Penetrance of Hypertrophic Cardiomyopathy in Children and Adolescents: A 12 Year Follow-Up Study of Clinical Screening and Predictive Genetic Testing

Running title: Jensen et al.; Penetrance of HCM during adolescence

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Abstract:

**Background**—The penetrance of hypertrophic cardiomyopathy (HCM) during childhood and adolescence has only been sparsely described. We studied the penetrance of HCM and the short- and long-term outcomes of clinical screening and predictive genetic testing of child relatives to patients with HCM.

**Methods and Results**—Ninety probands and 361 relatives were included in a family-screening program for HCM (1994-2001). Eleven sarcomere genes, CRYAB, α-GAL and Titin were screened. Sixty-six relatives and four probands were <18 years of age at inclusion. Twelve child relatives were mutation carriers (age 12±5 years), and 26 had unknown genetic status, i.e. relatives from families without identified mutations (n=21) or not tested (n=5) (age 11±5 years). Twenty-eight (42%) non-carriers (age 10±4 years) served as controls. Two out of 38 (5%) child relatives at risk of developing HCM fulfilled diagnostic criteria for HCM at inclusion. After 12±1 years follow-up two out of the 36 (6%, CI 2-18%) at risk child relatives, who were phenotype-negative at inclusion, had developed the HCM phenotype at the age of 26 and 28 years. During follow-up none of the child relatives experienced serious cardiac events.

Participation in the screening program had no long-term negative psychological impact.

**Conclusions**—The penetrance of HCM in phenotype-negative child relatives at risk of developing HCM was 6% after 12 years follow-up. The finding of phenotype conversion in the mid-twenties warrants continued screening to extend into adulthood. Forty-two percent of the child relatives were non-carriers, and repeated clinical follow-up could safely be limited to the remaining children.

**Key words:** children; family screening; penetrance; pre-symptomatic testing; sarcomere gene mutation
Introduction

Hypertrophic cardiomyopathy (HCM) is most often autosomal dominantly inherited with incomplete penetrance and variable expressivity. The manifestations of this genetically heterogeneous disease vary from asymptomatic or mildly symptomatic patients to patients with severe heart failure symptoms, angina, arrhythmia, syncope or sudden cardiac death (SCD). SCD may be the first manifestation of the disease. The main purposes of family screening as recommended by ACC (American College of Cardiology) and AHA (American Heart Association) are to identify undiagnosed HCM patients to allow for clinical management of early symptoms and identification of patients with an increased risk of SCD. A disease-causing mutation can be identified in sarcomere genes in 30-60% of HCM patients. Predictive genetic testing of relatives identifies mutation carriers at risk of developing HCM as well as non-carriers without such a risk.

The timing of clinical and genetic screening of children is based mainly on consensus opinions and clinical judgments and has been addressed in a few studies only. It is largely unknown, how many relatives under the age of 18 years are affected by HCM. The general recommendation is to screen child relatives from the age of 12 years. The prognostic value of identifying sarcomere gene mutations in children without phenotypic manifestations of HCM is unclear.

We studied the outcome of clinical screening and predictive genetic testing of child relatives (<18 years) from families with HCM, and assessed the age-related penetrance of HCM during 12 years follow-up in these young relatives.

Methods

Ethics
The local Ethics Committee approved the clinical and genetic family screening study (V92-213) and allowed clinical screening and predictive genetic testing of all children regardless of age after written informed consent had been obtained from parents or guardians. All families were offered genetic counseling. The children were included in the decision process according to their intellectual and emotional maturity, and were given the opportunity to reject participation. The parents/guardians/child decided whether or not to accept the offered genetic test, and whether or not they wanted to be informed about the genetic test result. These options were important to ensure that the study did not violate the “best interest” of the children\textsuperscript{12}. Parents or guardians were informed about the children’s “right not to know”\textsuperscript{13} before deciding on the level of participation.

