Clinical Manifestations and Long-Term Mortality in *Lamin A/C* Mutation Carriers From a Japanese Multicenter Registry

Kenzaburo Nakajima, MD; Takeshi Aiba, MD, PhD; Takeru Makiyama, MD, PhD;
Suguru Nishiuchi, MD; Seiko Ohno, MD, PhD; Koichi Kato, MD, PhD; Yuta Yamamoto, PhD;
Takahiro Doi, MD, PhD; Satoshi Shizuta, MD, PhD; Kenji Onoue, MD, PhD;
Nobue Yagihara, MD, PhD; Taisuke Ishikawa, PhD; Ichiro Watanabe, MD, PhD;
Hiroshi Kawakami, MD, PhD; Yasushi Oginosawa, MD, PhD; Nobuyuki Murakoshi, MD, PhD;
Akihiko Nogami, MD, PhD; Kazutaka Aonuma, MD, PhD; Yoshihiko Saito, MD, PhD;
Takeshi Kimura, MD, PhD; Satoshi Yasuda, MD, PhD; Naomasa Makita, MD, PhD;
Wataru Shimizu, MD, PhD; Minoru Horie, MD, PhD; Kengo Kusano, MD, PhD

Background: Mutation in the lamin A/C gene (*LMNA*) is associated with several cardiac phenotypes, such as cardiac conduction disorders (CCD), atrial arrhythmia (AA), malignant ventricular arrhythmia (MVA) and left ventricular dysfunction (LVD), leading to sudden cardiac death (SCD) and/or end-stage heart failure. We investigated how these phenotypes are associated with each other and which of them are most important for total mortality.

Methods and Results: A multicenter registry included 110 *LMNA* mutation carriers (age, 43±15 years, male: 62%) from 60 families. After genetic diagnosis of *LMNA* mutation (missense: 27%, non-missense: 73%), patients or subjects were followed to evaluate the manifestations of their phenotypes and the risk of total mortality; 90 patients could be followed (median: 5 [0–35] years). Prevalence of the 4 clinical phenotypes was significantly increased during follow-up. Among these phenotypes, AA was significantly associated with MVA. CCD was significantly associated with LVD. LVD, meanwhile, was significantly associated with CCD and MVA. Male sex was significantly associated with MVA. Furthermore, during follow-up, 17 patients died: 12 end-stage heart failure, 4 SCD and 1 stroke. LVD was the only independent predictor for all-cause death (OR: 41.7, 95% CI: 4.1–422.3; P=0.0016).

Conclusions: Several cardiac phenotypes were age-dependently increased in *LMNA* mutation carriers, suggesting that ICD or CRT-D could suppress SCD after middle age; however, LVD leading to end-stage heart failure was the only independent predictor for total mortality.

Key Words: Arrhythmias; Heart failure; Lamin A/C; Phenotypes; Prognosis

Lamin A/C gene (*LMNA*) mutations are associated with a variety of clinical phenotypes, including not only major cardiac abnormalities¹ but also premature aging, axonal neuropathy Charcot-Marie-Tooth type 2, and skeletal muscle diseases such as Emery-Dreifuss muscular dystrophy and limb-girdle muscular dystrophy. The most common cardiac manifestations associated with

Editorial p 2699

LMNA mutations are cardiac conduction disorders (CCD), atrial arrhythmias (AA), malignant ventricular arrhythmias (MVA) and left ventricular dysfunction (LVD) sometimes leading to end-stage heart failure (HF).² The natural

