Gene-Based Risk Stratification for Cardiac Disorders in *LMNA* Mutation Carriers

（ラミン遺伝子変異キャリアにおける遺伝子型を用いた心疾患リスクの層別化）

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Original Article

Gene-Based Risk Stratification for Cardiac Disorders in *LMNA* Mutation Carriers

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Background—Mutations in *LMNA* (*lamin A/C*), which encodes lamin A and C, typically cause age-dependent cardiac phenotypes, including dilated cardiomyopathy, cardiac conduction disturbance, atrial fibrillation, and malignant ventricular arrhythmias. Although the type of *LMNA* mutations have been reported to be associated with susceptibility to malignant ventricular arrhythmias, the gene-based risk stratification for cardiac complications remains unexplored.

Methods and Results—The multicenter cohort included 77 *LMNA* mutation carriers from 45 families; cardiac disorders were retrospectively analyzed. The mean age of patients when they underwent genetic testing was 45±17, and they were followed for a median 49 months. Of the 77 carriers, 71 (92%) were phenotypically affected and showed cardiac conduction disturbance (81%), low left ventricular ejection fraction (<50%; 45%), atrial arrhythmias (58%), and malignant ventricular arrhythmias (26%). During the follow-up period, 9 (12%) died, either from end-stage heart failure (n=7) or suddenly (n=2). Genetic analysis showed truncation mutations in 58 patients from 31 families and missense mutations in 19 patients from 14 families. The onset of cardiac disorders indicated that subjects with truncation mutations had an earlier occurrence of cardiac conduction disturbance and low left ventricular ejection fraction, than those with missense mutations. In addition, the truncation mutation was found to be a risk factor for the early onset of cardiac conduction disturbance and the occurrence of atrial arrhythmias and low left ventricular ejection fraction, as estimated using multivariable analyses.

Conclusions—The truncation mutations were associated with manifestation of cardiac phenotypes in *LMNA*-related cardiomyopathy, suggesting that genetic analysis might be useful for diagnosis and risk stratification. **(*Circ Cardiovasc Genet*. 2017;10:e001603. DOI: 10.1161/CIRCGENETICS.116.001603.)**

Key Words: arrhythmia ■ cardiomyopathies ■ death, sudden, cardiac ■ heart failure ■ lamin type A ■ mutation ■ prognosis

The *LMNA* (*lamin A/C*) gene encodes the A-type lamin proteins, lamin A and C—the major components of the nuclear membrane in vertebrates—and mutations in *LMNA* have been reported to cause a variety of clinical phenotypes, including cardiac disorders,[1] Emery–Dreifuss muscular dystrophy,[2,3] limb-girdle muscular dystrophy,[4] Charcot–Marie–Tooth type 2,[5] familial partial lipodystrophy,[6,7] and premature aging.[8,9] The cardiac phenotypes associated with *LMNA* mutations are characterized by cardiac conduction disturbance (CCD), atrial fibrillation, ventricular tachyarrhythmia (VT), and dilated cardiomyopathy.[10–14]

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Risk stratification for *LMNA*-related cardiomyopathy has been assessed previously in some reports. Approximately 90% of *LMNA* mutation carriers >30 years of age have some type of arrhythmia, and the subjects have a risk of sudden arrhythmic death before the development of cardiac failure[15] Recently, Kumar et al[16] reported the long-term follow-up data of 122 consecutive *LMNA* mutation carriers and showed that *LMNA*-related heart diseases had a malignant course; most

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patients experienced arrhythmia, heart block, embolic events, or heart failure within 7 years of diagnosis.

Several studies have also explored genotype–phenotype correlations; frameshift mutations were often associated with the cardiac disorders in laminopathies,[17] and splice-site mutations were one of the independent risk factors for sudden cardiac death.[13] Recently, van Rijsingen et al[18] revealed that nonmissense mutations were (ins-del/truncating or mutations affecting splicing) one of the major independent risk factors for malignant ventricular arrhythmias (MVAs) in a large European cohort of 269 *LMNA* mutation carriers. These results indicated that patients with truncation *LMNA* mutations, which result in haploinsufficiency of A-type lamins, might be at significantly higher risk of sudden cardiac death because of MVAs, than missense mutation carriers. However, other cardiac complications, such as left ventricular systolic dysfunction and CCD, in *LMNA* mutation carriers have not yet been fully investigated in terms of the type of mutations. Based on the available literature, we hypothesized that truncation mutations might be associated with poor prognosis in *LMNA* mutation carriers because of CCD, MVAs, and heart failure. To test this hypothesis, we performed a multicenter retrospective cohort study.

Methods

Study Design

The cohort for the present study consisted of genotyped *LMNA* mutation carriers and their relatives who had been introduced to the Kyoto University Hospital (Kyoto, Japan), Shiga University of Medical Science (Shiga, Japan), Nagasaki University Graduate School of Biomedical Sciences (Nagasaki, Japan), Niigata University Graduate School of Medical and Dental Sciences (Niigata, Japan), University of Tsukuba (Ibaraki, Japan), and the National Cerebral and Cardiovascular Center (Osaka, Japan) for genetic screening of *LMNA* between April 2008 and October 2015. Carriers of compound heterozygous mutations in *LMNA* and *SCN5A* were not included in this cohort. All subjects willingly provided informed consent for participating in this study. After providing informed consent, the patients were subjected to clinical screening and underwent peripheral blood sampling for genetic testing. Detailed clinical information was obtained from each subject by cardiologists in the respective institutes. After the genetic diagnosis, *LMNA* mutation carriers were followed up by a cardiologist from one of the participating institutes or by a private doctor. The primary end point in this analysis was all-cause death during the follow-up period. The secondary end point was a composite of cardiac events and diagnosis, including the first occurrence of CCD, atrial arrhythmias, low left ventricular ejection fraction (LVEF), or MVAs. The protocol of this study was approved by the Institutional Ethics Committee and performed in accordance with its guidelines (Kyoto University, G194; National Cerebral and Cardiovascular Center, M24-031-4).

Genetic Analysis

The methods followed for DNA extraction, for generating the PCR primers, and for mutational screening are described in Appendix in the Data Supplement and Table I in the Data Supplement. The mutations were divided into 2 groups: truncation (including splice site, frameshift insertion, frameshift deletion, and nonsense mutations) and missense mutations. We also classified the mutations based on the site: mutations located upstream (nuclear localization signal [NLS] class 1) of the spanning residues 416 to 423 and mutations located downstream (NLS class 2; Figure 1).[13,19] If a frameshift or nonsense mutation was located upstream of the NLS residue, or a missense mutation disturbed the base sequence of the NLS residue, it was defined as an NLS-disturbed mutation. All the other mutations that did not disturb the NLS sequence were defined as NLS-conserved mutations.

Bioinformatic Analysis

Variants in *LMNA* were screened by genetic variant databases, a splicing site prediction tool, and in silico predictions (details in Appendix in the Data Supplement). Additionally, we confirmed the pathogenicity of mutations by using cascade screening, after accommodating relatives. Of the 84 carriers from 51 families genotyped by screening, 7 carriers from 6 families were excluded (Table 1; Table II in the Data Supplement) because unreported *LMNA* variants without enough clinical or genetic data of relatives were considered variants of unknown significance.

Clinical Definitions

Low LVEF was defined as LVEF <50%. dilated cardiomyopathy was defined as low LVEF or left ventricular enlargement, as per published normal values.[20] MVAs were defined as sustained VT, ventricular fibrillation, sudden cardiac death, cardiopulmonary resuscitation, and appropriate implantable cardioverter defibrillator (ICD) treatment (an ICD discharge for termination of ventricular fibrillation/VT and antitachycardia pacing for sustained VT). Nonsustained VT was defined as ≥3 consecutive ventricular beats at >120 bpm[21] and a duration for <30 seconds. A history of sudden cardiac death within a family was considered positive, if at least 1 relative (up to the fourth degree) had died suddenly before the age of 60 years. Atrioventricular blocks were classified into first, second, or third degree. First-degree atrioventricular block was defined by a PQ interval >200 ms. Sinus node dysfunction was defined as heart rate <45 per minute or sinus pause >3 seconds. CCD was defined as having sinus node dysfunction or any degree of atrioventricular block. Atrial arrhythmia included atrial fibrillation, atrial flutter, and paroxysmal supraventricular tachycardia.

