Prevalence and Spectrum of Thin Filament Mutations in an Outpatient Referral Population With Hypertrophic Cardiomyopathy

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**Background**—Thin filament mutations are reported to cause ≈20% of cases of hypertrophic cardiomyopathy (HCM), and they have been associated with specific phenotypes. However, the frequency of these mutations and their associated phenotype(s) from a large tertiary referral center population are unknown.

**Methods and Results**—DNA was obtained from 389 unrelated patients with HCM. A mutational analysis of all protein coding exons of cardiac troponin T, cardiac troponin I, α-tropomyosin, and cardiac actin was performed using polymerase chain reaction, denaturing high-performance liquid chromatography, and DNA sequencing. The clinical data were extracted from patient records and maintained independent of the patient genotype. Overall, only 18 patients (4.6%) harbored isolated thin filament mutations: 8 had troponin T mutations, 6 had troponin I mutations, 3 had α-tropomyosin mutations, and 1 had an actin mutation. Of the 12 unique missense mutations identified, 9 (75%) were novel mutations. As a group, patients with thin filament mutations were not significantly different from the rest of the cohort in age at diagnosis, left ventricular wall thickness, left ventricular outflow tract obstruction, or family history of HCM or sudden cardiac death.

**Conclusions**—Mutations in genes encoding thin filament proteins are less prevalent in HCM than previously estimated. Patients with mutations in troponin T, troponin I, α-tropomyosin, and actin do not invariably present with any distinct clinical feature, thus limiting the utility of gene status for risk stratification or of clinical phenotype in guiding individual genetic screening at this time. (*Circulation. 2003;108:445-451.)*

**Key Words:** hypertrophy ■ cardiomyopathy ■ genetics ■ death, sudden

Once thought to be a rare and deadly disease, it is now known that hypertrophic cardiomyopathy (HCM) is relatively common (prevalence, ≈0.2%) and leads to diverse clinical phenotypes.1,2 Patient outcomes range from an asymptomatic course with normal longevity to chronic progressive heart failure or unexpected sudden cardiac death (SCD). HCM remains the leading cause of cardiac death in young people.3 The first genetic cause for HCM was defined in 1990 as a mutation in the gene encoding β-myosin heavy chain (*MYH7*).4 Since that time, >150 different mutations have been defined in 10 different sarcomeric genes.3,5,6

Previous reports estimated that mutations in these sarcomeric genes account for ≈80% of all cases of HCM.7-9 Mutations in the thick filament (β-myosin heavy chain and the regulatory and essential light chains) and myosin binding protein-C are estimated to cause ≈50% of HCM. Thin filament mutations are estimated to cause ≈10 to 30% of HCM. Cardiac troponin T (*TNNT2*) mutations have been cited to cause up to 20% of HCM,10,11 cardiac troponin I (*TNNT3*) ≈5%,12,13 α-tropomyosin (*TPM1*) mutations ≈5%,11,14,15 and cardiac actin (*ACTC*) <5%.16,17 Mutations in the thin filament genes have been associated with specific clinical phenotypes.12-15,18-20

These estimated frequencies and phenotypic associations were based on the data from early linkage studies of small, selected cohorts, usually from expansive families with high penetrance and disease expressivity.7-9 However, these patients may not be representative of the outpatient population, which represents the majority of patients with HCM in a tertiary referral center. These estimates of mutation frequency may also be subject to publication bias, because small cohorts with negative genetic screening results may not be reported. Therefore, it was our aim to determine the frequency of thin filament HCM present in a large cohort of unrelated HCM patients seen at a tertiary referral center through a comprehensive mutational analysis of the 4 thin filament genes.
implicated in the pathogenesis of HCM. We also sought to determine the phenotypic correlates associated with HCM due to perturbations in the cardiac thin filament.

**Methods**

**Clinical Characterization of Unrelated HCM Cases**

During a 4.5-year period between April 1997 and December 2001, 434 patients were evaluated at the Mayo Clinic’s HCM Clinic in Rochester, Minnesota. With standard clinical assessment of family history, relatedness was excluded through three degrees (ie, first degree, parent or sibling; second degree, grandparent, aunt, or uncle; third degree, great-grandparent, cousin, etc). Accordingly, this assessment led to the exclusion of 45 relatives. When relatives were identified, the patient who was evaluated at this institution first was included for analysis. This study was therefore confined to 389 unrelated patients (age, 41.3 \pm 19 years; 215 men). Each of these subjects met the clinical diagnostic criteria for HCM of a left ventricular wall thickness (LVWT) \(\geq 13\) mm in the absence of another confounding diagnosis. A blood sample was provided for molecular genetic testing after obtaining informed, written consent in accordance with study protocols approved by the Mayo Foundation Institutional Review Board.

