RESEARCH ARTICLE

Association of Hypoxia-Inducible Factor-2 Alpha Gene Polymorphisms with the Risk of Hepatitis B Virus-Related Liver Disease in Guangxi Chinese: A Case-Control Study

Liling Huang[1,2☯], Cunxu Liu[3☯], Yan Deng[1], Yanqiong Liu[1], Jiangyang Zhao[1], Xiuli Huang[1], Wenjun Tang[1], Yifan Sun[4], Xue Qin[1]*, Shan Li[1]*

1 Department of Clinical Laboratory, First Affiliated Hospital of Guangxi Medical University, Nanning, Guangxi, China, 2 Department of Clinical Laboratory, Longtan Hospital of Guangxi Zhuang Autonomous Region, Liuzhou, Guangxi, China, 3 Department of Infectious Disease, Longtan Hospital of Guangxi Zhuang Autonomous Region, Liuzhou, Guangxi, China, 4 Department of Clinical Laboratory, Third Affiliated Hospital of Guangxi University of Chinese Medicine, Liuzhou, Guangxi, China

☯ These authors contributed equally to this work.
* lis8858@126.com (SL); qinxue919@126.com (XQ)

Competing Interests: The authors have declared that no competing interests exist.

Abstract

Objective

Hypoxia-inducible factor-2 alpha (HIF-2a) plays a major role in the progression of disease, although the role of HIF-2α gene polymorphisms in hepatitis B virus (HBV)-related diseases remains elusive. The aim of this study is to determine whether HIF-2a rs13419896 and rs6715787 single-nucleotide polymorphisms (SNPs) are associated with susceptibility to chronic hepatitis B (CHB), liver cirrhosis (LC), or hepatocellular carcinoma (HCC).

Method

A case-control study of 107 patients with CHB, 83 patients with LC, 234 patients with HCC, and 224 healthy control subjects was carried out, and the HIF-2a rs13419896 and rs6715787 SNPs were genotyped by polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP).

Results

No significant differences were observed in the genotype or allele frequency of two HIF-2a SNPs between the cases and controls (all $p > 0.05$). However, in subgroup analysis by gender, the HIF-2a rs13419896 GA and AA genotypes were significantly associated with a risk of CHB (odds ratio [OR] = 3.565, 95% confidence interval [CI] = 1.123–11.314, $p = 0.031$ and OR = 12.506, 95% CI = 1.329–117.716, $p = 0.027$) in females, and the A allele of rs13419896 was associated with a risk of CHB (OR = 2.624, 95% CI = 1.244–5.537, $p = 0.011$) and LC (OR = 2.351, 95% CI = 1.002–5.518, $p = 0.050$) in females. The rs6715787 CG genotype polymorphism may contribute to a reduced risk of LC in the Guangxi Zhuang

Chinese population (OR = 0.152, 95% CI = 0.028–0.807, p = 0.027), as determined via subgroup analysis by ethnicity. Moreover, binary logistic regression analyses that were adjusted by drinking status indicated that the AA genotype of rs13419896 may contribute to an increased risk of LC in the non-alcohol-drinking population (OR = 3.124, 95% CI = 1.091–8.947, p = 0.034). In haplotype analysis, GG haplotype was significantly associated with a reduced risk of LC (OR = 0.601, 95% CI = 0.419–0.862, p = 0.005).

Conclusions

The HIF-2a rs13419896 polymorphism is associated with an increased risk of CHB and LC in the Guangxi Chinese population, especially in females and in the non-alcohol-drinking population, while the HIF-2a gene rs6715787 polymorphism is associated with a decreased risk of LC in the Guangxi Zhuang population.

Introduction

Chronic hepatitis B virus (HBV) infection is a major public health problem throughout the world, particularly in sub-Saharan Africa and in most of Asia [1]. Chronic HBV infection can lead to chronic hepatitis B (CHB) and liver cirrhosis(LC); moreover, it may eventually increase susceptibility to hepatocellular carcinoma (HCC) [2]. CHB, LC, and HCC are three progressive stages of acute and chronic HBV infection, which represent a complex biological process where the molecular and cellular mechanisms of pathogenesis remain unknown [3]. HCC is a multifactorial malignant tumor involving a complex interplay between the genetic and environmental. It has been revealed that many risk factors affecting HCC, such as HBV infection, hepatitis C virus (HCV) infection, aflatoxin exposure, smoking, alcohol drinking, and non-alcoholic fatty liver disease (NAFLD) [4, 5], which are mainly external factors. The internal factors mainly include immunity, inflammation, and host genetic susceptibility (e.g., single nucleotide polymorphisms [SNPs]). Although genetic susceptibility to HCC is relatively weak, it has also been found to be associated with many gene polymorphisms in the progression of HCC [6–11]. For instance, hypoxia-inducible factor (HIF)-2a gene polymorphism may play a major role.

Tian et al. discovered HIF-2a in endothelial cells in 1997; it is also called endothelial PASprotein-1 (EPAS1), HIF-like factor (HLF), and HIF-related factor (HRF) [12]. It is located on chromosome 2p21-p16, as a heterodimer transcription factor with a composition of "a" and "β" subunits. The "a" subunit was sensitive to oxygen, which has a unique oxygen-dependent degradation domain (ODDD) and determines the biological activities of HIF; this can be activated by a lack of oxygen, inflammatory cytokines, and tumor mutation. HIF was first identified for its role in erythropoietin regulation [13], but it was later discovered to also regulate genes involved in glycolysis, angiogenesis, cell differentiation, apoptosis, and other cellular pathways [14]. Three kinds of HIF family subtypes have been identified: namely HIF-l, HIF-2, and HIF-3. Among these, HIF-1 and HIF-2 have been considered to be the most important regulators of cellular responses to hypoxia [15]. Hypoxia and HIF-a can promote dedifferentiation in a variety of cells, including cancer cells [16, 17]. Studies have confirmed the relationship between hypoxia and tumor invasiveness [18], which has complex mechanics that may involve cytotoxic resistance, a decline in deoxyribonucleic acid (DNA) repair ability, insensitivity to radiation, the promotion of blood vessel formation, and the increase of metastasis potential

[4, 5, 19]. It was speculated for a long time that HIFs are associated with tumor angiogenesis, and this relationship became clear after the discovery of hypoxia promotion via the expression of vascular endothelial growth factor (VEGF) [20, 21]. Hohnquist-Mengelbier et al. [22] suggested that HIF-1 adjusts the expression of VEGF in the acute stage of oxygen restriction, while it is mainly regulated by HIF-2 in the process of long-term anoxia. HIF-1a and HIF-2a amino acids are 48% homologous, and both identify and combine the same DNA, but they regulate different downstream genes. HIF-la has been widely studied in clinical cancer cases. Initially, it was considered to be associated with tumor invasion, while HIF-2a overexpression has been demonstrated to be associated with tumor metastasis and poor prognosis for the past few years [23, 24]. It is probable that HIF-2a can serve as an effective biomarker for the early diagnosis and prognosis of tumors. Furthermore, Hepatitis B viral X protein (HBx)—a key protein of the HBV genome—has been identified as enhancing the stability of the HIF-2a protein through binding to the von Hippel-Lindau protein (pVHL) and activating the NF-κB signaling pathway. This leads to an overexpression of HIF-2 target genes [25], which may play an important role in hepatocarcinogenesis. However, few studies have investigated the correlations between HIF-2a gene polymorphisms and HBV-related disease.

