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ORIGINAL ARTICLE

# Association between single-nucleotide polymorphisms in six hypertensive candidate genes and hypertension among northern Han Chinese individuals

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**Hypertension is one of the leading risk factors for mortality. The renin–angiotensin–aldosterone system (RAAS) is a potent and powerful mediator in the homeostasis of hypertension. Here, the association between six candidate genes, *renin*, *adrenoceptor β3*, *angiotensinogen*, *aldosterone synthase*, *angiotensin II receptor type 1* and *angiotensin II receptor type 2*, that are related to RAAS and essential hypertension (EH) was evaluated and explored in northern Chinese Han individuals. A case–control study including 1090 EH cases and 700 controls was performed. Eight single-nucleotide polymorphisms (SNPs), rs699, rs4762, rs5707, rs5186, rs4994, rs1799998, rs5193 and rs5194, located in the six genes were genotyped with TaqMan real-time PCR method. Statistical analysis software (SPSS 17.0) was used for descriptive statistics and association analyses. Among the six genes related to RAAS, the frequencies of rs4994 (*ADRB3*) and rs5194 (*AGTR2*) were found to be significantly different between the EH cases and controls ($P<0.05$). Logistic regression analyses adjusted for covariates showed rs4994 to be closely associated with EH under the recessive ($P=0.019$, odds ratio (OR) = 0.373, 95% confidence interval (CI) 0.163–0.851) and homozygous ($P=0.028$, OR = 0.394, 95% CI 0.172–0.903) models. The association was also significantly close in the male subset ($P<0.05$). Significant association was also observed between rs1799998 (*CYP11B2*) and EH ($P<0.05$) in the dominant, additive and allelic models. These data demonstrated that *ADRB3* rs4994 and *CYP11B2* rs1799998 were significantly closely associated with EH in northern Han Chinese individuals. The CC of rs4994 and CC or C allele of rs1799998 might be protective genetic factors of hypertension.**
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**Keywords:** aldosterone synthase; candidate gene; CYP11B2; northern Han individual; polymorphism; RAAS

## INTRODUCTION

Hypertension is a common, complex disease. It has been intensively studied to identify susceptibility loci in humans. In 2000, an estimated 26.4% (972 million) of the world's adult population was recorded as being hypertensive. This number is projected to increase by 60%, to a total of 1.56 billion people, by 2025.[1] The high prevalence of hypertension and its consequent significant adverse economic and quality-of-life effects on both the individual and society highlight the importance of primary prevention of hypertension. Accordingly, there is a pressing need for a greater understanding of the genetic underpinnings of blood pressure (BP) regulation and dysregulation.[2] The mechanisms underlying hypertension remain unclear. Studies have demonstrated that BP is a genetically determined trait, with estimates of heritability ranging from 31 to 68%.[3] BP is regulated by hormones, the nervous system and body fluid volume. The renin–angiotensin–aldosterone system (RAAS) is a potent and powerful mediator of BP homeostasis.

RAAS is a peptidergic system with endocrine characteristics associated with the regulation of BP, water balance, electrolytic homeostasis and cardiac remodeling.[4] Major components of the circulation RAAS are prorenin, angiotensinogen, renin, angiotensin-converting enzyme, angiotensin I and angiotensin II.[5] Their matched receptors participate in RAAS regulation and are the remarkable aspects of the system. The observation that *angiotensinogen*, *renin* and the *angiotensin II receptor, type 1* are expressed in multiple tissues has prompted the suggestion of multiple

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local RAASs that act as independent entities from the systemic RAAS.[6]

Angiotensinogen (AGT), the protein encoded by the pre-angiotensinogen or angiotensinogen precursor genes, is expressed in the liver and cleaved by the enzyme renin in response to lowered BP. Based on the function of AGT in regulating BP, changes in its gene sequence are likely to play an important role in the pathogenesis of cardiovascular risk traits, such as hypertension, as well as manifestation of coronary artery disease.[7,8] Single-nucleotide polymorphisms (SNPs) rs4762 (NP 000020.1:p. Thr207Met) and rs699 (NP 000020.1:p. Met268Thr), which are located in the *AGT* gene, can affect the pharmacogenomics of angiotensinogen, and have been associated with susceptibility to various cardiovascular risk traits, such as primary hypertension and type 2 diabetes.[9–11] However, research on the effects of this gene in Chinese individuals has been scant.

Renin, an aspartyl protease, catalyzes the first step in the activation pathway of angiotensinogen, a cascade that can result in the release of aldosterone, vasoconstriction and higher BP. The genotype GG of the rs5707 gene (NM_000537.3:c.492 + 17T > G), which is located in an intronic region downstream of exon 4, has been reported to be associated with BP and the risk of hypertension in postmenopausal Spanish women.[12]

Angiotensin II receptor, type 1 mediates the cardiovascular actions of angiotensin II. It is a member of the G-protein-coupled receptor superfamily expressed in most tissues, where receptor activation leads to vasoconstriction, water retention and vascular smooth muscle cell proliferation and hypertrophy.[13] It can also stimulate reactive oxygen species within the cell nucleus.[14] A1166C polymorphism is involved in the development of coronary heart disease,[15] but data confirming the association between it and hypertension are limited, especially in the Chinese population.

