Original Research

The alleles of AGT and HIF1A gene affect the risk of hypertension in plateau residents

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Impact statement

Plateau essential hypertension (PEH) is a common chronic harmful disease of permanent residents in plateau areas. There are relatively few molecular level evidence showing the cause of PEH, and there are even fewer results uncovering the relationship between gene polymorphisms and hypertension in Tibetan nationality lived in Qinghai. This study carried out DNA sequencing in Qinghai Tibetan population to identify polymorphic loci and studied the relationship between PEH and susceptible SNPs. The results would uncover the potential roles of SNPs in the risk of Tibetan PEH, help to improve understanding of the pathogenesis, and serve as signatures for early diagnosis of PHE.

Abstract

Plateau essential hypertension is a common chronic harmful disease of permanent residents in plateau areas. Studies have shown some single nucleotide polymorphisms (SNPs) associations with hypertension, but few have been verified in plateau area-lived people. In this paper, we examined some hypertension-related gene loci to analyze the relationship between risk SNPs and plateau essential hypertension in residents in Qinghai-Tibet plateau area. We screened hypertension-related SNPs from the literature, Clinvar database, GHR database, GTR database, and GWAS database, and then selected 101 susceptible SNPs for detection. Illumina MiSeq NGS platform was used to perform DNA sequencing on the blood samples from 185 Tibetan dwellings of Qinghai, and bioinformatic tools were used to make genotyping. Genetic models adjusted by gender and age were used to calculate the risk effects of genotypes. Four known SNPs as well as a new locus were found associated with PHE, which were rs2493134 (AGT), rs9349379 (PHACTR1), rs1371182 (CYP2C56P-PRPS1P1), rs567481079 (CYP2C56P-PRPS1P1), and chr14:61734822 (HIF1A). Among them, genotypes of rs2493134, rs9349379, and rs567481079 were risk factors, genotypes of rs1371182 and chr14:61734822 were protective factors. The rs2493134 in AGT was found associated with an increased risk of the plateau essential hypertension by 3.24-, 3.24-, and 2.06-fold in co-dominant, dominant, and Log-additive models, respectively. The rs9349379 in PHACTR1 is associated with a 2.61-fold increased risk of plateau essential hypertension according to the dominant model. This study reveals that the alleles of AGT, HIF1A, and PHACTR1 are closely related to plateau essential hypertension risk in the plateau Tibetan population.

Keywords: Plateau essential hypertension, Qinghai Tibetan population, single nucleotide polymorphisms (SNPs), association analysis, AGT, HIF1A

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Introduction

Plateau essential hypertension (PEH), as a special kind of essential hypertension (EH), is a chronic disease that involves environmental and genetic factors.[1,2] The main victims of this disease are people who lived permanently in the plateau area. Parati *et al*.[3] had indicated that altitude played a crucial role in regulating blood pressure (BP). In the plateau region, the residents have a much higher prevalence of PEH, since the air temperature, atmospheric pressure, and air humidity are significantly decreased.[4,5] As a minority living in high-altitude areas for generations, Tibetans have a much higher prevalence of hypertension

than other groups.[6] A study had shown that hereditary factors also played a significant role in the incidence of hypertension in Tibetan nationality.[7]

The wide application of candidate gene linkage analysis and genome-wide association study (GWAS) has identified many hypertension susceptibility genes and revealed functions of related SNPs.[8–12] It has been reported that BP is related to above 30 known genes, with rare mutations leading to monogenic forms of hypotension or hypertension. 1477 common SNPs were related to the BP phenotype also.[13] Kato *et al.*[1] identified some common variation loci association with BP changes in East Asians and found four new gene loci located on ST7L-CAPZA1, FIGN-GRB14, ENPEP, and NPR3 through meta-analysis and GWAS. Yang *et al.*[14] identified 100 susceptibility genes of high blood pressure through GWAS and found 17 genes showed different allele frequencies expression values between case and control by permutation tests. A study also identified 13 novel susceptibility sites associated with high blood pressure through exome-wide association studies.[15] Some researches had confirmed the mutation A-6G, which is a substitution of adenine for a guanine 6 bp upstream from the locus of transcription initiation in the 5′ terminal regulation region of the angiotensin (AGT) gene correlated positively with EH in the Tibetan population, but this mutation had no correlation with EH in Han and Yi population.[16,17] Gesang *et al.*[7] found there was a significant relationship between the D allele of the angiotensin-converting enzyme (ACE) gene and hypertension in Tibetan females, but not in Tibetan males. Pandey *et al.*[18] proved four gene loci rs978906, rs6753921, rs10495582, and rs2230774 in the ROCK2 gene significantly related to EH in the population living in a high-altitude area.

