

# Homozygous Mutation in Cardiac Troponin T Implications for Hypertrophic Cardiomyopathy

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**Background**—Mutations in the gene that encode cardiac troponin T (cTnT) account for ≈15% of cases of familial hypertrophic cardiomyopathy (HCM). These mutations are associated with a particularly severe form of HCM characterized by a high incidence of sudden death and a poor overall prognosis, despite subclinical or mild left ventricular hypertrophy.

**Methods and Results**—We evaluated a family with HCM and multiple occurrences of sudden death in children. DNA samples were isolated from peripheral blood or paraffin-embedded tissue, and all protein-encoding exons of the cTnT gene were sequenced. A mutation was identified in exon 11 and is predicted to substitute a phenylalanine-for-serine mutation at residue 179 (Ser<sup>179</sup>Phe) in cTnT. Both parents and 3 of 4 surviving and clinically unaffected children were heterozygous for this mutation; another clinically unaffected child did not carry the mutation. Genetic analysis of DNA from a child who died suddenly at age 17 years demonstrated he was homozygous for this mutation. A review of his echocardiogram revealed profound left and right ventricular hypertrophy.

**Conclusions**—An homozygous Ser<sup>179</sup>Phe mutation in cTnT causes a severe form of HCM characterized by striking morphological abnormalities and juvenile lethality. In contrast, the natural history of the heterozygous mutation is benign. These studies emphasize the relevance of genetic diagnosis in hypertrophic cardiomyopathy and provide a new perspective on the clinical consequences of troponin T mutations. (*Circulation*. 2000;102:1950-1955.)

**Key Words:** cardiomyopathy ■ cardiac troponin T ■ genetics

Familial hypertrophic cardiomyopathy (HCM) is an autosomal dominant disorder caused by mutations in genes that encode sarcomere proteins, including cardiac  $\beta$ -myosin heavy chain,  $\alpha$ -tropomyosin, cardiac troponin T (cTnT), cardiac myosin-binding protein C, cardiac troponin I, and cardiac myosin regulatory and essential light chains.<sup>1-5</sup> In addition to this genetic diversity, the phenotypic expression of these mutations varies considerably, ranging from asymptomatic individuals with a normal life expectancy to those with sudden cardiac death or need for early heart transplantation. Clinical parameters such as degree of the left ventricular hypertrophy, the presence or absence of a left ventricular outflow tract gradient, and electrophysiology testing have not been predictive markers of poor prognosis.<sup>5-8</sup> In contrast, there has been a more consistent relationship between certain genetic mutations and clinical outcome, allowing for the classification of “benign” and “malignant” mutations.<sup>7-12</sup> This underscores the importance of genetic analysis and the potential role of genotype determination in assisting with patient management.

Mutations of cTnT are thought to account for ≈15% of familial HCM<sup>7</sup> and are associated with a particularly severe

form of disease characterized by a poor overall prognosis with a high incidence of sudden death despite only mild left ventricular hypertrophy.<sup>6,7</sup> However, this association is based on limited experience; to date, only 11 different mutations have been identified.<sup>2,7,13-15</sup> The identification and evaluation of families with cTnT mutations may be of particular importance to increase our understanding of the pathophysiology of severe forms of HCM. Furthermore, these data may provide greater information about the structure and function of this sarcomere protein.

We identified a family with HCM and multiple episodes of sudden death in children. Direct sequence analysis of the cTnT gene was performed to define the disease-causing mutation in this family. Identification of a novel mutation provided unique insights into a particularly malignant form of HCM. These insights both influence patient management and expand current understanding of the clinical nature of cTnT mutations in this disorder.

