1166 A/C Polymorphism of the Angiotensin II Type 1 Receptor Gene and the Response to Short-Term Infusion of Angiotensin II

Karl F. Hilgers, MD; Matthias R.W. Langenfeld, MD; Markus Schlaich, MD; Roland Veelken, MD; Roland E. Schmieder, MD

Background—Previous studies reported an association of the 1166 A/C polymorphism of the angiotensin II (Ang II) type 1 receptor gene with high blood pressure and cardiovascular disease. We tested the hypothesis that this polymorphism affects the blood-pressure, renal hemodynamic, and aldosterone response to infused Ang II.

Methods and Results—Young, male, white volunteers (n = 116) with normal (n = 65) or mildly elevated (n = 51) blood pressure on a high salt intake were genotyped for the 1166 A/C polymorphism. Two doses of Ang II (0.5 and 3 ng · kg⁻¹ · min⁻¹ over 30 minutes each) increased blood pressure, plasma aldosterone, glomerular filtration rate, and filtration fraction and decreased renal blood flow. The blood-pressure, renal hemodynamic, and aldosterone responses were not significantly different between subjects homozygous for the A allele (n = 56) and heterozygous subjects (n = 47) or subjects homozygous for the C allele (n = 13). Comparison of A allele homozygotes with all C allele carriers pooled (n = 60) or restriction of the analysis to normotensive volunteers also revealed no significant differences between genotypes.

Conclusions—The 1166 C variant of the Ang II type 1 receptor does not lead to a greater blood-pressure, aldosterone, or renal vascular response to infused Ang II in young, male, white subjects. We conclude that the 1166 A/C polymorphism does not have a major effect on these actions of Ang II. (Circulation. 1999;100:1394-1399.)

Key Words: ■ angiotensin ■ genes ■ kidney ■ blood pressure

The activity of the renin-angiotensin system (RAS) influences blood pressure and cardiovascular structure in humans.¹,² During the past 5 years, variants of genes of the RAS have been associated with cardiovascular disease. A deletion polymorphism of the ACE gene was associated with myocardial infarction³ and left ventricular hypertrophy (see Reference 4 for review). A variant of the angiotensinogen gene was associated with hypertension in several populations.⁵⁻⁹

In 1994, Bonnardeaux et al¹⁰ described a nucleotide substitution (A/C in position 1166) in the gene of the angiotensin II (Ang II) type 1 receptor (AT₁). These authors reported an increased prevalence of the C allele in hypertensives.¹⁰ These results were confirmed by some authors¹¹⁻¹³ but not by others.¹⁴⁻¹⁷ The A/C 1166 polymorphism was associated with aortic stiffness,¹⁸ left ventricular mass,¹⁵,¹⁹ and coronary vasoconstriction²⁰ and was reported to be a risk factor for myocardial infarction in synergism with the ACE gene deletion polymorphism.²¹ These associations could be due to an effect of the AT₁ gene variant or to an effect of an as yet unidentified gene locus in linkage disequilibrium with the AT₁ polymorphism.²² In that regard, it is important to determine whether or not the gene variant leads to an altered expression and/or function of the gene product.²² We are not aware of any investigation addressing these issues.

Therefore, we tested the hypothesis that the A/C 1166 polymorphism of the AT₁ Ang II receptor gene affects the physiological response to Ang II. Young, male, normotensive and never-treated mildly hypertensive white volunteers on a high-sodium diet were infused with 2 doses of Ang II. Blood pressure, renal plasma flow (RPF), and aldosterone release in response to Ang II were measured, and the AT₁ A/C 1166 genotype was determined.

Methods

Subjects

By announcement, we elicited the participation of young white male students at the campus of the University of Erlangen-Nürnberg. Blood pressure values were said to be mildly hypertensive if the average of all casual blood pressure readings taken 4 times on 2 different occasions in our outpatient clinic (at least 2 weeks apart) was ≥140 mm Hg systolic or ≥90 mm Hg diastolic, according to the WHO recommendations. The cuff size of the sphygmomanometer was adjusted according to the subject’s arm circumference, and
blood pressure was measured by specifically trained personnel with the participant seated after 5 minutes of rest.

