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Haplotype-based association study between PRCP gene polymorphisms and essential hypertension in Hani minority group from a remote region of China

Yanrui Wu[1*], Xingming Pan[2*] and Xiaoxiao Jin[1]

Abstract

Objective: Prolylcarboxypeptidase (PRCP) is both involved in the Kallikrein-Kinin system (KKS) and renin-angiotensin-aldosterone system (RAAS). This study aimed to determine the genetic impact of PRCP gene polymorphisms on essential hypertension (EH) in an isolated population from a remote region of China.

Methods: A haplotype-based study was investigated in 346 EH patients and 346 normal subjects and all samples were Hani minority residents in Southwest China. A total of 11 tag single nucleotide polymorphisms (SNPs) in PRCP gene were tested by polymerase chain reaction-restriction fragment length polymorphism method.

Results: Single site analysis found that PRCP gene 3′UTR SNP rs3750931 was associated with EH. The minor allele G of rs3750931 was more prevalent in the EH patients compared to control subjects after Bonferroni correction ($p < 0.05$). Moreover, the rs3750931 G allele carriers showed higher average blood pressure (BP) level among the subjects. The H2 (GAGCACTAACA) haplotype without rs3750931 G allele showed the protective effect for EH (OR = 0.68, 95 CI 0.54–0.85, $p = 0.001$).

Conclusion: The present study indicated PRCP gene rs3750931 was associated with the risk of EH. This SNP G allele could be considered as one of risk markers for EH in Hani population.

Keywords

Essential hypertension, PRCP, tag SNP, Hani population, RAAS

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Introduction

Essential hypertension (EH) is a complex disorder characterized by the elevated blood pressure (BP). It is an independent risk factor for cardiovascular and cerebrovascular diseases based on the Epidemiological studies.[1,2] It could be classified as a polygenic disease, despite the genetic mechanisms still not completely clear behind EH.[3,4] In fact, besides genetic background, environmental factors including lifestyles, living conditions and nutritional factors involved in the occurrence of EH are also complex. Thus, both environmental and genetic impact can be diverse and vary between populations. Genetic architecture of the isolated population can help reduce genetic diversity and track down genes in complex traits as it is expected for hypertension.[5,6]

In the present study, we recruited EH patients in the Hani ethnic minority, an isolated population reside in the remote rural area of Yunnan province in Southwest China. The Hani people have been living only in the valleys of HongHe and LanCang River regions of Yunnan province in China. They have their own language and share their own living environment due to geographic isolation. Thus, Hani ethnic minority has the low frequency of migration and intermarriage with other minorities.[7,8] This reduced genetic diversity in founder populations could increase the genetic study power to explore the susceptible gene markers for EH.

As a complex trait, BP are regulated by many pathways. The renin-angiotensin-aldosterone system (RAAS) plays a crucial role in the BP regulation.[9] The genes in this system,

[1]School of Basic Medical Sciences, Kunming Medical University, Kunming, Yunnan Province, P. R. China
[2]Human Resources Department of Kunming Medical University, Kunming, Yunnan Province, P. R. China

*These authors contributed equally to this article.

Corresponding author:
Yanrui Wu, School of Basic Medical Sciences, Kunming Medical University, #1168 N. West Chunrong Road, Kunming, Yunnan Province 650500, P. R. China.
Email: wyr102282@126.com

including angiotensinogen gene (AGT), angiotensin converting enzyme gene (ACE), aldosterone synthase gene (CYP11B2) and so on, have been well studied as important members for the pathogenesis analyses of EH.[10–15] In the RAAS, prolylcarboxypeptidase (PRCP) is involved in the metabolism processes of angiotensin II (Ang II) and angiotensin III (Ang III), which can metabolize Ang II to $Ang_{1–7}$ and Ang III to $Ang_{2–7}$.[16–18] PRCP also promotes the formation of kallikrein, which works on the complex of high weight kininogen (HK) and prekallikrein (PK) to release a potent vasodilator.[17,19–21] The PRCP gene is mapped on 11q14, including 10 exons.[22] However, studies between EH and PRCP gene have not been performed widely. The present study tried to explore the genetic impact of PRCP gene on EH in Hani isolated population from China.

Methods

Subjects

There are 692 Hani (346 EH patients and 346 normal subjects) samples and each subject was the resident of the remote mountainous area from Yunnan Province in China. During the sample collection, BP levels were measured using standard mercury sphygmomanometers on the right arm of the subjects seated quietly after 30 min rest. Three measurements were obtained for each subject at 5 min intervals and the average BP values were taken for analysis.

