Angiotensin II Type 1 Receptor A1166C Gene Polymorphism Is Associated With an Increased Response to Angiotensin II in Human Arteries

Peter Paul van Geel, Yigal M. Pinto, Adriaan A. Voors, Hendrik Buikema, Margreeth Oosterga, Harry J.G.M. Crijns, Wiek H. van Gilst

Abstract—An adenine/cytosine (A/C) base substitution at position 1166 in the angiotensin II type 1 receptor (AT1R) gene is associated with the incidence of essential hypertension and increased coronary artery vasoconstriction. However, it is still unknown whether this polymorphism is associated with a difference in angiotensin II responsiveness. Therefore, we assessed whether the AT1R polymorphism is associated with different responses to angiotensin II in isolated human arteries. Furthermore, we evaluated whether inhibition of the renin-angiotensin system modifies the effect of the AT1R polymorphism. One hundred twelve patients who were undergoing coronary artery bypass graft surgery were prospectively randomized to receive an ACE inhibitor or a placebo for 1 week before surgery. Excess segments of the internal mammary artery were exposed to angiotensin II (0.1 nmol/L to 1 \mu mol/L) and KCl (60 mmol/L) in organ bath experiments. Patients homozygous for the C allele (n = 17) had significantly greater angiotensin II responses (percentage of this maximal KCl-induced response) than did patients genotyped with AA+AC (n = 95, P < 0.05). Although ACE inhibition increased the response to angiotensin II, the difference in the response to angiotensin II, between CC and AA+AC patients remained intact in ACE inhibitor–treated patients. These results indicate increased responses to angiotensin II in patients with the CC genotype. The mechanism is preserved during ACE inhibition, which in itself also increased the response to angiotensin II. This reveals that the A1166C polymorphism may be in linkage disequilibrium with a functional mutation that alters angiotensin II responsiveness, which may explain the described relation between this polymorphism and cardiovascular abnormalities. (Hypertension. 2000;35:717-721.)

Key Words: angiotensin II receptors, angiotensin II arteries genetics risk factors polymorphism

Increased levels of angiotensin II have been suggested to be involved in the pathophysiology of cardiovascular disease. Angiotensin II acts mainly via the angiotensin II type 1 receptor (AT1R) as an acute vasoconstrictor that regulates systemic blood pressure and vascular tone. Furthermore, angiotensin II is involved in cardiac and vascular growth processes. Genetic variations in components of the renin-angiotensin system (RAS), the enzymatic cascade that generates angiotensin II, have been associated with cardiovascular disease. An ACE insertion/deletion polymorphism is associated with myocardial infarction, left ventricular hypertrophy, and cardiomyopathy; this seems to be related to the increased activity of this enzyme in plasma and cardiac tissue. Similarly, a genetic polymorphism (MT235) in the angiotensinogen gene has been related to cardiovascular diseases and to increased angiotensinogen levels. A polymorphism in the AT1R (an adenine/cytosine [A/C] base substitution at position 1166) has been associated with essential hypertension, increased artery vasoconstriction, and cardiac hypertrophy. However, this association is more difficult to interpret, because it is still unknown whether this variation in a noncoding region is associated with a difference in angiotensin II responsiveness.

Therefore, to evaluate whether the AT1R polymorphism is associated with a functional alteration, in a prospective study we assessed whether this polymorphism is associated with different responses to angiotensin II in isolated human arteries. Because it is known that ACE inhibition and a decrease in angiotensin II increase responsiveness to angiotensin II, we also assessed whether the effects of the C allele were modified by ACE inhibition.

Methods

Patients and Materials

Human isolated vessel material was obtained as excess material from patients undergoing coronary artery bypass graft surgery (CABG).
During the operation, the arteries were dissected free in such a way that they remained in their original anatomic environment, and remaining segments were collected. Permission to use this excess graft tissue was granted by the Human Ethics Committee of the University Hospital Groningen (Groningen, the Netherlands). All patients (n=187) were scheduled to undergo elective CABG with an internal mammary artery (IMA) and were eligible to participate in the study. Patients were recruited between October 1994 and February 1997. The study was defined to include ≥100 IMAs from 100 different patients that could be evaluated; this was based on an expected frequency of homozygosity for the C allele of ≥80%, so analysis would be done on at least 8 subjects homozygous for the C allele. All patients who were scheduled to undergo elective CABG were enrolled in a prospective randomized study. The aim of the study was to evaluate the effects of ACE inhibition on angiotensin II responses in human vasculature. Patients were randomized to receive blind oral treatment with quinapril (40 mg QD), captopril (50 mg BD), or placebo. Patients were eligible for enrollment if they were able to take the study drug for ≥7 days immediately before surgery. Patients were not included if they had used an ACE inhibitor or angiotensin II antagonist in the past 2 years. Patients with a known intolerance to ACE inhibitors; a history of angioedema, symptomatic heart failure, aortic stenosis, hypertrophic obstructive cardiomyopathy, left ventricular hypertrophy, severe renal impairment, functional renal artery stenoses, a renal transplant, primary aldosteronism, hypokalemia, severe hypertension, hypotension, atrial fibrillation, or obstructive pulmonary disease; or a clinically significant hematological or biochemical abnormality were not included. Treatment with diuretics, antiarrhythmics, digitals, or tricyclic antidepressants was not allowed. The present study was a prospectively defined substudy.

