Renin-Angiotensin System Genetic Polymorphisms and Salt Sensitivity in Essential Hypertension

Vicente Giner, Esteban Poch, Ernesto Bragulat, Josep Oriola, Daniel González, Antonio Coca, Alejandro de la Sierra

Abstract—We evaluated the association between salt-sensitive hypertension and 3 different genetic polymorphisms of the renin-angiotensin system. Fifty patients with essential hypertension were classified as salt sensitive or salt resistant, depending on the presence or absence of a significant increase (P<0.05) in 24-hour ambulatory mean blood pressure (BP) after high salt intake. 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 were determined with the use of standard polymerase chain reaction methods. Twenty-four (48%) patients with significantly increased (P<0.05) 24-hour mean BP with high salt intake (from 107.3±9.4 to 114.8±10.6 mm Hg) were classified as salt sensitive. In the remaining 26 patients (52%), high salt intake did not significantly modify 24-hour mean BP (from 107.6±10 to 107.8±9 mm Hg), and they were classified as having salt-resistant hypertension. We did not find any significant association between either M235T AGT or A1166C AT1 receptor genotypes and the BP response to high salt intake. However, patients with essential hypertension homozygous for the insertion allele of the ACE gene (II) had a significantly higher BP increase with high salt intake (9.8±8.1 mm Hg for systolic BP and 5.2±4.2 mm Hg for diastolic BP) than that observed in patients homozygous for the deletion allele (DD) (1.2±5.9 mm Hg for systolic BP; P=0.0118 and −0.2±4.2 mm Hg for diastolic BP; P=0.0274). Heterozygous patients (ID) exhibited an intermediate response. The prevalence of salt-sensitive hypertension also was significantly higher (P=0.012) in II (67%) and DI patients (62%) compared with DD hypertensives (19%). We conclude that a significant association exists between the I/D polymorphism of the ACE gene and salt-sensitive hypertension. Patients with II and DI genotypes have significantly higher prevalence of salt sensitivity than DD hypertensives. (Hypertension. 2000;35[part 2]:512-517.)

Key Words: angiotensin-converting enzyme ▪ genes ▪ angiotensin II ▪ blood pressure monitoring, ambulatory

The blood pressure (BP) response to increased dietary salt is heterogeneous among individuals, a phenomenon known as salt sensitivity. Normotensive and hypertensive salt-sensitive subjects tend to exhibit familial history of hypertension more frequently than salt-resistant subjects.1,2 This suggests the existence of genetic determinants that influence BP sensitivity to sodium chloride. More than 10 years ago, Weinberger et al3 reported a significant relation between haptoglobin 1-1 phenotype and BP response to intravenous sodium overload both in normotensive and hypertensive subjects.

The renin-angiotensin system (RAS) has a central role in controlling BP and sodium homeostasis.4 In the last decade, several authors have investigated RAS polymorphisms as genetic determinants of essential hypertension and end-organ damage. Although the results obtained are controversial, the presence of the D allele in intron 16 of the angiotensin-converting enzyme (ACE) gene appears to be associated with a higher risk for development of both macrovascular and microvascular disease in hypertensive individuals.5 Moreover, the presence of the T allele in exon 2 of the angiotensinogen (AGT) gene appears to be associated with a higher risk for development of hypertension.6 Finally, the association of the T allele of the AGT gene and the D allele of the ACE gene has a synergistic effect on the incidence of cerebrovascular disease,7 and the association of the D allele of the ACE gene and the C allele of angiotensin II type 1 (AT1) receptor gene appears to increase the risk of myocardial infarction.8

The RAS also is implicated in the BP response to salt intake. Low-renin hypertensives show an increased BP response to NaCl load,9 and salt-sensitive individuals exhibit a blunted response of the RAS when they switch from low to high salt intake compared with salt-resistant subjects.10 Moreover, plasma levels of ACE and angiotensinogen differ in subjects with different ACE and angiotensinogen genotypes.11,12

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The relation between ACE gene polymorphism and salt sensitivity has been tested in 2 Japanese hypertensive groups, with controversial results. In 1994, Kojima et al\textsuperscript{13} showed no relation between the insertion/deletion (I/D) ACE gene polymorphism and salt sensitivity (diagnosed by an oral test) in 75 Japanese patients with essential hypertension. However, Hiraga et al\textsuperscript{14} found that patients with the insertion allele were more sensitive to the pressor effect of increased salt intake.

