Brief Rapid Communication

Myosin Light Chain Mutation Causes Autosomal Recessive Cardiomyopathy With Mid-Cavitary Hypertrophy and Restrictive Physiology

Timothy M. Olson, MD; Margaret L. Karst, BA; Frank G. Whitby, PhD; David J. Driscoll, MD

Background—Autosomal dominant hypertrophic cardiomyopathy (HCM) is caused by inherited defects of sarcomeric proteins. We tested the hypothesis that homozygosity for a sarcomeric protein defect can cause recessive HCM.

Methods and Results—We studied a family with early-onset cardiomyopathy in 3 siblings, characterized by mid-cavitary hypertrophy and restrictive physiology. Genotyping of DNA markers spanning 8 genes for autosomal dominant HCM revealed inheritance of an identical paternal and maternal haplotype at the essential light chain of myosin locus by the affected children. Sequencing showed that these individuals were homozygous for a Glu143Lys substitution of a highly conserved amino acid that was absent in 150 controls. Family members with one Glu143Lys allele had normal echocardiograms and ECGs, even in late adulthood, whereas those with two mutant alleles developed severe cardiomyopathy in childhood. These findings, coupled with previous studies of myosin light chain structure and function in the heart, suggest a loss-of-function disease mechanism.

Conclusions—Distinct mutations affecting the same sarcomeric protein can cause either dominant or recessive cardiomyopathy. Electrostatic charge reversal of a highly conserved amino acid may be benign in the heterozygous state as the result of compensatory mechanisms that preserve cardiac structure and function. By contrast, homozygous carriers of a sarcomeric protein defect may have a malignant course. Recognizing recessive inheritance in children with cardiomyopathy is essential for appropriate family counseling. (Circulation. 2002;105:2337-2340.)

Key Words: genetics ■ cardiomyopathy ■ myosin

Heterozygous mutations in genes that encode sarcomeric proteins cause autosomal dominant hypertrophic cardiomyopathy (HCM). Functional studies of these mutant proteins have shown a dominant-negative effect on contractile force dynamics.1,2 Nonfamilial HCM presenting in childhood may be caused by malignant de novo mutations in the same genes that are defective in familial HCM.3 Alternatively, children with familial HCM may have more severe disease than their adult relatives because of a second inherited or de novo mutation, resulting in compound heterozygosity4 or homozygosity.5-7 We studied a family with recessive HCM to determine if an inherited sarcomeric protein defect could be clinically silent in the heterozygous state, causing HCM only in the presence of 2 mutant alleles.

Methods

Clinical Evaluation and DNA Extraction

Informed written consent was obtained from study participants under a protocol approved by the Institutional Review Board of the Mayo Clinic. Family members were evaluated by electrocardiography and echocardiography, with normal values based on body surface area and age.8 Medical records were acquired for the proband’s deceased siblings, as well as a detailed medical history for the extended family. Genomic DNA was extracted from either whole blood samples or paraffin-embedded cardiac tissue from biopsy (QIAamp DNA Mini Kit, QIAGEN, Inc).

Genotyping and Haplotype Analyses

We selected 3 polymorphic dinucleotide repeat markers spanning each of the 8 gene loci for autosomal dominant HCM: troponin T, essential light chain of myosin, myosin-binding protein C, regulatory light chain of myosin, β-myosin heavy chain, cardiac actin, α-tropomyosin, and troponin I. Markers were identified from online genetic databases and maps (Map Viewer9 and GeneMap'9910 [National Center for Biotechnology Information] and The Genome Database11) and amplified by the polymerase chain reaction (PCR), as previously described.12 Genotypes were scored and haplotypes were constructed with the goal of identifying the inheritance of an identical paternal and maternal haplotype among the siblings with cardiomyopathy.

