Genetic Polymorphisms of the Renin-Angiotensin System and Atheromatous Renal Artery Stenosis

Oliviero Olivieri, Elisabetta Trabetti, Silvia Grazioli, Chiara Stranieri, Simonetta Friso, Domenico Girelli, Carla Russo, Pier Franco Pignatti, Giancarlo Mansueto, Roberto Corrocher

Abstract—Genes that influence the renin-angiotensin system have been investigated in recent years as potential etiologic candidates of cardiovascular and renal diseases. In atheromatous renal artery stenosis (RAS), a condition characterized by persistent activation of the renin-angiotensin system, the study of these genes may be of particular relevance. We evaluated angiotensin-converting enzyme (ACE) insertion/deletion, angiotensinogen (AGT) M235T, and angiotensin II receptor (ATR) A1166C polymorphisms in relation to the occurrence of RAS. We studied 58 patients with angiographically documented RAS; 102 normotensive subjects with normal coronary arteries and no history or clinical or instrumental evidence of atherosclerosis in other vascular districts were considered the control group. Patients had a significantly higher D allele frequency (0.70 versus 0.55; $\chi^2$ 6.88, $P=0.01$; odds ratio [OR] 1.9, 95% CI 1.17 to 3.07) than did the control population; 48.3% of patients were homozygous for DD ($\chi^2$ 6.62, $P<0.05$; OR 2.04, 95% CI 1.05 to 3.95); and only 8.6% carried the II genotype (OR 0.34, 95% CI 0.19 to 1.47). No significant association was found for AGT M235T and ATR A1166C. Our results suggest a predisposing role for ACE genetic polymorphism in the development and progression of atheromatous RAS. (Hypertension. 1999;34:1097-1100.)

Key Words: renal artery $\bullet$ angiotensin-converting enzyme $\bullet$ angiotensinogen $\bullet$ angiotensin II receptor polymorphism

Atheromatous renal artery stenosis (RAS) is generally considered a localization of a more generalized vascular disease.1–3 The estimated prevalence of RAS varies according to the various studies and the clinical features of the patients, ranging from 1% to 2% in the overall hypertensive population to 30% to 40% in selected hypertensive patients with overt atherosclerotic disease (14% to 38% in patients with aorto-occlusive or peripheral vascular disease and 11% to 30% in patients with coronary artery disease) (for a recent review, see Reference 4). In contrast, very low prevalences (<1%) are reported in hypertensive patients without clinical evidence of vascular disease.1–5,9

Unlike the acquired vascular risk factors that have frequently been associated with this condition,1,5–9 the role of genetic predisposition to RAS has been studied inadequately. The only report published on the topic to date demonstrated an increased frequency of deletion polymorphism of the renin-angiotensin system and the development of vascular damage in individual subjects.

Plasma and cellular ACE levels are stable within individuals but show marked interindividual variability; $\approx 50\%$ of this variability is accounted for by a major gene effect.11,12 An insertion/deletion (I/D) polymorphism of a 287-bp sequence near the 3’ end of intron 16 of the ACE gene appears to be responsible for this variability.14 Specifically, it has been demonstrated that the presence of the D allele correlates with higher plasma and cellular ACE levels compared with the levels observed in subjects carrying the I allele, with the result that different ACE polymorphisms have graduated effects on phenotypical ACE expression (DD>IID=II).

Plasma AGT concentrations are also related to a polymorphic variant of the corresponding gene, the M235T mutation, with individuals homozygous for the T allele having the highest and individuals homozygous for the M allele having the lowest plasma AGT levels.13 Polymorphisms have also been described in the gene encoding subtype 1 of the angiotensin II receptor (ATR), but these mutations have not yet been linked to any biological phenotype.15 Nonetheless, it has been postulated that the A1166C polymorphism of the ATR gene may potentiate the cardiovascular risk carried by the ACE D allele and contribute to aortic stiffness in hypertensive patients.16,17
In the present study, we evaluated the renin-angiotensin system ACE I/D, AGT M235T, and ATR A1166C polymorphisms in relation to the occurrence of RAS.

