Effects of Endothelial Nitric Oxide Synthase, α-Adducin, and Other Candidate Gene Polymorphisms on Blood Pressure Response to Hydrochlorothiazide

Stephen T. Turner, Arlene B. Chapman, Gary L. Schwartz, and Eric Boerwinkle

Background: Pharmacogenetic discoveries may enable greater individualization of antihypertensive drug therapy. We investigated polymorphisms in the genes encoding endothelial nitric oxide synthase (Glu298→Asp), α-adducin (Gly460→Trp), the β1-adrenoceptor (Arg389→Gly), β2-adrenoceptor (Arg16→Gly), and lipoprotein lipase (Ser447→Stop) for their potential influences on blood pressure (BP) response to a thiazide diuretic.

Methods: The sample consisted of 291 unrelated non-Hispanic African American adults (150 women and 141 men) and 294 unrelated non-Hispanic white adults (126 women and 168 men) who were between 30 and 59.9 years of age and who had essential hypertension. Previous antihypertensive drug therapy was withdrawn for at least 4 weeks, and subjects were then treated with hydrochlorothiazide (25 mg daily) for 4 weeks to determine BP response.

Results: The covariates of ethnicity, gender, age, and waist-to-hip ratio accounted for 26% of interindividual variation in systolic BP response and 11% of interindividual variation in diastolic BP response. After adjustment for covariates, the endothelial nitric oxide synthase Glu298→Asp polymorphism made an additional statistically significant contribution to predicting diastolic BP response to hydrochlorothiazide, accounting for another 1% of interindividual variation in response (P = .034). In contrast, the other polymorphisms, including the α-adducin Gly460→Trp polymorphism, made no statistically significant contributions to prediction of BP response.

Conclusions: Although we reject the null hypothesis of no genetic effects on BP response to hydrochlorothiazide, the influence of variation at single sites is likely to be small. More extensive characterization of genetic variation is required for pharmacogenetic approaches to become clinically useful in tailoring antihypertensive drug therapy for individual patients.

Key Words: Polymorphism, genetics, diuretic, pharmacology, blood pressure, hypertension.

Among hypertensive individuals treated with blood pressure (BP) lowering medications, <40% have their BP levels controlled to <140/90 mm Hg. Interindividual variation in BP responses to antihypertensive drug therapies has been well documented. Typically, for any given monotherapy, the standard deviation of response is comparable to the mean response (approximately 5 to 10 mm Hg) and the range of responses is more than four times greater. Most of this variation is due to pharmacodynamic, not pharmacokinetic, differences and reflects the heterogeneity of pathophysiologic mechanisms contributing to elevation of BP in individual patients. Except for consideration of comorbid conditions, drug selection remains largely empiric—a trial-and-error process that all too often achieves less than optimal lowering of BP.

Mapping and sequencing of the human genome, followed by increasingly detailed characterizations of DNA sequence variation, offers the promise that pharmacogenetic approaches may lead to new predictors of drug responses and may allow greater individualization (and efficacy) of antihypertensive therapy. Several candidate gene studies have provided initial “proof of concept” by rejecting the null hypothesis of no genetic effects on an...
ti hypertensive drug responses. The most compelling reports have involved genetic predictors of responses to thiazide diuretics.5,6

We previously reported that a C825T polymorphism in the gene encoding the β3-subunit of G-proteins and polymorphisms in genes encoding components of the renin-angiotensin-aldosterone system predicted interindividual differences in BP responses to hydrochlorothiazide in community-based African American and non-Hispanic white individuals with essential hypertension.7,8 The present report communicates findings regarding BP response to hydrochlorothiazide and additional candidate gene polymorphisms that have been associated with interindividual differences in BP: endothelial nitric oxide synthase Glu298→Asp, α-adducin Gly460→Trp, β1-adrenoceptor Arg389→Gly, β2-adrenoceptor Arg16→Gly, and lipoprotein lipase Ser447→Stop. Another objective was to assess additive and interactive effects among all of the measured polymorphisms in this sample.