Mutational Analyses

Once the haplotype analyses implicated the essential light chain of myosin (MYL3) as a candidate gene for autosomal recessive cardiomyopathy, mutational analyses of the gene were performed. Genomic structure was first determined by performing an online Human Genome Basic Local Alignment Search Tool (BLAST) search13 with mRNA sequence (GenBank accession number NM_000258), identifying a single genomic contig harboring MYL3.
Performed as previously described. DNA cycle sequencing of exon amplification and single-strand polymorphism (SSCP) analyses were performed with use of a Thermo Sequenase kit (Amersham Life Sciences, Inc). Sequence homology between human essential light chain of myosin and homologous proteins was determined with the MaxHom multiple sequence alignment program on the Predict-Protein server.

Figure 1. A, Pedigree structure of family with autosomal recessive cardiomyopathy caused by mutation of MYL3. Pedigree symbols represent the following: circles indicate females; squares, males; diagonal lines, deceased; filled, cardiomyopathy; shaded, uncertain cardiac phenotype; double line, consanguineous mating; digit within symbol, number of siblings of specified sex; +, mutant allele (Lys143); and −, normal allele (Glu143). B, Sequence alignment of myosin essential light chains demonstrating conservation of the glutamic acid residue at position 143. Amino acids identical to the ventricular isoform (GenBank accession number NT_029949). Oligonucleotide primer pairs were designed for PCR amplification of coding and splice junction regions for the 6 translated exons of MYL3 by use of Oligo 6.5 Primer Analysis Software (National Biosciences). PCR amplification and single-strand polymorphism (SSCP) analyses were performed as previously described. DNA cycle sequencing of exon 4 was performed with the use of a Thermo Sequenase kit (Amersham Life Sciences, Inc). Sequence homology between human essential light chain of myosin and homologous proteins was determined with the MaxHom multiple sequence alignment program on the Predict-Protein server.

Results

Family History

The family pedigree structure is shown in Figure 1A. The proband (V.3) and his sister (V.4) were referred for cardiac evaluation after the death of 2 siblings. The oldest brother (V.1) developed dyspnea and peripheral edema at age 13 and died a year later after a thromboembolic event. The other brother (V.2) developed fatigue, cardiomegaly, and hepatomegaly at age 12 and died 2 years later after operation to remove an intracardiac thrombus. Intraoperative needle biopsies of both ventricles demonstrated mild-moderate myocyte hypertrophy and interstitial fibrosis. Echocardiograms were not available for either brother, but their medical records indicated they had cardiomyopathy with dilated atria, suggesting restrictive heart disease.

Notably, the proband’s parents are consanguineous, related as second cousins. They are also half–first cousins once removed, according to the paternal grandmother’s and maternal grandfather’s lineages (not shown on pedigree). Neither parent had symptoms of heart disease, suggesting the proband’s deceased brothers may have inherited a recessive form of cardiomyopathy. Recessive, not dominant, inheritance was further supported by the absence of history of heart disease in the grandparents (III.1 to 4; ages [in years] ranging from 60s to 80s) and 10 living aunts and uncles (IV.2 to 3, IV.6 to 13; ages ranging from 40s to 60s). A paternal aunt (IV.1) died at age 7 of an unknown cause.

Clinical Evaluation

Because an inherited form of cardiomyopathy was suspected, echocardiograms and ECGs were performed on the nuclear family to screen for presymptomatic disease (Table). The proband had left ventricular hypertrophy and repolarization abnormalities on his ECG and a markedly abnormal echocardiogram (Figure 2). An unusual variant of HCM was identified, characterized by mid-cavity left ventricular hypertrophy with mild dynamic obstruction in systole. The right ventricular apex was also hypertrophied. Systolic function was normal, but restrictive physiology was diagnosed on the basis of Doppler measurements, severe bialtrial enlargement, and mild pulmonary hypertension. He was treated with propranolol and warfarin prophylactically. His echocardiographic findings 2 years later were notable for an increase in peak intracavitary left ventricular gradient from 16 to 41 mm Hg and marked increase in left atrial dimension. No arrhythmia was detected by 24-hour Holter monitoring and exercise testing. Echocardiographic findings were normal for the proband’s paternal grandparents, his parents, and his sister. Similarly, ECGs were normal in relatives, except for T-wave abnormalities in the paternal grandmother, who subsequently was found not to carry the mutation.

