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ORIGINAL PAPER

Genetic polymorphisms in hypoxia-inducible factor-1a gene and its association with HBV-related hepatocellular carcinoma in a Chinese population

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Abstract Previous studies have demonstrated that hypoxia-inducible factor-1a (HIF-1a) may play a vital role in the pathogenesis of hepatocellular carcinoma (HCC). However, the relationship between *HIF-1a* polymorphisms and HCC has not been thoroughly investigated. The aim of this study is to determine whether *HIF-1a* polymorphisms are associated with HCC through a case–control study. Two polymorphisms in the *HIF-1a* gene (*rs11549465* and *rs115494657*) were examined in 157 hepatitis B virus (HBV)-related HCC patients and 173 healthy controls using the polymerase chain reaction–restriction fragment length polymorphism method. DNA sequencing was used to validate genotype results. There were no significant differences in the genotype and allele frequencies of *HIF-1a rs11549465* and *rs115494657* polymorphisms between the HBV-related HCC patients and healthy controls. However, the data revealed that subjects with the CG haplotype have a higher susceptibility to HBV-related HCC [odds ratio (OR) = 2.327, 95 % confidence interval (CI) = 1.578–4.721, $P = 0.008$]. In contrast, the CA haplotype was associated with a significantly decreased risk of HBV-related HCC (OR = 0.416, 95 % CI = 0.172–0.910, $P = 0.025$). *HIF-1a rs11549465* and *rs115494657* polymorphisms appeared to be irrelevant to HBV-related HCC. However, the *HIF-1a* CG and CA haplotypes might be a risk factor and a protective marker, respectively, for HBV-related HCC in a Chinese population. Further investigations with a larger sample size may be required to validate the genetic effects of *HIF-1a* polymorphisms on HBV-related HCC.

Keywords Hypoxia-inducible factor-1a · Polymorphism · Hepatocellular carcinoma

Introduction

Hepatocellular carcinoma (HCC) is a common malignant neoplasm, with an estimated 782,000 new cancer cases occurring worldwide in 2012 (50 % in China alone) [1]. HCC ranks as the fifth most common incident cancer in men and the ninth in women and, owing to its poor prognosis, it is the second most common cause of death from cancer worldwide [1]. All of these data highlight the importance of a better understanding of the risk factors related to HCC development. However, HCC is a multifactorial disease involving a complex interplay between genetic and environmental factors. Epidemiological research has revealed that hepatitis B virus (HBV) and hepatitis C virus infections, smoking, aflatoxin exposure, and excessive alcohol intake are the major etiological factors affecting HCC [2–4]. However, the molecular and cellular mechanisms of HCC pathogenesis remain largely unknown and need to be further elucidated.

Many intracellular and extracellular factors are involved in carcinogenesis, cancer growth, and metastasis. A well-recognized critical component for cancer progression is the capability of cancer cells to sense hypoxia and enhance cell

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proliferation, invasion, and metastasis [5]. Hypoxia-inducible factor-1a (HIF-1a) is a key regulator of cellular response to hypoxia [6] and has been identified to play an important role in regulating angiogenesis and cell adhesion, metastatic spread, and apoptosis [7, 8]. Further, an overexpression of HIF-1a has been observed in most neoplastic hepatocytes [9–11] and has been associated with increased microvessel density and hepatocarcinogenesis [9].

Genetic polymorphisms are considered as the main genetic elements involved in the development of common and complex diseases [12]. The *HIF-1a* gene is located on chromosome 14q21–24 [13]. Several single nucleotide polymorphism (SNP) sites have been found in *HIF-1a*, among which *rs11549465* (encoding P582S) and *rs11549467* (encoding A588T) polymorphisms have been widely investigated. Previous studies have suggested that these genetic mutations resulted in promoted transcription activities under both normoxic and hypoxic conditions and increased the expression of HIF-1a [5, 14, 15]. Furthermore, many studies have investigated the association between genetic polymorphisms and susceptibility to various cancers such as breast, prostate, gastric, oral, colorectal, and renal cell carcinoma [16–18]. Nevertheless, to date, little effort has been made to investigate the potential role of *HIF-1a* genetic polymorphisms in the development of HCC [19]. The purpose of this study is to further evaluate the association between these two widely studied *HIF-1a* polymorphisms (*rs11549465* and *rs11549467*) and the risk of the development of HBV-related HCC in a Guangxi, southern Chinese population.

