Angiotensin II Type 2 Receptors and Cardiac Hypertrophy in Women With Hypertrophic Cardiomyopathy

Jaap Deinum, Jeanette M.G. van Gool, Marcel J.M. Kofflard, Folkert J. ten Cate, A.H. Jan Danser

Abstract—The development of left ventricular hypertrophy in subjects with hypertrophic cardiomyopathy (HCM) is variable, suggesting a role for modifying factors such as angiotensin II. Angiotensin II mediates both trophic and antitrophic effects, via angiotensin II type 1 (AT₁-R) and angiotensin II type 2 (AT₂-R) receptors, respectively. Here we investigated the effect of the AT₂-R gene A/C³¹²³ polymorphism, located in the 3’ untranslated region of exon 3, on left ventricular mass index (LVMI) in 103 genetically independent subjects with HCM (age, 12 to 81 years). LVMI and interventricular septum thickness were determined by 2D echocardiography. Extent of hypertrophy was quantified by a point score (Wigle score). Plasma prorenin, renin, and ACE were determined by immunoradiometric or fluorometric assays, and genotyping was performed by polymerase chain reaction. In men, no associations between AT₂-R genotype and any of the measured parameters were observed, whereas in women, LVMI decreased with the number of C alleles (211±19, 201±18, and 152±10 g/m² in women with the AA, AC, and CC genotype, respectively; P=0.015). Similar C allele–related decreases in women were observed for interventricular septum thickness (P=0.13), Wigle score (P=0.05), plasma renin (P=0.03), and plasma prorenin (P=0.26). Multiple regression analysis revealed that the AT₂-R C allele–related effect on LVMI (β=−30.7±11.1, P=0.010) occurred independently of plasma renin, the AT₁-R gene A/C¹¹⁶⁶ polymorphism, or the ACE gene I/D polymorphism. In conclusion, AT₂-Rs modulate cardiac hypertrophy in women with HCM, independently of the circulating renin-angiotensin system. These data support the contention that AT₂-Rs mediate antitrophic effects in humans. (Hypertension. 2001;38:1278-1281.)

Key Words: cardiomyopathy ■ hypertrophy ■ receptors, angiotensin II ■ renin

Hypertrophic cardiomyopathy (HCM) is characterized by idiopathic myocardial hypertrophy. It often occurs as an autosomal dominant disorder, but sporadic cases exist. Mutations in 8 different genes, all coding for sarcomeric proteins, have been identified in patients with HCM.¹ Patients vary considerably by phenotype, even if they have identical causative genotypes. This has led to the idea that trophic and mitotic factors modify the clinical manifestations of HCM.²

One of these factors is angiotensin (Ang) II. Ang II is generated by ACE from Ang I, which is formed by renin from angiotensinogen. Angiotensin production in the heart depends on kidney-derived renin and/or prorenin.³,⁴ Both are taken up from the circulation, either through diffusion into the cardiac interstitium or by binding to cardiac cells, and prorenin is activated to renin in cardiac cells.⁵,⁶ The extent of hypertrophy in subjects with HCM is associated with the ACE I/D polymorphism and the angiotensinogen M235T polymorphism,⁷,⁸ although the association may depend on the underlying disease gene mutation.⁹ Moreover, the Ang II type 1 receptor (AT₁-R) A/C¹¹⁶⁶ polymorphism also modulates the phenotypic expression of hypertrophy in subjects with HCM.¹⁰ AT₁-Rs mediate most, if not all, of the known effects of Ang II, including vasoconstriction and growth stimulation. Taken together, these data support the concept of Ang II modifying cardiac hypertrophy in HCM.

In addition to its effects mediated via AT₁-Rs, Ang II also stimulates Ang II type 2 receptors (AT₂-Rs). Although AT₂-Rs are upregulated in the human heart under pathological conditions,¹¹,¹² their effects in man are currently unknown. Animal studies suggest that AT₂-R stimulation may oppose AT₁-R–mediated effects, ie, may result in vasodilation and growth inhibition.¹³–¹⁵ AT₂-R stimulation will occur in patients during treatment with AT₁-R antagonists, because the latter drugs increase the levels of Ang II.

