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Published on: 02 Dec 1999 - The New England Journal of Medicine (Massachusetts Medical Society)

Topics: LMNA, Laminopathy, Emery–Dreifuss muscular dystrophy, Cardiac conduction and Cardiomyopathy

MISSENSE MUTATIONS IN THE ROD DOMAIN OF THE LAMIN A/C GENE AS CAUSES OF DILATED CARDIOMYOPATHY AND CONDUCTION-SYSTEM DISEASE

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ABSTRACT

Background Inherited mutations cause approximately 35 percent of cases of dilated cardiomyopathy; however, few genes associated with this disease have been identified. Previously, we located a gene defect that was responsible for autosomal dominant dilated cardiomyopathy and conduction-system disease on chromosome 1p1–q21, where nuclear-envelope proteins lamin A and lamin C are encoded by the *LMNA* (lamin A/C) gene. Mutations in the head or tail domain of this gene cause Emery–Dreifuss muscular dystrophy, a childhood-onset disease characterized by joint contractures and in some cases by abnormalities of cardiac conduction during adulthood.

Methods We evaluated 11 families with autosomal dominant dilated cardiomyopathy and conduction-system disease. Sequences of the lamin A/C exons were determined in probands from each family, and variants were confirmed by restriction-enzyme digestion. The genotypes of the family members were ascertained.

Results Five novel missense mutations were identified: four in the α-helical rod domain of the lamin A/C gene, and one in the lamin C tail domain. Each mutation caused heritable, progressive conduction-system disease (sinus bradycardia, atrioventricular conduction block, or atrial arrhythmias) and dilated cardiomyopathy. Heart failure and sudden death occurred frequently within these families. No family members with mutations had either joint contractures or skeletal myopathy. Serum creatine kinase levels were normal in family members with mutations of the lamin rod but mildly elevated in some family members with a defect in the tail domain of lamin C.

Conclusions Genetic defects in distinct domains of the nuclear-envelope proteins lamin A and lamin C selectively cause dilated cardiomyopathy with conduction-system disease or autosomal dominant Emery–Dreifuss muscular dystrophy. Missense mutations in the rod domain of the lamin A/C gene provide a genetic cause for dilated cardiomyopathy and indicate that this intermediate filament protein has an important role in cardiac conduction and contractility. (N Engl J Med 1999;341:1715-24.)

DILATED cardiomyopathy, a myocardial disorder characterized by dilatation of the cardiac chambers and impaired systolic contraction, is a major cause of congestive heart failure worldwide. Despite advances in therapy, mortality due to dilated cardiomyopathy remains high.[1,2] Dilated cardiomyopathy can result from coronary artery disease, myocarditis, or systemic diseases but can also result from a primary (idiopathic) disorder of the myocyte's contractile apparatus, cytoskeleton, or both.[3] The cellular and molecular basis of primary dilated cardiomyopathy remains poorly understood.

Approximately one third of cases of idiopathic dilated cardiomyopathy are inherited.[4-6] Although this disorder can be transmitted as a recessive or X-linked trait, autosomal dominant inheritance occurs most frequently and exhibits both clinical variability and genetic heterogeneity. Adult-onset dilated cardiomyopathy, without accompanying conduction disease or skeletal-muscle abnormalities, has been mapped to six loci, on chromosomes 9q13–q22,[7] 10q21–q23,[8] 1q32,[9] 2q31,[10] 2q11–q22,[11] and 15q14.[12] Of these, only one disease-linked gene, that for cardiac actin (on chromosome 15), has been identified. Dilated cardiomyopathy is often accompanied by conduction-system disease, and disease loci on chromosomes 3p22–p25[13] and 1p1–q21[14] have been identified. Linkage studies further indicate that the chromosome 1p1–q21 locus accounts for an important fraction of cases of dilated cardiomyopathy and conduction-system disease (unpublished data).

Emery–Dreifuss muscular dystrophy results in the

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onset of contractures of the elbows, Achilles tendons, and postcervical muscles in childhood, with slowly progressive wasting and weakness of the muscles in a humeroperoneal distribution. By adulthood, affected persons have conduction-system disease, most commonly heart block[15,16]; left ventricular dilatation and contractile dysfunction are very rare.[17-19] Emery–Dreifuss muscular dystrophy occurs as an X-linked trait, caused by emerin mutations,[20,21] or as an autosomal dominant disorder resulting from mutations in the gene encoding lamins A and C.[22]

Like emerin, lamins A and C are components of the nuclear envelope but are located in the lamina, a multimeric structure associated with the nucleoplasmic surface of the inner nuclear membrane (Fig. 1). These highly conserved proteins are transcribed from a single gene, *LMNA* (referred to hereinafter as the lamin A/C gene), which is encoded on chromosome 1q21.2–q21.3.[29] Lamins are structurally homologous with other intermediate filaments and consist of a central-rod domain flanked by globular amino and carboxyl domains (Fig. 1B).[30,31] Hydrophobic repeats within the central rod promote formation of an α-helical coiled-coil dimer, and charged residues along the surface of the coiled-coil dimer promote interactions between rod dimers, thereby producing complex assembly of the filaments.[30,32] Lamins A, C, and B2 are expressed in a wide range of tissues, including adult heart and skeletal muscle.[33,34] The mechanism by which lamin A/C mutations cause Emery–Dreifuss muscular dystrophy is unknown.

On the basis of the chromosomal location of the lamin A/C gene and the cardiovascular phenotype of some adults with Emery–Dreifuss muscular dystrophy, we hypothesized that distinct mutations in the lamin A/C gene caused dilated cardiomyopathy and conduction-system disease in the absence of skeletal myopathy. To test this hypothesis, we sequenced the lamin A/C gene in probands of 11 families and evaluated affected family members for clinical manifestations of Emery–Dreifuss muscular dystrophy.

