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ORIGINAL PAPER

Interleukin-17A and *interleukin-17F* gene polymorphisms and hepatitis B virus-related hepatocellular carcinoma risk in a Chinese population

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Abstract Interleukin (IL)-17A and IL-17F are inflammatory cytokines, which play a critical function in inflammation. Genetic variations in the *IL-17A* and *IL-17F* genes may be associated with a risk of hepatitis B virus (HBV)-related hepatocellular carcinoma (HCC), which is a typical inflammation-related cancer. However, their relationship with HBV-related HCC has not been thoroughly investigated. We conducted a case–control study including 155 patients with HBV-related HCC and 171 healthy controls to assess the association between *IL-17A rs4711998*, *IL-17A rs2275913*, and *IL-17F rs763780* polymorphisms and risk of HCC. Genotypes were determined by polymerase chain reaction–restriction fragment length polymorphism and DNA sequencing. There were no significant differences in the genotype and allele frequencies of *IL-17A rs4711998*, *IL-17A rs2275913*, and *IL-17F rs763780* polymorphisms between the HBV-related HCC patients and healthy controls. However, our results revealed a statistically significant association between the ACA haplotype and increased HCC risk [odds ratio (OR) 1.820, 95 % confidence interval (CI) 1.181–2.624, $P = 0.013$]. In contrast, the GCG haplotype was associated with a significantly decreased risk of HBV-related HCC (OR 0.454, 95 % CI 0.112–0.898, $P = 0.035$). Our results suggest that *IL-17A rs4711998*, *IL-17A rs2275913*, and *IL-17F rs763780* polymorphisms do not contribute to HBV-related HCC susceptibility independently. However, the ACA and GCG haplotypes in the *IL-17* gene might be a risk factor and a protective marker, respectively, for HBV-related HCC in a Chinese population.

Keywords Hepatocellular carcinoma · Haplotype · Interleukin-17 · Polymorphism

Xue-E Xi and Yanqiong Liu have contributed equally to this work.

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Introduction

Hepatocellular carcinoma (HCC) is the fifth most common cancer and ranks as the second most common cause of death from cancer worldwide [1]. In 2012, there were 782,000 estimated new cases (50 % in China alone), and nearly 746,000 deaths from HCC (9.1 % of the total) occurred worldwide [1]. Therefore, it is clear that despite decades of advances in its prevention and treatment, HCC remains a substantial cause of death. It is widely accepted that hepatitis B virus (HBV) and hepatitis C virus infections, smoking, aflatoxin exposure, and excessive alcohol intake are the major contributors to HCC [2–4]. However, HCC is a multifactorial disease involving a complex interplay between genetic and environmental factors. The etiology of HCC, however, remains largely elusive. Thus, further study of the risk factors related to HCC development merits additional consideration. Genetic variations in inflammation-related genes, in particular cytokines, are thought to play a role in the progression of HCC. Recent studies have focused on the roles of inflammation-related genes such as *IL-1*, *IL-2*, *IL-4*, *IL-6*, *IL-8*, *IL-10*, *IL-18*, *IFN-γ*, and *TNF-α* [5–9].

Interleukin-17 (IL-17) is a novel family of pro-inflammatory cytokines composed of six IL-17 family ligands, namely IL-17A (the founding member), IL-17B, IL-17C, IL-17D, IL-17E, and IL-17F [10]. Among the IL-17 family members, IL-17A and IL-17F share the highest amino acid sequence identity (50 %), bind to the same receptor [11], and have a similar function [12, 13]. IL-17A and IL-17F, which are responsible for the pathogenic activity of Th17 cells—a distinct lineage of $CD4^{+}$ effector cells [14]—play a role in a number of biological activities through the induction of multiple pro-inflammatory mediators, including chemokines, cytokines, and metalloproteinases, in epithelial and fibroblast cells [15]. Recently, several studies have demonstrated that over-expression of Th17 and IL-17A levels is associated with HCC development, progression, and poor prognosis [16–19].

