

MUTATIONS IN THE GENES FOR CARDIAC TROPONIN T AND α -TROPOMYOSIN IN HYPERTROPHIC CARDIOMYOPATHY

HUGH WATKINS, M.R.C.P., WILLIAM J. MCKENNA, M.D., LUDWIG THIERFELDER, M.D.,
H. JACQUELINE SUK, B.A., RYUICHIRO ANAN, M.D., ANNIE O'DONOGHUE, R.N., PAOLO SPIRITO, M.D.,
AKIRA MATSUMORI, M.D., CHRISTINE S. MORAVEC, PH.D., J.G. SEIDMAN, PH.D.,
AND CHRISTINE E. SEIDMAN, M.D.

Abstract Background. Familial hypertrophic cardiomyopathy can be caused by mutations in the genes for β cardiac myosin heavy chain, α -tropomyosin, or cardiac troponin T. It is not known how often the disease is caused by mutations in the tropomyosin and troponin genes, and the associated clinical phenotypes have not been carefully studied.

Methods. Linkage between polymorphisms of the α -tropomyosin gene or the cardiac troponin T gene and hypertrophic cardiomyopathy was assessed in 27 families. In addition, 100 probands were screened for mutations in the α -tropomyosin gene, and 26 were screened for mutations in the cardiac troponin T gene. Life expectancy, the incidence of sudden death, and the extent of left ventricular hypertrophy were compared in patients with different mutations.

Results. Genetic analyses identified only one α -tropomyosin mutation, identical to one previously described. Five novel mutations in cardiac troponin were identified, as well as a further example of a previously described

mutation. The clinical phenotype of four troponin T mutations in seven unrelated families was similar and was characterized by a poor prognosis (life expectancy, approximately 35 years) and a high incidence of sudden death. The mean (\pm SD) maximal thickness of the left ventricular wall in subjects with cardiac troponin T mutations (16.7 ± 5.5 mm) was significantly less than that in subjects with β cardiac myosin heavy-chain mutations (23.7 ± 7.7 mm, $P < 0.001$).

Conclusions. Mutations in α -tropomyosin are a rare cause of familial hypertrophic cardiomyopathy, accounting for approximately 3 percent of cases. Mutations in cardiac troponin T account for approximately 15 percent of cases of familial hypertrophic cardiomyopathy in this referral-center population. These mutations are characterized by relatively mild and sometimes subclinical hypertrophy but a high incidence of sudden death. Genetic testing may therefore be especially important in this group. (N Engl J Med 1995;332:1058-64.)

FAMILIAL hypertrophic cardiomyopathy is clinically variable and genetically heterogeneous. The extent and pattern of ventricular hypertrophy and the prognosis for affected persons, particularly the risk of sudden death, vary markedly.^{1,2} Hypertrophic cardiomyopathy may be caused by mutations at any one of four disease loci on chromosomes 1, 11, 14, and 15³⁻⁶; the existence of further loci can be inferred from the existence of families with hypertrophic cardiomyopathy that is not linked to any of the known loci (unpublished data). The disease gene at three of these loci has been shown to encode a sarcomeric contractile protein.^{7,8} Approximately 30 percent of cases of familial hypertrophic cardiomyopathy, and some cases of sporadic hypertrophic cardiomyopathy, are caused by missense mutations in the β cardiac myosin heavy-chain gene on chromosome 14.^{9,10} More recently, disease-causing mutations have been identified in the genes for α -tropomyosin (chromosome 15) and cardiac troponin T (chromo-

some 1). Thus, mutations in components of both the thick and thin filaments of cardiac striated muscle can cause hypertrophic cardiomyopathy. Two missense mutations in α -tropomyosin and two missense mutations and one splice-site mutation in cardiac troponin T have been described, each found in one family.⁸ The proportion of familial hypertrophic cardiomyopathy attributable to mutations in these two genes is unknown.

Differences in the clinical manifestations of hypertrophic cardiomyopathy may be due in part to allelic (intragenic) or nonallelic (intergenic) heterogeneity in this condition. Different mutations within the β cardiac myosin heavy-chain gene appear to correlate with significantly different rates of survival among affected persons,¹¹⁻¹³ but in one study they were not obviously associated with differences in the morphologic features of the disease.¹⁴ Studies of hypertrophic cardiomyopathy in two families with α -tropomyosin mutations revealed a different finding: significantly less cardiac hypertrophy was associated with the mutation involving the substitution of glycine for glutamic acid at position 180 (Glu180Gly) than with the mutation involving the substitution of asparagine for aspartic acid at position 175 (Asp175Asn), although the life expectancy of patients with either mutation was similar.^{5,8} Collectively, these findings indicate that both the disease gene and the specific mutation within it can influence clinical expression.

