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Original Article

Polymorphisms in ASTN2 and VHL are associated with risk of high altitude polycythemia in Han Chinese immigrants at Qinghai-Tibetan plateau

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Abstract: High altitude polycythemia (HAPC) is a common disease among Han Chinese immigrants at Qinghai-Tibetan plateau, while the genetic basis inside is poorly understood. A total of 481 male Han individuals (232 HAPC patients and 249 matched healthy controls) were enrolled in this study. Single nucleotide polymorphisms rs10983334, rs10983337, rs10983339, rs2119687, rs10733620 and rs10759856 in astrotactin 2 (ASTN2), rs457414 in the downstream region of the Von Hippel-Lindau (VHL), rs699 in angiotensinogen (AGT) and rs5186 in angiotensin II receptor type 1 (AGTR1) were genotyped by polymerase chain reaction-ligase detection reaction (PCR-LDR). The accuracy of PCR-LDR was confirmed by PCR products sequencing. HAPC patients showed lower pulse oxygen saturation than healthy controls. Significant differences were observed in genotype frequency or allele frequency between HAPC and healthy controls, including rs10983334, rs10983337, rs2119687, rs10733620 and rs10759856 in ASTN2 as well as rs457414 in the downstream region of VHL. C allele of rs10983334, T allele of 10983337, A allele of rs10759856, and G allele of rs457414 were protective factors, while T allele of rs10733620 and A allele of rs2119687 were risk factors of HAPC in Han Chinese population. Haplotypes of ASTN2 showed significant differences between HAPC and controls. We concluded that genetic susceptibility existed in Han Chinese immigrants at Qinghai-Tibetan plateau, and SNPs rs10983334, rs10983337, rs2119687, rs10733620 and rs10759856 in ASTN2 as well as SNP rs457414 in the downstream region of VHL were associated with HAPC risk in Han Chinese immigrants.

Keywords: High altitude polycythemia, single nucleotide polymorphism, susceptibility, ASTN2, VHL

Introduction

High altitude polycythemia (HAPC) is a common disease among Han Chinese immigrants at Qinghai-Tibetan plateau, where the average altitude is above 4000 meters. The diagnostic criteria of HAPC is based on hemoglobin (females: Hb≥190 g/L; males: Hb≥210 g/L) [1]. Although hypoxia is the main cause of HAPC, the pathogenesis inside is poorly understood, and the genetic basis is rarely investigated. Tibetans and Han Chinese immigrants showed significant difference in prevalence of HAPC. For Tibetans, the prevalence of HAPC was 1.21% [2], while in Han Chinese immigrants it was more than 24.0% [3]. Compared with Han Chinese immigrants from low-altitude areas, Tibetan showed more resistance to high altitude environment. Recently, genomics studies of Tibetans revealed that genetic factors play an important role in high altitude adaptation of Tibetans, and further analysis indicated that polymorphisms in Endothelial PAS domain protein 1 (EPAS1) and Egl nine homolog 1 (EGLN1) were associated with low Hb in Tibetans [4, 5]. Hence, we believed that genetic factors may also contribute to the development of HAPC in Han Chinese immigrants at Qinghai-Tibetan plateau.

The genetic basis of HAPC in Han Chinese immigrants were rarely reported [6-8]. Genes in the hypoxia inducible factor (HIF) pathway, including genes regulated HIF, genes interacted with HIF and target genes of HIF might function in the pathogenesis of HAPC. Hence, variations in these genes might be genetic basis of HAPC. In normoxia environment, the oxygen dependent degradation domain of HIF-1α is bound by Von Hippel-Lindau (VHL) protein, then this complex

is degraded by E3 ligase through polyubiquitinated procedure. This process is blocked in hypoxia environment, so HIF is able to activate the transcription of genes downstream [9]. Obviously, this progress is affected by the oxygen dependent degradation domain of HIF-1α and function of VHL protein. Chen reported that two mutations in the oxygen dependent degradation domain of HIF-1α showed no association with HAPC risk in Han Chinese immigrants [6], and mutations in VHL gene were associated with polycythemia under normoxia conditions [10-13]. Hence, it seems that VHL may function in the development of HAPC in Han Chinese immigrants.

