

MUTATIONS IN THE GENE FOR CARDIAC MYOSIN-BINDING PROTEIN C AND LATE-ONSET FAMILIAL HYPERTROPHIC CARDIOMYOPATHY

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ABSTRACT

Background Mutations in the gene for cardiac myosin-binding protein C account for approximately 15 percent of cases of familial hypertrophic cardiomyopathy. The spectrum of disease-causing mutations and the associated clinical features of these gene defects are unknown.

Methods DNA sequences encoding cardiac myosin-binding protein C were determined in unrelated patients with familial hypertrophic cardiomyopathy. Mutations were found in 16 probands, who had 574 family members at risk of inheriting these defects. The genotypes of these family members were determined, and the clinical status of 212 family members with mutations in the gene for cardiac myosin-binding protein C was assessed.

Results Twelve novel mutations were identified in probands from 16 families. Four were missense mutations; eight defects (insertions, deletions, and splice mutations) were predicted to truncate cardiac myosin-binding protein C. The clinical expression of either missense or truncation mutations was similar to that observed for other genetic causes of hypertrophic cardiomyopathy, but the age at onset of the disease differed markedly. Only 58 percent of adults under the age of 50 years who had a mutation in the cardiac myosin-binding protein C gene (68 of 117 patients) had cardiac hypertrophy; disease penetrance remained incomplete through the age of 60 years. Survival was generally better than that observed among patients with hypertrophic cardiomyopathy caused by other mutations in the genes for sarcomere proteins. Most deaths due to cardiac causes in these families occurred suddenly.

Conclusions The clinical expression of mutations in the gene for cardiac myosin-binding protein C is often delayed until middle age or old age. Delayed expression of cardiac hypertrophy and a favorable clinical course may hinder recognition of the heritable nature of mutations in the cardiac myosin-binding protein C gene. Clinical screening in adult life may be warranted for members of families characterized by hypertrophic cardiomyopathy. (N Engl J Med 1998;338:1248-57.)

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HYPERTROPHIC cardiomyopathy, a disorder occurring in approximately 1 of every 500 people, causes a broad spectrum of pathological findings and clinical manifestations.^{1,2} Early observations^{2,3} emphasized the morphologic features of this disease (e.g., marked septal hypertrophy and subaortic obstruction) and its unfavorable natural history (e.g., progressive symptoms, serious arrhythmias, heart failure, and sudden death). Today the anatomical and clinical expression of the disease is recognized to encompass a wider range of phenotypes, including mild or focal hypertrophy, limited symptoms, and a good prognosis.

Molecular genetic studies of familial hypertrophic cardiomyopathy have demonstrated that this autosomal dominant condition is caused by mutations in the genes encoding sarcomere proteins.⁴⁻⁷ More than 100 different disease-causing mutations have been identified in components of the thick filaments (e.g., cardiac β -myosin heavy chain and ventricular essential and regulatory myosin light chains), com-

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ponents of the thin filaments (cardiac troponin T, troponin I, and α -tropomyosin), and cardiac myosin-binding protein C. Analyses of the clinical consequences of these distinct genetic defects have focused largely on particularly severe manifestations of disease: the early onset of marked hypertrophy (associated with defects in the gene for cardiac β -myosin heavy chain) and poor survival (associated with some mutations, termed "malignant," in the gene for cardiac β -myosin heavy chain and most mutations in the gene for cardiac troponin T⁸⁻¹¹).

Genetic linkage analyses indicate that 15 percent of cases of hypertrophic cardiomyopathy are due to a mutation on chromosome 11p11.2, where the cardiac myosin-binding protein C gene is encoded.^{6,7,12} By binding to myosin heavy chain and the cytoskeleton protein titin, cardiac myosin-binding protein C contributes to the structural integrity of the sarcomere¹³⁻¹⁶; it may also regulate cardiac contractility in response to adrenergic stimulation.¹⁷

The 1274 amino acid residues in human cardiac myosin-binding protein C are encoded by 24,000 base pairs, organized into at least 37 exons.¹⁸ This structure has hindered comprehensive screening for disease-causing mutations; hence, the clinical consequences of such defects are largely unknown. The recent definition of the gene structure by us (unpublished data) and others¹⁹ has permitted the use of automated DNA sequencing as a direct approach to the identification of mutations that cause hypertrophic cardiomyopathy. In this article we report 12 novel mutations in the gene for cardiac myosin-binding protein C that have caused hypertrophic cardiomyopathy in 16 families.

These studies indicate that patients with cardiac myosin-binding protein C gene mutations have a unique and favorable clinical profile, characterized by late-onset hypertrophic cardiomyopathy and a good prognosis. We suggest that the development of cardiac hypertrophy in middle-aged or elderly persons may indicate the presence of an inherited defect in the gene for cardiac myosin-binding protein C.

