Renin-Angiotensin System Genetic Polymorphisms and Cerebral White Matter Lesions in Essential Hypertension

Cristina Sierra, Antonio Coca, Elisenda Gómez-Angelats, Esteban Poch, Javier Sobrino, Alejandro de la Sierra

Abstract—It has been reported that both the DD genotype of the angiotensin converting enzyme (ACE) gene and the presence of cerebral white matter lesions (WML) may represent risk factors for the development of stroke. The present study investigates a possible association between 3 different genetic polymorphisms of the renin-angiotensin system and the presence of WML in 60 never-treated essential hypertensive patients (36 men, 24 women), aged 50 to 60 years, without clinical evidence of target organ damage. All patients underwent brain magnetic resonance imaging to establish the presence or absence of WML. The insertion/deletion (I/D) ACE gene, the M235T angiotensinogen (AGT) gene, and the A1166C angiotensin II type 1 receptor gene polymorphisms were determined by standard polymerase chain reaction. Twenty-five hypertensive patients (41.6%) were found to have WML on brain magnetic resonance imaging. No significant association between the M235T angiotensinogen or A1166C angiotensin II type 1 receptor genotypes and the presence of WML was found. However, the frequency of the DD genotype in patients with WML (64%) was significantly higher than that observed in patients without WML (28.6%; \( P = 0.022 \)). The DD genotype odds ratio for the presence of WML was 4.44 (95% confidence interval: 1.48 to 13.3). Likewise, the proportion of the D allele in patients with WML (74%) was significantly higher (\( P = 0.014 \)) than that observed in patients without WML (51.4%). We conclude that the presence of the DD genotype and/or the D allele of the ACE gene may be a predisposing factor for developing WML in essential hypertensive patients. However, because of the small sample size, these results require confirmation in a larger study. (Hypertension. 2002;39[part 2]:343-347.)

Key Words: genetics ■ ACE gene ■ angiotensinogen gene ■ angiotensin II type 1 receptor gene ■ white matter lesions ■ stroke

Cerebral white matter lesions (WML) represent a subclinical form of ischemic brain damage. Although their pathogenesis is poorly understood, various studies have shown that age, hypertension, diabetes mellitus, and a history of stroke or heart disease are the most important factors related to the presence of WML.\(^1\) In addition, a recent study of elderly twins indicated that susceptibility to white matter hyperintensities was largely determined by genetic factors.\(^2\) The identification of hereditary factors is important because the presence of WML is an important prognostic factor for the development of stroke\(^3–5\) and also for cognitive impairment.\(^6–11\) The association between the presence of WML and arterial hypertension\(^1\) suggests that genes involved in the regulation of blood pressure (BP) may also contribute to the development of this cerebral abnormality. Essential hypertension has a well-known familial aggregation, and it has been calculated that BP variation is approximately 40% genetically determined.\(^12\) The renin-angiotensin system (RAS) plays a central role in the regulation of BP,\(^13\) and the genes involved in the activity of this enzyme cascade are potential candidates for essential hypertension and some associated clinical conditions.

In the past decade, several authors have investigated RAS polymorphisms as genetic determinants of essential hypertension and cardiovascular complications, with controversial results. Evidence implicating the insertion/deletion (I/D) angiotensin-converting enzyme (ACE) gene, the M235T angiotensinogen (AGT) gene, and the A1166C angiotensin II type 1 (AT1) receptor gene polymorphisms in essential hypertension has been reported in some studies\(^14–16\) but not in others.\(^17,18\) A recent meta-analysis by Staessen et al\(^17\) reported that the D allele of the ACE gene was not related to hypertension but seemed to be associated with a higher risk of atherosclerotic complications (coronary heart disease, myocardial infarction, and stroke). Conversely, the same group also reported that the T allele of the AGT gene was not associated with atherosclerotic complications but seemed to be related to hypertension.\(^19\) With respect to possible inter-
action among genes, the association of the T allele of the AGT gene and the D allele of the ACE gene has been reported to have a synergistic effect on the incidence of cerebrovascular disease, and the association of the D allele of the ACE gene and the C allele of AT1 receptor gene seems to increase the risk of myocardial infarction.