In the present study we have analyzed the relation between salt sensitivity and the 3 main reported RAS polymorphisms: the I/D ACE gene, the M235T AGT gene, and the A1166C AT1 receptor gene polymorphisms in a group of patients with essential hypertension.

### Methods

#### Patient Selection

Fifty outpatients with essential hypertension were consecutively recruited from the Hypertension Clinic of the Hospital Clinic, Barcelona (Spain). The diagnosis of essential hypertension was considered on the basis that no known cause of high BP could be detected after complete clinical, biochemical, and radiologic examination. None of the patients included had renal impairment, papilledema, cardiac, coronary, or cerebrovascular diseases, diabetes, or pregnancy. All the patients had at least 3 office BP measurements >140/90 mm Hg after 4 weeks of an unrestricted salt diet and without antihypertensive medication. “White coat” hypertension was ruled out by ambulatory BP monitoring (ABPM). Written informed consent was obtained from all the participants.

#### Diagnosis of Salt-Sensitive Hypertension

A low-salt diet containing 50 mmol sodium daily was administered to all participants for 14 days. The diet was designed by the hospital dietary department and contained 62 g of protein, 234 g of carbohydrate, 108 g of fat, 60 mmol of potassium, and 20 mmol of calcium per day. The amount of calories remained constant for the whole study period, with slight individual modifications for individual needs. This baseline diet was supplemented in a single-blind fashion by placebo tablets during the first period of 7 days (low-salt period) and by 12 tablets containing 17.8 mmol of sodium each, for the second period of seven days (high-salt period), to achieve a total NaCl intake of 260 mmol daily. Compliance with diet was assessed by measuring the 24-hour urinary Na\textsuperscript+ excretion on the last day of each period.

On the last day of each period, 24-hour ABPM was carried out with the use of an automated, noninvasive oscillometric device (Spacelabs 90207, Space Labs Inc.). The appropriate cuff was placed on the nondominant arm, and BP was registered automatically at 15-minute intervals for a 24-hour period. Mean values and standard deviations of systolic, mean, and diastolic BP and heart rate were obtained from each record in the 24-hour period.

Unedited records obtained from ABPM carried out during either low-salt and high-salt periods were individually analyzed by means of BMDP Statistical Software. The normal distribution of 24-hour BP values for individual records was tested by the Shapiro–Francia test. If both records of the same subject were normally distributed, they were compared by an independent Student’s \textit{t} test. If one or both records of the same subject were not normally distributed, they were compared by the nonparametric Mann-Whitney test.

The BP change in the whole group of patients with essential hypertension studied followed a gaussian distribution, ranging from a decrease of 13 mm Hg to an increase of 19 mm Hg. There was not a clear cutoff in the BP change that allowed us to define patients as having salt-sensitive or salt-resistant hypertension. Thus the selection of a minimal increase in 24-hour BP would have been completely arbitrary. To eliminate this arbitrariness, we chose the mathematical criterion of the statistical significance of the variation. Thus salt sensitivity was diagnosed when the increase in 24-hour mean BP from the low-salt period to the high-salt period was statistically significant (\textit{P}<0.05).\textsuperscript{18} It is important to note that all subjects classified as salt sensitive presented an increase of at least 4 mm Hg in 24-hour mean BP.