**Study cohort**

From 1994 to 2001 90 child and adult HCM probands were consecutively included in the family screening program at The Unit for Inherited Heart Diseases, The Heart Center, in close collaboration with The Department of Clinical Genetics both at The National University Hospital, Rigshospitalet, Copenhagen, Denmark\textsuperscript{3,14-18}. In 32 of the 90 (36\%) probands 38 disease-causing mutations were identified. Each family was offered screening, and a total of 361 relatives (all ages) were included in the screening program. Four probands and 66 relatives (from 26 families) < 18 years of age at the time of inclusion in the screening program (Figure 1) were included in the present long-term follow-up study.

**Clinical and genetic family screening**

The first step in the screening process was clinical examination including electrocardiogram (ECG) recording and echocardiography in probands and relatives.

The second step was genetic testing of the probands. All coding regions of 11 sarcomere
genes (MYH7, MYL2, MYL3, MYBPC3, TNNI3, TNNT2, TPM1, ACTC, CSRP3, TCAP, TNNC1), CRYAB, α-GAL and five exons of Titin were analyzed in all probands\(^3,14-18\).

A sequence variant was considered disease-causing if this had been shown in previous reports or if the criteria (1-3) were fulfilled: 1. The mutation resulted in a missense mutation causing a non-synonymous amino acid substitution, or resulted in a frame-shift or interference with a canonical splice-site; 2. The mutation affected an evolutionarily conserved amino acid; 3. The mutation was not found in 500 control alleles or in dbSNP. Additionally, the mutations had to co-segregate with HCM in the family. In small families, where assessment of familial co-segregation was not possible, a disease-association was presumed if the other criteria were met.

In the case of a missense mutation in CRYAB the pathogenicity was further supported by showing interference with correct folding of the protein, assessed by circular dichroism spectroscopy, and changes in amyloid properties measured by changes in interaction with Congo red.

If a disease-causing gene mutation was identified in the proband, a selective genetic test for the identified mutation was offered to 1\(^{st}\) degree relatives. Relatives carrying a gene mutation and 1\(^{st}\) degree relatives from families without an identified gene mutation were referred for routine follow-up after the initial screening process. Non-carriers were reassured and no further clinical follow-up was offered.

**Carriers, non-carriers and child relatives with unknown genetic status**

On the basis of the genetic testing the 66 child relatives were divided into three groups. The first group included 28 child relatives, who were not carrying the gene mutation identified in their family (non-carriers). This group served as controls (Figure 1). The second group included 12 gene mutations carriers. The third group of 26 child relatives with unknown genetic status
included 21 child relatives from families without identified gene mutations and five child relatives from families with identified gene mutations, but who declined genetic testing. Together, the second and third group included 38 child relatives at risk of developing HCM.

**Follow-up assessment**

The follow-up assessment included clinical, ECG and echocardiographic evaluations as well as questionnaire assessments of the long-term psychological impact of participation in the family screening program\textsuperscript{19-22}. Vital status was available for all probands and relatives.

**Echocardiography**

At the time of inclusion 2-dimensional (2D) echocardiography, M-mode and Doppler evaluations and measurements were performed. Analyses were performed according to the ASE guidelines\textsuperscript{23} using the Echopac Dimension software version 8. Echocardiograms were analyzed for left ventricular wall thickness, cavity dimensions, structural abnormalities, systolic anterior movement of mitral leaflets (SAM) and outflow tract gradients at rest and during Valsalva-maneuver. At the time of inclusion and at follow-up left ventricular wall thickness was evaluated in all segments including the apical segments. The maximal wall thickness (MWT) was defined as the maximal thickness of the left ventricular wall in any of the segments. In children (< 18 years) the MWT was adjusted for body surface area (BSA) using the formula by Dubois\textsuperscript{24}.

**Electrocardiogram**

Electrocardiograms (ECG) were recorded using commercially available 12 lead ECG recorders at the time of inclusion and at follow-up. The ECG’s were analyzed for Q-waves, T-wave abnormalities and time intervals. The presence of Sokolow-Lyon voltage ≥35 mV\textsuperscript{25}, a Cornell product >2440\textsuperscript{26} or a Romhilt-Estes score ≥ 5\textsuperscript{27} were considered suggestive of hypertrophy in adults.
Diagnostic criteria for hypertrophic cardiomyopathy

In child relatives (< the 18 years of age) a BSA adjusted MWT > mean BSA adjusted wall thickness + 2 × standard deviation (SD) (i.e. Z-score > 2) in the control group was considered diagnostic for HCM. In adult relatives (≥18 years of age) a MWT ≥ 13 mm was considered diagnostic for HCM according to guidelines. These criteria were used at inclusion and at follow-up.

