A Newly Created Splice Donor Site in Exon 25 of the MyBP-C Gene Is Responsible for Inherited Hypertrophic Cardiomyopathy With Incomplete Disease Penetrance

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Background—Hypertrophic cardiomyopathy is a myocardial disorder resulting from inherited sarcomeric dysfunction. We report a mutation in the myosin-binding protein-C (MyBP-C) gene, its clinical consequences in a large family, and myocardial tissue findings that may provide insight into the mechanism of disease.

Methods and Results—History and clinical status (examination, ECG, and echocardiography) were assessed in 49 members of a multigeneration family. Linkage analysis implicated the MyBP-C gene on chromosome 11. Myocardial mRNA, genomic MyBP-C DNA, and the myocardial proteins of patients and healthy relatives were analyzed. A single guanine nucleotide insertion in exon 25 of the MyBP-C gene resulted in the loss of 40 bases in abnormally processed mRNA. A 30-kDa truncation at the C-terminus of the protein was predicted, but a polypeptide of the expected size (≈95 kDa) was not detected by immunoblot testing. The disease phenotype in this family was characterized in detail: only 10 of 27 gene carriers fulfilled diagnostic criteria. Five carriers showed borderline hypertrophic cardiomyopathy, and 12 carriers were asymptomatic, with normal ECG and echocardiograms. The age of onset in symptomatic patients was late (29 to 68 years). In 2 patients, outflow obstruction required surgery. Two family members experienced premature sudden cardiac death, but survival at 50 years was 95%.

Conclusions—Penetrance of this mutation was incomplete and age-dependent. The large number of asymptomatic carriers and the good prognosis support the interpretation of benign disease. **(*Circulation*. 2000;101:1396-1402.)**

Key Words: genes ■ cardiomyopathy ■ diagnosis

Familial hypertrophic cardiomyopathy (HCM) is an inherited primary heart muscle disease characterized by myocardial hypertrophy in the absence of secondary causes.[1] Heterogeneous mutations in genes coding for cardiac muscle proteins are known to account for HCM.[2] Mutations often occur in the genes encoding β-myosin heavy chain, cardiac troponin T, and myosin binding protein-C (MyBP-C) on chromosomes 14, 1, and 11, respectively.[3–5] Less frequently, mutations will be found in the genes specifying α-tropomyosin, regulatory and essential myosin light chains, and cardiac troponin I on chromosomes 15, 12, 3, and 19, respectively.[4,6,7] Recently, a mutation in the cardiac α-actin gene on chromosome 15 was described.[8] An unknown HCM gene has been localized on chromosome 7.[9]

Mutations in MyBP-C are responsible for ≈15% of cases of HCM.[10] This protein binds with its C-terminus to myosin heavy chain and to titin.[11] It seems to be involved in thick filament assembly[12,13] and may also regulate cardiac contractility in response to adrenergic stimulation.[14] At this point, >20 mutations in this gene have been described.[5,10,15–18] Apart from a few missense mutations,[5,10,18,19] most are predicted to result in a truncation of the protein.[5,10,15–17] The loss of C-terminal myosin and titin binding sites suggests a pathogenesis based on distorted filament assembly. However, molecular details of the dysfunction are not understood.

In this article, we report on a large family with HCM in which a mutation in exon 25 of the MyBP-C gene (insertion of a single G base pair) was identified on the basis of linkage to chromosome 11. Because the affected cardiac tissue was available, the predicted consequences of the mutational change (truncation of the protein by almost one-third) were investigated in detail. Unexpectedly, the shortened protein

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The Methods section of this article can be found at http://www.circulationaha.org

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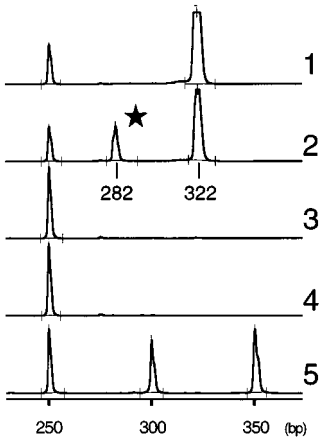

