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Linkage of Angiotensinogen Gene Polymorphisms with Hypertension in a Sibling Study of Hong Kong Chinese

Yu-Jing Fang^{a,b}, Han-Bing Deng^a, G Neil Thomas^{a,c}, Chi Hung Tzang^d, Cai-Xia Li^e, Zong-Li Xu^f, Mengsu Yang^d, and Brian Tomlinson^a

^aDepartment of Medicine and Therapeutics, The Chinese University of Hong Kong, Hong Kong SAR

^cDepartment of Public Health and Epidemiology, University of Birmingham, Birmingham, UK

^dDepartment of Biology and Chemistry, City University of Hong Kong, Hong Kong SAR

^eDepartment of Medical Statistics, Sun Yat-sen University, Guangzhou, China

^fEpidemiology Branch, National Institute of Environmental Health Sciences, USA.

Abstract

Objective—The angiotensinogen gene has been linked with human essential hypertension in Caucasians but the relationship in Asian populations has been less consistent. This study aimed to examine genetic associations between hypertension and the M235T, T174M, and G-217A polymorphisms of the angiotensinogen gene in Chinese siblings.

Methods—We studied members of 126 families with a hypertensive proband, including 434 siblings, of which 178 were hypertensive. Parental history of hypertension was recorded. The M235T, T174M, and G-217A polymorphisms were examined using a microarray method, validated by sequencing. The transmission disequilibrium test was applied to identify whether the genetic polymorphism loci were related to hypertension. Haplotype analysis of the combined polymorphisms was applied using the TRANSMIT program. Linkage study was conducted by applying the affected pedigree member method.

Results—A significant over-transmission was observed for the T235 allele at the M235T polymorphism and hypertension ($\chi^2=4.41$, $p=0.036$), but not for the T174M and G-217A polymorphisms. The haplotype analysis showed a significant association with the haplotypes of paired markers (T174 and T235) with χ^2 value of 8.131, $p=0.004$ (global test $\chi^2=9.131$, $p=0.028$). Linkage between M235T and hypertension was detected ($T=-2.25$, $P=0.019$), and a tendency for linkage with central obesity-related hypertension was found for the M235T and T174M polymorphisms ($P=0.0087$ and $P=0.01$).

Conclusion—The M235T and T174M variants, especially the T235 allele, contribute to an increased risk of hypertension in these Chinese subjects.

Keywords

angiotensinogen; hypertension; sibling study

Corresponding author: Professor Brian Tomlinson Division of Clinical Pharmacology Department of Medicine and Therapeutics Prince of Wales Hospital Shatin, Hong Kong Tel: (852) 2632 3139 Fax: (852) 2632 3139 btomlinson@cuhk.edu.hk.

^bcurrent address: Department of Colorectal Surgery, State Key Laboratory of Oncology in South China, Cancer Center, Sun Yatsen University, Guangzhou, China

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Introduction

Large population based studies, adoption studies, and twin studies have suggested that about 30-60% of human blood pressure (BP) variance is genetically determined [1]. Genome-wide association studies have identified over 100 quantitative trait loci attributed to hypertension across the genome, particularly in chromosomes 1, 2, 3, 17 and 18 which contain multiple loci with overlapping limits [2]. These broad loci suggest the possibility that only a limited number of genetic variants with large effects account for essential hypertension (EHT). However, identifying the BP-related genetic variants in those broad loci regions could be challenging and requires large samples. Some variants with relatively smaller impact may not be detected in a large general population sample. Investigating candidate genes in sib-pairs or family-based settings provides an efficient alternative to identify the genetic variants with moderate to weak risk. The candidate genes for hypertension were selected based on the understanding of different roles of encoded proteins in BP regulation or Mendelian hypertension (autosomal dominant inheritance of hypertension). Most of these genes are, directly or indirectly, coupled to renal salt handling, being included in the renin-angiotensin system (RAS), or steroid-hormone metabolism.

Condensed abstract

The relationships between hypertension and M235T, T174M, and G-217A polymorphisms of the angiotensinogen gene were investigated in 126 Hong Kong Chinese families with a hypertensive proband, including 434 siblings, of which 178 were hypertensive. A significant over-transmission was observed for the T235 allele at the M235T polymorphism and hypertension, but not for the T174M and G-217A polymorphisms. The haplotype analyses showed a significant association of hypertension with the haplotypes of paired markers (T174 and T235) with χ^2 value of 8.131, $p=0.004$ (global test $\chi^2=9.131$, $p=0.028$). Linkage between M235T and hypertension was detected ($T=-2.25$, $P=0.019$) and linkage appeared more closely with central obesity-related hypertension for M235T and T174M ($P=0.0087$ and $P=0.01$, respectively). In conclusion, the M235T and T174M variants, especially the T235 allele, contribute to an increased risk of hypertension in these Hong Kong Chinese subjects.