Subjects were consecutively enrolled in the study if they fulfilled all the inclusion criteria, i.e., age between 20 and 40 years, male sex, no current or previous treatment for arterial hypertension, no cardiovascular disease, no secondary hypertension, and no WHO stage III of hypertensive disease. Therefore, exclusion criteria were advanced hypertensive fundoscopic changes, myocardial infarction or any other evidence of coronary artery disease, congestive heart failure (New York Heart Association classes II through IV), previous cerebrovascular event, or hepatic or renal insufficiency.

Each participant underwent a routine clinical workup. In particular, a 12-lead ECG at rest was performed, as well as a fundoscopic evaluation, sonography of the kidneys and adrenal glands, Doppler sonography of renal arteries, and routine laboratory tests. Detailed evaluation of hormones and endocrine metabolites was conducted if indicated. The study protocol was approved by our Clinical Investigation Committee, and written informed consent was obtained from each participant before the study.

Ambulatory 24-hour blood pressure measurements were taken with an automatic portable device (SpaceLab No. 90207). Measurement intervals were every 15 minutes during daytime (defined from 6 AM to 10 PM) and every 30 minutes during nighttime. Dietary salt intake was estimated with participants on their usual diet by measurement of sodium excretion in urine collected over a 24-hour period repeated twice. To ensure complete collection of urine, all samples containing <600 mL and/or the expected creatinine/kg body wt were excluded. One week before Ang II infusion, the participants were advised to consume an oral dietary intake of 13 g salt/d either by increasing their nutritional intake or by using salt tablets. The exogenous Ang II is more pronounced and can be detected better than under low-salt conditions.

Response to Ang II Infusion

At 10:00 AM, blood pressure (mean of 10 automated measurements over a period of 10 minutes) (Dinamap, Criticon), Ang II, aldosterone, glomerular filtration rate (GFR), and RPF were measured after 1 hour of rest in the supine position. We applied the constant-infusion technique to determine RPF (para-aminomhipturate clearance) and GFR (inulin clearance) without urinary sampling, as previously described in detail. This method may overestimate RPF by 20%, but changes from baseline in each subject are not affected by this potential bias. The filtration fraction was calculated by expressing GFR as a percentage of RPF. After 2 hours of rest in the supine position, a constant infusion of Ang II (Hypertensin, Ciba-Geigy) was administered, beginning with 0.5 ng ⋅ kg⁻¹ ⋅ min⁻¹. After 30 minutes, blood pressure, aldosterone, and renal hemodynamics were measured again. Subsequently, the dose of Ang II was increased to 3.0 ng ⋅ kg⁻¹ ⋅ min⁻¹ for another 30-minute period. At the end of this period, blood pressure, serum aldosterone, and renal hemodynamics were measured again.

Hormone Measurements

Blood samples for the determination of plasma Ang II were collected in prechilled syringes containing enzyme inhibitors as described previously. The samples were centrifuged for 10 minutes at 4°C immediately after collection, and plasma was stored rapidly after centrifugation at −21°C and analyzed within 3 months. Peptides were extracted from plasma with Bond Elut PH cartridges (PK 100, ICT-ASS-Chem). Ang II was measured by radiolmunoassay as described previously. Cross-reactivity was 1.2% for Ang I and 100% for Ang III and Ang IV, respectively. All measurements were done in duplicate, and the mean value is given. The coefficient of variation was 8.8%.

Samples for aldosterone measurements were also collected in prechilled tubes, immediately centrifuged, and stored at −26°C. Serum aldosterone was measured by a commercially available radioimmunoassay kit (Aldosterone Maia 12254, Serono Diagnostics). Measurements were done in duplicate, and the mean value is given. The coefficient of variation was <10%.