## Methods

### Clinical Evaluation

After informed consent was obtained in accordance with the guidelines of the Brigham and Women's Hospital Human Subjects

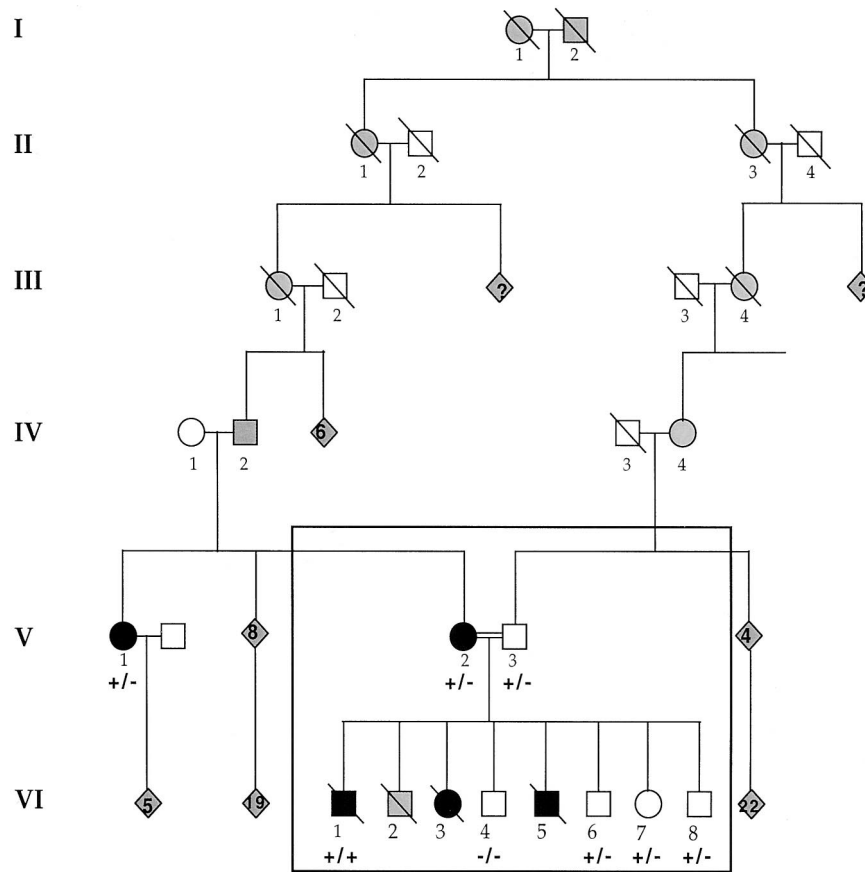
Received April 21, 2000; revision received May 31, 2000; accepted May 31, 2000.

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**Figure 1.** Clinical and genetic status of a family with HCM and multiple occurrences of sudden death. Pedigree symbols denote clinically affected individuals (filled), clinically unaffected individuals (open), and those of unknown clinical status (gray). Slashes indicate deceased individuals; numbers within symbols represent number of offspring. Boxed portion of pedigree indicates individuals screened for mutations in cTnT gene. Genotypes: +/- indicates heterozygous for mutant (+) and wild-type (-) alleles; -/-, genetically unaffected; +/+, homozygous for mutant alleles.

Committee, blood was drawn for genetic analyses from members of a family with HCM. Family members were evaluated through history, physical examination, and ECG<sup>16</sup> and echocardiographic<sup>17</sup> assessments. Clinical information on deceased children was obtained from their parents and medical records.

**Genetic Analysis**

**Identification of a Mutation in the Cardiac TnT Gene**

Genomic DNA was isolated from peripheral blood samples as described previously.<sup>18</sup> Exons 2 through 16 of the cTnT gene were

