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Association between Visfatin Gene rs9034 and rs2505568 Polymorphisms with the Risk of HBV-Related Hepatocellular Carcinoma in a Chinese Population

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Keywords:

Visfatin, gene polymorphisms; Chronic hepatitis B; Liver cirrhosis; Hepatocellular carcinoma

Abbreviations:

NAMPT: Nicotinamide phosphoribosyltransferase; SNPs: single nucleotide polymorphisms; SBE: single-base extension; ORs: Odds ratios; Cis: confidence intervals; HBV: Hepatitis B virus; HCC: Hepatocellular carcinoma; CHB: chronic hepatitis B; LC: liver cirrhosis; NAFLD: non-alcoholic fatty liver disease; IL: interleukin; TNF-α: tumor necrosis factor-alpha; AFP: alpha-fetoprotein; CT: computed tomography; PCR: polymerase chain reaction; ANOVA: analysis of variance; HWE: Hardy–Weinberg equilibrium; LD: linkage disequilibrium

1. Abstract

1.1. Background and Aims: The aim of current study was to investigate whether Visfatin gene polymorphisms could contribute to the susceptibility of HBV-related liver diseases in the Guangxi Chinese population.

1.2. Material and Methods: Two Visfatin single nucleotide polymorphisms (SNPs), rs9034 and rs2505568, were genotyped using the single-base extension method in 140 chronic hepatitis B (CHB) patients, 143 hepatitis B virus (HBV)-related liver cirrhosis (LC) patients, 151 HBV-related hepatocellular carcinoma (HCC) patients, and 132 healthy subjects.

1.3. Results: Subjects carrying the rs2505568 AT genotype showed a significantly greater risk of CHB, LC, and HCC than those carrying the TT genotype. Similarly, carriers of the rs2505568 A allele had elevated risk for CHB, LC, and HCC, in comparison to carriers of the T allele. However, no significant differences in Visfatin rs9034 polymorphisms was observed between the healthy subjects and the patients.

1.4. Conclusion: Our results suggested that Visfatin rs2505568 polymorphisms significantly associate with HBV-related CHB, LC, and HCC risk in the Guangxi Chinese population.

2. Introduction

Hepatocellular carcinoma (HCC) is the most common form of primary liver cancer, accounting for ~90% of all primary liver cancers worldwide, imposing a huge economic burden on both the patients and the society. It is the sixth most common cancer and the fourth leading cause of cancer-related death [1]. The prevalence of HCC varies globally, with a higher incidence in Southeast Asia and sub-Saharan Africa, and a lower incidence in USA and Europe [2]. China alone accounts for approximately 50% of all HCC case [3]. Multiple factors including chronic infection with hepatitis B virus (HBV) or hepatitis C virus (HCV), liver cirrhosis (LC), aflatoxin exposure, alcohol addiction, smoking, obesity, and non-alcoholic fatty liver disease (NAFLD) are believed to increase the risk of HC [4]. Furthermore, recent studies on genetic mutations during HCC development have revealed potential genetic factors that may account for the development of the cancer [5]. Particularly, single nucleotide polymorphism (SNP) changes have been widely studied for association with the risk of HCC development [6].

Numerous candidate-gene studies have reported associations between single nucleotide polymorphisms (SNPs) and the presence of HCC. Genetic variations in inflammation-related genes, particularly cytokine genes, are involved in HCC progression [7]. For instance,

multiple studies have shown that variations in many cytokine genes, including interleukin (IL)-6[8], IL-17, IL-21[9], IL-37[10], and tumor necrosis factor-alpha (TNF-α) [11], are associated with HCC risk. Visfatin, also known as nicotinamide phosphoribosyltransferase (NAMPT) or pre-B-cell colony-enhancing factor, is a proinflammatory and immunomodulating adipocytokine secreted mostly from visceral adipocytes. It can stimulate the expression of TNF-α, IL-1B, and IL-6 and enhance B cells differentiation [12]. In addition, studies have shown that Visfatin expression is up-regulated in patients with HCC and other gastrointestinal cancers [13]. However, the roles of Visfatin polymorphisms and its relationship in HCC have not yet been demonstrated.

