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# Myosin Binding Protein C Mutations and Compound Heterozygosity in Hypertrophic Cardiomyopathy

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**OBJECTIVES** We sought to determine the frequency and phenotype of mutations in myosin binding protein C (*MYBPC3*) in a large outpatient cohort of patients with hypertrophic cardiomyopathy (HCM) seen at our tertiary referral center.

**BACKGROUND** Mutations in *MYBPC3* are one of the most frequent genetic causes of HCM and have been associated with variable onset of disease and prognosis. However, the frequency of mutations and associated clinical presentation have not been established in a large, unrelated cohort of patients.

**METHODS** Using deoxyribonucleic acid from 389 unrelated patients with HCM, each protein coding exon of *MYBPC3* was analyzed for mutations by polymerase chain reaction, denaturing high-performance liquid chromatography, and direct deoxyribonucleic acid sequencing. Clinical data were extracted from patient records blinded to patient genotype.

**RESULTS** Of 389 patients with HCM, 71 (18%) had mutations in *MYBPC3*. In all, 46 mutations were identified, 33 of which were novel (72%). Patients with *MYBPC3* mutations did not differ significantly from patients with thick filament-HCM, thin filament-HCM, or genotype-negative HCM with respect to age at diagnosis, degree of hypertrophy, incidence of myectomy, or family history of HCM or sudden death. Patients with multiple mutations (n = 10, 2.6%) had the most severe disease presentation.

**CONCLUSIONS** This study defines the frequency and associated phenotype for *MYBPC3* and/or multiple mutations in HCM in the largest cohort to date. In this cohort, unrelated patients with *MYBPC3*-HCM virtually mimicked the phenotype of those with mutations in the beta-myosin heavy chain. Patients with multiple mutations had the most severe phenotype. (J Am Coll Cardiol 2004;44:1903–10) © 2004 by the American College of Cardiology Foundation

Hypertrophic cardiomyopathy (HCM), clinically defined as thickening of the myocardial wall in the absence of any other cause for left ventricular hypertrophy, affects 1 in 500 individuals and is the leading cause of sudden cardiac death (SCD) in the young (1). Genetic causes are diverse, with over 200 published disease-associated mutations scattered among 10 sarcomeric genes (2). One of the most common genetic causes for HCM in many populations studied involves mutations in *MYBPC3*, the gene encoding myosin binding protein C (3–6). Over 60 HCM-causing mutations in *MYBPC3* have been reported. Unlike the other sarcomeric genes, where missense mutations overwhelmingly predominate, approximately one-third of all *MYBPC3* mutations are single amino acid substitutions (i.e., missense mutations), approximately one-third are frameshift mutations, and the remaining mutations cause premature stop codons, in-frame insertions or deletions, or affect splicing (4).

Several studies have sought to define the phenotype associated with mutations in *MYBPC3*. Variability in the onset of disease and prognosis have been observed, but, in general, from the findings of studies of large families and specific patient subgroups, *MYBPC3*-HCM has been associated with later onset, less hypertrophy, lower penetrance, and a better prognosis than HCM caused by mutations in the beta-myosin heavy chain gene (*MYH7*) (7–10). These data suggested that *MYBPC3* mutations may be the predominant genetic substrate for HCM in elderly patients, among whom the natural history is generally favorable (11). In addition, it has been suggested that patients with protein truncations in *MYBPC3* manifested HCM earlier in life and required more invasive therapy than those harboring either missense or in-frame mutations (12). We sought to determine the frequency of *MYBPC3* mutations in a large cohort of unrelated patients and establish genotype-phenotype correlations for this outpatient tertiary referral center.

## METHODS

**Cohort.** Between April 1997 and December 2001, 389 unrelated patients who were evaluated and diagnosed at Mayo Clinic with unequivocal and unexplained HCM provided written informed consent and were enrolled in sarcomeric genetic testing. Patients were eligible for enroll-

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**Abbreviations and Acronyms**

DHPLC = denaturing high-performance liquid chromatography
DNA = deoxyribonucleic acid
HCM = hypertrophic cardiomyopathy
ICD = implantable cardioverter-defibrillator
LVOTO = left ventricular outflow tract obstruction
LVWT = left ventricular wall thickness
MYBPC3 = myosin binding protein C
MYH7 = beta-myosin heavy chain
SCD = sudden cardiac death

ment on the basis of: 1) being seen and evaluated in the HCM clinic; 2) having an unequivocal diagnosis of HCM; and 3) being the first family member seen during this time period. By definition, each subject met the clinical diagnostic criteria for HCM by having a maximum left ventricular wall thickness (LVWT) >13 mm in the absence of another confounding diagnosis. Purgene deoxyribonucleic acid (DNA) extraction kits (Gentra, Inc., Minneapolis, Minnesota) were used to extract total genomic DNA for subsequent mutational analysis. This single-institution cohort was genotyped previously for mutations in genes encoding the sarcomeric proteins comprising the thick filament (*MYH7* and the regulatory and essential light chains [*MYL2* and *MYL3*]) and the thin filament (troponin-T [*TNNT2*], troponin-I [*TNNI3*], alpha-tropomyosin [*TPM1*], and alpha-actin [*ACTC*]) (13,14).

