Hypertrophic cardiomyopathy: towards an optimal strategy
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Risk stratification in hypertrophic cardiomyopathy

3.5 Septum thickness in Dutch MYBPC3 mutation carriers is influenced by the M235T-AGT polymorphism, increasing age, and male sex.

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Submitted

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Abstract

Background
Hypertrophic cardiomyopathy (HCM) is the most common inherited cardiac disorder characterized by profound clinical heterogeneity, even in patients carrying the same pathogenic HCM mutation. The RAAS system is a plausible candidate for modifying expression of left ventricular hypertrophy (LVH) because of its regulatory role in cardiac function, blood pressure, and electrolyte homeostasis.

Methods
Family based association analyses were performed on five RAAS polymorphisms (DD-ACE, CC-AGTR1, AA-CMA, M235T-AGT, and CC-CYPB11B2) in 403 subjects carrying functional identical truncating mutations in the MYBPC3 gene.

Results
Septum thickness was influenced by increasing age and male sex. Analyzing the five RAAS SNPs separately corrected for sex, age, and proband status showed that only the M235T-AGT SNP had a significant (P=6.1E-04) effect on septum thickness and was significantly (P=8.83E-04) associated with a thickened septum. The association analyses for septum thickness according to pro-LVH score showed no significant effect in the probands or relatives.

Conclusions
Septum thickness in Dutch MYBPC3 mutations carriers is influenced by the M235T-AGT polymorphism, progressive age and male sex. The pro-LVH score did not influence hypertrophy.
**Introduction**

Hypertrophic cardiomyopathy (HCM) is the most common inheritable cardiac disorder with a phenotypic prevalence of 1:500. It is defined by the presence of left ventricular hypertrophy (LVH) in the absence of loading conditions (hypertension, valve disease) sufficient to cause the observed abnormality. Mutations in more than 14 genes, mostly encoding sarcomeric proteins, are known to cause HCM and are found in up to 60% of cases. In the Netherlands, approximately one third of all HCM cases are caused by the truncating c.2373_2374insG, c.2864_2865delCT and c.2827C>T founder mutations in the myosin binding protein C gene (MYBPC3). These mutations lead to haplo-insufficiency and are thought to be functionally identical.

Several studies have tried to establish genotype-phenotype relations; these have been hampered by the clinical heterogeneity of HCM and the private nature of HCM mutations. Even individuals with the same genetic substrate express a wide spectrum of phenotypes. This indicates that disease course is not solely determined by the pathogenic gene and environmental factors and genetic modifiers must play an important role. The most important subgroup of polymorphisms studies to date involves the major components of the renin-angiotensin-aldosterone system (RAAS). The RAAS system contributes to LVH through effects mediated by circulating angiotensin as well as local activation of RAAS in the myocardium. Angiotensin (Ang) I is produced from angiotensinogen (AGT) which is converted to Ang II predominantly by angiotensin-converting enzyme (ACE) and partly by chymase (CMA). Ang II binds primary to Ang II type 1 receptor (AGTR1) to promote cell growth and hypertrophy. It is also converted to aldosterone by aldosterone synthase (CYP11B2) which promotes fluid retention and cardiac fibrosis. Polymorphisms in the RAAS pathway (DD-ACE, CC-AGTR1, AA-CMA, M235T-AGT, and CC-CYP11B2) appear to influence the severity of LVH in HCM patients. Furthermore, a combined ‘pro-LVH’ profile of 5 RAAS polymorphisms was associated with a higher degree of LVH in one particular, founder MYBPC3 HCM pedigree and in a large cohort of myofilament-negative patients.

Previous studies were hampered by small numbers and genetic heterogeneity. We used a large cohort of subjects carrying functional identical truncating mutations in the MYBPC3 gene, to analyze whether RAAS polymorphisms are modifiers of disease severity in MYBPC3 gene associated HCM.

**Methods**

**Subjects**

In the Netherlands genetic counseling and genetic testing are offered to all HCM patients visiting cardiogenetics outpatient clinics. After the detection of the causal mutation in the proband predictive genetic testing of relatives is possible (cascade screening). For the current study, 403 subjects carrying a truncating mutation in the MYBPC3 gene (both probands and mutation carrying relatives) were included from two university hospitals in the Netherlands; the Amsterdam Medical Centre and the Erasmus Medical Centre in Rotterdam. All subjects provided informed written consent. The study complies with the declaration of Helsinki and the local review board did approve the study.