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Department of Cardiovascular Medicine (K.N., T.A., S.Y., K. Kusano), Department of Advanced Arrhythmia and Translational Medical Science (T.A.), National Cerebral and Cardiovascular Center, Suita; Department of Advanced Cardiovascular Medicine, Graduate School of Medical Sciences Kumamoto University, Kumamoto (K.N., S.Y., K. Kusano); Department of Cardiovascular Medicine, Kyoto University Graduate School of Medicine, Kyoto (T.M., S.N., Y.Y., T.D., S.S., T.K.); Department of Cardiovascular and Respiratory Medicine, Shiga University of Medical Science, Otsu (S.O., K. Kato, M.H.); Department of Cardiovascular Medicine, Nara Medical University, Kashihara (K.O., Y.S.); Department of Cardiovascular Biology and Medicine, Niigata University Graduate School of Medical and Dental Sciences, Niigata (N.Y.); Department of Molecular Physiology, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki (T.I., N. Makita); Division of Cardiology, Department of Medicine, Nihon University School of Medicine, Tokyo (I.W.); Department of Cardiology, Pulmonology, Hypertension and Nephrology, Ehime University Graduate School of Medicine, Toon (H.K.); The Second Department of Internal Medicine, University of Occupational and Environmental Health, Kitakyushu (Y.O.); Cardiovascular Division, Faculty of Medicine, University of Tsukuba, Tsukuba (N. Murakoshi, A.N., K.A.); and Department of Cardiovascular Medicine, Nippon Medical School, Tokyo (W.S.), Japan

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Table 1. Baseline Characteristics of 110 Patients With *LMNA* Mutations

No. of probands	60
No. of relatives	50
Asymptomatic relatives	7
M/F	68 (62)/42 (38)
Type of mutation	
Missense	30 (27)
Non-missense	80 (73)
Family history in 60 families	
Cardiac disease	54 (90)
SCD	26 (43)
CCD	52 (87)
LVD	22 (37)
MVA	31 (52)

Values are n (%). CCD, cardiac conduction disorders; LVD, left ventricular dysfunction; MVA, malignant ventricular arrhythmia; SCD, sudden cardiac death.

history of each of these cardiac phenotypes typically consists of progressive decline towards end-stage HF and MVA.^{3,4} Although implantable cardioverter-defibrillator (ICD) therapy is effective for the prevention of sudden cardiac death (SCD) in *LMNA* mutation carriers,⁵ treatment for HF is the next biggest concern affecting patient prognosis.

Although *LMNA*-related cardiomyopathy seems to be rare, it has been suggested that 5–10% of patients with familial dilated cardiomyopathy may actually have an *LMNA* mutation.^{6,7} We recently reported that patients with *LMNA* truncation mutations presented worse clinical outcomes than those with missense mutations.⁸ However, how the 4 clinical phenotypes are associated with each other and which of them are the most important for total mortality in *LMNA* mutation carriers are still unclear. It is clinically important to clarify the detailed prevalence and outcomes of each type of cardiac event associated with *LMNA* mutation over long-term follow-up. In this multicenter registry study, we assessed the presentation and progression of each cardiac phenotype and the long-term follow-up prognostic outcomes of *LMNA* mutation carriers.

Methods

Study Population and Design

This retrospective cohort consisted of 110 *LMNA* mutation carriers (60 probands and 50 relatives, 68 (62%) men, mean entry age: 43±15 years) (Table 1) who had been introduced to the registry from the following Japanese institutions: National Cerebral and Cardiovascular Center (Suita), Kyoto University Hospital (Kyoto), Shiga University of Medical Science (Otsu), Nara Medical University (Kashihara), Nagasaki University Graduate School of Biomedical Sciences (Nagasaki), Niigata University Graduate School of Medical and Dental Sciences (Niigata); Ehime University Hospital (Toon) University of Tsukuba (Tsukuba), University of Occupational and Environmental Health

(Kitakyushu) and Nihon University School of Medicine (Tokyo) between April 2008 and December 2017. All had pathogenic *LMNA* mutations or variants previously associated with cardiac laminopathy in published reports or newly identified *LMNA* variants with clinical or family evidence of possible cardiac laminopathy (Table S1).^{8–10} Unreported *LMNA* variants with insufficient clinical or genetic data were considered insignificant variants and were excluded.