METHODS

Clinical Evaluation

Informed consent was obtained from all participants in accordance with the requirements of the human-research committees of participating centers (Brigham and Women's Hospital, Boston; Baylor College of Medicine, Houston; Toronto General Hospital, Toronto; the University of Manitoba, Winnipeg; St. George's Hospital Medical School, London; and the Minneapolis Heart Institute Foundation, Minneapolis). Probands had been given a diagnosis of familial hypertrophic cardiomyopathy (left-ventricular-wall thickness, >13 mm in the absence of a confounding diagnosis), as described previously,^{8,11} without prior knowledge of their genotype. Family members found to have a cardiac myosin-binding protein C mutation were also evaluated clinically. Mutations in adults 20 years of age or older who had a maximal left-ventricular-wall thickness of less than 13 mm or in children or adolescents (age, <20 years) with normal ventricular-wall thick-

ness for body-surface area were considered nonpenetrant. The results in patients with cardiac hypertrophy and confounding diagnoses (such as systemic hypertension) were considered indeterminate. Deaths of patients who participated in genetic studies and deaths of obligate carriers were classified as noncardiac, as related to the disease, or as sudden, as described previously.⁸ Study families were denoted by sequential letters of the alphabet or numbers.

Analyses of Disease Penetrance and Survival

Disease penetrance (with disease defined by a ventricular-wall thickness greater than 13 mm) in patients with mutations in the cardiac myosin-binding protein C gene was compared with disease penetrance in patients with representative mutations in the genes for β -myosin heavy chain^{11,20} and cardiac troponin T⁸ at different ages. Differences between the mean values for maximal left-ventricular-wall thickness were compared, and two-tailed P values were calculated with Student's t-test with use of Statview software (Abacus Concepts, Berkeley, Calif.).

Kaplan-Meier product-limit survival curves were constructed and compared according to the log-rank method, as described previously.^{8,21,22}

Genetic Studies

Two genomic clones containing the 5' or 3' portions of cardiac myosin-binding protein C (data not shown) were isolated from a human genomic P1 library with use of primers designed from complementary DNA (cDNA)¹⁸ and sequences from the European Molecular Biology Laboratory data bank (accession no. X84075), as previously described.²³

Three *EcoRI*-*Bam*HI fragments and one *Bam*HI fragment were subcloned from P1 DNA. The nucleotide sequences of these subclones were determined with the Sequenase version 2.0 DNA-sequencing kit (United States Biochemical, Cleveland), an ABI PRISM dye-terminator cycle-sequencing kit (Perkin-Elmer, Foster City, Calif.), or both. Intron-exon boundaries were ascertained by comparison of genomic and cDNA sequences.¹⁸ The entire sequence of cardiac myosin-binding protein C was entered into the GenBank data base (accession no. U91629).

Identification of Mutations in the Cardiac Myosin-Binding Protein C Gene

We obtained 5 to 30 ml of peripheral blood from each proband; DNA was isolated as previously described.¹⁰ Exons 2 through 35, which encode protein sequences, were amplified from genomic DNA with use of primers designed from intron sequences (available on the Internet at <http://genetics.med.harvard.edu/~seidman>). Genomic DNA fragments amplified with the polymerase chain reaction (PCR) were purified with the QIAquick PCR Purification kit (QIAGEN, Santa Clarita, Calif.) to remove the residual primers and sequenced with the ABI PRISM dye-terminator cycle-sequencing kit (Perkin-Elmer).

Confirmation of Mutations and Family Genotyping

Variants in cardiac myosin-binding protein C sequences were independently confirmed in DNA samples from probands by restriction-enzyme digestion, oligonucleotide-specific hybridization, or the amplification refractory mutation system. The same method was then used to determine the genotype (i.e., the presence or absence of the sequence variant) in DNA from family members of the probands and in normal controls (with use of primers and oligonucleotides shown at <http://www.genetics.med.harvard.edu/~seidman>).

Restriction-Enzyme Digestion

Seven sequence variants (Tables 1 and 2) predicted to alter a restriction-enzyme site were confirmed by PCR amplification of exons, digestion, and size fractionation on a 3 percent NuSieve-1 percent agarose gel. Mutations Glu258Lys (in Family BM) and Int24DSG+1T (Family BO) abolish *Hph*I sites. Mutations

TABLE 1. MISSENSE MUTATIONS IN CARDIAC MYOSIN-BINDING PROTEIN C THAT CAUSE FAMILIAL HYPERTROPHIC CARDIOMYOPATHY.

MUTATION	FAMILY	NUCLEOTIDE CHANGE	NO. OF PERSONS WITH MUTATION*	PHENOTYPE†		NO. OF DISEASE-RELATED DEATHS‡
				AGE <20 YR	AGE ≥20 YR	
Glu258Lys	BM	G→A at 804	7	1/2	4/4	1
Glu451Gln	D	G→C at 1383	20	0/0	10/16	4
Arg495Gln	DQ	G→A at 1516	6	1/1	2/3	0
Arg502Gln	BD, XX	G→A at 1537	16	0/4	9/11	1

*The mutation was identified by genetic screening or deduced on the basis of the genotype of offspring or the phenotype.

†For each mutation, the values shown are the numbers of persons with cardiac hypertrophy divided by the numbers of persons who could be clinically assessed and who had the mutation.

‡The values shown are the numbers of disease-related deaths in persons with the mutation.