To assess the effect of defects in thin filaments on the clinical features of hypertrophic cardiomyopathy, we analyzed the α -tropomyosin and cardiac troponin T genes in affected persons and compared the resultant disease phenotypes. These studies demonstrate that

From the Howard Hughes Medical Institute, Boston (H.W., L.T., H.J.S., R.A., J.G.S., C.E.S.); the Cardiovascular Division, Brigham and Women's Hospital, Boston (H.W., R.A., C.E.S.); the Department of Cardiological Sciences, St. George's Hospital Medical School, London (H.W., W.J.M., A.O.); the Department of Genetics, Harvard Medical School, Boston (L.T., H.J.S., J.G.S.); Franz Volhard Klinik and Max Delbrück Centrum, Berlin, Germany (L.T.); the Division of Cardiology, Ospedale Galliera, Genoa, Italy (P.S.); the Department of Internal Medicine, Kyoto University, Kyoto, Japan (A.M.); and the Center for Anesthesiology Research, Cleveland Clinic Foundation, Cleveland (C.S.M.). Address reprint requests to Dr. Watkins at the Department of Genetics, Harvard Medical School, 200 Longwood Ave., Boston, MA 02115.

Supported by grants from the British Heart Foundation (to Drs. Watkins and McKenna), Thelethon-Italy (No. 600, to Dr. Spirito), the Ministry of Health and Ministry of Education, Science, and Culture, Japan (to Dr. Matsumori), the National Institutes of Health and Howard Hughes Medical Foundation (to Drs. Seidman and Seidman), and Bristol-Myers Squibb Company. Dr. Watkins is the recipient of a British Heart Foundation Clinical Scientist Fellowship.

α -tropomyosin mutations are a rare cause of familial hypertrophic cardiomyopathy. Defects in the cardiac troponin T gene cause hypertrophic cardiomyopathy less commonly than do mutations in the β cardiac myosin heavy-chain gene but are not infrequent. These defects are also associated with subtle hypertrophic manifestations but a particularly poor prognosis.

METHODS

Clinical Evaluation

After informed consent was obtained in accordance with the guidelines of the Brigham and Women's Hospital Human Subjects Committee, blood was drawn from members of families with hypertrophic cardiomyopathy for genetic analyses. Study families were denoted by sequential letters of the alphabet. All family members underwent clinical, electrocardiographic, and echocardiographic assessments as described previously.³ Maximal thickness of the left ventricular wall was measured in diastole in the region of greatest hypertrophy. A diagnosis of hypertrophic cardiomyopathy was based on the demonstration of a maximal left-ventricular-wall thickness of at least 13 mm in the absence of other causes of ventricular hypertrophy. Subjects 16 years of age or older with a maximal left-ventricular-wall thickness below 13 mm and without electrocardiographic features of hypertrophic cardiomyopathy^{1,2} but who were later shown to carry a gene defect were classified as having a nonpenetrant mutation. Clinical or postmortem records (in some cases) and family histories were obtained to determine the number of disease-related deaths and sudden deaths (a subgroup of disease-related deaths in which unexpected cardiac arrest or abrupt circulatory collapse led to death within one hour) and the age at death or current age of all affected members in each family. The disease status of living family members was determined on the basis of genotype. Deceased family members who had an affected parent were considered to have been affected if they had transmitted the disease to their children, were shown to have hypertrophic cardiomyopathy at autopsy, or died suddenly with no other cause of death identified. Kaplan-Meier product-limit survival curves were constructed as described elsewhere,^{15,16} and compared according to the log-rank method of Peto and Peto.¹⁷ We compared survival among subjects with cardiac troponin T mutations with survival among subjects with representative β cardiac myosin heavy-chain mutations using our published data.¹¹ We used qualitative categories, as described previously,¹³ to characterize mutations associated with high and low rates of disease-related death as "malignant" and "benign," respectively. Differences for mean values of maximal left-ventricular-wall thickness were compared by Student's *t*-test. All *P* values were two-tailed.

Linkage Analyses

DNA samples from family members were genotyped for the intragenic short-tandem-repeat polymorphism HTM α_{CA} to detect linkage to the α -tropomyosin gene.⁸ The flanking marker D15S108 was used to analyze haplotypes at the α -tropomyosin locus.³ Linkage to the cardiac troponin T gene was assessed with flanking markers FIIIIB⁴ and MYBP.¹⁸ Analyses that supported linkage prompted screening for a mutation in the cardiac troponin T gene. When a sequence variant was identified in a proband, it was used to assess genetic linkage between the disease status of all family members and the cardiac troponin T gene. Family members with nonpenetrant mutations were considered to have an unknown diagnosis for linkage analyses. Lod scores were calculated with the MLINK program,¹⁹ for a recombination fraction (θ) of 0, with a penetrance of 0.95 and an allele frequency of the sequence variant of 0.01. The lod scores of families with the same mutation were combined. A lod score of more than +1.3 indicates that the odds in support of linkage are greater than 20 to 1.

Detection of Mutations

Screening for mutations was performed with previously described techniques in two members of each family in which hypertrophic car-

diomyopathy was linked to a known disease gene and in a panel of unrelated probands with familial hypertrophic cardiomyopathy. Individual exons of the striated-muscle isoform of α -tropomyosin were amplified from genomic DNA by the polymerase chain reaction and screened by cycle sequencing.⁸ The entire coding sequence of cardiac troponin T was amplified in two sections by a nested polymerase chain reaction with complementary DNA (cDNA) from transformed lymphocytes (Fig. 1).^{8,21} Cardiac troponin T cDNA sequences were screened by either cleavage of RNA-DNA hybrids at mutation sites by RNase A or by cycle sequencing.^{8,21} Mutations were denoted by the normal amino acid residue and its position,²⁰ followed by the mutant amino acid (e.g., Arg92Gln).

