Genetic association study of prolylcarboxypeptidase polymorphisms with susceptibility to essential hypertension in the Yi minority of China: A case–control study based on an isolated population

Yanrui Wu¹,²*, Hongju Yang³ and Chunjie Xiao²

Abstract
Objective: Prolylcarboxypeptidase (PRCP) is a negative regulator of the pressor actions of the renin–angiotensin–aldosterone system. It is also involved in the kallikrein–kinin system. This gene has an important role in blood pressure (BP) regulation.

Methods: A case–control study was performed for 615 Yi participants (303 cases and 312 controls) from a remote mountainous area in Yunnan Province of China. For the PRCP gene, 11 tag single-nucleotide polymorphisms were genotyped using the polymerase chain reaction-restriction fragment length polymorphism method.

Results: The PRCP gene rs12290550 was associated with the occurrence of essential hypertension (EH) and BP traits. Logistic regression analysis indicated that the rs12290550 T allele was significantly linked to the risk of EH (odds ratio (OR) = 1.85, 95% confidence interval (CI) 1.44–2.39, p = 0.2 × 10⁻⁵). Under Bonferroni correction, the H7 TAGCACTAACA haplotype containing the risk allele rs12290550 T increased the risk of EH (OR = 4.53, 95% CI 2.29–8.93, p = 0.2 × 10⁻⁵).

Conclusions: The findings of this study demonstrate the strong association of the PRCP gene with EH. rs12290550 may be a useful genetic predictor of EH in the Yi minority.

Keywords
Essential hypertension, PRCP, polymorphisms, Yi minority, isolated population

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Introduction
As a major risk factor for cardiovascular and cerebrovascular diseases, essential hypertension (EH) is the top cause of death worldwide.¹,² EH is commonly described as a complex disease indicated by a chronic elevation of blood pressure (BP) with no clear cause. It is a classic example of a multifactorial trait caused by the inheritance of susceptibility genes and multiple environmental factors.¹,³ Efforts to identify the genes responsible for the occurrence and development of EH are useful for understanding the genetic and pathogenic mechanisms behind it. However, genetic contributions are difficult to elucidate for EH. Genetic and environmental factors are diverse, and vary among populations or within a population, which will affect the research findings of linkage and association studies.⁴ Isolated populations with reduced genetic diversity might better facilitate identification of susceptible genes for hypertension.⁵–⁷

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The Yi population is the largest minority group of Yunnan Province and lives in the HongHe and ChuXiong Prefecture in northwestern Yunnan, a remote region in China. They retain their own language and written symbols. Individuals of the Yi minority commonly share similar living environments due to geographic isolation and a remarkably stable culture. This minority group has a low frequency of migration and intermarriage with other groups. The above conditions minimize the influence of confounding environmental factors and reduce genetic diversity in founder populations.8,9 A genetic association study based on such an isolated population could increase the chances of identifying genetic factors contributing to EH. We recruited hypertensive and normal individuals from the Yi minority population in this study, and attempted to explore the association of the prolylcarboxypeptidase (PRCP) gene with EH.

The human PRCP gene is on chromosome 11 and is part of the renin–angiotensin–aldosterone system (RAAS).10 It encodes a product that contains 496 amino acid residues and is widely expressed in liver, kidney and many other tissues.11–13 PRCP is a serine protease that can cleave C-terminal amino acids linked to proline in peptides, such as angiotensin (Ang) II and Ang III.14–17 Besides participating in the metabolic processes of Ang II and Ang III, PRCP is also an activator in the production of kallikrein, which acts on the complex of high-weight kininogen and prekallikrein to release bradykinin.11,18–20 Ang II, Ang III, kallikrein and bradykinin are all associated with BP levels. Thus, PRCP is connected to EH through several routes. However, studies examining the potential of a link between EH and PRCP gene polymorphisms have not been performed widely. Using tag single-nucleotide polymorphisms (SNPs) as markers, the present study aimed to investigate the genetic contribution of the PRCP gene to EH in the Yi minority in southwestern China.

Methods

Participants

A total of 615 participants were enrolled in the present study and all samples were from the Human Genetics Center of Yunnan University. There were 303 cases and 312 controls. All implicated participants were individuals from the Yi minority from Yunnan Province and gave informed consent. The study protocol was approved by the ethics committee of the School of Medicine of Yunnan University. In this case-control study, the EH patients and normal individuals were selected based on the criteria described previously.21 According to the World Health Organization criteria, hypertension was defined as systolic BP (SBP) ≥ 140 mm Hg and/or diastolic BP (DBP) ≥ 90 mm Hg. Participants with secondary hypertension, diabetes mellitus, myocardial infarction, cerebrovascular accident and other serious diseases were excluded. Unrelated healthy villagers with BP < 140/90 mm Hg were recruited to match EH patients for age and gender to serve as the control group. The control group members were without a history of hypertension and other diseases. All participants did not have antihypertensive treatment.