#### RAS Polymorphism Determination

Samples for DNA analysis were obtained from frozen peripheral leukocytes as previously described.\textsuperscript{16} The I/D polymorphism of the ACE gene was assessed by detecting the presence (allele \textit{I}, insertion) or absence (allele \textit{D}, deletion) of a 287-bp fragment in exon 2 of the ACE gene in chromosome 17 with the polymerase chain reaction (PCR) technique and agar electrophoresis as described by Rigat et al.\textsuperscript{11} Because the \textit{D} allele is preferentially amplified in heterozygous subjects,\textsuperscript{17} each sample found to be \textit{DD} was reanalyzed by a second independent PCR amplification with a primer that specifically recognizes an insertion-specific sequence.\textsuperscript{18} Depending on the genotype, subjects were classified as \textit{DD}, \textit{DI}, or \textit{II}.

The M235T polymorphism of the angiotensinogen gene was determined by PCR amplification of a 303-bp fragment in exon 2 of the angiotensinogen gene. After that, a restriction-endonuclease digestion with the \textit{SfiI} enzyme was performed. In the presence of coding ATG, the enzyme produces a 266-bp fragment corresponding with the M allele. When the fragment is not digested, the allele identified is \textit{T}.\textsuperscript{19} Depending on the presence of alleles, subjects were classified as \textit{MM}, \textit{MT}, or \textit{TT}.

The A1166C polymorphism of the AT1 receptor was determined by PCR. The mutation (\textit{C}) creates a restriction for the enzyme \textit{DdeI}. The undigested wild-type (\textit{A}) allele was visible as a fragment of 519 bp and the digested \textit{C} allele occurred with 2 bands at 134 and 384 bp.\textsuperscript{20} Patients were classified as \textit{AA}, \textit{AC}, or \textit{CC}, depending on their genotype.

#### Statistical Analysis

Allele frequencies were estimated by the gene-counting method, and Hardy-Weinberg equilibrium was tested by the \textit{\chi}\textsuperscript{2} test. One-way ANOVA was used to compare means among different genotype groups. The association of salt sensitivity with genotype distribution was tested by the \textit{\chi}\textsuperscript{2} test. The relation of the \textit{I/D} alleles with salt sensitivity also was tested, considering a dominant effect of the insertion allele (\textit{II}+\textit{DI} patients compared with \textit{DD}), and a recessive effect for the same allele (\textit{II} patients compared with \textit{DI}+\textit{DD}).\textsuperscript{18} Odds ratios for these comparisons were calculated.

Values are expressed as mean±SD, and a value of \textit{P}<0.05 was considered statistically significant.

#### Results

#### Diagnosis of Salt-Sensitive Hypertension

Salt-sensitive hypertension was diagnosed in 24 (48%) patients with the use of the aforementioned criteria. Their mean increase in 24-hour systolic and diastolic BP was 10.2±5.6 mm Hg and 5.5±4.8 mm Hg, respectively (Table 1). Conversely, 26 (52%) patients with essential hypertension were considered as having salt-resistant hypertension. The BP increase with high-salt intake in this group was 0.3±5.9 mm Hg for systolic BP and ~0.6±4.1 mm Hg for diastolic BP. Table 2 shows baseline characteristics of patients with salt-sensitive and salt-resistant essential hypertension. No differences were observed in terms of age, gender, body mass index, renal function, or baseline 24-hour BP.

#### Allele and Genotype Frequencies

The genotype distribution and derived allele frequencies are shown in Table 3. When the obtained genotype frequencies

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for the I/D ACE gene (0.327, 0.429, and 0.245 for DD, DI, and II, respectively), M235T AGT gene (0.422, 0.444, and 0.133 for MM, MT, and TT, respectively), and A1166C AT1 receptor gene (0.522, 0.413, and 0.065 for AA, AC, and CC, respectively) were compared with the expected frequencies predicted by the Hardy-Weinberg equilibrium, both ACE I/D polymorphism and A1166C AT1 receptor polymorphism exhibited a nonsignificant difference \((P=0.9211 \text{ for I/D polymorphism and } P=0.0873 \text{ for A1166C polymorphism})\), whereas M235T AGT genotype frequencies deviated slightly from the Hardy-Weinberg equilibrium \((P=0.0409)\). The D allele (0.540), M allele (0.645), and A allele (0.728) were the most frequently observed.