METHODS

Clinical Evaluation

Written informed consent was obtained from all participants in accordance with the requirements of the human-research committee of Brigham and Women's Hospital. All the family members underwent clinical evaluation, including history taking, physical examination, 12-lead electrocardiography, and transthoracic echocardiography. Initial clinical evaluations were performed without knowledge of genotype status. Family members were considered to be affected if cardiac studies demonstrated sinoatrial dysfunction, atrioventricular conduction-system abnormalities, or left ventricular dysfunction (increased end-diastolic diameter, reduced fractional shortening, or both) in the absence of other known causes.[14] The disease status of deceased family members was determined on the basis of a review of their medical records. Deaths were classified as disease-related or as due to noncardiac causes, as described previously.[10] Family members with lamin A/C mutations underwent further evaluations, including a review of their medical history, clinical neuromuscular examination, measurement of serum creatine kinase, and skeletal-muscle biopsy.

Genetic Studies

We obtained 5 to 30 ml of peripheral blood from each family member who was evaluated. DNA was isolated from whole blood or lymphocytes transformed by Epstein–Barr virus as described elsewhere.[14] Refined physical mapping of polymorphisms of chromosome 1 has recently extended the disease locus at chromosome 1p1–1q1[14] to q21. Genetic linkage to the 1p1–q21 locus was ascertained in Families A and B with the use of the polymorphic markers D1S305 and D1S506. Two-point lod scores were calculated with the use of the MLINK program.[14]

Identification of Mutations in the Lamin A/C Gene

Protein-encoding sequences from exons 1 through 12 were amplified from genomic DNA with the use of primers derived from intron sequences. The sequences are available on the Internet (at http://genetics.med.harvard.edu/~seidman/lamin.html) or from the National Auxiliary Publications Service (NAPS).* Intron–exon boundaries for lamin A/C were obtained from genomic sequences in the GenBank data base (accession numbers L12399, L12400, and L12401). Genomic DNA fragments amplified with the polymerase chain reaction (PCR) were purified with a PCR purification kit (QIAquick, Qiagen, Santa Clarita, Calif.) to remove the residual primers and sequenced with a dye-terminator cycle-sequencing system (ABI PRISM 377, Perkin–Elmer Applied Biosystems, Foster City, Calif.).

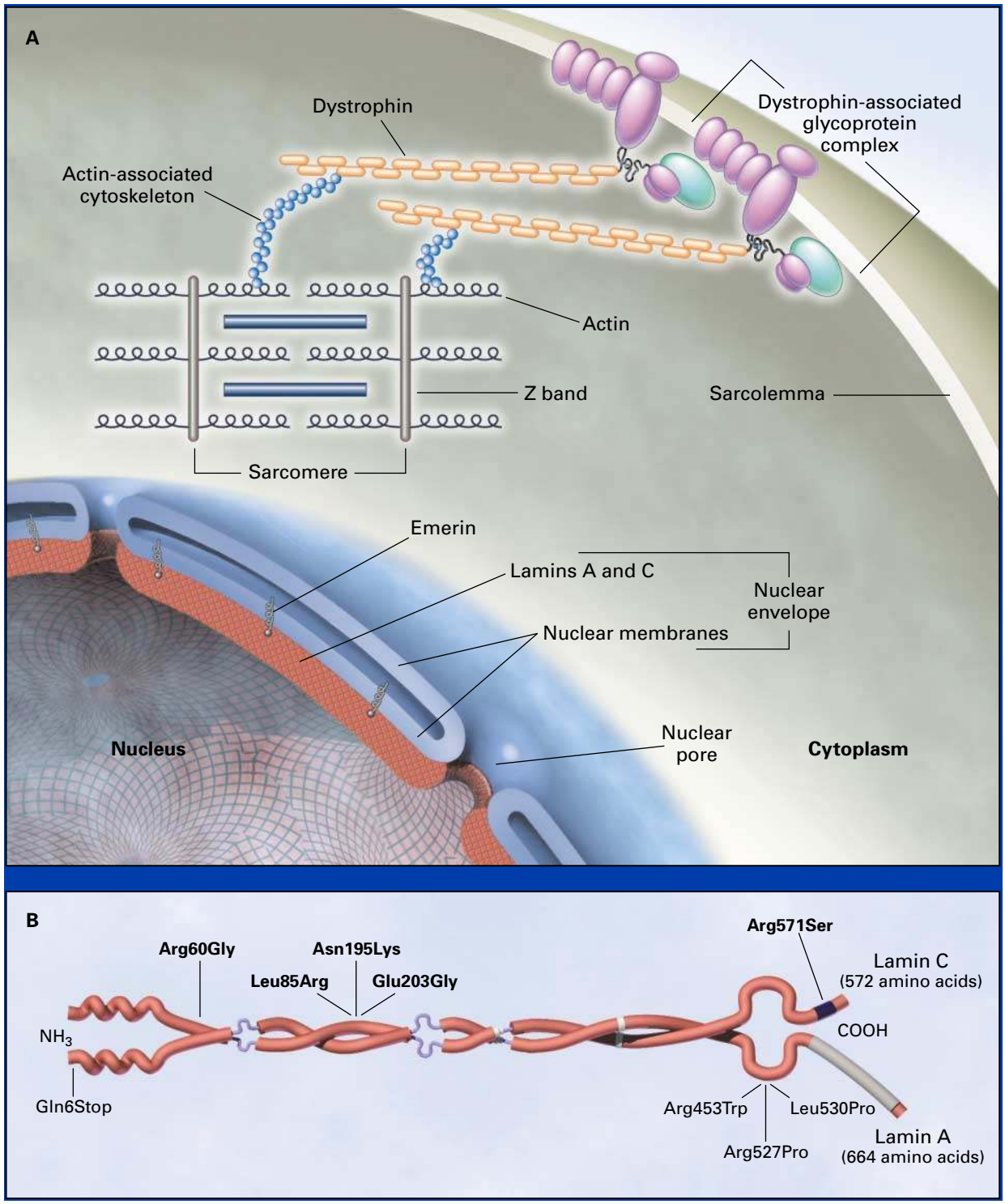
Confirmation of Mutations and Family Genotypes

Five variants in the lamin A/C gene sequences (Fig. 2A) were identified by sequence analysis and independently confirmed by restriction-enzyme digestion. Exons were amplified by PCR with the use of primers available on the Internet (at http://genetics.