All subjects gave informed consent prior to genetic analysis and clinical data acquisition. They then underwent clinical screening and peripheral blood sampling for genetic testing. After 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. Secondary end points were a composite of cardiac events and diagnosis including the first occurrence of CCD, AA, LVD or MVA. The protocol of this study was approved by the institutional ethics committees and performed in accordance with guidelines (Kyoto University, G194; National Cerebral and Cardiovascular Center, M24-031-4).

Genetic Analysis for *LMNA* Mutation

Details of the methods used for the genetic screening of *LMNA* have been described previously.⁸ In brief, genomic DNA was extracted from peripheral blood leukocytes to generate polymerase chain reaction primers to amplify the protein-coding exons of *LMNA* for mutational screening. Variants in *LMNA* were screened by genetic variant databases, a splicing site prediction tool, and in silico predictions.

Definition of Clinical Manifestations

The definition of each clinical manifestation was based on our previous report.⁸ LVD was defined as either left ventricular ejection fraction (LVEF) <50% and/or LV enlargement with a diameter of 5.5 cm according to published normal values. MVA was defined as sustained ventricular tachycardia (VT), ventricular fibrillation (VF), SCD, cardiopulmonary resuscitation, and appropriate ICD treatment (ICD discharge for termination of VF/VT and antitachycardia pacing for sustained VT). Nonsustained VT was defined as ≥3 consecutive ventricular beats with a rate >120 beats/min and a duration <30 s. The definition AA included paroxysmal, persistent, permanent atrial fibrillation (AF), atrial flutter (AFL) and atrial tachycardia (AT). Atrioventricular block (AVB) was classified as first, second or third degree. First-degree AV block was defined as a P-Q interval >200 ms. Sinus node dysfunction was defined as a heart rate <45 beats/min and/or sinus pause >3 s. CCD was defined as sinus node dysfunction and/or any degree of AVB. Family history of SCD was considered positive if at least 1 family member (up to the fourth degree) had died suddenly under the age of 60 years.

We assessed the manifestation and progression of each cardiac phenotype, the correlations among the phenotypes and their contribution to the prognostic outcomes of *LMNA* mutation carriers.

A list of participating institutes and investigators according to subject prevalence is provided in Appendix S1.

Mailing address: Takeshi Aiba, MD, PhD, Department of Advanced Arrhythmia and Translational Medical Science, National Cerebral and Cardiovascular Center, 5-7-1 Fujishiro-dai, Suita 565-8565, Japan. E-mail: aiba@ncvc.go.jp

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Table 2. Manifestation of Phenotypes and Therapies During Follow-up of 110 LMNA Mutation Carriers

	First clinical contact (n=110)	End of follow-up (n=90)	P value
Age (years)	43±15	49±17	—
AA (n, %)	35 (32)	58 (64)	<0.0001*
PAF	13	5	
Persistent AF	19	49	
AT/AFL	3	4	
Stroke (n, %)	6 (5)	11 (12)	0.088
MVA (n, %)	20 (18)	38 (42)	0.0002*
Sustained VT	18 (16)	32 (36)	
VF	3 (3)	14 (16)	
CCD (n, %)	79 (72)	80 (89)	0.0029*
SSS (n, %)	27 (25)	30 (33)	0.17
AVB (≥I) (n, %)	62 (56)	71 (79)	0.0008*
I AVB	22 (20)	16 (18)	—
II AVB	11 (10)	8 (9)	—
III AVB	29 (26)	47 (52)	—
QRS width (ms)	109±28	131±32	<0.0001*
Heart failure			
LVD: LVEF <50% (n, %)	22 (20)	47 (52)	<0.0001*
LVEF (%)	56±12	47±17	0.0004*
LVDd (mm)	52±7	53±7	0.50
LVDs (mm)	36±8	39±9	0.025*
LV enlargement (n, %)	29 (26)	30 (33)	0.29
LADs (mm)	38±7	43±10	0.0076*
NYHA classification ≥3 (n, %)	8 (7)	30 (34)	<0.0001*
Medications			
β-blocker (n, %)	18 (16)	33 (37)	0.0010*
ACEI/ARB (n, %)	27 (25)	37 (42)	0.0106*
Antithrombotic therapy	22 (20)	37 (41)	0.0011*
Common diseases			
CAD (n, %)	2 (2)	2 (2)	0.83
HT (n, %)	10 (9)	13 (14)	0.24
DM (n, %)	1 (1)	2 (2)	0.45
All-cause death (n, %)	—	17 (19)	
End-stage heart failure death (n, %)	—	12 (13)	
SCD (n, %)	—	4 (4)	
Stroke (n, %)	—	1 (1)	

