Novel Cardiac Troponin T Mutation as a Cause of Familial Dilated Cardiomyopathy

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Background—Familial dilated cardiomyopathy (FDCM) and hypertrophic cardiomyopathy (FHCM) are the 2 most common forms of primary cardiac muscle diseases. Studies indicate that mutations in sarcomeric proteins are responsible for FHCM and suggest that mutations in cytoskeletal proteins cause FDCM. Evidence is evolving, however, that such conclusions are premature.

Methods and Results—A novel missense mutation in the cardiac troponin T gene was identified by direct sequencing and confirmed by endonuclease restriction analysis in a large family with FDCM that we had previously mapped to chromosome 1q32. The mutation substitutes tryptophan for a highly conserved amino acid, arginine, at amino acid residue 141 (Arg141Trp). The mutation occurs within the tropomyosin-binding domain of cardiac troponin T and alters the charge of the residue. This mutation cosegregates with the disease, being present in all 14 living affected individuals. The mutation was not found in 100 normal control subjects. Clinical features were congestive heart failure with premature deaths. The age of onset and severity of the disease are highly variable, with incomplete penetrance. Because 15 mutations in troponin T are known to cause FHCM, 219 probands with FHCM were screened, and none had the mutation.

Conclusions—Thus, the novel cardiac troponin T mutation Arg141Trp is responsible for FDCM in our family. Because several mutations in troponin T have already been recognized to be responsible for FHCM, it appears that the phenotype, whether it be hypertrophy or dilatation, is determined by the specific mutation rather than the gene. (Circulation. 2001; 104:2188-2193.)

Key Words: cardiomyopathy ■ genetics ■ molecular biology

Cardiomyopathy, a group of primary cardiac muscle disorders including dilated (DCM) and hypertrophic (HCM) cardiomyopathy, is one of the major causes of heart failure and sudden death.1,2 The genetic basis for familial hypertrophic cardiomyopathy (FHCM) has progressed rapidly since the discovery of the first FHCM gene in 1990.3 with >100 mutations identified in genes, all of which encode for sarcomeric proteins.1,4 In contrast, identification of genes responsible for familial dilated cardiomyopathy (FDCM) has been difficult, partly because of multiple phenocopies.4 DCM is characterized clinically by the manifestations of heart failure or sudden death and is diagnosed on the basis of ventricular chamber enlargement and systolic dysfunction. The pathology is also nonspecific, consisting of elongated myocytes and microscopic areas of fibrosis without the characteristic hypertrophy or myocyte disarray observed in FHCM.1,5 The cause of hypertrophy other than FHCM, such as hypertension or valvular disease, can usually be detected, whereas causes of cardiac dilatation such as previous viral myocarditis during childhood cannot be differentiated from genetically induced forms of DCM. The diagnosis, essential to genetic approaches for identifying disease-causing genes, remains a conundrum.6

The most common familial form of DCM is the autosomal dominant, for which 15 loci have been mapped. Fewer than one half of the genes residing at these loci have been identified. The first gene to be identified for pure DCM was cardiac actin in 1998,7 followed by desmin,8 which suggested that genes encoding for proteins that transmit force would be responsible for FDCM, analogous to the sarcomere proteins that generate force and are responsible for FHCM. Lamin A/C, identified to be responsible for FDCM with a conduction disorder,9 however, is a filament of the inner layer of the nuclear membrane; although its function is not known, it is
probably not involved with mechanical force. Then came a major surprise, when 2 of the genes responsible for FHCM, \( \beta \)-myosin heavy chain and cardiac troponin T (cTnT), were shown to have mutations responsible for FDCM. This has significantly compounded the theoretical framework on which to postulate a common mechanism responsible for FDCM.

In 1995, we mapped the first locus responsible for pure idiopathic DCM in a large family to 1q32. We now report a novel missense mutation in cTnT to be responsible for the disease in this family. We screened 219 probands with FHCM, and none had this mutation. Thus, despite several mutations in cTnT being responsible for FHCM, namely, the hypertrophic growth response, our mutation appears to stimulate only cardiac dilatation.