The human Visfatin gene maps to a 34.7 kb region on chromosome 7q22.2 and consists of 11 exons and 10 introns [14]. Approximately 52 SNPs in Visfatin gene have been identified [15]. Of these SNPs, rs9034 and rs2505568 located in the 3'untranslated region (3'-UTR) are two important polymorphisms which may influence Visfatin expression [16]. In recent years, several studies have reported an association of Visfatin rs9034 and rs2505568 polymorphisms with the risk of diverse cancers, including bladder cancer [17], esophageal squamous cell carcinoma [18], and pancreatic ductal adenocarcinoma [19]. However, the correlation between Visfatin rs9034 and rs2505568 with the risk of HCC has not been examined. Here, we aim to evaluate whether these two polymorphisms of Visfatin gene associate with HCC risk among the Chinese population in the Guangxi Zhuang Autonomous Region, China.

3. Materials and Methods

3.1. Subjects

All included subjects were Chinese from Guangxi Zhuang Autonomous Region. Prior to enrollment, written consent was collected from all participants. The cohort includes 140 patients with chronic hepatitis B (CHB), 143 patients with HBV-related LC, 151 patients with HBV-related HCC, and 132 healthy participants. All subjects were enrolled in the study from August to December 2019 in the Eighth Affiliated Hospital of Guangxi Medical University. All subjects in the three patients' groups (CHB, LC and HCC) had chronic HBV infections for more than six months, determined by seropositive HBV surface antigen (HBsAg), hepatitis B core antibody (HBcAb), and hepatitis B e antibody or e antigen (HBeAb or HBeAg). Furthermore, elevated levels of alanine aminotransferase (>40 IU/mL) or aspartate aminotransferase (>40 IU/mL) was used to confirm CHB. LC patients was confirmed by morphologic diagnosis based on ultrasonography examination or computed tomography (CT) examination, in combination with laboratory diagnosis. HCC patients were confirmed based on typical cytologic evaluation or histological diagnosis or elevated alpha-fetoprotein (AFP) levels (>400 ng/mL) in combination with CT or ultrasonography. All the included HCC patients were newly diagnosed and free of other type of cancer. 132 healthy subjects were randomly selected from the Health Examination Center. All the healthy individuals had normal liver function and were free of HBV infection.

The study was reviewed and approved by the ethics committee of the Eighth Affiliated Hospital of Guangxi Medical University (Guangxi, China) and all methods were conducted in accordance with the relevant guidelines.

DNA extraction and SNP genotyping

Approximately 2 mL of venous blood was collected from each subject using EDTA-K2 anticoagulant tubes and stored at −20 °C until analysis. Genomic DNA was prepared from the blood samples using a blood DNA extraction (Corning Biotechnology, Jiangsu, China) as described previously [20]. Target genomic DNA fragments were amplified by polymerase chain reaction (PCR), using the following PCR primers: rs9034 (forward: GCAATAGAAGCCAAATGAGA, reverse: ATTATTTAGCCTCCTCCCTT) and rs2505568 (forward: CAAGGGGCAGTAAGGTTAG, reverse: TCTTTTCCAGTGTTTAGGTGA).

Single-base extension (SBE) method was used to genotype the Visfatin SNPs with the SNaPshot Multiplex Kit (Applied Biosystems). The SBE primers for rs9034 and rs2505568 were TTTTTTTTTTTTTTTTTATTAACCTGCCCTTTACACACAAAAT and TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTGCTTTATGTTTATATTTAACTTGTATTTTTGT, respectively. GeneMarkerV1.91 was used for data collection and analysis.