Psychological evaluation

See supplemental material for a detailed description of the psychological testing.

Statistics

Data was analyzed using the SAS statistical software version 9.1. Data is presented as mean ± SD. Unpaired comparisons were performed using the Student’s t-test after confirmation of normal distribution. Normal values for the left ventricular wall thicknesses are available in the literature for the basal segments of the inter-ventricular septum and posterior wall, but not for other segments. Therefore, Z-scores for BSA adjusted MWT were calculated on the basis of the SD in controls. A Z-score = 2 was considered the upper normal limit for BSA adjusted MWT in controls. The evaluation scores from questionnaires were handled as continuous variables, which enabled comparisons using the unpaired Students t-test. Proportions are presented as percentages (%) and whenever relevant confidence intervals (CI) are reported. Proportions were compared using the Chi-square test or Fisher’s exact test. A two-sided probability of less than 0.05 was considered significant. Bonferroni’s correction of p-values was used to adjust for multiple comparisons leaving the level of significance unchanged at 0.05. Only corrected p-values are given.
Results

Seventy children and adolescents (<18 years) (66 relatives and 4 probands) from 26 families were included in the family screening program (Figure 1). In 15 (58%) of these families, a disease-causing sarcomere gene mutation was identified in the proband, and genetic testing was performed in 89% of their child relatives. In 87% of the genetically tested child relatives parents/children wanted to know the genetic test result.

Genetic findings

In the 12 carriers from 9 families we identified mutations in three different genes (MYH7, MYBPC3, MYL2) (Table 1). One carrier had two mutations as did the proband in the family (family T). The disease-causing mutation in the family was not present in 28 children from 10 families (controls). Two of the four child probands had MYBPC3 mutations, and one child proband had a CRYAB mutation. In the last child proband no mutations were found (Table 2).

Clinical findings at inclusion

There were no differences in BSA-adjusted MWT between controls (6.0±1.6 mm/m²) and carriers (5.7±1.0 mm/m²; p=ns) and relatives with unknown genetic status (5.7±2.0 mm/m²; p=ns). None of the carriers but two of 26 child relatives with unknown genetic status fulfilled the diagnostic criteria for HCM at the time of inclusion (Z-score: 2.7 and 2.5; age 5 and 7 years, respectively). Both were from families with known disease-causing sarcomere gene mutations, but these two children had not been genetically tested. In total, two out of 38 (5%, CI 1-17%) child relatives at risk of developing HCM fulfilled the diagnostic criteria for HCM at inclusion. Applying published and institutional references values for IVSd and LVPWd to the LV wall thicknesses measured in other projections did not change the diagnosis in any of the child relatives at risk of developing HCM.
Clinical findings at follow-up

All child relatives were alive at follow-up, and none had experienced any cardiovascular symptoms or any cardiac events during follow-up. Follow-up was performed in all carriers and all relatives with unknown genetic status (Table 3). Twenty-three controls were available for follow-up.

At follow-up, two of the 12 carriers (17%, CI 5-45%) had developed HCM with a MWT of 15 and 17 mm, respectively (Table 1, Figure 2, Panel B). One relative with unknown genetic status, diagnosed at inclusion, still fulfilled the diagnostic criteria for HCM at follow-up (MWT = 15 mm) (6%, CI 1-19%). The other relative with unknown genetic status, fulfilling criteria for HCM at inclusion (age 5 years), did not fulfill the diagnostic criteria for HCM at follow-up (MWT =12 mm, Z-score = -0.75, age 17 years, weight = 110 kg). In total, two out of 36 (6%, CI 2-18%) child relatives at risk of developing HCM, who were phenotype-negative at inclusion, developed HCM during 12±1 years follow-up.