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). A 428-bp fragment of the AT1 gene, corresponding to nucleotides 959 to 1387 of the human AT1 mRNA, was amplified with the primers 5′-TCTCCCCAAA-GCCAAATCCAC-3′ and 5′-CAGGCTAGGGAATGCTTTC-TGTCGAG-3′. Thirty-seven polymerase chain reaction (PCR) cycles were performed with a TouchDown thermocycler (Hybaid Ltd) using a “touchdown” approach: the temperature during the 1-minute annealing step decreased from 72°C to 64°C over the first 12 cycles and remained at 64°C for the remaining cycles. Denaturation was 94°C for 40 seconds and extension was 72°C for 1 minute for all cycles.

The PCR product was subjected to restriction digestion with 15 U of the enzyme DdeI (New England Biolabs, Inc) at 37°C for 4 hours and subsequent gel electrophoresis. In addition to the genotype-specific DdeI site created by the 1166 C polymorphism, the 428-bp PCR fragment contained a second DdeI site (at position 1023 of the AT1 mRNA) present in all genotypes that was used as an internal control for the completeness of the restriction digestion. For the A allele, DdeI cleaved the 428-bp PCR fragment into a 64-bp and a 364-bp fragment, whereas 3 fragments were generated for the 1166 C allele: 64, 221, and 143 bp, respectively (see Figure 1).

Statistics

The sample size was estimated by use of power calculations based on variances derived from earlier studies. Sample size was chosen to allow detection of a different response of mean arterial pressure by ≥4 mm Hg, RPF of ≥40 ml/min, and aldosterone of ≥40 pg/mL with power of 0.75. Examples of power calculations based on actual variances are given in the Results section.

Paired t tests were used to test the significance of changes after Ang II infusion compared with baseline. This test was performed to ensure significant responses to Ang II. ANOVA was performed to compare groups stratified according to genotypes. The statistical analysis was performed on both the absolute values and the differences between baseline and Ang II. To increase the power to detect smaller differences, comparisons were also made between AA homozygotes and pooled C allele carriers (AC and CC) by unpaired t test. In addition, a separate analysis restricted to normotensive participants only was also performed to exclude any influence of the frequency of hypertensives in the different groups.

Analysis was carried out with SPSS software. A 2-tailed value of P<0.05 was considered significant. Values are given as mean±SD.

Results

All subjects could be genotyped unambiguously for the 1166 A/C polymorphism of the AT1 gene (Figure 1). In hypertensive subjects (29 AA homozygotes, 3 CC homozygotes, and 19 AC heterozygotes) classified according to the WHO criteria by casual blood pressure measurements, the allele frequency of the C allele was 0.25 compared with 0.37 in normotensives (27 AA, 10 CC, 28 AC). However, many subjects classified as hypertensive according to the WHO criteria displayed only mild hypertension (or white-coat hypertension) during ambulatory 24-hour measurements and follow-up casual blood pressure measurements. Systolic 24-hour ambulatory blood pressure was 120±7 mm Hg in normotensive versus 133±10 mm Hg in hypertensive subjects (P<0.05); diastolic ambulatory blood pressure was 72±6 mm Hg in normotensive versus 79±9 mm Hg in hypertensive volunteers (P<0.05).
If subjects were stratified for genotypes, there were no differences between groups regarding blood pressure and other clinical characteristics (Table 1). Baseline measurements of blood pressure did not differ between genotypes (Figure 2). The AT1 gene polymorphism did not affect the pressor response to Ang II at both doses of the peptide (Figure 2). Furthermore, no significant differences were detected between AA homozygotes and all C allele carriers (AC and CC), regardless of whether all participants were included (Table 2) or exclusively normotensive participants (Table 3). For the higher dose of Ang II, a difference of $\pm 3.8$ mm Hg in the response to Ang II can be excluded with a power of 0.75 and a difference of $\pm 6.1$ mm Hg with a power of 0.99.