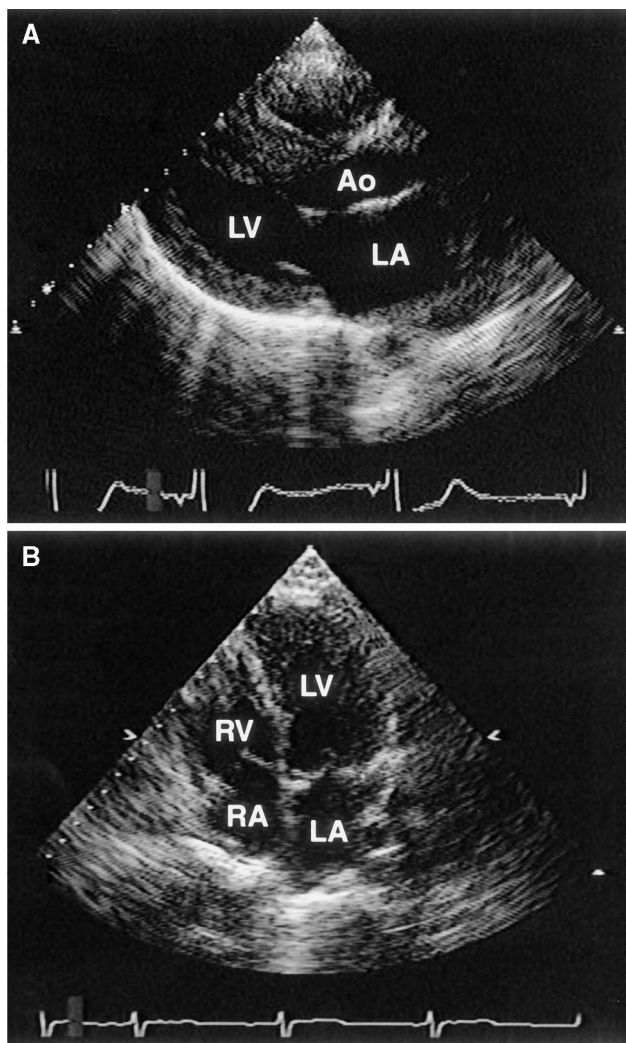
amplified with PCR from 100 ng genomic DNA through the use of primers designed from flanking intron sequences (10 to 15 pmol/ primer; sequences are available on the Internet at <http://genetics.med.harvard.edu/~seidman>). Amplified fragments were purified with the QIAquick PCR Purification kit (QIAGEN). DNA sequences were determined through automated DNA sequencing with the ABI Prism dye-terminator cycle-sequencing kit (Applied Biosystems/Perkin-Elmer Cetus) and compared with the published genomic sequence of the cTnT gene (EMBL accession numbers X98477, X98478, X98479, X98480, X98481, X98482, X98483, Y09626, Y09627, Y09628, and AF00412).

**Summary of Clinical Features of Evaluated Family Members**

Pedigree	Date of Birth	Sex	Symptoms	ECG Data			Echocardiographic Dimensions, mm	
				LVH	STΔ	Q	IVS	PW
V-1	Unknown	F	Exertional chest pain and dyspnea	N	Y	Y	(+) LVH	
V-2	5/30/55	F	Exertional chest pain and dyspnea	N	Y	N	13	11
V-3	4/5/50	M	Asymptomatic	N	N	Y	10	10
VI-1	1/4/76	M	Sudden death at age 17 while ambulating	Y	N	Y	25	11
VI-3	12/9/78	F	Chest pain, sudden death at age 15 while at rest		NA		(+) LVH	
VI-4	8/30/80	M	Asymptomatic	N	N	N	10	9
VI-5	8/27/82	M	Sudden death at age 12 during soccer practice		NA		NA	
VI-6	12/8/84	M	Asymptomatic; AICD placed August 1998	N	N	Y	7	7
VI-7	10/17/87	F	Asymptomatic	N	N	Y	7	6
VI-8	7/27/91	M	Asymptomatic	N	N	Y	5	4

LVH indicates left ventricular hypertrophy; STΔ, ST-segment changes; Q, the presence of Q waves; IVS, thickness of the interventricular septum; PW, thickness of the posterior wall.

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**Figure 2.** Echocardiography of individual VI-1 shows striking right and left ventricular hypertrophy associated with homozygous mutant alleles (phenylalanine at residue 179). A, Parasternal long-axis view. Ao indicates aorta; LA, left atrium; and LV, left ventricle. B, Apical 4-chamber view. Left ventricle, right ventricle (RV), left atrium, and right atrium (RA) are indicated.