Figure 1. Demonstration of internal truncation in mRNA/cDNA. PCR products of cDNA were determined using primers F-2148 and R-2450; total expected length of normal fragment is 322 bp. Lane 1, Wild-type fragment (322 bp) using cDNA of unaffected (unrelated) person. Lane 2, Wild-type allele (322 bp) of cDNA obtained from mRNA isolated from myectomy tissue of patient IV-22. ⋆ indicates truncated allele (282 bp). Lanes 3 and 4, Controls (RT-PCR without RNA and reverse transcriptase, respectively). Lane 5, Length markers (in bp). All DNA fragments were fluorescence-labeled (Cy5).

was not detected in the myectomy tissue. The significance of this finding remains unclear. Another major point of interest was the assessment of the clinical phenotype associated with this mutation and the comparison of this phenotype with that of other HCM-related mutations. Because of the size of the family (27 gene carriers), the clinical consequences associated with the altered splicing of exon 25 could be reliably assessed as incomplete penetrance, late onset of disease, and low cardiac mortality. However, the overall benign character of the disease does not prevent the occurrence of a typical HCM phenotype once symptoms have developed.

Results

Mutation in the MyBP-C Gene

A fragment length difference in amplified myocardial mRNA-derived cDNA from affected tissue suggested an altered sequence in a region including exons 24 to 26 of the MyBP-C gene (exon numbers according to Reference [10]). By reverse transcriptase polymerase chain reaction (RT-PCR), a predicted 322-bp fragment and a fragment shorter by ≈40 bp were produced (Figure 1). When sequencing both amplified and cloned exon 25 cDNA, a stretch of 40 bp at the 3′ end of this exon was identified as missing, a result suggesting modified splicing (Figure 2A). In cloned genomic exon 25 DNA, a single base insertion (G) preceding codon 792 (or in codon 791) was identified. The additional G in this position converts a AGTGGG into a AGGTGGG motif, which is apparently used as a 5′ consensus splice site, with AG determining the 3′ end of the exon, and GTGGG defining the beginning of an unscheduled intron (Figure 2B). PCR-single strand conformation polymorphism (SSCP) screening of all 35 coding exons of the gene revealed altered mobility of exon 25 (Figure 3) in the DNA of those diagnosed as having symptomatic or borderline HCM and in 11 asymptomatic individuals (Table 1). This change was not observed in exon 25 DNA of 100 unrelated healthy individuals from the general population.

Immunoblotting

Unscheduled splicing of exon 25 of MyBP-C mRNA would result in a reading frame shift with a stop in exon 26, which in turn should lead to translational termination in position 816. The predicted size of the protein resulting from a loss of the C-terminus is 95 kDa, 30% less than the normal 137-kDa protein.[5] With immunoblotting (using cardiac tissue from patient IV-22), only the native (not truncated) form of the MyBP-C gene was observed (Figure 4).

Clinical Characterization

The clinical identification of the index patient (IV-20, see Figure 6, see online Methods) occurred after presentation at age 46 with symptoms of HCM in association with left ventricular outflow obstruction, which was treated by surgical myectomy. Other affected family members were—in generation III—the proband's mother, her sister, 2 half-siblings and 3 cousins, and—in generation IV—the proband's brother (IV-22) and 6 of her cousins (see Figure 6, online). The

