Genetic Determinants of Nonmodulating Hypertension

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Abstract—We sought to determine whether genes of the renin-angiotensin-aldosterone system can predict the nonmodulating intermediate phenotype in essential hypertension. Aldosterone responses to angiotensin II were assessed in 298 subjects with hypertension. Subjects were genotyped at the angiotensinogen M235T, angiotensin-converting enzyme I/D, aldosterone synthase C−344 T, renin, angiotensin II type 1 receptor, and adducin loci. The data were analyzed by Student t test, ANOVA, stepwise linear regression and general linear model or GENMOD regression techniques, and χ² analysis odds ratios (ORs). Aldosterone response varied by genotype for angiotensin and aldosterone synthase but not for the other loci. The combination of angiotensinogen 235 TT and angiotensin-converting enzyme DD showed further reduction (P=0.0377) when compared with angiotensinogen 235 TT alone, an example of genetic epistasis. When the subject was required also to possess the CYP11B2 −344 TT genotype, there was a further substantial reduction. Of these 3 loci, only angiotensinogen 235 TT significantly increased the OR of predicting the nonmodulating hypertensive phenotype (OR, 2.00; 95% confidence interval, 1.152 to 3.51). However, when angiotensin-converting enzyme DD was combined with angiotensinogen 235 TT, the OR nearly doubled to 3.74, with a further increase to 5.36-fold when the subject possessed all 3 genotypes. Thus, the angiotensinogen, angiotensin-converting enzyme, and aldosterone synthase genotypes identified individuals with the nonmodulating phenotype with an increasing degree of fidelity. For this subclass of essential hypertension, it is likely that genotyping can be substituted for complex phenotyping for therapeutic and preventive decision making. (Hypertension. 2003;42:901-908.)

Key Words: hypertension, genetic ■ hypertension, nonmodulating ■ aldosterone ■ genetics ■ gene expression

For the past decade, intensive efforts have focused on identifying genes involved in the pathogenesis of human hypertension. However, these efforts, which have used classic linkage analysis, have been hampered by the complex regulation of arterial pressure, the presence of multiple “susceptibility” genes, and the multifactorial nature of hypertension. An alternative approach is based on the assumption that hypertension is not a disease but a syndrome. Thus, by subdividing the hypertensive population into more homogeneous genetic subgroups, intermediate phenotypes, there will be an increased probability that only a limited number of genes will be involved.1 Such an intermediate phenotype involves nonmodulation (NM) of adrenal and renal vascular responses to stimulation by angiotensin II (Ang II).2 As initially described nearly 20 years ago, these patients had 2 principal characteristics: a reduced renal blood flow response to Ang II infusion when studied during a period of high sodium intake and a reduced aldosterone (ALDO) response to Ang II infusion during low salt intake.3 In a study primarily comprising normotensive subjects, we have previously reported that a single-nucleotide polymorphism in the coding region (cSNP) of the angiotensinogen (AGT) gene is associated with a decreased renal blood flow response to Ang II, suggesting that this gene might be associated with NM hypertension.4 Recently, we extended this observation in 3 ways: (1) a similar but stronger association is present in subjects with hypertension; (2) the same relation exists between the AGT polymorphism and renal blood flow responsiveness in hypertensive subjects with a different SNP in the regulatory region of the gene; and (3) we documented that in the same subject, the polymorphism in this same SNP is also associated with decreased ALDO responsiveness to Ang II.5 Thus, we assumed that the genes of the renin-angiotensin-ALDO system could be potential candidates for genetic determinants of NM. We therefore assessed the role of polymorphism in several of these genes (renin, AGT, angiotensin-converting enzyme [ACE], angiotensin receptor type 1...
[\(\text{AT}_1\)], and ALDO synthase [CYP11B2]) and as a control, the adducin gene, which is not associated with this pathway. We first assessed the effect of these genes on ALDO responses to Ang II as a quantitative trait. Then we determined the likelihood that genetic variants of genes that had a significant effect could identify the NM intermediate phenotype.