#### Allele-Specific Oligonucleotide Hybridization

The presence of a single-base pair sequence variation was confirmed with PCR amplification and allele-specific oligonucleotide hybridization as described previously.<sup>19</sup> Exon 11 sequences were amplified with PCR, transferred to 2 nylon membranes (GeneScreen Plus; NEN Life Science Products), and hybridized separately with <sup>32</sup>P-labeled wild-type (5'-GCTTTGTCCAACATG-3') or mutant (5'-GCTTTGT7CAACATG-3') oligonucleotides. The hybridized nylon membranes were washed in 6× SSC (1× SSC is 0.15 mol/L sodium chloride and 0.015 mol/L sodium citrate) and 0.05% sodium pyrophosphate for 20 minutes at 48°C, and the hybridization signal was quantified with a PhosphorImager (Molecular Dynamics).

#### Extraction of DNA From Fixed, Paraffin-Embedded Tissue

A paraffin ribbon containing five 10- $\mu$ m-thick tissue sections was digested in proteinase K solution (50 mmol/L Tris-Cl, pH 8.5 at 25°C, 1 mmol/L EDTA, 0.5% Tween 20, proteinase K 200  $\mu$ g/ $\mu$ L) at 65°C overnight. A slurry (1:1 wt/vol mixture of Chelex 100 [50 to 10 mesh beads; Bio-Rad] in water) was added to remove heavy metal ions, and the mixture was boiled and centrifuged. The isolated DNA solution was used directly for PCR amplification.<sup>20</sup>

#### Restriction Enzyme Digest Analyses

Confirmation of the sequence variant in exon 11 was confirmed with modified restriction enzyme digestion.<sup>21</sup> A partial *Xmn*I site, created by the point mutation in exon 11, was completed by introducing 2 mismatched nucleotides (underlined) with PCR and primers: F: 5'-GAGGCCCGGAAGAAGAAGAATTTGT-3'; and R: 5'-GG-ACCTGACCTAAAGTCTACCTGC-3'.

The resultant 153-bp fragment was digested with *Xmn*I. The presence of the C→T transition at nucleotide residue 77 in exon 11 allows cleavage (yielding a 131-bp fragment), whereas the wild-type allele is not cut.

