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Variants and Haplotypes in Angiotensinogen Gene Are Associated With Plasmatic Angiotensinogen Level in Mexican Population

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Abstract

Introduction—The plasmatic angiotensinogen (AGT) level has been associated with essential hypertension. Linkage analysis has found a relationship between the *AGT* gene locus and hypertension in the Mexican-American population, but studies have failed to identify genetic variants associated with hypertension or plasma AGT levels. This study analyzes the relationship between polymorphisms in the *AGT* gene and plasmatic AGT levels in Mexican population.

Methods—Nine polymorphisms in *AGT* gene were genotyped, and plasma AGT level was determined by enzyme-linked immunosorbent assay.

Results—Differences in AGT plasma levels were associated with 2 polymorphisms: T-20G, TT = 25.3 ± 8.3 versus TG + GG = 21.6 ± 8.8 $\mu\text{g/mL}$; $P = 0.008$ and C3389T (T174M), CC = 25.8 ± 9.9 versus TC + TT = 20.5 ± 5.4 $\mu\text{g/mL}$; $P = 0.0002$. Haplotype 2 was associated with low plasma AGT (-5.1 $\mu\text{g/mL}$ [95% confidence interval: -8.6 to -1.6], $P = 0.004$) and Haplotype 8 was associated with high plasma AGT (6.5 $\mu\text{g/mL}$ [95% confidence interval: 2.5 to 10.6], $P = 0.001$). This association remained after adjustment for covariates. A Likelihood Ratio Test for haplotype-phenotype association adjusted for covariates resulted in $\chi^2 = 38.9$, $P = 0.0005$. The total effect of the haplotypes on plasma AGT level variance was 19.5%. No association was identified between haplotypes and quantitative traits of blood pressure.

Conclusions—Two polymorphisms (T-20G and C3389T) and 2 haplotypes (H2 and H8) showed an association with plasma AGT levels in Mexican population.

Keywords

Angiotensinogen gene; Genetics; Haplotypes; Polymorphism; Plasma angiotensinogen

The renin-angiotensin system (RAS) has a central role in regulating blood pressure (BP), and different genetic polymorphisms in the RAS have been reported to be associated with essential hypertension, including those in the angiotensinogen gene (*AGT*). *AGT* is a logical candidate gene because its product, angiotensinogen (AGT), is the substrate of the potent

vasoactive angiotensin II, and its circulating plasma level has been positively related to BP. In addition, the intravenous administration of pure AGT into salt-depleted rats increased their BP,¹ whereas the injection of anti-AGT antibodies decreased their BP.² In humans, there is a high positive correlation between plasma AGT levels and BP ($r = 0.39$, $P < 0.00001$).³ Plasma AGT is elevated in hypertensive individuals and in the offspring of hypertensive parents compared with normotensive parents.^{4,5} Genetic linkage studies have demonstrated this relationship involving the *AGT* locus in white, African Caribbean, Asian and Mexican-American populations.^{6–9} Nevertheless, the identification of causal genetic variants or genetic markers of intermediate traits or disease for most populations has been difficult. The single-nucleotide polymorphism (SNP) C4072T (M235T) has been associated with both plasma levels of AGT and essential hypertension in the white population.^{6,10–12} This association has been reported as borderline in Japanese individuals¹³ but has failed to be replicated in Arab Gulf and African American populations.^{7,14,15} The C4072T (M235T) polymorphism itself does not explain the mechanism involved in the increased risk to essential hypertension or the increased plasma AGT levels.¹⁶ Haplotype-based association studies suggest that a polymorphism in the *AGT* promoter such as the G-6A variant, which is in almost complete linkage disequilibrium (LD) with C4072T (M235T), could be the causal polymorphism. *In vitro* studies have shown differences in the level of transcription induced by *AGT* promoters containing G-6A.¹⁷ Other polymorphisms such as T-20G and G-217A, however, have shown to be associated with differences in plasma AGT levels.^{18,19} Although the *AGT* locus has shown linkage to hypertension in Mexican-American and African-Caribbean families, the C4072T (M235T) polymorphism was not been significantly associated with either plasma AGT levels or essential hypertension.^{9,20} This lack of replication across intercontinental populations suggests an important role for population genetic diversity in the control of intermediate traits. Recent data from the analysis of genomic diversity in the Mexican Mestizo population have shown significant differences with that of the HapMap populations.²¹