### Methods

#### Study Subjects

Data obtained for this report came from subjects studied by the international HyperPath (Hypertension Pathotypes) group. Details of the protocol and study design used by this group have been reported previously. In brief, the group consists of investigators at several sites in the United States and Europe. They studied hypertensive and normotensive subjects on a precise protocol designed to control environmental factors so as to identify intermediate phenotypes for hypertension. The database used by these investigators is continually expanding. Some of the subjects are part of a sibling pair or triplet, whereas others are singletons. Although data from some of these patients have been published, we have previously analyzed the data reported herein for all studies reported here.

Data from 298 hypertensive subjects, 101 singletons and 197 individuals who belonged to 101 pedigrees, form the basis of this report. These subjects were selected from the data set because they had the following 4 items available for analysis: (1) plasma ALDO increment in response to a 3 ng · kg\(^{-1}\) · min\(^{-1}\) infusion of Ang II on a low-sodium diet, defined as a 24-hour sodium excretion of <30 mEq/d; (2) AGT M 235 T genotype; (3) ACE ID genotype; and (4) CYP11B2 T–344 C genotype. Most also had renin intron 4, adducin G460W, and AT\(_1\) receptor A1116C genotypes. Patients were drawn from the following sites: Brigham and Women’s Hospital in Boston (n = 120), the University of Utah in Salt Lake City (n = 54), Hospital Broussais in Paris (n = 102), and the University La Sapienza in Rome (n = 22). The protocol was approved by the Institutional Review Board at each site, and informed, written consent was obtained from each subject. There were no site-associated effects related to the studies reported herein.

#### Protocol

In most subjects, antihypertensive therapy was discontinued between 2 and 4 weeks before the study began. When the patient had been taking an ACE inhibitor, it was discontinued 3 months before the study. When antihypertensive therapy was needed in the ensuing 2 to 2.5 months, an antihypertensive, non–ACE inhibitor drug was administered. Each subject was admitted to a Clinical Research Center and placed on a constant isocaloric diet containing 10 mmol sodium, 100 mmol potassium, 800 mg calcium, and 2000 to 2500 mL fluid. Some subjects began their low-salt diet as outpatients. Daily 24-hour urine collections were obtained for measurement of sodium, potassium, and creatinine to determine external balance. The primary analysis used was the general linear model (GLM) procedure (SAS 6.12, SAS Institute) with statistical comparisons by least-squares means. The effect of sib pairs in the data set was assessed with the GENMOD procedure, also in the SAS statistical package. The reliability of the allele distribution for each gene was assessed with the Hardy-Weinberg equilibrium test. Conditions for statistical analysis were as follows: \(p\) for significance was 0.05 or less. To ensure the overall protection level, only probabilities associated with preplanned comparisons were used in the GLM procedure.

#### Laboratory Procedures

Blood samples were drawn from an intravenous catheter located in the arm opposite the infused arm. Samples were placed on ice and immediately centrifuged. Serum or plasma and urine samples were stored at \(-20^\circ\text{C}\) until assay. Serum and urine sodium and potassium levels were measured by flame photometry. PRA and ALDO levels were measured by radioimmunoassay techniques, as previously described. DNA was obtained from stored leukocytes. As previously described, single-nucleotide polymorphisms (SNPs) were determined by either polymerase chain reaction (PCR) and restriction enzymes or PCR and allele-specific oligonucleotide probe techniques in the following genes: AGT at codon 235, CYP11B2 at –344, adducin at 460, renin at 387, and AT\(_1\) at 1116. An insertion-deletion (ID) polymorphism at intron 16 of the ACE gene was determined by nested PCR, as described by Rigat et al.

#### Phenotype Classification

Subjects were classified as low-renin hypertensive when their PRA response to upright posture was \(<2.4 \text{ ng} \cdot \text{mL}^{-1} \cdot \text{h}^{-1}\). The non–low renin subjects were further classified as modulators or NMs based on their ALDO response to Ang II. An increment \(<15 \text{ ng/dL}\) defined the NM phenotype.