Values are n (%). *P<0.05. AA, atrial arrhythmias; ACEI, angiotensin-converting enzyme inhibitor; AF, atrial fibrillation; AFL, atrial flutter; ARB, angiotensin II receptor blocker; AT, atrial tachycardia; AVB, atrioventricular block; CAD, coronary artery disease; DM, diabetes mellitus; HT, hypertension; LADs, left atrial diameter; LVDd, left ventricular end-diastolic dimension; LVDs, left ventricular end-systolic dimension; LVEF, left ventricular ejection fraction; PAF, paroxysmal AF; SSS, sick sinus syndrome; VT, ventricular tachycardia; VF, ventricular fibrillation. Other abbreviations as in Table 1.

Statistical Analysis

JMPpro 12.2.0 software (SAS Institute Inc., Cary, NC, USA) was used for statistical analysis. Continuous variables are expressed as mean±SD if normally distributed, or as the median with interquartile range if not. Categorical variables are reported as numbers and percentages. Comparison of means was by Student's t-test. Proportions were compared by χ^2 test or Fisher's exact test. Logistic regression analysis determined the predictors of each end-point and the relationship with each phenotype. Two-sided P<0.05 was considered statistically significant.

Results

Study Population and Clinical Characteristics

As shown in **Table 1**, 54 (90%) probands had a history of cardiovascular disease; of these, 26 (43%) had a family history of SCD. In contrast, 24 subjects (9 probands and 15 relatives) were phenotype-negative at the time of study entry, but all 9 probands and 8 relatives showed some phenotype at the last follow-up. During follow-up (median 5 years; 0–35 years), 17 patients died with a mean age at death of 53±14 years. Cause of death was HF death (HFD) in 12 subjects (mean age at death, 57±14 years), SCD in 4 subjects (42±8 years), and stroke in 1 (66 years). The rate of each of the 4 clinical phenotypes (AA, CCD, MVA and

LVD) was significantly increased during follow-up (AA: 35 [32%] to 58 [64%], CCD: 45 [75%] to 80 [89%], MVA: 20 [18%] to 38 [42%], LVD: 22 [20%] to 47 [52%], $P<0.01$ in all) (Table 2). In probands, the prevalence of each major phenotype also increased from study entry to the end of

follow-up (AA: 28 [47%] to 44 [80%], CCD: 45 [75%] to 51 [93%], MVA: 16 [27%] to 28 [51%], LVD: 15 [25%] to 36 [66%], $P<0.05$ in all) (Table S2).

Table 3. Interactions Among Major Cardiac Phenotypes in 110 LMNA Mutation Carriers