3.2. Statistical Analysis

The statistical analyses were performed using SPSS 25.0 as described previously[21]. For continuous variables, between group variation was analyzed by one-way ANOVA. Chi-square tests or Fisher's exact tests were performed to evaluate categorical variables, including the differences in the genotype and allele distribution among the different groups. Direct counting method was used to determine the genotypic frequencies of the rs9034 and rs2505568 SNPs. The probability of Hardy–Weinberg equilibrium (HWE) was assessed for each SNP by goodness-of-fit chi-square test. The odds ratios (ORs) and 95% confidence intervals (CIs) were calculated utilizing a binary logistic regression model. Then the obtained ORs and CIs were adjusted by age and gender, and were used in association analysis between Visfatin polymorphisms and the risk for HBV-related liver diseases. The haplotype analyses were conducted using the SHEsis program (http://analysis.bio-x.cn/ myAnalysis.php). $P < 0.05$ was considered statistically significant, and all statistical tests were two-sided.

4. Results

4.1. Demographic Characteristics of the Subjects

Table 1 shows the correlation between demographic and clinical characteristics of the patients. Baseline characteristics for gender were matched in all patients and healthy subjects ($P>0.05$). However, there was a statistically significant variation in the mean age between the CHB group and the rest groups. The patients with CHB were overall six years younger, in comparison to the patients with LC or HCC and healthy individuals ($P<0.001$) (Table 1).

Table 1: Basic characteristic of the study population

Variable	Healthy controls (n=132)	CHB patients (n=140)	*P*-value	LC patients (n=143)	*P*-value	HCC patients (n=151)	*P*-value
Age(year,mean±SD)	52.23±14.10	46.44±14.42	**0.001**	54.84±11.75	0.098	53.47±12.19	0.427
Gender,N(%)							
Male	97 (73.48)	108 (77.14)	0.484	108 (75.52)	0.624	124 (82.12)	0.08
Female	35 (26.52)	32 (22.86)		35 (24.48)		27 (17.88)	

4.2. Association between Visfatin SNPs and the Risk of HBV-Related HCC

The allele and genotype frequencies of Visfatin rs9034 and rs2505568 SNPs in the studied groups are provided in Table 2. The frequencies of rs9034 genotype were consistent in all 4 groups with the prediction under HWE (all P>0.05). In contrast, the frequencies of rs2505568 genotype in the 4 groups differed from the prediction under HWE (all P<0.001). Table 2 showed the frequencies in genotype and allele of Visfatin rs9034 and rs2505568 SNPs between the patients groups and the healthy group. After being adjusted by age and gender, binary logistic regression analysis revealed no significant distribution variation of allele and genotype between patients and healthy subjects, for the Visfatin rs9034 polymorphisms. Therefore, there is no association between the Visfatin rs9034 polymorphisms and the risk of CHB, LC, or HCC.

For Visfatin rs2505568, we only found two genotypes: TT and AT. This is consistent with earlier reports that a homozygote for the minor allele at rs2505568 was found absent in Chinese population [22]. Therefore, the association analysis for rs2505568 was only assessed using a dominant genetic model in this study. We found that the carriers of the rs2505568 AT genotype showed a significantly greater risk of CHB, LC, and HCC, compared with the carriers of the TT genotype, with adjusted ORs of 4.009 (95% CI=2.314–6.945, P<0.001), 2.022 (95% CI=1.236–3.308, P=0.005), and 2.101 (95% CI=1.290–3.422, P=0.003), respectively. Similarly, compared with the T allele carriers, the carriers of the rs2505568 A allele displayed increased CHB, LC, and HCC risk, with adjusted ORs of 1.992 (95% CI=1.370–2.897, P<0.001), 1.498 (95% CI=1.033–2.172, P=0.033), and 1.529 (95% CI=1.058–2.210, P=0.024), respectively.