Some studies have reported that common functional polymorphisms in β-adrenergic receptor genes might be associated with heart failure phenotypes. An Indian study found that *adrenoceptor beta 3* C190T (rs4994) may be involved in the complex pathophysiology of coronary artery disease, and the CC genotype might indicate the genetic risk.[16] In a Brazilian-Caucasian population, researchers found that rs4994 was associated with type 2 diabetes.[17] However, the relationship between rs4994 and hypertension in Chinese is unclear.

In recent years, genome-wide association studies have been a relatively new way to identify genes involved in human diseases. This method searches the whole genome for causal variations. However, different genome-wide association studies identified different variants in different races.[18] In addition, these variants are mostly located in non-gene regions, and their functions are difficult to explain. We therefore conducted an association study of eight variants from six notable candidate genes related to RAAS, *REN (renin)*, *ADRB3* (*adrenoceptor β3*), *AGT* (*angiotensinogen*), *CYP11B2* (*aldosterone synthase*), *AGTR1* (*angiotensin II receptor, type 1*) and *AGTR2* (*angiotensin II receptor, type 2*) among Han Chinese individuals without diabetes (1790 samples in total).

## METHODS

### Study population

All of the participants were screened at a medical center and hypertension clinic at Anzhen Hospital. A total of 700 normotensive participants (control group) and 1090 essential hypertension (EH) patients (case group) were screened. BP was measured according to The Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (JNC-VII). Normotensive controls were defined as follows: systolic blood pressure (SBP) < 130 mm Hg and diastolic blood pressure (DBP) < 85 mm Hg without antihypertensive treatment and age ⩾ 40 years, and the hypertensive group was defined as follows: SBP ⩾ 140 mm Hg or/and DBP ⩾ 90 mm Hg and all patients were diagnosed with EH.

None of the participants had diabetic disease, primary valvular disease, active myocarditis, history of myocarditis, hypertrophic or restrictive cardiomyopathy, pericardial disease, arrhythmia, or primary hepatic, renal, neurological, pulmonary or endocrine disease. This study complied with the Declaration of Helsinki. All participants signed a consent form, and the study was approved by the hospital's ethics committee.

### DNA genotyping

DNA was extracted from whole blood by lysing RBCs and digesting the remaining white cell pellet with proteinase K. The SNP genotyping kits were obtained from Applied Biosystems (Foster City, CA, USA). The kits matched to the SNPs were as follows: C___1985481_20, rs699; C___1985480_20, rs4762; C___2215549_20, rs4994; C___1842206_20, rs5707; C__11880452_10, rs5193; C___1841567_20, rs5194; C___3187716_10, rs5186; C___8896484_10, rs1799998. The genotyping kits, TaqMan PCR Master Mix (Life Technologies, Foster City CA, USA), No AmpErase UNG (Life Technologies), and ~5 ng genomic DNA were used in a final volume of 5 μl. A 7300 Real-Time PCR system (Life Technologies) was used for amplification. The first step of the thermocycle was initial denaturation and activation at 95 °C for 15 s and 60 °C for 1 min.

### Statistics

Continuous variables were expressed as the mean ± s.e. The association of genotypes with hypertension was analyzed using the $\chi^2$ test. To compare indexes in the two groups, *t*-test was used. The five genetic models (dominant, recessive, additive, homozygous and allelic) were used for both the univariate and multivariate association analyses. Multinomial logistic regression was used to study the effects of eight SNPs on the hypertension status with other variables adjusted as covariates. Linear regression was used to analyze the relationship between SNPs and BP. SPSS 17.0 (SPSS, Chicago, IL, USA) was used for descriptive statistics and association analyses. The linkage disequilibrium analyses and the Hardy–Weinberg equilibrium (HWE) test were conducted using Plink (Harvard University, Cambridge, MA, USA). A *P*-value of $<0.05$ was considered to be significant.

## RESULTS

### Basic characteristics of the studied subjects

Table 1 shows the basic characteristics of the studied participants, including the EH group and the normotensive control (NT) group. A total of 1790 unrelated subjects were recruited in the study that consisted of 1090 EH patients (660 men and 389 women) and 700 NT subjects (418 men and 282 women). In the EH group (1090 EH participants), there were 622 EH participants under antihypertensive therapy. Participants in the EH group had greater values ($P<0.05$) with respect to age, weight, body mass index, SBP, DBP, blood glucose, total cholesterol, triglyceride, uric acid and blood urea nitrogen than participants in the NT group. Participants in the EH group had greater values with respect to drinking and family hypertension history ($P<0.05$). However, the EH group had lower average high-density lipoprotein cholesterol ($P<0.05$).