Tag SNP selection and genotyping

SNPs are preferred for studies seeking disease associations, because of their large abundance in the human genome and accessibility to high-throughput genotyping.22,23 Tag SNPs are a small set of informative SNPs that can predict the rest of SNPs, and are sufficient to capture most haplotypes. Moreover, the genotyping burden can be greatly reduced with sufficient power for associated studies based on tag SNPs.24 Here, 11 tag SNPs of the PRCP gene were selected according to the criteria and method described previously.21 The 11 tag SNPs and their relative positions are shown in Figure 1.

Figure 1. The structure of the prolylcarboxypeptidase gene and relative positions of 11 tag single-nucleotide polymorphisms. UTR: untranslated region.
the selection criterion of tag SNPs \( r^2 \geq 0.8 \), these 11 tag SNPs could predict the remaining common SNPs with minor allele frequency \( \geq 1\% \).

Using the standard phenol–chloroform method, DNA was isolated from all the samples. \(^\text{25}\) Eleven tag SNPs were genotyped by the method of polymerase chain reaction (PCR)-restriction fragment length polymorphism. PCR was performed in a volume of 20 \( \mu \text{L} \) with 1 \( \mu \text{M} \) of each primer and 10 \( \mu \text{L} \) Premix Taq (TaKaRa Taq™ Version 2.0 plus dye, TaKaRa Biotechnology Co. Ltd, Japan). PCR conditions were: initial denaturation at 95°C for 5 minutes; 35 cycles of denaturation at 95°C for 30 seconds, at the annealing temperature (annealing) for 30 seconds and at 72°C (extension) for 30 seconds; and final extension at 72°C for 5 min. PCR products were digested with restriction enzymes. Genotypes were identified by 3% agarose gel electrophoresis with ethidium bromide staining. The restriction endonuclease digestion results of all SNPs are in the supplemental material S1. PCR primers, annealing temperatures and restriction enzymes are shown in Table 1.

**Table 1.** Primers and restriction enzymes used in prolylcarboxypeptidase gene single-nucleotide polymorphism identification.

| SNP       | Primers                                                                 | Annealing temperature (°C) | Product (bp) | Enzyme         |
|-----------|------------------------------------------------------------------------|-----------------------------|--------------|----------------|
| rs12290550| F: 5′-CATGTTTGTGGGAGAATGGGA-3′<br>R: 5′-TAGTCTGGGAGATGGGATC-3′         | 57                          | 189          | BamHI          |
| rs17144371| F: 5′-AAACAAGCTTGTGTTACTGCTA-3′<br>R: 5′-ATGCTTTGTACGCTCTACT-3′       | 56                          | 347          | TaqI           |
| rs6592086 | F: 5′-TGAAAGGAGAGTGAAGTCTTT-3′<br>R: 5′-TGCTCTGGTTCCTCGGA-3′          | 56                          | 129          | ScaI           |
| rs7104980 | F: 5′-CAGGGAGGTAGTTGCTGA-3′<br>R: 5′-CTTATTTCAGTTTTGAGC-3′           | 56                          | 117          | Sall           |
| rs2298668 | F: 5′-AAGTTATCTCAGTGAGGGA-3′<br>R: 5′-AGATGCTCTTTTGTCTGGC-3′          | 57                          | 302          | BmgT120I       |
| rs13306597| F: 5′-AGCAGAGAGAAGAACACCTCA-3′<br>R: 5′-AGTGCGATTGCTAGTAA-3′          | 56                          | 115          | VspI           |
| rs10792653| F: 5′-AAGAGATGCTCCACCCATG-3′<br>R: 5′-CTCGTGTACGTTAAGGC-3′           | 56                          | 203          | Haelll         |
| rs4084193 | F: 5′-CCCTCACACCAGAGAAGG-3′<br>R: 5′-TTTGTTATGTTTGTACGAT-3′           | 56                          | 184          | Mbol           |
| rs4759    | F: 5′-ATGCAAGGTATTTTCTTTTGCAGAT-3′<br>R: 5′-GATGAGAGTTTCTAGCAGA-3′    | 56                          | 182          | PstI           |
| rs3750931 | F: 5′-CTTGGCTCTTACCCCTAC-3′<br>R: 5′-GGGAAAGACGAGCTGATGATT-3′         | 57                          | 175          | Rsal           |
| rs7272    | F: 5′-GCTACCTCATCCTGAGAAGA-3′<br>R: 5′-CCCTTTGTTAAGTCCTGAC-3′         | 57                          | 317          | MspI           |

F: forward primer; R: reverse primer; SNP: single-nucleotide polymorphism.