With the discovery of more and more hypertension-susceptibility genes and SNPs, targeted medicines, such as tamoxifen targeted at ESR1 and statins for HMGCR, have changed the clinical practice of hypertension treatment and improved outcomes and reduced side effects.[13,19–21] Nowadays, different diagnosis and precise treatments for single-gene hypertension, especially refractory hypertension, have been guided through specific genotypes, and the approach was estimated to reduce total three-year costs by 47%.[19,22] It is believed that with the integrated researches on multiple genetic perspectives, the pathogenesis of essential hypertension will be accelerated uncover.

Qinghai province is located in the northeast of Qinghai-Tibet Plateau in western China, with an average altitude of more than 3000 meters. The unique environment in this region and the relatively primitive and isolated lifestyle of the Tibetan residents offer a favorable opportunity to study the PEH in the high-altitude area. Although the researches on the mechanism of hypertension in high-altitude areas are increasing gradually, there are relatively few molecular level results uncovering the relationship between PEH and genotypes in Qinghai-Tibet plateau-lived people. In this study, we aimed at the reported hypertension-related SNPs, and then carried out gene sequencing in the Qinghai Tibetan population to identify polymorphic loci, study the relationship between PEH and susceptible SNPs, and furthermore, discover the potential roles of SNPs in the risk of Tibetan PEH. The results will help to understand the pathogenesis of PEH, and the identified gene loci may also serve as markers for PHE diagnosis and candidate therapy targets.

Materials and methods

Study participants

All samples were collected from First People's Hospital of Xining City, Qinghai Province. Two hundred participants were recruited, and the number of both hypertensive patients and healthy controls was 100. The participants were Tibetan nationality who had lived in Huangnan County, Qinghai Province, for at least three generations and had no history of emigration. All patients had no other diseases causing hypertension. For each participant, 5 mL of peripheral blood sample was collected by venipuncture, kept in blood collection tubes containing sodium heparin, and froze in a refrigerator at −80°C. After removing the damaged blood samples, the remaining 185 participants were 87 hypertension samples and 98 healthy control samples. We used the unified questionnaire to collect the clinical information, including age, gender, height, weight, body mass index, systolic blood pressure (SBP), diastolic blood pressure (DBP), total cholesterol, triglycerides, low-density lipoprotein, high-density lipoprotein, and so on. The study was approved by the ethics committee of Xining First People's Hospital. All participants were well informed and signed the informed consent.

SNPs selection and high-throughput DNA sequencing

More and more SNPs associated with hypertension had been reported, some belong to non-coding loci in intron regions, and some are related to genes involved in different signaling channels or metabolic processes. In this study, we screened the hypertension-related gene loci from literature as well as databases including Clinvar, GHR, GTR, and GWASdb. Furthermore, we examined the frequency distribution, allele frequency, surrounding sequence and, primer sequence homology of each locus. Finally, 101 SNPs in different BP-related pathways were selected for further detection.

One hundred and one pairs of primers were designed to amplify the target SNPs sites by PCR (Table S1), and a library was then constructed for paired-end sequencing with the reads' length of 2×150 bp. The experiment was carried by Shanghai Yihe applied Biotechnology Ltd. using Illumina-MiSeq platform.

Data processing

We separated each sample from raw sequence data according to the barcode. Then, the adaptor sequences of each were removed by cutadapt program. We used hg38 as reference genome and BWA-MEM[23] for read alignment. Samtools and Genome Analysis Toolkit (GATK)[24] was used to remove PCR duplicates, call variants, and filter SNPs. Vcftools[25] was used to perform data-preprocessing according to sequencing depth, allele counts, minimum

allele frequency (MAF), and individual genotype missing rate. Mutation loci with MAF < 0.05 and Hardy-Weinberg equilibrium (HWE) $<1 \times 10^{-6}$ were excluded.

Statistical analysis

We used Plink 1.9[26,27] and SNPstats[28] (https://www.snpstats.net/) to perform statistical analysis. Chi-square test was used to analyze the correlation between the gene loci and PEH risk according to odds ratio (OR),[29] 95% confidence intervals (CIs), and p-value. SNPstats was used to calculate the relevance between genotype frequency and PEH risk in the genetic model and adjusted the risk effect by gender and age.

Results

Characteristics of the study population

One hundred and eighty-five subjects were involved in this study, including 87 PEH patients (35 females and 52 males) and 98 healthy controls (56 females and 42 males). The clinical characteristics of the subjects are listed in Table 1.

