Heliyon 10 (2024) e23660

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

The effect of FOXP3 genetic polymorphisms on correlations with hepatitis B virus-hepatocellular carcinoma: A case-control study

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ARTICLE INFO

Keywords:
FOXP3
Hepatocellular carcinoma
SNP
Case–control study

ABSTRACT

Background: Previous studies have reported that transcription factor forkhead box protein 3 (FOXP3) polymorphisms are correlated with the progress of some cancers, but the relationships between the FOXP3 polymorphisms and hepatocellular carcinoma (HCC) risk remain unclear.
Method: Genotypes were detected in156 hepatitis B virus (HBV)-HCC patients, 109 HBV-liver cirrhosis (LC) patients, 125 chronic hepatitis B (CHB) patients, and 188 healthy controls. The FOXP3 rs3761547 and rs3761548 polymorphisms were genotyped by polymerase chain reaction (PCR) combined with restriction fragment length polymorphism, and the rs2232365 polymorphism was genotyped using PCR with sequence-specific primers.
Results: We did not obtain any significant results with the FOXP3 rs3761547, rs3761548, and rs2232365 polymorphisms in groups of patients compared to healthy controls (all $p > 0.05$), no matter the overall group or subgroup.
Conclusions: Our findings suggest that the FOXP3 polymorphisms at rs3761547, rs3761548, and rs2232365 were not related to HBV-HCC risk in the Chinese population.

1. Introduction

Hepatocellular carcinoma (HCC) is the 3rd major cause of cancer death and 6th most common cancer worldwide accounting for an estimated 830,180 deaths and 905,677 new cases, in accordance with the International Agency for Research on Cancer Global Cancer Statistics 2020 [1]. China is a region with a high prevalence of HCC. It was estimated that there were 391,152 deaths and 410,038 new cases of HCC in China in 2020, making up 45.3 % of cancers diagnosed and 47.1 % of deaths worldwide, respectively [2]. Therefore, it is crucial to identify risk factors to develop early prevention strategies and promote a better understanding of the pathogenesis of HCC. Currently, the key risk factors for HCC consist of the infection of HBV or HCV, food contaminated with aflatoxin, obesity, heavy alcohol consumption, smoking, and type 2 diabetes [3–5]. However, only a fraction of people eventually develop HCC after exposure to these recognized risk factors, may be due to differences in age, sex, host genetics, and other environmental mediators. More powerful and novel approaches for determining the genetic factors of predisposition are expected to offer novel insights into the tumor microenvironment participating in tumorigenesis and progression.

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https://doi.org/10.1016/j.heliyon.2023.e23660
Received 30 March 2023; Received in revised form 23 November 2023; Accepted 9 December 2023
Available online 12 December 2023

Regulatory T cells (Treg cells) are key components of the tumor microenvironment (TME), and Treg cells in TME are suggested as checkpoint that block tumor immune surveillance and inhibit anti-tumor immune responses [6,7]. The forkhead box protein 3 (FOXP3) belongs to the forkhead transcription factor family and is mainly localized in the nucleus [7]. The FOXP3 is considered to be a particular molecular marker of the Treg cells [8]. It is suggested that the modulation, activation, differentiation as well as function of Treg cells depend on the expression of FOXP3 [9]. Moreover, aberrant FOXP3 expression is associated with the progression of a variety of lesions, including Crohn's disease, coronary artery disease, and various cancers [10–12]. In 2017, Shi et al. suggested that FOXP3 was upregulated in HCC and acted as a suppressor gene that reduced the HCC proliferation and invasion in vitro and suppressed the growth of tumor in vivo. Additionally, significantly upregulated FOXP3 expression was related to low levels of serum alpha-fetoprotein (AFP), non-vascular invasion, superior survival together with lower recurrence rates, and served as an independent prognostic factor for patients with HCC [13]. FOXP3 serves a critical role in the progression of HCC, and this finding underscores the significance of identifying FOXP3 gene variants, as single nucleotide polymorphisms (SNPs) can change protein expression and gene function [14].