**Methods**

**Clinical Evaluation**

Informed consent was obtained according to the guidelines of Baylor College of Medicine. The probands and the family members were evaluated by detailed history and physical examination, 12-lead ECG, M-mode and 2D echocardiography, and Doppler ultrasonography. The diagnostic criteria for DCM were defined as an ejection fraction <50% on echocardiographic analysis, regional fractional shortening <25% on M-mode analysis, or both in the presence of a left ventricular internal diastolic diameter ≥2.7 cm/m² of body surface area. A diagnosis of HCM was based on the demonstration of a left ventricular wall thickness ≥13 mm by echocardiography measured in diastole in the region of greatest hypertrophy. In addition, other conditions that may simulate DCM or HCM were excluded, including coronary heart disease, myocarditis, hypertension, and valvar heart disease.

**Preparation of DNA**

Blood was collected from each family member. DNA was extracted from the white blood cells, and lymphoblastoid cell lines were developed as previously reported.

**Genotyping and Delineation of the Critical Chromosomal Region**

In the initial report, we had mapped the locus to 1q32 with a region of 20 cM. Recently, 4 additional affected individuals were identified, and DNA was analyzed for 20 additional markers located between D1S238 and D1S227 in the 1q32 region. Two-point linkage analysis was conducted on a personal computer with version 5.2 of the LINKAGE program.

**Mutation Analysis**

Candidate genes were selected from current public computer databases, including GeneMap99 (www.ncbi.nlm.nih.gov/genemap99/map.cgi). The screening for mutations consisted of sequencing candidate genes amplified from 2 affected and 2 normal individuals. The primer sequences used in this study are available on request. Polymerase chain reactions (PCR) were carried out with standard protocol as previously described. For some exons with unusual GC-rich contents, Hotstart Taq polymerase and Q solution (Qiagen) were used. PCR products were examined on agarose gel and purified with a Qiagen PCR purification kit. The purified PCR products were sequenced on an ABI model 310 genetic analyzer with a Big Dye Terminator sequencing kit (Perkin Elmer). A Sequencer program was used to facilitate mutation detection, in addition to careful manual examination of individual sequences.

Once the cTnT mutation Arg141Trp was identified and shown to abolish an HpaII restriction site, the mutation was confirmed by screening the whole family with HpaII restriction digestion of the PCR product amplified from a genomic region flanking the mutation. The primer sequences used for the PCR are as follows: forward, 5'-ATGCAGGTTTCTGTACCTGCGATG-3' ; reverse, 5'-TGATGATGAATAGAGAGGGGCCTG-3'. PCR reactions were carried out with Hotstart Taq polymerase (Qiagen), and the cycling parameters were as follows: 35 cycles of 95°C for 20 seconds, 64°C for 30 seconds, 72°C for 30 seconds after initial activation, and denaturing at 95°C for 15 minutes. Restriction endonuclease HpaII was obtained from New England Biolabs, and the digestion was conducted at 37°C for 2 hours and separated by electrophoresis. The resulting PCR product is 237 bp and is normally cut into 2 fragments of 112 and 125 bp. The mutant allele cannot be digested, however, and remains unchanged as a product of 237 bp.

**Homologous Sequence Search and Alignment**

A BLASTP search was conducted via NCBI search launcher to identify proteins homologous to human cTnT. The CLUSTALW algorithm (version 1.7; Thomas et al, 1994; can be found at http://www.ncbi.nlm.nih.gov) was used to perform multiple sequence alignment with the protein sequences of significant homology to human cTnT.

**Results**

**Genetic Analysis**

Genotyping of the newly identified 4 affected individuals in this 5-generation family (Figure 1), followed by haplotype
tryptophan (Arg141Trp).

dicted to change normal amino acid arginine at residue 141 to

tide 471 of human cTnT (GenBank No. NM_000364) that is pre-

patients with FDCM. Arrow points to C-to-T transition at nucleo-

tide 471 (GenBank No. NM_000364) predicted to change normal amino acid arginine at residue 141 to tryptophan (Arg141Trp). Sequence analysis of exon 10 was performed in all family members, and the mutation was demonstrated to be present in 20 individuals (Figure 1). The diagnosis of FDCM had been given to 14 members, and 1 other was considered indeterminate. The remaining 5 are phenotypically normal. The mutation cosegregates with the disease in the family, because all of the affected individuals inherited the mutation (Figure 1). Furthermore, evaluation of 100 unrelated control individuals (200 chromosomes) and 219 probands with FHCM failed to detect this base variation. In addition, the amino acid substitution in human cTnT is strictly conserved across many species, including mouse, rat, chicken, quail, and nematode, as well as among the slow or fast skeletal muscle isoform of TnT (Figure 3). This further confirms that the missense mutation in the cTnT gene is responsible for DCM in this family.

