Prevalence of Sarcomere Protein Gene Mutations in Preadolescent Children With Hypertrophic Cardiomyopathy

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Background—Hypertrophic cardiomyopathy (HCM) in infants and children is thought to be commonly associated with metabolic disorders and malformation syndromes. Familial disease caused by mutations in cardiac sarcomere protein genes, which accounts for most cases in adolescents and adults, is believed to be a very rare cause of HCM.

Methods and Results—Seventy-nine consecutive patients diagnosed with HCM aged 13 years or younger underwent detailed clinical and genetic evaluation. The protein-coding sequences of 9 sarcomere protein genes (MYH7, MYBPC3, TNNI3, TNNT2, TPM1, MYL2, MYL3, ACTC, and TNNC1), the genes encoding desmin (DES), and the γ-2 subunit of AMP kinase (PRKAG2) were screened for mutations. A family history of HCM was present in 48 patients (60.8%). Forty-seven mutations (15 novel) were identified in 42 (53.2%) patients (5 patients had 2 mutations). The genes most commonly implicated were MYH7 (48.9%) and MYBPC3 (36.2%); mutations in TNNT2, ACTC, MYL3, and TNNI3 accounted for <5% of cases each. A total of 16.7% of patients with sarcomeric mutations were diagnosed before 1 year of age. There were no differences in clinical and echocardiographic features between those children with sarcomere protein gene mutations and those without or between patients with 2 mutations and those with 1 or no mutations.

Conclusions—This study shows that familial disease is common among infants and children with HCM and that, in most cases, disease is caused by mutations in cardiac sarcomere protein genes. The major implication is that all first-degree relatives of any child diagnosed with HCM should be offered screening. Furthermore, the finding that one sixth of patients with sarcomeric disease were diagnosed in infancy suggests that current views on pathogenesis and natural history of familial HCM may have to be revised. (Circ Cardiovasc Genet. 2009;2:436-441.)

Key Words: cardiomyopathy ■ death ■ sudden ■ genes ■ myocardial contraction ■ pediatrics

Hypertrophic cardiomyopathy (HCM) is a heterogeneous condition, defined by the presence of left ventricular hypertrophy (LVH) in the absence of congenital heart disease or abnormal loading conditions sufficient to cause the observed degree of hypertrophy. Numerous studies have shown that, in most adults and adolescents, HCM is inherited as an autosomal dominant trait caused by mutations in the genes encoding a range of cardiac sarcomere proteins. In contrast, studies in infants and children report familial disease in <20% of cases. In some young patients, LVH is associated with disorders of metabolism, neuromuscular disease, and congenital malformation syndromes, but recently published studies suggest that these conditions account for <10% of cases. The demonstration that LVH in older patients, with familial HCM frequently develops during adolescence, has led to an assumption that sarcomeric protein gene mutations are a very rare cause of HCM in preadolescent children. The aim of this study, therefore, was to determine the prevalence of sarcomere protein disease in a consecutively referred cohort of infants and children diagnosed with HCM at an age of 13 years or younger.

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Methods

Patients

Between 1989 and 2007, 140 unrelated patients first diagnosed with HCM at an age of 13 years or younger were evaluated in a dedicated HCM clinic based at St George’s Hospital (1989–2003), The Heart Hospital (2003–2007), and Great Ormond St Hospital (1989–2007), London, United Kingdom. Of these, 25 had syndromic or metabolic disease (Noonan/LEOPARD syndrome, n=14; Friedreich ataxia, n=3; AMP kinase disease, n=3; Danon disease, n=1; undiagnosed metabolic disorder, n=1; mitochondrial disease, n=3) and were excluded. In 36 patients, no blood was available for analysis or patients refused to participate in the study, and these patients were also excluded. The final study cohort comprised 79 patients. All patients fulfilled conventional diagnostic criteria for HCM (LVH ≥2 SDs corrected for body surface area or more than the normal upper limit of the patient’s age). Patients underwent noninvasive assessment including clinical history, physical examination, 12-lead ECG,
and 2D, M-mode, and Doppler echocardiography. Data collected at the initial visit and during follow-up outpatient consultations were entered into a relational database. Left atrial and left ventricular (LV) internal dimensions, maximal LV wall thicknesses, and resting LV outflow tract gradients were determined using standard 2D and M-mode echocardiographic views, as described previously.13 Where patients’ height and weight were available (n=68), echocardiographic parameters were expressed as a deviation from the body surface area-corrected mean (z score) based on previously published normal values in our laboratory.14