Statistical Analysis

The JMP Pro 12.2.0 software (SAS Institute, Inc, Cary, NC) was used for statistical analyses. Continuous variables were expressed as the mean and SD or as the median with interquartile range. Categorical variables were expressed as numbers and percentages. We compared continuous variables using the Student *t* test or the Welch *t* test, based on the type of distribution. We compared categorical variables using the Pearson χ^2 test when appropriate or the Fisher exact test. Logistic regression models were used to investigate the association of the type of mutation with events. Odds ratios and 95% confidence intervals were calculated. All statistical analyses were 2-tailed, and *P* values <0.05 were considered statistically significant.

Results

Study Population and Clinical Characteristics

This study included a multicenter cohort of 45 probands with pathogenic *LMNA* mutations and 32 genotypically affected relatives (77 carriers in total from 45 different families). Of these 77 *LMNA* mutation carriers, 49 (63.6%) were men, and the mean age at entry was 45±16 years for men and 41±17 years for women. The cascade screening identified truncation mutations in 58 subjects (75%) from 31 families and missense mutations in 19 subjects (25%) from the remaining 14 families (Table 1). As shown in Table 2, there were no significant differences in the baseline characteristics, except for mean age between truncation and missense mutation groups. However, the prevalence of low LVEF (<50%) was significantly higher in the truncation mutation group than in the missense mutation group among the probands, at first clinical contact (Table III in the Data Supplement).

Genotype Analysis

In the cohort of this study, 11 missense mutations in 14 probands and 26 truncation mutations in 31 probands were

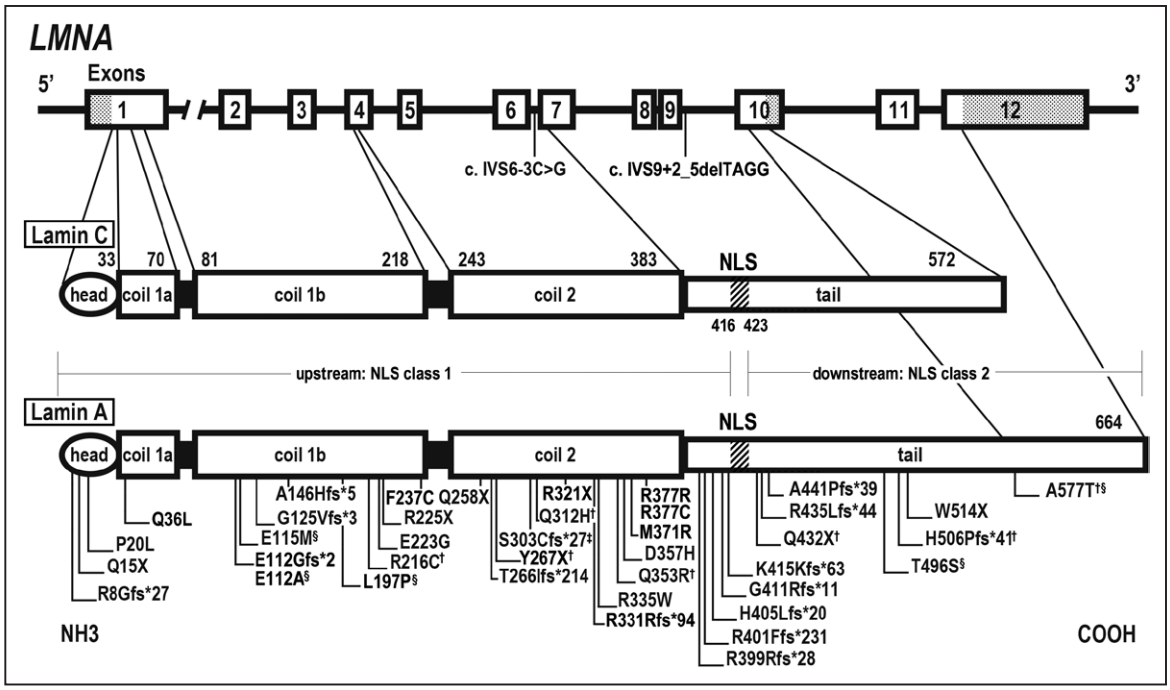

Figure 1. Structure of *LMNA* (*lamin A/C*) (**top**) and lamin A (**bottom**) and C (**middle**) protein. *LMNA* consists of 12 exons. The lamin A and C proteins comprise the N-terminal globular head domain, α-helical rod domain (coil 1a, 1b, and 2 were separated by linkers: black boxes), and C-terminal globular tail domain, which included the nuclear localization signal (NLS) sequence that spans residues 416 to 423 (diagonal stripes). The noncoding regions are indicated by the black dotted patterns. The locations of 37 mutations identified in the subjects and the 5 excluded variants are shown in the topology of the lamin A and C proteins. †Mutations identified in 2 probands, ‡mutations identified in 4 probands, §variants excluded because of unknown significance.

identified, including 9 (20%) nonsense, 3 (6.7%) splice site, 16 (35.6%) frameshift deletion, and 3 (6.7%) frameshift insertion mutations (Table 1). Among the identified mutations, 23 (62%) were novel: 12 frameshift deletion, 3 frameshift insertion, 4 nonsense, 3 splice site, and 1 missense mutation (novel mutations are indicated by boldface in Table 1). All identified missense variants were screened using the databases of Human Genetic Variation Database, Exome Aggregation Consortium, and ClinVar (Table II in the Data Supplement). We found no available data in Exome Aggregation Consortium, whereas 4 variants were listed on ClinVar, of which 2 were considered pathogenic. Thirty-seven mutations identified in 45 probands, with 5 variants excluded, were located in the topology of the LMNA protein (Figure 1). Among these 37 mutations, 30 (81.1%) were located upstream of the spanning residue (NLS class 1), and the remaining 7 (18.9%) mutations were located downstream (NLS class 2). In 25 probands, the deletion, frameshift, or occurrence of stop codon was predicted to cause disturbance in the NLS residue of the mutant allele.

All 26 identified truncation mutations in *LMNA* from the 31 families were considered to be pathogenic because of the premature termination codon generated by frameshift, aberrant splice site, or nonsense mutations. As for the 11 missense mutations in the 14 families, the pathogenicity of 5 had been confirmed in several previous studies[22–25] and using Single Nucleotide Polymorphism database. Another novel missense mutation was deemed pathogenic after cascade screening and using in silico prediction tools (Table II in the Data Supplement).

Manifestation of Cardiac Phenotypes

Missense Versus Truncation Mutations

The age of onset in cardiac disorders revealed that patients in the CCD and low LVEF developed at significantly younger age in truncation mutation group compared with the missense mutation group (Figure 2). To exclude the bias of the number of family members, we further compared the age of onset only in the probands between truncation and missense mutations. As shown in Figure 3, a significant difference was only observed in the CCD because of a small number of patients, especially in the missense mutation, but outcomes show the same tendency.

When the prevalence of cardiac disorders at last follow-up (median follow-up period, 49.0 months; 11.1–95.9) was analyzed, we found no significant difference in the baseline characteristics, except for mean age, of the truncation and missense mutation groups (Table 3). However, regarding the age-dependent penetrance of cardiac disorders, the prevalence was higher at age <40 (CCD), <50 (CCD, atrial arrhythmias, lower LVEF, and MVA), and <60 (atrial arrhythmias, lower LVEF, and MVAs) in the truncation mutation carriers compared with the missense mutation carriers (Figure 4). When only probands were considered, prevalence of low LVEF was significantly higher in the truncation mutation group than in the missense mutation group (Tables III and IV in the Data Supplement).