Clinical Features of the cTnT Mutation

Clinical evaluation of 72 members of a large family spanning 5 generations (Figure 1) identified 14 living members affected with typical DCM (Table). Among the 14 living affected individuals, 1 (subject II:1) has lived beyond normal life expectancy with NYHA class III heart failure. In contrast, echocardiographic manifestations of the disease were present in ≥2 individuals before the age of 10 years (subjects IV:20 and IV:21). For the majority, the onset of cardiac symptoms generally occurred in the second decade. Two needed heart transplants at the ages of 20 and 24 years. Seventeen individuals died of heart failure, presumably due to DCM, before the study was undertaken. Five children died of the disease between the ages of 1 and 15 years (subjects II:5, IV:1, IV:31, IV:32, and IV:35). One of them (subject IV:1) died at the age of 2.5 years, and the autopsy analysis confirmed the diagnosis of DCM. There were 3 peripartum deaths (subjects II:6, III:16, and IV:25) in this family. The medical histories of other deceased persons showed that the majority of them developed a cardiac condition in the second decade of life. The disease progressed to overt heart failure at 30 to 50 years of age. There was no documented case of sudden cardiac death. The incomplete right bundle-branch blocks in 2 individuals were not present initially but developed secondary to progression of the disease. One family member (subject III:10) had mild cardiac symptoms and borderline cardiac enlargement, but an ejection fraction of 60%, and thus did not meet our diagnostic criteria and was

Figure 3. Alignment of human cTnT protein sequence flanking mutation Arg141Trp. Protein sequences were obtained from GenBank with following accession numbers: human TNNT2, NM_000364; bovine, AF175558; rabbit TNNT2, A25345; chicken TNNT2, M10013; rat TNNT2, M26051; mouse TNNT2, AB052890; human TNNT1, NM_003283; chicken TNNT1, D85105; mouse TNNT1, NM_011618; human TNNT3, NM_006757; mouse TNNT3, NM_011620; quail Tnt, M26600; and ascidian Tnt, D58867. TNNT2 indicates cTnT; TNNT1, slow skeletal Tnt; and TNNT3, fast skeletal Tnt.
designated indeterminate. Other family members did not have any clinical manifestations or echocardiographic abnormalities of DCM and were designated normal. Among the normal members, 5 (subjects IV:12, IV:22, IV:24, V:1, and V:2) inherited the mutation. Thus, DCM caused by the cTnT mutation in this family exhibits a phenotype variable with moderate to high penetrance.

Discussion

We identified a large pedigree spanning 5 generations with FDCM segregating as an autosomal dominant trait. We previously mapped the responsible locus to 1q32 12 with a critical region of 20 cM and subsequently narrowed the region to 8.6 cM. Using the positional candidate gene approach, we identified a novel missense mutation in the tail of the cTnT and showed that it was responsible for the disease in this family. The mutation induced a substitution of tryptophan for a highly conserved amino acid, arginine, at amino acid residue 141. The predominant clinical features are cardiac failure and premature death. The age of onset and the severity of the disease are highly variable. One individual died of heart failure at age 2.5 years and was confirmed on autopsy to have DCM, yet other individuals in their sixth or seventh decades of life with the disease are minimally symptomatic. The majority of the deceased developed a cardiac condition in the second decade of life and had severe congestive heart failure in the third to fifth decade. Five individuals varying in age from 1 to 47 years have the mutation without clinical manifestations. Thus, FDCM in this family has a phenotype primarily of heart failure that is variable in age of onset and severity with moderate to high penetrance.

