Scientific Contributions

Angiotensinogen Genotype, Sodium Reduction, Weight Loss, and Prevention of Hypertension

Trials of Hypertension Prevention, Phase II

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Abstract—The angiotensinogen gene has been linked to essential hypertension and increased blood pressure. A functional variant believed to be responsible for hypertension susceptibility occurs at position −6 in the promoter region of the gene in which an A for G base pair substitution is associated with higher angiotensinogen levels. To test whether an allele within the angiotensinogen gene is related to subsequent incidence of hypertension and blood pressure response to sustained sodium reduction, 1509 white male and female subjects participating in phase II of the Trials of Hypertension Prevention were genotyped at the angiotensinogen locus. Participants had diastolic blood pressures between 83 and 89 mm Hg and were randomized in a 2×2 factorial design to sodium reduction, weight loss, combined intervention, or usual care groups. Persons in the usual care group with the AA genotype at nucleotide position −6 had a higher 3-year incidence rate of hypertension (44.6%) compared with those with the GG genotype (31.5%), with a relative risk of 1.4 (95% confidence interval [0.87, 2.34], test for trend across all 3 genotypes, \( P=0.10 \)). In contrast, the incidence of hypertension was significantly lower after sodium reduction for persons with the AA genotype (relative risk = 0.57 [0.34, 0.98] versus usual care) but not for persons with the GG genotype (relative risk = 1.2 [0.79, 1.81], test for trend \( P=0.02 \)). Decreases of diastolic blood pressure at 36 months in the sodium reduction group versus usual care showed a significant trend across all 3 genotypes (\( P=0.01 \)), with greater net blood pressure reduction in those with the AA genotype (−2.2 mm Hg) than those with the GG genotype (+1.1 mm Hg). A similar trend across the 3 genotypes for net systolic blood pressure reduction (−2.7 for AA versus −0.2 mm Hg for GG) was not significant (\( P=0.17 \)). Trends across genotypes for the effects of weight loss on hypertension incidence and decreases in blood pressure were similar to those for sodium reduction. We conclude that the angiotensinogen genotype may affect blood pressure response to sodium or weight reduction and the development of hypertension. ([Hypertension. 1998;32:393-401.])

Key Words: blood pressure ■ clinical trials ■ genetics ■ interaction ■ prospective study ■ renin

Basic research and genetic studies in human populations have implicated the angiotensinogen gene in the development of elevated blood pressure and hypertension. Genetic linkage analysis has shown that the angiotensinogen gene is linked to hypertension in Utah, French, and English sib pairs.1,2 Multiple polymorphisms were examined for association with hypertension in an attempt to identify a specific mutation that could cause increased expression of angiotensinogen levels leading to increased blood pressure. The two polymorphisms receiving the most attention were a methionine-to-threonine amino acid substitution at amino acid 235 (M235T) in the mature angiotensinogen protein and a threonine-to-methionine substitution at amino acid 174 (T174 M).1,3 These amino acid substitutions arise from nucleotide substitutions at positions +704 and +521, respectively, from the transcription start site. The strongest evidence for association has been with the M235T polymorphism. However, this polymorphism is not near the cleavage site where renin acts on angiotensinogen to form angiotensin I, nor is it thought that this amino acid substitution plays a functional role. Because of this and the lack of consistency in association studies of M235T with hypertension,3,4–6 it was suspected that there may be another site in linkage disequilibrium with M235T that is a causal mutation.7 Recently, evidence was published that an A for G nucleotide substitution in the promoter region of the angioten-
Angiotensinogen gene 6 nucleotides upstream from the start site of transcription appears to be a functional mutation. The A substitution alters the binding of a nuclear protein, resulting in increased gene transcription compatible with increased angiotensinogen levels. The G-6A alleles are in nearly complete linkage disequilibrium with the M235T alleles. Angiotensinogen is expressed in tissues involved in blood pressure regulation, such as kidney, adrenal, and brain tissue. Angiotensinogen gene duplication in the mouse has resulted in increased angiotensinogen levels and increased blood pressure. In humans, it has also been shown that increased angiotensinogen levels correlate with increased blood pressure. Injection of angiotensinogen increases blood pressure, whereas antibodies against angiotensinogen decrease blood pressure. Hypertensive persons with the TT genotype compared with the MM genotype at M235T had higher systolic and diastolic blood pressure and plasma angiotensinogen levels, and they were 1.6 times more likely to use an antihypertensive medication and 2.1 times more likely to use 2 antihypertensive medications. In addition to the genetic evidence from the above studies, it has been suggested that the angiotensinogen gene influences the salt sensitivity of blood pressure. Therefore, subjects involved in an ongoing clinical trial of blood pressure reduction were tested for both the M235T and G(-6)A angiotensinogen loci variants. This allowed hypotheses to be tested about whether the form of the gene that shows increased transcription leads to an increased incidence of hypertension over 3 years of follow-up and whether sodium reduction can protect persons with this genotype from hypertension. This article reports on a substudy conducted in a randomized clinical trial of blood pressure reduction at the 36-month follow-up visit. The primary hypothesis of TOHP was that diastolic blood pressure would be significantly decreased by sodium reduction and by weight loss. The primary hypothesis of our substudy was that the diastolic and systolic blood pressure reductions resulting from sodium reduction at the 36-month follow-up visit in the trial would differ among angiotensinogen genotypes. We also were able to test whether there were differences in the incidence of hypertension among angiotensinogen genotypes. Secondary hypotheses for this substudy included testing whether weight loss and/or combined weight and sodium reduction would show similar results to sodium reduction. Differences in results across different clinic visits were not an original hypothesis and should be considered as post hoc analyses.

