Association of CYP17A1 Genetic Polymorphisms and Susceptibility to Essential Hypertension in the Southwest Han Chinese Population

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Background: The CYP17A1 gene encodes for cytochrome P450 enzyme CYP17A1, which is involved with the steroidogenic pathway including mineralocorticoids. The CYP17A1 polymorphisms might affect enzyme activity, then leading to a state of mineralocorticoid 11-deoxycorticosterone excess characterized by hypertension, suppressed plasma renin activity, and low aldosterone concentrations. The aim of this study was to investigate the contribution of CYP17A1 polymorphisms in inducing the susceptibility to essential hypertension among the Southwest Han Chinese population.

Material/Methods: Eight single nucleotide polymorphisms of CYP17A1 were genotyped in a case-control study for samples by polymerase chain reaction-restriction fragment length polymorphism analysis.

Results: The polymorphisms rs11191548 and rs4919687 were significantly associated with hypertension risk, which was confirmed by systolic and diastolic blood pressure distribution analyses between different genotype groups, and these two polymorphisms were found in linkage disequilibrium. The rs4919687 polymorphism was estimated to cause the destruction of exonic splicing silencer (ESR and Motif 3) sites and to transform the transcription factor AREB6 binding site, respectively, in the bioinformatics analyses. The haplotypes rs4919686A-rs3740397G-rs4919687C-rs743572C-rs11191548C and rs4919686A-rs3740397G-rs4919687T-rs743572C-rs11191548T were found to be susceptible to essential hypertension.

Conclusions: Our findings suggest that the CYP17A1 polymorphisms could be a genetic risk factor for essential hypertension among the Yunnan Han Chinese population, which would have implications for the treatment of this complex disorder.

MeSH Keywords: Genetic Association Studies • Genetics, Population • Hypertension • Polymorphism, Single Nucleotide

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Background

Essential hypertension (EH) has become a major public-health concern in the world, and it is supposed to be associated with the increased risk of cardiovascular and kidney disease [1–3]. In China, about 153 million (18%) Chinese adults were hypertensive in 2002 [4]. It is estimated that 29.2% (range, 28.8–29.7%) of the world population will have hypertension by 2025, increasing by about 60% [2]. EH is considered to be a multifactorial inherited disease impacted with the interactions of genetic (polygenic predisposition) and environmental (obesity, alcohol excess, smoking, dietary sodium intake) factors [5]. Nearly 30–50% of the blood pressure (BP) variation among the population can be attributed to the genetic risks [6]. Over the last decade, scientists have found many gene variants associated with EH [7,8].

The CYP17A1 gene encodes a member of the cytochrome P450 superfamily of enzymes. The cytochrome P450 proteins were supposed to be involved with endogenous metabolites, such as aldosterone, and sex hormones, e.g., 17α-hydroxyprogesterone, 11-deoxycorticosterone, and corticosterone, almost all patients with EH are believed to have hypertension [3]. This work is supported by the National Natural Science Foundation of China (51371110), and the tag SNPs were screened under the standard of http://www.broad.mit.edu/hapmap/.

Clinical measurements

For the sample collection, questionnaires were used to collect information involved with the history of hypertension, medications, and anthropometric parameters of patients. After at least 30 minutes of rest, measurements were performed by using standard mercury sphygmomanometers on the right arm of subjects in the sitting position. SBP was determined by the onset of the “tapping” Korotkoff sound and DBP by the fourth Korotkoff sound. The BP measurements were performed at 5-minute intervals 3 times, and the average value was used for analysis.

Selection of single nucleotide polymorphisms (SNPs)

The common SNPs (minor allele frequency [MAF] >1%) of CYP17A1 were obtained from the Han Chinese data set of the International HapMap Consortium (http://www.hapmap.org/index.html.js). The tag SNPs were screened under the standard of r² >0.8 by using Haplovew4.1 software (http://www.broad.mit.edu/hapmap/).
The analysis of covariance (ANCOVA) was used to compare the analyses, because the frequency of AA genotype for this polymorphism P<0.001. The rs755443 was also ruled out for the further analyses, because they were not in the Hardy-Weinberg equilibrium. rs1004467 and rs762563 were ruled out for the further analyses because they were not in the Hardy-Weinberg equilibrium. The quantitative variables and categorical variables were expressed as the mean ± standard deviation and percentages, respectively. The Hardy-Weinberg equilibrium analyses for each SNP were calculated by using the chi-square test. The Hardy-Weinberg equilibrium analyses for each SNP were calculated by using the chi-square test (respectively. The Hardy-Weinberg equilibrium analyses for each SNP were calculated by using the chi-square test (respectively). The genotype call rates for all eight SNPs were 98.9% (1009/1020). To guarantee the accuracy of genotyping, 50 randomly selected samples were repeated for each SNP, and 100% concordance was observed between the results of the two tests. In addition, 50 samples were randomly selected for direct sequencing, and 100% concordance was observed between these two genotyping methods.

