A Complex Double Deletion in LMNA Underlies Progressive Cardiac Conduction Disease, Atrial Arrhythmias, and Sudden Death

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**Background**—Cardiac conduction disease is a clinically and genetically heterogeneous disorder characterized by defects in electrical impulse generation and conduction and is associated with sudden cardiac death.

**Methods and Results**—We studied a 4-generation family with autosomal dominant progressive cardiac conduction disease, including atrioventricular conduction block and sinus bradycardia, atrial arrhythmias, and sudden death. Genome-wide linkage analysis mapped the disease locus to chromosome 1p22-q21. Multiplex ligation-dependent probe amplification analysis of the LMNA gene, which encodes the nuclear-envelope protein lamin A/C, revealed a novel gene rearrangement involving a 24-bp inversion flanked by a 3.8-kb deletion upstream and a 7.8-kb deletion downstream. The presence of short inverted sequence homologies at the breakpoint junctions suggested a mutational event involving serial replication slippage in trans during DNA replication.

**Conclusions**—We identified for the first time a complex LMNA gene rearrangement involving a double deletion in a 4-generation Dutch family with progressive conduction system disease. Our findings underscore the fact that if conventional polymerase chain reaction–based direct sequencing approaches for LMNA analysis are negative in suggestive pedigrees, mutation detection techniques capable of detecting gross genomic lesions involving deletions and insertions should be considered. (Circ Cardiovasc Genet. 2011;4:280-287.)

**Key Words:** LMNA conduction disease ■ gene rearrangement ■ complex deletion ■ MLPA

Inherited cardiac conduction disease is characterized by genetically determined defects in electrical impulse generation and conduction. The electric impulse initiates in the sinoatrial node within specialized pacemaker cells and travels through the atria to the atrioventricular (AV) node. The impulse then conducts across the AV junction through the His-bundle, which bifurcates into the left and right bundle branches and subsequently spreads throughout the ventricles through Purkinje ramifications. Electroanatomic impairment of the conduction system predisposes affected individuals to life-threatening arrhythmias and sudden cardiac death. Sudden death may be the first clinical manifestation of the disease.1,2

**Clinical Perspective on p 287**

Genetic testing plays an important role in identifying patients at risk. Conduction disease is often progressive in nature, and young individuals presenting with a normal ECG may be at risk of arrhythmia and sudden death as they get older. Genetic testing allows for implementation of preventive measures in asymptomatic patients carrying the genetic defect, whereas patients with a negative genetic test can be reassured.

The genetic basis of cardiac conduction disorders is heterogeneous. To date, mutations have been identified in genes encoding ion channel subunits (SCN5A, HCN4, TRPM4, and SCN1B) as well as in genes involved in the regulation of heart development (NKX2.5 and TBX5), in which mutations are also associated with congenital cardiac malformations.3-9 A complex phenotype of conduction disease associated with dilated cardiomyopathy and a variety of other (extracardiac) disorders (referred to as “laminopathies”) have been associated with mutations the LMNA gene (MIM No. 150330), encoding for the inner nuclear membrane protein lamin A/C.10

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In this study, we investigated a 4-generation Dutch family with progressive cardiac conduction disease, including atrioventricular block and sinus bradycardia, atrial arrhythmias, mainly atrial flutter, and sudden death. Using a genome-wide linkage approach, we mapped the disease locus to chromosome 1p12-q21. Candidate gene analyses identified a complex rearrangement in the LMNA gene.

**Methods**

**Clinical Analysis of the Pedigree**

Clinical data including medical history, physical examination, 12-lead ECG, 24-hour Holter recordings, and echocardiographic data were collected and evaluated. Individuals were classified as “affected” if they showed any one of the following symptoms or a combination thereof: sinus node dysfunction, atrial arrhythmias (atrial flutter and/or fibrillation), atrioventricular conduction block, or intraventricular conduction defect. Individuals were classified as “unaffected” when they showed no ECG abnormalities. Rhythm and conduction abnormalities were defined according to established criteria. Sinus node dysfunction was defined as sinus bradycardia, sino-atrial exit block, and/or sinus arrest. Dilated cardiomyopathy (DCM), defined by ventricular dilation and diminished (left ventricular) contractile function, was based on the presence of left ventricular ejection fraction <45% and/or left ventricular fractional shortening <25% and left ventricular end-diastolic dimensions >95th percentile indexed for body surface area and age.