Methods

Sample

The sample consisted of 291 unrelated non-Hispanic African American adults (150 women and 141 men) from Atlanta, GA, and 294 unrelated non-Hispanic white adults (126 women and 168 men) from Rochester, MN, who were between 30 and 59.9 years of age and who had previously diagnosed with essential hypertension. In Atlanta, study candidates were identified through lists of registered voters and other clinic, hospital, and community-based sources.7,9 In Rochester, candidates were identified through a diagnostic index maintained by the Mayo Clinic for all residents of Olmsted County.9–11 Subjects were required to have systolic BP <180 and diastolic BP <110 mm Hg; to be in good general health; and to be able to discontinue drugs that could influence BP level or the renin-angiotensin-aldosterone system or that could antagonize the effect of thiazide diuretics. Postmenopausal women were allowed to continue hormone replacement therapy. Participants were required to sign a written consent form. The institutional review boards of Emory University and the Mayo Clinic approved all study procedures, which were carried out in the General Clinical Research Center of each institution in accordance with institutional guidelines.

Protocol

The study protocol has been described previously.9 Briefly, subjects had their antihypertensive medications withdrawn and other contraindicated drugs were discontinued. A dietitian instructed each subject to ingest a standard sodium intake of 2 mmol/kg body weight/day. At a minimum of 4 weeks after discontinuing antihypertensive drug therapy, subjects were given 25 mg of hydrochlorothiazide orally each day for the next 4 weeks. Subjects’ weight and BP, their 24-h urine sodium, potassium, and aldosterone excretions, and their serum sodium and potassium concentrations were measured at the end of the washout period and after 4 weeks of diuretic therapy. The responses to diuretic therapy were calculated as differences between values at these two time points. Oral potassium supplements (20 to 40 mmol/day) were prescribed after 2 weeks of diuretic therapy if serum potassium was <3.6 mmol·L−1. At the end of the drug-free and the diuretic therapy periods, subjects slept overnight in the General Clinical Research Center. At approximately 6 AM the next morning, blood for measurement of plasma aldosterone concentration and renin activity was drawn from subjects in the seated position after a 30-min period of ambulation.

Laboratory Procedures

Electrolytes and aldosterone and renin activity were determined by ion selective electrode and radioimmunoassays, respectively, as previously described.7 Selected polymorphisms in the genes encoding nitric oxide synthase, α-adducin, the β1-adrenoceptor, β2-adrenoceptor, and lipoprotein lipase were genotyped using the TaqMan assay (Applied Biosystems, Foster City, CA), which uses PCR amplification of the region of interest and a fluorescent allele-specific ligation reaction, Allele detection and genotype calling were performed using the ABI 7700 and the Sequence Detection System software (Applied Biosystems). The sequences of all primers and probes are available from the authors on request.

Statistical Methods

Genotype and allele frequencies were calculated for each ethnic group and for each gender within ethnic groups. The χ² contingency test was used to assess differences in relative frequencies between groups. For quantitative traits, data was initially summarized by calculating means and variances for each ethnic–gender group. One-way analysis of variance was used to contrast means across the four groups, followed by the Scheffé method of multiple comparisons to contrast means between genders within each ethnic group and between ethnic groups within each gender. Multiple linear regression was used to construct models explaining interindividual differences in BP response to hydrochlorothiazide based on consideration of covariate traits and genotypic variation. Given low frequencies of the less common alleles for the endothelial nitric oxide synthase Glu298→Asp, α-adducin Gly460→Trp, and lipoprotein lipase Ser447→Stop polymorphisms, homozygotes and heterozygotes carriers were combined for these analyses, thereby reducing the number of genotype classes from three to two. Test statistics with values of P < .05 were considered to be statistically significant. All analyses were performed using SAS version 8 (SAS Institute Inc, Cary, NC).
Table 1. Descriptive characteristics