### Statistical analysis

The quantitative variables and categorical variables were expressed as the mean ± standard deviation and percentages, respectively. The Hardy-Weinberg equilibrium analyses for each SNP were calculated by using the chi-square test (χ² test). The rs1004467 and rs762563 were ruled out for the further analyses, because they were not in the Hardy-Weinberg equilibrium. The rs755443 was also ruled out for the further analyses, because the frequency of AA genotype for this polymorphism was close to 0, leading to the inaccurate results. The analysis of covariance (ANCOVA) was used to compare the differences in BP levels among different genotype and allele groups with age, sex, and body mass index (BMI) as covariates. Logistic regression analysis was applied to test the association between each SNP and hypertension risk. In genetic model analyses (co-dominant, dominant, recessive, overdominant, and additive), the minor allele of each SNP was regarded as the risk factor, and each analysis was adjusted by age, gender, and BMI. Linkage disequilibrium (LD) analysis was performed in the case and control groups using SHEsis software online (http://analysis.bio-x.cn/myAnalysis.php). To explore whether these five variants had combined effects on hypertension, haplotype analysis was also performed by using SHEsis software. The Bonferroni correction was applied to assess the probability of a spurious association by multiple comparisons (P<0.05/5). A 2-tailed probability value of P<0.05 was considered as statistically significant. All analyses were carried out by using SPSS (version 16.0; SPSS Inc., Chicago, Illinois, USA) and adjusted for age, sex, and BMI.

### Bioinformatics analysis

P-match is a kind of functional prediction tool (http://www.gene-regulation.com/cgi-bin/pub/programs/pmatch/bin/pmatch.cgi), which is used to predict the effects of polymorphisms on the sequence structures of potential binding sites for transcription factors (TFs). This software is involved with a library of mononucleotide weight matrices as well as sets of aligned known TF binding sites from TRANSFACR. The available TF binding sites around the SNPs localized in the promoter and intron region of the target gene are considered in this software.

Human splicing finder (HSF) software, a kind of Bioinformatics tool (http://www.umd.be/HSF/), is used to predict the effects of mutations on splicing signals and to identify splicing motifs in any human sequence. This software contains all the genes and alternative transcripts, as well as intronic sequences extracted from the Ensembl human genome database, to study the potential effects of SNPs on splicing.

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Table 1. The tag SNPs of CYP17A1 gene.

| Tag SNP | Captured SNP |
|---------|--------------|
| rs743572 | rs10786712 rs6163 rs6162 rs3781287 rs743572 |
| rs1004467 | rs17115100 rs3824755 rs1004467 |
| rs4919687 | rs10883783 rs4919687 |
| rs755443 | rs755443 |
| rs3740397 | rs3740397 |
| rs4919686 | rs4919686 |
| rs762563 | rs762563 |

The r² of each Tag SNP with its captured SNPs were more than 0.8.

edu/mpg/haploview). Seven tag SNPs (rs743572, rs1004467, rs4919687, rs755443, rs3740397, rs4919686, rs762563) and another well-studied polymorphism (rs11191548) were selected (Table 1) for this study. The CYP17A1 gene structure and relative location of these eight SNPs are shown in Figure 1.

**Figure 1.** CYP17A1 (cytochrome P450, family 17, subfamily A, polypeptide 1) structure and relative position of 8 tag SNPs. Black boxes indicate exons, lines indicate introns, and gray boxes indicate 5’-UTR and 3’-UTR.
Table 2. Oligonucleotides and restriction enzymes of polymorphic sites for genotyping.

| Locus       | Location | Long PCR | Nest PCR | Restriction enzyme | Digested bands (bp) |
|-------------|----------|----------|----------|-------------------|---------------------|
|             |          | Primer sequence (5'–3') | TA | Primer sequence (5'–3') | TB |                     |
| rs4919686   | Intron6  | F: GTCAAGGACGACGATAGGAC 56°C | 56°C | F: GTACCCGTAACGTTCACTTC 56°C | NmuCl | A: 122, 208; C: 330 |
|             |          | R: TGGATGAGCTGAACTGCTGGT 56°C | 56°C | R: GCTGGTCCTGAAACCCCTG 56°C |                     |                     |
| rs3740397   | Intron5  | F: ACTCGAGGACGACGATAGGAC 56°C | 56°C | F: AACCACATTCAAGCCATAG 57°C | Munl | G: 172, 75; C: 247 |
|             |          | R: TGGATGAGCTGAACTGCTGGT 56°C | 56°C | R: GGGTCGCAAAGAAAATGCTGGT 56°C |                     |                     |
| rs1004467   | Intron3  | F: GAGGGACCACTGTGCTGGT 56°C | 56°C | F: CGTTCGCTAGGGCTGAAC 56°C | Rsal | T: 19, 193 C: 212 |
|             |          | R: CAGGAGTAAACTGCTGGTATA 56°C | 56°C | R: GGGGCAATGTCCAGGGTTG 56°C |                     |                     |
| rs755443    | Intron2  | F: AGAGTTGCCTTCCTGTGGTC 56°C | 56°C | F: AGGTAAGTCCTAAGGCTTAG 57°C | HapI | G: 190, 20 A: 210 |
|             |          | R: CAGGATGAACTGCTGGTATA 56°C | 56°C | R: AGATGAGGCGAGGGAAC 57°C |                     |                     |
| rs4919687   | Intron1  | F: AGAGTTGCCTTCCTGTGGTC 56°C | 56°C | F: AGGTAAGTCCTAAGGCTTAG 57°C | Kpn I | C: 153, 81; T: 234 |
|             |          | R: CAGGATGAACTGCTGGTATA 56°C | 56°C | R: AGATGAGGCGAGGGAAC 57°C |                     |                     |
| rs762563    | Exon1    | F: CCCTGAAAAGTGCACTGTGAGAA 56°C | 56°C | F: GGGTTAAGTGGCCACCATG 56°C | Ncol | C: 16,177; G: 193 |
|             |          | R: CCAGATACCCCTCGACT 56°C | 56°C | R: GTCAGGGTGGAAGTCCAGGATG 56°C |                     |                     |
| rs743572    | Exon1    | F: CCCTGAAAAGTGCACTGTGAGAA 56°C | 56°C | F: GGGTTAAGTGGCCACCATG 56°C | Ncol | C: 16,177; G: 193 |
|             |          | R: CCAGATACCCCTCGACT 56°C | 56°C | R: GTCAGGGTGGAAGTCCAGGATG 56°C |                     |                     |
| rs11191548  | 5’ UTR   | F: TTCTGTGTCGTCGGAGGATGTC 56°C | 56°C | F: TGGATGGTTCGTTGGTATG 56°C | Taq I | C: 65,276 T: 341 |
|             |          | R: TGAGGAGCCACCTTGCCAC 56°C | 56°C | R: ACCAGAAATACGCTGGAGACA 56°C |                     |                     |