Linkage and Haplotype Analyses

On the basis of consanguinity and results of clinical screening, we hypothesized that a homozygous mutation was the likely mechanism for recessive HCM in this family and that similar genes might be responsible for both dominant and recessive forms of HCM. Accordingly, genotypes for 3 polymorphic DNA markers spanning each of 8 loci for autosomal dominant HCM were scored for living family members (III.1, III.2, IV.4, IV.5, V.3, and V.4) and for one of the deceased brothers (V.2). At least 2 of 3 markers at each locus were informative, and haplotypes were constructed to determine segregation of each chromosomal region harboring a known HCM gene. We looked for segregation patterns in which the 2 affected siblings (V.2, V.3) inherited identical paternal and maternal haplotypes. These analyses excluded a homozygous recessive mutation in 7 of the 8 genes. For the
MYL3-associated haplotype on chromosome 3p21.3-p21.2 (D3S1581-D3S1568-D3S1578), linkage to HCM was established assuming a homozygous recessive model of disease (data not shown). On the basis of these findings, we sought to identify a mutation in the gene encoding the essential light chain of myosin.

**Mutational Analyses**

SSCP analysis of exon 4 in MYL3 identified an anomalous conformer in the family samples (data not shown). No anomalous bands were identified in the other 5 exons. Clinically unaffected family members were either heterozygotes (III.2, IV.4, IV.5, V.4) or lacked the anomalous allele (III.1). The 2 siblings with cardiomyopathy were homozygous for the anomalous allele (V.2, V.3). DNA sequencing demonstrated that they were homozygous for a G-to-A point mutation in codon 143, resulting in a Glu143Lys substitution in both copies of the protein (Figure 1A, Table). Consistent with the SSCP data, unaffected individuals were either heterozygous for the mutation or had normal sequence. The anomalous conformer corresponding to the Glu143Lys substitution was absent in 150 normal controls. Sequence comparisons revealed that the Glu143Lys substitution involves an amino acid that is highly conserved among species (Figure 1B).

**Phenotypic Data**

<table>
<thead>
<tr>
<th>Pedigree Number</th>
<th>Age, y</th>
<th>LVDD, mm</th>
<th>EF, %</th>
<th>SEPT/PW, mm</th>
<th>Left Atrial Dimension, mm</th>
<th>Electrocardiogram</th>
<th>Glu143Lys Mutation in MYL3</th>
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</thead>
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<tr>
<td>III.1</td>
<td>70</td>
<td>42</td>
<td>58</td>
<td>12/11</td>
<td>33</td>
<td>Flat T waves V5-V6</td>
<td>Normal</td>
</tr>
<tr>
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<td>70</td>
<td>50</td>
<td>61</td>
<td>10/10</td>
<td>35</td>
<td>Normal</td>
<td>Heterozygous</td>
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<tr>
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<td>41</td>
<td>51</td>
<td>65</td>
<td>7/8</td>
<td>37</td>
<td>Normal</td>
<td>Heterozygous</td>
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<tr>
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<td>Heterozygous</td>
</tr>
<tr>
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<td>14</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>Unknown</td>
</tr>
<tr>
<td>V.2</td>
<td>14</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
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<td>Homozygous</td>
</tr>
<tr>
<td>V.3*</td>
<td>11</td>
<td>43</td>
<td>69</td>
<td>8/9 (25 mm mid-LV)</td>
<td>39 (55 mm at age 13)</td>
<td>LVH, ST elevation V5, T-wave inversion V5-V6</td>
<td>Homozygous</td>
</tr>
<tr>
<td>V.4</td>
<td>7</td>
<td>31</td>
<td>73</td>
<td>9/9</td>
<td>25</td>
<td>Normal</td>
<td>Heterozygous</td>
</tr>
</tbody>
</table>

LVDD indicates left ventricular diastolic dimension; EF, ejection fraction; SEPT, left ventricular septal thickness; PW, left ventricular posterior wall thickness; LVH, left ventricular hypertrophy; and NA, not available.