**Echocardiographic evaluation**

Echocardiographic studies were recorded from all subjects using commercially available
equipment. The acquired data were digitally stored and subsequently analyzed by 2 physicians, who were blinded for the clinical and genetic data. Interventricular septum thickness (IVS) was measured in diastole from the parasternal short-axis view at the level of the papillary muscles. For subjects ≥ 16 years a IVS thickness ≥ 13 mm was abnormal. For subjects < 16 years IVS thickness was corrected for length and height and abnormal if the z-score was > 2. The extent of hypertrophy was assessed by a semiquantitative score method developed by Wigle et al. A maximum of 10 points are given: 1 to 4 points for IVS hypertrophy based on magnitude of thickness, 2 points for extension of hypertrophy beyond the level of the papillary muscles (basal two thirds of the IVS), 2 points for extension of hypertrophy to the apex (total IVS involvement), and 2 points for extension of hypertrophy into the lateral wall.

**SNP selection and genotyping**
Five 'pro-LVH' RAAS polymorphisms previously described in the literature (DD-ACE, CC-AGTR1, AA-CMA, M235T-AGT, and CC-CYPB11B2) were genotyped in 403 subjects. The five SNPs had a call rate >95%.

**Statistical analyses**
Phenotypic data for mutation carriers were normally distributed and are reported as the mean ± standard deviation. Pedigree information was available for all related subjects. To correct for the relatedness of our subjects we used a family based association program, a linear mixed effect model function (lmekin) in the Kinship package (version 1.1.0-22, Atkinson and Therneau, 2008) in R. Initially, we assumed that each SNP-phenotype relationship had an additive genetic model. To check our assumption we added a heterozygosity indicator variable to the additive model. In the cases were there was a significant (P<0.1) deviation from additivity, depending on the direction of the deviation a more fitting model was selected, either a dominant or recessive genetic model. Our model was adjusted for covariates sex, age and proband status. The significance threshold was calculated by dividing α (0.05) by 5 SNPs and the 'pro-LVH' score x 4 phenotypes and x 2 models, resulting in a (bonferroni corrected) significance threshold of P<1.04E-03.

**Results**

**Study population**
For this study, DNA and echo data were available for 403 subjects; 113 probands and 290 relatives. The age distributions in probands and relatives were similar. As expected, probands had more profound hypertrophy according to IVS thickness and Wigle score. There also was a higher frequency of males in the proband group. Only 11 subjects (10 probands) had an IVS thickness ≥ 30 mm. There were 26 children under the age of 16 which were analyzed separately (Table 1).

**Influence of age, sex and proband status**
Both age and gender had a significant effect on IVS thickness in our population. On average men had a thicker IVS than women, and IVS thickness increased with age in both sexes. In family based association studies, probands are usually more heavily affected than their relatives. This can result in an ascertainment bias due to non-random sampling of cases. In this study a large proportion of subjects are probands and this resulted in a big ascertainment
Effect of RAAS polymorphisms on septum thickness

bias effect. To compensate for this ascertainment bias we corrected for proband status.

**Association analyses**

The association analyses for septum thickness according to 'pro-LVH' score showed no significant effect in probands or relatives (Table 2). Analyzing the five RAAS SNPs separately corrected for sex, age, and proband status showed that only the M235T-AGT SNP has a significant (P=6.1E-04) effect on septum thickness and was significantly associated (P=8.83E-04) with a thickened septum (Table 3). Interactions analyses between the five SNPs were negative.

The DD-ACE SNP significantly influenced the Wigle score, but not the IVS thickness. Extreme IVS thickness ≥ 30 mm was present in 3 subjects with the DD-ACE polymorphism, 4 subjects with the DI-ACE polymorphism and 4 subjects with the II-ACE polymorphism. Profound hypertrophy indicated by a Wigle score > 8 was present in 10 subjects; 4 subjects with the DD-ACE polymorphism, 2 subjects with the DI-ACE polymorphism and 4 subjects with the II-ACE polymorphism.