*See NAPS document no. 05534 for 4 pages of supplementary material. To order, contact NAPS, c/o Microfiche Publications, 248 Hempstead Tpk., West Hempstead, NY 11552.

Figure 1. Components of Myocyte Cytoarchitecture (Panel A) and Mutations Causing Dilated Cardiomyopathy and Conduction-System Disease or Autosomal Dominant Emery–Dreifuss Muscular Dystrophy (Panel B).

Mutations in the rod domain of the lamin A/C gene cause isolated dilated cardiomyopathy and conduction-system disease, presumably through perturbed interactions with nuclear or cytoplasmic constituents (Panel A). Other cytoskeletal molecules implicated in the pathophysiology of human dilated cardiomyopathy include actin, dystrophin, and the dystrophin-associated glycoprotein complex.[12,23-28] Interactions between lamins A and C and cytoskeletal or sarcomere proteins are unknown. Conduction-system disease is a common feature of Emery–Dreifuss muscular dystrophy caused by defects in the head or tail domain of the lamin gene or by emerin mutations. Mutations causing dilated cardiomyopathy and conduction-system disease or autosomal dominant Emery–Dreifuss muscular dystrophy are distributed in distinct domains of the lamin dimer (Panel B). Lamins A and C have identical structures throughout the amino-terminal head (NH_3), α-helical rod domain, and proximal carboxyl-terminal tail (COOH), but they differ in their distal amino acids (lamin A is shown in gray, and lamin C is shown in black). Mutations in the rod domain (Arg60Gly, Leu85Arg, Asn195Lys, and Glu203Gly) cause dilated cardiomyopathy and conduction-system disease without skeletal myopathy; the mutation at the carboxyl terminal (Arg571Ser) is associated with subclinical skeletal-muscle disease. Mutations that cause Emery–Dreifuss muscular dystrophy (Gln6Stop, Arg453Trp, Arg527Pro, and Leu530Pro) do not affect the α-helical rod domain.



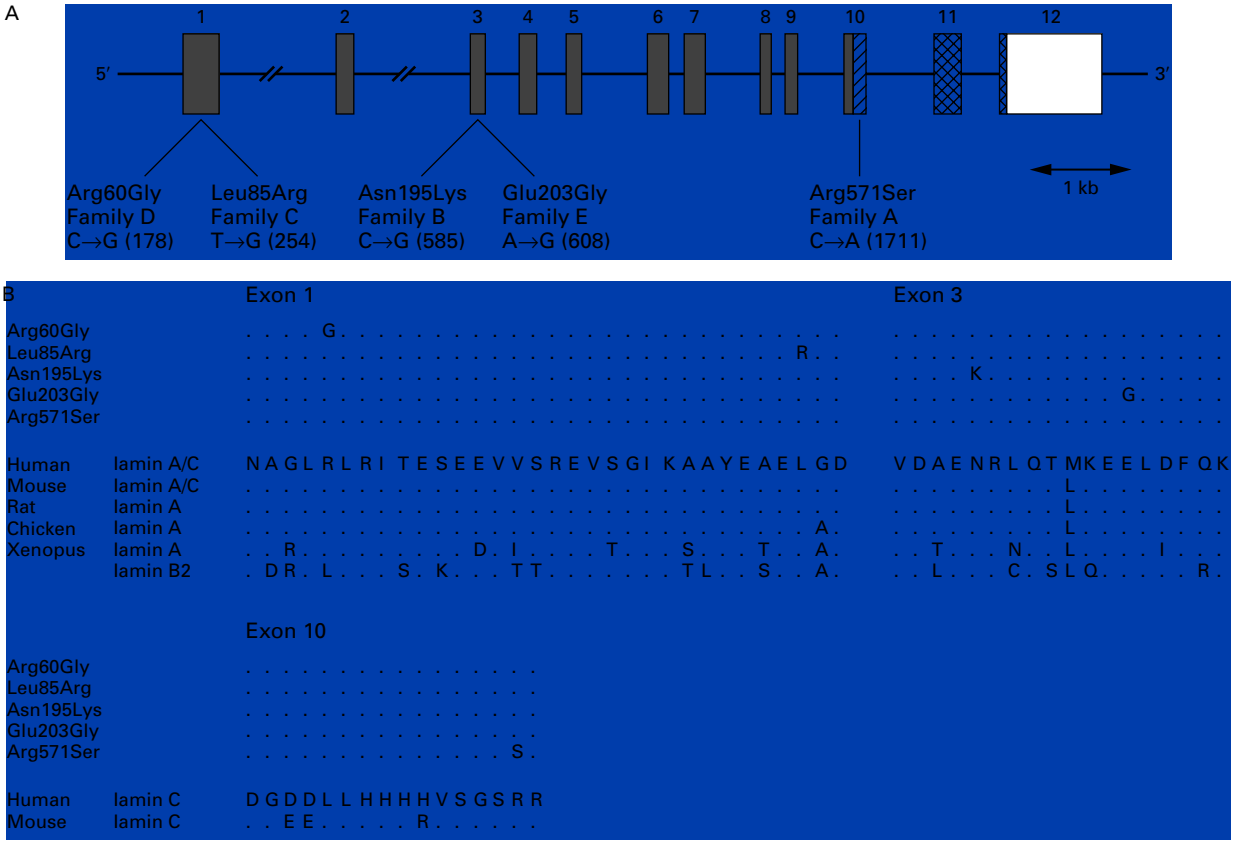


Figure 2. Isoforms of the Lamin A/C Gene and Sequences of Lamin Proteins.