#### Statistics

The primary analysis used was the general linear model (GLM) procedure (SAS 6.12, SAS Institute) with statistical comparisons by least-squares means. The effect of sib pairs in the data set was assessed with the GENMOD procedure, also in the SAS statistical package. The reliability of the allele distribution for each gene was assessed with the Hardy-Weinberg equilibrium test. Conditions for statistical analysis were as follows: \(p\) for significance was 0.05 or less. To ensure the overall protection level, only probabilities associated with preplanned comparisons were used in the GLM procedure.

#### Results

**Effect of Polymorphisms in Genes of the Renin-Angiotensin-ALDO System on ALDO Responses to Ang II**

For polymorphisms in each of the 6 genes tested, the allele frequencies satisfied the Hardy-Weinberg equilibrium law. Two genes that can modify Ang II production had contrasting effects on ALDO responses to infused Ang II. For the ACE gene, neither homozygotes for either allele nor heterozygotes differed in their responses (\(I D, 23.5 \pm 2.1 \text{ ng/dL}\); \(I D, 22.1 \pm 1.6 \text{ ng/dL}\); and \(D D, 24.2 \pm 2.6 \text{ ng/dL}\)). In contrast, among the AGT 235 genotypes, a significant difference was observed (\(M M, 24.2 \pm 2.1 \text{ ng/dL}\); \(M T, 22.8 \pm 1.2 \text{ ng/dL}\); and \(T T, 19.2 \pm 2.2 \text{ ng/dL}\); \(P = 0.016\), ANOVA; Figure 1, left). The effect of the CYP11B2 –344 genotype on response was also significant, but only marginally (\(C C, 23.8 \pm 2.2 \text{ ng/dL}\); \(C T, 23.6 \pm 1.5 \text{ ng/dL}\); and \(T T, 20.1 \pm 1.9 \text{ ng/dL}\); \(P = 0.042\). Poly-
morphisms in the renin, adducin, and AT1 genes had no effect on ALDO responses to Ang II and therefore, were not further assessed.

Principally, the significant effect among AGT genotypes was driven by the homozygous TT. There were no differences in ALDO responses to Ang II in heterozygotes when compared with homozygotes for the M allele. Thus, in our modeling procedures, the MM + MT genotypes are contrasted with the TT AGT genotype. For consistency, the same approach was used for the other 2 genotypes: the combined ACE II/DD response was contrasted with the ACE D/D response, and the combined CYP11B2 −344 CC + CT response was contrasted with the CYP11B2 TT response. With this approach, AGT 235 TT was associated with a significant (P=0.004) reduction in ALDO response to Ang II when compared with the rest of the hypertensive patients (Figure 1, right). In contrast, the ACE D/D genotype was not associated with such a change (P=0.775; Figure 2, left). CYP11B2 −344 TT subjects also had significantly (P=0.0275) reduced ALDO responses, although these were less than in subjects with AGT 235 TT (Figure 2, right).

The data were then reanalyzed to determine the effect on ALDO responses to Ang II when 2 or more genotypes were combined. The combinations that produced significantly increased effects over the single genes were the AGT 235 TT plus ACE DD combination (Figure 3, middle) and the triple combination by adding CYP11B2 −344 TT to the other 2 (Figure 3, right).

### Comparison of Clinical Characteristics by AGT Genotype

Because from the aforementioned analysis the AGT genotype greatly influenced ALDO responses to Ang II, we performed an assessment of the clinical characteristics of our population divided by AGT genotype (Table 1). There were no significant differences for any of the following parameters: age, gender, body mass index (BMI), serum potassium, urine sodium and potassium, basal serum ALDO, PRA, or diastolic blood pressure. The only statistically different parameter was a slightly higher systolic blood pressure in the AGT 235 TT group compared with the MM + MT group (P<0.031). Blood