Genetic polymorphisms are considered as the main genetic elements involved in the development of common and complex diseases [20]. The human *IL-17A* and *IL-17F* encoding genes are located on human chromosome 6p12.2. Recent genetic studies have investigated the association between polymorphisms in *IL-17A* gene to the susceptibility of various malignancies such as breast [21, 22], lung [23, 24], gastric [25, 26], cervical [27], and colorectal cancers [28, 29]. Nevertheless, to date, little effort has been made to investigate the potential role of *IL-17A* and *IL-17F* genetic polymorphisms in the development of HCC. The purpose of this study is to further evaluate the association between two widely studied *IL-17A* polymorphisms (*rs4711998* and *rs2275913*) and one *IL-17F* polymorphism (*rs763780*), and the risk for the development of HBV-related HCC in a Guangxi, southern Chinese population.

Materials and methods

Study population

This study consisted of 171 control subjects and 155 HBV-related HCC patients. All patients were selected from the First Affiliated Hospital of Guangxi Medical University between May and December 2013. The inclusion and exclusion criteria have been previously described [30]; briefly, all patients were confirmed to have a history of HBV infection of more than 6 months. Cases were diagnosed 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. There were no other hepatitis virus infections such as hepatitis C or hepatitis E. Patients were excluded from the study if they had any of the following conditions: (1) concomitant causes of liver disease or mixed etiologies (hepatitis A virus, hepatitis C virus, hepatitis D virus, or hepatitis E virus, autoimmune hepatitis, primary biliary cirrhosis, alcoholic hepatitis); (2) had a history of autoimmune or inflammatory diseases such as systemic lupus erythematosus, diabetes mellitus, rheumatoid arthritis, or inflammatory bowel disease; (3) had a family history of HCC or other cancers; or (4) were undergoing treatment with antiviral agents, such as nucleotides, or immunomodulators such as interferon-α. 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 had smoked up to 1 year before the date of diagnosis for cases, or up to the date of interview for controls.

The control subjects, confirmed to be HBV free and 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 over the same time period for a 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). Informed consent for genetic analysis was obtained from all participants, and the study was approved by the Ethics Committee of the First Affiliated Hospital of Guangxi Medical University.

IL-17A and *IL-17F* genotyping

Genomic DNA was extracted from 2 mL peripheral blood by using a QIA amp DNA blood mini kit (QIAGEN GmbH, Hilden, Germany), and the samples with absorbance values from 1.8 to 2.0 at the length of A260/A280 were selected for PCR amplification.

The SNPs of *IL-17A rs4711998*, *IL-17A rs2275913*, and *IL-17F rs763780* were retrieved by polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP). 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 Biotech Co., Ltd., China), and 8.5 μL of nuclease-free water. The primers used for amplification and the cycling conditions are listed in Table 1.

For *IL-17A rs4711998*, 10 μL aliquots of the PCR products were digested at 65 °C for 2 h with 1 μL of *Taq*I restriction enzymes. For *IL-17A rs2275913* and *IL-17F rs763780*, 10 μL aliquots of the PCR products were digested at 37 °C for 3 h with 1 μL of *Xmn*I or *Nla*III restriction enzymes, respectively. Digested fragments were separated by electrophoresis in 2 % agarose gel containing GoldView I (Beijing Solarbio Science & Technology Co., Ltd., China) and visualized using a UV transilluminator. To control the quality of genotyping, a negative control with a

Table 1 Primer sequence and the reaction condition for genotyping *IL-17* polymorphisms

