β2-Adrenergic receptor genotype affects the renin-angiotensin-aldosterone system response to the Dietary Approaches to Stop Hypertension (DASH) dietary pattern

Bei Sun, Jonathan S Williams, Laura P Svetkey, Nikheel S Kolatkar, and Paul R Conlin

ABSTRACT

Background: β2-Adrenergic receptor (β2-AR) is a susceptibility locus for hypertension, and polymorphisms at this site relate to salt sensitivity and low plasma renin activity (PRA). The Dietary Approaches to Stop Hypertension (DASH) dietary pattern lowers blood pressure and appears to interact with the renin-angiotensin-aldosterone system (RAAS).

Objective: We hypothesized that the DASH diet associates with increased RAAS activity, and genotype status at β2-AR G46A modifies this response.

Design: We genotyped participants in the DASH-Sodium study (n = 372) at β2-AR G46A to determine the association with blood pressure, RAAS components, and consumption of the DASH diet. We used 2-way mixed linear regression and an additive model for all primary analyses.

Results: Mean (±SEM) PRA was significantly higher in participants in the DASH group than in participants in the control group (0.68 ± 0.03 compared with 0.54 ± 0.03 ng·mL⁻¹·h⁻¹, P = 0.002). Serum aldosterone, urinary aldosterone, and urinary potassium concentrations were also significantly higher in the DASH group (P < 0.01 for all). We observed significant gene-diet interactions for changes in systolic blood pressure (SBP) and concentrations of aldosterone and urinary potassium (P for interaction = 0.048, 0.017, and 0.001 for SBP and aldosterone and urinary potassium concentrations, respectively). There was an association between the A allele of β2-AR G46A and greater blood pressure reduction and blunted aldosterone and PRA responses to the DASH diet.

Conclusions: Our results indicate that the DASH diet lowers blood pressure and increases PRA and aldosterone concentrations. There is an association between the G46A polymorphism of β2-AR and blood pressure and RAAS responses to the DASH diet, which suggests that β2-AR may be a genetic modifier of DASH-diet responsiveness. This trial was registered at clinicaltrials.gov as NCT00000608.

INTRODUCTION

The β2-adrenergic receptor (β2-AR) functions as a mediator of the vasodilatory response to adrenergic agonists. Several genome-wide association studies suggested that the β2-AR is a susceptibility locus for blood pressure (BP) control and the risk of hypertension (1–3). A blunted vasodilation in response to β2-AR stimulation occurs in white hypertensive patients (4, 5) and normotensive African Americans (6). Furthermore, the β2-AR may affect volume homeostasis through increased renin secretion (7–9). Thus, altered vasodilation and renin secretion via the β2-AR may have a role in the pathogenesis of hypertension.

One missense mutation of the β2-AR, G46A (Gly16Arg), has been extensively studied. In vitro and in vivo studies that investigated vascular hemodynamics in patients with the β2-AR G46A polymorphism showed impaired agonist-mediated receptor down-regulation and desensitization (10, 11). The Bergen Blood Pressure Study revealed a higher frequency of Arg16 in first-born offspring of hypertensive parents (12). A study showed that 46AA (16 Arg-Arg), along with 79CC (27Gln-Gln) were also associated with a higher mortality rate after acute coronary syndrome when discharged with β-blockade (13), which added more significance to the clinical implications of this single nucleotide polymorphism (SNP). Arg16 has also been associated with a subset of hypertensive patients with low plasma renin activity (PRA) and salt sensitivity of BP (14). An association of the β2-AR G46A polymorphism with salt sensitivity and low renin hypertension may relate to the important role of sympathetic innervation in the control of renin synthesis and secretion, which is modulated by postjunctional β1 receptors and prejunctional β2 receptors (7). To our knowledge, prior studies have not examined whether the β2-AR G46A polymorphism influences BP through its effects on renin secretion.

The Dietary Approaches to Stop Hypertension (DASH) dietary pattern, which is rich in potassium, magnesium, and calcium, lowers BP at normal concentrations of sodium intake (15). The DASH-Sodium study investigated the combined effects of so-

1 From the Division of Endocrinology, Diabetes, and Hypertension, Brigham and Women’s Hospital and Harvard Medical School, Boston, MA (BS, JSW, and PRC); the Duke Hypertension Center, Duke University Medical Center, Durham, NC (LPS); the Cardiovascular Metabolic Medicines Development Centre, GlaxoSmithKline Research & Development, King of Prussia, PA (NSK); the Philadelphia Veterans Affairs (VA) Medical Center, University of Pennsylvania School of Medicine, Philadelphia, PA (NSK); and the VA Boston Healthcare System, Boston, MA (PRC).
2 BS and JSW contributed equally to this study.
3 Supported by grants R01 HL57114, R01 HL77234, K24 DK63214, and K23 HL084236 from the National Institutes of Health.
4 Address correspondence to JS Williams, Division of Endocrinology, Diabetes, and Hypertension, Brigham and Women’s Hospital, 221 Longwood Avenue, Boston, MA 02115. E-mail: jwilliams5@partners.org.