RESULTS

We used genetic-linkage techniques to define the disease locus responsible for hypertrophic cardiomyopathy in families with multiple affected first-degree relatives. Twenty-seven kindreds with hypertrophic cardiomyopathy in which the disease was not linked to the β cardiac myosin heavy-chain gene were identified. These families were of diverse racial and ethnic origins: 14 from Europe, 10 from North America, and 1 each from South America, Africa, and India.

Studies of linkage between the disease status and the α -tropomyosin gene (chromosome 15) were not informative in seven families (lod scores, -1.3 to +1.3). In none of the other 20 families was hypertrophic cardiomyopathy linked to the α -tropomyosin gene (lod scores, less than -1.3).

Studies of linkage between the cardiac troponin T gene (chromosome 1) and hypertrophic cardiomyopathy were uninformative in three families. Hypertrophic cardiomyopathy was not linked to the cardiac troponin T gene in 19 families but was linked to this disease locus in 5 families (Families AU, AW, BA, C, and CJ) (data not shown). Nucleotide sequences of cardiac troponin T cDNA from affected members in these five families were then determined (Fig. 1).⁸ In each, a variant of the normal sequence was identified (Table 1).

In addition to studies in these families, unrelated patients with familial hypertrophic cardiomyopathy were studied by searching directly for mutations. The coding sequence of the α -tropomyosin gene was determined in 100 probands who had not undergone prior linkage studies or genetic analyses of other genes. A sequence variant was identified in only one proband (Proband DB). This sequence variant encoded a missense mutation (Asp175Asn) previously reported to cause hypertrophic cardiomyopathy. To our knowledge, this missense mutation and one other remain the only identified mutations in this gene.⁸

Cardiac troponin T cDNA sequences were analyzed in 26 probands with familial hypertrophic cardiomyopathy who were not found to have mutations in the β cardiac myosin heavy-chain gene (data not shown). These probands were from smaller kindreds, all with more than one affected member, and were of diverse racial and ethnic origins: 16 from Europe, 4 from North America, 3 from Japan, and 1 each from China, Southeast Asia, and Pakistan. Variant nucleotide sequences were identified in six probands (Probands CK, AL, AQ, AG, DA, and WW); in each instance all avail-

able first-degree relatives were then checked for the variant.

A total of 11 different sequence changes were identified in the cardiac troponin T gene. Three appeared to be polymorphisms that do not cause hypertrophic cardiomyopathy. Two of these are silent DNA polymorphisms (a change from guanine to adenine at position 219 and a change from thymine to cytidine at position 330) that do not alter the encoded amino acid and appear frequently in the normal population (data not shown). Another sequence variant was identified in two families that altered the normal amino acid (Lys253Arg) but did not segregate with disease. The Lys253Arg mutation was also present in 2 of 100 unrelated normal subjects and therefore is assumed to be an uncommon neutral polymorphism.

Eight of the variant troponin T sequences exhibited the features expected of disease-causing mutations and thus are not silent polymorphisms (Fig. 2). Three of these sequence changes have been described previously as disease-causing mutations⁸: a G→A transition

in the 5' splice donor site of intron 15 that results in truncated cDNA transcripts in Family AU (designated intron 15 G₁→A) and missense mutations Ile79Asn and Arg92Gln present in Families AW and BA, respectively.⁸ Four novel missense mutations were identified: Phe110Ile (Fig. 1), Glu163Lys, Glu244Asp, and Arg278Cys (Table 1). Another mutation, designated ΔGlu160, is caused by the deletion of three nucleotides that correspond to an entire glutamic acid codon and therefore does not cause a frame shift. These sequence variants are believed to be disease-causing mutations

