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Natural History of Dilated Cardiomyopathy Due to Lamin A/C Gene Mutations

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OBJECTIVES We examined the prevalence, genotype-phenotype correlation, and natural history of lamin A/C gene (*LMNA*) mutations in subjects with dilated cardiomyopathy (DCM).

BACKGROUND Mutations in *LMNA* have been found in patients with DCM with familial conduction defects and muscular dystrophy, but the clinical spectrum, prognosis, and clinical relevance of *laminopathies* in DCM are unknown.

METHODS A cohort of 49 nuclear families, 40 with familial DCM and 9 with sporadic DCM (269 subjects, 105 affected), was screened for mutations in *LMNA* using denaturing high-performance liquid chromatography and sequence analysis. Bivariate analysis of clinical predictors of *LMNA* mutation carrier status and Kaplan-Meier survival analysis were performed.

RESULTS Mutations in *LMNA* were detected in four families (8%), three with familial (R89L, 959delT, R377H) and one with sporadic DCM (S573L). There was significant phenotypic variability, but the presence of skeletal muscle involvement ($p < 0.001$), supraventricular arrhythmia ($p = 0.003$), conduction defects ($p = 0.01$), and "mildly" DCM ($p = 0.006$) were predictors of *LMNA* mutations. The *LMNA* mutation carriers had a significantly poorer cumulative survival compared with non-carrier DCM patients: event-free survival at the age of 45 years was 31% versus 75% in non-carriers.

CONCLUSIONS Mutations in *LMNA* cause a severe and progressive DCM in a relevant proportion of patients. Mutation screening should be considered in patients with DCM, in particular when clinical predictors of *LMNA* mutation are present, regardless of family history. (J Am Coll Cardiol 2003;41:771–80) © 2003 by the American College of Cardiology Foundation

Dilated cardiomyopathy (DCM) is a severe disease of heart muscle characterized by progressive ventricular dilation and impaired systolic function. Population surveys estimated a prevalence of 1 DCM case in 2,500 individuals (1). In recent years the contribution of genetic mutations to the etiology of DCM has been appreciated. Familial dilated cardiomyopathy (FDC) is now estimated to account for as many as 50% of all cases of "idiopathic" DCM (2,3). Mutations in 10 cytoskeletal/sarcomeric protein-encoding genes have been identified in FDC, including dystrophin (4,5), desmin (6), lamin A/C (7–9), cardiac actin (10), tafazzin (11), cardiac beta-myosin heavy chain and cardiac troponin T (12), alpha-tropomyosin (13), delta-sarcoglycan (14), and titin (15).

The lamin A/C gene (*LMNA*) maps on the long arm of chromosome 1 (1q21.2-q21.3) and encodes two main isoforms by alternative splicing (16), lamin A and C. Lamin proteins are type V intermediate filaments, major components of the nuclear lamina (a filamentous structure that supports the inner nuclear membrane), and are classified as A-type or B-type (17,18). A-type lamins (lamin A and C) are expressed exclusively in differentiated non-proliferating cells (18,19). Mutations in *LMNA* cause DCM, frequently complicated by conduction system defects and variable skeletal muscle involvement (7,9,20–22). The *LMNA* mutations have also been reported in autosomal dominant (23) and recessive (24) Emery-Dreifuss muscular dystrophy (EDMD), autosomal dominant limb-girdle muscular dystrophy (LGMD) (8), sensory and motor axonal neuropathy Charcot-Marie-Tooth type 2 (25), and familial partial lipodystrophy, characterized by the development of abnormal patterns of adipose tissue distribution (26,27).

The prevalence of *LMNA* mutations in patients with DCM, the genotype-phenotype correlations, and the im-

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Abbreviations and Acronyms

DCM = dilated cardiomyopathy
DHPLC = denaturing high-performance liquid chromatography
DNA = deoxyribonucelic acid
EDMD = Emery-Dreifuss muscular dystrophy
FDC = familial dilated cardiomyopathy
LGMD = limb-girdle muscular dystrophy
LMNA = lamin A/C gene
MDDC = dilated cardiomyopathy with muscle disease
PCR = polymerase chain reaction
RNA = ribonucelic acid
SNP = single-nucleotide polymorphism

pact on survival are still unknown. Over the past two decades, we have studied families with both FDC and sporadic DCM (28,29). Here we report the results of our comprehensive genetic screening of *LMNA* in 49 unrelated families comprised of 40 familial and 9 sporadic cases of DCM.

METHODS

Patient population. The 49 families of patients with DCM (269 subjects, 105 affected) were evaluated by the investigators in Europe and the U.S. Subjects (probands and family members) with DCM were recruited from general cardiology clinics at the University of Colorado Health Sciences Center and Children's Hospital (Denver, Colorado) and the University of Trieste (Trieste, Italy) from 1991 to 2001 (28,29). Subjects were identified solely on the basis of having DCM and were not prescreened or preselected for any neuromuscular, cardiac conduction, or any other non-cardiac phenotype. Detailed clinical information was obtained from each subject, including family history; age of presentation; initial symptoms of heart failure; New York Heart Association classification; physical examination; serum creatine kinase (MM isoform); electrocardiograms, echocardiograms, and, when appropriate, Holter monitoring; treadmill testing; invasive examination (right and left heart catheterization, ventriculography, coronary angiogram, and endomyocardial biopsy); and neuromuscular investigations (electromyography and skeletal muscle biopsy).