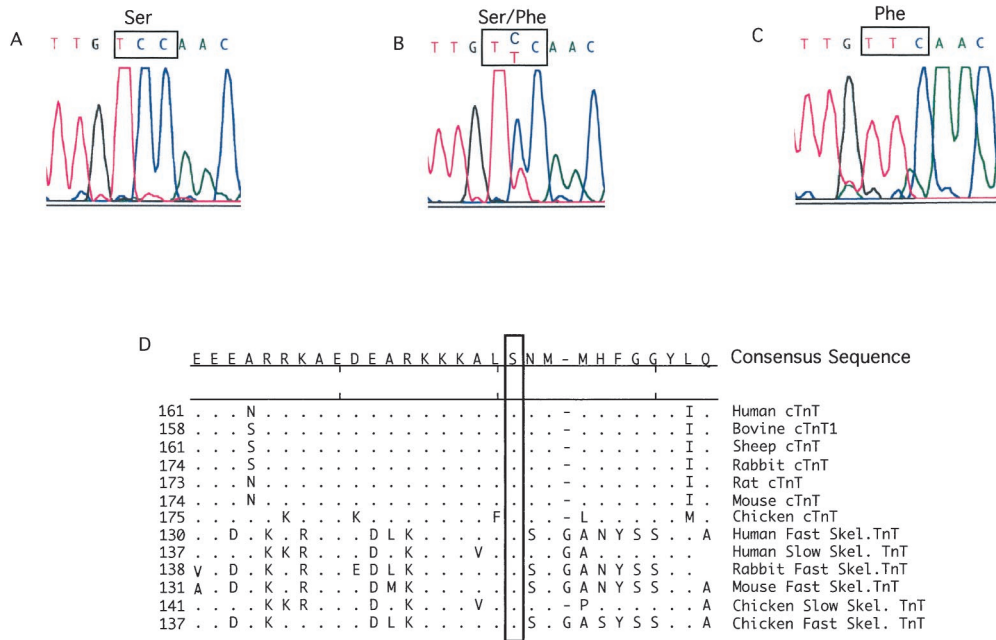
## Results

#### Family Characteristics

A kindred from Kuwait (Figure 1) was referred to one of the authors (R.D.) for further evaluation and management of HCM after the occurrence of sudden death in 3 children. The clinical features of the studied family members are summarized in the Table. Individual VI-2, the son of V-2 and V-3, died at 1 year of age after a brief febrile illness. In 1993, the 12-year-old son (VI-5) died suddenly during soccer practice. Neither had a postmortem examination, and further clinical information is unavailable. This second death prompted an evaluation of the 6 surviving children: 2 had clinical findings consistent with HCM. Individual VI-3 reported intermittent chest discomfort, and an echocardiogram (primary data not available) showed marked left ventricular hypertrophy; later that same year, she died suddenly at the age of 15 years while conversing with her family. Individual VI-1 was diagnosed with HCM based on ECG (striking voltage in the precordial leads) and echocardiography findings. The latter showed characteristic features of HCM, including marked septal hypertrophy (maximal septal thickness of 25 mm) with reverse septal curvature. A striking degree of right ventricular hypertrophy was also present with right ventricular free wall thickness of  $\approx$ 18 mm (Figures 2A and 2B). Left ventricular systolic function was preserved, and there was no evidence of dynamic outflow tract obstruction or valvular abnormalities. In 1993, at the age of 17 years, he died suddenly while ambulating.

The children's mother (individual V-2) and her sister (individual V-1) also had HCM. Limited information was available on individual V-1 (age  $\approx$ 48 years). She was reported to have mild exertional symptoms, ECG findings of Q waves in leads I and aVL, and evidence of hypertrophy on echocardiography (primary data unavailable). Individual V-2 (age 40 years) reported a long history of mild exertional chest pain and dyspnea. Physical examination and exercise testing were unremarkable. ECG showed left-axis deviation, Q waves in leads I and aVL, and T-wave inversion in lead III. Echocardiography demonstrated mild left ventricular hypertrophy with a maximal wall thickness of 13 mm. She and her husband, V-3, are distantly related via a common great-great-grandparent. He (V-3) was entirely asymptomatic and had an unremarkable physical examination. His ECG showed nondiagnostic Q waves in leads II, III, and aVF. Echocardiographic studies showed normal ventricular dimensions and function.

The 4 remaining children ranged in age from 4 to 14 years. All were asymptomatic and had normal development and unremarkable physical examinations. Echocardiography in



**Figure 3.** DNA sequence of a portion of exon 11 of cTnT gene demonstrates a single-base mutation of nucleotide residue 77. A, Wild-type sequence (codons 178 to 180) of cTnT gene (obtained from DNA from individual VI-4) encodes a serine residue at codon 179 (TCC). B, A single-base pair sequence variant on 1 allele (cytosine to thymidine, second position) encodes a mutant cTnT that contains phenylalanine at residue 179 (DNA sample from individuals V-1 and V-2). Individuals V-1, V-2, V-3, VI-6, VI-7, and VI-8 are heterozygous for Ser<sup>179</sup>Phe cTnT mutation. C, cTnT sequences (exon 11) in DNA derived from individual VI-1 demonstrate mutant (TTC) sequence on both alleles, indicating a homozygous (Ser<sup>179</sup>Phe) mutation. D, Conservation of serine 179 throughout vertebrate evolution in cardiac and skeletal (Skel.) TnT.

each defined normal cardiac structures and measurements appropriate for age and body surface area. Individual VI-4 (age 14 years) had a normal ECG. Nonspecific ECG findings were present in the 3 other children. Individual VI-6 (age 10 years) had inferior Q waves; VI-7 (age 7 years) had mild left-axis deviation, Q waves, and inverted T waves in lead III; and VI-8 (age 4 years) had inferior Q waves and a biphasic T wave in lead V<sub>1</sub>. Although these 3 children did not fulfill echocardiographic criteria for HCM,<sup>11,17,22</sup> the nonspecific ECG findings in individuals VI-6, VI-7, and VI-8 were tentatively considered indicative of a genetic predisposition for disease.

