Effect of the Angiotensin II Type 2-Receptor Gene (+1675 G/A) on Left Ventricular Structure in Humans

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OBJECTIVES
Our study goal was to analyze whether gene variants of angiotensin II type 2-receptor (AT2-R) modulate the effects of angiotensin II on the left ventricle (LV).

BACKGROUND
Experimental data suggest that angiotensin II modulates ventricular growth responses via angiotensin II type 1-receptors (AT1-R) and AT2-R.

METHODS
In 120 white, young male subjects with normal or mildly elevated blood pressure, we assessed plasma angiotensin II and aldosterone concentrations (RIA), 24-h urinary sodium excretion, 24-h ambulatory blood pressure and LV structure (two-dimensional guided M-mode echocardiography). The intronic +1675 G/A polymorphism of the X-chromosomal located AT2-R gene was investigated by single-strand conformational polymorphism analysis and DNA-sequencing.

RESULTS
Hypertensive subjects with the A-allele had a greater LV posterior (11.0 ± 1.3 vs. 9.9 ± 1.3 mm, p < 0.001), septal (11.8 ± 1.4 vs. 10.1 ± 1.2 mm, p < 0.001) and relative wall thickness (0.44 ± 0.06 vs. 0.39 ± 0.06, p < 0.01) as well as LV mass index (138 ± 23 vs. 120 ± 13 g/m², p < 0.001) than those with the G-allele. Confounding factors (i.e., body mass index and surface area, plasma angiotensin II, sodium excretion, systolic and diastolic ambulatory blood pressure) were similar between the two genotypes. In normotensive subjects, relative wall thickness (0.36 ± 0.05 vs. 0.35 ± 0.05) and LV mass index (115 ± 21 vs. 112 ± 17 g/m²) were nearly identical across the two genotypes, with similar confounding variables.

CONCLUSIONS
Our data indicate that the X-chromosomal located +1675 G/A-polymorphism of the AT2-R gene is associated with LV structure in young male humans with early structural changes of the heart due to arterial hypertension. (J Am Coll Cardiol 2001;37:175–82) © 2001 by the American College of Cardiology

Left ventricular (LV) structure and function are important predictors of cardiovascular morbidity and mortality in cardiac patients and in the general population (1,2). Echocardiographic LV hypertrophy increases the risk of cardiovascular morbidity events in primary and secondary hypertension (3–5) but also indicates a higher risk for the development of arterial hypertension in normotensive subjects (6,7). Constitutional and environmental factors have been identified that influence cardiac structural adaptation to an increased preload and afterload imposed on the LV (8–11). Studies of monozygotic and dizygotic twins have suggested that inherited factors are also of pathogenic relevance (12,13).

The activity of the renin angiotensin system (RAS) profoundly influences blood pressure (BP) and cardiovascular disease (14–17). Experimental studies have documented the growth stimulating and regulating effects of angiotensin II on myocardial cells (14,15). In hypertensive subjects impaired suppression of the RAS or, conversely, increased sensitivity to angiotensin II appeared to act as a stimulus for LV hypertrophy (18,19).

Over the last 5 years various genes of the RAS have been associated with cardiovascular disease (20–25). However, these studies yielded conflicting results: some found a positive association of the risk for myocardial infarction with the angiotensin-converting enzyme D-allele, whereas others did not (20,21). Similarly, the T235-variant of the angiotensinogen gene was found to be associated with essential hypertension, but subsequent studies in monozygotic and dizygotic twin pairs did not confirm the earlier observation (22,23). More recent studies have focused on variants of genes downstream of the angiotensinogen and angiotensin-converting enzyme in the RAS cascade.