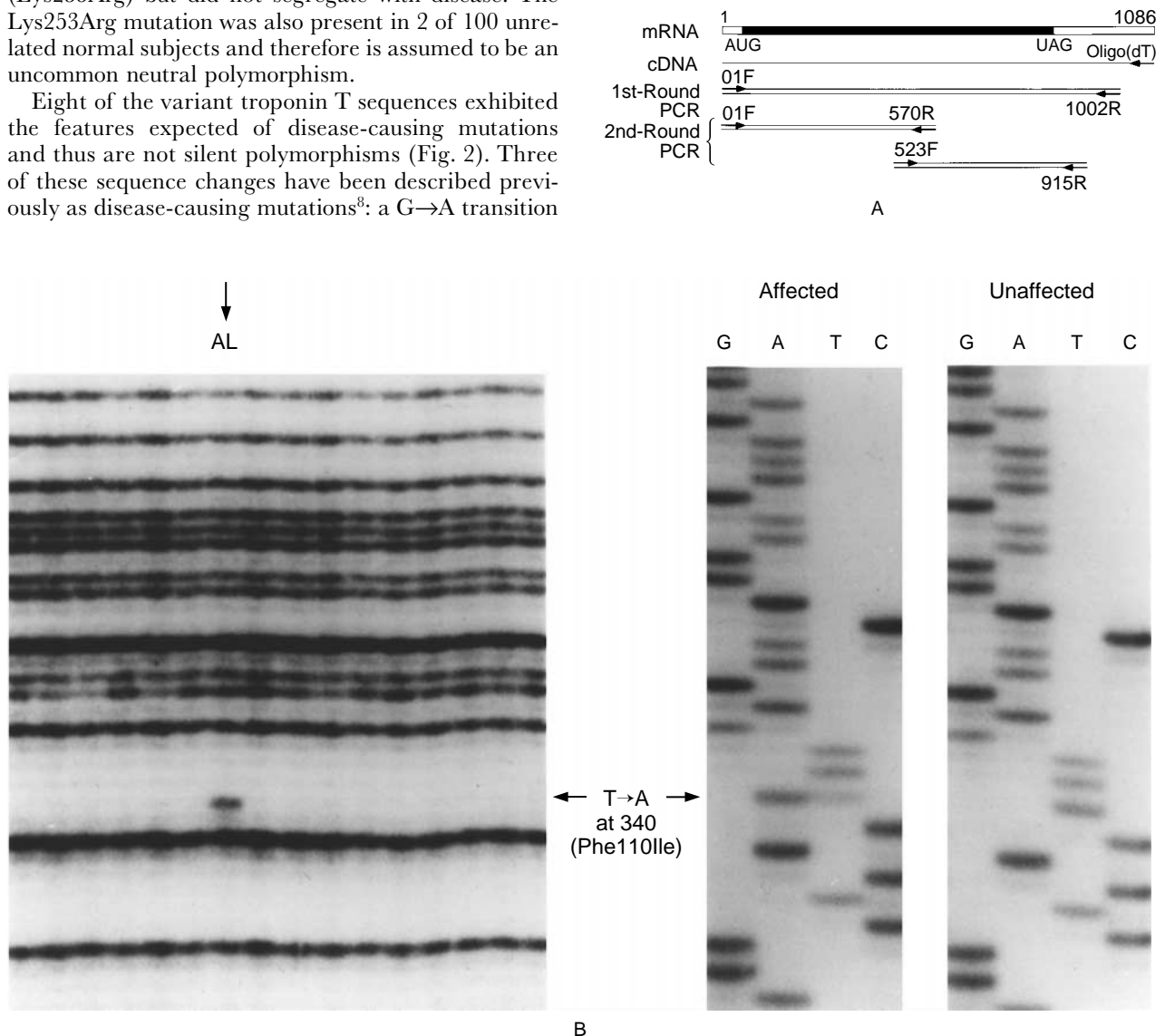


Figure 1. Amplification of Cardiac Troponin T Sequences and Detection of Mutations.

In Panel A, the cardiac troponin T messenger RNA (mRNA) — shown with the coding sequence shaded — was reverse transcribed with an oligo(dT) primer to generate a cDNA that was used as a template for a nested polymerase chain reaction (PCR). The initiation (AUG) and termination (UAG) codons are indicated. Two overlapping fragments of cDNA (1 to 570 and 523 to 915) were amplified from a dilution of a first-round product amplified by primers 01F and 1002R (arrows; the primers are numbered according to the 5' residue²⁰). F denotes forward, and R reverse. The left-hand side of Panel B shows dideoxy-ATP reactions of cycle sequencing of the cardiac troponin T cDNA fragment 1 to 570 from 16 unrelated probands. An abnormal A residue in proband AL (arrow) indicates a T→A transversion at position 340. The right-hand side of Panel B shows cycle sequencing of exon 9 genomic DNA derived from Proband AL (Affected) and a relative (Unaffected), confirming heterozygosity for the mutation.

Table 1. Mutations in the Cardiac Troponin T Gene in Families with Hypertrophic Cardiomyopathy.

	Ile79Asn	Arg92Gln	Phe110Ile	Δ Glu160	Glu163Lys	Glu244Asp	Intron 15 G ₁ →A	Arg278Cys
Nucleotide change	T→A at 248	G→A at 287	T→A at 340	Δ GAG	G→A at 499	G→T at 744	G ₁ →A	C→T at 844
Charge change*	0	-1	0	+1	+2	0	NA	-1
Conservation	+	+	+	+	+	+	+	+
Families affected	AW	BA, C, CK	AL	AQ, CJ	AG	DA	AU	WW
Lod score	2.0	8.3	0.8	7.2	1.3	0.0	11.8	0.6

*Changes in net charge of the polypeptide, based on amino acid charges at pH 7. NA denotes not applicable.

on the basis of several findings. First, each sequence variant initially identified in a proband was also present in all affected family members. Second, these variants were absent in chromosomes from more than 100 normal unrelated subjects (data not shown). Third, in all families of sufficient size, linkage was demonstrated between hypertrophic cardiomyopathy and these sequence variants (lod score, >1.3) (Table 1). Fourth, each variant affects a residue that is conserved among all known sequences of cardiac troponin T,²⁵ thereby implying that these differences have structural or functional consequences.