The study samples were selected according to the method reported previously.[7,23] The cases were diagnosed with EH according to the World Health Organization criteria for hypertension (systolic BP ⩾ 140 mm Hg and/or a diastolic BP ⩾ 90 mm Hg) and participants with secondary hypertension and other serious diseases were removed from this study. The controls with BP < 140/90 mm Hg had no history of hypertension and chronic diseases who were randomly chosen based on comparable gender and age matching hypertensive subjects. All the enrolled subjects did not receive any anti-hypertensive drug therapy. This study was approved by the ethics committee of Kunming Medical University, and all subjects involved in this study gave the informed consent.

SNP selection and genotype determination

A total of 11 tag SNPs (rs12290550, rs17144371, rs6592086, rs7104980, rs2298668, rs13306597, rs10792653, rs4084193, rs4759, rs3750931, rs7272) were selected to predict the other SNPs with $r^2 \geqslant 0.8$ by Haploview 4.2 software based on the Chinese data from the International HapMap data set. The selection method and criterion were described previously in published paper.[24,25] The relative location of these tags SNPs was listed in Figure 1.

Genomic DNA samples were extracted from peripheral blood leukocytes by the phenol-chloroform method.[26] Using the method of polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP), genotypes of 11 tag SNPs were identified. The PCR amplification protocol, length of target fragments and related restriction endonuclease are followed by a previously study.[23]

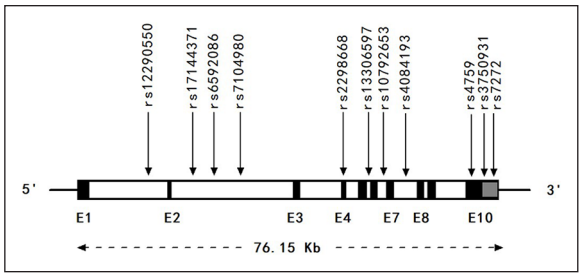

Figure 1. The *PRCP* gene structure and relative position of 11 tag SNPs. Black boxes indicate exons, blank boxes indicate introns, and gray box indicate 3′-UTR.

Table 1. Characteristics of the study subjects.

Parameters	Hani population	
	Normotensive	Hypertensive
Gender(male/female)	185/161	212/134
Age (years)	49.3 ± 11.1	50.2 ± 10.6
BMI (kg/m^2)	20.8 ± 2.4	22.9 ± 2.7*
SBP (mm Hg)	101.8 ± 6.3	153.8 ± 12.7*
DBP (mm Hg)	67.1 ± 4.8	93.9 ± 10.1*

SBP: systolic blood pressure; DBP: diastolic blood pressure; BMI: body mass index.
Continuous variable is expressed as mean ± SD.
*$p < 0.01$, with statistical difference compared between Normotensive and Hypertensive groups.

Statistical analysis

Experiment data were analyzed using SPSS software (version 16.0) and the online SHEsis analysis tool (http://analysis.bio-x.cn/myAnalysis.php). The Chi-square test (χ2-test) was carried out to assessment the Hardy-Weinberg equilibrium and compare the categorical variables between groups. Genotypes and alleles distributions of all SNPs were calculated by SPSS. Lewontin's D′ (|D′|) and r^2 between each pair of tag SNPs were speculated by Haploview 4.2 software. SHEsis tool was used to construct haplotypes according to the tag SNPs genotype data. Finally, logistic regression analysis was performed to evaluate the association between PRCP gene and the risk of EH under adjustment for gender, age and body mass index (BMI). Statistical significance was established at $p < 0.05$. The Bonfferoni correction was performed in this present study.

Results

Characteristics of study participants

The general characteristics of the enrolled subjects are shown in Table 1. Hypertensive patients exhibited significantly higher BP and BMI than control subjects ($p < 0.01$).

Table 2. Single SNP genotype and allele distributions between EH patients and controls.