**DNA Extraction and Mutation Analysis**

Genomic DNA from probands and family members was extracted from peripheral blood samples using a QIAamp DNA Mini kit (Qiagen, Hilden, Germany). Using standard protocols, the protein-coding sequences of the following sarcomeric genes were amplified by polymerase chain reaction (AmpliTaq Gold, Applied Biosystems, Foster City, Calif., USA): β-myosin heavy chain (MYH7); cardiac myosin-binding protein C (MYBPC3); troponin I (TNNI3); troponin T (TNNT2); α-tropomyosin (TPM1); regulatory myosin light chain (MYL2); essential myosin light chain (MYL3); α-cardiac actin (ACTC); and troponin C (TNC). In addition, the entire coding sequence of the desmin gene (DSC3) and the gene encoding the γ-2 subunit of AMP kinase (PRKAG2) were also amplified. Polymerase chain reaction conditions and primer sequences are available on request. All polymerase chain reaction fragments were subsequently directly sequenced on an ABI 3130 genetic analyzer using BigDye Terminator chemistry v3.1 (Applied Biosystems). Sequences were analyzed with Seqscape 2.5 software (Applied Biosystems). Following the same methodology, a cohort of 200 unrelated healthy volunteers served as controls for every novel sequence variation found in patients with HCM.

**Ethics**

The study was approved by the local research ethics committee, and informed written consent was obtained for genetic testing from all participants aged 16 years or older or from the parents of those younger than 16 years. The investigation conforms to the principles outlined in the Declaration of Helsinki.

**Statistical Analysis**

SPSS (version 11.0) was used for all statistical analyses. Normally distributed data are expressed as mean (95% CI); data not normally distributed are expressed as median (interquartile range). Differences between means were compared using the Student t test or 1-way ANOVA. The chi-square test was used for comparison of categorical data. The Mann–Whitney U test was used to analyze nonnormally

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**Table 1. Clinical Characteristics at First Evaluation**

<table>
<thead>
<tr>
<th></th>
<th>All Patients</th>
<th>Mutation Positive</th>
<th>Mutation Negative</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>79</td>
<td>42</td>
<td>37</td>
<td>...</td>
</tr>
<tr>
<td>Age at diagnosis, y</td>
<td>8.6 (2.0 to 11.0)</td>
<td>9.4 (2.5 to 11)</td>
<td>7.0 (8.0 to 11)</td>
<td>0.731</td>
</tr>
<tr>
<td>Age at 1st evaluation, y</td>
<td>10.1 (5.9 to 13.1)</td>
<td>10.9 (5.9 to 12.7)</td>
<td>10.6 (5.6 to 13.9)</td>
<td>0.552</td>
</tr>
<tr>
<td>Male/female</td>
<td>49/30 (62:38)</td>
<td>26/16 (61:9:38.1)</td>
<td>23/14 (62.2:37.8)</td>
<td>0.981</td>
</tr>
<tr>
<td>Symptoms at diagnosis</td>
<td>27 (34.2)</td>
<td>11 (28.2)</td>
<td>16 (43.2)</td>
<td>0.111</td>
</tr>
<tr>
<td>NYHA I</td>
<td>65 (82.3)</td>
<td>34 (81.0)</td>
<td>31 (83.8)</td>
<td></td>
</tr>
<tr>
<td>NYHA II</td>
<td>10 (12.7)</td>
<td>5 (11.9)</td>
<td>5 (13.5)</td>
<td></td>
</tr>
<tr>
<td>NYHA III/IV</td>
<td>4 (5.1)</td>
<td>3 (7.1)</td>
<td>1 (2.7)</td>
<td>0.662</td>
</tr>
<tr>
<td>Chest pain</td>
<td>12 (15.2)</td>
<td>6 (14.3)</td>
<td>6 (16.2)</td>
<td>0.687</td>
</tr>
<tr>
<td>Syncope</td>
<td>3 (3.8)</td>
<td>2 (4.8)</td>
<td>1 (2.7)</td>
<td>0.769</td>
</tr>
<tr>
<td>Presyncope</td>
<td>4 (5.1)</td>
<td>2 (4.8)</td>
<td>2 (5.4)</td>
<td>0.769</td>
</tr>
<tr>
<td>Palpitation</td>
<td>9 (11.4)</td>
<td>4 (9.5)</td>
<td>5 (13.5)</td>
<td>0.559</td>
</tr>
<tr>
<td>FHx HCM</td>
<td>48 (60.8)</td>
<td>28 (66.7)</td>
<td>20 (54.1)</td>
<td>0.252</td>
</tr>
<tr>
<td>FHx SCD</td>
<td>28 (35.4)</td>
<td>15 (35.7)</td>
<td>13 (35.1)</td>
<td>0.957</td>
</tr>
</tbody>
</table>