### Salt Sensitivity and RAS Polymorphisms

We found no relation between AGT M235T polymorphism or AT1 receptor A1166C polymorphism and BP response to increased salt intake (Table 4). Conversely, the BP elevation induced by high salt intake was significantly different, depending on the I/D ACE genotype. Table 4 shows the systolic and diastolic BP increases after high salt intake with respect to different RAS genotypes. As can be seen, the increase in 24-hour BP in patients homozygous for the insertion allele \((II)\) was 9.8±8.1 mm Hg for systolic BP and 5.2±4.2 mm Hg for diastolic BP, significantly higher \((P=0.0118 \text{ and } P=0.0274, \text{ respectively})\) than that observed for patients homozygous for the deletion allele \((DD)\) (1.2±5.9 mm Hg for systolic BP and −0.2±4.2 mm Hg for diastolic BP). Heterozygous patients \((ID)\) exhibited an intermediate response (5.0±7.3 mm Hg for systolic BP and 2.4±6.1 mm Hg for diastolic BP) (Table 4). The prevalence of salt-sensitive hypertension also was significantly different, depending on ACE genotype (18.75% for DD patients, 61.9% for DI patients, and 66.7% for II hypertensives; \(P=0.012\)).

### Discussion

The present study shows an association between the insertion/deletion polymorphism of the ACE gene and salt sensitivity in essential hypertension. Patients with essential hypertension homozygous for the insertion allele \((II)\) present a significantly higher BP response to high salt intake compared with patients homozygous for the deletion allele \((DD)\). Heterozygous hypertensives show an intermediate response. Moreover, the prevalence of salt-sensitive hypertension in II patients were compared with DI+DD pooled together (recessive effect for the insertion allele), the relative frequencies of salt-sensitive hypertension were not significantly different (67% vs 43%; \(P=0.158\)).
of these 2 polymorphisms on the association between ACE gene I/D polymorphism and salt-sensitive hypertension.

Essential hypertension has a well-known familial aggregation and has been calculated to be $\approx 40\%$ genetically determined.\(^21\) In the last decade, the development of PCR methodology has made possible the study of several genetic determinants of essential hypertension. The RAS has a central role in the regulation of BP and sodium homeostasis,\(^4\) and genes that are involved in the activity of this enzyme cascade are potential candidate genes for essential hypertension or some associated clinical features. Two recent meta-analyses have evaluated the relation of both I/D polymorphism of the ACE gene\(^5\) and M235T angiotensinogen gene polymorphism\(^22\) with essential hypertension and cardiovascular diseases. ACE I/D gene polymorphism is not associated with hypertension, but individuals homozygous for the deletion allele appear to have an increased risk of macrovascular and microvascular complications.\(^8\) However, the T allele encoding angiotensinogen appears to be a marker for hypertension, at least in white subjects.\(^23\)

The phenomenon of salt sensitivity, present in $\approx 50\%$ of patients with essential hypertension, may have some familial influence.\(^23,24\) In fact, the BP response to salt overload, an indirect measure of salt sensitivity, has been reported to be increased in normotensive sons of hypertensive parents.\(^24\) However, another study showed negative results in normotensive sons of subjects with high and low BPs with 4 weeks of low and high salt intakes.\(^25\) One of the problems regarding the assessment of salt sensitivity is a lack of uniform criteria. Different approaches with saline overload and low- and high-salt diets in ascending or random order have been used (see Reference 26 for review). It has been suggested that the physiological adaptation when subjects switch from low to high salt intake may differ from that observed when they switch from high to low salt intake.\(^26\) Moreover, it has been demonstrated that ABPM has minimal or no placebo effect, and it is important to note that the analysis of all ABPM records was performed by the same investigator, who did not participate in the clinical care of patients. Taking into account these considerations, we believe that the order of diets did not affect the diagnosis of salt-sensitive hypertension.