	No. of patients with phenotype	Univariate		Multivariate	
	Cumulative incidence (%)				
	Parameter positive vs. negative	OR (95% CI)	P value	OR (95% CI)	P value
AA (n=65)					
CCD	60 (63%) vs. 5 (33%)	3.4 (1.1–11.8)	0.030	2.3 (0.60–10.2)	0.23
LVD	41 (80%) vs. 24 (41%)	6.0 (2.6–14.8)	<0.0001	2.6 (0.94–7.1)	0.065
MVA	35 (83%) vs. 30 (44%)	6.3 (2.6–17.3)	<0.0001	3.9 (1.4–11.8)	0.0081*
Male	41 (60%) vs. 24 (58%)	1.1 (0.49–2.4)	0.74		
Proband	47 (78%) vs. 18 (36%)	6.4 (2.8–15.4)	<0.0001	4.1 (1.6–10.7)	0.0030*
CCD (n=95)					
AA	60 (92%) vs. 35 (78%)	3.4 (1.1–11.8)	0.030	2.0 (0.61–7.5)	0.25
LVD	49 (96%) vs. 46 (78%)	6.9 (1.8–45.8)	0.0035	5.3 (1.2–36.5)	0.023*
MVA	38 (90%) vs. 57 (83%)	1.8 (0.58–7.0)	0.31		
Male	60 (88%) vs. 35 (83%)	1.5 (0.49–4.5)	0.47		
Proband	54 (90%) vs. 41 (82%)	2.0 (0.66–6.3)	0.22		
LVD (n=51)					
AA	41 (63%) vs. 10 (22%)	6.0 (2.6–14.8)	<0.0001	2.6 (0.96–7.2)	0.059
CCD	49 (52%) vs. 2 (13%)	6.9 (1.8–45.8)	0.0035	6.1 (1.3–45.3)	0.020*
MVA	29 (69%) vs. 22 (32%)	4.7 (2.1–11.0)	0.0002	2.9 (1.1–7.6)	0.027*
Male	36 (53%) vs. 15 (36%)	2.0 (0.93–4.5)	0.077		
Proband	38 (63%) vs. 13 (26%)	4.9 (2.2–11.5)	<0.0001	3.0 (1.2–7.9)	0.020*
MVA (n=42)					
AA	35 (54%) vs. 7 (16%)	6.3 (2.6–17.3)	<0.0001	4.3 (1.5–13.2)	0.0057*
CCD	38 (40%) vs. 4 (27%)	1.8 (0.58–7.0)	0.31		
LVD	29 (57%) vs. 13 (22%)	4.7 (2.1–11.0)	0.0002	2.4 (0.95–6.3)	0.065
Male	32 (47%) vs. 10 (24%)	2.8 (1.2–6.9)	0.013	2.8 (1.1–7.6)	0.028*
Proband	30 (50%) vs. 12 (24%)	3.2 (1.4–7.4)	0.0047	1.5 (0.53–4.0)	0.45

*P<0.05. CI, confidence interval; OR, odds ratio. Other abbreviations as in Tables 1,2.

Age at First Manifestation of Each Cardiac Phenotype

Although not all mutation carriers showed the 4 cardiac phenotypes during follow-up, we could compare the ages at first clinical manifestation among each cardiac phenotype. Age at manifestation of LVD and MVA was significantly older than that of CCD and AA, whereas CCD and AA (44±11 and 45±13 years old; P=0.058 by paired t-test) and LVD and MVA (48±12 and 49±12 years old; P=0.065 by paired t-test) occurred almost simultaneously (**Figure S1**). Thus, we combined these as CCD/AA, and LVD/MVA and compared the ages at first clinical manifestation among each cardiac phenotype during follow-up. As **Figure 1** shows, age at first manifestation was significantly older in the order of HFD, LVD/MVA, CCD/AA and phenotype-negative.

Age-Dependent Changes in AVB and AA

The degree of AVB and the pattern of AA changed naturally with increasing patient age. As shown in **Figure 2A** and **Table 2**, 44% of our subjects did not have AVB at study entry, yet half of these exhibited type III AVB at the last follow-up date. As shown in **Figure 2A**, among patients with AVB, no one improved the severity of AVB during follow-up.