The three child relatives, who fulfilled the diagnostic criteria at follow-up, had been evaluated repeatedly during the clinical follow-up. The two carriers, who developed HCM during follow up, converted to the HCM phenotype at the age of 26 and 28 years (Figure 3).

In the ECG recordings abnormal Sokolow-Lyon voltage criteria, Cornell products or Romhilt-Estes scores were present in a number of the at-risk children with normal echocardiograms, and also in some of the controls (Table 3). These false positive findings indicate a reduced diagnostic value of ECG recordings in the screening program.

Psychological findings at follow-up

We did not find any differences in anxiety, depression, type D personality or overall psychological impact of participation in the family screening program between the three groups.
(carriers, controls and relatives with unknown genetic status (Supplemental material Table 1)).

Discussion

To our knowledge this study is the largest series of child relatives to HCM probands followed for the longest time period. Follow up after 12 years revealed a very low penetrance of HCM in childhood and in early adulthood and no cardiac events were seen in the child relatives. The low penetrance challenges the general perception that HCM predominantly develops during the growth spurt in childhood or adolescence\(^\text{10}\).

Diagnostic yield and penetrance

The initial screening revealed no affected carriers (n=12), two affected children with unknown genetic status, and no affected controls. Two carriers (17\%, CI 5-45\%) converted to the HCM phenotype during follow-up, but not until the age of 26 and 28 years, respectively. At follow-up only one child with unknown genetic status (6\%, CI 1-19\%), already diagnosed at inclusion at the age of 5 years, fulfilled diagnostic criteria. Thus, only 3 (5\%, CI 2-13\%) out of the total group of child relatives (n=66) or 3 (8\%, CI 3-21\%) out of the child relatives considered at risk of developing HCM, i.e. carriers (n=12) and relatives with unknown genetic status (n=26), fulfilled the diagnostic criteria for HCM after 12 years follow-up. By genetic screening we identified 28 (42\%) non-carriers. These children were reassured and further clinical follow-up was ceased. Our youngest child proband was diagnosed the age five years.

The observation that the HCM phenotype may not be present at birth, but may develop during childhood was initially reported in 1986 by Maron et al.\(^\text{10}\), showing that in a group of phenotype-negative child relatives at a mean age of 11 years 5 out of 16 child relatives (31\%, CI 14-56\%) developed significant left ventricular hypertrophy during 4 years follow-up.
Considering the small study populations in the available studies the presently observed low penetrance (6%, CI 2-18%) during childhood and adolescence may not differ significantly from the phenotype conversion rate reported by Maron et al. However, the presently observed penetrance seems more in line with recent findings by Pasquale et al.\textsuperscript{28} of the penetrance of cardiac troponin T gene mutations in children < 16 years. They report development of HCM in only two out of 13 (15%, CI 4-42%) children at the age of 19 and 29 years, respectively, during 6.7±3 years follow-up. Taken together, in the majority of cases the HCM phenotype does not seem to develop until adulthood.

**Cardiac events**

The risk of SCD is the major driving force for cascade screening in HCM families. The observation that none of our child relatives experienced any cardiac events supports the general perception that cardiac events are rarely seen in phenotype-negative sarcomere gene mutation carriers.\textsuperscript{29,30} This is reflected in the contemporary guidelines only recommending risk stratification in phenotype-positive patients.\textsuperscript{5} The low event rates in phenotype-negative relatives are in contrast to high event rates in child probands as illustrated in the present study (Table 2) and reported by others.\textsuperscript{9,28} Ostman-Smith et al.\textsuperscript{9} have shown that SCD in symptomatic HCM children peaks at the age of 8-9 years, which is before the generally recommended age for commencement of clinical screening of approximately 12 years.\textsuperscript{5} However, a recent nationwide Danish study indicated a generally low risk of SCD in children with diagnosed or un-diagnosed HCM of < 0.1% per person-year.\textsuperscript{31} Nevertheless, it is an ongoing lingering concern that children carrying a sarcomere gene mutation may be at risk of SCD even in the absence of any phenotypic manifestations. Pasquale et al.\textsuperscript{28} reported one cardiac event in one out of 18 phenotype-negative children carrying Troponin T mutations without echocardiographic signs of
hypertrophy; a boy aged 16 years with a MWT of 9 mm died suddenly. No data on symptoms, ECG findings or risk factors for SCD in this case was reported. This illustrate that even though the risk of major cardiac events is low in phenotype-negative mutation carriers fatal cases can be seen, but at present we have no tools to predict this risk.

