jraas

Journal of the Renin-Angiotensin-Aldosterone System
April-June 2020: 1–9

DOI: 10.1177/1470320320919586
journals.sagepub.com/home/jra
SAGE

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 Wu[1,2], Hongju Yang[3] and Chunjie Xiao[2]

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, $p = 0.2 \times 10^{-5}$). 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, $p = 0.2 \times 10^{-5}$).

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

Date received: 13 January 2020; accepted: 6 March 2020

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]

[1]Cell Biology and Genetics Department, Kunming Medical University, China
[2]School of Medicine, Yunnan University, China
[3]The First Affiliated Hospital of Kunming Medical University, Kunming Medical University, China

Corresponding authors:
Yanrui Wu, Cell Biology and Genetics Department, Kunming Medical University, #1168 N. West Chunrong Road, Kunming, Yunnan Province 650500, China.
Email: wyr102282@126.com

Chunjie Xiao, School of Medicine, Yunnan University, #2 N. Cuihu Road, Kunming, Yunnan Province 650091, China.
Email: chunjiexiao2006@126.com

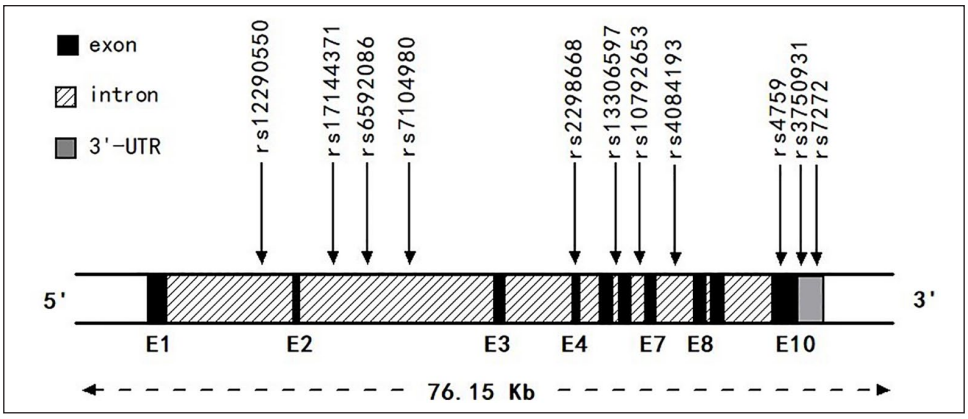

Figure 1. The structure of the prolylcarboxypeptidase gene and relative positions of 11 tag single-nucleotide polymorphisms.
UTR: untranslated region.

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) $\geqslant$ 140 mm Hg and/or diastolic BP (DBP) $\geqslant$ 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. Based on

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′-CAGTCTTATGGGGAATAGGGA-3′ R: 5′-TAGTCTGCGGTGATAGGGATC-3′	57	189	BamHI
rs17144371	F: 5′-AAACACTCTTTGCTTTACTGCTA-3′ R: 5′-ATGGTCTTTCCGACTTTACTACT-3′	56	347	TaqI
rs6592086	F: 5′-TGGAAGGAAGGTGGAGTTTAG-3′ R: 5′-TGCTCTCTGATCTTGTCCGA-3′	56	129	SacI
rs7104980	F: 5′-CACGGAGTACTTAGATGGTCGA-3′ R: 5′-CGTATTTCCAGTTGTTTAGCAC-3′	56	117	SalI
rs2298668	F: 5′-AAGTTATCTCACAGTGGGGCA-3′ R: 5′-GAGTGCTCTTTTTGTTCTGGC-3′	57	302	BmgT120I
rs13306597	F: 5′-AGACAGAAGCCAGAAACCTCA-3′ R: 5′-AAGTGCATTTGTACTGGAGATTAA-3′	56	115	VspI
rs10792653	F: 5′-AAGGATAGTCCACCATTGCC-3′ R: 5′-TGTGAAGAATAAATGATCTGTAAG-3′	56	203	HaeIII
rs4084193	F: 5′-CCTCCACCACCAGAAGAAG-3′ R: 5′-TTTATGATATTTGATTTGTCACAGAT-3′	56	184	MboI
rs4759	F: 5′-ATCACCCTCTATTCTATCTCAACT-3′ R: 5′-GATGAGAGATTTCTATGACACTGC-3′	56	182	PstI
rs3750931	F: 5′-CACTTGCTCTTACCGTCATCAC-3′ R: 5′-GGGAAAGCAGCACTGAGGTA-3′	57	175	RsaI
rs7272	F: 5′-GCCAACATCCCAGAACTAAGA-3′ R: 5′-CCCATTTGTAAGTCCCCATC-3′	57	317	MspI