<table>
<thead>
<tr>
<th></th>
<th>Non-Hispanic African Americans (n = 291)</th>
<th>Non-Hispanic Whites (n = 294)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Women (n = 150)</td>
<td>Men (n = 141)</td>
</tr>
<tr>
<td>Age, y</td>
<td>47.7 ± 5.7</td>
<td>47.9 ± 6.5</td>
</tr>
<tr>
<td>BMI, kg · m⁻²</td>
<td>33.1 ± 7.3†</td>
<td>30.0 ± 5.1</td>
</tr>
<tr>
<td>Waist-hip ratio</td>
<td>0.84 ± 0.07</td>
<td>0.91 ± 0.06§</td>
</tr>
<tr>
<td>PRA, ng · mL⁻¹ · h⁻¹</td>
<td>0.95 ± 1.01</td>
<td>0.83 ± 0.87</td>
</tr>
<tr>
<td>PAC, ng · dL⁻¹</td>
<td>20.5 ± 10.3§**</td>
<td>13.4 ± 8.4</td>
</tr>
<tr>
<td>Uₘₐₓ,V, μg · 24h⁻¹</td>
<td>9.9 ± 6.4</td>
<td>8.3 ± 5.7</td>
</tr>
<tr>
<td>Uₙₐ,V, mmol · 24h⁻¹</td>
<td>156 ± 58</td>
<td>178 ± 74</td>
</tr>
<tr>
<td>Uₙ,V, mmol · 24h⁻¹</td>
<td>46 ± 18</td>
<td>55 ± 23*</td>
</tr>
<tr>
<td>Systolic BP, mm Hg</td>
<td>153 ± 15†**</td>
<td>147 ± 15¶</td>
</tr>
<tr>
<td>Diastolic BP, mm Hg</td>
<td>97 ± 5¶</td>
<td>97 ± 6</td>
</tr>
</tbody>
</table>

Data are means ± standard deviation. BMI = body mass index; BP = blood pressure; PAC = plasma aldosterone concentration; PRA = plasma renin activity; Uₘₐₓ,V = urinary aldosterone excretion; Uₙₐ,V = urinary sodium excretion; Uₙ,V, urinary potassium excretion. P values are given for one-way analysis of variance contrast of means across the four ethnic-gender groups. Statistical significance for pairwise contrasts of means between genders within ethnic groups is denoted by: * P < .05; † P < .01; ‡ P < .001; § P < .0001; and for contrasts of means between ethnic groups within genders by: ¶ P < .05; ¶ P < .01; #P < .001; ** P < .0001.

Results

Sample Description

The mean age of subjects varied from 47.7 ± 5.7 in African American women to 49.7 ± 6.4 years in white women. Except for urinary aldosterone excretion, means for the descriptive characteristics differed significantly across ethnic–gender groups (Table 1). As expected, in both ethnic groups, mean waist-to-hip ratio was significantly greater in men than women; and in both genders, mean plasma renin activity was significantly lower in African American than white individuals. In contrast, mean systolic BP was significantly greater in African American than in white individuals.

For each polymorphism, relative genotype and allele frequencies differed significantly between ethnic groups (Table 2). In particular, the nitric oxide synthase Asp298 allele, α-adducin Trp460 allele, β₂-adrenoceptor Gly16 allele, and lipoprotein lipase 447Stop allele were each