Methods

Subects

TOHP, phase II, was designed to study the efficacy of sodium reduction and weight loss in reducing diastolic blood pressure levels. This 3-year trial recruited 2382 men and women between the ages of 30 and 54 years who were moderately overweight (110% to 160% of desirable body weight) and had mean diastolic blood pressures between 83 and 89 mm Hg averaged over 3 baseline visits. Subjects were randomly assigned to a usual care group, a sodium reduction group, a weight reduction group, or a combined sodium and weight reduction group. The intervention goal was a dietary sodium intake of ≤80 mmol/L per day in the sodium reduction group and a 4.5 kg reduction in weight in the weight reduction group. Both goals were used for individuals in the combined sodium and weight loss reduction group. These goals were to be met by the 6-month clinic visit. Additional details of study subjects and intervention protocols can be found in the publication of the main study results. Each study center received Institutional Review Board approval, and written informed consent was obtained from each participant.

Clinical Variables

Sitting blood pressures were measured by random zero sphygmomanometers at baseline and at follow-up visits every 6 months for 36 months, or, for some subjects, 42 or 48 months. The average of 9 measurements (3 from each of 3 visits) was used to calculate the blood pressure at baseline, the 18-month visit, and the 36-month visit. Blood pressures for those on antihypertensive medications were set equal to the measurement at the last unmedicated study visit. Blood pressures of persons on medications affecting blood pressure for reasons other than hypertension and of pregnant women were assumed missing for those visits. Hypertension was defined as an average systolic blood pressure ≥140 mm Hg, an average diastolic blood pressure ≥90 mm Hg, or diagnosis and drug treatment of hypertension. Blood was drawn only at the 36-month or last examination. Angiotensinogen levels or plasma renin activity were not measured. Urinary sodium excretion from a 24-hour sample was obtained routinely at baseline and at 18 and 36 months. In addition, a 25% sample had urinary sodium measurements obtained at the 6-month visit. Body weight in kilograms was also measured at each visit.

Angiotensinogen Genotyping

White cells were obtained from all study subjects who attended one of the participating clinics after the start of this substudy and gave consent to have their blood drawn. White cells were separated from plasma and sent on ice to the University of Utah, where genotypes at 2 loci of the angiotensinogen gene were obtained by assays performed at Myriad Genetics, Inc, Salt Lake City. Genotypes were determined at amino acid 235 coded for in exon 2 and at position -6, 6 nucleotides upstream from the transcription start site in the promoter region of the gene located on chromosome 1. Multiplex polymerase chain reaction (PCR) for the sequences around codon 235 in exon 2 and the promoter region of the human angiotensinogen gene were performed with the following primers: 5' AGC CAG CAG AGA GTT TTG 3' and 5' AGT GCT ATG CAG GCT GTG 3' for M235T; 5' GGT CCA AGC GTG AGT GTC 3' and 5' CGG CTT ACC TTC TGC TGT A 3' for G-6A. The sizes of the two products are 126 bp and 200 bp, respectively. PCR reactions were performed in a Perkin-Elmer 9600 with the following cycling parameters: 95°C for 5 minutes; 5 cycles of 95°C for 10 seconds, 62°C for 60 seconds; followed by 35 cycles of 95°C for 10 seconds, 58°C for 10 seconds, and 72°C for 60 seconds. Denatured PCR products were spotted onto 4 nylon membranes and hybridized with g-32P end-labeled allele-specific oligonucleotide probes corresponding to variants of the AGT gene. The probe sequences were 235T: 5' CCT GAC GAC GGC AGG AGC CAG T 3'; 235M: 5' AGC CAG GTC CAT CAG G 3'; 235A: 5' GCC AGG GGA AGA AG 3'; and -6G: 5' GCC GGG GGA AGA AG 3'. The membranes were prehybridized (M and T filters at 50°C, A and G filters at 45°C) in hybridization solution (0.5 mol/L Na-PO4, pH 7.2, 7% SDS, 1 mmol/L EDTA) for 10 minutes to 1 hour. The membranes were then hybridized with 1 μL of labeled probe in 3 mL hybridization solution for 2 hours to overnight at the same temperatures. The membranes were rinsed briefly in 2×SSC at room temperature and then 30 minutes in 2×SSC at 52°C (M and T) or 47°C (A and G). The membranes were drained briefly to remove excess buffer, then wrapped in plastic wrap. They were placed in an x-ray cassette with a sheet of Kodak XAR5 x-ray film for 10 minutes to 2 hours, then developed in an x-ray film processor.
TABLE 1. Percent (n) Concordance of M235T and G(−6)A Angiotensinogen Genotypes in White Subjects: TOHP, Phase II