### TABLE 1. Clinical and Study Parameters of Subjects During Low-Salt Intake

<table>
<thead>
<tr>
<th>Variable</th>
<th>AGT M 235 T Genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MM and MT</td>
</tr>
<tr>
<td>n (N=298)</td>
<td>205</td>
</tr>
<tr>
<td>Male/female, n/n</td>
<td>141/92</td>
</tr>
<tr>
<td>Age, y</td>
<td>47.5±0.6</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>27.3±0.2</td>
</tr>
<tr>
<td>Serum potassium, mmol/L</td>
<td>4.2±0.03</td>
</tr>
<tr>
<td>Urinary sodium, mmol/24 h</td>
<td>13.1±0.4</td>
</tr>
<tr>
<td>Urinary potassium, mmol/24 h</td>
<td>70.3±1.4</td>
</tr>
<tr>
<td>Basal Aldo, ng/dL</td>
<td>19.9±1.8</td>
</tr>
<tr>
<td>Basal PRA, ng/mL, per h</td>
<td>2.6±0.2</td>
</tr>
<tr>
<td>Basal systolic BP, mm Hg</td>
<td>134.6±1.2</td>
</tr>
<tr>
<td>Basal diastolic BP, mm Hg</td>
<td>81.9±0.7</td>
</tr>
</tbody>
</table>

BP indicates blood pressure.

*By Student t test, unless stated otherwise.
†χ² test.
pressures in these 298 hypertensive subjects on a low-salt diet after hospitalization (135.0±1.0/82.4±0.6 mm Hg) were lower than at the screening visit (155.4±1.2/95.6±0.9 mm Hg), when many subjects were still taking their antihypertensive medications. However, they were still highly significantly different from blood pressures in 30 normotensive subjects studied on an identical protocol during the same time period (108.7±1.5/66.0±1.0 mm Hg; P<0.0001). Thus, the subjects enrolled in this study had, on average, a systolic blood pressure 28 mm Hg higher than normotensive subjects and a diastolic blood pressure 16 mm Hg higher.

Effect of Gender and Age on the Influence of Genotype on ALDO Responses to Ang II

Because we have previously shown that the adrenal response to Ang II was influenced by gender,19 the influence of age and gender on the effect of genotype on ALDO responses to Ang II was determined. To accomplish this, a GLM procedure was used that contained all 3 genotypes and gender as dichotomous variables and age as a continuous variable. Adding gender but not age substantially increased the predictability of the model (model F value=13.00, P<0.0001 with gender and 2.36, P=0.0234 without), thus enhancing the magnitude of the effect of AGT genotype on ALDO responses to Ang II (Table 2). It also improved the magnitude of CYP11B2’s effect on ALDO responses (from P=0.042 to P=0.0199) but had no effect on the ACE genotype influence on the response. On the basis of these findings, we then reanalyzed the data by gender. The relation between ALDO response to Ang II and AGT (P=0.0055) and CYP11B2 (P=0.0417) genotypes in men was similar to that in the entire data set. However, there was no relation between these parameters in women.

Gene-Gene Interaction (Epistasis)

The potential of epistatic gene interaction was assessed by expanding the components in the GLM to include interactions between 3 genotypes, thereby producing 4 interacting terms. However, only the AGT×ACE combination reached statistical significance. Therefore, the 3 other interacting terms were dropped, and the modified model was reanalyzed (Table 3). With the addition of the interaction term, there was still a significant effect of AGT TT and CYP11B2 −344 TT and no appreciable effect of ACE DD on the change in ALDO responses to Ang II. In addition, those individuals who possessed both the AGT TT and the ACE DD genotypes had a significant effect independent of that of the parent genotypes (P=0.0377). When the data for men only were analyzed, an even more significant effect was observed (P=0.0057). In women, there was no effect of combined genotype on response (P=0.73). CYP11B2 in combination with the other 2 genotypes did not add significantly to the GLM. However, by the “least-squares means for effect parameter” of the GLM, there was a significant difference in the ALDO increment in subjects who had the CYP11B2 −344 TT and AGT TT genotypes versus the parent genotypes when gender was included in the model. Thus, compared with AGT TT alone, the reduction in the ALDO increment was significantly less when AGT TT and CYP11B2 −344 TT were both present (P=0.0471). Similarly, the combination produced a smaller increment than did CYP11B2 −344 TT alone (P=0.0202).