When we separated the patients into male and female groups to perform analysis, we noted that male rs2505568 AT carriers were at increased risk of CHB, LC and HCC, compared to the TT carriers, with adjusted ORs of 3.933 (95% CI=2.103–7.354, P<0.001), 2.524 (95% CI=1.415–4.503, P=0.002), and 2.242 (95% CI=1.292–3.890, P=0.004), respectively. In addition, in males, the male T allele carriers had significantly decreased risk of CHB, LC and HCC, in comparison with the rs2505568 A allele carriers, with adjusted ORs of 1.995 (95% CI=1.294–3.077, P=0.002), 1.680 (95% CI=1.092–2.584, P=0.018), and 1.592 (95% CI=1.047–2.421, P=0.03), respectively. Similarly, the female rs2505568 AT carriers had a significantly increased risk for CHB, but not for LC or HCC, compared to the TT carriers (OR=4.365, 95% CI=1.369–13.924, P=0.013). Moreover, we did not observed variation in the genotype and allele distributions of Visfatin rs9034 among male and female between the disease groups and the healthy group (Table 3 and Table 4).

Considering that the genetic background of Visfatin may be differ in different population, the distribution of genotypes and alleles of rs9034 and rs2505568 SNPs in our control participants were further compared with those in different ethnicities from the 1000 Genomes Project (https://www.ncbi.nlm.nih.gov/variation/tools/1000genomes/). As show in Table 5, the distributions of rs2505568 in our study is dramatically different from all the ethnicities we chosen, including Utah residents with northern and western European ancestry (CEU), Han Chinese in Beijing (CHB), Gujarati Indian from Houston, Texas (GIH), Japanese in Tokyo, Japan (JPT), and Yoruba in Ibadan, Nigeria (YRI) (all p<0.05). As for rs9034, the genotype frequency is similar with that from CEU and JPT, but dramatically different from that in CEU, GIH, YRI.

Table 2: Genotype and allele frequencies of rs9034 and rs2505568 SNPs between HBV-related patients and healthy controls

Polymorphisms	Healthy controls, N=132(%)	CHB patients, N=140(%)	LC patients, N=143(%)	HCC patients, N=151(%)	CHB patients vs. Healthy controls OR (95%CI)[a]	P_{OR}	LC patients vs. Healthy controls OR (95%CI)[a]	P_{OR}	HCC patients vs. Healthy controls OR (95%CI)[a]	P_{OR}
rs9034										
CC	111(84.09)	114(81.43)	120(83.92)	120(79.47)	1		1		1	
CT	20(15.15)	26(18.57)	21(14.68)	28(18.54)	1.172(0.611-2.248)	0.634	0.956(0.490-1.866)	0.896	1.310(0.694-2.470)	0.405
TT	1(0.76)	0(0.00)	2(1.40)	3(1.99)	—	—	2.045(0.179-23.372)	0.565	3.575(0.358-35.691)	0.278
Dominant model[b]	21(15.91)	26(18.57)	23(16.08)	31(20.53)	1.104(0.580-2.104)	0.763	0.85(0.49-1.47)		1.406(0.759-2.604)	0.279
Recessive model[c]	131(99.24)	140(100)	141(98.60)	148(98.01)	—	—	2.057(0.180-23.470)	0.562	3.409(0.342-33.931)	0.296
C allele	242(91.67)	254(90.71)	261(91.26)	268(88.74)	1		1		1	

T allele	22(8.33)	26(9.29)	25(8.74)	34(11.26)	1.025(0.559-1.880)	0.935	1.054(0.578-1.924)	0.864	1.458(0.825-2.575)	0.194
P-$_{HWE}$	0.924	0.226	0.342	0.376						
rs2505568										
Dominant model										
TT	65(49.24)	28(20.00)	46(32.17)	48(31.79)	1		1		1	
AT	67(50.76)	112(80.00)	97(67.83)	103(68.21)	**4.009(2.314–6.945)**	**<0.001**	**2.022（1.236–3.308）**	**0.005**	**2.101（1.290–3.422）**	**0.003**
AA	0(0.00)	0(0.00)	0(0.00)	0(0.00)	—	—	—	—	—	—
T allele	197(74.62)	168(60.00)	189(66.08)	199(65.89)	1		1		1	
A allele	67(25.38)	112(40.00)	97(33.92)	103(34.11)	**1.992（1.370-2.897）**	**<0.001**	**1.498(1.033-2.172)**	**0.033**	**1.529(1.058-2.210**	**0.024**
P-$_{HWE}$	<0.001	<0.001	<0.001	<0.001						

[a]Adjusted by age and gender;

[b]Dominant model: CT+TT versus CC;

[c]Recessive model: TT versus CC+CT.