<table>
<thead>
<tr>
<th></th>
<th>M235T</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AA</td>
<td>AG</td>
<td>GG</td>
<td>Total</td>
<td></td>
</tr>
<tr>
<td>TT</td>
<td>19% (294)</td>
<td>1% (10)</td>
<td>0% (0)</td>
<td>20% (304)</td>
<td></td>
</tr>
<tr>
<td>MT</td>
<td>1% (12)</td>
<td>46% (700)</td>
<td>1% (8)</td>
<td>48% (720)</td>
<td></td>
</tr>
<tr>
<td>MM</td>
<td>0% (0)</td>
<td>1% (15)</td>
<td>31% (470)</td>
<td>32% (485)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>20% (306)</td>
<td>48% (725)</td>
<td>32% (478)</td>
<td>100% (1509)</td>
<td></td>
</tr>
</tbody>
</table>

Gene frequency: A and T = .44, G and M = .56.

Statistical Analysis

Genotypes were obtained on all but 7 samples received, giving a total sample size of 1894 subjects: 1509 white subjects, 323 African Americans, and 62 of other races. Persons of other races were excluded from analysis because of small numbers. Also, because only 3% (n = 10 of 323) of African Americans had the GG genotype, the full analysis testing for genotype interactions with intervention on blood pressure could only be performed on the 1509 white subjects. In studies finding an association of a marker at amino acid 235 of the angiotensinogen gene with hypertension, the T allele is present in persons with higher angiotensinogen levels, higher blood pressures, and hypertension.1 The corresponding allele at the −6 position is an A. Therefore, the TT genotype at position 235 is comparable to an AA genotype at −6. These two genotypes are in nearly complete linkage disequilibrium, with only 3.0% of the sample from white subjects not showing genotypic concordance (Table 1). The discordance in white subjects was similar to that in African Americans (2.8%) and agrees with previously published numbers.1 Because of such strong linkage disequilibrium, the results were the same when analyzing each of the two loci, and only the analyses of the −6 genotypes are presented. Gene frequencies of the A (and T) allele are greatly increased in African Americans (q = .85) compared with white subjects (q = .44), as has been reported in other studies.5,18

Statistical analyses were the same as for the main trial,17 with additional terms in the model for angiotensinogen gene effects. Changes in study variables were calculated by subtracting the baseline from the follow-up value. At each time point, a regression model was fit to the mean changes with an interaction term included to test genotype by intervention after adjusting for gender, age, and baseline blood pressure. A repeated-measures analysis of variance was fit to the data to test for a genotype by intervention by time interaction, with blood pressure changes at each clinic visit as the repeated measure, and age, gender, and baseline blood pressure as adjustment covariates. Baseline measurements were compared by analysis of variance. Percentages were compared by χ² analysis. Relative risks of hypertension incidence for each genotype represent hazard rate ratios and were calculated from a Cox regression model. Tests for trends were done by using an ordinal variable in the regression equation representing the 3 genotypes (1, 2, 3; with 3 as the AA genotype). One-degree-of-freedom tests were calculated for trends across genotypes. Tests of trends rather than 2-degrees-of-freedom F tests were used because it has been shown that angiotensinogen levels of the AG genotype persons are intermediate between AA (TT) at position −6 (amino acid 235), whereas 32% was GG (MM) (Table 1). Table 2 shows the baseline sample size, gender, age, blood pressure, urinary sodium excretion, and weight for the 4 study groups by AGT genotype. There were no significant differences either for tests between means or for tests of trends for any variable except for gender in the sodium intervention group. After combining the 4 intervention groups, there was a small but significant increase in baseline mean systolic blood pressure across the GG, AG, and AA angiotensinogen genotypes (126.8, 127.5, 127.8 mm Hg, respectively; P = .02). The diastolic blood pressure trend across genotypes (85.8, 86.0, 86.1 mm Hg, respectively) was only of borderline significance (P = .08).