**Results**

**Clinical characteristics of the study population**

The general characteristics of the case and control groups are given in Table 2. The average ages and the gender distributions presented no significant differences between the two groups. This balanced distribution of characteristics between the two groups minimized the influence of age and gender covariates during logistic regression analysis. It is beneficial to assess whether the genetic variation was independently associated with EH. EH patients exhibited significantly higher SBP, DBP and BMI than normal controls \( p < 0.01 \).

SPSS Inc., Chicago, IL, USA). The haplotypes were constructed based on the genotyped data using the SHEsis software (http://analysis.bio-x.cn/myAnalysis.php),\(^\text{26}\) and at the same time, frequencies of tag SNPs and haplotypes were also calculated by this software. Haplotypes with frequencies \( > 1\% \) (main haplotypes) were considered for further analysis. The genetic impacts of single sites and haplotypes on the risk of EH were evaluated by logistic regression analysis using SPSS 16.0 adjusted for gender, age and body mass index (BMI). A \( p < 0.05 \) was considered statistically significant and the Bonferroni correction was performed.
Single-SNP analysis

There was no significant deviation from the Hardy–Weinberg equilibrium for each SNP in the control group. Table 3 shows that both the genotype and allele frequencies of PRCP rs12290550 exhibited significantly different distributions between the control and EH groups after Bonferroni correction ($p < 0.0045, 0.05/11$). The association analysis also showed that the rs12290550 T allele carriers had higher average BP levels than participants with the GG genotype after Bonferroni correction ($p < 0.0045, 0.05/11$) (Table 4). There were no statistically significant associations between other SNPs and BP level following Bonferroni correction ($p > 0.0045, 0.05/11$).

Logistic regression analysis was performed to evaluate the risk impact of SNPs on the occurrence of EH under genetic models (Table 5). By adjusting for gender, age and BMI, both the rs12290550 T allele and the TT genotype were tightly linked to the risk of EH after Bonferroni correction (dominant model $GT + TT$ vs. $GG$: odds ratio (OR) $=2.05$, 95% CI (confidence interval) $1.48–2.84$, $p = 0.2 \times 10^{-4}$; recessive model $TT$ vs. $GG + GT$: OR $= 2.20$, 95% CI $1.29–3.76$, $p = 0.004$; multiplicative model $T$ vs. $G$: OR $= 1.85$, 95% CI $1.44–2.39$, $p = 0.2 \times 10^{-5}$).

Haplotype analysis

A total number of 12 main haplotypes with frequencies $>1\%$ were constructed with 11 tag SNPs of the PRCP gene (Table 6). The haplotype of H2 GAGCACTAACA was the most prevalent type among all participants. Haplotypes of H1 GACGCTGCGCG, H2 GAGCACTAACA and H10 GAGGACGAGCA had higher frequencies ($0.068$, $0.365$ and $0.031$) in the control group compared to the EH group ($0.040$, $0.291$ and $0.012$) ($p = 0.040$, $0.009$ and $0.031$, respectively), but without the statistical significance after Bonferroni correction ($p > 0.004$, $0.05/12$). Two other haplotypes, H7 TAGCACTAACA and H8 TAGGACGAGCA, showed increased risk contribution to EH, but only H7 retained statistical significance after Bonferroni correction (OR $= 4.53$, 95% CI $2.29–8.93$, $p = 0.2 \times 10^{-5}$).

Table 2. Characteristics of the study groups.

| Characteristics       | Control group | Hypertension group |
|-----------------------|---------------|--------------------|
| Gender (male/female)  | 175/137       | 181/122            |
| Age (years)           | 42.8 ± 10.4   | 44.2 ± 9.7         |
| BMI (kg/m²)           | 21.2 ± 2.1    | 23.1 ± 2.2*        |
| SBP (mm Hg)           | 101.3 ± 5.7   | 147.7 ± 16.1*      |
| DBP (mm Hg)           | 67.8 ± 3.9    | 95.8 ± 9.9*        |

BMI: body mass index; DBP: diastolic blood pressure; SBP: systolic blood pressure.