Materials and methods

Ethics statement

The study protocol was approved by the ethics committee of the First Affiliated Hospital of Guangxi Medical University. All of the involved patients and all healthy volunteers provided written informed consent.

Cases and controls

The study included 157 HBV-related HCC patients consecutively selected from the First Affiliated Hospital of Guangxi Medical University in Guangxi, China, between May and December 2013. The inclusion criteria for the HBV-related HCC group were as follows: (1) confirmed to be positive for hepatitis B surface antigen, positive for hepatitis B virus core antibody, and positive for hepatitis B e antigen or hepatitis B e antibody for at least 6 months; (2) diagnosis based on either histological or cytological findings, or on elevated serum alpha fetoprotein levels >400 ng/mL combined with at least one positive liver image on computed tomography, magnetic resonance imaging, or ultrasonography; (3) there were no other hepatitis virus infections such as hepatitis C or hepatitis E. We only included newly diagnosed HCC patients; patients with a medical history of HCC or other cancers were excluded. An alcohol drinker was defined as someone who consumed alcoholic beverages at least once per week for more than 6 months. Subjects were considered smokers if they smoked up to 1 year before the date of diagnosis for cases, or up to the date of interview for controls.

A total of 173 controls without clinical evidence of hepatic disease or tumor were randomly selected from a pool of healthy volunteers who visited the general health checkup centers of the same hospital during the same time period for routine scheduled physical examination. To control for the effects of potential confounders, controls were individually matched to cases based on sex and age (±5 years).

DNA extraction

Peripheral blood samples (2 mL) were collected from all of the subjects in ethylenediaminetetraacetic acid-coated vials and stored at −80 °C until DNA extraction. Genomic DNA was extracted from white blood cell fractions using QIAamp DNA Blood Mini Kit (Qiagen GmbH, Hilden, Germany), according to the manufacturer's instructions. DNA concentration was determined spectrophotometrically.

PCR amplification

Two well-studied functional SNPs in the *HIF-1a* gene, *rs11549465* and *rs1154946*, were selected from the genotyping list in this study due to their important functions in regulating HIF-1a expression. Genotyping was performed using the polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP) method. The presence of the *rs11549465* polymorphisms of *HIF-1a* was detected by amplifying genomic DNA with the following oligonucleotide primers: forward, 5′-TGTGGCCATTGTAAAAA CTCA-3′; reverse, 5′-CTTGCGGAACTGCCTTCTAA-3′. For the *rs1154946*, the primers used were: forward, 5′-TCCTTCGATCAGTTGTCACCA-3′; reverse, 5′-GGCT GTCCGACTTTGAGTATC-3′. The PCR was performed in a total volume of 25 μL, consisting of 2 μL of genomic DNA, 1 μL of each primer, 12.5 μL of Green PCR Master Mix (Shanghai Sangon Biological Engineering Technology and Services Co., Ltd., China), and 8.5 μL of nuclease-free double-distilled water. The PCR protocol included an initial denaturation step at 95 °C for 5 min, followed by 35 cycles of 30 s at 95 °C (denaturation), 30 s at 55 °C (annealing), and 60 s of elongation at 72 °C, followed by a final elongation step at 72 °C for 10 min.