Table 2. Genotype and allele frequencies

<table>
<thead>
<tr>
<th>Gene</th>
<th>Polymorphism</th>
<th>Ethnicity</th>
<th>n</th>
<th>Genotype, %</th>
<th>P</th>
<th>Allele, %</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-adducin</td>
<td>Gly460Trp</td>
<td>African American</td>
<td>290</td>
<td>GG 85.2; GT 13.8; TT 1.0</td>
<td>&lt;.0001</td>
<td>Gly 92.1; Trp 7.9</td>
<td>&lt;.0001</td>
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<tr>
<td></td>
<td></td>
<td>White</td>
<td>291</td>
<td>GG 66.3; GT 27.8; TT 5.8</td>
<td></td>
<td>Gly 80.2; Asp 19.8</td>
<td></td>
</tr>
<tr>
<td>Nitric oxide</td>
<td>Glu298Asp</td>
<td>African American</td>
<td>289</td>
<td>GG 78.2; GT 21.1; TT 0.7</td>
<td>&lt;.0001</td>
<td>Gly 88.7; Asp 11.3</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>synthese</td>
<td></td>
<td>White</td>
<td>290</td>
<td>GG 52.4; GT 39.0; TT 8.6</td>
<td></td>
<td>Gly 71.9; Asp 28.1</td>
<td></td>
</tr>
<tr>
<td>β₁-adrenoceptor</td>
<td>Arg389Gly</td>
<td>African American</td>
<td>270</td>
<td>CC 39.3; CG 43.7; GG 17.0</td>
<td>&lt;.0001</td>
<td>Arg 61.1; Gly 38.9</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>White</td>
<td>274</td>
<td>CC 57.6; CG 35.8; GG 6.6</td>
<td></td>
<td>Arg 75.5; Gly 24.5</td>
<td></td>
</tr>
<tr>
<td>β₂-adrenoceptor</td>
<td>Arg16Gly</td>
<td>African American</td>
<td>289</td>
<td>CC 27.3; CG 47.4; GG 25.3</td>
<td>&lt;.0003</td>
<td>Arg 51.0; Gly 49.0</td>
<td>.0006</td>
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<tr>
<td></td>
<td></td>
<td>White</td>
<td>293</td>
<td>CC 16.7; CG 48.5; GG 34.8</td>
<td></td>
<td>Arg 41.0; Gly 59.0</td>
<td></td>
</tr>
<tr>
<td>Lipoprotein</td>
<td>Ser447Stop</td>
<td>African American</td>
<td>290</td>
<td>CC 89.0; CG 10.7; GG 0.3</td>
<td>.047</td>
<td>Ser 94.3; Stop 5.7</td>
<td>.02</td>
</tr>
<tr>
<td>lipase</td>
<td></td>
<td>White</td>
<td>291</td>
<td>CC 81.8; CG 17.9; GG 0.3</td>
<td></td>
<td>Ser 90.7; Stop 9.3</td>
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</tbody>
</table>

P values are for contrasts of genotype and allele frequencies between ethnic groups. Within ethnic–gender groups, the genotype frequencies did not differ significantly from those predicted by Hardy-Weinberg equilibrium, except for the α-adducin genotypes in white men (P = .025).
significantly more common in white than in African American subjects, whereas the \( \beta_1 \)-adrenoceptor Gly389 allele was significantly more common in African American than in white individuals.

**Prediction of BP Response to Hydrochlorothiazide**

The covariates of ethnicity, gender, age, and waist-to-hip ratio accounted for 26% of interindividual variation in systolic BP response and 11% of interindividual variation in diastolic BP response to hydrochlorothiazide (analyses not shown). When each polymorphism was considered alone (that is, without adjustment for effects of the covariates), the \( \alpha \)-adducin Gly460→Trp polymorphism and the nitric oxide synthase Glu298→Asp polymorphism were each associated with statistically significant differences in BP responses to hydrochlorothiazide (Table 3). The \( \alpha \)-adducin Trp460 allele was associated with a significantly greater decline in systolic BP, and the nitric oxide synthase Asp298 allele was associated with significantly greater declines in both systolic and diastolic BP.

After adjustment for the covariates, only the association of the Glu298→Asp polymorphism with diastolic BP response remained statistically significant. The mean adjusted decline in diastolic BP (± SEM) was \(-8.6 \pm 0.4 \) mm Hg in Asp298 homozygotes \( v \) \(-7.1 \pm 0.6 \) mm Hg in the combined group of Glu298 homozygotes + Glu298/Asp298 heterozygotes (Fig. 1). Before adjustment for the covariates, this polymorphism explained 2% of interindividual variation in diastolic BP response and afterward it accounted for 1%. There was no evidence of interaction between effects of either of these polymorphisms with the covariates (analyses not shown).

There was no evidence that any of the other measured polymorphisms in the \( \beta_1 \)-adrenoceptor, the \( \beta_2 \)-adrenoceptor, or lipoprotein lipase influenced interindividual differences in BP responses to hydrochlorothiazide (Table 3). Additional models that considered the effects of polymorphisms in two or more genes together did not significantly improve the ability to explain interindividual differences in BP responses to hydrochlorothiazide (analyses not shown). Finally, there was no evidence of interaction with polymorphisms in the \( \beta_3 \)-subunit of G-proteins or genes of the renin-angiotensin system previously reported in these subjects\(^7,8\) (analyses not shown).

