Electrophysiological and Histopathological Characteristics of Progressive Atrioventricular Block Accompanied by Familial Dilated Cardiomyopathy Caused by a Novel Mutation of Lamin A/C Gene

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Conduction Defect Caused by Lamin A/C Gene Mutation. ***Introduction:*** **Mutations of lamin A/C gene (*LMNA*) cause dilated cardiomyopathy (DCM) with atrioventricular (AV) conduction defect, although the electrophysiological and histological profiles are not fully understood.**

Methods and Results: **We analyzed a large Japanese family (21 affected and 203 unaffected members) of DCM with AV block. The responsible *LMNA* mutation of IVS3–10A>G was novel and caused an aberrant splicing. The first clinical manifestation was low-grade AV block or atrial fibrillation (AF), which developed in affected members aged ≥30 years. We observed that the AV block progressed to third-degree within several years. The electrophysiological study of the four affected members revealed an impairment of intra-AV nodal conduction. Because of advanced AV block, pacemakers were implanted in 14 out of 21 affected members at the mean age of 44 years. Three affected members died suddenly and two affected members died of heart failure and/or ventricular tachycardia (VT) even after the pacemaker implantation. Postmortem examination showed conspicuous fibrofatty degeneration of the AV node. Endomyocardial biopsies showed remarkably deformed nuclei and substantial glycogen deposits in the subsarcolemma.**

Conclusion: **The clinical phenotype in this family was characterized by (1) the first manifestation of the prolonged PQ interval or AF in adolescence, (2) progressive intra-AV nodal block to the third degree in several years, and (3) progressive heart failure after pacemaker implantation. Histological study revealed preferential degeneration at the AV node area and novel cellular damages in the working myocardium.**
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lamin A/C, dilated cardiomyopathy, atrioventricular block, atrial fibrillation, gene

Introduction

Two polypeptides, lamin A and lamin C, are encoded by the lamin A/C gene (*LMNA*). These arise through alternative splicing of the same primary transcript.[1,2] Lamin A and lamin C proteins are components of the nuclear envelope, forming the inner and the outer nuclear membranes, the nuclear pore complex, and the nuclear lamina.[3] It has been reported that mutations in the *LMNA* cause various genetic diseases referred to as "laminopathy."[4] To date, seven different types of laminopathies have been established: autosomal dominant[5]/recessive[6] Emery-Dreifuss muscular dystrophy, limb-girdle muscular dystrophy 1B,[7] familial partial lipodystrophy,[8] sensory and motor axonal neuropathy Charcot-Marie-Tooth disorder type 2,[9] mandibuloacral dysplasia,[10] Hutchinson-Gilford progeria syndrome,[11] and autosomal dominant dilated cardiomyopathy (DCM) with conduction defect.[12]

The laminopathies have two major cardiac phenotypes, progressive atrioventricular (AV) block, and myocardial damage. The AV block in the laminopathies is mostly accompanied by cardiomyopathy, but isolated AV block was reported in limb girdle muscular dystrophy 1B[7,13] and autosomal dominant DCM with conduction defect.[14] We found a large family with DCM with progressive AV block caused by a novel mutation of *LMNA* and provide the clinical, genetic, electrophysiological, and histopathological findings concerning this particular familial DCM.

Methods

Participation of the Family

This familial DCM was first identified by the high incidence of pacemaker implantation (PMI). We started the investigation of the family because the preliminary study revealed a

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high incidence of not only PMI but also DCM (Fig. 1A). Written informed consent was obtained from all participants under approval of the local ethics committee. The family members showing DCM on echocardiogram were designated as affected irrespective of the presence of the conduction defect. The members who died suddenly or died of heart failure without otherwise known causes were also regarded as affected. We collected clinical data of the affected members from the medical records, which included 12-lead electrocardiogram (ECG), transthoratic echocardiogram, and serum creatine kinase. The data of cardiac catheterization and postmortem examination were also evaluated when available. Neurological assessment was performed for clinically affected members by review of medical records, blood examination of serum creatine kinase, and clinical neuromuscular examination evaluated by neurological experts. The family members ($\geq$30 years of age) showing no cardiac phenotypes were designated as unaffected. We examined ECG, echocardiogram, and serum creatine kinase in some unaffected members.