**Genetic Analysis**

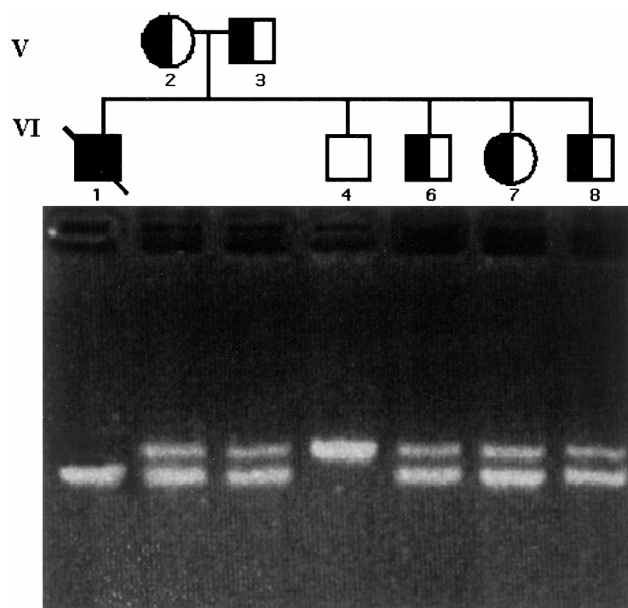
The high incidence of sudden death in the family and the mild left ventricular hypertrophy evident in the affected mother appeared consistent with clinical features of cTnT gene mutations. Therefore, a strategy of direct automated DNA sequencing (see Methods) of all protein-encoding exons of the cTnT gene was initiated without prior linkage analyses. Samples derived from 2 affected adult family members (V-1 and V-2) were initially studied. In both samples, a single heterozygous sequence variant, cytosine to thymidine, was found at nucleotide 77 in exon 11 (Figures 3A and 3B). This sequence variation is predicted to substitute a phenylalanine for serine at residue 179 (Ser<sup>179</sup>Phe). Subsequent analysis of samples from all surviving family members (V-2, V-3, VI-4, VI-6, VI-7, and VI-8) surprisingly demonstrated that both parents (V-2 and V-3) and 3 children (VI-6, VI-7, and VI-8) were heterozygous for the C→T base pair change. Only VI-4 carried the

normal sequence on both alleles (Figures 3A and 3B). The presence of this sequence variant was confirmed with allele-specific oligonucleotide hybridization (data not shown), which verified the presence of the transition in all family members except individual VI-4. This sequence variant was not found in >150 normal and unrelated alleles screened in this manner. Given the probable functional consequences of a Ser<sup>179</sup>Phe substitution in cTnT, we conclude that the C→T transition is a disease-causing mutation that caused HCM in this family.

Based on the unexpected finding that both parents and 3 of the 4 surviving children were heterozygous, we hypothesized that the 3 children who died suddenly and exhibited striking morphological evidence of HCM were homozygous for the mutant allele. The discovery of a lymph node biopsy from deceased individual VI-1 made it possible to test this conjecture. DNA was isolated from paraffin-embedded tissue, and cTnT sequences were determined. Both alleles of exon 11 exhibited the C→T transition (Figure 3C), indicating that individual VI-1 was indeed homozygous for this mutation. The finding of homozygosity was confirmed with restriction endonuclease typing (Figure 4).

**Discussion**

We report that a mutation in cTnT that, when homozygous, causes a severe form of familial HCM. A single-nucleotide change in codon 179, resulting in a serine-to-phenylalanine amino acid substitution, causes mild clinical phenotype in the heterozygous state. In contrast, homozygosity for cTnT Ser<sup>179</sup>Phe produced marked biventricular hypertrophy and juvenile lethality. This first description of a homozygous



**Figure 4.** cTnT genotypes of family members illustrated by modified restriction enzyme digestion. Pedigree symbols reflect genotype: open symbols indicate homozygote wild type; half-filled, heterozygote; filled, homozygote mutant. Modified restriction digestion (see Methods) of wild-type allele results in a 153-bp fragment (top band). Presence of thymidine at residue 77 in exon 11 results in a 131-bp fragment (bottom band). Note that both parents (V-2 and V-3) and 3 of 4 surviving children (VI-6, VI-7, and VI-8) are heterozygous for mutation. Samples from 1 deceased child (VI-1) demonstrate mutant allele on both chromosomes. Only VI-4 is genetically unaffected (wild-type allele present on both chromosomes).

cTnT mutation provides new insights into the spectrum of HCM caused by this disease gene.

