Genotype-Phenotype Correlations in Hypertrophic Cardiomyopathy
Insights Provided by Comparisons of Kindreds With Distinct and Identical β-Myosin Heavy Chain Gene Mutations

Lameh Fananapazir, MD, FRCP, Neal D. Epstein, MD

Background We have previously described two distinct mutations in the β-myosin heavy chain gene with markedly different clinical presentations and outcome: The 908Leu→Val mutation was associated with a low disease penetrance and a benign prognosis. In contrast, the 403Arg→Gln mutation in a Caucasian kindred was associated with a 100% disease penetrance and high incidence of sudden cardiac death. Recently, another mutation, 606Val→Met, has been reported to be associated with “near normal survival” and offered as evidence for the benign nature of neutral charge substitutions.

Methods and Results We report (1) a large kindred (245 family members at risk of inheriting the disease gene) with a 256G→C mutation characterized by a similar disease penetrance in adults and in children (56% and 60%, respectively) and a cumulative sudden cardiac death rate of only 2% at 50 years of age, (2) a kindred with the 606Val→Met mutation with four sudden cardiac deaths in eight affected individuals, and (3) a Korean kindred with the 403Arg→Gln mutation. Although the disease occurred early and was associated with a high prevalence of myocardial ischemia in both of our kindreds with the 403Arg→Gln mutation, no sudden cardiac death or syncope has occurred in the Korean kindred. Furthermore, in the Caucasian kindred, all patients had nonobstructive hypertrophic cardiomyopathy, but most of the patients in the Korean kindred had left ventricular outflow obstruction.

Conclusions The conclusions are as follows: (1) Although several sudden cardiac deaths are sufficient to establish that a mutation is malignant, study of a large kindred is necessary to be certain that a mutation is benign. To date, only the 908Leu→Val and the 256G→C mutations satisfy this requirement. (2) The 256G→C mutation demonstrates that not all mutations that result in a charge change are malignant. (3) Conversely, the 606Val→Met mutation is malignant in some kindreds; hence, despite the absence of a charge change, minor substitutions in critical regions of β-myosin heavy chain protein may also have serious consequences. (4) The diverse ethnic origins of the two 403Arg→Gln kindreds provide evidence suggesting that the identical mutation occurred independently and was associated with different genetic backgrounds. Their distinct phenotypes underline the importance of modifying genes and nongenetic factors.

Key Words • hypertrophic cardiomyopathy • genetics • sudden death • missense mutation • myosin heavy chain

Hypertrophic cardiomyopathy (HCM) is a primary myopathy with an autosomal pattern of inheritance, characterized by increased left ventricular (LV) wall thickness in the absence of another cause for the increased cardiac mass. Patients with HCM are often symptomatic and are prone to arrhythmias and sudden cardiac death. In some kindreds, the disease has been linked to the β-myosin heavy chain (β-MHC) gene locus on chromosome 14q1. Hypertrophic cardiomyopathy has been associated with substitutions of single highly conserved amino acids in the head or head-rod junction of the β-MHC molecule. We have previously reported that clinical expression has differed significantly in two kindreds with distinct β-MHC gene mutations: The 908Leu→Val mutation has been associated with low disease penetrance and a benign prognosis. In contrast, the 403Arg→Gln mutation has been associated with expression of the phenotype in all individuals with the disease allele and a high incidence of premature sudden cardiac death. Recently, another mutation, 606Val→Met, has been reported to be associated with “near normal survival” and offered as evidence for the benign nature of neutral charge substitutions compared with β-MHC gene mutations that result in a charge change. The present study reports on further insights that are gained by characterizing the clinical expression of HCM in kindreds with a distinct β-MHC gene and comparing kindreds with identical missense mutations in this gene.

See p 499

Methods

Informed consent for the phenotype-genotype studies was obtained in every patient in accordance with study protocols (87-H-57 and 91-H-50) approved by the institute review board of the National Heart, Lung, and Blood Institute.

Determination of Phenotype

Evaluation of the phenotype was completed before determination of the genotype. Family members at risk of inheriting the disease gene were assessed by 12-lead ECG and M-mode, two-dimensional, and Doppler echocardiography. A real-time pulsed-array 90° ultrasonic scanner (Sonos 1000, Hewlett-Packard) with 2.5- and 5.0-MHz transducers was used to record the echocardiograms. Standard transducer positions

Received June 30, 1993; revision accepted October 19, 1993.
From the Inherited Cardiac Diseases Section, Cardiology Branch, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, Md.
Reprint requests to Lameh Fananapazir, MD, FRCP, Codirector, Cardiac Diseases Section, Head, Clinical Electrophysiology Laboratory, Cardiology Branch, Room 7B14, Bldg 10, National Institutes of Health, Bethesda, MD 20892.
were used to obtain two-dimensional images in a number of cross-sectional planes. The distribution of LV hypertrophy was assessed primarily in the parasternal short-axis plane, although parasternal long-axis apical two- and four-chamber views were also used to integrate the information obtained from the short-axis images. Because the 403AA→Gln mutation has been associated with a poor prognosis,16,17 patients with this mutation were evaluated additionally by radionuclide angiography, exercise thallium scintigraphy, cardiac catheterization, and electrophysiological study, as part of ongoing efforts to risk-stratify HCM patients (study protocol 84-H-232). Details of these investigations have been reported previously.30-33