Data statistics

The total number of reads generated in this study was 68,633,082, after removed the adaptor sequences, 41,330,937 reads were aligned to the reference. After filtering by GATK and Vcftools, 239 genetic loci were obtained.

Table 1. Clinical characteristics of the participants.

Variable	Cases (n = 87)	Controls (n = 98)
Male	52 (55.32)	42 (44.68)
Female	35 (38.46)	56 (61.54)
Age (year)	58.86 ± 12.14	36 ± 12.51
Height (cm)	165.95 ± 11.50	164.54 ± 12.34
Weight(kg)	74.45 ± 11.98	64.48 ± 12.26
BMI (kg/m2)	28.06 ± 14.17	23.68 ± 3.74
SBP (mmHg)	161.6 ± 16.11	117.61 ± 9.64
DBP (mmHg)	103.19 ± 10.91	71.18 ± 9.14
TC (mmol/L)	5.20 ± 1.70	3.97 ± 1.03
TG (mmol/L)	1.70 ± 0.84	1.75 ± 0.93
LDL-C (mmol/L)	2.56 ± 0.74	2.24 ± 0.86
HDL-C (mmol/L)	1.44 ± 0.19	1.12 ± 0.45

BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; TC: total cholesterol; TG: triglycerides; LDL-C: low-density lipoprotein; HDL-C: high-density lipoprotein cholesterol.
Note: The categorical variables are presented as number (%). Continuous variables are expressed as mean ± SD.

Thirteen genetic loci were removed due to deviation from the Hardy-Weinberg equilibrium, and 143 genetic loci were removed due to the low MAF. Finally, 83 mutation loci were kept for further investigation.

Associations between SNPs and PEH

After analysis, we found five gene loci associated with PEH risk (rs2493134 in AGT, p = 0.009, OR = 1.88, 95% CI = 1.169–3.021; rs9349379 in PHACTR1, p = 0.015, OR = 1.909, 95%CI = 1.128–3.232; rs1371182 between CYP2C56P and PRPS1P1, p = 0.026, OR = 0.594, 95% CI = 0.375–0.941; rs567481079 between CYP2C56P and PRPS1P1, p = 0.015, OR = 2.552, 95%CI = 1.171–5.560; chr14:61734822 in HIF1A, p = 0.045, OR = 0.375, 95% CI = 0.140–1.005). Other gene loci in these samples were not significantly relative to PEH risk. Table 2 shows the basic information of the five candidate gene loci in this study.

The associations between genotype models of hub SNPs and PEH

We calculated the Co-dominant, Dominant, Recessive, and Log-additive models of candidate loci, and adjusted them according to age and gender (Table 3). We found the rs2493134 in the AGT gene associated with a 3.24-fold increased risk of PEH in Co-dominant model (p = 0.037, OR =3.24, 95%CI =0.85–12.36 for the "T/T" genotype), 3.24-fold increased risk in Dominant model (p = 0.01, OR = 3.24, 95%CI = 1.28–8.21 for the "T/C-T/T" genotype), and 2.06-fold increased risk in Log-additive model (p = 0.021, OR = 2.06, 95%CI = 1.09–3.90), respectively. The rs9349379 in the PHACTR1 gene is associated with a 2.61-fold increased risk of PEH in Co-dominant model (p = 0.042, OR = 2.61, 95%CI = 1.02–6.73 for the "A/G-A/A" genotype). We did not find statistically significant relevance between other genetic models of candidate loci and PEH risk.

Discussion

More than 140 million people live at an altitude of more than 2500 meters worldwide.[30] As a common chronic disease, PEH is harmful to health of plateau-lived residents. Qinghai Tibetans show a higher prevalence of hypertension than other ethnic groups living in high-altitude areas, but the treatment rate, control rate, and prevention rate are lower due to economic or other reasons. To reduce the

Table 2. MAF and OR for PEH risk genotypes.

CHR	SNP	Gene	Position	Alleles (A1/A2)	MAF		OR (95%CI)	p
					Case	Control		
1	rs2493134	AGT	230713613	T/C	0.349	0.222	1.88 (1.169–3.021)	0.009
6	rs9349379	PHACTR1	12903725	A/G	0.303	0.185	1.909 (1.128–3.232)	0.015
2	rs1371182	CYP2C56P-PRPS1P1	164242705	T/C	0.259	0.371	0.594 (0.375–0.941)	0.026
2	rs567481079	CYP2C56P-PRPS1P1	164242698	C/T	0.128	0.054	2.552 (1.171–5.560)	0.015
14	chr14:61734822	HIF1A	61734822	G/A	0.067	0.160	0.375 (0.140–1.005)	0.045

MAF: minor allele frequency, MAF > 0.05; OR: odds ratio, 95%CI: confidence interval.
*p < 0.05 indicates statistical significance.

