Hypertrophic cardiomyopathy (HCM) is a common cardiac condition inherited as an autosomal dominant trait with a prevalence of approximately 1:500 (1). The diagnosis relies on demonstration of unexplained myocardial hypertrophy (2–4). Genetic investigations have identified disease-causing mutations in eight cardiac sarcomeric contractile protein genes in 50% to 60% of all patients with HCM (5–11). Sequence variations in three additional sarcomeric genes have also been described (12–14).

Recent developments in molecular genetic methods and equipment for mutation analysis have made it technically possible to provide genetic diagnosis in HCM (15). However, the use of genetic diagnosis in clinical management of affected HCM families is dependent on detailed information about the genotype-phenotype correlation in relation to age of onset, penetrance, characteristics of disease expression, and risk of sudden death. Several studies have suggested a number of possible genotype-phenotype associations, but most have been performed in small numbers of individual patients (proband) or families selected for gene identification studies. Proband studies have provided information about disease expression in single individuals but have not contained information on disease presentation in families. Gene identification studies require large families with many affected individuals but have not contained information on disease presentation in families. Gene identification studies require large families with many affected individuals, which are rare and may not resemble the disease expression in the smaller families most frequently seen in a clinical setting (10,16–28). In this study, we present a detailed analysis of the relation between genotype and clinical phenotype in families with mutations in the gene encoding cardiac troponin I (TNNI3) identified by mutation analysis of 748 consecutive patients with HCM and their relatives. The aim was to examine the potential value of genetic diagnosis for management, counseling, and follow-up of HCM families with TNNI3 mutations.
**Abbreviations and Acronyms**

ASH  = asymmetrical septal hypertrophy  
ECG  = electrocardiogram/electrocardiographic  
F-SSCP = fluorescent SSCP  
HCM = hypertrophic cardiomyopathy  
LV = left ventricle/ventricular  
RCM = restrictive cardiomyopathy  
TNNI3 = cardiac troponin I

**METHODS**

**Clinical investigations.** The study cohort consisted of 748 consecutive and apparently unrelated HCM probands who were evaluated in a dedicated cardiomyopathy clinic during a 10-year period at St. George’s Hospital (London, United Kingdom). Relatives of HCM probands in whom a TNNI3 mutation was identified and at risk of having inherited the disease gene were invited for evaluation. All probands and relatives underwent physical examination, 12-lead electrocardiography, 48-h Holter recording, transthoracic two-dimensional echocardiography, and Doppler studies (29). Cine cardiac magnetic resonance was performed in 30 mutation carriers (30). The study was approved by the local research ethics committee, and informed consent was obtained from all participants.

Hypertrophic cardiomyopathy was diagnosed in probands when echocardiography identified unexplained left ventricular (LV) hypertrophy ≥13 mm or LV wall thickness >2 standard deviations for age, size, and gender or in relatives who fulfilled proposed diagnostic criteria within the context of familial HCM (2,3). The pattern of hypertrophy was classified as asymmetrical septal hypertrophy (ASH), concentric, or predominant apical as previously described (31); HCM with restrictive physiology was diagnosed as reported recently (32). Before the present study, we were not aware that the patients with HCM and restrictive cardiomyopathy (RCM) in family H816 and H805 were related, and they were listed independently in two separate study cohorts, one for HCM patients and one for RCM patients, respectively. Only individual II:3 (family H816) and individual II:3 (family H805) with RCM have been reported previously (32).

Supraventricular tachycardia was defined as three or more consecutive supraventricular premature beats at a rate of >120 beats/min. Nonsustained ventricular tachycardia was defined as three or more consecutive ventricular ectopics at a rate of >120 beats/min lasting <30 s.

**Genetic investigations.** Penetrance was defined as the percentage of clinically affected mutation carriers (clinically affected + clinically unaffected mutation carriers). Probands were included in the calculation.

Genomic deoxyribonucleic acid for mutation analysis was obtained and protein-encoding exons (including splice sites) of TNNI3 were amplified as previously described. Direct sequencing and fluorescent SSCP (F-SSCP) analysis was performed using standard protocols (15,32).

The prevalence of TNNI3 mutations was established by direct sequencing of all eight protein-encoding exons of the gene in 185 HCM probands. An additional 95 HCM probands were investigated by F-SSCP analysis of all exons, and abnormal conformers were subjected to direct sequencing. Finally, 468 HCM probands were screened by F-SSCP analysis of exons 5, 7, and 8, and abnormal conformers sequenced. All mutations identified by direct sequencing were detectable by F-SSCP analysis implying a sensitivity of F-SSCP of 100% (15).

