Familial Dilated Cardiomyopathy Locus Maps to Chromosome 2q31

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Background—Inherited gene defects are an important cause of dilated cardiomyopathy. Although the chromosome locations of some defects and 1 disease gene (actin) have been identified, the genetic etiologies of most cases of familial dilated cardiomyopathy remain unknown.

Methods and Results—We clinically evaluated 3 generations of a kindred with autosomal dominant transmission of dilated cardiomyopathy. Nine surviving and affected individuals had early-onset disease (ventricular chamber dilation during the teenage years and congestive heart failure during the third decade of life). The disease was nonpenetrant in 2 obligate carriers. To identify the causal gene defect, linkage studies were performed. A new dilated cardiomyopathy locus was identified on chromosome 2 between loci GCG and D2S72 (maximum logarithm of odds [LOD] score = 4.86 at \( \theta = 0 \)). Because the massive gene encoding titin, a cytoskeletal muscle protein, resides in this disease interval, sequences encoding 900 amino acid residues of the cardiac-specific (N2-B) domain were analyzed. Five sequence variants were identified, but none segregated with disease in this family.

Conclusions—A dilated cardiomyopathy locus (designated CMD1G) is located on chromosome 2q31 and causes early-onset congestive heart failure. Although titin remains an intriguing candidate gene for this disorder, a disease-causing mutation is not present in its cardiac-specific N2-B domain. (Circulation. 1999;99:1022-1026.)

Key Words: cardiomyopathy ■ genetics ■ mapping

Dilated cardiomyopathy, a disorder characterized by ventricular dilatation and systolic contractile dysfunction, is an important cause of heart failure. With an estimated prevalence in the United States of 36.5 per 100,000 individuals,1 dilated cardiomyopathy is a major cause of morbidity and mortality, contributing to >10,000 deaths annually.2 Clinical presentations include congestive heart failure, thromboembolism, arrhythmia, and sudden death. Although toxic, metabolic, and infectious agents can cause dilated cardiomyopathy, inherited gene defects account for \( \approx 35\% \) of cases.3

Familial dilated cardiomyopathy exhibits significant genetic and clinical heterogeneity. This disorder can be transmitted as an autosomal dominant or a recessive, matrilineal (mitochondrial), or X-linked trait; dominant inheritance occurs most frequently. Six different dominant disease loci have been identified.4–9 CMD1A on chromosome 1q, and CMD1E on chromosome 3p3 cause dilated cardiomyopathy and conduction system disease. CMD1F, on chromosome 6, causes dilated cardiomyopathy and adult-onset limb girdle dystrophy.6 Isolated dilated cardiomyopathy is caused by mutations in cardiac actin10 and unknown gene mutations encoded at loci CMD1B (chromosome 1q32), CMD1C (chromosome 9p), and CMD1D (chromosome 10q21).11

We report a new dilated cardiomyopathy locus on chromosome 2q31 (designated CMD1G) that causes dominant transmission of early-onset disease. This genomic location implicated titin as a candidate disease gene; however, no mutation was identified in sequences encoding the cardiac-specific N2-B domain of this massive cytoskeletal muscle protein.

Methods

Clinical Evaluation

Studies were performed in accordance with the Brigham and Women’s Hospital committee for the protection of human subjects from research risks. Family members were evaluated by the use of clinical history, physical examination, 12-lead ECG, and 2-dimensional echocardiography. Echocardiographic measurements of wall thickness and cavity dimensions were determined from M-mode and 2-dimensional views. Individuals were considered affected if the left ventricular end-diastolic dimension was >2.7 cm/m² or >5.6 cm or if the left ventricular fractional shortening was <27%.11,12 in the absence of known systemic or other cardiovascular disease. The disease status of deceased individuals was based on

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medical records. The disease status of clinically unaffected individuals younger than 16 years was considered to be unknown.

Genetic Studies
Genetic analyses were performed with DNA samples extracted from whole blood or Epstein-Barr virus–transformed lymphocytes, as described previously. Linkage studies used short tandem repeat markers (STRs) spaced ~10 cM apart, with a polymorphic content of ~0.70. Polymerase chain reaction (PCR) amplification of genomic DNA with selected STR primers was performed in a 10-μL reaction containing 100 ng genomic DNA; 40 ng polynucleotide kinase 32P-labeled oligonucleotide primer; 40 ng reverse complement primer; a 200 μmol/L concentration each of dATP, dCTP, dGTP, and dTTP; Taq polymerase; and commercial buffer (Boehringer Mannheim). Thirty cycles of PCR amplification were performed with the following conditions: denaturing at 94°C for 30 seconds, annealing at 55°C for 30 seconds, and extension at 72°C for 40 seconds. PCR products were resolved on 6% denaturing polyacrylamide gels (BioRad) and visualized with the use of autoradiography. Genotypes were determined without knowledge of the patient’s clinical status.

Linkage Analysis
Two-point logarithm of odds (LOD) scores were calculated using the program MLINK (version 5.1), and multipoint LOD scores were calculated using the program LINKMAP. LOD score calculations were performed with a disease penetrance of either 0.75 and 0.90 and a phenocopy rate of 0.001. Allele frequencies were assessed in a population of unrelated individuals.