Received November 10, 2009. Accepted for publication April 30, 2010. First published online June 2, 2010; doi: 10.3945/ajcn.2009.28924.
ium restriction and the DASH diet on BP in individuals with prehypertension and stage 1 hypertension (16). Although much is known about the physiologic response to sodium restriction, the mechanisms underlying the BP response to the DASH diet remain elusive. Clues to potential mechanisms involving the renin-angiotensin-aldosterone system (RAAS) include that 1) the DASH diet enhances the BP response to an angiotensin receptor blockade (17), with larger effects seen in those with higher baseline PRA, and 2) the DASH diet is associated with enhanced natriuresis (18).

Given the possible interaction of the DASH diet with the RAAS and, in particular, PRA, we hypothesized that polymorphisms in the β2-AR would modify the BP and RAAS response to the DASH diet. To test this hypothesis, we examined the PRA and aldosterone responses to the DASH diet and assessed whether genotype status at G46A of the β2-AR gene modified the effects of the DASH diet on 1) BP and 2) PRA and aldosterone secretion.

SUBJECTS AND METHODS

Subjects and protocol

The DASH genetics study seeks to understand the genetic underpinnings of responsiveness to the DASH diet by using a candidate-gene approach. Genotyping was performed from DNA obtained from participants of both the DASH and DASH-Sodium studies, but for this specific project, we used data only from the DASH-Sodium study because its design allowed more stringent assessment of sodium intake in relation to RAAS activity. The DASH-Sodium study started in February 1997 and completed in July 2002. The study was approved by the Human Subjects Committees for each study location, and written informed consent was obtained from each participant. The details of the study have been described elsewhere (16). Briefly, at entry participants were ≥22 y of age with a systolic BP (SBP) of 120–159 mm Hg and diastolic BP of 80–95 mm Hg. After a 2-wk run-in period on a control diet that was based on the typical American intake, study participants were randomly assigned to 1 of 2 dietary patterns, the control diet or the DASH diet, for 3 consecutive 30-d periods of dietary intervention. Each diet period consisted of a fixed sodium content of 50, 100, or 150 mmol Na/d assigned in a random sequence. For the purposes of this analysis, we focused only on the high-salt (HS) intervention diet. For the purposes of this study, the DASH diet consisted of a fixed sodium content of 50, 100, or 150 mmol Na/d assigned in a random sequence. For the purposes of this analysis, we focused only on the high-salt (HS) intervention diet.

The BP response to the DASH diet was defined as the difference between the baseline (the average of 5 sets of preintervention readings) and the end of the DASH HS diet feeding period. The BP response to the control diet was similarly defined as the difference between the baseline and the end of the control diet feeding period. Results for PRA, aldosterone, and 24-h urine collections for aldosterone, sodium, and potassium were compared at baseline and at the end of the intervention feeding periods. Blood samples were collected on ice and centrifuged immediately, and the plasma was frozen until the time of assay. PRA and aldosterone were assayed by radioimmunoassay techniques as previously described (19). Urine sodium and potassium concentrations were measured by flame photometry, with lithium as an internal standard.

Genotyping

Primer sequences for G46A (rs1042713) and C79G (rs1042714) were obtained from the SNP database dbSNP (http://www.ncbi.nlm.nih.gov/projects/SNP). Genomic DNA was extracted from stored peripheral leukocytes by using standard procedures. Genotyping was performed with the MassARRAY matrix-assisted laser desorption/ionization time-of-flight mass spectrometry platform (Sequenom, San Diego, CA). Duplicate genotyping was performed in 10% of randomly selected samples to assess genotyping quality.