Five of the mutations identified were confirmed to change restriction enzyme sites by polymerase chain reaction amplification of the relevant exon, restriction enzyme digest, followed by size fractionation using 3% agarose gel electrophoresis. Arg145Trp abolished an AcI site, Arg145Gly abolished an AcI site, Ala157Val abolished a CfoI site, Arg162Gln abolished a MspI site, Asp196Asn abolished a ClaI site, Ser199Asn created a DdeI site, and Gly203Arg abolished an HaeIII site.

To investigate if families carrying identical mutations were related, haplotype analysis was performed using microsatellite markers defining the TNNI3 locus. In accordance with chromosome 19 sequencing data available at NCBI, TNNI3 is localized between the proximal flanking marker D19S926 and the distally flanking markers D19S891 and D19S887; TNNI3 and D19S926 are part of contig NT011225, while D19S891 and D19S887 are part of contig NT011104 (33).

**RESULTS**

**Genetic investigations.** Mutation analysis of all eight protein-encoding exons of TNNI3 in 280 consecutive HCM probands identified eight families with five different mutations localized in exon 7 or 8 (frequency: 2.9%) (Fig. 1). The frequency of mutations identified by direct sequencing and F-SSCP was the same. Subsequent mutation screening of 468 consecutive HCM probands was limited to analysis of exons 5, 7, and 8, due to the fact that no amino acid substitutions have been identified in the remaining exons after mutation analysis of the entire TNNI3 gene in a total of 1,081 HCM patients investigated in this and previous studies (10,14,21,22,27,28,32). Mutation analysis of exons 7 and 8 identified another eight different mutations in 15 families (frequency: 3.2%). In total, 23 families with 13 different mutations were identified of which 6 were novel. All mutations were identified in patients of Caucasian descent except for H305 who was Asian and H945 who was of Arabic origin.

The mutations identified in exons 7 and 8 were all considered to be disease-causing because: 1) the amino acid substitution identified in the proband was present in all relatives with the disease; 2) no sequence variations led to amino acid substitutions in 150 ethnically matched control chromosomes;
3) identical mutations appeared in two or three separate families (Arg141Gln, Arg145Trp, Ala157Val, Arg162Gln, Arg186Gln, Asp196Asn, Ser199Asn) and/or had been reported previously (Arg141Gln, Arg145Gln, Ala157Val, Arg162Gln, Ser166Phe, Arg186Gln, Asp196Asn); 4) mutations in this study appeared in codons previously reported to harbor similar disease-causing amino acid substitutions (Arg145Trp, Arg162Gln, Lys183Glu, Gly203Arg); and 5) all mutations were located in functionally important and conserved regions of the gene (Fig. 1, Table 1) (10,14, 21,22,27,28,32).

Haplotype analysis with highly polymorphic markers defining the locus for \textit{TNNI3} was performed in families carrying identical mutations to investigate if the same mutation had an independent origin in separate families or was likely to have arisen from a common ancestor (founder effect). A unique haplotype for each of the following mutations was identified: Arg145Trp in family H816 and H805; Arg162Gln in family H15, H72, H772; Asp196Asn in family H25, H655; Ser199Asn in family H578, H375 (Fig. 2). This indicates that a common founder of the mutations in these families was likely as opposed to the remaining families with identical mutations present on different haplotypes (Ala157Val in family H201, H886, H167; Arg186Gln in family H136, H305). No detectable relationship was present in families sharing common haplotypes.

Two sequence variants identified in exon 5 led to amino acid changes (Fig. 1). An HCM patient of Asian descent was identified with an Arg79Cys amino acid substitution.

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|c|c|c|c|c|c|c|}
\hline
Protein Encoding Exon &  \multicolumn{6}{|c|}{Exon 7} &  \multicolumn{3}{|c|}{Exon 8} \\
\hline
Amino Acid Position & Human Cardiac Troponin I & Arg & 145 & 157 & 162 & 166 & 183 & Arg & 196 & 199 & 202 & 203 \\
\hline
H81, H945 & & Trp & . & . & . & . & & . & . & . & . & . \\
H402 & & Glu & . & . & . & . & & . & . & . & . & . \\
H12 & & & & & & & & & & & & \\
H1136, H305 & & Gln & . & . & . & . & & . & . & . & . & . \\
Rat cardiac troponin I & & & & & & & & & & & & \\
Mouse cardiac troponin I & & & & & & & & & & & & \\
Chicken cardiac troponin I & & Lys & Gly & . & . & & . & . & . & . & . \\
Quail cardiac troponin I & & Lys & Gly & . & . & & . & . & . & . & . \\
\hline
\end{tabular}
\caption{Conservation of Cardiac Troponin I Amino Acid Residues Affected by Missense Mutations}
\end{table}