TABLE 2. TRUNCATION MUTATIONS IN CARDIAC MYOSIN-BINDING PROTEIN C THAT CAUSE FAMILIAL HYPERTROPHIC CARDIOMYOPATHY.*

MUTATION	FAMILY	LOCATION OF MUTATION	NO. OF PERSONS WITH MUTATION†	PHENOTYPE‡		NO. OF DISEASE-RELATED DEATHS§
				AGE <20 YR	AGE ≥20 YR	
Int8DSG+1A	DP	Intron 8, donor site, G→A res +1	9	1/2	4/4	0
Int12ASA-2G	I, R	Intron 12, acceptor site, A→G res -2	26	0/4	11/16	5
DelC698	BK	C deletion in codon 698, nt 2125	11	0/1	4/7	2
Int24DSG+1T	BO	Intron 24, donor site, G→T res +1	7	0/1	5/6	1
InsG791	153, BL, BW	Insertion G in codon 791, nt 2405	115	1/19	44/72	10
DelCT955	262	CT deletion in codon 955, nt 2896	18	2/4	6/8	3
InsAA1042	DS	Insertion AA in codon 1042, nt 3156	15	1/2	5/7	1
Int33DSG+1A	CT	Intron 33, donor site, G→A res +1	25	1/6	9/12	3

*The location of the nucleotide (nt) change in the cardiac myosin-binding protein C intron or cDNA is shown; res denotes residue, DS donor splice site, AS acceptor splice site, Int intron, Ins insertion, and Del deletion. Positive numbers indicate residues following exons; negative numbers indicate residues preceding exons.

†The mutation was identified by genetic screening or deduced on the basis of the genotype of offspring or the phenotype.

‡For each mutation, the values shown are the numbers of persons with cardiac hypertrophy divided by the numbers of persons who could be clinically assessed and who had the mutation.

§The values shown are the numbers of disease-related deaths in persons with this mutation.

tions Int8DSG+1A (Family DP), Arg495Gln (Family DQ), Arg502Gln (Family BD and Family XX), and Int33DSG+1A (Family CT) abolish a *Bsa*AI, *Sma*I, *Hpa*II, and *Syl*I site, respectively. Mutation Glu451Gln (Family D) creates an *Mae*II site.

Oligonucleotide-Specific Hybridization

Four sequence variants (Int12ASA-2G in Families I and R; DelC698 in Family BK; InsG791 in Families 153, BL, and BW; and DelCT955 in Family 262) (Tables 1 and 2) were confirmed by PCR amplification of exons and oligonucleotide-specific hybridization as described elsewhere.²⁴ Amplified products were transferred to two nylon membranes (GeneScreen Plus, NEN Life Science Products, Boston) and hybridized separately with

³²P-labeled wild-type or mutant oligonucleotides. The hybridized nylon membranes were washed in 6× saline sodium citrate (SSC) (1× SSC is 0.15 M sodium chloride and 0.015 M sodium citrate) and 0.05 percent sodium pyrophosphate for 60 minutes at 48°C (or 30 minutes at 61°C, in the case of mutation DelC698), and the hybridization signal was quantified with a PhosphorImager (Molecular Dynamics, Sunnyvale, Calif.).

Amplification Refractory Mutation System

The mutation InsAA1042 (Table 2) was independently confirmed with the amplification refractory mutation system.^{25,26} Amplified products were identified on a 3 percent NuSieve-1 percent agarose gel.

Analyses of Cardiac Myosin-Binding Protein C in Lymphocyte RNA

Cardiac myosin-binding protein C sequences were amplified from lymphocyte RNA with two rounds of PCR amplification, as described previously.²⁷ Normal and mutant products were size-fractionated on a 3 percent NuSieve-1 percent agarose gel, purified, and sequenced.

RESULTS

Genetic Studies

DNA samples from 29 unrelated patients with familial hypertrophic cardiomyopathy were studied. Previous screening of the β -myosin heavy-chain and cardiac troponin T genes had failed to identify a disease-causing mutation in 17 of these samples (unpublished data). Linkage studies had indicated that hypertrophic cardiomyopathy was due to a mutation of chromosome 11 in Families 153 and D (maximal lod scores of 7.12 and 2.1, respectively).

Protein-encoding sequences of the gene for cardiac myosin-binding protein C were determined in DNA samples from 29 probands. Twelve sequence

variants (Fig. 1) were identified in 16 samples; three were shared by probands from different families. Each sequence variant was independently confirmed by restriction-enzyme analyses, by oligonucleotide-specific hybridization, or with the amplification refractory mutation system. These techniques were then used to determine the genotypes of family members (Fig. 2). Because each of these sequence variants was found in clinically affected patients but was absent in more than 200 chromosomes from normal controls (data not shown) and was predicted to alter the encoded protein, all were considered mutations that caused familial hypertrophic cardiomyopathy.