Statistical analyses

This project was ancillary to the main DASH-Sodium trial; all statistical analyses were performed by the authors rather than by the study’s coordinating center. Group mean BP and serum aldosterone and PRA responses to each diet (DASH and control) were compared on the basis of the genotype at G46A of β2-AR. Genotyping was performed in 412 participants. Hardy-Weinberg equilibrium was assessed by using the chi-square test and performed in each genotype subgroup. A 2-way mixed linear regression (PROC MIXED) with an exchangeable covariance matrix was used for all primary analyses assuming an additive inheritance model. The presence of a significant interaction by using the likelihood ratio test with 1 df was considered to indicate that genotype status–influenced response to the DASH diet. The changes of SBP, PRA and serum aldosterone and 24-h urinary potassium concentrations were the outcome variables. Because the BP response to the DASH diet is enhanced in African-Americans (20), we included an adjustment for race in our analyses. Additional adjustments included baseline values for SBP, PRA, aldosterone and urinary potassium concentrations, sex, and age, which were presented in the model as fixed effects, and the study site was included as a random effect. Statistical analyses were performed with SAS version 9.1 (SAS Institute, Cary, NC). Statistical significance required a 2-sided P value <0.05.

RESULTS

Complete genotypes were obtained in 372 of 412 study participants (215 African Americans and 157 whites) with a concordance rate of >99%. The distribution of demographic characteristics according to β2-AR G46A genotype in the DASH and control diet groups is shown in Table 1. Demographic characteristics and baseline SBP, PRA, and serum aldosterone concentrations were similar across β2-AR G46A genotypes except for 24-h urinary potassium concentrations in the DASH diet (P = 0.03). The frequency of the AA genotype was substantially higher in African Americans (27%) than in whites (16%) (P = 0.03), which was consistent with previous reports (21). Hardy-Weinberg equilibrium was confirmed in the 2 diet groups (P > 0.4; chi-square) and 2 racial groups (P > 0.3; chi-square).

The absolute values of BP and RAAS components at baseline and at the end of diet intervention periods are shown in Table 2. There were no differences in baseline characteristics of the DASH and control groups except for urinary potassium concentrations, which were higher in the DASH group than in the control group (P = 0.048). At the end of diet, participants...
reached a steady state sodium balance, with no difference in sodium concentrations between the 2 groups. BP was significantly lower on the DASH diet than on the control diet after adjusting for the baseline BP. In addition, participants who consumed the DASH diet had a significantly higher PRA than those on the control diet (mean ± SEM: 0.68 ± 0.03 compared with 0.54 ± 0.03 ng·mL⁻¹·h⁻¹; P = 0.002). Furthermore, serum aldosterone, urinary aldosterone, and urinary potassium concentrations were significantly higher on the DASH diet than on the control diet (P < 0.01 for each).

There was a significant gene-diet interaction between the β2-AR and diet (DASH compared with control) for SBP and aldosterone and urinary potassium concentrations. The PRA shared the same qualitative differences but was not significant (P = 0.11) (Figure 1). Specifically, the A allele was associated with greater SBP reduction with the DASH diet, and the GG genotype had no significant SBP change. Furthermore, although the AA genotype showed no response in aldosterone concentrations and PRA, the GG genotype had a significant increase in each of them with the DASH diet. Corresponding trends were observed for urinary potassium because subjects with the GG genotype had the highest urinary potassium concentrations and subjects with the AA genotype had the lowest urinary potassium concentrations. The removal of covariates for race, age, and sex from the model did not materially change the interaction between G46A genotype and diet for the above phenotypes.

We also genotyped another closely related SNP C79G (Gln27Glu). The 2 SNPs A46G (Arg16Gly) and C79G (Gln27Glu) were in strong linkage disequilibrium (r² = 0.70 and D' = 1.0 for whites and r² = 0.42 and D' = 0.96 for African Americans). The effects of SNP C79G on BP and RAAS responses to the DASH diet were qualitatively the same as A46G, as genotype 79CC (27Gln) had the greatest SBP reduction and blunted RAAS activation. When we examined the 2 loci

<table>
<thead>
<tr>
<th>Variable</th>
<th>DASH diet (n = 189)</th>
<th>Control diet (n = 183)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>End of diet</td>
</tr>
<tr>
<td>SBP (mm Hg)</td>
<td>134.9 ± 0.6</td>
<td>127.7 ± 0.5</td>
</tr>
<tr>
<td>DBP (mm Hg)</td>
<td>85.4 ± 0.3</td>
<td>81.3 ± 0.4</td>
</tr>
<tr>
<td>PRA (ng·mL⁻¹·h⁻¹)</td>
<td>0.62 ± 0.04</td>
<td>0.68 ± 0.03</td>
</tr>
<tr>
<td>Serum aldosterone (ng/dL)</td>
<td>9.8 ± 0.4</td>
<td>10.7 ± 0.4</td>
</tr>
<tr>
<td>Urinary aldosterone (mg/24 h)</td>
<td>8.1 ± 0.5</td>
<td>10.1 ± 0.4</td>
</tr>
<tr>
<td>Urinary sodium (mmol/24 h)</td>
<td>163.0 ± 5.4</td>
<td>146.3 ± 3.9</td>
</tr>
<tr>
<td>Urinary potassium (mmol/24 h)</td>
<td>57.7 ± 1.6</td>
<td>76.5 ± 1.5</td>
</tr>
</tbody>
</table>