Period indicates amino acid identity. Protein accession numbers for rat, mouse, chicken, and quail: NP_055840, NP_033432, P27673, and A41030, respectively (33).
Figure 2. Pedigrees of hypertrophic cardiomyopathy (HCM) families with TNNI3 mutations. Squares = male family members; circles = female family members; symbols with slash = deceased individuals; open symbols = unaffected individuals; solid symbols = individuals affected; question mark = unknown clinical status; plus sign = presence of mutation; minus sign = absence of mutation. No clinical data were available in H375, II:6, but she was assumed to have HCM due to the fact that she was an obligate mutation carrier and had symptoms of disease preceding her unexplained sudden death.
previously shown to be present in healthy controls of same ethnicity (10). Also, we identified a previously reported Pro82Ser amino acid substitution in an Afro-Caribbean HCM patient (14). However, we identified the same sequence variant in 3% of healthy Afro-Caribbean controls. Both amino acid substitutions localized in exon 5 were likely to be polymorphisms and not disease-causing mutations.

Clinical presentation. Genetic investigations of the 23 families with TNNI3 mutations identified a total of 100 mutation carriers of which 48 individuals (23 probands, 25 relatives) fulfilled HCM diagnostic criteria (Table 2, Fig. 2). A total of 43 of the 48 patients with HCM had both echocardiography recordings and electrocardiograms (ECGs) available for analysis, and 27 had hypertrophy at initial evaluation (Table 2). The majority of affected individuals had asymmetrical septal (n = 14) or predominant apical (n = 11) hypertrophy of the LV. Three mutation carriers (H201, II:4; H167, I:1; H136, I:2) had impaired LV systolic function (fractional shortening <25%) with an LV wall thickness ranging between 9 to 14 mm; II:4, H201, was diagnosed at the age of 15 years after a cardiac arrest from which she was successfully resuscitated, whereas I:1, H167, and I:2, H136, were diagnosed due to symptoms of heart failure and angina at the ages of 34 and 55 years, respectively (Fig. 2, Table 2). Biopsy or postmortem microscopy of cardiac tissue from these individuals revealed myocyte hypertrophy, interstitial fibrosis, and myofibrillar disarray consistent with a diagnosis of “end-stage” dilated HCM.

The finding of the same mutation (Arg145Trp) in eight affected individuals provided the opportunity to assess heterogeneity of disease expression within and between families (H215, H805, H816). In family H816, the proband (III:2) developed severe biventricular hypertrophy with biaxial dilation over a 12-year period. She died from right- and left-sided heart failure at the age of 40. Her uncle (II:3) presented with clinical features of RCM (31). In family H805, III:4 had severe hypertrophy (30 mm), with midcavity LV obstruction at the age of 49 (Fig. 3C), whereas a brother (III:6) seen at age 50 had ECG abnormalities with a normal two-dimensional echocardiogram. Their father (II:4) was diagnosed at the age of 61 with ASH of 15 mm when he presented in atrial flutter, while an aunt (II:3) had previously been diagnosed with RCM (32). In family H215, the proband (II:3) was diagnosed with ASH of 21 mm at the age of 30, whereas her father (I:1) had a normal echocardiogram at the age of 61 but an abnormal ECG with T-wave inversion in precordial leads (V2 through V6) and paroxysmal atrial fibrillation. He never developed morphologic signs of HCM and died from pneumonia at the age of 74.

The heterogeneous cardiac morphology in these three families was representative of the clinical findings in other HCM families with different mutations (Fig. 3). Two patients who carried an identical mutation (Arg162Gln) had markedly different distribution of myocardial hypertrophy (Figs. 3A and 3B). Two patients had biventricular hypertrophy with the most severe disease expression in a patient who was homozygous for an Arg141Gln mutation (H945, II:1) (Fig. 3D). His remaining family members were not available for evaluation, but both parents and several of his siblings had symptoms of cardiac disease, and one brother had died suddenly at the age of 20. A patient with an Arg186Gln mutation was diagnosed at the age of 29 with severe ASH of 35 mm and developed LV wall thinning and dilation over 13 years (Fig. 3E).