Search for polymorphisms responsible for AGT plasma levels in the Mexican Mestizo population would be important to elucidate the interethnic variability in plasma AGT levels and determining the role of AGT in essential hypertension. To map the genetic polymorphisms associated with this intermediate trait, we analyzed data from 149 Mexicans without effect of hypertensive drugs.

MATERIALS AND METHODS

Participants and Phenotype

DNA samples and plasma were selected from individuals without medical history of essential hypertension and none of them were receiving antihypertensive drugs. BP, which was measured by a trained nurse and supervised by a certified cardiologist, was taken at rest (sitting) by mercury sphygmomanometer. The final BP was an average of 3 separate measurements taken 5 minutes apart. All individuals signed the informed consent approved by the Research and Ethics Commissions of the National Institute of Genomic Medicine (IN-MEGEN) and Central North Hospital of PEMEX in Mexico. A total of 149 individuals (systolic BP [SBP]/diastolic BP [DBP] <140/90 mm Hg) of self-defined Mexican Mestizo ancestry from the Mexico City metropolitan area were recruited from the outpatient clinic of the Central North Hospital of PEMEX, Mexico.

Genotyping

Genomic DNA was obtained from whole blood using the Maxi-Kit Qiagen protocol (Qiagen, Chatsworth, CA). Nine SNPs at the *AGT* locus were genotyped using the 5' nuclease method (TaqMan) on a 7900HT RT-PCR System (Applied Biosystems, Foster

City, CA). Those SNPs were C-532T (rs5046), G-217A (rs5049), T-20G (rs5050), T-6C (rs5051), C3389T-T174 M (rs4762), C4072T-M235T (rs699), C6309T (rs2493132), C11535A (rs7079) and G12775A (rs943580). Only samples with a genotyping call rate exceeding 95% per SNP were included for analysis, and 40 independent duplications were performed to identify discordant genotypes.

Assay Procedures

Plasma AGT levels were quantified using a sandwich enzyme-linked immunosorbent assay method.²²

Statistical Analysis

For SNP analysis, the allele frequencies of the 9 SNPs were estimated using direct allele counting. Hardy-Weinberg equilibrium was calculated for each SNP using χ^2 with 1 degree of freedom (*df*). Analysis of single-marker associations was performed by pairwise analysis between genotypes and AGT plasma levels and was reported as a mean $\pm$ standard deviation. The AA genotype was used as the reference group, and the other genotypes (AB and BB) were used for comparison. Student *t* test was used to identify differences between genotypes, and 2-tailed *P* value < 0.005 was considered statistically significant after adjusting for 9 comparisons (Bonferroni's correction). Haplotype inference and size effect on plasma AGT levels were carried out using the maximum likelihood model with the Stochastic-EM algorithm implemented by THESIAS software.²³ By definition, the reference haplotype corresponded to the most frequent haplotype and was considered to be the intercept in linear regression analysis. For LD pairwise analysis (*D'*, LOD and r^2), a 2-marker EM algorithm, implemented by Haploview software,²⁴ was used to estimate the maximum-likelihood values of the 4 gametes. Haplotypes associated with plasma AGT levels were adjusted by regression analysis for the possible covariates of age, waist and body mass index (BMI). The Likelihood Ratio Test was calculated for haplotype-phenotype associations adjusted for covariates (age, waist and BMI) with its *df* and the *P* value calculated with χ^2 . The potential effects of amino acid changes in the function of AGT were predicted *in silico* using the PolyPhen and SIFT software.^{25,26} A correlation analysis between plasma AGT levels and other phenotypic variables was performed to identify any quantitative associations, using Pearson's correlation coefficient (*r*) and the coefficient of determination (r^2), both with a 95% confidence interval (CI) and *P* value for r^2 , assuming $\rho = 0$ for the null hypothesis. Plasma AGT levels were considered as a continuous variable for statistical power calculation. Sample-based calculation was established on comparison of means and standard deviation between genotypes of associated SNPs using Stata 10.1 (Stata Corp). This dataset achieves 98% and 69% power at 2-tailed $\alpha = 0.05$ for C3389T (T174M) and T-20G, respectively.

RESULTS

Population Characteristics

The demographic data of the 149 subjects included in this study are listed in Table 1. Data were reported to detect differences by gender. No differences in age, BMI, SBP, DBP, pulse pressure (PP) and median arterial pressure (MAP) were observed between men and women. However, differences in plasma AGT levels were observed: $22 \pm 6.1 \mu\text{g/dL}$ for men and $26.6 \pm 8.6 \mu\text{g/dL}$ for women, $P < 0.001$.

Association Between SNPs and Plasmatic AGT

All alleles and genotypes were in Hardy-Weinberg equilibrium, as demonstrated by the respective χ^2 tests. Pairwise analysis between the AA genotype as a reference group and the

other genotypes was performed. The results of this bivariate analysis are listed in Table 2. Differences in the AGT plasma levels were observed for the genotypes of SNP T-20G (TT = 25.3 ± 8.3 versus TG = 22.1 ± 7.1 $\mu\text{g/mL}$; $P = 0.03$) and SNP C3389T (T174M) (CC = 25.8 ± 9.9 versus CT = 20.7 ± 5.5 $\mu\text{g/mL}$; $P = 0.0005$). Although the GG genotype of T-20G SNP had a low frequency with only 8 carriers, the analysis was still performed: TT = 25.3 ± 8.3 versus TG + GG = 21.6 ± 8.8 $\mu\text{g/mL}$; $P = 0.008$. The same was done for C3389T (T174M), which had only 2 individuals with the TT genotype: CC = 25.8 ± 9.9 versus TC + TT = 20.5 ± 5.4 $\mu\text{g/mL}$; $P = 0.0002$. The association of these SNPs with plasma AGT levels was tested by linear regression and persisted after full adjustment for covariates (age, gender and BMI). $P = 0.01$ and $P < 0.001$ for T-20G and C3389T, respectively. Based on the LD map of the AGT locus shown in Figure 1, there is high LD between T-20G and C3389T (T174M) ($r^2 = 0.68$; $D' = 1$; 95% CI: 0.91–1.0; LOD = 27.8).

Haplotypes and Plasmatic AGT: Effects on Hypertension-Related Traits

Ten haplotypes accounted for 92% of all potential combinations. Two haplotypes showed an association with plasma AGT levels by a comparison with the intercept (Table 3): haplotype 2 (H2: CGGTTCCCG) was associated with decreased plasma AGT levels (-5.1 $\mu\text{g/mL}$ [95% CI: -8.6 to -1.6], $P = 0.004$), and haplotype 8 (H8: CGTCCTCCG) was significantly associated with increased plasma AGT levels (6.5 $\mu\text{g/mL}$ [95% CI: 2.5 – 10.6], $P = 0.001$). Furthermore, after analysis of the effect of each SNP in this haplotype background, 1 SNP was found to be related to AGT plasma levels: C3389T (T174M), -11.1 $\mu\text{g/mL}$ (95% CI: -14.2 to -2.2 , $P = 0.007$). The prevalence of H2 in the sample was 14% and 2% for H8. The association of these haplotypes with AGT plasma levels persisted after adjustment for covariates (age, waist and BMI). The total effect of the haplotypes on AGT plasma level variance was 19.5%. A Likelihood Ratio Test for haplotype-phenotype association adjusted for covariates (age, gender and BMI) resulted in a χ^2 of 38.9 with 11 *df* ($P = 0.0005$). These haplotypes were also analyzed for any potential effects on the hypertension-related traits, SBP, DBP, MAP, PP and double product (DP), but no association was found (Table 4).

Correlation Analysis: Plasmatic AGT and Hypertension and Obesity-Related Traits

The correlation analysis between plasma AGT levels and hypertension-related traits (SBP, DBP, PP, PAM and DP) did not show any significant association. There was a marginally positive correlation between plasma AGT levels and MBF: $r = 0.24$, (95% CI: 0.08 – 0.39) and $r^2 = 0.06$ (95% CI: 0.002– 0.18), $P = 0.02$ but not with age, height or other obesity-related traits (waist, hip, weight, BMI and body fat percentage).

In Silico Analysis for C3389T (T174M) Polymorphism

The amino acid sequence around the position 174 of the AGT protein is shown in Figure 2. A multispecies amino acid sequence comparison showed that the T174 allele is highly conserved throughout hominids. The PholyPhen/SIFT predictions of the effects of nonsynonymous polymorphisms with a minor allele frequencies (MAFs) ≥ 0.01 that are included in The Single Nucleotide Polymorphism Database (dbSNP) are listed in Table 5. The *in silico* modeling analysis for the C3389T (T174M) indicated “possibly damaging/tolerated” suggesting a potential modification in the function of AGT, with a PSIC/SIFT score of 1.575/0.13.

CONCLUSIONS

The precise relationship among AGT genetic polymorphisms, plasma AGT levels and the risk of essential hypertension remains uncertain. Several reports have found an association between C4072T (M235T) and T-6C with plasma AGT levels and the risk of developing essential hypertension. In Mexican-American families, Atwood et al⁹ reported linkage of the

AGT locus with essential hypertension but did not find association between plasma AGT levels and C4072T (M235T).

In Mexican Mestizo, the variant C3389T (T174M) was strongly associated with the trait, whereas T-20G was marginally associated. No association was identified between C4072T (M235T) and T-6C. A similar study carried out in a Japanese population²⁷ also identified variability in the genetic control of plasma AGT, which was later mapped to 9 SNPs within the *AGT* gene, with no association observed with C4072T (M235T) but a significant association with G-1074T. In an African-derived population, differences have been found in hypertension-related traits such as BP and mean AGT levels.²⁸ In African American children, plasma AGT levels were not associated with C4072T (M235T) or C3389T (T174M).²⁹

The low frequency of the 4072T (235M) allele in African (0.10) and African American (0.20) populations has been an argument against its negative association in African-derived populations.³⁰ However, this argument is not valid for the Mexican sample, in which the observed frequency of the 4072T (235M) allele was 0.30.

Data from Mexicans showed that the MAF allele 3389T (174M) was associated with decreased AGT plasma levels. The high frequency of the C3389 (T174) allele in all human populations and its homozygosity in other hominids suggest that the C3389 allele is the ancestral allele and that it is associated with high plasma levels of AGT. The C4072 (T235) allele associated with plasma AGT levels in white populations is also the ancestral allele.^{16,17} The threonine in the 174-position is highly conserved throughout hominids, suggesting evolutionary positive selection in the first human ancestors. The correlation of ancestral alleles with plasma AGT levels may provide selective advantages to carriers for improved salt retention and BP in human groups with a vegetable-based diet.

We observed high degree of LD analysis between T-20G and C3389T (T174M) suggesting a unique locus effect. In white populations, plasma AGT levels was directly correlated with SBP and DBP.³¹ Bloem et al¹⁰ reported a correlation of these variants with DBP and BMI in a sample of white and African American children. However, in Mexican population, we did not find significant correlation between plasma AGT levels and hypertension-related traits and only a weak correlation with BFM was observed. This could be explained by the exclusion of individuals with essential hypertension. Limited conclusions can be driven from these observations due to our sample size; however, they will be tested as part of an on-going association case-control study in the Mexican Mestizo population.

Two haplotypes were identified by regression analysis as being associated with plasma AGT levels. The H2, which contains -20G at the third position and 3389T (174M), correlated with the lowest plasma AGT levels. Both alleles correspond to the MAF of their associated SNPs. In contrast, the H8, which contains -20T at the third position and 3389C (T174) at the fifth position, correlated with the highest plasma AGT levels. This haplotype has a prevalence of 2% in this Mexican Mestizo sample. The biological significance on BP of individuals who harbor these haplotypes and higher plasma AGT levels has not been established, and no potential haplotype-phenotype association with BP traits, including DBP, SBP, MAP, PP and DP, could be established in this sample. However, plasma AGT, the only known substrate for renin, is the rate-limiting enzyme of the RAS. Because the level of plasma AGT is close to the Michaelis-Menten constant for renin, not only renin levels but also AGT levels can control the activity of the RAS, and the upregulation of AGT levels may lead to elevated angiotensin peptide levels and increases in BP.^{32,33} Recent studies on experimental animal models and transgenic mice have documented the involvement of AGT in the activation of the RAS and development of hypertension.^{30,34,35}

Genetic manipulations that lead to overexpression of AGT have consistently been shown to cause hypertension.^{37,38} Thus, plasma AGT levels play an important role in the development and progression of hypertension and kidney diseases.³⁹ The lack of association among BP traits could reflect the limitation from 3 morning measurements. Monitoring during 24-hour periods could be more sensitive to identify the effect of plasma AGT levels on blood pressure pattern. Also, clinical relationship could potentially arise after the inclusion of individuals with essential hypertension.

This lack of association with BP traits could reflect the lack of a relationship between plasma AGT levels and BP in this sample or a relationship that could not be identified due to the limitations of the study. A clinical relationship could potentially arise after the inclusion of individuals with essential hypertension.

If we consider the direct significance of a bivariate analysis and size effect on haplotype background, variation C3389T (T174M) is the more consistent polymorphism associated with plasma AGT levels in Mexican population. This is the first report that demonstrates association of C3389T (T174M) with plasma AGT levels. This polymorphism has been associated with hypertension in some studies involving white^{6,39,40} and Asian populations,^{42,43} but this association has failed to be replicated in other white, Asian, Arab and African populations.^{7,14,44–47}

Results from a meta-analysis by Pereira et al,⁴⁸ including 11,079 subjects (5,271 hypertensive and 5,808 normotensive), underlined the association between T174 M and hypertension in Asian and diverse-ancestry populations but not in European-derived populations. The functional mechanism by which T174 M increases BP among carrier individuals is unknown, and several studies have failed to show association of this polymorphism with plasma AGT levels.^{6,30,47} Nevertheless, *in silico* modeling analysis using PolyPhen and SIFT software suggests that the C3389T (T174M), which changes a nonpolar amino acid (methionine) into a polar amino acid (threonine), is probably damaging/not tolerated, which could result in the abnormal functioning of AGT. On the other hand, this genetic variant may only represent a marker in strong LD with the true causal genetic variant.

The role of the AGT locus in the genetic risk to hypertension is supported by linkage and association studies across most intercontinental populations. The inconsistency of findings could be related to differences in the genomic structure among populations. This issue has been described for the AGT locus by Pereira et al⁴⁸ who found pattern differences in the size, number and frequency of haplotype blocks among 3 populations with distinct ancestry. Also, differences in LD across *AGT* among white and Japanese populations have been described.⁴⁹

The Mexican Mestizo population is a recently admixed population composed of Amerindian, European and, to a lesser degree, African ancestries, making this group a useful resource for analyzing the genetic basis of complex diseases such as hypertension. The association observed in the Mexican Mestizo population may be due to the particular genomic structure of this population and its distinct interactions with particular environmental factors. A recent study showed a decreased diversity in LD patterns, and the long-range haplotype diversity is reduced in the Mexican Mestizo population, a characteristic that could be attributed to the Amerindian contribution to admixture.²¹ It is becoming clear that such association studies must be interpreted within the context of the genetic structure of the populations being studied and within patterns of variation within specific genomic regions.