Four point mutations (Glu258Lys, Glu451Gln, Arg495Gln, and Arg502Gln; Table 1), identified in five families, were predicted to be missense mutations and to alter one amino acid in cardiac myosin-binding protein C. These four mutations are clustered in a 244-amino-acid segment that spans the phosphorylation domain of the molecule. The prox-

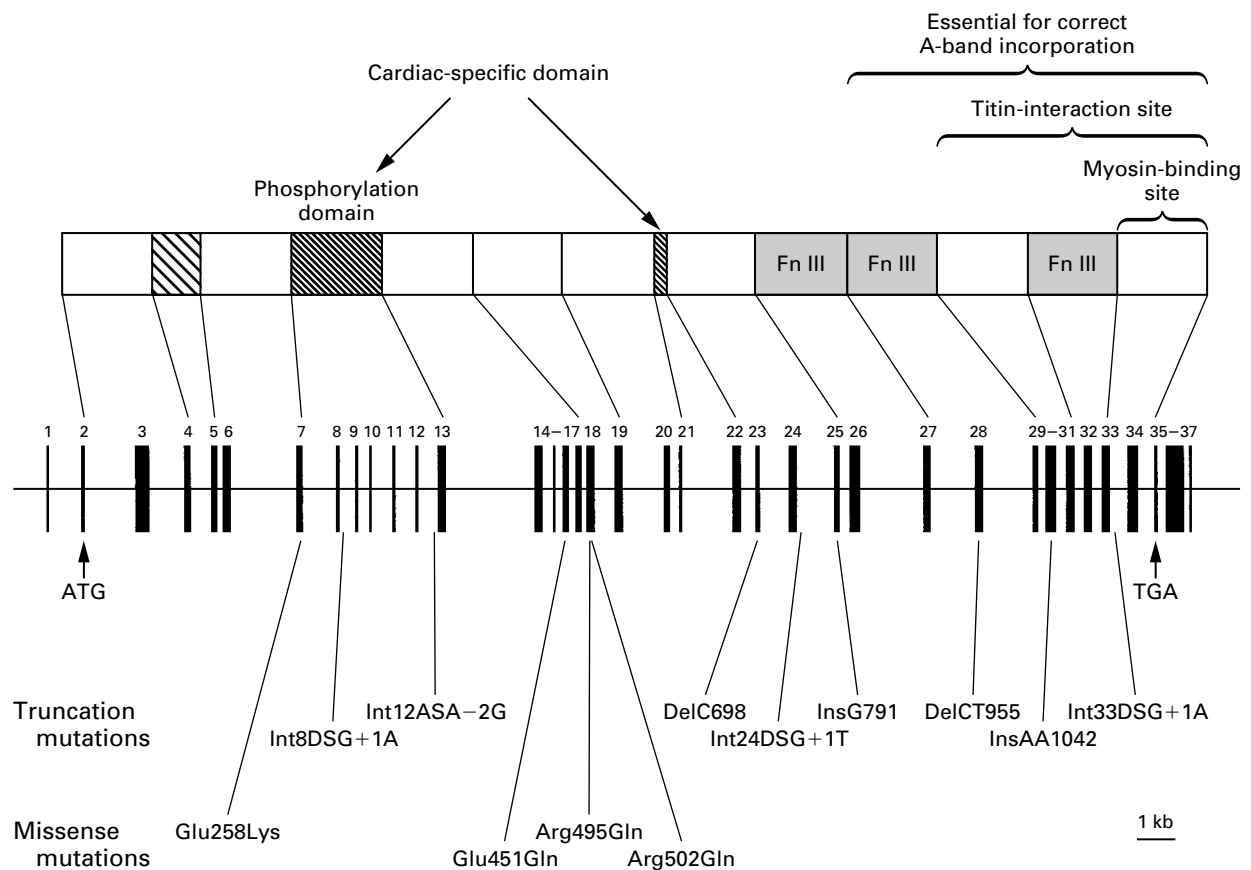
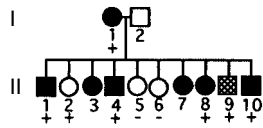


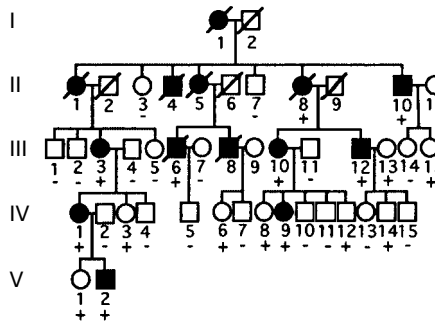
Figure 1. The Human Cardiac Myosin-Binding Protein C Polypeptide and Gene and 12 Mutations Causing Familial Hypertrophic Cardiomyopathy.

FN denotes fibronectin-like motif, Int intron, DS donor splice site, AS acceptor splice site A, Del deletion, and Ins insertion. Positive numbers indicate residues following exons; negative numbers indicate residues preceding exons.

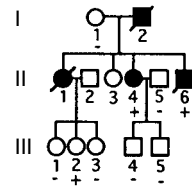
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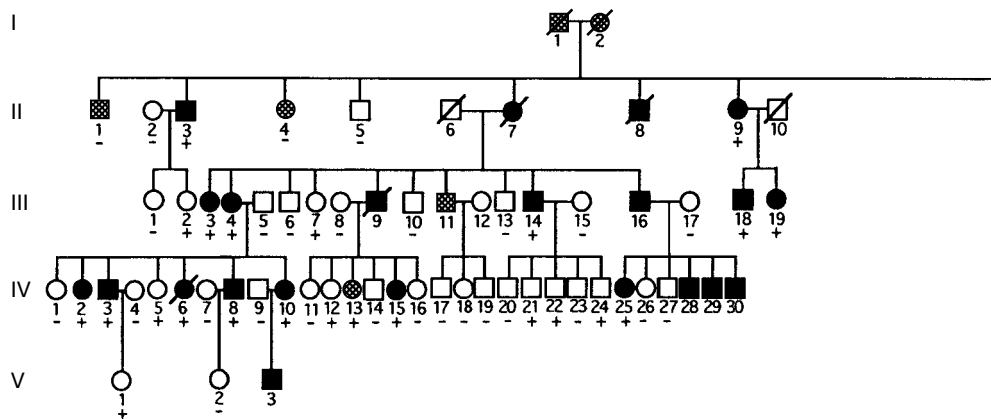
Family I