The diagnostic criteria for FDC followed the *Guidelines for the Study of Familial Dilated Cardiomyopathies* (30). In brief, individuals were classified as affected on the basis of major and minor criteria. The major criteria were: 1) left ventricular ejection fraction <45% or frameshift <25%, and 2) left ventricular end-diastolic dimension >117% of the predicted value corrected for age and body surface area (31). Individuals were classified as healthy when found to be normal or affected by known diseases and unknown when isolated minor cardiac or skeletal muscle abnormalities were observed, as described in detail previously (30). The final study population included 40 unrelated families with FDC, and 9 with non-familial sporadic DCM (Table 1). Families with X-linked DCM due to dystrophin gene mutations (32,33) were excluded from the mutation analysis. Written informed consent was obtained from all subjects.

Genetic analysis. Genomic deoxyribonucelic acid (DNA) was extracted from blood leukocytes according to standard techniques. Polymerase chain reaction (PCR) primers (Life Technologies, Carlsbad, California) were designed to amplify all protein coding exons of *LMNA*, and denaturing high-performance liquid chromatography (DHPLC) analysis was carried out on a WAVE DNA fragment analysis system (Transgenomic, San Jose, California). Elution profiles that differed from wild-type patterns were selected for sequence analysis, purified using QIAquick PCR Purification Kits (Qiagen, Valencia, California), and then sequenced bidirectionally using an ABI 377 DNA sequencer (Applied Biosystem, Foster City, California). Putative disease-causing mutations were evaluated for segregation with DCM phenotype within a family and were analyzed for change in predicted amino acid sequence, predicted protein secondary and tertiary structure, and for alterations in ribonucelic acid (RNA) splicing. In the cases of predicted changes in amino acid sequence, the altered residues were analyzed for conservation across different species. A genetic change was considered to be a putative disease-causing mutation when it altered the predicted amino acid sequence, cosegregated with the disease within the family, was absent in over 150 normal, ethnically matched controls (300 chromosomes, $p < 0.05$) (34) (data not shown), was altering

Table 1. Phenotypic Characterization (29) and Molecular Epidemiology of the Study Population

Phenotype	Number of Families	Subjects Examined	Affected Subjects	Families With *LMNA* Mutation
Familial DCM, total	40	236	95	3
Autosomal dominant	25	163	62	1
Autosomal recessive	4	15	7	—
With conduction defect	3	18	8	—
With muscle disease	4	27	11	2
X-linked	2	5	3	—
Unclassifiable	2	8	4	—
Sporadic DCM	9	33	10	1
Total	49	269	105	4

DCM = dilated cardiomyopathy; *LMNA* = lamin A/C gene.

