Genetic risk factors for common and rare cardiac rhythm disorders
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A complex double deletion in LMNA underlies progressive cardiac conduction disease, atrial arrhythmias and sudden death

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ABSTRACT

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

Methods and Results
We here studied a four-generation family with autosomal dominant progressive cardiac conduction disease, including atrioventricular conduction block, sinus bradycardia, atrial arrhythmias and sudden death. Genome-wide linkage analysis mapped the disease locus to chromosome 1p22-q21. Multiplex ligation-dependent probe amplification (MLPA) 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.

Conclusion
We identified for the first time a complex LMNA gene rearrangement involving a double deletion in a four-generation Dutch family with progressive conduction system disease. 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.
INTRODUCTION

Inherited cardiac conduction disease is characterized by genetically determined defects in electrical impulse generation and conduction. The electrical 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. Electro-anatomical 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

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 electrocardiogram (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 while 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, SCN1B) as well as in genes involved in the regulation of heart development (NKX2.5, TBX5) where 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 (extra-cardiac) disorders (referred to as ‘laminopathies’) have been associated with mutations the LMNA gene (MIM# 150330), encoding for the inner nuclear membrane protein lamin A/C.10

In this study, we investigated a four-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.

METHODS

Clinical analysis of the pedigree
Clinical data including medical history, physical examination, 12-lead electrocardiogram (ECG), 24-hr 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.11

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.12,13
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\textsuperscript{14} running Merlin v1.0.1.\textsuperscript{15} 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 males and females. 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 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\textsuperscript{16} 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 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, USA) chemistry.

Multiplex ligation-dependent probe amplification (MLPA) analysis
MLPA analysis of the \textit{LMNA} gene, for detection of gene rearrangements, was performed as described in detail previously.\textsuperscript{17} In brief, MPLA was performed with a set of custom probes (Salsa MLPA kit P048-B1, MRC- Holland, the Netherlands) according to the manufacturer’s protocol. The kit contains probes for all 12 exons of the \textit{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.

Breakpoint characterization
Fine mapping of the deletion breakpoints in the \textit{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’-GAGTGAAGTGGATAGGGATCCTGACC-3’; reverse primer downstream of exon 12, 5’-TTTCTGGTTCCTACGTTATCTCCTC-3’ (Figure 2). PCR was performed using TaKaRa LA Taq (TaKaRa Bio Inc., Shiga, Japan), and the purified PCR products were sequenced using BigDye Terminator 3.1 (Applied Biosystems, Foster City, CA, USA) 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 \textit{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, 5µm sections were stained with Haematoxylin and Eosin (H&E) 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, MA, USA). Control myocardial tissue of a patient with ischemic cardiomyopathy was used as reference material and immunostained following the same protocol. Third, a 1mm 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 four generations. Conduction disease displayed an autosomal dominant pattern of inheritance. Analysis of clinical data from family members identified 10 affected individuals (8 males; 2 females), 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 (two known to be clinically affected, II-2, III-3; and one 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 Table 1. Besides II-3, another two males (II-2 and III-3) died suddenly, respectively at 59 and 50 years of age. These two individuals died during physical activity and one (III-3) carried a pacemaker at the time of death. Six individuals showed sinus node dysfunction, nine individuals had various degrees of atrioventricular block, five subjects showed atrial flutter/atrial fibrillation and four displayed
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<th>Conduction disease</th>
<th>Atrial Fib/Flutter</th>
<th>VT Age at echo</th>
<th>LVEF / FS (95th percentile)</th>
<th>LA (mm)</th>
<th>LVESd (mm)</th>
<th>LVEDd (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(BSA)1/3-0.03 (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.
intraventricular conduction defect. Furthermore, two subjects (III-1 and III-3) displayed episodes of ventricular arrhythmia (bi/trigeminy, couplets and sustained tachycardia). Pacemakers have been implanted in three individuals. In two individuals the pacemaker was replaced by an implantable defibrillator (ICD). Four individuals received an ICD device. 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 nine individuals, revealed mildly increased LV diastolic diameters in two individuals (III-1 and III-3) with normal values of left ventricular function, however these echocardiographic indices did not conform to diagnostic criteria for DCM. Mild to moderate left atrial enlargement was manifest in seven individuals. None of the individuals had left ventricular dysfunction.

**Linkage analysis**

Genome-wide linkage analysis taking a conservative approach considering only affected individuals identified two loci with maximal LOD scores suggestive of linkage. These loci were on chromosome 1p22-q21 and chromosome 2q35 (LOD = 2.7 for each) and in aggregate spanned 36 cM.

