Lamin A/C Gene Mutation Associated With Dilated Cardiomyopathy With Variable Skeletal Muscle Involvement

Gary L. Brodsky, PhD; Francesco Muntoni, MD; Snjezana Miocic, MD; Gianfranco Sinagra, MD; Caroline Sewry, PhD; Luisa Mestroni, MD

Background—Dilated cardiomyopathy is a form of heart muscle disease characterized by impaired systolic function and ventricular dilation. Familial transmission of the disease is frequently observed, and genetic heterogeneity is indicated by clinical and morphological variability in the disease phenotype. In the family MDDC1 reported here, the disease phenotype is severe and characterized by an autosomal dominant pattern of transmission. In addition, the majority of affected family members show signs of mild skeletal muscle involvement.

Methods and Results—On the basis of the clinical observation of both cardiac and skeletal muscle abnormalities in the MDDC1 family, the lamin A/C gene was examined in this kindred. Coding regions were polymerase chain reaction–amplified from genomic DNA and sequenced. A single nucleotide deletion was identified within exon 6, and all affected individuals were found to be heterozygous for this deletion.

Conclusions—Heterozygosity for a single nucleotide deletion in exon 6 of lamin A/C segregates with both the cardiac and skeletal abnormalities observed in the MDDC1 family. (Circulation. 2000;101:473-476.)

Key Words: cardiomyopathy • genetics • molecular biology • muscles

Dilated cardiomyopathy (DCM) is a form of heart muscle disease characterized by impaired systolic function and ventricular dilation of the left, or both, ventricles. Clinical and molecular genetic studies have resulted in the identification of 11 candidate disease loci for inherited DCM. However, only 4 disease genes responsible for DCM have been identified. These include cardiac actin, dystrophin, tafazzin, and desmin.

The family MDDC1 has a severe DCM phenotype that is inherited in an autosomal dominant pattern. The majority of affected family members demonstrate a mild form of skeletal muscle involvement. Cardiac abnormalities have been reported in Duchenne, Becker, and limb-girdle (LGMD) muscular dystrophy. In autosomal dominant Emery-Dreifuss muscular dystrophy (EDMD2), skeletal muscle weakness and wasting is often associated with conduction defects and myocardial dysfunction.

Recently, mutations in the lamin A/C gene have been identified in 5 EDMD2 families. The phenotypic similarities shared by EDMD2 patients and affected individuals in the MDDC1 pedigree led to the consideration of the lamin A/C gene as a candidate for the disease phenotype observed in this family. To test this hypothesis, we analyzed the lamin A/C gene for mutations and examined the segregation of lamin A/C mutations in the MDDC1 pedigree.

Methods

Subjects

After informed consent and approval by the institutional review committee, 14 members of the MDDC1 family were examined as previously described. Patients were evaluated by history, physical examination, ECG, and echocardiography (M-mode, 2D, and Doppler). Subjects II-1 and II-5 underwent cardiac catheterization, including ventriculography, coronary angiography, and endomyocardial biopsy. Neuromuscular investigations included electromyography and skeletal muscle biopsy. DCM status was classified as affected, unaffected, or unknown according to previously published criteria.

Lamin A/C Mutation Analysis

The coding regions of the lamin A/C gene from patient II-1 were amplified from genomic DNA isolated from MDDC1 family members with primers flanking the exon (5'-ATCCTGGAGAGATGCCAG-3' and 5'-TCTAGTCAAGGCCAGTTGCC-3'). The resulting PCR amplimers were sequenced in both directions. Single-strand conformation polymorphism (SSCP)/heteroduplex (HDX) analysis was performed on the MDDC1 amplimers and amplimers from 50 ethnically matched control subjects (100 chromosomes).

Results

Fourteen members of the MDDC1 family were examined (Figure 1A), and 5 of these individuals were classified as affected. The main clinical features observed are summarized in the Table. Patient II-5 displayed a pure DCM phenotype,
Figure 1. Heterozygous lamin A/C mutations in MDDC1 patients. A, Pedigree of MDDC1 family with individuals indicated by generation and pedigree number. Solid squares (males) and circles (females) indicate affected status, open symbols indicate unaffected status, and shaded symbols indicate unknown disease status (patient II-10 has isolated left ventricular conduction delay). B, SSCP/HDX analysis showing homoduplex (a), heteroduplex (b), and SSCP (c) bands. Heteroduplex band cosegregates with disease within family. C, DNA sequencing fluorograms demonstrating heterozygosity for lamin A/C nt 959 deletion. Forward sequencing reaction of exon 6 from an unaffected MDDC1 family member (a) yields a single DNA sequence. Same sequencing reaction performed on an affected MDDC1 family member (b) yields a single DNA sequence through nt 959. Heterozygosity for T959 deletion is indicated by presence of overlapping wild-type lamin A/C sequence and a lamin A/C sequence shifted 1 nt to left (5' end) beyond nt 959. Below fluorograms are predicted wild-type (a) and mutant (b) protein sequences, starting at codon 317. Novel mutant peptide sequences caused by frame shift are shown in blue along with resulting premature termination (Stop) codon. Nuclear localization signal, which is absent in mutant protein, is shown in red.