**Linkage Analysis**

Informed consent was obtained from all subjects. Genomic DNA of 25 family members, including spouses, was extracted from peripheral blood according to standard procedures. Genotyping was performed at 610,000 single-nucleotide polymorphism sites, using the Illumina Human610-Quad v1 array. Phenotype, genotype, and pedigree information were combined for multipoint linkage analysis using the easyLinkage software package running Merlin v1.0.1. with the assumption of an autosomal dominant pattern of inheritance, a disease-allele frequency of 0.0001, and a disease penetrance of 90%. Gene frequency was assumed to be equal between male and female subjects. Three individuals (II-2, II-3, and III-3, Figure 1) were classified as obligate carriers. One individual whose phenotype was uncertain was not included in the linkage analysis. Multipoint logarithm of odds (LOD) scores were calculated, and a LOD score of >2.5 was considered as suggestive linkage and >3 was considered linkage.

**Candidate Gene Analysis**

Bioinformatics-based text-mining analysis was used to identify candidate genes within regions displaying (suggestive) linkage, using the concept “cardiac conduction disease.” Genes identified in this way were analyzed for coding region mutations. These regions were polymerase chain reaction (PCR)-amplified using primers complementary to flanking intronic sequences, and the purified PCR products were sequenced using BigDye Terminator 3.1 (Applied Biosystems, Foster City, CA) chemistry.

**Multiplex Ligation-Dependent Probe Amplification Analysis**

Multiplex ligation-dependent probe amplification (MLPA) analysis of the LMNA gene, for detection of gene rearrangements, was performed as described in detail previously. In brief, MLPA was performed with a set of custom probes (Salsa MLPA kit P048-B1, MRC-Holland, Amsterdam, The Netherlands) according to the manufacturer’s protocol. The kit contains probes for all 12 exons of the LMNA gene as well as 13 control probes that hybridize to other human genes. Data were normalized by dividing each probe’s peak area by the average peak area of the control probes of the sample. To calculate copy-number ratios, the normalized peak patterns were divided by the average peak area of all samples in the same experiment. A copy-number ratio of 1.0 indicates the presence of two alleles. Copy-number ratio alterations were considered significant with thresholds of 0.7 and 1.3 for deletions and insertions, respectively. MLPA analysis was repeated for all samples in which an aberrant peak pattern was observed.

**Break Point Characterization**

Fine mapping of the deletion break points in the LMNA gene was carried out by PCR in deletion carriers using primers designed to anneal to regions flanking the deletion. The following primer combination was used: forward primer in intron 2, 5'-GAGTGTGAGTGTAGTGCTCTGACC-3'; reverse primer down-stream of exon 12, 5'-TTTCTGTTTTCTACGTTATCTCCTCCC-3' (Figure 2). PCR was performed using TaKaRa LA Taq (TaKaRa Bio Inc, Japan).
Shiga, Japan), and the purified PCR products were sequenced using BigDye Terminator 3.1 (Applied Biosystems, Foster City, CA) chemistry.

The identified complex deletion was named according to the latest nomenclature guidelines on the Human Genome Variation Society web page (http://www.hgvs.org/mutnomen), with GenBank accession number NG_008692.1 (transcript NM_170707.2) used as the reference cDNA sequence for LMNA.

Pathology
Review of patient data revealed that an autopsy was performed on one of the deceased patients (III-3). The autopsy report and 2 available filed paraffin embedded tissue blocks of the heart were retrieved and used for further studies. First, 5-μm sections were stained with hematoxylin and eosin stain and Masson trichrome stain, respectively, for histomorphologic observation. Second, one section was immunostained with Lamin A/C antibody (clone, dilution 1:200, Cell Signaling Technology Inc, Danvers, MA). Control myocardial tissue of a patient with ischemic cardiomyopathy was used as reference material and immunostained following the same protocol. Third, a 1-mm biopsy was taken out of the paraffin blocs from a light microscopically, preselected site and worked up for transmission electron microscopy.