Table 3. Relationships between polymorphism and PEH risk.

					Before adjusted		Adjusted by sex + age			
SNP	**Model**	**Genotype**	**control**	**case**	**OR (95% CI)**	**p**	**OR (95% CI)**	**p**	**AIC**	**BIC**
rs2493134	Co-dominant	C/C	58 (64.4%)	41 (49.4%)	1	0.066	1	**0.037***	133.1	148.8
		T/C	24 (26.7%)	26 (31.3%)	1.53 (0.77–3.04)		3.24 (1.16–9.06)			
		T/T	8 (8.9%)	16 (19.3%)	2.83 (1.11–7.23)		3.24 (0.85–12.36)			
	Dominant	C/C	58 (64.4%)	41 (49.4%)	1	**0.045***	1	**0.01***	131.1	143.7
		T/C-T/T	32 (35.6%)	42 (50.6%)	1.86 (1.01–3.42)		3.24 (1.28–8.21)			
	Recessive	C/C-T/C	82 (91.1%)	67 (80.7%)	1	**0.047***	1	0.26	136.4	149
		T/T	8 (8.9%)	16 (19.3%)	2.45 (0.99–6.07)		2.01 (0.58–6.93)			
	Log-additive	–	–	–	1.64 (1.07–2.51)	**0.02***	2.06 (1.09–3.90)	**0.021***	132.3	145
rs9349379	Co-dominant	G/G	54 (66.7%)	38 (50%)	1	0.059	1	0.13	124	139.3
		A/G	24 (29.6%)	30 (39.5%)	1.78 (0.90–3.50)		2.61 (0.96–7.10)			
		A/A	3 (3.7%)	8 (10.5%)	3.79 (0.94–15.22)		2.62 (0.39–17.88)			
	Dominant	G/G	54 (66.7%)	38 (50%)	1	**0.034***	1	**0.042***	122	134.3
		A/G-A/A	27 (33.3%)	38 (50%)	2.00 (1.05–3.81)		2.61 (1.02–6.73)			
	Recessive	G/G-A/G	78 (96.3%)	68 (89.5%)	1	0.089	1	0.5	125.7	138
		A/A	3 (3.7%)	8 (10.5%)	3.06 (0.78–11.99)		1.85 (0.29–11.83)			
	Log-additive	–	–	–	1.86 (1.10–3.14)	**0.018***	2.06 (0.95–4.47)	0.058	122.6	134.8
rs1371182	Co-dominant	C/C	41 (44.1%)	45 (55.6%)	1	0.073	1	0.76	136.8	152.6
		T/C	35 (37.6%)	30 (37%)	0.78 (0.41–1.49)		0.93 (0.36–2.40)			
		T/T	17 (18.3%)	6 (7.4%)	0.32 (0.12–0.89)		0.59 (0.14–2.46)			
	Dominant	C/C	41 (44.1%)	45 (55.6%)	1	0.13	1	0.69	135.2	147.8
		T/C-T/T	52 (55.9%)	36 (44.4%)	0.63 (0.35–1.15)		0.84 (0.35–2.01)			
	Recessive	C/C-T/C	76 (81.7%)	75 (92.6%)	1	**0.031***	1	0.47	134.8	147.4
		T/T	17 (18.3%)	6 (7.4%)	0.36 (0.13–0.96)		0.61 (0.16–2.39)			
	Log-additive	–	–	–	0.63 (0.41–0.98)	**0.035***	0.82 (0.43–1.54)	0.53	134.9	147.6
rs567481079	Co-dominant	T/T	82 (89.1%)	67 (77.9%)	1	**0.032***	1	0.11	139.6	155.5
		C/T	10 (10.9%)	16 (18.6%)	1.96 (0.83–4.60)		2.54 (0.59–10.97)			
		C/C	0 (0%)	3 (3.5%)	NA (0.00–NA)		NA (0.00–NA)			
	Dominant	T/T	82 (89.1%)	67 (77.9%)	1	**0.042***	1	0.095	139.2	151.9
		C/T-C/C	10 (10.9%)	19 (22.1%)	2.33 (1.01–5.34)		3.21 (0.78–13.21)			
	Recessive	T/T-C/T	92 (100%)	83 (96.5%)	1	**0.036***	1	0.1	139.2	152
		C/C	0 (0%)	3 (3.5%)	NA (0.00–NA)		NA (0.00–NA)			
	Log-additive	–	–	–	2.38 (1.11–5.11)	**0.019***	3.26 (0.90–11.78)	0.056	138.3	151
chr14:61734822	–	A/A	34 (68%)	39 (86.7%)	1	**0.028***	1	0.081	67.5	77.7
		G/A	16 (32%)	6 (13.3%)	0.33 (0.11–0.93)		0.28 (0.06–1.23)			