Mutational Analysis of LMNA

We obtained 5–10 mL blood samples from each family member. Genomic DNA was extracted from the whole blood in 25 family members or heart tissue obtained at autopsy. Because the *LMNA*-mutated DCM with conduction defect was known, we first screened for mutations of *LMNA*.[12] Twelve coding exons of *LMNA* were amplified by polymerase chain reaction (PCR) in the proband (IV-70 in Fig. 1A) as described.[12] Direct sequencing analysis was performed with an automated fluorescence sequencer (ABI PRISM 310, PE-Biosystems, Foster City, CA) by the dye-primer cycle sequencing method as described.[15] The PCR-based restriction fragment length polymorphism (PCR-RFLP) analysis was performed in family members and in 100 chromosomes from 50 normal volunteers (randomly selected from our control genomic store, $\geq$30 years of age) to test whether the mutation was a polymorphism by using a modified forward primer, 5′-CCAGCACTCAGCTCCCAGGT-3′ and a reverse primer, 3′-TGGTCTCACGCAGCTCCTGGAAGCG-5′. The underlined nucleotide represents an introduced mismatch, which generated a recognition site for *HhaI* in DNA fragments amplified from the mutant allele. The thermoprofile consisted of initial denaturing of 95°C for 3 min, 35 cycles of 98°C or 10 seconds, 55°C for 30 seconds, and 72°C for 30 seconds, followed by 72°C for 7 min. A total of 10 μL of PCR products were digested with 10 units of *HhaI* at 37°C overnight followed by 4% agarose gel electrophoresis. The DNA flagments were stained with ethidium bromide and visualized on a UV transilluminator.

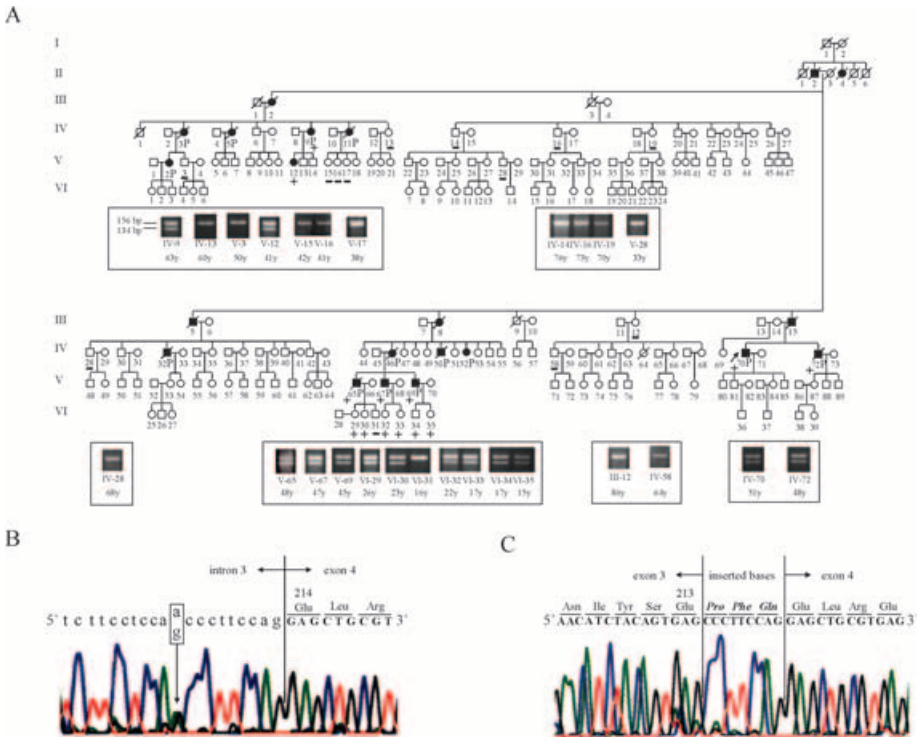

Figure 1. *The family pedigree and LMNA mutation. (A) The family pedigree with DCM with conduction defect caused by the LMNA mutation. Circles and squares indicate male and female, respectively. Closed circles and squares indicate affected members. Roman and arabic numerals indicate generation and case numbers. The letter P indicates affected members who received pacemaker implantation. Slash means deceased. Plus and minus indicate presence and absence of the mutation, IVS3–10A>G, respectively. An arrow indicates the proband. The photographs in boxes show electrophoresis of HhaI-digested PCR by PCR-RFLP analysis. Two DNA fragments of 156 bp and 134 bp show the presence of the mutation, IVS3–10A>G, while only the fragment of 156 bp shows the absence of the mutation. (B) Direct sequencing analysis of the LMNA mutation. The proband had a heterozygous base substitution from adenine (a) to guanine (g) at 10-base upstream from the junction between intron 3 and exon 4 (IVS3–10A>G, arrow points). Numbers above the amino acid symbols indicate the codon number. (C) A cDNA analysis of the LMNA mutation. Direct sequencing analysis of a cDNA fragment of 158 bp reveals insertion of nine bases, 5′-CCCTTCCAG-3′, in the junction between exon 3 and exon 4. Amino acid symbols in bold and italic represent three codons inserted into the exon 3/exon 4 junction.*

Analysis of LMNA mRNA of a Lymphoblast Cell Line Established from a Patient

A lymphoblast cell line was established from a blood sample of the proband by Epstein-Barr virus infection. Total RNA was purified from the lymphoblasts with RNeasy Mini kit (Qiagen, Germany) and subjected to reverse transcription as described.[15] A cDNA fragment containing the exon 3/exon 4 junction was amplified by PCR with a forward primer, 5′-GAGAACAGGCTGCAGACCAT-3′, and a reverse primer, 3′-TTGTCAATCTCCACCAGTC G-5′. The RT-PCR products were size-separated by 5% NuSieve GTG agarose (FMC BioProducts, Rockland) gel electrophoresis, and cDNA fragments of 149 bp and 158 bp in size were excised for direct sequencing analysis as described above.