Genetic polymorphisms are considered the main genetic elements involved in the development of various kinds of diseases [26]. To date, several studies have reported associations between HIF-2a gene polymorphisms and renal cell carcinoma [27], prostate cancer [28], lungadenocarcinoma [29], non-small-cell lung cancer [30], acute mountainsickness [31], and maximum metabolic performance in elite endurance athletes [32]. However, HIF-2a gene polymorphisms' involvement in HBV-related diseases remains elusive. Moreover, the latest study by Yanqiong Liu et al. [33] estimated the association between HIF-1a rs11549465 and rs11549467 gene polymorphisms and the risk of HBV-related HCC. They found the CG haplotype to be associated with a significantly increased susceptibility to HCC, whereas the CA haplotype was found to be associated with a significantly decreased risk of HCC in a Chinese population. We hypothesized that the HIF-2a gene polymorphisms also influence one's susceptibility to HBV-related disease in a Chinese population. In the present study, we selected two HIF-2a gene tag SNPs (rs13419896 and rs6715787), referring to previous research, which interrogated the NT-022184.14 sequence of the NCBI database with Hapview software, and we conducted a case-control study to explore the associations between the two SNP polymorphisms and HBV-related disease in a Chinese population.

Materials and Methods

Study subjects

A total of 648 subjects were recruited in this case-control study, including 107 patients with CHB, 83 patients with HBV-related LC, 234 patients with HBV-related HCC, and 224 healthy controls. All subjects were recruited at the First Affiliated Hospital of Guangxi Medical University from 1 June to 1 October 2014. The patients in the case group had tested positive for the hepatitis B surface antigen (HBsAg). Patients were included in the CHB group and LC group following the prevention guide of CHB diagnostic criteria (2010, China), while they were included in the HCC group following the standard of hepatocellular carcinoma diagnosis and treatment criteria (2011, China). To control for the effects of potential confounders, healthy participants were individually matched to the cases based on gender and age (±5 years).

The patients infected with other hepatitis viruses, such as hepatitis C or hepatitis E, as well as those with liver diseases caused by non-viral infection, were excluded. None of the subjects had received treatment (antivirals, surgery, radiation, or chemotherapy) before seeing a doctor.

The study protocol was approved by the Ethics Committee of the First Affiliated Hospital, Guangxi Medical University (S1 File), and written informed consent was obtained from all participants after a full explanation of the study.

DNA extraction and SNP genotyping

All operations were carried out in strict accordance with the standard operating procedure. Genomic DNA was extracted from venous blood using the standard phenol-chloroform method. The genotyping of HIF-2a rs13419896 and rs6715787 SNPs was performed via polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP). Polymerase chain reaction (PCR) was used to amplify the target DNA, with the 5'−TTCCCTGTTCCCTCCTCCTTT−3' forward primer, 5'−TCCTACCCTGTGGTTGCCTCG−3' reverse primer of rs13419896 and 5'−TCAGTTGAGCATCCCTAACCC−3' forward primer, 5'−CCAGCCCTTGTCAGCATCTTT−3' reverse primer of rs6715787. Primer sequences and reaction conditions for the genotyping of HIF-2a polymorphisms are shown in Table 1. The PCR amplification was carried out in a final volume of 25μL of a reaction mixture, consisting of 2 μL of genomic DNA (60 ng/μL), 1 μL of forward primer (10 μmol/L), 1 μL of reverse primer (10 μmol/L), 12.5 μL of DreamTaq Green PCR Master Mix (Thermo Fisher Scientific), and 8.5 μL of nuclease-free deionized water using a Perkin-Elmer thermocycler (2700, Applied Biosystems, Foster City, CA, USA). PCR amplification products of HIF-2a genes rs13419896 and rs6715787 were digested with HaeII restriction enzymes and ECORI restriction enzymes, respectively. Their enzyme-digested products were separated by 3% agarose gel electrophoresis and visualized with an ultraviolet (UV) transilluminator. Enzyme-digested products of rs13419896 included three digested fragments of 373bp, 306bp, and 67bp (Fig 1A), and those of rs6715787 included three digested fragments of 358bp, 263bp, and 95bp (Fig 1B). To confirm the accuracy of genotyping, about 10% of specimens were selected at random for direct DNA sequencing by the Sangon Biotech Company (Shanghai, China). The results of DNA sequencing and PCR–RFLP were 100% concordant.

Statistical analysis

The distribution of the general demographic and clinical features between cases and controls was evaluated using the one-way analysis of variance test and the $\chi 2$ test for continuous and categorical variables, respectively. Hardy–Weinberg equilibrium (HWE) was employed with a goodness-of-fit χ^2 test to verify the crowd representativeness of the study subjects. The χ^2 test and Fisher exact test were used to analyze the distribution of the genotype and allele frequencies among the cases and controls. The independent risk and protective genotypes and alleles were selected by binary logistic regression analyses adjusting for age, gender, smoking status, drinking status, ethnicity, and body mass index (BMI). Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated to assess the relative risk. SHEsis online software [34] was applied for the haplotype analysis of HIF-2a gene polymorphism (http://analysis.bio-x.cn/myAnalysis.php).

Table 1. Primer sequence and the reaction condition for genotyping HIF-2a polymorphisms.

SNPs	Primer sequence (5'-3')	Annealing temperature	Restriction enzyme	Product size (bp)
HIF-2a	Forward:TTCCCTGTTCCCTCCTCCTTT	60°C 5min	HaeII	GG:306+67
rs13419896	Reverse:TCCTACCCTGTGGTTGCCTCG		37°C for 15min	GA:373+306+67
				AA:373
HIF-2a	Forward:TCAGTTGAGCATCCCTAACCC	60°C 5min	ECORI	CC:263+95
rs6715787	Reverse:CCAGCCCTTGTCAGCATCTTT		37°C for 3h	CG:358+263+95
				GG:358

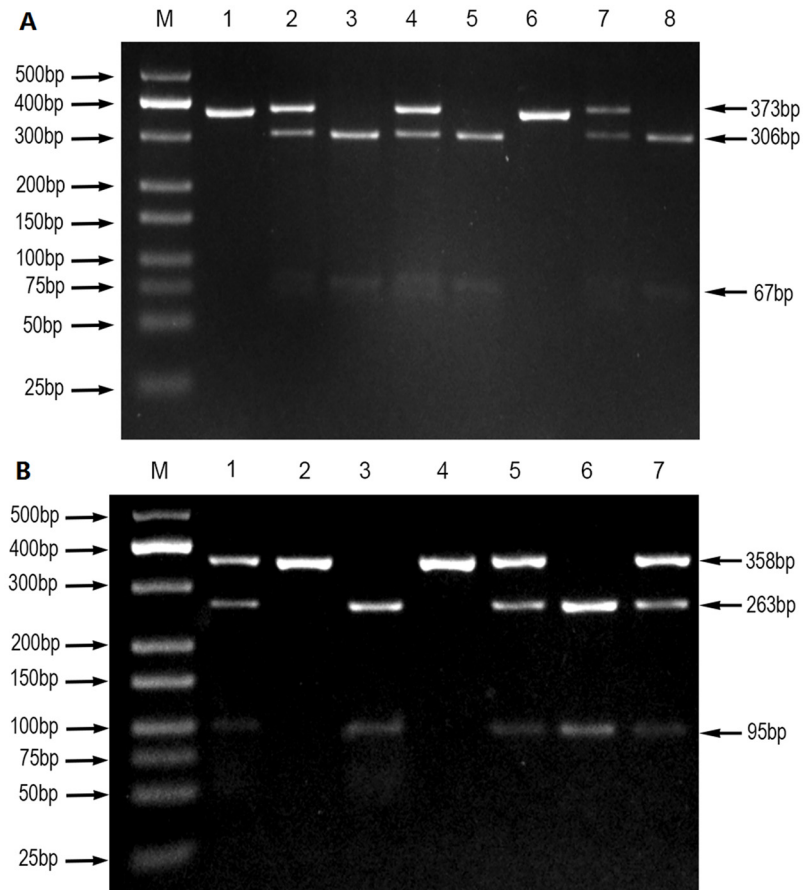doi:10.1371/journal.pone.0158241.t001

Fig 1. PCR-RFLP assay for analyzing the rs13419896 and rs6715787 polymorphisms of the HIF-2a gene. (A) rs13419896- lane M shows DNA marker; lanes 1 and 6 show AA genotype; lanes 2, 4, and 7 show GA genotype; lanes 3, 5, and 8 show GG genotype. (B) rs6715787- lane M shows DNA marker; lanes 2 and 4 show GG genotype; lanes 3 and 6 show CC genotype; lanes 1, 5, and 7 show CG genotype.

doi:10.1371/journal.pone.0158241.g001

All of the statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS, version13.0). Statistical significance was assumed at two-sided values of $p < 0.05$.