Note: values are expressed as mean ± SD; $^* p < 0.01$, statistical difference between control and hypertension groups.

Table 3. Genotype and allele distributions of single-nucleotide polymorphisms between hypertension patients and controls.

| Variant     | Allele¹ | Group | Genotype (frequency)² | p     | Allele (frequency)² | p     |
|-------------|---------|-------|-----------------------|-------|---------------------|-------|
| rs12290550  | G/T     | Control | 192 (0.615) 97 (0.311) 23 (0.074) | 481 (0.771) 143 (0.229) |
|             |         | Case   | 135 (0.446) 123 (0.406) 45 (0.149) | 393 (0.649) 213 (0.351) |
| rs17144371  | A/C     | Control | 299 (0.958) 13 (0.042) 0 (0.000) | 611 (0.979) 13 (0.021) |
|             |         | Case   | 289 (0.954) 14 (0.046) 0 (0.000) | 592 (0.977) 14 (0.023) |
| rs6592086   | G/C     | Control | 232 (0.744) 71 (0.228) 9 (0.029) | 335 (0.857) 89 (0.143) |
|             |         | Case   | 238 (0.785) 62 (0.205) 3 (0.010) | 353 (0.888) 68 (0.112) |
| rs7104980   | C/G     | Control | 151 (0.484) 127 (0.407) 34 (0.109) | 388 (0.640) 218 (0.360) |
|             |         | Case   | 122 (0.403) 144 (0.475) 37 (0.122) | 361 (0.600) 216 (0.390) |
| rs2298668   | A/C     | Control | 244 (0.782) 62 (0.199) 6 (0.019) | 550 (0.881) 74 (0.119) |
|             |         | Case   | 248 (0.818) 51 (0.168) 4 (0.013) | 547 (0.903) 59 (0.097) |
| rs13306597  | C/T     | Control | 207 (0.663) 90 (0.288) 15 (0.048) | 504 (0.808) 120 (0.192) |
|             |         | Case   | 223 (0.736) 75 (0.248) 5 (0.017) | 521 (0.860) 85 (0.140) |
| rs10792653  | T/G     | Control | 137 (0.439) 136 (0.436) 39 (0.125) | 410 (0.657) 214 (0.343) |
|             |         | Case   | 113 (0.373) 150 (0.495) 40 (0.132) | 376 (0.620) 230 (0.380) |
| rs4084193   | A/C     | Control | 218 (0.699) 84 (0.269) 10 (0.032) | 520 (0.833) 104 (0.167) |
|             |         | Case   | 230 (0.759) 69 (0.228) 4 (0.013) | 529 (0.873) 77 (0.127) |
| rs4759      | A/G     | Control | 85 (0.272) 155 (0.497) 72 (0.231) | 325 (0.521) 299 (0.479) |
|             |         | Case   | 86 (0.284) 156 (0.515) 61 (0.201) | 328 (0.541) 278 (0.459) |
| rs3750931   | C/G     | Control | 274 (0.878) 34 (0.109) 4 (0.013) | 582 (0.933) 42 (0.067) |
|             |         | Case   | 264 (0.871) 35 (0.116) 4 (0.013) | 563 (0.929) 43 (0.071) |
| rs7272      | A/G     | Control | 139 (0.446) 140 (0.449) 33 (0.106) | 418 (0.670) 206 (0.330) |
|             |         | Case   | 165 (0.545) 113 (0.373) 25 (0.083) | 443 (0.731) 163 (0.269) |

¹The major allele is referred to as allele 1 and the minor allele as allele 2.
Studies have found that starting from an SBP of 115/75 mm Hg, each elevation by 20 mm Hg may confer a double increase of death from stroke and ischemic heart disease.\textsuperscript{28} Great effort is necessary to explore the underlying molecular mechanisms and risk factors for hypertension. BP is a continuous trait and many pathways are involved in regulating its formation. The important role of the RAAS in BP regulation is supported by the fact that it is targeted by first-line drugs for antihypertension therapy, for example angiotensin converting enzyme (ACE) inhibitors and mineralocorticoid receptor antagonists.\textsuperscript{29} Genes of this system, such as ACE, renin, angiotensin receptor type 1 and angiotensin, have been extensively investigated as main candidate targets to explore the pathogenesis of hypertension.\textsuperscript{30–34} In addition to the above well-known genes of the RAAS, the human PRCP gene is also worthy of further close investigation, and in fact this gene was considered to be a candidate gene for EH as early as 1997.\textsuperscript{10} In the RAAS, Ang II and Ang III can constrict blood vessels and raise BP. \textsuperscript{35} As a negative regulator of the pressor actions of the RAAS, PRCP could counteract the effects of Ang II and Ang III by degrading them to Ang1-7 and Ang2-7, respectively.\textsuperscript{14,17} Thus, inhibiting PRCP might cause a hypertensive state as a result of elevated levels of Ang II and Ang III. Animal models of PRCP-deficient mice produced via a gene trap method exhibited a hypertensive phenotype and had a heightened risk for arterial thrombosis,\textsuperscript{36,37} which demonstrates the important role of PRCP in BP regulation.