The pathophysiological mechanisms related to salt-sensitive essential hypertension are not completely understood. However, the RAS has a pivotal role.\(^9,10\) Salt-sensitive hypertensive patients tend to have low renin values.\(^9\) Moreover, the RAS response to high salt intake is blunted in salt-sensitive subjects and is inversely correlated with the BP response.\(^10\) Thus it is logical to suppose that salt-sensitive hypertensive patients may differ in genotypes encoding the different steps of the RAS cascade.

The relation of salt-sensitive hypertension and I/D ACE gene polymorphism has been previously evaluated in 2 Japanese studies.\(^13,14\) Kojima et al.\(^13\) reported in 1994 a lack of association between the ACE I/D genotype and salt sensitivity in 75 Japanese patients with essential hypertension. Conversely, Hiraga et al.\(^14\) studying another 66 Japanese patients with essential hypertension, reported a significant association between salt sensitivity and the presence of the insertion allele of the ACE gene. Our results in white patients with hypertension confirm those obtained by Hiraga et al.\(^14\) in Japanese patients. We have observed that patients with $II$ or $DI$ genotypes have a significantly higher prevalence of salt sensitivity (67% and 62%, respectively) than hypertensives with the $DD$ genotype (19%). Furthermore, BP response to high salt intake has been found to be significantly higher in $II$ patients compared with $DD$ hypertensives. Heterozygous

### TABLE 3. Genotype Distribution and Derived Allele Frequencies

<table>
<thead>
<tr>
<th>Polymorphism</th>
<th>I/D ACE Gene ($n=49$)</th>
<th>M235T AGT Gene ($n=45$)</th>
<th>A1166C AT1 Receptor Gene ($n=46$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotype</td>
<td>DD (n=16)</td>
<td>DI (n=20)</td>
<td>MT (n=20)</td>
</tr>
<tr>
<td></td>
<td>16 (32.7)</td>
<td>21 (42.9)</td>
<td>20 (44.4)</td>
</tr>
<tr>
<td></td>
<td>DI (n=20)</td>
<td>II (n=12)</td>
<td>TT (n=6)</td>
</tr>
<tr>
<td></td>
<td>12 (24.5)</td>
<td>9.8 ± 8.1</td>
<td>3.8 ± 5.9</td>
</tr>
<tr>
<td></td>
<td>19 (42.2)</td>
<td>5.9 ± 3.5</td>
<td>6.5 ± 3.5</td>
</tr>
<tr>
<td></td>
<td>20 (44.4)</td>
<td>5.1 ± 6.4</td>
<td>5.1 ± 5.1</td>
</tr>
<tr>
<td>Allele</td>
<td>D (n=45)</td>
<td>I (n=45.9)</td>
<td>M (n=35.5)</td>
</tr>
<tr>
<td></td>
<td>53 (54.1)</td>
<td>45 (45.9)</td>
<td>32 (35.5)</td>
</tr>
<tr>
<td></td>
<td>58 (64.5)</td>
<td>4.5 ± 8.9</td>
<td>6.5 ± 3.8</td>
</tr>
<tr>
<td></td>
<td>67 (72.8)</td>
<td>25 (27.2)</td>
<td>0.9371</td>
</tr>
<tr>
<td></td>
<td>24 (52.2)</td>
<td>19 (41.3)</td>
<td>0.6231</td>
</tr>
</tbody>
</table>