The lamin A/C gene encodes four isoforms by alternative splicing of the 12 exons that make up the gene (Panel A). Exons 1 through 10 contain sequences shared by all isoforms (stippled bars). Exons 11 and 12 contain sequences (cross-hatched shading) that are found in transcripts of lamin A, and exon 10 contains sequences (hatched shading) that encode lamin C isoforms. The 3′ untranslated region (open area) becomes part of the lamin A messenger RNA. The precise sizes of introns 1 and 2 are not known. The nucleotide residues mutated in the lamin A/C gene that were identified in study families and their predicted amino acid substitutions in lamin peptides are indicated in parentheses. A comparison of sequences of lamin proteins among various species (Panel B) indicates that human mutations that cause dilated cardiomyopathy and conduction-system disease occur in highly conserved residues. Dots denote amino acid identity with the human sequence (shown as a single-letter code). There is considerable divergence among species in the sequences of lamin B2 as compared with those of lamins A and C. (The lamin sequences for humans, mice, rats, chickens, and xenopus were obtained from the GenBank data base. In cases in which human lamin B2 sequences were not available, murine sequences are provided.)

med.harvard.edu/~seidman/lamin.html) or from NAPS*, digested with restriction enzyme, and size-fractionated on a 3 percent Nusieve–1 percent agarose gel. The presence or absence of sequence variants in DNA samples from family members and from 75 normal controls was also ascertained by analyses of the results of restriction-enzyme digestion. The Leu85Arg mutation (in Family C) abolishes a *Sac*I restriction-enzyme site. Mutations Arg60Gly (Family D), Asn195Lys (Family B), Glu203Gly (Family E), and Arg571Ser (Family A) create *Sau*96I, *Mbo*II, *Bsm*FI, and *Alw*NI restriction-enzyme sites, respectively.

RESULTS

Genetic Studies

Autosomal dominant dilated cardiomyopathy and conduction-system disease in two families (Families A and B) were assessed for linkage to the disease locus at chromosome 1p1–q21. Analyses of polymorphic loci *D1S305* and *D1S506* indicated that there was linkage of disease to this genomic location in the families (likelihood of coinheritance, 1 in 100 and 1 in 125, respectively). Exon sequences of the lamin A/C gene (Fig. 2A) were then analyzed in samples from the probands in these and nine other, unrelated families with autosomal dominant dilated cardiomyopathy and conduction-system disease. A unique sequence variant was identified in the genomes of five unrelated probands, each of which was predicted to alter one amino acid in the lamin protein. Each sequence variant was independently confirmed by analysis of the results of restriction-enzyme digestion. None of the five sequence variants were found in more than 150 chromosomes from normal persons, demonstrating

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that these sequence variants are not common polymorphisms.

Four of the five sequence variants alter 1 of 566 amino acids common to isoforms of lamin A and C: Arg60Gly and Leu85Arg are encoded in exon 1; Asn195Lys and Glu203Gly are encoded in exon 3. These variants were predicted to alter residues in the α-helical rod domain (coils 1A and 1B) of the peptide. In contrast, the mutation Arg571Ser (in exon 10) alters a residue specific to the carboxyl tail of the lamin C isoform. These five sequence variants were designated as disease-causing mutations because they were found in affected family members but not in unaffected relatives more than 30 years of age, because they altered the charge of the encoded amino acid and therefore would be expected to perturb the lamin structure considerably, and because each altered residue is highly conserved throughout evolution (Fig. 2B), indicating that these defects probably have functional consequences.

Clinical Features of Lamin A/C Mutations

Clinical evaluations of 85 members of the five families with the sequence variants identified 39 with cardiovascular disease (Table 1 and Fig. 3). Genetic studies in 26 clinically affected family members demonstrated that each was heterozygous for a lamin missense mutation. DNA samples were not available from 12 deceased family members and 1 affected living member. Twelve family members who were less than 30 years of age were heterozygous for a lamin A/C missense mutation but had neither signs nor symptoms of heart disease.

The natural history of disease was assessed on the basis of clinical histories of affected members (Table 1) in the five families. The onset of disease typically occurred early in middle age (mean age, 38 years; range, 19 to 53), and in multiple instances the initial presentation was asymptomatic electrocardiographic abnormalities in cardiac rate and rhythm, which were detected during routine physical examination. Progressive abnormalities in cardiac conduction and atrial fibrillation or flutter became evident with increasing age (Fig. 4A). Thirty-four of the 39 affected family members (87 percent) had sinus-node dysfunction or disturbances in atrioventricular conduction (sinus bradycardia or first-, second-, or third-degree heart block); 23 affected members (59 percent) had atrial fibrillation or flutter. Twenty-one (54 percent) had pacemakers implanted because of high-grade atrioventricular block or bradyarrhythmias (sinus bradycardia or atrial fibrillation with a slow ventricular response).

