

Effects of Angiotensinogen and Angiotensin II Type I Receptor Genes on Blood Pressure and Left Ventricular Mass Trajectories in Multiethnic Youth

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The objective of this study was to evaluate the impact of variations of the angiotensinogen (AGT) and angiotensin II type I receptor (AGTR1) genes on progression of blood pressure (BP) and left ventricular mass (LVM) in multiethnic youth. The study was longitudinal involving 581 European American (EA) and African American (AA) youth with 12 assessments over a 15-year period. AGT M235T and three AGTR1 polymorphisms (C-521T, L191L and A1166C) were genotyped and individual growth curve modeling analyses were conducted. Single nucleotide polymorphism (SNP) analyses found a significant 3-way interaction between M235T, ethnicity and gender on BP levels. Systolic BP (SBP) levels were 5.8 mmHg ($p = .00003$) and diastolic BP (DBP) levels were 2.6 mmHg ($p = .005$) lower in carriers versus noncarriers of the M235 allele in AA males only. Furthermore, the AGTR1 L191 allele showed a SBP lowering effect in subjects with a high socioeconomic status (SES; $p = .048$) and a DBP lowering effect in AAs ($p = .038$). Haplotype analyses identified a protective haplotype (C-521, 191L and A1166) for LVM levels ($p = .03$). LVM in individuals homozygous for this haplotype was 12.9 g lower than those homozygous for the most common haplotype (-521T, 191L and A1166). No significant interactions were found between the AGT M235T polymorphism and any of the single SNPs or haplotypes of the AGTR1 gene. Our results in multiethnic youth uncover an ethnicity and gender-specific effect of the AGT M235T polymorphism and a SES or ethnicity-specific effect of the AGTR1 L191L polymorphism on the progression of hypertension risk. A protective AGTR1 haplotype for LVM was also identified.

renin it produces angiotensin I, which in turn is cleaved by angiotensin-converting enzyme to produce the active octapeptide pressor hormone angiotensin II. In humans, most of the known effects of angiotensin II are mediated by the angiotensin II type I receptor (AGTR1). Over the last decade a series of reports have investigated the effect of AGT and AGTR1 genes on blood pressure (BP) regulation and cardiac structure with the most direct evidence provided by genetically engineered mouse models. Deletion or overexpression of these two genes in mice can lead to the corresponding lower BP or higher BP phenotypes compared with their wild type littermates (Kim et al., 1995; Le et al., 2003). In addition, one recent study showed that transgenic mice overexpressing human AGTR1 displayed significant cardiac hypertrophy (Paradis et al., 2000).

The human AGT gene is located on chromosome 1 and evidence of linkage between a GT repeat in the 3'-flanking region of the AGT gene and essential hypertension (EH) has been found both in white people and Afro-Caribbean black people (Caulfield et al., 1994; Caulfield et al., 1995). A variant within this gene, M235T, was the first polymorphism reported to be associated with EH (Jeunemaitre et al., 1992). The 235 T allele carriers were also observed to have higher plasma AGT levels (Jeunemaitre et al., 1992). Studies involving only white subjects have assessed the association of M235T with left ventricular mass (LVM) in athletes, normotensive subjects and hypertensive patients, and have produced conflicting findings (Karjalainen et al., 1999; Kauma et al., 1998; Linhart et al., 2000). The human AGTR1 gene is

The renin-angiotensin-aldosterone system (RAAS) plays a critical role in the cardiovascular system. Angiotensinogen (AGT) is the first gene product in the RAAS physiological cascade. When cleaved by

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Table 1
Descriptive Characteristics of the BP and LVM Samples by Ethnicity and Gender

	EAs		AAs	
	Males	Females	Males	Females
Number of subjects	159	139	130	153
Mean age at first visit, y	12.2 ± 3.7	11.7 ± 3.8	12.3 ± 3.5	12.0 ± 3.2
BMI at first visit, kg/m ²	19.9 ± 4.6	20.2 ± 5.3	22.0 ± 6.2	23.2 ± 7.6
Father's education level (y)	14.1 ± 2.5	13.9 ± 2.1	12.6 ± 2.4	13.0 ± 2.0
Single-parent household, %	13.9	12.4	28.5	35.3
John Henryism	47.2 ± 5.4	47.9 ± 5.7	47.9 ± 5.7	49.6 ± 4.3
SBP at first visit, mmHg	106.8 ± 9.7	102.9 ± 8.3	110.7 ± 12.4	107.2 ± 9.8
DBP at first visit, mmHg	56.6 ± 5.5	57.3 ± 5.8	59.8 ± 6.1	61.1 ± 6.5
Subjects with ≥ 6 BP measures, %	80	87	88	89
LVM at first visit*, g	121.9 ± 41.5	102.3 ± 31.2	134.6 ± 41.1	109.3 ± 31.4
Subjects with ≥ 5 LVM measures, %	81	82	81	81

Note: Values are mean ± SD. BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; LVM, left ventricular mass; EA, European Americans; AA, African Americans.

**n* = 580.

mapped to chromosome 3 and a genome-wide scan suggested that this locus is the most significant contributor to EH in Finnish populations (Kainulainen et al., 1999). Bonnardeaux et al. (1994) screened exon 5 and the 3' untranslated region (UTR) for mutations and identified several frequent polymorphisms, one of which, A1166C, was found to be associated with EH in white people. Since this initial study, conflicting results have been obtained concerning association of this polymorphism with EH or with BP levels in white people (Castellano et al., 1996; Schmidt et al., 1997). Only two studies concerning this polymorphism were conducted in black people (Barbeau et al., 2002; Hindorff et al., 2002), with negative findings on both BP levels and EH. Apart from the A1166C, the Etude Cas-Temoins de l'Infarctus du Myocarde (ECTIM) study identified several additional single nucleotide polymorphisms (SNPs) in other exons and the 5' untranslated region (Poirier et al., 1998). Of these variants, C-521T is located in the promoter region and one study observed that it was associated with platelet AII binding in white normotensive pregnant women (Plummer et al., 2004) and another study (Henderson et al., 2004) found that the T allele increased the risk of EH in African Americans (AAs). L191L is a synonymous base substitution in exon5 and Zhu et al. (2003) found that it was associated with EH in AAs. A few studies assessed the association of A1166C with LVM, with negative findings in white subjects (Castellano et al., 1996; Kuznetsova et al., 2004).

To date, all the AGT and AGTR1 gene association studies have been cross-sectional, which prevents examinations of the impact of genetic susceptibility on interindividual differences in development of BP and LVM over time. We previously observed effects of a positive family history of EH on systolic BP (SBP) and

LVM trajectories in European American (EA) and AA youth (Dekkers et al., 2003). Longitudinal studies involving genetic markers are warranted. Furthermore, using serial measurements of a phenotype at multiple time points may provide better insight into the influence of genes on BP or LVM levels by diminishing measurement errors and minimizing the effect of short-term fluctuations. Accordingly, the main purpose of the present study was to examine the effect of the AGT M235T polymorphism and the AGTR1 gene C-512T, A1166C and L191L polymorphisms, individually and/or as haplotypes on BP and LVM trajectories from childhood into early adulthood by utilizing an established cohort of 581 AA and EA youth who have been evaluated 12 times over a 15-year period.

Methods

Subjects

Subjects are among participants in ongoing longitudinal studies evaluating the development of cardiovascular risk factors in youth. The data encompass 12 assessments over a 15-year period (1989–2004). This cohort has been described in detail previously (Dekkers, Treiber, et al., 2002; Dekkers, Snieder, et al., 2002; Dekkers et al., 2003). Descriptive characteristics by ethnicity and gender at first evaluation among those with available DNA (*n* = 581) are shown in Table 1. The correlations between SBP and diastolic BP (DBP), SBP and LVM, and DBP and LVM were .48 (*p* < .001), .42 (*p* < .001) and .03 (*p* = *ns*), respectively. The data set is complicated as not all subjects had the same number of visits, with subjects recruited into the study at different ages and different years. However, over 80% of subjects had five or more visits with data on LVM and six or more visits on BP,

making this data set very informative for the study of LVM and BP changes over time.

Subjects were classified as AA or EA according to criteria described previously (Dekkers, Snieder, et al., 2002). At baseline evaluation, subjects were normotensive for age and gender and were apparently healthy based on parental reports of the child's medical history. Eleven subjects began to take antihypertensive medication during the study, and the data obtained during this period were excluded from analyses.

Subject recruitment, evaluation and attrition rate have been previously described (Dekkers, Snieder, et al., 2002). The Institutional Review Board at the Medical College of Georgia had given approval for the study. The fact that 129 of the total number of subjects for BP or LVM sample were siblings may have affected the significance of observed effects. However, when siblings were excluded from the analyses, results were virtually unchanged, so results for the entire sample are reported here.

Measurements

On each laboratory visit after obtaining informed consent, anthropometric, resting hemodynamic, and cardiac structure evaluations were conducted as described elsewhere (Dekkers, Treiber, et al., 2002; Dekkers, Snieder, et al., 2002; Dekkers et al., 2003). SBP and DBP were measured with the Dinamap Vital Signs Monitor (model 1864 SX; Criticon Incorporated, Tampa, FL). BP measurements were taken at 11, 13, and 15 minutes, during a 15-minute supine relaxation period. The average of the last two readings was used to represent SBP and DBP values. LVM was calculated using the necropsy-validated formula of Devereux et al. (1986). Intra- and inter-rater coefficients of variation for all cardiac structures assessed were less than 10%.

Socioeconomic status (SES) was used as a proxy for environmental stress exposure (Dekkers, Snieder, et al., 2002) and indexed by father's education level (low education level < 12 years, medium education level \geq 12 and < 16 years, or high education level \geq 16 years) and marital status (single-parent household — single, divorced, widowed, separated, or 2-parent household — married), as father's education level was the most influential SES variable affecting SBP and LVM and marital status was the only SES variable affecting DBP in previous studies involving this cohort (Dekkers, Treiber, et al., 2002; Dekkers, Snieder, et al., 2002).

Stress-related coping styles play an important role in moderating deleterious effects of stress on cardiovascular health (Snieder, Harshfield, et al., 2002; Wang et al., 2005). One such stress-related coping style is 'John Henryism', which is characterized by a strong behavioral disposition to actively handle psychosocial and environmental stresses of daily living (James et al., 1983). Studies (Duijkers et al., 1988; James et al., 1983) have shown that individuals who score high on John Henryism and have few resources

for effective coping such as low level of education or SES tend to have higher BP and are more likely to have EH than others. In this study, John Henryism was measured with the 12-item John Henryism Active Coping Scale (James et al., 1983) on visits 4, 5, 6, 7 and 8. Sample items from this scale are: 'Once I make up my mind to do something, I stay with it until the job is completely done' and 'When things don't go the way I want them to, that just makes me work even harder'. Response options for each question range from 'completely true' (score = 5) to 'completely false' (score = 1). For each available visit a *z* score transformation was applied. These were subsequently averaged across the five visits to yield one John Henryism score taken to represent all 12 visits.

Genotyping

All polymorphisms were detected by polymerase chain reaction with restriction fragment length polymorphism (PCR-RFLP), as previously described elsewhere with minor modifications (Poirier et al., 1998; Russ et al., 1993). To prevent observer bias, the investigators were unaware of sample origin and all gels were cross-checked by a separate investigator.

Statistical Analyses

The purposes of our analyses were to test (1) the effect of the one AGT and three AGTR1 SNPs and AGTR1 haplotypes on the BP and LVM development from childhood to adulthood, (2) whether the effects of these SNPs and/or haplotypes on BP and LVM were moderated by ethnicity, gender, SES, John Henryism and/or adiposity, and (3) the possible interactions between the AGT M235T and the three AGTR1 SNPs.

Growth Curve Modeling and Haplotype Trend Regression

All analyses in this study were conducted by using individual growth curve modeling within a multilevel framework, a technique particularly suited for longitudinal data analysis (Dekkers, Treiber, et al., 2002; Dekkers, Snieder, et al., 2002). In growth curve modeling a curve is fitted for each individual subject. These curves (BP or LVM development with age) are characterized by their intercept (or level) and slope (rate of change). Addition of independent variables to the model, such as SNP and haplotype variation, is aimed at explaining between-subject variation (in level and slope) of the growth curves.

To test the effects of statistically inferred haplotypes of AGTR1 gene on changes in BP and LVM over time, we incorporated the haplotype trend regression (HTR) method (Zaykin et al., 2002) into the growth curve modeling framework. Assuming additive effects of the haplotypes on the trait, the HTR approach tests for the contribution of individual haplotypes rather than haplotype pairs. It is more powerful than analysis of variance methods and naturally extends to the case where haplotype frequencies are not directly observed. HTR is based on the regression of a trait on a design matrix that includes the

expected proportions of haplotypes. The contributions of haplotypes are weighted with the design matrix, such that unambiguous pairs of haplotypes are coded 1 for the haplotypes of homozygotes, .5 for each of the haplotypes of a heterozygote and 0 for all the other haplotypes. However, the contributions of ambiguous pairs of haplotypes in the design matrix are based on the probabilities of haplotype pairs as estimated by PHASE2.0 software (Stephens & Donnelly, 2003). Subjects with at least genotype data on two of the three AGTR1 SNPs were used for haplotype reconstruction. Haplotypes with estimated frequencies below 5% in all the subjects were pooled together and included in the model as one term. The most frequent haplotype was used as the baseline haplotype with which effects of the other haplotypes were contrasted.

Analytical Strategy and Software

Analyses were done separately for each of the SNPs and followed up by haplotype analyses and gene–gene interactions. For single SNP analysis, codominant (three genotype groups), dominant (carriers of rare allele vs. homozygotes of common allele) and recessive models (homozygotes of rare allele vs. carriers of common allele) were tested. We first built the most parsimonious environmental model for BP and LVM separately to maximize the variance explained by environmental covariates without unnecessarily increasing the number of estimated parameters as described in detail previously (Dekkers, Treiber, et al., 2002; Dekkers, Snieder, et al., 2002). In brief, we first specified the unconditional growth model, in which fixed and random linear and quadratic trends were fitted by adding, respectively, age and age² to the intercept-only model. Age was expressed as a deviation from its mean of 16 years for the BP data set and 17 years for the LVM data set. Ethnicity and gender were then added to the unconditional growth model to test the effects on BP or LVM level (i.e., intercept) as well as on the rate of change (i.e., slope, modeled as interactions with age and age²). In the next step, BMI, SES and John Henryism were separately added to the model to get the effect of these variables on the intercept and slope of BP and LVM. BMI was centered at its mean of 24.0 kg/m², SES was coded as a dummy variable and the *z* score–transformed John Henryism score was used. The interactions of these variables with ethnicity and gender were also tested. In the final step, all variables that had significant effects on the BP and LVM development in the previous models were entered simultaneously in a model to get the most parsimonious full ‘environmental’ model including only significant terms. After arriving at this model, SNPs or haplotypes were added to the model to test their main effects on the level of the growth curve. Effects on the slope of BP and LVM curves were modeled as interactions of SNPs or haplotypes with age and age². In the next step, the two-way interactions of SNPs or haplotypes with ethnicity, gender, BMI, SES and John Henryism were modeled to examine whether the effect

of the gene on BP and LVM was moderated by these factors. For LVM, SBP was also added as a predictor (Dekkers, Treiber, et al., 2002). Finally, the interaction between AGT and AGTR1 was tested by introducing M235T-AGTR1 single SNP or M235T-AGTR1 haplotype interaction terms into the most parsimonious dominant model reached for AGT M235T polymorphism. To increase statistical power, only the dominant model was used in this step. Likelihood ratio tests were used to determine the significance of the effects that were added to the model in each of the analysis steps. This test yields the difference between two models of their deviance (–2 log likelihood), which is asymptotically χ^2 distributed, with the number of degrees of freedom (*df*) equal to the difference in number of estimated parameters between the two models.

All multilevel modeling was performed using MLwiN software (Rasbash et al., 2000). Hardy-Weinberg equilibrium (HWE) was tested separately in AAs and EAs by a χ^2 test with 1 *df*. Ethnic differences in allele and genotype frequencies were tested with χ^2 tests of 1 and 2 *df*, respectively. To prevent inflated significance, these tests were performed in data including only one of the sibs, chosen at random. We used *D'* to describe the pattern of pairwise linkage disequilibrium (LD), calculated using 2LD software (Zhao, 2004). Haplotype frequencies for AGTR1 SNPs were estimated using PHASE 2.0 (Stephens & Donnelly, 2003). All subjects were used for estimates of haplotype frequencies and LD.

Results

Table 2 shows genotype and allele frequencies of the four polymorphisms in EAs and AAs. As can be seen from Table 2, total number of subjects genotyped for each polymorphism varied slightly and was somewhat lower than 581. This was due to unsuccessful amplification of the target sequences for some samples (1.5% for M235T, 1.9% for C-521T, 2.4% for L191L, and 3.9% for A1166C, respectively). No significant departure from HWE was found, except for M235T in EAs ($p = .04$) and L191L in AAs ($p = .005$). The fact that the genotype distributions of M235T in AAs and L191L in EAs were in HWE and that the investigators were unaware of sample origin indicated that the deviations from HWE were not due to genotyping error. Significantly different allele and genotype frequencies were observed between AAs and EAs for all four polymorphisms ($p < .001$). Compared to EAs, the 235T allele (46.4% vs. 76.8%), –521T allele (33.6% vs. 88.7%) and 191L allele (50.2% vs. 77.5%) were more common and the 1166C allele (31.2% vs. 6.7%) was less common in AAs. The LD patterns among these polymorphisms were also different between ethnic groups: strong LD between the C-521T and A1166C loci was observed in AAs but not in EAs (Table 3).

Table 4 displays the results for the analyses of single SNP effects on SBP, DBP and LVM levels based

Table 2

Genotype and Allele Frequencies of AGT M235T and AGTR1 Gene Polymorphisms in European and African Americans

SNPs	Ethnicity	N	Genotype			p^*	Allele freq.	p^*
			11	12	22			
M235T	EAs	294	74	167	53		.536/.464	
	AAs	278	14	101	163	< .001	.232/.768	< .001
C-521T	EAs	295	137	118	40		.664/.336	
	AAs	275	5	52	218	< .001	.113/.887	< .001
L191L (T573C)	EAs	292	74	143	75		.498/.502	
	AAs	275	23	78	174	< .001	.225/.775	< .001
A1166C	EAs	288	130	136	22		.688/.312	
	AAs	270	234	36	0	< .001	.933/.067	< .001

Note: EA, European Americans; AA, African Americans.

* Excluded sibs.

on the most parsimonious full 'environmental' models shown in the footnote. For these four polymorphisms, 235T, -521T, 191L and A1166 were the common alleles in all subjects. Under the three models (codominant [Table 4], dominant and recessive models [data not shown]), none of main effects of the four SNPs reached statistical significance for any of the three phenotypes. Also, none of the four polymorphisms showed significant interactions with age and age², that is, these polymorphisms did not affect the slope of the BP or LVM curves. However, a significant interaction between M235T and gender as well as a significant interaction between M235T and ethnicity on both SBP and DBP levels were observed. Further testing the interaction among M235T, ethnicity and gender discovered a significant three-way interaction on both SBP ($p = .004$) and DBP ($p = .046$) levels. Stratification of the sample in four different ethnicity by gender groups showed that the effect of this locus was only significant in AA males, with SBP levels 5.8 mmHg ($p = .00003$) and DBP levels 2.6 mmHg lower ($p = .005$) in the M235 allele carriers compared to noncarriers at age 16 (Figure 1). These effects represent an upward shift of the entire growth curve of SBP and DBP in AA male M235 carriers that is independent of age, as no effect of this SNP on the slope was detected. That is, redefining the age centering at

12 or 27 years did not lead to any differences in the observed effects. Compared with the full environmental models of SBP and DBP in AA males, this locus explained an additional 9.6% and 5.1% between-subject variation of SBP and DBP, respectively. We also observed a significant interaction between L191L and SES on SBP levels ($p = .048$). Separate analyses in low, medium and high SES groups showed that this polymorphism only had effect within the high SES group ($p = .038$), with lower SBP levels in the L191 homozygotes ($\beta = -3.12$ compared with 191L carriers). Similarly, a significant interaction between this polymorphism and ethnicity on DBP levels ($p = .036$) was found and homozygotes of the L191 allele only had significant ($p = .015$) lower DBP levels ($\beta = -2.59$ compared with 191L carriers) in AAs.

The inferred haplotype frequencies of the three AGTR1 polymorphisms in EAs were significantly different from those in AAs ($p < .001$). Only three common haplotypes (> 5%) comprising 91% of the total in AAs and six common haplotypes comprising 97.6% of the total in EAs were observed (Table 5). Haplotype 4, 5 and 6 are quite common in EAs (> 10%), but their frequencies are less than 5% in AAs. Since the frequencies of these three haplotypes in all subjects are greater than 5%, we kept these haplotypes as independent terms in the regression model, with all the other rare haplotypes pooled together as one term. After adding the haplotype or haplotype interactions with age, age², gender, ethnicity, BMI, SES into the full 'environmental' models of SBP and DBP, no significant overall changes of χ^2 values were observed (data not shown). That is, no significant main effect of AGTR1 haplotypes or effects of interactions on BP were found. Separate analyses in AAs by pooling the haplotype 4, 5 and 6 with the other rare haplotypes in a rest category did not change the results (data not shown). Although the overall contribution of haplotype variation to the LVM model did not reach statistical significance, a significant main effect of haplotype 6 ($p = .03$) on LVM levels was observed with a beta coefficient of -12.94

Table 3

Pairwise Linkage Disequilibrium Coefficients of AGTR1 Gene Polymorphisms in European and African Americans

SNPs	Distance between adjacent SNPs	C-521T	L191L (T573C)	A1166C
C-521T	—		.342*	-.456*
L191L (T573C)	44208bp	.284*		1.000
A1166C	593bp	.075	.844*	

Note: EA, European Americans; AA, African Americans.

D' for EAs below diagonal and D' for AAs above diagonal.

* $p < .001$.

Table 4
Results of Growth Curving Modeling Analysis of Individual Polymorphisms on BP and LVM

Locus	Genotype	β value		
		SBP (mmHg)*	DBP (mmHg)*	LVM (g)*
M235T	MT vs. TT	-0.290	-0.604	-1.322
	MM vs. TT	-0.389	0.465	-0.506
C-521T	CT vs. TT	-0.670	-0.904	-1.210
	CC vs. TT	0.355	-0.032	-1.448
L191L (T573C)	TC vs. CC	0.627	-0.065	1.723
	TT vs. CC	0.664	-0.956	4.488
A1166C	CA vs. AA	-1.107	0.199	-0.957
	CC vs. AA	-0.191	0.111	-2.608

Note: SBP, systolic blood pressure; DBP, diastolic blood pressure; LVM, left ventricular mass.

* Based on the full environment models:

SBP = 114.7 (cons) + 0.73 (age) - 0.058 (age²) + 2.67 (ethnicity) - 7.11 (gender) + 0.26 (ethnicity × age) - 0.64 (gender × age) + 0.04 (gender × age²) + 0.30 (BMI) - 0.020 (BMI × age) + 0.002 (BMI × age²) - 1.74 (medium education level) - 0.66 (high education level) + 0.66 (John Henryism).

DBP = 56.4 (cons) + 0.41 (age) + 0.05 (age²) + 2.94 (ethnicity) + 1.87 (gender) + 0.09 (gender × age) + 0.19 (ethnicity × age) - 0.026 (gender × age²) + 0.021 (ethnicity × age²) - 0.15 (BMI) - 0.007 (BMI × age) + 0.08 (BMI × ethnicity) + 1.05 (single-parent household).

LVM = 145.5 (cons) + 3.31 (age) - 0.42 (age²) + 3.76 (ethnicity) - 36.1 (gender) - 2.96 (gender × age) + 0.34 (gender × age²) + 2.73 (BMI) - 0.67 (BMI × ethnicity) + 0.31 (SBP).

(Table 6). This means that the level of the LVM growth curve shows a downward shift of on average 12.94 g for individuals who are homozygous for the haplotype 6 compared to individuals who are homozygous for the haplotype 1.

No significant interaction was found between AGT M235T polymorphism and any of the polymorphisms or the haplotype variants of AGTR1 gene, neither for the BP or LVM levels, nor for the slopes.

Discussion

This study examined the effect of AGT M235T and AGTR1 C-521T, L191L and A1166C polymorphisms on the BP and LVM development from childhood into early adulthood in a large group of healthy AA and EA males and females. Strong associations between the M235T polymorphism and both SBP and DBP levels were found in AA males. We also observed a SBP level lowering effect of the AGTR1 L191 allele in

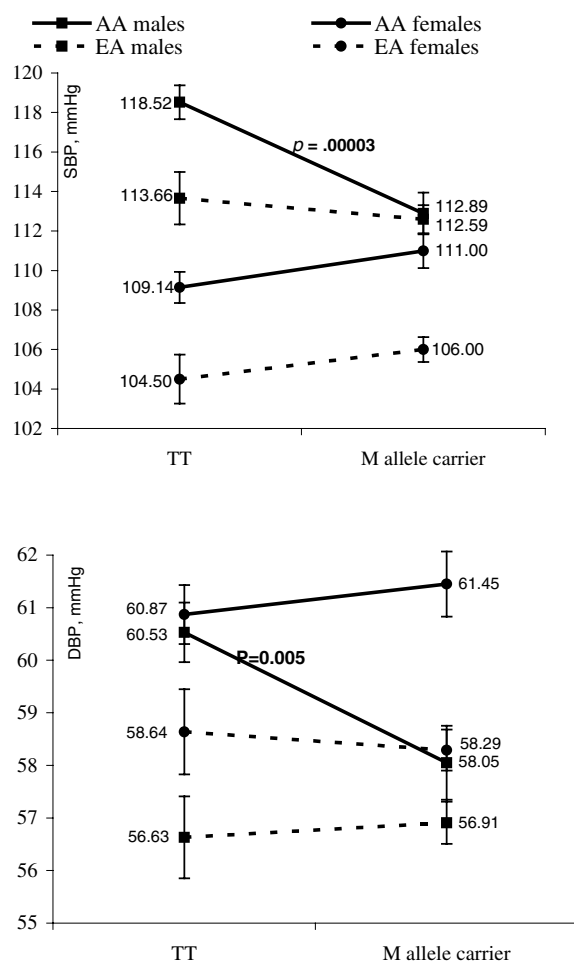
subjects from a high SES background and a DBP level lowering effect in AAs. Further haplotype analyses based on the three polymorphisms of AGTR1 gene identified a protective haplotype C-521/191L/A1166 for LVM levels.

Although the relationship between EH and M235T (as well as G-6A, which is in almost perfect LD with M235T in different populations) has been studied extensively both in black people and in white people, information on the association of this variant with BP as a quantitative trait is limited and conflicting, especially in black people. A recent meta-analysis (Sethi et al., 2003) based on nine studies in white people has reported no overall effect of the 235T allele on either SBP or DBP, which is in agreement with the current findings in EAs. To the best of our knowledge, only three studies included black subjects. Tiago et al. (2003) found no association between the M235T and BP in 231 newly diagnosed AA hypertensive patients

Table 5
Haplotype Frequencies of AGTR1 Gene Polymorphisms Constructed by PHASE2.0 in European and African Americans

Haplotype	C-521T	L191L (T573C)	A1166C	Frequency in EAs (N = 586)	Frequency in AAs (N = 552)
1	T	C	A	.104	.687
2	C	T	A	.361	.055
3	T	T	A	.112	.169
4	C	C	C	.180	.034
5	T	C	C	.110	.032
6	C	C	A	.109	.023
7	C	T	C	.018	.000
8	T	T	C	.007	.000

Note: EA, European Americans; AA, African Americans.


Figure 1

Mean (\pm SE) SBP and DBP levels by AGT gene M235T polymorphism in different ethnic by gender groups at age 16.

Note: AA, African American; EA, European American.

aged 51 (\pm 11) years who had never received therapy. A study of Afro-Caribbeans aged between 18 and 60 years by Robinson et al. (2004) observed that M235T was significantly associated with blood pressure only within one specific Rh blood group. The study by Province et al. (2000) which assessed the effect of the G-6A polymorphism on BP in the NHLBI family blood pressure program was the only study that involved both EAs and AAs and did observe 6G allele carriers had significantly lower levels of both SBP and DBP in AAs of one network group (HyperGen), although the meta-analysis based on the total of four network groups did not reach significance. In contrast with Province's study in which tests were done separately in EAs and AAs, our study is the first study to conduct formal tests of ethnic differences and observed quite a large effect of the M235T polymorphism on both SBP and DBP levels only in AA males. Compared with the full environmental models of SBP and DBP in AA males, this locus explained an additional 9.6% and 5.1% between-subject variation of

SBP and DBP, respectively. The fact that M allele carriers had lower BP is also consistent with previous findings (Jeunemaitre et al., 1992) showing that the M allele is associated with lower plasma AGT levels.