At the end of the follow-up period, the prevalence of phenotypically affected carriers was 71 of the 77 carriers (92%) with a mean age of 48±17 years. A total of 36 (47%) patients were affected by low LVEF, and 20 subjects (26%) had experienced at least 1 episode of MVAs. Nine subjects (12%) died because of end-stage heart failure with a mean age of 58

Table 1. Genomic Characteristics and Clinical Phenotypes in 51 Families (Including 6 Excluded Families)

LMNA Gene Mutation, codon	Protein	Exon	NLS Class	Proband Phenotype (No. of Probands, >1)	No. of Families	No. of Subjects	No. of Affected Subjects at Last Follow-Up
Truncation mutation							
Frameshift deletion mutation							
16_32del*	R8Gfs*27*	1	1	AVB, DCM, VT, AF	1	1	1
374del*	G125Vfs*3*	2	1	SSS	1	4	3
436_446del*	A146Hfs*5*	2	1	AVB, DCM, VF, AF	1	2	2
797del*	T266Ifs*214*	4	1	AVB, DCM, AF	1	1	1
908_909del	S303Cfs*27	5	1	AVB (4), SSS, DCM (2), VT (3), AF (4)	4	14	12
1201_1205del*	R401Ffs*231*	7	1	AVB, DCM, VT, AF	1	1	1
1212_1213del*	H405Lfs*20*	7	1	AVB, DCM, AF	1	1	1
1231_1241del*	G411Rfs*11*	7	1	AVB, DCM, VT, AF	1	1	1
1249del*	K415Kfs*63*	7	1	AVB, SSS, DCM, AF	1	1	1
1304_1307del*	R435Lfs*44*	7	2	AVB, DCM, VF, AF	1	1	1
1321del*	A441Pfs*39*	7	2	AF	1	1	1
1516del*	H506Pfs*41*	8	2	SSS, DCM, AF	1	3	3
1517del*	H506Pfs*41*	8	2	AVB, SSS, VT, AF	1	2	2
Frameshift insertion mutation							
335insG*	E112Gfs*2*	1	1	SSS, DCM, VT, AF	1	1	1
992insG*	R331Rfs*94*	6	1	AV, DCM	1	1	1
1196_1199dup*	R399Rfs*28*	7	1	AVB, SSS, DCM	1	1	1
Nonsense mutation							
43C>T*	Q15X*	1	1	AVB, SSS, DCM, VT, AF	1	1	1
673C>T	R225X	4	1	AVB, VT	1	2	2
772C>T*	Q258X*	4	1	AVB, DCM, VT, VF, AF	1	2	2
801T>A*	Y267X*	4	1	DCM, AF (2), VT	2	4	4
961C>T	R321X	6	1	VT, AF, DCM	1	1	1
1294C>T	Q432X	7	2	VT (2), SSS, DCM, AF	2	6	4
1542G>A*	W514X*	8	2	SSS, DCM, AF	1	1	1
Splice-site mutation							
1131C>T*	R377R*	6	1	AVB, DCM, AF	1	1	1
IVS6-3C>G*	…*	intron 6	1	AVB	1	2	1
IVS9+2_5delTAGG*	…*	intron 9	2	AVB, DCM, AF, VT	1	2	2
Missense mutation							
59C>T	P20L	1	1	AVB	1	1	1
107A>T	Q36L	1	1	AVB, SSS, AF	1	1	1
335A>C*†	E112A*	1	1	AVB, VT	1	1	1
[343G>A;344A>T]*†	E115M*	1	1	AVB, DCM, VT, AF	1	1	1
590T>C*†	L197P*	3	1	AVB, SSS, DCM, VT, AF	1	1	1
646C>T	R216C	4	1	VF, AVB	2	2	2
668A>G*	E223G*	4	1	SSS	1	5	5
710T>G	F237C	4	1	SSS, DCM	1	1	1
936G>C	Q312H	5	1	AVB, DCM, VT, AF	2	2	2

(*Continued*)

Table 1. Continued

LMNA Gene Mutation C	p.	Exon	NLS Class	Proband Phenotype (No. of Probands, >1)	No. of Families	No. of Subjects	No. of Affected Subjects at Last Follow-Up
1003C>T	R335W	6	1	SSS, DCM	1	2	2
1058A>G	Q353R	6	1	AF, VT	2	2	2
1069G>C	D357H	6	1	AVB, VT, AF	1	1	1
1112T>G	M371R	6	1	AVB, AF	1	1	1
1129C>T	R377C	6	1	AVB, AF, DCM	1	1	1
1486A>T*†	T496S*	8	2	VT	1	1	1
1729G>A*†	A577T*	11	2	VT, VF, AMI	2	3	2

NLS class: classification described in Methods. AF indicates atrial fibrillation; AMI, acute myocardial infarction; AVB, atrioventricular block; DCM, dilated cardiomyopathy; *LMNA*, *lamin A/C* gene; NLS, nuclear localization signal; SSS, sick sinus syndrome; VF, ventricular fibrillation; and VT, ventricular tachycardia.

*Novel variants.

†Variants excluded because of unknown significance.

years, and 2 men carrying the truncation mutations died suddenly. The 6 phenotypically unaffected mutation carriers were relatively younger than the affected relatives (17±13 versus 34±18 years old; P=0.002). In addition, we found no correlation between the type of mutations and the manifestation of cardiac phenotypes in relatives.

Device Therapy in LMNA-Related Cardiomyopathy

During the follow-up period, additional 7 subjects developed CCD, 7 showed a progression in CCD, 12 received a pacemaker, 9 underwent an ICD implantation, and 24 underwent cardiac resynchronization therapy defibrillator implantation (including upgraded cases) in all carriers. Among the 28 patients initially implanted with pacemaker, 25 of them had not been genetically diagnosed as carriers of *LMNA* mutations. After the genetic diagnosis, 12 of these 28 were upgraded to defibrillator during the follow-up period. The remaining 16 patients were alive without any MVA event, but 1 patient died because of end-stage heart failure. The percentage of patients with implantable devices increased substantially with age, and the proportion of cardiac resynchronization therapy defibrillator–implanted patients increased in the older population (Figure 5).

Probands Versus Relatives

The age of onset in cardiac disorders was comparable between probands and relatives (Figure I in the Data Supplement). The prevalence of cardiac disorders also did not differ between them at the last follow-up.

NLS-Disturbed Mutations Versus NLS-Conserved Mutations

Regarding the site of mutations, the majority of subjects with cardiomyopathy and CCD had mutations upstream of the NLS, spanning residues 416 to 423. This region was reported to be essential for the structure and the translocational regulation of the nucleus.[19] In this study, the onset of cardiac disorders was not associated with disturbance of the NLS residue (Figure II in the Data Supplement).

Sex Difference

Male sex was reported to be at higher risk for MVAs and end-stage heart failure in laminopathy.[18,26] We also assessed the impact of sex differences on clinical outcome (Figure III in the Data Supplement); however, the onset of cardiac disorders was comparable between male and female carriers. Therefore, sex difference did not affect the clinical outcomes in this cohort.

Risk Stratification

We performed multivariable analysis using logistic regression model to evaluate which clinical character, such as type of mutations, sex, and probands, is actually associated with occurrence of cardiac disorders. As shown in Figure 4, the penetrance of CCD at age <60 years was ≈100% both in the truncation and missense mutations. Therefore, only for CCD, we evaluated those with age <50 years. As a result of multivariable analysis, the truncation mutation carriers were at higher risk for the occurrence of CCD under 50-year-old and that of atrial arrhythmias and low LVEF under 60-year-old compared with the missense carriers (Table 4). The event rate of MVAs in missense mutations was small during the follow-up period in this study, and risk evaluation for MVAs was not fully convinced by results of this study. On the contrary, proband and male sex did not reach statistical significance.

Discussion

Our multicenter cohort study showed the age-dependent and high prevalence of cardiac manifestations in *LMNA* mutation carriers. To the best of our knowledge, this is the first study on *LMNA*-related cardiomyopathy in an Asian country to provide extensive information on the progressive course of cardiac phenotypes. The high penetrance in *LMNA* mutation carriers found in this study is similar to that found in other studies on caucasians.[13,27,28] Furthermore, truncation mutations carriers showed a significantly earlier manifestation of the cardiac phenotypes of laminopathy than missense mutation carriers, suggesting that genetic analysis may play an important role in both the diagnosis and risk stratification in probands with *LMNA* mutations and their relatives.

Genotype–Phenotype Correlations

Previous reports have suggested that the type or site of *LMNA* mutations might be related to the occurrence and prognosis of the cardiac phenotypes.[13,16,18,19] According to results of this study, the truncation mutations were associated with the onset of CCD, atrial arrhythmia, and low LVEF (Figure 2; Table 4).