Taken together, by repeated clinical and echocardiographic screening from the youngest age, at which child relatives have been diagnosed by family screening, i.e. early school age, it is possible to identify the few children with manifest HCM who are at risk of cardiac events. The recommended follow-up at 12-18 months intervals in children and adolescents\textsuperscript{5} seems prudent, and the phenotype conversion in the twenties rather than during adolescence found in this and other studies\textsuperscript{28} warrants continued screening to extend into adulthood. This study does not provide data regarding the optimal screening intervals.

**Psychological impact of screening**

Data enlightening the concerns of whether family screening programs and predictive genetic testing present a threat to the psychological development of children has been requested\textsuperscript{32}.

Based on formal testing we did not identify apparent negative impact of participating in the screening on the general psychological states in terms of anxiety, depression and personality (Supplemental Table 1). Thus, screening of children for HCM and possibly other inherited heart diseases may be considered safe if performed in an appropriate clinical setting and in the best interest of the child\textsuperscript{12}.

**Role of genetic testing for the clinical management**

The decision on medical, ICD or surgical treatment of patients with HCM is not based on genetic findings, but relies entirely on symptoms, clinical findings and presence of risk factors for SCD. At present the value of genetic testing mainly relates to the decision on continuation or
discontinuation of clinical and echocardiographic follow-up. In this study 42% of the child relatives were reassured of their non-carrier status and follow-up could safely be limited to the remaining 58% of the child relatives. From clinical experience a genotype negative test-result in a child relative is of major importance and causes considerable relief in these families.

Genetic testing in child relatives depends on the ability to identify the disease-causing mutation in the proband, which at present is possible in ~50% of cases. Thus, genetic testing is only technically possible in half of the child relatives, but for families without an identified disease-causing mutation it should be emphasized that based on the present findings and other data, there is no evidence that genetic testing per se improves safety or outcome in the children. Thus, whereas the clinical screening and follow-up is clearly justified in child relatives, predictive genetic testing is useful to differentiate between those with and those without a need for clinical follow-up. However, it is important to consider postponing the predictive genetic testing until adulthood in order to ensure the autonomy of the child and for legal reasons. It seems reasonable to suggest that the present findings provide some support for a general notion that genetic testing and clinical follow-up of children from families with inherited cardiac diseases are unlikely to have a significant negative impact on the psychological development of the child.

It should be emphasized that the present findings were obtained in a close multidisciplinary teamwork between expert cardiologist and clinical geneticists.

Limitations

The generalizability of the present findings is limited by the relatively small number of child relatives at risk of developing HCM included in the study. It should also be noted that phenotype conversion rates and timing might depend on a number of factors, e.g. differences in genotype,
ethnicity or gender. In this study the genotype-positive child relatives had mutations in the two most frequently involved genes in HCM, i.e. MYBPC3 and MYH7, all included children were Caucasians and around 1/3 of children at risk of developing HCM were male. However, even though this is the largest series of child relatives followed for the longest period, larger and more mixed populations of child relatives at risk of developing HCM should be followed during childhood and adolescence to assess the timing and rate of phenotype conversion with more accuracy. This is of major importance for further development of recommendations for screening of child relatives to HCM patients.

Conclusion
The penetrance of HCM in phenotype-negative child relatives at risk of developing HCM was 6% after 12 years follow-up. The observations of serious cardiac events in the few children with HCM strongly support clinical screening from early school age. The finding of phenotype conversion in the mid-twenties warrants continued screening to extend into adulthood. By contemporary genetic testing almost half of the child relatives were excluded from being at risk of developing HCM. It was safe to limit the repeated clinical follow-up to the remaining children.

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Conflict of Interest Disclosures: None.
References:


Table 1. Identified sarcomere gene mutations and phenotypic status after 12 years follow-up of child relatives included in the family screening program for hypertrophic cardiomyopathy.