**Determination of Genotype**

**Polymerase chain reaction and Southern blot analysis.** Each family member was phlebotomized, and DNA was extracted from isolated nuclei of peripheral white blood cells by procedures described previously.34 The published sequence of the human β-MHC gene35 was used to design a set of intronic primers, each of which encompassed one of the 40 β-MHC gene exons and yielded a single unique fragment of the expected size in a polymerase chain reaction (PCR) amplification. Intronic primers were used to avoid coamplification of the highly homologous α-MHC gene. PCR was performed in a 100-μL volume using the AmpliTaq enzyme (Perkins Elmer Cetus, Norwalk, Conn) according to the manufacturer’s recommendations. The denaturation, annealing, and extension segments were for 1, 2, and 3 minutes, respectively. Thirty-five cycles were performed. Annealing temperatures of ≥61°C were used. Radioactive labeling of the amplified fragment was accomplished through the addition of 0.1 μCi of [α-32P]dCTP (3000 Ci/mmol, Amer sham, Chicago, Ill) to the 100-μL reaction volume.

**Single-strand conformation polymorphism detection.** Polymorphisms were detected by single-strand conformation polymorphism (SSCP) analysis of PCR-amplified fragments encompassing each of the 40 β-MHC gene exons using a modification of the procedure described by Orita et al.36 Briefly, 1 μL of a 100-μL reaction was diluted with 9 μL of a denaturing solution (95% formamide, 20 mM/μL EDTA, 0.05% bromophenol blue, and 0.05% xylene cyanol), heated to 80°C, plunged into an ice bath, and resolved on a 5% polyacrylamide and 10% glycerol gel run at 20 W at room temperature and also on a 5% polyacrylamide gel run at 4°C.

**Sequencing.** The PCR fragments showing polymorphisms by SSCP analysis were sequenced without subcloning through the chain-termination technique using a modification of a method described previously37 and a Sequenase kit (USB, Cleveland, Ohio). Briefly, a biotin phosphoramidite (Midland Reagent Co, Midland, Tex) was used to add a biotin molecule to the 5’ end of an oligonucleotide primer in the last step in its synthesis on the nucleic acid synthesizer (model 380B, Applied Biosystems, Foster City, Calif). This primer, together with a second primer that was not biotin-labeled, was used to generate the PCR-amplified fragment to be sequenced. Twenty microliters of this product was then incubated at room temperature with 20 μL of magnetic beads bound to streptavidin (Dynal, Oslo, Norway) for 15 minutes. The product, now bound to beads, was then denatured with 0.2N NaOH and washed with 1× Tris/EDTA and then water. After collection with a magnet, the beads were resuspended in 7 μL of water, and the bound template was sequenced using the complementary primer according to the manufacturer’s recommendations.

**Statistics**

Cumulative survival was determined by product-limit survival analysis using premature (<50 years of age) sudden cardiac death and syncope accompanied by discharge by an implantable defibrillator as time variables. Two product-limit survival functions were compared using the log-rank test. Contingency tables were evaluated by χ² and Fisher’s exact tests. A value of P<.05 was considered significant.

**Results**

**Clinical Consequences of the 256G→C Mutation in Kindred 2280**

We have reported the 256G→C mutation previously.38 To determine the phenotypic consequences of this mutation in kindred 2280, we evaluated 245 family members at risk of inheriting the 256G→C mutation. A subset of this pedigree is depicted in Fig 1.

The disease allele was demonstrated in 39 individuals: 34 adults (aged 23 to 82 years) and 5 children (aged 1 to 11 years). Three family members in whom the disease allele was absent were excluded from the study because of the presence of aortic valve disease (n=1) and atrial septal defect (n=2).

**Disease penetrance.** Fig 2 shows the relation between LV wall thickness as a function of age in individuals with the disease mutation and family members in whom the mutation was absent. The maximal LV wall thickness in 117 individuals aged ≥20 years in whom the disease allele was absent ranged from 4 to 14 mm. Notably, the maximal LV wall thickness was ≤14 mm in 15 (44%) of the 34 adult individuals with the 256G→C mutation and ranged from 15 to 27 mm in the remaining 19 individuals with the disease allele (Fig 2). The pattern of LV hypertrophy suggested that the LV wall thickness did not increase significantly beyond the age of 20 years (Fig 2). There was clinical and echocardiographic evidence of LV outflow tract obstruction in only 5 (13%) of these patients. Of the 15 individuals with the 256G→C muta-
tion who had LV wall dimensions that were within the normal range, two had enlarged left atria (45 and 49 mm, respectively). In no patient has the disease progressed to LV wall thinning and diminished LV systolic function. LV hypertrophy was present in 3 of the 5 young individuals with the disease allele (Fig 2). Two of these children had abnormal 12-lead ECGs. The 12-lead ECG was abnormal in all of the 7 adults with normal echocardiograms in whom the disease allele was present and who were aged ≥45 years. However, the 12-lead ECG was also abnormal in 13 (39%) of 33 individuals without the mutation who were aged ≥45 years. By comparison, the 12-lead ECG was abnormal in 2 of the 8 individuals aged <45 years with the disease allele who had normal echocardiograms and in one (prolonged PR interval) of the 172 individuals aged <45 years without the mutation.