Baseline values of RPF were not different between genotypes (Figure 3). RPF was decreased by 0.5 and 3.0 ng·kg$^{-1}$·min$^{-1}$ Ang II infusion (Figure 3). The RPF response was not affected by genotype, regardless of whether the analysis was performed considering all 3 genotypes separately (Figure 3) or by comparing AA homozygotes with C allele carriers among all participants (Table 2) or normotensive subjects (Table 3). A difference in RPF of $\pm 43.7$ mL/min can be excluded with a power of 0.75 and a difference of $\pm 54.6$ mL/min with a power of 0.99. Baseline values of GFR were not different between genotypes (AA 121±12, AC 116±13, and CC 115±14 mL/min). Both doses of Ang II caused small but significant increases of GFR, but there were no significant differences between genotypes (Tables 2 and 3). Likewise, filtration fraction was not different between genotypes at baseline (AA 19.3±3.0%, AC 19.1±2.9%, and CC 18.9±2.2%), and it increased significantly in response to both doses of Ang II in all groups (Tables 2 and 3). The filtration fraction response was not affected by the genotype (Tables 2 and 3).

Baseline serum aldosterone was not significantly different between genotypes (Figure 4). Aldosterone was increased significantly in all groups by both doses of Ang II, and there were no differences between genotypes (Figure 4, Tables 2 and 3).

### TABLE 1. Subject Characteristics

<table>
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<tr>
<th>Genotype</th>
<th>n</th>
<th>Body weight, kg</th>
<th>Body mass index, kg/m$^2$</th>
<th>Age, y</th>
<th>Ambulatory systolic pressure, mm Hg</th>
<th>Ambulatory diastolic pressure, mm Hg</th>
<th>Sodium excretion, mmol/d</th>
<th>Plasma Ang II, fmol/mL</th>
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<tbody>
<tr>
<td>AA</td>
<td>56</td>
<td>80±11</td>
<td>24.3±3.0</td>
<td>26±3</td>
<td>132±11</td>
<td>79±7</td>
<td>248±93</td>
<td>7.4±3.9</td>
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<tr>
<td>AC</td>
<td>47</td>
<td>78±10</td>
<td>23.7±2.6</td>
<td>26±3</td>
<td>128±10</td>
<td>77±8</td>
<td>224±70</td>
<td>7.7±3.9</td>
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<tr>
<td>CC</td>
<td>13</td>
<td>76±10</td>
<td>23.0±2.8</td>
<td>25±2</td>
<td>128±10</td>
<td>78±7</td>
<td>236±57</td>
<td>7.5±3.3</td>
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Values are mean±SD. There were no significant differences between genotypes.
TABLE 2.  AA and Pooled AC/CC Genotypes: All Participants

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<th>Genotype</th>
<th>AA</th>
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<td>Ang II 0.5 ng · kg⁻¹ · min⁻¹</td>
<td>5.2±4.9</td>
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<td>Ang II 3.0 ng · kg⁻¹ · min⁻¹</td>
<td>15.7±7.8</td>
<td>15.0±7.4</td>
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<th>RPF response, ml/min</th>
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<td>Ang II 0.5 ng · kg⁻¹ · min⁻¹</td>
<td>-41.6±51.9</td>
<td>-41.0±57.0</td>
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<tr>
<td>Ang II 3.0 ng · kg⁻¹ · min⁻¹</td>
<td>-130.2±63.0</td>
<td>-123.3±70.8</td>
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<td>Ang II 0.5 ng · kg⁻¹ · min⁻¹</td>
<td>5.3±6.4</td>
<td>3.7±3.7</td>
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<tr>
<td>Ang II 3.0 ng · kg⁻¹ · min⁻¹</td>
<td>4.6±4.7</td>
<td>4.4±6.5</td>
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<th>FF response, %</th>
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<td>Ang II 0.5 ng · kg⁻¹ · min⁻¹</td>
<td>2.2±1.8</td>
<td>1.9±1.6</td>
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<tr>
<td>Ang II 3.0 ng · kg⁻¹ · min⁻¹</td>
<td>5.8±2.2</td>
<td>5.6±2.9</td>
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</tbody>
</table>

<table>
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<th>Aldosterone response, pg/ml</th>
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<tbody>
<tr>
<td>Ang II 0.5 ng · kg⁻¹ · min⁻¹</td>
<td>22.2±46.0</td>
<td>18.1±48.8</td>
</tr>
<tr>
<td>Ang II 3.0 ng · kg⁻¹ · min⁻¹</td>
<td>101.4±76.7</td>
<td>80.9±65.3</td>
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Values are mean±SD. There were no significant differences between genotypes.