Variant	Allele[a] 1/2	Group	Genotype (frequency)			OR[b] (95%CI), *p*	Allele (frequency)		OR[b] (95%CI), *p*
			1/1	1/2	2/2	Dominant model 1/2 + 2/2 versus 1/1	1	2	Multiplicative model 2 versus 1
rs12290550	G/T	Control	252 (0.728)	86 (0.249)	8 (0.023)		590 (0.853)	102 (0.147)	
		Case	236 (0.682)	93 (0.269)	17 (0.049)	1.27 (0.92–1.77), 0.150	565 (0.816)	127 (0.184)	1.34 (1.00–1.78), 0.047
rs17144371	A/C	Control	339 (0.980)	7 (0.020)	0 (0.000)		685 (0.990)	7 (0.010)	
		Case	337 (0.974)	9 (0.026)	0 (0.000)	1.27 (0.46–3.45), 0.645	683 (0.987)	9 (0.013)	1.26 (0.47–3.42), 0.647
rs6592086	G/C	Control	277 (0.801)	64 (0.185)	5 (0.014)		618 (0.893)	74 (0.107)	
		Case	245 (0.708)	89 (0.257)	12 (0.035)	1.65 (1.16–2.35),0.006	579 (0.837)	113 (0.163)	1.62 (1.18–2.23), 0.005
rs7104980	C/G	Control	173 (0.500)	133 (0.384)	40 (0.116)		479 (0.692)	213 (0.308)	
		Case	152 (0.439)	152 (0.439)	42 (0.121)	1.28 (0.95–1.73), 0.107	456 (0.659)	236 (0.341)	1.17 (0.93–1.47), 0.173
rs2298668	A/C	Control	278 (0.803)	63 (0.182)	5 (0.014)		619 (0.895)	73 (0.105)	
		Case	256 (0.740)	84 (0.243)	6 (0.017)	1.44 (1.00–2.06), 0.049	596 (0.861)	96 (0.139)	1.36 (0.98–1.89), 0.064
rs13306597	C/T	Control	198 (0.572)	126 (0.364)	22 (0.064)		522 (0.754)	170 (0.246)	
		Case	184 (0.532)	140 (0.405)	22 (0.064)	1.17 (0.86–1.58), 0.318	508 (0.734)	184 (0.266)	1.11 (0.87–1.41), 0.412
rs10792653	T/G	Control	128 (0.370)	157 (0.454)	61 (0.176)		413 (0.597)	279 (0.403)	
		Case	124 (0.358)	165 (0.477)	57 (0.165)	1.04 (0.77–1.43), 0.783	413 (0.597)	279 (0.403)	1.00 (0.81–1.25), 0.970
rs4084193	A/C	Control	221 (0.639)	108 (0.312)	17 (0.049)		550 (0.795)	142 (0.205)	
		Case	203 (0.587)	130 (0.376)	13 (0.038)	1.22 (0.89–1.66), 0.211	536 (0.775)	156 (0.225)	1.11 (0.86–1.44), 0.408
rs4759	A/G	Control	115 (0.332)	176 (0.509)	55 (0.159)		406 (0.587)	286 (0.413)	
		Case	105 (0.303)	177 (0.512)	64 (0.185)	1.14 (0.82–1.57), 0.436	387 (0.559)	305 (0.441)	1.13 (0.91–1.40), 0.260
rs3750931	C/G	Control	294 (0.850)	48 (0.139)	4 (0.012)		636 (0.919)	56 (0.081)	
		Case	262 (0.757)	73 (0.211)	11 (0.032)	1.91 (1.29–2.82), 0.001	597 (0.863)	95 (0.137)	1.89 (1.33–2.69), 0.0004
rs7272	A/G	Control	177 (0.512)	136 (0.393)	33 (0.095)		490 (0.708)	202 (0.292)	
		Case	159 (0.460)	147 (0.425)	40 (0.116)	1.23 (0.91–1.67), 0.169	465 (0.672)	227 (0.328)	1.19 (0.95–1.50), 0.132

OR: odds ratio; CI: confidence interval.

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

[b]OR estimated by logistic regression analysis, adjusted for gender, age, BMI.

Table 3. Association of rs3750931 with blood pressure level in Hani population.

SNP	Genotype (n)	SBP	*p*	DBP	*p*
rs3750931	GG (15)	142.47 ± 23.03		89.80 ± 13.59	
	CG (121)	133.00 ± 28.62		82.85 ± 17.54	
	CC (556)	126.29 ± 28.62	0.009	79.72 ± 15.85	0.012

SBP: systolic blood pressure; DBP: diastolic blood pressure.

The distributions of age and gender showed no significant difference between the cases and controls.