Hypertension Incidence

The Figure and Table 3 show the incidence of hypertension in each angiotensinogen genotype by intervention group. Three-year hypertension incidence in the usual care group was
greater (but not statistically significant) in the AA genotype than the GG genotype, with the heterozygotes having an incidence rate nearly that of the AA group (test for trend, \( P = 0.10 \)). The hypertension incidence rate in the sodium reduction group was significantly reduced below the usual care group at 36 months among those with the AA genotype and the AG genotype. There was no significant effect of sodium reduction intervention on hypertension incidence in those with the GG genotype. There was a significant trend of hypertension incidence rate reduction across genotypes.

Similarly, the weight loss intervention decreased hypertension incidence over the course of the study in the AA genotype group compared with the usual care group. The heterozygotes also showed a significant reduction in risk. There was no significant effect of weight loss intervention on hypertension incidence in the GG group. There was a trend in reduction of risk across genotypes that was of borderline significance (\( P = 0.07 \)). Combined sodium reduction and weight loss did not show a significant reduction in incidence in the AA genotype group but did show a significant reduction in incidence in the heterozygote group. The test for trend was not significant (\( P = 0.23 \)).

**Blood Pressure Reduction**

As a reference for the genotype-specific results, the main study found net 2.9/1.6 and 1.2/0.7 mm Hg decreases in systolic/diastolic blood pressure for sodium reduction after 6 and 36 months, respectively, 3.7/2.7 and 1.3/0.9 mm Hg decreases for weight reduction, and 4.0/2.8 and 1.1/0.6 mm Hg reductions in the combined reduction group. Table 4 shows crude blood pressure changes after 36 months for each genotype and intervention group. These crude blood pressure changes were used to calculate the net blood pressure change between intervention and usual care groups.

Table 5 shows net adjusted blood pressure changes from baseline for the sodium reduction group compared with the usual care group. The trend across genotypes in the net intervention effect after adjustment for age, gender, and baseline blood pressure was significant at the 36-month visit for diastolic blood pressure (\( P = 0.01 \)) but not for systolic blood pressure (\( P = 0.17 \)). Trends were not significant for the 6- and 18-month visits for either systolic or diastolic blood pressure. There was a significant 3-way interaction effect between time, genotype, and intervention group (sodium reduction versus usual care) on change in diastolic blood pressure (\( P = 0.01 \)). The corresponding interaction was of borderline significance for systolic blood pressure change (\( P = 0.08 \)). It appeared that persons with the AA genotype were able to maintain blood pressure reduction at each time point, whereas persons with the GG genotype were not, despite maintenance of similar sodium reduction. In models...
with additional control for any interaction of intervention group with baseline blood pressure, results by genotype were nearly identical. Net sodium excretion changes did not vary significantly across genotypes (Table 5) at any time point. Average adjusted net weight change in the sodium intervention versus usual care groups ranged from −0.4 to +0.6 kg among genotype groups (data not shown).

The difference between the AA and GG homozygote genotypes in the adjusted net effect of sodium reduction on systolic and diastolic blood pressure change after 36 months was 2.5/3.3 mm Hg. Mean diastolic blood pressure tended to increase in the sodium reduction group relative to the usual care group for the GG genotype, but the difference was not significant. This increase occurred as a result of the usual care group showing a greater decrease in blood pressure than the intervention group rather than an actual increase in mean blood pressure in the intervention group itself (see Table 4).

For weight reduction compared with usual care at the 36-month visit (Table 6), the trend across genotypes in the adjusted net intervention effect across genotypes was significant for diastolic blood pressure ($P=0.05$) but not for systolic blood pressure ($P=0.23$). Change in weight was not significantly different among the 3 genotype groups, ranging from a net 1.7 to 2.7 kg weight loss compared with usual care at the 36-month follow-up visit (Table 6). The average adjusted net sodium change ranged from −8 to +6 mmol/24 hours among genotype groups (data not shown). There were no significant trends across genotype in blood pressure reduction at the 6- and 18-month visits despite the greater weight loss that was observed at these visits compared with 36 months. Non-significant 3-way interactions of time, genotype, and intervention group (weight loss versus usual care) were seen for both systolic ($P=0.15$) and diastolic ($P=0.10$) blood pressure change. At 36 months, there was a net systolic and diastolic blood pressure difference between AA and GG homozygote genotypes of 2.4/2.7 mm Hg.