**Mutation detection.** Polymerase chain reaction primers were designed to amplify all exons and flanking intronic sequences for each of the 34 protein-coding exons of *MYBPC3*. Primers and methods are available upon request. Each patient's DNA sample was amplified for each exon, and amplicons were analyzed for sequence variants using denaturing high-performance liquid chromatography (DHPLC) (WAVE, Transgenomic, Omaha, Nebraska) (15). All samples with abnormal DHPLC elution profiles were characterized by direct DNA sequencing (ABI Prism 377; Applied Biosystems, Foster City, California) to determine the precise sequence variation present. Nonsynonymous variants were reconfirmed by sequencing from stock DNA samples. All candidate disease-associated mutations were searched for in 100 healthy black and 100 healthy white DNA samples (400 reference alleles) obtained from Coriell Laboratories (Camden, New Jersey) to exclude the variant as a common polymorphism.

**Statistical analysis.** Analysis of variance tests were used to assess differences between continuous variables, followed by Fisher Protected Least Significant Difference post-hoc testing for pairwise differences. Contingency tables or z-tests were used as appropriate to analyze nominal variables. Probability values $< 0.05$ were considered statistically significant.

## RESULTS

**Clinical analysis of cohort.** This single institution cohort comprising 389 unrelated patients (215 male) with HCM was diagnosed at a mean age of 41.3 ± 19 years. At presentation to Mayo Clinic, 216 (55.5%) had cardiac symptoms, 120 (31%) had a family history of HCM, and 56 (14%) had a SCD event in a first-degree relative. The mean maximum LVWT was 21.5 ± 7 mm, and mean peak gradient for left ventricular outflow tract obstruction (LVOTO) was 46.6 ± 42 mm Hg. Of 389 patients, 297 (76%) had resting, labile, or mid-cavitary obstruction; 161 (41%) had undergone a surgical myectomy; and 60 (15%) had received an implantable cardioverter-defibrillator (ICD).

**Spectrum of *MYBPC3* mutations.** In this cohort, 46 different *MYBPC3* mutations were identified in 71 of 389 patients (18%). Thirteen of the identified mutations had been published previously, and the remaining 33 (72%) were novel (Fig. 1, Table 1). Mutations were identified in 20 of 33 exons studied. Each of the novel mutations identified was not found in the 400 reference alleles. Twenty-one (46%) of the mutations identified altered single amino acids (missense mutations), 15 (33%) were insertions or deletions causing a frameshift, 6 (13%) coded for premature stop codons (nonsense mutations), 3 (7%) were putative splice donor or acceptor site mutations located in the introns, and 1 (2%) was an in-frame deletion (Fig. 1, Table 1). No statistically significant difference in clinical phenotype was attributable to the specific type of *MYBPC3* mutation present (i.e., missense vs. premature truncations resulting from frameshift and nonsense mutations [data not shown]).

In addition to the putative pathogenic mutations identified, 7 amino-acid altering variants in *MYBPC3* were identified in patient samples as well as our 400 reference alleles (Table 1). In addition, three such variants were identified in 5 individual patients (S236G, R326Q, and V896M), were not seen in our 400 reference alleles, but were previously reported as common polymorphisms with allele frequencies >0.5% (6). Therefore, these three variants were not considered pathogenic, and these five patients were not included in the *MYBPC3*-HCM subgroup.

**Phenotype of *MYBPC3*-HCM.** When patients with a single mutation in *MYBPC3* (n = 63, excluding those harboring multiple mutations in one or more genes) (Fig. 2) were compared with those patients with single mutations involving the thick filament (*MYH7* or light chains, n = 61), there were no statistically significant differences with respect to age at diagnosis (37.6 ± 15 years vs. 33.0 ± 17 years), LVWT (22.5 ± 5 mm vs. 23.5 ± 7 mm), frequency of myectomy (35% vs. 56%), or frequency of ICD placement (29% vs. 21%) (Table 2). The phenotype ascribed to thick filament-HCM is not affected by removal of individuals with mutations in one of the two genetic components of the thick filament, namely mutations in the regulatory light chain encoded by *MYL2* (data not shown). Compared with

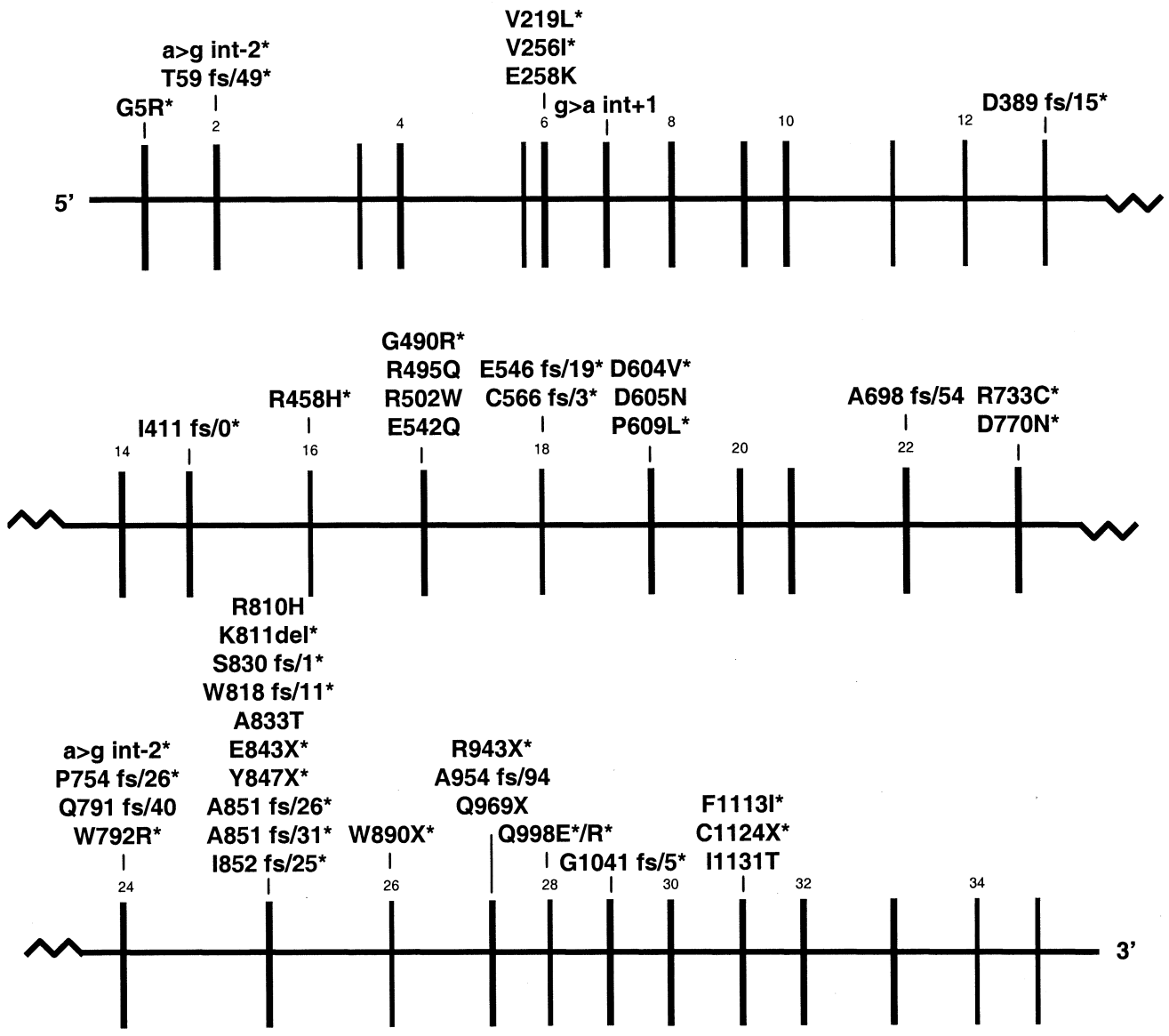


**Figure 1.** Schematic diagram of mutations identified in *MYBPC3*. **Vertical lines** represent exons, and mutations identified in this cohort are indicated above their exonic location. *Novel mutation.

patients with thin filament mutations (alpha-actin, alpha-tropomyosin, troponin-T, or troponin-I, n = 13), patients with *MYBPC3*-HCM were not statistically different in these same clinical parameters (Table 2, Figs. 3 and 4). With regard to HCM morphology (resting obstruction, labile obstruction, mid-cavitary obstruction, apical, and nonobstructive HCM), again, no statistically significant differences were present between *MYBPC3*-HCM, thick filament-HCM, thin filament-HCM, and multiple mutation-HCM (data not shown).

**Phenotype of patients with no identified mutation.** In this cohort of 389 patients, 242 (62.2%) have no mutations in the eight genes studied to date (Fig. 2). This subset of patients (genotype negative) is significantly older at diagnosis than patients with an identifiable sarcomere defect (genotype positive) (Table 2, Fig. 3). In fact, patients with genotype-negative HCM were older at diagnosis than even those with *MYBPC3*-HCM (45.4 ± 19 years vs. 37.6 ± 15 years, $p < 0.003$). These genotype-negative patients also have significantly less hypertrophy than patients with thick filament or multiple mutations and trended toward less hypertrophy than patients with *MYBPC3*-HCM (Table 2, Fig. 4).

**Phenotype of patients with multiple sarcomeric mutations.** In all, 10 of 389 patients were identified with multiple sarcomeric mutations (i.e., compound heterozygosity, 2.6% of total cohort, 7% of the genotyped subset) (Fig. 2). One patient had mutations in *MYH7* and troponin-T (R453C and Q191del, respectively), and one patient had two *MYH7* mutations (R719Q plus T1513S). Multiple *MYBPC3* mutations were identified in two patients (G5R plus R502W, E258K plus A954fs/94); *MYBPC3* and

**Table 1.** Putative HCM-Causing Mutations and Nonpathogenic, Nonsynonymous Polymorphisms Identified in *MYBPC3*

| Mutation or SNP # | Exon | Nucleotide Change | Variant | Mutation Type (Heterozygote Frequency for Polymorphisms)† |
|---|---|---|---|---|
| 1* | 1 | ggg>cgg | G5R | Missense mutation |
| 2* | 2 | a>g int-1 | splice | Splice mutation |
| 3* | 2 | del gggcacacggc | T59 fs/49 | Frameshift mutation |
| 4* | 6 | gtc>ctc | V219L | Missense mutation |
| 5* | 6 | gtc>atc | V256I | Missense mutation |
| 6 | 6 | gag>aag | E258K | Missense mutation |
| 7 | 7 | g>a int+1 | splice | Splice mutation |
| 8* | 13 | del c | D389 fs/15 | Frameshift mutation |
| 9* | 15 | del tt | I411 fs/0 | Frameshift mutation |
| 10* | 16 | cgc>cag | R458H | Missense mutation |
| 11* | 17 | ggg>agg | G490R | Missense mutation |
| 12 | 17 | cgg>cag | R495Q | Missense mutation |
| 13 | 17 | cgg>tgg | R502W | Missense mutation |
| 14 | 17 | gaa>caa | E542Q | Missense mutation |
| 15* | 18 | del gt | E546 fs/19 | Frameshift mutation |
| 16* | 18 | del ga | C566 fs/3 | Frameshift mutation |
| 17* | 19 | gac>gtc | D604V | Missense mutation |
| 18* | 19 | gac>aac | D605N | Missense mutation |
| 19* | 19 | cct>ctt | P609L | Missense mutation |
| 20 | 22 | del c | A698 fs/54 | Frameshift mutation |
| 21* | 23 | cgc>tgc | R733C | Missense mutation |
| 22* | 23 | gac>aac | D770N | Missense mutation |
| 23* | 24 | a>g int−2 | splice | Splice mutation |
| 24 | 24 | ins g | Q791 fs/40 | Frameshift mutation |
| 25* | 24 | tgg>cgg | W792R | Missense mutation |
| 26* | 24 | del g | P794 fs/26 | Frameshift mutation |
| 27 | 25 | cgc>cac | R810H | Missense mutation |
| 28* | 25 | del aag | K811del | Deletion mutation |
| 29* | 25 | del atgcg | W818 fs/11 | Frameshift mutation |
| 30* | 25 | ins t | S830 fs/1 | Frameshift mutation |
| 31 | 25 | gcg>acg | A833T | Missense mutation |
| 32* | 25 | gag>tag | E843X | Truncation mutation |
| 33* | 25 | tac>tag | Y847X | Truncation mutation |
| 34* | 25 | del c | A851 fs/26 | Frameshift mutation |
| 35* | 25 | ins t, ggc>tgc | A851 fs/31 | Frameshift mutation |
| 36* | 25 | del g | I852 fs/25 | Frameshift mutation |
| 37* | 26 | tgg>tga | W890X | Truncation mutation |
| 38* | 27 | cga>tga | R943X | Truncation mutation |
| 39 | 27 | del ct | A954 fs/94 | Frameshift mutation |
| 40 | 27 | caa>taa | Q969X | Truncation mutation |
| 41* | 28 | cag>gag | Q998E | Missense mutation |
| 42* | 28 | cag>cgg | Q998R | Missense mutation |
| 43* | 29 | ins aa | G1041 fs/5 | Frameshift mutation |
| 44* | 31 | ttc>atc | F1113I | Missense mutation |
| 45* | 31 | tgc>tga | C1124X | Truncation mutation |
| 46* | 31 | att>act | I1131T | Missense mutation |
| 1* | 4 | gtg>atg | V58M | Polymorphism (11%) |
| 2 | 6 | acg>ggc | S236G | Polymorphism (20%)‡ |
| 3 | 12 | cgg>cag | R326Q | Polymorphism (6%)‡ |
| 4* | 13 | cgg>tgg | R382W | Polymorphism (6%) |
| 5* | 15 | ggt>agt | G416S | Polymorphism (2%) |
| 6* | 17 | ggg>agg | G507R | Polymorphism (4%) |
| 7* | 18 | ctg>atg | L545M | Polymorphism (1%) |
| 8 | 26 | gtg>atg | V896M | Polymorphism (5%)‡ |
| 9 | 33 | cag>tag | Q1233X | Polymorphism (2%) |
| 10* | 33 | dup gggggcatctatgtctgc | GGIYVC 1248–1253dup | Polymorphism (1%) |

*Indicates a novel mutation or variant; †in 100 African American and 100 Caucasian deoxyribonucleic acid samples, unless otherwise noted; ‡previously published frequency (6).

HCM = hypertrophic cardiomyopathy; SNP = single nucleotide polymorphism.

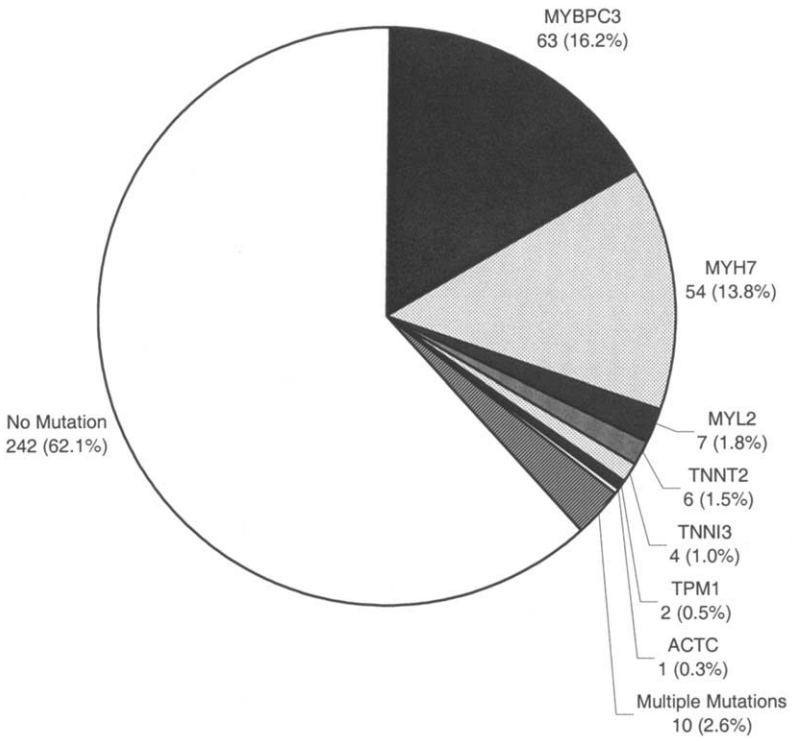


**Figure 2.** Distribution of sarcomeric mutations in patients with hypertrophic cardiomyopathy. The relative frequency of each genotype identified in the cohort of 389 unrelated patients is indicated as n (%). Note that each genotype is exclusive of patients with multiple mutations, who are included only in the "multiple mutations" subgroup.

*MYH7* in two patients (D605N plus E894G, Q791fs/40 plus R694C); *MYBPC3* and troponin-T in two patients (V256I plus R92W, A833T plus R286H); *MYBPC3* and troponin-I in one patient (R943X plus S166F); and *MYBPC3* and α-tropomyosin in one patient (F1113I plus I172T). These 10 patients were significantly younger at diagnosis than any other subgroup, had the most hypertrophy, and had the highest incidence of myectomy and ICD placement, 3 of the 4 of which were placed due to the strong family history of SCD (Table 2, Figs. 3 and 4).