Results

Demographic characteristics of the study population

A total of 648 participants (224 controls and 424 cases) were recruited in this case-control study. The demographic characteristics of the study are shown in Table 2 and S2 File. We carried out comparative analysis of the four groups using the following data: age, gender, nationality, BMI, smoking, and drinking. The data in Table 2 show that the male and female sex ratios in the CHB, LC, and HCC groups were 3.1:1, 4.2:1, and 11.3:1, respectively. These results are consistent with those of a previous report [35], which indicated that the sex ratio in liver cancer roughly fluctuated between 1.4 and3.3, and the higher the incidence of liver cancer, the greater the sex ratio. Our data verify that the Guangxi region of China is an endemic area for HCC. The difference in HCC sex ratios are considered to relate to the hormones and living habits of males and females. Another previous report suggested that the onset age shows a sharp increase after age 20 and peaks at 50 years old in areas characterized by a high incidence of liver cancer in southeast Asia and on the west coast of Africa [36]. The average ages in the control, CHB,

Table 2. Demographic characteristics of the study population.

Variables	Controls	CHB	p	LC	p	HCC	p
Total number	224	107		83		234	
Gender							
Male	125	81	<0.001	67	<0.001	215	<0.001
Female	99	26		16		19	
Ethnicity							
Han	95	60	0.071	47	0.087	147	<0.001
Zhuang	116	43		33		83	
Others	13	4		3		4	
Tobacco smoking							
Yes	72	44	0.112	42	0.003	80	0.692
No	152	63		41		154	
Alcohol drinking							
Yes	63	54	<0.001	31	0.127	79	0.225
No	161	53		52		155	
Age(mean±SD)	46.71±7.05	37.56±11.58	<0.001	49.30±12.07	0.068	48.45±11.03	0.044
BMI(mean±SD)	22.44±3.49	22.08±3.58	0.382	22.83±5.04	0.513	22.01±3.60	0.194

CHB, chronic hepatitis B; LC, liver cirrhosis; HCC, hepatocellular carcinoma; SD, standard deviation; BMI = body mass index.

doi:10.1371/journal.pone.0158241.t002

LC, and HCC groups were 46.71±7.05, 37.56±11.58, 49.30±12.07, and 48.45±11.03 years, respectively, in our study, which is also consistent with previous reports.

Association analysis of HIF-2a gene polymorphisms

The genotype and allele frequencies of the HIF-2a gene rs13419896 and rs6715787 SNPs in the control and patient groups are described in Table 3. The data on genotype frequencies among the control, CHB, LC, and HCC groups were all in agreement with the HWE. No significant differences were observed in the genotype distribution or in the allele frequency of the two SNPs between cases and controls (all $p>0.05$). To explore the impact of risk factors on CHB, LC, and HCC subjects, we conducted stratified analysis of confounding factors, such as gender, age, ethnicity, smoking, and alcohol drinking. Binary logistic regression analyses adjusted by gender are shown in Table 4, indicating that the GA and AA genotypes of rs13419896 loci in females were significantly associated with a risk of CHB (OR = 3.565, 95% CI = 1.123–11.314, p = 0.031 and OR = 12.506, 95% CI = 1.329–117.716, p = 0.027). Moreover, the A allele was also associated with a risk of CHB (OR = 2.624, 95% CI = 1.244–5.537, p = 0.011) and LC (OR = 2.351, 95% CI = 1.002–5.518, p = 0.050) in females. These results revealed that the GA and AA genotypes of rs13419896 may be risk factors for CHB in females in Guangxi, while the A allele genotype of rs13419896 may be a risk factor for CHB and LC in women in Guangxi. For the rs6715787 polymorphism, the CG genotype may contribute to a reduced risk of LC in the Guangxi Zhuang population (OR = 0.152, 95% CI = 0.028–0.807, p = 0.027), as shown in Table 5, suggesting that it may be a protective factor in this population. Binary logistic regression analyses adjusted by alcohol drinking status indicated that the AA genotype of rs13419896 may contribute to an increased risk of LC in the non-alcohol-drinking population (OR = 3.124, 95% CI = 1.091–8.947, p = 0.034) (Table 6). No significant differences were found after adjusting for age and smoking found by binary logistic regression analyses.

Table 3. Association analysis of HIF-2a gene polymorphisms between HBV-related patients and healthy controls.

SNPs	Control(n = 224)	CHB(n = 107)	LC(n = 83)	HCC(n = 234)	CHB vs. Control OR(95% CI)*	P	LC vs. Control OR(95%CI)*	P	HCC vs. Control OR (95%CI)*	P
rs13419896										
GG	115	47	35	122	1 ref		1 ref		1 ref	
GA	93	50	39	99	1.502(0.845–2.668)	0.165	1.333(0.760–2.339)	0.316	0.904(0.591–1.382)	0.64
AA	16	10	9	13	2.091(0.768–5.693)	0.149	1.916(0.741–4.954)	0.18	0.574(0.249–1.323)	0.192
G	323	144	109	343	1 ref		1 ref		1 ref	
A	125	70	57	125	1.439(0.951–2.178)	0.085	1.344(0.899–2.010)	0.15	0.835(0.606–1.151)	0.271
rs6715787										
CC	11	4	9	6	1 ref		1 ref		1 ref	
CG	84	39	32	89	1.604(0.230–11.185)	0.633	0.377(0.106–1.341)	0.132	2.461(0.608–9.972)	0.207
GG	129	64	42	139	2.770(0.403–19.035)	0.3	0.414(0.119–1.438)	0.165	2.952(0.740–11.78)	0.125
C	106	47	50	101	1 ref		1 ref		1 ref	
G	342	167	116	367	1.451(0.817–2.577)	0.204	0.829(0.511–1.345)	0.448	1.261(0.866–1.835)	0.227

CHB, chronic hepatitis B; LC, liver cirrhosis; HCC, hepatocellular carcinoma; SNPs, single nucleotide polymorphisms.

*Adjusted for gender, age, ethnicity, smoking, alcohol drinking status, and BMI (body mass index) by logistic regression model.

doi:10.1371/journal.pone.0158241.t003

Table 4. Association analysis of HIF-2a gene polymorphisms between HBV-related patients and healthy controls in female population.

SNPs	CHB vs. Control OR (95% CI)*	P	LC vs. Control OR (95% CI)*	P	HCC vs. Control OR (95% CI)*	P
rs13419896						
GG	1 ref		1 ref		1 ref	
GA	3.565(1.123–11.314)	0.031	2.284(0.617–8.455)	0.216	1.747(0.572–5.338)	0.328
AA	12.506(1.329–117.716)	0.027	4.109(0.493–34.266)	0.192	0(0)	0.999
G	1 ref		1 ref		1 ref	
A	2.624(1.244–5.537)	0.011	2.351(1.002–5.518)	0.050	1.102(0.459–2.647)	0.828
rs6715787						
CC	1 ref		1 ref		1 ref	
CG	1.338(0.054–33.406)	0.859	0.725(0.083–6.296)	0.771	0.245(0.018–3.359)	0.292
GG	1.183(0.048–28.934)	0.918	0.589(0.070–4.981)	0.627	0.141(0.142–1.912)	0.141
C	1 ref		1 ref		1 ref	
G	0.893(0.385–2.069)	0.791	0.789(0.323–1.925)	0.602	0.475(0.204–1.106)	0.084

CHB, chronic hepatitis B; LC, liver cirrhosis; HCC, hepatocellular carcinoma; SNPs, single nucleotide polymorphisms.