It has been reported that in hypertensive patients, the presence of the DD genotype of the ACE gene is a risk factor for stroke and also for the development of renal and cardiac abnormalities. Nevertheless, the relationship between the RAS polymorphisms and the existence of early brain damage in essential hypertension, such as the presence of WML, has not been fully explored. Only Amar et al. have reported an association between the DD genotype and WML in a group of patients with dementia, whereas Schmidt et al. found a relationship between the TT genotype of the AGT gene and WML in elderly individuals.

The relationship between the 3 main reported RAS polymorphisms—the I/D ACE gene, the M235T AGT gene, and the A1166C AT1 receptor gene polymorphisms—and their possible synergistic effect and the presence of silent cerebral WML in hypertensive patients is not well known. The present study analyzes this relationship in a group of middle-aged never-treated essential hypertensive patients.

**Methods**

**Patient Selection**

Sixty never-treated essential hypertensive patients of both genders, aged 50 to 60 years, were consecutively selected from the Hypertension Unit of the Hospital Clinic, Barcelona, Spain. The diagnosis of essential hypertension was considered on the basis that no known cause of high blood pressure (BP) could be detected after complete clinical, biochemical, and radiological examination. All patients had systolic BP (SBP) ≥140 mm Hg and/or diastolic BP (DBP) ≥90 mm Hg in at least 3 different measurements at 1-week intervals. Exclusion criteria included type 2 diabetes mellitus (fasting plasma glucose > 6.6 mmol/L), carotid stenosis > 50% measured by ultrasonography, alcohol intake > 30 g of pure ethanol per day, clinical evidence of cerebrovascular or coronary heart diseases, cardiac failure, papilledema, and renal impairment (serum creatinine > 115 µmol/L). All patients gave informed consent, and the study was approved by the Ethics Committee of the Hospital Clinic and by the Spanish Health Authority.

**Cerebral Magnetic Resonance Imaging and WML Classification**

Magnetic resonance imaging (MRI) of the brain was performed using a 1.5 Tesla Siemens MAGNETON SP machine. The recordings were analyzed by 2 investigators who were blinded to all clinical information. Details of the image interpretation protocols used were the same as the Rotterdam Study. Patients were classified by consensus as having (1) normal scans: no or slight periventricular hyperintensity (small caps or pencil-thin lining), fewer than 5 focal lesions, and no confluent lesions; or (2) WML: moderate or severe periventricular hyperintensity, 5 or more focal lesions, or confluent lesions.

**RAS Polymorphism Determination**

Samples for DNA analysis were obtained from frozen peripheral leukocytes as previously described. The I/D polymorphism of the ACE gene was assessed by detecting the presence (allele I, insertion) or absence (allele D, deletion) of a 287-bp sequence in intron 16 of the ACE gene in chromosome 17 with the polymerase chain reaction (PCR) technique and agar electrophoresis as described by Rigat et al. Because the D allele is preferentially amplified in heterozygous subjects, each sample found to be DD was reanalyzed by a second independent PCR amplification with a primer that specifically recognizes an insertion-specific sequence. Depending on the genotype, subjects were classified as DD, DI, or II.

The M235T polymorphism of the AGT gene was determined by PCR amplification of a 303-bp fragment in exon 2 of the AGT gene. Subsequently, a restriction-endonuclease digestion with the SfaNI enzyme was performed. In the presence of codon ATG, the enzyme produces a 266-bp fragment corresponding to the M allele. When the fragment is not digested, the allele identified is T. The undigested wild-type (A) allele was visible as a fragment of 519-bp, and the digested C allele occurred with 2 bands at 134 and 384 bp. Patients were classified as AA, AC, or CC, depending on their genotype.