The angiotensinogen (AGT) gene has been described as a strong candidate associated with the onset of EHT [3]. The human AGT gene is located on chromosome 1q42-43 and contains five exons spanning 13 kb [4]. AGT is cleaved by renin to the decapeptide angiotensin (Ang) II precursor, Ang I and des (Ang I)-AGT [4]. A number of associated single nucleotide polymorphisms (SNPs) have been identified including M235T (rs669), T174M (rs4762), and G-217A (rs5049) since Jeunemaitre et al. showed an excess allele sharing in the AGT gene among hypertensive sib-pairs in 1992 [3]. Meta-analyses suggested that the 235T and T174 allele increased the risk of EHT by about 20% [5-7]. Asian carriers with the 235T allele appeared to have a higher risk of hypertension than white carriers (Odds ratios: 1.60 vs. 1.19) [8]. However, associations between polymorphisms in the AGT gene and EHT are less consistent in Asian populations, especially in Han Chinese. The Harvard-Anhui study comprising 110 concordant-affected sib-pairs of 53 Han Chinese hypertensive families failed to replicate the association between the AGT gene and hypertension [9]. Case-control studies in Hong Kong Han Chinese and later in a large Beijing Han Chinese population found no linkage between the M235T variant and EHT [10,11]. We initiated the Hong Kong Han Chinese hypertensive sibling study to investigate the hypertension-related genetic variants in families with at least one hypertensive proband. In the present analysis the relationships between EHT and the M235T (rs669), T174M (rs4762), and G-217A (rs5049) polymorphisms in AGT gene were examined.

Methods

Hypertensive subjects, aged 20 to 60 years, were recruited if they had a family history of hypertension, and had at least one sibling living in Hong Kong. All subjects underwent clinical and biochemical investigation to exclude those with either secondary hypertension, impaired glucose tolerance or type 2 diabetes mellitus. Treatment for hypertension was withdrawn to enable the determination of off-treatment BP. Following withdrawal of antihypertensive medication subjects were monitored weekly to check their BP did not exceed the upper exclusion limit and to confirm that they were not at risk from severe hypertension. After the two to four week washout period, the sitting BP was recorded in triplicate after a 10 to 15 minute resting period. The baseline BP, anthropometric measurements, and the blood samples for routine assays were taken at this time [12]. The 24-hour urine sample taken over the previous 24 hours was processed. Hypertension was defined as systolic BP (SBP) of at least 140 mmHg and/or diastolic BP (DBP) of at least 90 mmHg at baseline [13]. General obesity was defined as body mass index (BMI) $\geq 25 \text{ kg/m}^2$ [14], and central obesity was defined as waist-to-hip ratio (WHR) ≥ 0.9 for males, and ≥ 0.85 for females [15]. DNA was extracted from white blood cells. The AGT gene polymorphisms were examined by multiplex polymerase chain reaction (PCR) amplification and microarray provided by Applied Research Centre for Genomic Technologies, City University of Hong Kong. For microarray scanning and image analysis we used the GenePix4000 scanner and the GenePix Pro4.0 software.

The study was approved by the Joint Chinese University of Hong Kong and New Territories East Cluster Clinical Research Ethics Committee, Hong Kong. Written informed consent was obtained from the subjects.

Statistical analysis

It has been shown that for common diseases the sibling-based transmission disequilibrium test (TDT) could achieve power similar to that of the parents-proband trio-based TDT if at least one extra sibling was available for genotyping [16,17]. We estimated the genetic power using the M235T polymorphism as an example, a TDT would have 80% power to detect linkage between hypertension (prevalence 0.272 in Hong Kong [18]) and T allele (frequency 0.85 in Hong Kong Chinese [10]) at type I error of 0.05 when the sample includes 115 families with at least one hypertensive proband and one unaffected sibling [19]. We also performed post-hoc exploratory analyses to examine the relationships of the polymorphisms with general and central obesity in the hypertensive siblings. A genetic power estimation showed that 173 discordant sib-pairs also had greater than 80% power to detect linkage between central obesity (prevalence 70.8% in hypertensive patients in the present study) and M235T variant (T allele frequency 0.85 in Hong Kong Chinese [10]). Data from normally distributed parameters are presented as mean $\pm$ S.D., whereas those parameters with a skewed distribution were logarithmically (base 10) transformed and are presented as geometric means with 95% confidence intervals of the mean. Differences in the levels of anthropometric and biochemical parameters between the hypertensive and normotensive siblings were assessed using the paired t-test. The Conditional Logistic Regression Model was applied to examine the association between the disease trait and all the covariates measured by using the matched sib-pairs. The χ^2 -test was used to identify any differences in the genotype or allele distribution of the AGT polymorphisms between the normotensive and hypertensive populations and to assess for deviations from the Hardy-Weinberg equilibrium for each cohort. The transmission disequilibrium test (TDT) was applied to evaluate whether the genetic polymorphism loci were related to hypertension. Haplotype analysis of the combined polymorphisms was applied using the TRANSMIT program. The program produces the following asymptotic chi-squared tests: 1. For each haplotype or

allele, a test on 1-df for excess transmission of that haplotype. 2. A global test for association on $H-1$ df, where H is the number of haplotypes for which transmission data are available [20]. The affected pedigree member identity-by-state (APM-IBS) linkage analysis method (sib-pair program, version 0.99.9, by D.L. Duffy, <http://www2.qimr.edu.au/davidD/>) [21] was applied to examine whether the hypertensive members of a pedigree have an excess sharing of alleles of the AGT gene polymorphisms. A P value less than 0.05 was indicative of statistical significance. Fundamentals and rationale of the TDT and APMIBS methods have been described previously [12].

Results

Clinical characteristics of the study siblings

In this study, 126 hypertensive probands were identified and a total of 434 siblings were recruited resulting in 327 discordant sib-pairs with some siblings being used in more than one pair. Overall, the proportion of female siblings was 56.9%, of hypertensive was 41%, of generally obese (defined by BMI) was 54.1%, and of centrally obese (defined by WHR) was 56.5%. For hypertensive siblings, the proportion with obesity (defined by BMI and/or WHR) was 70.8%. In addition to having higher BP ($151 \pm 13.8/94 \pm 10$ mmHg vs. $117 \pm 10.2/70 \pm 9.8$ mmHg), the hypertensive siblings had a higher proportion of males, increased age, greater levels of general and central obesity (BMI, and WHR), higher plasma sodium, higher 24 hour urinary sodium, more adverse lipid parameters (higher total cholesterol, LDL-cholesterol and triglycerides and lower HDL-cholesterol), and higher fasting plasma glucose (FPG), although hyperglycaemic subjects were excluded from the study (Table 1).

Allele and genotype frequencies

The genotype and allele frequencies of the M235T, T174M and G-217A polymorphisms for hypertensive and normotensive siblings are shown in Table 2. There were no statistically significant differences for the genotype and allele frequencies of these 3 polymorphisms between hypertensive and normotensive siblings, but the frequencies of the variant alleles T235, M174 and A-217 were somewhat higher than those reported in other ethnic groups. The A allele carriers of G-217A had higher blood pressure than the G allele homozygotes (SBP 135 ± 19.2 mmHg vs. 129 ± 2.4 mmHg, $P = 0.007$; DBP 83 ± 15.5 mmHg vs. 78 ± 14.9 mmHg, $P = 0.007$). There was no significant difference in blood pressure, body weight, lipid profile or urinary sodium among the three genotypes of either the M235T or the T174M polymorphism.

Association of hypertension with M235T, T174M and G-217A polymorphisms

A total of 327 discordant sib-pairs were included in the analyses. Conditional logistic regression analysis, incorporating the significant phenotypic covariates and the AGT polymorphisms, showed that BMI ($P < 0.0001$, odds ratio=1.39, 95% CI: 1.22-1.58), FPG ($P = 0.015$, odds ratio=2.43, 95% CI: 1.19-4.95), and plasma sodium ($P = 0.046$, odds ratio=1.29, 95% CI: 1.01-1.67) were significantly related to having EHT after adjustment for age and gender. The AGT gene G-217A (A allele) ($P = 0.015$, odds ratio=4.21, 95% CI: 1.33-13.33) and the AGT gene M235T (T allele) ($P = 0.017$, odds ratio=2.26, 95% CI: 1.09-8.79) were also significantly related to having EHT. However, there was no significant relationship between the T174M polymorphism and having EHT (Table 3).

Association analyses were also performed by a TDT, a family-based method for the determination of preferential transmission of a specific allele or haplotype [22]. The TDT was implemented using the software package TRANSMIT v. 2.5, which uses additional genotype information from unaffected siblings to infer missing parental genotypes and

reconstruct multilocus haplotypes [20,23]. A global χ^2 -test for transmission distortions was performed across alleles.

The transmission of alleles of M235T, T174M, G-217A separately and as haplotypes was tested using TRANSMIT. We estimated the haplotype frequencies of the three SNPs, and a total of three haplotypes were detected, with one common haplotype (-217G, 174T, 235T) with a frequency of 82.1%. TRANSMIT indicated that there was only a significant over-transmission of the allele 235T with Global χ^2 test, on 1 degree of freedom = 4.4, $P = 0.0036$ (Table 4). We also analyzed haplotypes of paired markers for transmission disequilibrium with TRANSMIT. The results indicated evidence for linkage disequilibrium with the 235T-174T haplotype of the AGT gene with Global χ^2 -test on 3 degrees of freedom at 9.13, $P = 0.02$ (Table 5).

Linkage between hypertension and M235T, T174M and G-217A polymorphisms

Using the APM method, 110 hypertensive–hypertensive sib-pairs of 53 families and 327 hypertensive–normotensive sib-pairs of 117 families were analyzed using three weighting functions. There was a tendency for linkage between the M235T polymorphism and hypertension with $T = -1.005$, $P = 0.014$ for weighting function $f(p) = 1$, $T = -2.25$, $P = 0.019$ for weighting function $f(p) = 1/\sqrt{p}$, and $T = -1.56$, $P = 0.0026$ for weighting function $f(p) = 1/p$ for the discordant sib-pairs, but there were no significant findings in the concordant sib-pairs. For the T174M and G-217A polymorphisms, there was no evidence of linkage with hypertension among these families.

Linkage between central obesity with M235T, T174M and G-217A polymorphisms

No linkage between these three SNPs and general obesity (defined by BMI) was observed. However, there was some evidence that the polymorphisms of the AGT gene might be related to central obesity by the APM method. When the 75 sib-pairs concordant and 173 sib-pairs discordant for central obesity were analyzed, evidence of linkage was found between the M235T and T174M polymorphisms and central obesity in the discordant sib-pairs ($T = -1.49$, $P = 0.058$; $T = -2.23$, $P = 0.0087$; $T = -1.58$, $P = 0.0095$; and $T = -0.75$, $P = 0.06$; $T = -1.16$, $P = 0.01$; $T = -0.9$, $P = 0.008$, respectively, for three weighting functions), but not in the concordant sib-pairs. For the G-217A polymorphism, we did not find significant linkage with central obesity among these families.

Discussion

Significant evidence supporting the genetic susceptibility of the AGT locus for hypertension has been provided by a number of studies in various ethnic groups. In the present study, the frequencies of M235T, T174M and G-217A genotypes were similar to those reported for Japanese, Hong Kong Chinese and Taiwanese Chinese but differed markedly from those among Caucasians and African Americans.

The plasma level of AGT correlates with BP and tracks through families, suggesting it has a role in EHT [5]. The AGT gene has been linked to EHT in Caucasian siblings from the USA and France [3], and in British [24], African Caribbean [25], and Mexican American patients [26]. In our study, the TDT test showed the 235T allele was transmitted more frequently from parents to hypertensive offspring. This agreed with the association we found between the 235T allele and hypertension using conditional logistic regression. In this study we used TDT rather than a case-control study design, thereby eliminating the possibility of biased results due to poorly matched controls. Furthermore, TDT can be much more powerful than allele-sharing methods, such as sib-pair analysis for genes with modest effects [27].

We found a positive relationship between polymorphisms in the AGT gene and hypertension in 327 discordant affected-unaffected sib-pairs of 117 hypertensive families using the APM method. In the Harvard-Anhui study, Niu et al. applied an APM analysis in 310 hypertensive sib-pairs of 248 sib-ships recruited from Anqing district in Anhui province [9]. There was no significant linkage found in 110 concordant-affected sib-pairs of 53 hypertensive families. Comparing the study populations between the present study and the Harvard-Anhui study they differ in terms of socio-economic status, nutrition profile, prevalence of tobacco use, and physical activity level. In our study, all subjects were Hong Kong Han Chinese who live in a highly urbanized environment with higher prevalence of sedentary lifestyle and low-fiber high-energy density diet. In contrast, Anqing is a rural area, with cotton and grain as its major agricultural products. The Chinese population in the Anqing area are more physically active but with a higher consumption of tobacco, alcohol and salt [28]. Given the above dissimilarity in lifestyle of these two Chinese populations, even if the genetic make-up is similar there may be a different interaction with the environmental factors in the development of hypertension and cardiovascular diseases.

Compared with another case-control study in the Hong Kong Chinese population [10], our family-based design is less influenced by the effects of population stratification or improper controls [7]. The sib-paired controls from the same ancestry of hypertensive siblings provided higher statistical power to detect a moderate to weak suspected risk [27].

In the present study, there was a significant relationship between the M235T and the T174M polymorphisms and central obesity defined by WHR. The WHR is a marker of visceral fat accumulation. Increased visceral fat, even in normal weight individuals, has been strongly associated with insulin resistance [29], deranged lipid metabolism [30], and high BP [31]. Differential AGT gene expression in visceral adipose versus subcutaneous adipose tissue has been suggested as a link between central obesity and hypertension [32-34]. An excess of visceral adipose tissue leads to increased generation of AGT, increased renin activity, stimulation of aldosterone production, and decline of adiponectin [35,36]. Meanwhile, high activity of the sympathetic nervous system (SNS) induced by visceral adiposity results in increased renal sodium retention and peripheral resistance, and consequently hypertension [35,36]. A dietary intervention study showed that a reduction of waist circumference was strongly correlated to the decline in plasma AGT in menopausal women [37]. What is less clear is whether the variants of the AGT gene affect both obesity and hypertension. The HERITAGE Family Study suggested a positive relationship between the AGT M235T polymorphism and body fatness measured by under-water weighing in Caucasian women [38]. The TT genotype was associated with resting and exercise DBP in a manner dependent on body fatness [38]. A study in Japanese women also showed the TT genotype was significantly related to visceral obesity in terms of waist circumference and percentage of body fat, although the relationship of the M235T polymorphism and BP was not significant [39]. Vasku et al. [40] reported that hypertensive carriers with the M allele of T174M and/or T allele of M235T were more prevalent in the Czech subjects with BMI>25kg/m². However, no relationships between the M235T and T174M genotypes and BMI or body weight were detected in the present study.

Based on the TDT, the 235T variant of the AGT gene was significantly over transmitted to hypertensive offspring, but there was no evidence of over transmission of the 174M allele of the T174M variant. However, the haplotype of the two variants 235T and 174T combined was over transmitted to hypertensive offspring. This could be related to linkage disequilibrium between the two variants rather than to the mutation itself.

In conclusion, the 2 common variants in the AGT gene may contribute to a small degree to the risk of hypertension in this sibling study in Chinese subjects. The 235T allele showed the

most consistent relationship with hypertension and this may be mediated by a relationship of this polymorphism with central obesity.

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Abbreviations

EHT	essential hypertension
NT	normotension
BP	blood pressure
SBP	systolic blood pressure
DBP	diastolic blood pressure
RAS	renin-angiotensin system
AGT	angiotensinogen
SNP	single nucleotide polymorphism
TDT	Transmission disequilibrium test
APM	affected pedigree member
IBS	identity-by-state
BMI	body mass index
WHR	waist-to-hip ratio
LDL	low-density lipoprotein
HDL	high-density lipoprotein
FPG	fasting plasma glucose

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Table 1

Association between hypertension blood pressure and covariates in 167 discordant sib-pairs

	HT	NT	P
Females (%)	52.7	68.9	0.001
Age (years)	42±0.5	40±0.6	<0.0001
Weight (kg)	69±1.1	60±0.8	<0.0001
WHR (ratio)	0.87±0.006	0.83±0.005	<0.0001
BMI (kg/m ²)	26.7±0.3	23.7±0.3	<0.0001
Body fat %	32.0±0.6	31.6±0.5	0.65
Total cholesterol (mmol/L)	5.5±0.08	5.1±0.07	0.0002
Triglycerides (mmol/L)	1.7±0.1	1.1±0.06	<0.0001
HDL-cholesterol (mmol/L)	1.3±0.03	1.4±0.03	0.011
LDL-cholesterol (mmol/L)	3.5±0.07	3.2±0.07	0.0038
TC/HDL-c ratio	4.5±0.1	4.0±0.1	0.006
LDL-c/HDL-c ratio	2.8±0.08	2.6±0.1	0.038
FPG (mmol/L)	5.2±0.07	4.9±0.04	0.0024
Plasma sodium (mmol/L)	141±0.2	140±0.1	0.02
Plasma potassium (mmol/L)	4.2±0.04	4.2±0.03	0.08
Urine sodium (mmol/day)	188.3±6.1	162.8±5.3	0.0007
Urine potassium (mmol/day)	49.1±2.3	45.5±1.2	0.14

P value from paired t test

Table 2

The AGT gene polymorphisms genotype/allele frequencies of hypertensive and normotensive siblings

	HT (n=178)	NT (n=256)	Males		Females	
			HT (n=90)	NT (n=97)	HT (n=88)	NT (n=159)
M235T (rs669)						
MM	7 (3.9)	8 (3.1)	7 (7.8)	5 (5.2)	0	3 (1.9)
MT	50 (28.1)	72 (28.1)	31 (34.4)	27 (27.8)	19 (21.6)	45 (28.3)
TT	121 (68)	176 (68.8)	52 (57.8)	65 (67)	69 (78.4)	111 (69.8)
M	64 (18)	88 (17.2)	45 (25)	37 (19.1)	19 (10.8)	51 (16)
T	292 (82)	424 (82.8)	135 (75)	157 (80.9)	157 (89.2)	267 (84)
T174M (rs4762)						
TT	146 (82)	209 (81.6)	68 (75.6)	76 (78.4)	78 (88.6)	133 (83.6)
MT	25 (14.1)	37 (14.5)	17 (18.9)	14 (14.4)	8 (9.1)	23 (14.5)
MM	7 (3.9)	10 (3.9)	5 (5.6)	7 (7.2)	2 (2.3)	3 (1.9)
T	317 (89)	455 (88.9)	153 (85)	166 (85.6)	164 (93.2)	289 (90.9)
M	39 (11)	57 (11.1)	27 (15)	28 (14.4)	12 (6.8)	29 (9.1)
G-217A (rs5049)						
GG	112 (62.9)	186 (72.7)	61 (67.8)	73 (75.3)	51 (58)	113 (71.1)
AG	52 (29.2)	50 (19.5)	22 (24.4)	14 (14.4)	30 (34)	36 (22.6)
AA	14 (7.9)	20 (7.8)	7 (7.8)	10 (10.3)	7 (8)	10 (6.3)
G	276 (77.5)	422 (82.4)	144 (80)	160 (82.5)	132 (75)	262 (82.4)
A	80 (22.5)	90 (17.6)	36 (20)	34 (17.5)	44 (25)	56 (17.6)

Table 3

Association between blood pressure, covariates and genetic factors in siblings (analyzed by conditional logistic regression)

	DF	Parameter estimate	SE	χ^2	P	odds ratio	95% CI	
							lower	upper
AGT-(A-217)	1	1.437	0.589	5.956	0.0147	4.21	1.33	13.33
AGT-(T235)	1	1.356	0.568	5.693	0.017	2.26	1.09	8.79
BMI	1	0.327	0.067	23.951	<0.0001	1.39	1.22	1.58
FPG	1	0.888	0.363	5.982	0.0145	2.43	1.19	4.95
Plasma sodium	1	0.258	0.129	3.998	0.0456	1.29	1.01	1.67

Transmission Disequilibrium Test (TDT) analysis for transmission of proposed hypertension-linked alleles from candidate gene

Table 4

Polymorphism (allele)	TDT transmission of proposed disease-linked allele to affected siblings		Var (O-E) ^c	P value ^d	Frequency in probands (%)
	Observed ^a	Expected ^b			
AGT M235T (T)	292	281.02	27.35	0.0036	82.1
AGT T174M (M)	41	48.09	18.10	0.096	13.5
AGT G-217A (A)	80	79.27	24.55	0.88	21.8

^aObserved and expected transmission of haplotypes from parents to the probands.

^bObserved and expected transmission of haplotypes from parents to the probands.

^cVariance (difference between observed and expected transmissions).

^dGlobal χ^2 -test p value.

Table 5

Transmission Disequilibrium Test (TDT) analysis for transmission of proposed hypertension-linked haplotypes from the AGT gene

Polymorphism (haplotype)	TDT transmission of proposed disease-linked haplotype to affected siblings		Var (O-E)	P	informative families
	Observed	Expected			
* AGT T174M-M235T	266.55	250.06	33.45	0.0043	124
174M-235T	21.45	26.36	9.414	0.11	
# AGT G-217A-M235T	222.95	215.07	35.72	0.19	121
-217A-235T	59.05	56.10	18.32	0.49	

* Global χ^2 -test test, on 3 degrees of freedom = 9.13, p=0.028

Global χ^2 -test, on 3 degrees of freedom = 7.56, p=0.056