F – forward primer; R – reverse primer; TA – temperature of long-PCR annealing; TB – the temperature of nest-PCR annealing.

Results

Population characteristics

The general characteristics of the study subjects are summed up in Table 3. The distribution of age and gender showed no significant difference between the case and control groups, whereas the BP and BMI levels were significantly higher in the patient group than in the control group. As the development of hypertension would be affected by multiple confounding factors, the confounding factors (including gender, age, and BMI) were adjusted as covariants in the further analyses.

The genotype distributions of eight SNPs in the case-control groups are described in Table 4. Of the eight variants examined in the total population, six showed no indication of a deviation from Hardy-Weinberg equilibrium in normotensive controls (P<0.05). Because the minor allele frequency of rs755443 was 0 in the control group, leading to the inaccurate results for analysis, this SNP was also ruled out for the further analysis. Genotyping was successfully done for 504 out of the 510 (98.8%) EH patients and for 505 out of the 510 (99.0%) controls.

Single-site analyses

The results of single-site analyses for the five SNPs localized in the CYP17A1 region are shown in Tables 5–7. The minor allele-T rate of rs4919687 (P=0.0202, OR=1.283) was found to be significantly greater in the patient group than in the control group, and the minor allele-T of rs4919687 (mean difference [MD]=3.045, P=0.0106) was also found to be significantly associated with increased SBP levels. However, the minor allele-C frequency of rs11191548 (P=0.0002, OR=0.675) was found to be significantly smaller in the patient group than in the control group, and the minor allele-T of rs4919687 (mean difference [MD]=3.354, P=0.0041, MD=1.917, P=0.0066) was also found to be significantly associated with decreased BP levels (Table 5).

The differences in BP levels among the groups with different genotypes were analyzed by ANCOVA with multiple comparison correction, and the results are shown in Table 6. Via adjusting for age, gender, and BMI, the SBP level of the subjects with CT+TT genotype of rs4919687 was significantly higher than that of the subjects with CC genotype (P=0.002), before or after Bonferroni correction. Indeed, the carriers with T alleles had higher SBP levels compared with those with the CC
Table 3. The basic characteristics of the Yunnan Han Chinese population.

| Parameters       | Normotensive | Hypertensive | p-value |
|------------------|--------------|--------------|---------|
| Number           | 510.00       | 510.00       |         |
| Males (%)        | 65.69        | 65.69        | 1.000   |
| Age (years)      | 53.46±10.44  | 53.81±10.23  | 0.588   |
| Current smokers (%) | 38.82      | 37.84        | 0.747   |
| Current drinkers (%) | 26.47      | 27.45        | 0.724   |
| SBP (mm HG)      | 111.5±11.09  | 147.92±20.67 | <0.001  |
| DBP (mm HG)      | 73.53±7.07   | 94.75±13.04  | <0.001  |
| BMI (Kg/m²)      | 22.98±2.79   | 24.64±2.81   | <0.001  |

SBP – systolic blood pressure; DBP – diastolic blood pressure; BMI – body mass index. Data were presented as mean±standard deviation. p-value was calculated on comparison on hypertension cases and controls and p<0.05 was considered statistical significance.

Table 4. Exact test for Hardy-Weinberg equilibrium (n=1009).

| Region      | Variant | Allele *1/2 | Case-hypertensive | Control-normotensive | p-value |
|-------------|---------|-------------|-------------------|----------------------|---------|
| Intron_6    | rs4919686 | A/C        | 0.170 348 141 15 | 0.871 0.144 370 125 |   0.001 |
| Intron_5    | rs3740397 | C/G        | 0.480 142 240 122 | 0.332 0.505 127 246 |   0.590 |
| Intron_3    | rs1004467 | T/C        | 0.361 244 156 104 | <0.0001 0.432 197 180 |   0.001 |
| Intron_2    | rs755443  | G/A        | 0.031 475 27 2 | 0.074 0.000 499 6 |   0.001 |
| Intron_1    | rs4919687 | C/T        | 0.273 261 211 32 | 0.260 0.245 292 179 |   0.401 |
| Exon_1      | rs762563  | C/G        | 0.003 502 1 1 | 0.004 0.004 503 0 |   0.001 |
| 5' UTR      | rs743572  | C/T        | 0.439 163 240 101 | 0.473 0.416 177 236 |   0.411 |
| 5' near     | rs11191548 | T/C        | 0.241 280 205 19 | 0.014 0.305 243 216 |   0.401 |

MAF – minor allele frequency. p-value >0.05 was considered to be consistent with Hardy-Weinberg equilibrium. * 1 represented major allele, 2 represented minor allele.