Polymorphism	Primer sequence (5′–3′)	The cycling conditions (30 cycles)	Restriction enzyme	Product size (bp)
IL-17A rs4711998	F:5′-TTACACTCCAGCCATTGAGTTG-3′	94 °C, 5 min	*Taq*I	AA 382
	R:5′-TGAAAATGGGGATAGAGACTGG-3′	94 °C, 45 s		AG 382 + 206 + 176
		54 °C, 30 s		GG 206 + 176
		72 °C, 30 s		
		72 °C 10 min		
IL-17A rs2275913	F:5′-CAGAAGACCTACATGTTACT-3′	95 °C, 5 min	*Pdm*I (*Xmn*I)	AA 344
	R:5′-GTAGCGCTATCGTCTCTCT-3′	95 °C, 45 s		AG 344 + 213 + 131
		58.5 °C, 30 s		GG 213 + 131
		72 °C, 40 s		
		72 °C, 5 min		
IL-17F rs763780	F:5′-GCTGGGAATGCAAACAAACACC-3′	94 °C, 5 min	*Hin*1II (*Nla*III)	CC 410
	R:5′-TTCCCATCCAGCAAGAGACCCT-3′	95 °C, 45 s		CT 410 + 291 + 119
		55 °C, 30 s		TT 291 + 119
		72 °C, 30 s		
		72 °C, 10 min		

PCR-amplified DNA product but without the restriction enzymes was used for each genotyping assay.

To confirm the genotyping results, a total of 33 specimens (10 %) were randomly selected and genotyped by DNA sequencing with an ABI Prism 3100 (Shanghai Sangon Biotech Co., Ltd., China). A 100 % concordance rate was achieved.

Statistical analysis

Demographic and clinical data were compared by χ^2 test (or Fisher's exact test, if required) and Student's *t* test for categorical and continuous variables, respectively. Hardy–Weinberg equilibrium was tested with χ^2 test with one degree of freedom to compare the observed genotype frequencies with the expected genotype frequencies among the subjects. Genotype, allele, and haplotype distributions of IL-17 were compared with controls using the χ^2 test and Fisher's exact test, when appropriate. Linkage disequilibrium (LD) and haplotype analyses were performed using SHEsis software [31]. Odds ratio (OR) and 95 % confidence intervals (CIs) were calculated by binary logistic regression and adjusted for age, gender, smoking, and drinking to evaluate the relationship between the polymorphisms and risks of HBV-related HCC. In addition, the frequencies of the *rs4711998*, *rs2275913*, and *rs763780* single-nucleotide polymorphism (SNP) genotypes in the 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 16.0).

Results

Characteristics of the study population

The demographic and clinical characteristics of the cases and control subjects are shown in Table 2. The mean ages (±SD) of the control and HCC groups were 47.72 ± 11.71 and 49.03 ± 11.28, respectively. There was no significant difference between the two groups with respect to age and gender. There were no significant differences for gender and age distributions, smoking status, or alcohol consumption status,

Table 2 Characteristics of the study population

Groups	Controls	HBV-related HCC patients	*P* value
Overall	171	155	
Age (years, mean ± SD)	47.72 ± 11.74	49.03 ± 11.28	0.817
Sex [*N* (%)]			0.636
Male	147 (86.0)	136 (87.7)	
Female	24 (14.0)	19 (12.3)	
Smoking (%)			0.721
No	125 (73.1)	116 (74.8)	
Yes	46 (26.9)	39 (25.2)	
Drinking (%)			0.913
No	119 (69.6)	107 (69.0)	
Yes	52 (30.4)	48 (31.0)	

indicating that the case data were comparable with that of the controls (all $P > 0.05$).

Association of *IL-17A* and *IL-17F* gene polymorphisms and HBV-related HCC risk

The genotype and allele frequencies of *IL-17A rs4711998*, *IL-17A rs2285913*, and *IL-17F rs763780* polymorphisms in the HBV-related HCC patients and the healthy control subjects are shown in Table 3. Genotype distributions of these three SNPs in the case and control subjects were in agreement with those expected under the Hardy–Weinberg equilibrium (all $P > 0.05$). The three SNPs, *IL-17A rs4711998*, *IL-17A rs2285913*, and *IL-17F rs763780*, all showed no significant differences in genotype distribution between the cases and controls ($P > 0.05$).