The human FOXP3 gene is mapped to the X chromosome at Xp11.23, and has been confirmed to have numerous SNPs in the exon, intron, and promoter regions [10,15]. Previous studies on different ethnic groups have shown that polymorphisms in the FOXP3 gene are associated with the development of various cancers, such as prostate cancer [16], colorectal cancer [17], breast cancer [18], gastric adenocarcinoma [19], thyroid cancer [20], non-small cell lung cancer [21], endometrial cancer [22], and cervical cancer [23]. To date, only one study has investigated the underlying role of two FOXP3 promoter region SNPs (rs3761549 and rs2280883) in the progression of HCC in a Chinese population [24]. However, the influences of the other three FOXP3 promoter region SNPs (rs3761547, rs3761548, and rs2232365) to HCC progression remained not well-investigated. In present study, we focused on three SNPs of FOXP3 rs3761547, rs3761548, and rs2232365, and try to explore their correlations to CHB, HBV-LC, and HBV-HCC risk in the population from Guangxi (southern China).

2. Materials and methods

2.1. Study population

Our research consisted of 390 patients with HBV-associated liver disease who were evaluated at the First Affiliated Hospital of Guangxi Medical University (Guangxi, China). Of these, 156, 109, and 125 for patients with HBV-associated HCC, HBV-associated LC, and CHB, respectively. All of those patients were identified as having positive hepatitis B surface antigen (HBsAg) results for at least 6 months. CHB was described further as HBV-DNA positivity and continued elevated levels of alanine aminotransferase (ALT) or aspartate aminotransferase (AST). The diagnosis of HBV-LC was on the basis of a combination of pathological and imaging examinations, and all these HBV-LC patients had backgrounds of suffering from CHB. All HBV-HCC patient diagnoses were confirmed by either pathological or cytological findings or AFP level $>$400 ng/mL along with a minimum of one positive liver imaging finding on ultrasonography, magnetic resonance imaging and computed tomography, and all these HBV-HCC patients had backgrounds of suffering from HBV-LC.

Patients were excluded if they had other factors causing liver disease (e.g., alcoholic hepatitis, autoimmune liver disease, primary biliary cirrhosis, or alcoholic cirrhosis), or suffered from other types of viral hepatitis (e.g., hepatitis A/C/D/E), or had other concomitant malignancies. Patients enrolled in this research had not received antiviral therapy, immunotherapy, radiotherapy, or chemotherapy before hospitalization.

During patient recruitment, 188 healthy volunteers who underwent routine physical examinations at a Physical Examination Center were randomly selected as the control group. The controls included in this study were required to meet the following conditions: testing negative for HBsAg and HBV-DNA, showing no clinical evidence of liver disease, and displaying no history of tumors or other major diseases.

The demographic and laboratory parameters of each subject were collected from their electronic medical records. The demographic parameters included age, gender, body mass index (BMI), smoking status, drinking status, and ethnicity. The laboratory parameters included serum levels of AFP, AST, ALT, total bilirubin (TBIL), gamma glutamyl transpeptidase (GGT), total protein (TP), as well as albumin (ALB). Smokers were defined as those who smoked one or more cigarettes per day for six consecutive months. The subjects were considered alcohol drinkers if they drank for up to one year before they were enrolled in our study. All of the participants gave their written informed consent. The research procedures were approved by the ethics committee of the First Affiliated Hospital of Guangxi Medical University (Guangxi, China).

2.2. DNA extraction

After overnight fasting, all the subjects' peripheral venous blood samples were gathered in EDTA-K2 anticoagulated tubes. Genomic DNA was separated from the venous blood through applying a phenol-chloroform methodology as described before [25] and kept under a temperature of -20 °C for the further genotyping.