### Genotype and allele frequencies of six genes and association analyses

Among the participants, the genotyping success rates for the locus were as follows: rs699, 98.5%; rs4762, 98.8%; rs5707, 98%; rs5186, 99.4%; rs4994, 98.5%; rs1799998, 98.5%; rs5193, 98%; and rs5194, 98.2%. Some missing values existed in the present study, such as gender, but other information about genotypes was available. In the NT group, no deviation from HWE was observed for any of the eight SNPs except rs5193 and rs5194 (which were in X chromosome and

**Table 1 Anthropometric and clinical characteristics (mean ± s.e.) of participants**

| | *Hypertensive* (n = *1090*) | | | *Normotensive* (n = *700*) | | | |
|---|---|---|---|---|---|---|---|
| *Characteristics* | *Total* | *Male* | *Female* | *Total* | *Male* | *Female* | *Overall P-value* |
| Age (years) | 51.94 ± 0.44 | 49.43 ± 0.81 | 53.00 ± 0.79 | 51.81 ± 0.93 | 49.55 ± 0.82 | 56.49 ± 0.96 | 0.075 |
| Gender (male/female) | 660/389 | 660 | 389 | 418/282 | 418 | 282 | 0.192 |
| Height (cm) | 165.78 ± 0.32 | 170.97 ± 0.41 | 158.68 ± 0.53 | 164.93 ± 0.33 | 171.41 ± 0.61 | 159.14 ± 0.66 | 0.626 |
| Weight (kg) | 72.98 ± 0.49 | 77.99 ± 0.78 | 64.79 ± 0.93 | 67.67 ± 0.48 | 71.67 ± 1.02 | 59.66 ± 0.99 | <0.001 |
| BMI ($kg\,m^{-2}$) | 26.47 ± 0.14 | 26.65 ± 0.23 | 25.70 ± 0.33 | 24.82 ± 0.14 | 24.37 ± 0.30 | 23.60 ± 0.41 | <0.001 |
| Smoking (yes/no) | 265/748 | 226/416 | 39/332 | 123/322 | 112/165 | 11/157 | 0.563 |
| Drinking (yes/no) | 282/730 | 243/396 | 39/334 | 70/377 | 60/217 | 10/160 | <0.001 |
| SBP (mm Hg) | 141.77 ± 1.07 | 139.46 ± 1.26 | 145.82 ± 1.91 | 115.66 ± 0.88 | 117.89 ± 1.10 | 112.69 ± 1.36 | <0.001 |
| DBP (mm Hg) | 90.85 ± 0.70 | 92.07 ± 0.92 | 88.70 ± 1.01 | 75.62 ± 0.65 | 77.87 ± 0.79 | 72.60 ± 0.99 | <0.001 |
| GLU ($mmol\,l^{-1}$) | 5.43 ± 0.04 | 5.48 ± 0.06 | 5.36 ± 0.06 | 5.17 ± 0.06 | 5.22 ± 0.09 | 5.11 ± 0.07 | <0.001 |
| HR (b.p.m.) | 71.81 ± 0.37 | 69.04 ± 0.64 | 70.25 ± 0.96 | 72.06 ± 0.45 | 66.32 ± 1.01 | 67.72 ± 0.95 | 0.438 |
| CHO ($mmol\,l^{-1}$) | 6.01 ± 0.24 | 5.89 ± 0.34 | 6.21 ± 0.30 | 5.12 ± 0.07 | 5.09 ± 0.11 | 5.16 ± 0.10 | <0.001 |
| TG ($mmol\,l^{-1}$) | 2.09 ± 0.08 | 2.26 ± 0.11 | 1.79 ± 0.09 | 1.43 ± 0.06 | 1.59 ± 0.08 | 1.22 ± 0.05 | <0.001 |
| LDL-C ($mmol\,l^{-1}$) | 3.42 ± 0.05 | 3.37 ± 0.06 | 3.51 ± 0.09 | 3.48 ± 0.06 | 3.56 ± 0.09 | 3.38 ± 0.09 | 0.331 |
| HDL-C ($mmol\,l^{-1}$) | 1.16 ± 0.04 | 1.12 ± 0.05 | 1.22 ± 0.04 | 1.40 ± 0.13 | 1.16 ± 0.02 | 1.72 ± 0.30 | <0.001 |
| UA | 387.91 ± 5.66 | 421.82 ± 6.70 | 328.41 ± 7.64 | 350.80 ± 6.85 | 392.92 ± 8.06 | 294.42 ± 7.43 | 0.026 |
| Family hypertension history (yes/no) | 604/431 | 382/273 | 222/158 | 148/330 | 98/196 | 50/134 | <0.001 |
| CRE ($mmol\,l^{-1}$) | 82.98 ± 1.17 | 91.41 ± 1.44 | 68.20 ± 1.08 | 77.64 ± 1.16 | 86.18 ± 1.23 | 66.20 ± 1.05 | 0.902 |
| BUN ($mmol\,l^{-1}$) | 5.73 ± 0.09 | 5.99 ± 0.11 | 5.28 ± 0.14 | 5.59 ± 0.40 | 5.44 ± 0.12 | 5.80 ± 0.94 | <0.001 |

Abbreviations: BMI, body mass index; BUN, blood urea nitrogen; CHO, total cholesterol; CRE, serum creatinine; GLU, blood glucose; HDL-C, high-density lipoprotein cholesterol; HR, heart rate; LDL-C, low-density lipoprotein cholesterol; SBP, systolic blood pressure; TG, triglyceride; UA, uric acid.
Note that overall *P*-value was the result from both the hypertensive group and the normotensive group.