1 All values are means ± SEMs. DASH, Dietary Approaches to Stop Hypertension; SBP, systolic blood pressure; DBP, diastolic blood pressure; PRA, plasma renin activity. Comparisons between DASH and control groups were made by using the general linear model. All baseline variables were adjusted for age, sex, and race, and additional adjustment for baseline values was made for end-of-diet measurements. When DASH and control groups were compared at the end of diet, DASH participants had significantly lower SBP and DBP and higher PRA and concentrations of serum aldosterone, 24-h urinary aldosterone, and 24-h urinary potassium (P < 0.01). There were no differences in baseline characteristics of participants between DASH and control groups except that 24-h urinary potassium concentrations were higher in the DASH group than in the control group (P = 0.048).

2 Participants reached a steady state sodium balance, and there were no differences in end-of-diet 24-h urinary sodium concentrations between DASH and control groups.
DISCUSSION

Although the DASH diet has been clearly shown to significantly reduce BP, the underlying mechanisms of its effect have yet to be defined to our knowledge. The current study provides clues to the interaction between the DASH diet and the RAAS, specifically through increases in PRA and aldosterone concentrations. These responses are consistent with a possible natriuretic effect of the DASH diet (18). We also observed an interaction between the genotype at the β2-AR (G46A) and the aldosterone and BP responses to the DASH diet. Individuals carrying the A allele displayed greater BP lowering in response to the DASH diet, whereas those with the GG genotype had no significant effect. This suggests that the β2-AR may be a genetic modifier of BP and RAAS responses to the DASH diet. Of note, the apparent lack of SBP reduction in the GG genotype was accompanied by an increased RAAS activity.

The DASH diet is an important lifestyle intervention in the treatment of hypertension (15, 22), but specific mechanisms that underlie its effects on BP are largely unknown. Several factors support RAAS involvement in the DASH effect on the cardiovascular system. The angiotensinogen gene G-6A polymorphism, which is associated with an enhanced BP response to sodium restriction and converting enzyme inhibition, is associated with increased BP responsiveness to the DASH diet (23). The BP lowering effect of the DASH diet is enhanced by an angiotensin receptor blocker, which correlates with higher concentrations of baseline PRA (17). In this study we observed an increase in PRA and serum and urine aldosterone and urinary potassium concentrations in response to the DASH diet. Together, these data provide a strong rationale for investigating the physiologic and genetic mechanisms that underlie the DASH effect.

β2-AR is a reasonable candidate to investigate for involvement in the pathogenesis of hypertension. β2-AR might influence BP through sympathetic nervous system-mediated effects on vascular tone and cardiac contractility as well as regulation of renin expression and release (4, 9, 24–26). There is a growing body of evidence that shows that β2-AR A46A homozygotes have blunted receptor function compared with the receptor function of 46GG homozygotes. For example, the AA genotype is associated with attenuated vasodilation in response to local infusion of the α agonist isoproterenol (10, 27). The effect of A46G on the arterial response to isoproterenol was shown to be nitric oxide dependent (28) and could be modulated by salt restriction (29). We observed an association between a blunted PRA and aldosterone response and the 46AA and AG genotypes among individuals who consumed the DASH diet. This may imply that the DASH diet, via direct or indirect effects on renal sympathetic nerves and its affects on local nitric oxide formation, increases RAAS activity in individuals with the GG genotype. We posit that these responses are actually counter-regulatory and therefore blunt the BP response to the diet. In contrast, those with the A allele do not manifest the same degree of counterregulatory effects, and their BP response is greater. Thus, the A allele of the β2-AR G46A polymorphism may be associated with an underlying defect in the renin response to changes in intravascular volume or sodium intake. As further support of this hypothesis, G46A has been associated with low-renin hypertension and salt sensitivity of BP (14). This association between the presence of the A allele, a greater SBP response to