Renin angiotensin aldosterone system (RAAS) functions as regulators of blood volume and pressure, which participate into high altitude adaption in Andeans, and genes belongs to RAAS were involved in high altitude acclimatization and high altitude diseases [14]. Although the I/I genotype of ACE gene was associated with higher oxygen saturation and lower Hb in Andeans [15], this polymorphism was not associated with risk of HAPC in Han Chinese immigrants, and the genotypes of ACE I/D polymorphism showed no correlation with Hb or oxygen saturation in HAPC patients or healthy controls [6]. The angiotensinogen (AGT) gene and angiotensin II receptor type 1 (AGTR1) gene are important components of RAAS. The T235M polymorphism (rs699) in AGT gene significantly affects AGT function, and the A1066C allele (rs5186) in the 3' untranslated regions of AGTR1 was associated with AGTR1 expression which finally would influence its effect [16]. Activation of AGTR1 promotes a series of signal transduction that mediate multiple processes including cell proliferation, pro-inflammatory reaction and immune responses that could contribute to the development of HAPC. Besides, the rs699 was reported to be associated with risk of HAPC in Tibetans [17], and rs5186 was involved in early inflammatory changes [18] as well as pulmonary arterial hypertension [19]. Therefore, both rs699 in AGT and rs5186 in AGTR1 were also investigated in this study.

Except for searching candidate genes based on literatures, we also explored the genetic basis of HAPC with small sample size in Han Chinese immigrants. Our preliminary experiment carried out by SNP array indicated that single nucleotide polymorphisms (SNPs) rs10983334, rs10983337, rs10983339, rs10733620, rs2119687 and rs10759856 in ASTN2, rs457414 in the downstream region of VHL were associated with the risk of HAPC in Han Chinese immigrants. To better understand the genetic basis of HAPC, we carried out a case-control study with a larger sample size to evaluate the association between polymorphisms including rs10983334, rs10983337, rs10983339, rs10733620, rs2119687 and rs10759856 in ASTN2, rs457414 in the downstream region of VHL, rs699 in AGT as well as rs5186 in AGTR1 and risk of HAPC in Han Chinese immigrants.

Materials and methods

Study population

Due to the poor conditions at high altitude, only a total of 481 young male Han Chinese subjects including 232 HAPC patients and 249 healthy controls were enrolled in this study. All subjects had lived at an altitude above 4000 m for at least 3 months. The incidence of HAPC in female was much lower than that in male, so only male individuals were recruited. Subjects with pulmonary diseases were excluded from this study according to the diagnostic criteria of HAPC. 5 ml of venous blood was collected for blood tests and DNA extraction. Hb concentration was measured by the cyanmethemoglobin determination method [8], and heart rate as well as SpO_2 (pulse oxygen saturation) were detected by pulse blood oxygen saturation monitor at resting state. Genomic DNA was extracted using a blood DNA isolation kit (Omega Bio-Tek, Inc., Norcross, GA, USA). The quality and concentration of DNA were determined by NanoDrop-1000 spectrophotometer (Isogen Life Science, IJsselstein, Netherlands) and the DNA were stored in -20°C for further use. All subjects signed informed consent forms before this study was carried out. The research procedure was approved by the ethics committee of General Hospital of Lanzhou Military Region.

Genotyping

The characteristics of target SNPs are listed in **Table 1**. Genotyping of theses polymorphisms were based on polymerase chain reaction-ligase detection reaction (PCR-LDR) [20, 21]. PCR was performed in 20 μL reactions. The

Table 1. Information of genotyped SNPs

SNP No	NCBI rs	Gene	Chromosome position (hg38)	Location	Base change	MAF$^{\Delta}$
1	rs10983334	ASTN2	chr9: 116741463	Intron 12	A/C	0.215
2	rs10983337	ASTN2	chr9: 116755225	Intron 12	A/T	0.474
3	rs10983339	ASTN2	chr9: 116759977	Intron 12	A/G	0.241
4	rs10733620	ASTN2	chr9: 116762213	Intron 12	C/T	0.489
5	rs2119687	ASTN2	chr9: 116764342	Intron 12	A/G	0.496
6	rs10759856	ASTN2	chr9: 116766649	Intron 12	A/G	0.474
7	rs457414	VHL	chr3: 10161200	3' intergenic region to VHL	A/C	0.223
8	rs699	AGT	chr1: 230710048	Exon 2	C/T	0.208
9	rs5168	AGTR1	chr19: 44945298	3'UTR	A/T	NA

Δ: Minor Allele Frequencies (MAF) of CHB in HapMap release #28 data (http://hapmap.ncbi.nlm.nih.gov/cgi-perl/gbrowse/hapmap28_B36/; HapMap Data Rel 28 Phase II+III, August 10, on NCBI B36 assembly, dbSNP b126). Position of target SNPs were acquired from UCSC genome browser (Dec, 2013, GRch38/hg38) (http://genome.ucsc.edu/cgi-bin/hgGateway).