Titin Sequence Analyses
The genomic organization of the cardiac-specific N2-B region of titin was determined as follows: Oligonucleotide primers 12242 F (5’-GCCGGGCTTTGCAAGCAGCCGTGG-3’) and 12589 R (5’-GGAAGACACTTGGACTTTTCTTA-3’) generated from the titin cDNA sequence (accession No. X90568) were used to isolate BAC clone 211P22 (Genome Systems). BamHI/XbaI restriction fragments of 211P22 were subcloned into Bluescript, and nucleotide sequences were determined. Intronic/exon boundaries of the N2-B domain of titin were deduced from comparison of genomic and cDNA sequences. The 2 exons encoding the N2-B domain were amplified from patient DNA and sequenced using an ABI automated DNA sequencer and the following primer pairs: 10355 (intron) F, ATAAAGCAGAAAAGGCCATCAAC; 11192 R, TCAGGAGCCTCTGGTGTGTACCTTT; 11451 F, CAGTATTCACTTTCAGCCTCTCAA; 13135 (intron) R, CAGGGCAGTAAGGGAAAAGGTGAG; 10434 F, ACTTGAACTTTTGTCTGAATCTCC; and 11940 R, TTCTGTGATGTTCTGAAGG.

Results
Clinical Studies
Three generations of a Native American kindred (designated family MAO, Figure 1) were studied. Individuals II-1 and II-2 died at ages 79 and 46, respectively; neither had a history of heart disease. Twelve of their descendants had dilated cardiomyopathy (Table 1); none had antecedent conduction system disease or skeletal muscle dysfunction. Five affected individuals (II-1, II-2, III-1, III-2, and
III-8) died prematurely from congestive heart failure, and 2 individuals (III-3 and III-9) underwent cardiac transplantation for end-stage heart failure. Explanted hearts and postmortem examinations demonstrated moderate-to-massive cardiac enlargement without evidence of significant coronary artery disease. Histological studies showed myocyte hypertrophy without myofibrillar disarray and diffuse interstitial fibrosis without inflammation. Clinical data for other surviving and affected individuals indicated early-onset disease with a rapidly progressive course: 2 children aged 9 and 13 years had significant ventricular chamber dilation, and 2 young women (aged 16 and 21 years) required treatment for congestive heart failure. In contrast, 2 adult fathers (II-5 and II-8) with affected children had neither signs nor symptoms of cardiomyopathy, although individual II-8 died suddenly at age 50 from circulatory collapse with Rocky Mountain spotted fever.

Four individuals were assigned an indeterminate disease status: 3 are children (IV-3, IV-6, and IV-9) with normal ventricular function, and 1 is an adult (III-5) with normal ventricular dimensions but reduced fractional shortening (27%).

### TABLE 1. Clinical Features of Affected Individuals

<table>
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<tr>
<th>ID</th>
<th>Sex</th>
<th>Age, y</th>
<th>NYHA Class</th>
<th>LVED, cm</th>
<th>LVES, cm</th>
<th>LVED/BSA, cm/m²</th>
<th>FS, %</th>
<th>Comment</th>
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<td>45</td>
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<td>...</td>
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<tr>
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<td>38</td>
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<td>...</td>
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<td>...</td>
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<tr>
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<td>I</td>
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<td>I</td>
<td>5</td>
<td>3.4</td>
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<td>32</td>
<td>Septic shock†</td>
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<td>20</td>
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<td>...</td>
<td>CHF†</td>
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<td>...</td>
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<td></td>
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<tr>
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<td>3.5</td>
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</table>

ID indicates family identification number in Figure 1; NYHA, New York Heart Association; LVED, left ventricular end-diastolic dimension; LVES, left ventricular end-systolic dimension; BSA, body surface area; FS, percent fractional shortening; and CHF, congestive heart failure.

†Deceased.

### TABLE 2. Linkage Between CMD1G and Chromosome 2q Loci

<table>
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<th>Map,*</th>
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<tr>
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<tr>
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<td>175</td>
<td>D2S2330</td>
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<td>181</td>
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<tr>
<td>184</td>
<td>D2S326</td>
<td>3.60</td>
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<td>3.11</td>
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<td>188</td>
<td>D2S1244</td>
<td>4.86</td>
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<tr>
<td>188</td>
<td>HOXD8</td>
<td>4.86</td>
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<tr>
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<tr>
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<tr>
<td>205</td>
<td>D2S72</td>
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</tr>
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*Distances of loci from the 2p telomere are taken from http://www.genome.wi.mit.edu/cgi-bin/contig/phys_map (information as of January 1999).