In the present study, we set out to study the role of AT₂-Rs in man by investigating the association between a polymorphism in the 3’ untranslated region of exon 3 (A/C³¹²³) of the X chromosome–located AT₂-R gene¹⁶ and the extent of cardiac hypertrophy in 103 HCM patients.

Methods

Patients
One hundred sixteen patients with HCM (age, 21 to 81 years) visiting the HCM Clinic at the Academic Hospital Dijkzigt between

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1994 and 1997 for a routine follow-up were included. HCM had been diagnosed on the basis of echocardiographic criteria showing a nondilated, hypertrophied left ventricle (any wall thickness >15 mm) in the absence of known causes of left ventricular hypertrophy.\(^1\) Patients using ACE inhibitors (n = 7) were excluded from the study because of interference with the ACE measurement. Of the remaining 109 subjects, 41 had a sporadic form of HCM and 50 had at least one other affected first-degree family member. The family history of HCM was unknown in 18 patients. To avoid potential bias introduced by the presence of genetically dependent patients, of whom 30 were receiving a β-adrenergic antagonist, 44 a calcium-channel blocker, and 8 a diuretic. The study was approved by the internal review board, and patients gave informed consent.

**Echocardiographic Methods**

Two-dimensional echocardiography was performed with commercially available equipment (Toshiba Sonolayer). Images were recorded on videotape for offline analysis by 2 physicians who were blinded to the genotyping results. Interventricular septal thickness and left ventricular mass (LVM) were determined as described before.\(^3\) LVM was indexed (LVMi) to body surface area.

Peak left ventricular outflow tract gradient at rest was estimated using the modified Bernoulli equation.\(^1\) Because echocardiographic measurement of LVMi may not truly reflect the extent of hypertrophy and the involvement (or lack thereof) of the distal (apical) half of the septum or lateral wall, the extent of hypertrophy was also assessed by a semiquantitative point score (range, 0 to 10) method developed by Wigle et al.\(^4\)

**Biochemical Measurements**

Prorenin and renin were quantified in peripheral venous blood using a commercial kit (ACE Color).\(^2\) Prorenin are expressed as mU/L, using the human kidney renin developed by Wigle et al.\(^3\) Prorenin and renin were quantified in peripheral venous blood using the modified Bernoulli equation.\(^1\) Because echocardiographic measurements of LVMi were not truly reflective of the extent of hypertrophy and the involvement (or lack thereof) of the distal (apical) half of the septum or lateral wall, the extent of hypertrophy was also assessed by a semiquantitative point score (range, 0 to 10) method developed by Wigle et al.\(^4\)

**Genetic Analysis**

Peripheral leukocytes were used to isolate genomic DNA in H\(_2\)O using the QIAamp Bloodkit (QIAGEN Inc). The ACE I/D polymorphism and the AT\(_1\)-R A/C\(^{116}\) polymorphism were determined as described before.\(^1\) The AT\(_2\)-R A/C\(^{121}\) polymorphism, an Alu restriction fragment length polymorphism, was determined according to Katsuya et al.\(^6\)

**Statistical Analysis**

Data are expressed as mean±SEM. Analysis was performed with the SPSS 9.0 statistical package. Hardy-Weinberg equilibrium was tested by χ\(^2\) test. Univariate and multiple regression analyses were conducted to determine the percentage of explained variance in LVMi that is accounted for by the genotypes of the candidate modifier genes and other variables. In the multiple regression analysis, the renin-angiotensin system gene polymorphisms, age, peak left ventricular outflow tract gradient, and renin concentration were tested as independent variables. Prorenin and ACE were excluded from this analysis because of their high correlations with renin (r = 0.680, P < 0.001) and ACE genotype (r = 0.389, P = 0.003), respectively.