Twenty-five of the 39 clinically affected family members (64 percent) had dilated cardiomyopathy. The age at onset and the severity of left ventricular dysfunction within each family were variable; impairment of left ventricular contraction was mild to moderate in 12 persons, but heart failure developed in 13. Progression of disease was rapid in six members, who required cardiac transplantation within three years after diagnosis; two of these six died from accelerated coronary atherosclerosis in the transplanted heart. Eleven family members with dilated cardiomyopathy died suddenly between the ages of 30 and 59 years: the only prior arrhythmia of one of these was paroxysmal atrial tachyarrhythmia without electrocardiographic evidence of atrioventricular conduction delay (Fig. 4B).

Pathological examination of heart tissue explanted during transplantation or obtained at autopsy from affected members of Families B, C, and E identified four-chamber dilatation, myocyte hypertrophy, and fibrosis without inflammation. Detailed histopathological studies[35] of the conduction system of a member of Family E revealed marked fibrosis and fatty metamorphosis of the sinoatrial and atrioventricular nodes, as well as the atrioventricular bundle.

Differences in clinical manifestations of disease were apparent among the five families. The affected members of Families B and C had a more severe phenotype than the affected members of Families A, D, and E, and many had atrial fibrillation (91 percent of affected members of Family B and 100 percent of affected members of Family C) (Table 1), dilated cardiomyopathy (73 percent and 100 percent, respectively), or sudden death (45 percent and 40 percent). Embolic events occurred in three members of Family E (38 percent) but in only one from Family A and one from Family B. Affected members of Family A had a relatively mild phenotype, with a low prevalence of atrial fibrillation (38 percent) and dilated cardiomyopathy (38 percent) and no sudden death.

Affected members of each family were evaluated for the presence of skeletal-muscle involvement indicative of Emery–Dreifuss muscular dystrophy, a disorder also caused by lamin A/C mutations.[22] Mutations in the α-helical rod domain of lamins A and C produced no skeletal-muscle dysfunction, no weakness or wasting, and no joint contractures in any members of Families B, C, D, and E. The serum creatine kinase levels in 15 clinically affected members from these four families were normal. Skeletal-muscle–biopsy specimens from three members of Family D were normal. A biopsy of the vastus lateralis obtained from one member of Family B, an active jogger, showed mild, nonspecific changes with minimal variation in fiber size and few internal nuclei (Fig. 5A and 5B). Inflammatory cells were not present. Sections exposed to ATPase showed a normal checkerboard pattern of type 1 and 2 fibers. Sections exposed to NADH dehydrogenase, periodic acid–Schiff, myophosphorylase, and oil red O were also normal. The marked variations in the size of muscle fibers and in the degree of fibrofatty replacement typically

TABLE 1. CLINICAL CHARACTERISTICS OF PERSONS WITH LAMIN A/C MUTATIONS.

FAMILY AND MEMBER	AGE AT CLINICAL PRESENTATION (YR)	CONDUCTION-SYSTEM DISEASE	ATRIAL FIBRILLATION	DILATED CARDIOMYOPATHY	CAUSE OF DEATH	COMMENTS*
Family A						
III-1	51	Third-degree atrioventricular block, permanent pacemaker	No	Yes	—	Creatine kinase, 181 U/liter (<210 U/liter)
III-5	39	Second-degree atrioventricular block	No	No	—	Creatine kinase, 180 U/liter (<140 U/liter)
III-8	42	Sinus bradycardia, permanent pacemaker	Yes	Yes	—	Arterial embolus; creatine kinase, 170 U/liter (<140 U/liter)
III-9	40	Third-degree atrioventricular block, permanent pacemaker	No	Yes	Lymphoma	Heart failure
III-11	46	Second-degree atrioventricular block	Yes	No	—	Creatine kinase, 360 U/liter (<140 U/liter)
III-13	43	Sinus bradycardia, first-degree atrioventricular block	Yes	No	—	Creatine kinase, 89 U/liter (<210 U/liter)
III-14	40	First-degree atrioventricular block	No	No	—	Creatine kinase, 167 U/liter (<270 U/liter)
III-15	39	Third-degree atrioventricular block	No	No	—	
IV-1, 2, 3	<30	No	No	No	—	Clinically unaffected; IV-1: creatine kinase, 270 U/liter (<140 U/liter)
Family B						
III-1	19	Yes	Yes	Yes	Sudden death	Heart failure
III-3	39	Slow atrial fibrillation, permanent pacemaker	Yes	Yes	Sudden death	
III-11	53	Yes; permanent pacemaker	Yes	Yes	—	Embolic stroke; creatine kinase, 72 U/liter (<220 U/liter)
III-13	39	Yes	Yes	Yes	Sudden death	Heart failure
IV-1	29	Yes	Yes	Yes	Sudden death	Heart failure
IV-4	31	Slow atrial fibrillation, permanent pacemaker	Yes	No	—	Left atrial enlargement; creatine kinase, 161 U/liter (<250 U/liter)
IV-8	40	Slow atrial fibrillation	Yes	Yes; received heart transplant	—	Renal failure
IV-10	50	No	Yes	Yes	—	Creatine kinase, 128 U/liter (<170 U/liter)
IV-16	34	Second-degree atrioventricular block	Yes	No	—	Creatine kinase, 83 U/liter (<220 U/liter)
V-1	29	Yes	Yes	Yes	Sudden death	Creatine kinase, 248 U/liter (<270 U/liter)
V-2	22	Sinus bradycardia	No	No	—	
V-4	15	No	No	No	—	Clinically unaffected
Family C						
II-1	40s	Yes; permanent pacemaker	Yes	Yes	—	Creatine kinase, 73 U/liter (<170 U/liter)
II-2	40s	Yes; permanent pacemaker	Yes	Yes	Sudden death	
II-4	38	Yes; permanent pacemaker	Yes	Yes	Sudden death	
III-2	40	Slow atrial fibrillation, permanent pacemaker	Yes	Yes; received heart transplant	—	Creatine kinase, 43 U/liter (<270 U/liter)
III-4	30	Slow atrial fibrillation	Yes	Yes; received heart transplant	Rejection of heart transplant	Creatine kinase, 18 U/liter (<50 U/liter)
IV-1, 2	<30	No	No	No	—	Clinically unaffected
Family D						
II-1	35	Yes; permanent pacemaker	No	Yes	Sudden death	Heart failure
II-4	52	No	No	No	Sudden death	
II-5	40	Yes; permanent pacemaker	No	No	No details	
III-2	37	Sinus bradycardia, permanent pacemaker	No	Yes; received heart transplant	Rejection of heart transplant	Creatine kinase, 12 U/liter (<70 U/liter)
III-3	38	Third-degree atrioventricular block, permanent pacemaker	No	Yes; received heart transplant	—	Creatine kinase, 83 U/liter (<220 U/liter)
III-6	28	Third-degree atrioventricular block, permanent pacemaker	No	Yes	—	Creatine kinase, 33 U/liter (<270 U/liter)
III-7	45	Yes; permanent pacemaker	Yes	No	—	Creatine kinase, 60 U/liter (<270 U/liter)
IV-2, 4, 5	<30	No	No	No	—	Clinically unaffected; creatine kinase, 83–111 U/liter (<270 U/liter)