**Methods**

We studied 58 consecutive patients referred to our Hypertension Clinic from May 1996 to September 1998 and diagnosed as having RAS. All but 2 patients had a significant RAS (at least 70% lumen reduction) were enrolled; the 2 remaining patients refused to give blood for DNA evaluation. Patients referred to our Hypertension Clinic ranged from 250 to 300 patients per year, and RAS patients represented 10% to 12% of the total. Patients with renal artery lumen narrowing <70% were excluded in order to compare only phenotypically extreme conditions.

All the patients included in the present study underwent angiographic evaluation and were found to have significant monolateral or bilateral RAS (at least 70% lumen reduction). All of them were examined for severe hypertension requiring >2 drugs and/or associated with clinically evident vascular disease, mainly affecting the lower extremities. Angiographic study was performed when a moderate or high index of clinical suspicion, defined according to Mann and Pickering,6 was recognized.

Routine biochemical and clinical data were collected on the occasion of the first examination, venous blood samples for genetic analysis were taken after renal angiography, and the patient’s informed consent was obtained.

Because renal angiography was regarded as unacceptable in control subjects for ethical reasons, we exploited the possibility offered by our recently performed large case-control study in subjects with an angiographically documented normal or pathologic coronary bed.18 One hundred two subjects with valvular heart disease and normal coronary arteries who proved normotensive at 3 consecutive evaluations over a 3-month period were considered as a control group. Hypertension was defined as systolic blood pressure >140 mm Hg or diastolic blood pressure >95 mm Hg. Moreover, control subjects were enrolled providing that they had both a normal coronary angiogram at cardiac catheterization and no history or clinical or instrumental evidence of atherosclerosis in vascular districts other than the coronary bed. With these selection methods, the prevalence of renal artery disease can be considered extremely low or close to zero.1,5–9

**Mutation Analysis**

Mutation analysis (as well as routine biochemical analysis) was conducted as a study that was blinded as to whether the sample came from an RAS or a control subject. Genomic DNA was extracted from whole blood by use of standard methods.

To determine the ACE genotype, genomic DNA was amplified by polymerase chain reaction (PCR) with the hace3 primer pair that recognized only the I allele as described by Lindpaintner et al.19 Each sample with the DD genotype was subjected to a second independent PCR amplification with the hace3 primer pair that recognized only the I allele as described by Shanmugam et al.20

Analysis of M235T polymorphism in the human AGT gene was performed by PCR and Tth 111 I restriction, as described by Russ et al.21 A1166C polymorphism of the ATR gene was detected by PCR and Ddel digestion, as reported by Katsuya et al.22

**Statistical Analysis**

Statistical analysis was performed by the Systat 5.2.1 package working on a Macintosh Performa 5300. Quantitative data were analyzed with the Student t test or ANOVA with the Tukey post hoc comparison of the means when appropriate. Qualitative data were analyzed by $\chi^2$ test. A value of $P<0.05$ was considered significant.

**Results**

The relevant characteristics of the population studied are summarized in Table 1. As expected, the RAS patients presented more conventional cardiovascular risk factors (higher levels of glucose, triglycerides, and LDL cholesterol and lower HDL cholesterol levels) and higher (although not yet statistically significant) serum creatinine levels than did the control subjects.

The distribution of the genotypes for the 3 polymorphisms in the control population was compatible with a Hardy-Weinberg equilibrium. Table 2 indicates the allele frequencies and genotype distributions in RAS patients and control subjects. The allele frequencies of AGT M235T and the ATR A1166C polymorphisms were not statistically different between patients and controls. The frequency of the D allele in the RAS group was significantly higher than that in the control group (0.70 versus 0.55; $\chi^2$ 6.88, $P=0.01$; odds ratio [OR] 1.9, 95% CI 1.17 to 3.07). An increased frequency of the DD genotype was also evident (DD 28 of 58 patients in the RAS group versus 32 of 102 subjects in the control group; $\chi^2$ 6.62, $P<0.05$; OR 2.04, 95% CI 1.05 to 3.95).