Results
Clinical Data and Pedigree
Figure 1 shows the pedigree of the family studied spanning 4 generations. Conduction disease displayed an autosomal dominant pattern of inheritance. Analysis of clinical data from family members identified 10 affected individuals (8 male; 2 female) and 11 unaffected using the described classification. One individual (III-11) with atrial fibrillation but no conduction system disease was classified as “uncertain.” Three individuals were obligate carriers (2 known to be clinically affected, II-2, III-3; and 1 who died suddenly at 49 years of age but for whom no further clinical details were available, II-3). The clinical characteristics of the affected individuals are summarized in the Table. Besides II-3, another 2 male subjects (II-2 and III-3) died suddenly, at 59 and 50 years of age, respectively. These 2 individuals died during physical activity and 1 (III-3) carried a pacemaker at the time of death. Six individuals showed sinus node dysfunction, 9 individuals had various degrees of atrioventricular block, 5 subjects showed atrial flutter/atrial fibrillation, and 4 displayed intraventricular conduction defect. Furthermore, 2 subjects (III-1 and III-3) displayed episodes of ventricular arrhythmia (bi/trigeminy, couplets and sustained tachycardia). Pacemakers were implanted in 3 individuals. In 2 individuals, the pacemaker was replaced by an implantable cardioverter-defibrillator. Four individuals received an implantable cardioverter-defibrillator. In 2 individuals (IV-5 and III-5), a typical right-sided cavo-tricuspid isthmus–dependent atrial flutter was ablated with acute success and subsequent clinical improvement.

Echocardiography, performed in 9 individuals, revealed mildly increased left ventricular diastolic diameters in 2
### Clinical Characteristics of Family Members

<table>
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<tr>
<th>Pedigree ID</th>
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<th>Conduction Disease</th>
<th>Atrial Fib/Flutter</th>
<th>Age at Echo</th>
<th>LVEF/FS, %</th>
<th>LA, mm</th>
<th>LVEDd (95th percentile), mm</th>
<th>LVESd, mm</th>
<th>IVS, mm</th>
<th>LVPW, mm</th>
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- AVB indicates atrioventricular block; SND, sinus node dysfunction; IVCD, intraventricular conduction defect; atrial fib/flutter, atrial fibrillation/atrial flutter; VT, ventricular tachycardia; LVEF, left ventricular ejection fraction (normal value >45%); FS, fractional shortening (normal value >25%); LA, left atrium (reference range, 27 to 40); LVEDd, left ventricular end-diastolic diameter (reference range, 39 to 59); 95th percentiles for LVEDd, based on body surface area and age, are given in parentheses. The following formula was used: 95th percentile for LVEDd (mm) = 45.3(BSA1/3) – 0.03 (age) – 7.2 + 12%. LVESd indicates left ventricular end-systolic diameter; IVS, interventricular septal thickness (reference range, 6 to 10); LVPW, left ventricular posterior wall thickness (reference range, 6 to 10); ICD, implantable cardioverter-defibrillator; PM, pacemaker; and NA, not available.

### Linkage Analysis

Genome-wide linkage analysis, taking a conservative approach considering only affected individuals, identified 2 loci with maximal LOD scores suggestive of linkage. These loci were on chromosome 1p22-q21 and chromosome 2q35 with maximal LOD scores suggestive of linkage. These loci approach considering only affected individuals, identified 2 loci in aggregate spanning 36 cM.

On repeating the linkage analysis, this time including both affected as well as unaffected individuals, a maximal LOD score of 5.1 at the chromosome 1 locus was yielded and the linkage area at this locus reduced from 34 to 11 cM (chr 1p12-q21). The LOD score for the chromosome 2q35 locus decreased.