**Study Design**

CABG was performed at the University Hospital Groningen and the St Antonius Hospital Nieuwegein (the Netherlands). CABG was performed with a standardized anesthesia regimen for all patients at both centers. During surgery, segments of the IMA were harvested and transported immediately to the Department of Clinical Pharmacology at the University of Groningen, where measurements of in vitro vascular function took place within 3 hours after harvesting. The chickens in vivo view of both participating centers approved the protocol. Written informed consent was obtained from all patients.

**Measurements of In Vitro Vascular Function**

During CABG, segments of the left or right IMA were obtained as excess graft material from 139 patients. The segments were dissected free, cleansed from surrounding tissues, and cut into several rings (2 mm) with a sharp razor blade. The rings were mounted in 15-mL organ baths containing a buffer solution of the following composition (in mmol/L): NaCl 120.4, KCl 5.9, CaCl\(_2\) 2.5, MgCl\(_2\) 1.2, Na\(_2\)PO\(_4\) 1.2, glucose 11.5, and NaHCO\(_3\) 25.0. The medium was continuously aerated with 95% O\(_2\)/5% CO\(_2\) and maintained at 37°C. The rings were connected to an isometric displacement transducer, which gave a preload of 14 mN. The rings were allowed to equilibrate for 1 hour before they were primed and checked for viability through repeated stimulation (3 or 4 times) with 10 μmol/L phenylephrine and intermediate washing and stabilization periods.

For measurements of dose-response curves to increasing concentrations of angiotensin II, rings were preincubated with 100 μmol/L \(N^\omega\)-monomethyl-L-arginine (L-NMMA) for 30 minutes. Then, with the L-NMMA still present, rings were stimulated with increasing concentrations of angiotensin II (0.1 μmol/L to 1 μmol/L). After the final dose of angiotensin II, a response was evoked by stimulation with 60 mmol/L KCl in the presence of L-NMMA. Results are presented as a percentage of this maximal KCl-induced response (%KCl). The concentration agonist that induced 50% of the maximal response, expressed as the negative log mol/L (pEC\(_{50}\)), was obtained graphically from individual concentration-response curves. To evaluate the effects of ACE inhibition on the response to angiotensin II in CC and AA+AC patients, pEC\(_{50}\) and the maximal response to 0.3 μmol/L angiotensin II (angiotensin II\(_{\text{max}}\)) were also determined separately in placebo- and ACE inhibitor–treated patients. Angiotensin II for functional measurements was obtained from Novartis Pharma BV. All other chemicals and reagents were obtained from Sigma Chemical Co. Measurements of in vitro vascular function were performed by the same analyst and were analyzed in a blinded fashion at the University of Groningen.

**Genetic Analyses**

Genomic DNA was extracted from white blood cells. The AT\(_R\) polymorphism was identified by a mismatch-polymerase chain reaction/restriction fragment length polymorphism strategy.\(^{14}\) Digested products were separated with agarose gel electrophoresis.

**Statistical Analysis**

All statistical analyses were conducted with a statistical analysis system package (SAS version 6.12; SAS Institute Inc). Student’s t test, Fisher’s exact test, \(\chi^2\), or Wilcoxon test statistics were applied for baseline comparisons of patient characteristics. An ANOVA for repeated measures was used to compare vascular function at different concentrations of angiotensin II. To determine differences between the AT\(_R\) genotypes, a 2-way ANOVA was used, with the main effects of AT\(_R\) polymorphism and treatment. In this way, the effect of the AT\(_R\) polymorphism was corrected for a possible treatment effect. All analyses were 2-tailed, and a value of \(P<0.05\) was considered statistically significant.

**Results**

Excess graft material was obtained from 139 patients. No control responses could be evoked in 27 of these patients through repeated stimulation with phenylephrine (AA, n=16; AC, n=9; CC, n=2). Of the remaining 112 patients, 60 (54%) were genotyped as AA, 35 (31%) as AC, and 17 (15%) as CC. Baseline characteristics did not differ between CC- and AA+AC–genotyped patients, except for HDL cholesterol (Table 1).