Electrophysiological and Histological Examination

Conventional electrophysiological (EP) study was performed in four affected members because of AV block. Three standard electrode catheters were positioned in the high right atrium, His bundle region, and RV. A quadripolar-catheter or octapolar-catheter was inserted into the coronary sinus and positioned to record the coronary sinus electrogram using each pair of electrodes. The EP study included standard diagnostic protocols for sinus nodal function, AV nodal function, and ventricular tachycardia (VT) induction.[16] Briefly, in the induction of VT, up to three extrastimuli were induced (coupling intervals $\geq$ 180 ms) from the apex and the outflow tract of the RV after conditioning pacing (basic cycle length = 600 or 400 ms). If negative, we repeated the protocol above under intravenous administration of isoproterenol (started at 0.5 μg/min, a target heart rate was 20% increase from the control).[17]

Histopathological examination was performed in two postmortem heart samples obtained from affected members (IV-72, V-65) and in four biopsied samples of endomyocardial tissues taken from the septum and apex of the RV. Light and electron microscopical analysis was performed.

Results

Clinical Characteristics of Affected Family Members

The clinical characteristics of 21 affected members are summarized in Table 1. Information concerning the first manifestation was available in six affected members: first- or second-degree AV block and/or atrial fibrillation (AF) initially developed in five of the six affected members, IV-70, V-12, V-65, V-67, and V-69, while affected member IV-72 presented congestive heart failure with AF and third-degree

TABLE 1

Clinical Characteristics of Clinically Affected Members in the Family with Lamin A/C Gene Mutation

		Clinical Course			Echocardiogram					
Case No.	Gender	Onset (age)	PMI (age)	Cause of Death (age)	Age	LVEDD/ ESD (mm)	LVEF (%)	LAD (mm)	MD	Comment
II-2*	M	ND	No	53 y CHF					ND	
II-4*	F	ND	No	51 y SD					ND	
III-2*	F	ND	No	58 y CHF					ND	
III-5*	M	ND	No	55 y CHF					ND	
III-8*	F	ND	No	45 y SD					ND	
III-15*	M	ND	No	59 y CHF					ND	
IV-3*	F	ND	50 y	71 y cause unknown					ND	
IV-5*	F	ND	50 y	59 y apoplexy					ND	
IV-9	F	ND	49 y	64 y live					ND	
IV-11*	F	ND	40 y	58 y CHF and VT					ND	
IV-32*	M	ND	37 y	55 y SD					ND	
IV-46*	F	ND	45 y	62 y SD					ND	
IV-50*	M	ND	Yes	Age and cause unknown					ND	
IV-52*	F	ND	Yes	Live; age unknown					ND	
IV-70	M	42 y first- to second-degree AVB and AF (QRS = 0.10 sec)	45 y high-degree AVB and AF	53 y live	42 y 45 y 53 y	55/38 61/42 61/45	68 69 60	35 38 47	NM	43 y Holter ECG; four consecutive PVCs CK 79 (mU/mL) (<197 mU/mL)
IV-72	M	47 y CHF and third-degree AVB with AF (QRS = 0.10 sec)	47 y third-degree AVB with AF	50 y SD	47 y 49 50	69/56 66/65 76/67	36 33 24	55 55 58	NM	Diabetes mellitus, 48 y spontaneous sustained VT, CK 155 (mU/mL) (<200 mU/mL)
V-2*	F	ND	38 y	54 y live					ND	DCM
V-12	F	41 y first-degree AVB (PR = 0.22 sec, QRS = 0.08 sec)	No	42 y live	41 y	43/30	65	23	NM	41 y Holter ECG; couplet PVCs, Ergometer; PVC increased
V-65	M	42 y paroxysmal AF (QRS = 0.10 sec)	45 y high-degree AVB and AF	48 y CHF and VT	45 y 48 y	59/44 60/53	51 23	42 43	NM	47 y spontaneous sustained VT (LBBB+inferior axis type) CK 134 (mU/mL) (<195 mU/mL)
V-67	M	37 y paroxysmal AF (QRS = 0.10 sec)	43 y high-degree AVB and AF	49 y live	43 y 47 y	56/47 62/43	31 58	43 49	NM	47 y spontaneous sustained VT (RBBB+superior axis type) CK 134 (mU/mL) (<195 mU/mL)
V-69	M	30 y first- to second-degree AVB (QRS = 0.11 sec)	37 y third-degree AVB	47 y live	39 y 44 y	52/45 60/47	31 41	43 41	NM	38 y spontaneous sustained VT (RBBB+inferior axis type) 42 y performed catheter ablation for VT, CK 49 (mU/mL) (< 195 mU/mL)

*Clinically affected member whose DNA was not available for the study.