Table 2. Characteristics of 77 Carriers at First Clinical Contact

	Total	Truncation	Missense	
	n=77	n=58	n=19	*P* Value
Age, y		41.1±16.5	52.2±12.8	0.009*
Men	49	36 (62)	13 (68)	0.78
Probands/families	45/32	31/27	14/5	0.18
Symptom				
Syncope	9	6 (10)	3 (15)	0.68
NYHA classification ≥3	9	7 (12)	2 (11)	1.0
Arrhythmia				
Atrial arrhythmia	37	28 (48)	9 (47)	1.0
Ventricular arrhythmia	21	17 (29)	4 (21)	0.57
CCD	55	39 (67)	16 (84)	0.24
SSS	20	12 (21)	8 (42)	0.08
AV block (≥1)	41	33 (57)	8 (42)	0.29
Cardiomyopathy				
LVEF <50%	26	22 (38)	4 (20)	0.26
LV enlargement	21	17 (29)	4 (20)	0.56
Comorbidities				
Coronary artery disease	2	2 (3)	0 (0)	1.0
Hypertension	9	5 (9)	4 (21)	0.21
Diabetes mellitus	1	0 (0)	1 (5)	1.0
PM implantation	15	10 (17)	5 (26)	0.50
ICD/CRT-D implantation	3	3 (5)	0 (0)	0.57
Medication				
Anticoagulant	18	15 (26)	3 (16)	0.53
β-Blocker	18	14 (24)	4 (21)	1.0
ACE-I inhibitor or ARB	24	16 (28)	8 (42)	0.26

Number of subjects are expressed as n (%). Continuous variables are shown as mean±SD. ACE indicates angiotensin converting enzyme; ARB, angiotensin receptor blocker; AV, atrioventricular; CCD, cardiac conduction disturbance; CRT-D, cardiac resynchronization therapy defibrillator; ICD, implantable cardioverter defibrillator; LV, left ventricular; LVEF, left ventricular ejection fraction; NYHA, New York Heart Association; PM, pacemaker; and SSS, sick sinus syndrome.

*P value <0.05.

The mechanism behind the earlier occurrence of cardiac disorders in the truncation mutation group might be related to the haploinsufficiency of A-type lamins or to the dominant negative fashion because of the truncated amino terminus of lamin proteins. A previous report on a heterozygous mouse model harboring the exon 8 to 10 deletion in *LMNA*[29] showed that an abnormal expression of lamin protein directly impaired contractility and caused early-onset programmed cell death of atrioventricular nodal cardiomyocytes.[30] For missense mutations that do not cause a dominant negative effect or haploinsufficiency, a previous analysis of the structural and dynamic properties using molecular dynamics simulations had indicated that the severity of a functional problem, such as

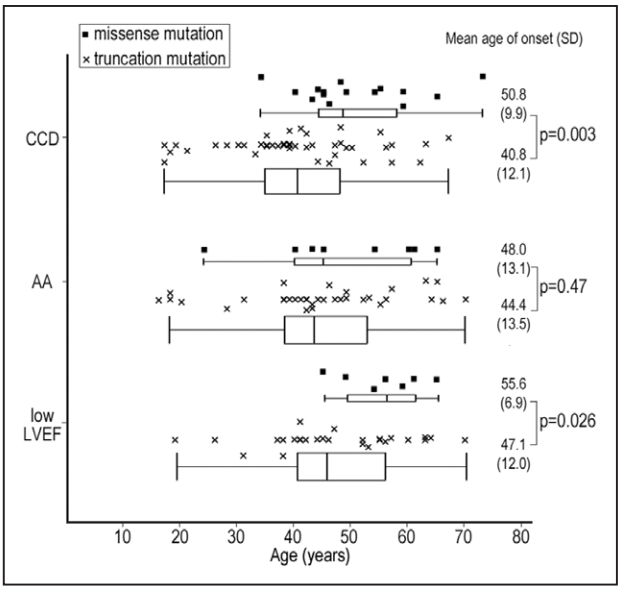

Figure 2. Age of onset in each cardiac phenotype in 77 *LMNA* (*lamin A/C*) mutation carriers with truncation mutation (x mark) and missense mutation (solid square mark). AA indicates atrial arrhythmias; CCD, cardiac conduction disturbance; and LVEF, left ventricular ejection fraction.

a protein stability, of A-type lamins could depend on the type or site of the mutations.[31] It has been speculated that some missense mutations might retain the partial function of wild-type A-type lamins and thus would not have a dominant negative effect. Therefore, the prognosis of the cardiac phenotypes might be better in the missense mutation group than in the truncation mutation group. Thus, the genetic analyses would be useful not only for diagnosing the laminopathy before the manifestation of the phenotype but also for stratifying the prognosis of carriers.

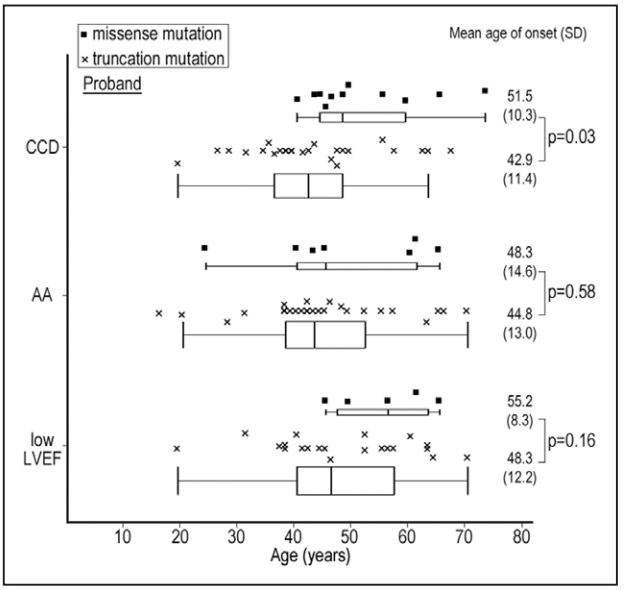

Figure 3. Onset of major cardiac phenotypes in 45 probands with truncation mutation (x mark) and missense mutation (solid square mark). AA indicates atrial arrhythmias; CCD, cardiac conduction disturbance; and LVEF, left ventricular ejection fraction.

Table 3. Characteristics of 77 Carriers at Last Follow-Up

	Total	Truncation	Missense	
	n=77	n=58	n=19	*P* Value
Age, y		44.5±16.9	58.1±13.1	0.002*
Men	49	36 (62)	13 (68)	0.78
Proband	45	31 (53)	14 (74)	0.18
Symptom				
Syncope	14	9 (16)	5 (26)	0.32
NYHA classification ≥3	26	18 (31)	8 (42)	0.41
Arrhythmia				
Atrial arrhythmias	45	36 (62)	9 (47)	0.29
Onset of atrial arrhythmias, y	45±13	44±14	48±13	0.47
Ventricular arrhythmia	35	27 (47)	8 (42)	0.79
CCD	62	46 (79)	16 (84)	0.75
SSS	24	16 (28)	8 (42)	0.26
AV block (≥1)	48	40 (69)	8 (42)	0.06
Cardiomyopathy				
LVEF <50%	36	29 (50)	7 (37)	0.43
LV enlargement	23	18 (31)	5 (26)	0.78
Comorbidities				
Coronary artery disease	3	2 (3)	1 (5)	1.0
Hypertension	12	8 (13)	4 (21)	0.48
Diabetes mellitus	2	0 (0)	2 (11)	0.06
PM implantation	16	11 (19)	5 (26)	0.52
ICD/CRT-D implantation	33	24 (41)	9 (47)	0.79
Medication				
Anticoagulant	28	18 (31)	10 (53)	0.11
β-Blocker	26	19 (33)	7 (37)	0.78
ACE-I inhibitor or ARB	33	25 (43)	8 (42)	1.0
Major cardiac events				
VF/sustained VT	19	17 (29)	2 (11)	0.13
Appropriate ICD shock	8	8 (14)	0 (0)	0.19
Cardiopulmonary resuscitation	2	2 (3)	0 (0)	1.0
All-cause death	9	7 (12)	2 (11)	0.82
Death because of end-stage heart failure	7	5 (9)	2 (11)	1.0
SCD	2	2 (3)	0 (0)	1.0
Unaffected	6	6 (10)	0 (0)	0.32

Number of subjects is expressed as n (%). Continuous variables are shown as mean±SD. ACE indicates angiotensin converting enzyme; ARB, angiotensin receptor blocker; AV, atrioventricular; CCD, cardiac conduction disturbance; CRT-D, cardiac resynchronization therapy defibrillator; ICD, implantable cardioverter defibrillator; LV, left ventricular; LVEF, left ventricular ejection fraction; NYHA, New York Heart Association; PM, pacemaker; SCD, sudden cardiac death; SSS, sick sinus syndrome; VF, ventricular fibrillation; and VT, ventricular tachyarrhythmia.

