4   | 57167   | spalt-like transcription factor 4       | 20q13.2    |
| SCN1B   | 6324    | sodium voltage-gated channel beta subunit 1 | 19q13.1    |
| SCN2B   | 6327    | sodium voltage-gated channel beta subunit 2 | 11q23      |
| SCN3B   | 55800   | sodium voltage-gated channel beta subunit 3 | 11q23.3    |
| SCN4B   | 6330    | sodium voltage-gated channel beta subunit 4 | 11q23.3    |
| SCN5A   | 6331    | sodium voltage-gated channel alpha subunit 5 | 3p21       |
| SC02    | 9997    | SC02 cytochrome c oxidase assembly protein | 22q13.33   |
| SDHA    | 6389    | succinate dehydrogenase complex flavoprotein subunit A | 5p15       |
| SEPN1   | 57190   | selenoprotein N, 1                     | 1p36.13    |
| SGCB    | 6443    | sarcoglycan beta                       | 4q12       |
| SGCD    | 6444    | sarcoglycan delta                      | 5q33.3     |
| SGCG    | 6445    | sarcoglycan gamma                      | 13q12      |
| SHOC2   | 8036    | SHOC2 leucine-rich repeat scaffold protein | 10q25      |
| SLC25A4 | 291     | solute carrier family 25 member 4      | 4q35       |
| SLC2A10 | 81031   | solute carrier family 2 member 10      | 20q13.1    |
| SMAD3   | 4088    | SMAD family member 3                   | 15q22.33   |
| SMAD4   | 4089    | SMAD family member 4                   | 18q21.1    |
| SNTA1   | 6640    | syntrophin alpha 1                     | 20q11.2    |
| SOS1    | 6654    | SOS Ras/Rac guanine nucleotide exchange factor 1 | 2p21       |
| SREBF2  | 6721    | sterol regulatory element binding transcription factor 2 | 22q13      |
| TAZ     | 6901    | Tafazzin                               | Xq28       |
| TBX20   | 57057   | T-box 20                               | 7p14.3     |
| TBX3    | 6926    | T-box 3                                | 12q24.21   |
| TBX5    | 6910    | T-box 5                                | 12q24.1    |
| TCAP    | 8557    | titin-cap                              | 17q12      |
| TGFBR2  | 7042    | transforming growth factor beta 2       | 1q41       |
| TGFBR3  | 7043    | transforming growth factor beta 3       | 1q42       |
| TGFBR1  | 7046    | transforming growth factor beta receptor 1 | 9q22       |
| TGFBR2  | 7048    | transforming growth factor beta receptor 2 | 3p22       |
| TMEM43  | 79188   | transmembrane protein 43               | 3p25.1     |
| TMPO    | 7112    | thymopoietin                           | 12q22      |
| TNN1    | 7134    | troponin C1, slow skeletal and cardiac type | 3p21.1     |
| TNN3    | 7137    | troponin I3, cardiac type              | 19q13.4    |
| TNNT2   | 7139    | troponin T2, cardiac type              | 1q32       |
| TPM1    | 7168    | tropomyosin 1 (alpha)                  | 15q22.1    |
| TRDN    | 10345   | triadin                                | 6q22.31    |
| TRIM63  | 84676   | tripartite motif containing 63         | 1p34-p33   |
| TRPM4   | 54795   | transient receptor potential cation channel subfamily M member 4 | 19q13.33   |
| TTN     | 7273    | titin                                  | 2q31       |
| TTR     | 7276    | transthyretin                          | 18q12.1    |
| TXNRD2  | 10587   | thioroxygen reductase 2                | 22q11.21   |
| VCL     | 7414    | vinculin                               | 10q22.2    |
| ZBTB17  | 7709    | zinc finger and BTB domain containing 17 | 1p36.13    |
| ZHX3    | 23051   | zinc fingers and homeoboxes 3          | 20q12      |
| ZIC3    | 7547    | Zic family member 3                    | Xq26.2     |
Figure 2. Evidence of severe cardiac conduction disease and dilated cardiomyopathy in the proband (III-1). A: Results of a 12-lead electrocardiogram (ECG) showing third-degree atrioventricular block at 33 years of age. B: Results of a 12-lead ECG during ventricular tachycardia (VT) at 42 years of age. VT was suppressed by a combination therapy of antiarrhythmic drugs (200 mg amiodarone and 5 mg bisoprolol). C: Chest radiography showed cardiomegaly and congestive heart failure. A pacemaker was implanted in the left chest wall. D: Echocardiographic apical 4-chamber view at end diastole (left panel) and end systole (right panel) showing severe left ventricular cavity dilatation with reduced ejection fraction, and left atrial dilatation.