AF = atrial fibrillation; AVB = atrioventricular block; CHF = congestive heart failure; CK = creatine kinase; DCM = dilated cardiomyopathy; F = female; LAD = left atrial diameter; LBBB = left bundle branch block; LVEDD/ESD = left ventricular end-diastolic/end-systolic diameter; LVEF = left ventricular ejection fraction; M = male; MD = skeletal muscle disorder; ND = not detectable; y = year; NM = no manifestation; PMI = permanent pacemaker implantation; PVC = premature ventricular contraction; RBBB = right bundle branch block; SD = sudden death; VT = ventricular tachycardia.

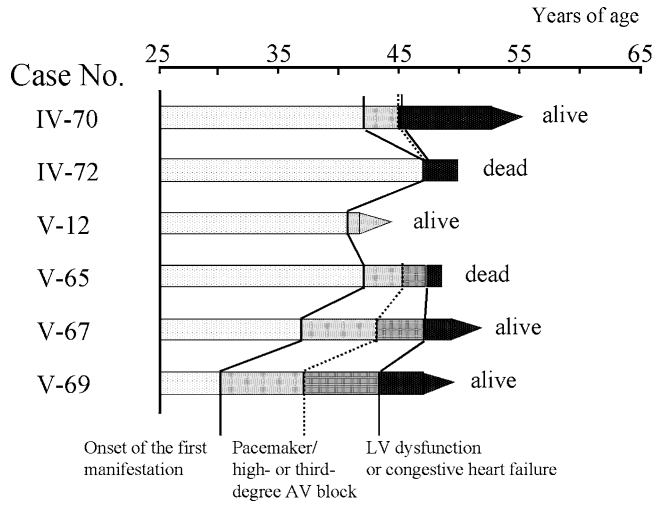

Figure 2. *Onset and time course of AV block and LV dysfunction. The onset and the time course of AV block and LV dysfunction are illustrated. The first manifestation, including first- or second-degree AV block and/or AF, occurred at 30 to 47 years of age. In four out of six affected members, AV block progressed to third-degree AV block, and these members received pacemaker implantation within 3 to 7 years. In two dead affected members, the first manifestation was more likely to occurr later and progressed rapidly. LV dysfunction was defined by LV diameter ≥ 60 mm or LV ejection fraction ≤ 40%.*

AV block at the onset of the disease (Fig. 2). The age of onset ranged from 30 to 47 years with a mean age of 40 years. At the first manifestations, all affected members had normal width of QRS complex, and intraventricular conduction delay was not found. Because of the progression to advanced AV block, 14 of the 21 the affected members received pacemakers, and six affected members died before pacemakers were clinically available. One affected member, V-12, was 42 years old and had only first-degree AV block. The mean age of PMI was 44 years with a range of 37–50 years. The PMI was needed within 7 years from the first manifestations such as first- or second-degree AV block and/or AF (Fig. 2). The width of QRS complex remained normal at the PMI. Fourteen of the 21 affected members have died, and the affected members with pacemakers died at a mean age of 57 (48–71) years, while the affected members without pacemakers died at a mean age of 53 (45–59) years. Sudden death was observed in five of these 14 affected members and six affected members died of congestive heart failure or fatal VT. The affected members, IV-70, V-12, V-65, V-67, and V-69, showed no clinical symptoms of congestive heart failure at the first manifestation. In affected members, IV-70 and V-12, echocardiography revealed normal LV dimensions and cardiac function. After the first manifestation, affected member IV-70 showed the progression of LV dilatation within 3 years. Echocardiogarphical LV dysfunction (LV diameter ≥ 60 mm, or LV ejection fraction ≤40%) was found 6 years after the first manifestation in affected member V-65, 6 years later in affected member V-67, and 9 years later in affected member V-69. The affected member, IV-72, first visited a hospital for clinical heart failure with third-degree AV block. No documentation was available concerning whether heart failure was followed by AV block (Fig. 2). In the general neurological assessment, no skeletal muscle abnormalities were found by neurological experts or in the review of medical records of clinically affected members in the family. Four genetically affected members (IV-70, IV-72, V-65, and V-69) were extensively evaluated by neurological experts and no manifestation such as Emery-Dreifuss muscular dystropy, limb-girdle muscular dystrophy, or Charcot-Marie-Tooth disorder was found. Serum creatine kinase was within the normal range in some affected members (IV-70, IV-72, V-65, and V-69).

Identification of LMNA Mutation

All coding regions of *LMNA* were screened for mutations in a DNA sample of the proband (IV-70). Direct sequencing analysis did not reveal any base change in each exon, but a heterozygous base substitution from adenine to guanine was detected 10-base upstream of the intron 3/exon 4 junction (IVS3–10A>G) (Fig. 1B). We screened for the IVS3–10A>G by direct sequencing and PCR-RFLP analysis in 19 members (≥30 years of age), which included seven affected (IV-9, IV-70, IV-72, V-12, V-65, V-67, V-69) and 12 unaffected members (III-12, IV-13, IV-14, IV-16, IV-19, IV-28, IV-58, V-3, V-15, V-16, V-17, V-28), and in seven members (<30 years of age) showing no cardiac phenotype (VI-29, VI-30, VI-31, VI-32, VI-33, VI-34, VI-35) whose fathers are affected members. The IVS3–10A>G mutation was detected in all the seven members, but not in all the 12 unaffected members. Furthermore, the mutation was detected in six of the seven members (<30 years of age) showing no cardiac phenotype. Results of the PCR-RFLP analysis are shown in Figure 1A. On the other hand, the mutation was not detected in 100 control alleles (data not shown). These results suggested that the mutation was tightly associated with the disease and that there were mutant carriers (<30 years of age) before the onset of the disease.