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

the selection criterion of tag SNPs ($r^2 \geqslant 0.8$), these 11 tag SNPs could predict the remaining common SNPs with minor allele frequency $\geqslant 1\%$.

Using the standard phenol–chloroform method, DNA was isolated from all the samples.[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 μL with 1 μM of each primer and 10 μL 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.

Statistical analysis

The Hardy–Weinberg equilibrium was tested by the chi-square test. Numerical data of participants were analyzed by one-way analysis of variance using SPSS (version 16.0; 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),[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.

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 ($p < 0.01$).

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).

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/m^2)	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.

Logistic regression analysis was performed to evaluate the risk impact of SNPs on the occurrence of EH under genetic models (Table 5).[27] 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, $p = 0.2\times10^{-4}$; 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, $p = 0.2\times10^{-5}$).

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, $p = 0.2\times10^{-5}$).

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

Variant	Allele[a] 1/2	Group	Genotype (frequency)[a]			*p*	Allele (frequency)[a]		*p*
			1/1	1/2	2/2		1	2	
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)	0.5×10^{-4}	393 (0.649)	213 (0.351)	0.2×10^{-4}
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)	0.784	592 (0.977)	14 (0.023)	0.786
rs6592086	G/C	Control	232 (0.744)	71 (0.228)	9 (0.029)		535 (0.857)	89 (0.143)	
		Case	238 (0.785)	62 (0.205)	3 (0.010)	0.169	538 (0.888)	68 (0.112)	0.110
rs7104980	C/G	Control	151 (0.484)	127 (0.407)	34 (0.109)		429 (0.688)	195 (0.313)	
		Case	122 (0.403)	144 (0.475)	37 (0.122)	0.126	388 (0.640)	218 (0.360)	0.079
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)	0.504	547 (0.903)	59 (0.097)	0.231
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)	0.033	521 (0.860)	85 (0.140)	0.014
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)	0.238	376 (0.620)	230 (0.380)	0.182
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)	0.120	529 (0.873)	77 (0.127)	0.050
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)	0.675	328 (0.541)	278 (0.459)	0.473
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)	0.124	563 (0.929)	43 (0.071)	0.801
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)	0.048	443 (0.731)	163 (0.269)	0.019

[a]The major allele is referred to as allele 1 and the minor allele as allele 2.

Table 4. Associations between single-nucleotide polymorphisms and blood pressure levels in the Yi minority.

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.[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.[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.[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.[10] In the RAAS, Ang II and Ang III can constrict blood vessels and raise BP.[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 Ang_{1-7} and Ang_{2-7}, respectively.[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,[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.[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.[38] Aiming to evaluate the association

Table 5. Logistic regression analysis under genetic models.