**Mutational Analysis**

Purgene DNA extraction kits (Gentra, Inc) were used to extract patient genomic DNA from peripheral blood lymphocytes. Previsously published intron/exon-based primers or novel primers were used to amplify the protein-coding exons of the 4 thin filament genes (TNNT2, TNNI3, TPM1, and ACTC) from genomic DNA from each of the 389 patients by polymerase chain reaction. Sequence variations were detected by denaturing high performance liquid chromatography (DHPLC) using the Transgenomic WAVE system, as previously described. Primer sequences and conditions for polymerase chain reaction and DHPLC are indicated in the Data Supplement Table. Abnormal DHPLC elution profiles were further characterized by automated dye terminator cycle-sequencing using an ABI Prism 377 (Applied Biosystems). When novel sequence variations were identified, DNA samples from 100 healthy white and 100 healthy black individuals (Coriell Cell Repositories) were analyzed to exclude the variant’s presence as a common polymorphism in unaffected individuals.

**Statistical Analysis**

Differences between continuous variables were assessed using unpaired t tests. Nominal variables were analyzed using contingency tables or z-tests, where appropriate. A probability value <0.05 was considered statistically significant.

**Results**

**Characterization of the HCM Cohort**

Table 1 summarizes the demographics for the 389 unrelated individuals with HCM. The mean age at diagnosis was
41.3 ± 19 years. At presentation to Mayo Clinic, 216 patients (55.5%) had cardiac symptoms. Approximately one-third (30.8%) had a family history of HCM involving a first-degree relative (parent, sibling, or offspring), and 14.4% had a family history of unexplained SCD involving a first-degree relative. The mean maximal LVWT was 21.5 ± 7 mm. Of the 389 patients, 161 patients underwent a surgical myectomy (41.4%), and 60 patients have an implanted cardioverter-defibrillator (15.4%).

Spectrum of Thin Filament Mutations

Figure 1 depicts the genes encoding the 4 thin filament proteins and the locations of the mutations identified. Overall, 12 unique missense mutations were identified, including 9 novel mutations. Each of these 9 novel mutations was absent in 400 reference alleles.

### Troponin T

Five missense mutations in TNNT2 were identified in 8 patients with HCM (2.1%). Two were novel mutations: D86A (1 patient) and R286H (2 patients). The remaining 3 mutations, R92W (1 patient), R278P (1 patient) and R278C (3 patients), have previously been reported in association with HCM. In these 8 patients, HCM was diagnosed at 37.2 ± 18 years. This subset of patients had a mean LVWT of 23.4 ± 6 (range, 15 to 32 mm), which was not statistically different from the remainder of the cohort. In the present study, no patient with a TNNT2 mutation had SCD in a first-degree relative.

### Tropomyosin

Three novel TNNI3 missense mutations were identified in 6 patients: R162Q (1 patient), R141Q (2 patients), and S166F (3 patients). In these 6 patients, HCM was diagnosed at 37.2 ± 18 years. This subset of patients had a mean LVWT of 23.4 ± 6 (range, 15 to 32 mm), which was not statistically different from the remainder of the cohort. In the present study, no patient with a TNNI3 mutation had SCD in a first-degree relative.

### Actin

Three novel ACTC missense mutations were identified in 3 patients: A232V (1 patient), I172T (1 patient), and M281T (1 patient). In these 3 patients, HCM was diagnosed at 37.2 ± 18 years. This subset of patients had a mean LVWT of 23.4 ± 6 (range, 15 to 32 mm), which was not statistically different from the remainder of the cohort. In the present study, no patient with an ACTC mutation had SCD in a first-degree relative.
(3 patients). Five of the 6 patients had obstructive HCM with a mean resting peak gradient of 88 ± 21 mm Hg. All 5 required a septal myectomy. Of the 6 patients with TNNI3 mutations, 3 were diagnosed before 30 years of age. None of the 34 patients with apical hypertrophy in our study had a TNNI3 mutation, and the age at onset was not statistically different from the cohort as a whole (44.3 ± 24 versus 41.3 ± 19 years).