Table II. Clinical Characteristics and Phenotype of Family Members

| Subject       | Sex | Age at first evaluation (years/old) | Age at last evaluation (years/old) | Conduction defect | Arrhythmia | LVEF at last evaluation (%) | NYHA functional class | Age at death (years/old) | Manifestation |
|---------------|-----|-------------------------------------|-----------------------------------|-------------------|------------|-----------------------------|-----------------------|-------------------------|--------------------------|
| III-1 Proband | M   | 30                                  | 42                                | SSS               | AF, VT     | 29                          | IV                    | -                       | CCD + DCM                |
| III-2 Sister  | F   | 30                                  | 36                                | 1st-degree AVB    | None       | 64                          | I                     | -                       | CCD                     |
| II-1 Uncle    | M   | 40                                  | 52                                | SSS               | AF, VT     | 26                          | IV                    | 53                      | CCD + DCM                |
| II-2 Mother   | F   | 54                                  | 67                                | 3rd-degree AVB    | AF         | 49                          | II                    | -                       | CCD + DCM                |
| II-3 Farther  | M   | -                                   | 68                                | SSS               | None       | None                        | -                     | -                       | Healthy                 |

AF indicates atrial fibrillation; AVB, atrioventricular block; CCD, cardiac conduction disease; CRT-D, cardiac resynchronization therapy defibrillator; DCM, dilated cardiomyopathy; LVEF, left ventricular ejection fraction; SSS, sick sinus syndrome; and VT, ventricular tachycardia.

Racemic echocardiography showed mild left ventricular dilatation and a mild reduced left ventricular ejection fraction (LVEF) of 49%. During follow-up, he developed deterioration in systolic function and the onset of paroxysmal atrial fibrillation. At 42 years of age, he underwent his first emergency hospitalization for worsening heart failure with ventricular tachycardia (VT) (Figure 2B). Chest radiography showed cardiomegaly and congestive heart failure (Figure 2C) and transthoracic echocardiography revealed a decreased LVEF of 29% (Figure 2D). His dual-chamber (DDD) pacemaker was therefore upgraded to a cardiac resynchronization therapy defibrillator (CRT-D).

His younger sister (III-2) had only first-degree AVB and no cardiac event without medications. Her 12-lead ECG and echocardiography at the start and end of the 6-year follow-up are shown in Figure 3. The PQ interval in her 12-lead ECG was gradually prolonged without deterioration of LVEF during the follow-up period.

Two subjects in the parent generation of the proband have both CCD and DCM. His uncle (II-1) had CCD and severe DCM. Although bradycardia due to sick sinus syndrome and second-degree AVB were identified in a health
check at 41 years of age, the subject rejected detailed investigation and treatment. However, he was admitted to hospital because of worsening heart failure and third-degree AVB at the age of 49, and required a DDD pacemaker. At that time, his LVEF had decreased to 40%. Despite the pacemaker implantation and medication, he experienced recurrent events of worsening heart failure. His LVEF decreased to <30% within several months, and an upgrade to a CRT-D was performed at 52 years of age (Figure 4). Unfortunately, CRT did not result in amelioration of his condition and he passed away at the age of 53 years from multiple organ failure resulting from heart failure. This subject’s sister (II-2), the mother of the proband, required a DDD pacemaker because of third-degree AVB at 54 years of age, but her cardiac function condition was relatively mild. Although her LVEF gradually decreased during follow-up, she did not require emergency hospitalization for worsening heart failure.