The angiotensin II type 1-receptor (AT1-R) appears to be the primary receptor that mediates the vasoconstrictor and growth promoting effects of angiotensin II in humans (15). The gene that encodes AT1-R has been intensively investigated as a risk factor for hypertension and cardiovascular disease (23,26). The nucleotide substitution (A/C in position +1166) in the gene of the AT1-R is located in the 3’ untranslated part of the gene, and increased frequency of the C-allele has been reported in patients with arterial hypertension, increased aortic stiffness, LV hypertrophy and coronary vasoconstriction (24,25). However, other reports did not confirm such an association between the +1166 A/C polymorphism and cardiovascular disorders (23).
AT2 is essential for the development of pressure overload- 
fibrogenic (39) and growth responses (40). The signaling via 
vascular smooth muscle cells (15,35–38) and modifies LV 
BP, exerts antiproliferative effects on endothelial and vas- 
receptors differ among species with the AT2-R being the 
expression patterns and regulation of the angiotensin II 
to a few tissues in adults. It is upregulated in the endothe- 
lyum under pathological circumstances, such as new intima 
formation after vascular injury and in the heart after 
myocardial infarction or during cardiac remodeling (28–30). 
The expression patterns and regulation of the angiotensin II 
receptors differ among species with the AT2-R being the 
moderator receptor subtype in the human heart (30–32). 
AT2-R gene is located on the X-chromosome and spans about 5 kb (33). The gene structure and the complete 
nucleotide sequence of the human AT3-R gene, which 
includes the promoter region, was elucidated in 1995 (33). 
Regulatory elements are located in the first intron in 
addition to the promoter region (34). The AT2-R decreases 
BP, exerts antiproliferative effects on endothelial and vas- 
cular smooth muscle cells (15,35–38) and modifies LV 
fibrogenic (39) and growth responses (40). The signaling via 
AT2 is essential for the development of pressure overload- 
mediated cardiac hypertrophy in transgenic mice (41), 
whereas in isolated adult rat hearts AT2 inhibition amplifies 
the LV growth response to angiotensin II (40).

The pathogenic effects of the AT2-R for cardiac struc- 
tural processes in humans are far from being understood. To 
the best of our knowledge no data have been reported in 
humans so far. We thought that the functional character- 
ization of the human AT2-R genotypes is an attractive 
approach to elucidate the pathogenic role of AT2-R in 
cardiovascular diseases, particularly since no AT2-R agonist 
or antagonist is available for human use, and, thus, no 
pharmacological experiments have been carried out to elu- 
cidate the physiological and pathophysiological role of 
AT2-R. Therefore, we tested whether the +1675G/A 
polymorphism is associated with cardiac structural adapta- 
tion to an increased afterload in a homogeneous study 
cohort of young male white subjects.

METHODS

Study population. By advertising we elicited the partici- 
pation of young white male subjects at the campus of the 
University Erlangen-Nürnberg. One-hundred twenty sub- 
jects were consecutively enrolled in the study up to a number 
of 60 subjects in the normotensive range and 60 subjects 
with mildly elevated BP. According to the World Health 
Organization/International Society of Hypertension 
(WHO/ISH) criteria, an average BP of \( \geq 140 \text{ mm Hg} \) 
systolic or \( \geq 90 \text{ mm Hg} \) diastolic was said to be hypertensive 
(42). The study protocol was approved by the clinical 
investigation and ethics committees of the University 
Erlangen-Nürnberg, Germany, and informed consent was 
given before study inclusion.

Study inclusion criteria were: age between 20 and 40 
years, male gender, white, no current or previous treatment 
for arterial hypertension, no cardiovascular disease (with the 
exception of mildly elevated BP) and no secondary hyper- 
tension or WHO stage III hypertensive disease. Therefore, 
exclusion criteria were: advanced hypertensive fundoscopic 
changes, myocardial infarction or any other evidence of 
coronary artery disease, congestive heart failure or previous 
cerebrovascular event and hepatic or renal insufficiency. 
Each participant underwent a thorough clinical work-up 
described elsewhere in detail (19).

BP measurements. To obtain correct BP readings a stan- 
ard sphygmomanometer was used, and the cuff size was 
adjusted according to the participant’s arm circumference. 
Four casual BP readings were taken in our outpatient clinic 
on at least two different occasions (at least four weeks apart) 
after 5 min of rest in a standardized fashion. Subjects who 
had been allocated to one of the two groups according to the 
screening BP, but later failed to fulfill all study entry criteria, 
were replaced. 