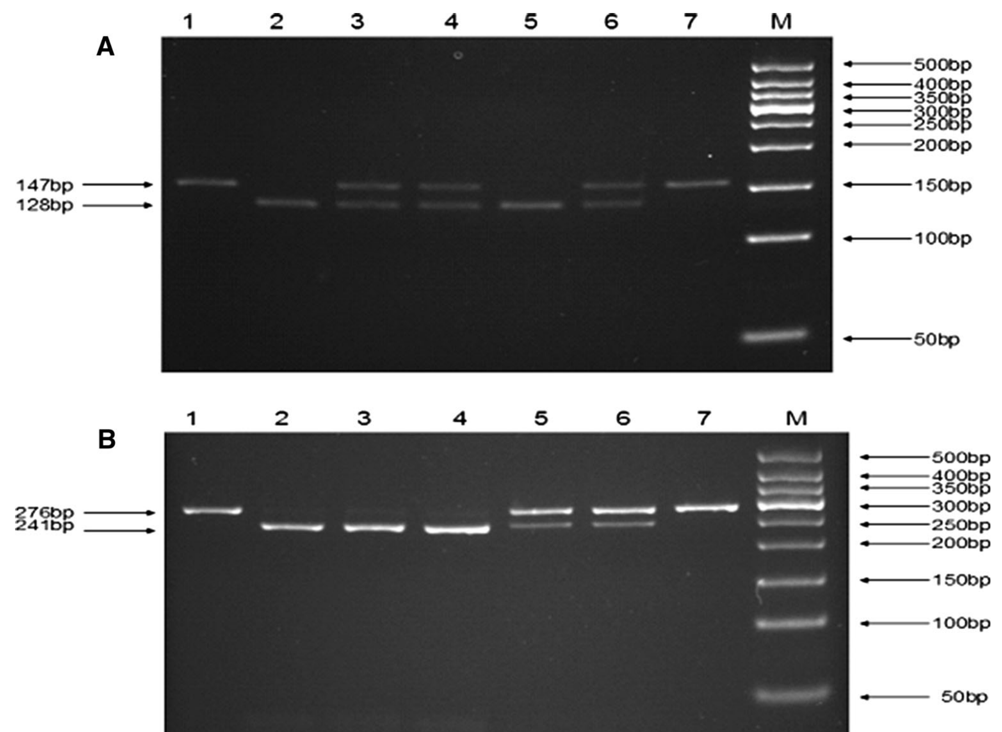

Fig. 1 PCR–RFLP assay to analyze the *rs11549465* and *rs11549467* polymorphisms of the *HIF-1a* gene. **a** *rs11549465* (19-bp band is invisible): *Lane M* is the DNA Marker; *line 1* is the *negative* control (the *negative* control utilized a PCR-amplified DNA product but without the restriction enzymes); *lines 2* and *4* are the CC genotype; *lines 3*, *4*, and *6* are the CT genotype; *line 7* is the TT genotype). **b** *rs11549467* (35-bp band is invisible): *Lane M* is the DNA Marker; *line 1* is the *negative* control; *lines 2–7* are the samples; *lines 2*, *3*, and *4* are the GG genotype; *lines 5* and *6* are the GA genotype; *line 7* is the AA genotype

Polymorphism genotyping

For *rs11549465* and *rs1154946*, 10 µL aliquots of the PCR products was digested at 37 °C for 3 h with 1 µL of BslI or AciI restriction enzymes, respectively. Digested fragments were separated by electrophoresis in 2 % agarose gel containing ethidium bromide and fragments and visualized using a UV transilluminator. To control the quality of genotyping, a negative control with a PCR-amplified DNA product but without the restriction enzymes was used for each genotyping assay.

The *rs11549465* polymorphism homozygous wild-type CC genotype yielded 128- and 19-bp products, the heterozygous CT genotype yielded 147-, 128-, and 19-bp products, and the homozygous mutant TT allele yielded a 147-bp product. The *rs11549467* polymorphism homozygous wild-type GG genotype yielded 241- and 35-bp products, the heterozygous GA genotype yielded 276-, 241-, and 35-bp products, and the homozygous mutant AA genotype yielded a 276-bp product (Fig. 1).

DNA sequencing

To confirm the genotyping results, a total of 33 specimens (10 %) were randomly selected and genotyped by DNA sequencing with an ABI Prism 3100 (Applied Biosystems, Shanghai Sangon Biological Engineering Technology and Services Co., Ltd., China). The results of DNA sequencing were 100 % concordant with those identified by PCR–RFLP.