Table 2. Basic information of HAPC patients and healthy controls

Individual characteristics		HAPC (n = 232) (mean ± SD)	Controls (n = 249) (mean ± SD)	*p* value
Hb (g/L)		224.2±12.1	181.2±9.0	<0.001
Age (years)		25.1±5.6	25.5±5.8	0.60
SpO_2 (%)		87.1±3.3	89.7±3.0	<0.001
Heart rate (bpm)		86.2±14.1	83.1±12.8	0.003
Smoking status	Yes	121	124	0.61
	No	111	125	
Drinking status	Yes	22	35	0.12
	No	210	214	

reaction mixture contained 1 μL of DNA (50 ng), 2 μL of 1 × buffer, 0.6 μL of Mg^{2+} (3 mM), 2 μL of dNTPs (200 μM each), 0.2 μL of Taq (5 U/μL), 2 μL of primer mix (0.5 pM) and 12.2 μL of sterilized deionized distilled water (ddH_2O). The PCR was performed on a PTC-200 DNA Engine thermal cycler (Bio-Rad, Hercules, CA, USA) with a protocol of 95°C for 2 minutes; 40 cycles of 30 seconds at 94°C, 90 seconds at 56°C and 30 seconds at 65°C, and a final hold at 65°C for 10 minutes.

The PCR products were detected by agrose gel electrophoresis. A multiple LDR was performed after confirmation of the PCR products. The LDR was carried out in 10 μL reactions, and the mixture contained 1 μL of 1 × buffer, 0.05 μL of Taq DNA ligase (2 U/μL), 1 μL of probe mix (0.2 pmol/μL), 4 μL of PCR products and 4 μL of ddH_2O. The LDR was performed on a PTC-200 DNA Engine thermal cycler with a protocol of 95°C for 2 minutes; 40 cycles of 15 seconds at 94°C, 25 seconds at 50°C. LDR products were analyzed in PRISM 3730 (ABI, USA) and genotypes were identified by Genemapper in ABI system. Genotyping were performed again if the process was failed or genotypes were unclear.

DNA sequencing

After genotyping, 49 subjects (10% of all samples) were sequenced to assure the accuracy of the PCR-LDR analysis. Sequenced samples included each genotype were randomly selected from both HAPC patients and healthy controls.

Statistical analysis

Statistical analysis was applied by SPSS version 18.0 software (SPSS Inc, Chicago, IL). Results were shown as mean ± S.D. Means between HAPC patients and healthy controls were analyzed by independent sample test. Hardy-Weinberg equilibrium (HWE) in healthy controls, smoking and drinking status distribution, genotypes and alleles distribution between HAPC patients and healthy controls were analyzed by the chi-square test. The odds ratio (OR), 95% confidence interval and *p* values were calculated to confirm the power of gene-disease association. Haplotype analysis was performed using SHEsis online software (http://analysis.bio-x.cn) [22]. The significant level was set at $p<0.05$ and all *p* values were two-tailed. All results with *p* value below 0.05 were reported without corrections for multiple comparisons [23].