<table>
<thead>
<tr>
<th>Family</th>
<th>Gender</th>
<th>Gene</th>
<th>cDNA change</th>
<th>Protein</th>
<th>Age at follow-up (years)</th>
<th>Age at HCM diagnosis (years)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T</td>
<td>M</td>
<td>MYL2</td>
<td>c.37G&gt;A</td>
<td>p.A13T</td>
<td>22</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MYH7</td>
<td>c.3981C&gt;A</td>
<td>p.N1327K</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>M</td>
<td>MYH7</td>
<td>c.5134C&gt;T</td>
<td>p.R1712W</td>
<td>26</td>
<td>-</td>
</tr>
<tr>
<td>A</td>
<td>F</td>
<td>MYH7</td>
<td>c.569G&gt;C</td>
<td>p.R190T</td>
<td>19</td>
<td>-</td>
</tr>
<tr>
<td>A</td>
<td>M</td>
<td>MYH7</td>
<td>c.569G&gt;C</td>
<td>p.R190T</td>
<td>27</td>
<td>26</td>
</tr>
<tr>
<td>G</td>
<td>F</td>
<td>MYH7</td>
<td>c.2080C&gt;T</td>
<td>p.R694C</td>
<td>23</td>
<td>-</td>
</tr>
<tr>
<td>G</td>
<td>M</td>
<td>MYH7</td>
<td>c.2080C&gt;T</td>
<td>p.R694C</td>
<td>22</td>
<td>-</td>
</tr>
<tr>
<td>ZJ</td>
<td>M</td>
<td>MYBPC3</td>
<td>c.682G&gt;A</td>
<td>p.D228N</td>
<td>29</td>
<td>-</td>
</tr>
<tr>
<td>XD</td>
<td>F</td>
<td>MYH7</td>
<td>c.958G&gt;A</td>
<td>p.V320M</td>
<td>26</td>
<td>-</td>
</tr>
<tr>
<td>ZL</td>
<td>M</td>
<td>MYBPC3</td>
<td>c.1153_1168del16</td>
<td>p.Val385Metfs*16</td>
<td>22</td>
<td>-</td>
</tr>
<tr>
<td>ZL</td>
<td>M</td>
<td>MYBPC3</td>
<td>c.1153_1168del16</td>
<td>p.Val385Metfs*16</td>
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<td>28</td>
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<tr>
<td>ZH</td>
<td>M</td>
<td>MYBPC3</td>
<td>c.3491-14_3491-8delCCTGTCA</td>
<td>Splice variant</td>
<td>17</td>
<td>-</td>
</tr>
<tr>
<td>ZV</td>
<td>M</td>
<td>MYBPC3</td>
<td>c.281delT</td>
<td>p.Val94Alafs*2</td>
<td>13</td>
<td>-</td>
</tr>
</tbody>
</table>

HCM: hypertrophic cardiomyopathy, M: male, F: female, -: normal phenotype at follow-up.

Ref.seq: MYL2: NM_000432.3; NP_000423.2
MYH7: NM_000257.2; NP_000248.2
MYBPC3: NM_000256.3; NP_000247.2
Table 2. Characteristics and clinical events during 12 years follow-up in 4 child probands with hypertrophic cardiomyopathy.

<table>
<thead>
<tr>
<th>Family</th>
<th>Age (years)*</th>
<th>Gender</th>
<th>MWT (mm)*</th>
<th>Gene</th>
<th>Mutation</th>
<th>Events during follow-up</th>
</tr>
</thead>
<tbody>
<tr>
<td>ZV</td>
<td>8</td>
<td>M</td>
<td>20</td>
<td>MYBPC3</td>
<td>g2430 delG</td>
<td>Myectomy/ICD</td>
</tr>
<tr>
<td>ZJ</td>
<td>15</td>
<td>F</td>
<td>13</td>
<td>MYBPC3</td>
<td>Asp230Asn</td>
<td>No events</td>
</tr>
<tr>
<td>YA</td>
<td>17</td>
<td>M</td>
<td>30</td>
<td>CRYAB</td>
<td>Q26X</td>
<td>Sudden death</td>
</tr>
<tr>
<td>ZN</td>
<td>5</td>
<td>F</td>
<td>14</td>
<td>-</td>
<td>-</td>
<td>ICD</td>
</tr>
</tbody>
</table>

* At inclusion. MWT, Maximal wall thickness. ICD, implantable cardioverter defibrillator. M, male. F, female. The CRYAB mutation was a de novo mutation with confirmed maternity and paternity.