Mutations in cTnT have been established to be the third most common cause of FHCM. FHCM caused by cTnT mutations usually exhibits mild cardiac hypertrophy but a high incidence of sudden death. A recent surprise finding by Kamisago et al showed that deletion of 1 amino acid from cTnT (Δ210Lys) caused FDCM in 2 families; notably, both were associated with a high frequency of sudden death. In our family, the clinical features are typical for DCM, namely, depressed contractile function and ventricular dilatation resulting in the symptoms of congestive heart failure. No case of sudden death has been documented in our family. None of the echocardiograms or ECGs showed ventricular hypertrophy. Our family does not represent HCM that has evolved to end-stage dilatation, because several members of the family developed DCM during childhood, and 1 child who died at age 2.5 years was confirmed on autopsy to have had DCM. Furthermore, we screened more than 200 probands with FHCM for the Arg141Trp mutation, and none were identified. Similarly, the mutation Δ210Lys in cTnT is not associated with FHCM; furthermore, no mutation responsible for FHCM has ever been identified in either of these exons (Figure 4). One conclusion is that the growth response of the heart, whether it is hypertrophy or dilatation, is mutation-specific rather than gene-specific.

It is very perplexing that mutations in the same gene can induce what appear to be 2 apposing phenotypes, namely,

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Sex/Age, y</th>
<th>NYHA Class</th>
<th>LVEF, %</th>
<th>LVEDD, cm/m²</th>
<th>LVSF, %</th>
<th>LVWT &gt;13 mm</th>
<th>COMMENTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>II:1</td>
<td>M/84</td>
<td>III</td>
<td>25</td>
<td>3.6</td>
<td>N/A</td>
<td>None</td>
<td>S bradycardia</td>
</tr>
<tr>
<td>III:2</td>
<td>M/40</td>
<td>II</td>
<td>&lt;20</td>
<td>3.4</td>
<td>8</td>
<td>None</td>
<td>S tachycardia, IRBBB</td>
</tr>
<tr>
<td>III:4</td>
<td>M/42</td>
<td>II</td>
<td>40</td>
<td>2.6</td>
<td>N/A</td>
<td>None</td>
<td>Inferior MI</td>
</tr>
<tr>
<td>III:6</td>
<td>M/46</td>
<td>I</td>
<td>45</td>
<td>2.9</td>
<td>30</td>
<td>None</td>
<td>S bradycardia</td>
</tr>
<tr>
<td>III:10</td>
<td>M/69</td>
<td>II</td>
<td>60</td>
<td>2.7</td>
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<td>None</td>
<td></td>
</tr>
<tr>
<td>IV:5</td>
<td>F/27</td>
<td>I</td>
<td>38</td>
<td>3.2</td>
<td>17</td>
<td>None</td>
<td>IRBBB</td>
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<tr>
<td>IV:10</td>
<td>F/18</td>
<td>I</td>
<td>61</td>
<td>3.0</td>
<td>N/A</td>
<td>None</td>
<td>S arrhythmia</td>
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<tr>
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<td>F/15</td>
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<td>51</td>
<td>2.6</td>
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<td></td>
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<tr>
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<td>F/15</td>
<td>II</td>
<td>32</td>
<td>4.0</td>
<td>21</td>
<td>None</td>
<td>S arrhythmia</td>
</tr>
<tr>
<td>IV:19</td>
<td>M/17</td>
<td>IV</td>
<td>&lt;20</td>
<td>3.7</td>
<td>N/A</td>
<td>None</td>
<td>Heart transplant</td>
</tr>
<tr>
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<td>F/9</td>
<td>I</td>
<td>40</td>
<td>4.7</td>
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<td>None</td>
<td>Prolonged QT</td>
</tr>
<tr>
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<td>I</td>
<td>45</td>
<td>2.8</td>
<td>26</td>
<td>None</td>
<td>Prolonged QT</td>
</tr>
<tr>
<td>IV:22</td>
<td>M/47</td>
<td>II</td>
<td>55</td>
<td>2.6</td>
<td>N/A</td>
<td>None</td>
<td></td>
</tr>
<tr>
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<td>M/42</td>
<td>I</td>
<td>60</td>
<td>2.1</td>
<td>N/A</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td>IV:27</td>
<td>F/37</td>
<td>II</td>
<td>40</td>
<td>2.7</td>
<td>33</td>
<td>None</td>
<td>Prolonged QT</td>
</tr>
<tr>
<td>IV:38</td>
<td>M/21</td>
<td>IV</td>
<td>&lt;20</td>
<td>3.5</td>
<td>N/A</td>
<td>None</td>
<td>Heart transplant</td>
</tr>
<tr>
<td>IV:39</td>
<td>F/22</td>
<td>II</td>
<td>49</td>
<td>3.1</td>
<td>28</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td>IV:43</td>
<td>M/60</td>
<td>II</td>
<td>38</td>
<td>2.7</td>
<td>25</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td>V:1</td>
<td>F/1</td>
<td>I</td>
<td>71</td>
<td>N/A</td>
<td>34</td>
<td>None</td>
<td>ST and T wave abn.</td>
</tr>
<tr>
<td>V:2</td>
<td>F/29</td>
<td>II</td>
<td>57</td>
<td>2.5</td>
<td>N/A</td>
<td>None</td>
<td>S arrhythmia</td>
</tr>
</tbody>
</table>