The RT-PCR study revealed two cDNA fragments of 149 and 158 bp in size in the proband (data was not shown), while only the 149-bp fragment was observed in the control (data was not shown). Direct sequencing analysis showed that a nine-base fragment, 5′-CCCTTCCAG-3′, was inserted in the junction between exon 3 and exon 4 in the 158-bp fragments (Fig. 1C). These results suggested that the IVS3–10A > G altered a splicing site to the 10th nucleotide (a/g heterozyzous) upstream from the 3′ end of the intron 3 from the junction between the intron 3 and exon 4 (Fig. 1B).

TABLE 2

Electrophysiological Characteristics of Affected Members with Lamin A/C Gene Mutation

			Electrophysiological Conductive Parameters							Induced Arrythmia	
Case No.	Gender	Age	P Duration (ms)	PR Interval (ms)	PA Interval (ms)	AH Interval (ms)	HV Interval (ms)	QRSS Duration (ms)	Wenckebach Rate at Atrial Pacing (beats/min)	Atrial Arrhythmia	Ventricular Arrhythmia
IV-70	M	45	110	470	30	370	55	100	100	AF (before EPS)	NP
V-65	M	45	120	565	30	460	55	100	50	AF (before EPS)	Sustained VT (LBBB+inferior axis type)
V-67*	M	43	AF	AF	AF	AF	50	100	AF	AF	NI
V-69	M	42	140	Wenckebach	80	Wenckebach	40	110	During sinus rhythm	NP	Sustained VT (RBBB+inferior axis type)

* During sinus rhythm before EPS, administration of 1.0 mg of atropine sulphate improved Wenckebach-type AV block to one to one AV conduction.
AF = atrial fibrillation; EPS = electrophysiological study; LBBB = left bundle branch block; M = male; NI = not induced; NP = not performed; RBBB = right bundle branch block; VT = ventricular tachyardia.

Electrophysiological and Histopathological Characteristics

EP study was performed in four affected members, IV-70, V-65, V-67, and V-69, because of bradycardia and/or spontaneous VT (Table 2). The EP study indicated that affected members, IV-70 and V-65, had AV nodal dysfunction with marked prolongation of the AH interval. In contrast, the HV interval and QRS duration were normal in all four affected members, indicating that the conduction systems of the infra-His bundle to Purkinje fiber and intraventricles might not be impaired. Although affected member, V-65, had AF (Fig. 3A), the AV conductivity was evaluated after DC defibrillation. He demonstrated Wenckebach-type second-degree AV block (Fig. 3B) with prolonged AH interval (Fig. 3C). Furthermore, VT could be reproducibly induced by programmed stimulation to the outflow tract of the RV (Fig. 3D).

Heart specimens were obtained at autopsy from affected members, IV-65 and V-72. Microscopical examination disclosed marked fibrofatty degeneration in the AV junction and