Changes in LVEF in all four subjects with CCD are shown in Figure 5. Intra-familial clinical variability and gender differences were observed in this family. CCD onset was earlier in male subjects compared to female subjects, and the DCM phenotypes were notably more severe in affected males than those found in affected females (Figure 5).

**Genetic analysis:** After filtering and data analysis using VariantStudio, a single rare variant associated with inherited cardiovascular disease was detected in this family, a novel heterozygous truncating LMNA mutation (c.774delG) was identified in all four subjects (II-1, II-2, III-1, and III-2) with CCD, and confirmed by Sanger sequencing (Figure 6). Based on our *in silico* analysis, this mutation is predicted to cause a frameshift mutation resulting in premature termination (p.Gln258HisfsTer222) of LMNA. Because this mutation was found in an amino acid region that is highly conserved among species, and is localized within a functionally important domain, we therefore believe that this LMNA mutation was the causal mutation in this Japanese family with CCD and laminopathies.

In addition, a heterozygous RBM20 variant (c.224C>T, p. Ser75Leu) was identified in three of these subjects (II-1, II-2, and III-1). This variant affects an amino acid that is highly conserved among species and was localized within a functionally important domain, and PolyPhen and SIFT analyses suggested this variant was probably damaging or deleterious. However, the ClinVar archives (https://www.ncbi.nlm.nih.gov/clinvar/variation/202052) described too that this mutation was observed in 1.1% of Japanese ancestry in the 1000 genomes database, which indicates it
Figure 4. Evidence of severe cardiac conduction disease and dilated cardiomyopathy in the uncle (II-1) of the proband. A: Results of the 12-lead electrocardiogram (ECG) after implantation of a cardiac resynchronization therapy defibrillator (CRT-D). The ECG showed atrial fibrillation and all biventricular pacing; the QRS morphologies were wide and there was a QS pattern in leads V1-V6. B: Chest radiography showed significant cardiomegaly and congestive heart failure. A CRT-D was implanted in the left chest wall. C and D: Echocardiographic apical 4-chamber view at (C) end diastole and (D) end systole showing severe left ventricular cavity dilatation with reduced ejection fraction, and left atrial dilatation. E: Pulsed-wave Doppler ultrasonography showing a tall E wave (E = 112 cm/s). The A wave was not observed because of persistent atrial fibrillation. F: Tissue Doppler ultrasonography showing severely reduced early diastolic tissue Doppler velocity (e’ = 4.3 cm/s). E/e’ = 26.

Discussion

In the present study, we discovered from targeted re-sequencing of 174 genes a novel heterozygous truncating LMNA mutation (c.774delG) in a Japanese family with CCD and DCM. In addition, we found intra-familial clinical variability between the carriers of this LMNA mutation that was associated with gender differences in clinical severity.

LMNA is one of the most common causal genes of CCD and DCM.3,9,10,12,19-21 This newly discovered c.774 delG mutation causes a predicted frameshift mutation (p. Gln258HisfsTer222) resulting in a truncated protein. Laminopathies primarily result from missense mutations; it is less common for them to be because of nonsense or splice-site mutations or insertions/deletions (indels).21-24 In LMNA mutation carriers, non-missense mutations may result in more severe cardiac events such as malignant ventricular arrhythmias than from missense mutations.21,23 Van Rijsingen et al evaluated risk factors for malignant ventricular arrhythmias in a multicenter cohort of 269 LMNA mutation carriers and found that non-missense mutations (indels, truncating mutations, or mutations affecting splicing) were an independent risk factor for malignant ventricular arrhythmias.23 In the present study, we found that our subjects had a high risk of VT, with two subjects implanted with CRT-D pacemakers because of VT and heart failure with dyssynchrony. Although we did not find VT in two other female subjects in this family, it may subsequently develop as these conditions have a late age-of-onset. Anselme, et al evaluated a prophylactic strategy of implantable cardioverter-defibrillator (ICD) implantation in LMNA mutation carriers with significant CCD,25 and found that even when LVEF was preserved, malignant ventricular arrhythmias were common in these subjects. It may be a benign variant in Japanese people. Therefore, the clinical significance of this variant is uncertain in this Japanese family. From our genetic analysis, no other predicted or possibly disease modifying mutations including synonymous mutations were identified apart from these two variants.
Figure 5. Clinical course and changes in left ventricular ejection fraction in the affected members of this family. Note that gender may be associated with clinical severity of the cardiac conduction disorder and dilated cardiomyopathy found in affected individuals. Affected males (II-1 and III-1) experienced an earlier onset of CCD and more severe DCM compared to affected females (II-2 and III-2).