**Results**

Table 1 lists the characteristics of the HCM patients by AT\(_2\)-R genotype. Genotype frequencies were in agreement with Hardy-Weinberg equilibrium. The percentage of patients taking β-adrenergic antagonists, calcium channel blockers, or diuretics did not differ between the various groups (data not shown). In men, no genotype-related differences were observed with regard to any of the measured parameters. Similarly, in women no relationship between AT\(_1\)-R genotype and age, body surface area, or plasma ACE was observed. However, LVMi, Wigle score, and plasma renin in women decreased in parallel with the number of C alleles. Peak left ventricular outflow tract gradient was higher in women carrying the C allele. Univariate regression analysis showed that AT\(_2\)-R genotype accounted for 10.5%, 17.8%, 8.8%, and 12.9% of the variability of interventricular septal thickness (r = 0.32; P = 0.044), LVMi (r = 0.42; P = 0.007), plasma renin (r = 0.30; P = 0.063), and peak left ventricular outflow tract gradient (r = 0.36, P = 0.023), respectively.

Subdivision of men and women by both AT\(_1\)-R and AT\(_2\)-R genotypes (Table 2), to further investigate the previously described AT\(_1\)-R C allele–related effect on LVMi, revealed that the latter effect was restricted to male carriers of the AT\(_2\)-R A allele. In women, using 2-factor ANOVA, no interaction could be demonstrated between the AT\(_1\)-R C allele and the AT\(_2\)-R C allele with regard to LVMi.

Multiple regression analysis (Table 3) showed that age, AT\(_2\)-R genotype, and peak left ventricular outflow tract...
The concept of the net effect of Ang II being the result of a balance between AT-1-Rs and AT-2-Rs, in a gender-specific manner, in that the AT-1-R C allele -related effect is observed in men carrying the AT-2-R A allele only, whereas the AT-2-R C allele-related effect is observed in women only, irrespective of their AT-1-R genotype. The concept of the net effect of Ang II being the result of the balance between AT-1-Rs and AT-2-Rs, AT-2-Rs opposing the growth-stimulatory effects of AT-1-Rs, originates from studies in whole animals and isolated cells.

The polymorphic markers that we tested in the AT-1-R and AT-2-R genes, respectively, are located in untranslated regions. Consequently, their association with LVMI must be explained by a linkage disequilibrium with a functional variant of the 2 genes. Both receptors are expressed in the heart, with AT-1-Rs predominating under normal conditions. Initially, it was thought that AT-2-Rs are widely expressed in the fetal heart and disappear after birth, to return only under pathological conditions. More recent studies in adult animals, however, have shown that this may not be true. In cardiomyopathic hamsters, AT-2-Rs exert an anti-AT-1-R action on the progression of interstitial fibrosis during cardiac remodelling, by inhibiting both fibrillar collagen metabolism and growth of cardiac fibroblasts, whereas in the infarcted rat heart, AT-2-R blockade abolishes the beneficial effects of AT-1-R blockade. In the line of these animal data and the findings of the present study, it is logical to assume that AT-2-R stimulation in HCM protects against left ventricular hypertrophy, particularly in women with the CC phenotype.

The gender-specificity of the association of the AT-1-R and AT-2-R genotypes with LVMI is not readily explained. It may relate to the fact that the AT-1-R is located on the X-chromosome and thus is present twice in women only. The AT-2-R promoter region, unlike the angiotensinogen promoter region, does not contain estrogen-responsive elements, which suggests that if estrogens play a role in the development of left ventricular hypertrophy through AT-2-Rs, a third factor is involved, eg, an estrogen-dependent transcription factor. One may argue that the estrogen-induced higher angiotensinogen levels in women, via increased cardiac Ang II generation, have resulted in more intense AT-2-R stimulation. However, the lower plasma renin levels in women do not support this possibility.