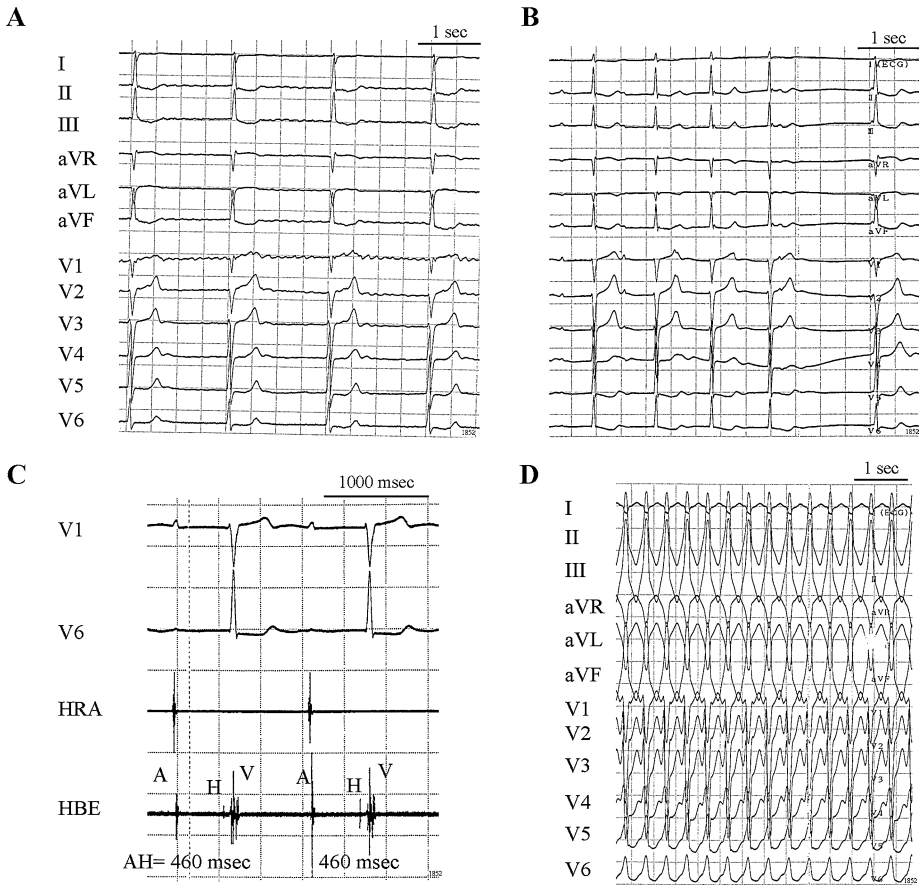

Figure 3. *Twelve-lead ECGs and intracardiac electrogram. Cardiograms were recorded during the electrophysiological study of affected member, V-65. At the beginning of the electrophysiological study, (A) AF with complicated third-degree AV block (30 ventricular beats/min) was found. (B) After a direct current defibrillation, the sinus rhythm appeared in association with Wenckebach-type second-degree AV block. (C) In the intracardiac electrogram a prolonged AH interval (460 ms) was detected during the sinus rhythm. (D) Sustained VT could be induced by programmed stimulations at the out-flow tract of the RV. HRA and HBE indicate high right atrium and His bundle electrogram, respectively.*