Analysis of Singletons and Sibling Pairs

Our data consisted of individuals who were singletons and those who were part of a pedigree. The GLM treats the data from each subject as being independent and does not consider the “relatedness” of some of our subjects. This could influence our results. Thus, the data were reanalyzed with the GENMOD procedure, a statistical comparison that does consider sibships (Table 3). The comparison between the GLM and GENMOD analyses is in agreement with the conclusions of McGue et al.20 If anything, exclusion of a familial component to the data was the more not the less conservative way of analyzing the results.

Relations Between Gene Polymorphism and NM

Given the influence of genotype on ALDO responses to Ang II as a continuous variable, the data also were analyzed to determine the reliability of genotype to predict the NM hypertension phenotype as a dichotomous variable by both χ² and logistic regression analyses. First, the hypertensive subjects were classified as NMs or not by the aforementioned criteria. Then, the clinical and study characteristics were examined by comparing the 2 groups of hypertensive subjects (Table 4). As previously reported,19 there was a highly significant difference in the female to male ratio (P<0.001; percent females: 15%; 95% confidence interval [CI], 9% to 26% in NMs] vs 49% [95% CI, 29% to 84% in the remaining hypertensives]). NMs also had a significantly higher basal

<table>
<thead>
<tr>
<th>Category</th>
<th>Mean Data ALDO, SEM F P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>15.8 1.47</td>
</tr>
<tr>
<td>Female</td>
<td>28.1 1.66 40.49 &lt;0.0001</td>
</tr>
<tr>
<td>AGT genotype</td>
<td></td>
</tr>
<tr>
<td>MM−MT</td>
<td>25.3 1.27</td>
</tr>
<tr>
<td>TT</td>
<td>18.6 2.10 8.43 0.0028</td>
</tr>
<tr>
<td>ACE genotype</td>
<td></td>
</tr>
<tr>
<td>II+ID</td>
<td>22.1 1.39</td>
</tr>
<tr>
<td>DD</td>
<td>21.8 2.02 0.02 0.88</td>
</tr>
<tr>
<td>CYP11B2 gene</td>
<td></td>
</tr>
<tr>
<td>CC+CT</td>
<td>24.8 1.44</td>
</tr>
<tr>
<td>TT</td>
<td>19.1 1.99 5.48 0.020</td>
</tr>
</tbody>
</table>

The dependent variable was the ALDO response on a low-salt diet to Ang II infusion as a change from baseline. The classes were gender and AGT, ACE, and CYP11B2 genotypes. Each was treated as a dichotomous variable: AGT TT vs all others, ACE DD vs all others, and CYP11B2 TT vs all others. The model had 8 df and an F value of 13.0 (P<0.0001). P values in the table reflect the interaction of each variable on all others, specifically, on the effect of gender in modifying the genotype relation to the ALDO response to Ang II. The effects of genotype independent of gender are shown in Figures 1 and 2. The Aldo increments are reported as least-squares means and, therefore, vary somewhat from those shown in Figures 1 through 3.

The potential of epistatic gene interaction was assessed by expanding the components in the GLM to include interactions between 3 genotypes, thereby producing 4 interacting terms. However, only the AGT×ACE combination reached statistical significance. Therefore, the 3 other interacting terms were dropped, and the modified model was reanalyzed (Table 3). With the addition of the interaction term, there was still a significant effect of AGT TT and CYP11B2 −344 TT and no appreciable effect of ACE DD on the change in ALDO responses to Ang II. In addition, those individuals who possessed both the AGT TT and the ACE DD genotypes had a significant effect independent of that of the parent genotypes (P=0.0377). When the data for men only were analyzed, an even more significant effect was observed (P=0.0057). In women, there was no effect of combined genotype on response (P=0.73). CYP11B2 in combination with the other 2 genotypes did not add significantly to the GLM. However, by the “least-squares means for effect parameter” of the GLM, there was a significant difference in the ALDO increment in subjects who had the CYP11B2 −344 TT and AGT TT genotypes versus the parent genotypes when gender was included in the model. Thus, compared with AGT TT alone, the reduction in the ALDO increment was significantly less when AGT TT and CYP11B2 −344 TT were both present (P=0.0471). Similarly, the combination produced a smaller increment than did CYP11B2 −344 TT alone (P=0.0202).
TABLE 3. GENMOD Procedure Assessing the Effect of Gender and Genotypes, Singly and in Combination on ALDO Responses to Ang II