needed no HWE test); the SNPs all obeyed HWE in gender-specific subgroups. The genotype and allele frequencies of the eight SNPs are shown in Table 2. Univariate analyses indicated that rs4994 was associated with EH, and the frequency of the CC genotype was slightly lower in the EH group ($P = 0.019$; odds ratio (OR) = 0.466, 95% confidence interval (CI) 0.235–0.922). This finding indicated that the CC genotype is protective; rs5194 was associated with EH in women, and the AA genotype was more common in the EH group than in the NT group (OR = 1.372, 95% CI 1.107–1.701, $P = 0.01$). The EH group had more individuals with at least one copy of the A-allele individuals and lesser G-allele individuals than the NT group ($OR_A = 1.163$, 95% CI 1.055–1.282; $OR_G = 0.819$, 95% CI [0.723–0.928, $P = 0.002$). Although no significant differences in rs5193 genotypes were found among female participants, TT genotypes were more common in the EH group than in the NT group (OR = 2.69, 95% CI 1.105–6.545, $P = 0.059$; Table 2).

Logistic regression analyses were performed and adjusted for confounding covariates including age, gender, body mass index, blood glucose, smoking status and drinking status. The results of the logistic regression analyses are shown in Table 3. The rs4994 was found to be closely associated with EH under the recessive ($P = 0.019$, OR = 0.373, 95% CI 0.163–0.851) and homozygous ($P = 0.028$, OR = 0.394, 95% CI 0.172–0.903) models. The difference was also significant in the recessive ($P = 0.025$, OR = 0.316, 95% CI 0.116–0.866) and homozygous ($P = 0.024$, OR = 0.311, 95% CI 0.113–0.857) models in males. However, in females, significant differences were only observed in the dominant model ($P = 0.047$, OR = 1.671, 95% CI 1.006–2.776).

A significant association was detected between rs1799998 and EH. Adjusted significant differences in the prevalence of rs1799998 were observed in the dominant ($P = 0.011$, OR = 0.626, 95% CI 0.437–0.898), additive ($P = 0.036$, OR = 0.751, 95% CI 0.575–0.982) and allelic ($P = 0.037$, OR = 0.755, 95% CI 0.58–0.983) models. Table 2 shows that the EH group had more TT genotype or T allele. In the male subgroup, results under the dominant ($P = 0.007$, OR = 0.476, 95% CI 0.299–0.757), additive ($P = 0.007$, OR = 0.628, 95% CI 0.447–0.88) and allelic ($P = 0.007$, OR = 0.63, 95% CI 0.451–0.88) models were similar (Table 3). However, no significant differences were observed among females.

The SNPs in the same gene, such as rs699 and rs4762 in *AGT* and rs5193 and rs5194 in *AGTR2*, were used to perform linkage disequilibrium analyses. No obvious linkage disequilibrium was observed ($P > 0.05$).

### Analysis of the correlation between loci and BP

The correlation analyses were performed between the eight SNPs and BP (SBP and DBP). The rs5186 was closely correlated with SBP in NT males ($r = -0.105$, $P = 0.033$), and rs5186 was significantly correlated with DBP in the female NT subgroup ($r = 0.161$, $P = 0.008$). The rs5194 was significantly correlated with SBP in the female NT subgroup ($r = -0.132$, $P = 0.03$). Among EH females, rs5194 was found to be closely correlated with DBP ($r = -0.106$, $P = 0.039$). No obvious correlations were observed between other SNPs and BPs.

## DISCUSSION

EH is a risk because it is an inducing factor for many other diseases, such as diabetes, coronary heart disease and nephropathy. EH can seriously affect a patient's physical and financial health. In the present study, the relationships among EH-related traits (EH, SBP and DBP) and eight notable candidate genetic loci were found to be closely related to the RAAS. The present research was conducted on 1790 northern Han Chinese individuals without diabetes, including 1090 EH patients and 700 normotensive healthy controls.

The rs4994 is a SNP in *ADRB3* (*adrenoceptor beta 3*) gene. The protein encoded by this gene is part of the β-adrenergic receptor family that mediates catecholamine-induced activation of adenylate

**Table 2 Distribution of genotypic and univariate association analyses of SNPs in RAAS**

| *Located gene* | *SNP ID rs no.* | *Chr. position* | *Genotypes/alleles* | *Hypertension,* N *(%)* | *Control,* N *(%)* | *Overall* P*-value* | *OR* | *95% CI* |
|---|---|---|---|---|---|---|---|---|
| *AGT* | rs699 | 1q42.2 | CC | 695 (64.6) | 438 (63.8) | 0.284 | 1.013 | 0.943–1.088 |
| | | | CT | 334 (31.0) | 228 (33.2) | | 0.935 | 0.814–1.074 |
| | | | TT | 47 (4.4) | 21 (3.1) | | 1.429 | 0.862–2.369 |
| | | | C | 1724 (80.1) | 1104 (80.3) | 0.897 | 0.997 | 0.964–1.031 |
| | | | T | 428 (19.9) | 270 (19.7) | | 1.012 | 0.883–1.160 |
| | rs4762 | 1q42.2 | CC | 918 (84.9) | 584 (84.9) | 0.208 | 1 | 0.96–1.041 |
| | | | CT | 145 (13.4) | 99 (14.4) | | 0.932 | 0.735–1.181 |
| | | | TT | 18 (1.7) | 5 (7) | | 2.291 | 0.855–6.143 |
| | | | C | 1981 (91.6) | 1267 (92.1) | 0.66 | 0.995 | 0.975–1.015 |
| | | | T | 181 (8.4) | 109 (7.9) | | 1.057 | 0.842–1.327 |
| *REN* | rs5707 | 1q32 | GG | 147 (13.8) | 109 (15.9) | 0.456 | 0.867 | 0.69–1.09 |
| | | | GT | 529 (49.6) | 336 (49.0) | | 1.012 | 0.918–1.116 |
| | | | TT | 391 (36.6) | 241 (35.1) | | 1.043 | 0.917–1.186 |
| | | | G | 823 (38.6) | 554 (40.4) | 0.288 | 0.955 | 0.878–1.038 |
| | | | T | 1311 (61.4) | 818 (59.6) | | 1.03 | 0.975–1.089 |
| *AGTR1* | rs5186 | 3q24 | CC | 4 (0.4) | 1 (0.1) | 0.42 | 2.556 | 0.286–22.822 |
| | | | AC | 103 (9.5) | 76 (11.0) | | 0.866 | 0.654–1.147 |
| | | | AA | 979 (90.1) | 617 (88.9) | | 1.014 | 0.981–1.048 |
| | | | C | 111 (5.1) | 78 (5.6) | 0.54 | 0.909 | 0.686–1.205 |
| | | | A | 2061 (94.9) | 1310 (94.4) | | 1.005 | 0.989–1.022 |
| *ADRB3* | rs4994 | 8p12 | TT | 745 (68.9) | 489 (71.6) | 0.019 | 0.963 | 0.905–1.024 |
| | | | CT | 322 (29.8) | 175 (25.6) | | 1.163 | 0.993–1.36 |
| | | | CC | 14 (1.3) | 19 (2.8) | | 0.466 | 0.235–0.922 |
| | | | T | 1812 (83.8) | 1153 (84.4) | 0.671 | 0.993 | 0.964–1.023 |
| | | | C | 350 (16.2) | 213 (15.6) | | 1.038 | 0.888–1.214 |
| *CYP11B2* | rs1799998 | 8q21-q22 | CC | 105 (9.8) | 71 (10.3) | 0.329 | 0.944 | 0.71–1.256 |
| | | | CT | 459 (42.7) | 314 (45.7) | | 0.933 | 0.839–1.039 |
| | | | TT | 512 (47.6) | 302 (44.0) | | 1.082 | 0.974–1.202 |
| | | | C | 669 (31.1) | 456 (33.2) | 0.195 | 0.937 | 0.849–1.033 |
| | | | T | 1483 (68.9) | 918 (66.8) | | 1.031 | 0.984–1.081 |
| *AGTR2* | rs5193[a] | Xq22-q23 | GG | 263 (69.4) | 194 (69.8) | 0.059 | 0.994 | 0.898–1.101 |
| | | | GT | 94 (24.8) | 78 (28.1) | | 0.884 | 0.683–1.143 |
| | | | TT | 22 (5.8) | 6 (2.2) | | 2.69 | 1.105–6.545 |
| | | | G | 620 (81.8) | 466 (83.8) | 0.376 | 0.976 | 0.929–1.026 |
| | | | T | 138 (18.2) | 90 (16.2) | | 1.125 | 0.883–1.433 |
| | rs5194[a] | Xq22-q23 | AA | 161 (41.7) | 83 (30.4) | 0.01 | 1.372 | 1.107–1.701 |
| | | | AG | 150 (38.9) | 121 (44.3) | | 0.877 | 0.73–1.052 |
| | | | GG | 75 (19.4) | 69 (25.3) | | 0.769 | 0.576–1.025 |
| | | | A | 472 (61.1) | 287 (52.6) | 0.002 | 1.163 | 1.055–1.282 |
| | | | G | 300 (38.9) | 259 (47.4) | | 0.819 | 0.723–0.928 |

Abbreviations: *ADRB3*, *adrenoceptor β3*; *AGT*, *angiotensinogen*; *AGTR1*, *angiotensin II receptor, type 1*; *AGTR2*, *angiotensin II receptor, type 2*; CI, confidence interval; Chr., chromosome; *CYP11B2*, *aldosterone synthase*; OR, odds ratio; RAAS, renin–angiotensin–aldosterone system; *REN*, *rennin*; SNP, single-nucleotide polymorphism.
[a]Only female genotype statistics.

cyclase through the action of G proteins. Studies of the Trp64Arg (rs4994 T/C) missense mutation have shown Trp64 alleles to be linked to elevated BP in young normotensive men.[19] Trp64Arg polymorphism has been reported to be associated with obesity, central obesity, weight gain, insulin resistance, type 2 diabetes mellitus, hypereuricemia[20] and metabolic syndrome.[21] Cohort studies have shown rs4994 to be a genetic risk factor for cardiovascular disease in hypertensive patients.[22] However, the effect of rs4994 remains unclear.[23] Studies performed on Chinese Han individuals remain rare. The present study was performed in corroboration with a longitudinal study conducted by Dr K Masuo. The present study showed the frequency of the CC rs4994 genotype to be higher in the NT group than in the EH group. Chinese individuals were shown to have lower C allelic (64Arg) frequency (only 15–16% in the present study) than Japanese individuals (20.24%).[24] Although there have been several studies about the relationship between rs4994, hypertension and metabolism, the exact mechanisms remain unclear. ADRB3 was found to belong to the β-adrenergic receptor family. These receptors mediate catecholamine-induced activation of adenylate cyclase through the action of G proteins. ADRB3 is located mainly in the adipose tissue and is involved in the regulation of lipolysis and thermogenesis. In addition to its role in metabolic functions, ADRB3 regulates cardiac inotropy, angiogenesis and endothelium-dependent vasorelaxation in the coronary microvasculature.[25] Some experimental data showed that ADRB3 can modulate peripheral vascular tone and increase BP.[26] Recently, Kumar *et al.*[27] reported that *ADRB3* rs4994 polymorphism was associated with a higher risk of coronary artery disease in Indian

**Table 3 The results from logistic regression analyses of the eight SNPs under the five models**

| | | | *Total* | | | | *Male* | | | | *Female* | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *SNP ID* | *Modle type* | *Comparison* | $P_k$-*value* | P-*value* | *OR* | *95% CI* | $P_k$ -*value* | P-*value* | *OR* | *96% CI* | $P_k$-*value* | P-*value* | *OR* | *97% CI* |
| rs699 | Dominant | TT+CT *vs.* CC | 0.722 | 0.415 | 0.894 | 0.682–1.172 | 0.435 | 0.614 | 0.916 | 0.653–1.286 | 0.676 | 0.722 | 0.919 | 0.575–1.466 |
| | Recessive | TT *vs.* CT+CC | 0.204 | 0.318 | 1.437 | 0.706–2.926 | 0.294 | 0.278 | 1.571 | 0.694–3.556 | 0.629 | 0.832 | 1.173 | 0.267–5.147 |
| | Additive | TT *vs.* CT *vs.* CC | 0.284 | 0.728 | 0.96 | 0.762–1.209 | 0.276 | 0.99 | 0.998 | 0.754–1.321 | 0.734 | 0.722 | 0.919 | 0.575–1.466 |
| | Homozygous | TT *vs.* CC | 0.247 | 0.423 | 0.749 | 0.37–1.518 | 0.441 | 0.351 | 0.68 | 0.302–1.531 | 0.478 | 0.847 | 0.865 | 0.198–3.776 |
| | Allele | T/C | 0.897 | 0.729 | 1.042 | 0.827–1.311 | 0.784 | 0.868 | 1.024 | 0.774–1.355 | 0.562 | 0.801 | 1.054 | 0.7–1.586 |
| rs4994 | Dominant | CC+CT *vs.* TT | 0.24 | 0.291 | 1.166 | 0.877–1.549 | 1 | 0.643 | 1.086 | 0.766–1.541 | 0.037 | 0.047 | 1.671 | 1.006–2.776 |
| | Recessive | CC *vs.* CT+TT | 0.03 | 0.019 | 0.373 | 0.613–0.851 | 0.111 | 0.025 | 0.316 | 0.116–0.866 | 0.25 | 0.25 | 2.382 | 0.543–10.442 |
| | Additive | CC *vs.* CT *vs.* TT | 0.019 | 0.809 | 1.032 | 0.802–1.327 | 0.188 | 0.273 | 0.841 | 0.617–1.146 | 0.025 | 0.146 | 1.4 | 0.89–2.203 |
| | Homozygous | CC *vs.* TT | 0.047 | 0.028 | 0.394 | 0.172–0.903 | 0.111 | 0.024 | 0.311 | 0.113–0.857 | 0.385 | 0.349 | 2.029 | 0.462–8.917 |
| | Allele | C/T | 0.671 | 0.812 | 0.97 | 0.758–1.243 | 0.675 | 0.261 | 1.186 | 0.88–1.599 | 0.122 | 0.15 | 0.72 | 0.461–1.126 |
| rs4762 | Dominant | TT+CT *vs.* CC | 1 | 0.849 | 0.953 | 0.578–1.569 | 0.793 | 0.871 | 1.053 | 0.566–1.959 | 0.913 | 0.462 | 0.713 | 0.29–1.756 |
| | Recessive | TT *vs.* CT+CC | 0.13 | 0.232 | 2.997 | 0.496–18.12 | 0.391 | 0.242 | 4.482 | 0.363–55.41 | 0.207 | 0.696 | 1.655 | 0.132–20.72 |
| | Additive | TT *vs.* CT *vs.* CC | 0.208 | 0.862 | 1.042 | 0.653–1.665 | 0.563 | 0.023 | 1.381 | 1.047–1.822 | 0.757 | 0.623 | 0.828 | 0.391–1.756 |
| | Homozygous | TT *vs.* CC | 0.13 | 0.227 | 1.725 | 0.712–4.183 | 0.39 | 0.267 | 2.009 | 0.586–6.882 | 0.209 | 0.72 | 1.588 | 1.26–19.97 |
| | Allele | T/C | 0.66 | 0.857 | 1.021 | 0.81–1.287 | 0.572 | 0.644 | 1.1 | 0.801–1.432 | 0.765 | 0.596 | 0.896 | 0.597–1.345 |
| rs5707 | Dominant | GG+GT *vs.* TT | 0.541 | 0.875 | 0.971 | 0.671–1.404 | 0.511 | 0.756 | 1.077 | 0.675–1.717 | 0.934 | 0.352 | 0.74 | 0.398–1.395 |
| | Recessive | GG *vs.* GT+TT | 0.239 | 0.442 | 0.819 | 0.492–1.363 | 0.521 | 0.52 | 0.804 | 0.414–1.561 | 0.234 | 0.76 | 0.85 | 0.366–1.976 |
| | Additive | GG *vs.* GT *vs.* TT | 0.456 | 0.61 | 0.934 | 0.717–1.217 | 0.395 | 0.919 | 0.983 | 0.688–1.38 | 0.468 | 0.394 | 0.824 | 0.528–1.285 |
| | Homozygous | TT *vs.* GG | 0.226 | 0.454 | 0.794 | 0.435–1.451 | 0.423 | 0.543 | 0.78 | 0.351–1.736 | 0.344 | 0.973 | 0.983 | 0.358–2.698 |
| | Allele | T/G | 0.288 | 0.622 | 0.938 | 0.726–1.211 | 0.41 | 0.923 | 0.984 | 0.711–1.363 | 0.494 | 0.753 | 0.925 | 0.569–1.504 |
| rs5186 | Dominant | AA+AC *vs.* CC | 0.425 | 0.522 | 1.214 | 0–618–2.199 | 0.683 | 0.203 | 1.644 | 0.765–3.534 | 0.52 | 0.318 | 0.425 | 0.522 |
| | Recessive | CC+AC *vs.* AA | 0.654 | 0.913 | 0.654 | 0.405– 1.454 | 1 | | | | 1 | | 0.654 | 0.913 |
| | Additive | CC *vs.* AC+AA | 0.42 | 0.531 | 1.207 | 0.671–2.172 | 0.752 | 0.202 | 1.645 | 0.766–3.532 | 0.594 | 0.314 | 0.42 | 0.531 |
| | Homozygous | CC *vs.* AC *vs.* AA | 0.655 | 0.911 | 0.789 | 0.012–51.19 | 1 | | | | 1 | | 0.655 | 0.911 |
| | Allele | CC *vs.* AA | 0.54 | 0.541 | 1.281 | 0.69–2.376 | 0.768 | 0.214 | 1.677 | 0.763–3.354 | 0.622 | 0.325 | 0.54 | 0.541 |
| rs1799998 | Dominant | CC+CT *vs.* TT | 0.142 | 0.011 | 0.626 | 0.437–0.898 | 0.086 | 0.007 | 0.476 | 0.299–0.757 | 0.875 | 0.368 | 0.581 | 0.179–1.893 |
| | Recessive | CC *vs.* CT+TT | 0.685 | 0.692 | 0.892 | 0.505–1.574 | 0.489 | 0.415 | 0.748 | 0.372–1.503 | 0.662 | 0.838 | 1.073 | 0.544–2.116 |
| | Additive | CC *vs.* CT *vs.* TT | 0.329 | 0.036 | 0.751 | 0.575–0.982 | 0.095 | 0.007 | 0.628 | 0.447–0.88 | 0.963 | 0.804 | 0.936 | 0.556–1.576 |
| | Homozygous | CC *vs.* TT | 0.441 | 0.261 | 0.839 | 0.617–1.14 | 0.247 | 0.114 | 0.725 | 0.486–1.08 | 0.762 | 0.47 | 1.565 | 0.464–5.278 |
| | Allele | C/T | 0.195 | 0.037 | 0.755 | 0.58–0.983 | 0.098 | 0.007 | 0.63 | 0.451–0.88 | 1 | 0.806 | 1.066 | 0.837–1.784 |
| rs5193 | Dominant | TT+GT *vs.* GG | | | | | | | | | 0.932 | 0.074 | 1.792 | 0.911–3.525 |
| | Recessive | TT *vs.* GT+GG | | | | | | | | | 0.03 | 0.199 | 2.796 | 0.582–13.426 |
| | Additive | TT *vs.* GT *vs.* GG | | | | | | | | | 0.36 | 0.055 | 1.696 | 0.968–2.972 |
| | Homozygous | TT *vs.* GG | | | | | | | | | 0.03 | 0.144 | 1.847 | 0.811–4.21 |
| | Allele | T/G | | | | | | | | | 0.376 | 0.044 | 1.809 | 1.016–3.223 |
| rs5194 | Dominant | GG+AG *vs.* AA | | | | | | | | | 0.003 | 0.231 | 0.683 | 0.367–1.273 |
| | Recessive | GG *vs.* AG+AA | | | | | | | | | 0.085 | 0.546 | 0.777 | 0.343–1.761 |
| | Additive | GG *vs.* AG *vs.* AA | | | | | | | | | 0.004 | 0.258 | 0.783 | 0.513–1.196 |
| | Homozygous | GG *vs.* AA | | | | | | | | | 0.007 | 0.33 | 0.795 | 0.501–1.262 |
| | Allele | G/A | | | | | | | | | 0.002 | 0.229 | 0.759 | 0.485–1.19 |

Abbreviations: CI, confidence interval; OR, odds ratio; SNP, single-nucleotide polymorphism.
Note that $P_k$-value was the result from $\chi^2$ test; *P*-value was the result from logistic regression analysis; and OR and 95% CI were also the result from logistics.

individuals. The rs4994 was found to be associated with EH, but no association was found between BP levels (including SBP and DBP).