**FIGURE 1.** Systolic blood pressure (SBP), plasma renin activity (PRA), serum aldosterone (ALDO), and 24-h urinary potassium (UK) responses to each diet by genotype status at β2-AR A46G. Open and closed bars represent data for the control diet and DASH diet, respectively. P values for genotype-diet interaction are shown at the top of each panel. ***DASH diet compared with the control diet: *P < 0.05, **P < 0.001 (2-way mixed linear regression and additive model).**

together among those who consumed the DASH diet, the change of SBP was greatest between homozygotes for 46AA (Arg16 + 47CC [Gln27]) compared with 46GG (Gly16) + 47GG (Glu27) (change in SBP: −7.90 ± 1.54 compared with −6.03 ± 2.10 mm Hg). Subjects with 46GG + 79CC homozygotes had an intermediate response (change in SBP: −6.38 ± 1.93 mm Hg). These differences were not significant. PRA and aldosterone responses were qualitatively similar but significantly different. Subjects with 46GG + 79GG homozygotes had significantly greater changes than subjects with 46AA + 79CC homozygotes (change in PRA: 0.46 ± 0.11 compared with 0.09 ± 0.07 ng·mL⁻¹·h⁻¹; P = 0.01; change in aldosterone concentrations: 5.49 ± 0.16 compared with 0.37 ± 0.87 ng/dL; P = 0.001).
the DASH diet, and a lower PRA may imply that individuals who are salt sensitive would also respond favorably to the DASH diet.

It has been suggested that $\beta_2$-AR influences renin release in animal studies (7–9). However, there is very limited evidence for a role of $\beta_2$-AR on renin secretion in humans. Indeed, $\beta_1$-AR primarily mediates direct effects of catecholamines on juxtaglomerular cells and renin release. Because the DASH diet produces such large systemic BP effects, it is very likely that many compensatory mechanisms are engaged, and these could include the activation of the sympathetic nervous system and the RAAS, and these effects may intersect at the level of the juxtaglomerular cell. For example, the activation of $\beta_2$-AR may have indirect effects on renin secretion because presynaptic $\beta_2$-AR regulate norepinephrine release at multiple levels of the sympathetic nervous system. Altered signaling at these presynaptic sites, either within or external to the kidney, could modify the known postsynaptic $\beta_1$ effects on renin release. However, aside from the observations made in this study, any speculation on specific mechanisms affected by the DASH diet must be inferential.

The results of this analysis should be considered in the context of its retrospective design and the heterogeneity of the study population. Although we did find more of the AA genotype in African Americans than in whites, there was no evidence of an interaction between race and genotype for any of the outcome phenotypes in this study. Further, we conducted a stratification analysis in each racial group that showed similar trends as seen in the combined groups but with less significance because of inadequate power. The controlled-feeding protocol of the DASH-Sodium study reduced possible confounding on the assessment and correlation of RAAS activity that occurs with uncontrolled dietary sodium intake. The aldosterone and urinary potassium results support the hypothesis that an interaction between RAAS activity and $\beta_2$-AR polymorphisms exists; more substantial evidence of a specific DASH relation would require replication in a similarly studied population.

In conclusion, our results show that the $\beta_2$-AR G46A polymorphism is associated with increased BP responsiveness to the DASH diet and altered activation of the RAAS, which suggest that there is an interaction of this genotype with renin release. Additional investigation is required to link altered $\beta_2$-AR activity and the BP response to the DASH diet and whether genotyping at $\beta_2$-AR may be used to predict DASH-diet responsiveness.

We thank the study participants for their sustained commitment to the study, the DASH Collaborative Research Group for their dedication to the successful completion of the DASH-Sodium trial, and William M Vollmer for his very thoughtful advice in preparing this manuscript for publication.

The authors’ responsibilities were as follows—BS, JSW, and NSK: data collection and analysis; BS and JSW: writing of the manuscript; JSW, LPS, and PRC: study design and supervision for his very thoughtful advice in preparing this manuscript for publication.

The DASH Collaborative Research Group for their dedication to the successful completion of the DASH-Sodium trial, and William M Vollmer for his very thoughtful advice in preparing this manuscript for publication.

The authors’ responsibilities were as follows—BS, JSW, and NSK: data collection and analysis; BS and JSW: writing of the manuscript; JSW, LPS, and PRC: study design and supervision for his very thoughtful advice in preparing this manuscript for publication.

The DASH Collaborative Research Group for their dedication to the successful completion of the DASH-Sodium trial, and William M Vollmer for his very thoughtful advice in preparing this manuscript for publication.

The authors’ responsibilities were as follows—BS, JSW, and NSK: data collection and analysis; BS and JSW: writing of the manuscript; JSW, LPS, and PRC: study design and supervision for his very thoughtful advice in preparing this manuscript for publication.