Table 3. Genotypes distribution between HAPC patients and healthy controls

Gene	Polymorphisms	Genotypes	HAPC patients		Controls		OR (95% CI)	χ^2	*p* value
			n	Frequency (%)	n	Frequency (%)			
ASTN2	rs10983334	A/A	23	9.9	13	5.2	1.0 (ref)	7.10	0.029▲
		A/C	102	44.0	95	38.2	0.61 (0.29-1.27)		
		C/C	107	46.1	141	56.6	0.43 (0.21-0.89)		
	rs10983337	A/A	86	37.1	67	26.9	1.0 (ref)	6.84	0.033▲
		A/T	102	44.0	117	47.0	0.68 (0.45-1.03)		
		T/T	44	18.9	65	26.1	0.53 (0.32-0.87)		
	rs10983339	G/G	24	10.3	25	10.0	1.0 (ref)	4.76	0.093
		A/G	110	47.4	95	38.2	1.21 (0.65-2.25)		
		A/A	98	42.3	129	51.8	0.79 (0.43-1.47)		
	rs10733620	C/C	47	20.3	66	26.5	1.0 (ref)	8.77	0.012▲
		C/T	101	43.5	123	49.4	1.15 (0.73-1.82)		
		T/T	84	36.2	60	24.1	1.97 (1.19-3.24)		
	rs2119687	G/G	46	19.8	68	27.3	1.0 (ref)	5.62	0.06
		A/G	104	44.8	114	45.8	1.35 (0.85-2.13)		
		A/A	82	35.4	67	26.9	1.81 (1.10-3.00)		
	rs10759856	G/G	85	36.6	68	27.3	1.0 (ref)	6.36	0.04▲
		A/G	103	44.4	115	46.2	0.72 (0.47-1.09)		
		A/A	44	19.0	66	26.5	0.53 (0.32-0.88)		
VHL	rs457414	T/T	147	63.4	130	52.2	1.00 (ref)	6.87	0.03▲
		G/T	77	33.2	103	41.4	0.66 (0.45-0.97)		
		G/G	8	3.4	16	6.4	0.44 (0.18-1.08)		
AGT	rs699	T/T	16	6.9	11	4.4	1.00 (ref)	1.90	0.38
		C/T	65	28.0	79	31.7	0.57 (0.25-1.30)		
		C/C	151	65.1	159	63.9	0.65 (0.29-1.45)		
AGTR1	rs5186	A/A	209	90.1	225	90.4	1.00 (ref)	0.002	0.96
		A/C	23	9.9	24	9.6	0.99 (0.54-1.79)		

▲: $p<0.05$ compared with healthy controls.

Results

Study population

Basic information of HAPC patients and controls are shown in **Table 2**. The SpO_2 of HAPC patients were significantly lower than that in healthy controls, indicating that the HAPC patients were in terrible hypoxemia. Besides, no differences were observed in the distribution of smoking and drinking status in HAPC patients or healthy controls. This result was in accordance with former reports [6-8]. It seemed that Hb in HAPC patients may not be affected by smoking or drinking status.

Genotyping and DNA sequencing

According to the results of genemapper, genotypes of target SNPs were easily distinguished. Genotypes of 49 sequenced subjects were consistent with results of genotyping by PCR-LDR, suggesting the sensitivity and specificity of the PCR-LDR analysis in this study were both 100%. Therefore, genotyping results of all SNPs could be taken into further analysis.

HWE test

HWE tests of genotype distributions in healthy controls are listed below: rs10983334 (0.558), rs10983337 (0.342), rs10983339 (0.232), rs10733620 (0.856), rs2119687 (0.183) and rs10759856 (0.229) in ASTN2, rs457414 (0.461) in VHL, rs699 (0.766) in AGT and rs5186 (0.505) in AGTR1. Hence, the genotypic distribution of target SNPs in healthy controls were in accordance with HWE, and genotypic data of target SNPs can be taken into further analysis.

Table 4. Alleles distribution between HAPC patients and healthy controls

Gene	Polymorphisms	Alleles	HAPC patients		Controls		OR (95% CI)	χ^2	*p* value
			n	Frequency (%)	n	Frequency (%)			
ASTN2	rs10983334	A	148	31.9	121	24.3	1.0 (ref)	6.89	0.009Δ
		C	316	68.1	377	75.7	0.69 (0.52-0.91)		
	rs10983337	A	274	59.1	251	50.4	1.0 (ref)	7.25	0.007Δ
		T	190	40.9	247	49.6	0.71 (0.55-0.91)		
	rs10983339	G	158	34.1	145	29.1	1.0 (ref)	2.71	0.10
		A	306	65.9	353	70.9	0.80 (0.61-1.05)		
	rs10733620	C	195	42.0	255	51.2	1.0 (ref)	8.13	0.004Δ
		T	269	58.0	243	48.8	1.45 (1.12-1.89)		
	rs2119687	G	196	42.2	250	50.2	1.0 (ref)	6.12	0.013Δ
		A	268	57.8	248	49.8	1.38 (1.07-1.78)		
	rs10759856	G	273	58.8	251	50.4	1.0 (ref)	6.89	0.009Δ
		A	191	41.2	247	49.6	0.71 (0.55-0.92)		
VHL	rs457414	T	371	80.0	363	72.9	1.00 (ref)	6.63	0.01Δ
		G	93	20.0	135	27.1	0.67 (0.50-0.91)		
AGT	rs699	T	97	20.9	101	20.3	1.00 (ref)	0.06	0.81
		C	367	79.1	397	89.7	0.96 (0.70-1.32)		
AGTR1	rs5186	A	441	95.0	474	95.2	1.00 (ref)	0.01	0.92
		C	23	5.0	24	4.8	1.03 (0.57-1.85)		

Δ: p<0.05 compared with controls.