**Echocardiography**

<table>
<thead>
<tr>
<th></th>
<th>All Patients</th>
<th>Mutation Positive</th>
<th>Mutation Negative</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>MLWWT, mm</td>
<td>17 (11 to 23)</td>
<td>17 (11 to 20)</td>
<td>19 (13 to 27)</td>
<td>0.184</td>
</tr>
<tr>
<td>MLWWT z score</td>
<td>14.5 (6.9 to 22.3)</td>
<td>13.6 (6.3 to 18.1)</td>
<td>15.2 (8.1 to 28.0)</td>
<td>0.144</td>
</tr>
<tr>
<td>LVEDD, mm</td>
<td>36 (30 to 40)</td>
<td>36 (31 to 40)</td>
<td>36 (30 to 42)</td>
<td>0.911</td>
</tr>
<tr>
<td>LVEDD z score</td>
<td>−1.8 (−2.8 to −0.8)</td>
<td>−1.7 (−2.7 to −0.8)</td>
<td>−1.9 (−3.0 to −0.6)</td>
<td>0.973</td>
</tr>
<tr>
<td>LVESD, mm</td>
<td>19 (16 to 23)</td>
<td>19 (16 to 23)</td>
<td>19 (14 to 24)</td>
<td>0.966</td>
</tr>
<tr>
<td>LVESD z score</td>
<td>−3.1 (−4.7 to −1.5)</td>
<td>−2.9 (−4.8 to −1.5)</td>
<td>−3.3 (−4.7 to −1.5)</td>
<td>0.727</td>
</tr>
<tr>
<td>FS, %</td>
<td>45 (38 to 54)</td>
<td>45 (37 to 54)</td>
<td>46 (40 to 54)</td>
<td>0.495</td>
</tr>
<tr>
<td>LA dimension, mm</td>
<td>33 (27 to 39)</td>
<td>31 (27 to 38)</td>
<td>34 (27 to 40)</td>
<td>0.637</td>
</tr>
<tr>
<td>LA dimension z score</td>
<td>2.1 (1.0 to 4.3)</td>
<td>2.1 (1.0 to 3.2)</td>
<td>2.7 (0.9 to 5.6)</td>
<td>0.179</td>
</tr>
<tr>
<td>LVOT gradient, mm Hg</td>
<td>10 (6 to 31)</td>
<td>9 (5 to 17)</td>
<td>12 (7 to 61)</td>
<td>0.054</td>
</tr>
<tr>
<td>Pattern of LVH</td>
<td>ASH</td>
<td>63 (79.7)</td>
<td>36 (85.7)</td>
<td>27 (73.0)</td>
</tr>
<tr>
<td></td>
<td>Concentric</td>
<td>11 (13.9)</td>
<td>4 (9.5)</td>
<td>7 (18.9)</td>
</tr>
<tr>
<td></td>
<td>Eccentric</td>
<td>5 (6.3)</td>
<td>2 (4.8)</td>
<td>3 (8.1)</td>
</tr>
</tbody>
</table>
distributed continuous data. Survival curves and estimates were calculated using the Kaplan–Meier method for time since diagnosis. Group differences in survival were assessed using the log-rank test. A \( P < 0.05 \) was considered significant.

**Results**

**Clinical Characteristics**

The clinical characteristics of the study population at first evaluation are shown in Table 1: 89.9% of patients were white; 3.8% were Asian; 2.5% were black; 2.5% were Middle Eastern; and 1 was Chinese. Seventeen patients (21.5%) were diagnosed in infancy (1 year younger or younger; Figure 1). Thirty-one patients (39.2%) were diagnosed after the incidental detection of a murmur or another abnormal finding during clinical examination; 29 patients (36.7%) were diagnosed as a result of clinical screening for a family history of HCM; 16 patients (20.3%) were diagnosed during investigation of cardiovascular symptoms; and 3 patients (3.8%) were diagnosed after an out-of-hospital cardiac arrest. Thirty-three patients were diagnosed at our institution, whereas 46 were diagnosed elsewhere and referred to us for further management. Fifty patients were initially evaluated at our institution within 2 years of diagnosis; in the remaining patients, referral to our unit occurred between 2 and 29 years after diagnosis. Of these, only 13 patients were older than 13 years and only 4 were older than 15 years at the time of the initial evaluation in our unit.

