Aldosterone Synthase (CYP11B2) −344 C/T Polymorphism Is Associated With Left Ventricular Structure in Human Arterial Hypertension

Christian Delles, MD,* Jeanette Erdmann, PhD,† Johannes Jacobi, MD,* Karl F. Hilgers, MD,* Eckart Fleck, MD,† Vera Regitz-Zagrosek, MD,† Roland E. Schmieder, MD, FACC*

Nürnberg and Berlin, Germany


© 2001 by the American College of Cardiology ISSN 0735-1097/01/$20.00
Journal of the American College of Cardiology Vol. 37, No. 3, 2001

The presence of left ventricular hypertrophy worsens the prognosis of cardiovascular disease (1,2). In arterial hypertension, left ventricular structure is determined by hemodynamic (e.g., blood pressure) and nonhemodynamic factors such as angiotensin II (3,4) and aldosterone (5–7). Aldosterone stimulates cardiac collagen synthesis and thus contributes to the development of cardiac fibrosis (8–10). Aldosterone acts via the mineralocorticoid type I receptor, which has been found in both the rat heart (11–13) and, more recently, in the human heart (14).

The human aldosterone synthase (CYP11B2) gene is located on chromosome 8, band 8q22, closely related to CYP11B1 (15,16). Gene variants of CYP11B2 are associated with high urinary sodium excretion and elevated sympathetic activity (22,23), but another study reports an elevated urinary aldosterone excretion in T-allele carriers (20). Blood pressure might also be influenced by the −344 C/T polymorphism. There are, however, inconsistent results about whether the T-allele (21,24) or the C-allele (20) is associated with arterial hypertension.

In a recent study, Kupari et al. (25) found an association between the −344 C/T polymorphism and left ventricular structure: the greater the number in C-alleles, the greater left ventricular mass (LVM) and left ventricular end-systolic and end-diastolic diameter (25). In contrast, a most recent study by Schunkert et al. (26) could not confirm these findings in a larger study cohort. However, the pattern of cardiac morphology in subjects with the CC genotype in the study of Kupari et al. (25) is similar to that found in a state of volume load that can be the result of high dietary sodium intake (27,28); consequently, a correlation between dietary sodium intake and LVM index was found in C-allele carriers only (25).
The present study was conducted to provide further insight into the modulation of left ventricular structure by the aldosterone synthase −344 C/T polymorphism. We hypothesized that differences in renal sodium handling may be the cause for differences in cardiac structure across the genotypes.

MATERIALS AND METHODS

Study Cohort

Through announcements at the campus of the University Erlangen-Nürnberg, we elicited the participation of young, white male students who had never been on any cardiovascular medication. Subjects were screened for normal or mildly elevated blood pressure and gave written consent prior to study inclusion. Screening of participants was continued until a total of 120 subjects fulfilled all inclusion criteria. The study protocol was approved by the Clinical Investigation and Ethics Committee of the University Erlangen-Nürnberg, Germany.

All subjects underwent a thorough clinical workup, including fundoscopy, 12-lead electrocardiography at rest, sonography of the kidneys, and routine laboratory tests (blood and urine). Inclusion criteria were male gender, Caucasian race, and age between 20 and 40 years. Exclusion criteria were renal, hepatic, and any cardiovascular disease other than mild arterial hypertension; any secondary form of hypertension; any current medication and any history of cardiovascular medication including antihypertensive treatment.

Blood Pressure Measurement and Urine Sampling

Casual blood pressure was measured according to World Health Organization (WHO) recommendations (29). In particular, to obtain correct blood pressure readings, a standard sphygmomanometer was used with the cuff size adjusted to the participant’s arm circumference. Measurements were performed seated after 5 min of rest in standardized fashion by specially trained personnel. Four casual blood pressure readings were taken in our outpatient clinic on at least two different occasions (at least four weeks apart). None of the participants followed any specific dietary guidelines before the blood pressure, endocrine and echocardiographic measurements.

