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.

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

Received January 20, 2001; first decision April 16, 2001; revision accepted June 13, 2001.
From the Cardiovascular Research Institute of the Erasmus University Rotterdam (COEUR), Departments of Internal Medicine (J.D., J.M.G.v.G.), Cardiology (M.J.M.K., F.J.t.C.), and Pharmacology (A.H.J.D.), Erasmus University Rotterdam, Rotterdam, The Netherlands.
Correspondence to Dr A.H.J. Danser, PhD, Department of Pharmacology, Room EE1418b, Erasmus University Rotterdam, Dr. Molewaterplein 50, 3015 GE Rotterdam, The Netherlands. E-mail danser@farma.fgg.eur.nl
© 2001 American Heart Association, Inc.
Hypertension is available at http://www.hypertensionaha.org
by guest on May 3, 2017 http://hyper.ahajournals.org/ Downloaded from

Described before. 10 The AT2-R A/C3123 polymorphism, an immunoradiometric assay kit (Nichols Institute). 19 Renin and prorenin are expressed as mU/L, using the human kidney renin standard MRC 68/356 as a reference. ACE activity was measured with a commercial kit (ACE Color). 20

Genetic Analysis
Peripheral leukocytes were used to isolate genomic DNA in H2O using the QIAamp Bloodkit (QIAGEN Inc). The ACE I/D polymorphism and the AT1-R A/C106 polymorphism were determined as described before. 10 The AT2-R A/C123 polymorphism, an Alul restriction fragment length polymorphism, was determined according to Katsuya et al. 16

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. 17 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 samples (relatives), we randomly selected 1 patient per family. One patient was excluded because he had Klinefelter’s syndrome (XXY genotype). This resulted in a final cohort of 103 genetically independent 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. 10 LVM was indexed (LVMI) to body surface area.

Peak left ventricular outflow tract gradient at rest was estimated to 15 mm) in the absence of known causes of left ventricular hypertrophy. 11022 diagnosing 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. 17 Patients using ACE inhibitors (n = 7) were excluded from the study because of interference with the ACE measurement.

Statistical Analysis
Data are expressed as mean ± SEM. Analysis was performed with the SPSS 9.0 statistical package. Hardy-Weinberg equilibrium was tested by χ2 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 AT2-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 AT2-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 AT2-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 AT1-R and AT2-R genotypes (Table 2), to further investigate the previously described AT1-R C allele–related effect on LVMI, revealed that the latter effect was restricted to male carriers of the AT2-R A allele. In women, using 2-factor ANOVA, no interaction could be demonstrated between the AT1-R C allele and the AT2-R C allele with regard to LVMI.

Multiple regression analysis (Table 3) showed that age, AT2-R genotype, and peak left ventricular outflow tract

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Men AT2-R Genotype</th>
<th>Women AT2-R Genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A (n = 31)</td>
<td>C (n = 32)</td>
</tr>
<tr>
<td>Age, y</td>
<td>43 ± 6</td>
<td>48 ± 4</td>
</tr>
<tr>
<td>Body surface area, m²</td>
<td>1.97 ± 0.02</td>
<td>1.92 ± 0.02</td>
</tr>
<tr>
<td>IVS, mm</td>
<td>21.7 ± 0.8</td>
<td>21.8 ± 0.9</td>
</tr>
<tr>
<td>LVMI, g/m²</td>
<td>170 ± 10</td>
<td>182 ± 10</td>
</tr>
<tr>
<td>Wigle score (1–10)</td>
<td>6.4 ± 0.4</td>
<td>6.5 ± 0.4</td>
</tr>
<tr>
<td>Gradian, mm Hg</td>
<td>36.6 ± 7.1</td>
<td>46.8 ± 7.1</td>
</tr>
<tr>
<td>Prorenin, μU/L</td>
<td>222 ± 41</td>
<td>191 ± 21</td>
</tr>
<tr>
<td>Renin, μU/L</td>
<td>28.1 ± 6.2</td>
<td>23.7 ± 2.1</td>
</tr>
<tr>
<td>ACE, U/L</td>
<td>10.1 ± 0.5</td>
<td>10.8 ± 0.6</td>
</tr>
</tbody>
</table>

Values are mean ± SEM. IVS indicates interventricular septum thickness; Gradient, peak left ventricular outflow tract gradient.
cardiac remodelling, by inhibiting both fibrillar collagen metabolism and growth of cardiac fibroblasts,\textsuperscript{21} whereas in the infarcted rat heart, AT\textsubscript{2}-R blockade abolishes the beneficial effects of AT\textsubscript{1}-R blockade.\textsuperscript{22} In the line of these animal data and the findings of the present study, it is logical to assume that AT\textsubscript{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\textsubscript{1}-R and AT\textsubscript{2}-R genotypes with LVMI is not readily explained. It may relate to the fact that the AT\textsubscript{2}-R is located on the X-chromosome and thus is present twice in women only. The AT\textsubscript{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\textsubscript{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\textsubscript{2}-R stimulation. However, the lower plasma renin levels in women do not support this possibility.\textsuperscript{26}

The present study may be important from a pharmacotherapeutic point of view. If AT\textsubscript{2}-R stimulation is indeed partly responsible for the increased LVM in subjects with HCM, the use of ACE inhibitors or AT\textsubscript{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.\textsuperscript{27} The antihypertrophic effect of AT\textsubscript{2}-Rs, based on the current and previous data, raises the possibility that AT\textsubscript{1}-R antagonists should be preferred above ACE inhibitors, because the former drugs, unlike ACE inhibitors, will result in AT\textsubscript{2}-R stimulation. In support of this concept, Lim et al\textsuperscript{28} recently demonstrated that AT\textsubscript{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\textsubscript{2}-R genotypes in other hypertrophic conditions like hypertensive left ventricular hypertrophy and postinfarction remodelling. Schmieder et al\textsuperscript{29} studied the A/G\textsuperscript{1675} variant of the AT\textsubscript{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\textsubscript{2}-R genotype in women, and a similar trend was observed for the plasma levels of prorenin. Plasma renin levels, however, did not contribute independently to LVMI. These findings suggest that AT\textsubscript{2}-Rs, like AT\textsubscript{1}-Rs, affect the plasma levels of renin, but that at the same time cardiac Ang II generation (and thus cardiac AT\textsubscript{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.\textsuperscript{5,6} The decrease of
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 AT 1 -Rs and AT 2 -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
This study was supported by a grant from The Netherlands Heart Foundation, No. NHS 97.186.

References
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 and A.H. Jan Danser

Hypertension. 2001;38:1278-1281
doi: 10.1161/hy1101.096114

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2001 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/38/6/1278

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at:
http://hyper.ahajournals.org/subscriptions/