It is well known that the prevalence of essential hypertension in AAs is more than twice that of EAs and ethnic differences in BP levels are already present in childhood (Dekkers, Snieder, et al., 2002). Although our previous twin study observed that relative contributions of genetic and environmental factors to BP variability in AA youth were similar to those in EA youth (Snieder et al., 2003), it does not exclude the possibility that the actual genes (or their number) responsible for this heritability differ between ethnic groups. The present finding in AAs and our previous study observing significant effects of β 2-adrenergic receptor gene on BP control only in EAs (Snieder, Dong, et al., 2002) are consistent with the above speculation. Fejerman et al. (2004) recently resequenced the AGT gene in 57 Nigerian males and found that M235T was not only in perfect LD with G-6A, but also in perfect LD with four other polymorphisms including T68C, A507G, C5093A and A5593G. Haplotype network analysis showed that these SNPs were all in the same branch that led to a specific haplotype, which can be defined by the rare allele of each of these SNPs (M235, G-6, T68, A507, C5093 and A5593). Further analysis found that this was the only haplotype associated with lower plasma AGT levels. This effect might be due to one of these six SNPs or any additional unknown SNPs in LD with these SNPs or the multiple cis-acting interactions among these polymorphisms. Further functional studies are required to explore this.

In both ethnic groups, the observed allele frequencies of the three polymorphisms in AGTR1 gene were similar to those reported by previous studies (Barbeau et al., 2002; Henderson et al., 2004; Plummer et al., 2004; Zhu et al., 2003). The same LD pattern in EAs was also observed in the ECTIM study (Plummer et

Table 6

Haplotype Analysis of AGTR1 Gene Polymorphisms with LVM

Main Effects	Haplotype	LVM	
		β	p^{\dagger}
1	TCA*	—	—
2	CTA	1.08	ns
3	TTA	3.21	ns
4	CCC	-1.47	ns
5	TCC	-4.34	ns
6	CCA	-12.94	.03
Overall p value for haplotype		—	.13

Note: LVM, left ventricular mass.

* Estimates are contrasts with this most common haplotype.

\dagger Based on the full environment model.

al., 2004). One study by Zhu et al. (2003) found the L191 allele lowered the risk of EH in AAs, which is consistent with our findings that the L191 allele showed a DBP lowering effect in AAs. However, we also observed that the AGTR1 L191 allele showed a SBP-level lowering effect in subjects from a high SES background. SES is usually strongly confounded with ethnicity, and our study was no exception with EAs overrepresented in the high-SES category and AAs in the low-SES category (Dekkers, Snieder, et al., 2002). Since we included both variables in our models allowing us to adjust for their potential confounding effects, the finding of the L191 allele having a beneficial influence on BP but moderated by ethnicity and SES needs to be replicated in other populations.

Haplotypes can improve power to detect disease susceptibility regions if they are directly responsible for the observed variation in the trait or if they are in much higher LD with the functional polymorphism than the individual markers (Bader, 2001), especially when the functional polymorphism has minor allele frequency lower than 5% (de Bakker et al., 2005). In this study, a significant main effect of haplotype 6 on LVM levels was observed with a beta coefficient of -12.94 , which means that the level of the LVM growth curve shows a downward shift of on average 12.94 g for individuals who are homozygous for the haplotype 6 compared to individuals who are homozygous for the most common reference haplotype 1. The differentiating characteristic of haplotype 6 is the C-521 allele at position 1 as compared to the most common haplotype TCA. Since we did not find the effect of C-521T on LVM levels in single SNP analysis, we speculate that haplotype 6 is in LD with a functional variant in or around this gene.

The present results for the AGT and AGTR1 genes extend the findings of previous studies in at least three important ways. First, this study was conducted in youth, which means that important etiologic relationships have not been masked by the development of target organ damage. Second, this study examined a wider range of potential moderating variables including adiposity and several stress-related parameters that have previously been associated with BP and/or LVM and could partially account for the inconsistencies in the literatures. For example, this is the first longitudinal study to explore the effect of John Henryism on BP and LVM development from childhood into early adulthood and we observed that subjects scoring high on John Henryism had higher SBP levels. The inclusion of John Henryism in the SBP model could remove the potential confounding effects caused by any unbalanced distribution of John Henryism among different genotype groups. Third, all the studies focusing on the effect of AGT and AGTR1 on BP or LVM have been cross-sectional in nature, and the short-term fluctuations and measurement errors of BP or LVM levels may substantially influence estimates of the gene effect. In this study, a BP or LVM growth curve is

fitted for each individual. Not only do the intercepts of these curves provide more reliable estimates of BP or LVM levels based on multiple measurements, the slope of these curves made it possible to explore the effect of genes on the rate of BP or LVM change with age. However, in this study none of the four polymorphisms showed significant interactions with age, that is, these polymorphisms did not affect the slope of the BP or LVM curves.