Table 3: Genotype and allele frequencies of rs9034 and rs2505568 SNPs between HBV-related patients and healthy controls in males

Polymorphisms	Healthy controls, N=97(%)	CHB patients, N=108(%)	LC patients, N=108(%)	HCC patients, N=124(%)	CHB patients vs. Healthy controls		LC patients vs. Healthy controls		HCC patients vs. Healthy controls	
					OR (95%CI)[a]	P_{OR}	OR (95%CI)[a]	P_{OR}	OR (95%CI)[a]	P_{OR}
rs9034										
CC	82(84.54)	87(81.43)	92(85.19)	100(80.64)	1		1		1	
CT	14(14.43)	21(18.57)	14(12.96)	23(18.55)	1.274((0.599-2.710)	0.529	0.895(0.402-1.995)	0.787	1.346(0.651-2.783)	0.422
TT	1(1.03)	0(0.00)	2(1.85)	1(0.81)	-	-	1.994(0.175-22.761)	0.579	0.893(0.054-14.737)	0.937
Dominant model[b]	15(15.46)	21(19.44)	16(14.81)	24(19.35)	1.177(0.560-2.473)	0.667	0.964(0.447-2.079)	0.926	1.317(0.649-2.676)	0.446
Recessive model[c]	96(98.97)	108(100)	106(98.15)	123(99.19)	-	-	2.025(0.178-23.058)	0.57	0.851(0.052-13.997)	0.91
C allele	178(91.75)	195(90.28)	198(91.67)	223(89.92)	1		1		1	
T allele	16(8.25)	21(9.72)	18(8.33)	25(10.08)	1.062(0.531-2.125)	0.865	1.034(0.510-2.096)	0.926	1.258(0.651-2.430)	0.495
rs2505568										
Dominant model										
TT	49(50.52)	22(20.37)	31(32.17)	39(31.45)	1		1		1	
AT	48(49.48)	86(79.63)	77(67.83)	85(68.55)	**3.933 (2.103-7.354)**	**<0.001**	**2.524 (1.415-4.503)**	**0.002**	**2.242 (1.292-3.890**	**0.004**
AA	0(0.00)	0(0.00)	0(0.00)	0(0.00)	—	—	—	—	—	—
T allele	146(75.26)	130(60.19)	139(64.35)	163(65.73)	1		1		1	
A allele	48(24.74)	86(39.81)	77(35.65)	85(34.27)	**1.995 (1.294-3.077)**	**0.002**	**1.680 (1.092-2.584)**	**0.018**	**1.592 (1.047-2.421)**	**0.03**

[a]Adjusted by age and gender;

[b]Dominant model: CT+TT versus CC;

[c]Recessive model: TT versus CC+CT.

Table 4: Genotype and allele frequencies of rs9034 and rs2505568 SNPs between HBV-related patients and healthy controls in females

Polymorphisms	Healthy controls, N=35(%)	CHB patients, N=32(%)	LC patients, N=35(%)	HCC patients, N=27(%)	CHB patients vs. Healthy controls		LC patients vs. Healthy controls		HCC patients vs. Healthy controls	
					OR (95%CI)[a]	P_{OR}	OR (95%CI)[a]	P_{OR}	OR (95%CI)[a]	P_{OR}
rs9034										
CC	29(82.86)	27(81.43)	28(80.00)	20(74.07)	1		1		1	
CT	6(17.14)	5(18.57)	7(20.00)	5(18.52)	0.896(0.239-3.356)	0.871	1.057(0.307-3.640)	0.93	1.200(0.319-4.522)	0.787
TT	0(0.00)	0(0.00)	0(0.00)	2(7.41)	—	—	—	—	—	—
Dominant model[b]	6(17.14)	5(18.57)	7(20.00)	7(25.93)	0.905(0.257-3.36)		1.057(0.307-3.640)	0.93	1.702(0.493-5.877)	0.4
Recessive model[c]	35(100.00)	32(100.00)	35(100.00)	25(92.59)	—	—	—	—	—	—
C allele	64(91.43)	59(92.19)	63(90.00)	45(83.33)	1		1		1	