TABLE 1. CONTINUED.

FAMILY AND MEMBER	AGE AT CLINICAL PRESENTATION (YR)	CONDUCTION-SYSTEM DISEASE	ATRIAL FIBRILLATION	DILATED CARDIOMYOPATHY	CAUSE OF DEATH	COMMENTS*
Family E						
II-2	46	Second-degree atrioventricular block, permanent pacemaker	Yes	Yes	—	Embolic stroke; creatine kinase, 34 U/liter (<160 U/liter)
II-4	37	Slow atrial fibrillation, permanent pacemaker	Yes	Yes	Heart failure	Embolic stroke
II-6	30s	Yes; permanent pacemaker	Yes	Yes	Sudden death	
II-8	48	Second-degree atrioventricular block, permanent pacemaker	Yes	Yes	Sudden death	Heart failure; creatine kinase, 93 U/liter (<270 U/liter)
II-9	47	Yes; permanent pacemaker	No	Yes; received heart transplant	—	Embolic stroke
III-1	35	First-degree atrioventricular block	No	No	—	Creatine kinase, 45 U/liter (<135 U/liter)
III-4	28	First-degree atrioventricular block	No	No	—	
III-8	28	First-degree atrioventricular block	No	No	—	
III-3, 10, 12	<30	No	No	No	—	Clinically unaffected

*Values in parentheses are the upper limits of the normal laboratory reference values.

observed in patients with Emery–Dreifuss muscular dystrophy were absent (Fig. 5C).

The mutation in the lamin C carboxyl tail in Family A caused neither signs nor symptoms of muscular dystrophy. However, serum creatine kinase levels were elevated in three of six clinically affected family members, as well as in one genetically affected, asymptomatic member (Table 1). Skeletal-muscle biopsies were not performed for Family A.

DISCUSSION

In the families we studied, lamin A/C mutations caused familial dilated cardiomyopathy associated with conduction-system disease. Defects were identified in 5 of 11 affected families, indicating that defects in the lamin A/C gene are a major cause of this clinical phenotype. Because missense mutations in the tail regions of lamins A and C cause Emery–Dreifuss muscular dystrophy[22] and rod mutations cause isolated myocardial disease, we speculated that these domains participate in unique interactions in skeletal or cardiac muscle. These data define an important role of nuclear-membrane biology in cardiac conduction and contraction.

Alternate splicing of the 12 exons that make up the lamin A/C gene produces at least four different types of RNA that encode closely related proteins: lamins A, Aδ10, C, and C2.[32] Lamins A and C are coexpressed in the nuclear envelope of many tissues, including heart and skeletal muscle.[34] The first 566 amino acids of lamins A and C (encoded in exons 1 through 10) are identical, whereas the carboxyl terminal of these peptides differs in length and amino acid sequence (Fig. 1B).[30]

The Arg571Ser mutation, which selectively alters the carboxyl terminal of lamin C, caused a relatively milder cardiac phenotype than mutations in the rod domain of the lamin A/C gene and subclinical disease affecting skeletal muscle. Although affected members of Family A had no skeletal-muscle symptoms, some had elevated serum creatine kinase levels, including one asymptomatic family member with the genotype associated with the disease. The Arg571Ser mutation affects only lamin C isoforms, whereas previously described defects causing Emery–Dreifuss muscular dystrophy perturb both lamin A and lamin C isoforms. Perhaps mutations in the carboxyl terminal perturb peptide stability or prevent assembly of lamin filaments, a process of head-to-tail polymerization.[32,36] Selective muscle involvement might then reflect a graded response to deficiencies of lamin C or of lamins A and C. Alternatively, restricted expression of the Arg571Ser mutation in lamin C or distinct functions of lamin A could account for the disparity in skeletal-muscle involvement.