Prognosis. Only one premature sudden cardiac death has occurred in this large kindred. Thus, despite the positive charge change, prognosis has been excellent in patients with this mutation: 2% cumulative incidence of cardiac events at 50 years of age.

Clinical Consequences of the 606\textsuperscript{Val}→\textsuperscript{Met} β-MHC Gene Mutation

We studied the clinical consequences of the 606\textsuperscript{Val}→\textsuperscript{Met} mutation, illustrated in Fig 3, in kindred 2206 consisting of 17 family members. Four sudden cardiac deaths have occurred between the ages of 15 to 27 years in 8 patients with HCM (Fig 4). In addition, a 45-year-old highly symptomatic patient has developed chronic atrial fibrillation and LV wall thinning associated with diminished ejection fraction. At electrophysiological study, performed because of recurrent syncope and the strong family history of sudden cardiac death, a sustained monomorphic ventricular tachycardia was induced with two premature extrastimuli from the right ventricular apex. Subsequently, there have been two episodes of syncope accompanied by discharges by an implantable defibrillator device. Thus, despite the neutral charge change, the prognosis for patients with the 606\textsuperscript{Val}→\textsuperscript{Met} mutation in kindred 2206 was poor: a 71% cumulative cardiac event rate at 50 years of age (Fig 5).

We have demonstrated this mutation in another unrelated kindred. This kindred has not as yet been expanded, but the index case, a 45-year-old man, has had recurrent syncope related to atrial and ventricular arrhythmias.

Comparison of Clinical Expression in Four Kindreds With Distinct β-MHC Gene Mutations (256\textsuperscript{Gly}→\textsuperscript{Gln}, 606\textsuperscript{Val}→\textsuperscript{Met} 908\textsuperscript{Leu}→\textsuperscript{Val} and 403\textsuperscript{Arg}→\textsuperscript{Gln})

To examine further mutation-specific natural histories, we compared phenotypic expression and prognosis in four HCM kindreds with distinct mutations: kindred 2280 with the 256\textsuperscript{Gly}→\textsuperscript{Gln} mutation, kindred 2206 with the 606\textsuperscript{Val}→\textsuperscript{Met} mutation, kindred 2755 with the 908\textsuperscript{Leu}→\textsuperscript{Val} mutation, and kindred 2002 with the 403\textsuperscript{Arg}→\textsuperscript{Gln} mutation. The latter two kindreds have been described previously.\textsuperscript{16}

Phenotypic expression and disease penetrance. The phenotype in family members in whom the disease mutation was absent was significantly different in kindred 2280 compared with kindreds 2755 and 2002. In kindred 2280, the maximal LV wall thickness in all family members in whom the disease allele was absent was ≤14 mm. In contrast, in kindreds 2755 and 2002, the maximal LV wall thickness was ≤12 mm. Hence, to determine the ability of the 12-lead ECG and echocardiogram to identify individuals with the disease allele (Table 1) and to estimate disease penetrance (Table 2), it was necessary to use two different echocardiographic criteria for LV hypertrophy: ≥15 mm for kindred 2280 and ≥13 mm for kindreds 2755 and 2002. By these criteria, more than a third of the adults with the 256\textsuperscript{Gly}→\textsuperscript{Gln} and the 908\textsuperscript{Leu}→\textsuperscript{Val} mutations did not have echocardiographic evidence of HCM. In contrast, all adults and children over the age of 5 years with the 403\textsuperscript{Arg}→\textsuperscript{Gln} and 606\textsuperscript{Val}→\textsuperscript{Met} mutations manifested the HCM phenotype.

Prognosis. The cumulative age-related cardiac event-free rate in this kindred was similar to that reported for kindred 2755 with the 908\textsuperscript{Leu}→\textsuperscript{Val} (neutral charge change)
mutation by a patient with hypertrophic cardiomyopathy in kindred 2206 and from a normal control subject are shown. The G→A transition resulted in the substitution of a methionine for a highly conserved valine at codon 606. The affected individual, a heterozygote, has both the normal G and the mutant A (absent in the control). βMHC indicates β-myosin heavy chain. A, Nla III digest of the exon 16 containing PCR-amplified fragments from a subset of kindred 2206 is shown. A G→A transition at nucleotide 1902 (codon 606) creates a Nla III restriction enzyme site. The two aberrant smaller fragments (indicated by arrows) cosegregated with the disease in this kindred. M indicates marker lanes.

Fig 3. A, Portions of the sequence in the polymerase chain reaction (PCR)-amplified fragment containing exon 9 from a patient with hypertrophic cardiomyopathy in kindred 2206 and from a normal control subject are shown. The G→A transition resulted in the substitution of a methionine for a highly conserved valine at codon 606. The affected individual, a heterozygote, has both the normal G and the mutant A (absent in the control). βMHC indicates β-myosin heavy chain. A, Nla III digest of the exon 16 containing PCR-amplified fragments from a subset of kindred 2206 is shown. A G→A transition at nucleotide 1902 (codon 606) creates a Nla III restriction enzyme site. The two aberrant smaller fragments (indicated by arrows) cosegregated with the disease in this kindred. M indicates marker lanes.