Table 5. The associations between minor-alleles of CYP17A1 SNPs and the risk of EH and BP levels.

| SNP          | Allele *1/2 | MAF  | Risk of minor allele | SBP | DBP |
|--------------|-------------|------|----------------------|-----|-----|
|              |             | Control | Case     | OR** | 95% CI** | p** | MD** | 95% CI** | p** | MD** | 95% CI** | p** |
| rs4919686    | A/C         | 0.144 | 0.170 | 1.298 | 1.008–1.673 | 0.0435 | 2.657 | -0.157–5.471 | 0.0642 | 0.550 | -1.150–2.250 | 0.5265 |
| rs3740397    | C/G         | 0.505 | 0.480 | 0.886 | 0.738–1.064 | 0.1959 | -1.038 | -3.082–1.006 | 0.3194 | -1.273 | -2.506–(–0.040) | 0.0430 |
| rs4919687    | C/T         | 0.245 | 0.273 | 1.283 | 1.040–1.583 | 0.0202 | 3.045 | 0.712–5.378 | 0.0106** | 0.976 | -0.434–2.386 | 0.1748 |
| rs743572     | C/T         | 0.416 | 0.439 | 1.096 | 0.911–1.318 | 0.3308 | 0.713 | -1.351–2.778 | 0.4980 | 1.321 | 0.076–2.566 | 0.0376 |
| rs11191548   | T/C         | 0.305 | 0.241 | 0.675 | 0.549–0.830 | 0.0002** | -3.354 | -5.642–(–1.066) | 0.0041** | -1.917 | -3.299–(–0.536) | 0.0066*** |

SBP – systolic blood pressure; DBP – diastolic blood pressure; MAF – minor allele frequency; MD – mean difference. MD and 95% confidence intervals (CI) were analyzed by ANCOVA between the groups with different alleles; odds ratio (OR) and 95% CI were analyzed by logistic regression between the case and control groups; p<0.05 was considered statistical significance. * 1 represented major allele, 2 represented minor allele; ** adjusted for sex, age and BMI; *** indicated that the p-value remained significant after Bonferroni correction (p<0.05/5).
Table 6. The associations of 5 SNPs with the BP levels in this population.

| SNP          | Genotype | N   | SBP*      | p-value* | DBP*      | p-value* |
|--------------|----------|-----|-----------|----------|-----------|----------|
| rs4919686    | AA       | 718 | 128.91±24.54 |          | 83.73±14.84 |          |
|              | AC+CC    | 291 | 131.93±23.59 | 0.064    | 84.33±14.26 | 0.547    |
| rs3740397    | CC       | 269 | 132.09±24.01 |          | 85.88±14.47 |          |
|              | CG+GG    | 740 | 129.02±24.13 | 0.067    | 83.23±14.53 | 0.080    |
| rs4919687    | CC       | 553 | 127.69±24.15 |          | 83.42±14.63 |          |
|              | CT+TT    | 456 | 132.34±23.85 | 0.002**  | 85.04±14.46 | 0.071    |
| rs743572     | CC       | 340 | 129.64±23.90 |          | 83.04±14.42 |          |
|              | CT+TT    | 669 | 129.90±24.26 | 0.870    | 84.35±14.64 | 0.163    |
| rs11191548   | TT       | 523 | 131.17±24.13 |          | 85.04±14.57 |          |
|              | TC+CC    | 486 | 128.10±23.94 | 0.038    | 83.19±14.46 | 0.037    |

SBP – systolic blood pressure; DBP – diastolic blood pressure. Continuous variables were expressed as the mean ±S.D. p-value <0.05 was considered statistical significance. * Adjusted for age, gender and BMI; ** indicated that the p-value remained significant after Bonferroni correction.

In order to find a comprehensive view of the effects mediated by the genetic factors on the development of EH, different genetic models were used to identify the associations between these polymorphisms and the risk of EH (Table 7). After adjustment by age, sex, and BMI, the SNP rs4919687 was found to be significantly associated with hypertension risk (dominant: OR=1.47, 95% CI=1.13–1.92, P=0.0038), and the rs11191548 was also found to be significantly associated with hypertension risk (codominant model: P=0.001, TC vs. TT: OR=0.76, 95% CI=0.58–1.00, CC vs. TT: OR=0.31, 95% CI=0.17–0.55; dominant: OR=0.68, 95% CI=0.52–0.88, P=0.0036; recessive: OR=0.35, 95% CI=0.19–0.62, P=0.0002; log-additive: OR=0.66, 95% CI=0.53–0.81, P=0.0001). These results remained statistically significant after Bonferroni correction.