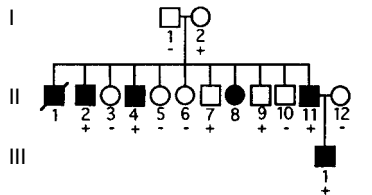
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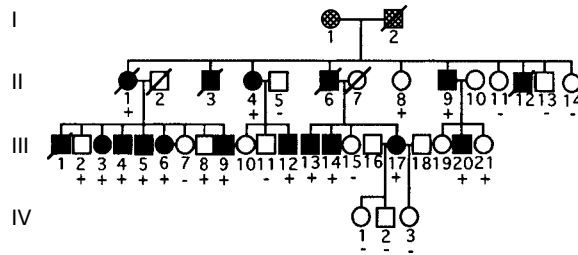
Family 153



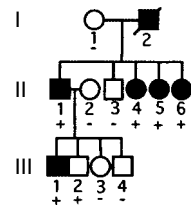
Family BL



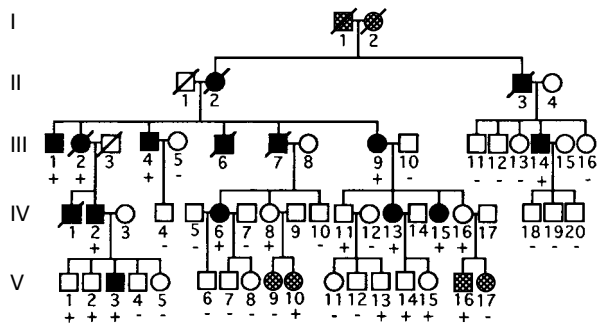
Family BW



Family BM



Family CT



Family D

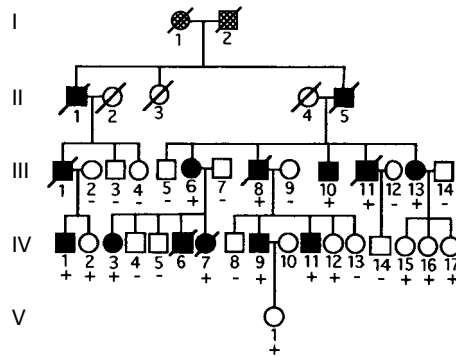
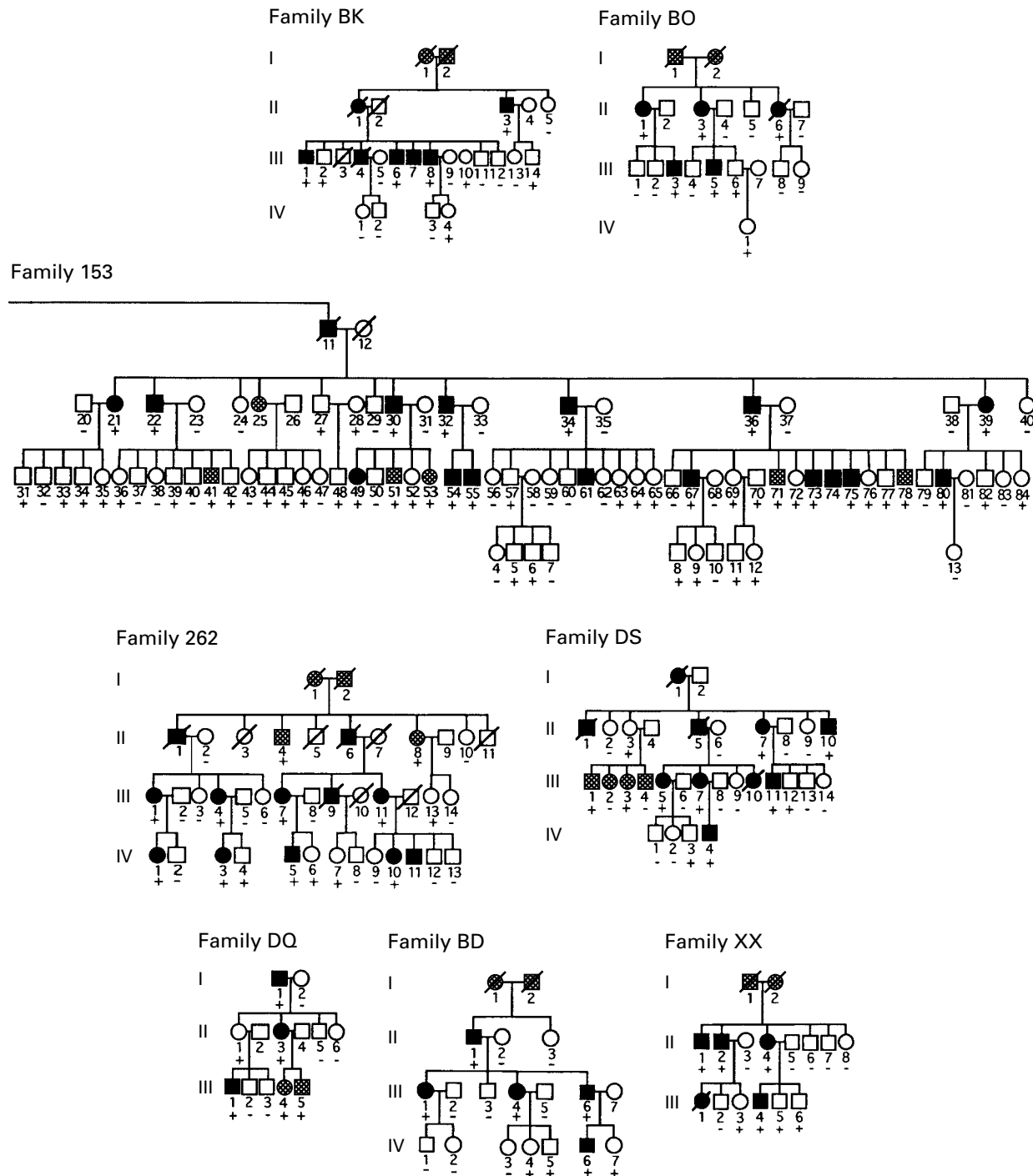