A missense mutation, rs2298668 (E112D), was studied repetitively to explore the association of the PRCP gene with hypertension, coronary heart disease and other metabolic syndromes.\textsuperscript{38,39} Based on two stratified groups, the DD genotype of E112D jointly with chronic hypertension indicated the highest risk effect for pre-eclampsia in female American patients.\textsuperscript{38} Aiming to evaluate the association

| SNP          | Genotype (n) | SBP          | p         | DBP          | p         |
|--------------|--------------|--------------|-----------|--------------|-----------|
| rs12290550   | TT (68)      | 130.63 ± 20.83 |           | 86.49 ± 15.87 |           |
|              | GT (220)     | 127.48 ± 26.38 |           | 83.52 ± 16.61 |           |
|              | GG (327)     | 120.49 ± 25.20 | 0.001     | 79.33 ± 16.26 | 0.0005    |
| rs17144371   | AC (27)      | 123.33 ± 24.11 |           | 83.26 ± 17.29 |           |
|              | AA (588)     | 124.15 ± 25.55 | 0.871     | 81.54 ± 16.50 | 0.598     |
| rs6592086    | CC (12)      | 115.83 ± 25.19 |           | 74.00 ± 12.00 |           |
|              | CG (133)     | 123.05 ± 24.72 |           | 81.51 ± 16.90 |           |
|              | GG (470)     | 124.62 ± 25.69 | 0.430     | 81.84 ± 16.49 | 0.267     |
| rs7104980    | GG (71)      | 123.69 ± 24.75 |           | 81.83 ± 16.43 |           |
|              | CG (271)     | 126.41 ± 26.84 |           | 82.76 ± 16.94 |           |
|              | CC (273)     | 121.94 ± 24.11 | 0.122     | 80.43 ± 16.10 | 0.259     |
| rs2298668    | CC (10)      | 121.10 ± 23.51 |           | 78.50 ± 17.33 |           |
|              | AC (113)     | 122.97 ± 26.80 |           | 80.84 ± 17.88 |           |
|              | AA (492)     | 124.43 ± 25.23 | 0.801     | 81.86 ± 16.20 | 0.701     |
| rs13306597   | TT (20)      | 108.55 ± 20.00 |           | 73.8 ± 16.32  |           |
|              | CT (165)     | 122.05 ± 25.24 |           | 80.97 ± 17.21 |           |
|              | CC (430)     | 125.62 ± 25.53 | 0.006     | 82.23 ± 13.21 | 0.070     |
| rs10792653   | GG (79)      | 123.61 ± 24.42 |           | 81.34 ± 17.09 |           |
|              | GT (286)     | 125.70 ± 25.77 |           | 82.81 ± 16.22 |           |
|              | TT (250)     | 122.45 ± 25.42 | 0.331     | 81.62 ± 16.52 | 0.220     |
| rs4084193    | CC (14)      | 112.93 ± 23.83 |           | 71.36 ± 11.83 |           |
|              | AC (153)     | 122.41 ± 24.78 |           | 80.64 ± 16.87 |           |
|              | AA (448)     | 125.04 ± 25.69 | 0.137     | 82.27 ± 16.43 | 0.036     |
| rs4759       | GG (133)     | 122.29 ± 25.33 |           | 80.47 ± 16.50 |           |
|              | AG (311)     | 124.74 ± 25.98 |           | 81.91 ± 17.29 |           |
|              | AA (171)     | 124.38 ± 24.69 | 0.640     | 81.98 ± 15.11 | 0.666     |
| rs3750931    | GG (8)       | 124.63 ± 24.48 |           | 86.63 ± 19.21 |           |
|              | CG (69)      | 124.33 ± 24.19 |           | 80.25 ± 15.06 |           |
|              | CC (538)     | 124.07 ± 25.68 | 0.995     | 81.72 ± 16.67 | 0.541     |
| rs7272       | GG (58)      | 119.45 ± 25.16 |           | 80.41 ± 17.86 |           |
|              | AG (253)     | 122.27 ± 25.25 |           | 80.62 ± 16.83 |           |
|              | AA (304)     | 126.53 ± 25.53 | 0.049     | 82.68 ± 15.97 | 0.290     |

DBP: diastolic blood pressure; SBP: systolic blood pressure; SNP: single-nucleotide polymorphism.

**Discussion**

Studies have found that starting from an SBP of 115/75 mm Hg, each elevation by 20 mm Hg may confer a double increase of death from stroke and ischemic heart disease.\textsuperscript{28} Great effort is necessary to explore the underlying molecular mechanisms and risk factors for hypertension. BP is a continuous trait and many pathways are involved in regulating its formation. The important role of the RAAS in BP regulation is supported by the fact that it is targeted by first-line drugs for antihypertension therapy, for example angiotensin converting enzyme (ACE) inhibitors and mineralocorticoid receptor antagonists.\textsuperscript{29} Genes of this system, such as ACE, renin, angiotensin receptor type 1 and angiotensin, have been extensively investigated as main candidate targets to explore the pathogenesis of hypertension.\textsuperscript{30–34} In addition to the above well-known genes of the RAAS, the human PRCP gene is also worthy of further close investigation, and in fact this gene was considered to be a candidate gene for EH as early as 1997.\textsuperscript{10} In the RAAS, Ang II and Ang III can constrict blood vessels and raise BP.\textsuperscript{35} As a negative regulator of the pressor actions of the RAAS, PRCP could counteract the effects of Ang II and Ang III by degrading them to Ang1-7 and Ang2-7, respectively.\textsuperscript{14,17} Thus, inhibiting PRCP might cause a hypertensive state as a result of elevated levels of Ang II and Ang III. Animal models of PRCP-deficient mice produced via a gene trap method exhibited a hypertensive phenotype and had a heightened risk for arterial thrombosis,\textsuperscript{36,37} which demonstrates the important role of PRCP in BP regulation.

A missense mutation, rs2298668 (E112D), was studied repetitively to explore the association of the PRCP gene with hypertension, coronary heart disease and other metabolic syndromes.\textsuperscript{38,39} Based on two stratified groups, the DD genotype of E112D jointly with chronic hypertension indicated the highest risk effect for pre-eclampsia in female American patients.\textsuperscript{38} Aiming to evaluate the association...
### Table 5. Logistic regression analysis under genetic models.