*Adjusted for age, ethnicity, smoking and drinking status by logistic regression model.

doi:10.1371/journal.pone.0158241.t004

Table 5. Association analysis of HIF-2a gene polymorphisms between HBV-related patients and healthy controls in Zhuang nationality population.

SNPs	CHB vs. Control		LC vs. Control		HCC vs. Control	
	OR (95% CI)*	P	OR (95% CI)*	P	OR (95% CI)*	P
rs13419896						
GG	1 ref		1 ref		1 ref	
GA	1.793(0.689–4.663)	0.231	1.107(0.466–2.628)	0.818	0.529(0.270–1.034)	0.063
AA	0.524(0.070–3.918)	0.529	1.329(0.266–6.640)	0.729	0.443(0.121–1.627)	0.220
G	1 ref		1 ref		1 ref	
A	1.121(0.568–2.213)	0.741	1.116(0.596–2.090)	0.731	0.614(0.372–1.014)	0.056
rs6715787						
CC	1 ref		1 ref		1 ref	
CG	1.083(0.138–8.510)	0.939	0.152(0.028–0.807)	0.027	1.350(0.130–13.974)	0.801
GG	1.247(0.163–9.541)	0.831	0.211(0.044–1.018)	0.053	1.468(0.146–14.733)	0.744
C	1 ref		1 ref		1 ref	
G	1.127(0.542–2.344)	0.749	0.728(0.376–1.409)	0.346	1.094(0.649–1.846)	0.736

CHB, chronic hepatitis B; LC, liver cirrhosis; HCC, hepatocellular carcinoma; SNPs, single nucleotide polymorphisms.

*Adjusted for age, smoking and drinking status by logistic regression model.

doi:10.1371/journal.pone.0158241.t005

Haplotype analysis of the HIF-2α gene polymorphisms

As the haplotype-based analysis is considered to have more power than SNP genotyping does, SHEsis online software was applied for the haplotype analysis of HIF-2α gene polymorphism (rs13419896 and rs6715787). We calculated the linkage disequilibrium in both the case and the control for the two SNP sites, and the value of the lowest frequency threshold for haplotype analysis is 0.03. According to the results, a total of four haplotypes (CG, CA, GG, and GA) were derived from the observed genotypes. The haplotype distribution in the CHB, LC, and HCC patients and healthy controls are shown in Table 7. The GG haplotype was the most common haplotype, followed by GA, CG, and CA, in turn. The GG haplotype was a protective

Table 6. Association analysis of HIF-2a gene polymorphisms between HBV-related patients and healthy controls in non-alcohol population.

SNPs	CHB vs. Control		LC vs. Control		HCC vs. Control	
	OR (95% CI)*	P	OR (95% CI)*	P	OR (95% CI)*	P
rs13419896						
GG	1 ref		1 ref		1 ref	
GA	1.417(0.677–2.965)	0.355	1.317(0.647–2.678)	0.448	0.895(0.540–1.483)	0.667
AA	2.992(0.813–11.005)	0.099	3.124(1.091–8.947)	0.034	0.859(0.324–2.273)	0.759
G	1 ref		1 ref		1 ref	
A	1.528(0.900–2.594)	0.117	1.596(0.989–2.577)	0.056	0.916(0.627–1.337)	0.650
rs6715787						
CC	1 ref		1 ref		1 ref	
CG	4.037(0.319–51.136)	0.281	0.492(0.140–1.732)	0.269	1.089(0.282–4.199)	0.902
GG	4.306(0.348–53.282)	0.255	0.382(0.109–1.340)	0.133	1.117(0.295–4.225)	0.871
C	1 ref		1 ref		1 ref	
G	1.237(0.677–2.260)	0.488	0.689(0.411–1.152)	0.155	1.035(0.688–1.556)	0.868

CHB, chronic hepatitis B; LC, liver cirrhosis; HCC hepatocellular carcinoma; SNPs, single nucleotide polymorphisms.

*Adjusted for age, smoking by logistic regression model; ref refers to the reference genotype.

doi:10.1371/journal.pone.0158241.t006

Table 7. Analysis of HIF-2a gene rs13419896 and rs6715787 haplotype frequencies with the risk of CHB, LC, and HCC.

Haplotype	Control	CHB	LC	HCC	CHB vs. Control		LC vs. Control		HCC vs. Control	
					OR(95% CI)*	P	OR(95% CI)*	P	OR(95%CI)*	P
CG	0.188	0.142	0.249	0.155	0.718(0.457–1.127)	0.149	1.440(0.942–2.200)	0.091	0.795(0.563–1.122)	0.192
CA	0.049	0.078	0.052	0.061	1.627(0.842–3.146)	0.145	1.059(0.471–2.377)	0.89	1.254(0.707–2.221)	0.438
GG	0.533	0.531	0.407	0.578	0.989(0.714–1.371)	0.948	0.601(0.419–0.862)	0.005	1.197(0.922–1.554)	0.176
GA	0.311	0.25	0.292	0.206	1.114(0.762–1.629)	0.577	1.378(0.923–2.057)	0.116	0.871(0.636–1.192)	0.387

CHB, chronic hepatitis B; LC, liver cirrhosis; HCC, hepatocellular carcinoma.

*Adjusted for sex, age, ethnicity, smoking and drinking status by logistic regression model.

doi:10.1371/journal.pone.0158241.t007

factor and significantly decreased the LC risk in the Guangxi population (OR = 0.601, 95% CI = 0.419–0.862, p = 0.005).

Comparison of genotype distributions with the HapMap project data

Considering that the HIF-2α rs13419896 and rs6715787 genotype frequencies might be distinct among different populations, the genotype and allele frequencies of the two polymorphisms in our control group were further compared with those populations from the Haplotype Map (HapMap) project. The data in Table 8 show that the genotype and allele frequencies of rs13419896 in our control group were similar to those in the CHB (Han Chinese in Beijing, China), JPT (Japanese in Tokyo, Japan), and YRI (Yoruba in Ibadan, Nigeria)populations, but they were significantly different from those in CEU (Utah residents with northern and western European ancestry) populations. In the current study, for the rs6715787 site, the genotype and allele distributions were similar to those in the CHB and JPT populations, but they were significantly different from those of the CEU and YRI populations. This revealed that the genotype frequencies of HIF-2α rs13419896 and rs6715787 show regional and ethnic differences.

Table 8. Comparison of genotype and allele frequencies in the control subjects of our study and those from the HapMap Project.

SNPs	Sample	Genotype frequency (%)				Allele Frequency (%)		
rs13419896		AA	GA	GG	P	A	G	P
Present study†	224	16(7.1)	93(41.5)	115(51.3)	1	125(27.9)	323(72.1)	1
CHB*	82	6(7.3)	38(46.3)	38(46.3)	0.729	50(30.5)	114(69.5)	0.545
JPT*	172	30(17.4)	68(39.5)	74(43.0)	0.006	128(37.2)	216(62.8)	0.006
CEU*	226	0(0)	4(1.8)	222(98.2)	<0.001	4(0.9)	448(99.1)	<0.001
YRI*	226	14(6.2)	104(46.0)	108(47.8)	0.612	132(29.2)	320(70.8)	0.712
rs6715787		CC	CG	GG	P	C	G	P
Present study†	224	11(4.9)	84(37.5)	129(57.6)	1	101(21.6)	367(78.4)	1
CHB*	82	12(14.6)	28(34.1)	42(51.2)	0.025	52(31.7)	112(68.3)	0.011
JPT*	172	12(7.0)	62(36.0)	98(57.0)	0.685	86(25.0)	258(0.75)	0.273
CEU*	226	120(53.1)	84(37.2)	22(9.7)	<0.001	324(71.7)	128(28.3)	<0.001
YRI*	226	58(25.7)	126(55.8)	42(18.6)	<0.001	242(53.5)	210(46.5)	<0.001

SNPs, single nucleotide polymorphisms; CHB, Han Chinese in Beijing, China; JPT, Japanese in Tokyo, Japan; CEU, Utah residents with northern and western European ancestry; YRI, Yoruba in Ibadan, Nigeria.