*$p < 0.05$ indicates statistical significance.

burden on the public health of Tibetans in the plateau, researches are needed to both uncover the mechanism of PEH and take new methods to prevent and treat PEH. A study had shown that the heritability of BP was about 30%–50%[11]; however, there are relatively few studies aimed at PEH in Qinghai Tibetans. The main pathogenesis causing PEH of Qinghai Tibetans is still not clear. This study took the Qinghai Tibetan population as the research object and studied the relationship between the 101 SNPs, along with their surrounding loci, and PEH. Our results proved that SNPs rs2493134, rs9349379, rs1371182, and rs567481079, together with a new locus (chr14:61734822) may associate with the risk of PEH in Qinghai Tibetan population.

The rs2493134 locates in the AGT gene. AGT is one of the most effective vasoconstrictors, a precursor of angiotensin II (Ang II).[31] A previous study had found the A/G SNP (rs2493134) associated with SBP and DBP.[10] Kolifarhood *et al.*[32] also found that rs2493134 was associated with risk of SBP and DBP in a study on whether familial-hereditary disease or environmental risk factors were associated with the increased risk of high blood pressure. In this study, we found that the rs2493134 had a significant association with PEH in Qinghai Tibetans. In the allele model, the rs2493134 is associated with a 1.88-fold increased risk of PEH ($p = 0.009$, 95%CI = 1.169–3.021). In the genetic models, the rs2493134 is associated with an increased risk of PEH in the Co-dominant, Dominant, and Log-additive model. This is consistent with the results of previous studies. The AGT gene can interact with genes such as ACE, ACE2, and ATP6AP2 to form an interaction network (Figure 1). Genes in the network are enriched in the renin-angiotensin-system (RAS) and renin secretion pathway (Table S2), and they are mainly involved in biological processes such as regulation of systemic arterial BP and regulation of vasoconstriction.

AGT gene is in the RAS system, which has peptidergic and endocrine properties that regulate BP and hydro-electrolytic balance. In classic RAS, the renin enzyme cleaves its substrate AGT to form decapeptide angiotensin I, which is in turn cleaved by ACE to produce Ang II. Ang II activates its AT1 receptor, which is the main receptor mediating most of the known effects of Ang II in the kidney, such

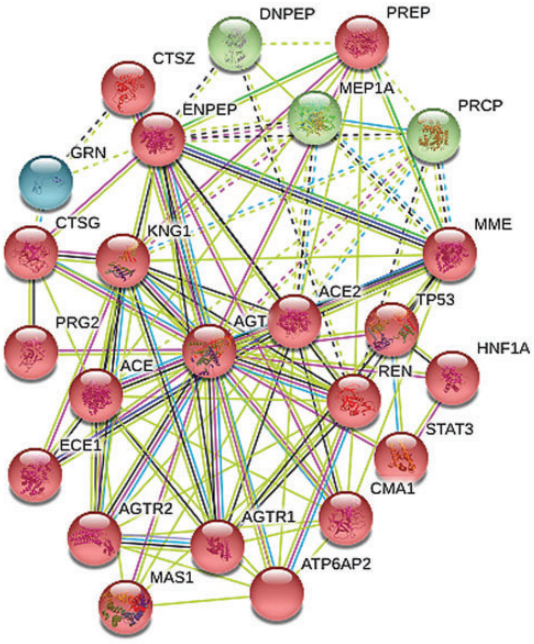

Figure 1. The protein interaction map of AGT gene co-expressed with ACE, ACE2, ATP6AP2, and other genes. (A color version of this figure is available in the online journal.)

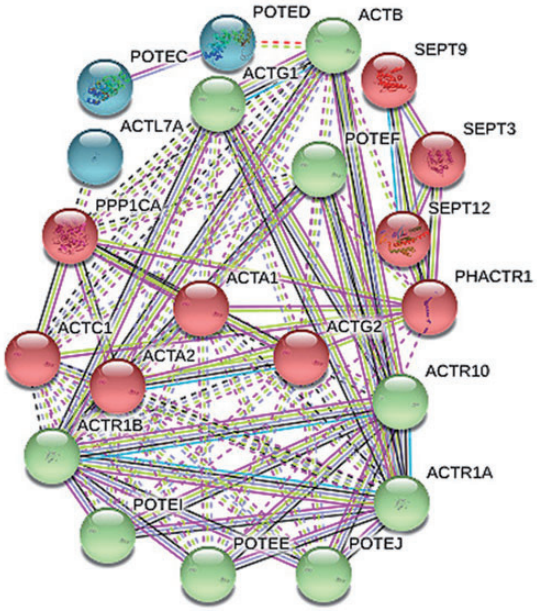

Figure 3. The protein interaction map of PHACTR1 gene co-expressed with ACTA1, ACTC1h, PPP1CA, and other genes. (A color version of this figure is available in the online journal.)