| Variant   | Allele | Genetic model | OR (95% CI)        | p          |
|-----------|--------|---------------|--------------------|------------|
| rs12290550 | G/T    | Dominant model | 2 ½ vs. 1 | 2.05 (1.48–2.84) | 0.2 × 10⁻⁴ |
|           |        | Recessive model | 2/2 vs. 1/1 | 2.20 (1.29–3.76) | 0.004      |
|           |        | Multiplicative model | 2 vs. 1 | 1.85 (1.44–2.39) | 0.2 × 10⁻⁵ |
| rs17144371 | A/C    | Dominant model | 1/2 vs. 1 | 1.14 (0.52–2.47) | 0.748      |
|           |        | Recessive model | 2/2 vs. 1 | — | —         |
|           |        | Multiplicative model | 2 vs. 1 | 1.13 (0.53–2.44) | 0.751      |
| rs6592086  | G/C    | Dominant model | 1/2 vs. 1 | 0.80 (0.55–1.16) | 0.231      |
|           |        | Recessive model | 2/2 vs. 1 | 0.32 (0.09–1.22) | 0.095      |
|           |        | Multiplicative model | 2 vs. 1 | 0.76 (0.54–1.07) | 0.112      |
| rs7104980  | C/G    | Dominant model | 1/2 vs. 1 | 1.18 (0.72–1.94) | 0.515      |
|           |        | Recessive model | 2/2 vs. 1 | 0.69 (0.19–2.49) | 0.374      |
|           |        | Multiplicative model | 2 vs. 1 | 0.81 (0.56–1.37) | 0.260      |
| rs2298668  | A/C    | Dominant model | 1/2 vs. 1 | 0.81 (0.54–1.20) | 0.289      |
|           |        | Recessive model | 2/2 vs. 1 | 0.69 (0.19–2.49) | 0.574      |
|           |        | Multiplicative model | 2 vs. 1 | 0.81 (0.56–1.37) | 0.260      |
| rs13306597 | C/T    | Dominant model | 1/2 vs. 1 | 0.72 (0.51–1.01) | 0.060      |
|           |        | Recessive model | 2/2 vs. 1 | 0.32 (0.11–0.89) | 0.029      |
|           |        | Multiplicative model | 2 vs. 1 | 0.69 (0.51–0.93) | 0.016      |
| rs10792653 | T/G    | Dominant model | 1/2 vs. 1 | 1.34 (0.97–1.85) | 0.080      |
|           |        | Recessive model | 2/2 vs. 1 | 1.04 (0.65–1.67) | 0.868      |
|           |        | Multiplicative model | 2 vs. 1 | 1.17 (0.93–1.48) | 0.177      |
| rs4084193  | A/C    | Dominant model | 1/2 vs. 1 | 0.74 (0.52–1.06) | 0.097      |
|           |        | Recessive model | 2/2 vs. 1 | 0.39 (0.12–1.27) | 0.118      |
|           |        | Multiplicative model | 2 vs. 1 | 0.73 (0.53–1.00) | 0.051      |
| rs4759     | A/G    | Dominant model | 1/2 vs. 1 | 0.95 (0.66–1.35) | 0.771      |
|           |        | Recessive model | 2/2 vs. 1 | 0.83 (0.56–1.22) | 0.334      |
|           |        | Multiplicative model | 2 vs. 1 | 0.92 (0.73–1.15) | 0.455      |
| rs3750931  | C/G    | Dominant model | 1/2 vs. 1 | 1.03 (0.64–1.67) | 0.903      |
|           |        | Recessive model | 2/2 vs. 1 | 1.03 (0.25–4.19) | 0.964      |
|           |        | Multiplicative model | 2 vs. 1 | 1.03 (0.66–1.60) | 0.900      |
| rs7272     | A/G    | Dominant model | 1/2 vs. 1 | 0.65 (0.47–0.90) | 0.009      |
|           |        | Recessive model | 2/2 vs. 1 | 0.76 (0.44–1.31) | 0.320      |
|           |        | Multiplicative model | 2 vs. 1 | 0.73 (0.57–0.94) | 0.014      |

CI: confidence interval; OR: odds ratio.

*The major allele is referred to as allele 1 and the minor allele as allele 2.

1OR estimated by logistic regression analysis, adjusted for gender, age and body mass index.

### Table 6. The distributions of haplotypes and logistic regression analysis.

| Name   | Haplotype | Control (frequency) | Case (frequency) | OR (95% CI) | p          |
|--------|-----------|---------------------|-----------------|------------|------------|
| H1     | GACGCTGCGCG | 42 (0.068)          | 24 (0.040)     | 0.59 (0.35–0.98) | 0.040      |
| H2     | GAGCATAACA  | 228 (0.365)         | 176 (0.291)    | 0.71 (0.55–0.92) | 0.009      |
| H3     | GAGCATAAGA  | 7 (0.012)           | 10 (0.017)     | 1.47 (0.57–3.80) | 0.424      |
| H4     | GAGCAGCAGA  | 13 (0.020)          | 15 (0.025)     | 1.00 (0.62–1.77) | 0.755      |
| H5     | GAGCAGCAGG  | 62 (0.100)          | 43 (0.070)     | 0.69 (0.46–1.05) | 0.081      |
| H6     | GAGCAGTGGG  | 15 (0.025)          | 10 (0.016)     | 0.66 (0.29–1.50) | 0.320      |
| H7     | TAGCATAACA  | 11 (0.017)          | 43 (0.071)     | 4.53 (2.29–8.93) | 0.2 × 10⁻⁵ |
| H8     | TAGGAGGAGCA | 54 (0.086)          | 77 (0.128)     | 1.61 (1.11–2.34) | 0.011      |
| H9     | TAGGAGGAGCA | 10 (0.016)          | 9 (0.015)      | 0.99 (0.40–2.46) | 0.988      |
| H10    | TAGGAGGAGCA | 19 (0.031)          | 8 (0.012)      | 0.40 (0.17–0.95) | 0.031      |
| H11    | TAGGAGGAGCA | 8 (0.013)           | 7 (0.012)      | 0.96 (0.35–2.62) | 0.935      |
| H12    | TAGGAGGAGCA | 5 (0.008)           | 9 (0.015)      | 0.55 (0.19–1.65) | 0.281      |

CI: confidence interval; OR: odds ratio.