## DISCUSSION

This study provides mutational analysis of the largest cohort of unrelated patients derived from a single institution to date for the most common genetic cause of HCM seen in this cohort, *MYBPC3*, and completes the comprehensive mutational analysis of the eight most common sarcomeric subtypes of HCM. In this cohort, *MYBPC3*-HCM was statistically indistinguishable from other sarcomeric causes of HCM by clinical parameters of LVWT, LVOTO, or incidence of myectomy.

It was observed previously that *MYBPC3* mutations were associated with variable onset of disease and prognosis, including late-onset HCM and benign disease (7–9). In our cohort, the age at diagnosis for *MYBPC3* mutation carriers was not statistically different than patients with thick filament-HCM, thin filament-HCM, or genotype-negative HCM. Similarly, these unrelated *MYBPC3*-HCM patients have not as yet experienced a more benign course than those with other HCM genotypes or those with genotype-negative HCM, as there was no lower frequency of myectomy or ICD in these patients, or SCD among their first-degree relatives. Long-term follow-up of these patients is necessary to establish disease course; however, this cross-sectional study suggests, not surprisingly, that data from linkage studies and large pedigrees may not always translate to the individual patient in the clinical setting.

Previous studies have found significantly earlier disease manifestations and a higher incidence of invasive procedures in *MYBPC3*-HCM patients with mutations leading to protein truncation (frameshift or nonsense mutations) than in their counterparts with missense or in-frame mutations (3,16). However, in our comparatively larger cohort, there was no difference between these two groups for any clinical variable studied, likely due to a difference in study design. In this study, only unrelated probands were included to avoid skewing of the data by any single large pedigree manifesting either a benign or malignant disease course. Although large pedigree studies are clearly of crucial importance, the current study design suggests that the findings from specific pedigrees cannot be extended to a series of unrelated patients.

As perhaps anticipated, patients with multiple sarcomeric mutations had the most severe phenotype: youngest at diagnosis, greater degree of hypertrophy, and highest surgical intervention rate of the subgroups studied here. Over-

**Table 2.** Clinical Characteristics of Patients With Single, Multiple, or No Sarcomeric Mutations

| | *MYBPC3* Mutation | Thick Filament Mutation | Thin Filament Mutation | Multiple Mutations | No Sarcomeric Mutation | ANOVA p Value |
|---|---|---|---|---|---|---|
| Number of individuals | 63 | 61 | 13 | 10 | 242 | |
| Male/female | 41/22 | 25/36 | 10/3 | 5/5 | 134/108 | 0.04 |
| Age at diagnosis | | | | | | |
| Mean (yrs) | 37.6 ± 15 | 33.0 ± 17 | 42.9 ± 16 | 20.7 ± 11 | 45.4 ± 19 | <0.0001 |
| Range | 3.4–75.2 | 0.1–70.6 | 22.6–72.5 | 0.2–37.4 | 0.0–89.5 | |
| >25 yrs, n (%) | 50 (79%) | 41 (67%) | 12 (92%) | 3 (30%) | 202 (83%) | <0.0001 |
| Presentation | | | | | | |
| Cardiac symptoms, n (%) | 37 (59%) | 30 (49%) | 10 (77%) | 4 (40%) | 134 (55%) | 0.08 |
| Family history* | | | | | | |
| HCM, n (%) | 28 (44%) | 27 (44%) | 5 (38%) | 5 (50%) | 55 (23%) | 0.01 |
| SCD, n (%) | 13 (21%) | 11 (18%) | 3 (23%) | 2 (20%) | 27 (11%) | 0.35 |
| Echocardiography | | | | | | |
| LVWT (mm) | 22.5 ± 5 | 23.5 ± 7 | 21.5 ± 4 | 25.2 ± 12 | 20.8 ± 6 | 0.01 |
| Severe hypertrophy†, n (%) | 4 (6%) | 9 (15%) | 0 (0%) | 2 (20%) | 14 (6%) | 0.07 |
| Peak LVOT gradient (mm Hg) | 41.4 ± 38 | 52.3 ± 46 | 34.9 ± 42 | 46.3 ± 44 | 47.3 ± 42 | 0.60 |
| >30 mm Hg at rest, n (%) | 31 (49%) | 36 (59%) | 5 (38%) | 6 (60%) | 128 (53%) | 0.79 |
| Treatment | | | | | | |
| Myotomy/myectomy, n (%) | 22 (35%) | 34 (56%) | 3 (23%) | 6 (60%) | 95 (39%) | 0.04 |
| PM, n (%) | 12 (19%) | 13 (21%) | 2 (15%) | 0 (0%) | 40 (17%) | 0.55 |
| Myectomy or PM, n (%) | 25 (40%) | 37 (61%) | 4 (31%) | 6 (60%) | 117 (48%) | 0.10 |
| ICD, n (%) | 18 (29%) | 13 (21%) | 2 (15%) | 4 (40%) | 23 (10%) | 0.003 |

No significant differences were defined between *MYBPC3*-HCM and thick filament-HCM subsets. *In a first degree relative; †LVWT ≥30 mm.