**TABLE 1. Main Clinical Features of RAS Patients and Control Subjects**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Patients (n = 58)</th>
<th>Controls (n = 102)</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age, y</td>
<td>66.2±8.3</td>
<td>64±12</td>
<td>NS</td>
</tr>
<tr>
<td>Gender, M/F</td>
<td>45/13</td>
<td>77/25</td>
<td>NS</td>
</tr>
<tr>
<td>Smokers, Y/N</td>
<td>26/32</td>
<td>37/65</td>
<td>NS</td>
</tr>
<tr>
<td>SBP, mm Hg</td>
<td>166±8.6</td>
<td>128±7</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>DBP, mm Hg</td>
<td>101±12</td>
<td>82±8</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>5.85±1.1</td>
<td>5.62±1.15</td>
<td>NS</td>
</tr>
<tr>
<td>LDL cholesterol, mmol/L</td>
<td>4.1±0.9</td>
<td>3.72±1</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>HDL cholesterol, mmol/L</td>
<td>1.15±0.27</td>
<td>1.47±0.47</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td>1.98±0.7</td>
<td>1.42±0.6</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Glucose, mmol/L</td>
<td>6.3±2.5</td>
<td>5.5±0.7</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Uric acid, mmol/L</td>
<td>0.38±0.1</td>
<td>0.36±0.1</td>
<td>NS</td>
</tr>
<tr>
<td>Creatinine, μmol/L</td>
<td>106±29</td>
<td>100±16</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are mean±SD or numbers of patients. SBP indicates systolic blood pressure; DBP, diastolic blood pressure, and NS, not significant.

*Student t test or $\chi^2$ test was performed as appropriate.

**TABLE 2. Allele and Genotype Frequencies of ACE I/D and Allele Frequencies of AGT and ATR Polymorphisms in Patients and Control Subjects**

<table>
<thead>
<tr>
<th>Polymorphisms</th>
<th>Patients n (%)</th>
<th>Controls n (%)</th>
<th>$\chi^2$</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alleles</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>81 (70)</td>
<td>112 (55)</td>
<td>6.88</td>
<td>0.01</td>
</tr>
<tr>
<td>I</td>
<td>35 (30)</td>
<td>92 (45)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Genotypes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DD</td>
<td>28 (48.3)</td>
<td>32 (31.4)</td>
<td>6.62</td>
<td>0.05</td>
</tr>
<tr>
<td>ID</td>
<td>25 (43.1)</td>
<td>48 (47)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>5 (8.6)</td>
<td>22 (21.6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alleles</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AGT 235T</td>
<td>59 (51)</td>
<td>100 (49)</td>
<td>0.11</td>
<td>NS</td>
</tr>
<tr>
<td>AGT 235M</td>
<td>57 (49)</td>
<td>104 (51)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alleles</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ATR 1166C</td>
<td>31 (26.7)</td>
<td>68 (33)</td>
<td>1.51</td>
<td>NS</td>
</tr>
<tr>
<td>ATR 1166A</td>
<td>85 (73.3)</td>
<td>136 (67)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
genotype was associated with a lower relative risk of RAS (OR 0.34, 95% CI 0.19 to 1.47). There was no preferential association between ACE and AGT or ATR genotypes in the patients (Table 3).

The distribution of the main vascular risk factors (smoking and levels of total, LDL, and HDL cholesterol, triglycerides, uric acid, and glucose) and serum creatinine levels were not affected by I/D polymorphism (data not shown).

### Discussion

The main result of the present study was the demonstration that patients affected by atheromatous RAS had a significantly higher D allele frequency than did the control population; about half of them were DD homozygotes and only 8.6% were II homozygotes compared with 31% and 22% in the control population, respectively (Tables 2 and 3). In contrast, AGT and ATR polymorphism alleles and genotypes were similarly distributed between the 2 populations and were not associated with the occurrence of renal vascular pathology (Table 2).

Despite the current interest in the genetics of the renin-angiotensin system in relation to the vascular and renal risk profile, RAS, a clinical condition characterized by persistent activation of this system, has not been extensively studied. Because of the relative scarcity of reports, comparison of our results with previously published data are objectively limited. To the best of our knowledge, the only article on this topic is the study by Missouris et al., who evaluated ACE polymorphism in 56 English RAS patients; AGT and ATR polymorphisms were not analyzed. Besides the geographical origin, a relevant difference vis-à-vis the present study was that Missouris et al used subjects from the general population as a control group, without any objective angiographic information about their coronary arteries. With our approach, however, we were able to rule out the possibility that the controls might have had substantial (though not clinically manifest) coronary atherosclerosis; thus, the occurrence of atheromatous RAS would also be highly unlikely. Having considered objective conditions, we therefore feel confident that we reduced the likelihood of spurious results. Apart from this difference, however, the D allele frequency observed in the English population was similar to that found in our Italian patients.