**Statistical Analysis**

Allele frequencies were estimated by the gene-counting method, and Hardy-Weinberg equilibrium was tested by the χ² test. The association of the presence of WML with genotype distribution was tested by the χ² test. The relation of the I/D alleles with the presence of WML was also tested, considering both a recessive effect of the deletion allele (DD patients compared with DI + II) and a dominant effect for the same allele (DD + DI patients compared with II). Odds ratios for these comparisons were calculated. One-way ANOVA and Student’s t test were used to compare BP among different genotypes and WML groups, respectively. Finally, the presence of possible synergistic effects of the 3 RAS gene polymorphisms on WML was assessed by multiple logistic regression analysis.

**Results**

Essential hypertensive patients were classified into 2 groups on the basis of the presence (25 patients) or absence (35 patients) of WML on brain MRI. As shown in Table 1, main baseline characteristics including age, gender distribution, BMI, plasma glucose and cholesterol, and renal function did not differ between groups. In contrast, essential hypertensive patients with WML showed significantly higher values of SBP (171.0 ± 10.6 mm Hg versus 160.3 ± 14.0 mm Hg; P = 0.003) and DBP (102.5 ± 6.2 mm Hg versus 97.8 ± 6.4 mm Hg; P = 0.009) compared with hypertensive patients without WML.

**TABLE 1. Baseline Characteristics of Essential Hypertensive Patients With and Without Cerebral WML**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Without WML (n=35)</th>
<th>With WML (n=25)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>53.5 (3.6)</td>
<td>55.3 (4.3)</td>
<td>0.083</td>
</tr>
<tr>
<td>Sex, M/F</td>
<td>21/14</td>
<td>15/10</td>
<td>0.606</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>28.1 (2.7)</td>
<td>29.0 (3.7)</td>
<td>0.313</td>
</tr>
<tr>
<td>Smokers, %</td>
<td>25.7</td>
<td>20.0</td>
<td>0.760</td>
</tr>
<tr>
<td>SBP, mm Hg</td>
<td>160.3 (14.0)</td>
<td>171.0 (10.6)</td>
<td>0.003</td>
</tr>
<tr>
<td>DBP, mm Hg</td>
<td>97.8 (6.4)</td>
<td>102.5 (6.2)</td>
<td>0.009</td>
</tr>
<tr>
<td>Serum creatinine, µmol/L</td>
<td>80.8 (14.8)</td>
<td>85.6 (14.8)</td>
<td>0.141</td>
</tr>
<tr>
<td>Serum cholesterol, mmol/L</td>
<td>5.4 (1.0)</td>
<td>5.1 (0.8)</td>
<td>0.383</td>
</tr>
<tr>
<td>Serum glucose, mmol/L</td>
<td>5.4 (0.5)</td>
<td>5.3 (0.5)</td>
<td>0.436</td>
</tr>
</tbody>
</table>

Values are mean (SD). BMI indicates body mass index.
Allele and Genotype Frequencies

The genotype distribution and derived allele frequencies are shown in Table 2. When the genotype frequencies obtained for the I/D ACE gene (0.188, 0.304, and 0.377 for II, DI, and DD, respectively), M235T AGT gene (0.275, 0.478, and 0.116 for MM, MT, and TT, respectively), and A1166C AT1 receptor gene (0.377, 0.377, and 0.116 for AA, AC, and CC, respectively) were compared with the expected frequencies predicted by the Hardy-Weinberg equilibrium, the 3 polymorphisms exhibited a nonsignificant difference ($P=0.095$ for I/D, $P=0.565$ for M235T, and $P=0.905$ for A1166C AT1 receptor polymorphism). The D allele (0.608), M allele (0.592), and A allele (0.650) were the most frequently observed.

Cerebral WML and RAS Polymorphisms

We found no relationship between AGT M235T polymorphism or A1166C AT1 receptor polymorphism and the presence of cerebral WML, as shown in Table 3. Conversely, with respect to I/D ACE gene polymorphism, the frequency of the DD genotype in hypertensive patients with WML (64%) was significantly higher ($P=0.022$) than in those without WML (28.6%). This association remained significant ($P=0.049$) after controlling for both SBP and DBP. Moreover, the proportion of the D allele in hypertensive patients with WML (74%) was also significantly higher ($P=0.014$) than in those without WML (51.4%).