Figure 2. Pedigrees of 16 Families with Hypertrophic Cardiomyopathy Caused by Cardiac Myosin-Binding Protein C Mutations. Circles indicate female family members, squares male family members, solid symbols affected family members, open symbols unaffected family members, stippled symbols family members whose status was indeterminate, and symbols with a slash mark those who had died. The presence (+) or absence (-) of a cardiac myosin-binding protein C mutation is indicated for persons whose DNA was tested for the mutation segregating in their family.



imity of two of the point mutations (Glu258Lys and Glu451Gln) to splice signals might also alter RNA splicing. To examine this possibility, cardiac myosin-binding protein C cDNA was amplified by reverse-transcription PCR from samples derived from two affected persons in Family D. Four amplified cDNA products were gel-purified and sequenced (data not shown). Two products varied only at residue 1383 (guanine or cytosine), corresponding to the wild-type transcript and a transcript encoding a Glu451Gln missense mutation. Two products resulted from aberrant RNA splicing: one contained sequences in intron 16 that would encode five novel amino acids followed by a premature termination signal. The other product deleted 40 nucleotides by juxtaposing residues 1343 and 1384, thereby encoding 14 novel amino acids followed by a premature termination signal.

Eight mutations (Table 2), identified in 11 families, were predicted to truncate the encoded cardiac myosin-binding protein C. Four defects (Int8DSG+1A, Int12ASA-2G, Int24DSG+1T, and Int33DSG+1A) occur in donor or acceptor splice sequences. Cardiac myosin-binding protein C RNA was aberrantly spliced in lymphocytes derived from persons with the Int12ASA-2G, Int24DSG+1T, or Int33DSG+1A mutation (data not shown); lymphocytes were not available for analyses of the Int8DSG+1A defect. Two mutations identified in exon 25 (InsG791) and exon 30 (InsAA1042) were insertions of a single base pair and two base pairs, respectively. Deletions of one and two nucleotides in exon 23 (DelC698) and exon 28 (DelCT955) were also detected. Although the specific changes resulting from these defects varied, each caused a frame shift in the corresponding RNA, which encoded novel amino acids and a premature termination signal. Carboxyl amino acids, which are required for the incorporation of cardiac myosin-binding protein C into sarcomere A bands, titin interaction, and myosin binding,^{14,15} are predicted to be absent or mutated in each of these eight defects.

Several families shared the same mutation causing hypertrophic cardiomyopathy. Affected members of Families 153, BL, and BW shared a single base-pair insertion (InsG791); affected members of Families I and R shared an adenosine-to-guanine transversion in intron 12 (Int12ASA-2G), which alters RNA splicing. A common disease haplotype was present in affected persons (data not shown), indicating that a founding mutation caused hypertrophic cardiomyopathy, in Families 153, BL, and BW, as well as in Families I and R. In contrast, the Arg502Gln defect in Families BD and XX arose on different haplotypes, indicating that independent mutation events occurred in these families.

Three marriages occurred between related persons with identical cardiac myosin-binding protein C mu-

tations. Genotypes of the offspring of Subjects III-27 and III-28 (in Family 153), Subjects IV-69 and IV-70 (in Family 153), and Subjects III-12 and III-13 (in Family I) demonstrated no homozygosity for any of these mutations (Fig. 2).

Clinical Features of Cardiac Myosin-Binding Protein C Mutations

Genotyping of 574 family members at risk of inheriting the mutations causing hypertrophic cardiomyopathy identified the defect in 212 persons. Genetic studies confirmed previously recognized clinical disease in 8 children and adolescents (age, <20 years) and 113 adult patients (≥ 20 years). The extent and distribution of clinical symptoms and signs of cardiac hypertrophy in these patients appeared similar to those observed in patients with other mutations causing hypertrophic cardiomyopathy (data not shown).