*Data from HapMap Project;

†Data from previous reports

doi:10.1371/journal.pone.0158241.t008

Discussion

HIF-2, an important transcription factor in the hypoxic condition, was recently recognized as controlling the activation state and key microbicidal functions of immune cells [37]. A growing body of evidence has shown that HIF-2a overexpression in primary and metastatic tumors [38], and the expression level has a positive correlation with the formation of tumor blood vessels and patient mortality[39]. A high expression of HIF-2a is closely related to some tumors and indicates a poor prognosis for patients [22, 40–43]. A previous study [25] identified that HBV enhances HIF-2a expression through HBx binding to the pVHL and activating the NF-κB signaling pathway, which may play an important role in hepatocarcinogenesis. Although HIFs can undergo degradation or stabilization, polymorphisms or mutations in HIFs can also influence their activity [44]. We therefore performed this study.

This is the first case-control study to reveal whether HIF-2a rs13419896 and rs6715787 polymorphisms are associated with susceptibility to CHB, LC, or HCC in the Guangxi population. In the present study, we attempted to explore the association between the HIF-2a gene rs13419896 and rs6715787 polymorphisms and HBV-related liver disease risk in a Chinese population. As a result, we found no significant differences in the genotype distribution or allele frequency of two HIF-2a SNPs between cases and controls. However, some confounding factors, such as gender, age, smoking, drinking, ethnicity, and BMI, probably play a role in the development of HBV-related liver disease, so we stratified our population to analyze the effect of these factors. In subgroup analysis, we showed that the GA and AA genotypes and the A allele of HIF-2a rs13419896 polymorphisms were associated with an elevated risk of HBV-related liver disease in female and non-alcohol-drinking populations, respectively. For the rs6715787 polymorphism, the CG genotype may contribute to a reduced risk of LC in the Guangxi Zhuang population. No significant differences were found after adjusting for age and smoking by binary logistic regression analyses. Furthermore, haplotype analysis indicated that the GG haplotype might be a protective factor for LC in the Guangxi population. According to our latest studies [33, 45] on the association between HIF-1a genetic polymorphisms (rs11549465 and rs115494657) and the risk of HBV-related liver disease, we did not find an association between HIF-1a rs11549465 and rs11549467 polymorphisms and HBV-related liver disease. However, the HIF-1a CG and CA haplotypes might be a risk factor and a protective marker, respectively, for HBV-related HCC in the Guangxi population. As can be seen from the above, our studies suggest that HIF-1α and HIF-2α gene polymorphisms may not be important contributors in HBV-related disease, but haplotypes of these polymorphisms were observed to be significantly associated with HBV-related disease. Although HIF-1a and HIF-2a subunits are very similar in their DNA binding and dimerization domains but differ in their transactivation domains, a similar result was observed. Compared with our previous studies, the present study involves performing a more detailed subgroup analysis and has some new findings.

With regard to rs13419896, which is located within intron1 of the gene, one study by Putra et al. [30], focusing on 76 Japanese non-small-cell lung cancer patients, explored the relationships between HIF-2αgene rs13419896 polymorphism and the risk of non-small-cell lung cancer with bioinformatics analysis in 2015. The researchers showed that the A allele at rs13419896 SNP may contribute to the overexpression of HIF-2α through the alteration of the binding affinity of the activator protein-1(AP-1), especially in the presence of overexpressed c-Fos or c-Jun. Moreover, the A allele of HIF-2α SNP is associated with a poorer prognosis in lung cancer patients. A similar result was observed in the present study, where the GA and AA genotypes and the A allele of HIF-2a rs13419896 polymorphisms were associated with an elevated risk of HBV-related liver disease in subgroup analysis, which indirectly confirmed the

above argument. With a larger sample size (107 CHB patients, 83 LC patients, 234HCC patients, and 224 healthy controls), our results had a much greater statistical power than did the previous study. However, our result seems unexpected because the male sex and alcohol drinking are closely related to the incidence of liver cancer [35, 46]; this is an interesting phenomenon worthy of further discussion. A previous study [29] suggested that HIF-2a rs4953354 SNP is a potentially susceptible marker for the development of lung adenocarcinoma, especially in female never-smokers. Lung cancer that has developed among never-smokers represents a unique subset of cancers. Thus, we speculated that the female and non-alcohol-drinking populations represent a unique subset of HBV-related liver disease, with a different etiology and natural history. Our data were supported, but the mechanisms underlying the high risk were not fully determined. As mentioned above, a potential explanation was that the GA and AA genotypes and the A allele of HIF-2a rs13419896 might be involved in altering the binding activity of transcription factors through NF-kB, mitogen-activated protein kinase (MAPK) and extracellular signal-regulated kinase (ERK) pathways [47–49], or through HBx binding to the pVHL and activating the NF-κ B signaling pathway HIF signals [25], leading to an overexpression of HIF-2a. This could increase the risk of HBV-related liver disease in female or non-alcohol-drinking populations. Another possible reason for this effect may be the limited sample size in our research. Notwithstanding, our study confirmed that HIF-2a gene rs13419896 polymorphism is associated with an elevated risk of HBV-related liver disease; studies with larger samples would further confirm these results.

With regard to rs6715787, unfortunately, no research has investigated the association of HIF-2a rs6715787 polymorphism with various diseases, except for a report on living high-exercise high-training low (HiHiLo). However, one study showed that exon2 of the HIF-2a gene is the key to the DNA binding heterodimer [50]. Since rs6715787 polymorphism is located within intron 2 of the gene, near the exon2 loci, this may affect the DNA binding heterodimer probably through the NF-κB signaling pathway, thereby altering the expression of the HIF-2a target gene, such as Oct4 [25]. Since relevant research data are less, the potential mechanism is still not fully clear. In our study, the CG genotype of HIF-2a rs6715787 may contribute to a reduced risk of LC in the Guangxi Zhuang population, indicating that the SNP may contribute to reducing the expression of HIF-2a and may be considered a protective marker. In contrast, a comparison of genotype distributions with the HapMap project data suggested that the CG genotype was significantly higher in the YRI population, while no significant difference in genotypes was found between the remaining populations and the current study. The CG genotype distribution exhibits regional and ethnic differences, so further studies of other ethnic populations are necessary.

A haplotype is a set of closely linked genetic markers present on one chromosome; such markers tend to be inherited together more frequently than expected by chance and could better explain the observed associations compared with each polymorphism independently. Therefore, further haplotype analysis should be performed to explore the associations between the HIF-2a gene and the risk of HBV-related liver disease. Our result indicated that the GG haplotype might be a protective factor for LC in the Guangxi population; it may induce splice-site variants [51] or affect microRNA regulation [52], which could alter the expression level of HIF-2a target genes or influence the gene-gene interaction, thus leading to a reduced risk of LC.