Statistical analysis

The distribution of the general demographic and clinical features between cases and controls was evaluated by using the Student's t test and the χ^2 test (or Fisher's exact test, if required) for continuous and categorical variables, respectively. The Hardy–Weinberg equilibrium was tested with a goodness-of-fit χ^2 test with one degree of freedom to compare the observed genotype frequencies among the subjects with the expected genotype frequencies. Genotype, allele, and haplotype distributions of *HIF-1a* were compared with controls using the χ^2 test and Fisher's exact test, when appropriate. Haplotype analysis was performed using SHEsis software [20]. Binary logistic regression models were used to estimate ORs with corresponding 95 % CIs to test the association of the genotypes and HCC risk. All ORs were adjusted for age, gender, and smoking and alcohol consumption status. In addition, the frequencies of the *rs11549465* and *rs11549467* SNP genotypes in our control group were compared with those from the HapMap Project dbSNP database (http://www.ncbi.nlm.nih.gov/snp/) using the χ^2 test or Fisher's exact test. Statistical significance was assumed at two-sided values at the $P < 0.05$ level. All of the statistical analyses were performed using the Statistical Package for Social Sciences (SPSS, version 13.0).

Table 1 Demographic characteristics of the study population

Variables	Healthy control ($n = 173$) n (%)	HCC patients ($n = 157$) n (%)	P value
Age (mean ± SD)	47.78 ± 11.71	49.03 ± 11.28	0.821
Gender			
Male	149 (86.1)	138 (87.9)	0.633
Female	24 (13.9)	19 (12.1)	
Smoking			
No	127 (73.4)	116 (73.9)	0.922
Yes	46 (26.6)	41 (26.1)	
Alcohol			
No	121 (69.9)	107 (68.2)	0.725
Yes	52 (30.1)	50 (31.8)	

Results

Characteristics of the study population

The characteristics of the cases and control subjects are shown in Table 1. The mean ages (SD) of the control and HCC group were 47.78 ± 11.71 and 49.03 ± 11.28, respectively. There were no significant differences for sex, mean age, and smoking status or alcohol consumption status, indicating that the case data were comparable with those of the controls (all $P > 0.05$).

Association between *rs11549465* polymorphisms and HBV-related HCC risk

The genotype and allele frequencies of *rs11549465* polymorphisms among the healthy control subjects and the HBV-related HCC patients are shown in Table 2. The observed genotype frequencies in controls conformed to the Hardy–Weinberg equilibrium ($P = 0.666$). The frequencies of the CC, CT, and TT genotypes of *rs11549465* were 93.6, 6.4, and 0.0 % in healthy controls and 96.8, 2.6, and 0.6 % in HCC patients, respectively. In logistic regression analyses adjusted by age, sex, smoking, and drinking, the *rs11549465* CT and TT genotypes were not associated with HCC risk compared with the CC genotype ($P = 0.204$ and $P = 1.00$). In addition, compared with reference subjects carrying the *rs11549465* T allele, carriage of the C allele was not associated with susceptibility to HBV-related HCC (OR = 0.851, 95 % CI = 0.273–2.652, $P = 0.780$). Further, the dominant model genotype CT + TT was also not associated with the risk of HBV-related HCC. When analyses of genotype and allele frequencies were stratified by gender, no significant differences in the distributions of *HIF-1a* polymorphisms among patients with HCC and control groups were observed (Table 2).

Association between *rs11549467* polymorphisms and HBV-related HCC risk

The genotype and allele distributions of the *rs11549467* sites in the *HIF-1a* gene of the control subjects and HCC patients are described in Table 2. According to the Hardy–Weinberg equilibrium test, the observed genotype distributions in control subjects were not significantly different from the expected distributions ($P = 0.773$). The frequencies of the GG, AG, and AA genotypes of *rs11549467* were 87.3, 12.1, and 0.6 % in healthy controls and 93.6, 6.4, and 0.0 % in HCC patients, respectively. Further, no significant differences were observed between the genotype and allele frequencies of the *rs11549467* polymorphism and HBV-related HCC risk after adjusting for age, sex, smoking, and drinking by binary logistic regression analyses, even after stratification of the study groups by gender.

Haplotype analyses of *HIF-1a* gene polymorphisms and HCC risk

We further performed a haplotype analysis using SHEsis software to evaluate the haplotype frequencies of polymorphisms located in the same chromosome regions in order to derive haplotypes specifically correlated with HCC. A total of three haplotypes were derived from the observed genotypes. The haplotype distribution in the HCC patients and healthy controls are shown in Table 3. We found one protective haplotype: CA (OR = 0.416, 95 % CI = 0.172–0.910, $P = 0.025$). In contrast, the CG haplotype in HCC patients was associated with a significantly increased risk of HCC (OR = 2.237, 95 % CI = 1.578–4.721, $P = 0.008$). The remaining haplotypes were not associated with HCC risk.