Clinical Features of MDDC1 Family Members Heterozygous for the Lamin A/C Deletion Mutation

<table>
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<tr>
<th>ID</th>
<th>II-1</th>
<th>II-5</th>
<th>III-1</th>
<th>III-3</th>
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<td>F</td>
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<tr>
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<td>N</td>
<td>3×</td>
<td>4×</td>
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<tr>
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<td>AF, VT</td>
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<td>SVT, PVC</td>
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<td>AVB</td>
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<td>LAH</td>
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<td>36</td>
<td>33</td>
<td>37</td>
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<tr>
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<td>48</td>
<td>...</td>
<td>49</td>
<td>63</td>
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<td>Minimal weakness</td>
<td>Slight weakness of limb-girdle</td>
<td>Slight rigidity of spine, elbow, Achilles tendon</td>
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<td>Aspecific</td>
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<td>Mild EDMD</td>
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<td>LVEF, %</td>
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</table>

CK-MM indicates muscle creatine kinase; N, normal; NSVT, nonsustained ventricular tachycardia; SVT, supraventricular tachycardia; AF, paroxysmal atrial fibrillation; VT, ventricular tachycardia; PAC, premature atrial contractions; PVC, premature ventricular contractions; AVB 2°–3°, second- and third-degree atrioventricular block; LBBB, left bundle-branch block; LAH, left anterior hemiblock; LVEDD 1°, first degree left ventricular end-diastolic dimension; FS, fractional shortening; EF, ejection fraction; HF, heart failure; and SD, sudden death.
whereas patients II-1, III-3, and III-4 had mild skeletal muscle involvement with slight calf hypertrophy and mild proximal limb girdle muscle weakness. Patient III-4 displayed mild atrophy of the biceps brachii and peroneal muscles and the supraspinatus and infraspinatus muscles, as well as spinal rigidity. Endomyocardial biopsies were performed on II-1 and II-5 and showed significant abnormalities compatible with acute myocarditis in the proband (II-5) and healing myocarditis in his brother (II-1)7 (Figure 2A). Muscle pathology indicated mild dystrophic changes in patients II-1, III-3, and III-4 (Figure 2B). Therefore, patient II-5 was classified as having pure DCM, whereas patients II-1 and III-3 displayed symptoms of LGMD and patient III-4 displayed symptoms characteristic of EDMD (Table). Serum creatine kinase levels were above normal limits in 3 of the 5 affected family members (Table).

DNA sequence analysis of the complete lamin A/C coding region from patient II-1 revealed heterozygosity for a single nucleotide deletion at position 959, which is within exon 6 (Figure 1C). Sequence analysis of exon 6 in all available family members demonstrated that all affected individuals are heterozygous for the exon 6 deletion, whereas all unaffected family members are homozygous for the normal lamin A/C allele. SSCP/HDX analysis demonstrated the presence of a heteroduplex band formed by the wild-type and deletion alleles (Figure 1B). SSCP/HDX analysis was then used to demonstrate that the nt 959 deletion was not present in 100 control chromosomes from unaffected individuals (data not shown).

Discussion
The present study identifies a lamin A/C gene mutation that segregates with the disease phenotype in a family with severe DCM and variable skeletal muscle involvement. The single nucleotide deletion identified in MDDC1 results in pure DCM with conduction defects, DCM with EDMD-like skeletal muscle abnormalities, and DCM with LGMD-like skeletal muscle dystrophy. These findings demonstrate that a lamin A/C gene mutation can result in a pure DCM phenotype without skeletal muscle involvement and that

![Image](https://example.com/image.png)
LGMD1-B, which maps to the same genetic locus, is likely to be due to mutations in the lamin A/C gene. Finally, the identification of this mutation in the lamin A/C gene further supports the model that DCM is caused by mutations in cytoskeletal proteins.

The lamin A/C gene encodes 2 proteins that are members of the intermediate filament class of cytoskeletal proteins. The nt 959 deletion is predicted to result in a mutant protein that shares its 318-amino-acid N-terminal sequence with that of lamin A and C. The frame shift causes the addition of 158 novel amino acids to the C-terminal end of the mutant protein. The mutation is predicted to leave part of the rod domain, which is involved in dimerization, intact while removing the C-terminal globular domain, lamin A processing domain, and nuclear localization signal. The unique C-terminal end of the mutant protein shows no homology to previously identified protein sequences.

The variable phenotype of skeletal muscle involvement in MDDC1 suggests that the mutation responsible for this disease differs in mechanism from the mutations resulting in AD-EDMD. However, it remains to be determined whether haploinsufficiency, due to mutant mRNA or protein instability, or a poison peptide effect is responsible for DCM in this family. Although lamin A localization was found to be normal in cardiac myocytes isolated from EDMD2 patients carrying a nonsense mutation in codon 6, the abundance of the protein was not assayed and expression of the mutant protein was not specifically examined.

In conclusion, the results described here demonstrate that a single mutation in the lamin A/C gene can result in a phenotype of DCM with a history of conduction defects, or DCM with EDMD-like symptoms, or DCM with LGMD-like symptoms. These results further support the hypotheses that DCM arises from mutations in cytoskeletal proteins and that LGMD1-B is also due to mutations in the lamin A/C gene. Although the identification of this mutation enhances the possibilities for early diagnosis and prevention of DCM, characterization of the mechanism by which this mutation causes the MDDC1 phenotype is likely to provide significant insights into the molecular basis of both DCM and muscular dystrophy.

Acknowledgments

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References


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