Cardiac myosin-binding protein C mutations were also identified in samples from 91 family members who had no clinical manifestations of hypertrophic cardiomyopathy. Thirty-eight were less than 20 years of age, but surprisingly, 53 adults did not fulfill the diagnostic criteria for affected status (Tables 1 and 2). To determine whether the penetrance of cardiac myosin-binding protein C mutations remained low throughout life, we assessed the presence of cardiac hypertrophy in genetically affected persons in different decades of life (Fig. 3). As previously observed, hypertrophy caused by cardiac β -myosin heavy-chain or cardiac troponin T mutations was more often found in adults than in teenagers or children. However, only 60 percent of persons with cardiac myosin-binding protein C gene defects had hypertrophy, as compared with nearly 100 percent of those with either cardiac β -myosin heavy-chain or cardiac troponin T mutations (Fig. 3). That is, cardiac myosin-binding protein C gene defects caused disease later than mutations in either cardiac β -myosin heavy-chain or cardiac troponin T. Disease penetrance of cardiac myosin-binding protein C defects remained incomplete through the fifth decade of life (Fig. 3).

Deaths from cardiac causes were documented in 36 of 281 persons with a cardiac myosin-binding protein C defect; 34 of these deaths were sudden, and death frequently occurred during vigorous exercise. The incidence of sudden death among persons with cardiac myosin-binding protein C mutations was similar to that observed among persons with six mutations in cardiac troponin T (39 sudden deaths and 50 deaths from cardiac causes). However, life expectancy (assessed using Kaplan-Meier product-limit survival curves, Fig. 4) among persons with hypertrophic cardiomyopathy caused by truncation and missense mutations in the gene for cardiac myosin-binding protein C was longer than that

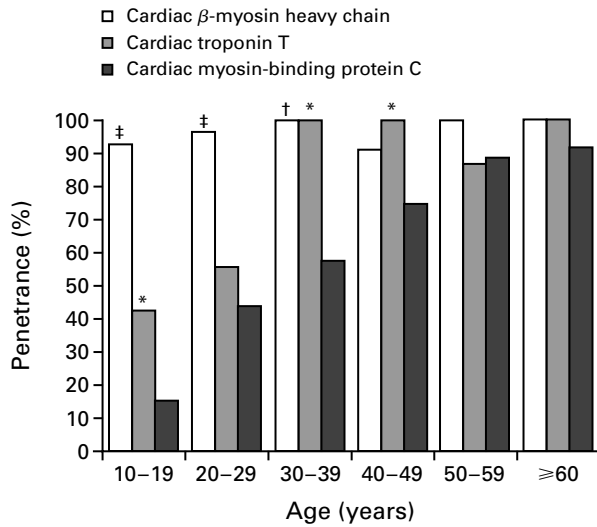


Figure 3. Age-Related Penetrance of Familial Hypertrophic Cardiomyopathy Caused by Mutations in the Genes for Cardiac Myosin-Binding Protein C, Cardiac Troponin T, and Cardiac β -Myosin Heavy Chain.

Solid bars denote the percentage of persons with both cardiac myosin-binding protein C mutations and cardiac hypertrophy. Comparable clinical data for cardiac troponin T and cardiac β -myosin heavy chain are from Watkins et al.,⁸ Solomon et al.,^{11,20} and our unpublished data. Significant differences in the penetrance of familial hypertrophic cardiomyopathy caused by cardiac myosin-binding protein C mutations and hypertrophic cardiomyopathy caused by mutations in cardiac troponin T or cardiac β -myosin heavy chain are indicated as follows: asterisks denote $P < 0.05$, the dagger $P < 0.005$, and double daggers $P < 0.001$.

observed among persons with either cardiac troponin T or malignant cardiac β -myosin heavy-chain mutations ($P < 0.001$).^{8,21}

DISCUSSION

We determined the clinical consequences of 12 novel mutations in the gene for cardiac myosin-binding protein C that cause familial hypertrophic cardiomyopathy. Although the cardiac phenotypes resulting from these defects resembled those produced by other mutations in genes for sarcomere proteins, important differences were also observed. All cardiac myosin-binding protein C mutations exhibited reduced penetrance until the fifth decade of life, whereas hypertrophic cardiomyopathy caused by mutations in other genes are almost completely penetrant by the second or third decade (Fig. 4). Furthermore, survival of patients with cardiac myosin-binding protein C mutations was better than that observed with cardiac troponin T mutations or malignant cardiac β -myosin heavy-chain mutations. Collectively, these data indicate that cardiac myosin-binding protein C mutations account for the milder

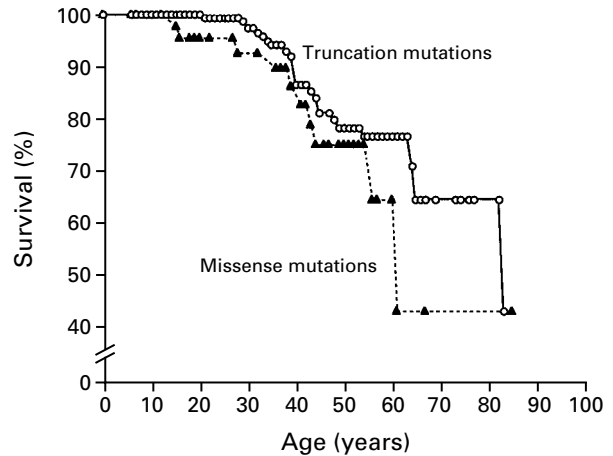


Figure 4. Kaplan-Meier Survival Curves for Persons with Familial Hypertrophic Cardiomyopathy Caused by Cardiac Myosin-Binding Protein C Mutations.