We tested for a possible synergistic effect of the 3 RAS polymorphisms studied on the presence of WML and found that the inclusion of M235T AGT gene polymorphism or the A1166C AT1 receptor gene polymorphism or both had no synergistic effect on the association of I/D ACE gene polymorphism and the presence of WML. Furthermore, no significant differences in BP values were observed with respect to ACE genotype, as shown in Table 4.

Finally, the association between ACE gene polymorphism and the presence of WML was tested assuming both a recessive effect (comparing DD versus DI+II genotypes) and a dominant effect (comparing DD+DI versus II genotypes) for the D allele. The prevalence of WML was significantly higher ($P=0.009$) in DD (61.5%) patients compared with DI+II genotypes (26.5%), whereas no differences were observed assuming a dominant effect (DD+DI: 44.7% versus II: 30.8%; $P=0.527$). The odds ratio of the DD genotype for the presence of WML was 4.44 (95% confidence interval: 1.48 to 13.32).

Discussion

The present study shows an association between the I/D polymorphism of the ACE gene and the presence of silent cerebral WML in middle-aged patients with uncomplicated essential hypertension. Hypertensive patients who were homozygous for the deletion allele (DD) presented a significantly higher prevalence of WML compared with patients who were homozygous for the insertion allele (II) or heterozygous (DI). Conversely, no association was found between WML and either the M235T polymorphism of the AGT gene or the A1166C polymorphism of the AT1 receptor gene. In addition, the multivariate analysis showed no significant synergistic effect of these 2 polymorphisms on the association between ACE gene I/D polymorphism and the presence of WML. The association between DD genotype and WML is consistent as shown by a high odds ratio. However, the study is limited by a small sample size, and it would be desirable achieve confirmation in a larger study.

The mechanism responsible for the effect of different ACE gene polymorphisms on the development of WML remains speculative. ACE is thought to play an important role in vascular remodeling in the arteries of stroke-prone spontaneously hypertensive rats and in human WML. It has been reported that plasma levels of ACE differ in subjects with different ACE genotypes, and, in fact, the D allele of the ACE gene seems to be associated with a higher risk of atherosclerotic complications. Cerebral WML probably rep-

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### Table 2. Genotype Distribution and Derived Allele Frequencies

<table>
<thead>
<tr>
<th>Polymorphism</th>
<th>I/D ACE Gene (n=60)</th>
<th>M235T AGT Gene (n=60)</th>
<th>A1166C AT1, Receptor Gene (n=60)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotype</td>
<td>II</td>
<td>DI</td>
<td>DD</td>
</tr>
<tr>
<td>N (%)</td>
<td>13 (21.7)</td>
<td>21 (35.0)</td>
<td>26 (43.3)</td>
</tr>
<tr>
<td></td>
<td>19 (31.7)</td>
<td>33 (55.0)</td>
<td>8 (13.3)</td>
</tr>
<tr>
<td>Allele</td>
<td>I</td>
<td>D</td>
<td>M</td>
</tr>
<tr>
<td>N (%)</td>
<td>47 (39.2)</td>
<td>73 (60.8)</td>
<td>71 (59.2)</td>
</tr>
<tr>
<td></td>
<td>49 (40.8)</td>
<td>78 (65)</td>
<td>42 (35)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Table 3. Genotype Distribution among Hypertensive Patients With and Without Cerebral WML