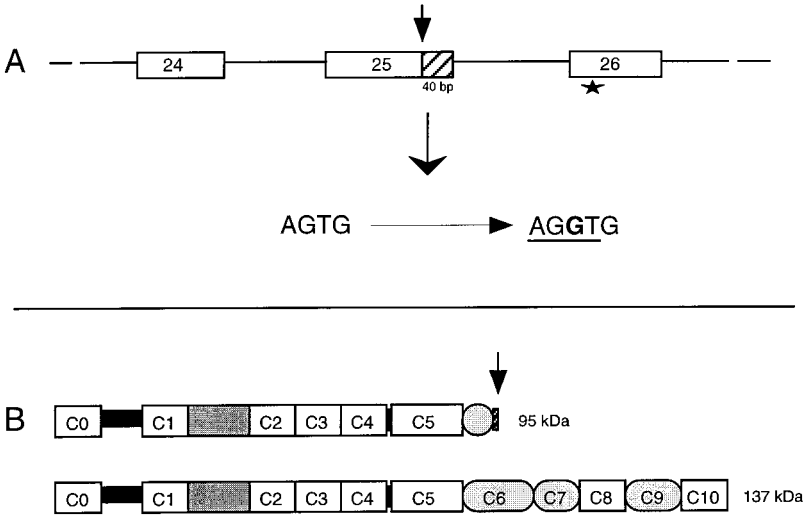

Figure 2. Mutation in MyBP-C gene. A, Position of inserted guanine (G) in exon 25 indicated by top arrow, together with mutated sequence of potential splice donor site, resulting in internal loss of 40 bp at 3′ end of exon 25. Premature stop in exon 26 is indicated by ⋆. B, Postulated truncation would result in 95-kDa protein (wild-type protein is 137 kDa) with break in C6 domain as indicated by the arrow (domains as published previously[20]). Myosin binding is specified by C10 domain and titin binding by C9 and C10 domains.

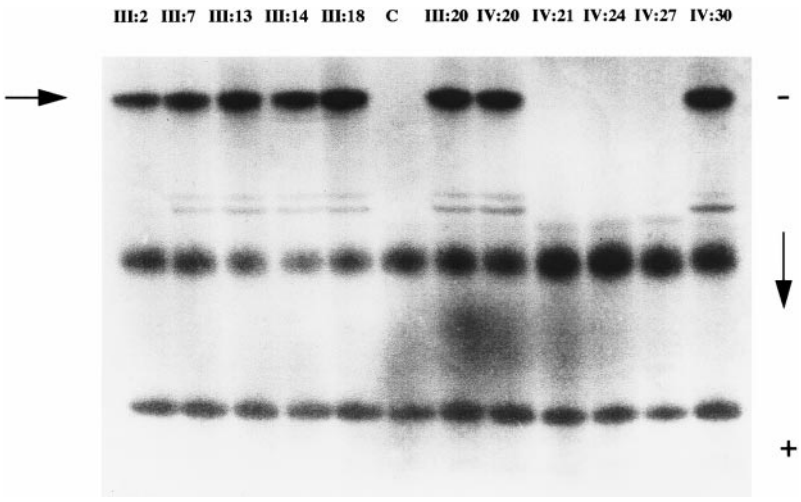

Figure 3. Single-strand conformation analysis of exon 25 segment of MyBP-C gene. Amplification products (215 bp) of 8 mutation carriers and 3 nonaffected members of family were analyzed in nondenaturing polyacrylamide gels. C indicates unrelated control person. Left arrow indicates carrier status. Right arrow indicates DNA migration from − to +.

brother developed symptoms at age 38 and also had a myectomy. In 5 individuals symptoms were mild (Group B, see Table 1).

A total of 27 members of the family were identified as gene carriers, of which only 10 persons (group A in Table 1) were clearly symptomatic according to the criteria used (clinical evidence, echocardiography, and electrocardiography[21]). This group was older and had more obvious manifestations of HCM (symptoms, echocardiogram, and arrhythmia) than the other gene carriers (Tables 1 and 2). The age of onset of HCM-related symptoms was 29 to 68 years (mean, 44). Of the other gene carriers, 5 (group B) had borderline HCM, and 12 (group C) were asymptomatic without clinical HCM. Three patients with longstanding hypertension in group C had increased septum thickness (13 to 14 mm). After an assessment that was blinded to genotype, the ECG and echocardiographic changes in these 3 patients were thought to reflect hypertension rather than HCM.