*P value <0.05.

Sex-Specific Differences

Significant sex-based differences in the mortality of human idiopathic dilated cardiomyopathy have been reported.[32] In a clinical cohort study on *LMNA*-related cardiomyopathy, men were reported to be at higher risk for MVAs and end-stage heart failure.[18,26] The sex-specific differences in *LMNA* carriers were comparable with those found previously in a mouse model with a homozygous H222P-*LMNA* mutation. The study had

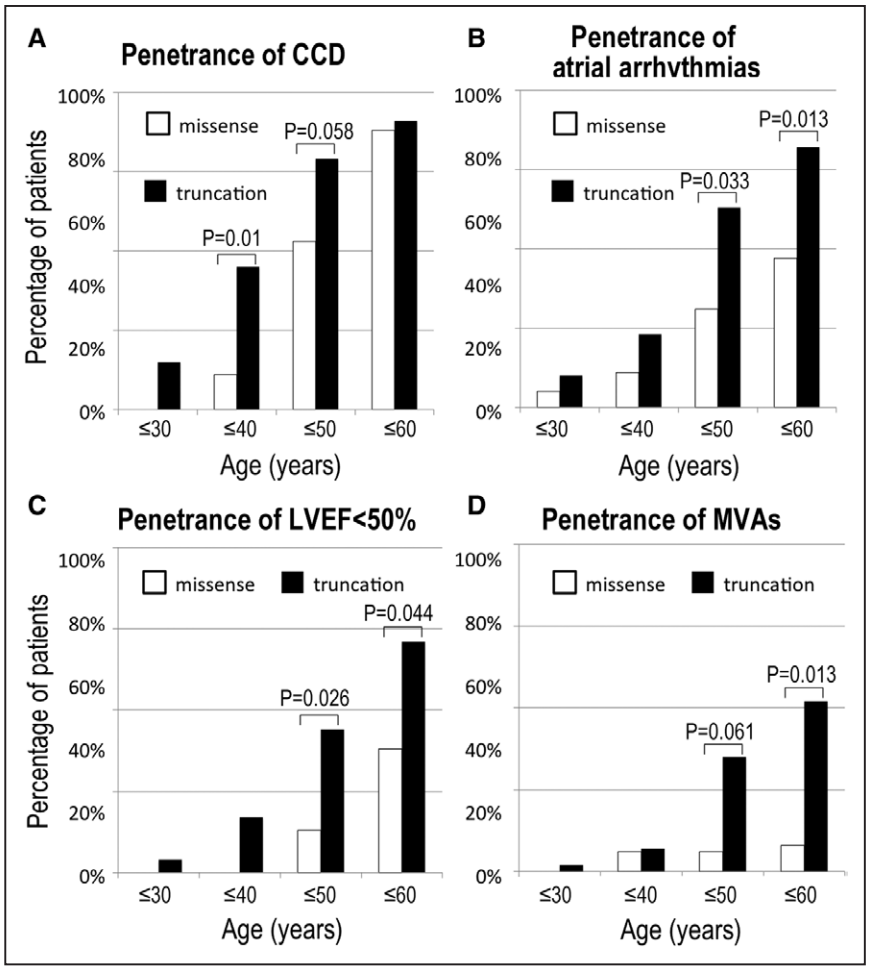

Figure 4. Penetrance of cardiac disorders in each age range. The unmarked pairs did not show any significant differences. CCD indicates cardiac conduction disturbance; LVEF, left ventricular ejection fraction; and MVA, malignant ventricular arrhythmia.

demonstrated a nuclear accumulation of androgen receptors and its coactivators and confirmed the relationship between testicular hormone and disease progression by a castration and treatment with testosterone or an androgen receptor antagonist.[33] Another possible reason for the sex difference might be the adverse effect of androgens. Although some reports[34,35] have demonstrated an influence of androgens on cardiac hypertrophy and fibrosis in mice, their role in the progression of cardiac

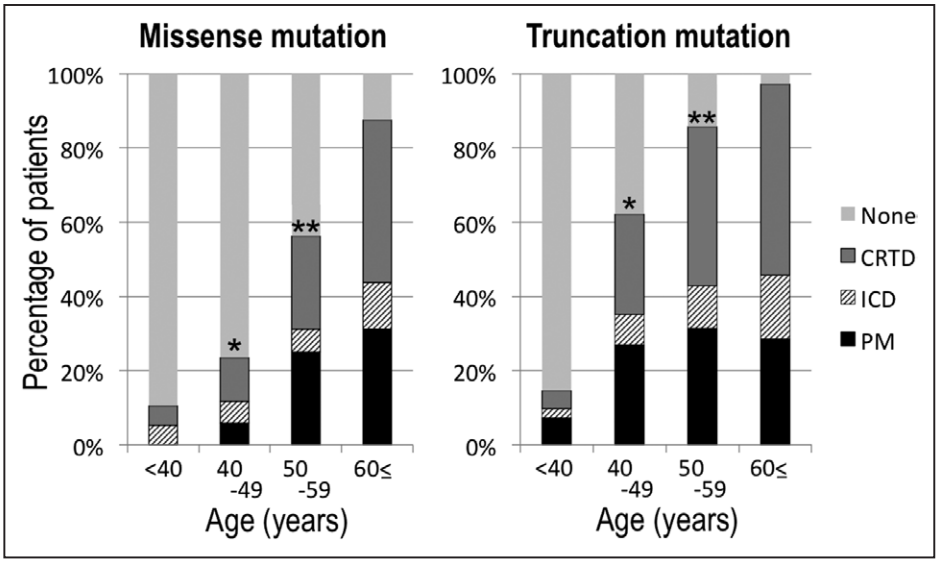

Figure 5. Percentage of patients with implantable arrhythmia devices. Total patients with implantable arrhythmia devices of truncation vs missense mutations: *, ***P* value <0.05. Unmarked other pairs did not show any significant differences. CRTD indicates cardiac resynchronization therapy defibrillator; ICD, implantable cardioverter defibrillator; and PM, pacemaker.

Table 4. Parameters Related to Cardiac Disorders at Age <60 y Evaluated by Logistic Regression Model Analyses

	No. of Patients With Phenotype / Cumulative Incidence (%) / Parameter: Positive vs Negative	OR (95% CI)	*P* Value
CCD (<50 y)*			
Truncation mutation	38 (79) vs 9 (53)	3.55 (1.06–12.33)	0.04*
Proband	29 (71) vs 18 (75)	0.94 (0.27–3.09)	0.91
Men	29 (67) vs 18 (82)	0.43 (0.10–1.47)	0.18
Atrial arrhythmias			
Truncation mutation	31 (82) vs 7 (47)	5.18 (1.42–20.38)	0.013*
Proband	28 (72) vs 10 (71)	1.13 (0.24–4.95)	0.87
Men	25 (69) vs 13 (76)	0.62 (0.13–2.56)	0.52
Low LVEF (≤50%)			
Truncation mutation	24 (71) vs 5 (38)	3.92 (1.03–16.55)	0.045*
Proband	22 (67) vs 7 (50)	2.28 (0.59–9.33)	0.23
Men	18 (58) vs 11 (69)	1.76 (0.46–7.62)	0.41

CCD indicates cardiac conduction disturbance; CI, confidence interval; LVEF, left ventricular ejection fraction; and OR, odds ratio.

*P value <0.05.

diseases has not fully been elucidated. However, *LMNA*-null or heterozygous knock-out mice models and a knock-in mouse model with an *LMNA* mutation, p.delK32,[36] showed no apparent sex-related differences in cardiac phenotypes or prognosis,[37] suggesting that all of *LMNA* mutations were not associated with the sex differences. Further experiments would be needed to clarify the mechanisms behind sex differences.