**α-Tropomyosin**

Three novel mutations were identified in TPM1 (0.8%), each in a single patient (I172T, L185R, and M281T). Two of these 3 individuals had a family history of HCM and SCD in a first-degree relative. One previously reported patient (index case 16) was asymptomatic at 42 years, but 2 of his 3 mutation-positive children died of SCD due to HCM before 9 years of age.\(^{23}\)

**Actin**

One actin mutation was identified (A232V) in a 48-year-old female patient. She was diagnosed at 41 years when she presented with angina and dyspnea. She had a first-degree relative with HCM and a 40-year-old sibling who died from SCD.

**Discussion**

**Cohort Analysis**

The 389 patients evaluated in this study represent unrelated patients seen at a tertiary referral center with known expertise in the surgical management of HCM. When compared with a cohort of 744 patients seen in 3 regional centers, the age at initial evaluation and LVWT are not significantly different.\(^{24}\) However, our cohort has a larger representation of patients with outflow tract obstruction (53% versus 22% in regional
centers) and myectomy (41% versus 5% in regional centers). Therefore, our cohort is comparable in age and degree of hypertrophy to the population of patients with HCM, but may be over-represented with patients having significant outflow tract gradients secondary to surgical referral bias.

Frequency of Thin Filament Mutations in a Tertiary HCM Referral Center

In the present study, we found that <5% of unrelated HCM patients had thin filament mutations. It has been suggested that as many as 20% of HCM cases may be due to mutations in the \( TNNT2, TNNI3, TPM1, \) or \( ACTC \) genes.\(^7\) However, these estimates have been drawn from the pioneering sentinel studies that established HCM as a molecular disease of the sarcomere.\(^11,12\) By necessity, these linkage studies involved selected, multigenerational pedigrees having a high penetrance of the underlying HCM genotype.

Studies of mutation frequency in large, unselected cohorts are lacking. Analysis of a small cohort of Finnish patients for \( TNNI3 \) (37 unrelated patients) and \( ACTC \) (40 unrelated patients) revealed no causative mutations in either of these genes.\(^25,26\) Our analysis of a large cohort of unrelated patients seen in a tertiary HCM referral center confirms the relative rarity of thin filament HCM.

Phenotype of Patients With Thin Filament HCM

Several genotype-phenotype correlations have been suggested for genes of the thin filament.\(^7\) \( TNNT2 \)-HCM has been associated with minimal hypertrophy and a high risk for SCD.\(^18\) In the present study, the 8 \( TNNT2 \)-positive individuals did not have significantly less hypertrophy than the rest of the cohort, and none of the 8 had a family history of SCD.

\( TNNI3 \) mutations have been associated with apical HCM and elderly onset of disease.\(^12,13\) Of the 6 patients identified with \( TNNI3 \) defects, none had apical hypertrophy, and 3 were diagnosed before 30 years of age. HCM due to perturbations of \( TPM1 \) have been associated with variable outcomes.\(^14,15,19,20\) In our cohort, 2 of the 3 individuals with \( TPM1 \)-HCM had a family history of SCD. Finally, several reported cases of \( ACTC \)-HCM have presented with apical hypertrophy.\(^17\) The one patient with the \( ACTC \) mutation in this cohort did not have apical hypertrophy.

The results of the present study warrant caution for application of prior genotype-phenotype associations for individual HCM patients based solely on the HCM-causing gene mutation. This is consistent with our previous studies, which demonstrated that the presence or absence of specific HCM mutations previously annotated as “malignant” or “benign” should not be used for prognosis or risk stratification.\(^27,28\)

From our previous studies, it also seems that the phenotype may not be a reliable predictor of the specific causative mutation.\(^27,28\) In the subgroup of individuals harboring thin filament defects (\( n = 18 \)), no statistical difference was evident when compared with the rest of the cohort. Our findings suggest that individuals with thin filament mutations had a similar degree of hypertrophy, age at presentation, and incidence of a family history of SCD as those without such mutations. Because of the rarity of thin filament mutations, however, definitive phenotypic correlations will be difficult to establish until mutation identification becomes more technically feasible for additional large cohorts. Presently, it cannot be concluded on the basis of this large cohort of unrelated HCM patients whether or not thin filament HCM is “thinner” than HCM mediated by thick filament mutations, as has been suggested previously.\(^29\)