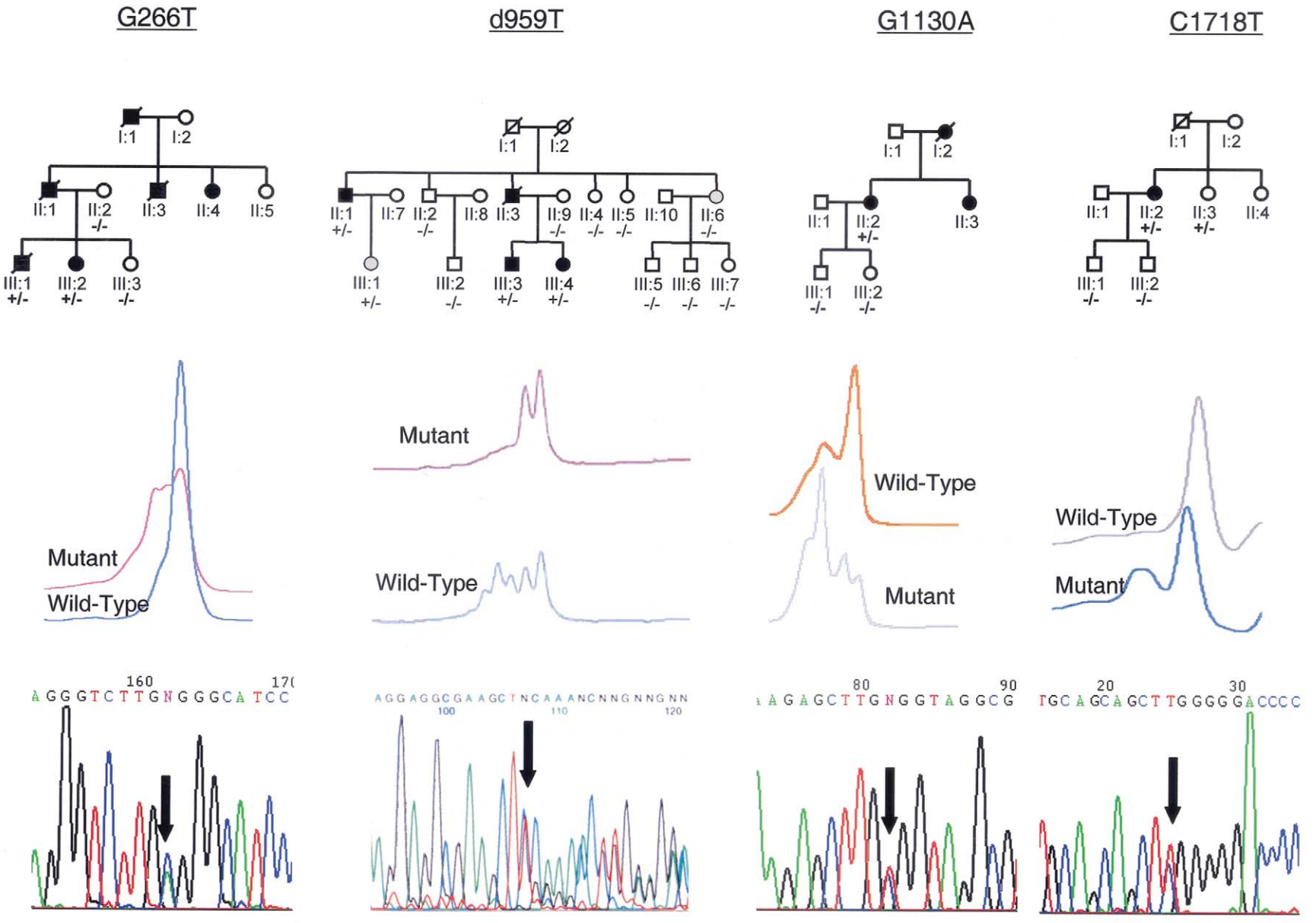

Figure 1. The denaturing high performance liquid chromatography elution profiles for lamin A/C gene (*LMNA*) mutations detected in three families with dilated cardiomyopathy. **(Top)** Pedigree of the families: individuals are indicated by generation and pedigree number. Affected status is indicated by **filled symbols**, unaffected by **clear symbols**. The **(+)** and **(−)** symbols indicate the presence of the mutant allele and the wild type, respectively. **(Middle)** Elution profiles for the wild-type and patients carrying the *LMNA* mutations (G266T: III-2; 959delT: II-1; G1130A: II-2; C1718T: II-2). **(Bottom)** The direct sequencing results illustrate the corresponding nucleotide substitutions (reverse sequence shown for G1130A mutation).

Table 2. Phenotype and Genotype of 12 Patients with DCM Heterozygous for *LMNA* Mutations

Family	Mutation	Codon	Exon	ID	Age	Gender	NYHA	CK-MM	Arrhythmia
TSFDC13	G266T	R89L	1	II-2	39	M	4	N	Atrial fibrillation
				II-2	23†	F	3	N	Sinus rhythm
				II-1	22	M	4	Upper N	Ventricular tachycardia Atrial ectopy
MDDC1§	959delT	320	6	II-1	22	M	2	N-3X	Ventricular and supraventricular tachycardia
				II-5	30	M	3	N	Atrial fibrillation Ventricular tachycardia
				III-1	4	F	1	N	Atrial ectopy
				III-3	16	M	1	3X	Supraventricular tachycardia Ventricular ectopy
				III-4	18	F	1	4X	Supraventricular tachycardia
DNFDC33	G1130T	R377H	6	I-2	40	F	2		
				II-2	31	F	3	Upper N	Atrial fibrillation Ventricular tachycardia Ventricular fibrillation
SDCM8	C1718T	S573L	11	II-2	50	F	3	N	Ventricular tachycardia Ventricular ectopy
				II-3	60	F	1	N	Sinus rhythm

*At autopsy. †Peri-partum. ‡At heart transplant. §Reported by Brodsky et al. (9).

CK-MM = creatine kinase, MM isoform; DCM = dilated cardiomyopathy; EDMD = Emery-Dreifuss muscular dystrophy; LVEF = ejection fraction (%); FS = fractional shortening; ID = pedigree identification number; LGMD = limb-girdle muscular dystrophy; *LMNA* = lamin A/C gene; LVEDD = left ventricular end-diastolic dimension (31); N = normal; NYHA = New York Heart Association (class).

a highly conserved residue throughout evolution, and was predicted to alter any combination of protein structure, charge, or function.

Statistical analysis. Comparison of predictor variables and carrier status was done by Fisher exact test for binary predictors and by the Wilcoxon rank-sum test for continuous predictors.