Genetic association analysis

The number and frequencies of genotypes and alleles are presented in **Tables 3** and **4**, respectively. Compared with healthy controls, genotypic distribution analysis showed that rs10983334, rs10983337, rs10733620 and rs10759856 in ASTN2 as well as rs457416 in VHL were associated with the risk of HAPC in Han Chinese immigrants. Allelic distribution analysis indicated that C allele of rs10983334, T allele of rs10983337, A allele of rs10759856 and G allele of rs457414 were protective factors of HAPC, whereas T allele of rs10733620 and A allele of rs2119687 were risk factors of HAPC in Han Chinese immigrants. Genotypes and alleles of rs10983339, rs699 and rs5186 showed no significant difference in frequency distribution between HAPC patients and healthy controls, indicating three SNPs mentioned above were not associated with the risk of HAPC in Han Chinese immigrants in this study.

ASTN2 haplotype analysis

Haplotypes were constructed by SHEsis online software based on the genotypic data of ASTN2 acquired in this study. Unsuccessfully genotyped subjects were excluded, and frequencies of haplotypes below 0.03 were not taken into further analysis as the website indicated. The SNP order of haplotype was rs10983334, rs10983337, rs10983339, rs10733620, followed by rs2119687 and rs10759856. Four haplotypes including A-A-G-T-A-G, C-A-A-T-A-G, C-A-G-T-A-G and C-T-A-C-G-A were constructed. Frequency of the A-A-G-T-A-G haplotype was significantly higher in the HAPC patients than that in the healthy control (OR = 1.471, 95% CI = 1.084-1.996; p = 0.013), the frequency of the C-T-A-C-G-A haplotype was significantly lower in the HAPC patients than that in the healthy control (OR = 0.674, 95% CI = 0.516-0.879; p = 0.003), whereas the haplotypes frequency of C-A-A-T-A-G and C-A-G-T-A-G showed no significant difference between HAPC patients and the healthy control (**Table 5**).

Discussion

The Qinghai-Tibet railway has been in service since 2006, and a great number of Han Chinese individuals immigrated to Qinghai-Tibetan plateau from low-altitude regions year by year. These Han Chinese immigrants are in great danger of developing HAPC. Up to now, the most effective treatment of HAPC is getting

Table 5. Haplotype analysis for ASTN2 in HAPC patients and healthy controls

	HAPC [n (%)]	Control [n (%)]	χ^2	*p* value	Odds Ratio [95% CI]
A-A-G-T-A-G	124.46 (27.7)	99.24 (20.4)	6.183	0.013$^{\Delta}$	1.471 [1.084~1.996]
C-A-A-T-A-G	105.96 (23.5)	98.69 (20.3)	1.174	0.279	1.189 [0.869~1.625]
C-A-G-T-A-G	19.38 (4.3)	23.25 (4.8)	0.162	0.688	0.881 [0.475~1.634]
C-T-A-C-G-A	177.64 (39.5)	233.44 (48.0)	8.484	0.003★	0.674 [0.516~0.879]

$^{\Delta}$Comparison of A-A-G-T-A-G haplotype vs. others in HAPC and controls; ★Comparison of C-T-A-C-G-A haplotype vs. others in HAPC and controls.

back to low-altitude areas, while this therapy could not be realized for many reasons. Hence, it is reasonable to screen subjects who show great resistance to high altitude environment before they get into high-altitude areas. In this study, rs10983334, rs10983337, rs10733620, rs2119687 and rs10759856 in ASTN2 as well as rs457414 in the downstream region of VHL were found to be associated with HAPC risk in Han Chinese immigrants. However, rs1098339 in ASTN2, rs699 in AGT and rs5186 in AGTR1 did not show such an association. So far this is the first time that all of SNPs in this study were investigated to evaluate their association with HAPC risk in Han Chinese immigrants, and this is the first report of associations between polymorphisms of rs10983334, rs10983337, rs10733620, rs2119687 and rs10759856 as well as rs457414 and HAPC risk in Han Chinese immigrants at Qinghai-Tibetan plateau.