In logistic regression analyses, the *rs4711998* AG and GG genotypes were not associated with HCC risk compared with the AA genotype ($P = 0.793$ and $P = 0.202$), even after adjustment for age, gender, and smoking and drinking status. In addition, no significant differences were found between the *rs4711998* polymorphism and HCC risk under both the dominant and recessive models ($P = 0.393$ and $P = 0.200$, respectively). Similarly, no significant differences were observed between *IL-17A rs2285913* and *IL-17F rs763780* genotypes and HBV–HCC risk after

Table 3 Genotype and allele frequencies of three SNPs in the *IL-17* gene between HBV–HCC patients and healthy controls

SNPs/model	Allele/genotype	Controls $n = 171$ (%)	HCC $n = 155$ (%)	Logistic regression analysis			
				Crude OR (95 % CI)	*P*	Adjusted OR (95 % CI)*	*P**
rs4711998							
Allele	A	273 (79.8)	250 (80.6)	1.0[ref]		1.0[ref]	
	G	69 (20.2)	60 (19.4)	0.950 (0.645–1.397)	0.793	0.920 (0.599–1.414)	0.704
Codominant	AA	107 (62.6)	104 (67.1)	1.0[ref]		1.0[ref]	
	AG	59 (34.5)	42 (27.1)	0.732 (0.454–1.182)	0.202	0.686 (0.402–1.172)	0.168
	GG	5 (2.9)	9 (5.8)	1.852 (0.601–5.710)	0.277	1.873 (0.545–6.430)	0.319
Dominant	AA	107 (62.6)	104 (67.1)	1.0[ref]		1.0[ref]	
	AG + GG	64 (37.4)	51 (32.9)	0.820 (0.519–1.294)	0.393	0.778 (0.467–1.294)	0.333
Recessive	AG + AA	166 (97.1)	146 (94.2)	1.0[ref]		1.0[ref]	
	GG	5 (2.9)	9 (5.8)	2.047 (0.671–6.245)	0.200	2.113 (0.623–7.164)	0.230
rs2275913							
Allele	A	182 (53.2)	163 (52.6)	1.0[ref]		1.0[ref]	
	G	160 (46.8)	147 (47.4)	1.026 (0.754–1.396)	0.871	1.052 (0.746–1.485)	0.772
Codominant	AA	46 (26.9)	46 (29.7)	1.0[ref]		1.0[ref]	
	AG	90 (52.6)	71 (45.8)	0.789 (0.472–1.318)	0.365	0.688 (0.385–1.230)	0.207
	GG	35 (20.5)	38 (24.5)	1.086 (0.587–2.008)	0.793	1.148 (0.582–2.263)	0.609
Dominant	AA	46 (26.9)	46 (29.7)	1.0[ref]		1.0[ref]	
	AG + GG	125 (73.1)	109 (70.3)	0.872 (0.538–1.413)	0.578	0.816 (0.476–1.400)	0.461
Recessive	AG + AA	136 (79.5)	117 (75.5)	1.0[ref]		1.0[ref]	
	GG	35 (20.5)	38 (24.5)	1.262 (0.749–2.126)	0.381	1.449 (0.815–2.576)	0.206
rs763780							
Allele	T	273 (79.8)	246 (79.4)	1.0[ref]		1.0[ref]	
	C	69 (20.2)	64 (20.6)	1.029 (0.703–1.507)	0.882	0.976 (0.639–1.491)	0.911
Codominant	TT	105 (61.4)	100 (64.5)	1.0[ref]		1.0[ref]	
	CT	63 (36.8)	46 (29.7)	0.767 (0.480–1.225)	0.266	0.263 (0.062–1.116)	0.070
	CC	3 (1.8)	9 (5.8)	3.150 (0.829–11.970)	0.077	0.344 (0.083–1.418)	0.140
Dominant	TT	105 (61.4)	100 (64.5)	1.0[ref]		1.0[ref]	
	CT + CC	66 (38.6)	55 (35.5)	0.875 (0.558–1.373)	0.561	0.871 (0.527–1.440)	0.591
Recessive	TT + CT	168 (98.2)	146 (94.2)	1.0[ref]		1.0[ref]	
	CC	3 (1.8)	9 (5.8)	3.452 (0.917–12.992)	0.052	3.190 (0.782–13.006)	0.106

Ref reference group, *CI* confidence interval, *OR* odds ratio, *SNP* single-nucleotide polymorphism, *n* number

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

binary logistic regression analyses adjusted by age, sex, and smoking and drinking status ($P > 0.05$).