<table>
<thead>
<tr>
<th>Pro-LVH score</th>
<th>&lt;16 probands</th>
<th>&gt;16 probands</th>
<th>&lt;16 relatives</th>
<th>&gt;16 relatives</th>
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<tbody>
<tr>
<td>0</td>
<td>11 ± 14</td>
<td>21 ± 5</td>
<td>10 ± 6</td>
<td>12 ± 4</td>
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<tr>
<td>1</td>
<td>-</td>
<td>22 ± 6</td>
<td>8 ± 3</td>
<td>13 ± 5</td>
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<tr>
<td>2</td>
<td>30 ± 14</td>
<td>21 ± 4</td>
<td>9 ± 4</td>
<td>12 ± 4</td>
</tr>
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<td>3</td>
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<td>18 ± 3</td>
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<td>12 ± 2</td>
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<td>14 ± 0</td>
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<td>19 ± 16</td>
<td>21 ± 5</td>
<td>9 ± 4</td>
<td>12 ± 4</td>
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<tr>
<td>P-value</td>
<td>0.12</td>
<td>0.62</td>
<td>0.27</td>
<td>0.91</td>
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</table>

Table 2. Septum thickness according to pro-LVH score.
Risk stratification in hypertrophic cardiomyopathy

Discussion

In a large cohort of subjects carrying functional identical truncating mutations in MYBPC3 IVS thickness was significantly related with the M235T-AGT polymorphism, increasing age, and male sex. DD-ACE had a significant effect on the Wigle score, but not on the IVS thickness. There was no effect of the previously described ‘pro-LVH’ score on any parameter.\textsuperscript{11}

The M235T-AGT polymorphism has been described as a predisposing factor for cardiac hypertrophy in hypertension patients, endurance athletes and in sporadic cases of HCM.\textsuperscript{17-19} In our study of familial HCM it had a significant effect on IVS thickness. Angiotensinogen is a glycoprotein synthesized mainly in hepatocytes and secreted into the circulation. Jeunemaitre et al. showed that a specific variant leading to the substitution of a methionine (M) for a threonine (T) at the codon 235 was associated with elevated AGT serum concentrations.\textsuperscript{20} The concentration of AGT is rate limiting in the production of Ang I, which is further converted in Ang II, the biologically vasoconstrictive peptide with potent myoptrophic action.

Tissue levels of ACE are increased in patients with DD-ACE and DD-ACE is considered to be a pro-LVH genotype.\textsuperscript{21} In accordance with a previous report, describing 63 HCM patients with single mutations in the MYBPC3 gene, DD-ACE is a significant modifier of the extent of hypertrophy as described by the Wigle score.\textsuperscript{12} However, DD-ACE did not have an influence on the IVS thickness in our large and genetically homogeneous cohort. In contrast to the previous report, subjects with extreme hypertrophy (IVS thickness ≥ 30 mm), which is known to be a risk factor for sudden cardiac death, were equally divided among subjects carrying DD-ACE, ID-ACE and II-ACE polymorphisms.\textsuperscript{12, 22} In accordance with this previous study. we did not find an effect of the RAAS ‘pro-LVH’ score in this group with an identified myofilament mutation.\textsuperscript{12} As has been previously reported IVS thickness in HCM mutation carriers increases with progressive age.\textsuperscript{6, 7} Males and females differ in their presentation of HCM, with cohorts usually having a predominance of males.\textsuperscript{23, 24} Estrogens are known to play a protective role in the hypertrophic response, while there is evidence that exposure of cardiac myocytes to androgen results in hypertrophy.\textsuperscript{25, 26} Furthermore the HCM phenotype is influenced by sex hormone receptor variants.\textsuperscript{27}

The present study has several limitations. Although the approach to look at these five SNPs is plausible, we are currently not informed on the presents of other genetic variations and their effect on septum thickness.

Overall this study shows that the HCM phenotype is in part influenced by the M235T-AGT and DD-ACE RAAS polymorphisms and that identification of RAAS modifier genes may help to risk-stratify patients with HCM and possibly provide therapeutic options with the use of ACE-inhibitors or AGTR1-antagonists in selected patients.

<table>
<thead>
<tr>
<th>Trait</th>
<th>SNP</th>
<th>P-value</th>
<th>Model</th>
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<tr>
<td>Septum &gt; 13 mm</td>
<td>AGT</td>
<td>8.83E-04</td>
<td>d</td>
</tr>
<tr>
<td>Septum thickness</td>
<td>AGT</td>
<td>6.12E-04</td>
<td>d</td>
</tr>
</tbody>
</table>

Table 3. Significant associations.
Effect of RAAS polymorphisms on septum thickness

References


Risk stratification in hypertrophic cardiomyopathy