**Linkage disequilibrium analysis**

Linkage disequilibrium (LD) analysis demonstrated that two SNPs (rs11191548 and rs4919687) were in LD in both control (/D’=0.82) and case (/D’=0.92) groups (Figures 2, 3).

**Bioinformatics analyses**

Seven SNPs (including rs11191548, rs743572, rs4919687, rs755443, rs1004467, rs3740397, and rs4919686) excluding the exonic polymorphisms of the CYP17A1 gene were further analyzed for the influence on the sequence structures of TF binding sites by p-match. The results indicated that the sequence (AGGCA GTACCTG) containing the major allele G of rs4919687 (c.298-99C>T) could be captured by the TF AREB6, while the sequence (AGGCA GTACCTG) containing the minor allele A of rs4919687 could not be captured by the TF AREB6, suggesting that the single nucleotide substitution of G→A at position 298–299 in intron 1 of the CYP17A1 gene could change the sequence structure of the TF binding site. No TF binding site was found around the loci rs11191548, rs743572, rs755443, rs3740397, and rs4919686.

These SNPs (rs762563, rs4919687, rs755443, rs1004467, rs3740397, rs4919686) localized in the exons or introns of the CYP17A1 gene were also analyzed for the effects on the process of mRNA splicing by HSF software. The results revealed that the rs4919687 (c.298-99C>T) polymorphism localized at position 298-299 of intron 1 of CYP17A1 led to the destruction of ESS (including ESR and Motif 3) sites; the rs755443 (c.437-83C>T) polymorphism localized in intron 2 of CYP17A1 led to the creation of ESE (EIEs and ESR) sites, possibly enhancing the process of splicing; the rs762563 (c.66C>G) polymorphism localized at the 66 nucleotide of exon 1 of CYP17A1 led to the creation of the new exonic splicing silencer (ESS, including Motif 2, Fas, IIEs, ESR) sites and the destruction of the exonic splicing enhancer (ESE, including SC35, EIEs, SF2/ASF(lg)) sites, thereby weakening the process of splicing.

**Haplotype analyses**

The summarized results of haplotype analyses for the combined effect of rs4919686, rs3740397, rs4919687, rs743572, and rs11191548 in the Yunnan Han Chinese population are shown in Table 8. Twenty-three haplotypes of the CYP17A1 gene were...
Table 7. The associations of 5 SNPs with the hypertension risk in this population.

| SNP       | Allele *1/2 | Model                      | Genotype status | n=1009 | OR** (95% CI) | p***-value |
|-----------|-------------|----------------------------|-----------------|--------|---------------|------------|
| rs4919686 | A/C         | Codominant                 | 1/1             | 1.00   |               |            |
|           |             |                            | 1/2             | 1.29 (0.96–1.73) | 0.1300    |            |
|           |             |                            | 2/2             | 1.77 (0.75–4.18) |           |            |
|           |             | Dominant                   | 1/1             | 1.00   |               |            |
|           |             |                            | 1/2–2/2         | 1.32 (0.99–1.76) | 0.0580    |            |
|           |             | Recessive                  | 1/1–1/2         | 1.00   |               |            |
|           |             |                            | 2/2             | 1.65 (0.70–3.87) | 0.2400    |            |
|           |             | Overdominant               | 1/1–2/2         | 1.00   |               |            |
|           |             |                            | 1/2             | 1.26 (0.94–1.69) | 0.1200    |            |
|           |             | Log-additive               | ---             |        | 1.30 (1.01–1.68) | 0.0430    |
| rs3740397 | C/G         | Codominant                 | 1/1             | 1.00   |               |            |
|           |             |                            | 1/2             | 0.85 (0.62–1.16) | 0.4100    |            |
|           |             |                            | 2/2             | 0.79 (0.55–1.14) |           |            |
|           |             | Dominant                   | 1/1             | 1.00   |               |            |
|           |             |                            | 1/2–2/2         | 0.83 (0.62–1.11) | 0.2100    |            |
|           |             | Recessive                  | 1/1–1/2         | 1.00   |               |            |
|           |             |                            | 2/2             | 0.88 (0.65–1.19) | 0.4100    |            |
|           |             | Overdominant               | 1/1–2/2         | 1.00   |               |            |
|           |             |                            | 1/2             | 0.95 (0.73–1.23) | 0.6800    |            |
|           |             | Log-additive               | ---             |        | 0.89 (0.74–1.07) | 0.2000    |
| rs4919687 | C/T         | Codominant                 | 1/1             | 1.00   |               |            |
|           |             |                            | 1/2             | 1.53 (1.16–2.01) | **0.0100*** |            |
|           |             |                            | 2/2             | 1.20 (0.70–2.05) |           |            |
|           |             | Dominant                   | 1/1             | 1.00   |               |            |
|           |             |                            | 1/2–2/2         | 1.47 (1.13–1.92) | **0.0038*** |            |
|           |             | Recessive                  | 1/1–1/2         | 1.00   |               |            |
|           |             |                            | 2/2             | 1.00 (0.59–1.69) | 0.9900    |            |
|           |             | Overdominant               | 1/1–2/2         | 1.00   |               |            |
|           |             |                            | 1/2             | 1.50 (1.14–1.96) | 0.0320    |            |
|           |             | Log-additive               | ---             |        | 1.29 (1.04–1.59) | 0.0190    |
constructed, but only eight of them had a frequency above 1% in the case and control groups. The results showed that the frequency of haplotype 1 (H1 ACCTT) was higher than that of the other haplotypes. Thus, taking H1 as a reference, the relative risk of EH related to different haplotypes was evaluated by logistic analyses. After adjusting for sex, age, and BMI, H2 AGCCC (P=0.0014, OR=0.68) displayed a significant association with decreased risk of EH; the H6 AGTCT (P=0.0013, OR=4.27) had a significant association with increased risk of EH. These results remained statistically significant via Bonferroni corrections.