Haplotype analyses of *IL-17* gene polymorphisms and HCC risk

It is believed that haplotype-based analysis may have greater power than SNP genotyping. Therefore, linkage disequilibrium (LD) and haplotype analysis of *IL-17* SNPs was performed 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. Moderate to minor LD was found between the alleles of *rs4711998* and *rs2275913* ($D' = 0.110$), *rs4711998* and *rs763780* ($D' = 0.296$), and *rs2275913* and *rs763780* ($D' = 0.004$). The haplotype distributions in the HBV-related HCC patients and healthy control subjects are shown in Table 4. A total of seven haplotypes were derived from the observed genotypes. The most frequent haplotype in both control subjects and HCC patients was ATA (39.5 and 34.0 %, respectively), but no difference between patients and controls was observed ($P = 0.124$). We found one protective haplotype: GCG (OR 0.454, 95 % CI 0.112–0.898, $P = 0.035$). In contrast, the ACA haplotype was associated with a significantly increased risk of HCC (OR 1.820, 95 % CI 1.181–2.624, $P = 0.013$). The remaining haplotypes were not associated with HCC risk.

The genotype frequencies of *rs4711998*, *rs2275913*, and *rs763780* in the control group were compared with those from the Haplotype Map (HapMap) project [32] (http://www.ncbi.nlm.nih.gov/snp/). Data in Table 5 show the genotype frequencies of the control subjects in the present study and in HCB (Han Chinese in Beijing, Asia), JPT (Japanese in Tokyo, Asia), CEU (Utah residents with Northern and Western European ancestry, Europe), and YRI (Yoruba in Ibadan, Africa) populations. The results indicated that there were no significant differences in the genotype frequencies of these three SNPs in our study compared to those of HCB ($P = 0.304$, 0.165, 0.075, respectively), but were inconsistent with those in the various other ethnic populations. The AA genotype frequency among the healthy controls in the *rs4711998* site in the present study was 62.6 %, which was higher than those of the JPT (50 %), CEU (6.2 %), and YRI (37.2 %) populations. The frequency of the *rs2275913* AA genotypes among the healthy controls was 26.9 %, which was similar to the frequency observed in the healthy JPT population (20.9 %), but significantly higher than those of YRI and CEU populations (12.4 % and 0.0 %, respectively). On the contrary, the TT genotype frequency among the healthy controls in the *rs763780* site in the present study was 61.4 %, which was lower than those of the JPT (75.6 %), CEU (90.3 %), and YRI (84.1 %) populations.

Table 4 Analysis of *IL-17* haplotype frequencies with the risk of HCC

Haplotype	Control frequency	HCC frequency	OR (95 %CI)	*p*
ACA	0.072	0.132	1.820 (1.181–2.624)	**0.013**
ACG	0.074	0.075	1.002 (0.558–1.797)	0.995
ATA	0.395	0.340	0.777 (0.564–1.072)	0.124
ATG	0.257	0.284	1.139 (0.805–1.611)	0.464
GCG	0.055	0.023	0.454 (0.112–0.898)	**0.035**
GTA	0.045	0.064	1.524 (0.773–3.006)	0.221
GTG	0.102	0.082	0.989 (0.595–1.645)	0.967