Comparison of Clinical Expression in Kindreds With Identical β-MHC Gene Mutations

To compare clinical expression in kindreds with identical β-MHC gene mutation, we compared the phenotype and prognosis in three unrelated kindreds with the 403 Arg→Gln mutation and two kindreds with the 606 Val→Met mutation.

403 Arg→Gln β-MHC gene mutation. The clinical findings in two unrelated HCM kindreds (Caucasian and Korean) with the 403 Arg→Gln mutation are compared in Table 3. In several respects, the clinical findings were similar in both kindreds: (1) Disease penetrance was 100% in adults and children in both kindreds. (2) There was a high prevalence of myocardial ischemia (Fig 6).

Fig 4. Diagrammatic representation of kindred 2206, in which the valine→methionine substitution is present at amino acid residue 606. Four premature sudden cardiac deaths have occurred in eight family members with the mutation. Additionally, the disease has progressed to left ventricular wall thinning and diminished left ventricular systolic function in one patient (+) who presented with recurrent syncope and ventricular arrhythmias. Ages of individuals are shown below symbols. **Premature birth; ***sudden infant death syndrome (genetic and phenotype information are not available in these two children).

(3) The disease progressed to chronic atrial fibrillation associated with LV wall thinning and/or markedly diminished systolic LV function in some adult patients.

(4) Programmed ventricular stimulation did not demonstrate an arrhythmogenic LV substrate except in one patient who presented with syncope and in whom a sustained ventricular tachycardia was induced (Tables 3 and 4). In all the remaining patients, symptoms of impaired consciousness (syncope and presyncope) were related to myocardial ischemia. Furthermore, the high incidence of sudden cardiac death in kindred 2002 was similar to that reported in a kindred with the identical 403 Arg→Gln mutation (Table 3).13,17

However, there were some significant differences between the 403 Arg→Gln kindreds: (1) None of the patients in our two kindreds had echocardiographic evidence of right ventricular hypertrophy, but 45% of the patients in the kindred reported by Seidman et al13,17 had right ventricular hypertrophy. (2) Most of the patients in the Korean family had LV outflow obstruction, but all patients in kindred 2002 had nonobstructive
HCM ($P<.05$). (3) All but three children in kindred 2002 have either died suddenly or had syncope or presyncope, but none of the Korean kindred have died or had symptoms of impaired consciousness (Table 4).

606<sup>Val</sup>-<sup>Met</sup> $\beta$-MHC gene mutation. The poor outcome in our kindred with the 606<sup>Val</sup>-<sup>Met</sup> mutation is in sharp contrast to the benign prognosis reported for this mutation in three small families (Table 4). However, it is noteworthy that the sudden cardiac death reported in the latter series occurred in a young patient aged 13 years.

### Discussion

The phenotype is markedly varied in HCM. Although the LV wall thickness often predominantly affects the interventricular septum, many other morphological types have been described. Most patients have nonobstructive HCM, but in 25% there is evidence of obstruction to LV outflow. This is commonly subvalvular but occasionally is at the level of midcavity and is often associated with LV apical aneurysm. The hypertrophy may involve the right ventricle, and there may be right ventricular outflow obstruction. Although both allelic and nonallelic heterogeneity have been demonstrated in HCM, even within the same family, the severity of symptoms, cardiac morphology, LV systolic and diastolic functions, and type of arrhythmias differ greatly. Therefore, an important question is the extent to which the genotype accounts for this phenotypic diversity.

HCM has been shown to be associated with missense mutations in the $\beta$-MHC gene. To date, about 15 mutations have been reported, and an additional 5 mutations have been identified (authors' unpublished observations). A deletion of the 3' end of the $\beta$-MHC gene has also been reported in an HCM patient, but its significance is unclear. That the disease is caused by these missense mutations is supported by the following observations: (1) The mutations have only been found in HCM kindreds and not in the general population. (2) Recent findings have shown that mutant cardiac $\beta$-myosin message and protein are present in skeletal muscle and that cardiac $\beta$-myosin purified from skeletal muscle, as well as from isolated single skeletal myofibers, has an abnormal function in a variety of assays. (3) Skeletal myofibers containing mutant $\beta$-myosin are hypertrophied and show other myopathic features, resembling central core disease, a rare nonprogressive

### Table 1. Ability of the 12-Lead ECG and Echocardiogram to Identify Individuals With the Disease Allele in Kindreds 2280, 2755, and 2002 In Which Hypertrophic Cardiomyopathy Was Associated With Three Distinct $\beta$-Myosin Heavy Chain Gene Mutations (256<sup>Arg</sup>-<sup>Glu</sup>, 908<sup>Val</sup>-<sup>Met</sup>, and 403<sup>Arg</sup>-<sup>Val</sup>, Respectively)