Discussion

Hypertension is a main factor causing high rates of global morbidity and mortality [1]. The CYP17A1 gene is emerging as a risk factor for EH [33,34]. Since there are limited data on the CYP17A1 genetic determinants of EH for the patients among the Han Chinese population in Yunnan province, our study sought to determine the contribution of eight SNPs localized in the CYP17A1 gene region (including rs743572, rs1004467, rs4919687, rs755443, rs3740397, rs4919686, rs762563, and rs11191548) in inducing susceptibility to EH in this population. We found that (1) among the Chinese Han population in Yunnan province the rs11191548 and rs4919687 SNPs were significantly associated with EH, and these two polymorphisms were found in linkage disequilibrium; (2) in the bioinformatics analyses, the rs4919687 polymorphism was found to cause the destruction of exonic splicing silencer (including ESR and Motif 3) sites and to transform the sequence structure of the transcription factor AREB6 binding site, respectively, thereby functionally affecting the expression level

Table 7 continued. The associations of 5 SNPs with the hypertension risk in this population.

| SNP      | Allele *1/2 | Model     | Genotype status | n=1009 | OR** (95% CI) | p**-value |
|----------|-------------|-----------|-----------------|--------|---------------|-----------|
| rs743572 | C/T         | Codominant| 1/1             | 1.00   |               |           |
|          |             |           | 1/2             | 1.15 (0.86–1.54) | 0.5800     |
|          |             |           | 2/2             | 1.18 (0.81–1.70) |           |
|          |             | Dominant  | 1/1             | 1.00   |               |           |
|          |             |           | 1/2–2/2         | 1.16 (0.88–1.52) | 0.3000     |
|          |             | Recessive | 1/1–1/2        | 1.00   |               |           |
|          |             |           | 2/2             | 1.09 (0.78–1.51) | 0.6200     |
|          |             | Overdominant| 1/2–2/2 | 1.00 | 1.08 (0.83–1.40) | 0.5600 |
|          |             | Log-additive|        | 1.09 (0.91–1.31) | 0.3400 |
| rs11191548| T/C         | Codominant| 1/1             | 1.00   |               |           |
|          |             |           | 1/2             | 0.76 (0.58–1.00) | 0.0001*** |
|          |             |           | 2/2             | 0.31 (0.17–0.55) |           |
|          |             | Dominant  | 1/1             | 1.00   |               |           |
|          |             |           | 1/2–2/2         | 0.68 (0.52–0.88) | 0.0036*** |
|          |             | Recessive | 1/1–1/2        | 1.00   |               |           |
|          |             |           | 2/2             | 0.35 (0.19–0.62) | 0.0002*** |
|          |             | Overdominant| 1/1–2/2 | 1.00 |               |           |
|          |             | Log-additive|        | 0.86 (0.66–1.12) | 0.2600 |

OR – odd ratio; CI – confidence interval. Note: OR and 95% CI was calculated by logistic regression analysis model; p-value<0.05 was considered statistical significance. * 1 represented major allele, 2 represented minor allele; ** estimated by logistic regression analysis adjusted for sex, age and BMI; *** Indicated that the p-value remained significant after Bonferroni correction.
of the CYP17A1 gene; and (3) the haplotype-rs4919686A, rs3740397G, rs4919687C, rs11191548C and haplotype-rs4919686A, rs3740397G, rs4919687T, rs743572C, rs11191548T were found to be susceptible to EH among the Han Chinese population in Yunnan province.

Several reports have investigated the relationship of the rs11191548 polymorphism (g.111886A>G) to the development of EH among different populations. Most of those studies claimed that the T allele of rs11191548 might be a risk factor for EH. Two large-scale GWAS studies among European ancestry from the Global BPgen consortium and CHARGE consortium identified that the allele-T of rs11191548 was significantly associated with increased hypertension risk and BP levels [10,12]. An associated study conducted in Europeans also found that minor alleles-C of rs11191548 were associated with a lower probability of orthostatic hypotension [18]. Research among Japanese [13] and Korean [15] populations indicated that individuals carrying the allele-T of rs11191548 experienced significantly increased BP levels and hypertension risk compared to the respective non-carriers. A recent hypertension study among East Asians also exhibited the significant association between the T-allele of this polymorphism and the increased hypertension risk and BP levels [20,22,24]. However, other related studies

Table 8. Associations between the CYP17A1 gene haplotypes and the hypertension risk in this population.

| Name | Haplotype   | n=1009    | Frequency | OR (95 CI%) | p-value |
|------|-------------|-----------|-----------|-------------|---------|
| H1   | ACCTT       | 0.3977    | 1.00      |             |         |
| H2   | AGCCC       | 0.2558    | 0.68 (0.53–0.86) | 0.0014*   |
| H3   | CGTCT       | 0.1454    | 1.17 (0.87–1.56) | 0.3000    |
| H4   | ACTCT       | 0.0734    | 0.95 (0.66–1.38) | 0.7900    |
| H5   | AGCCT       | 0.0597    | 0.87 (0.58–1.29) | 0.4800    |
| H6   | AGTCT       | 0.0117    | 4.27 (1.77–10.29) | 0.0013*   |
| H7   | ACTTT       | 0.0119    | 0.66 (0.25–1.74) | 0.4000    |
| H8   | ACCCT       | 0.0116    | 1.69 (0.68–4.22) | 0.2600    |

CI – confidence interval; OR – odds ratio; SNP – single-nucleotide polymorphism. Note: The SNP order of constructing haplotype was as follows: rs4919686(A/C), rs3740397(C/G), rs4919687(C/T), rs743572(C/T), rs11191548(T/C); OR and 95% CI were calculated by a haplotype-based logistic regression analysis. Haplotypes with frequencies<0.01 were not included in this table; p-value<0.05 was considered statistical significance. * Indicated that the p-value remained significant after Bonferroni correction (p<0.05/8).
argued against them and indicated that the rs11191548 C-allele might be a risk factor for EH. A study among the Shanghai Han Chinese population found that the risk C-allele was associated with increased SBP levels and increased hypertension risk (in an additive genetic model) [21]. Another study conducted in the adult She ethnic minority from Ningde City in Fujian province of China came to the same conclusion [23]. In our present study, by adjusting for age, gender, and BMI, the minor C-allele of rs11191548 was found to be significantly associated with decreased EH risk and BP levels, and the subjects with TC+CC genotypes were found to have lower EH risk and BP levels than the subjects with TT genotype. Indeed, individuals who carried the C allele of rs11191548 could be less susceptible to EH among the Yunnan Han population. Our finding among the Yunnan Han Chinese population was in accordance with the reports of Newton-Cheh et al. [10,12,13,15,18,20,22,24]. However, this result was in contrast to what Li et al. [21,23] found in their study. This inconsistency could be attributed to the different genetic backgrounds, living habits, and environmental factors among different populations. It was not hard to see that people from different ethnic groups or just from different regions in the same country could also demonstrate certain genotype differences. Even though the researches were conducted in one country, our study conducted in the Yunnan Han Chinese population was in contrast to the She population in Fujian [23] and the Han population in Shanghai [21].

Detection of intron 1 rs4919687 (c.298-99C>T) polymorphism in our samples indicated that the minor T-allele and TT+CT genotype of this polymorphism were found to be significantly associated with the increased risk of EH and level of SBP among the Yunnan Han Chinese population. Indeed, the T-allele of this polymorphism seemed to be a risk factor for EH. The TC genotype of rs4919687 was found to be a protective genetic marker for EH among the Uighur population [36]. Previous studies have shown that the polymorphisms localized in the introns and exons close to the splice site would have effects on the process of mRNA splicing [37]. In the bioinformatics analysis, it was observed that the c.298-99C>T (rs4919687) led to the destruction of ESS (including ESR and Motif 3) sites, thereby enhancing the process of splicing. It was also known that the SNPs localized in regulatory regions within introns or in the regions between genes were called regulatory SNPs, and that these regulatory SNPs affected gene regulation by changing transcription factor (TF) binding affinities to genomic sequences [38]. Also, the functional effect of this intrinsic polymorphism on the expression level of the CYP17A1 gene was predicted by p-match. The result indicated that the single nucleotide substitution of G→A at position 298–299 in intron 1 of CYP17A1 could transform the sequence structure of the TF AREB6 binding site, thereby influencing TF AREB6 binding affinities to its binding site localized in this gene. Taken together, our findings suggested that the C→T single nucleotide substitution of the intronic polymorphism rs4919687 was strongly associated with EH in our samples by affecting the process of CYP17A1 mRNA splicing and by changing binding affinity with TF AREB6.

The rs743572 (c.-34T>C) was localized at the 5′ untranslated region of the CYP17A1 gene. Currently, the relation of rs743572 polymorphism and EH has not been clarified domestically or abroad. An associated study among European ancestry participants from the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) Consortium indicated that polymorphism rs743572 was associated with BP traits [39]. Another recent study conducted in patients with arterial hypertension and cardiac left ventricular ejection fraction revealed that no significant association was found between SNP rs743572 and mean 24-h systolic or diastolic BP [40]. In our study, no association was observed between this polymorphism and EH among the Han Chinese population.

The polymorphism rs1004467 (c.666+35T>C) localized in intron 3 of the CYP17A1 gene has been investigated in lots of association studies of complex polygenic disease. Several hypertensive association studies among Japanese [17] and Shanghai Han Chinese [20] have reported that the minor allele-A of rs1004467 could be a risk factor for EH. On the contrary, insignificant associations with EH or BP traits were reported in the populations of Beijing Chinese children [25] and of Han and Uighur in Xinjiang province of China [36]. In our study, no association was observed between this polymorphism and EH among the Han Chinese population in Yunnan province. The failure to find an association among our study sample could be attributed to the insufficient sample size. Further study in a larger sample size from the Yunnan Han Chinese population is needed to identify its relationship with EH. The bioinformatics analyses found that the sequence (CTAGTAGACC) containing the major/minor allele of rs1004467 could be captured by the TF c-Rel, but this mutation could not affect the binding affinity between this TF c-Rel and its binding site.