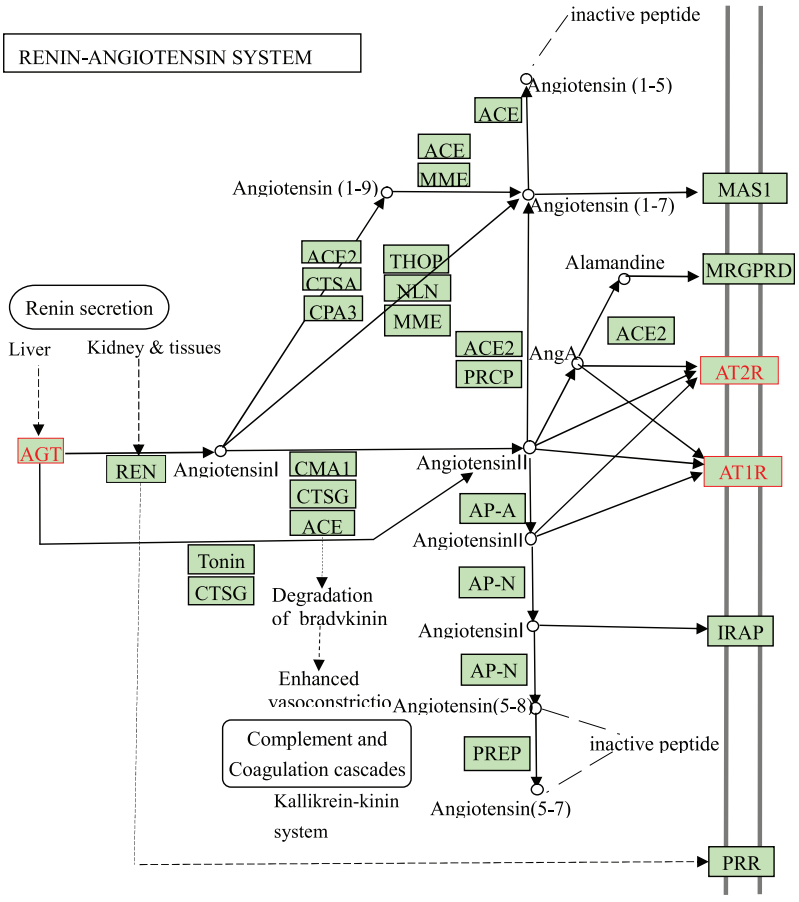

Figure 2. The metabolic pathway maps of the renin-angiotensin system (RAS). The figure shows the endocrine processes of the RAS system that regulates the electrolyte balance and blood pressure. The encoded product of AGT is angiotensinogen, an angiotensin precursor, and plays a crucial role in regulating vascular tension, heart, and vascular remodeling as the sole substrate of renin in RAS. (A color version of this figure is available in the online journal.)
Rectangle: gene product; circle: chemical compound, DNA, and other molecules; rounded rectangle: map; solid arrow: molecular interaction or relation; dashed arrow: indirect link or unknown reaction; red gene in rectangular boxes: disease-associated gene variants.

as vasoconstriction, aldosterone secretion, renal sodium (Na+) reabsorption, which increasing BP and promoting the development of hypertension (Figure 2). The rs2493134 locates in the intron of the AGT gene (Figure S1) where regulatory sequences may exist. Although the SNP does not directly change the protein coding, it possibly influences the gene function by changing genes transcription activity.

The rs9349379 is in the coding region of the Phosphoase and ActinRegulator 1 (PHACTR1) (Figure S2), which may affect the coding of the translated protein. PHACTR1 plays a crucial role in renal tubule formation and endothelial cell survival. Studies have shown rs9349379 may increase the risk of coronary artery disease,[33] reduce the risk of migraine, carotid dissection, and fibromuscular dysplasia,[34,35] and reduce the potential risk variants of hypertension.[36] We found rs9349379 to be significantly associated with PEH in Qinghai Tibetans. In the allele model, the rs9349379 associated with a 1.909-fold increased risk of PEH ($p = 0.015$, 95%CI = 1.128–3.232). In the genetic model, rs9349379 is associated with an increased risk of PEH in the Dominant model. Our results showed that rs9349379 increased the risk of PEH in Qinghai Tibetans, while Schiffrin[37] reported that rs9349379 may reduce the risk of hypertension. The protein interaction map shows the PHACTR1 gene co-expressed with ACTA1, ACTC1h, and PPP1CA (Figure 3) which are mainly involve in actomyosin structure organization, actin cytoskeleton organization, and the function of protein phosphatase 1 binding (Table S3). How the PHACTR1 affects PEH is not clear, but in annotation databases, it encodes as many as 54 different mRNAs or proteins. We presumed this gene affected PEH through different transcripts and proteins (Figure S3).