Table 3. Follow-up characteristics of relatives included during childhood in the family screening program for hypertrophic cardiomyopathy.

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>Unknown genetic status</th>
<th>Carriers</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N=23</td>
<td>N=26</td>
<td>p*</td>
</tr>
<tr>
<td><strong>Demographics</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age at follow-up (years)</td>
<td>22±4</td>
<td>22±6</td>
<td>ns</td>
</tr>
<tr>
<td>Follow-up (years)</td>
<td>12±1</td>
<td>13±2</td>
<td>ns</td>
</tr>
<tr>
<td>Gender (female)</td>
<td>25%</td>
<td>38%</td>
<td>ns</td>
</tr>
<tr>
<td>BSA at follow-up (m²)</td>
<td>1.9±0.3</td>
<td>1.9±0.2</td>
<td>ns</td>
</tr>
<tr>
<td><strong>Echocardiography</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MWT (inclusion)</td>
<td>7±2</td>
<td>8±2</td>
<td>ns</td>
</tr>
<tr>
<td>MWT (follow-up)</td>
<td>10±1</td>
<td>10±1</td>
<td>ns</td>
</tr>
<tr>
<td>BSA adjusted MWT</td>
<td>5.3±1</td>
<td>5.1±1</td>
<td>ns</td>
</tr>
<tr>
<td>IVSd (mm)</td>
<td>9±2</td>
<td>10±2</td>
<td>ns</td>
</tr>
<tr>
<td>LVEDd (mm)</td>
<td>46±4</td>
<td>49±5</td>
<td>ns</td>
</tr>
<tr>
<td>LVPWd (mm)</td>
<td>9±1</td>
<td>10±2</td>
<td>ns</td>
</tr>
<tr>
<td>LA dimension (mm)</td>
<td>36±5</td>
<td>38±6</td>
<td>ns</td>
</tr>
<tr>
<td>LVEF (%)</td>
<td>56±7</td>
<td>54±8</td>
<td>ns</td>
</tr>
<tr>
<td><strong>Electrocardiogram</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sokolow-Lyon &gt;35 mm</td>
<td>13%</td>
<td>15%</td>
<td>ns</td>
</tr>
<tr>
<td>Cornell product &gt;2440</td>
<td>4%</td>
<td>10%</td>
<td>ns</td>
</tr>
<tr>
<td>Romhilt-Estes score &gt;5</td>
<td>13%</td>
<td>5%</td>
<td>ns</td>
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</table>

*P-values for comparisons with the control group, BSA body surface area, MWT maximal wall thickness, IVSd interventricular septum thickness (diastolic), LVEDd left ventricular end-diastolic dimension (diastolic), LVPWd left ventricular posterior wall thickness (diastolic), LA left atrium, LVEF left ventricular ejection fraction.
Figure Legends:

Figure 1. Study outline. Ninety probands with HCM and 361 relatives were included. Sixty-six relatives and 4 proband were <18 years of age. G-: Genetically tested child relatives not carrying the disease-causing gene mutation (see Methods) identified in their family (i.e. non-carriers or controls). G+: Genetically tested child relatives carrying the disease-causing gene mutation identified in their family. G null: Child relatives from genetically tested families, where a disease-causing gene mutation was not identified. White boxes represent study subgroups at follow-up.

Figure 2. Echocardiographic images of two carriers with MYH7 mutation at follow-up. Panel A: Age 23 years, MWT = 9 mm. Panel B: Age 27 years, MWT 17 mm. Left: Parasternal long axis view. Right: Sagittal axis view.

