Prevalence and Age-Dependence of Malignant Mutations in the Beta-Myosin Heavy Chain and Troponin T Genes in Hypertrophic Cardiomyopathy

A Comprehensive Outpatient Perspective

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OBJECTIVES

The goal of this study was to determine the prevalence of “malignant” mutations in hypertrophic cardiomyopathy (HCM).

BACKGROUND

Previous genotype-phenotype studies have implicated four mutations (R403Q, R453C, G716R and R719W) as highly malignant defects in the beta-myosin heavy chain (MYH7). In the cardiac troponin T gene (TNNT2), a specific mutation (R92W) has been associated with high risk of sudden death. Routine clinical screening for these malignant mutations has been suggested to identify high-risk individuals.

METHODS

We screened 293 unrelated individuals with HCM seen at the Mayo Clinic in Rochester, Minnesota, between April 1997 and October 2000. Deoxyribonucleic acid (DNA) was obtained after informed consent; amplification of MYH7 exons 13 (R403Q), 14 (R453C) and 19 (G716R and R719W), and TNNT2 exon 9 (R92W) was performed by polymerase chain reaction. The mutations were detected using denaturing high-performance liquid chromatography and automated DNA sequencing.

RESULTS

The mean age at diagnosis was 42 years with 53 patients diagnosed before age 25. The mean maximal left ventricular wall thickness was 21 mm. Nearly one-third of cases were familial and one-fourth had a family history of sudden cardiac death. Only 3 of the 293 patients possessed one of the five “malignant” mutations, and all 3 patients were <25 years of age at presentation (p < 0.006).

CONCLUSIONS

This finding underscores the profound genetic heterogeneity in HCM. Only 1% of unrelated individuals seen at a tertiary referral center for HCM possessed one of the five “malignant” mutations that were examined. Routine clinical testing for these specific mutations is of low yield.

Hypertrophic cardiomyopathy (HCM) is defined as the presence of a hypertrophied, nondilated left ventricle in the absence of another causative disease (1). It is estimated to affect 1 in 500 persons, with highly variable clinical and pathologic presentations and penetrance (2,3). The severity of disease varies from a lifelong asymptomatic course to a sentinel event of sudden cardiac death (SCD) at a young age. A growing realization that the implanted cardioverter defibrillator (ICD) is effective in the primary and secondary prevention of SCD has provided an added impetus to discover new approaches for the identification and risk stratification of susceptible individuals in whom the prophylactic implantation of an ICD might be life saving (4).

In addition to this phenotypic variability, there is profound heterogeneity in the genetic substrate for HCM. To date, nine genes encoding various components of the cardiac sarcomere have been implicated in HCM: cardiac beta-myosin heavy chain (MYH7) (5–8), troponin T (TNNT2) (9,10), alpha troponymosin (TPM1), myosin binding protein C3 (MYBPC3), cardiac ventricular myosin light chain (MYL2), cardiac myosin alkali light chain (MYL3) (11), troponin I (TNNI3) (12), alpha cardiac actin (ACTC) (13,14) and titin (TTN) (15). The familial HCM mutation database lists over 150 unique mutations scattered throughout these sarcomeric genes (16).

Despite this considerable phenotypic and genotypic diversity, it was hoped that through genotype-phenotype correlative studies genetic testing would identify those individuals at high risk for SCD to facilitate primary prevention (17–19). Mutations in MYH7 are the most commonly described defects in HCM and account for...
approximately 35% to 50% of all cases of familial HCM (17,19–22). Although it is well-established that no particular clinical or prognostic phenotype is mutation specific, four MYH7 mutations, R403Q (exon 13), R453C (exon 14) and G716R and R719W (exon 19), have been associated particularly with a high incidence of SCD and are considered “malignant” mutations in comparison with other HCM-causing mutations (17,23,24). Mutations in cardiac troponin T, particularly R92W (exon 9), have been associated with a high incidence of SCD in spite of minimal hypertrophy (10,23).