After the evaluation at our institution, the total number of patients who had clinical evidence of familial disease was 48 (60.8%). In 42 patients, at least one first-degree relative was affected; in the remaining 6 patients, a second-degree relative was known to be affected, but the probands’ parents had normal ECGs and echocardiograms and were thus considered to be obligate carriers.

**Genetic Analysis of the Proband**

A total of 47 mutations were identified in 42 (53.2%) patients (5 patients with 2 mutations; Figure 2) including 4 missense mutations found in >1 proband (R453C [n=3], G716R [n=2], and G741R [n=2] in MYH7; R502W [n=4] in MYBPC3). Mutations were most frequent in MYH7 (48.9% of cases) and MYBPC3 (36.2%); mutations in TNNT2, ACTC, MYL3, and TNNI3 accounted for <5% of cases each. No mutations were identified in TPM, TNNC1, MYL2, PRKAG2, or DES. A list of mutations is shown in Table 2. Of the 5 double heterozygotes, 2 had mutations in the same gene (MYBPC3 in both cases). In one case, the mutations were on different copies of the MYBPC3 gene as each mutation was inherited from a different parent. In the other case, the parents of the affected individual could not be genotyped, as samples were unavailable for analysis.

There were no significant differences in clinical or echocardiographic characteristics between those individuals in whom sarcomere protein gene mutations were identified and those without mutations (Table 1). Furthermore, there were no differences between individuals with 2 mutations and those with 1 or no mutations (data not shown).

**Pathogenicity of Novel Sequence Alterations**

Of the 47 mutations identified, 25 have been previously reported in patients with HCM and are considered disease causing. Fifteen were novel, and we assessed their possible pathogenicity using the following criteria: absence in 200 control samples; segregation with disease in families (where appropriate and feasible); and, in particular for missense sequence changes, whether the affected amino acid residue is evolutionarily conserved and located in functionally important regions of the protein. Conservation of amino acid residues was determined by multiple alignment of orthologues in various species using the Clustal W computer program.15 A review of the literature was the source of information on the function of specific regions in sarcomeric proteins, using the following search terms on PubMed: sarcomeric proteins; genes; function; mutation; and sarcomere.

In more detail, 6 novel missense mutations were detected in MYH7: 7847G>A, D239N; 7986C>A, H251N; 10100G>T, D382Y; 13436C>G, P710R; 14523A>G, K847E; and 15310G>A, E903K. These changes result in substitution of...
highly conserved amino acids in functionally important regions of the β-myosin protein. Four of these mutations are located in the head domain (D239N and H251N, active site; D382Y, actin-binding domain; and P710R, light-chain–binding domain), whereas K847E and E903K are in the head-rod junction.

In MYBPC3, we identified 2 novel deletions: 5810_5816delGC-CTTCC and 17645delC. The first results in a frame shift deletion of 2 amino acid residues (A270 and F271) and a premature termination codon in exon 27 (P870fsX878). We also detected 2 novel missense mutations (18584C→T, P961L and 20069A→G, Y1172C) that are located in the fibronectin type 3 C7 domain and the titin-binding domain of MYBPC3, respectively.

In ACTC, we identified a novel deletion (2385_2387delTCT) in exon 2, which is predicted to delete a phenylalanine residue at position 92 and a missense mutation (4645C→T, R314C) in the subdomain 3 of the actin protein.

In MYL3, 2 novel missense mutations were found (4940G→A, E152K and 4949C→G, Y1172C) that are located in the fibronectin type 3 C7 domain and the titin-binding domain of MYBPC3, respectively.

Finally, in TNNI3, a novel missense mutation was identified (g.4769G→A, R170Q) within the actin-binding domain of the troponin I protein in exon 7, which is predicted to increase the inhibitory effect of troponin I.

Of the 15 novel mutations identified, only one occurred in a nonwhite patient (P961L in MYBPC3). This patient had mild, concentric LV hypertrophy, diagnosed after the sudden death of his sibling, in whom the diagnosis of HCM was made at autopsy. Tissue was not available from the sibling for analysis, but the same sequence alteration was identified in the proband’s father, who had a normal echocardiogram but an abnormal 12-lead ECG.

**Genetic Evaluation of the Probands’ Relatives**

Peripheral blood samples from first-degree relatives were available in 30 (71.4%) of the 42 families in which a mutation was identified. Where blood samples were unavailable, this was because the first-degree relatives were not alive or could not be contacted, or they refused to provide a blood sample. Of these 30 families, 24 were known to have a family history of HCM from pedigree analysis and family screening. The prevalence of inherited and de novo mutations is shown in Figure 3. There was no difference in the frequency of sarcomere protein gene mutations between individuals with a family history of HCM and those without ($P=0.252$).