Fractional shortening was within normal limits in all gene carriers. In 17 of the 27 gene carriers, 24-hour Holter monitoring was performed (Table 1). One patient experienced episodes of paroxysmal atrial fibrillation and nonsustained ventricular tachycardia. Episodes of supraventricular tachycardia were recorded in 7 of 19 gene carriers. A total of 8 of the 27 gene carriers had a history of documented hypertension. The incidence of hypertension among non-gene carriers in the family was 26% (5 of 19 persons), which was similar to that of the gene carriers.

A comparison of symptomatic patients (group A in Table 1) with mildly symptomatic or asymptomatic carriers (groups B and C combined) and 22 healthy noncarriers in the family (Table 2) helped characterize the disease. The late clinical manifestation of HCM in this family is underscored by the more advanced age (mean, 54.7±4.5 years) in group A compared with the other groups (group B, 45.2±16.9 years; group C, 37.8±13.6 years). Furthermore, borderline cases and asymptomatic carriers of the mutation exhibited signs of a cardiac condition on a preclinical level, as shown by the frequency of mild symptoms and echocardiographic and ECG abnormalities.

Survival

Kaplan-Meier product-limit analyses of survival (heart-related mortality) in all 27 gene carriers and the 5 deceased family members assumed to be carriers are depicted in Figure 5. In 4 siblings in generation II (no clinical data available), presumed cardiac death occurred. Because 3 of them had affected progeny, their HCM carrier status was safely assumed. One (II-6) died suddenly at the age of 35 years. Predeath echocardiography and postmortem examination confirmed HCM in another case of sudden death at the age of 33 (IV-9). Survival of carriers of the MyBP-C mutation at 50 years was 95%, which is similar to that of carriers of the Asp175Asn mutation in the α-tropomyosin gene but unlike that of carriers of the Arg403Gln mutation of the β-myosin heavy chain gene ($P<0.0001$).

Discussion

The genetic cause and the phenotypic expression of HCM in a large, multigeneration family with one mutation was investigated.

The Mutation and Its Consequences

The genetic change was a single base (G) insertion in exon 25 of the cardiac MyBP-C gene on chromosome 11, which resulted in a sequence suitable to serve as a 5′ splice donor site (AG GTGGG). This mutation was also recently reported in a nonrelated family of the same ethnic group in North America.[10] We showed that the mutation resulted in the loss of 40 bp at the 3′ end of exon 25 in mRNA extracted from affected myocardial tissue. This loss resulted in a premature translational stop, which was then predicted to result in a truncated protein of 95 kDa and the loss of the C-terminal binding sites for myosin heavy chain and titin.[20] A reading shift would also occur if mRNA splicing were unaffected by the inserted G in exon 25. In this case, the shift resulting from the additional G would lead to a premature stop 40 residues away from the 3′ terminal of codon 792. However, such mutated mRNA was not observed (data not shown).

The shortened 95-kDa MyBP-C protein was not detected in nondenaturing gels (data not shown) or by immunoblotting the total protein of affected tissue using 2 different