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Least-Squares Mean Change in ALDO, ng/dL</th>
<th>SEM</th>
<th>χ²</th>
<th>GLM P</th>
<th>GENMOD P</th>
</tr>
</thead>
<tbody>
<tr>
<td>AGT MM+MT</td>
<td>24.31</td>
<td>1.35</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TT</td>
<td>17.61</td>
<td>2.23</td>
<td>8.38</td>
<td>0.0038</td>
<td>0.0006</td>
</tr>
<tr>
<td>ACE II+ID</td>
<td>21.34</td>
<td>1.47</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DD</td>
<td>20.59</td>
<td>2.59</td>
<td>1.46</td>
<td>0.2274</td>
<td>0.2056</td>
</tr>
<tr>
<td>CYP11B2 −344 CC+CT</td>
<td>23.35</td>
<td>1.52</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>−344 TT</td>
<td>18.58</td>
<td>2.12</td>
<td>4.86</td>
<td>0.0275</td>
<td>0.0301</td>
</tr>
<tr>
<td>AGT×ACE</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>235 TT</td>
<td>25.02</td>
<td>1.22</td>
<td>4.32</td>
<td>0.0377</td>
<td>0.0279</td>
</tr>
<tr>
<td>ACE DD</td>
<td>13.17</td>
<td>3.71</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Because only the combination of AGT×ACE had a significant effect, the other combinations were eliminated. Because the changes in ALDO values are least-squares means and, therefore, do not precisely agree with the data depicted in Figures 1 and 2, two P values are shown. The GLM P considers each subject independently. The GENMOD value, which uses an exchangeable correlation structure, takes into account that some subjects are siblings. Gender (data not shown) had a highly significant effect in both models (P<0.0001).

PRA level (3.3±0.2 vs 2.3±0.1 ng · mL⁻¹ · h⁻¹; P=0.029) despite the same baseline plasma ALDO levels (19.6±1.1 vs 19.8±0.8 ng/dL; P=0.883). As would be anticipated from the data reported earlier, the T allele of AGT 235 was significantly associated with NM (66% in NMs and 48% in other hypertensives). Neither the ACE D allele (56% in NMs vs 60% in the remaining hypertensives) nor the CYP11B2 −344 T allele (55% in NMs and 61% in other hypertensives) was associated with the NM phenotype. Figure 4 depicts the odds ratios of having the NM phenotype, given various genotype combinations. ACE DD and CYP11B2 −344 TT, whether alone or in combination, did not increase the ability to identify the NM. However, when a hypertensive subject had the AGT 235 TT genotype, there was a 2-fold increase in the odds that the subject would be an NM (95% CI, 1.15 to 3.50; P=0.013). Requiring the individual to also have the CYP11B2 −344 TT genotype did not appreciably alter the odds ratio. Adding ACE DD to AGT 235 TT increased the odds of the subject’s being an NM to 4-fold (nearly double what it was with AGT 235 TT alone; 95% CI, 1.62 to 8.60; P=0.001). Finally, the triple combination of AGT 235 TT, ACE DD, and CYP11B2 −344 TT genotypes resulted in an even further increased likelihood (odds ratio, 5.36) that the subject would be an NM, although the sample size is too small to achieve statistical significance over the double genotype.

The data were also evaluated by logistic regression analysis. The initial model used the 3 genotypes, gender, age, and BMI. Only gender and AGT genotype were significant. With this approach, the odds ratio that male gender was associated with the NM phenotype was 10.2 (P=0.00001), whereas the odds ratio for the AGT 235 TT genotype was 2.43 (P=0.006), similar to the χ² analysis (Figure 4). The model was then simplified by eliminating age and BMI and analyzing the effect of each genotype, alone and in various combinations. The combination of AGT and ACE genotypes was a significantly better predictor of NM (P=0.0067) than either genotype alone.