Ambulatory 24-h BP measurements were taken with an 
automatic portable device (Spacelab No. 90207, Redmont, 
California). Measurement intervals were every 15 min 
during the day (defined from 7:00 to 22:00 h) and every 
30 min during the night (43). In parallel, a 24-h urinary 
sodium excretion, which represents a rough but valuable 
estimate of daily sodium intake, was measured (18,19).

Parameters of the RAS. Blood samples for the determi- 
nation of plasma angiotensin II and serum aldosterone levels 
were collected from patients who were in the supine 
position after 1 h of complete rest. For plasma angiotensin 
II measurements, blood was collected into prechilled 10 ml 
syringes, prepared with EDTA 125 mmol and phenantro- 
line 26 mmol (Merck, Darmstadt, Germany) to inhibit the 
angiotensin-converting enzyme. The samples were cen- 
trifuged for 10 min at 4°C immediately after collection and 
rapidly stored after centrifugation at –21°C, but analyzed 
within three months. Plasma samples were extracted, and, 
after purification of the samples, immunoreactive angioten-
sin II was measured in duplicate by radioimmunoassay with 
antiserum, as previously described (19). The coefficient of 
variation was <5%. Serum aldosterone was measured by a 
commercially available radioimmunoassay kit (Aldosterone 
Maia, Serono, Freiburg, Germany).

Echocardiography. Two-dimensional guided M-mode 
echocardiography was performed using an ultrasonoscope 
(Picker–Hitachi CS 192; Tokyo, Japan) with a 2.5 MHz 
probe (for details see [18,19]). All echocardiographic read-
ings were evaluated by two investigators independently. The echocardiographic reading was done blindly with respect to other clinical data and, in particular, to the genotyping of our subjects (as indicated by different locations of the echocardiographic and genetic evaluation). Relative wall thickness taken as a parameter for concentric LV hypertrophy was calculated as two times posterior wall thickness divided by end-diastolic diameter. Left ventricular mass was calculated according to the American Society of Echocardiography recommendations (44) but was then corrected according to the suggestions of Devereux and coworkers (45). Coefficients of variation for all structural parameters were less than 10%.

**Mutation screening and genotyping.** Genomic DNA was extracted from 2 to 5 ml of whole blood by standard methods using a commercially available kit (QIAamp Blood Midi Kit, QIAGEN GmbH, Hilden, Germany). In a previous study the complete coding region of the human AT2-R gene (1,092 base pair [bp]) as well as intron 1 (152 bp) were screened in 92 patients with cardiovascular diseases. Eight overlapping PCR-fragments of 142 to 297 bp were used. SSCP was run at 4°C and room temperature on 10% polyacrylamide gels (49:1; acrylamide: bisacrylamide). Two variants significantly altered the migration pattern of the corresponding PCR-products. Sequencing of aberrant products revealed one frequently occurring intronic polymorphism (+1675 G/A) (Fig. 1) and one rare amino acid substitution (Gly-21−Val).

Since the +1675 G/A polymorphism does not alter any known enzyme restriction site, the genotyping in larger samples was based on repeated SSCP analysis. In brief, after amplification using primers AT2.intron 1F (5'-ATT ACG TCC CAG CGT CTG AG-3') and AT2.intron1R (5'-ATA AAT CAG CTT GCT TAG TGC C-3'), a PCR-fragment of 255 bp was produced. SSCP-gels were run for 16 to 18 h at 7 V/cm at 4°C. Bands were visualized by silver staining (46).

Three different DNA samples that represented the three genotypes (G−, A−, GA) were analyzed as controls on every gel (Fig. 1). All ambiguous samples were analyzed a second time, as proposed by others (47,48), and a second analysis was also performed for every 10th sample for control reasons.

**Statistical analysis.** A power calculation was used to calculate the sample size with a 95% confidence (type I error: α = 0.05) and 90% power (type II error: β = 0.10) assuming that a difference of δ = 12 g/m² for LV mass index (with a standard deviation of σ = 20 g/m²) is of clinical relevance (49).