HIFs contribute to inflammatory functions in various other components of innate immunity, such as mast cells, dendritic cells, and epithelial cells [53], which plays a major role in the progression of disease. Indeed, HIF and its interaction with innate immunity in liver diseases have been observed [54, 55]. Toll-like receptors (TLR) play a critical role in innate immunity response, and our recent study showed that the TT genotype of TLR3 rs3775290 was associated

with a decreased risk for CHB, LC, and HCC [56]. The polymorphisms of cytokines (e.g., *IL-4*, *IL-6*, *IL-17*, *IL-23*, *INF-γet al.*), which have crucial roles in innate immunity, are also related to the risk of CHB, LC, and HCC in our previous studies [3, 57–60]. On the basis of this study and our previous work, genetic variants are clearly a key factor influencing innate immunity in HBV-related diseases, suggesting that they may be useful therapeutic targets for treating immune system disorders in the development of CHB, LC, and HCC.

Our study has several limitations. First, the positive results were shown only in subgroup analysis. The potential skewing of results due to the relatively small sample size should been considered when interpreting the results. Second, the current study selected only two rather than all SNPs of the HIF-2α gene. In addition, we did not measure the serum HIF-2α levels both in patients and controls; thus, the relationship between the expression of HIF-2α and HIF-2α gene polymorphisms was unclear. Considering HIF-2a SNP as a useful clinical prognostic marker of HBV-related liver disease patients, much more extensive studies on mechanisms at the cellular and molecular levels are needed in the future. Finally, the study sample was limited to the Guangxi population. Because the genotype distribution exhibits regional and ethnic differences, further studies involving other ethnic populations are necessary for systematical study.

In conclusion, HIF-2a rs13419896 polymorphism is associated with an increased risk of CHB and LC in the Guangxi Chinese population, especially in females or non-alcohol drinkers; meanwhile, the HIF-2a gene rs6715787 polymorphism is associated with a decreased risk of LC in the Guangxi Zhuang population. In addition, the GG haplotype of the two SNPs might be a protective factor for LC in this population. An effective risk prediction model should be constructed to further clarify the connection between HIF-2a polymorphism and HBV-related liver disease.

Supporting Information

S1 File. Ethical Review Committee Approval Notice.
(PDF)

S2 File. Original data.
(XLS)

S3 File. STROBE Statement—checklist of items that should be included in reports of observational studies.
(PDF)

Acknowledgments

We thank Scribendi.com for its linguistic assistance during the preparation of this manuscript.

Author Contributions

Conceived and designed the experiments: SL XQ. Performed the experiments: LH YD. Analyzed the data: YL JZ XH. Contributed reagents/materials/analysis tools: SL XQ WT. Wrote the paper: LH CL. Designed the software used in analysis: YS.

References

1. Te HS, Jensen DM. Epidemiology of hepatitis B and C viruses: a global overview. Clinics in liver disease. 2010; 14(1):1–21, vii. Epub 2010/02/04. doi: 10.1016/j.cld.2009.11.009 PMID: 20123436.

2. Harkisoen S, Arends JE, van Erpecum KJ, van den Hoek A, Hoepelman AI. Hepatitis B viral load and risk of HBV-related liver disease: from East to West? Annals of hepatology. 2012; 11(2):164–71. Epub 2012/02/22. PMID: 22345332.

3. Lu Y, Wu Z, Peng Q, Ma L, Zhang X, Zhao J, et al. Role of IL-4 gene polymorphisms in HBV-related hepatocellular carcinoma in a Chinese population. PloS one. 2014; 9(10):e110061. Epub 2014/10/09. doi: 10.1371/journal.pone.0110061 PMID: 25295591; PubMed Central PMCID: PMCPmc4190355.

4. Erler JT, Bennewith KL, Nicolau M, Dornhofer N, Kong C, Le QT, et al. Lysyl oxidase is essential for hypoxia-induced metastasis. Nature. 2006; 440(7088):1222–6. Epub 2006/04/28. doi: 10.1038/nature04695 PMID: 16642001.

5. Lofstedt T, Fredlund E, Holmquist-Mengelbier L, Pietras A, Ovenberger M, Poellinger L, et al. Hypoxia inducible factor-2alpha in cancer. Cell cycle (Georgetown, Tex). 2007; 6(8):919–26. Epub 2007/04/04. PMID: 17404509.

6. Sang N, Stiehl DP, Bohensky J, Leshchinsky I, Srinivas V, Caro J. MAPK signaling up-regulates the activity of hypoxia-inducible factors by its effects on p300. The Journal of biological chemistry. 2003; 278(16):14013–9. Epub 2003/02/18. doi: 10.1074/jbc.M209702200 PMID: 12588875; PubMed Central PMCID: PMCPmc4518846.

7. Zhong H, De Marzo AM, Laughner E, Lim M, Hilton DA, Zagzag D, et al. Overexpression of hypoxia-inducible factor 1alpha in common human cancers and their metastases. Cancer research. 1999; 59 (22):5830–5. Epub 1999/12/03. PMID: 10582706.

8. Maxwell PH, Dachs GU, Gleadle JM, Nicholls LG, Harris AL, Stratford IJ, et al. Hypoxia-inducible factor-1 modulates gene expression in solid tumors and influences both angiogenesis and tumor growth. Proceedings of the National Academy of Sciences of the United States of America. 1997; 94(15):8104–9. Epub 1997/07/22. PMID: 9223322; PubMed Central PMCID: PMCPmc21564.

9. Semenza GL. Involvement of hypoxia-inducible factor 1 in human cancer. Internal medicine (Tokyo, Japan). 2002; 41(2):79–83. Epub 2002/03/01. PMID: 11868612.

10. Jia ZZ, Jiang GM, Feng YL. Serum HIF-1alpha and VEGF levels pre- and post-TACE in patients with primary liver cancer. Chinese medical sciences journal = Chung-kuo i hsueh k'o hsueh tsa chih / Chinese Academy of Medical Sciences. 2011; 26(3):158–62. Epub 2012/01/03. PMID: 22207924.

11. Li S, Yao D, Wang L, Wu W, Qiu L, Yao M, et al. Expression characteristics of hypoxia-inducible factor-1alpha and its clinical values in diagnosis and prognosis of hepatocellular carcinoma. Hepatitis monthly. 2011; 11(10):821–8. Epub 2012/01/10. doi: 10.5812/kowsar.1735143X.771 PMID: 22224081; PubMed Central PMCID: PMCPmc3234574.

12. Tian H, McKnight SL, Russell DW. Endothelial PAS domain protein 1 (EPAS1), a transcription factor selectively expressed in endothelial cells. Genes & development. 1997; 11(1):72–82. Epub 1997/01/01. PMID: 9000051.

13. Semenza GL, Wang GL. A nuclear factor induced by hypoxia via de novo protein synthesis binds to the human erythropoietin gene enhancer at a site required for transcriptional activation. Molecular and cellular biology. 1992; 12(12):5447–54. Epub 1992/12/01. PMID: 1448077; PubMed Central PMCID: PMCPmc360482.

14. Nizet V, Johnson RS. Interdependence of hypoxic and innate immune responses. Nature reviews Immunology. 2009; 9(9):609–17. Epub 2009/08/26. doi: 10.1038/nri2607 PMID: 19704417; PubMed Central PMCID: PMCPmc4343208.

15. Loboda A, Jozkowicz A, Dulak J. HIF-1 and HIF-2 transcription factors—similar but not identical. Molecules and cells. 2010; 29(5):435–42. Epub 2010/04/17. doi: 10.1007/s10059-010-0067-2 PMID: 20396958.

16. Gustafsson MV, Zheng X, Pereira T, Gradin K, Jin S, Lundkvist J, et al. Hypoxia requires notch signaling to maintain the undifferentiated cell state. Developmental cell. 2005; 9(5):617–28. Epub 2005/11/01. doi: 10.1016/j.devcel.2005.09.010 PMID: 16256737.

17. Holmquist L, Lofstedt T, Pahlman S. Effect of hypoxia on the tumor phenotype: the neuroblastoma and breast cancer models. Advances in experimental medicine and biology. 2006; 587:179–93. Epub 2006/12/14. PMID: 17163166.