The present study may be important from a pharmacotherapeutic point of view. If AT-1-R stimulation is indeed partly responsible for the increased LVM in subjects with HCM, the use of ACE inhibitors or AT-1-R antagonists in this disease might be reconsidered. Both are currently not widely used in HCM, although they are very effective in regressing and preventing ventricular hypertrophy in hypertension and after myocardial infarction. Prevention of hypertrophy in HCM is desirable in view of the increased risk of sudden death with higher LVM in subjects with HCM. The antihypertrophic effect of AT-2-Rs, based on the current and previous data, raises the possibility that AT-1-R antagonists should be preferred above ACE inhibitors, because the former drugs, unlike ACE inhibitors, will result in AT-2-R stimulation. In support of this concept, Lim et al recently demonstrated that AT-2-R blockade reverses myocardial fibrosis in a transgenic mouse model of human HCM.

In view of our present findings, one may also speculate on the role of AT-2-R genotypes in other hypertrophic conditions like hypertensive left ventricular hypertrophy and postinfarction remodelling. Schneider et al studied the A/G variant of the AT-2-R gene in 120 young males with normal or mildly elevated blood pressure and found LVMI to be higher in hypertensives with the A allele than in hypertensives with the G allele. It would be of interest to repeat this study in a larger population and preferably also in women.

Finally, the plasma levels of renin were partly determined by the AT-2-R genotype in women, and a similar trend was observed for the plasma levels of prorenin. Plasma renin however did not contribute independently to LVMI. These findings suggest that AT-2-Rs, like AT-1-Rs, affect the plasma levels of renin, but that at the same time cardiac Ang II generation (and thus cardiac AT-2-R stimulation) does not correlate directly with plasma renin levels. The latter might be explained on the basis of differences in the cardiac uptake of circulating renin, as well as differences in the uptake and local activation of circulating prorenin. The decrease of

### TABLE 2. LVMI (g/m²) According to AT-1-R and AT-2-R Genotype

<table>
<thead>
<tr>
<th>AT-2-R Genotype</th>
<th>AA</th>
<th>AC + CC</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Men</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>143±5(n=17)</td>
<td>202±17(n=14)</td>
</tr>
<tr>
<td>C</td>
<td>184±20(n=14)</td>
<td>180±11(n=18)</td>
</tr>
<tr>
<td><strong>Women</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>207±31(n=5)</td>
<td>216±4(n=3)</td>
</tr>
<tr>
<td>AC</td>
<td>196±24(n=7)</td>
<td>205±27(n=8)</td>
</tr>
<tr>
<td>CC</td>
<td>155±11(n=13)</td>
<td>142±21(n=4)</td>
</tr>
</tbody>
</table>

Values are mean±SEM. In men, LVMI variation is explained for 6.2% (P=0.04) by the AT-1-R genotype. In women, LVMI variation is explained for 18.5% (P=0.03) by the AT-2-R genotype.

### TABLE 3. Multiple Regression Analysis of Factors With Potential Effect on LVMI in Women

<table>
<thead>
<tr>
<th>Parameter</th>
<th>β</th>
<th>SEM</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>−1.794</td>
<td>0.488</td>
<td>0.001</td>
</tr>
<tr>
<td>ACE genotype, No. of D alleles</td>
<td>−9.837</td>
<td>11.529</td>
<td>0.400</td>
</tr>
<tr>
<td>AT-1-R genotype, No. of C alleles</td>
<td>5.862</td>
<td>10.696</td>
<td>0.587</td>
</tr>
<tr>
<td>AT-2-R genotype, No. of C alleles</td>
<td>−30.691</td>
<td>11.148</td>
<td>0.010</td>
</tr>
<tr>
<td>Renin, μIU/L</td>
<td>0.065</td>
<td>0.488</td>
<td>0.895</td>
</tr>
<tr>
<td>Gradient, mm Hg</td>
<td>0.445</td>
<td>0.180</td>
<td>0.019</td>
</tr>
</tbody>
</table>
LVMI with age, and its increase with peak left ventricular outflow tract gradient have been described before.10,30

In summary, this paper suggests that the variability and/or extent of cardiac hypertrophy in HCM patients is partly determined by the balance between stimulation of AT1-Rs and AT2-Rs, with different effects in men and women. This may have therapeutic consequences for the prevention of hypertrophy in these patients, which may be important in view of the association of hypertrophy with sudden death in HCM.

Acknowledgment
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