Figure 3. Age-related penetrance of hypertrophic cardiomyopathy in sarcomere gene mutation carriers (carriers) and child relatives with unknown genetic status (unknown).
Penetrance of Hypertrophic Cardiomyopathy in Children and Adolescents: A 12 Year Follow-Up Study of Clinical Screening and Predictive Genetic Testing
Morten K. Jensen, Ole Havndrup, Michael Christiansen, Paal S. Andersen, Birgitte Diness, Anna Axelsson, Flemming Skovby, Lars Køber and Henning Bundgaard

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SUPPLEMENTAL MATERIAL

Psychological evaluation

The possible psychological impact of including children and adolescents into the family screening program was evaluated by four self-reporting questionnaires. Primarily, the psychological impact of knowing the genetic test result (positive or negative) was evaluated. Secondly, the psychological impact of experiencing cardiovascular events in the family was evaluated. The questionnaires were sent to the relatives after they were informed about the long-term follow-up study and had accepted participation. Anxiety was evaluated by the State Trait Anxiety Inventory (STAI)\(^1\). STAI evaluates the temporary short-term (state) anxiety and the personality-related tendency to respond with anxiety (trait anxiety) with a cut off value of 42 indicating clinically significant levels of anxiety\(^1\). Anxiety was also evaluated by the Hospital Anxiety and Depression Scale (HADS) that evaluates depression as well\(^2\). For both anxiety and depression a HADS score of 0-7 was considered normal and >11 was considered highly suggestive of a mood disorder. The Impact of Event Scale (IES) was used to evaluate current subjective distress related to the family screening program\(^2,3\). The IES was analyzed with respect to avoidance behavior, intrusive thoughts and hyper-arousal, and a higher score reflects a more affected psychological state. The DS14 questionnaire was used to evaluate negative affectivity and social inhibition, and a higher score reflects a more affected psychological state. The prevalence of type D personality\(^4\) was estimated from these scores. Type D personality has been associated with increased cardiovascular risk\(^5,6\).

We used questionnaires developed for evaluation of adults. STAI is available for children, but the majority of relatives tested in this study were >18 years and only three children were < 15 years of age at follow-up. The adult STAI used in this study was written in a 6\(^{th}\) grade reading level. HADS and IES were available in Danish. The STAI and DS14 were translated into Danish. To validate the
translation it was re-translated back into English by a second person, blinded with respect to the original English version. The original English version and the re-translated English version were compared by a third person (American with Danish as his second language). Disagreements between the two versions were then discussed in detail until consensus on a final Danish version was reached. When evaluating the psychological impact of the family screening program, persons not informed about the result of the genetic screening test were allocated to the group of relatives with unknown genetic status.
Table 1. Results of psychological evaluation of child relatives 12 years after inclusion to family screening for hypertrophic cardiomyopathy.

<table>
<thead>
<tr>
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<tr>
<td></td>
<td>Controls</td>
<td>genetic status</td>
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<tr>
<td>N</td>
<td>24</td>
<td>31</td>
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<tr>
<td>Impact of Event Scale (IES)</td>
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<tr>
<td>Intrusion</td>
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<td>3±5</td>
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<td>Avoidance behavior</td>
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<td>7±6</td>
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<td>Hyper-arousal</td>
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<td>2±3</td>
</tr>
<tr>
<td>IES</td>
<td>6±7</td>
<td>12±12</td>
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<tr>
<td>Hospital Anxiety and Depression Scale (HADS)</td>
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<tr>
<td>Anxiety score</td>
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<td>12±2</td>
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<tr>
<td>Depression score</td>
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<td>9±1</td>
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<tr>
<td>DS14</td>
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<tr>
<td>Negative affectivity</td>
<td>7±5</td>
<td>7±5</td>
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<tr>
<td>Social inhibition</td>
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<td>10±4</td>
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<tr>
<td>Type D personality</td>
<td>24%</td>
<td>22%</td>
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<tr>
<td>State Trait Anxiety Inventory (STAI)</td>
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</tr>
<tr>
<td>STAI</td>
<td>45±5</td>
<td>45±4</td>
</tr>
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</table>

All differences were non-significant after Bonferroni correction.
Supplemental references:


