

Multilocus effects of the renin–angiotensin–aldosterone system genes on blood pressure response to a thiazide diuretic

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ABSTRACT

Background: The renin–angiotensin–aldosterone (RAA) system regulates blood pressure (BP) levels and influences responses to antihypertensive medications. Variation in RAA system genes has been reported to influence interindividual differences in BP levels and the occurrence of hypertension (HTN).

Methods: We evaluated the relationship between variation in genes of the RAA system and interindividual differences in BP response to a thiazide diuretic. Analyses were carried out in a race- and gender-specific manner in 255 unrelated hypertensive African-Americans (125 men and 130 women) and 246 unrelated hypertensive non-Hispanic Whites (133 men and 113 women).

Results: The angiotensin II receptor (AT₁R) A1166C and angiotensinogen G-6A polymorphisms had a significant effect on systolic BP response to the diuretic in African-American women. Multilocus analyses indicated that the effects of these genes combined additively to influence response. Results of a permutation test to adjust for multiple comparisons and the possible nonindependence among genotypes remained significant at the $P=0.003$ level.

Conclusions: Among African-American women, particular gene variations in the RAA system have additive effects on BP response to a thiazide diuretic. *The Pharmacogenomics Journal* (2004) 4, 17–23. doi:10.1038/sj.tpj.6500215

Keywords: hypertension; diuretics; genetics; RAA system; African-Americans; gender; polymorphisms

INTRODUCTION

Hypertension (HTN), a major risk factor for cardiovascular disease, stroke, and renal disease, affects approximately 50 million Americans.¹ Pharmacologic management of HTN is not universally effective.² Part of the blood pressure (BP)-management challenge in patients with HTN is inter-individual variation in responses to antihypertensive medications. For thiazide diuretics, which are the most frequently prescribed class of antihypertensive drugs,³ the inter-individual standard deviation in BP change in response to diuretic monotherapy is approximately equal to the mean BP change.⁴ In addition, BP response to a diuretic differs with race and gender, with African-Americans and women having greater BP responses than their non-Hispanic White and male counterparts.⁵

Drug therapies that target the renin–angiotensin–aldosterone (RAA) system have proven useful in HTN control,⁶ and, because of that, the system has been the subject of genetic studies of BP and HTN.⁷ The RAA system regulates BP levels and fluid and electrolyte balance through multiple actions of angiotensin II, a potent vasoconstrictor hormone that also enhances renal tubular sodium reabsorption through direct and indirect (via aldosterone) effects on proximal and distal nephron segments, respectively.⁸ Each of the genes of the RAA system: angiotensinogen (AGT), renin (REN), angiotensin-converting enzyme (ACE), angiotensin II receptor (AT₁R), and aldosterone synthase (CYP11B2) have been mapped, sequenced, and systematically characterized for genetic variation.⁹ Since the activity of the RAA system, which has been inversely related to BP response to a diuretic, is regulated in a coordinated fashion with feedback control,¹⁰ analyses of the influence of RAA system gene variation on response to diuretics should take into account multiple genes and the possibility of gene–gene interaction. In this analysis, we have investigated the role of variation in five structural genes encoding components of the RAA system on BP response to a thiazide diuretic. The polymorphisms selected for study were those previously associated with BP levels and the diagnosis of HTN. The results indicate that RAA system genetic variation is significantly associated with BP response to a thiazide diuretic in African-American females, and that the significant gene effects combine additively to influence BP response.

RESULTS

The study sample was comprised of 255 unrelated hypertensive African-Americans (125 men and 130 women) recruited at the Emory University in Atlanta, and 246 unrelated hypertensive non-Hispanic Whites (133 men

and 113 women) recruited at the Mayo Clinic in Rochester, MN (Table 1). The mean age of all study participants was 48 years (SD = 6.7). Significant gender and ethnic differences were observed for the waist–hip ratios ($P = 0.0001$); the mean waist–hip ratios were 0.84 ± 0.07 and 0.91 ± 0.06 for non-Hispanic African-American females and males, respectively, and 0.89 ± 0.10 and 0.98 ± 0.06 for non-Hispanic White females and males, respectively. The mean baseline systolic BP was 146 mmHg (SD = 14 mmHg), and the mean duration of HTN was 6.4 years (SD = 7.0). The mean baseline systolic BP was 154 mmHg (SD = 16 mmHg) for African-American females, 147 mmHg (SD = 15 mmHg) for African-American males, 144 mmHg (SD = 12 mmHg) for White females, and 140 mmHg (SD = 12 mmHg) for White males. These values were significantly different among the four ethnic gender groups ($P < 0.0001$). The initial study results regarding the clinical predictors of systolic and diastolic BP responses have been previously published.⁴ Genotype and allele frequencies differed significantly between African-Americans and Whites for each RAA system gene (Table 2). Since the REN (A7174G) gene was not polymorphic in Whites, analysis of BP response in Whites did not consider the effects of this locus.