Survival was similar for persons with missense mutations and those with truncation mutations; survival was significantly better ($P < 0.001$) than that observed among persons with mutations in cardiac troponin T or malignant mutations in cardiac β -myosin heavy chain.

forms of hypertrophic cardiomyopathy in many patients with the disease.

A comparison of the clinical phenotype and genetic status of 16 families (Fig. 2) demonstrated that many young and middle-aged persons with a mutation in the gene for cardiac myosin-binding protein C had neither signs nor symptoms of disease. Although it is possible that some of these mutations will remain nonpenetrant throughout life, pedigree analyses indicate concordance between genotype and phenotype in the oldest members of each family. We therefore hypothesize that disease penetrance among persons with cardiac myosin-binding protein C defects increases with age.

Although it is often considered a disease of the young, hypertrophic cardiomyopathy is also diagnosed in the elderly. Older patients with hypertrophic cardiomyopathy present with typical manifestations of disease, including dyspnea, angina, and syncope, but often these symptoms are attributed to other disorders, such as coronary disease, valvular heart disease, and hypertension, that become more common with increasing age.^{27,28} The echocardiographic findings characteristic of hypertrophic cardiomyopathy in the elderly (e.g., increased wall thickness, decreased left-ventricular-cavity size, and supranormal systolic function) can be similar to those reported in young patients, but some morphologic differences (e.g., septal curvature and distortion of the left ventricular outflow tract) have also been observed.²⁹⁻³¹ Because few data are available from detailed family studies of hypertrophic cardio-

myopathy in the elderly, it is not known whether this disease has a genetic cause. Given the natural history and clinical manifestations of cardiomyopathy caused by cardiac myosin-binding protein C mutations, some cases of hypertrophic cardiomyopathy in the elderly are probably inherited and caused by mutations in this sarcomere-protein gene.

Cardiac myosin-binding protein C is an abundant myofibrillar protein that does not directly participate in force generation but has unique functions within the sarcomere. Expression of the protein during embryogenesis corresponds to the appearance of cross-striations,³² implying a developmental role in thick-filament alignment. The 372-amino-acid carboxyl residues of the protein are required for incorporation into A-band thick filaments,¹⁵ where the peptide binds myosin heavy chain and titin. Eight defects reported here (Table 2) and several reported previously^{6,7,19,33} are splice-site mutations, insertions, and deletions. These defects are predicted to encode truncated peptides, which may be unable to be incorporated into sarcomere A-bands. The resulting insufficiency of cardiac myosin-binding protein C could impair the structural integration of the contractile unit with the myocyte cytoskeleton. This model further implies that complete lack of the protein could be lethal, thereby providing one explanation for the absence of homozygous mutations in any offspring of three consanguineous marriages.

Cardiac myosin-binding protein C also regulates contraction, by stimulating actin-activated cardiac myosin ATPase^{13,17} and by influencing myofibril tension generation and contractile velocity.³⁴ Phosphorylation of cardiac myosin-binding protein C by a catecholamine-sensitive pathway¹⁷ may provide dynamic regulation of these processes. Four novel missense mutations (Table 1) and one previously reported mutation¹⁹ are clustered in sequences flanking the phosphorylation domain (Fig. 1). These missense mutations are likely to act through a dominant negative mechanism, similar to that caused by defects in cardiac β -myosin heavy chain^{35,36} or cardiac troponin T.³⁷ (Given the proximity of mutations Gly258Lys and Arg502Gln to RNA splice signals, these mutations may also encode truncated proteins.) If dominant negative mutations adversely affect the phosphorylation of cardiac myosin-binding protein C, sarcomere function could be particularly impaired during states of heightened adrenergic tone. We observed a higher incidence of sudden death in some families with missense mutations; many of these events occurred during heavy exertion. A better understanding of adrenergic regulation of the function of cardiac myosin-binding protein C may provide insights that are relevant for the development of genotype-selective therapies for this disorder.

The distinct consequences of cardiac myosin-bind-

ing protein C mutations further define the broad clinical spectrum of hypertrophic cardiomyopathy. Other mutations in sarcomere-protein genes are often silent during childhood^{38,39} but produce clinically important disease early in adult life. In contrast, the effects of mutations in the cardiac myosin-binding protein C gene are often subclinical until middle age and beyond. We speculate that the delayed penetrance of cardiac myosin-binding protein C mutations, combined with the good survival of patients with hypertrophic cardiomyopathy caused by the mutations, has made estimates of the incidence of these gene defects in the population relatively inaccurate. Whether aging, alone or in conjunction with other factors, causes the phenotypic expression of cardiac myosin-binding protein C mutations will require further study. Our results indicate that clinical screening of persons at risk for hypertrophic cardiomyopathy should be continued throughout adult life. Furthermore, one cause of late-onset disease is a heritable gene defect, a fact that should prompt evaluations of family members of affected patients.

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