In recent years, there were only a few studies about the interactions between two polymorphisms, i.e., rs4919686 (1139+19T>G) and rs3740397 (969+75C>G), and occurrence of complex diseases. The AC genotype of rs4919686 was found to be a protective genetic marker for EH among the Uighur population [36]. Our present study among Han Chinese populations showed no association of these two polymorphisms with EH.

Regarding rs755443 (c.437-83C>T) and rs762563 (c.66C>G) polymorphisms, no studies have been reported about these two variants. Our present study among the Yunnan Han Chinese population showed no association of these two polymorphisms with EH.
In order to enhance the study power, haplotype analysis was performed. Few studies have been done to investigate the effects of haplotypes localized in the CYP17A1 gene on EH. An associated study conducted among the Uighur population indicated that the distributions of the haplotypes established by rs4919686–rs4919687, rs4919686–rs10786712, rs4919686–rs4919687–rs2486758, and rs4919686–rs10786712–rs2486758 were significantly different between the EH patients and control subjects [36]. In our present study, H2 (rs4919686A, rs3740397G, rs4919687C, rs743572C, rs11191548C) was found to significantly decrease the risk of EH and seemed to be protective, whereas H6 (rs4919686A, rs3740397G, rs4919687T, rs743572C, rs11191548T) was found to significantly increase the risk of EH and seemed to be risky. Indeed, the risk effect of rs4919686-T was shown in haplotype H6 and not shown in H2, whereas the protective effect of rs11191548-C was shown in H2 and not shown in H6. Coincidentally, H6 showed a significant association with the increased risk of EH, whereas H2 showed a significant association with the decreased risk of EH. The result of linkage disequilibrium analysis, that the rs11191548 and rs4919687 were found in linkage disequilibrium in both control and case groups among the Yunnan Chinese Han population, was in accordance with the haplotype analysis. Indeed, these two analyses suggested an additive effect of the rs11191548 and rs4919687 polymorphisms.

It is tempting to suggest that the mutations of the CYP17A1 gene might be a genetic risk factor for EH among the Chinese Han population. Indeed, cytochrome P450C17 (steroid 17α-hydroxylase/17, 20-lyase; EC 1.14.99.9) is a single microsomal enzyme, which has catalytic activities for the synthesis of mineralocorticoids and sex steroids, respectively [41]. Human expression of CYP17A1 in the fasciculate pregnenolone is converted into 17α-hydroxypregnenolone, which is oxidized to 17α-hydroxyprogesterone by 3β-hydroxysteroid dehydrogenase, followed by 21-hydroxylation by CYP21A2 to 11-deoxycortisol. 11-Deoxycortisol and 11-deoxycorticosterone then enter the mitochondria, where they are acted upon by the CYP11B1 enzyme that is specific to the zona Fasciculata to generate cortisol and corticosterone, respectively [42]. The single nucleotide polymorphism of the CYP17A1 gene may lead to deficiency of the P450C17 enzyme activity, which forces steroidogenesis to corticosterone rather than cortisol via 11-deoxycorticosterone. 11-Deoxycorticosterone, which in human beings is normally a very minor adrenal product. 11-Deoxycorticosterone, however, is a kind of mineralocorticoid, which is slightly less potent than aldosterone [30]. The state of mineralocorticoid 11-deoxycorticosterone excess is characterized by hypertension, hypokalemia, suppressed plasma renin activity, and low aldosterone concentrations [43]. As both renin and aldosterone play vital roles in the renin-angiotensin-aldosterone system (RAAS), SNPs in this gene might result in a suppressed RAAS characterized by abnormal blood pressure [44]. Taken together, our findings suggested that these two CYP17A1 polymorphisms rs11191548 and rs4919687 being in linkage disequilibrium could be a risk factor for EH among the Yunnan Han Chinese population, which provided new insights into the genetics and biology of blood pressure, and pointed out potential novel therapeutic pathways for cardiovascular disease prevention.

Some potential limitations of our study should be noted. Firstly, we did not collect data on antihypertensive medicine levels and drug compliance. Secondly, this study was limited by the relatively small sample size; a large number of clinical samples are needed in future studies.

**Conclusions**

In conclusion, this is the first study to investigate the contribution of CYP17A1 variants in inducing the susceptibility to EH among the Southwest Han Chinese Population in Yunnan province, and is the first haplotype-based case-control study to correlate its association with EH. Two polymorphisms, i.e., rs11191548 and rs4919687, of the CYP17A1 gene were found to be significantly associated with EH in the Yunnan Han population of China. The C-allele of rs11191548 could be a protective genetic marker for EH, and the T-allele of rs4919687 could be a risk genetic marker for EH among this population. However, future studies should be performed with a large sample size.

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**Conflicts of interest**

The authors declare that they have no competing interests.

**Statement**

This study was approved by the ethics committee of the School of Medicine of Yunnan University. Informed consent was obtained from all individual participants included in the study, and the consent authorization for publication was on file.
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