Tag SNPs analysis

The distributions of 11 tag SNPs in PRCP gene were consistent with Hardy-Weinberg equilibrium in the normal group ($p > 0.05$). Table 2 listed the distributions of genotype and allele data. The minor rate allele G of rs3750931 was more common in the EH group compared to the control group after Bonferroni correction ($p < 0.05$). At the same time, this minor rate allele was also associated with BP level in Hani population ($p < 0.05$). Table 3 showed the association of PRCP rs3750931 with the BP level.

In order to evaluate the impact mediated by the genetic risk factors on the development of EH, the single site data results were also analyzed under genetic models.[27] In Hani population, the associated single site influencing the risk of EH was rs3750931 after Bonferroni correction (dominant model CG + GG vs CC: OR = 1.91, 95%CI 1.29–2.82, $p = 0.001$; multiplicative model G vs C: OR = 1.89, 95%CI 1.33–2.69, $p = 0.0004$).

Haplotype analysis

The different patterns of linkage disequilibrium in the PRCP gene were shown in Figure 2. Compared to the control group, the level of linkage disequilibrium in EH group showed a decline trend. As tag SNPs are sufficient to capture most of the haplotype, the haplotypes were constructed with 11 tag SNPs. Eight haplotypes with the frequencies above 1% both in cases and controls were shown in Table 4. The most common haplotype was H2 GAGCACTAACA. This haplotype had a significantly lower incidence in EH group than in the control group after Bonferroni correction ($p < 0.05$) and decreased the risk of EH (OR = 0.68, 95%CI 0.54–0.85).

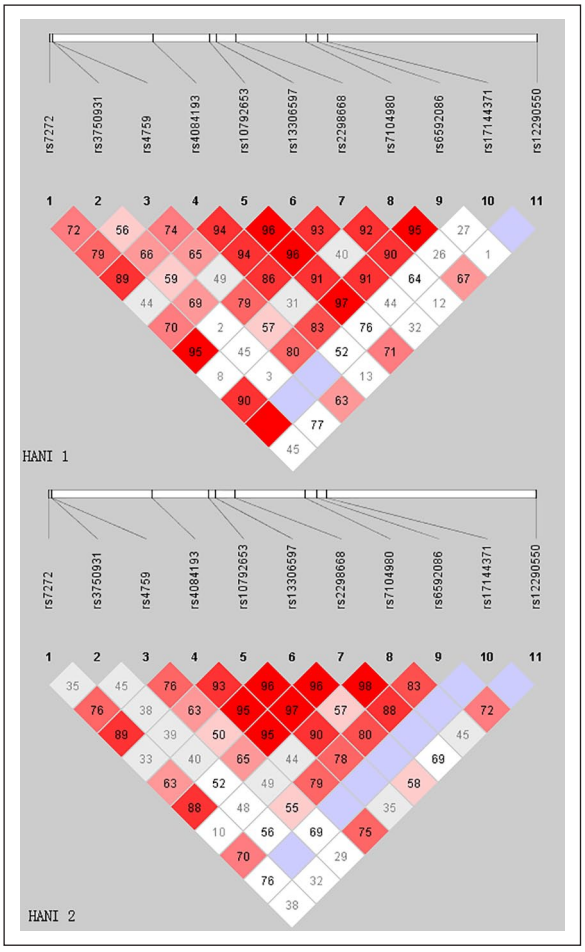

Figure 2. The linkage disequilibrium patterns of tag SNPs in PRCP gene. HANI 1 and HANI 2 showed the control and EH groups respectively.

Discussion

Ang II is the principal active vasoconstrictor peptide of the RAAS and it causes vasoconstriction leading to increases in arterial BP.[18] Like Ang II, Ang III is also a pressor agent which exerts vasoconstriction effects through angiotensin type 1 (AT1) receptors. As the vasodilator, PRCP acts the opposite effects to Ang II and Ang III by degrading these two peptides.[16] Moreover, PRCP has been found be involved in Kallikrein-Kinin system (KKS) which is closely associated with the RAAS in the cardiovascular system.[17,18,28] Experimental evidence in PRCP-deficient mice suggested the crucial impact of PRCP in contributing to regulate BP.[29,30]

The genetic association study found a nonsynonymous mutant (Glu→Asp) in exon4 of PRCP gene (rs2298668, E112D) coupled with chronic hypertension suggested an increased risk for preeclampsia in American women population.[31] Another study found this exon SNP was associated with the antihypertensive efficacy of an ACE inhibitor in a Northern Chinese population,[32] and the frequencies of

Table 4. Association between PRCP Haplotypes and EH.