TABLE 1. Clinical Features of 27 Family Members Identified as Gene Carriers

			Symptoms and Clinical Profile						Echo Data				ECG Data		
Family Member	Age/Sex	Age at Onset*	NYHA Class	CP	Syncope	HBP	Clinical Events	NSVT/SVT†	IVST, mm	SAM	LVDD, mm	FS, %	T-Wave Inversion	Abnormal Q-Waves	LVH
Group A: symptomatic/clinical HCM															
III-2	72/F	42	II	−	−	+		NA	15	−	32	41	+	−	−
III-13	75/F	68	II–III	+	+	−		NSVT+SVT	24	+	36	56	+	−	+
III-14	73/F	63	III	−	−	+		−	15	++	43	37	−	−	−
III-16	66/F	NA	II–III	−	−	−	NCD	NA	24	++	36	61	−	−	−
III-18	54/F	29	III	−	+	−		−	15	++	47	49	+	−	−
IV-9	33/M	NA	NA	NA	−	NA	SCD‡	NA	26	++	43	NA	−	−	+
IV-11	37/M	32	I–II	−	−	−		−	25	−	44	39	+	−	+
IV-20	54/F	46	III	+	−	−	M+M	−	28	+++	31	48	−	+	+
IV-22	45/M	38	III	+	−	−	M+M	SVT	26	−	41	49	+	−	+
IV-24	38/M	33	I–II	−	−	−		SVT	16	++	41	49	−	−	+
Group B: borderline HCM															
III-7	65/F	63	II	+	−	−		SVT	12	−	33	48	−	−	−
III-20	62/F	52	II	−	−	−		−	12	−	46	48	−	−	−
IV-13	32/M	30§	I	−	+	+		NA	12	−	48	40	+	−	+
IV-18	30/F	20	I–II	−	−	−		SVT	10	−	41	46	+	−	−
IV-30	37/F	19	(I–II)	(+)	−	−		−	12	−	43	44	+	−	−
Group C: clinically normal gene carriers															
III-11	59/F	asympt.	I	−	−	−		NA	9‖	−	40	45	−	−	−
III-22	58/F	asympt.	I	−	−	+		NA	14¶	+¶	47	43	+¶	−	−
III-23	55/M	asympt.	I	−	−	+		−	11	−	48	37	−	−	−
IV-1	46/F	asympt.	I	−	−	+		NA	12	−	38	42	−	−	−
IV-4	36/M	asympt.	I	−	−	+		NA	13¶	−	45	42	−	+¶	−
IV-6	29/F	asympt.	I–II	(+)	−	−		SVT	10	−	43	40	−	−	−
IV-29	39/F	asympt.	I	−	−	−		−	9	−	41	41	−	−	−
IV-31	32/M	asympt.	I	−	−	−		−	12#	−	46	39	−	−	−
IV-35	22/F	asympt.	I	−	−	−		NA	10	−	47	45	−	−	−
IV-36	25/F	asympt.	I	−	−	+		−	14¶	−	45	38	−	−	−
IV-37	23/F	asympt.	I	−	−	−		NA	11	−	48	38	−	−	−
V-1	30/F	asympt.	I	−	−	−		SVT	7	−	48	50	−	−	−

asympt. indicates asymptomatic; CP, chest pain (if in parentheses, atypical chest pain); Echo, echocardiogram; F, female; HBP, high blood pressure (hypertension); IVST, interventricular septal thickness; LVH, ECG typical of left ventricular hypertrophy; LVDD, left ventricular diastolic diameter; M, male; M+M, Morrow-myectomy; NA, not assessed; NCD, non-cardiac death; NSVT, nonsustained ventricular tachycardia; SVT, supraventricular tachycardia; NYHA, New York Heart Association; SAM, systolic anterior motion of the mitral valve (+, incomplete; ++, short contact with the septum; +++, prolonged contact with the septum); and SCD, sudden cardiac death.

*Age at onset was defined as age at which symptoms were experienced by patients for the first time.

†NSVT and SVT were assessed by Holter monitoring for ≥24 hours.

‡Diagnosis made at autopsy.

§Age at onset defined by syncope.

‖Septum thickness not accurately assessed and investigation could not be repeated.

¶The mild echocardiogram/ECG abnormalities seen in some patients in group C were thought to be more consistent with systemic hypertension than to reflect the expression of the HCM mutation.

#Abnormal echocardiogram but normal ECG.

polyclonal antibodies. The absence of detectable amounts of the truncated MyBP-C protein in the biopsy cannot be readily explained. The sensitivity of the staining method allows the detection of MyBP-C of at least 1/64th of the amount of protein used in the actual loading.[17] The epitopes of the polyclonal antibodies cover 55 kDa of the N-terminal region of MyBP-C. The absence of a visible band at the 95-kDa position suggests that the protein is either expressed at levels below the detection limit of ≈1% of wild-type protein or is absent (limit based on separate loading controls; data not shown). Absence could be the result of insufficient synthesis or rapid degradation. If the truncated MyBP-C protein were unable to contribute to filament assembly in the sarcomere, it could be rapidly degraded, as has been reported in cystic fibrosis, in which a deletion of phenylalanine in position 508 of CFTR results