We first considered the single-locus effects on the systolic BP response of each RAA system gene. In African-American females, BP-lowering response increased progressively and significantly with increasing number of AGT –6A alleles (–11.3 mmHg for GG, –18.2 mmHg for AG, and –22.2 mmHg for AA) ($P = 0.032$). The M235T allele of the AGT gene was also significant, but did not add additional information beyond the –6 polymorphism, because of their strong linkage disequilibrium (data not shown). The same type of relationship was observed at the AT₁R locus for the number of AT₁R 1166A alleles (–14 mmHg for CC, –15.6 mmHg for AC, and –22.5 mmHg for AA) ($P = 0.038$). Similar trends were observed for White females, but the

Table 1 Descriptive characteristics

	Non-hispanic blacks		Non-hispanic whites		P-value
	Women n = 130	Men n = 125	Women n = 113	Men n = 133	
Age (years)	48.1 ± 5.5	47.5 ± 6.5	49.6 ± 6.5	47.9 ± 8.0	0.08
BMI (kg/m ²)	33.6 ± 7.5	30.1 ± 5.1	31.1 ± 6.2	30.8 ± 5.0	<0.0001
Waist–hip ratio	0.84 ± 0.07	0.91 ± 0.06	0.89 ± 0.10	0.98 ± 0.06	<0.0001
Duration of HTN (years)	8.3 ± 7.1	7.8 ± 7.6	3.7 ± 5.0	5.5 ± 6.5	<0.0001
<i>Baseline blood pressure (mmHg)</i>					
Systolic	154 ± 16	147 ± 15	144 ± 12	140 ± 12	<0.0001
Diastolic	97 ± 5	97 ± 6	94 ± 5	96 ± 5	<0.0001
<i>Blood pressure response (mmHg)</i>					
Systolic	–21.0 ± 14.0	–14.9 ± 12.5	–11.9 ± 12.2	–9.0 ± 10.9	<0.0001
Diastolic	–10.6 ± 8.3	–8.7 ± 8.7	–6.6 ± 7.9	–5.7 ± 6.9	<0.0001

Table entries are means ± SD. P-values are given for one-way ANOVA contrast of means across the four ethnic-gender group.

Table 2 Renin–angiotensin–aldosterone system genotype and allele frequencies*

Gene	Polymorphism	Ethnicity	n	Genotype (%)			P-value	Allele (%)		P-value
				AA	AG	GG		A	G	
AGT (angiotensinogen)	G-6A	African-American	255	73.7	24.7	1.6	<0.0001	86.1	13.9	<0.0001
		White	246	29.7	39.4	30.9		49.4	50.6	
AT ₁ R (angiotensin II receptor, type 1)	A1166C	African-American	255	80.0	18.4	1.6	<0.0001	89.2	10.8	<0.0001
		White	246	41.9	50.0	8.1		66.9	33.1	
ACE (angiotensin-converting enzyme)	Insertion/deletion	African-American	255	II	ID	DD	0.01	I	D	0.04
		White	246	33.3	51.8	14.9		59.2	40.8	
CYP11B2 (aldosterone synthase)	C-344T	African-American	255	CC	CT	TT	<0.0001	C	T	<0.0001
		White	246	3.5	33.7	62.8		20.4	79.6	
REN (renin)	A7174G	African-American	255	AA	AG	GG	0.0002	A	G	<0.0001
		White	246	93.3	6.3	0.4		100.0	0	

*P-values shown are for a χ^2 test of differences in genotype or allele frequencies between African Americans and Whites.