In contrast with the findings from a previous report by van Rijsingen et al,[26] we found no significant differences in the onset of low LVEF and MVAs between male and female subjects in our study. This discrepancy might have resulted from differences in race-specific genetic backgrounds and the percentage of truncation mutation carriers between this study on Japanese patients and the previous study on European patients (Table 1). Truncation mutation carriers comprised 75% of our cohort but only 45% of the European cohort.[26]

Clinical Implications

The results of our study provided the risk stratification for *LMNA* mutation carriers. A high prevalence of cardiac disorders and major cardiac events was found in our cohort of *LMNA* mutation carriers, and continuous follow-up was necessary for all of them. Furthermore, more intensive follow-up should be recommended in patients carrying truncation mutations.

Regarding the indication for device-based therapy, recent Heart Rhythm Society/European Heart Rhythm Association/Asia Pacific Heart Rhythm Society statement[38] suggests that ICD implantation can be useful for *LMNA* mutation carriers with LV dysfunction or MVAs. Based on the percentage of MVAs observed in the truncation mutation group of our cohort, we suggested that subjects carrying truncation mutations should be recommended for ICD implantations. However, because this study was not designed to determine the effect of ICD therapy, we cannot definitively predict the efficacy of ICD implants in the early phase. During the follow-up period, 8 patients received appropriate ICD therapies, and 6 of them underwent implantation with ICD or cardiac resynchronization therapy defibrillator for primary prevention. All 6 of these subjects had truncation mutations, and it seemed that ICD could be potentially lifesaving for *LMNA* mutation carriers, especially if they are truncation mutation carriers. Further prospective study would be necessary to confirm appropriate indications for ICD implantation for *LMNA* mutation carriers.

Study Limitations

The retrospective observational study design might include confounders. There was a potential source of bias in subject selection. In Japan, genetic testing for cardiomyopathy and CCD is not common, and those with typical phenotypes of *LMNA*-related cardiomyopathy, such as familial dilated cardiomyopathy or CCD, might be preferentially referred for genetic testing. Therefore, this study does not entirely clarify the full view of how *LMNA* mutations manifest.

Second, *LMNA* mutation carriers were not screened for sarcomeric genes, which might have influenced the dilated cardiomyopathy phenotype. Third, some of the mutations had been observed in a large number of subjects, which might be another potential source of bias. Fourth, the cohort was derived from several institutes, which might have introduced unforeseen bias. Yet, another limitation is the accuracy of information on the family history collected. We did not include the unexpected deaths of relatives without medical records because of the lack of conclusive evidence, which might have led to an underestimation of cardiac events.

Conclusion

This multicenter, retrospective cohort study demonstrated that, compared with missense mutation carriers, truncation mutation carriers had a significantly worse prognosis because of earlier onset of CCD, atrial arrhythmias, and left ventricular systolic dysfunction. Further studies are needed to enable better risk stratification of *LMNA* mutation carriers and to clarify the mechanisms behind some of the trends observed in this study.

Appendix

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Disclosures

None.

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CLINICAL PERSPECTIVE

Mutations in *LMNA* (*lamin A/C*) gene are associated with several cardiac phenotypes, including cardiac conduction disturbance, atrial or ventricular tachyarrhythmias, and dilated cardiomyopathy, resulting in heart failure or sudden cardiac death; however, risk stratification for *LMNA*-related cardiomyopathy has been still controversial. This multicenter cohort study has shown a high prevalence of these cardiac disorders and major cardiac events in the *LMNA* mutation carriers, and multivariable analyses revealed that the truncation mutation in *LMNA* gene was associated with a risk for earlier onset of cardiac conduction disturbance and the occurrence of atrial arrhythmias and low left ventricular ejection fraction, suggesting more intensive follow-up is necessary in patients carrying truncation mutations. On the contrary, even though a missense mutation, the *LMNA* mutation carriers have some risk for the cardiac disorders and significant cardiac mortality. Moreover, this study included only for subjects with typical *LMNA*-related cardiomyopathy called laminopathy and their relatives but not included forme fruste *LMNA* carriers. Therefore, this study does not entirely clarify the full view of how *LMNA* mutations manifest. Regarding the indication for device-based therapy, we would recommend the subjects carrying *LMNA* truncation mutations to receive implantable cardioverter defibrillators (or cardiac resynchronization therapy defibrillators) because of their high incidence of ventricular tachyarrhythmias observed over 40 years old. However, this study was not designed to determine the effect of implantable cardioverter defibrillator therapy on mortality or quality of daily activity, we cannot completely define the efficacy of prophylactic implantable cardioverter defibrillator implantation in the early age. Further prospective study may disclose appropriate indications for implantable cardioverter defibrillator (or cardiac resynchronization therapy defibrillator) implantation for *LMNA* mutation carriers.

SUPPLEMENTAL MATERIAL

Methods

Genetic analysis

Genomic deoxyribonucleic acid was extracted from peripheral blood leukocytes using a DNA isolation kit for Mammalian Blood (Roche Diagnostics, Basel, Switzerland). Standard polymerase chain reaction (PCR) primers were derived from intronic sequences (**Table S1**) to amplify the 12 protein-coding exons of *LMNA*. Mutational screenings of PCR amplicons were performed by direct sequencing on an ABI PRISM 3130x Genetic Analyzer (Thermo Fisher Scientific, Waltham, Massachusetts) by using BigDye Terminator chemistry (v1.1 or 3.1) according to standard protocols. Reference sequences used in this study are as follows: *LMNA* gene: NCBI NC_000001; *LMNA* messenger ribonucleic acid: NCBI NM_170707; lamin A protein: NCBI NP_733821; *LMNC* messenger ribonucleic acid: NCBI NM_005572; lamin C protein: NCBI NP_005563. Mutations were categorized in two ways based on type and site.

Bioinformatic analysis

Mutations present in the dbSNP build 146 or published in the literature were identified. All non-matching variants were filtered using a minor allele frequency threshold <0.3% based on the Human Genetic Variation Database (http://www.genome.med.kyoto-u.ac.jp/SnpDB/), which included the Japanese population, Exome Aggregation Consortium (http://exac.broadinstitute.org), and ClinVar (http://www.ncbi.nlm.nih.gov/clinvar/). All single base substitutions without changes in the coding amino acid were screened by a splicing site prediction tool (Berkeley Drosophila Genome Project: http://www.fruitfly.org), and the possibility of aberrant splicing was estimated. The candidate variant was considered as a pathogenic mutation if it generated a stop codon, a frameshift of the open reading frame,

or an aberrant splice site. The pathogenicity of novel missense variants was screened by *in silico* predictions using Polyphen2 (http://genetics.bwh.harvard.edu/pph2/), SIFT (http://sift.jcvi.org/), and Condel (http://bg.upf.edu/fannsdb/). A novel missense variant was considered pathogenic if classified as 'probably damaging' by Polyphen2, 'damaging' by SIFT, or predicted to be 'deleterious' by Condel.

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Table S1.

Oligonucleotide primers of PCR amplifying *LMNA*

Exon	Sense (5' to 3')	Antisense (3' to 5')	Product (bp)	Annealing (℃)
1	CCCAGATCCCGAGGTCCGAC	CCTCTCCACTCCCCGCCA	574	55
2	TGCCCTCTCCTGGTAATTGC	AGGGCCTAGGTAGAAGAGTG	352	58
3	CCTTCCAGTTCTTGTGTTCTGTGAC	CCTAGCCCAGCCCAAGTCTGTC	250	58
4	GGCCTCCCAGGAACTAATTCTG	CTCCCTGCCACCATCTGC	334	58
5	GCAGTGATGCCCAACTCAGG	TGCATCCGGCCCAGACTCTA	257	58
6	GCCAAGACTATGTTTAGAGCTTG	GGTCTAGTCAAGGCCAGTTG	466	58
7	AGTGTCCTCTGGCCGGCAAC	TCACCCTGGTCCACCCTCTG	400	72
8-9	GAGGCCTCAATTGCAGGCAGGC	CTCGTCCAGCAAGCAGCCAG	452	72
10	GTAAGCAGCAGGCCGGACAAAG	CACAGGAATATTCCATGGCATC	459	58
11	GGAGCCTGCAGGAGCCTGGAGC	GCTGCGGAAGAGAAGGCAGGCTC	465	72
12	CTTGTCTGAGCCCCAGACTGGAG	AGGGAAAAGGAAGGGAGGAGAAAT	436	58

Table S2.