18. Bertout JA, Patel SA, Simon MC. The impact of O2 availability on human cancer. Nature reviews Cancer. 2008; 8(12):967–75. Epub 2008/11/07. doi: 10.1038/nrc2540 PMID: 18987634; PubMed Central PMCID: PMCPmc3140692.

19. Reddy KR, Guan Y, Qin G, Zhou Z, Jing N. Combined treatment targeting HIF-1alpha and Stat3 is a potent strategy for prostate cancer therapy. The Prostate. 2011; 71(16):1796–809. Epub 2011/04/12. doi: 10.1002/pros.21397 PMID: 21480310.

20. Shweiki D, Itin A, Soffer D, Keshet E. Vascular endothelial growth factor induced by hypoxia may mediate hypoxia-initiated angiogenesis. Nature. 1992; 359(6398):843–5. Epub 1992/10/29. doi: 10.1038/359843a0 PMID: 1279431.

21. Forsythe JA, Jiang BH, Iyer NV, Agani F, Leung SW, Koos RD, et al. Activation of vascular endothelial growth factor gene transcription by hypoxia-inducible factor 1. Molecular and cellular biology. 1996; 16 (9):4604–13. Epub 1996/09/01. PMID: 8756616; PubMed Central PMCID: PMCPmc231459.

22. Holmquist-Mengelbier L, Fredlund E, Lofstedt T, Noguera R, Navarro S, Nilsson H, et al. Recruitment of HIF-1alpha and HIF-2alpha to common target genes is differentially regulated in neuroblastoma: HIF-2alpha promotes an aggressive phenotype. Cancer cell. 2006; 10(5):413–23. Epub 2006/11/14. doi: 10.1016/j.ccr.2006.08.026 PMID: 17097563.

23. Chen Z, He X, Xia W, Huang Q, Zhang Z, Ye J, et al. Prognostic value and clinicopathological differences of HIFs in colorectal cancer: evidence from meta-analysis. PloS one. 2013; 8(12):e80337. Epub 2013/12/11. doi: 10.1371/journal.pone.0080337 PMID: 24324596; PubMed Central PMCID: PMCPmc3855620.

24. Gong L, Zhang W, Zhou J, Lu J, Xiong H, Shi X, et al. Prognostic value of HIFs expression in head and neck cancer: a systematic review. PloS one. 2013; 8(9):e75094. Epub 2013/09/24. doi: 10.1371/journal.pone.0075094 PMID: 24058651; PubMed Central PMCID: PMCPmc3772872.

25. Hu JL, Liu LP, Yang SL, Fang X, Wen L, Ren QG, et al. Hepatitis B virus induces hypoxia-inducible factor-2alpha expression through hepatitis B virus X protein. Oncology reports. 2016; 35(3):1443–8. Epub 2015/12/10. doi: 10.3892/or.2015.4480 PMID: 26647960.

26. Rannala B. Finding genes influencing susceptibility to complex diseases in the post-genome era. American journal of pharmacogenomics: genomics-related research in drug development and clinical practice. 2001; 1(3):203–21. Epub 2002/06/27. PMID: 12083968.

27. Purdue MP, Johansson M, Zelenika D, Toro JR, Scelo G, Moore LE, et al. Genome-wide association study of renal cell carcinoma identifies two susceptibility loci on 2p21 and 11q13.3. Nature genetics. 2011; 43(1):60–5. Epub 2010/12/07. doi: 10.1038/ng.723 PMID: 21131975; PubMed Central PMCID: PMCPmc3049257.

28. Ciampa J, Yeager M, Amundadottir L, Jacobs K, Kraft P, Chung C, et al. Large-scale exploration of gene-gene interactions in prostate cancer using a multistage genome-wide association study. Cancer research. 2011; 71(9):3287–95. Epub 2011/03/05. doi: 10.1158/0008-5472.can-10-2646 PMID: 21372204; PubMed Central PMCID: PMCPmc3085580.

29. Iwamoto S, Tanimoto K, Nishio Y, Putra AC, Fuchita H, Ohe M, et al. Association of EPAS1 gene rs4953354 polymorphism with susceptibility to lung adenocarcinoma in female Japanese non-smokers. Journal of thoracic oncology: official publication of the International Association for the Study of Lung Cancer. 2014; 9(11):1709–13. Epub 2014/12/02. doi: 10.1097/jto.0000000000000309 PMID: 25436804.

30. Putra AC, Eguchi H, Lee KL, Yamane Y, Gustine E, Isobe T, et al. The A Allele at rs13419896 of EPAS1 Is Associated with Enhanced Expression and Poor Prognosis for Non-Small Cell Lung Cancer. PloS one. 2015; 10(8):e0134496. Epub 2015/08/12. doi: 10.1371/journal.pone.0134496 PMID: 26263511; PubMed Central PMCID: PMCPmc4532412.

31. Guo LI, Zhang J, Jin J, Gao X, Yu J, Geng Q, et al. Genetic variants of endothelial PAS domain protein 1 are associated with susceptibility to acute mountain sickness in individuals unaccustomed to high altitude: A nested case-control study. Experimental and therapeutic medicine. 2015; 10(3):907–14. Epub 2015/12/02. doi: 10.3892/etm.2015.2611 PMID: 26622413; PubMed Central PMCID: PMCPmc4533176.

32. Henderson J, Withford-Cave JM, Duffy DL, Cole SJ, Sawyer NA, Gulbin JP, et al. The EPAS1 gene influences the aerobic-anaerobic contribution in elite endurance athletes. Human genetics. 2005; 118 (3–4):416–23. Epub 2005/10/07. doi: 10.1007/s00439-005-0066-0 PMID: 16208515.

33. Liu Y, Sui J, Zhai L, Yang S, Huang L, Huang L, et al. Genetic polymorphisms in hypoxia-inducible factor-1a gene and its association with HBV-related hepatocellular carcinoma in a Chinese population. Medical oncology (Northwood, London, England). 2014; 31(10):200. Epub 2014/09/10. doi: 10.1007/s12032-014-0200-8 PMID: 25195037.

34. Shi YY, He L. SHEsis, a powerful software platform for analyses of linkage disequilibrium, haplotype construction, and genetic association at polymorphism loci. Cell research. 2005; 15(2):97–8. Epub 2005/03/03. doi: 10.1038/sj.cr.7290272 PMID: 15740637.

35. Bosch FX, Ribes J, Diaz M, Cleries R. Primary liver cancer: worldwide incidence and trends. Gastroenterology. 2004; 127(5 Suppl 1):S5–s16. Epub 2004/10/28. PMID: 15508102.

36. Mittal S, El-Serag HB. Epidemiology of hepatocellular carcinoma: consider the population. Journal of clinical gastroenterology. 2013; 47 Suppl:S2–6. Epub 2013/05/02. doi: 10.1097/MCG.0b013e3182872f29 PMID: 23632345; PubMed Central PMCID: PMCPmc3683119.

37. Bhandari T, Nizet V. Hypoxia-Inducible Factor (HIF) as a Pharmacological Target for Prevention and Treatment of Infectious Diseases. Infectious diseases and therapy. 2014. Epub 2014/08/20. doi: 10.1007/s40121-014-0030-1 PMID: 25134687; PubMed Central PMCID: PMCPmc4269623.

38. Rohwer N, Cramer T. Hypoxia-mediated drug resistance: novel insights on the functional interaction of HIFs and cell death pathways. Drug resistance updates: reviews and commentaries in antimicrobial and anticancer chemotherapy. 2011; 14(3):191–201. Epub 2011/04/07. doi: 10.1016/j.drup.2011.03.001 PMID: 21466972.

39. Qing G, Simon MC. Hypoxia inducible factor-2alpha: a critical mediator of aggressive tumor phenotypes. Current opinion in genetics & development. 2009; 19(1):60–6. Epub 2009/01/27. doi: 10.1016/j.gde.2008.12.001 PMID: 19167211; PubMed Central PMCID: PMCPmc3146034.