Name	Haplotype	Control(freq)	Case(freq)	OR (95%CI)	*p*
H1	GACGCTGCGCG	41 (0.059)	58 (0.083)	1.49 (0.98–2.27)	0.059
H2	GAGCACTAACA	316 (0.457)	252 (0.365)	0.68 (0.54–0.85)	0.001*
H3	GAGCACTAGCG	47 (0.068)	46 (0.066)	1.00 (0.65–1.52)	0.983
H4	GAGCATGCGGG	29 (0.042)	34 (0.049)	1.21 (0.73–2.01)	0.464
H5	TAGGACGAGCA	70 (0.101)	57 (0.083)	0.82 (0.57–1.18)	0.282
H6	GAGGACGAGCA	28 (0.041)	17 (0.025)	0.62 (0.34–1.15)	0.125
H7	GAGGATGAACA	20 (0.029)	24 (0.034)	1.20 (0.66–2.20)	0.549
H8	GACGCTGCACG	13 (0.018)	9 (0.013)	0.75 (0.32–1.76)	0.504

OR: odds ratio; CI: confidence interval. The haplotype structure of the *PRCP* gene 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 not included in the table.

E112D genotypes were similar to those tested in the present study. However, the association was not found between E112D and EH in Hani population. The previous haplotype-based study in Chinese Han population in southwestern China reported the G allele of PRCP intron SNP rs7104980 was associated with EH, and rs7104980 probably effect on the BMI in hypertensive subjects.[24,33] The prevalence of rs7104980 G allele between Han Chinese and Hani populations had the similar frequencies. Researchers explored if E112D and rs7104980 of PRCP gene were associated with cardiovascular disease, and they found the G allele of rs7104980 but not E112D predicted an increased trend of having a history of percutaneous transluminal coronary angioplasty although without the statistical significance after Bonferroni correction.[34] In this present study, rs7104980 did not show any association with EH in Hani population and the frequency of rs7104980 G allele was lower than that in Caucasian population.[34] It seems like that different genetic background population had the specific genetic markers within the same gene, like other EH candidate genes.[15,35] The implication of this inconsistency may be the different genetic background result from the distinctive customs and living habits, living environment and other factors.

In this present study, rs3750931 which located in 3′UTR of PRCP gene was associated with EH, and the G allele may be the risk factor for the occurrence of EH in Hani population. One of the most notable features of this study is the samples who come from the remote rural area of Yunnan Province in China, and all subjects in this study did not have the anti-hypertension therapy. Therefore, it presents an opportunity to objectively reflect the genotype-phenotype correlation of EH. PRCP rs3750931 G allele carriers had higher average BP level than the subjects with CC genotype. This further proved the risk contribution of rs3750931 G allele in Hani minority to EH.

The haplotype analysis exhibited strong association between PRCP gene and EH in Hani minority. H2 GAGCACTAACA haplotype showed the significantly protective effect to EH. This risk reduction effect may be associated with the contribution of rs3750931, since H2 did not contain the risk allele G of it. Here, the haplotype results were consistent with the single SNP analysis results, which indicated that rs3750931 G allele could be used as the risk genetic marker for EH in Hani group. As one mutant in untranslated region, rs3750931 might be a non-functional variant. It is noteworthy that SNPs located in 3′UTR could regulate gene expression by blocking miRNA binding the target sites.[36] Further investigations are necessary to be performed to better understand the contribution of rs3750931 for BP.

Some potential limitations should be noted to this study. The present study samples reside in the remote rural area, so we could not obtain the detailed clinical and biochemical index data of subjects. Additionally, other genotyping methods, such as sequencing and gene chip may be more convenient in SNP genotyping under optimized condition. Taking the isolated population for research objects, this haplotyped-based study minimizes the influence of confounding environmental factors, such as high frequency of migration and intermarriage with other minorities, and raises the power to explore the genetic contribution to EH. This was the highlights of the present study.

In conclusion, both single SNP site and haplotype analysis results suggested that PRCP gene was associated with EH in Hani population. The analysis between phenotype and genotype also showed the determinate of the susceptible SNP to the occurrence of EH in Hani population. The present results indicated that PRCP gene rs3750931 can be considered as one of risk markers for EH in Hani population from Yunnan province in China. Since EH is commonly recognized as a polygenic disorder, further studies in a large sample size on the level of gene-gene interaction are warranted to confirm the genetic contribution to EH.

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Declaration of conflicting interests

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ORCID iD

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

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