Four missense mutations in the rod domains of isoforms of lamins A and C that caused isolated cardiac disease would be expected to function through a dominant negative mechanism. Amino acid substitutions that alter charge, hydrophobicity, or both should disrupt α-helical–rod structure, but such defects are unlikely to function as null alleles. This hypothesis is supported by clinical findings: the null allele G1n6Stop[22] causes autosomal dominant Emery–Dreifuss muscular dystrophy, whereas defects in the rod domain of the lamin A/C gene have no consequence in terms of skeletal-muscle function. Family members with missense mutations in the rod domain of the lamin A/C gene had normal muscle strength, normal muscle morphology, no joint con-

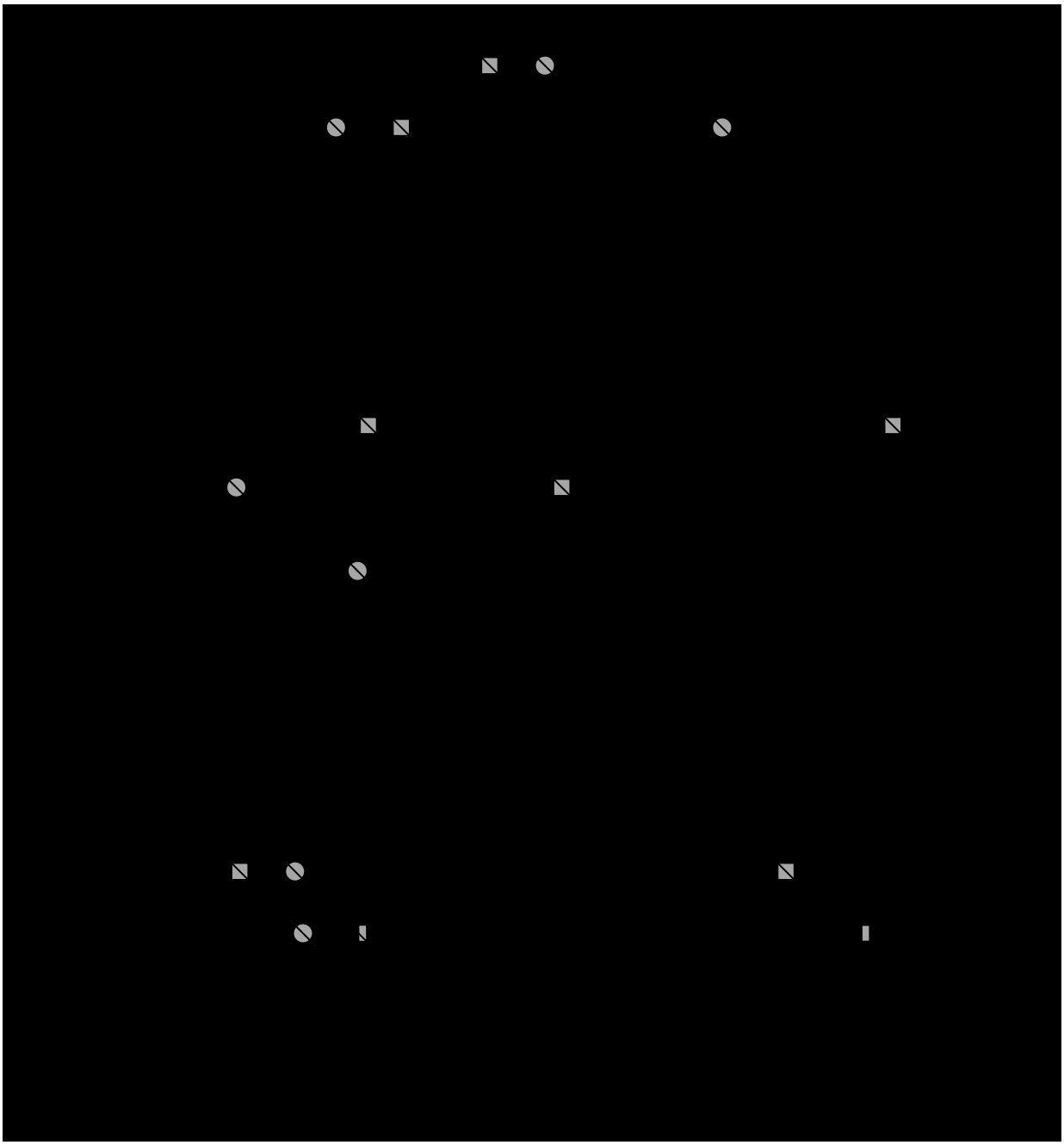

Figure 3. Pedigrees of Five Families with Lamin A/C Mutations.

Squares indicate male family members, circles female family members, and symbols with a slash mark deceased family members. Rhythm disturbance is shown by symbols that are solid on the left-hand side, and dilated cardiomyopathy by symbols that are solid on the right-hand side. Totally solid symbols indicate the presence of both rhythm disturbance and cardiomyopathy. Open symbols indicate unaffected family members, and shaded symbols those whose status was indeterminate. The presence (+) or absence (−) of a lamin A/C mutation is indicated for persons with DNA samples available for testing.

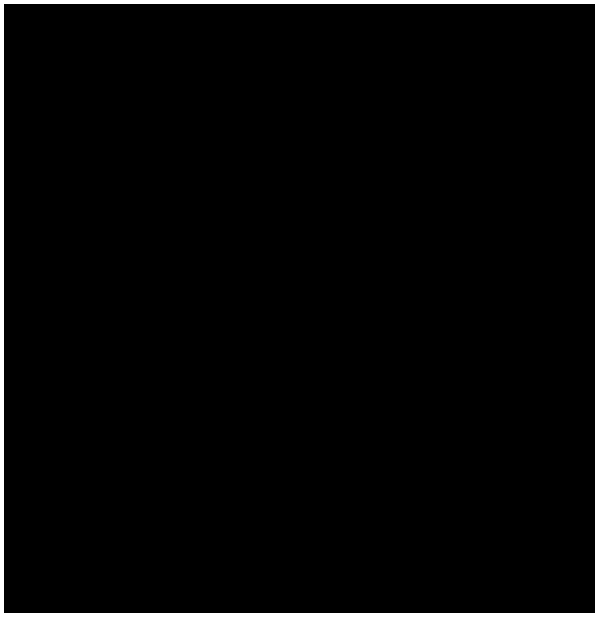

Figure 4. Electrocardiographic Tracings of Affected Members of Family B.