Variant	Allele[a] 1/2	Genetic model		OR[b] (95% CI)	*p*
rs12290550	G/T	Dominant model	½ + 2/2 vs. 1/1	2.05 (1.48–2.84)	0.2×10^{-4}
		Recessive model	2/2 vs. 1/1 + 1/2	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 + 2/2 vs. 1/1	1.14 (0.52–2.47)	0.748
		Recessive model	2/2 vs. 1/1 + 1/2	—	—
		Multiplicative model	2 vs. 1	1.13 (0.53—2.44)	0.751
rs6592086	G/C	Dominant model	1/2 + 2/2 vs. 1/1	0.80 (0.55–1.16)	0.231
		Recessive model	2/2 vs. 1/1 + 1/2	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 + 2/2 vs. 1/1	1.46 (1.06–2.02)	0.021
		Recessive model	2/2 vs. 1/1 + 1/2	1.18 (0.72–1.94)	0.515
		Multiplicative model	2 vs. 1	1.28 (1.01–1.62)	0.043
rs2298668	A/C	Dominant model	1/2 + 2/2 vs. 1/1	0.81 (0.54–1.20)	0.289
		Recessive model	2/2 vs. 1/1 + 1/2	0.69 (0.19–2.49)	0.574
		Multiplicative model	2 vs. 1	0.81 (0.56–1.17)	0.260
rs13306597	C/T	Dominant model	1/2 + 2/2 vs. 1/1	0.72 (0.51–1.01)	0.060
		Recessive model	2/2 vs. 1/1 + 1/2	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 + 2/2 vs. 1/1	1.34 (0.97–1.85)	0.080
		Recessive model	2/2 vs. 1/1 + 1/2	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 + 2/2 vs. 1/1	0.74 (0.52–1.06)	0.097
		Recessive model	2/2 vs. 1/1 + 1/2	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 + 2/2 vs. 1/1	0.95 (0.66–1.35)	0.771
		Recessive model	2/2 vs. 1/1 + 1/2	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 + 2/2 vs. 1/1	1.03 (0.64–1.67)	0.903
		Recessive model	2/2 vs. 1/1 + 1/2	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 + 2/2 vs. 1/1	0.65 (0.47–0.90)	0.009
		Recessive model	2/2 vs. 1/1 + 1/2	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.
[a]The major allele is referred to as allele 1 and the minor allele as allele 2.
[b]OR 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	GAGCACTAACA	228 (0.365)	176 (0.291)	0.71 (0.55–0.92)	0.009
H3	GAGCACTAAGA	7 (0.012)	10 (0.017)	1.47 (0.57–3.80)	0.424
H4	GAGCACTAGCA	13 (0.020)	15 (0.025)	1.30 (0.61–2.77)	0.500
H5	GAGCACTAGCG	62 (0.100)	43 (0.070)	0.69 (0.46–1.05)	0.081
H6	GAGCATGCGGG	15 (0.025)	10 (0.016)	0.66 (0.29–1.50)	0.320
H7	TAGCACTAACA	11 (0.017)	43 (0.071)	4.53 (2.29–8.93)	0.2×10^{-5}
H8	TAGGACGAGCA	54 (0.086)	77 (0.128)	1.61 (1.11–2.34)	0.011
H9	TAGGACTAACA	10 (0.016)	9 (0.015)	0.99 (0.40–2.46)	0.988
H10	GAGGACGAGCA	19 (0.031)	8 (0.012)	0.40 (0.17–0.95)	0.031
H11	GAGCACTAACG	8 (0.013)	7 (0.012)	0.96 (0.35–2.62)	0.935
H12	TAGCACTAACG	9 (0.015)	5 (0.008)	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.[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.[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.[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 much 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. It might be merely a marker that is 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 GAGCACTAACA. Logistic regression analysis revealed that the H1 GACGCTGCGCG, H2 GAGCACTAACA and H10 GAGGACGAGCA 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 TAGCACTAACA 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 TAGCACTAACA 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.

Acknowledgements

We would like to thank all the participants enrolled in this study and all the staff in the Human Genetics Center of Yunnan University.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This work was supported by the Hundred-Talent Program of Kunming Medical University (60117190439) and the Foundation of the Education Department of Yunnan Province (2018JS151).

ORCID iD

Yanrui Wu https://orcid.org/0000-0001-5008-4300

Supplemental material

Supplemental material for this article is available online.