The association between specific MYH7 and TNNT2 defects with an adverse prognosis raised the exciting possibility that genotyping alone may identify individuals at high risk of SCD and direct potentially lifesaving ICD therapy (19,21,25). The success of genotype-phenotype correlative studies is dependent on the interactions with modifier genes and environmental influences, but the notion that molecular genetic testing may direct clinical treatment persists (26,27). Moreover, from a cost-effective standpoint, an estimation of the prevalence of malignant mutations in the overall perspective of HCM is of utmost relevance. For these reasons, we determined the prevalence of these “malignant” mutations among a diverse, unselected group of patients with HCM seen in a subspecialty clinic within a large tertiary referral center.

METHODS

Patient population. From April 1997 through October 2000, 293 unrelated individuals seen in the Mayo Medical Center HCM Clinic and diagnosed with HCM provided written informed consent for genomic evaluation in this study approved by the Mayo Foundation Institution Review Board.

Extraction and amplification of MYH7 and TNNT2. Genomic deoxyribonucleic acid (DNA) was extracted from peripheral blood lymphocytes using the Purgene DNA extraction kit from Gentra, Inc. (Minneapolis, Minnesota). Protein-encoding exons containing the previously reported “malignant” mutations (exons 13, 14 and 19) of the cardiac beta-myosin heavy chain, MYH7, were amplified from genomic DNA by polymerase chain reaction (PCR) using the full-length genomic sequence and previously published intron/exon-based primers (28). exon 9 of cardiac troponin T, TNNT2, was amplified using a forward primer designed in our lab (5’ CTAGCCACCCCATCTCCTCC 3’) and the published 9R280 reverse primer sequence (5’ GGATGAGACAGACTGGCCATCAG 3’) (29).

Mutational analysis. Sequence variations were detected by denaturing high-performance liquid chromatography (DHPLC) using the Transgenicome WAVE system (Omaha, Nebraska), as previously described (30). In brief, PCR products were injected into the denaturing column and eluted with increasing concentrations of acetonitrile. Homozygotes have only homoduplex DNA. Heterozygotes form both homoduplexes and heteroduplexes. Heteroduplexes are eluted from the column and detected before homoduplexes. Most sequence variations yield a unique elution profile and a characteristic chromatogram pattern. The precise nature of the sequence variation was determined by manual, radiolabeled ThermoSequenase sequencing (Amersham Life Science, Cleveland, Ohio) and independently confirmed by dye-terminator cycle-sequencing (ABI Prism 377) (31).

Mutations are denoted using accepted nomenclature (32). For proteins, the single letter amino acid code is utilized. To indicate an amino acid missense mutation, the format R403Q is used. Here, the “wild-type” amino acid (R = arginine) is given before and the mutant amino acid (Q = glutamine) is provided after the codon number , 403.

R403Q mutation–specific Ddel restriction enzyme assay. Ddel restriction enzyme digests were performed to screen for the R403Q mutation in MYH7. Restriction digests were performed on PCR products for 2 h at 37°C, and then the digests were analyzed on a 3% agarose gel.

Statistical analysis. Tests for associations between the presence or absence of malignant mutations and clinical variables (i.e., gender, age-dependence, left ventricular wall thickness [LVWT]) were performed with Fisher exact test for categorical variables and the Wilcoxon-Mann-Whitney test for continuous and ordered variables. Reported p values are two-sided, and a p value <0.05 is considered statistically significant.

RESULTS

Table 1 summarizes the demographics of this HCM cohort. Between April 1997 and October 2000, 293 unrelated individuals diagnosed with HCM were seen at the HCM Clinic and investigated for the presence of a “malignant” mutation. Over half of the patients sought medical evaluation because of the presence of cardiac symptoms including angina, syncope, dyspnea or out-of-hospital cardiac arrest (n = 160, 54.6%). The remaining patients were diagnosed during evaluation of a family history of HCM, SCD or during a routine medical evaluation. A positive family history for HCM was elicited in 95 patients (32.4%). A total of 69 patients (23.5%) had a family history of at least one

Abbreviations and Acronyms

<table>
<thead>
<tr>
<th>Acronym</th>
<th>Description</th>
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<tr>
<td>DHPLC</td>
<td>Denaturing high-performance liquid chromatography</td>
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<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
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<td>HCM</td>
<td>Hypertrophic cardiomyopathy</td>
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<td>ICD</td>
<td>Implanted cardioverter defibrillator</td>
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<td>LVOTO</td>
<td>Left ventricular outflow tract obstruction</td>
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<td>LVWT</td>
<td>Left ventricular wall thickness</td>
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<tr>
<td>MYH7</td>
<td>Cardiac beta-myosin heavy chain</td>
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<td>PCR</td>
<td>Polymerase chain reaction</td>
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<td>SCD</td>
<td>Sudden cardiac death</td>
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<td>TNNT2</td>
<td>Troponin T</td>
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**Acknowledgments**

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SCD before the age of 40. Overall, 40 patients (13.7%) presented with cardiac symptoms and a positive family history of SCD. The average age at diagnosis was 42.5 years. A total of 53 patients (18%) were diagnosed with HCM before age 25.

Figure 1 displays the anatomic phenotype of HCM expressed in this cohort. A total of 155 patients (53%) had “typical” HCM characterized by asymmetric septal hypertrophy and resting left ventricular outflow tract obstruction (LVOTO). In this subset with resting obstruction, the peak gradient at rest was approximately 60 mm Hg. For the entire cohort, the average maximal LVWT was 21 mm. The greatest LVWT recorded was 46 mm. A total of 18 patients (6.1%) had extreme hypertrophy (LVWT >30 mm). With respect to therapies rendered, 83 patients (28.3%) had a surgical myectomy, and 25 patients (8.5%) had received an ICD.

Malignant mutations. Only 3 of 293 patients (1%) were found to possess one of the previously published “malignant” mutations (Fig. 2A to 2C). All three individuals harboring a malignant mutation were diagnosed with HCM before age 25 (3/53 vs. 0/240, p < 0.006). Table 2 summarizes the clinical profiles of the three patients found to possess a “malignant” mutation.

None of the 293 patients had the R403Q mutation by DHPLC. The absence of this specific mutation in this HCM cohort was confirmed using a mutation-specific restriction enzyme assay (data not shown). No patients were identified with the R719W mutation either. However, one patient had a published R719Q mutation indicating that DHPLC can resolve sequence variations at this site (data not shown).

Case 1. The R453C-MHY7 defect was found in an 11-year-old male patient despite no family history of HCM or sudden death (Fig. 2A). He was diagnosed during infancy after evaluation of a murmur. Calcium channel and beta-blocker therapy was initiated at age 8 after serial observation of increasing LVWT. He had extreme hypertrophy (38-mm septum) with maximal obstruction extending 4 cm below the aortic valve. At 11 years of age, a surgical myectomy was attempted, but the gradient was not eliminated completely. Two months later, he had a documented episode of symptomatic ventricular tachycardia. He was re-evaluated, and ventricular tachycardia was induced during an electrophysiology study. An ICD was implanted in March 2000. Thus far, there have been no shocks delivered.

Case 2. The G716R-MHY7 defect was found in a 32-year-old man with a strong family history of both HCM and SCD (Fig. 2B). He was known to have a murmur in childhood and was diagnosed with obstructive HCM in his early 20s after presenting with palpitations. He has never had a syncopal episode. Of his 10 siblings, 4 have echocardiographic documentation of obstructive HCM with asymmetric septal hypertrophy. There have been three sudden deaths in his immediate family including his mother at age 46 and two sisters—ages 16 and 42. His echocardiogram demonstrates a resting gradient of 77 mm Hg and severe septal hypertrophy with a maximal LVWT of 26 mm. He is now on beta-blockers and has received an ICD as primary prevention.

Case 3. A third patient possessed the R92W-TNNI2 mutation (Fig. 2C). This 24-year-old woman was diagnosed at 20 years of age after experiencing palpitations and mild chest pain. She had a syncopal episode. Of her 10 siblings, 4 have echocardiographic documentation of obstructive HCM with asymmetric septal hypertrophy. There have been three sudden deaths in his immediate family including his mother at age 46 and two sisters—ages 16 and 42. His echocardiogram demonstrates a resting gradient of 77 mm Hg and severe septal hypertrophy with a maximal LVWT of 26 mm. He is now on beta-blockers and has received an ICD as primary prevention.
dyspnea with exercise. Despite her TNNT2 substrate, she has echocardiographic evidence of extreme hypertrophy involving the entire septum but without any obstruction (LVWT = 33 mm). There is no family history of SCD. Her mother was diagnosed clinically with a “mild variant of HCM” based upon her echocardiogram at age 56. She has the R92W mutation as well. In contrast to her daughter, the hypertrophy was mild (16 mm), nonobstructive and localized to the midseptal region.