Examination of the pedigree (Figure 1) revealed a minimum of 7 obligate heterozygotes (I-1 or I-2, II-1, II-3, III-1, III-4, IV-2, and IV-4) and at least 64 other individuals at risk of being a carrier of the mutation. Despite these large numbers, there was no other family history of sudden death or heart failure. Of the many relatives outside of the branch studied, only 1 premature death had occurred: a brother of V-3 died in a motor vehicle accident at age 17 years. This family history demonstrates that survival in HCM due to the heterozygous Ser<sup>179</sup>Phe mutation is far better than that previously reported with most other cTnT mutations. Previously described malignant cTnT and  $\beta$ -MHC missense mutations were associated with a change in the charge of the substituted residue.<sup>7,8</sup> Therefore, the benign clinical consequences of the heterozygous Ser<sup>179</sup>Phe mutation may reflect conservation of charge, although this substitution replaces a polar residue for a hydrophobic residue.

The data presented here support a gene dosage effect for this cTnT mutation, a phenomenon seen in other genetic disorders, such as familial hypercholesterolemia. Homozygous mutations associated with HCM have not been widely reported and have been thought to be potentially lethal. There is 1 prior description in humans of a homozygous  $\beta$ -MHC mutation<sup>23</sup> that was associated with a more severe phenotype than when heterozygous. Nematodes homozygous for the missense MHC mutation *unc54 (d)* are nonviable,<sup>24</sup> and

genetically engineered mice homozygous for the malignant  $\beta$ -MHC mutation Arg<sup>403</sup>Gln die shortly after birth.<sup>25</sup> Homozygosity for Ser<sup>179</sup>Phe caused severe morphological abnormalities at an early age, including striking left and right ventricular hypertrophy (Figure 2), as well as sudden death during childhood. Because heterozygosity is associated with a relatively benign clinical phenotype with a seemingly normal life expectancy, we presume that heterozygosity for Ser<sup>179</sup>Phe does not have a dramatic impact on protein folding and only mildly perturbs interaction with other components of the thin filament and overall sarcomere function. The severity of the homozygous phenotype suggests that this mutation disrupts an important cTnT function that can be largely complemented by the presence of 1 normal allele.

The precise functional consequences of the Ser<sup>179</sup>Phe mutation are difficult to predict. TnT links the troponin complex to tropomyosin and therefore plays a central role in the control of striated muscle contraction by regulating the interaction of thick and thin filaments in response to intracellular calcium concentration. Exon 11 is thought to reside within the putative  $\alpha$ -tropomyosin binding domain of TnT,<sup>26–28</sup> and Ser<sup>179</sup> has been highly conserved throughout vertebrate evolution (Figure 3D), suggesting that it may be a critical residue. However, the 3-dimensional structure of cTnT is not known, and detailed structure-function relationships of the thin filament components remain to be elucidated.

The clinical manifestations of disease in this family illustrate the usefulness of determining genetic causes in the management of patients with HCM. The identification of subtle ECG abnormalities in 3 surviving children was interpreted as possible evidence of the prehypertrophic phase of disease. Given the multiple sudden deaths of siblings, these findings caused serious concern in both parents and physicians about each child's risk for sudden death. Indeed, this issue prompted the implantation of an implantable cardioverter-defibrillator in the second eldest surviving child (individual VI-6). In contrast, genetic studies indicate that these heterozygous children have a vastly reduced estimate of risk. This information should not only alter the clinical management of the surviving children but also provide their parents with more realistic expectations about their future.

### Acknowledgments

This work was supported by grants from the Howard Hughes Medical Institutes and the National Institutes of Health (Drs J. and C. Seidman). We are indebted to Hideshi Niimura for invaluable technical advice and to Lars Ericsson for providing clinical information on the children.

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*Circulation*. 2000;102:1950-1955

doi: 10.1161/01.CIR.102.16.1950

*Circulation* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231

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Print ISSN: 0009-7322. Online ISSN: 1524-4539

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