Reported frequencies of the DD genotype in “control” populations vary from 16% to 28% in Japanese subjects to ≈ 22% to 32% in Caucasians. Our data confirm that the range of frequency of DD polymorphism in Northern Italy is of the same order of magnitude as that generally reported for Caucasian populations (homozygosity in our control subjects was 31.37%). At this level, any possible selection bias deriving from a “spurious” low frequency in the control population and leading to an erroneous statistical difference in favor of the RAS group should be excluded. In addition, the distribution of the ACE genotypes in the control population was compatible with a Hardy-Weinberg equilibrium.

Another possible source of bias lies in the power of the study. The present study analyzing 320 alleles had a predicted 80% power (5% probability) of detecting an allelic difference such as that observed in D allele frequency between the RAS and control groups (Table 2); thus, the results should be reliable.

The problem of the power of the study is also of major relevance with regard to the negative results that we observed for AGT and ATR polymorphisms. For this reason, our findings do not allow us to completely rule out an association between AGT and ATR polymorphisms and RAS risk; therefore, firm conclusions should be avoided. However, it should be borne in mind that the allele frequency of AGT was so balanced in the 2 groups (Table 2) that a positive association with this polymorphism appears highly improbable.

The predisposing role of ACE I/D polymorphism in cardiovascular disease has been a subject of controversy and debate in recent years: both positive and negative associations with coronary atherosclerotic disease (CAD) and myocardial infarction (MI) have been reported (for review see References 27, 28, 31, and 32). Controversial findings have also been reported for several other pathological conditions such as cerebrovascular disease (for a review see Reference 33), left ventricular hypertrophy, and diabetic or nondiabetic nephropathy (for review see References 37 and 38). Note that very few of the numerous published studies reported an elevated DD genotype frequency such as that found in the present study. If we also take into account the results reported by Missouris et al., an estimated frequency of DD in RAS patients should range from 41% to 48.3% (mean 44.7%, 51 of 114 patients), and the estimated D allele frequency should range from 0.64 to 0.70 (mean 0.67).

As already stated, unsuspected RAS is commonly found in patients with angiographically documented CAD, with a prevalence ranging from 11% to 30% (the mean for 11 published reports was 23%). If ≈ 50% of patients affected by RAS carry a DD genotype, then 5.5% to 15% (mean 11.5%) of DD genotypes observed in a population of CAD patients may vary in relation to the relative proportion of unsuspected RAS patients included in the study. Because patients affected by CAD or MI were not angiographically checked for RAS in the previous studies, negative or positive statistical associations with I/D polymorphism might depend on this unrecognized confounding factor. Similarly, one can speculate regarding the reported increase in D allele frequency in cerebrovascular disease or myocardial hypertrophy, which commonly complicate the clinical history of the patients with RAS.

### Table 3. Distribution of AGT and ATR Genotypes According to ACE Polymorphism in RAS Patients

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>II (n=5)</th>
<th>ID (n=25)</th>
<th>DD (n=28)</th>
<th>χ²</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>AGT 235MM</td>
<td>2 (40%)</td>
<td>4 (16%)</td>
<td>7 (25%)</td>
<td>1.71</td>
<td>NS</td>
</tr>
<tr>
<td>AGT 235MT</td>
<td>2 (40%)</td>
<td>15 (60%)</td>
<td>14 (50%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AGT 235TT</td>
<td>1 (20%)</td>
<td>6 (24%)</td>
<td>7 (25%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ATR 1166AA</td>
<td>2 (40%)</td>
<td>11 (44%)</td>
<td>16 (57.1%)</td>
<td>1.43</td>
<td>NS</td>
</tr>
<tr>
<td>ATR 1166AC</td>
<td>3 (60%)</td>
<td>13 (52%)</td>
<td>11 (39.3%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ATR 1166CC</td>
<td>0 (4%)</td>
<td>1 (4%)</td>
<td>1 (3.6%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are number (percentage) of patients.

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In conclusion, several lines of evidence suggest a close link between ACE polymorphism, activation of the renin-angiotensin system, and the causes of RAS. Reported controversial associations between D allele frequency and various forms of cardiovascular pathology (eg, coronary artery disease) should be reconsidered in light of this potentially confounding factor.

Acknowledgments
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References

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