Table 3 Average systolic BP response by genotype in each of the four groups

Gene	Ethnicity	BP response: mean (SD) (mmHg)							
		Genotype		Genotype		Genotype		P	
AGT	AA female	A/A	N	A/G	N	G/G	N		0.032
		–22.2 (14.4)	95	–18.2 (12.8)	32	–11.3 (8.0)	3		
		AA male	93	–17.6 (12.5)	31	–4.0	1	0.752	
		White female	30	–11.7 (11.1)	49	–9.9 (10.7)	34	0.403	
AT ₁ R	AA female	A/A	N	A/C	N	C/C	N	0.038	
		–22.5 (14.5)	101	–15.6 (11.5)	26	–14.0 (4.4)	3		
		AA male	103	–16.5 (12.6)	21	–39	1		0.106
		White female	50	–11.4 (12.4)	54	–8.4 (8.8)	9		0.673
ACE	AA female	D/D	N	D/I	N	I/I	N	0.962	
		–19.8 (14.5)	39	–21.9 (13.7)	74	–19.7 (14.8)	17		
		AA male	46	–14.8 (12.9)	58	–12.9 (11.4)	21		0.588
		White female	33	–11.2 (10.2)	51	–14.5 (11.9)	29		0.135
CYP	AA female	C/C	N	C/T	N	T/T	N	0.841	
		–14.2 (12.5)	5	–21.7 (12.7)	45	–21.0 (14.8)	80		
		AA male	4	–14.3 (11.4)	41	–15.0 (13.0)	80		0.658
		White female	27	–11.3 (11.6)	54	–10.0 (12.1)	32		0.438
REN	AA female	A/A	N	A/G	N	G/G	N	0.251	
		–20.4 (13.6)	119	–28.8 (17.7)	10	–14.0	1		
		AA male	119	–19.8 (15.1)	6	—	0		0.469
		White male	21	–9.7 (10.6)	77	–8.0 (12.4)	35		0.930

P-values are univariate values that are a contrast of means among genotypes adjusted for waist–hip ratio, age, and baseline BP.

Table 4 Multiple regression analysis of BP response by paired genotypes in each of the four groups

African-American female	P-value #1	P-value #2	R ²	African-American male	P-value #1	P-value #2	R ²
Covariates alone			0.18	Covariates alone			0.17
AGT+AT ₁ R	0.041	0.048	0.23*	AGT+AT ₁ R	0.583	0.094	0.19*
AGT+ACE	0.033	0.995	0.21	AGT+ACE	0.723	0.574	0.17
AGT+CYP	0.033	0.852	0.21	AGT+CYP	0.778	0.676	0.17
AGT+REN	0.039	0.314	0.22	AGT+REN	0.762	0.474	0.17
AT ₁ R+ACE	0.038	0.847	0.21	AT ₁ R+ACE	0.104	0.564	0.19
AT ₁ R+CYP	0.037	0.737	0.21	AT ₁ R+CYP	0.114	0.757	0.19
AT ₁ R+REN	0.026	0.162	0.22	AT ₁ R+REN	0.119	0.553	0.19
ACE+CYP	0.952	0.840	0.18	ACE+CYP	0.551	0.611	0.17
ACE+REN	0.963	0.253	0.19	ACE+REN	0.529	0.429	0.17
CYP+REN	0.927	0.259	0.19	CYP+REN	0.608	0.443	0.17

White female	P-value #1	P-value #2	R ²	White male	P-value #1	P-value #2	R ²
Covariates alone			0.18	Covariates alone			0.28
AGT+AT ₁ R	0.416	0.705	0.19*	AGT+AT ₁ R	0.649	0.532	0.28*
AGT+ACE	0.336	0.118	0.21	AGT+ACE	0.705	0.916	0.28
AGT+CYP	0.381	0.413	0.20	AGT+CYP	0.675	0.896	0.28
AT ₁ R+ACE	0.618	0.130	0.21	AT ₁ R+ACE	0.548	0.929	0.28
AT ₁ R+CYP	0.728	0.462	0.19	AT ₁ R+CYP	0.559	0.980	0.28
ACE+CYP	0.147	0.486	0.21	ACE+CYP	0.859	0.941	0.28

*Pairwise genotype R²'s have been adjusted for waist-hip ratio, age, baseline BP. The first P-value corresponds to the first gene in the pair, and the second P-value corresponds to the second gene.

differences for either single-locus genotype did not reach statistical significance in this group (Table 3). In multiple linear regression analyses of BP response that controlled for the effects of waist-hip ratio, age, and baseline BP, the polymorphisms in the AT₁R gene (P=0.038) and the AGT gene (P=0.032) each remained significant additional predictors of systolic BP response in African-American females.

The next step was to determine the multilocus effects of the RAA system genes on systolic BP response to the diuretic. Initially, all pairwise combinations of loci were considered (Table 4). The only two-locus combination of polymorphisms significantly influencing BP response was that involving the AT₁R and AGT genes in African-American females. Results of a permutation test used to adjust for multiple comparisons while taking into account the non-independence among genotypes remained significant at the P=0.003 level. Further analysis indicated that there was no evidence of interaction between the effects of the AT₁R and AGT polymorphisms. In other words, the effects of these two loci appeared to combine additively to influence BP response. In African-American females, BP response increased with the number of AGT -6A and AT₁R 1166A alleles (Figure 1).