<table>
<thead>
<tr>
<th>ECG (≥45 y)</th>
<th>Sensitivity, %</th>
<th>Specificity, %</th>
<th>Positive Predictive Value, %</th>
<th>Negative Predictive Value, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>256&lt;sup&gt;Arg&lt;/sup&gt;-&lt;sup&gt;Glu&lt;/sup&gt;</td>
<td>100</td>
<td>61</td>
<td>61</td>
<td>100</td>
</tr>
<tr>
<td>908&lt;sup&gt;Val&lt;/sup&gt;-&lt;sup&gt;Met&lt;/sup&gt;</td>
<td>92</td>
<td>100</td>
<td>100</td>
<td>89</td>
</tr>
<tr>
<td>403&lt;sup&gt;Arg&lt;/sup&gt;-&lt;sup&gt;Val&lt;/sup&gt;</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Echocardiogram (≥20 y)</th>
<th>Sensitivity, %</th>
<th>Specificity, %</th>
<th>Positive Predictive Value, %</th>
<th>Negative Predictive Value, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>256&lt;sup&gt;Arg&lt;/sup&gt;-&lt;sup&gt;Glu&lt;/sup&gt;</td>
<td>56</td>
<td>100</td>
<td>100</td>
<td>90</td>
</tr>
<tr>
<td>908&lt;sup&gt;Val&lt;/sup&gt;-&lt;sup&gt;Met&lt;/sup&gt;</td>
<td>63</td>
<td>100</td>
<td>100</td>
<td>80</td>
</tr>
<tr>
<td>403&lt;sup&gt;Arg&lt;/sup&gt;-&lt;sup&gt;Val&lt;/sup&gt;</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>ECG (&lt;45 y)</th>
<th>Sensitivity, %</th>
<th>Specificity, %</th>
<th>Positive Predictive Value, %</th>
<th>Negative Predictive Value, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>256&lt;sup&gt;Arg&lt;/sup&gt;-&lt;sup&gt;Glu&lt;/sup&gt;</td>
<td>58</td>
<td>99</td>
<td>92</td>
<td>96</td>
</tr>
<tr>
<td>908&lt;sup&gt;Val&lt;/sup&gt;-&lt;sup&gt;Met&lt;/sup&gt;</td>
<td>43</td>
<td>100</td>
<td>100</td>
<td>86</td>
</tr>
<tr>
<td>403&lt;sup&gt;Arg&lt;/sup&gt;-&lt;sup&gt;Val&lt;/sup&gt;</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Echocardiogram (&lt;20 y)</th>
<th>Sensitivity, %</th>
<th>Specificity, %</th>
<th>Positive Predictive Value, %</th>
<th>Negative Predictive Value, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>256&lt;sup&gt;Arg&lt;/sup&gt;-&lt;sup&gt;Glu&lt;/sup&gt;</td>
<td>60</td>
<td>100</td>
<td>100</td>
<td>98</td>
</tr>
<tr>
<td>908&lt;sup&gt;Val&lt;/sup&gt;-&lt;sup&gt;Met&lt;/sup&gt;</td>
<td>15</td>
<td>100</td>
<td>100</td>
<td>84</td>
</tr>
<tr>
<td>403&lt;sup&gt;Arg&lt;/sup&gt;-&lt;sup&gt;Val&lt;/sup&gt;</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

### Table 2. Disease Penetrance and Prognosis in Three Hypertrophic Cardiomyopathy Kindreds With Distinct Mutations in the $\beta$-Myosin Heavy Chain Gene

<table>
<thead>
<tr>
<th>Disease penetrance</th>
<th>256&lt;sup&gt;Arg&lt;/sup&gt;-&lt;sup&gt;Glu&lt;/sup&gt; (Kindred 2280)</th>
<th>908&lt;sup&gt;Val&lt;/sup&gt;-&lt;sup&gt;Met&lt;/sup&gt; (Kindred 2755)</th>
<th>403&lt;sup&gt;Arg&lt;/sup&gt;-&lt;sup&gt;Val&lt;/sup&gt; (Kindred 2002)</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥20 y</td>
<td>19/34 (56%)</td>
<td>17/27 (63%)</td>
<td>10/10 (100%)</td>
</tr>
<tr>
<td>&lt;20 y</td>
<td>3/5 (60%)</td>
<td>2/13 (15%)</td>
<td>5/5 (100%)</td>
</tr>
<tr>
<td>Cumulative sudden cardiac death rate at age 50 y</td>
<td>... 2%</td>
<td>... 8%</td>
<td>... 100%</td>
</tr>
</tbody>
</table>
skeletal myopathy characterized by loss of mitochondria from the center of some of the type I (slow) myofibers. 38

The prevalence of HCM due to β-MHC gene mutations is unknown. By our methods using SSCP analysis for all 40 exons, only 10% of the representative members of unrelated kindreds with HCM have demonstrable β-MHC gene mutations (authors’ unpublished observations). Although it is not possible to be certain of the percentage of mutations that are missed by the SSCP analysis, the fact that five rare mutations identified by us have also been reported by other groups (403Met–Glu, 741Arg–Glu, 606Val–Met, 719Arg–Trp, and 908Leu–Val) indicates that our technique is sufficiently sensitive to detect many of the known mutations. 13–26

Indeed, it is evident from the proportion of kindreds with β-MHC gene mutations found by all investigators taken together that, in at least 60% to 70% of the kindreds, HCM is caused by genes as yet unidentified, 15,18,19 such as those recently localized to chromosome 1q3, 11p13–q13, and 1q2.42–44