**Vascular Responses to Angiotensin II**

The angiotensin II\(_{\text{max}}\) response was significantly higher in CC patients (CC 304±48 μm, AA+AC 161±13 μm, \(P<0.001\)). The Figure shows the average of individual angiotensin II dose-response curves (in %KCl) for the CC versus the AA+AC group. Contraction, which was induced by increasing concentrations of angiotensin II, was significantly higher in the CC group (\(P<0.05\)). The pEC\(_{50}\) of the dose-response curve to angiotensin II did not differ between CC- and AA+AC–genotyped patients (pEC\(_{50}\): CC 7.86±0.12 –log mol/L, AA+AC 7.75±0.05 –log mol/L, \(P=0.41\)).

Tables 2 and 3 show the angiotensin II\(_{\text{max}}\) response (in %KCl) and the pEC\(_{50}\) of the dose-response curve to angiotensin II, respectively, in CC and AA+AC patients for placebo- (ACEI−) and ACE inhibitor– (ACEI+) treated patients. Overall, there was a significantly higher angiotensin II\(_{\text{max}}\) response in CC than in AA+AC patients (\(P=0.045\)), whereas pEC\(_{50}\) did not differ (\(P=0.41\)). Moreover, ACE inhibition did not modify the effect of AT\(_R\) genotype on the angiotensin II\(_{\text{max}}\) response (interaction \(P=0.58\) or pEC\(_{50}\) interaction \(P=0.95\)).

**Discussion**

The present study is the first to demonstrate that the AT\(_R\) A1166C polymorphism is associated with an increased response to angiotensin II in isolated human arteries. The
A1166C mutation is located in a nontranslated region of the gene, and it has been shown that the frequency of the C allele is increased in patients with hypertension.9 This suggests that the A1166C polymorphism may be in linkage disequilibrium with a functional mutation that alters angiotensin II responsiveness. In contrast to many other studies on genetic polymorphisms, our findings were obtained in a prospectively defined study in which vascular experiments were performed and evaluated before genotype or treatment status was known.

If these results are confirmed, the situation for subjects with the CC genotype resembles that of patients with the deletion polymorphism in the ACE gene or the MT235 polymorphism in the angiotensinogen gene: these polymorphisms are also associated with an increased activity of the RAS. Therefore, all polymorphisms in the RAS that are associated with cardiovascular disease seem to be associated with the activation of this system. This underlines the large body of evidence that suggests an adverse role for inappropriate activation of the RAS. Individual heterogeneity in activity of the RAS seems to modify the relative role of the RAS in cardiovascular disease.15,16

We also studied the effects of ACE inhibition on the response to angiotensin II. The findings make 2 points. First, ACE inhibition increased the responsiveness to angiotensin II. The effect of the C allele on angiotensin II responsiveness was comparable to that of the ACE inhibitor, providing an idea of the physiological magnitude of the effects of the C allele. A second point is that the effect of the AT,R C allele is independent of the effect of ACE inhibition. Chronic ACE inhibition increases vascular responsiveness to angiotensin II,13 possibly due to receptor upregulation.17 If the CC genotype causes a fixed increase in receptor numbers, we would expect the increased responsiveness in CC patients to be masked by the increase that is due to ACE inhibition. However, that is not what we observed. We saw essentially the same difference for CC and AA+AC patients, regardless of the use of an ACE inhibitor. This suggests that the A1166C polymorphism is

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**TABLE 1. Baseline Characteristics According to AT,R Genotype**

<table>
<thead>
<tr>
<th>AT,R Genotype</th>
<th>AA+AC</th>
<th>CC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients, n</td>
<td>95</td>
<td>17</td>
</tr>
<tr>
<td>Age, y</td>
<td>63±1</td>
<td>66±2</td>
</tr>
<tr>
<td>Gender, M/F</td>
<td>77/18</td>
<td>16/1</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>26.0±0.3</td>
<td>27.0±0.7</td>
</tr>
<tr>
<td>Current/past/nonsmokers, %</td>
<td>16/68/16</td>
<td>6/65/29</td>
</tr>
<tr>
<td>Family history of CAD, %</td>
<td>38</td>
<td>38</td>
</tr>
<tr>
<td>Myocardial infarction, %</td>
<td>37</td>
<td>29</td>
</tr>
<tr>
<td>NYHA functional class</td>
<td>2.5±0.1</td>
<td>2.5±0.2</td>
</tr>
<tr>
<td>Diabetes mellitus, %</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td>Current hypertension, %</td>
<td>18</td>
<td>12</td>
</tr>
<tr>
<td>SBP, mm Hg</td>
<td>142±2</td>
<td>146±6</td>
</tr>
<tr>
<td>DBP, mm Hg</td>
<td>82±1</td>
<td>81±2</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>6.2±0.1</td>
<td>6.2±0.3</td>
</tr>
<tr>
<td>LDL cholesterol, mmol/L</td>
<td>4.1±0.1</td>
<td>4.3±0.3</td>
</tr>
<tr>
<td>HDL cholesterol, mmol/L</td>
<td>1.05±0.03</td>
<td>1.31±0.12*</td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td>2.0±0.2</td>
<td>1.5±0.2</td>
</tr>
<tr>
<td>Aspirin, %</td>
<td>77</td>
<td>71</td>
</tr>
<tr>
<td>Coumarins, %</td>
<td>20</td>
<td>6</td>
</tr>
<tr>
<td>β-Blockers, %</td>
<td>84</td>
<td>94</td>
</tr>
<tr>
<td>Calcium antagonists, %</td>
<td>69</td>
<td>71</td>
</tr>
<tr>
<td>Nitrates, %</td>
<td>75</td>
<td>65</td>
</tr>
<tr>
<td>Lipid-lowering drugs, %</td>
<td>42</td>
<td>29</td>
</tr>
</tbody>
</table>