### Discussion
ALDO’s response to Ang II is a well-defined physiologic characteristic that is substantially influenced by environmental factors. Maximizing the degree of responsiveness by sodium restriction reduces the background noise and enhances the likelihood that genetic components contributing to this characteristic can be identified. With this approach, several
studies have documented that ALDO’s response in non-low-renin hypertensive subjects has a bimodal distribution, with the lower mode being different from that in normotensives without a family history of hypertension.²⁶⁻²⁹ These subjects have been termed NMs. This abnormality is heritable and therefore, is an intermediate phenotype for essential hypertension.²³ Furthermore, there are several genes that could contribute to variability in this characteristic in individual subjects. The present report documents that the degree of ALDO response varies according to polymorphisms in 3 genes—AGT and ACE that modify Ang II levels and CYP11B2 that codes for ALDO synthase. Polymorphisms in 2 of them individually modify ALDO’s response. Hypertensive subjects who were homozygous for the T allele at amino acid 235 of AGT had a significantly reduced response compared with heterozygotes and those homozygous for the M allele. Likewise, those who were homozygous for the T allele at –344 of CYP11B2 had a decreased response. The polymorphisms in the ACE gene that were tested were not associated with a variable response. However, when the subject was required to have both the AGT 235 TT alleles and the ACE DD alleles, the combination was associated with a reduction in ALDO response that was significantly greater than that which occurred with the AGT gene alone—another example of genetic epistasis. There was also a suggestion that the number of subjects in our sample who were homozygous for the mutant allele at all 3 genetic loci might interact in modifying ALDO’s response to Ang II. Finally, with a case-control approach, polymorphisms in these 3 genes substantially increase the probability of identifying the NM in a hypertensive population.

Intriguingly, the results of the present study raise the potential that genotype can predict phenotype in patients with hypertension. In 1983, Shoback and colleagues¹ reported that a subset of the hypertensive population lacked the normal dietary sodium–mediated changes in vascular and adrenal responses to Ang II. Because of the absence of this dietary sodium–dependent modulation, these patients were termed “nonmodulators.” During the past 20 years, this subset has been further defined by several investigators throughout the world.²⁻⁴,⁶⁻¹²,¹⁹⁻²¹,²³⁻²⁴,²⁸ Relevant to the present report, SNP markers in both the coding and the 5’–regulatory regions of the AGT gene are associated with the 2 most specific characteristics defining the NM phenotype: ALDO and renal plasma flow responses to Ang II. The present study confirms that the NM phenotype is associated with the mutant allele at the 235th codon of the AGT gene. There was a 2-fold increased likelihood that an individual possessing the AGT 235 TT genotype might be an NM than with any other AGT genotype. The impact of the other 2 genotypes on predicting the NM phenotype was also assessed. Although the ACE DD polymorphic marker in the 16th intron by itself did not predict NM, it substantially increased the predictive value of the AGT 235 TT genotype when subjects were required to be homozygous for the mutant allele in both genes. With that constraint, the odds ratio of identifying an NM increased to nearly 4-fold. Finally, the SNP in the ALDO synthase gene’s promoter region at –344 also contributed to identifying NMs. However, the statistical relevance of this increased predictability could not be documented, likely owing to the small number of subjects in our sample who were homozygous for the mutant form at all 3 genetic loci.

Previous studies have suggested that the NM phenotype is secondary to altered production of tissue Ang II. The AGT 235 TT and ACE DD genotypes have been reported to be associated with increased AGT and/or Ang II formation.¹⁶,²⁹ Therefore, the association of these genotypes with the NM phenotype is not surprising. Intriguing is the interdependence of their effects in modifying ALDO’s response to Ang II. Alone, the ACE DD genotype had no effect on ALDO response, but it greatly enhanced the effect of the AGT TT genotype. Two caveats are apparent. First, decreased ALDO response to Ang II, at least in hypertensive individuals on a low-salt diet, is associated with genes leading to an increased production of Ang II, presumably locally in the adrenals as well as in other tissues, eg, the kidney. Second, the ACE DD genotype contributes to this decreased adrenal responsiveness but only in the presence of a gene that produces increased formation of its substrate. Thus, the influence of the enhanced conversion of Ang I to Ang II physiologically is only manifested pathophysiologically in the presence of an increased level of AGT.