Boldfaced values indicate a significant difference

Discussion

Carcinogenesis of HCC is a complex, multistep, and multifactorial process involving a complex interplay between genetic and environmental factors [2]. Due to the polygenic model of HCC susceptibility, many association studies between SNPs and HCC have been performed [30, 33, 34]. We have previously demonstrated that the *IL-4*-590C/T and *IL-4*-33C/T [5], *IL-2*+114T/G [35], *IL-6R rs6684439* [36], *IL-23R rs1884444* [37], *hypoxia-inducible factor-1a* [30], and *estrogen receptor alpha rs2234693* [38] markedly elevated the risk of HCC in a high-prevalence region of China (Guangxi). In this study, we identified the relationship between *IL-17A* and *IL-17F* gene polymorphisms and HCC in a high-risk Chinese population. The data revealed that there were no significant differences between HCC patients and controls in terms of the distributions of *IL-17A rs4711998*, *IL-17A rs2275913*, and *IL-17F rs763780* genotypes and alleles (all $P > 0.05$). However, we found that the ACA haplotype was associated with a significantly increased risk of HCC (OR 1.820, 95 % CI 1.181–2.624, $P = 0.013$), whereas the GCG haplotype decreased the susceptibility of HCC (OR 0.454, 95 % CI 0.112–0.898, $P = 0.035$).

IL-17, a relatively novel family of proinflammatory cytokines, plays a role in coordinating local tissue inflammation by inducing release of proinflammatory and neutrophil-mobilizing cytokines [2]. IL-17A and IL-17F are members of the IL-17 cytokine family, which is responsible for the pathogenic activity of IL-17 cells, the lineage of $CD4^{+}$ effector cells, and multiple proinflammatory mediators [14]. *IL-17A* and *IL-17F* are both located at human chromosome 6p12.2 and share similar functions as the basis of their ability to induce chemokines, which are important in neutrophil recruitment and activation [22].

Table 5 Genotype frequencies in healthy control subjects in present study and from the HapMap project

SNPs	Present study N = 171 (%)	HCB N = 86 (%)	JPT N = 172 (%)	CEU N = 226 (%)	YRI N = 226 (%)
rs4711998 genotypes					
AA	107 (62.6)	50 (58.1)	86 (50.0)	14 (6.2)	84 (37.2)
AG	59 (34.5)	30 (34.9)	72 (41.9)	70 (31.0)	116 (51.3)
GG	5 (2.9)	6 (0.7)	14 (8.1)	142 (62.8)	26 (11.5)
P value		0.304	**0.020**	**<0.001**	**<0.001**
rs2275913 genotypes					
AA	46 (26.9)	14 (16.3)	36 (20.9)	28 (12.4)	0 (0.0)
AG	90 (52.6)	52 (60.5)	84 (48.8)	104 (46.0)	30 (13.3)
GG	35 (20.5)	20 (23.3)	52 (30.2)	94 (41.6)	196 (86.7)
P value		0.165	0.093	**<0.001**	**<0.001**
rs763780 genotypes					
TT	105 (61.4)	64 (74.4)	130 (75.6)	204 (90.3)	190 (84.1)
CT	63 (36.8)	22 (25.6)	40 (23.3)	22 (9.7)	36 (15.9)
CC	3 (1.8)	0 (0.0)	2 (1.2)	0 (0.0)	0 (0.0)
P value		0.075	**0.018**	**<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

Boldfaced values indicate a significant difference

SNPs can alter gene functions and protein expression, influencing cell proliferation and increasing cancer risk. The *IL-17A rs4711998*, *IL-17A rs2275913*, and *IL-17F rs763780* polymorphisms are the most common loci associated with IL-17 activity and cancer risk. A few epidemiological studies have been conducted to investigate the association between the *IL-17* gene polymorphisms and the risk of different cancer types. In agreement with our study, Wu et al. [39] reported that there were no apparent relationships between the *IL-17A rs2275913* polymorphism and gastric cancer risk. Furthermore, other studies were also unable to find any significant association between the *IL-17F rs763780* polymorphism and gastric [40, 41], cervical [27], or breast cancer [21] risk. In contrast to the current study, several studies have reported that the *IL-17A rs2275913* and *IL-17F rs763780* polymorphisms were significantly associated with gastric [41], bladder [22], breast [21], and cervical cancer [27]. A recent comprehensive meta-analysis by Niu et al. [42], which included ten relevant case–control studies involving 4,516 cases and 5,645 controls, indicated that the *IL-17A rs2275913* and *IL-17A rs763780* polymorphisms were significantly associated with increased cancer risk, particularly gastric cancer. In 2014, Li et al. conducted the first study reporting a positive relationship between HCC risk and the *IL-17A rs2275913* polymorphism in a Chinese population (GG vs. AA genotype: OR 3.317, 95 % CI 1.663–6.617; G vs. A allele: OR 1.844, 95 % CI 1.311–2.595) [43].