**DISCUSSION**

**Genetic heterogeneity in HCM.** Hypertrophic cardiomyopathy is the final common pathway of several different sarcomeric defects. To date, over 150 mutations have been reported in nine different genes encoding various elements of the sarcomere. Mutations are scattered throughout these genes. Unlike diseases like cystic fibrosis where a single mutation (F508del) within a single gene (CFTR) causes the majority of cases, an HCM “hot-spot” does not exist (33). It would appear that new mutations are being discovered continually in the nine known HCM-causing genes, and it is likely that for many families with HCM the specific disease-causing mutation will be a unique one. In this respect, our own exon-targeted screening of MYH7 and TNNT2 revealed four novel mutations (unpublished data, M. J. Ackerman, January 2001). These factors will limit the widespread application of a future HCM gene chip containing only those mutations already identified. In addition to the complexities introduced by genotype-specific interactions with environmental influences and gene modifiers, significant technological challenges must be surmounted before routine HCM genotyping using gene chips becomes a clinical reality.

**Malignant versus benign mutations.** There is continued debate over the prognostic significance of HCM causing mutations. For every genotype-phenotype association there are exceptions, and these constitute a major impediment to the use of genotyping alone as a clinical and prognostic tool for the individual patient. Initially, the four mutations in MYH7 were assigned the malignant phenotype based on studies involving a limited number of families: five families with R403Q, one family with R453C, one family with G716R and four families with R719W (17,19,20,23,24). Several exceptions to these associations have been found. Marian et al. (21) reported a R403Q family where some affected members had only mild symptoms of HCM. Another R403Q Korean kindred has been reported having no SCD in the family (34). The G716R mutation has been reported in another small family with no history of SCD (17). The R92W-TNNT2 mutation is associated with minimal hypertrophy and high risk of SCD (10). However, in our study, the R92W-TNNT2 proband had extreme hypertrophy while her similarly affected mother did not, and there have been no sudden deaths in the family. Other confounding factors include the variability and phenotypic expression within individual families, the small size of many family studies, modifier genes, the role of polymorphisms and other nongenetic factors. Taken together, these observations weaken the premise that risk for SCD can be associated with any certain mutation. We do not yet have the understanding of HCM necessary to determine which mutation, combinations of mutations or combinations of mutations and environmental factors portend an ominous clinical outcome.

In this study, 1% of the HCM patients possessed one of the more frequently described “malignant” mutations involving the MYH7 and TNNT2 genes. Interestingly, only age predicted the presence of one of these “malignant” mutations. Three of 53 patients <25 years (5.7%) had 1 of 5 “malignant” mutations compared with none of the 240 patients over 25 years. No other factors including degree of hypertrophy, family history of HCM or sudden death were associated with this small subset of patients. Of the 40 patients presenting with cardiac symptoms and a family history of SCD, 1 had a malignant mutation (Case 2). Of the 18 patients manifesting extreme left ventricular hypertrophy (>30 mm), malignant mutations were identified in 2 patients. Moreover, the individual with the most significant number of sudden deaths (4) in the family did not have any of the five mutations analyzed for in this series. Given the profound genetic heterogeneity of HCM, the variability of clinical presentation, despite the same mutation profile, and the exceedingly low prevalence of “malignant” mutations in a high-risk tertiary center for HCM, genetic screening is not an appropriate test for risk assessment.

**Study limitations.** The proportion of patients in this cohort with obstructive HCM (53%) is higher than previously reported in population-based studies. This discrepancy likely stems from our institution’s experience in surgical myectomy/myotomy and referral patterns for surgical treatment of LVOTO. Previous reports of TNNT2 mutations indicate a phenotype of minimal hypertrophy (35). Therefore, TNNT2 mutations may be under-represented in our cohort.