In kindreds with distinct β-MHC gene mutations, it is possible to correlate the phenotype with the genotype and to develop mutation-specific natural histories. These types of studies suggest that the disease penetrance and prognosis differ in kindreds with distinct β-MHC gene mutations. 16,17 For example, in two kindreds, the 403Met–Glu mutation has been associated with a high disease penetrance in adults and children and a 60% to 100% cumulative incidence of sudden cardiac death at 50 years of age. 16,17 Conversely, although many patients in a large kindred with the 908Leu–Val mutation have marked cardiac hypertrophy, the disease penetrance has been low (63% in adults with the mutation), and the incidence of sudden cardiac death has been only 8% at 50 years of age. 16 The benign prognosis associated with the 908Leu–Val mutation could be attributed to the absence of a charge change, and indeed, the 606Val–Met mutation has recently been reported to be associated with a low incidence of premature sudden cardiac death. This has been offered as evidence of the benign nature of neutral charge change mutations compared with missense mutations that result in a charge change, such as the 249Asp–Glu, 403Met–Glu, and 453Lys–Glu mutations (Fig 7). 17 However, the large number of premature sudden cardiac deaths in our kindred with the identical 606Val–Met mutation indicates that neutral charge change amino acid substitutions in critical regions of the β-myosin molecule may be associated with a malignant prognosis in some kindreds. Furthermore, the 256Thr–Glu mutation demonstrates that not all mutations associated with a charge change have a poor prognosis (Fig 7).

Determination of the genotype provides an opportunity for reexamining the definitions of cardiac hypertrophy in HCM. A finding of the present study with important implications for the diagnosis of HCM and estimation of disease penetrance is that, ideally, cardiac hypertrophy needs to be assessed not by reference to an arbitrary value of LV wall thickness but in the context of the cardiac dimensions of family members who do not have the disease allele. For example, in kindred 2755 (908Leu–Val mutation), with most members of Irish origin, the upper limit of normal LV wall thickness was significantly less than that found in kindred 2280 (256Thr–Glu mutation), mostly of Germanic extraction.

The present study also shows that, although HCM patients with the identical β-MHC gene mutation share certain clinical features, they differ in other important respects, underlining the importance of modifying

### TABLE 3. Comparison of Clinical Findings in Two Unrelated Hypertrophic Cardiomyopathy Kindreds With the 403Met–Glu β-Myosin Heavy Chain Gene Mutation

<table>
<thead>
<tr>
<th></th>
<th>Kindred 2002</th>
<th>Kindred 2258</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients with HCM</td>
<td>15</td>
<td>6</td>
<td>21</td>
</tr>
<tr>
<td>Premature sudden cardiac death</td>
<td>6</td>
<td>0*</td>
<td>6 (29%)</td>
</tr>
<tr>
<td>Surviving patients</td>
<td>9</td>
<td>6</td>
<td>15</td>
</tr>
<tr>
<td>Syncope/presyncope</td>
<td>7</td>
<td>0*</td>
<td>7 (47%)</td>
</tr>
<tr>
<td>Low LV EF plus atrial fibrillation</td>
<td>1/9 (11%)</td>
<td>1/6 (17%)</td>
<td>2 (13%)</td>
</tr>
<tr>
<td>RV hypertrophy</td>
<td>0/9 (0%)</td>
<td>0/6 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>LV outflow obstruction*</td>
<td>0/9 (0%)</td>
<td>4/6 (67%)*</td>
<td>4 (27%)</td>
</tr>
<tr>
<td>LV diastolic pressure &gt;15 mm Hg</td>
<td>8/9 (100%)</td>
<td>1/4 (25%)</td>
<td>9 (69%)</td>
</tr>
<tr>
<td>RV diastolic pressure &gt;10 mm Hg</td>
<td>8/9 (67%)</td>
<td>1/4 (25%)</td>
<td>7 (54%)</td>
</tr>
<tr>
<td>Myocardial ischemia†</td>
<td>5/9 (56%)</td>
<td>3/6 (50%)</td>
<td>8 (53%)</td>
</tr>
<tr>
<td>Ventricular tachycardia on Holter</td>
<td>0/9 (0%)</td>
<td>0/6 (0%)</td>
<td>0 (0%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Electrophysiological findings</th>
<th>Kindred 2002</th>
<th>Kindred 2258</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abnormal sinus node function</td>
<td>5/8 (63%)</td>
<td>0/3 (0%)</td>
<td>5/11 (45%)</td>
</tr>
<tr>
<td>Abnormal AV node function</td>
<td>2/8 (25%)</td>
<td>1/4 (25%)</td>
<td>3/12 (25%)</td>
</tr>
<tr>
<td>HV interval ≥55 ms</td>
<td>1/8 (13%)</td>
<td>0/4 (0%)</td>
<td>1/12 (8%)</td>
</tr>
<tr>
<td>Induction of VT</td>
<td>1/8 (13%)</td>
<td>0/4 (0%)</td>
<td>1/12 (8%)</td>
</tr>
</tbody>
</table>

HCM indicates hypertrophic cardiomyopathy; LV, left ventricular; EF, ejection fraction (<50%); RV, right ventricular; AV, atrioventricular; and VT, ventricular tachycardia.