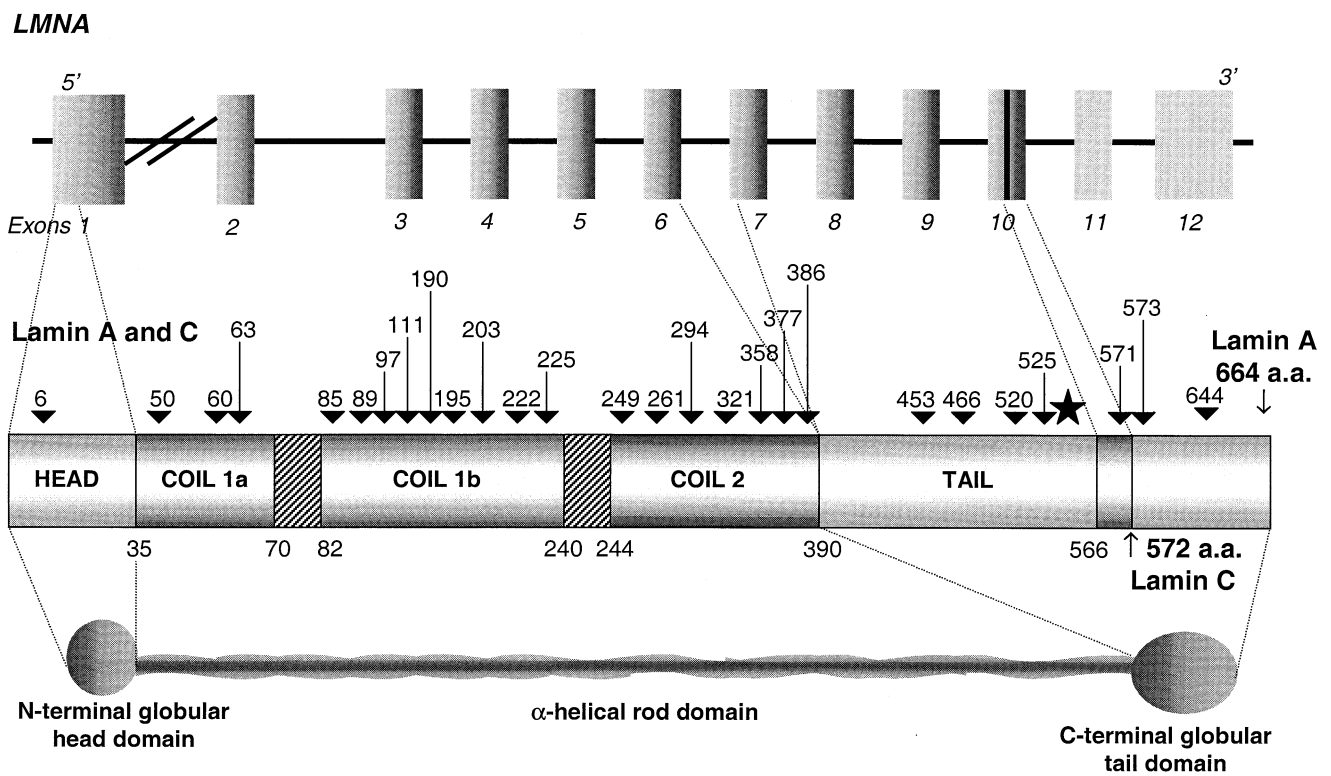

Figure 2. Structure of lamin A/C gene (*LMNA*) and of the lamin A/C protein. *LMNA* encodes lamin A (664 amino acids) and lamin C (572 amino acids) by alternative splicing in exon 10. The region common to both lamins includes the globular head, the α-helical domain (coils 1a, 1b, and 2, separated by linkers depicted as **diagonal stripes**), and part of the tail (566 amino acids). The mutations identified in patients with DCM, the corresponding nucleotide, and their position on the protein are indicated (**star** = splice site mutation).

Table 2 Continued

Conduction	LVEDD	FS	LVEF	Muscular Disease	Outcome	Age
N	Dilation*			Muscular atrophy*	Congestive heart failure, death	41
N	Dilation‡			no	Transplant	25
1° Atrioventricular block	6.3	10	27	no	Congestive heart failure Sudden death	23
2°–3° Atrioventricular block	5.9	12	30	Mild LGMD	Transplant	33
Left bundle-branch block	Restrictive pattern					
2°–3° Atrioventricular block	6	24	48	no	Congestive heart failure Sudden death	32
Left bundle-branch block						
N	2.8	36		Minimal weakness	Left ventricular dysfunction	6
N	4.8	33	49	Mild LGMD	Transplant	20
Left anterior hemiblock	4.5	37	63	Mild EDMD	Stable	21
				Mild LGMD	Congestive heart failure, death	62
Cardiac defibrillator	4.18		30	Mild EDMD	Cardiac arrest	56
Pacemaker	Restrictive pattern				Congestive heart failure, death	
N	7	17	20	no	Stable	60
N	N	N	N	no	Stable	

Event-free survival analyses used 2 × 2 contingency tables for occurrence of event and the Cox proportional hazards model for time from birth to event, comparing by carrier status. Three survival events were used: major cardiac event, transplant, and cardiovascular death. A major cardiac event was defined as: 1) hospitalization for worsening heart failure, 2) hospitalization for major arrhythmia (hypokinetic or hyperkinetic), or 3) a thromboembolic event. As these survival events constituted competing risks, analyses used only the following outcomes: composite of major cardiac event, transplant, and death; composite of transplant and death; and death alone. For composite outcomes, survival time was time to first occurring event.

The primary survival method used was the Cox model utilizing the SAS procedure PHREG, with the family-specific variable "carrier status" as the predictor of interest. The 105 affected subjects are not independent, but nested in 49 families. As several families had data for only a single affected individual, it was not possible to control for the nesting in the final statistical analysis. The primary analysis compared *LMNA* mutation "carrier" versus "non-carrier" status for the entire group of affected individuals; analyses restricted to the probands only provided secondary results.

Statistical software used was SAS Version 6.12 (SAS Institute, Cary, North Carolina). Unless otherwise stated, data are given as means ± SEM. Two-sided statistical significance was taken as $p < 0.05$. Expected left ventricular end-diastolic dimension was calculated by correcting for body surface area and age (31). Survival curves were estimated by the Kaplan-Meier method.

RESULTS

Genotype analysis. Mutation screening found putative mutations in 4 of 49 families (8%) (Fig. 1, Table 2). In exon 1, a G266T missense mutation was found in one Italian family with autosomal dominant FDC. The change predicts an arginine (basic) to leucine (hydrophobic) substitution (R89L) in a conserved residue located (16) in the coil 1b of the alpha-helical rod domain (Fig. 2). In exon 6, a single base deletion (959delT) was identified in another Italian family with autosomal dominant FDC with muscle disease (MDDC). As previously reported (9), this mutation predicts a frameshift that is expected to completely disrupt the lamin A and C sequences starting from codon 320. Also in exon 6, a *LMNA* mutation was found in an American family of British descent with MDDC. This G1130A missense mutation predicts an arginine to histidine substitution (R377H) at a highly conserved residue. All mutations involved conserved residues (Fig. 3), cosegregated with the disease within the families, and were absent in 300 control chromosomes.

A missense mutation (C1718T) was found in one of the nine sporadic cases of DCM, in a family of Italian origin. A serine (hydrophilic) to a leucine (hydrophobic) (S573L) substitution is predicted to affect the carboxyl tail of the lamin A isoform (Fig. 2). This residue is also highly conserved (Fig. 3), and the mutation was absent in 300 control chromosomes and in a total of 450 chromosomes. The mutation was absent in the proband's two unaffected offspring, whereas it was found in one clinically unaffected sister.