**Discussion**

This study provides additional evidence that genetic variation influences antihypertensive responses to a thiazide diuretic. Before adjustment for covariates, the evidence was limited to two of the five polymorphisms measured,
namely, those in the genes encoding nitric oxide synthase and \( \alpha \)-adducin. Afterward the evidence was limited to an effect of the Glu298→Asp polymorphism of nitric oxide synthase, which explained 1% of interindividual variation in the adjusted diastolic BP response to hydrochlorothiazide.

The endothelial nitric oxide synthase Glu298→Asp polymorphism was a logical candidate to investigate for possible influence on antihypertensive drug response. Endothelial dysfunction, manifest by impairment of synthesis and release of nitric oxide, is an initiating or contributing event in most cardiovascular disease states including hypertension. Moreover, in rats with deoxycorticosterone acetate (DOCA)–salt hypertension, BP lowering response to a thiazide diuretic has been associated with increased renal nitric oxide production.12 Conversely, BP increases in response to dietary sodium have been associated with endothelial dysfunction in humans.13 As the Asp298 allele has been associated with greater enzyme susceptibility to proteolytic cleavage,14 presumably resulting in less nitric oxide, these previous reports are consistent with our finding of a smaller mean decline in diastolic BP in response to hydrochlorothiazide in subjects carrying the Asp298 allele.

Variation in the genes encoding the cytoskeletal protein adducin is responsible for 50% of the difference in BP levels between Milan hypertensive and normotensive rats.15 The effect seems to be mediated through an increase in renal tubular sodium reabsorption, leading to a volume-expanded, low-renin form of hypertension. A study of Italian families, which used a highly polymorphic marker for the gene encoding \( \alpha \)-adducin, reported evidence of linkage to a gene influencing BP level and diagnostic category, and the \( \alpha \)-adducin Gly460→Trp polymorphism predicted a twofold mean difference in BP response to hydrochlorothiazide among hypertensive subjects.5 In the present sample of African-Americans and non-Hispanic white Americans of European descent, after withdrawal of previous antihypertensive drug therapy we could not confirm an association of the Gly460→Trp polymorphism with BP levels among hypertensive subjects (analyses not shown) or with the subsequent BP responses to hydrochlorothiazide. It is also noteworthy that after covariate adjustments, the Family Blood Pressure Program did not consistently confirm evidence of linkage or association with BP levels or diagnostic category across multiple ethnic groups.16

The other polymorphisms that we investigated (namely, \( \beta_1 \)-adrenocceptor Arg389→Gly, \( \beta_2 \)-adrenocceptor Arg16→Gly, and lipoprotein lipase Ser447→Stop) have been associated with interindividual differences in BP in previous studies17–19 and therefore were also reasonable candidates to influence antihypertensive responses to hydrochlorothiazide. However, inability to detect statistically significant associations may result from several factors. First, measurement of variation at a single site within the candidate gene captures only a small proportion of total variation in the gene. In most cases, the measured variation serves only as a marker and may itself have no functional effect. Moreover, it may not be in linkage disequilibrium with variants elsewhere that are functional. Second, variation in most polymorphic genes is expected to have only a small effect on a complex phenotype such as antihypertensive drug response. This is because of interactions among effects of many other genetic and environmental factors, which act in a homeostatic network of redundant counterbalancing influences.20 Third, the small effect of variation in a single gene may be study-specific, depending on factors such as study design, sample selection, and laboratory methodologies. For these reasons, in assessing negative results in the present study we cannot exclude the possibility that variation in the genes encoding \( \alpha \)-adducin, the \( \beta_1 \) and \( \beta_2 \)-adrenocceptors, and lipoprotein lipase influences BP response to hydrochlorothiazide.

In summary, results from this and other candidate genes studies serve to reject the null hypothesis of no genetic effects on BP response to antihypertensive drug therapy. However, it is also apparent that the effects of single site polymorphisms in candidate genes are small. Thus, for pharmacogenetic approaches to achieve clinical utility will require more extensive characterization of variation within single genes as well as variation in multiple genes.

Acknowledgments
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References