†Two-point LOD scores were calculated at several recombination fractions with a penetrance of 0.75 and population-adjusted allele frequencies.
Genetic Studies
Pedigree analyses indicated that the dilated cardiomyopathy found in family MAO was transmitted as an autosomal dominant trait that was expressed with incomplete penetrance. To identify the chromosome location of this disease gene, polymorphic loci throughout the genome were studied. Analyses of 290 polymorphic loci excluded 70% of the genome. Linkage between the dilated cardiomyopathy in family MAO was found with locus D2S1244 (LOD score 5.486 at \( \theta = 0 \)). These data and genotypes at flanking loci (Table 2) indicated a likelihood of 30,000:1 that the disease gene was located on chromosome 2q31. Multipoint linkage analysis using data from loci D2S2157, D2S346, and D2S324 demonstrated that the maximum LOD scores achieved were robust to variations in penetrance and allele frequency (Figure 2 and data not shown).

A disease haplotype (Figure 1) based on data from 6 loci was identified in all affected individuals and obligate carriers (II-5 and II-8). One other clinically unaffected adult also carried the entire disease haplotype (individual II-6); 3 adults carried a portion of this haplotype (individuals II-4, II-7, and IV-3).

Candidate Gene Analysis
The titin gene has been mapped to YACs 764H11, 930H10, 955D4, and 963D11, each of which is contained within the disease interval. Because titin is a critical component of the cytoskeleton of cardiac and skeletal myocytes, the massive gene encoding this protein was considered a candidate for mutations that caused dilated cardiomyopathy in family MAO. To screen for disease-causing mutations, the cardiac-specific region of titin, N2-B, was analyzed. A BAC clone (211P22; Genome Systems) containing the N2-B region was isolated, its nucleotide sequence was determined, and the genomic organization was deduced by comparison with titin cDNA sequences.\(^4\) The N2-B domain is encoded in 2 exons of 2781 and 279 bp (designated exons A and B in Figure 3). Sequence comparison of the N2-B region in DNA samples derived from 2 affected and unaffected members of family MAO revealed no disease-causing mutations in exon A or B (data not shown). Five sequence variants were identified in exon A; 3 did not alter the encoded amino acids, whereas 2 sequences predicted the substitution of a threonine to a proline and a leucine to a phenylalanine. These latter 2 sequence variants create novel restriction enzyme sites that were identified in family MAO individuals and unrelated, unaffected individuals (data not shown). We conclude that neither are disease-causing mutations.

Discussion
We demonstrate that an autosomal dominant dilated cardiomyopathy disease locus, CMD1GI, is located on chromosome 2q31. Although most affected individuals exhibited early-onset disease with rapidly progression to heart failure, the gene defect was nonpenetrant in some. Whether this variable expression reflects differences in modifying genes or lifestyle differences remains an important question.

To date, 6 different familial dilated cardiomyopathy genes have been mapped, but only 1 disease gene, actin,\(^10\) has been defined. Although it is possible that each of these disease loci encodes proteins that participate in related processes, several lines of investigation indicate that different mutations are likely to perturb distinct pathways. Aberrant myocyte signaling cascades are potential etiologies, given the altered expression of \( \beta \)-adrenergic receptors in patients with heart failure\(^15\) and the cardiomyopathic phenotype of transgenic mice expressing an activated \( \alpha \) subunit of G protein.\(^16\) Defects in the

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**Figure 2.** Multipoint linkage analysis of STR markers D2SS2157, D2S1244, and D2S324 at penetrances 0.90 (○) and 0.75 (□). Distances along chromosome 2 are indicated.

**Figure 3.** Schematic of titin and the sarcomere (adapted from Labeit and Kolmerer\(^14\)) and genomic organization of titin cardiac-specific N2-B region. Single titin molecules stretch from Z disk to M line of sarcomere crossing I and A bands. These molecules are thought to interact with sarcomere proteins: cardiac myosin heavy chain (myosin), myosin binding protein C (MyBP-C), and cardiac actin. N2-B region is encoded in 2 exons (indicated) consisting of 2781 and 279 bp separated by 782-bp intron.
components of the myocyte cytoskeleton are another likely cause of this disorder: actin mutations cause familial dilated cardiomyopathy,10 and targeted disruption of genes encoding cytoskeletal proteins δ-sarcoglycan17 or muscle LIM18 result in a similar phenotype in mice.

On the basis of these data, 2 cytoskeletal genes (encoding nebulin and titin), located in the CMD1GI disease interval, were initially considered to be candidates for disease-causing mutations. Because the muscle protein nebulin has not been found in the heart,19 it was excluded from further analyses. The protein titin remained an excellent candidate given its role in specifying sarcomere length during myofibrillogenesis,17,18 contributions to passive tension generation,20 and altered expression in failing human hearts.21 Analyses of titin exons encoding the cardiac-specific domain, N2-B, failed to demonstrate a disease-causing mutation. Although a mutation in another regions of this massive (3 MDa) sarcomere protein has not been excluded, a defect located in titin domains that are expressed in all muscles would most likely cause both skeletal and cardiac muscle dysfunction. Based on the cardiac-restricted phenotype found in our study family, we therefore hypothesize that the likelihood of a disease-causing mutation elsewhere in titin sequences is low.

Eventual identification of the gene defect at CMD1GI and other dilated cardiomyopathy loci should improve diagnosis of this heterogeneous disorder. Recognition of the clinical profile associated with each of these may help to guide management and improve survival of patients with dilated cardiomyopathy.

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