Age is the subject’s age at diagnosis. LVEF indicates left ventricular ejection fraction; LVEDD, LV end-diastolic dimension; LVSF, LV shortening fraction; LVWT, LV wall thickness; S, cardiac sinus; IRBBB, incomplete right bundle-branch block; MI, myocardial infarction; and ST and T wave abn., ST-segment abnormality and T-wave inversion in the ECG.
hypertrophy or dilatation. The normal compensatory response of the heart to physiological or pathological stimuli is hypertrophy or dilatation or both.1 We have shown that a cTnT mutation (Arg92Gln) responsible for FHCM expressed in transgenic mice is incorporated into the cardiac sarcomere myofibrils,20 exhibits myocyte disarray21 and increased fibrosis,20 and is associated with upregulation of growth factors such as insulin-like growth factor and transforming growth factor,22 as is observed in human HCM.23 Similar upregulation of growth factors is observed in the transgenic rabbit model of human HCM.22,24 Most investigators agree that the hypertrophy is a secondary phenotype, which is further confirmed by our recent findings showing reversal of the fibrosis in the mouse model with losartan25 and reversal of the hypertrophy is a secondary phenotype, which is further confirmed by our recent findings showing reversal of the fibrosis in the mouse model with losartan25 and reversal of the hypertrophy or dilatation or both. We have shown that a cTnT mutation (Arg141Trp), this compensatory growth response is absent. It is expected that cardiac contractility will be impaired, because residue 141 is required for the troponin-tropomyosin complex to bind to actin.27,28 The growth factors will most likely be upregulated, but why no growth? A likely reason stems from recent studies showing that the amino terminus (residues 1 to 153) of cTnT is essential for filament and sarcomere assembly. In vitro site-directed mutagenesis of the tail of cTnT showed that increasing elimination of residues 1 to 153 correlated with increasing to virtually complete elimination of the tropomyosin binding to actin.28 It appears that the amino-terminal tail of cTnT is essential for assembly and anchoring of the troponin-tropomyosin complex onto the thin filament. Thus, despite mitotic and trophic simulation, the autosomal dominant inherited cTnT (Arg141Trp) acts as a poison peptide to inhibit filament assembly, which stymies the growth response. The chronic impaired contractility leads to increasing left ventricular filling pressure and wall stress, resulting in further mechanical dilatation and ultimately cardiac failure and death. These postulates are based on incomplete data at this time, particularly given that a 3D model of TnT is not available.29 Nevertheless, many of these hypotheses can be addressed in genetic animal models, which could provide insights fundamental to the ultimate goal of modulating cardiac growth. Documentation of mutations in the same gene resulting in dilatation or hypertrophy has intriguing implications. Genetic animal models should provide insight into understanding the fundamental difference between these responses. It is potentially possible to induce FHCM and FDCM in the same animal model with the same gene but with different mutations. Comparison of the growth factors or other genes upregulated during hypertrophy versus dilatation on the same genetic background should provide insight into the mediators of both cardiac growth responses heretofore not possible.

**Acknowledgments**

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**References**


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