Twenty-four hour urinary sodium excretion was measured in parallel to ambulatory 24-h blood pressure monitoring. Samples with a volume <600 ml/24 h and those containing less than the expected amount of creatinine per kilogram body weight were excluded to ensure complete collection of urine (30).

Parameters of the Renin-Angiotensin-Aldosterone System

Blood samples for the determination of plasma angiotensin II concentration and serum aldosterone levels were drawn from patients 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 125 mmol/liter ethylenediaminetetraacetic acid and 26 mmol/liter phenantroline (Merck, Darmstadt, Germany) to inhibit angiotensin-converting enzyme and angiotensinases. The samples were centrifuged 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 angiotensin II was measured by radioimmunassay with antiserum as previously described (30). Measurements made by this method are accurate within a range from 1.9 to 32 pg angiotensin II per milliliter. All determinations of immunoreactive angiotensin II were made in duplicate, and the mean values are given in the Results section. The coefficient of variation was <5%. Cross-reactivity was 1.2% for angiotensin I and 100% for angiotensin III and angiotensin IV, respectively. Serum aldosterone was measured by a commercially available radioimmunoassay kit (Aldosterone Maia, Serono, Freiburg, Germany). Measurements were performed in duplicate, and the mean values are given in the Results section. The coefficient of variation was <10%.

Echocardiography

Either before or after the resting period for blood sampling, two-dimensional-guided M-mode echocardiography was performed using an ultrasonoscope (Picker-Hitachi CS 192, Tokyo, Japan) with a 2.5-MHz probe by one of two skilled physicians who were unaware of the subject’s blood pressure status. According to recommendations of the American Society for Echocardiography (ASE), recordings were done at rest in the third or fourth intercostal space lateral to the left sternal border, with the subject lying in a supine or half left-sided position (31). All echocardiographic readings were evaluated by two investigators independently. Raw parameters of LVM included left ventricular end-diastolic diameter and left ventricular posterior wall thickness. If the measurements of the raw parameters differed by more than 10%, a third investigator evaluated the echocardiograms. The LVM was calculated according to the ASE recommendations (31), but it was then corrected by the formula of Devereux et al. (32).

Intervention

After the baseline examinations outlined above, participants were advised to increase their daily sodium intake by salt tablets (6 g/day) while remaining on their usual diet. One
week later, 24-h urine collection and standard laboratory tests were performed again.

**Analysis of the −344 C/T Polymorphism of the Human Aldosterone Synthase Gene**

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). Subjects were genotyped for the −344 C/T polymorphism with primers 5′-CAGGAGGAGACCATGT-GAC-3′ (sense) and 5′-CCTCCACCCTGTTCAGCCC-3′ (antisense) according to the method used by Kupari et al. (25). Standard polymerase chain reaction (PCR) was carried out in a 25-µl volume containing 60 ng genomic DNA; 10 pmol/liter of each primer; 50 mmol/liter KCl; 10 mmol/liter TRIS–HCl (pH 8.3); 1.5 mmol/liter MgCl₂; 0.01% gelatin; 200 µmol/liter of each dNTP; and 1 U Taq DNA polymerase (Gibco BRL, Germany). Samples were processed in a GeneAmp PCR System 9600 (Perkin-Elmer Cetus, Norwalk, Connecticut). After an initial 5-min denaturation at 94°C, 35 temperature cycles were carried out consisting of 20 s at 94°C, 20 s at 64°C, 20 s at 72°C, followed by a final step of 10 min at 72°C. Next, 8 µl DNA–product was digested with 5 U of restriction endonuclease *Hae*III in accordance with the manufacturer’s recommendation (New England Biolabs, Schwalbach, Germany). The digested products were subjected to electrophoresis in 1% agarose gels. The −344 T allele lacks an *Hae*III site present in the −344 C allele, so −344 T alleles are detected as *Hae*III fragments of 273 bp and −344 C alleles are detected as fragments of 202 bp (plus smaller fragments in each case).