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		Exon 1: R89L		Exon 6: R377H		Exon 11: S573L	Genbank Accession Number
Organism	Protein	Sequence		Sequence		Sequence	
Human	Lamin A/C	YEAELGDA**R**KTLDSVAK	•••	LDMEIHAY**R**KLLEGEEE	•••	HGSHCSS**S**GDPAEYNL	AAH00511.1
Mouse	Lamin A/C	YEAELGDA**R**KTLDSVAK	•••	LDMEIHAY**R**KLLEGEEE	•••	H*R*GSHCS**G**SGDPAEYNL	BAA08569
Rat	Lamin A/C	YEAELGDA**R**KTLDSVAK	•••	LDMEIHAY**R**KLLEGEEE	•••	H*R*GSHCSS**S**GDPAEYNL	CAA53945.1
Chicken	Lamin A	YEAELADA**R**KTLDSVAK	•••	LDMEINAY**R**KLLEGEEE	•••	H-SGCSG**S**ADPAEYNL	CAA34762.1
Xenopus	Lamin A	YETELADA**R**KTLDSVAK	•••	LDMEINAY**R**KLLEGEEE	•••	HDGQ-NS**S**GDPGEYNL	CAA29652.1
Human	Lamin B1	YETELADA**R**RALDDTAR	•••	LDMEISAY**R**KLLEGEEE	•••		AAC37575.1
Mouse	Lamin B1	FETELADA**R**KTLDDTAR	•••	LDMEISAY**R**KLLESEEE	•••		CAA34677.1
Rat	Lamin B1	YETELADA**R**RALDDTAR	•••	LDMEISAY**R**KLLEGEEE	•••		AAB09600.1
Chicken	Lamin B1	YETELADA**R**RALDDTAR	•••	LDMEISAY**R**KLLEGEEE	•••		CAA34761.1
Human	Lamin B2		•••	LDMEINAY**R**KLLEGEEE	•••		AAA80979.1
Mouse	Lamin B2	YESELADA**R**RVLDETAR	•••	LDMEISAY**R**KLLEGEEE	•••		CAA38032.1
Chicken	Lamin B2	YESELADA**R**RVLDETAK	•••	LDMEISAY**R**KLLEGEEE	•••		CAA34763.1
Xenopus	Lamin LI	YETELADA**R**RSLDDTAR	•••	LDMEISAY**R**KLLEGEEE	•••		CAA29651.1
Xenopus	Lamin LII	YESELADA**R**KVLDETAR	•••	LDLEINAY**R**KLLEGEEE	•••		CAA38033.1
D. mel	Lamin C	YEKELAAA**R**KLLDETAK	•••		•••		AAF58237.1

Figure 3. Amino acid sequences alignment for lamin proteins from various species. The conservation of the residues corresponding to the R89L, R377H, and S573L mutations is shown. The corresponding GenBank accession numbers are listed in the **column at far right**.

A total of five exonic (silent) mutations, six intronic single-nucleotide polymorphisms (SNPs) substitutions, and one SNP in the 3′ untranslated region were each found in a proportion of the 76 study subjects and are reported in the National Center for Biotechnology Information Single Nucleotide Polymorphism database. None of these nucleotide changes predicted a change in amino acid sequence, cosegregated with the disease within the family pedigrees, or predicted altered RNA splicing.

Phenotype analysis. The mutations were found in families with autosomal dominant isolated FDC, autosomal dominant FDC with variable muscle disease (MDDC), and in sporadic DCM (Table 1). The expressed phenotype in the 12 *LMNA* mutation carriers was highly variable (Table 2). The age of onset of DCM ranged from 4 to 59 years. Serum creatine kinase was elevated or "high-normal" in five cases. Seven patients had clinical skeletal muscle involvement, ranging from modest proximal weakness in a 4-year-old subject, to mild signs compatible with an EDMD diagnosis (Fig. 4), to disabling LGMD in a 56-year-old subject. Conduction defects were present in 5 of 12 subjects.

Genotype-phenotype correlation. Clinical variables and outcome events were compared between *LMNA* mutation carriers (n = 12) and DCM patients without *LMNA* mutations (n = 93). In carriers, there was a trend toward a younger age of disease onset (27 ± 5 years vs. 37 ± 2 years, $p = 0.08$) and a greater prevalence of females (58% vs. 34%, $p = 0.12$). Bivariate clinical predictors of carrier status were the presence of supraventricular arrhythmia (73% vs. 26%, $p = 0.003$), conduction disease (including atrioventricular blocks and need of a pacemaker; 50% vs. 11%, $p = 0.01$), and skeletal muscle involvement (including clinical or histological signs of muscular dystrophy, or increased serum creatine kinase; 67% vs. 7%, $p < 0.002$), where the respective proportions are for carriers and non-carriers.

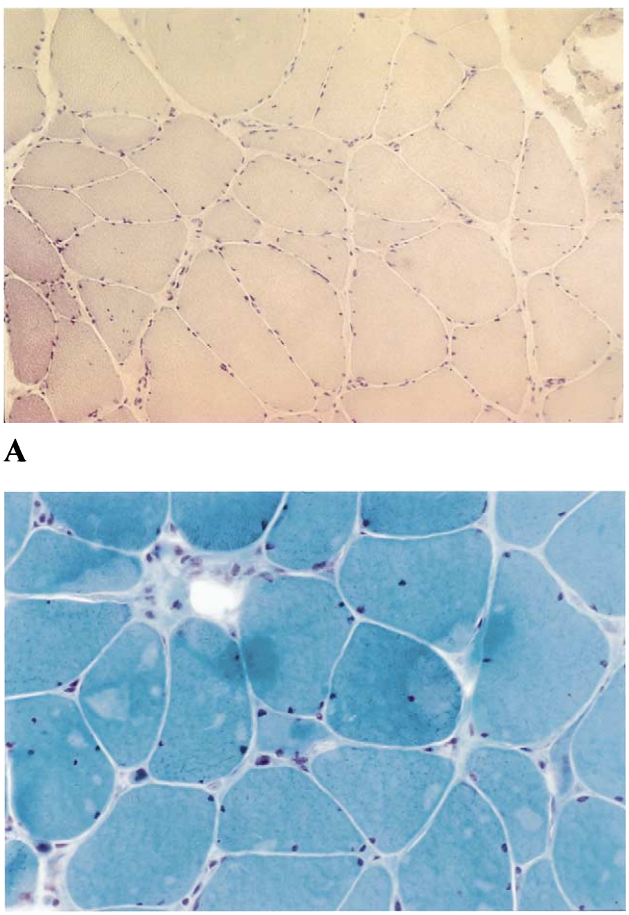

Figure 4. Skeletal muscle biopsy in dilated cardiomyopathy due to laminopathy. **(A)** Family MDDC1, Patient III-4 (hematoxylin-eosin, 40×), and **(B)** family DNFDC33, Patient II-2 (trichrome, 40×). Vastus lateralis muscle biopsies showing variability in fiber size, some angulated fibers, and several internal nuclei.