### TABLE 4. Blood Pressure Response to High Salt Intake With Respect to Different Genotypes

<table>
<thead>
<tr>
<th>Genotype</th>
<th>24-H Systolic BP Increase, mm Hg</th>
<th>24-H Diastolic BP Increase, mm Hg</th>
</tr>
</thead>
<tbody>
<tr>
<td>I/D ACE gene polymorphism</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DD (n=16)</td>
<td>1.2 ± 5.9</td>
<td>-0.2 ± 4.2</td>
</tr>
<tr>
<td>DI (n=20)</td>
<td>5.0 ± 7.3</td>
<td>2.4 ± 6.1</td>
</tr>
<tr>
<td>II (n=12)</td>
<td>9.8 ± 8.1</td>
<td>5.2 ± 4.2</td>
</tr>
<tr>
<td>ANOVA P value</td>
<td>0.0118</td>
<td>0.0274</td>
</tr>
<tr>
<td>M235T AGT gene polymorphism</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MM (n=19)</td>
<td>5.8 ± 9.6</td>
<td>2.9 ± 6.4</td>
</tr>
<tr>
<td>MT (n=20)</td>
<td>3.9 ± 6.6</td>
<td>1.1 ± 4.6</td>
</tr>
<tr>
<td>TT (n=6)</td>
<td>3.8 ± 5.9</td>
<td>3.5 ± 5.1</td>
</tr>
<tr>
<td>ANOVA P value</td>
<td>0.7542</td>
<td>0.4804</td>
</tr>
<tr>
<td>A1166C AT1 receptor gene polymorphism</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AA (n=24)</td>
<td>4.5 ± 8.9</td>
<td>2.9 ± 6.5</td>
</tr>
<tr>
<td>AC (n=19)</td>
<td>5.0 ± 7.0</td>
<td>1.2 ± 4.2</td>
</tr>
<tr>
<td>CC (n=3)</td>
<td>6.1 ± 2.9</td>
<td>1.7 ± 3.8</td>
</tr>
<tr>
<td>ANOVA P value</td>
<td>0.9371</td>
<td>0.6231</td>
</tr>
</tbody>
</table>
subjects exhibit an intermediate response. The analysis of the mode of inheritance suggests that the effect is dominant for the insertion allele regarding the prevalences of salt-sensitive hypertension in II and DI patients, significantly higher than that observed in DD hypertensives. Differences between both the present study and Hiraga and colleagues' study, compared with the negative results obtained by Kojima et al, may be related to the protocol used for the assessment of salt sensitivity, which in the study by Kojima et al was based on the BP response to salt restriction and the fact that antihypertensive medication was stopped only 10 days before the beginning of the protocol.

The relation of the insertion allele of the ACE gene with salt sensitivity does not agree with previous observations that related both salt sensitivity and DD genotype with a higher cardiovascular risk. We do not have a complete explanation for this apparent contradiction. The presence of salt sensitivity has been associated with an increased left ventricular mass, worse lipid profile, more insulin resistance, and microalbuminuria. Moreover, a recent study reports an increased rate of cardiovascular events in Japanese salt-sensitive patients. Conversely, the relation of the DD genotype of the ACE gene with cardiovascular complications is controversial. Although the article by Staessen et al suggests an association with macrovascular and microvascular complications, the same authors recommend a cautious interpretation of this meta-analysis because of a bias of publication of negative studies. In fact, it has been clearly demonstrated that the DD genotype is not associated with hypertension nor with left ventricular hypertrophy.

We have found no relation between the 2 other polymorphisms studied (the M235T angiotensinogen gene and the A1166C AT1 receptor gene polymorphisms) and salt sensitivity. Two recent studies have been published examining the AGT gene and BP response to sodium intake. Hunt et al reported that patients homozygous for the M allele had a lesser decrease in BP after mild sodium restriction than hypertensives with TT or MT genotypes. Conversely, Schorr et al found no relation between salt sensitivity and AGT genotype in a detailed study including 200 healthy subjects. To our knowledge, no other studies have been published up to now examining the relation between A1166C AT1 receptor gene polymorphism and salt sensitivity.

Finally, we have examined a possible synergistic effect of these 3 polymorphisms on salt sensitivity similar to those reported for coronary and cerebrovascular diseases. The addition of AGT gene polymorphism or AT1 receptor gene polymorphism or both did not significantly affect the relation between ACE gene polymorphism and salt sensitivity.

In conclusion, salt-sensitive essential hypertension is associated with the insertion/deletion polymorphism of the ACE gene. This observation strengthens the hypothesis that salt sensitivity is partially determined by genetic predisposition. This association may have clinical implications because of the relatively low (<20%) prevalence of salt sensitivity in patients with essential hypertension homozygous for the D allele.

References


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