40. Raval RR, Lau KW, Tran MG, Sowter HM, Mandriota SJ, Li JL, et al. Contrasting properties of hypoxia-inducible factor 1 (HIF-1) and HIF-2 in von Hippel-Lindau-associated renal cell carcinoma. Molecular and cellular biology. 2005; 25(13):5675–86. Epub 2005/06/21. doi: 10.1128/mcb.25.13.5675-5686.2005 PMID: 15964822; PubMed Central PMCID: PMCPmc1157001.

41. Kondo K, Klco J, Nakamura E, Lechpammer M, Kaelin WG Jr. Inhibition of HIF is necessary for tumor suppression by the von Hippel-Lindau protein. Cancer cell. 2002; 1(3):237–46. Epub 2002/06/28. PMID: 12086860.

42. Kondo K, Kim WY, Lechpammer M, Kaelin WG Jr. Inhibition of HIF2alpha is sufficient to suppress pVHL-defective tumor growth. PLoS biology. 2003; 1(3):E83. Epub 2003/12/24. doi: 10.1371/journal.pbio.0000083 PMID: 14691554; PubMed Central PMCID: PMCPmc300692.

43. Giatromanolaki A, Koukourakis MI, Sivridis E, Turley H, Talks K, Pezzella F, et al. Relation of hypoxia inducible factor 1 alpha and 2 alpha in operable non-small cell lung cancer to angiogenic/molecular profile of tumours and survival. British journal of cancer. 2001; 85(6):881–90. Epub 2001/09/15. doi: 10.1054/bjoc.2001.2018 PMID: 11556841; PubMed Central PMCID: PMCPmc2375073.

44. Kondo K, Kaelin WG Jr. The von Hippel-Lindau tumor suppressor gene. Experimental cell research. 2001; 264(1):117–25. Epub 2001/03/10. doi: 10.1006/excr.2000.5139 PMID: 11237528.

45. Lu Y, Sui J, Liu Y, Li R, Ma L, Zhang X, et al. Association between hypoxia-inducible factor-1alpha gene polymorphisms and risk of chronic hepatitis B and hepatitis B virus-related liver cirrhosis in a Chinese population: a retrospective case-control study. Gene. 2015; 564(1):96–100. Epub 2015/04/01. doi: 10.1016/j.gene.2015.03.053 PMID: 25824382.

46. Morgan TR, Mandayam S, Jamal MM. Alcohol and hepatocellular carcinoma. Gastroenterology. 2004; 127(5 Suppl 1):S87–96. Epub 2004/10/28. PMID: 15508108.

47. Karapetsas A, Giannakakis A, Pavlaki M, Panayiotidis M, Sandaltzopoulos R, Galanis A. Biochemical and molecular analysis of the interaction between ERK2 MAP kinase and hypoxia inducible factor-1alpha. The international journal of biochemistry & cell biology. 2011; 43(11):1582–90. Epub 2011/08/03. doi: 10.1016/j.biocel.2011.07.007 PMID: 21807114.

48. Frede S, Stockmann C, Freitag P, Fandrey J. Bacterial lipopolysaccharide induces HIF-1 activation in human monocytes via p44/42 MAPK and NF-kappaB. The Biochemical journal. 2006; 396(3):517–27. Epub 2006/03/15. doi: 10.1042/bj20051839 PMID: 16533170; PubMed Central PMCID: PMCPmc1482811.

49. Sumbayev VV. PI3 kinase and direct S-nitrosation are involved in down-regulation of apoptosis signal-regulating kinase 1 during LPS-induced Toll-like receptor 4 signalling. Immunology letters. 2008; 115(2):126–30. Epub 2007/12/07. doi: 10.1016/j.imlet.2007.10.013 PMID: 18054391.

50. O'Rourke JF, Tian YM, Ratcliffe PJ, Pugh CW. Oxygen-regulated and transactivating domains in endothelial PAS protein 1: comparison with hypoxia-inducible factor-1alpha. The Journal of biological chemistry. 1999; 274(4):2060–71. Epub 1999/01/16. PMID: 9890965.

51. Antonacopoulou AG, Floratou K, Bravou V, Kottorou A, Dimitrakopoulos FI, Marousi S, et al. The survivin -31 snp in human colorectal cancer correlates with survivin splice variant expression and improved overall survival. Cellular oncology (Dordrecht). 2011; 34(4):381–91. Epub 2011/05/04. doi: 10.1007/s13402-011-0038-4 PMID: 21538024.

52. Gong J, Tong Y, Zhang HM, Wang K, Hu T, Shan G, et al. Genome-wide identification of SNPs in microRNA genes and the SNP effects on microRNA target binding and biogenesis. Human mutation. 2012; 33(1):254–63. Epub 2011/11/03. doi: 10.1002/humu.21641 PMID: 22045659.

53. Imtiyaz HZ, Simon MC. Hypoxia-inducible factors as essential regulators of inflammation. Current topics in microbiology and immunology. 2010; 345:105–20. Epub 2010/06/03. doi: 10.1007/82_2010_74 PMID: 20517715; PubMed Central PMCID: PMCPmc3144567.

54. Arias-Loste MT, Fabrega E, Lopez-Hoyos M, Crespo J. The Crosstalk between Hypoxia and Innate Immunity in the Development of Obesity-Related Nonalcoholic Fatty Liver Disease. BioMed research international. 2015; 2015:319745. Epub 2015/10/23. doi: 10.1155/2015/319745 PMID: 26491664; PubMed Central PMCID: PMCPmc4600870.

55. Imtiyaz HZ, Williams EP, Hickey MM, Patel SA, Durham AC, Yuan LJ, et al. Hypoxia-inducible factor 2alpha regulates macrophage function in mouse models of acute and tumor inflammation. The Journal

of clinical investigation. 2010; 120(8):2699–714. Epub 2010/07/21. doi: 10.1172/jci39506 PMID: 20644254; PubMed Central PMCID: PMCPmc2912179.

56. Huang X, Li H, Wang J, Huang C, Lu Y, Qin X, et al. Genetic polymorphisms in Toll-like receptor 3 gene are associated with the risk of hepatitis B virus-related liver diseases in a Chinese population. Gene. 2015; 569(2):218–24. Epub 2015/05/31. doi: 10.1016/j.gene.2015.05.054 PMID: 26024592.

57. Xi XE, Liu Y, Lu Y, Huang L, Qin X, Li S. Interleukin-17A and interleukin-17F gene polymorphisms and hepatitis B virus-related hepatocellular carcinoma risk in a Chinese population. Med Oncol. 2015; 32 (1):355. Epub 2014/11/29. doi: 10.1007/s12032-014-0355-3 PMID: 25429834.

58. Sun Y, Lu Y, Xie L, Deng Y, Li S, Qin X. Interferon gamma polymorphisms and hepatitis B virus-related liver cirrhosis risk in a Chinese population. Cancer cell international. 2015; 15:35. Epub 2015/04/11. doi: 10.1186/s12935-015-0184-2 PMID: 25861244; PubMed Central PMCID: PMCPmc4389711.

59. Mo CJ, Peng QL, He Y, Wang J, Xie L, Li TJ, et al. Positive association between IL-16 rs11556218 T/G polymorphism and cancer risk: a meta-analysis. Asian Pacific journal of cancer prevention: APJCP. 2014; 15(11):4697–703. Epub 2014/06/28. PMID: 24969906.

60. Peng Q, Qin Y, Chen Z, Deng Y, Xu J, Li S, et al. Correlation between interleukin23 receptor gene polymorphisms and risk of hepatitis B virus infection in patients. Molecular medicine reports. 2013; 8 (2):613–20. Epub 2013/06/29. doi: 10.3892/mmr.2013.1558 PMID: 23807368.