Table 3. Analysis of Clinical Variables as Predictors of *LMNA* Mutations in Patients With DCM

Variable	Non-Carriers (n = 93)	Carriers (n = 12)	p Value
Male gender (%)	66	42	0.12
Age of onset (yrs)	38 (2)*	28 (5)	0.08
NYHA functional class	1.76 (0.10)	2.23 (0.37)	0.20
Skeletal muscle involvement (%)	5	58	0.00001
Supraventricular arrhythmia (%)	26	73	0.003
Conduction defects (%)	10	62	0.01
LVEF (EF unit)	32 (1.5)	38 (6)	0.23
LVEDD (cm)	6.41 (0.12)	5.02 (0.49)	0.008
LVEDD, % of expected†	140 (2)	112 (9)	0.006
CI (L/m/m^2)	4.3 (0.4)	2.9 (0.3)	0.07
PCWP (mm Hg)	12 (1)	13 (1)	0.46
CK-MM (U/ml)	85 (11)	288 (88)	0.003

*Standard error in parentheses. †Expected based on predicted value for age and body surface area.

CI = cardiac index; LVEF = left ventricular ejection fraction; PCWP = pulmonary capillary wedge pressure. Other abbreviations as in Table 2.

Indexes of myocardial function, including ejection fraction and hemodynamic variables, were not different in the two groups. However, carriers had a lesser degree of dilation of the left ventricle than expected, also after correction for body surface area and age (p = 0.006) (31) (Table 3).

Survival analysis. Carriers were characterized by a significantly worse prognosis compared with non-carriers. By analysis of contingency tables, the relative risk was 2.6 (5/12 vs. 15/93, p = 0.05) for cardiovascular death, 3.4 (8/12 vs. 18/93, p = 0.001) for cardiovascular death or transplant, and 2.2 (9/12 vs. 32/93, p = 0.01) for cardiovascular death, transplant, or major event. Kaplan-Meier survival curves for time to each of three outcomes are shown in Figure 5. By age 45, event-free survival in carriers compared with non-carriers was 62% versus 90%, 45% versus 89%, and 31% versus 75% (panels A, B, and C, respectively). Using the Cox proportional hazards models, for the three outcomes considered, the hazard ratios for experiencing an event for *LMNA* mutation carriers compared with non-carriers were 4.3 (p = 0.007), 5.6 (p = 0.0001), and 3.7 (p = 0.0008), respectively. These results were in agreement with results from a secondary analysis restricted only to probands: 5.9 (p = 0.04), 6.1 (p = 0.01), and 4.2 (p = 0.01), respectively.

DISCUSSION

Prevalence of *LMNA* mutations in DCM. We have extensively characterized the *LMNA* gene in a series of DCM families using a sensitive and efficient method of screening based on DHPLC and sequence analysis. A clinically significant frequency of disease-causing mutations was found in the overall population (8% of the families), both in the familial (7%) and in the sporadic (11%) forms. Mutations of *LMNA* were associated with a wide range of phenotypes, and the *LMNA* mutation carriers had a severe prognosis and had higher event-rates compared with the non-carriers. Our findings suggest that *LMNA* mutations