This study was designed as an antemortem study to determine the frequency of “malignant” mutations in individuals still living. The low prevalence of “malignant” mutations detected may be due to screening a cohort of HCM patients alive with their disease at an average age at diagnosis of 42.5 years. In this study, all three patients having a “malignant” mutation were diagnosed before 25 years of age. However, based upon the published literature, these “malignant” mutations should have been represented in our cohort. The life expectancy for individuals with R403Q (MYH7), R719W (MYH7) and R92W (TNNT2) mutations was 33 to 38 years old (17,23,25). The mean age at SCD was 30 years for both R453C and G716R (MYH7) mutations (19,24). Moreover, 95 individuals in our cohort had a positive family history of HCM, and 69 probands had family members who had died suddenly due to their HCM. However, even in this high-risk subset with a
Figure 2. “Malignant” mutation detection by denaturing high-performance liquid chromatography. Depicted are the elution profiles for normal samples and the “malignant” mutations identified in exon 14 (A), exon 19 (B) of the cardiac beta-myosin heavy chain (MHY7) gene and exon 9 (C) of the troponin T (TNNT2) gene.
declared malignant phenotype, only 1 of 69 probands (G716R, 1.4%) possessed a “malignant” mutation. The other four “malignant” mutations were absent in this high-risk subset.

Nonetheless, this patient cohort reflects the individuals who at presentation are seeking genetic testing for prognostic purposes. Thus, in this patient population, these “malignant” mutations are rare. Perhaps those individuals harboring one of these malignant mutations had already died and were not represented (35). Only a future study involving a molecular autopsy for these particular mutations in deceased patients with HCM could determine if there is a difference in the frequency of these so-called “malignant” mutations among the living and the dead.

CONCLUSIONS

Hypertrophic cardiomyopathy is the final common pathway for a large number of sarcomeric perturbations. The exact mechanisms mitigating hypertrophy and SCD remain unknown. Although it has been suggested that routine screening should be done for “malignant” mutations, our evaluation of a tertiary referral population of individuals with HCM suggests that such a targeted screen will elucidate very few cases. Informing a patient that they have a “benign” or “malignant” mutation has serious clinical implications. Because the genotype/phenotype associations are not unequivocally known, the clinical decision to proceed with

Table 2. Patient Profiles

<table>
<thead>
<tr>
<th>Index Case</th>
<th>Age/Gender</th>
<th>Age at Dx</th>
<th>Presentation</th>
<th>LVWT (mm)</th>
<th>Peak Gradient (mm Hg)</th>
<th>FH of HCM</th>
<th>FH of SCD</th>
<th>Treatment(s)</th>
<th>Mutation</th>
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<td>murmur</td>
<td>38</td>
<td>70</td>
<td>neg</td>
<td>neg</td>
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<td>R453C-MYH7</td>
</tr>
<tr>
<td>2</td>
<td>32/M</td>
<td>24 yrs</td>
<td>palpitations</td>
<td>26</td>
<td>80</td>
<td>pos</td>
<td>pos</td>
<td>prophylactic ICD, beta-blocker</td>
<td>G716R-MHY7</td>
</tr>
<tr>
<td>3</td>
<td>24/F</td>
<td>20 yrs</td>
<td>palpitations</td>
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<td>None</td>
<td>pos</td>
<td>neg</td>
<td>none</td>
<td>R92W-TNNT2</td>
</tr>
</tbody>
</table>

Dx = diagnosis; FH = family history; HCM = hypertrophic cardiomyopathy; ICD = internal cardioverter defibrillator; LVWT = left ventricular wall thickness; MYH7 = cardiac beta-myosin heavy chain; SCD = sudden cardiac death; TNNT2 = troponin T.
primary prevention ICD therapy should not consider the patient’s fundamental disease-causing genetic substrate.

Despite this present limitation in molecular genetic testing for HCM, yesterday’s unraveling of the molecular basis for HCM as a primary genetic disease of the sarcomere combined with new insights from genotype-phenotype correlative studies have provided the framework for tomorrow’s improved understanding of the natural history and prognosis for a patient diagnosed with HCM. Although not yet a routine clinical test, HCM genotyping is already making a profound impact in the preclinical diagnosis of family members where, in fact, the HCM-causing mutation has been identified. In this situation, an unambiguous identification of relatives who do or do not possess the underlying genetic substrate for HCM is not only possible, but also clinically invaluable.

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REFERENCES