**Statistical Analysis**

One-way analysis of variance was used to compare means among the three genotypes of the aldosterone synthase promoter. Groups that were significantly different from each other were identified by post hoc analysis using the Student *t* test with Bonferroni correction for multiple tests for normally distributed parameters and Duncan’s multiple range test for parameters not following normal distribution, respectively. To exclude the influence of potentially confounding cofactors, analysis of covariance was performed. Chi-square tests were used to compare allele frequencies. Multiple stepwise linear regression analyses with significance levels of 0.05 (entry of a variable) and 0.10 (removal of a variable) at each forward step were calculated where indicated. For all tests, a two-tailed *p* value ≤0.05 was considered significant. All values are given as means ± SD.

**RESULTS**

**Characteristics of the Study Cohort**

When classified according to WHO recommendations, a group of 65 normotensive and a group of 55 hypertensive subjects resulted. On average, hypertensive subjects had greater body weight (83 ± 11 vs. 75 ± 8 kg, *p < 0.001*), greater body surface area (2.04 ± 0.15 vs. 1.95 ± 0.13 m², *p < 0.001*) and greater body mass index (25.0 ± 3.1 vs. 23.0 ± 2.2 kg/m², *p < 0.001*) than normotensive participants.

Further stratification was performed according to the aldosterone synthase genotype. These data are given in Table 1. Frequencies for the T- and C-allele were 52% and 48% in normotensive, and 54% and 46% in hypertensive subjects, respectively. Allele frequency was not significantly different between normotensive and hypertensive subjects, and the observed numbers were not significantly different from expected values according to the Hardy-Weinberg equation. Within the normotensive and hypertensive groups, no significant differences were seen in clinical parameters but for two exceptions: In the normotensive group, subjects with the CT genotype consumed more alcohol than those with the CC genotype (Table 1). In the hypertensive group, casual diastolic blood pressure in subjects with the TT genotype was greater than in those with the CC genotype (Table 1).
Echocardiographic parameters

Echocardiographic parameters for normotensive and hypertensive subjects are given in Table 2. Noteworthy, within the normotensive group and the hypertensive group, LVM was similar across the aldosterone synthase promoter genotypes (all \( p > 0.20 \)). In the normotensive group, all other listed parameters were also not significantly different across the aldosterone synthase genotypes (all \( p > 0.20 \)).

In contrast, the \(-344\) C/T polymorphism of the aldosterone synthase promoter was associated with left ventricular structure in hypertensive subjects. Subjects with the CC genotype had a greater left ventricular end-diastolic diameter than those with the TT genotype (\( p < 0.05 \)). Similarly, left ventricular end-systolic diameter was found to be greater in subjects with the CC genotype than in TT homozygous subjects (\( p < 0.05 \)). These findings were confirmed by regression analysis. When the entire study cohort was analyzed, aldosterone synthase \(-344\) C/T polymorphism was the only parameter in the final model to determine left ventricular end-diastolic diameter (\( \beta = 0.20, p = 0.031 \); variables not in the equation: systolic blood pressure, urinary sodium excretion at baseline, body mass index, and alcohol consumption). Subjects with the CC genotype had a lower relative wall thickness than subjects with the TT genotypes (\( p < 0.05 \)) (Fig. 1).

### Parameters of the Renin-Angiotensin-Aldosterone System and Response to Increased Oral Sodium Load

Baseline serum aldosterone and plasma angiotensin II levels were similar across the aldosterone synthase genotypes in Table 2.