One mutation in α -tropomyosin and two mutations in cardiac troponin T were identified in more than one family (Table 1). To determine whether these mutations arose independently or reflected a founder effect, the haplotype associated with each gene defect was identified (Table 2). For the α -tropomyosin mutation alleles were determined at the intragenic polymorphism HTM α_{CA} and a flanking short tandem repeat; for the cardiac troponin T mutations alleles were defined at the two neutral intragenic polymorphisms described above (G→A at position 219 and T→C at position 330). Because the haplotypes were different in the families with the α -tropomyosin mutation Asp175Asn, and also in the families with the cardiac troponin T mutation Arg92Gln, we conclude that these defects arose independently in each family. The latter mutation may define a mutational hot spot²⁶ in the cardiac troponin T gene, since this C→T transition in a cytidine phosphate guanosine dinucleotide must have occurred three times. In contrast, the families with the Δ Glu160 deletion in cardiac troponin T had the same haplotype. This may reflect a founder mutation in an ancestor common to Families AQ and CJ, both of which are of English origin. However, the shared haplotype may be coincidental, since it was also identified in over 50 percent of unrelated subjects.

The clinical phenotypes associated with two mutations in the α -tropomyosin gene have been described previously.^{3,8} To assess the effect of cardiac troponin T mutations on the clinical manifestations of hypertro-

phic cardiomyopathy, life expectancy and left-ventricular-wall thickness were studied in more than 100 patients with these mutations. When available, data were combined for members of unrelated families to minimize the influence of other shared genetic and environmental factors on the expression of disease. Data on three families with the Arg92Gln mutation and two families with the Δ Glu160 mutation yielded similar results when analyzed independently. Sufficient numbers of genetically affected patients were available to allow product-limit survival analysis for four mutations (Fig. 3). The life expectancy of patients with the Ile79Asn, Arg92Gln, Δ Glu160, or intron 15 G₁→A mutation was comparable to that of patients with hypertrophic cardiomyopathy caused by malignant myosin mutation Arg403Gln, Arg453Gln, or Arg719Trp.¹¹⁻¹³ Survival was significantly shorter than that associated with the benign myosin mutations Val606Met, Phe513Cys, and Leu908Val ($P < 0.03$ for the comparison of each troponin T mutation with the Val606Met mutation¹¹ and $P = 0.006$ for the comparison with all troponin T mutations).

Survival analyses also showed that as compared with patients with β cardiac myosin heavy-chain mutations, patients with cardiac troponin T mutations had a higher incidence of death before the age of 30 years (Fig. 3), and a significantly higher proportion of these deaths

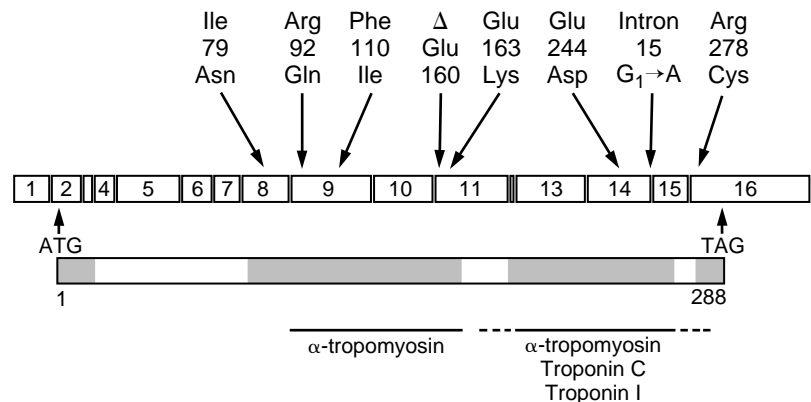


Figure 2. Eight Mutations in Cardiac Troponin T that Cause Familial Hypertrophic Cardiomyopathy.

The schematic illustration of the cardiac troponin T gene is based on the rat genomic structure²² and our unpublished data. Exons are indicated by boxes; the location of each mutation is shown. The initiation (ATG) and termination (TAG) codons are indicated. The peptide is represented below; shaded areas indicate high levels of conservation between cardiac and skeletal isoforms.²³ The postulated binding sites are indicated for α -tropomyosin, troponin C, and troponin I.^{23,24}

were sudden ($P=0.012$). Of 50 disease-related deaths in patients with cardiac troponin T mutations, 39 were deemed sudden deaths and 11 were attributed to other cardiac causes, mainly congestive heart failure. There were no significant differences in the proportion of sudden deaths among patients with different cardiac troponin T mutations. Similar analyses of 75 disease-related deaths in patients with β cardiac myosin heavy-chain mutations^{11,13} showed that 42 were deemed sudden deaths.