None of the polymorphisms in the other three RAA system genes significantly improved the predictive ability of the two-locus combination of the AGT and AT₁R polymorphisms in African-American females or any of the other groups. The multilocus effects of the RAA system genes on

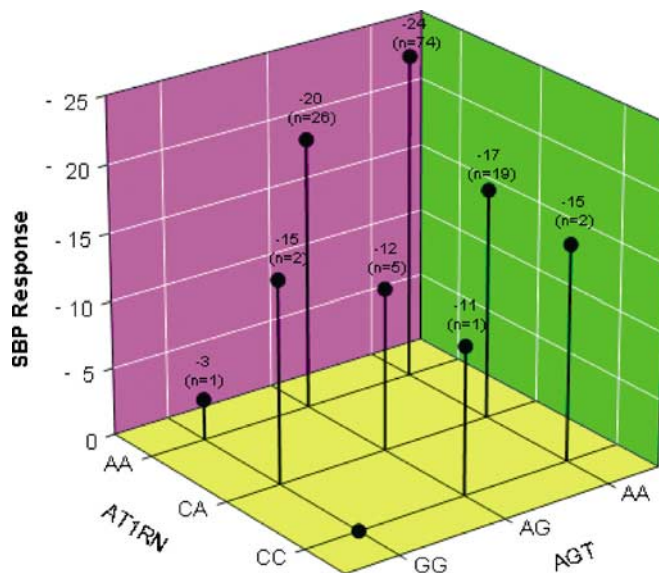


Figure 1 Combined effects of AGT and AT₁R on systolic BP response to a thiazide diuretic in African-American females. Adjusted for waist-hip ratio, age, baseline BP.

the diastolic BP response to the diuretic were not significant in non-Hispanic Whites or African-Americans in either gender.

DISCUSSION

The data presented here suggest that, among African-American females, variation in the AGT and AT₁R genes influences response to the BP-lowering effects of a thiazide diuretic. Multilocus analyses indicate that the effects of variation in the two genes combine additively to influence BP response. Variation in the REN, ACE, and CYP genes, which have previously been associated with interindividual variation in BP levels and the diagnosis of HTN,^{11–13} did not significantly influence BP response to the diuretic in any ethnic-gender group. However, these data cannot rule out the possibility that other DNA sequence variations in these genes may influence response to the diuretic. The AGT G-6A and AT₁R A1166C polymorphisms may influence BP responses through effects on the generation or action of angiotensin II.¹⁴ Typically hypertensive patients who have greater BP lowering in response to diuretics have smaller increases in renin and aldosterone, and presumably angiotensin II, that is, consistent with less counter-regulatory activation of the RAA system in response to the initial sodium-volume reduction following initiation of diuretic therapy.¹⁵ The observed association of the AGT –6A allele with greater BP response to hydrochlorothiazide is unexpected, since this variant was previously associated with greater angiotensinogen generation, which would be predicted to enhance counter-regulatory response to the diuretic. However, this unexpected association of the –6A allele with greater BP response to hydrochlorothiazide mirrors its similarly unexpected association with greater BP response to dietary sodium restriction.¹⁶ In contrast, the AT₁R –1166C variant has been associated with a greater number of binding sites or receptor affinity for angiotensin II at the cellular level,¹⁴ implying enhanced angiotensin II activity.¹⁷ Consequently, the observed association of the –1166C allele with diminished BP response is consistent with the conventional notion of an inverse relationship between BP lowering in response to a diuretic and activity of the RAA system.

The knowledge base on associations of the AGT and AT₁R gene variations with BP levels and HTN status is extensive.^{18,14} Accumulating data from other studies on the effects of single gene studies of the RAA system on BP measures indicate that effects of the insertion/deletion polymorphism of the ACE gene may be gender specific.¹⁹ Polymorphisms in the AGT have been reported to predict the differences in BP response to ACE inhibition,²⁰ and the AT₁R gene polymorphisms have been reported to predict the differences in renal hemodynamic responses to angiotensin II receptor blockade.²¹ Evidence of gene–gene interaction among effects of polymorphisms in these three genes of the RAA system has been reported with respect to occurrence of HTN.²² This study is the first to report an association between the combined effects of polymorphisms in the AGT and AT₁R genes, and BP response to a thiazide diuretic.