Note: the haplotype structure was rs12290550 (G/T), rs17144371 (A/C), rs6592086 (G/C), rs7104980 (C/G), rs2298668 (A/C), rs13306597 (C/T), rs10792653 (T/G), rs4084193 (A/C), rs4759 (A/G), rs3750931 (C/G) and rs7272 (A/G); haplotypes with frequencies < 0.01 were excluded.
between the PRCP gene and the antihypertensive effect of benazepril, Zhang et al. tested the E112D polymorphism in hypertensive patients with daily treatment for 15 days and found that the D allele carrier patients were more sensitive to benazepril.\textsuperscript{40} In the present study, rs2298668 (E112D) showed no association with hypertension. This result is consistent with the investigations by Gittleman et al., who also showed no association of E112D with hypertension and angina.\textsuperscript{39} To date, reports on the functional implications of E112D are limited and it is unclear whether this polymorphism will lead to a functional change of the protein. One possible explanation is that E112D may be significant in interindividual variation in response to benazepril but not significantly related to hypertension. Further investigation should be performed to explore the functional information for E112D.

Here, our study samples were special. First, being limited by objective conditions, none of the participants had received any antihypertension drugs for treatment. Second, one of the most unique features of Yunnan Province in China is its ethnic pluralism, which is closely related to the geographic environment. Besides the Han population, there are 25 ethnic minorities in Yunnan Province. Because of the plateau landscape, different altitudes and region-isolated living conditions, these minorities, including the Yi group, usually have their own spoken and written languages. Moreover, they tend to not migrate or not intermarry with other minorities.\textsuperscript{8,9} Thus, the genetic backgrounds of samples in this study were very similar and the BP phenotypes were not affected by drugs. The above features raise the power of our evaluation of the contributions of genetic factors to EH and make our study findings more objective. Logistic regression analysis found that the T allele of rs12290550 may be a risk factor for EH in the Yi group. Risk correlations between rs12290550 and EH under three genetic models all reached statistical significance after Bonferroni correction. In the PRCP gene, rs12290550 is an intronic mutation and may not cause a functional change of the PRCP protein directly. One possible explanation is that E112D may be significant in linkage disequilibrium with a true functional variant or may alter the function of a nearby regulatory element. The correlation between the rs12290550 genotype and the BP phenotype was also estimated here. The average BP levels of rs12290550 T carriers were significantly higher compared to GG homozygous individuals, which further confirmed the role of the rs12290550 T allele in the risk for EH in the Yi minority.

We further detected the haplotypes of the PRCP gene, which were constructed by 11 tag SNPs. Among the 12 main haplotypes, the highest-frequency haplotype was H2 GAGCAGCTGCG, H2 GAGCAGCTAACA and H10 GAGGAGGAGGCA haplotypes decreased the risk of EH, and that none of them contained the susceptible allele of rs12290550 T. However, the risk correlations did not exist via strict Bonferroni correction. A harmful effect of H8 TAGGACGAGCA containing the risk allele rs12290550 T regarding EH was detected, although no risk association was found after Bonferroni correction. Notably, individuals with the H7 TAGCAGCTAACA haplotype were more likely to have EH and this risk correlation reached a conclusive level of statistical significance both before and after Bonferroni correction. Moreover, the susceptibility rs12290550 T allele was contained within the H7 TAGCAGCTAACA haplotype. Taken together, our haplotype analysis findings suggested that haplotypes containing the rs12290550 T allele could increase the risk of EH, which confirms the risk effect of rs12290550 T for EH in the Yi minority.

The participants in the present study resided in a remote rural area and, due to the limitation of objective conditions, we could not get complete information about the physiological values of participants. This was the limitation of the present study. In summary, the design of the present study based on the isolated population could help track down genetic factors in EH. The T allele of rs12290550 might contribute to the risk of EH in the Yi minority. Its susceptibility to EH was confirmed based on haplotype analysis. Moreover, the correlation analysis between the rs12290550 genotype and BP phenotype also indicated its risk effect. The findings consistently demonstrated a strong association of the PRCP gene with EH, and rs12290550 may be a useful genetic predictor of EH in the Yi minority. We hope this report will stimulate investigation of the relationship between the PRCP gene and EH. Future work should sequence the whole PRCP gene in related samples to find the causal mutations and functional examination is required to confirm the association of the PRCP gene with EH.

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Supplemental material

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