The effect of the combined intervention did not show a significant blood pressure trend across genotypes at any of the follow-up visits (Table 7). Although there were net blood pressure reductions in the AA and AG genotype groups compared with no net blood pressure reduction in the GG group at 36 months, these differences were not significant. At 36 months, the combined intervention group showed apparent differences in compliance with intervention between genotypes. The net urinary sodium decrease maintained at the 36-month visit in the combined intervention group with the AA genotype was only 22 mmol/24 hours, not significantly different from zero ($P=0.39$), compared with a net 44 mmol/24 hours decrease ($P<0.01$) for the heterozygote genotype. Similarly, net weight reduction in

### TABLE 5. Adjusted Net Effect of Sodium Reduction on Blood Pressure Change (±SE) Among White Subjects: TOHP, Phase II*

<table>
<thead>
<tr>
<th>Change in DBP</th>
<th>AA</th>
<th>AG</th>
<th>GG</th>
<th>Trend P</th>
</tr>
</thead>
<tbody>
<tr>
<td>6 mo</td>
<td>−1.0±1.1</td>
<td>−1.3±0.7</td>
<td>−1.5±0.8</td>
<td>0.73</td>
</tr>
<tr>
<td>18 mo</td>
<td>−2.2±1.0</td>
<td>−1.2±0.7</td>
<td>−0.6±0.8</td>
<td>0.18</td>
</tr>
<tr>
<td>36 mo</td>
<td>−2.2±1.1</td>
<td>−0.7±0.7</td>
<td>1.1±0.9</td>
<td>0.01</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Change in SBP</th>
<th>AA</th>
<th>AG</th>
<th>GG</th>
<th>Trend P</th>
</tr>
</thead>
<tbody>
<tr>
<td>6 mo</td>
<td>−2.3±1.4</td>
<td>−2.6±0.9</td>
<td>−2.7±1.1</td>
<td>0.81</td>
</tr>
<tr>
<td>18 mo</td>
<td>−2.5±1.2</td>
<td>−1.0±0.8</td>
<td>−2.2±0.9</td>
<td>0.89</td>
</tr>
<tr>
<td>36 mo</td>
<td>−2.7±1.4</td>
<td>−1.3±0.9</td>
<td>−0.2±1.1</td>
<td>0.17</td>
</tr>
</tbody>
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<table>
<thead>
<tr>
<th>Change in sodium, mmol/24 h</th>
<th>AA</th>
<th>AG</th>
<th>GG</th>
<th>Trend P</th>
</tr>
</thead>
<tbody>
<tr>
<td>6 mo</td>
<td>−90±21</td>
<td>−58±14</td>
<td>−57±17</td>
<td>0.25</td>
</tr>
<tr>
<td>18 mo</td>
<td>−48±12</td>
<td>−48±8</td>
<td>−62±9</td>
<td>0.38</td>
</tr>
<tr>
<td>36 mo</td>
<td>−53±12</td>
<td>−45±8</td>
<td>−45±9</td>
<td>0.55</td>
</tr>
</tbody>
</table>

DBP indicates diastolic blood pressure; SBP, systolic blood pressure.

*Change in blood pressure calculated by change in sodium reduction group minus change in usual care group adjusted for age, gender, and baseline level of the variable.

### TABLE 6. Adjusted Net Effect of Weight Reduction on Blood Pressure Change (±SE) Among White Subjects: TOHP, Phase II*