Serial tracings from one family member (Panel A) show progressive atrioventricular block (from 33 to 37 years of age), paroxysmal atrial fibrillation (at the age of 34 years), and sinus arrest with infrequent junctional escape beats (at the age of 37). Panel B shows a normal electrocardiographic tracing from another family member (V-1), with congestive heart failure and prior paroxysmal episodes of atrial fibrillation, which was obtained one week before his sudden death. There was no delay in atrioventricular conduction.

tractures, and normal serum creatine kinase levels, but they did have cardiac disease characterized by sinus-node dysfunction, atrioventricular-node dysfunction, or both and dilated cardiomyopathy with onset in adulthood. Although disturbances of cardiac rhythm develop in the majority of patients with Emery–Dreifuss muscular dystrophy,[15-19] important differences were found in those with the cardiac phenotypes caused by mutations in the rod domain of the lamin A/C gene. These defects are highly penetrant, and, by the fourth decade of life, they uniformly produced cardiac disease with hemodynamically important bradyarrhythmias and tachyarrhythmias (97 percent) and a high incidence of sudden death (28 percent), thromboembolic events (13 percent), and congestive heart failure (33 percent). Despite the related molecular causes of Emery–Dreifuss muscular dystrophy and dilated cardiomyopathy with conduction-system disease, we suggest that there is clinical value in recognizing each as a distinct disease entity.

How do lamin defects cause heart disease? Nuclear lamins contribute to the structural integrity of the nuclear envelope and provide mechanical support for the nucleus.[36] These molecules interact with nuclear components. In dividing cells, they have a dynamic role in the organization of interphase chromatin and the reassembly of nuclear membrane during mitosis.[37-39] In nondividing cells, lamins may participate in signal transduction by mediating molecular movement between the cytoplasm and the nucleus.[40] Hence, one possible effect of lamin mutations may be the disruption of nuclear function, resulting in cell death. Myocyte loss could account for the postmortem finding of extensive fibrofatty infiltration of the myocardium and conduction system in

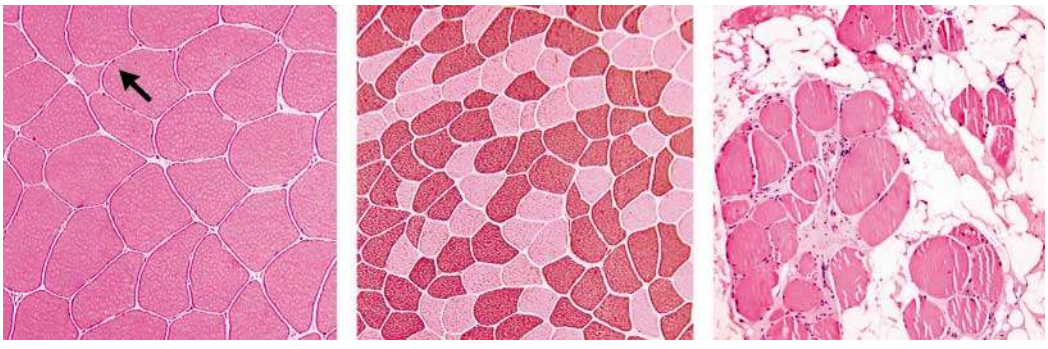

Figure 5. Skeletal-Muscle–Biopsy Specimens from a Member of Family B Who Had Dilated Cardiomyopathy and Conduction-System Disease Caused by the Lamin-Rod Mutation Asn195Lys (Panels A and B), as Compared with Typical Findings of Emery–Dreifuss Muscular Dystrophy (Panel C).

A transverse section of skeletal muscle (Panel A) shows slight variation in fiber size, rare internalized nuclei (arrow), and no evidence of increased endomysial connective tissue (hematoxylin and eosin, ×75). Panel B shows a normal checkerboard pattern of type 1 (light) and type 2 (dark) fibers evident on staining with ATPase (pH 9.4, ×40). Emery–Dreifuss muscular dystrophy (Panel C) causes widespread loss of muscle fibers, marked variation in fiber size, and extensive infiltrates of fibroadipose connective tissue (hematoxylin and eosin, ×40).

one member of Family E,[35] and for that found in the skeletal muscles of patients with Emery–Dreifuss muscular dystrophy (Fig. 5C). Alternatively, missense mutations in the rod domain of the lamin A/C gene may alter interactions with cytoplasmic proteins (in particular, intermediate filament components of the sarcomere, the actin-based cytoskeleton, and the sarcolemma) (Fig. 1A), some of which have been implicated in dilated cardiomyopathy,[12,23-28] although direct interactions between lamin A or lamin C and these components have not been demonstrated to date. Defining how lamin mutations alter cardiac-cell biology should ultimately increase our understanding of the pathophysiology of dilated cardiomyopathy. More immediately, these data implicate genes encoding other lamin proteins and associated components of the nuclear membrane as a cause of the cardiac or skeletal myopathy that occurs with conduction-system disease.

Supported by the Stanley J. Sarnoff Endowment and the Marshfield Medical Research Foundation and by grants from the National Institutes of Health and the Howard Hughes Medical Institute.

We are indebted to the participating families and their physicians, without whose invaluable assistance these studies would not have been possible; to Barend P. Lotz, M.D., Andrew J. Waclawik, M.D., and M. Shahriar Salamat, M.D., Ph.D., for preparation and analysis of muscle-biopsy specimens; to Spencer Kubo, M.D., D. Woodrow Benson, M.D., and Kristin Patton, M.D., for participating in the clinical evaluations; to Cathy Duffy for assistance in DNA analysis; and to Susanne Bartlett for assistance with the figures.

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