The rs1799998 (−344C/T) is upstream of the *CYP11B2* (*cytochrome P450, family 11, subfamily B, polypeptide 2*) gene on human chromosome 8 and has been reported to be linked to hypertension. The promoter genotype of −344C/T was found to have an effect on intima–media thickness in Greek individuals with untreated hypertension (see Androulakis *et al.*[28]). In the present study, the CC genotype and C-allele individuals had a lower risk of EH in the total population and in males, but no significant differences were found among females. There is evidence that male sex hormones contribute to the exacerbation of hypertension in spontaneously hypertensive rats by reducing pressure-natriuresis.[29] Russo *et al.*[30] found −344C/T to be associated with higher BP and prevalence of hypertension in younger men but not in women. Several other studies have also reported gender-specific effects of gene variants and gene-by-gender interactions in human hypertension.[31,32] Hypertension may be more linked to C allele of rs1799998 in males than in females. Male participants comprised a large proportion of the current study cohort, and this may have skewed the overall results toward the male subgroup. This outcome was not consistent with the results of a meta-analysis published in 2010.[33] In that systemic review of the relationship between rs1799998 and EH, Cheng and Xu[33] did not find any association between rs1799998 and susceptibility to hypertension in either gender in a meta-analysis mainly covering studies from 2003 to 2007. The sample size of each study was smaller than that of the current study. No significant association was found between rs1799998 and BP traits (SBP and DBP). The present results had some differences from a Japanese study published in 2012.[34] Relationships were detected only between rs1799998 and EH, and rs1799998 was found to be closely associated with EH, SBP and DBP. These differences became more significant after logistic regression analysis with covariates adjusted in the SNP rs1799998. This outcome may indicate the presence of feedback between rs1799998 and environment; more information on the molecular level and clinical evidence are required.[35] These issues may be addressed in another study. In the present study and in studies by Takeuchi *et al.*[34] and Li *et al.*,[36] significant associations were found between rs1799998 and EH and BPs. All of these studies involved ≈2000 samples, and in the 2010 meta-analysis there were only 4259 EH and 3213 controls in nine studies.

The frequencies of rs5194 were found to differ ($P_{rs5194}<0.01$) between the EH and NT subgroups in female. The AA genotype and A allele of rs5194 were found to be more common in the female EH subgroup than in other groups. The genotypes of rs5193 and rs5194 met the HWE test for females. However, no significant differences were found among EH and rs5193 or rs5194 in all genetic models except rs5193 under the allelic model ($P=0.044$, OR = 1.809, 95% CI 1.016–3.223). TT of rs5193 tended to be more common in the female EH group ($P=0.059$). However, this outcome does not fully prove an association between rs5193 and EH. Because no statistically significant differences were found among the other genetic models, and the lower confidence limit was near 1, rs5194 was found to be linked to SBP in the female NT subgroup and was associated with DBP in the female EH group. The present results were similar to those of another study about the association between rs5193, rs5194 and EH performed in a smaller sample of Cantonese individuals.[37] No association was found between rs5193/rs5194 and EH. The rs5193 and rs5194 are located in Xq22-q23, the untranslated regions 3′ of AGTR2. This receptor has been shown to mediate programmed cell death, and this apoptotic function may play an important role in developmental biology and pathophysiology. Mutations in this gene have been found to be associated with X-linked mental retardation (www.ncbi.nlm.nih.gov/SNP). Studies of the relationship between SNPs and EH are scarce. Some studies found the A allele of rs5194 to be associated with diminished angiotensin activity, but this phenomenon has not been verified.[38] Ellis *et al.*[39] found rs5194 to be closely associated with aortic stenosis in elderly individuals. Ouyang *et al.*[40] reported that rs5194 was associated with the risk of aldosterone-producing adenoma, and the A allele was associated with an especially high risk of aldosterone-producing adenoma. However, existing studies of rs5194 are not sufficient. The data observed in the current study show that some SNPs might affect the level of *AGTR2* expression and thus, both directly and indirectly, affect BP through mediation of apoptosis.

Using linear correlation, we found that among the six important candidate genes related to RAAS, only rs4994 (*ADRB3*), rs1799998 (*CYP11B2*), rs5186 (*AGTR1*), rs5193 (*AGTR2*) and rs5194 (*AGTR2*) were found to be linked to BP traits (including EH, SBP and DBP) across all eight SNPs, and some of them only showed significant differences in one gender subgroup or the other.

The data collected here demonstrated that *ADRB3* rs4994 and *CYP11B2* rs1799998 were closely associated with EH in northern Han Chinese individuals. The CC genotype of rs4994 and CC genotype and C allele of rs1799998 may be associated with a lower risk of EH. *AGTR1* rs5186 and *AGTR2* rs5194 were found to be closely correlated with BP in gender-specific subgroups.

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