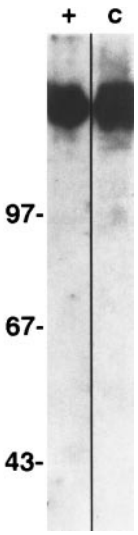

Figure 4. Immunoblot of total protein isolated from affected cardiac tissue. Tissue sample (≈0.5 mg) was boiled in presence of 1% sodium dodecyl sulfate. Protein (20 μg total) was loaded on 5% polyacrylamide gels containing 0.1% sodium dodecyl sulfate. Blotted protein was stained with polyclonal rabbit anti–MyBP-C IgG and visualized using biotinylated mouse anti-rabbit IgG and streptavidin-coupled peroxidase. Lanes show protein from HCM patient (+) and from unaffected myocardial control tissue (C). Left margin shows size markers (in kDa).

in defective processing and rapid degradation of partially glycosylated protein.[22] Protein degradation may, therefore, offer a plausible explanation for the absence of detectable mutant protein.

How does this relate to the pathogenesis of HCM? The presence or absence of the predicted truncated protein may provide insight into the mechanism of disease. Another hypothetical explanation (given in reference 15) may be cotranslational events coupling with the synthesis of MyBP-C to filament assembly.[23] Rapid degradation of the truncated protein may be the critical step preventing myofibrillogenesis. Filament formation rather than the function of assembled filaments would thus be disturbed. Although very low levels of mutant protein (below ≈1%) in the cells may exert a negative effect on filament assembly or maintenance, this seems unlikely because the interaction of the N-terminal regulatory domain of MyBP-C with the S2 region of myosin has a kDa of 5 μmol/L.[24] Low levels of mutant protein would, therefore, be insufficient to compete with the filament-associated normal protein. This suggests that a "poison polypeptide" (or dominant-negative) mechanism is less likely than a lack of protein (haploinsufficiency) to explain the mode of action of the mutation.

Experimental data from studies of transgenic mice do not provide an explanation either.[25] In mice with mutated MyBP-C lacking the myosin and titin binding sites, the mutated protein was expressed in detectable quantities and had detrimental effects on organization and contractile function of cardiac sarcomeres. We cannot explain the differences between the steady-state levels of truncated MyBP-C in transgenic mice and man. Either these differences are mutation-specific or human cardiomyocytes have an unknown mechanism to prevent the accumulation of truncated MyBP-C in cells.

Phenotype-Genotype Correlation

The second major point of this study was related to the clinical expression of disease associated with the MyBP-C mutation. The family was of sufficient size to examine penetrance, disease severity, and prognosis.

TABLE 2. Comparison Between Symptomatic HCM Patients, Borderline HCM Patients and Unaffected Gene Carriers, and Non-Gene Carriers in Family

	Symptomatic HCM (n=10)	Borderline HCM Patients or Unaffected Carriers* (n=17)	Non-Gene Carriers† (n=22)
Age, y	54.7±15.2	40±14.0	43±12.9
Sex, M/F	4/6	4/13	9/13
Symptoms			
Dyspnea, %	100	30	0
AP, %	35	18	0
Syncope, %	50	12	0
Hypertension, %	20	36	26
Echocardiography			
LVEDD, mm	39.4±5.3	43.9±4.2	43.9±4.2
LVESD, mm	20.4±4.8	25.2±3.4	25.2±3.4
Septum, mm	21.4±4.5	11.4±2.1	11.1±1.8
ECG abnormalities			
Abnormal ECG, %	80	30	0
LVH, %	60	6	0
T-wave inversion, %	50	23	0
Altered Q waves, %	10	6	0

Values are mean±SD unless otherwise indicated. AP indicates angina pectoris; F, female; M, male; LVEDD, left ventricular end-diastolic diameter; LVESD, left ventricular end-systolic diameter; and LVH, Sokolov index >3.5 mV.

*This group (borderline or unaffected gene carriers) combines the family members of categories B and C in Table 1.