### Mutation Screening

Mutation analysis of selected candidate genes from the chromosome 1p22-q21 locus (GJA5, CASQ2, and LMNA) did not identify any nonsynonymous coding region or splice site mutation. Because direct sequence analysis does not detect large gene rearrangements such as large duplications and deletions, MLPA of the LMNA gene was carried out. All family members were tested by MLPA. In 8 individuals, MLPA detected a significant decrease in copy-number ratio for exons 3 to 12, suggesting the presence of a large deletion of ~11 kb encompassing these exons (Figure 2). Three individuals in the pedigree (II-2, II-3, and III-3) who were not tested were obligate carriers of this deletion. The individual with the uncertain phenotype (III-11) proved to be a (partial) phenocopy because he did not carry this deletion.

### Validation of MLPA Result and Mapping of Break Points

Given that the MLPA results indicated a decreased ratio for the LMNA probes for exons 3 to 12, we attempted to capture the break points of the deletion by PCR (Figure 2). Using forward and reverse primers located upstream of exon 3 and downstream of exon 12, respectively, an amplification product of 566 bp that captured the break points was obtained in deletion carriers only. Sequencing of this 566-bp fragment revealed a complex double deletion with a break point in exon 3, 2 break points in intron 10, and another break point downstream of the LMNA gene (Figure 2). The 24-bp region in between the 2 break points in intron 10 was inverted. In brief, the inverted 24-bp region in intron 10 was flanked by a 3.8-kb deletion upstream and a 7.8-kb deletion downstream. Using current nomenclature, this complex deletion can be described as c.[1699 to 159_1995 trans] to c.[1699 to 159_1995]. Inspection of the sequences flanking the 4 breakpoint junctions revealed the presence of short inverted sequence homologies at the break points, suggesting a mutational mechanism consisting of serial replication slippage in trans involving slipped-strand mispairing at the replication fork, resulting in 2 putatively linked successive deletion events. The serial replication process...

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slippage model potentially underlying the generation of the complex gene rearrangement is presented in Figure 2.

Pathology
The autopsy report of family member III-3, who is an obligate carrier of the deletion and who died suddenly at the age of 50 years, mentioned a hypertrophic heart (weight, 560 g) with dilatation of both ventricles and a pacemaker lead in situ in the right ventricle. There was no significant atherosclerotic narrowing noticed in the coronary arteries. Microscopically, the tissue blocks were derived from the interventricular septum and the left ventricular free wall. Sections showed a severe degree of interstitial fibrosis and occasional small areas of fibrolipomatosis throughout the myocardium at both locations. Cardiomyocytes showed hypertrophic cytonuclear changes, sometimes cytoplasmic vacuolization, and irregular outlines of the nuclear contours. Myocardial disarray was notably absent. Antilam A/C immunostaining revealed immunoreactivity with interstitial cells in the myocardium but only faint staining of nuclear membranes of cardiomyocytes, which was often incomplete and sometimes almost absent. By contrast, immunostaining of control sections (derived from a patient with ischemic cardiomyopathy) showed distinct continuous immunostaining of cardiomyocyte nuclear membranes (Figure 3). Morphology of the ultrathin sections for transmission electron microscopy was poor because of prior paraffin embedding of the tissue. Nevertheless, nuclei were observed showing a highly irregular and convoluted shape with occasional small herniations of the nuclear membrane (blebs). Such pathological findings have been described recently in the setting of LMNA-related cardiomyopathy.\(^\text{20}\)

![Image](http://circgenetics.ahajournals.org/)

**Figure 3.** Microscopy of the myocardium of patient III-3. **A,** Distinct interstitial fibrosis of the left ventricular wall; light blue staining material depicts fibrosis surrounding purple staining myocytes; Masson trichrome stain, \(\times100\). **B,** Nuclear detail of cardiomyocyte showing convoluted nuclear shape and small bleb of the nuclear membrane (arrow). **C**, Lamin A/C immunostain on myocardial tissue from patient III-3; incomplete and absent staining of cardiomyocyte nuclei and intact staining of interstitial-vascular cell nuclei. **D,** Lamin A/C immunostain on control tissue from a patient with ischemic cardiomyopathy; distinct and continuous staining of cardiomyocyte nuclei and interstitial-vascular cell nuclei.