They concluded that ICD is an effective treatment and should be considered in LMNA mutation carriers with CCD. Therefore, regarding the family in the present study, we recommend that the mother and sister of the proband should be kept under careful observation.

During our investigation, we observed a gender difference in the family studied in which affected males experienced an earlier onset of CCD and a more severe DCM phenotype compared to affected females. Although laminopathies have been considered monogenic, they exhibit a remarkable degree of clinical variability in severity, penetrance, and age at onset. Clinical variability has

| Gene   | Description | Chromosome | Nucleotide change | Type      | Effect of protein               | Conserved sequence | Previously reported |
|--------|-------------|------------|-------------------|-----------|--------------------------------|--------------------|---------------------|
| LMNA   | lamin A/C   | 1          | c.774delG         | Truncation| p.Gln258HisfsTer222            | Yes                | Novel mutation      |

Figure 6. A: Electropherograms of direct sequencing showing (left panel) wild-type LMNA in a healthy control and (right panel) a novel heterozygous truncating LMNA mutation (c.774delG) leading to a predicted frameshift and premature stop codon (p.Gln258HisfsTer222). Arrows indicate the mutant nucleotide position. B: Detailed genetic information of the LMNA mutation.
been observed among family members with the same LMNA mutation; however, the cause of the intra-familial clinical variability has remained unclear. A recent study revealed significant gender differences in cardiac phenotypes such as higher mortality, and more severe cardiac dysfunction in males with DCM carrying an LMNA mutation. In addition, there have been several reports of adverse events occurring earlier and/or more frequently in male mutation carriers than in female carriers with inherited cardiomyopathy. Furthermore, Van Rijssingen, et al reported that male gender was an independent risk factor for severe ventricular arrhythmias in patients with laminopathies, whereas Herman, et al reported that males with DCM and mutations in titin (TTN) experienced adverse events at significantly earlier ages compared to that found in affected females. It has been suggested that gonadal hormones may explain the gender difference found in cardiac phenotypes. Results from in vitro and in vivo studies indicate that estrogen may play a pivotal role, in part because of its known protective effects, as evidenced by increased cardiovascular risk in women after menopause and by the cardiovascular benefits of estrogen replacement therapy. LVEF in the mother (II-2) of the proband gradually deteriorated after menopause, which would be consistent with a female hormone hypothesis. Indeed, nuclear accumulation of the androgen receptor has been associated with specific LMNA mutations that show a gender difference in the disease progression of DCM. The present study has a few limitations. First, the findings are from the analysis of a single family consisting of a small number of subjects with laminopathies. Further molecular and functional investigation is warranted to assess the predicted effects of rare variants associated with laminopathies. Second, the observed gender difference in this family remains tentative because the sister of the proband was young at the time of the investigation. Several reports have described DCM onset occurring after the age of 40 years. Therefore, long-term follow-up is needed to confirm whether there is a gender difference in this family. In addition, the influence of gender on the clinical severity of inherited cardiovascular disease caused by an autosomal monogenic mutation is still unclear and requires further investigation in a large cohort. Conclusion: We discovered using targeted resequencing a novel truncating LMNA mutation associated with CCD and DCM in a Japanese family. In addition, we observe there may be gender differences associated with disease onset and severity between the affected members of the family. Furthermore, we propose that at least some patients with laminopathy, clinical severity may be the result of multiple factors.

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Disclosures
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