CYP11B2 gene variance has been associated with alterations in ALDO production or levels,¹⁷,³⁰,³¹ with the CYP11B2 –344 TT variant being associated with reduced ALDO secretion. A relation to hypertension has not been reported. The present study confirmed these findings in relation to the ALDO response to Ang II and extended them. 
to a likely role in a subset of hypertensive patients. However, as with the ACE gene, the effect of the CYP11B2 gene polymorphism was only modestly significant by itself, with a substantial enhancement when the individual possessed certain polymorphisms in both the AGT and the CYP11B2 genes. This was particularly evident in the subjects who had the triple genotypes, although statistical superiority could not be documented because of the small sample size. This suggests that the epistasis between the 3 genes is primarily triggered by a change in the AGT level, presumably in the adrenal gland itself. In the absence of that, variability in the function of ACE or ALDO synthase does not appreciably alter the ALDO response to Ang II infusion.

Studies assessing the association and/or linkage of ACE polymorphisms to hypertension have been variable. Most have not found an association, and no linkage studies have been documented. One exception is the study by O’Donnell et al., where, in the Framingham Heart Study, they reported an association between hypertension and the ACE gene but only in men. The present report suggests that the lack of consistency in previous studies is likely related to genetic variability in the populations studied. The ACE and AGT genes are not involved in the hypertensive process in all subjects but only in a minority. Furthermore, the ACE gene probably does not contribute to hypertension by itself but does so only in the presence of a specific polymorphism in another gene, eg, AGT 235 TT. Thus, only if a study investigated the effect of the 2 genes simultaneously would the ACE gene’s effect on hypertension be consistently appreciated.

Only a few studies have examined the influence of gene–gene interaction on the risk of cardiovascular disease. Marre et al. reported that ACE II/D and AGT M 235 T polymorphisms interact significantly in the development of diabetic nephropathy. Individuals with either the ACE DD or ID genotype and homozygous for the T allele at position 235 of the AGT gene had significantly greater renal impairment than did those with the same ACE genotype but were homozygous for the AGT 235 M allele. Borecki et al. using data derived from the National Heart, Lung, and Blood Institute Family Heart Study, reported that there might be a gene–gene interaction in predicting severe hypertension in subjects homozygous for AGT 235 T and ACE D genotypes. However, not all studies have shown a relation between the AGT and ACE genes. Kiema et al. concluded that polymorphisms in the ACE and AGT genes do not confer an increased risk for the development of hypertension.

The results of the present study should be interpreted conservatively, because it is an association study. However, the controls in this study closely matched the cases, except for the differences in genotype. All subjects were drawn from a single pool of hypertensive patients who were recruited and evaluated under identical circumstances. The only difference between the 2 groups was the presence or absence of the NM intermediate phenotype and the specific genotypes at the AGT, ACE, and CYP11B2 loci. Specifically, 3 other genotypes assessed were not associated with the NM phenotype. It is unlikely that the differences observed were related to errors in genotyping. The phenotyping was performed by a standard protocol, which has previously documented a bimodal distribution in the ALDO response to Ang II in the hypertensive population. Given the relation between the ALDO response to Ang II and sodium intake, any misclassification would likely be in the direction of a false-positive, ie, a modulator or a low-renin hypertensive patient being classified as an NM.

**Perspectives**

The present study documents that specific markers at the AGT, ACE, and ALDO synthase gene loci predict NM essential hypertension. Because the pathophysiologic characteristics of NM hypertension are corrected by administration of an ACE inhibitor, it is likely that a double or triple genotype can be substituted with a high degree of fidelity for the complex physiologic characterization of NM for therapeutic and preventive decision making. Finally, the present study provides a specific example of genetic epistasis that might have relevance to understanding complex interaction of genes in producing changes in human physiologic function with relevance for disease prevention and management.

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