---

**Table 2. Echocardiographic Parameters of the Study Cohort**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Normotensives</th>
<th></th>
<th>Hypertensives</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TT (n = 20)</td>
<td>CT (n = 27)</td>
<td>CC (n = 18)</td>
<td>TT (n = 15)</td>
</tr>
<tr>
<td>Ventricular septal wall thickness (mm)</td>
<td>9.7 ± 0.9</td>
<td>9.7 ± 1.5</td>
<td>9.9 ± 1.2</td>
<td>11.4 ± 1.2</td>
</tr>
<tr>
<td>Posterior wall thickness (mm)</td>
<td>9.0 ± 1.1</td>
<td>9.0 ± 1.0</td>
<td>9.3 ± 1.0</td>
<td>11.0 ± 1.2</td>
</tr>
<tr>
<td>LV end-diastolic diameter (mm)</td>
<td>50.6 ± 3.6</td>
<td>51.7 ± 3.5</td>
<td>51.5 ± 3.2</td>
<td>50.3 ± 4.0</td>
</tr>
<tr>
<td>LV end-systolic diameter (mm)</td>
<td>33.8 ± 3.9</td>
<td>33.3 ± 3.6</td>
<td>32.7 ± 3.6</td>
<td>30.4 ± 3.8</td>
</tr>
<tr>
<td>Relative wall thickness</td>
<td>0.36 ± 0.05</td>
<td>0.35 ± 0.05</td>
<td>0.36 ± 0.04</td>
<td>0.44 ± 0.06</td>
</tr>
<tr>
<td>LVM (g)</td>
<td>214 ± 35</td>
<td>221 ± 41</td>
<td>228 ± 39</td>
<td>272 ± 57</td>
</tr>
<tr>
<td>Fractional fiber shortening (%)</td>
<td>35 ± 4</td>
<td>36 ± 4</td>
<td>36 ± 5</td>
<td>40 ± 6</td>
</tr>
<tr>
<td>Midwall fractional fiber shortening (%)</td>
<td>17 ± 2</td>
<td>18 ± 2</td>
<td>18 ± 3</td>
<td>17 ± 2</td>
</tr>
<tr>
<td>E/A velocity time integral ratio</td>
<td>0.28 ± 0.11</td>
<td>0.28 ± 0.09</td>
<td>0.24 ± 0.08</td>
<td>0.35 ± 0.17</td>
</tr>
<tr>
<td>E/A peak velocity ratio</td>
<td>0.55 ± 0.14</td>
<td>0.52 ± 0.12</td>
<td>0.56 ± 0.14</td>
<td>0.61 ± 0.16</td>
</tr>
<tr>
<td>Atrial filling fraction (%)</td>
<td>21 ± 7</td>
<td>22 ± 6</td>
<td>19 ± 5</td>
<td>25 ± 9</td>
</tr>
</tbody>
</table>

Subjects are stratified according to their blood pressure status and aldosterone synthase promoter genotype. Within one blood pressure group, * indicates significant differences from the TT genotype (\( p < 0.05 \)). E/A peak velocity ratio denotes ratio of maximal inflow velocity of passive over active diastolic filling.

LV = left ventricular; LVM = left ventricular mass.

---

**Figure 1.** Cardiac structure in hypertensive subjects stratified according to the aldosterone synthase promoter \(-344\) C/T polymorphism (filled circles, TT; filled squares, CT; filled triangles, CC). An asterisk (*) indicates significant differences across the respective genotypes (\( p < 0.05 \)).
Table 3. Serum Aldosterone, Plasma Angiotensin II Concentration, and Urinary Sodium Excretion at Baseline and After One Week of Increased Oral Sodium Uptake