All statistical analyses were carried out using SPSS software (SPSS for Windows, SPSS Inc., Chicago, Illinois). In particular, two-way analysis of variance was used to detect any significant difference between carriers of the A-allele vs. G-allele of the X-chromosomal located +1675 G/A polymorphism of the AT2-R gene in the normotensive versus the hypertensive group.

![Figure 1](image)

**RESULTS**

The clinical characteristics of our study population are given in Table 1. In our homogenous study cohort of young male white subjects, the frequency of the A-allele was 57% and of the G-allele 43%. When classified according to the WHO, the G-allele frequency was 46% in the hypertensive and 41% in the normotensive subjects, which was not significantly different (χ² test: p > 0.20).

In the whole study group LV structural parameters were linked to the +1675 G/A polymorphism of the AT2-R gene (Table 2). Subjects with the A-allele of the AT2-R gene had a greater posterior (p < 0.025) and septal wall thickness (p < 0.001) than those with a G-allele of AT2-R gene. Relative wall thickness and LV mass were greater in subjects with the A-allele than they were in those with the
G-allele of the AT2-R gene (p < 0.02). This difference in LV mass was not related to body surface area or body mass index, 24-h ambulatory systolic or diastolic BP, urinary sodium excretion, plasma angiotensin II or serum aldosterone concentrations. Hypertensive subjects (according to WHO criteria) with the A-allele of the AT2-R gene had a greater posterior wall thickness (p < 0.001), septal wall thickness (p < 0.001), relative wall thickness (p < 0.001) and LV mass index (p < 0.01) than hypertensive subjects with the G-allele of the AT2-R gene (Table 2). Accordingly, analysis of covariance revealed that the association of the +1675 G/A polymorphism of the AT2-R gene with LV mass (p = 0.003) and relative wall thickness (p = 0.014) were independent of body mass index, 24-h ambulatory systolic and diastolic BP, urinary sodium excretion, plasma angiotensin II and serum aldosterone concentrations.

Hypertensive subjects (according to WHO criteria) with the A-allele of the AT2-R gene had a greater posterior wall thickness (p < 0.001), septal wall thickness (p < 0.001), relative wall thickness (p < 0.001) and LV mass index (p < 0.01) than hypertensive subjects with the G-allele of the AT2-R gene (Tables 3 and 4, Fig. 2 and 3). Ambulatory and casual BP, body mass index, urinary sodium excretion, plasma angiotensin II and serum aldosterone concentrations were similar between the two genotypes of the AT2-R gene (Table 3). Analysis of covariance confirmed that the +1675 G/A polymorphism of the AT2-R gene was associated with LV mass (p < 0.001), relative wall thickness (p < 0.05), posterior (p < 0.01) and septal wall thickness (p < 0.001), independent of confounding factors. In contrast, no significant difference of LV structural parameters between the A- and G-allele carriers was found in the normotensive subgroups (Table 4).

Similarly, when our subjects were post hoc divided according to 24-h ambulatory BP criteria (≥130 mm Hg or ≥80 mm Hg [50]), the +1675 G/A polymorphism of the AT2-R modulated LV structure in the hypertensive (n = 46) but not in the normotensive individuals (n = 74). Hypertensive subjects with the A-allele of AT2-R revealed an increased LV mass index (132 ± 25 vs. 116 ± 14 g/m², p = 0.011) and relative wall thickness (0.43 ± 0.06 vs. 0.37 ± 0.06, p = 0.004) with similar confounding factors such as 24-h ambulatory systolic and diastolic BP, body mass index, urinary sodium excretion, plasma angiotensin II.