<table>
<thead>
<tr>
<th>Change in DBP</th>
<th>AA</th>
<th>AG</th>
<th>GG</th>
<th>Trend P</th>
</tr>
</thead>
<tbody>
<tr>
<td>6 mo</td>
<td>−3.1±1.1</td>
<td>−3.5±0.7</td>
<td>−2.7±0.8</td>
<td>0.73</td>
</tr>
<tr>
<td>18 mo</td>
<td>−2.0±1.0</td>
<td>−1.7±0.6</td>
<td>−0.9±0.8</td>
<td>0.39</td>
</tr>
<tr>
<td>36 mo</td>
<td>−2.4±1.2</td>
<td>−1.0±0.7</td>
<td>0.3±0.9</td>
<td>0.05</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Change in SBP</th>
<th>AA</th>
<th>AG</th>
<th>GG</th>
<th>Trend P</th>
</tr>
</thead>
<tbody>
<tr>
<td>6 mo</td>
<td>−3.8±1.3</td>
<td>−3.8±0.8</td>
<td>−4.7±1.0</td>
<td>0.54</td>
</tr>
<tr>
<td>18 mo</td>
<td>−1.8±1.3</td>
<td>−1.3±0.8</td>
<td>−2.2±1.0</td>
<td>0.74</td>
</tr>
<tr>
<td>36 mon</td>
<td>−3.5±1.4</td>
<td>−0.9±0.9</td>
<td>−1.1±1.1</td>
<td>0.23</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Change in weight, kg</th>
<th>AA</th>
<th>AG</th>
<th>GG</th>
<th>Trend P</th>
</tr>
</thead>
<tbody>
<tr>
<td>6 mo</td>
<td>−5.7±0.8</td>
<td>−4.9±0.5</td>
<td>−5.1±0.6</td>
<td>0.57</td>
</tr>
<tr>
<td>18 mo</td>
<td>−3.4±0.9</td>
<td>−2.9±0.6</td>
<td>−3.7±0.7</td>
<td>0.71</td>
</tr>
<tr>
<td>36 mo</td>
<td>−2.7±1.0</td>
<td>−1.7±0.6</td>
<td>−2.4±0.8</td>
<td>0.85</td>
</tr>
</tbody>
</table>

DBP indicates diastolic blood pressure; SBP, systolic blood pressure.

*Change in blood pressure calculated by change in weight loss group minus change in usual care group adjusted for age, gender, and baseline level of the variable.
TABLE 7. Adjusted Net Effect of Combined Sodium and Weight Reduction on Blood Pressure Change (±SE) Among White Subjects: TOHP, Phase II*

<table>
<thead>
<tr>
<th>Change in DBP</th>
<th>AA</th>
<th>AG</th>
<th>GG</th>
<th>Trend P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Change in weight, kg</td>
<td>6 mo</td>
<td>-4.3±0.7</td>
<td>-5.4±0.5</td>
<td>-4.4±0.5</td>
</tr>
<tr>
<td></td>
<td>18 mo</td>
<td>-2.9±0.9</td>
<td>-3.6±0.6</td>
<td>-3.3±0.6</td>
</tr>
<tr>
<td></td>
<td>36 mo</td>
<td>-1.2±1.0</td>
<td>-3.0±0.6</td>
<td>-1.9±0.7</td>
</tr>
</tbody>
</table>

DBP indicates diastolic blood pressure; SBP, systolic blood pressure.
*Change in blood pressure calculated by change in combined intervention group minus change in usual care group adjusted for age, gender, and baseline level of the variable.

Discussion

The design of TOHP, phase II, provided a unique opportunity to test whether hypertension incidence differed by genotype over 3 years of follow-up, since persons selected to be in this study had a high normal level of diastolic blood pressure averaging between 83 and 89 mm Hg at baseline. This substudy of TOHP found that after a 3-year period of follow-up in the usual care group, systolic and diastolic blood pressures and incidence of hypertension were higher, but with borderline significance, in persons with the AA genotype of the angiotensinogen gene than in those with the GG genotype. The heterozygotes had an intermediate level of blood pressure and had a higher incidence of hypertension than persons with the GG genotype. This reinforces the hypothesis that this nucleotide substitution in the promoter region of the gene predisposes individuals to hypertension.

Despite higher blood pressures and an increased incidence of hypertension in the control group participants with the AA genotype, there was a significant reduction in diastolic blood pressure and/or hypertension incidence in those assigned to the sodium reduction or weight loss interventions who had the AA and AG genotypes. It appears that although persons with the AA genotype develop hypertension to a greater degree than those with the other genotypes when there is no intervention, they respond more favorably to salt reduction or weight loss intervention. It appears that the GG genotype group may comprise primarily salt-insensitive individuals.

The net blood pressure decrease with sodium reduction in the AA genotype was 2.5/3.3 mm Hg greater than the decrease in the GG genotype after 36 months. This mild effect is not surprising, given that the genetic effect of the AA genotype versus the GG genotype raises angiotensinogen levels by only 20% to 30%. It is only the long-term elevation of angiotensinogen levels that would be expected to slowly increase blood pressure as compensatory mechanisms reset or fail, the rate of that increase depending on other genetic and environmental factors. All other things being equal and with no intervention, Table 4 suggests that over only 3 years the AA genotype group would differ from the GG genotype by 1.6 mm Hg diastolic blood pressure. Differences of 2 to 3 mm Hg in blood pressure response between genotypes can have an important public health impact. Analyses using data from the Framingham Heart Study and NHANES II showed that a 2 mm Hg reduction in diastolic blood pressure would be associated with a 17% decrease in the prevalence of hypertension, a 6% reduction in risk of coronary heart disease, and a 15% reduction in risk of stroke and transient ischemic attacks.16