The genotype and allele frequencies of *rs11549465* and *rs11549467* SNPs in our control group were compared with those from the Haplotype Map (HapMap) Project [21] (http://www.ncbi.nlm.nih.gov/snp/). Data in Table 4 show the genotype and allele differences between the control subjects in the present study and those of various ethnicities included in the HapMap Project. Through this comparison, we noted that the results presented herein were inconsistent with those of some previous epidemiological studies performed in different ethnic populations. The C allele frequency among the healthy controls in the *rs11549465* site in the present study was 0.968, which was similar to the frequencies observed in healthy HCB (Han Chinese in Beijing; 0.953), JPT (Japanese in Tokyo; 0.965), and YRI (Yoruba in Ibadan; 0.973) populations, but higher than those of the CEU (Utah residents with northern and western European ancestry; 0.925) population. The frequency of the *rs11549465* G allele among the healthy controls was 0.934, which was similar to the frequency

Table 2 Distributions of HIF-1a SNPs genotypes in each group and logistic regression analyses of associations between these polymorphisms and HBV-related HCC risk

Genotypes	Overall				Men				Women			
	Controls $n = 173$	HCC cases $n = 157$	OR (95 % CI)*	*P*	Controls $n = 149$	HCC cases $n = 138$	OR (95 % CI)*	*P*	Controls $n = 147$	HCC cases $n = 52$	OR (95 % CI)*	*P*
rs11549465												
CC	162 (93.6)	152 (96.8)	1.00[ref]		141 (94.6)	134 (97.1)	1.00[ref]		21 (87.5)	18 (94.7)	1.00[ref]	
CT	11 (6.4)	4 (2.6)	0.426 (0.114–1.592)	0.204	8 (5.4)	3 (2.2)	0.368 (0.078–1.725)	0.205	3 (12.5)	1 (5.3)	0.684(0.059–7.899)	0.761
TT	0 (0)	1 (0.6)	0.000	1.000	0 (0)	1 (0.7)	0.000	1.000	0 (0)	0 (0)	NC	NC
CT + TT	11(6.4)	5 (3.2)	0.484(0.165–1.427)	0.188	8 (16.3)	4 (2.9)	0.540 (0.159–1.833)	0.323	3 (12.5)	1 (5.3)	0.684(0.059–7.899)	0.761
C allele	335 (96.8)	308 (98.1)	1.00[ref]		290 (97.3)	271 (98.2)	1.00[ref]		45 (93.7)	37 (97.4)	1.00[ref]	
T allele	11(3.2)	6 (1.9)	0.851 (0.273–2.652)	0.780	8 (2.7)	5 (1.8)	0.919 (0.251–3.365)	0.898	3 (6.3)	1 (2.6)	0.700(0.065–7.544)	0.769
rs11549467												
GG	151 (87.3)	147 (93.6)	1.00[ref]		130 (87.2)	128 (92.8)	1.00[ref]		21 (87.5)	19 (100)	1.00[ref]	
AG	21 (12.1)	10 (6.4)	0.491 (0.197–1.226)	0.128	18 (12.1)	10 (7.2)	0.546 (0.214–1.393)	0.205	3 (12.5)	0 (0)	0.000	0.999
AA	1 (0.6)	0 (0)	0.000	1.000	1 (0.7)	0 (0)	0.000	1.000	0 (0)	0 (0)	NC	NC
AA + AG	22(12.7)	10 (6.4)	0.467(0.214–1.020)	0.056	19 (38.8)	10 (7.2)	0.568 (0.255–1.265)	0.166	3 (12.5)	0 (0)	0.000	0.999
G allele	323 (93.4)	304 (96.8)	1.00[ref]		278 (93.3)	266 (96.4)	1.00[ref]		45 (93.7)	38 (100)	1.00[ref]	
A allele	23 (6.6)	10 (3.2)	0.488 (0.201–1.185)	0.113	20 (6.7)	10 (3.6)	0.537 (0.217–1.327)	0.178	3 (6.3)	0 (0)	0.000	0.999

HCC hepatocellular carcinoma, *OR* odds ratio, *CI* confidence interval, *ref* reference, *NC* not calculated

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

observed in the healthy HCB population (0.930), but significantly lower than those of JPT, YRI, and CEU populations (0.971, 0.987, and 0.996, respectively).