Electrocardiograms from 96 mutation carriers were available for evaluation, of which 45 had abnormalities consistent with a diagnosis of HCM in the context of familial disease, including the presence of Q waves, ST segments, P waves, or axis (Fig. 4). Nine mutation carriers had ECG abnormalities with normal LV wall thickness on echocardiogram and no symptoms of disease (H805, III:6; H215, I:1; H201, II:2; H15, III:2, IV:1, IV:3; H136, III:2; H25, II:3; H512, III:2) (mean age 35 years, range 14 to 72 years) (Fig. 2, Table 2). All patients with abnormal echocardiography had ECG abnormalities and symptoms of disease with dyspnea and/or angina except for two patients who had ECG abnormalities, ASH of 24 mm, (H896, III:1), and apical hypertrophy of 18 mm (H25, II:1) (age 15 and 74 years, respectively) but no cardiac symptoms.

On Holter monitoring, 11 individuals had supraventricular tachycardia (mean age 52 years, range 17 to 70 years), whereas five individuals had episodes of nonsustained ventricular tachycardia (mean age of 32 years, range 15 to 47 years). Two individuals were successfully resuscitated after cardiac arrest (H201, II:4 age 15; H305, II:2 age 22 years), whereas four died suddenly (H15, IV:2 age 13; H136, II:2 age 34; H375, II:1 age 59 and II:6 age 51 years). Five of these individuals fulfilled HCM diagnostic criteria on echocardiography (H201, II:4; H305, II:2), postmortem examination (H15, IV:2; H136, II:2), and/or ECG recording (H375, II:1; H201, II:4; H305, II:2). No clinical data were available on II:6, H375, who was an obligate mutation carrier, but relatives reported she had symptoms of angina, dyspnea, and syncope before her otherwise unexplained sudden death. Six individuals experienced a cardiac-related death due to heart failure or stroke (H167, II:3; H816, III:2; H805, II:4; H12, I:1; H136, I:2; H578, I:2) (mean age 63 years, range 40 to 72 years).

Three clinically unaffected mutation carriers (H811, II:2; H201, I:2; H305, I:1) had affected offspring of whom two (H201, II:4; H305, II:2) were successfully resuscitated from cardiac arrest as previously mentioned.

Phenotypic appearance and age distribution of mutation carriers at first clinical evaluation. The age at diagnosis of 23 probands and 25 clinically affected relatives was similar and evenly distributed from the second to the eighth decade (Fig. 5). A total of 52% (52 of 100) of mutation carriers had no signs or symptoms of disease with normal ECGs and echocardiograms at initial clinical investigation. Their age distribution was comparable with that of clinically affected
<table>
<thead>
<tr>
<th>Mutation</th>
<th>Family</th>
<th>Individuals Fulfilling HCM Criteria/All Mutation Carriers</th>
<th>ASH</th>
<th>Echocardiography (Maximal Wall-Thickness in mm)</th>
<th>ECG Abnormal</th>
<th>Arrhythmia</th>
</tr>
</thead>
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<tr>
<td>Arg141Gln</td>
<td>H945§, H81</td>
<td>2/6</td>
<td>1 (16)</td>
<td>Apical 1 (34)</td>
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<td>AF 1</td>
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<td>Arg145Trp</td>
<td>H816, H805, H215</td>
<td>8/20</td>
<td>3 (22, 15–30)</td>
<td>Biventricular 1 (24)</td>
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<td>PAF 4</td>
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<tr>
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<td>H811</td>
<td>1/5</td>
<td>1 (21)</td>
<td>Dilution (FS%) 2 (22, 20–24)</td>
<td>5</td>
<td>NSVT 6</td>
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<tr>
<td>Ala157Val</td>
<td>H201, H886, H167</td>
<td>5/12</td>
<td>2 (24; 15–29)</td>
<td>RCM 1 (19)</td>
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<tr>
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<td>H15°, H772, H72</td>
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<td>1 (28)</td>
<td>ECG 1</td>
<td>1</td>
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<tr>
<td>Ser166Phe</td>
<td>H402</td>
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<td>1 (19)</td>
<td>Arrhythmia 1</td>
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<td>Lys183Glu</td>
<td>H12</td>
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<td>1 (13)</td>
<td>2 (22; 20–24)</td>
<td>3</td>
<td>1</td>
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<tr>
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<td>2 (24; 18–30)</td>
<td>4 (18; 16–20)</td>
<td>7</td>
<td>3</td>
</tr>
<tr>
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<td>4/7</td>
<td>2 (18; 18)</td>
<td>1 (19)</td>
<td>4</td>
<td>2#</td>
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<tr>
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<td>H578§, H375‡</td>
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<td>1 (25)</td>
<td>1</td>
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<td></td>
</tr>
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<td>14 (21; 13–34)</td>
<td>22 (29; 24–34)</td>
<td>3 (21; 20–24)</td>
<td>45</td>
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</table>