The LMNA gene, which maps to chromosome 1q21.2-q21.3, encodes the intermediate filament lamins A and C. These proteins are ubiquitously coexpressed in the nuclear membrane of many tissues, including skeletal and cardiac muscle. Mutations in LMNA have a pleiotropic effect and cause a wide spectrum of over 10 different clinical disease entities,\(^{21,22}\) which can be clinically classified in 4 groups: (1) diseases of striated muscle, including cardiac muscle and the conduction system, (2) lipodystrophy syndromes, (3) peripheral neuropathy, and (4) accelerated aging disorders.

A cardiac phenotype consisting of conduction disease with or without DCM is a feature often expressed in LMNA mutation carriers, whereas a significant subset displays cardiac disease without involvement of other organ systems. The conduction disease observed in this family is comparable to that observed previously in LMNA mutations carriers. In a meta-analysis of 299 patients with LMNA mutations, 92% of carriers \(>30\) years of age had conduction disease, including atrioventricular conduction disturbances, atrial arrhythmias, and ventricular arrhythmias.\(^{23}\) In addition, atrial tachyarrhythmias rarely occur isolated in families with LMNA mutations, but the onset of disease may be characterized by atrial fibrillation (ie, in the absence of preexisting DCM).\(^{24,25}\)

LMNA mutations are found at frequencies of 6% to 8% among patient populations with idiopathic or familial DCM.\(^{26,27}\) However, severe loss of cardiac contractility is a very common finding in LMNA mutation carriers, especially at more advanced ages. Therefore, heart failure is a common feature in families with cardiac manifestations of LMNA disease. Nevertheless, with the exception of individual III-3, who died suddenly at 50 years with overt dilatation and extensive interstitial fibrosis at postmortem histological examination, structural and functional abnormalities as determined by echocardiography were only scarcely found in the family we studied here. Extensive echocardiographic examination of mutation carriers in this family, including 3 individuals in the fifth and sixth decades of life, uncovered only mild left ventricular dilatation in the absence of left ventricular dysfunction. Left ventricular dilatation has been more

Discussion
We identified a complex deletion in the LMNA gene in a 4-generation Dutch family with progressive cardiac conduction disease, including atrioventricular conduction block and sinus bradycardia, atrial arrhythmias, and sudden death.
often reported not to occur in LMNA mutation carriers. A large family with a 674-bp deletion at the 5' end of the LMNA gene also manifested with limited dilatation in the presence of early-onset myocardial fibrosis, although echocardiographic examinations were not taken beyond the age of 47.28 In a large LMNA-related DCM cohort, the average age of onset of DCM was 42.8 ± 8.7 years, with a median of 42 years.27 Even when one considers an age-dependent component in the development of DCM, the degree of structural abnormalities in this family is surprisingly low. This observation is not in line with the most commonly observed presentation, which typically consists of an initial presentation of conduction disease and later development of DCM or conduction disease together with DCM at first presentation. Two of the sudden (cardiac) death victims died during physical activity, and one (III-3) participated actively in sports. Indeed, participation in competitive sports for 10 years or longer was demonstrated to be an independent risk factor for cardiac events (lifethreatening arrhythmias or end-stage heart failure) in a large cohort of LMNA mutation–positive individuals.29

Laminopathies result primarily from missense mutations.10,22 Nonsense mutations, splice-site mutations, small insertions/deletions,33 as well as large deletions28,34 are less common. The mutation that we describe is the first complex deletion in this gene reported. A large deletion encompassing exon 3 to 12 was described by Gupta et al14 in a 39-year-old woman with ventricular arrhythmias and mild dilatation and normal function. PCR analysis for the 566-bp fragment specific for our deletion on genomic DNA from this woman did not result in an amplification product, suggesting that although the mutation encompassed the same region, a different mutational event could underlie the disorder (Frédérique Tesson, personal communication). Identification of a large rearrangement further expands the LMNA mutational spectrum. Although the actual frequency of large genomic rearrangements in the LMNA gene may be low, the technology for detecting these mutations is well available (including MLPA and quantitative real-time PCR) and should be implemented in routine genetic diagnostic testing for this gene.