### Table 1. Characteristics of the Whole Study Cohort (n = 120), the Normotensive (n = 60) and Mildly Hypertensive Group (n = 60)

<table>
<thead>
<tr>
<th></th>
<th>Whole Study Cohort (n = 120)</th>
<th>Normotensive Group (n = 60)</th>
<th>Mildly Hypertensive Group (n = 60)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical data</td>
<td></td>
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</tr>
<tr>
<td>Age (yrs)</td>
<td>26 ± 3</td>
<td>26 ± 2</td>
<td>26 ± 3</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.81 ± 0.07</td>
<td>1.81 ± 0.07</td>
<td>1.82 ± 0.07</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>78.7 ± 10.4</td>
<td>75.2 ± 8.5</td>
<td>82.9 ± 11</td>
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<tr>
<td>Body surface area (m²)</td>
<td>1.99 ± 0.15</td>
<td>1.95 ± 0.13</td>
<td>2.04 ± 0.15</td>
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<tr>
<td>Body mass index (kg/m²)</td>
<td>23.9 ± 2.8</td>
<td>23.0 ± 2.2</td>
<td>24.9 ± 3.1</td>
</tr>
<tr>
<td>Casual systolic BP (mm Hg)</td>
<td>137 ± 17</td>
<td>123 ± 7</td>
<td>153 ± 9</td>
</tr>
<tr>
<td>Casual diastolic BP (mm Hg)</td>
<td>87 ± 11</td>
<td>79 ± 5</td>
<td>97 ± 8</td>
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<tr>
<td>Ambulatory systolic BP (mm Hg)</td>
<td>127 ± 10</td>
<td>122 ± 7</td>
<td>134 ± 10</td>
</tr>
<tr>
<td>Ambulatory diastolic BP (mm Hg)</td>
<td>76 ± 7</td>
<td>72 ± 5</td>
<td>79 ± 8</td>
</tr>
<tr>
<td>Plasma angiotensin II (pg/ml)</td>
<td>8.3 ± 3.9</td>
<td>7.9 ± 3.9</td>
<td>8.73 ± 3.9</td>
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<tr>
<td>Plasma aldosterone (pg/ml)</td>
<td>125 ± 39</td>
<td>121 ± 33</td>
<td>130 ± 45</td>
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<tr>
<td>Sodium excretion (mmol/day)</td>
<td>190 ± 70</td>
<td>191 ± 62</td>
<td>190 ± 80</td>
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<tr>
<td>Echocardiographic data</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Posterior wall thickness (mm)</td>
<td>9.8 ± 1.4</td>
<td>9.1 ± 1.0</td>
<td>10.5 ± 1.3</td>
</tr>
<tr>
<td>Septal wall thickness (mm)</td>
<td>10.3 ± 1.5</td>
<td>9.7 ± 1.2</td>
<td>11.0 ± 1.5</td>
</tr>
<tr>
<td>End-diastolic diameter (mm)</td>
<td>51.3 ± 3.5</td>
<td>51.3 ± 3.4</td>
<td>51.3 ± 3.6</td>
</tr>
<tr>
<td>End-systolic diameter (mm)</td>
<td>32.6 ± 3.6</td>
<td>32.9 ± 3.5</td>
<td>32.3 ± 3.7</td>
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<tr>
<td>Left ventricular mass (g)</td>
<td>241 ± 48</td>
<td>221 ± 38</td>
<td>265 ± 47</td>
</tr>
<tr>
<td>Left ventricular mass index (g/m²)</td>
<td>121 ± 22</td>
<td>114 ± 19</td>
<td>130 ± 21</td>
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<tr>
<td>Relative wall thickness (−)</td>
<td>0.38 ± 0.06</td>
<td>0.36 ± 0.05</td>
<td>0.41 ± 0.07</td>
</tr>
</tbody>
</table>

**BP = blood pressure; mean ± 1 SD.**

### Table 2. Clinical and Echocardiographic Data Stratified According to the X-Chromosomal Located +1675 G/A Polymorphism of the Angiotensin II AT2-receptor Gene