*IV:2 (family H15) and II:2 (family H136) (Fig. 2) died suddenly at ages 13 and 32, respectively. Autopsy revealed HCM. Both had symptoms of disease but no echocardiogram or ECG was available; †II:2 (family H15) (Fig. 2) echocardiogram not available; ‡I:2 (family H578) and II:1, II:3, II:6 (family H375) (Fig. 2); echocardiogram not available. No ECG available on II:6 (family H375). II:1 and II:6 (family H375) died suddenly at the ages of 59 and 51, respectively; §II:2 (family H945) (Fig. 2) was homozygous for the Arg141Gln mutation; ¶II:4 (family H201) was resuscitated from cardiac arrest at the age of 15. She had an implantable cardioverter-defibrillator implanted and has been defibrillated five times during a 20-year period; #II:2 (family H305) was resuscitated from cardiac arrest at the age of 22. She was treated with amiodarone and has not had ventricular arrhythmia for the past 12 years.

AF = atrial fibrillation; Apical = predominant apical ventricular wall hypertrophy; ASH = asymmetrical septal hypertrophy; Biventricular = hypertyrophy of both left and right ventricular walls; Dilution = impaired left ventricle function; FS% = fractional shortening; HCM = hypertrophic cardiomyopathy; NSVT = nonsustained ventricular tachycardia; PAF = paroxysmal atrial fibrillation; RCM = restrictive cardiomyopathy; SCD = sudden cardiac death.
Figure 3. Cine cardiac magnetic resonance (CMR) images of hypertrophic cardiomyopathy (HCM) patients with TNNI3 mutations (30). A wide range of HCM was present including: (A) asymmetrical anteroseptal hypertrophy (Arg162Gln), (B) apical hypertrophy (Arg162Gln), (C) midcavity obstruction (Arg145Trp), (D) extreme biventricular hypertrophy (Arg141Gln), (E) “end-stage dilation” (Arg186Gln), and (F) restrictive cardiomyopathy in a child six years of age (de novo Lys178Glu) as recently reported (did not participate in this study) (32). CMR images: (A and B) From left to right: four-chamber, basal short-axis, and apical short-axis, all in diastole; (C to F) four-chamber systole, four-chamber diastole, short-axis diastole. (A and B) first image = four-chamber view, diastole; second image = cross-sectional view, papillary muscle level, diastole; third image = cross-sectional view, apical level, diastole. (D to F) first image = four-chamber view, systole; second image = four-chamber view, diastole; third image = papillary muscle level, diastole. LA = left atrium; LV = left ventricle; RA = right atrium; RV = right ventricle.
Figure 4. Electrocardiograms of asymptomatic mutation carriers with normal left ventricular (LV) wall thickness on echocardiography. (A) H136, III:2 age 17 years: Q waves in III, V₃ through V₆; LV hypertrophy. (B) H15, III:2, age 36 years: left axis; Q waves in II, III, aVF; ST-segment abnormalities in II, III, V₂ through V₆. (C) H25, II:3, age 72 years: left axis; ST-segment abnormalities in I, aVL, V₅ through V₆; intraventricular conduction delay, V₃ through V₆.
relatives and probands. The disease penetrance including probands was 48% (48 of 100) and was incomplete in all families except one (H112), where all three mutation carriers had HCM. A total of 33% (25 of 77) of all relatives fulfilled HCM diagnostic criteria within the context of familial disease.