***In silico* bioinformatic information of 16 missense mutations**

LMNA gene mutation		dbSNP	MAF (HGVD, KyotoDB)	ExAC	ClinVar	PP2	SIFT	Condel	splice site prediction
codon	protein								
59C>T	P20L	na	0/1092	na	na	0.984/probably damaging	DAMAGING, 0	Deleterious,0.595857153013	negative
107A>T	Q36L	na	0/1098	na	na	0.842/probably damaging	DAMAGING, 0.1	Deleterious,0.676286467119	negative
335A>C[§]	**E112A**	na	0/1077	na	na	0.772/probably damaging	DAMAGING, 0	Deleterious,0.568292542996	negative
[343G>A;344A>T][§]	**E115M**	na	0/1072, 0/1070	na	na	0.999/probably damaging	DAMAGING, 0.05	na	negative
590T>C[§]	**L197P**	na	0/1100	na	na	0.891/probably damaging	DAMAGING, 0.01	Deleterious,0.634260076772	negative
646C>T	R216C	rs794728591	0/1086	na	Conflicting interpretations of pathogenicity	0.962/probably damaging	DAMAGING, 0	Deleterious,0.618111646	negative
668A>G	**E223G**	na	0/1089	na	na	0.996/probably damaging	DAMAGING, 0	Deleterious,0.540944714	negative
959T>G	F237C	na	0/990	na	na	0.847/probably damaging	DAMAGING, 0	Deleterious,0.595118982321	negative
936G>C	Q312H	na	0/853	na	na	0.96/probably damaging	DAMAGING, 0	Deleterious,0.648941685	negative

1003C>T	R335W	rs38613 4243	0/1078	na	Pathogenic/Like ly pathogenic	0.962/probably damaging	DAMAGING, 0	Deleterious,0.672 249193	negative
1058A>G	Q353R	na	0/1101	na	na	0.71/possibly damaging	DAMAGING, 0.01	Deleterious,0.630 732445	negative
1069G>C	D357H	rs267607 567	0/1101	na	not provided	0.995/probably damaging	TOLERATED, 0.07	Deleterious,0.525 178837	negative
1112T>G	M371R	na	0/1101	na	na	0.947/probably damaging	DAMAGING, 0	Deleterious,0.658 77859357	negative
1129C>T	R377C	rs397517 889	0/1101	na	Pathogenic/Like ly pathogenic	1/probably damaging	DAMAGING, 0	Deleterious,0.665 63497504	negative
1486A>T[§]	**T496S**	na	1/1102	na	na	0.998/ probably damaging	DAMAGING, 0.01	Deleterious,0.645 975169	negative
1729G>A[§]	**A577T**	na	0/865	na	na	0.273/ benign	DAMAGING, 0.03	Neutral,0.510797 817	negative

Novel mutations are indicated by boldface. §: variant excluded because of unknown significance. Results by in silico predictions were expressed as 'classification, score'. dbSNP build 146 database (http://www.ncbi.nlm.nih.gov/projects/SNP/); HGV (http://www.genome.med.kyoto-u.ac.jp/SnpDB/); ExAC (http://exac.broadinstitute.org); ClinVar (http://www.ncbi.nlm.nih.gov/clinvar/); Polyphen 2 (http://genetics.bwh.harvard.edu/pph2/); SIFT (http://sift.jcvi.org/); Condel (http://bg.upf.edu/fannsdb/); splice site prediction tool (Berkeley Drosophila Genome Project: http://www.fruitfly.org). HGV = Human Genetic Variation Database; *LMNA* = *lamin A/C* gene; MAF = minor allele frequency; na = not available.

Table S3.

Clinical characteristics of 45 Probands at first clinical contact

	Total n = 45	Truncation n = 31	Missense n = 14	*p* value
Age, yrs		46.3 ± 12.9	52.2 ± 13.6	0.17
Male	31	21 (68)	10 (71)	1.0
Symptom				
Syncope	7	6 (19)	1 (7)	0.41
NYHA classification ≥3	8	6 (19)	2 (14)	1.0
Arrhythmia				
atrial arrhythmia	27	20 (65)	7 (50)	0.51
ventricular arrhythmia	16	12 (39)	4 (29)	0.73
CCD	33	22 (71)	11 (79)	0.73
SSS	10	7 (23)	3 (21)	1.0
AV block (≥1)	26	18 (58)	8 (57)	1.0
Cardiomyopathy				
LVEF<50%	21	18 (58)	3 (21)	**0.028**
LV enlargement	18	14 (45)	4 (29)	0.34
Comorbidities				
Coronary artery disease	2	2 (6)	0 (0)	1.0
Hypertension	8	5 (16)	3 (21)	0.69
Diabetic mellitus	0	0 (0)	0 (0)	-
PM implantation	9	6 (19)	3 (21)	1.0
ICD/CRTD implantation	3	3 (10)	0 (0)	0.54
Medication				
Anticoagulant	15	12 (39)	3 (21)	0.32
Beta-blocker	14	10 (32)	4 (29)	1.0
ACEI inhibitor or ARB	19	13 (42)	6 (43)	1.0

Number of subjects is expressed as n(%). Continuous variables are shown as mean ± standard deviation. ; CCD = cardiac conduction disturbance; CRTD = cardiac resynchronization therapy defibrillator; SSS = sick sinus syndrome; AV block = atrio-ventricular block; LV = left ventricular; EF = ejection fraction; PM = pacemaker; ICD = implantable cardioverter defibrillator; ACE-I = angiotensin-converting enzyme inhibitor; ARB = angiotensin receptor blocker; VF = ventricular fibrillation

Table S4.

Clinical characteristics of 45 Probands at last follow-up

	Total n=45	Truncation n = 31	Missense n = 14	*p* value
Age, yrs		50.5±12.7	57.7±14.3	**0.048**
Male	31	21 (68)	10 (71)	1.0
Family history				
SCD	17	14 (45)	3 (21)	0.19
heart failure	13	10 (32)	3 (21)	0.72
CCD	31	21 (68)	10 (71)	1.0
Symptom				
syncope	12	9(29)	3(21)	0.73
NYHA classification≧3	18	12(39)	6(43)	1.0
Arrhythmia				
atrial arrhythmia	34	27(87)	7(50)	**0.02**
ventricular arrhythmia	27	19(61)	8(57)	1.0
CCD	38	27 (87)	11 (79)	0.66
SSS	13	10 (32)	3 (21)	0.72
AV block (≧1)	31	23 (74)	8 (57)	0.31
Cardiomyopathy				
LVEF<50%	29	24(77)	5(36)	**0.016**
LV enlargement	26	17(55)	9(64)	0.75
Comorbidities				
Coronary artery disease	2	2(6)	0(0)	1.0
Hypertension	9	6(19)	3(21)	1.0
Diabetic mellitus	1	0(0)	1(7)	0.31
PM implantation	11	7(23)	4(29)	0.72
ICD/CRTD implantation	25	17(55)	8(57)	1.0
Medication				
Anticoagulant	19	13(42)	6(43)	1.0
Beta-blocker	20	13(42)	7(50)	0.75
ACEI inhibitor or ARB	20	15(48)	5(36)	0.53
Major cardiac events				
VF/sustained VT	15	13(42)	2(14)	0.09
Appropriate ICD shock	5	5(16)	0(0)	0.30
Cardiopulmonary resuscitation	2	2(6)	0(0)	1.0
All-cause death	8	7(23)	1(7)	0.40
Death due to end-stage heart failure	6	5(16)	1(7)	0.65
SCD	2	2(6)	0(0)	1.0

Number of subjects is expressed as n (%). Continuous variables are shown as mean ± standard deviation. SCD = sudden cardiac death; CCD = cardiac conduction disturbance; CRTD = cardiac resynchronization therapy with defibrillator; SSS = sick sinus syndrome; AV block = atrio-ventricular block; LV = left ventricular; EF = ejection fraction; PM = pacemaker; ICD = implantable cardioverter defibrillator; ACE-I = angiotensin-converting enzyme inhibitor; ARB = angiotensin receptor blocker; VF = ventricular fibrillation

Supplemental Figure

Figure S1

Comparison of age at the onset of major cardiac phenotypes in 77 *LMNA* mutation carriers between probands and their relatives (blank triangle: probands vs. solid triangle: relatives). CCD = cardiac conduction disturbance; AA = atrial arrhythmias; LVEF = left ventricular ejection fraction.