*Proband.

Abnormal echocardiographic measurements are in bold font.

**Discussion**

The actin–myosin contractile apparatus consists of 5 thin filament proteins (actin, tropomyosin, and troponins T, I, and C) and 3 thick filament proteins (myosin heavy chain, essential light chain, and regulatory light chain). Myosin assembles into hexamers comprised of 2 heavy chains and 2 pairs of each light chain isoform.17,18 The globular head domains form crossbridges with actin filaments to generate contractile force, and the rod-like tails intertwine to form thick filaments. The light chains form a stabilizing collar around the α-helical neck of the heavy chain, a region of the myosin multimer thought to function as the lever arm.

A missense mutation in the gene encoding the essential light chain of myosin (Met149Val) was previously reported in a family with autosomal dominant HCM, characterized by mid-cavitary left ventricular hypertrophy.19 An in vitro motility assay of the mutant protein demonstrated increased velocity of actin–myosin translocation, indicating gain of function of the multimeric myosin complex. The in vivo effects of the mutation were subsequently investigated in transgenic mice. Impaired stretch-activation response of cardiac muscle and replication of the mid-cavitary left ventricular hypertrophy observed in humans were demonstrated.20 In a different strain of mice, mutant transgene expression did not result in cardiac hypertrophy, but impaired ventricular relaxation and hypercontractility were observed.21

Functional studies of heterozygous mutations causing autosomal dominant HCM have shown that mutant sarcomeric proteins have a dominant-negative effect, ie, disrupting the function of the normal protein.1-2 By contrast, loss-of-function is the likely mechanism by which the Glu143Lys substitution in the essential light chain of myosin causes recessive cardiomyopathy. This conclusion is based on multiple lines of reasoning. First, homozygotes for the mutation have severe, early-onset cardiomyopathy, whereas heterozygotes have normal cardiac structure and function, even as elderly adults. If the cardiomyopathy was caused by a dominant-negative mechanism, an intermediate phenotype in heterozygotes would be expected. Second, the substitution occurs in a surface-exposed loop of the essential light chain and is unlikely to disrupt protein conformation or stability.
Third, site-directed mutagenesis of the corresponding loop domain has no effect on binding between light and heavy chains, but actin-activated ATPase activity and in vitro motility are markedly reduced. Fourth, in vitro models in which the essential light chains are removed from the myosin complex (the equivalent of functional homozygosity) have an 80% reduction in actin–myosin sliding velocity and a 50% reduction in isometric force production. Moreover, studies in humans and mice suggest that the atrial isoform of the essential light chain in humans with both dominant and recessive HCM due to MYL3 mutations. Dysfunction of the essential light chain in humans with one mutant allele. In the homozygous state, adaptive mechanisms may either be inadequate or overcompensate for deficits in contractile force generation. Biochemical studies will be required to verify a loss-of-function mechanism.

From a clinical standpoint, several findings in the present study are noteworthy. A unique pattern of mid-cavity left ventricular hypertrophy has now been identified in families with both dominant and recessive HCM due to MYL3 mutations. Dysfunction of the essential light chain in humans and mice also seems to correlate with marked diastolic dysfunction and restrictive physiology. Our findings indicate that electrostatic charge reversal of a highly conserved amino acid may be clinically benign in the heterozygous state. This has important implications for potential use of genetic testing, through comprehensive screening of known HCM genes, for clinical decision-making and risk assessment. Finally, diagnosis of idiopathic cardiomyopathy in a child whose parents may share common ancestry should raise suspicion of recessive disease. Targeted genetic testing coupled with clinical screening of parents and siblings, regardless of symptomatic status, may provide critical information for medical management and family counseling.

Acknowledgments
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References
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