2.3. FOXP3 genotyping

The genotypes of the FOXP3 SNPs rs3761547 and rs3761548 were detected via employing polymerase chain reaction (PCR) in combination with the restriction fragment length polymorphism. The FOXP3 rs2232365 polymorphism was genotyped using PCR with sequence-specific primers. The PCR was performed with DreamTaq Green PCR Master Mix (2x) (12.5 μL, Thermo Scientific, Waltham,

MA, USA), nuclease-free water (7.5 μL), genomic DNA (3.0 μL), and primers (2 μL). The amplified product was digested with *Sse*9I and *Pst*I for the rs3761547 and rs3761548 polymorphisms, respectively. The primer sequences and reaction conditions used for the PCR are listed in Table 1 [26]. PCR products and restriction fragments were isolated with GoldView I on a 3 % agarose gel (Fig. 1). The genotype was assigned based on the product size.

Additionally, we randomly selected 60 samples (approximately 10 %) and genotyped them through DNA sequencing using an ABI-PRISM3730 instrument (Applied Biosystems, Foster City, CA, USA). DNA sequencing yielded 100 % concordance.

2.4. Statistical analysis

The data were analyzed with the SPSS 17.0 software, and $P < 0.05$ was deemed to be statistically significant. The differences in BMI, AFP, AST, ALT, TBIL, GGT, TP, and ALB were tested using the Kruskal-Wallis H test. An $\chi 2$ test was used to analyze the differences in terms of age, gender, drinking status, smoking status, ethnicity, and allele distribution among the controls and cases. Conformity of allele distribution to Hardy–Weinberg equilibrium (HWE) was tested by the Haploview software. The relationship between FOXP3 genetic polymorphisms and liver diseases was assessed by binary logistic regression, and the risk was evaluated with adjusted odds ratio (OR) and 95 % confidence intervals (CI).

3. Results

3.1. Population characteristics

In this study, 125 patients with CHB, 109 patients with HBV-associated LC, 156 patients with HBV-associated HCC, and 188 healthy volunteers were genotyped for the three polymorphisms in the FOXP3 gene. Table 2 illustrates the main demographic parameters of the study population. No significant body mass index (BMI) difference was found between the controls and cases ($P > 0.05$). Nonetheless, there were remarkable differences regarding the distributions of age, gender, smoking status, drinking status, and ethnicity among the four groups (all $P < 0.05$). In addition, the HBV-infected patients had markedly higher levels of AFP, AST, ALT, TBIL, and GGT and lower TP and ALB levels in comparison with controls (all $P < 0.001$).

3.2. FOXP3 polymorphisms and liver disease risk

The genotype and allele frequencies of the FOXP3 rs3761547, rs3761548, and rs2232365 polymorphisms in the controls and cases are displayed in Table 3. For male, they carry only one copy on the X chromosome for FOXP3 SNPs. The allele distributions of rs3761547 ($P = 0.451$) and rs3761548 ($P = 0.051$) were in HWE in the control group whereas the rs2232365 were not in HWE ($P < 0.05$). There were no apparent differences noted in the distribution of alleles of rs3761547, rs3761548, and rs2232365 among the four groups (all $P > 0.05$).

Binary logistic regression analyses have not revealed any remarkable correlations between the FOXP3 rs3761547 polymorphism and the risk of HCC, LC, and CHB after adjusting for age, BMI, gender, smoking, drinking, and ethnicity (CHB vs. Controls: OR = 1.735, 95 % CI = 0.985–3.057, $P = 0.056$; LC vs. Controls: OR = 1.018, 95 % CI = 0.590–1.757, $P = 0.949$; HCC vs. Controls: OR = 1.146, 95 % CI = 0.679–1.934, $P = 0.610$). Similarly, nonsignificant findings were also observed with respect to the FOXP3 rs3761548 (CHB vs. Controls: OR = 1.573, 95 % CI = 0.843–2.937, $P = 0.155$; LC vs. Controls: OR = 0.968, 95 % CI = 0.494–1.894, $P = 0.924$; HCC vs. Controls: OR = 1.141, 95 % CI = 0.604–2.154, $P = 0.684$) and rs2232365 (CHB vs. Controls: OR = 1.272, 95 % CI = 0.787–2.058, $P = 0.326$; LC vs. Controls: OR = 0.967, 95 % CI = 0.610–1.531, $P = 0.885$; HCC vs. Controls: OR = 0.926, 95 % CI = 0.590–1.453, $P = 0.739$) polymorphisms.

Table 1
PCR primer sequences and reaction conditions used for FOXP3 genotyping.

SNPs	Primer sequence (5′→3′)	Annealing temperature	Method	Product size, bp
rs3761547	F: CAGCCAGAGACCAGCAAATG R: TTGCCCTTCTACACTGAGCA	60 °C	PCR-RFLP	AA: 192 + 109 AG: 301 + 192 + 109 GG: 301
rs3761548	F: TGCTCAGTGTAGAAGGGCAA R: ACATGCCTCCATCATCACCA	62 °C	PCR-RFLP	CC: 220 + 134 CA: 354 + 220 + 134 AA: 354
rs2232365 (A Allele)	F: CCCAGCTCAAGAGACCCCA R: GGGCTAGTGAGGAGGCTATTGTAAC	64 °C	PCR-SSP	442
rs2232365 (G Allele)	F: CCAGCTCAAGAGACCCCG R: GCTATTGTAACAGTCCTGGCAAGTG	58 °C		427

SNP,single-nucleotide polymorphism; PCR-RFLP, polymerase chain reaction-restriction fragment length polymorphism.
PCR-SSP, polymerase chain reaction-sequence specific primer.

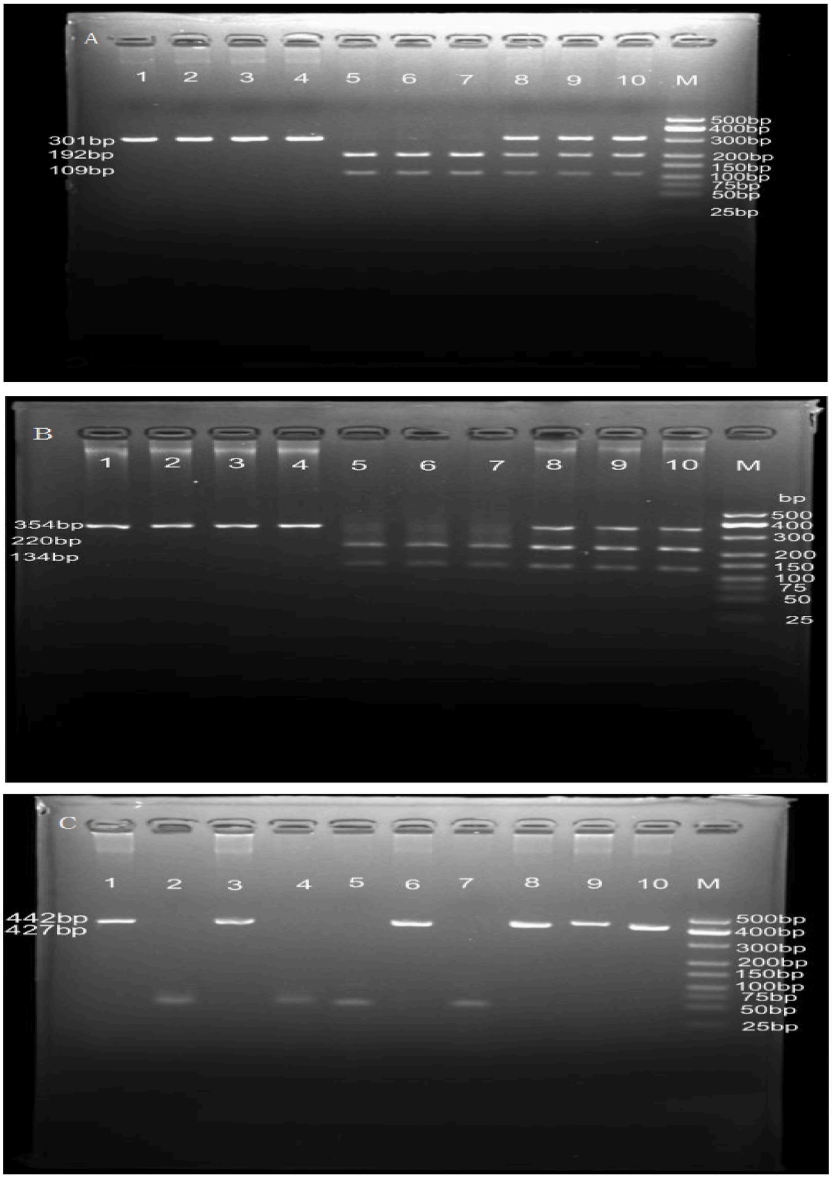

Fig. 1. Genotyping of the FXOP3 Gene. A, rs3761547: lanes 1, 2, 3, and 4 were GG genotype; lanes 5, 6, and 7 were AA genotype; lanes 8, 9, and 10 were AG genotype. B, rs3761548: lanes 1, 2, 3, and 4 were AA genotype; lanes 5, 6, and 7 were CC genotype; lanes 8, 9, and 10 were CA genotype. C, rs2232365: lanes 1and 2, 3 and 4 were AA genotype; lanes 5 and 6, 7 and 8 were GG genotype; lanes 9 and 10 was AG genotype; lane M was DNA marker.

3.3. Subgroup analyses

For the purpose of elucidating the effect of FOXP3 gene polymorphisms in HCC demographic parameters, the respective SNPS were optimized to identify their associations with the demographic parameters. Tables 4–8 reveal the findings of the subgroup analyses according to age, gender, smoking, drinking, and ethnicity. When participants were classified as subgroups according to age, logistic regression analyses showed no significant difference between subjects $\leq$50 years and >50 years after adjusting for BMI, gender, smoking, drinking, and ethnicity. Similarly, no effects of gender, smoking, drinking, or ethnicity on the correlation between the risk of HCC and the polymorphisms of FOXP3 were determined.