Each cardiac troponin T mutation produced a similar increase in the maximal thickness of the left ventricular wall (Table 3). Earlier studies of the maximal left-ventricular-wall thicknesses associated with six different mutations in the β cardiac myosin heavy-chain gene demonstrated that left-ventricular-wall thickness could not be used to distinguish the mutations.¹⁴ However, the degree of hypertrophy produced by cardiac troponin T mutations (mean maximal left-ventricular-wall thickness, 16.7 ± 5.5 mm) was significantly less ($P<0.001$) than that produced by β cardiac myosin heavy-chain mutations (mean maximal left-ventricular-wall thickness, 23.7 ± 7.7 mm).¹⁴

Clinical evaluations of family members identified several apparently unaffected members in whom genetic testing demonstrated a cardiac troponin T mutation. Of the 67 surviving subjects over the age of 16 years who had cardiac troponin T mutations, 16 (24 percent) did not fulfill our clinical diagnostic criteria for hypertrophic cardiomyopathy (Table 3). Eleven of the 16 had normal electrocardiograms and echocardiograms, and 5 had nondiagnostic abnormalities (with a maximal left-ventricular-wall thickness of less than 13 mm). Therefore, the penetrance of hypertrophic cardiomyopathy caused by cardiac troponin T mutations is estimated to be 75 percent, in contrast to the high penetrance (approximately 95 percent) of mutations in the β cardiac myosin heavy-chain gene with a comparable malignant phenotype.¹¹⁻¹³

DISCUSSION

We describe five novel mutations in the cardiac troponin T gene that cause hypertrophic cardiomyopathy. These mutations, and three previously described mutations, characteristically produce moderate hypertrophy

Table 2. Disease-Gene Haplotypes in Families with Identical Mutations Causing Hypertrophic Cardiomyopathy.

MUTATION AND FAMILY	HAPLOTYPE	
	G219A	T330C
Arg92Gln in troponin T gene		
BA	A	T
C	G	C
CK	G	T
Δ Glu160 in troponin T gene		
AQ	G	T
CJ	G	T
	HTM α_{ca}	D15S108
Asp175Asn in α -tropomyosin gene		
MI	3	1
DB	2	3

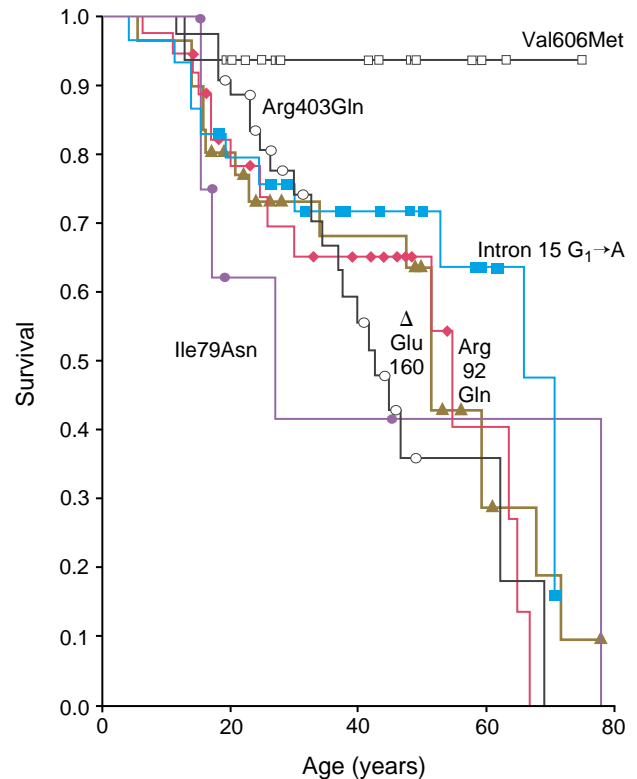


Figure 3. Kaplan-Meier Product-Limit Curves for Survival in Patients with Hypertrophic Cardiomyopathy Caused by Different Mutations.

Survival data are plotted for all persons carrying a given mutation. The survival of persons with hypertrophic cardiomyopathy caused by cardiac troponin T mutations (Intron 15 $G_1\rightarrow A$, Ile79Asn, Δ Glu160, and Arg92Gln) is similar to that in persons with a malignant β cardiac myosin heavy-chain mutation (Arg403Gln)¹¹ but significantly shorter than that in persons with a benign myosin mutation (Val606Met)¹¹ ($P<0.03$ for the comparison with each troponin T mutation and $P=0.006$ for the comparison with all troponin T mutations).

that in some instances appears clinically unimportant. Despite this, the hypertrophic cardiomyopathy caused by these mutations is associated with a poor prognosis and a high incidence of sudden death.

These studies allow an estimate of the proportion of familial hypertrophic cardiomyopathy attributable to mutations in three disease genes. Of 70 families with hypertrophic cardiomyopathy, 20 were previously shown to have a mutation in the β cardiac myosin heavy-chain gene^{11,13} (and unpublished data); 11 of the remaining 50 have a mutation in the cardiac troponin T gene. Independent analyses of the α -tropomyosin gene in more than 120 probands and families identified a mutation in only 3 (Proband DB and Families MI and MZ).⁸ Collectively, these data suggest that approximately 30 percent of cases of familial hypertrophic cardiomyopathy are caused by mutations in the β cardiac myosin heavy-chain gene; 15 percent are caused by mutations in the cardiac troponin T gene; and less than 3 percent are caused by mutations in the α -tropomyosin gene. These are conservative estimates because the data are largely based on direct screening methods, which may miss a

Table 3. Clinical Features of Hypertrophic Cardiomyopathy in Subjects with Cardiac Troponin T Mutations.