**Discussion**

The results of this study have important clinical implications for the investigation and management of infants and young children with LVH. The key findings are that familial HCM (not associated with neuromuscular, metabolic, or syndromic disorders) in this age group is caused by mutations in cardiac sarcomeric protein genes. The major implication of these findings is that clinical screening of all first-degree relatives should be an essential part of the management of any infant or child diagnosed with HCM.

**Causes of LVH in Infants and Children**

Most clinical reviews and guidelines suggest that LVH in infants and children is often associated with inborn errors of metabolism, mitochondrial dysfunction, neuromuscular conditions such as Friedreich’s ataxia, and disorders such as Noonan and LEOPARD syndromes. However, data from large population-based observational studies in Australia and 2 regions in the United States suggest that these conditions account for <10% of all cases of HCM in young patients,
including infants, the age group most likely to present with metabolic diseases or syndromes.\textsuperscript{7,8}

In a substantial proportion of adults with HCM, LVH is caused by mutations in the genes encoding the myofibrillar proteins of the cardiac sarcomere.\textsuperscript{2–6} Until recently, no study had examined the role of similar gene mutations in young children and infants with HCM. This deficiency is probably explained by observational studies that report a much lower frequency of familial disease in children,\textsuperscript{7,8} and the generally accepted view that LVH in individuals with familial HCM usually develops during the adolescent growth spurt.\textsuperscript{10} Recently, Morita et al\textsuperscript{16} reported sarcomere protein gene mutations in 55% of individuals diagnosed with HCM before the age of 15 years, including 49% of patients with apparently sporadic disease (although segregation data were only available in 44% of families). Together with this study, these results suggest that disease caused by sarcomeric protein gene mutations is at least as common as in adults. The fact that 7 of 42 patients (16.7%) with sarcomeric disease in our study were first diagnosed below the age of 1 year suggests that current views on pathogenesis and natural history of familial HCM may also have to be revised.

Implications for Family Screening

Current guidelines recommend that clinical screening of relatives of patients younger than 12 years with HCM should be optional, unless there is a malignant family history of sudden death, clinical suspicion of early LV hypertrophy, onset of symptoms, or the child is a competitive athlete involved in an intense training program.\textsuperscript{17} The findings in this study suggest that these guidelines should be revised to take into account the high incidence of disease expression in children.

Six percent of the patients in this study had 2 mutations, highlighting the importance of screening for other sarcomeric genes, even when a mutation in 1 gene has been identified.\textsuperscript{5} It has been suggested that double mutations are associated with earlier and more severe disease expression,\textsuperscript{5,18–21} but there was no difference in age at diagnosis or disease severity in patients with complex genotypes compared with those with single mutations or nonsarcomeric disease.

Limitations

In this study, we did not screen every gene implicated in familial HCM, and so it is quite possible that mutations in genes such as titin\textsuperscript{22} and myozienin,\textsuperscript{23} or even genes such as \textit{LAMP-2} associated with Danon disease\textsuperscript{24,25} or \textit{PTPN11} associated with Noonan syndrome,\textsuperscript{26} could explain some of the remaining idiopathic cases. However, most studies suggest that these account for only a very small number of cases.

Another limitation of this study is the lack of familial segregation data in 28% of patients in whom a sarcomeric mutation was identified, which does not allow us to determine the prevalence of nonpenetrant mutations in the parents of apparently sporadic cases. However, the prevalence of segregation data available in this study compares favorably with previously published reports.\textsuperscript{16}

The study population was derived from a tertiary referral center, including a proportion of patients who were referred because of a family history of HCM, which may introduce referral bias. However, this has not resulted in an overestimation of the prevalence of sarcomere protein disease in the HCM population, as there was no significant difference in the frequency of sarcomere protein mutations between patients with and without a family history.

An important issue arising from this study relates to the prevalence of HCM among children screened because of a family history, and it would be interesting to determine how many HCM families would need to be screened to identify one affected child. This is beyond the scope of this study but would be a topic of great interest for future studies.

Conclusions

This study shows that mutations in cardiac sarcomere protein genes are a common cause of idiopathic HCM in infants and children and expands the range of reported sarcomeric mutations in pediatric HCM. This has important implications for the management of HCM in the young and highlights the need for systematic screening of first-degree relatives of patients with HCM, even in those aged younger than 12 years.

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References
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