We report the association of 2 genetic variants in the *AGT* gene with plasma AGT levels in Mexican Mestizo population. These findings contribute to understanding the inter-ethnic variability related to the *AGT* locus and the control of an intermediate trait such as plasma AGT levels. Substantial evidence indicates an important role for *AGT* in the development of essential hypertension. Given these observations, determining the genetic control of this trait would provide a better understanding of genetic factors responsible for this complex disease.

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FIGURE 1.
Map of linkage disequilibrium in the angiotensinogen (AGT) locus.

FIGURE 2.
Variation of the C3389T (T174M) polymorphism in primates.

TABLE 1

Clinical characteristics of sample

Trait	Men (n = 73), Mean $\pm$ SD	Women (n = 76), Mean $\pm$ SD	P
Age (yr)	72.2 $\pm$ 8.1	70.1 $\pm$ 7.0	0.10
Waist (cm)	97.4 $\pm$ 10.2	91.5 $\pm$ 11.1	0.002
Hip (cm)	101 $\pm$ 8.9	96.5 $\pm$ 10.9	0.01
Height (cm)	163.4 $\pm$ 6.6	153.1 $\pm$ 6.2	<0.001
Weight (kg)	73.4 $\pm$ 11.5	62.4 $\pm$ 9.6	<0.001
BMI (kg/m ²)	27.5 $\pm$ 3.2	26.5 $\pm$ 3.5	0.12
Systolic BP (mm Hg)	127.2 $\pm$ 9.5	127 $\pm$ 13.9	0.90
Diastolic BP (mm Hg)	73.7 $\pm$ 6.8	71.5 $\pm$ 7.3	0.08
PP (mm Hg)	53 $\pm$ 11.9	55 $\pm$ 13.2	0.37
MAP (mm Hg)	91.6 $\pm$ 6.2	90.6 $\pm$ 8.1	0.44
Plasma AGT (μ g/mL)	22 $\pm$ 6.1	26.6 $\pm$ 8.6	<0.001

BMI, body mass index; BP, blood pressure; MAP, mean arterial pressure; PP, pulse pressure; AGT, angiotensinogen; SD, standard deviation.

TABLE 2

Analysis of 9 SNPs in *AGT* (genotype vs. *AGT* plasma levels)

SNPs	AGT (ng/mL)				Significance		
	AA	AB	BB		AA vs. AB ^a	AA vs. BB ^a	AA vs AB + BB ^a
C-532T	24.4 ± 8.0 (124)	25.1 ± 6.4 (23)	15.8 ± 6.6 (2)		0.67	—	0.98
G-217A	23.8 ± 8.1 (121)	26.0 ± 10.4 (27)	11.1 (1)		0.20	—	0.34
T-20G	25.3 ± 8.3 (100)	22.1 ± 7.1 (41)	19.4 ± 4.8 (8)		0.03	0.05	0.008
T-6C	24.3 ± 7.5 (77)	23.8 ± 10.3 (57)	26.8 ± 6.1 (15)		0.76	0.22	0.88
C3389T	25.8 ± 9.9 (110)	20.7 ± 5.5 (37)	18.1 ± 1.8 (2)		0.0005	—	0.0002
C4072T	24.5 ± 7.6 (74)	23.6 ± 8.9 (61)	26.7 ± 6.2 (14)		0.52	0.31	0.83
C6309T	24.4 ± 8.4 (68)	24.1 ± 5.9 (57)	24.5 ± 7.3 (24)		0.91	0.94	0.95
C11535A	24.4 ± 9.5 (110)	23.8 ± 8.7 (34)	26.1 ± 5.2 (5)		0.70	0.60	0.83
G12775A	24.0 ± 8.7 (84)	23.9 ± 7.4 (55)	26.1 ± 4.4 (10)		0.94	0.46	0.85

A, major allele; B, minor allele. Genotype with low prevalence (*t* test was omitted).^a Student *t* test; 2-tailed *P* ≤ 0.005 after Bonferroni's correction.

AGT, angiotensinogen; SNP, single-nucleotide polymorphism.

TABLE 3

Haplotypes effect on AGT plasma levels

N	Haplotype	Estimation	Standard error	t test	Difference	P ^a
1	CGTTCCCG	0.7	6.7	0.1		
2	CGGTTCCCG	-5.1	1.8	-2.8	-5.1 (-8.6 to -1.6)	0.004
3	CGTCCTTAA	1.1	1.4	0.7	1.1 (-1.7 to 3.9)	0.45
4	CGTCCTTCA	-0.5	2.1	-0.25	-0.5 (-4.7 to 3.7)	0.80
5	TATTCCTCG	-0.4	3.2	-0.11	-0.4 (-6.6 to 5.9)	0.90
6	CGGTCCCG	3.2	2.1	1.5	3.2 (-1.07 to 7.4)	0.14
7	CGTCCTTCG	-3.4	4.9	-0.7	-3.4 (-13.1 to 6.4)	0.50
8	CGTCCTCCG	6.5	2.0	3.1	6.5 (2.5 to 10.6)	0.001
9	CGGTCTCCG	-3.2	6.8	-0.5	-3.2 (-16.7 to 10.2)	0.63
10	CATTCTCG	0.63	3.9	0.16	0.63 (-6.9 to 8.2)	0.87

^a Haplotype effect by comparison with the reference, with 95% confidence interval

TABLE 4

Haplotype effects on hypertension-related traits

Trait	Phenotypic mean	Standard error	H2 ^a	H8 ^a
SBP	129.5	13.3	2.6 (−2.1 to 7.4) <i>P</i> = 0.28	4.6 (−4.9 to 14.0) <i>P</i> = 0.34
DBP	72.5	7.1	0.50 (−2.3 to 3.3) <i>P</i> = 0.73	2.3 (−3.3 to 7.9) <i>P</i> = 0.42
MAP	91.0	7.2	0.81 (−1.6 to 3.1) <i>P</i> = 0.50	3.1 (−2.4 to 8.5) <i>P</i> = 0.27
PP	54.1	12.5	0.30 (−4.9 to 5.4) <i>P</i> = 0.90	3.1 (−13.7 to 19.8) <i>P</i> = 0.72
DP	72.1	10.2	57.2 (−91.3 to 123.5) <i>P</i> = 0.09	89.1 (−35.5 to 213.8) <i>P</i> = 0.16

^aHaplotypic effect by comparison with the intercept with its 95% confidence interval.

SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; PP, pulse pressure; DP, double product; H2, haplotype 2; H8, haplotype 8; AGT, angiotensinogen.

TABLE 5

PolyPhen/SIFT prediction of nonsynonymous variants with MAF ≥ 0.01 in AGT (NP_000020.1; RefSeq)

rs	Heterozygosity	SNP	Position	Amino acid change	Prediction ^d	PSIC/SIFT score
4762	0.36	T/C	207 (174)	Met/Thr	+/-	1.575/0.13
699	0.67	C/T	268 (235)	Thr/Met	+/-	1.528/0.68
11568053	0.04	G/A	268	Ile/Met	-/-	0.06/0.19
34829218	0.08	T/C	137	Met/Thr	-/-	0.486/0.31
61751077	0.01	T/C	442	Phe/Ser	+/+	1.954/0.01
61731499	0.01	C/T	385	Val/Al	+/-	1.954/0.47

^a (-), benign or tolerated; (+) possibly damaging (PolyPhen) or deleterious with low confidence (SIFT) and (++) probably damaging (PolyPhen) or deleterious (SIFT).

SNP, single-nucleotide polymorphism; MAF, minor allele frequency; AGT, angiotensinogen.