CAD indicates coronary artery disease; NYHA, New York Heart Association; DBP, diastolic blood pressure; HDL, high-density lipoprotein; LDL, low-density lipoprotein; and SBP, systolic blood pressure. Values are mean±SEM. *P<0.05, CC vs AA+AC.

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**TABLE 2. Angiotensin IImax Response According to ACE Inhibition and AT,R Genotype**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>AA+AC</th>
<th>CC</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACEI−</td>
<td>25.8±2.3 (n=48)</td>
<td>32.5±4.5 (n=8)</td>
<td>29.1±3.5</td>
</tr>
<tr>
<td>ACEI+</td>
<td>30.8±2.8 (n=47)</td>
<td>42.7±6.6 (n=9)</td>
<td>36.8±3.2</td>
</tr>
<tr>
<td>Overall</td>
<td>28.3±1.8 (n=95)</td>
<td>37.6±4.4 (n=17)*</td>
<td>32.9±2.4</td>
</tr>
</tbody>
</table>

ACEI− indicates placebo-treated patients; ACEI+, ACE inhibitor–treated patients. Values are least squares mean±SEM, calculated with ANOVA. *P<0.045, CC overall vs AA+AC overall (2-way ANOVA).

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**TABLE 3. pEC50 According to ACE Inhibition and AT,R Genotype**

<table>
<thead>
<tr>
<th>pEC50, −log mol/L</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AA+AC</td>
</tr>
<tr>
<td>ACEI−</td>
<td>7.69±0.07 (n=48)</td>
</tr>
<tr>
<td>ACEI+</td>
<td>7.82±0.07 (n=47)</td>
</tr>
<tr>
<td>Overall</td>
<td>7.75±0.05 (n=95)</td>
</tr>
</tbody>
</table>

ACEI− indicates placebo-treated patients; ACEI+, ACE inhibitor–treated patients; pEC50, concentration of angiotensin II inducing 50% of the maximal response. Values are least squares mean±SEM, calculated with ANOVA. *P<0.41, CC overall vs AA+AC overall (2-way ANOVA).

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**Dose-response curves of increasing concentrations of angiotensin II (0.1 nmol/L to 1 µmol/L) in IMAs according to AT,R genotype.●, AA+AC genotype (n=95).○, CC genotype (n=17). Values are mean±SEM. P value represents ANOVA for repeated measures.**
in linkage disequilibrium with a mutation that dynamically increases the responsiveness to angiotensin II, on top of changes that are induced by exogenous stimuli. This is of interest because it provides the hypothesis that CC patients may still increase their response to angiotensin II, even under circumstances that by themselves cause increased angiotensin II responsiveness.

Recently, other studies have identified polymorphisms located in the coding or the 3′ and 5′ flanking region of the AT1R gene. Of these newly identified polymorphisms, only 573C was in complete linkage disequilibrium with 1166C. Although this single-base polymorphism is in the coding region of the gene, the mutation at this site does not alter the amino acid sequence of the encoded protein. Furthermore, none of the newly identified polymorphisms (including the single-base change at nucleotide 573) are associated with hypertension, and therefore it is less likely to be responsible for the associations found with the 1166C allele. As suggested by Poirier et al., newly identified polymorphisms in the AT1R gene that might explain the associations found with the 1166C allele are particularly worthwhile to further characterize when 3 conditions are met: linkage disequilibrium with the AT1R A1166C polymorphism, a similar or stronger interaction with the disease, and location in a region that would suggest a functional role.

The increased response of arteries from subjects homozygous for the C allele of the AT1R gene provides a possible explanation for the increased risk of cardiovascular disease in such subjects.

Acknowledgments

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References


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