The ASTN2 gene is highly expressed in brain, and protein encoded by this gene mainly functions in neuron migration by mediating formation of neuron and neuronal-glial adhesion release [24]. Genetic variations in ASTN2 were found to be associated with multiple neural disorders, such as attention-deficit/hyperactivity disorder [25], autism spectrum disorder [26], Alzheimer's disease [27] and schizophrenia as well as bipolar disorder [28]. Our results indicated that polymorphisms of ASTN2 were associated with risk of HAPC in Han Chinese immigrants. This result is unexpected, because ASTN2 is highly expressed in brain and may not function in erythropoiesis. More samples should be taken to confirm this association in further studies, and genes around ASTN2 could be also evaluated.

The VHL gene covers 12 kb regions, which is located in chromosome 3 from 3p25 to 3p26 with 3 exons [29]. Normal function of VHL is crucial to maintain the HIF signaling pathway in homeostasis, and dysfunction of HIF signaling pathway at high altitude environment may contribute to the development of HAPC. Because SNP rs457414 is located in the downstream region of VHL gene, so it is difficult to define the function caused by allele's change. However, we could get some clues from genetic studies of high altitude adaptation in Tibetans. SNP rs1868092 as well as other SNPs were located in 3' downstream region of EPAS1 gene, and these polymorphisms were found to be associated with low Hb in Tibetans [5]. Therefore we presume that this SNP might influence the VHL responsiveness with epigenetic mechanisms at hypoxia environment, because mutations in exons of VHL would induce polycythemia in normoxia environment, and all individuals included in this study were healthy before they went to high altitude. Perhaps variations in VHL gene show linkage disequilibrium with rs457414 would explain the mechanisms inside, and variations of VHL gene should be investigated in further analysis.

Variations of AGT and AGTR1 were seldom investigated in high altitude diseases. The 235 M allele of rs699 was a risk factor of acute mountain sickness in Han Chinese population and HAPC in Tibetans, while rs5186 was not [17]. In this study, rs699 and rs5186 showed no association with HAPC risk in Han Chinese immigrants. So far, ACE I/D polymorphism, AGT rs699 and AGTR1 rs5186 in RAAS were not associated with HAPC risk in Han Chinese immigrants. Although ACE I/D polymorphism, rs699 and rs5186 would influence ACE, AGT and AGTR1 function respectively, it seems that function changes caused by these polymorphisms were not enough to contribute to the development of HAPC in Han Chinese immigrants. More genes belong to RAAS including ACE-2, CYP11B2 and other variations in ACE, AGT and AGTR1 should be investigated in further analysis. Even though these variations alone may not be associated with HAPC, gene-

gene interaction analysis would provide more clues to explore the role of RAAS in pathogenesis of HAPC in Han Chinese immigrants.

Our sample size is relatively small compared with former HAPC genetic susceptibility studies in Han Chinese population, and function analysis of positive polymorphisms was not performed. Besides, expression levels and protein concentration of genes involved in HIF signaling pathway were not measured, such as VHL, HIF-1α and HIF-2α, etc.

In summary, this is the first study to investigate the association between polymorphisms of rs10983334, rs10983337, rs10983339, rs10733620, rs2119687 and rs10759856 in ASTN2, rs457414 in the downstream region of VHL, rs699 in AGT as well as rs5186 in AGTR1 and HAPC risk in Han Chinese immigrants at Qinghai-Tibetan plateau. Polymorphisms of rs10983334, rs10983337, rs10733620, rs2119687 and rs10759856 in ASTN2 as well as rs457414 in the downstream region of VHL were associated with risk of HAPC. The C allele of rs10983334, T allele of rs10983337, A allele of rs10759856, G allele of rs457414 and the C-T-A-C-G-A haplotype of ASTN2 were protective factors, while T allele of rs10733620, A allele of rs2119687 and the A-A-G-T-A-G of ASTN2 haplotype were risk factors. Further studies should take larger samples to confirm the association and to investigate correlation between the variations of VHL and risk of HAPC.

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Disclosure of conflict of interest

None.

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