T allele	6(8.57)	5(7.81)	7(10.00)	9(16.67)	0.905(0.257-3.191)	0.877	1.050(0.330-3.343)	0.934	2.168(0.716-6.566)	0.171
rs2505568 Dominant model										
TT	16(45.71)	6(18.75)	15(42.86)	9(33.33)	1		1		1	
AT	19(54.29)	26(81.25)	20(57.14)	18(66.67)	**4.365 (1.369-13.924)**	**0.013**	1.087 (0.419-2.821)	0.864	1.573 (0.547-4.521)	0.4
AA	0(0.00)	0(0.00)	0(0.00)	0(0.00)	—	—	—	—	—	
T allele	51(72.86)	38(59.38)	50(71.43)	36(66.67)	1		1		1	
A allele	19(27.14)	26(40.62)	20(28.57)	18(33.33)	1.981 (0.940-4.177)	0.072	1.053 (0.499-2.221)	0.893	1.288 (0.589-2.820)	0.526

Table 5: Comparison of genotype and allele frequencies in the healthy control subjects of our study and that from the 1000genomes.

Polymorphisms	Samples, N	Genotype frequency, n			Alleles frequency, n			P values
		CC	CT	TT	P values	C	T	
rs9034								
Present study	132	111(84.09)	20(15.15)	1(0.76)		242(91.67)	22(8.33)	
CEU	99	44(44.44)	40(40.40)	15(15.15)	**<0.001**	128(64.65)	70(35.35)	**<0.001**
CHB	103	87(84.47)	16(15.53)	0(0.00)	0.675	190(92.23)	16(7.77)	0.823
GIH	103	39(37.86)	54(52.43)	10(9.71)	**<0.001**	132(64.08)	74(35.92)	**<0.001**
JPT	104	90(86.54)	12(11.54)	2(1.92)	0.543	192(92.31)	16(7.69)	0.799
YRI	108	36(33.33)	50(46.30)	22(20.37)	**<0.001**	122(56.48)	94(43.52)	**<0.001**
rs2505568		TT	AT	AA		T	A	
Present study	132	65(49.24)	67(50.76)	0(0.00)		197(74.62)	67(25.38)	
CEU	99	15(15.15)	49(49.49)	35(35.35)	**<0.001**	79(39.90)	119(60.10)	**<0.001**
CHB	103	16(15.53)	56(54.37)	31(30.10)	**<0.001**	88(42.72)	118(57.28)	**<0.001**
GIH	103	13(12.62)	42(40.78)	48(46.60)	**<0.001**	68(33.01)	138(66.99)	**<0.001**
JPT	104	25(24.04)	38(36.54)	41(39.42)	**<0.001**	88(42.31)	120(57.69)	**<0.001**
YRI	108	29(26.85)	42(38.89)	37(34.26)	**<0.001**	100(46.30)	116(53.70)	**<0.001**

CEU, Utah residents with northern and western European ancestry; CHB, Han Chinese in Beijing, China; GIH, Gujarati Indian from Houston, Texas; JPT, Japanese in Tokyo, Japan; YRI, Yoruba in Ibadan, Nigeria.