ANOVA = analysis of variance; HCM = hypertrophic cardiomyopathy; ICD = implantable cardioverter-defibrillator; LVOT = left ventricular outflow tract; LVWT = left ventricular wall thickness; *MYBPC3* = myosin binding protein C; PM = permanent pacemaker; SCD = sudden cardiac death; thick filament = β-myosin heavy chain and regulatory myosin light chain; thin filament = troponin-T, troponin-I, α-tropomyosin, and α-actin.

all, 2.6% of the entire cohort had evidence for compound heterozygosity, and of those patients with an identified sarcomeric mutation, 7% were found to have two possible mutations. This finding is in accordance with a previous finding of ~5% frequency of complex genetic status in a smaller cohort (4). Because the high-throughput analysis of all HCM-associated genes has only recently become technically feasible, and the genetic cause for HCM remains to be elucidated for ~60% of our cohort, the relative risk associated with multiple mutations remains to be determined.

Importantly, this study design includes only one proband from each pedigree, defined as the first member of a family to be seen in our HCM clinic. Therefore, this study design does not provide direct information on mutation penetrance. In addition, because only individuals with clinical disease (and not asymptomatic carriers) are included, the genotype-phenotype correlations derived from this cohort may, in fact, overestimate the severity of disease ascribed to any particular genotype. However, our goal was to determine the frequency and phenotype of patients with *MYBPC3*-HCM seeking clinical evaluation, rather than defining the degree of non-penetrance associated with a particular HCM-causing genotype. In the subset of patients seeking clinical attention for their clinical disease, we have characterized the phenotype of HCM caused by *MYBPC3* mutations.

The veracity with which these data represent the true frequency of *MYBPC3* mutations and their phenotype in HCM may also be affected by bias present in this single-institution cohort. As a tertiary referral center known for surgical treatment of HCM, patients with obstructive disease treated by myectomy are overrepresented. However, in other clinical parameters, this cohort is similar to unselected regional center patients (17). In previous studies of *MYH7* mutations in HCM, we found no difference in mutation frequency in regional (Minnesota, Wisconsin, and Iowa residents) versus non-regional HCM patients in our cohort despite the fact that the non-regional HCM patients had a greater degree of obstruction and a higher incidence of myectomy (14). Similarly, in this cohort, there were no statistically significant differences in the frequency and phenotype of mutations in *MYBPC3* in regional versus non-regional patients (data not shown). In addition, it is possible that statistically significant differences do exist between genotyped subgroups, but are not apparent due to insufficient numbers of patients in each group. Future meta-analysis studies pooling the data from this and additional published genotyped cohorts may provide sufficient power to discern subtle differences between genotypes. However, from this cohort, it is apparent that there is a broad spectrum of clinical presentation in sarcomeric HCM, which may limit the clinical relevance of such findings.

Our estimation of *MYBPC3* mutation frequency is dependent upon the sensitivity of our mutation detection platform (DHPLC), the correct assignment of mutations as pathogenic, and the exclusion of related individuals from our cohort. The published sensitivity for DHPLC is established as >95%, and, for HCM mutation screening, 100% sensitivity has been reported (18–21). Regarding assignment of pathogenic mutations, every mutation identified in

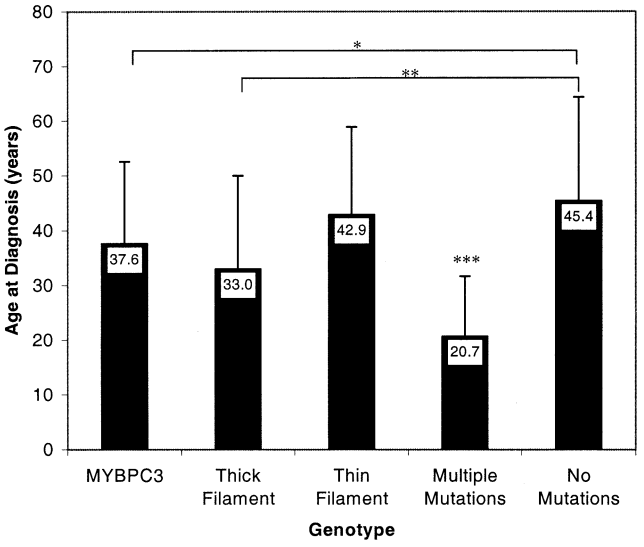


**Figure 3.** Age at diagnosis by genotyped subsets. Genotyped hypertrophic cardiomyopathy patients are grouped on the X-axis, and age at diagnosis is indicated on the Y-axis. **Error bars** = standard deviation. Unless noted, all other pairwise comparisons were not statistically significant. Thick filament = beta-myosin heavy chain and regulatory myosin light chain; thin filament = troponin-T, troponin-I, alpha-tropomyosin, and alpha-actin. *p = 0.003; **p < 0.0001; ***p < 0.05 vs. all other subgroups.