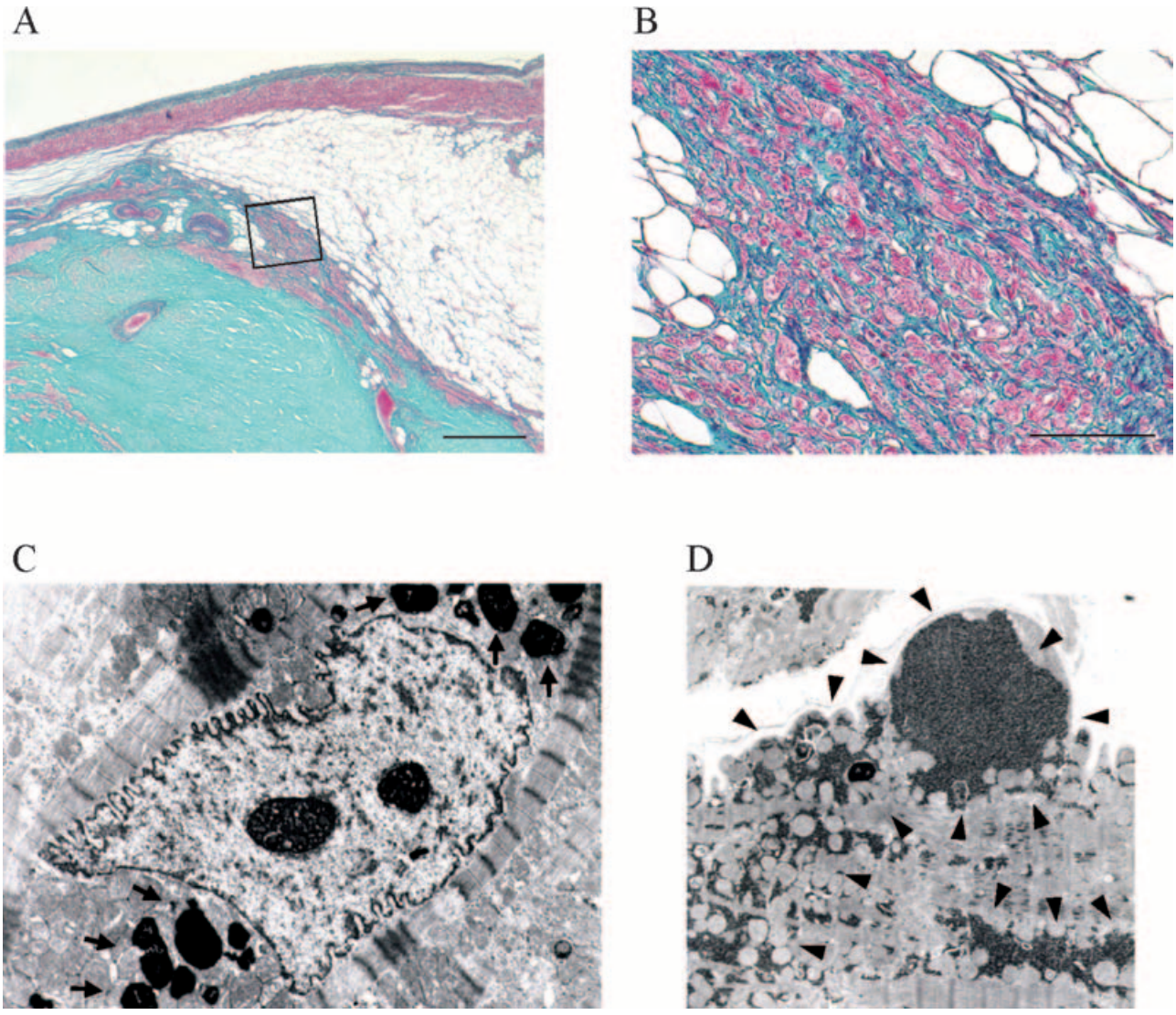

Figure 4. *Light and electron microgram. Light microscopic examination of the AV junction in affected member V-65 by Elastica-Masson staining revealed atrophy of the AV node with fibrofatty degeneration (Panel A). The boxed area in (Panel A) is shown with a higher magnification in (Panel B). The number of pacemaker cells appears to be decreased in the AV node in (Panel B). Electron microscopic analyses of myocardium samples obtained from affected member V-65 by biopsy showed deformed nuclei with increased lipofuscin (Panel C, arrows) and accumulated glycogen granules around the mitochondria and beneath the subsarcolemma (D, arrow heads). Scale bars in (Panel A) and (Panel B) represent 500 and 100 μm, respectively.*

advanced atrophy of the AV node (Fig. 4A), and fibrosclerosic degeneration in the AV node associated with decreased numbers of pacemaker cells (Fig. 4B). Endocardial biopsy of the RV was performed in affected members, IV-46, V-65, V-67, and V-69, and light microscopical examination revealed interstitial fibrosis and contraction band without the disarray arrangement (data are not shown). Electron microscopy demonstrated a large number of deformed nuclei with increased lipofuscin (Fig. 4C) and a prominent accumulation of glycogen deposits beneath the subsarcolemma and around the mitochondria (Fig. 4D). The nuclear membrane integrity was preserved in all of the examined specimens.

Discussion

We examined familial DCM with progressive AV block in a large Japanese family and identified a novel *LMNA* mutation of IVS3–10A>G. Affected members initially showed problems in ECG such as first- or second-degree AV block and/or AF at 30–40 years of age without detectable LV dysfunction. Progression to third-degree AV block was observed in 14 affected members, and they received pacemakers within several years of the first ECG finding. Unfortunately, seven affected members died suddenly or unexpectedly even after the pacemakers were implanted. Some affected members with PMI died from congestive heart failure. The EP study disclosed that the conduction defect occurred predominantly in the AV nodal area as compared with the ventricular area, which was supported by the histological findings that the fibrofatty degeneration was more striking in the AV node than in the atrial or ventricular wall.

The LMNA Mutation in Familial DCM with Conduction Defect

Since introns are noncoding sequences, a mutation in an intron does not usually cause an mRNA abnormality.

However, the present mutation of the intron (IVS3–10A>G) created an aberrant splicing site between intron 3 and exon 4. The missplicing arose at the 10th nucleotide (a/g) upstream of the 5′ end from the 3′ end of intron 3 (Fig. 1B), and consequently inserted nine bases to the 5′ end of exon 4. The genetic approach strongly indicated that this mutation has a close relation to the clinical phenotype. In fact, the mutation was never found in unaffected family members and 50 volunteers. Although we showed the insertion of the nine bases in the cDNA assay with RT PCR, we did not show the insertion at the protein level. Protein analysis would be important, especially for confirming the present interpretation and for future functional analyses of the protein.

Although the relationship between the mutation site of *LMNA* and the clinical phenotypes of the laminopathies remains unclear, this mutation was novel and first described in the present study. It also remains unknown why laminopathies due to the *LMNA* mutations have various clinical phenotypes. Further studies are necessary to elucidate the relationship between the phenotype and the prognosis of laminopathies and the *LMNA* mutations.[18–20]

The incidence of the *LMNA*-mutated DCM with AV conduction defect remains controversial. Fatkin[12] studied 11 families with autosomal dominant DCM with conduction defect and identified five *LMNA*-mutated DCM families (45%). Similarly, *LMNA*-mutated DCM was found in four of 49 families (8%) by Taylor,[20] two of 16 families (12.5%) by Jakobs,[21] and five of seven (71%) families by Arbustini.[22] Thus, the *LMNA* mutation may account for 19% of autosomal dominant DCM with conduction defect. Therefore, mutational analysis in *LMNA* should be first performed for the diagnosis of familial DCM associated with conduction defect.

Electrophysiological Characteristics

The first manifestation in the affected members was first- or second-degree AV block and/or AF, which developed at the mean age of 40 years (range 30–47 years). The EP study revealed AH block, that is, conduction defects in the AV node without those in the infra-Hisian conduction system. The PA interval, which may reflect the intraatrium conductivity, was within the normal range in affected members, IV-70 and V-65, but was prolonged in affected member V-69 whose diseased period had a long history (≥12 years).