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References

- Bader, J. S. (2001). The relative power of SNPs and haplotype as genetic markers for association tests. *Pharmacogenomics*, 2, 11–24.
- Barbeau, P., Kulharya, A., Harshfield, G., Snieder, H., Davis, H., & Treiber, F. (2002). Association between angiotensin II type I receptor polymorphism and resting hemodynamics in black and white youth. *Ethnicity and Disease*, 12, S1-68-71.
- Bonnardeaux, A., Davies, E., Jeunemaitre, X., Fery, I., Charru, A., Clauser, E., Tiret, L., Cambien, F., Corvol, P., & Soubrier, F. (1994). Angiotensin II type 1 receptor gene polymorphisms in human essential hypertension. *Hypertension*, 24, 63–69.
- Castellano, M., Muiesan, M. L., Beschi, M., Rizzoni, D., Cinelli, A., Salvetti, M., Pasini, G., Porteri, E., Bettoni, G., Zulli, R., & Agabiti-Rosei, E. (1996). Angiotensin II type 1 receptor A/C1166 polymorphism. Relationships with blood pressure and cardiovascular structure. *Hypertension*, 28, 1076–1080.
- Caulfield, M., Lavender, P., Farrall, M., Munroe, P., Lawson, M., Turner, P., & Clark, A. J. (1994). Linkage of the angiotensinogen gene to essential hypertension. *The New England Journal of Medicine*, 330, 1629–1633.
- Caulfield, M., Lavender, P., Newell-Price, J., Farrall, M., Kamdar, S., Daniel, H., Lawson, M., De Freitas, P., Fogarty, P., & Clark, A. J. (1995). Linkage of the angiotensinogen gene locus to human essential hypertension in African Caribbeans. *The Journal of Clinical Investigation*, 96, 687–692.
- de Bakker, P. I., Yelensky, R., Pe'er, I., Gabriel, S. B., Daly, M. J., & Altshuler, D. (2005). Efficiency and power in genetic association studies. *Nature Genetics*, 37, 1217–1223.
- Dekkers, C., Treiber, F. A., Kapuku, G., Van Den Oord, E. J., & Snieder, H. (2002). Growth of left ventricular mass in African American and European American youth. *Hypertension*, 39, 943–951.

- Dekkers, J. C., Snieder, H., Van den Oord, E. J., & Treiber, F. A. (2002). Moderators of blood pressure development from childhood to adulthood: A 10-year longitudinal study in African- and European American youth. *The Journal of Pediatrics*, *141*, 770–779.
- Dekkers, J. C., Treiber, F. A., Kapuku, G., & Snieder, H. (2003). Differential influence of family history of hypertension and premature myocardial infarction on systolic blood pressure and left ventricular mass trajectories in youth. *Pediatrics*, *111*, 1387–1393.
- Devereux, R. B., Alonso, D. R., Lutas, E. M., Gottlieb, G. J., Campo, E., Sachs, I., & Reichek, N. (1986). Echocardiographic assessment of left ventricular hypertrophy: Comparison to necropsy findings. *The American Journal of Cardiology*, *57*, 450–458.
- Duijkers, T. J., Drijver, M., Kromhout, D., & James, S. A. (1988). 'John Henryism' and blood pressure in a Dutch population. *Psychosomatic Medicine*, *50*, 353–359.
- Fejerman, L., Bouzekri, N., Wu, X., Adeyemo, A., Luke, A., Zhu, X., Ward, R., & Cooper, R. S. (2004). Association between evolutionary history of angiotensinogen haplotypes and plasma levels. *Human Genetics*, *115*, 310–318.
- Henderson, S. O., Haiman, C. A., & Mack, W. (2004). Multiple Polymorphisms in the renin-angiotensin-aldosterone system (ACE, CYP11B2, AGTR1) and their contribution to hypertension in African Americans and Latinos in the multiethnic cohort. *The American Journal of the Medical Sciences*, *328*, 266–273.
- Hindorff, L. A., Heckbert, S. R., Tracy, R., Tang, Z., Psaty, B. M., Edwards, K. L., Siscovick, D. S., Kronmal, R. A., & Nazari-Stewart, V. (2002). Angiotensin II type 1 receptor polymorphisms in the cardiovascular health study: Relation to blood pressure, ethnicity, and cardiovascular events. *American Journal of Hypertension*, *15*, 1050–1056.
- James, S. A., Hartnett, S. A., & Kalsbeek, W. D. (1983). John Henryism and blood pressure differences among black men. *Journal of Behavioral Medicine*, *6*, 259–278.
- Jeunemaitre, X., Soubrier, F., Kotelevtsev, Y. V., Lifton, R. P., Williams, C. S., Charru, A., Hunt, S. C., Hopkins, P. N., Williams, R. R., Lalouel, J. M., & Corvol, P. (1992). Molecular basis of human hypertension: Role of angiotensinogen. *Cell*, *71*, 169–180.
- Kainulainen, K., Perola, M., Terwilliger, J., Kaprio, J., Koskenvuo, M., Syvanen, A. C., Vartiainen, E., Peltonen, L., & Kontula, K. (1999). Evidence for involvement of the type 1 angiotensin II receptor locus in essential hypertension. *Hypertension*, *33*, 844–849.
- Karjalainen, J., Kujala, U. M., Stolt, A., Mantysaari, M., Viitasalo, M., Kainulainen, K., & Kontula, K. (1999). Angiotensinogen gene M235T polymorphism predicts left ventricular hypertrophy in endurance athletes. *Journal of the American College of Cardiology*, *34*, 494–499.
- Kauma, H., Ikaheimo, M., Savolainen, M. J., Kiema, T. R., Rantala, A. O., Lilja, M., Reunanen, A., & Kesaniemi, Y. A. (1998). Variants of renin-angiotensin system genes and echocardiographic left ventricular mass. *European Heart Journal*, *19*, 1109–1117.
- Kim, H. S., Krege, J. H., Kluckman, K. D., Hagaman, J. R., Hodgin, J. B., Best, C. F., Jennette, J. C., Coffman, T. M., Maeda, N., & Smithies, O. (1995). Genetic control of blood pressure and the angiotensinogen locus. *Proceedings of the National Academy Science USA*, *92*, 2735–2739.
- Kuznetsova, T., Staessen, J. A., Thijs, L., Kunath, C., Olszanecka, A., Ryabikov, A., Tikhonoff, V., Stolarz, K., Bianchi, G., Casiglia, E., Fagard, R., Brand-Herrmann, S. M., Kawecka-Jaszcz, K., Maljutina, S., Nikitin, Y., & Brand, E. (2004). Left ventricular mass in relation to genetic variation in angiotensin II receptors, renin system genes, and sodium excretion. *Circulation*, *110*, 2644–2650.
- Le, T. H., Kim, H. S., Allen, A. M., Spurney, R. F., Smithies, O., & Coffman, T. M. (2003). Physiological impact of increased expression of the AT1 angiotensin receptor. *Hypertension*, *42*, 507–514.
- Linhart, A., Sedlacek, K., Jachymova, M., Jindra, A., Beran, S., Vondracek, V., Heller, S., & Horvath, K. (2000). Lack of association of angiotensin-converting enzyme and angiotensinogen genes polymorphisms with left ventricular structure in young normotensive men. *Blood Press*, *9*, 47–51.
- Paradis, P., Dali-Youcef, N., Paradis, F. W., Thibault, G., & Nemer, M. (2000). Overexpression of angiotensin II type I receptor in cardiomyocytes induces cardiac hypertrophy and remodeling. *Proceedings of the National Academy Science USA*, *97*, 931–936.
- Plummer, S., Tower, C., Alonso, P., Morgan, L., Baker, P., Broughton-Pipkin, F., & Kalsheker, N. (2004). Haplotypes of the angiotensin II receptor genes AGTR1 and AGTR2 in women with normotensive pregnancy and women with preeclampsia. *Human Mutation*, *24*, 14–20.
- Poirier, O., Georges, J. L., Ricard, S., Arveiler, D., Ruidavets, J. B., Luc, G., Evans, A., Cambien, F., & Tiret, L. (1998). New polymorphisms of the angiotensin II type 1 receptor gene and their associations with myocardial infarction and blood pressure, the ECTIM study. Etude Cas-Temoin de l'Infarctus du Myocarde. *Journal of Hypertension*, *16*, 1443–1447.
- Province, M. A., Boerwinkle, E., Chakravarti, A., Cooper, R., Fornage, M., Leppert, M., Risch, N., & Ranade, K. (2000). Lack of association of the angiotensinogen-6 polymorphism with blood pressure levels in the comprehensive NHLBI Family Blood Pressure Program. National Heart, Lung and Blood Institute. *Journal of Hypertension*, *18*, 867–876.
- Rasbash, J., Browne, W., Goldstein, H., Yang, M., Plewis, I., Healy, M., Woodhouse, G., Draper, D., Langford, I., & Leavis, T. (2000). *A user's guide to MLwin*. London: University of London, Institute of Education.