Putative Functional Consequence of HCM-Causing Mutations in This Cohort

Figure 2 illustrates the normal structural relationships of the thin filament. HCM-causing mutations in the thin filament most often lie in domains lying at the interface of 2 proteins and lead to increased calcium sensitivity.\(^30-32\) The mutations reported herein also reside within established protein-protein binding domains. In \( TNNT2 \), the D86A and R92W mutations lie in the TPM1-binding region. R286H-\( TNNT2 \) and R278C/P-\( TNNT2 \) lie in the TPM1 and troponin-binding domain. The \( TNNI3 \) mutations identified occur in functionally significant domains. R141Q-\( TNNI3 \) lies within the “minimum inhibitory sequence” (residues 137 to 148), and both R162Q and S166F reside within the troponin C binding site.\(^21\) In \( TPM1 \), 2 of the novel mutations reported here (\( I172T \) and \( M281T \)) affect residues at the TPM1 dimer interface. However, the L185R-\( TPM1 \) mutation alters a residue on the outside surface of the fiber within the troponin-binding domain. The severe phenotype seen in the family with this mutation may be due to functionally distinct effects on troponin binding rather than filament stability.\(^23\) Finally, the A232V-\( ACTC \) mutation lies within the TPM1-binding domain.

Study Limitations

It is possible that our mutational analysis by DHPLC failed to detect mutations present in this cohort, which would account, in part, for the apparent low yield of thin filament HCM.
However, the sensitivity of DHPLC for mutation detection has been established as >95%. It is well known that the sensitivity of DHPLC is context-dependent. Therefore, for this study, DHPLC conditions for each amplicon were individually optimized. Of the 37 amplicons analyzed, 3 required >1 melting temperature to ensure comprehensive coverage of the amplicon by temperature-modulated heteroduplex analysis. Two GC-clamped primers were necessary. Our own internal quality control studies of our optimization methods using direct sequencing as the gold standard indicate a sensitivity of mutation detection by DHPLC of 100% (data not shown).

As previously noted, analysis of the clinical presentation of our cohort indicated an increased percentage of patients with significant outflow tract obstructions and a greater number of patients undergoing surgical myectomy for symptom relief. Otherwise, our cohort was similar to unselected regional center patients. Although it is important to recognize this selection bias, studies of selected cohorts have in the past provided the foundation for understanding HCM genetics. Similarly, careful analysis of our cohort provides the opportunity for a unique perspective and new insights for this heterogeneous disease.

Although we excluded individuals from the cohort who were clinically identified as first-, second-, or third-degree relatives, we did not use molecular haplotype analysis to rule out the possibility of relatedness in a more distant manner. Therefore, our 5% frequency of thin filament HCM could be an underestimate if there is a founder effect (ie, distant common ancestor) for several individuals in the cohort who were negative for thin filament mutations. However, if the individuals identified with identical thin filament mutations share a common ancestor, then the stated 5% frequency would overestimate the true prevalence of thin filament HCM.

Previous studies determined genotype-disease severity correlations based on survival curves for mutation-positive family members. Herein lies a major difference in the perspective brought by the present study. Our cohort included 389 unrelated individuals, and we searched for genotype-phenotype correlation in these individuals, who do not necessarily share genetic and environmental modifiers. This study design, although it precludes analyses such as Kaplan-Meier survival curves or mutation cosegregation within families, reveals that these modifiers may play a major role in the presentation and course of this disease, because correlations previously defined using related individuals were not found in this unrelated cohort. Although segregation data would provide additional evidence to substantiate the ascribed pathogenic status of the 9 novel mutations, none of these mutations have been identified in the healthy population, as based on previous studies, the National Center for Biotechnology Information database, and our own analysis of 400 reference alleles. In addition, the anticipated functional consequence of these mutations, based on their position in the protein product, is consistent with firmly established pathogenic mechanisms for HCM.

Finally, because of the rarity of mutations in the thin filament found in our study, it is difficult to achieve statistical significance for genotype-phenotype correlation in unrelated family members. Thus, despite evaluating nearly 400 unrelated cases of HCM, we are unable to statistically confirm or refute previous genotype-phenotype correlations implicated for thin filament HCM. As the technology for high-throughput genetic screening advances, the potential to elucidate meaningful associations between genetic cause and clinical presentation may become a reality.

**Conclusions**

In this cohort of 389 unrelated HCM patients from an outpatient tertiary referral center, thin filament mutations were uncommon (<5%) and were not distinguishable from non-thin filament causes of HCM by patient age at presentation, clinical course, family history, or anatomic type. Further study of genetic and environmental modifiers may reveal determinants mitigating the profound phenotypic variability seen in HCM.

**Note Added in Proof**

While this manuscript was in press, Richard and colleagues reported the distribution of HCM-causing mutations among 197 unrelated index cases and identified 16 patients (8%) with thin filament HCM.

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**References**


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