**DISCUSSION**

Previous studies have reported TNNI3 mutations in single patients or small numbers of HCM families with few affected individuals and limited clinical information (10,21,28). One study reported a heterogeneous phenotype and a high penetrance in several families affected by the same mutation (Lys183del) (22). Another classified TNNI3 mutations into malignant, intermediate, or benign based on clinical information in small numbers of mutation carriers (17 with 7 different TNNI3 mutations) (27). In this study, we demonstrate that the clinical expression of TNNI3 mutations is extremely heterogeneous within and between families with no apparent mutation or gene-specific disease pattern. The cardiac morphology observed in affected individuals ranged from mild ASH, apical and biventricular hypertrophy, to "end-stage" dilated HCM, and HCM with restrictive physiology. A similar degree of heterogeneity was observed in ECG abnormalities and the prevalence of arrhythmias. Although this study was not designed to establish the age of disease onset, it was remarkable that probands and affected relatives were diagnosed from the second to eighth decade of life. Moreover, clinically unaffected mutation carriers were present at all ages and comprised 52% of all genotype-positive individuals. These observations suggest that disease development may occur throughout life. However, prospective follow-up studies of clinically unaffected mutation carriers is needed to determine the precise age of disease onset as well as the true disease penetrance. The variable age of diagnosis and unpredictable disease expression of TNNI3 mutations indicate that life-long follow-up of clinically unaffected mutation carriers is warranted to identify and treat those at risk of developing disease-related complications. The data suggest that this follow-up could be undertaken by an ECG and evaluation of cardiac symptoms. The recommendation is based on the fact that all individuals who experienced disease-related complications and those who fulfilled diagnostic criteria by echocardiography also had ECG abnormalities and cardiac symptoms. Although ECGs were unavailable in three sudden deaths, they also had a history of cardiac symptoms, and two had hypertrophy at autopsy, making it likely that an ECG and symptom evaluation would have identified these individuals.

Other studies on probands and selected families have suggested that mutations in different HCM genes may be associated with a specific phenotype. It has been proposed that mutations in the gene for cardiac troponin T (TNNT2) may be associated with absent or mild cardiac hypertrophy and an adverse prognosis, whereas MYBPC3 mutations have been associated with a more favorable outcome (17,19). The marked clinical heterogeneity and relatively low penetrance observed with TNNI3 mutations illustrate that large numbers of consecutive genotype-positive families are needed to establish if specific genotype-phenotype relations are present in other HCM genes.

It is difficult to explain the variable disease expression based on existing functional studies and transgenic animal models expressing TNNI3 mutations, especially in families with identical mutations and a common ancestor present in this study. We have recently reported a large family affected by a TNNI3 mutation that had a more homogeneous phenotype with full disease penetrance and a high risk of sudden death (32). In contrast with most of the families in this study who were relatively small and from predominantly urban areas, this family originated from a rural area. It is likely that the genetic variation in such isolated areas is less diverse compared with open urbanized communities, which could help explain the more uniform phenotype (34,35). In addition, environmental factors are likely to influence the phenotypic expression of HCM, making it difficult to predict disease development and prognosis of individual patients with diverse genetic backgrounds.

The results of mutation analysis of all protein-encoding exons of TNNI3 in 1,081 HCM patients in this and previous studies have not revealed any disease-causing mutations outside exons 7 and 8 (10,14,27,28). Thus, the probability of identifying a mutation outside these exons would be <1:1,081 (<0.09%), and, therefore, it seems reasonable to limit mutation analysis of TNNI3 to exons 7 and 8. In addition, F-SSCP analysis was shown to be an appropriate method for mutation screening with the advantage of being cheaper, faster, and more suitable for high-throughput analysis than direct sequencing. Knowledge of the genetic status enhances the ability to provide accurate counseling of clinically unaffected relatives. Individuals who are shown to carry the mutation can be informed that disease penetrance is 48%, that age of diagnosis is variable, and that clinically unaffected carriers are at risk of having affected offspring who may experience disease-related com-
plications. It is important to emphasize that individuals without the mutation have no risk of developing or passing the disease on to their offspring. Thus, genetic diagnosis identifies relatives who require follow-up and enables termination of cardiac evaluation in individuals without the mutation, which is of great relief. This also facilitates cost-effective use of resources for clinical screening.

In summary, the severity of disease expression in HCM probands with \textit{TNNI3} mutations does not predict the severity or timing of disease development in genotype-positive offspring, siblings, and other relatives. The previous concept that knowledge of specific mutations would provide sufficient prognostic information to determine prophylactic treatment for sudden death was not substantiated. However, the fact that clinically unaffected mutation carriers may have offspring who present with sudden death and the overall unpredictability in disease development and complications suggest that genetic diagnosis is important, particularly in unaffected offspring. This will define the relevant subgroup of individuals who require clinical follow-up by ECG and monitoring of cardiac symptoms. Implementation of genetic diagnosis of \textit{TNNI3} is technically feasible, and the data support a potentially clinical utility in HCM.

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