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Normotensives</th>
<th>Hypertensives</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TT (n = 20)</td>
<td>CT (n = 27)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline serum aldosterone (pg/ml)</td>
<td>119 ± 28</td>
<td>122 ± 36</td>
</tr>
<tr>
<td>Serum aldosterone after sodium load (pg/ml)</td>
<td>114 ± 23</td>
<td>107 ± 32</td>
</tr>
<tr>
<td>Change in serum aldosterone (pg/ml)</td>
<td>−5 ± 40</td>
<td>−15 ± 53</td>
</tr>
<tr>
<td>Baseline plasma angiotensin II (pg/ml)</td>
<td>7.6 ± 3.2</td>
<td>8.0 ± 4.1</td>
</tr>
<tr>
<td>Plasma angiotensin II after sodium load (pg/ml)</td>
<td>7.1 ± 3.0</td>
<td>6.9 ± 3.2</td>
</tr>
<tr>
<td>Change in plasma angiotensin II (pg/ml)</td>
<td>−0.5 ± 3.1</td>
<td>−1.1 ± 3.9</td>
</tr>
<tr>
<td>Baseline urinary sodium excretion (mmol/liter/d)</td>
<td>192 ± 46</td>
<td>211 ± 55</td>
</tr>
<tr>
<td>Urinary sodium excretion after sodium load (mmol/liter/d)</td>
<td>213 ± 77</td>
<td>219 ± 77</td>
</tr>
<tr>
<td>Change in urinary sodium excretion (mmol/liter/d)</td>
<td>+22 ± 87</td>
<td>+1 ± 104</td>
</tr>
</tbody>
</table>

Within one blood pressure group, * indicates significant differences from the TT genotype. Within one genotype, † indicates significant changes from baseline due to oral sodium load. One symbol indicates p < 0.05, two symbols p < 0.01, and three symbols p < 0.001. Note that in hypertensive subjects, a significant suppression of serum aldosterone concentration after oral sodium load and a consequent increase in urinary sodium excretion are both found in the TT and CT genotypes only.

both the normotensive group and the hypertensive group (Table 3).

In the hypertensive group, response of the renin-angiotensin-aldosterone system and response of urinary sodium excretion to an elevated oral sodium intake differed across the aldosterone synthase genotypes. Decreased levels of serum aldosterone after an elevated daily sodium intake were found in subjects with the TT and the CT genotypes but not in subjects with the CC genotype. After an increased oral sodium intake, urinary sodium excretion increased only in subjects with the TT and CT genotypes (p < 0.01), whereas no significant increase of sodium excretion was observed in subjects with the CC genotype despite increased sodium intake (Table 3). Plasma angiotensin II levels were not different across the genotypes in hypertensive subjects after an elevated oral sodium intake (Table 3).

**DISCUSSION**

*Association Between the Aldosterone Synthase Promoter Genotype and Left Ventricular Structure*

Several cross-sectional studies have shown a relationship between serum aldosterone levels and LVM (5–7). Experimental studies suggest that this relationship might be due to direct action of aldosterone on the heart via cardiac mineralocorticoid receptors (11–14). Thus, aldosterone has been found to stimulate collagen synthesis in the heart, and hence it contributes to cardiac fibrosis (8–10). However, aldosterone exerts its “classic” action in the distal tubules and collecting ducts of the kidney where it regulates sodium and fluid homeostasis. Both sodium itself and the intravascular volume load have been found to modify cardiac structure (33,34); hence, aldosterone might influence cardiac structure indirectly via these mechanisms.

Up to now, only two studies have examined the relationship between gene variants of the aldosterone synthase and cardiac structure (25,26). In the first study by Kupari et al. (25), young subjects (age: 36 to 37 years) without cardiovascular disease were examined. Subjects with the CC genotype were characterized by greater LVM and, most strikingly, by greater left ventricular diameter. Meanwhile, the study by Schunkert et al. (26) in a larger cohort comprising of both young and elderly patients (mean age, 52 years; range, 25 to 74 years) without and with cardiovascular diseases has been published. The investigators could not confirm the previous finding of Kupari et al. (25). However, as the investigators state, this discrepancy could be due to different demographic and medical histories between the study cohorts.