4.3. Haplotype Analysis of the Visfatin SNPs and Risk of HBV-Related Diseases

Haplotype analysis the two polymorphisms was conducted in all 3 disease groups and the healthy control group using the SHEsis program. According to the determined genotypes, we constructed 4 haplotypes (CA, CT, TA, and TT). Very weak linkage disequilibrium (LD) was found between the rs9034 and rs2505568 SNPs within pairwise comparisons of the patient and control groups (all D'<0.3 and r2<0.02). We set the value of 0.03 as the lowest frequency threshold for haplotype analysis, thus the haplotypes with a frequency less than 0.03 were excluded in the analysis. The results showed that the CT was the most common haplotype, accounting for >50% in all four groups, followed by the CA haplotype accounting for 21%-40% in all groups (Table 6). The CA haplotype frequency significantly correlated with increased risk of CHB, LC, and HCC (OR=2.393, 95% CI=1.640–3.491, P<0.001; OR=1.774, 95% CI=1.210–2.600, P=0.003; OR=1.480, 95% CI=1.009–2.172, P=0.044). The TT haplotype also showed significant association with increased CHB risk (OR=2.058, 95% CI=1.025–4.132, P=0.039). In contrast, the CT haplotype associated with significantly decreased risk of CHB, LC, and HCC (OR=0.444, 95% CI=0.312–0.631, P<0.001; OR=0.600, 95% CI=0.422–0.853, P=0.004; OR=0.635, 95% CI=0.447–0.900, P=0.01). However, our data indicated no significant association between the CT haplotype and the risk of LC and HCC (Table 6). Similarly, no significant association was observed between TA haplotype and the risk of HBV-related liver diseases.

Table 6: Frequencies of the haplotypes formed by rs9034 and rs2505568 SNPs in HBV-related patients and healthy controls

Haplotype	Healthy control	CHB patients	OR (95%CI)	p	LC patients	OR (95%CI)	p	HCC patients	OR (95%CI)	p
CA	57(0.218)	112(0.400)	**2.393(1.640-3.491)**	**<0.001**	95 (0.331)	**1.774 (1.210-2.600)**	**0.003**	88 (0.292)	**1.480 (1.009-2.172)**	**0.044**
CT	185(0.699)	142(0.507)	**0.444(0.312-0.631)**	**<0.001**	167 (0.582)	**0.600 (0.422-0.853**	**0.004**	180 (0.596	**0.635 (0.447-0.900)**	**0.01**
TA	10(0.036)	0(0.000)	—	—	2 (0.009)	—	—	15 (0.051)	1.386 (0.604-3.182)	0.439
TT	12(0.047)	26(0.093)	**2.058(1.025-4.132)**	**0.039**	23 (0.079)	1.724 (0.845-3.515	0.13	19 (0.063)	1.362 (0.654-2.834)	0.407

5. Discussion

In this study, we evaluated the association between two selected Visfatin SNPs and the risk of HBV-related liver diseases (CHB, LC, and HCC) among the Chinese population in Guangxi Zhuang Autonomous Region, China. We found that there was a significant association between Visfatin rs2505568 SNP and the risk of CHB, LC, and HCC. Both the A allele and the AT genotype of Visfatin rs2505568 significantly associated with higher risk of CHB, LC, and HCC, compared with the T allele and TT genotype, especially among male individuals. In addition, we found one high-risk haplotype (CA) for CHB (OR=2.393, 95% CI=1.640–3.491, p<0.001), LC (OR=1.774, 95% CI=1.210–2.600, p=0.003), and HCC (OR=1.480, 95% CI=1.009–2.172, p=0.044); one protective haplotype (CT) for CHB (OR=0.444, 95% CI=0.312–0.631, p<0.001), LC (OR=0.600, 95% CI=0.422–0.853, p=0.004), and HCC (OR=0.635, 95% CI=0.447–0.900, p=0.01); and one CHB-specific high-risk haplotype (TT) (OR=2.058, 95% CI=1.025–4.132, p=0.039). However, no significant association was found between the rs9034 SNP and the risk of these liver diseases.