References

1. Timasheva Y, Nasibullin T, Imaeva E, et al. Genetic determinants of essential hypertension in the population of Tatars from Russia. *J Hypertens* 2017; 35: S16–S23.
2. Kearney PM, Whelton M, Reynolds K, et al. Global burden of hypertension: analysis of worldwide data. *Lancet* 2005; 365: 217–223.
3. Singh M, Singh AK, Pandey P, et al. Molecular genetics of essential hypertension. *Clin Exp Hypertens* 2016; 38: 268–277.
4. Hoffmann K, Planitz C, Ruschendorf F, et al. A novel locus for arterial hypertension on chromosome 1p36 maps to a metabolic syndrome trait cluster in the Sorbs, a Slavic population isolate in Germany. *J Hypertens* 2009; 27: 983–990.
5. Dominiczak AF, Delles C and Padmanabhan S. Are isolated populations better for studying genes that predispose to hypertension? *J Hypertens* 2009; 27: 939–940.
6. Southam L, Gilly A, Suveges D, et al. Whole genome sequencing and imputation in isolated populations identify genetic associations with medically-relevant complex traits. *Nat Commun* 2017; 8: 15606.
7. Ji W, Foo JN, O'Roak BJ, et al. Rare independent mutations in renal salt handling genes contribute to blood pressure variation. *Nat Genet* 2008; 40: 592–599.
8. Li Q, Sun L, Du J, et al. Risk given by AGT polymorphisms in inducing susceptibility to essential hypertension among isolated populations from a remote region of China: a case–control study among the isolated populations. *J Renin Angiotensin Aldosterone Syst* 2015; 16: 1202–1217.
9. Chen B, Nie S, Yue Z, et al. Haplotype-based case–control study of the human CYP11B2 gene and essential hypertension in Yi and Hani minorities of China. *Biochem Genet* 2011; 49: 122–137.
10. Watson B, Jr., Nowak NJ, Myracle AD, et al. The human angiotensinase C gene (HUMPCP) maps to 11q14 within 700 kb of D11S901: a candidate gene for essential hypertension. *Genomics* 1997; 44: 365–367.
11. Graham TH. Prolylcarboxypeptidase (PrCP) inhibitors and the therapeutic uses thereof: a patent review. *Expert Opin Ther Pat* 2017; 27: 1077–1088.
12. Yang HY, Erdos EG and Chiang TS. New enzymatic route for the inactivation of angiotensin. *Nature* 1968; 218: 1224–1226.
13. Jeong JK and Diano S. Prolyl carboxypeptidase mRNA expression in the mouse brain. *Brain Res* 2014; 1542: 85–92.
14. Luft FC. The renin-angiotensin system and prolylcarboxypeptidase. *J Mol Med (Berl)* 2017; 95: 461–463.
15. Tan F, Morris PW, Skidgel RA, et al. Sequencing and cloning of human prolylcarboxypeptidase (angiotensinase C). Similarity to both serine carboxypeptidase and prolylendopeptidase families. *J Biol Chem* 1993; 268: 16631–16638.
16. Odya CE, Marinkovic DV, Hammon KJ, et al. Purification and properties of prolylcarboxypeptidase (angiotensinase C) from human kidney. *J Biol Chem* 1978; 253: 5927–5931.
17. Mallela J, Yang J and Shariat-Madar Z. Prolylcarboxypeptidase: a cardioprotective enzyme. *Int J Biochem Cell Biol* 2009; 41: 477–481.
18. Shariat-Madar Z, Mahdi F and Schmaier AH. Recombinant prolylcarboxypeptidase activates plasma prekallikrein. *Blood* 2004; 103: 4554–4561.
19. Shariat-Madar Z, Rahimy E, Mahdi F, et al. Overexpression of prolylcarboxypeptidase enhances plasma prekallikrein activation on Chinese hamster ovary cells. *Am J Physiol Heart Circ Physiol* 2005; 289: H2697–H2703.
20. Schmaier AH. The kallikrein-kinin and the renin-angiotensin systems have a multilayered interaction. *Am J Physiol Regul Integr Comp Physiol* 2003; 285: R1–R13.
21. Wu Y, Yang H, Yang B, et al. Association of polymorphisms in prolylcarboxypeptidase and chymase genes with essential hypertension in the Chinese Han population. *J Renin Angiotensin Aldosterone Syst* 2013; 14: 263–270.
22. Zhang K, Qin ZS, Liu JS, et al. Haplotype block partitioning and tag SNP selection using genotype data and their applications to association studies. *Genome Res* 2004; 14: 908–916.
23. Zhang K, Qin Z, Chen T, et al. HapBlock: haplotype block partitioning and tag SNP selection software using a set of dynamic programming algorithms. *Bioinformatics* 2005; 21: 131–134.
24. Zhang K, Calabrese P, Nordborg M, et al. Haplotype block structure and its applications to association studies: power and study designs. *Am J Hum Genet* 2002; 71: 1386–1394.
25. Gross-Bellard M, Oudet P and Chambon P. Isolation of high-molecular-weight DNA from mammalian cells. *Eur J Biochem* 1973; 36: 32–38.
26. Li Z, Zhang Z, He Z, et al. A partition-ligation-combination-subdivision EM algorithm for haplotype inference with multiallelic markers: update of the SHEsis (http://analysis.bio-x.cn). *Cell Res* 2009; 19: 519–523.
27. Lewis CM. Genetic association studies: design, analysis and interpretation. *Brief Bioinform* 2002; 3: 146–153.
28. Lewington S, Clarke R, Qizilbash N, et al. Age-specific relevance of usual blood pressure to vascular mortality: a meta-analysis of individual data for one million adults in 61 prospective studies. *Lancet* 2002; 360: 1903–1913.
29. Seidel E and Scholl UI. Genetic mechanisms of human hypertension and their implications for blood pressure physiology. *Physiol Genomics* 2017; 49: 630–652.
30. Sun F, He N, Zhang K, et al. Association of ACE gene A2350G and I/D polymorphisms with essential hypertension in the northernmost province of China. *Clin Exp Hypertens* 2018; 40: 32–38.
31. Drummond GR, Vinh A, Guzik TJ, et al. Immune mechanisms of hypertension. *Nat Rev Immunol* 2019; 19: 517–532.
32. Parchwani DN, Patel DD, Rawtani J, et al. Analysis of association of angiotensin II type 1 receptor gene A1166C gene polymorphism with essential hypertension. *Indian J Clin Biochem* 2018; 33: 53–60.
33. Gunda P, Nagalingam S and Tirunilai P. Role of tagged SNPs of the AGT gene in causing susceptibility to essential hypertension. *Clin Exp Hypertens* 2016; 38: 520–525.
34. Zotova TY, Kubanova AP, Azova MM, et al. Analysis of polymorphism of angiotensin system genes (ACE, AGTR1, and AGT) and gene ITGB3 in patients with arterial hypertension in combination with metabolic syndrome. *Bull Exp Biol Med* 2016; 161: 334–338.
35. Shariat-Madar B, Shariat-Madar M and Shariat-Madar Z. On the mechanism of action of prolylcarboxypeptidase. In:

Atiq M (ed) *Recent advances in cardiovascular risk factors*. New York: InTech, 2012, pp.255–274.
36. Adams GN, LaRusch GA, Stavrou E, et al. Murine prolylcarboxypeptidase depletion induces vascular dysfunction with hypertension and faster arterial thrombosis. *Blood* 2011; 117: 3929–3937.
37. Maier C, Schadock I, Haber PK, et al. Prolylcarboxypeptidase deficiency is associated with increased blood pressure, glomerular lesions, and cardiac dysfunction independent of altered circulating and cardiac angiotensin II. *J Mol Med (Berl)* 2017; 95: 473–486.
38. Wang L, Feng Y, Zhang Y, et al. Prolylcarboxypeptidase gene, chronic hypertension, and risk of preeclampsia. *Am J Obstet Gynecol* 2006; 195: 162–171.
39. Gittleman HR, Merkulova A, Alhalabi O, et al. A cross-sectional study of KLKB1 and PRCP polymorphisms in patient samples with cardiovascular disease. *Front Med (Lausanne)* 2016; 3: 17.
40. Zhang Y, Hong XM, Xing HX, et al. E112D polymorphism in the prolylcarboxypeptidase gene is associated with blood pressure response to benazepril in Chinese hypertensive patients. *Chin Med J (Engl)* 2009; 122: 2461–2465.