<table>
<thead>
<tr>
<th></th>
<th>G-allele (n = 52)</th>
<th>A-allele (n = 68)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (kg)</td>
<td>77.6 ± 9.86</td>
<td>79.6 ± 10.7</td>
<td>&gt; 0.20</td>
</tr>
<tr>
<td>Body surface area (m²)</td>
<td>1.97 ± 0.13</td>
<td>2.00 ± 0.15</td>
<td>&gt; 0.20</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>23.7 ± 2.9</td>
<td>24.0 ± 2.8</td>
<td>&gt; 0.20</td>
</tr>
<tr>
<td>Casual systolic BP (mm Hg)</td>
<td>138 ± 18</td>
<td>136 ± 16</td>
<td>&gt; 0.20</td>
</tr>
<tr>
<td>Casual diastolic BP (mm Hg)</td>
<td>88 ± 11</td>
<td>87 ± 11</td>
<td>&gt; 0.20</td>
</tr>
<tr>
<td>Ambulatory systolic BP (mm Hg)</td>
<td>129 ± 10</td>
<td>126 ± 10</td>
<td>0.13</td>
</tr>
<tr>
<td>Ambulatory diastolic BP (mm Hg)</td>
<td>76 ± 7</td>
<td>75 ± 8</td>
<td>&gt; 0.20</td>
</tr>
<tr>
<td>Plasma angiotensin II (pg/ml)</td>
<td>8.4 ± 3.6</td>
<td>8.2 ± 4.2</td>
<td>&gt; 0.20</td>
</tr>
<tr>
<td>Serum aldosterone (pg/ml)</td>
<td>119 ± 34</td>
<td>130 ± 42</td>
<td>&gt; 0.20</td>
</tr>
<tr>
<td>Sodium excretion (mmol/day)</td>
<td>192 ± 74</td>
<td>188 ± 67</td>
<td>&gt; 0.20</td>
</tr>
<tr>
<td>Posterior wall thickness (mm)</td>
<td>9.4 ± 1.2</td>
<td>10.0 ± 1.5</td>
<td>0.025</td>
</tr>
<tr>
<td>Septal wall thickness (mm)</td>
<td>9.8 ± 1.3</td>
<td>10.8 ± 1.6</td>
<td>0.001</td>
</tr>
<tr>
<td>Diastolic diameter (mm)</td>
<td>51.6 ± 3.3</td>
<td>51.0 ± 3.7</td>
<td>&gt; 0.20</td>
</tr>
<tr>
<td>Relative wall thickness (−)</td>
<td>0.37 ± 0.06</td>
<td>0.39 ± 0.07</td>
<td>0.021</td>
</tr>
<tr>
<td>Left ventricular mass (g)</td>
<td>229 ± 37</td>
<td>250 ± 53</td>
<td>0.016</td>
</tr>
</tbody>
</table>

**BP = blood pressure; mean ± 1 SD.**
Table 3. Clinical Data Stratified According to the +1675 G/A Polymorphism of the Angiotensin II AT$_2$-Receptor Gene

<table>
<thead>
<tr>
<th></th>
<th>Normotensives (n = 60)</th>
<th>Hypertensives (n = 60)</th>
<th>Statistical Tests (p Value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body surface area (m$^2$)</td>
<td>1.93 ± 0.11</td>
<td>1.96 ± 0.14</td>
<td>2.02 ± 0.15</td>
</tr>
<tr>
<td>Body mass index (kg/m$^2$)</td>
<td>23.1 ± 2.3</td>
<td>22.9 ± 2.2</td>
<td>24.4 ± 3.3</td>
</tr>
<tr>
<td>Casual systolic BP (mm Hg)</td>
<td>123 ± 7</td>
<td>123 ± 7</td>
<td>154 ± 10</td>
</tr>
<tr>
<td>Casual diastolic BP (mm Hg)</td>
<td>79 ± 5</td>
<td>79 ± 6</td>
<td>97 ± 9</td>
</tr>
<tr>
<td>Ambulatory systolic BP (mm Hg)</td>
<td>124 ± 7</td>
<td>121 ± 6</td>
<td>134 ± 11</td>
</tr>
<tr>
<td>Ambulatory diastolic BP (mm Hg)</td>
<td>73 ± 5</td>
<td>72 ± 5</td>
<td>79 ± 7</td>
</tr>
<tr>
<td>Plasma aldosterone (pg/ml)</td>
<td>8.7 ± 3.8</td>
<td>7.3 ± 4.0</td>
<td>8.1 ± 3.5</td>
</tr>
<tr>
<td>Serum aldosterone (pg/ml)</td>
<td>116 ± 30</td>
<td>124 ± 35</td>
<td>121 ± 37</td>
</tr>
<tr>
<td>Sodium excretion (mval/day)</td>
<td>189 ± 59</td>
<td>190 ± 64</td>
<td>194 ± 90</td>
</tr>
</tbody>
</table>