The rs2505568 and rs9034 locate in the 3'untranslated region (3'-UTR) of the Visfatin gene. These two SNPs are potential binding sites for miRNAs which may affect the binding of miRNAs to target genes, leading to decreases or increases in translation of the target mRNA, thereby alternating disease susceptibility [23]. Thus far, only three studies have investigated the association of Visfatin rs9034 and rs2505568 polymorphisms with cancer risk [24]. The first study reported the association of Visfatin rs9034 and rs2505568 polymorphisms with bladder cancer risk [16]. In this study, a cohort of 407 bladder cancer patients and 316 healthy individuals was analyzed and the results showed that the A allele and AT genotype in rs2505568 could significantly decrease the risk of bladder cancer, whereas the association of Visfatin rs9034 polymorphism with bladder cancer risk was not statistically significant. However, rs9034 polymorphism was significantly associated with recurrence-free death of bladder cancer patients. The second study investigated the association between Visfatin gene polymorphisms and the incidence of esophageal squamous cell carcinoma (ESCC) in 405 patients with ESCC and 405 healthy controls in the Chinese population [25]. It was found that the Visfatin gene rs2505568 and rs9034 loci were all polymorphic, but the polymorphisms in rs2505568 and rs9034 were not significantly associated with the incidence of ESCC. The third study analyzed 273 patients with pancreatic ductal adenocarcinoma (PDAC) and 263 healthy controls and revealed no apparent relationships between Visfatin gene rs2505568 and rs9034 polymorphisms and PDAC risk [26]. In addition, Visfatin polymorphisms were found to be related to dilated cardiomyopathy (DCM) risk [16]. The T allele and CT genotype of rs9034 were associated with increased risk of DCM, whereas the A allele and AT genotype of rs2505568 were associated with decreased DCM risk. The reason for the different results on the association of Visfatin polymorphisms with diseases between these studies and ours may due to the different function of Visfatin in different type of cells and tissues, therefore the polymorphisms have different effects in type of diseases.

Many studies have demonstrated that HBV-related liver diseases are significantly more common in men than in women. In our current study, a sex stratified analysis demonstrated a gender-dependent effect on the degree to which the Visfatin rs2505568 polymorphism influences the risk of CHB, LC, and HCC. Male individuals carrying the rs2505568 AT genotype and A allele had a significant increase in risk of LC and HCC, whereas this association was not found in female individuals. The underlying mechanisms of this gender difference are unclear. It is possible that the limited number of patients in the study may account for the observed high risk in males with the polymorphism. In addition, male individuals tend to have more exposure to tobacco, excessive alcohol, which are known risk factors for HCC. As such, the differences in life style and/or gene-environment interactions between male and female patients may contribute to the differences observed.

Haplotype analysis is often more powerful than single-marker analysis for identification of genetic variants of complex diseases [27]. Therefore, further haplotype analyses were conducted to assess whether the rs2505568 and rs9034 polymorphisms associate with the risk of HBV-related diseases. Our data suggested that the CA haplotype is a potential risk factor for CHB, LC, and HCC, and the TT haplotype is a potential risk factor for CHB. In contrast, the CT haplotype might have protective effect against CHB, LC, and HCC. The variable influence of different haplotypes on disease association may due to the different function of these haplotypes in induction of splicing variants or microRNA binding, which in turn differentially regulate Visfatin expression or influence gene interactions, thus resulting in an increased or decreased disease risk association [28].

To our knowledge, this is the first study on the association between Visfatin rs9034 and rs2505568 polymorphisms and HBV-related liver diseases. However, there were some potential limitations in current study. First, the study participants were from a single hospital and were limited to the same region. Therefore, the findings of this study may not be applicable to other populations. Second, the relatively small sample size may limit the statistical power. In addition, only two SNPs of the Visfatin gene were investigated. Lastly, due to lack of information of dietary habits, lifestyle and environmental carcinogen exposure, we were unable to assess gene-environment interaction in terms of HBV-related liver disease susceptibility. Additional larger sample size studies from diverse populations and with comprehensive related information are warranted to corroborate our current findings.

In summary, the Visfatin rs2505568 polymorphism showed a strong association with risk of CHB, LC, and HCC, among the studied Chinese population in Guangxi district, especially among males. The CA haplotype might be a risk factor for CHB, LC, and HCC; the TT haplotype might be a risk factor for CHB; whereas the CT haplotype is

associated with decreased risk for CHB, LC, and HCC in the studied population. Therefore, the Visfatin gene rs2505568 polymorphism can serve as an important genetic marker for screening high-risk groups with HCC and could be a new target for genetic intervention or therapy for HBV-related diseases.

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