<table>
<thead>
<tr>
<th>Polymorphism</th>
<th>Without WML (n=35)</th>
<th>With WML (n=25)</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACE I/D</td>
<td>II</td>
<td>DI</td>
<td>DD</td>
</tr>
<tr>
<td></td>
<td>9 (25.7)</td>
<td>16 (45.7)</td>
<td>10 (28.6)</td>
</tr>
<tr>
<td></td>
<td>4 (16.0)</td>
<td>5 (20.0)</td>
<td>16 (64.0)</td>
</tr>
<tr>
<td>AGT M235T</td>
<td>MM</td>
<td>MT</td>
<td>TT</td>
</tr>
<tr>
<td></td>
<td>10 (28.6)</td>
<td>20 (57.1)</td>
<td>5 (14.3)</td>
</tr>
<tr>
<td></td>
<td>9 (36.0)</td>
<td>13 (52.0)</td>
<td>3 (12.0)</td>
</tr>
<tr>
<td>AT1, A1166C</td>
<td>AA</td>
<td>AC</td>
<td>CC</td>
</tr>
<tr>
<td></td>
<td>16 (45.7)</td>
<td>16 (45.7)</td>
<td>3 (8.6)</td>
</tr>
<tr>
<td></td>
<td>10 (40.0)</td>
<td>10 (40.0)</td>
<td>5 (20.0)</td>
</tr>
</tbody>
</table>

Values are n (%).
resresents an early marker of cerebrovascular disease, and it has been considered a risk factor for the development of stroke.\textsuperscript{3,5} Thus, the DD genotype, possibly through an increased ACE activity and angiotensin II formation, can lead to a high susceptibility of cerebrovascular disease and WML development.

The association between ACE gene polymorphism and cerebrovascular disease has been previously investigated. A meta-analysis by Sharma\textsuperscript{33} showed a moderate increase in the risk of stroke associated with the D allele under a recessive model of inheritance. Kario et al\textsuperscript{34} reported an association between the ACE deletion allele and silent lacunar infarct in Japanese hypertensive patients. The relationship between ACE gene polymorphism and the presence of WML has been evaluated in a study\textsuperscript{22} of 182 elderly patients with dementia or cognitive impairment, in which homozygosity for ACE gene deletion was significantly associated with WML only in patients with a previous cerebral infarct. The present study found a relationship between the presence of silent WML and the DD genotype and/or the D allele of the ACE gene in asymptomatic middle-aged hypertensive patients. Although BP did not differ significantly among ACE genotypes, patients with WML had higher BP values than those without, supporting previous studies showing an association between BP values and WML\textsuperscript{1} and the idea that, in addition to the severity of BP values, the development of WML is determined by genetic factors.

We found no relationship between the other 2 polymorphisms studied (the M235T AGT gene and the A1166C AT\textsubscript{1} receptor gene polymorphisms) and the presence of WML. With regard to the AGT gene, 2 studies\textsuperscript{20,35} evaluated the relationship between the M235T AGT gene polymorphism and stroke, with negative results. In contrast, Schmidt et al\textsuperscript{24} found an association between the TT genotype of the AGT gene and the presence of WML in 431 asymptomatic individuals (aged 50 to 75 years; 33\% were hypertensive). Our results in hypertensive patients did not find this association, possibly because of different sample characteristics. The subjects included in the study by Schmidt et al\textsuperscript{24} were older, and 37\% had a history of heart disease, which could influence the presence of WML.\textsuperscript{4} To our knowledge, no published studies have examined the relation between A1166C AT\textsubscript{1} receptor gene polymorphism and WML. We also examined a possible synergistic effect of these 3 polymorphisms on the presence of silent WML. The addition of AGT gene polymorphism or AT\textsubscript{1} receptor gene polymorphism or both did not significantly affect the relation between ACE gene polymorphism and the presence of WML. One study\textsuperscript{20} reported a positive M235T TT genotype interaction between ACE DD genotype and stroke, but no other studies have examined possible synergistic effect of these 3 polymorphisms on the presence of silent WML.

In conclusion, the presence of silent cerebral WML is associated with the I/D polymorphism of the ACE gene in middle-aged untreated essential hypertensive patients, suggesting that the presence of the DD genotype and/or the D allele of the ACE gene may be a predisposing factor for developing WML in essential hypertension. Neither the M235T AGT gene nor the A1166C AT\textsubscript{1} receptor gene is related to WML. In addition, we found no synergistic effect of these 3 polymorphisms and the presence of silent WML.

Acknowledgment

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