In this study, the additive effect of the AGT and AT₁R loci on the BP response to a thiazide diuretic was limited to African-American females. Although female hormones may contribute to gender differences in BP levels and responses

to antihypertensive drug therapy, additional unmeasured genetic and environmental effects may also interact to account for the gender × ethnic differences observed in this study. A gender interaction to BP response to a diuretic was first reported with the ACE I/D polymorphism, with the D allele associated with greater BP response to a diuretic in men and the I allele associated with greater response in women.²³ Previous studies have reported gender differences in AGT genotype and BP levels.²⁴ The Copenhagen City Heart Study²⁵ reported that women who were homozygous for the T allele of the M235T polymorphism had higher levels of angiotensinogen than heterozygotes or women who were noncarriers, and that the levels were positively correlated with BP in women, but not in men. The M235T polymorphism is in tight linkage disequilibrium with the functional AGT G-6A polymorphism in most populations²⁶ and in the sample analyzed in this study (data not shown). One conclusion emerging from this study and previously reported pharmacogenetic and pharmaco-epidemiologic data is that application of genetic and other predictive information to tailor drug therapies will need to take into account both gender and ethnic background.

MATERIAL AND METHODS

Subjects

African-Americans were recruited at the Emory University in Atlanta, GA, and non-Hispanic Whites were recruited at the Mayo Clinic in Rochester, MN, USA. The diagnosis of essential HTN was determined clinically by BP levels greater than 140/90 mmHg in the absence of known causes or a previous diagnosis of essential HTN in the presence of the current prescription antihypertensive drug therapy. The institutional review boards at all participating institutions approved the study protocol.

Details of the study protocol have been described previously.⁴ Briefly, the protocol included dietary counseling to stabilize the daily sodium intake at approximately 150 mmol/day, removal from previous BP-lowering medications for at least a 4-week washout period, followed by 4 weeks of diuretic therapy with 25 mg of hydrochlorothiazide. Subjects were counseled to maintain a dietary sodium intake of 2 mmol/kg/day. Dietary sodium was determined through urine sodium levels and 24-h recall diaries. Subjects experiencing symptoms related to HTN or systolic BP measurements above 180/110, or diastolic BPs remaining under 90 mmHg during the washout period were withdrawn from the study protocol. Physical, hemodynamic, and biochemical measurements were taken at the end of the ≥4-week washout period (baseline) and at the end of 4-week diuretic treatment period. BP measurements were taken with a mercury sphygmomanometer in the dominant arm in a seated position by nursing staff at the General Clinical Research Center. BP response to hydrochlorothiazide was the difference between the BP readings taken after 4 weeks of drug therapy and BP taken at baseline entrance after at least 4 weeks of withdrawal from previous antihypertensive medications.

Laboratory and genetic analyses

Typing of the AGT G(-6)A, AT₁R A1166C, CYP11B2 C(-344)T, and REN A7174G polymorphisms was carried out using the TaqMan assay (Applied Biosystems, Foster City, CA, USA), which uses PCR amplification of the region of interest followed by 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, Foster City, CA, USA). Typing of the ACE insert/deletion polymorphism was carried out using a two-stage PCR reaction to control for differential amplification of the insertion and deletion alleles, followed by gel electrophoresis. All gels were scored by two independent observers. The sequences of all primers and probes are available from the authors upon request.

Statistical analysis

All analyses were stratified by race and gender (four groups) because of differing allele frequencies between the races, and the possibility of gender-specific effects of the RAA system polymorphisms. *P*-values less than 0.05 were considered statistically significant. χ^2 contingency tests were used to analyze the differences in genotype and allele frequencies among groups.

Multivariable linear regression analysis, with the possibility of interaction terms, was used to assess the predictive ability of the RAA system gene variations. Single-site polymorphisms in each of the five RAA system candidate genes AGT, REN, ACE, AT₁R, and CYP were examined first individually and then in all paired combinations, controlling for baseline BP, age, and waist-hip ratio. For each paired combination with significant effects on BP response, each of the remaining individual gene polymorphisms was then added to the model to determine whether the predictive value of any additional locus was significant. In order to verify that the significant results of all the pairwise genotype-phenotype analyses were not the result of multiple comparisons, permutation methods were used to derive an adjusted *P*-value. The dependent variable, systolic BP response, was permuted among individuals 5000 times and all the 10 pairwise regressions carried out. The number of times that both *T*-values were greater than the observed *T*-value was tabulated, and is reported in the Results section.

DUALITY OF INTEREST

None declared.

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