Discussion

HCC is a multifactorial disease involving a complex interplay between genetic and environmental factors [2]. There are many association studies between SNPs and HCC due to the polygenic model of HCC susceptibility [22, 23]. Genes involved in angiogenesis and hypoxia are HCC susceptibility gene candidates. In this study, we investigated *HIF-1a* gene polymorphisms and determined whether these genetic factors are related to the occurrence of HCC in a Chinese population. The data revealed that there were no significant differences between HCC patients and controls in terms of the distributions of *rs11549465* and *rs11549467* genotypes and alleles (all $P > 0.05$). A haplotype is a set of closely linked genetic markers present on one chromosome which tend to be inherited together more frequently than expected by chance, in a block pattern owing to the presence of linkage disequilibrium. Herein, we conducted haplotype analysis for *HIF-1a rs11549465* and *rs11549467* polymorphisms and found the CG haplotype in the *HIF-1a* gene to be associated with a significantly increased susceptibility to HCC in our study population. In contrast, the CA haplotype might be a protective marker for HCC risk. Although the sample size is not large enough, this is the first study, to the best of our knowledge, to attempt an evaluation of the association between the *rs11549465* and *rs11549467* SNPs of the *HIF-1a* gene and HCC risk in a Chinese population.

HIF-1a, which is located on chromosome 14q21–24 [13], is a key regulator of cellular response to hypoxia and has been suggested to play an important role in tumorigenesis and metastasis [6, 24]. Genetic polymorphisms at *HIF-1a* have been reported to significantly increase transcription activities and expression levels of HIF-1a [5, 14]. Moreover, an increased expression of HIF-1a has been found in most neoplastic hepatocytes [9–11] and has been shown to be associated with increased microvessel density and hepatocarcinogenesis [9].

Several SNP sites have been found in *HIF-1a*. Among them, the *HIF-1a rs11549465* (C1772T) polymorphism, which results in an amino acid changing from proline to serine (P582S), has been widely investigated. Recently, several studies on various ethnicities have revealed that the *rs11549465* polymorphism in *HIF-1a* is associated with different types of malignant tumors, including breast [25], colorectal [26], gastric [27], lung [28], cervical [29], prostate [30], pancreatic [31], oral [32], and renal cell carcinoma [33].

Table 3 Haplotypes distribution in the patients with HCC and healthy controls

Haplotype	Controls ($2n = 346$)	HCC ($2n = 314$)	OR	*P*
CA	24 (0.07)	10 (0.03)	0.416	0.025
CG	311 (0.90)	298 (0.95)	2.327	0.008
TG	10 (0.03)	6 (0.02)	0.494	0.188
TA	0 (0)	0 (0)	–	–

Table 4 Genotype and allele frequencies in healthy control subjects in the present study and from the HapMap Project

SNPs	Present study ($n = 173$) (%)	HCB ($n = 86$) (%)	JPT ($n = 172$) (%)	CEU ($n = 226$) (%)	YRI ($n = 226$) (%)
rs11549465					
CC	162 (93.6)	80 (93.0)	160 (93.0)	194 (85.8)	214 (94.7)
CT	11 (6.4)	4 (4.7)	12 (7.0)	30 (13.3)	12 (5.3)
TT	0 (0)	2 (2.3)	0 (0.0)	2(0.9)	0 (0.0)
P		0.116	0.818	0.034	0.656
C allele	335 (96.8)	164 (95.3)	332 (96.5)	418 (92.5)	440 (97.3)
T allele	11(3.2)	8 (4.7)	12 (3.5)	34 (7.5)	12 (2.7)
P		0.401	0.821	0.008	0.661
rs11549467					
GG	151 (87.3)	74 (86.0)	162 (94.2)	220 (97.3)	224 (99.1)
AG	21 (12.1)	12 (14.0)	10 (5.8)	6 (2.7)	2 (0.9)
AA	1 (0.6)	0 (0.0)	0. (0.0)	0 (0.0)	0 (0.0)
P		0.720	0.008	<0.001	<0.001
G allele	323 (93.4)	160 (93.0)	334 (97.1)	446 (98.7)	450 (99.6)
A allele	23 (6.6)	12 (7.0)	10 (2.9)	6 (1.3)	2 (0.4)
P		0.888	0.021	< 0.001	< 0.001