Furthermore, though 68% of subjects did not have AF at study entry, more than half of them had persistent AF at the last contact (**Table 2**, **Figure 2B**). Interestingly, in *LMNA* mutation carriers, AF cannot be easily terminated, suggesting that, in most cases, AF (or AT/AFL) may

develop into a persistent pattern. A total of 6 (6%) patients had a history of ischemic stroke at first clinical contact, but during follow-up, new incidence of ischemic stroke was 9 of 90 patients; thus, a total of 11 (12%) patients had stroke events at the last contact, and 9 of the 11 patients with stroke events had persistent AF, even though 8 of them (73%) had taken oral anticoagulation drugs. AF was a significant risk for ischemic stroke (odds ratio [OR]: 9.2, 95% confidence interval [CI]: 1.1–74.7, P=0.038).

Phenotype Correlations and Risk for Cardiac Prognosis

To investigate the associations among the 4 phenotypes linked to *LMNA* mutations, we looked for correlations among them. As shown in **Table 3**, AA was significantly associated with MVA and tended to be associated with LVD. CCD was significantly associated with LVD. LVD, meanwhile, was significantly associated with CCD and MVA, and tended to be associated with AA. Finally, MVA was significantly associated with AA but not significantly associated with LVD.

Sex has been reported to affect clinical outcome, especially with regard to MVA and end-stage HF.^{9,10} In this cohort, there were no significant sex differences in AA, CCD and LVD, but ventricular arrhythmia was more frequent in male subjects (male vs. female; 29 (42%) vs. 9 (27%), P=0.023) (**Table S3**). Our logistic regression model revealed that male sex was a risk factor for the occurrence of MVA (**Table 3**). Even though there was no statistical

Table 4. Identification of Risk Factors for All-Cause Death				
All-cause death	Univariate		Multivariate	
	OR (95% CI)	P value	OR (95% CI)	P value
AA	2.6 (0.84–9.6)	0.10	0.89 (0.18–4.3)	0.89
III AVB	1.1 (0.41–3.2)	0.79	0.60 (0.18–2.0)	0.41
MVA	2.7 (0.96–8.1)	0.061	1.3 (0.35–5.0)	0.67
LVD	26.5 (5.1–488.8)	<0.0001	41.7 (4.1–422.3)	0.0016*
Male	2.2 (0.73–8.4)	0.16	1.4 (0.36–5.4)	0.63
Proband	1.2 (0.43–3.6)	0.70	0.42 (0.11–1.6)	0.20

*P<0.05. Abbreviations as in Tables 1–3.

Figure 3. Distribution of patients implanted with a cardiac device during follow-up, namely, pacemaker, implantable cardioverter-defibrillator (ICD), cardiac resynchronization therapy with defibrillator (CRT-D) or left ventricular assist device (LVAD). Values are n (%)

Figure 4. Schematic illustrating the relationship between each phenotype and all-cause death in *LMNA* mutation carriers. Solid arrow shows statistically significant relationship ($P<0.05$), dotted arrow shows a weak but not statistically significant correlation. One-way arrow indicates the phenotype is associated with another in the direction of the arrow. Two-way arrow indicates an association between phenotypes.

significance, SCD (n=4) occurred only in male patients during follow-up.

Furthermore, we investigated which of these clinical phenotypes was associated with all-cause death. Multivariate logistic regression analysis revealed that only LVD was an independent predictor for all-cause death (OR: 41.7, 95% CI: 4.1–422.3, $P=0.0016$) (Table 4).

Device Therapy in *LMNA* Cardiomyopathy

At first contact, only 20 patients (19%) had been implanted with cardiac devices, specifically, 18 pacemakers 1 ICD and 1 cardiac resynchronization therapy defibrillator (CRT-D). At the end of follow-up, 77 (86%) patients had been implanted with cardiac devices, specifically, 29 (32%) pacemakers, 12 (13%) ICD and 34 (38%) CRT-D (including 21 upgraded cases). Furthermore, 2 (2%) patients had been implanted with left ventricular assist devices (LVAD) (Figure 3). A total of 12 patients received appropriate ICD

therapy during follow-up; 1 patient suddenly died despite having been implanted with a CRT-D.