†This group (non-gene carriers as a control cohort) combines relatives and non-relatives (spouses) of the family.

Penetrance was assessed by evaluating the 26 gene carriers and the one case of premature sudden death that was considered to be a consequence of the mutation (27 total carriers). The overall nature of the disease was characterized by late onset, a moderate course, and a high proportion of completely asymptomatic or mildly affected carriers. Different phenotypes among gene carriers led to the assignment of affected family members into 1 of 3 groups: symptomatic clinical HCM (group A), borderline clinical HCM (group B), and clinically normal (group C).

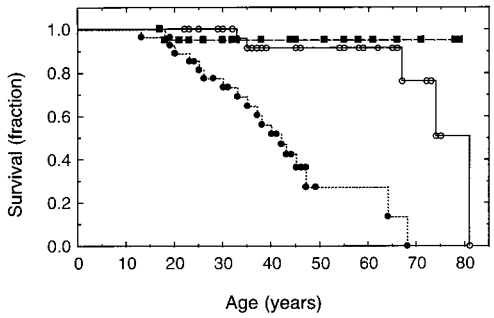

Figure 5. Kaplan-Meier survival curves. Comparison of cardiovascular mortality for patients with inherited HCM caused by guanine insertion in exon 25 of MyBP-C gene (○), Asp175Asn missense mutation in α-tropomyosin gene (■),[26] and Arg403Gln missense mutation in β-myosin heavy chain gene (●).[27]

The first group included 10 patients who fulfilled conventional diagnostic criteria for HCM.[1,21] In these patients, the mutation was judged to be fully penetrant. Three of the 5 members of the borderline HCM group (group B) fulfilled recently proposed criteria for the diagnosis of HCM within the context of familial disease with diagnostic ECG abnormalities, whereas the other 2 had disease-related symptoms. Septal thickness was in the upper range of normal in 4 of these 5 persons. There were 12 clinically normal gene carriers in whom the mutation was apparently not penetrant. A confounding factor in this group was the occurrence of systemic hypertension associated with concentric hypertrophy in 3 individuals (III-22, IV-4, and IV-36). In the absence of symptoms and typical morphological features of HCM, septal changes in these individuals (see Table 1) were thought to reflect chronic systemic hypertension rather than the action of the MyBP-C mutation. The prevalence of hypertension was comparable among mutation carriers (8 of 27; 30%) and noncarriers (6 of 23; 26%) in the family.

Overall, only 15 of 27 carriers (56%) showed features of the HCM phenotype. The older age in patients with phenotypic expression and the fact that the age at which symptoms first developed was >44 years confirms the late disease development of HCM caused by MyBP-C mutations.[10,28] A total of 95% of the carriers of the G insertion were alive at the age of 50 years (Figure 5). This is similar to the survival of carriers of the Asp175Asn mutation of the α-tropomyosin gene[26] and significantly better than the survival of most reported troponin T and β-myosin heavy chain families.[27,29,30] The relatively good prognosis seems to be consistent with the late-onset, benign HCM caused by MyBP-C mutations.

It is of interest, however, that patients with the morphological features of the HCM phenotype experienced syncope, supraventricular and ventricular arrhythmias, premature sudden cardiac death, and severe symptoms requiring surgical myectomy (Table 1). The perspective that HCM caused by MyBP-C mutations has a relatively benign prognosis arises in large part because of the late onset of disease expression. This family suggests that once the phenotype has developed, individual patients are at risk for the well-recognized complications of HCM.

In conclusion, we characterized a mutation in the MyBP-C gene leading to an internal truncation of the transcript and to a predicted premature translational stop in the message. The molecular details of the pathogenic mechanisms of this truncation require further investigation. Our phenotype analysis is in general agreement with the results of 2 recent studies[10,28] of a number of families suffering from different MyBP-C mutations, in particular regarding the late age of onset and the incomplete penetrance of the mutation. We wish to emphasize that despite the seemingly mild character of the disease, significant complications may occur once the phenotype has developed.

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