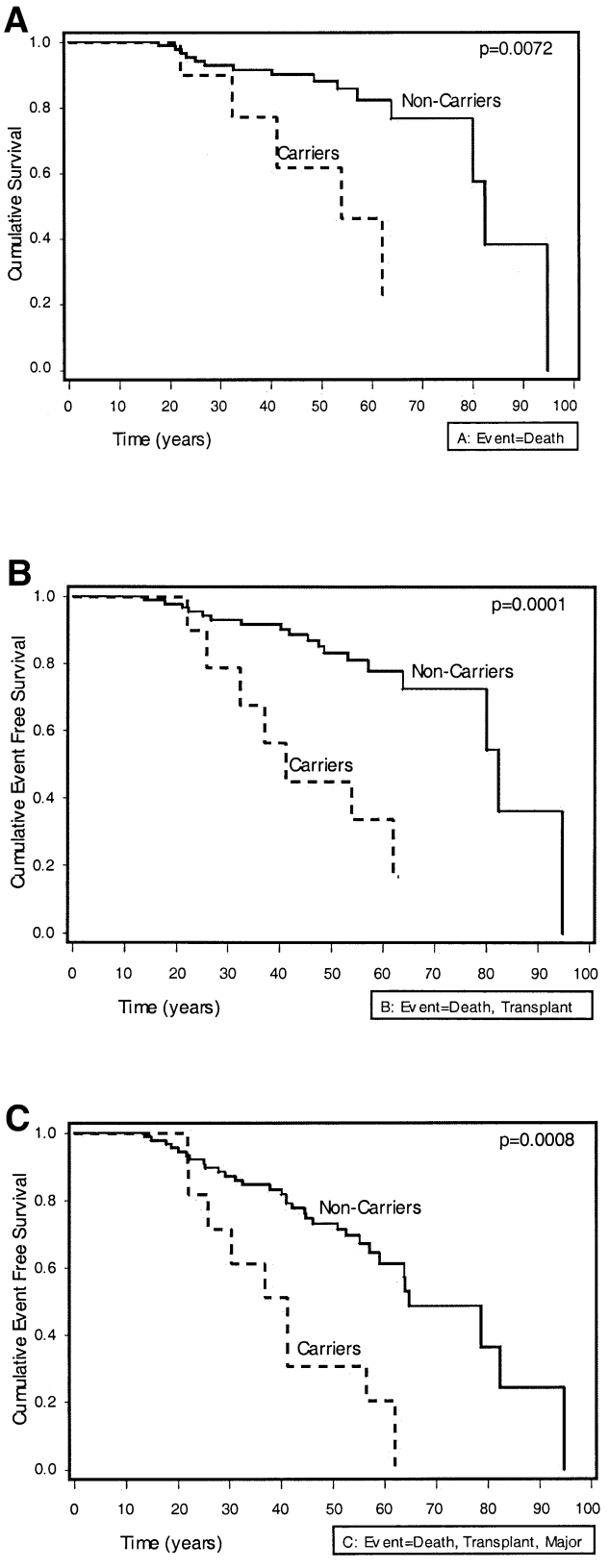

Figure 5. Kaplan-Meier cumulative survival curves for **(A)** cardiovascular death, **(B)** cardiovascular death or transplant, and **(C)** cardiovascular death, transplant, or major cardiac events in 105 patients with dilated cardiomyopathy, carriers of lamin A/C gene mutations **(dashed lines)**, and non-carriers **(solid lines)**. P values are derived by Cox regression.

Table 4. Summary of the Reported *LMNA* Mutations Leading to Cardiac Disease (DCM and/or Arrhythmia/Conduction Disease)

Exon	Nucleotide Change	Amino Acid Change	Domain	Cardiomyopathy	Arrhythmia/ Conduction Anomaly	Muscle Disease	Familial (F)/ Sporadic (S)	Reference Number
1	C16T	Q6X	Head	Y	Y	Y	F	(23)
1	G149C	R50P	Coil 1a	N	Y	Y	S	(35)
1	C178G	R60G	Coil 1a	Y	Y	N	F	(7)
1	T188G	I63S	Coil 1a	Y	Y	Y	S	(35)
1	T254G	L85R	Coil 1b	Y	Y	N	F	(7)
1	G266T	R89L	Coil 1b	Y	Y	N	F	this study
1	G289T	K97E	Coil 1b	Y	Y	N	F	(21)
1	A 331G	E111X	Coil 1b	Y	Y	N	F	(21)
3	C568T	R190W	Coil 1b	Y	Y	N	F	(21)
3	C585G	N195K	Coil 1b	Y	Y	N	F	(7)
3	A608G	E203G	Coil 1b	Y	Y	N	F	(7)
3	G607A	E203K	Coil 1b	Y	Y	N	F	(20)
3	ΔAAG 624-6	ΔK208	Coil 1b	Y	Y	Y	Y	(8)
4	A665C	H222P	Coil 1b	N	Y	Y	F	(35)
4	C673T	R225X	Coil 1b	Y	Y	N	F	(20)
4	G746A	R249Q	Coil 2	Y*/N	Y	Y	S	(24)
4	ΔAAG 783-5	ΔK 261	Coil 2	Y	Y	Y	S	(35)
5	A881C	Q294P	Coil 2	Y	Y	Y	S	(35)
6	G949A	E317K	Coil 2	Y	Y	N	F	(21)
6	959delT	FS R321	Coil 2	Y	Y	Y	F	this study†
6	G1072A	E358K	Coil 2	Y	Y	Y	S	(35)
6	T1112A	M371K	Coil 2	N	Y	Y	S	(35)
6	G1130A	R377H	Coil 2	Y	Y	Y	F	this study
6	G1130A	R377H	Coil 2	?	?	Y	F	(8)
6	G1157A	R386K‡	Coil 2	Y	Y	Y	F	(35)
7	C1357T	R453W	Tail	Y	Y	Y	S	(24)
7	C1357T	R453W	Tail	Y*/N	Y	Y	S	(35)
7	C1357T	R453W	Tail	N	Y	Y	S	(24)
8	1397delA	FS466	Tail	Y	N	N	F	(39)
8	T1406C	I469T	Tail	N	Y	Y	F	(24)
9	G1580C	R527P	Tail	N	Y	Y	S	(24)
9	C1583A	T528K	Tail	N	Y	Y	S	(24)
9	G1559C	W520S	Tail	Y	Y	Y	S	(35)
9	+ctgc	525ins	Tail	Y	Y	N	F	(21)
9	G1580C	R527P	Tail	N	Y	Y	F§	(23)
9	C1583A	T528K‖	Tail	N	Y	Y	S	(35)
Intron 9	G→C	Donor splice site	Tail	Y	Y	Y	F	(8)
10	C1711A	R571S	C-Tail	Y	Y	Y	F	(7)
11	C1718T	S573L	A-Tail	Y	Y	N	S	this study
11	C1930T	R644C	A-Tail	Y	N	N	F	(22)

*Identical mutation reported in two unrelated patients (one without cardiomyopathy and one with a mildly dilated cardiomyopathy). †Reported by Brodsky et al. (9). ‡Results in missense mutation at terminus of exon 6 with predicted disruption of normal exon 6 3′ splice site. §Identical mutation reported in two unrelated families. ‖Identical mutation reported in two unrelated patients.
× = termination nonsense; FS = frameshift. Other abbreviations as in Table 2.

are clinically relevant in a substantial subset of patients diagnosed with DCM.

Spectrum of *LMNA* mutations in DCM. To date, *LMNA* mutations causing DCM have been reported in all but two exons, 2 and 12 (Table 4) (7,8,35) and occur in protein coding regions (Fig. 2 and Table 4), with the exception of a splice donor site mutation in intron 9 (8). The DCM-causing mutations are distributed throughout the rod and tail domains of the protein, whereas in familial partial lipodystrophy, mutations have been reported exclusively in exon 8 (36).