Significant differences in diastolic blood pressure between the AA and GG genotypes for both the sodium and weight interventions were apparent only at the 36-month visit. The decrease in blood pressure at the 6-month visit was nearly identical for all 3 genotypes. Persons were less compliant with sodium reduction or weight loss at the 18- and 36-month visits, in which the blood pressure differences by genotype began to appear, although the differences in compliance were not significant. This might imply that the stronger intervention at 6 months was powerful enough to mask any underlying genotypic differences by temporarily overriding a resistance against blood pressure change in persons with the GG genotype. As the intervention became less strong, or as long-term control became equilibrated, only then were the genotypic differences observed. Because this time-dependent result was not a prior hypothesis, other studies will need to confirm the time course of blood pressure response to sodium or weight reduction. A smaller study on an older population suggested that angiotensinogen genotype differences in blood pressure change could be seen after only 6 months of sodium reduction and potassium supplementation (unpublished results). This suggests that age may play a critical role in how rapidly and how sustained a genotype-specific sodium effect may be.

Because there were significant genotype differences in blood pressure change in the sodium and weight reduction groups, it would be expected that similar or better results would be seen in the combined intervention group. However, for both the AA and GG genotype groups, the amounts of urinary sodium reduction in the combined
intervention group were only 22 and 25 mmol/L per 24 hours, respectively. Weight reduction was also the least in the AA combined intervention group. Because neither systolic nor diastolic blood pressure in those with the GG genotype in the sodium reduction group were responsive to long-term sodium reduction, the findings would not be expected to change if this genotypic group had been more compliant with the combined intervention. However, the low compliance in the AA group would be expected to have larger adverse effects on the blood pressure change estimate. If similar sodium compliance were reached in the AA group as in the AG group for the combined intervention, the blood pressure decrease and hypertension prevention may have at least paralleled the significant sodium reduction group intervention results. The argument that sodium reduction does not add any further beneficial effect to that of weight loss cannot be inferred from either the main study or this substudy because the study was not designed to detect such an effect and only limited reduction in sodium excretion was achieved in the combined intervention group.19

A meta-analysis of intervention trials suggested that a 21 to 70 mmol/L decrease in sodium intake produces reductions in systolic and diastolic blood pressures of 3.6/2.2 mm Hg.20 Another meta-analysis showed that median 77 and 76 mmol decreases in sodium were associated with systolic and diastolic blood pressure decreases of 4.8/2.5 and 1.9/1.1 mm Hg in hypertensive and normotensive persons, respectively.21 Infants who were randomized at birth to a low sodium diet for the first 6 months after birth had lower blood pressures 15 years later (3.6/2.2 mm Hg) than infants assigned to a normal sodium diet.22 In addition, a study in chimpanzees showed that adding salt within the normal human dietetic range to a baseline low salt diet increased systolic and diastolic blood pressure (33/10 mm Hg) over 20 months.23 The sodium-induced blood pressure changes were completely reversed within 6 months after removing the additional salt, and although most of the chimpanzees responded, there were clearly responders and nonresponders. In most human studies of salt sensitivity, there also has been a wide range of responses, even within the artificially defined subgroups of salt-sensitive and salt-resistant persons.24