	Ile79Asn	Arg92Gln	Phe110Ile	ΔGlu160	Glu163Lys	Glu244Asp	Intron 15 G ₁ →A	Arg278Cys
No. with mutation*	9	32	2	32	5	1	28	3
No. of disease-related deaths	5	15	0	17	0	0	12	1
No. of sudden deaths	4	11	0	14	0	0	9	1
No. studied clinically†	4	21	2	14	5	1	17	3
Maximal left-ventricular-wall thickness (mm)‡	13.4±4	15.0±6	17	17.5±5	19.8±8	—	17.5±5	16.3±6
Subjects ≥16 yr old with non-penetrant mutation	3	7	0	1	1	0	3	1

*The mutation was identified by screening or deduced on the basis of a family member's position in the pedigree or cause of death.

†Includes some deceased subjects.

‡Values are means ±SD.

small proportion of mutations (although previous studies document the high sensitivity of these methods).²⁷ Despite this, we conclude that a substantial proportion, up to 50 percent, of cases of familial hypertrophic cardiomyopathy are due to mutations in as yet unidentified disease genes. We speculate that these disease genes will encode other proteins involved in the structure or function of cardiac sarcomeres.⁸

The similarity between the clinical phenotypes found with four cardiac troponin T mutations is surprising, given the diversity of these gene defects. The splice-donor-site mutation (intron 15 G₁→A), which should result in a truncated protein⁸; the mutation involving a deleted codon (ΔGlu160) within a highly conserved motif; and two missense mutations would each be expected to exert different influences on the structure of cardiac troponin T. Perhaps the phenotype is similar because the mutant peptides result in common functional defects. Alternatively, the uniform consequences of these mutations may reflect a common intracellular mechanism by which dysfunctional thin filaments trigger cellular hypertrophy. Characterization of additional mutations should elucidate whether the phenotype described here is typical of all defects in the cardiac troponin T gene.

The mutations in cardiac troponin T reflect a dissociation between the severity of clinically demonstrable cardiac hypertrophy and the risk of sudden or disease-related death. Two cases are particularly illustrative: a 16-year-old boy with the Ile79Asn mutation who had normal findings on clinical evaluation nevertheless died suddenly, and a girl with the Arg278Cys mutation and normal ventricular-wall measurements was resuscitated after a cardiac arrest at the age of 17. Incomplete penetrance of hypertrophic cardiomyopathy has been reported with β cardiac myosin heavy-chain mutations that are associated with a good prognosis (for example, Leu908Val¹²). However, clinical data on more than 80 patients with the malignant mutation Arg403Gln, Arg453Gln, or Arg719Trp^{11-13,28} demonstrated obvious cardiac hypertrophy in all adults with these genotypes. We suggest that incomplete disease penetrance and poor prognosis may be a distinguishing feature of hypertrophic cardiomyopathy due to cardiac troponin T mutations. The substantial management issues posed by a difficult clinical diagnosis associated with a high risk of sudden death make genetic testing

for cardiac troponin T mutations particularly important. As genetic testing for the mutations becomes available, our ability to care for patients with hypertrophic cardiomyopathy should improve.

We are indebted to the participating families and their physicians, without whose invaluable assistance these studies would not have been possible; to Drs. Iain Findlay, Yutaka Furukawa, Michael Frenneaux, Huon Grey, Roger Lamas, Koh Ono, Masaki Kawanami, and Scott Solomon and to LifeBanc of Northeast Ohio for providing clinical data; and to Mohammed Miri for technical help.