Discussion

Major Findings

This is the largest *LMNA* cohort study in Japan or any other Asian countries showing age-dependent differences in the manifestation of cardiac phenotypes and prognosis. Our registry study showed that *LMNA* mutation carriers have a proclivity for clinically severe MVA and/or LVD leading to SCD or end-stage HF. Our findings are similar to those previously reported in Caucasian *LMNA* mutation carriers.^{2,9,10} On the other hand, this study is the first to report how the major clinical phenotypes seen in *LMNA* mutation patients are associated with each other. We found that LVD was the only independent predictor for all-cause death (Figure 4).

Manifestation of Phenotypes

LMNA mutation can cause familial cardiomyopathy with LV remodeling, early-age onset of advanced AVB, and lethal ventricular arrhythmias. Screening for *LMNA* mutations might be beneficial for risk stratification and clinical management of this type of familial cardiomyopathy. To date, however, only a small number of cases had been reported in Japan.^{11–13} Recently, we reported a gene-based risk stratification for cardiac disorders in 77 *LMNA* mutation carriers from 45 Japanese families, in which the truncation mutation carriers presented worse prognosis than those with a missense mutation.⁸

The clinical significance of *LMNA* mutation with regard to the heart is well known: the 4 most common phenotypes are CCD, AA, MVA and LVD.^{2,10} The rate of cardiac penetrance is reportedly quite high,¹⁴ reaching almost 100% at age 60.¹⁵ Yet *LMNA* mutation carriers often exhibit no disease phenotype until adolescence.⁴ In this study, most of the *LMNA* carriers under 20 years old were phenotype-negative, whereas those over 40 years old typically manifested cardiac phenotypes (**Figure 1**). During follow-up (median 5 [0–35] years), the incidence of each of these phenotypes increased not only in probands but also in relatives (**Table 2**). Furthermore, 9 of 46 mutations were identified from several different probands or families. As shown in the **Table S4**, the phenotype was quite similar among probands with the same mutation. On the other hand, among family members, particularly in younger carriers, phenotype manifestation was inconsistent, probably because of age. Thus, although there is some variation in the time course of these diseases, with some patients showing relatively late onset and mild phenotype,¹⁶ cardiac disorders in *LMNA* mutation carriers are definitely progressive over time.

Our results are in keeping with previous comprehensive data from 122 *LMNA* mutation carriers published by Kumar et al, in which the prevalence of each clinical manifestation increased during follow-up (median 7 years).² Age at first clinical contact and mean age at clinical appearance of AVB and AA in that study (approximately 43–44 years old) were quite similar to those in the present study. Age at first clinical manifestation of CCD and AA did not differ significantly in this study, yet the prevalence of CCD was much higher than that of AA at both first clinical contact and end of follow-up (CCD: 72% to 89% and AA: 32% to 64%, respectively). This might be because CCD (AVB ≥ 1) could be easily diagnosed during baseline ECG screening. Thus, as shown in **Table 1**, many of the *LMNA* mutation carriers were initially offered genetic screening because they had an ECG abnormality, especially CCD, and a family history of SCD and/or CCD and LVD.

AF and Prognosis

AA and especially AF are common in *LMNA* mutation carriers. Some *LMNA* carriers could be identified as familial AF patients with CCD.¹⁷ Yet *LMNA* mutations are not always the cause of AF even when conduction disease is present.^{18,19} *SCN5A* is another candidate gene that can be responsible for familial dilated cardiomyopathy with AF.²⁰ In this study, at the first contact, almost 70% of *LMNA* carriers were free from AF. During follow-up, however, only 32% of carriers remained AF free and more than 50% developed persistent AF. Furthermore, our phenotype correlation analysis revealed that AA was significantly associ-

ated with the manifestation of MVA and had a weak tendency to LVD (**Figure 4**) leading to SCD or end-stage HF. Catheter ablation of VT associated with *LMNA* cardiomyopathy in patients with already reduced LVEF did not always have a good outcome.²¹ Therefore, treatment of AF in *LMNA* carriers might be useful to retard the progression of cardiac laminopathy.