In the present study, we further evaluated the association between HCC risk and another two widely studied *IL-17* SNPs (*IL-17A rs4711998* and *IL-17F rs763780*) in a Guangxi, southern Chinese population. To the best of our knowledge, this is the first study to attempt an evaluation of the association between the SNPs of *IL-17A rs4711998* and *IL-17F rs763780* and HCC risk in a Chinese population. In contrast to previous studies, we did not find a significant association between HCC and *IL-17A rs4711998*, *IL-17F rs763780*, or *IL-17A rs2275913* polymorphisms. 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 LD. In addition, a haplotype harboring the risk alleles can better explain observed associations compared to each polymorphism independently. Herein, we found that the ACA haplotype was associated with a significantly increased risk of HCC (OR 1.820, 95 % CI 1.181–2.624), whereas the GCG haplotype decreased the susceptibility of HCC (OR 0.454, 95 % CI 0.112–0.898).

We further compared the genotype and allele frequencies of *rs4711998*, *rs2275913*, and *rs763780* SNPs in the control group with those from the HapMap Project. It was found that the genotype frequencies of these three SNPs in presented herein were consistent with those of the HCB population, but were significantly different from those in JPT, CEU, and YRI populations. The wild genotype (AA genotype) frequencies in the *rs4711998* and *rs2275913* sites among the healthy controls were significantly higher than those of the JPT, YRI, and CEU populations. On the other hand, the *rs763780* TT genotype frequency among the healthy controls was significantly lower than those of the JPT, YRI, and CEU populations. This suggests that the

distribution of *IL-17A* and *IL-17F* gene frequencies may vary among ethnic groups, which is why we chose the Guangxi population as the control subjects.

It is hard to decipher the exact reasons for the inconsistency of findings between the different studies. The various genetic backgrounds, variant frequencies between races, source of control subjects, sample size, and differing environmental factors (e.g., the various carcinogens that initiate different cancers and the diverse carcinogen exposure in the various populations) might have caused the discrepant results. We hypothesize that the allelic distribution of the *rs4711998*, *rs2275913*, and *rs763780* 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 *IL-17* polymorphisms in other ethnic populations would be beneficial.

Several potential limitations of this study must be acknowledged. First, the patient sample size was not large enough, and therefore, the study's statistical power may have been limited. Thus, additional studies with larger samples are desirable. Second, the study population was limited to the Guangxi population, and therefore, the findings may not be generalized to other populations. Continued study of the role of *IL-17* polymorphisms in patient susceptibility to HCC from other ethnic populations would also be of great value. Finally, subjects in the present study were recruited from only one hospital and may not be representative of the entire target population. Thus, the results presented herein must be interpreted with caution.

Conclusions

In conclusion, an association between *IL-17A rs4711998*, *IL-17A rs2275913*, and *IL-17F rs763780* polymorphisms and HBV-related HCC in a Chinese population was not observed. However, the ACA haplotype was found to be associated with a significantly increased susceptibility to HCC, whereas the GCG haplotype was found to be associated with a significantly decreased risk of HCC in a Guangxi, Chinese population. Further investigations with a larger sample size may be required to validate the genetic effects of *IL-17A* and *IL-17F* polymorphisms on HBV-related HCC.

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Conflict of interest None.

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