**Discussion**

We tested the hypothesis that the 1166 A/C polymorphism of the AT1 gene affects the response to infused Ang II. Renal hemodynamics, blood pressure, and aldosterone release were measured after 2 doses of Ang II in normotensive and mildly hypertensive volunteers. Our results provided no support for the hypothesis: We did not detect a major effect of the 1166 A/C polymorphism of the gene for the AT1 receptor on the short-term response to Ang II.

An association of a gene polymorphism with hypertension could be due to an effect of the genetic variant or to an effect of a yet unidentified gene locus in linkage disequilibrium with the polymorphism. In that regard, it is important to determine whether or not the gene variant leads to an altered expression and/or function of the gene product. For example, the deletion polymorphism of the ACE gene leads to increased serum ACE levels and cardiac tissue ACE activity. The angiotensinogen gene variant associated with high blood pressure is associated with altered gene transcription and increased serum levels of angiotensinogen.

The 1166 C variant of the AT1 gene was associated with hypertension, aortic stiffness, left ventricular mass, coronary vasconstriction, and myocardial infarction, but no data on the function of the AT1 gene product in subjects stratified for the AT1 A/C 1166 polymorphism have been reported to date. We tested the hypothesis that a variant of the AT1 gene affects 1 of the functions of its gene product, ie, the AT1-mediated response to Ang II.

We investigated some important parameters regulated by Ang II, namely, renal hemodynamics, aldosterone release, and blood pressure. The data did not show an augmented response in subjects with the 1166 C allele. Our study rests on the assumption that a physiologically important alteration in...
the AT1 gene would lead to an altered response to infused Ang II. This assumption has obviously not been tested in human subjects but is strongly supported by gene targeting studies in mice.33,34 There are several limitations of our study. We studied young, male white subjects without signs of target organ damage and on a high-sodium diet. Therefore, we do not know whether the results can be extended to other populations, eg, older patients with target organ injury, or patients with lower sodium intake. However, we believe that the use of a fairly homogeneous population is important to limit confounding sources of variation in the response to Ang II.

Our study was not designed to test for an association of the 1166 A/C polymorphism with hypertension. The allele frequencies measured in our hypertensive versus normotensive participants are not a valid association result, because we recruited volunteers and excluded patients with signs of organ damage. Some of our volunteers initially identified as hypertensive by the WHO criteria displayed either mild hypertension or white-coat hypertension on ambulatory blood pressure measurement. Therefore, we pooled hypertensive and normotensive subjects for the statistical analysis. If normotensive subjects were analyzed separately, identical results were obtained: the genotype did not affect the blood pressure, renal hemodynamic, or aldosterone response to Ang II. However, we cannot exclude the possibility that this type of analysis obscures a small effect of the AT1 genotype in hypertensive patients. Furthermore, we cannot rule out the possibility that the 1166 A/C polymorphism might play a role in long-term changes in cardiovascular structure, because we investigated only short-term responses to Ang II.

In summary, the 1166 A/C polymorphism of the AT1 gene did not affect the response to Ang II in our homogeneous group of young, male white subjects consuming a high salt intake. These results provide evidence against a major role of the 1166 A/C polymorphism for the short-term effects of Ang II. Other effects of the 1166 A/C polymorphism, eg, alterations of cardiac or vascular structure, may account for the reported associations with essential hypertension,10–13 aortic stiffness,19 left ventricular mass,15,19 coronary vasoconstriction,20 and myocardial infarction.21 Alternatively, other, as yet unidentified gene loci in linkage disequilibrium with the AT1 1166 C variant may account for these associations.

Acknowledgments

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References

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