We were able to confirm the findings from Kupari et al. (25) in our study population, which comprised young subjects without cardiovascular disease except mild arterial hypertension: the aldosterone synthase promoter CC genotype is associated with greater left ventricular diastolic and systolic diameter in hypertensive subjects. Our observation that relative wall thickness was smaller in hypertensive subjects with this genotype points toward an early state of eccentric hypertrophy that can be a result of volume load (27,28). However, two aspects must be stressed. First, in contrast to the data from Kupari et al., LVM was similar throughout the aldosterone synthase promoter genotypes in our study cohort. Second, we observed the association between left ventricular structure and the aldosterone synthase promoter genotype in hypertensive subjects only. What appears as a discrepancy at first glance can be explained by differences in the respective study cohorts. On the average, our study participants were 10 years younger than those in Kupari’s study (25). Both age and hypertension are determinants of left ventricular structure (35), and it appears as a consequence that in younger subjects another factor, namely hypertension, is necessary to amplify the effect of the aldosterone synthase promoter gene variants on left ventricular structure. Moreover, we have previously described differences in the regulation of the renin-angiotensin-aldosterone system between normotensive and hypertensive subjects (3,6) whose precise mechanism is also not yet determined.
Potential Mechanisms

A relation between daily sodium intake and LVM was only found in C-allele carriers but not in TT homozygous subjects in the study by Kupari et al. (25). To examine whether the aldosterone synthase genotype directly determines serum aldosterone levels, we measured serum aldosterone in all participants at baseline and after an elevated oral sodium intake. Two findings from our study might explain the differences in cardiac structure across the aldosterone synthase promoter genotypes in hypertensive subjects. First, the pattern of eccentric hypertrophy in CC homozygous subjects might be the result of a different response to oral sodium load as compared with TT homozygous subjects. Participants with the CC genotype did not adequately increase their urinary sodium excretion in response to an increased oral sodium uptake within the observation period of one week. Such disregulation of urinary sodium excretion in a short-term experiment might cause cardiac adaptation over a longer period similar to that observed in sodium overload (33,34).

Second, we found a different regulation of serum aldosterone concentration between TT and CC homozygous subjects in face of an increased oral sodium load. Serum aldosterone concentration has not been examined in the study by Kupari et al. (25). In our study we examined both baseline aldosterone levels and aldosterone levels in response to an increased oral sodium intake. We did not find any difference in aldosterone levels at baseline across the various aldosterone synthase promoter genotypes. However, while T-allele carriers had decreased levels of aldosterone after one week of increased sodium intake as compared with the baseline value, CC homozygous subjects did not downregulate aldosterone levels.

Again, what is seen in our short-term study with oral sodium load might be a model for a long-term disregulation of aldosterone secretion in subjects with the CC genotype. Inadequately high aldosterone secretion might lead to retention of both sodium and volume and might thus explain the pattern of eccentric left ventricular hypertrophy in hypertensive subjects with this genotype. However, it is a limitation of our study that we cannot provide data concerning urinary sodium excretion after a longer period of increased sodium uptake when a stable steady state is reached (37) and that our study was conducted without a metabolic ward.

It still remains unclear whether the aldosterone synthase promoter genotype itself is of functional relevance. Although a stronger binding of the transcription factor SF-1 to the T-allele is mentioned by Kupari et al. (25), this observation has not been published yet, and the relevance of this binding site is of limited importance (38). Furthermore, the −344 C/T polymorphism is in linkage disequilibrium with the intron-2 polymorphism of CYP11B2. As already stated by others (25,26), linkage disequilibrium with other genes, namely with the closely located CYP11B1, might be an alternative reason for our observations.

Finally, several studies have reported functional relevance of the aldosterone synthase promoter −344 C/T polymorphism (20–25). Our data underscore these findings: urinary sodium excretion, which is regulated by aldosterone, is also influenced by this polymorphism. Our findings support the hypothesis of a link among altered cardiac structure, biochemical (or physiological) parameters and variants of the aldosterone synthase gene.

Acknowledgments

We thank Ms. A. Friedrich for her skillful assistance throughout the clinical data collection.

Reprint requests and correspondence: Prof. Dr. med. Roland E. Schmieder, Universität Erlangen-Nürnberg, Medizinische Klinik IV/4, Klinikum Nürnberg Süd, Breslauer Strasse 201, 90471 Nürnberg, Germany. E-mail: roland.schmieder@rzmail.uni-erlangen.de.

REFERENCES