BP = blood pressure; mean ± 1 SD.

and serum aldosterone concentrations (data not shown). Again, analysis of covariance indicated an independent association of the AT$_2$-R gene with LV mass ($p < 0.02$), relative wall thickness ($p < 0.05$) and posterior ($p < 0.02$) and septal wall thickness ($p < 0.001$).

In the stepwise multiple regression analysis for posterior wall thickness, the +1675 G/A polymorphism of the AT$_2$-R gene ($p = 0.01$), urinary sodium excretion ($p = 0.002$), body mass index ($p < 0.001$) and 24-h ambulatory systolic BP ($p < 0.001$) emerged as independent parameters. Similarly, determinants for septal wall thickness were the +1675 G/A polymorphism of the AT$_2$-R ($p < 0.001$), urinary sodium excretion ($p = 0.03$), body mass index ($p = 0.001$) and 24-h ambulatory systolic BP ($p < 0.001$). Left ventricular mass was independently associated with the +1675 G/A polymorphism of the AT$_2$-R gene ($p = 0.006$), body mass index ($p < 0.001$) and 24-h ambulatory systolic BP ($p = 0.02$).

**DISCUSSION**

**AT$_2$-R gene and LV structure.** The principal finding of our study is that the +1675 G/A polymorphism of the X-chromosomal AT$_2$-R gene is associated with LV structural changes in young male subjects with mildly elevated BP. The A-allele carriers had a greater LV mass and relative wall thickness than G-allele carriers, independent of other determinants of LV structure. The association of LV mass with the AT$_2$-R gene polymorphism was most striking in hypertensive subjects, regardless of the definition used for arterial hypertension (Fig. 2 and 3). Elevated BP leads primarily to a thickening of the LV wall due to an increased afterload and, as a consequence, to a concentric remodeling of the LV indicated by an increased relative wall thickness and LV mass.

The association of the +1675 G/A polymorphism of the AT$_2$-R gene with LV mass was not affected by other confounding variables that are well known to modulate early structural processes of the LV to an increased pressure load (51). Similarly, body constitution known to modify the degree and pattern of LV hypertrophy in humans (11,52) did not bias our results.

**Biological relevance of AT$_2$-R gene.** Of the two 7-transmembrane angiotensin II receptor subtypes identified in humans up to now, most of the known effects of the renin angiotensin system are mediated by the AT$_1$-R (53). In contrast, regulation and signal transduction of the AT$_2$-R are still far from being understood. In the adult AT$_2$-R expression is restricted to a few tissues. It is upregulated under circumstances such as cardiac remodeling and infarction (28–30). The single copy human AT$_2$-R gene is located on the X-chromosome, spans about 5 kb and comprises two short noncoding exons (68 bp and 95 bp), 2 introns of 152 bp and 1,207 bp and exon 3 (>2,300 bp), which contains the entire protein coding region (33). Sequence elements located on the introns are necessary for efficient human AT$_2$-R transcription (34). The newly de-

Table 4. Echocardiographic Data Stratified According to the +1675 G/A Polymorphism of the Angiotensin II AT$_2$-Receptor Gene