All mutations observed in this study altered highly conserved regions of the *LMNA* gene. The R89L missense mutation may disrupt the heptad pattern of coil 1b which is characterized by hydrophobic residues in the first and fourth positions, charged residues in the fifth and seventh positions, and polar residues elsewhere (18,37). An interruption of this pattern predicts a disruption of the alpha-helix of the rod domain, which could affect the dimerization and assembly of lamins A and C. The single base deletion (959delT) (9) is predicted to disrupt the reading frame from exon 6, thereby generating a truncated mutant protein with a novel carboxy-terminus that would lack the C-terminal globular domain, the lamin A processing domain, and the nuclear localization signal (16). The missense mutation R377H occurs in a 30 amino acid segment at the C-terminal end of

the rod domain, which is conserved among lamins and cytoplasmic intermediate filaments and is critical for the higher order assembly of lamin polymers (18). The missense mutation S573L predicts a change of the highly conserved sequence and structure of the carboxyl terminal of lamin A, leaving lamin C intact. This region of the protein controls the lateral assembly of protofilaments and mediates the lamin network formation (18).

The majority of gene mutations causing DCM appear to be private mutations. However, the mutations G1130A, G746A, and C1357T have been recurrent in apparently unrelated individuals and may suggest possible hotspots or a distant founder effect (24). The R377H mutation (G1130A) found in one of our families has been reported in a LGMD1B family living in the Caribbean (8). Although a remote founder effect cannot be excluded, the ethnic backgrounds of the two families may suggest an independent mutational event. In keeping with this interpretation is the high rate of occurrence of *LMNA* de novo mutations that has been estimated in some studies to be higher than 50% (35).

The mechanism by which *LMNA* mutations cause DCM, conduction disease, and variable degrees of muscular dystrophy is still not well understood. The missense mutations observed in our population and by other authors (7,20,21) in a protein known to dimerize and form higher order assembly structures suggest a dominant negative mechanism. However, haploinsufficiency appears to be another potential mechanism in a family with EDMD due to a nonsense mutation in codon 6 (23). Lamin A and C are believed to have different complex functions: a current hypothesis is that *LMNA* mutations result in cellular and tissue fragility (intermediate filament-fragility syndrome) (17), particularly in tissues subjected to mechanical stress (heart and muscle). However, A-lamins have other important functions that could cause tissue damage, such as chromatin organization during cell division, signal transduction, differentiation maintenance, repair, and finally anchoring of other lamin-binding proteins, such as emerin, *LAP*, *BAF*, and *LBR* (19). Any of these functions may potentially lead to cell damage and apoptosis, and eventually alter myocardial and muscular function.

Genotype-phenotype correlation. Patients with DCM due to *LMNA* mutations frequently present with a "mildly" dilated form with severe dysfunction (38). The presence of any sign of skeletal muscle abnormality, such as increased serum creatine kinase, mild signs suggestive of an underlying muscular dystrophy, or family history suggestive of muscle disease in an affected relative, was a strong predictor for carrying a *LMNA* mutation in our study. The presence of atrioventricular blocks and atrial arrhythmia were also significantly associated with the carrier status. Contrary to other autosomal forms of familial DCM (29), we observed a trend toward an excess of females, perhaps suggesting the involvement of different "modifier" genes. The exon 11 subject's sister, who is ostensibly clinically healthy at age 60 years, illustrates an example of a nonpenetrant mutation carrier which has been reported previously (7,20). The occurrence of clinically unaffected carriers and the remarkable inter- and intra-familial variability in the expression of *LMNA* mutations (39) suggest the existence of additional genetic and/or environmental factors contributing to phenotype.

Prognosis of *LMNA* mutations. In our study we found that subjects with *LMNA* mutations had a significantly worse prognosis compared with the other DCM subjects. Eight of 12 carriers of *LMNA* mutations were deceased, required heart transplantation, or had severe worsening of myocardial function between the third and fifth decade. The cardiovascular mortality due to sudden death or heart failure was significantly higher in the carrier group compared with the DCM patients without *LMNA* mutations. In each of the three sets of curves shown in Figure 5, survival was significantly worse in *LMNA* mutation carriers compared with non-carriers with only 31% of event-free survival (panel C) at age 45 versus 75%.

A relatively milder phenotype with isolated DCM was observed in the sporadic family where the mutation involves only the lamin A isoform. Other authors have reported similar clinical features with mutations restricted to the lamin C isoform (7). These data suggest that the selective involvement of lamin C may have a more favorable outcome.

Study limitations. A potential source of ascertainment bias in the patient selection was that subjects were recruited in tertiary care referral centers. Statistical limitations included the fact that some variables had missing values distributed sporadically: this pattern precluded multivariate analyses. Many variables highly correlated with carrier status were themselves highly correlated: therefore, bivariate results should not be taken as additive.

Clinical implications. Results of this study provide clinical insights for genetic counseling and risk stratification of patients with DCM. Mutations of *LMNA* are frequent among patients with DCM, particularly when the disease is associated with skeletal muscle abnormalities, atrial arrhythmia, or conduction defects. Furthermore, a history of sporadic DCM does not necessarily exclude a *LMNA* gene defect. Taking into account the severity of prognosis in these patients and the possibility of early interventions to prevent the progression of heart failure, remodeling, and sudden death, our findings should prompt screening for *LMNA* in familial and sporadic cases of DCM with clinical indicators suggestive of laminopathy. The availability of DHPLC with targeted sequence analysis makes mutation screening efficient and feasible (40). Future studies should investigate the role of the nuclear envelope in the pathogenesis of DCM and the existence of modifier genes, which could alter the expressivity and severity of the disease, and could represent targets for novel therapeutic strategies.

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APPENDIX

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