4. Discussion

To the best of our knowledge, the current work is the first study to involve the association between the FOXP3 rs3761547, rs3761548, and rs2232365 polymorphisms and the incidence of HCC in the population from Guangxi. In the present case-control

Table 2
Characteristics of study population.

Parameters	Controls	CHB	LC	HCC	*P*
Overall	188	125	109	156	
Demographic parameters					
Age, n (%)					<0.05
≤50 years	129 (68.62)	106 (84.80)	58 (53.21)	93 (59.62)	
>50 years	59 (31.38)	19 (15.20)	51 (46.79)	63 (40.38)	
BMI (mean ± IQR)	22.62 ± 4.01	21.30 ± 5.32	22.58 ± 5.05	21.90 ± 4.98	0.087
Gender, n (%)					<0.05
Female	96 (51.06)	29 (23.2)	24 (22.02)	16 (10.26)	
Male	92 (48.94)	96 (76.8)	85 (77.98)	140 (89.74)	
Smoking, n (%)					<0.05
Yes	60 (31.91)	54 (43.20)	51 (46.79)	49 (31.41)	
No	128 (68.09)	71 (56.80)	58 (53.21)	107 (68.59)	
Drinking, n (%)					<0.05
Yes	47 (25.00)	62 (49.60)	41 (37.61)	48 (30.77)	
No	141 (75.00)	63 (50.40)	68 (62.39)	108 (69.23)	
Ethnicity, n (%)					<0.05
Han	87 (46.28)	70 (56.00)	67 (61.47)	88 (56.41)	
Zhuang	90 (47.87)	49 (39.20)	39 (35.78)	64 (41.03)	
Others	11(5.85)	6 (4.80)	3 (2.75)	4 (2.56)	
Laboratory parameters (median ± IQR)					
AFP (ng/mL)	3.25 ± 2.24	21.03 ± 78.27	8.79 ± 28.38	68.57 ± 3584.85	<0.001
AST (IU/L)	15.00 ± 8.00	65.00 ± 89.50	60.00 ± 58.5	46.50 ± 42.50	<0.001
ALT (IU/L)	15.00 ± 7.00	77.00 ± 157.5	39.00 ± 47.5	41.00 ± 31.75	<0.001
TBIL (mmol/L)	5.65 ± 3.38	39.3 ± 94.75	44.60 ± 81.75	15.75 ± 12.33	<0.001
GGT (IU/L)	20.00 ± 11.00	119.00 ± 117.5	71.00 ± 87.5	89.50 ± 130.25	<0.001
TP (g/L)	74.50 ± 9.30	65.70 ± 10.25	63.20 ± 12.75	65.40 ± 8.90	<0.001
ALB (g/L)	40.15 ± 4.93	35.30 ± 7.80	27.70 ± 7.00	36.30 ± 6.78	<0.001