*At echocardiography or at cardiac catheterization.
†Myocardial ischemia demonstrated by ECG changes during treadmill exercise test or rapid atrial pacing and/or by exercise thallium scintigraphy.

Fananapazir and Epstein Genotype-Phenotype Correlations in HCM Mutations 27
Right Atrial Pacing (CL=400 ms)  

S.P. 07/01/91

Fig 6. Evidence of myocardial ischemia in a 17-year-old boy with the 403Arg-Gln mutation who presented with syncope but without angina. At cardiac catheterization no left ventricular outflow gradient was recorded at rest or after provocation. At electrophysiological study, sinus node and atrioventricular node functions and His-Purkinje conduction were normal. Programmed electrical stimulation did not induce any atrial or ventricular arrhythmias. On 240 mg verapamil and 100 mg atenolol daily, the patient has not had any further episodes of impaired consciousness during a 3-year follow-up. A. The 12-lead ECG during right atrial pacing in this patient shows ischemic ST-T wave changes. CL indicates cycle length. B. Horizontal-axis and short-axis thallium tomograms during exercise stress and reinjection30 imaging show reversible inferior myocardial perfusion defect (arrow) in the same patient with the 403Arg-Gln mutation. C, Tracings show rapid onset of severe and prolonged hypotension during atrial pacing in the supine position. I, aVF, V1, and V6 are surface ECG leads; HIRA (high right atrium), HBE (His bundle), and RVA (right ventricular apex) are intracardiac electrograms.
genes and nongenetic factors. For example, disease penetrance was 100% in adults and children, and myocardial ischemia and biventricular diastolic dysfunction were prevalent in both of our kindreds with the 403Arg→Gln mutation. However, although most of the patients in one kindred have either died suddenly or presented with syncope or presyncope, no sudden cardiac death or symptoms of impaired consciousness have been noted in the second kindred. In addition, all patients in the first kindred have nonobstructive HCM, but two thirds of the patients in the second kindred have evidence of LV outflow obstruction. Significantly, in a kindred reported by Geisterfer-Lowrance et al.13 with the identical mutation, 9 of 20 affected individuals who were examined by echocardiography were judged to have right ventricular hypertrophy, but none of the 21 patients in our two kindreds with the 403Arg→Gln mutation have had abnormal right ventricular morphology at echocardiography.

There was a high incidence of sudden cardiac death in the kindred with the 403Arg→Gln mutation reported by Geisterfer-Lowrance et al.13 Hence, the apparent benign prognosis of the 403Arg→Gln mutation in kindred 2258 may be misleading because of the relatively small number of affected patients in this kindred. Indeed, the 606Val→Met mutation in kindred 2206 illustrates that although a number of premature sudden cardiac deaths are sufficient to establish that a mutation is associated with a poor prognosis, large kindreds must be studied to be certain that a mutation is benign. To our knowledge, the 256Glu→Gln and 908Leu→Val mutations in kindreds 2280 and 2755, respectively, are at present the only two β-MHC gene missense mutations that satisfy this requirement.

Knowledge of the molecular defect allows studies of mutation-specific causes of symptoms and sudden cardiac death. Although arrhythmias are probably the most common cause of sudden cardiac death in adults with

---

### Table 4. Prognosis in Hypertrophic Cardiomyopathy Kindreds With Identical β-Myosin Heavy Chain Gene Mutations

<table>
<thead>
<tr>
<th>Exon</th>
<th>Watkins et al.17</th>
<th>Kindred Groups</th>
<th>Watkins et al.17</th>
<th>Kindred Groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2206</td>
<td>2002</td>
<td>2258</td>
<td></td>
</tr>
<tr>
<td>Nucleotide change</td>
<td>G1902A</td>
<td>G1294A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amino acid residue</td>
<td>606</td>
<td>403</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amino acid change</td>
<td>Val→Met</td>
<td>Arg→Gln</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Charge change</td>
<td>No</td>
<td>Yes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kindreds, n</td>
<td>1</td>
<td>3</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Individuals with mutation, n</td>
<td>8</td>
<td>18</td>
<td>15</td>
<td>6</td>
</tr>
<tr>
<td>Disease-related deaths, n</td>
<td>5</td>
<td>2</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>Sudden deaths, n</td>
<td>4</td>
<td>1</td>
<td>6</td>
<td>. . .</td>
</tr>
<tr>
<td>Mean age at sudden death, y</td>
<td>20</td>
<td>13</td>
<td>30</td>
<td>. . .</td>
</tr>
<tr>
<td>Survival at 50 years of age, %</td>
<td>29</td>
<td>95</td>
<td>0</td>
<td>100</td>
</tr>
</tbody>
</table>