The rs567481079 is adjacent to the rs1371182 at a distance of 8 bp. They are all located on chromosome 2, upstream of the CYP2C56P gene and downstream of the PRPS1P1 gene. In the allele model, we found that rs1371182 associated with a 0.594-fold decreased risk of PEH ($p = 0.026$, 95% CI = 0.375–0.941), and rs567481079 associated with a 2.552-fold increased risk of PEH ($p = 0.015$, 95%

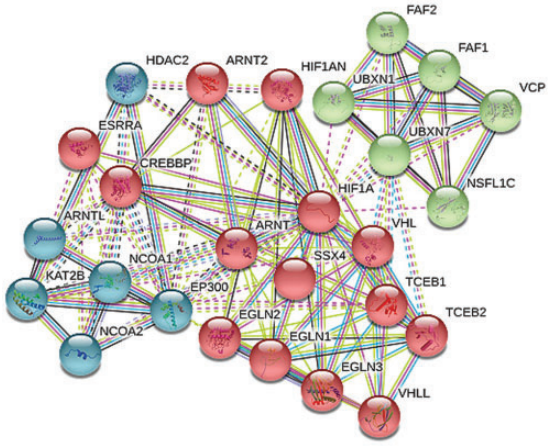

Figure 4. The protein interaction map of HIF1A gene co-expressed with ARNT, CREBBP, EGLN1, and other genes. (A color version of this figure is available in the online journal.)