Our study provides evidence that some of this variation is explained by genetic factors, specifically the angiotensinogen gene. Despite maintenance of sodium reduction, persons with the GG genotype may have become resistant to the blood pressure–lowering effects of sodium reduction, whereas persons with the AA genotype maintained their blood pressure response over 3 years. Constant genetically determined angiotensinogen elevations in persons with the AA genotype could explain this finding. However, because angiotensinogen levels were not available in this study, caution should be used before inferring that the angiotensinogen genotype findings prove an actual physiological role of angiotensinogen levels in blood pressure response to a sodium or weight loss intervention. There are known physiological mechanisms that could explain the above findings. The kidney normally compensates for increased blood pressure by increasing fluid and sodium excretion. This response curve is very steep, so that even small increases in blood pressure can return fluid and electrolyte balance to normal.15 Obesity shifts this curve to the right and may flatten the curve so that higher blood pressure levels are required to maintain fluid balance.25–27 A shift to the right without flattening, as seen in salt-insensitive subjects, means that blood pressures will be elevated but that natriuresis in response to blood pressure change will be nearly normal. A flattening of the response curve, as seen in more salt-sensitive individuals, requires a greater blood pressure increase to excrete a similar amount of sodium and fluid than in less salt-sensitive individuals. One mechanism proposed for the development of salt sensitivity is the failure to reduce angiotensin II to appropriate levels as sodium intake increases.28 The genetically determined higher angiotensinogen levels of the AA genotype on a normal (high) salt diet would make it more difficult for these individuals to lower angiotensinogen when needed, making them more salt sensitive. The resulting flattening of the pressure-natriuresis slope for the AA genotype would result in a greater blood pressure drop after sodium reduction than for the GG genotype, which would have the steeper response curve, resulting in a smaller blood pressure response.27 Under this model, one would expect very little change in blood pressure in the GG group because their angiotensinogen level would respond appropriately to salt reduction, maintaining the steep curve, and only temporarily lowering blood pressure. The AA genotype would also be expected to have a temporary drop until equilibrium is reached if there were not the constant higher genetically determined angiotensinogen levels that require a higher blood pressure to maintain the appropriate fluid volume and sodium concentration. If the salt change is a chronic change, then it may take time to reset some physiological set-point before blood pressure will return to normal levels. Over the 36-month period of intervention, a lower blood pressure was maintained in persons with the AA genotype despite a decrease in urinary sodium excretion between the 6-month and 36-month visits. For the GG group, however, net diastolic blood pressure was reduced only through 18 months, after which it returned to levels similar to those in the usual care group.

An important question is whether the effects of weight reduction operate through similar or different mechanisms as for sodium reduction. Persons on a normal salt diet who are 235T homozygotes have blunted renal plasma flow response to a 3 ng/kg per minute angiotensin II infusion compared with the other 2 genotypes.29 Also, greater body mass index was associated with significantly lower renal plasma flow in persons with the TT genotype ($r = -0.61$) than for the other 2 genotypes ($r = -0.38$). Therefore, weight loss may improve renal plasma flow to a greater extent in the TT genotype, resulting in reduced blood pressure.

An additional explanation may be that weight reduction results in loss of adipose tissue. Adipose tissue produces the second greatest amount of angiotensinogen next to the liver. Loss of fat mass would directly reduce local angio-
tensinogen levels, local vasoconstriction, and possibly resistance changes of a more central nature. Angiotensinogen levels have been correlated with blood pressure reduction caused by weight loss. A study with rats showed that fasting for 3 days reduced angiotensinogen amounts released per adipose cell to 33% of control animal levels. Refeeding increased angiotensinogen release to levels that were 41% to 83% higher than those in the control rats. Increased angiotensinogen release was limited to the local adipose tissue, since liver mRNA and central plasma levels of angiotensinogen were not affected by fasting or overfeeding.

Therefore, weight loss in the TOHP study participants could reduce both the amount of adipose tissue and the release of angiotensinogen per cell. This would be expected to have the greatest effects in persons with the AA genotype who already have greater angiotensinogen release. Reduction of angiotensinogen to near normal levels would remove local vasoconstriction and insulin resistance and reduce blood pressure to a greater extent than in persons with the GG genotype who already have near-normal levels of angiotensinogen. Further research is required to determine whether the mechanism relating weight loss to reduced blood pressure, especially in persons with the AA genotype, is a direct mechanism related to the amount of adipose tissue or whether it occurs by the same mechanism as for sodium reduction.

Possible Clinical Implications

Persons with a genetic predisposition to develop hypertension as a result of the angiotensinogen locus appear to have the greatest diastolic blood pressure decrease after either sodium reduction or weight loss. Because persons with the AA genotype had the greatest 3-year incidence of hypertension without intervention, they may be an important subgroup to target for effective diet counseling and weight loss. The data from this study suggest that weight or sodium intervention was not effective for persons with the GG genotype. Caution must be used, however, when interpreting these results. The confidence limits for blood pressure changes and relative risks do not exclude benefits in such persons, and the early benefit may have been maintained with better sustained adherence to the interventions.

It also appears that effective long-term maintenance of the intervention will be required before adequate control is reached, at least in younger subjects. Older subjects may have a more rapid response to sodium reduction or weight loss but may still need long-term changes in diet and exercise habits to maintain that response. This study did not have the power to specifically test whether the slight increase in relative risk for GG genotype individuals is real or has any clinical implications. Until further data are obtained, therefore, guidelines from the Sixth Report of the Joint National Committee on Detection, Evaluation, and Treatment of High Blood Pressure remain appropriate that all patients with elevated blood pressures should reduce sodium and weight. Our study only suggests that persons with the adverse angiotensinogen genotype who are being considered for lifetime antihypertensive therapy should be given even greater attention by health professionals.

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