<table>
<thead>
<tr>
<th></th>
<th>Normotensives (n = 60)</th>
<th>Hypertensives (n = 60)</th>
<th>Statistical Tests (p Value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Posterior wall thickness (mm)</td>
<td>8.95 ± 1.1</td>
<td>9.19 ± 1.0</td>
<td>9.94 ± 1.3</td>
</tr>
<tr>
<td>Septal wall thickness (mm)</td>
<td>9.49 ± 1.2</td>
<td>9.89 ± 1.2</td>
<td>10.1 ± 1.2</td>
</tr>
<tr>
<td>Diastolic diameter (mm)</td>
<td>51.4 ± 3.4</td>
<td>51.2 ± 3.5</td>
<td>51.8 ± 3.2</td>
</tr>
<tr>
<td>Relative wall thickness (%)</td>
<td>0.35 ± 0.05</td>
<td>0.36 ± 0.05</td>
<td>0.39 ± 0.06</td>
</tr>
<tr>
<td>Left ventricular mass (g)</td>
<td>216 ± 35</td>
<td>225 ± 40</td>
<td>243 ± 34</td>
</tr>
<tr>
<td>Left ventricular mass index (g/m$^2$)</td>
<td>112 ± 17</td>
<td>115 ± 21</td>
<td>120 ± 13</td>
</tr>
</tbody>
</table>

Mean ± 1 SD. Please note that a significant interaction between normotension/hypertension and G/A allele carriers for structural parameters of left ventricular structure was found ($p < 0.05$).
A protected intronic variant is located 29 bp before the start of exon 2, close to the region that is important for transcriptional activity of the human AT2-R gene (34).

Stimulation of the AT2-R has been shown to exert antiproliferative effects in rat coronary endothelial cells, vascular smooth muscle cells and adrenocortical cells (35–37). The signaling via AT2 is essential for the development of pressure overload-mediated cardiac hypertrophy in transgenic mice (41), whereas in adult hypertrophied rat hearts AT2-R blockade amplifies the immediate LV growth response to angiotensin II (40). In this study we observed that the AT2-R gene is associated with LV structural adaptive processes in hypertensive subjects prone to develop myocardial hypertrophy to an increased afterload. At the moment it is not yet understood whether the variant of the AT2-R gene directly affects the AT2-R function or gene expression or is in linkage disequilibrium with yet unknown polymorphisms in neighboring genes. However, since the 1675 G/A polymorphism is located in a gene region that is involved in transcriptional control of the AT2-R gene expression, it is tempting to speculate that it may affect gene expression and subsequently LV morphology. Interestingly, the antihypertrophic actions of AT2-R in experimental animals were more pronounced in hypertrophied hearts.

**Study limitations.** Our results are restricted to young male white subjects. Therefore, we cannot extrapolate that the +1675 G/A polymorphism is associated with LV structure in older subjects or in subjects with more severe arterial hypertension, that is, excessive increase in afterload imposed on the LV. Conversely, since we examined a homogenous study population, our study clearly has some advantages. None of the subjects included had ever received or was on any current antihypertensive or cardiovascular medication, thereby ruling out any potential effect of previous antihypertensive therapy (55,56). Moreover, the results are extremely unlikely to be due to an unexpected mixture of population in our study center because the local German population is ethnically homogenous, and subjects of other nationality have been excluded. Finally, we did not fully account for the exercise-induced physiological hypertrophy as a potential underlying confounding element. However, there is no rationale why the +1675 A genotype of the AT2-R gene should exercise more than the G-allele carriers.

**Conclusions.** The current data indicate that the +1675 G/A polymorphism of the AT2-R gene is associated with LV structure in young male subjects, in particular in those with mildly elevated BP. The influence of the G/A genotype of the AT2-R on the LV is independent of body size, plasma angiotensin II, serum aldosterone and the hemodynamic load as assessed by 24-h ambulatory BP monitoring.

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**Figure 2.** Left ventricular mass in normotensive and hypertensive subjects classified according to the X-chromosomal located +1675 G/A polymorphism of the angiotensin II AT2-receptor gene. **Solid circle** = A-allele; **solid square** = G-allele. Data are given by means (symbols) ± 95% confidence interval.

**Figure 3.** Posterior, septal and relative wall thickness in normotensive and hypertensive subjects classified according to the X-chromosomal located +1675 G/A polymorphism of the AT2-receptor gene. **Solid circle** = A-allele; **solid square** = G-allele. Data are given by means (symbols) ± 95% confidence interval.
Thus, our data suggest firstly that the AT$_2$-R subtype may be functional in humans and secondly that it may modulate LV morphology in mild essential hypertension.

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