Furthermore, *LMNA* mutation is reported as an independent risk factor for arterial and venous thromboembolic complications.²² In this cohort study, a total of 11 (10%) patients had experienced ischemic stroke episodes by the end of follow-up, representing a significantly elevated prevalence. Not all the *LMNA* patients had been treated with anticoagulants even if they had AF. Thus, though their risk of stroke remains fairly low, *LMNA* mutation carriers with AF should be started on oral anticoagulants.²³

MVA and LVD

Life-threatening VAs are common in patients with *LMNA* mutations and significant CCDs, even if LVEF is preserved. ICD is an effective treatment and should be considered in this patient population.⁵ Independent risk factors for MVA that should be considered are nonsustained VT, LVEF <45% at first clinical contact, male sex, and non-missense mutations (ins-del/truncating or mutations affecting splicing).⁹ In this study, MVA was found in 18% of the *LMNA* mutation carriers at first clinical contact and in 42% by the end of follow-up. Our pathway analysis also suggested that MVA was significantly associated with the manifestation of AA and partially associated with LVD but not directly with CCD (**Figure 4**).

Although some undiagnosed family members died suddenly during this study, many genetically diagnosed *LMNA* patients have been recently implanted with ICD, which, when administered appropriately, can prevent SCD. In this study, 42% of *LMNA* mutation carriers exhibited MVA by the end of follow-up; similar proportions of patients in previous reports have exhibited MVA.^{2,4,9} The incidence of VT was 49%, consisting of 34% with sustained VT, 14% with VF and 5% with SCD. Nevertheless, we were unable to show a link between MVA and prognosis ($P=0.68$, hazard ratio: 1.3). This might be because prophylactic ICD implantation is more frequently recommended²⁴ in expectation of progressive disease for *LMNA* mutation carriers with a previous pacemaker indication.

Our analysis of the relationships among the phenotypes revealed that LVD is linked to CCD and AA, but not to MVA. Furthermore, LVD is the only manifestation directly related to all-cause death. Our results suggested that, regardless of the presence or absence of MVA, LVD is the most crucial and inevitable manifestation associated with *LMNA* mutation that eventually leads to HFD. In this study, only 2 patients had LVAD implantation during follow-up; in another study in Norway, however, heart transplantation was performed in 15 of 79 (19%) *LMNA* patients (age 42 ± 16 years, LVEF: $45 \pm 13\%$) during 7.8 ± 6.3 years of follow-up.⁶

Study Limitations

The cohort was obtained from 8 academic referral centers, and this situation could have introduced a referral bias towards more severely affected cases. There might also be selection bias, and there were missing data that could have led to overestimation of the influence of the risks. The accuracy of the information collected regarding family his-

tory was also limited. We did not include unexpected deaths of relatives without medical records because of the lack of conclusive evidence. Finally, we could screen for *SCN5A* but not completely exclude the possibility of other genetic mutations except for *LMNA*.

Conclusions

This multicenter, retrospective cohort study demonstrated that Japanese *LMNA* mutation carriers have a high incidence of arrhythmic and cardiac dysfunctional penetration and age-dependent cardiac phenotypes. These results may enable us to identify specific cardiac phenotypes that warrant careful surveillance in advance of an expected malignant disease course.

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Supplementary Files

Supplementary File 1

Supplementary Methods

Appendix S1. List of Participating Institutes and Investigators

Figure S1. Correlation between age at first clinical manifestation of the cardiac phenotypes (CCD, AA, LVD, and MVA) in each mutation carrier.

Table S1. Genetic and clinical characteristics of 60 families (110 subjects) with *LMNA* mutations

Table S2. Manifestation of arrhythmia or heart failure during follow-up of 60 probands with *LMNA* mutations

Table S3. Manifestation of arrhythmia or heart failure at the end of follow-up in males and females

Table S4. Genetic and clinical characteristics of the same mutations among 45 *LMNA* mutations in 23 families

Please find supplementary file(s);
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