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**Figure 2** | Complex double deletion involving *LMNA* (A) and serial replication slippage model potentially underlying the generation of the complex gene rearrangement (B). (A) Panel (i) shows the wild-type *LMNA* gene at the top row and the *LMNA* gene with the deletion in red (Δ3-12) at the second row. Blue arrows indicate breakpoint mapping primers. Panel (ii) shows the sequence of the deletion breakpoints. (B) Panel (i) shows sequences at the breakpoints and the 24-bp inversion. The direction of replication of the nascent strand (5’-3’) is indicated by horizontal arrows. The breakpoint junctions are marked by dashed vertical lines and microhomologies at the breakpoint junctions are indicated in bold and underlined. The mutant sequence is shaded. Panel (ii) shows the proposed mechanism of SRS in *trans*. The three different steps of replication slippage are numbered sequentially. (1) 3’end of B. transiently dissociates from A. and misaligns with D. via homologous sequences; continued DNA synthesis against D. (2) 3’end of C. dissociates from D. and (3) re-anneals to A. via homologous sequences; continued DNA synthesis against A.
Upon 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, LMNA) did not identify any non-synonymous coding region or splice site mutation. As direct sequence analysis does not detect large gene rearrangements such as large duplications and deletions, multiplex ligation-dependent probe amplification (MLPA) of the LMNA gene was carried out. All family members were tested by MLPA. In eight individuals, MLPA detected a significant decrease in copy-number ratio for exons 3 to 12, suggesting the presence of a large deletion of ~11kb 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 since he did not carry this deletion.

**Validation of MLPA result and mapping of breakpoints**

Given that the MLPA results indicated a decreased ratio for the LMNA probes for exons 3 to 12, we attempted to capture the breakpoints of the deletion by PCR (Figure 2). Using forward and reverse primers located respectively upstream of exon 3 and downstream of

![Figure 3](image-url)  
**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, 100x. (B) Nuclear detail of cardiomyocyte showing convoluted nuclear shape and small bleb of the nuclear membrane (arrow). N=nucleus. Transmission Electron Microscopy, bar represents 2 µm. (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.
exon 12, an amplification product of 566 bp that captured the breakpoints was obtained in deletion carriers only. Sequencing of this 566-bp fragment revealed a complex double deletion with a breakpoint in exon 3, two breakpoints in intron 10, and another breakpoint downstream of the LMNA gene (Figure 2). The 24-bp region in between the two breakpoints 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.[1699-183_1699-160inv24; 568_1699-184del; 1699-159_1995+6997del]. Inspection of the sequences flanking the four breakpoint junctions revealed the presence of short inverted sequence homologies at the breakpoints suggesting a mutational mechanism consisting of serial replication slippage in trans involving slipped strand mispairing at the replication fork resulting in two, putatively linked successive deletion events.\textsuperscript{18,19} The model of serial replication slippage 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, mentioned a hypertrophic heart (weight 560 gram) with dilatation of both ventricles, and a pacemaker lead \textit{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. Anti Lamin 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 due to 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.\textsuperscript{20}

DISCUSSION
We identified a complex deletion in the LMNA gene in a four-generation Dutch family with progressive cardiac conduction disease, including atrioventricular conduction block, sinus bradycardia, atrial arrhythmias and sudden death.

The LMNA gene, which maps to chromosome 1q21.2-q21.3, encodes the intermediate filament lamins A and C. These proteins are ubiquitously co-expressed 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\textsuperscript{21,22} which can be clinically classified in four 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, while 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 over 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 (i.e. in the absence of pre-existing 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 echocardiographical examination of mutation carriers in this family, including 3 individuals in the fifth and sixth decade of life, uncovered only mild left ventricular dilatation in the absence of left ventricular dysfunction. Left ventricular dilatation has been more 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 (life-threatening arrhythmias or end-stage heart failure) in a large cohort of LMNA mutation positive individuals.29

Laminopathies result primarily from missense mutations.10,22 Nonsense30 mutations, splice-site31 mutations, small insertions2 / deletions33, as well as large deletions28,34 are less common. The mutation we describe here is the first complex deletion in this gene reported. A large deletion encompassing exon 3 to 12 was described by Gupta et al. in a 39-year-old female with ventricular arrhythmias and mild dilatation and normal function.34 PCR analysis for the 566-bp fragment specific for our deletion on genomic DNA from this female did not result in an amplification product, suggesting that although the mutation encompassed the same region, a different mutational event could underlie the disorder in this female (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 here, 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 onwards with the addition of 133 novel amino acids. This early out-of-frame deletion, in the first one-third of the primary protein sequence, most likely 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, while cardiomyopathy became overt in older mice (age>50 weeks). Missshapen 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 pre-symptomatic genetic diagnosis and early disease management. Disease management strategies of cardiac laminopathies fall into two 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.

**CLINICAL PERSPECTIVE**

Inherited cardiac conduction disease is characterized by genetically determined defects in electrical 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 electrocardiogram (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 while patients with a
negative genetic test can be reassured. In this study, we investigated a four-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 \textit{LMNA} gene. Our findings underscore the fact that if conventional PCR-based direct sequencing approaches for \textit{LMNA} analysis are negative in suggestive pedigrees, mutation detection techniques capable of detecting gross genomic lesions involving deletions and insertions should be considered. Furthermore, while heart failure is a common feature in families with cardiac manifestations of \textit{LMNA} disease, structural and functional abnormalities as determined by echocardiography were only scarcely found in the family we studied here. Even when one considers an age-dependent component in the development of DCM, the degree of structural abnormalities in this family is surprisingly low.

\textbf{ACKNOWLEDGEMENTS}

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REFERENCES