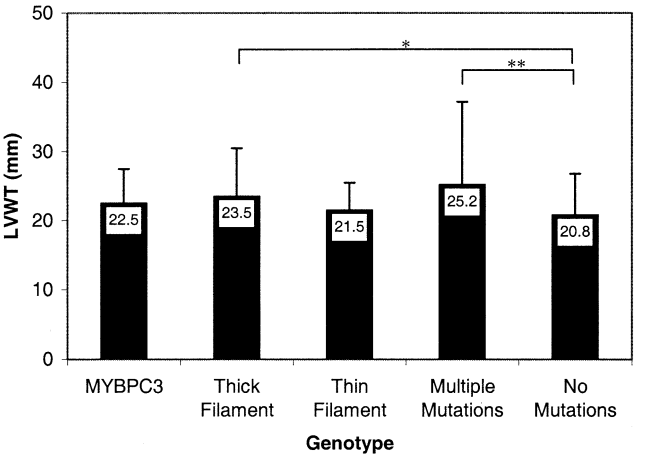


**Figure 4.** Degree of hypertrophy by genotyped subsets. Genotyped hypertrophic cardiomyopathy patients are grouped on the X-axis, and left ventricular wall thickness (LVWT) is indicated on the Y-axis. **Error bars** = standard deviation. Unless noted, all other pairwise comparisons were not statistically significant. Thick filament = beta-myosin heavy chain and regulatory myosin light chain; thin filament = troponin-T, troponin-I, alpha-tropomyosin, and alpha-actin. *p = 0.004; **p = 0.03.

this study is not present in 400 reference alleles (200 from ethnically matched Caucasian Americans, and 200 from ethnically diverse African Americans) and is not a reported polymorphism. Each variant alters a residue that is conserved across species and causes a change in the amino-acid sequence of the protein product, whether by substitution, truncation, or frameshift. The three variants identified in the introns occur within the invariant splice donor or acceptor sites within two nucleotides of the exon boundary. Due to the substantial size of the genotype-positive cohort, co-segregation of each mutation has not yet been determined. Such co-segregation data would provide further evidence that each mutation is correctly assigned, and this is the subject of ongoing investigation. It is of interest to note that seven nonsynonymous polymorphisms were identified in our panel of 400 reference alleles, one of which (Q1233X) was previously reported as an HCM-pathogenic mutation, based on co-segregation within a family and absence from 100 reference alleles (3). This finding highlights the difficulty in assessing whether a sequence variant identified is truly the pathogenic mutation for HCM, and the importance of adequate controls. Ongoing structure-function studies of *MYBPC3* are needed to characterize the function of this protein to assist in the assignment of mutations as independently pathogenic (HCM-causing) mutations, biologically relevant susceptibility variants, or irrelevant nonsynonymous polymorphisms.

Finally, clinical assessment was used to exclude relatedness to three degrees. If more distantly related individuals with or without *MYBPC3* mutations have been included in this cohort, our estimation of *MYBPC3* mutation frequency would be falsely high or low, respectively. However, using our clinical analysis, we were able to exclude 45 related individuals before analysis, proving the efficacy of the method. In addition, due to the large size of the cohort and subset with *MYBPC3* mutations, the influence of concealed relatives on the genotype-phenotype observations would be minimal.

**Conclusions.** This study establishes *MYBPC3* as the most common genetic cause for HCM in our tertiary referral center, with mutations found in 18% of patients with HCM. Patients with *MYBPC3* mutations were diagnosed at a younger age than those without sarcomeric mutations. Although prediction of the causative mutation based on age of onset or degree of hypertrophy has been suggested, patients with single *MYBPC3* mutations are not diagnosed later or with less severe hypertrophy than those with *MYH7* mutations, making such predictions impossible. Patients with multiple mutations have the most severe disease, suggesting that for individuals presenting early in life with extreme hypertrophy and a positive family history, the search for additional mutations should continue after the identification of an initial putative HCM-causing defect. Patients with no identifiable sarcomeric mutation were significantly older than those with sarcomeric mutations, suggesting an alternate genetic mechanism for HCM in elderly patients.

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