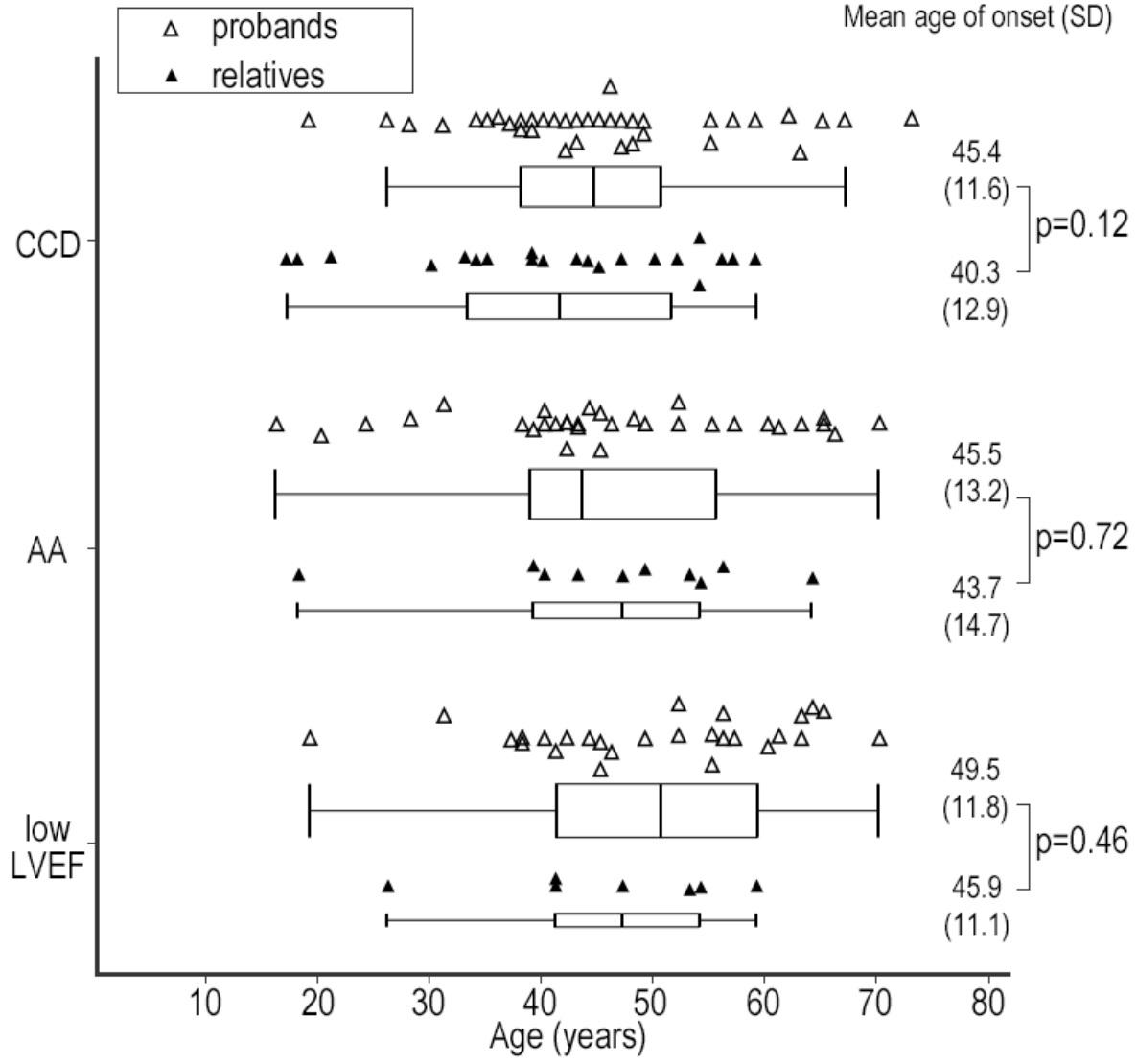

Figure S2

Comparison of age at the onset of major cardiac phenotypes in 77 *LMNA* mutation carriers between with NLS-conserved residue (blank inverted triangle) and NLS-disturbed residue (solid inverted triangle:). CCD = cardiac conduction disturbance; AA = atrial arrhythmias; LVEF = left ventricular ejection fraction; NLS = nuclear localization signal.

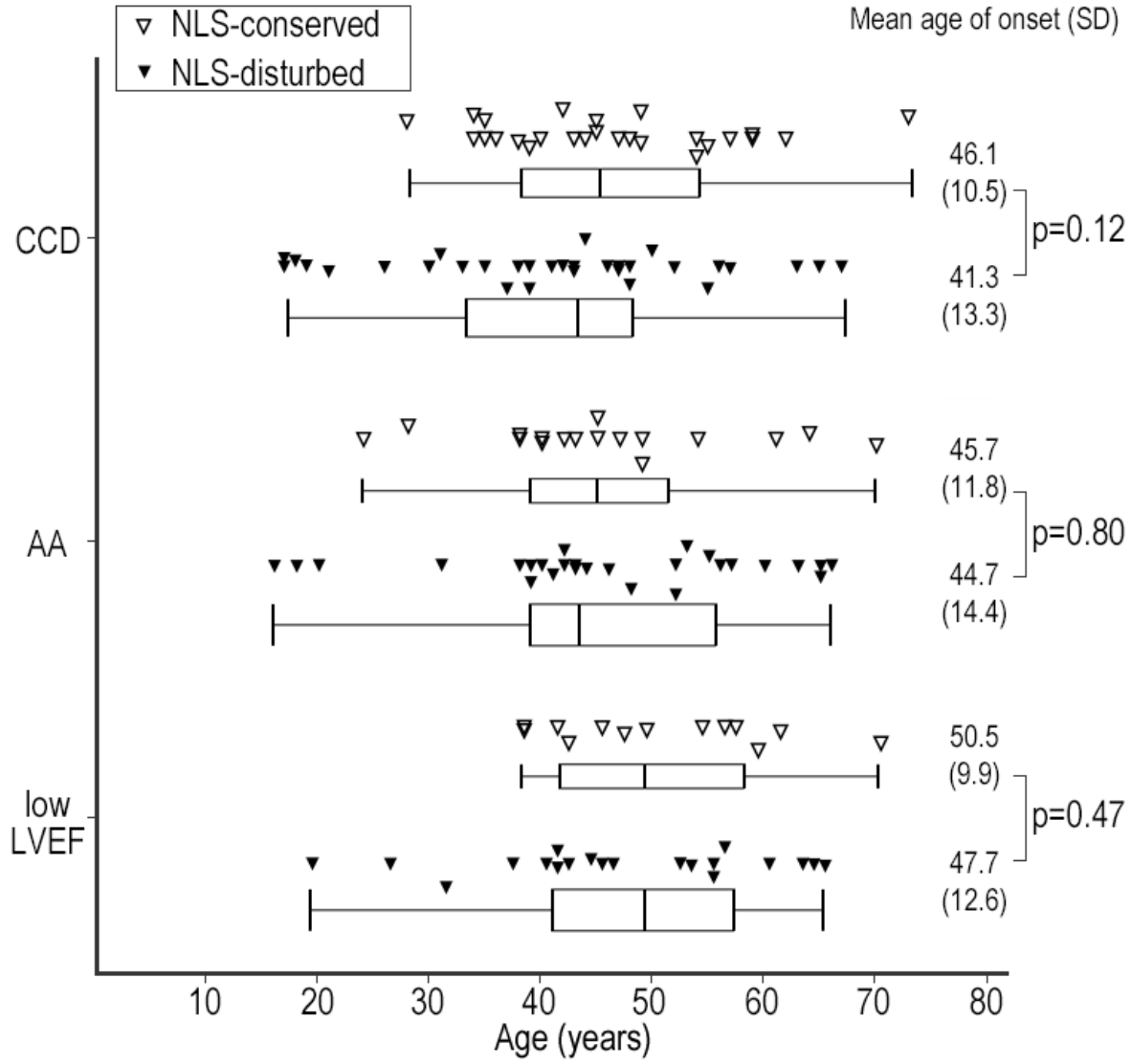

Figure S3

Comparison of age at the onset of major cardiac phenotypes in 77 *LMNA* mutation carriers by gender (blank circle: female vs. solid circle: male). CCD = cardiac conduction disturbance; AA = atrial arrhythmias; LVEF = left ventricular ejection fraction.