---

**Fig 7.** The relative positions and phenotypic consequences of known β-myosin heavy chain missense mutations. For charge change, + indicates positive charge change, −, neutral charge change. For strong family history (FH) of sudden cardiac death (SCD), + indicates poor prognosis; −, benign prognosis; and ?, undetermined natural history. Top row refers to National Institutes of Health families. Bottom row refers to kindreds reported in the literature.17,24,28 *Watkins et al.17; **NIH kindreds; ***Dausse et al.22; and ***Nishi et al.24
HCM,1-11 we have recently recognized that myocardial ischemia, often not accompanied by angina, is responsible for the majority of cardiac arrests, syncope, or presyncope in HCM children.46 Typically, in these patients, modest increases in heart rate due to sinus tachycardia or a tachyarrhythmia provoke hypotension accompanied by ECG changes that indicate myocardial ischemia. Occasionally, the myocardial ischemia is sufficiently severe to trigger malignant ventricular arrhythmias.45,46 The basis of the ischemia is not well understood. Although it may occur as the result of an imbalance between coronary blood flow and increased myocardial demand secondary to LV hypertrophy, young patients who present with this syndrome do not have unusually thick hearts. Endothelial hyperplasia of myocardial small blood vessels may be another explanation.48 However, this postmortem finding has not been correlated with myocardial ischemia that culminates in sudden cardiac death. In the present study, atrial pacing resulted in ischemic ST-T wave ECG changes associated with severe hypotension in three young members of kindreds 2002 with a history of exercise-induced light-headedness. This suggests that myocardial ischemia may be an early manifestation of HCM associated with the 403Arg-Glu mutation. Myocardial ischemia and secondary fibrosis may cause decreased ventricular compliance, reflected in elevated diastolic pressures. Myocardial scarring may, in the older patient, also result in LV wall thinning and reduced systolic function. Atrial tachyarrhythmias in such patients may consequently precipitate catastrophic hypotension and sudden cardiac death. Ventricular arrhythmias are likely to be an important cause of sudden cardiac death and syncope only during the latter stages of the disease, when extensive myocardial scarring is present. We have treated affected individuals in our two kindreds with the 403Arg-Glu mutation with β-blockers to prevent excessive tachycardia and verapamil to improve myocardial ischemia.49,50 With this therapy, no cardiac events have occurred in 15 patients with this “malignant” mutation during a 5-year follow-up period. Seven (47%) of these patients had presented with a history of exercise-induced syncope or presyncope. These preliminary observations promise that therapeutic strategies may be developed to address mutation-specific causes of sudden cardiac death in HCM.

HCM is often not associated with any symptoms and is therefore often undiagnosed.1 However, it is the most common cause of sudden cardiac death in otherwise healthy young individuals.2,3 In patients in whom the diagnosis is established, although several clinical features have been reported to indicate an increased risk for sudden cardiac death (eg. a history of cardiac arrest or syncope, young age, presence of ventricular tachycardia during ambulatory Holter monitoring, and a “malignant” family history of sudden cardiac death [sudden cardiac deaths in two or more first degree relatives]),1,4,6,9,51-53 these findings are not sufficiently sensitive or specific to aid in the management of individual HCM patients.50 Furthermore, individuals who carry the disease gene but who do not have cardiac hypertrophy or have mild disease that may go unrecognized may also be prone to arrhythmias and sudden cardiac death.54,55 Thus, the ability to identify individuals with distinct β-MHC gene mutations before clinical presentation of HCM46,56 and the reports of mutation-specific natural histories raise the important question of whether routine genotyping should be performed on children or athletes at risk for HCM.57 The answer hinges on the value of identifying the mutation and the contribution this makes to the prognosis of the disease in the genotyped individual. Although there seem to be broad characteristics, such as penetrance and frequency of sudden cardiac death, associated with kindreds with distinct mutations, a study of the mechanisms of sudden cardiac death in HCM patients has shown the weakness inherent in using this approach to an individual’s prognosis. Because within a single kindred sudden cardiac death associated with HCM is the result of one of several mechanisms (ventricular arrhythmias, atrial arrhythmias resulting in severe hypotension, bradyarrhythmias, severe LV outflow obstruction, or myocardial ischemia),7-11 individuals must be evaluated for their propensity to develop any of these possible events. Tendencies to develop these events is as varied within a kindred as between kindreds. We have reported here on the discrepancy in the broad characteristics between kindreds with the identical mutation. We have also demonstrated that even the background of what is normal ventricular thickness may vary from one kindred to another. It must also be remembered that β-MHC gene missense mutations account for only a minority of HCM in the unrelated kindreds that have been studied. Thus, although it is reasonable to genotype other family members when a mutation is discovered, we feel that until more is known about the correlations between the phenotype and genotype and mutation-specific causes of sudden cardiac death, routine genotyping of HCM patients will add little to their care and divert resources from more useful approaches to the diagnosis and treatment of this disease.

Acknowledgments

We would like to thank Dorothea McAarayev, Vasken Dilsizian, Peter Katsiyanis, Dorothy Tripodi, Judith B. Winkler, Shahin Hassanzadeh, Stephen Master, and Colleen LePore for their assistance.

References


Genotype-phenotype correlations in hypertrophic cardiomyopathy. Insights provided by comparisons of kindreds with distinct and identical beta-myosin heavy chain gene mutations.

L Fananapazir and N D Epstein

_Circulation._ 1994;89:22-32
doi: 10.1161/01.CIR.89.1.22

_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1994 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/89/1/22

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in _Circulation_ can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to _Circulation_ is online at:
http://circ.ahajournals.org/subscriptions/