HCB Han Chinese in Beijing, China; *JPT* Japanese in Tokyo, Japan; *CEU* Utah residents with northern and western European ancestry; *YRI* Yoruba in Ibadan, Nigeria

A recent comprehensive meta-analysis by He et al. [18], which included 10,186 cancer cases and 10,926 healthy controls in 37 case–control studies, suggests that substitution of the C allele with the T allele in the *HIF-1a rs11549465* polymorphism was a risk factor for overall cancer (TT + CT vs. CC OR = 1.23, 95 % CI = 1.03–1.47; TT vs. CT + CC OR = 2.51, 95 % CI = 1.54–4.09; TT vs. CC OR = 2.02, 95 % CI = 1.21–3.39), particularly for cervical (TT vs. CT + CC OR = 8.80, 95 % CI = 2.31–33.52; TT vs. CC OR = 11.49, 95 % CI = 2.21–59.67), head and neck (TT vs. CT + CC OR = 11.29, 95 % CI = 1.24–103.02; TT vs. CC OR = 2.24, 95 % CI = 1.14–4.39), pancreatic (TT vs. CT + CC OR = 4.13, 95 % CI = 1.57–10.86; TT vs. CC 3.39, 95 % CI = 1.28–8.97), and renal cell carcinoma (TT vs. CT + CC OR = 1.55, 95 % CI = 1.02–2.37), while not for colorectal, lung, breast, or prostate cancer.

To our knowledge, only the study by Hsiao et al. [19], focusing on 347 healthy controls and 102 patients with HCC, has estimated the relationship between *rs11549465* and *rs11549467* gene polymorphisms and the risk of HCC in a Taiwanese population. Hsiao et al. found that subjects heterozygous for GA had a 3.97-fold risk (95 % CI = 1.70–9.22) of HCC compared with GG homozygotes after adjusting for other confounders; however, there was no significant association between *rs11549465* gene polymorphisms and HCC susceptibility [19]. The results demonstrated herein corroborate those of Hsiao et al. in that no association between the *rs11549465* polymorphism and HCC was observed. However, no significant association was observed between *rs11549467* gene polymorphisms and HBV-related HCC risk. Furthermore, haplotype analysis revealed the CA haplotype to have a protective effect on the incidence risk of HCC (OR = 0.416, $P = 0.025$). In contrast, individuals with the CG haplotype in *HIF-1a* have a 2.33-fold risk ($P = 0.008$) of developing HCC in the Guangxi population.

We compared the genotype and allele frequencies of *rs11549465* and *rs11549467* SNPs in our control group with those from the HapMap Project. We found that, at the *rs11549465* site, the C allele frequency among the healthy controls in our population was higher than that of the CEU population. On the other hand, the *rs11549465* G allele frequency among the healthy controls was significantly lower than those of the JPT, YRI, and CEU populations. This suggests that the distribution of *HIF-1a* gene frequencies may vary among ethnic groups, which is why we chose the Guangxi population as the control subjects. We hypothesized that the allelic distribution of the *rs11549465* and *rs11549467* polymorphisms may be an important factor contributing to the varying HCC incidence in different regions of the world. Therefore, the findings of our study are valuable for the Guangxi population, and thus, further studies on *HIF-1a* polymorphisms in other ethnic populations would be beneficial.

However, there are some limitations to our study. First, the study population was limited to the Guangxi population, and therefore, our findings may not be generalized to other populations. Second, subjects in the present study were recruited from only one hospital and may not be representative of the entire target population. Third, the patient sample size was small, limiting the value of the data obtained. Fourth, the current research studied only two SNPs in the *HIF-1a* gene; the identification of more SNPs in the *HIF-1a* gene and their correlations with HBV-related HCC would be of interest. Thus, the results presented herein must be interpreted with caution.

Conclusions

In conclusion, we did not find an association between *rs11549465* and *rs11549467* polymorphisms and HBV-related HCC in a Chinese population. However, we 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. Further investigations with a larger sample size may be required to validate the genetic effects of *HIF-1a* polymorphisms on HBV-related HCC.

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Conflict of interest None.

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