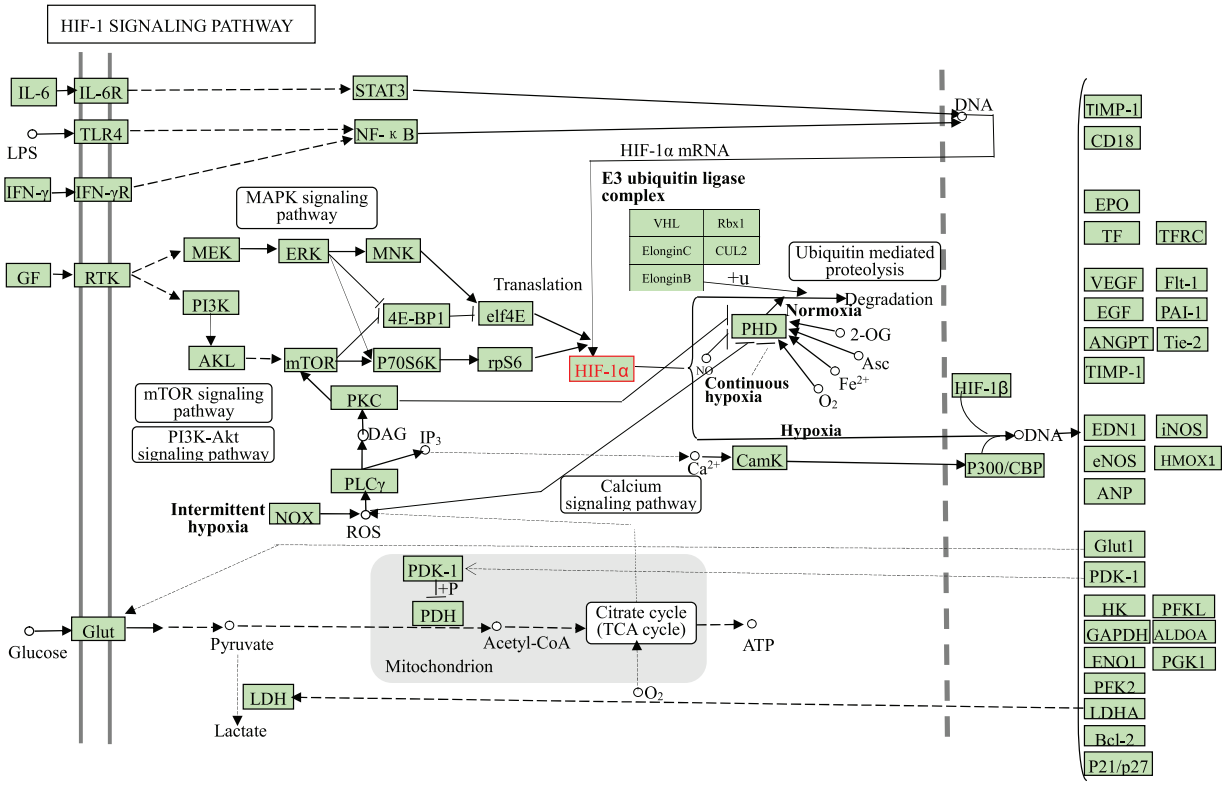

Figure 5. The metabolic pathway maps of the HIF-1 signaling. The figure shows the process of HIF-1 signaling pathway in regulating human oxygen homeostasis, in which HIF1A gene plays an important role. (A color version of this figure is available in the online journal.)

CI = 1.171–5.560). These two SNPs possibly influence blood pressure regulation by interfering with the normal expression of either upstream or downstream genes.

The chr14:61734822 is located in the intron region of hypoxia-inducible factor 1-alpha (HIF1A) (Figure S4). Our sequencing data revealed that there were multiple mutation loci around this locus (Figure S5). It suggests that the non-coding intron region composed of these loci might work together to affect the regulation of the HIF1A gene, which is a key link of blood pressure regulation. HIF1A encodes the α subunit of hypoxia-inducible factor-1 (HIF-1). Under hypoxic conditions, HIF1A can activate the transcription of more than 40 genes, including genes that increase oxygen delivery and promote hypoxia metabolic adaptation such as erythropoietin, glucose transporter, glycolytic enzyme, vascular endothelial growth factor, and other protein products. A recent study showed that HIF-1α gene polymorphism is associated with hypertensive left ventricular hypertrophy.[38] In this study, we found that the chr14:61734822 associated with a 0.375-fold decreased risk of PEH ($p = 0.045$, 95%CI = 0.140–1.005) from the allele model.

The interaction map of HIF1A is shown in Figure 4. HIF1A, together with ARNT, CREBBP, and EGLN1 are mainly involved in the regulation of vascular endothelial growth factor production, vasculature development, and other biological processes. These genes are also enriched in pathways such as renal cell carcinoma and HIF-1 signaling (Figure 5 and Table S4). HIF-1 target genes can encode proteins that increase O_2 transmission and mediate the adaptive response to O_2 deprivation. These signaling pathways could all affect blood pressure regulation.

Conclusions

In this study, we found that rs2493134, rs9349379, rs1371182, and rs567481079 were related to PEH risk in Qinghai Tibetan population. These SNPs are located near or in gene bodies of AGT, PHACTR1, CYP2C56P, and PRPS1P1. We also identified a novel PEH-related locus (chr14:61734822) in the intron of the HIF1A gene. Among these PEH-related genes, AGT and HIF1A play roles in RAS and HIF-1 signaling pathways, respectively, and thus link the two pathways to PEH. Some studies have indicated that hypertension is a metabolic disease. The two pathways were influenced by many genes' expression, which can in turn change metabolic elements such as enzymes, proteins, and organic molecules, and thus affect the regulation of BP. There are some limitations of this study. First, age in hypertensive and healthy samples was not strictly controlled. The mean age of the PEH group was 58.86 ± 12.14 years old, and that of the normal group was 36 ± 12.51 years old. Second, due to the difficulty of sample collection, the number of samples is small. We will continue collecting samples and verify our results. Also, public DNA sequencing data from other plateau regions will be analyzed and compared to our results to find the difference between populations. Last, to better understand the genetic mechanism of PEH, prospective studies taking pathway-related molecular quantification are needed for a deeper inspection, and basic biological experiments will be used to study the functions of target genes and SNP sites. Although with such limitations, this study has found new genetic features of PEH and provided a potential application of precision targeted diagnosis and therapy for hypertension in the future.

AUTHORS' CONTRIBUTIONS

ZL and XW conceived this study; JY, ZJ, CY, CS performed the recruitment and Investigation; ZL, XW, and CS performed the bioinformatics analysis; ZL, XW, and XH contributed in the writing of the report; ZL, XW, XH, LT, and JW contributed to the revision of the article; all the authors have reviewed and agreed to the final version of the article.

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.

ETHICAL APPROVAL

The study conforms to the ethical standards set by the Belmont Report: Ethical Principles and Guidelines for the Protection of Human Subjects of Research, and approved by the ethics committee of Xining First People's Hospital, and all the participants were well informed and signed the informed consent.

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SUPPLEMENTAL MATERIAL

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