REFERENCES

1. Maron BJ, Bonow RO, Cannon RO III, Leon MB, Epstein SE. Hypertrophic cardiomyopathy: interrelations of clinical manifestations, pathophysiology, and therapy. *N Engl J Med* 1987;316:780-9, 844-52.
2. McKenna WJ. Hypertrophic cardiomyopathy. In: Julian DG, Camm AJ, Fox KM, Hall RJC, Poole-Wilson PA, eds. *Diseases of the heart*. London: Bailliere Tindall, 1989:933-50.
3. Jarcho JA, McKenna W, Pare JAP, et al. Mapping of a gene for familial hypertrophic cardiomyopathy to chromosome 14q1. *N Engl J Med* 1989;321:1372-8.
4. Watkins H, MacRae C, Thierfelder L, et al. A disease locus for familial hypertrophic cardiomyopathy maps to chromosome 1q3. *Nat Genet* 1993;3:333-7.
5. Thierfelder L, MacRae C, Watkins H, et al. A familial hypertrophic cardiomyopathy locus maps to chromosome 15q2. *Proc Natl Acad Sci U S A* 1993;90:6270-4.
6. Carrier L, Hengstenberg C, Beckmann JS, et al. Mapping of a novel gene for familial hypertrophic cardiomyopathy to chromosome 11. *Nat Genet* 1993;4:311-3.
7. Geisterfer-Lowrance AA, Kass S, Tanigawa G, et al. A molecular basis for familial hypertrophic cardiomyopathy: a beta cardiac myosin heavy chain gene missense mutation. *Cell* 1990;62:999-1006.
8. Thierfelder L, Watkins H, MacRae C, et al. Alpha-tropomyosin and cardiac troponin T mutations cause familial hypertrophic cardiomyopathy: a disease of the sarcomere. *Cell* 1994;77:701-12.
9. McKenna WJ, Watkins HC. Hypertrophic cardiomyopathy. In: Scriver CR, Beaudet AL, Sly WS, Valle D, eds. *The metabolic and molecular bases of inherited disease*. 7th ed. Vol. 3. New York: McGraw-Hill, 1995:4253-72.
10. Hengstenberg C, Schwartz K. Molecular genetics of familial hypertrophic cardiomyopathy. *J Mol Cell Cardiol* 1994;26:3-10. [Errata, *J Mol Cell Cardiol* 1994;26:276 and 1994;26:277.]
11. Watkins H, Rosenzweig A, Hwang D-S, et al. Characteristics and prognostic implications of myosin missense mutations in familial hypertrophic cardiomyopathy. *N Engl J Med* 1992;326:1108-14.
12. Epstein ND, Cohn GM, Cyran F, Fananapazir L. Differences in clinical expression of hypertrophic cardiomyopathy associated with two distinct mutations in the beta-myosin heavy chain gene: a 908Leu→Val mutation and a 403Arg→Gln mutation. *Circulation* 1992;86:345-52.
13. Anan R, Greve G, Thierfelder L, et al. Prognostic implications of novel beta cardiac myosin heavy chain gene mutations that cause familial hypertrophic cardiomyopathy. *J Clin Invest* 1994;93:280-5.
14. Solomon S, Wolff S, Watkins H, et al. Left ventricular hypertrophy and morphology in familial hypertrophic cardiomyopathy associated with mutations of the beta-myosin heavy chain gene. *J Am Coll Cardiol* 1993;22:498-505.
15. Kaplan EL, Meier P. Nonparametric estimation from incomplete observations. *J Am Stat Assoc* 1958;53:457-81.
16. Lee ET. *Statistical methods for survival data analysis*. Belmont, Calif.: Lifetime Learning, 1980.
17. Cox DR, Oakes D. *Analysis of survival data*. London: Chapman and Hall, 1984.

18. Watkins H, MacRae C, Fischman D, Seidman JG, Seidman CE. A dinucleotide repeat polymorphism in the MYBPH gene. *Hum Mol Genet* 1994;3:2267.
19. Lathrop GM, Lalouel JM, Julier C, Ott J. Strategies for multilocus linkage analysis in humans. *Proc Natl Acad Sci U S A* 1984;81:3443-6.
20. Mesnard L, Samson F, Espinasse I, Durand J, Neveux JY, Mercadier JJ. Molecular cloning and developmental expression of human cardiac troponin T. *FEBS Lett* 1993;328:139-44.
21. Watkins H. Searching candidate genes for mutations: amplification of sequences from affected individuals: cleavage of RNA-DNA hybrids at mutation sites using RNase A. In: Dracopoli N, Haines J, Korf B, et al., eds. *Current protocols in human genetics*. New York: John Wiley, 1994:7.1.1-7, 7.2.1-5.
22. Jin JP, Huang QQ, Yeh HI, Lin JJ. Complete nucleotide sequence and structural organization of rat cardiac troponin T gene: a single gene generates embryonic and adult isoforms via developmentally regulated alternative splicing. *J Mol Biol* 1992;227:1269-76.
23. Pearlstone JR, Carpenter MR, Smillie LB. Amino acid sequence of rabbit cardiac troponin T. *J Biol Chem* 1986;261:16795-810.
24. Zot AS, Potter JD. Structural aspects of troponin-tropomyosin regulation of skeletal muscle contraction. *Annu Rev Biophys Biophys Chem* 1987;16:535-59.
25. Townsend PJ, Farza H, MacGeoch C, et al. Human cardiac troponin T: identification of fetal isoforms and assignment of the TNNT2 locus to chromosome 1q. *Genomics* 1994;21:311-6.
26. Cooper DN, Youssofian H. The CpG dinucleotide and human genetic disease. *Hum Genet* 1988;78:151-5.
27. MacRae CA, Watkins HC, Jarcho JA, et al. An evaluation of ribonuclease protection assays for the detection of beta-cardiac myosin heavy chain gene mutations. *Circulation* 1994;89:33-5.
28. Perryman MB, Yu QT, Marian AJ, et al. Expression of a missense mutation in the messenger RNA for beta-myosin heavy chain in myocardial tissue in hypertrophic cardiomyopathy. *J Clin Invest* 1992;90:271-7.