Progressive AV block prior to LV dysfunction was unique in affected members with *LMNA*-mutated DCM. Some investigators have reported a similar time-course of *LMNA*-mutated DCM, that is, the conduction defect, including AV block and AF, developed prior to LV dysfunction,[12,14,20–23] despite different sites of the mutation (Fig. 5). The present study indicated that the progressive AV block in *LMNA*-mutated DCM was caused by AV nodal impairment without infra-Hisian conduction defect.

AF frequently occurred in *LMNA*-mutated DCM.[24] However, AF may not be a particular phenotype of *LMNA* mutations, because LV dysfunction frequently causes AF. It remains unclear how *LMNA* and its locus, chromosome 1p1-q21, is related to familial AF.[25–29]

Sudden death accounted for 36% (5/14) of all causes of mortality in the *LMNA*-mutated DCM. Sudden death could not be prevented by PMI, and fatal VT appeared to cause sudden death. In fact, VT could be repeatedly induced by electrical stimulations of the RV in two affected members (V-65 and V-69). Affected member V-69 had spontaneous VT, and affected member V-65 developed spontaneous VT after the EP study. However, the incidence of sudden death is nearly identical to that of patients with DCM (approximately 40%).[30] Therefore, the susceptibility to VT and/or sudden deaths might not be specific to *LMNA*-mutated DCM; rather, it might be a common clinical feature of chronic heart failure with LV dysfunction.[31,32]

Histopathological Characteristics

The microscopical study revealed that fibrosclerotic degeneration occurred predominantly in the AV node. Less severe lesions were also observed in the sinoatrial node, atrium, and ventricular walls. These supported the findings of the clinical examination and EP study that the AV block preceded the other myocardial involvements. The electron microscopical study revealed the accumulation of glycogen deposits beneath the subsarcolemma in the working myocardium in all biopsy samples.

Arbustini[22] reported the fibrosclerotic and fibrofatty degeneration of the AV junction in *LMNA*-mutated DCM with conduction defect, and described the nuclear membrane damage showed by electron microscopical analysis.[22] Lamin A and lamin C null mice also showed obvious discontinuities in the nuclear envelopes of embryonic fibroblasts and hepatocytes, while those of wild-type and heterozygous mice were intact.[33] However, electron microscopical study in the present report revealed that the nuclear membrane remained intact. This may be related to the sampling

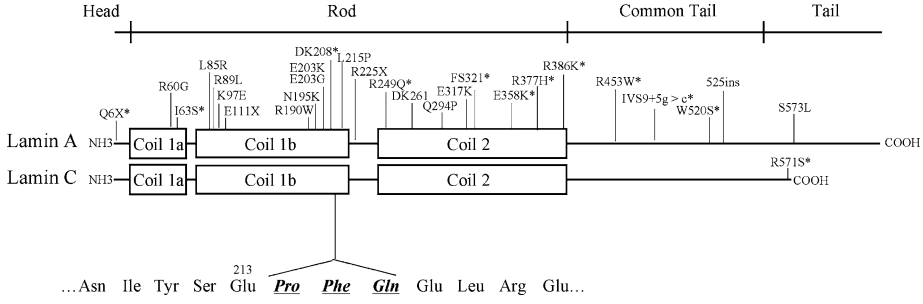

Figure 5. *Secondary structure of lamin A and lamin C proteins and sites of mutations of LMNA-mutated DCM with conduction defect with or without muscular dystrophy. Secondary structure of lamin A and lamin C proteins is illustrated. The previously reported LMNA mutations (above) and the mutation identified in this study (below) as the cause of the DCM with conduction defect are shown. Asterisks indicate cases with skeletal muscle symptoms. Amino acids with bold and italic indicate the insertions those identified in this study.*

site, that is, our biopsy samples were taken from the right ventricle.

Recently, it was reported that mutations in the gene coding the γ^2 regulatory subunit of AMP-activated protein kinase (*PRKAG2*), an enzyme that modulates glucose uptake and glycolysis, cause glycogen storage disease associated with cardiac hypertrophy and conduction defect including AV block.[34,35] Therefore, we may be able to propose the hypothesis that the *LMNA* mutation causes cellular damage by the intracellular accumulation of glycogen. However, it remains unknown whether a dysfunction of lamin A and lamin C proteins impairs glycogen metabolism. Some types of familial diseases preferentially develop cardiac conduction defects even with a heterogenous genetic background.[27,36,37] Understanding the actual molecular mechanism involved in the pathophysiology of familial diseases might enable us to understand the mechanisms of "common" cardiac conduction defects clinically recognized as "sporadic."

Conclusion

This is the first report of the electrophysiological profile of the progressive intra-AV nodal conduction impairment and its histopathological findings in *LMNA*-mutated DCM. The clinical phenotype of *LMNA*-mutated DCM in this study was characterized by (1) the first manifestation of the prolonged PQ interval or AF in adolescence, (2) progressive intra AV nodal block to the third degree in several years, and (3) progressive heart failure after PMI. Histological study revealed the preferential degeneration at the AV node area and novel cellular damages in the working myocardium.

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