Studies analyzing breakpoint junctions of complex genomic rearrangements underlying human genetic disease have led to the identification of a replication-based mechanism, termed “serial replication slippage” (SRS), characterized by the presence of microhomology junctions (≥2 bp) and sequence complexity (involving multiple deletions and interstrand inversions).18,19 SRS models have been proposed to explain the origin of complex genomic rearrangements involving deletions seen in a small but significant fraction of cystic fibrosis (≈1.5% of known CFTR gene lesions) and hemophilia A patients.35,36 For the complex double deletion described in the present study, we suggest a mutational mechanism invoking SRS in trans during DNA replication, which features annealing of single-stranded nascent leading-strand DNA to lagging strand DNA with minimal homology.

If translated, the mutated allele with the 11.6-kb deletion is predicted to disrupt the protein sequence from amino acid 189 onward with the addition of 133 novel amino acids. This early out-of-frame deletion, in the first one-third of the primary protein sequence, probably results in a null allele as a consequence of nonsense-mediated decay of the mutant mRNA.37 Defects in LMNA heterozygous knockout mice resemble deficiencies in individuals with LMNA mutations.38 In mice, lamin haploinsufficiency resulted in AV conduction defects as well as atrial and ventricular arrhythmias at 10 weeks, whereas cardiomyopathy became overt in older mice (age >50 weeks). Misshapen cell nuclei and abnormal lamin A/C immunostaining have been previously observed in cardiomyocytes from human LMNA patients with different mutations as well as in mice with LMNA haploinsufficiency.38,39 Similar observations were made in the postmortem tissue samples from subject III-3, in line with the causality of the LMNA mutation.

The pathogenetic mechanism associated with LMNA mutations is unknown, partly because the function of lamin A/C is currently undefined. Two hypotheses have been proposed to explain the cellular and molecular mechanism by which alterations in lamin A/C promotes (tissue-specific) pathology. The first implicates disruption of nuclear structural integrity by impaired lamin A/C function.40 A second theory focuses on lamin-dependent, tissue-specific gene regulation and DNA replication.40–42 Identification of the causal gene underlying inherited conduction disease and sudden death in this family enables presymptomatic genetic diagnosis and early disease management. Disease management strategies of cardiac laminopathies fall into 2 areas: prevention of potentially lethal arrhythmias and symptomatic treatment with a focus on conduction system disease, arrhythmias, and DCM. Our findings underscore the fact that if conventional PCR-based direct sequencing approaches for LMNA analysis are negative in suggestive pedigrees, mutation detection techniques capable of detecting gross genomic lesions involving deletions and insertions should be considered.

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Disclosures

None.

References


Inherited cardiac conduction disease is characterized by genetically determined defects in electric impulse generation and conduction. Genetic testing plays an important role in identifying patients at risk. Conduction disease is often progressive in nature, and young individuals presenting with a normal ECG may be at risk of arrhythmia and sudden death as they get older. Genetic testing allows for implementation of preventive measures in asymptomatic patients carrying the genetic defect, whereas patients with a negative genetic test can be reassured. In this study, we investigated a 4-generation Dutch family with progressive cardiac conduction disease, including atrioventricular conduction block, sinus bradycardia, atrial arrhythmias, mainly atrial flutter, and sudden death. Using a genome-wide linkage approach, we mapped the disease locus to chromosome 1p12-q21. Candidate gene analyses identified a complex rearrangement in the LMNA gene. Our findings underscore the fact that if conventional polymerase chain reaction–based direct sequencing approaches for LMNA analysis are negative in suggestive pedigrees, mutation detection techniques capable of detecting gross genomic lesions involving deletions and insertions should be considered. Furthermore, whereas heart failure is a common feature in families with cardiac manifestations of LMNA disease, structural and functional abnormalities as determined by echocardiography were only scarcely found in the family that we studied. Even when one considers an age-dependent component in the development of dilated cardiomyopathy, the degree of structural abnormalities in this family is surprisingly low.
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