- Robinson, M. T., Wilson, T. W., Nicholson, G. A., Grell, G. A., Etienne, C., Grim, C. M., Wilson, D., & Grim, C. E. (2004). AGT and RH blood group polymorphisms affect blood pressure and lipids in Afro-Caribbeans. *Journal of Human Hypertension*, *18*, 351–363.
- Russ, A. P., Maerz, W., Ruzicka, V., Stein, U., & Gross, W. (1993). Rapid detection of the hypertension-associated Met235: Thr allele of the human angiotensinogen gene. *Human Molecular Genetics*, *2*, 609–610.
- Schmidt, S., Beige, J., Walla-Friedel, M., Michel, M. C., Sharma, A. M., & Ritz, E. (1997). A polymorphism in the gene for the angiotensin II type 1 receptor is not associated with hypertension. *Journal of Hypertension*, *15*, 1385–1388.
- Sethi, A. A., Nordestgaard, B. G., & Tybjaerg-Hansen, A. (2003). Angiotensinogen gene polymorphism, plasma angiotensinogen, and risk of hypertension and ischemic heart disease, a meta-analysis. *Arteriosclerosis, Thrombosis, and Vascular Biology*, *23*, 1269–1275.
- Snieder, H., Dong, Y., Barbeau, P., Harshfield, G. A., Dalageogou, C., Zhu, H., Carter, N., & Treiber, F. A. (2002). Beta2-adrenergic receptor gene and resting hemodynamics in European and African American youth. *American Journal of Hypertension*, *15*, 973–979.
- Snieder, H., Harshfield, G. A., Barbeau, P., Pollock, D. M., Pollock, J. S., & Treiber, F. A. (2002). Dissecting the genetic architecture of the cardiovascular and renal stress response. *Biological Psychology*, *61*, 73–95.
- Snieder, H., Harshfield, G. A., & Treiber, F. A. (2003). Heritability of blood pressure and hemodynamics in African- and European-American youth. *Hypertension*, *41*, 1196–1201.
- Stephens, M., & Donnelly, P. (2003). A comparison of bayesian methods for haplotype reconstruction from population genotype data. *American Journal of Hypertension*, *73*, 1162–1169.
- Tiago, A. D., Badenhorst, D., Nkeh, B., Candy, G. P., Brooksbank, R., Sareli, P., Libhaber, E., Samani, N. J., Woodiwiss, A. J., & Norton, G. R. (2003). Impact of renin-angiotensin-aldosterone system gene variants on the severity of hypertension in patients with newly diagnosed hypertension. *American Journal of Hypertension*, *16*, 1006–1010.
- Wang, X., Trivedi, R., Treiber, F., & Snieder, H. (2005). Genetic and environmental influences on anger expression, John Henryism, and stressful life events, the Georgia Cardiovascular Twin Study. *Psychosomatic Medicine*, *67*, 16–23.
- Zaykin, D. V., Westfall, P. H., Young, S. S., Karnoub, M. A., Wagner, M. J., & Ehm, M. G. (2002). Testing association of statistically inferred haplotypes with discrete and continuous traits in samples of unrelated individuals. *Human Heredity*, *53*, 79–91.
- Zhao, J. H. (2004). 2LD, GENECOUNTING and HAP: Computer programs for linkage disequilibrium analysis. *Bioinformatics*, *20*, 1325–1326.
- Zhu, X., Chang, Y. P., Yan, D., Weder, A., Cooper, R., Luke, A., Kan, D., & Chakravarti, A. (2003). Associations between hypertension and genes in the renin-angiotensin system. *Hypertension*, *41*, 1027–1034.
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