CHB, chronic hepatitis B; LC, liver cirrhosis; HCC, hepatocellular carcinoma; BMI, body mass index; IQR, interquartile range.

research, we investigated the distribution of rs2232365, rs3761548 and rs3761547 SNPs in the FOXP3 gene together with their association with HCC, LC, and CHB in 578 Chinese subjects. However, we failed to find a correlation between the FOXP3 alleles and the HBV-infected patients. Our results suggest that FOXP3 genetic polymorphisms may not be an important contributor to HCC, at least in Guangxi Chinese subjects.

FOXP3 is expressed mostly on Treg cells and serves as a "master switch" for Treg cells polarization and repressive functions [27]. Elevated levels of FOXP3+ Treg cells in tumor tissues and peripheral blood have been confirmed in patients with multiple types of cancer [28–30]. Recently, FOXP3 has also been reported to be expressed in the tumor cells and exerts a vital role in cancer progression [31–33]. The function of FOXP3 is originated from the FOXP3 gene polymorphism. Genetic polymorphisms in FOXP3 may alter FOXP3 functionally or quantitatively, thereby modulating disease risk [26]. SNPs in FOXP3 gene have been detected in various cancers, for example, prostate cancer [16], colorectal cancer [17], breast cancer [18], gastric adenocarcinoma [19], thyroid cancer [20], non-small cell lung cancer [21], endometrial cancer [22], and cervical cancer [23].

Previous studies on the association between cancer risk and the polymorphism of FOXP3 gene have mainly focused on SNPs rs3761548, rs3761549, and rs2280883. In 2013, Zheng et al. from China identified that FOXP3 rs2294021 polymorphism was related to the breast cancer risk but found no considerable relationship for the SNPs rs3761547, rs3761548, rs3761549, and rs2280883 [34]. In2014, Chen et al. found that subjects carrying a minimum of one FOXP3 rs3761548 A allele was linked to colorectal carcinogenesis among Chinese population [17]. Additionally, a case-control research of controls and patients with lung cancer in 2015 in southern Iran revealed that the rs3761549 T allele enhanced the occurrence of lung cancer and that the rs2280883 T allele was linked to a high lung cancer risk in elderly patients [35]. Furthermore, Jiang et al. demonstrated that FOXP3 rs3761548 AA/AC genotype was related to a high risk of the differentiated thyroid cancer, whereas genotype CC/CT in cases of rs2280883 decreased the aforementioned risk in a 2017 study of Han people in China [20]. In addition, You et al. indicated that the FOXP3 rs3761548 and rs5902434 polymorphisms were connected to a lower endometrial cancer risk among Han Chinese women [22]. The inconsistency of the above findings was then addressed by a comprehensive meta-analysis [36], which showed no significant results for the rs3761548 polymorphism in the overall population and breast cancer group, although an elevated cancer risk was found for the A allele and AA genotype in the Chinese population (A vs. C: OR = 1.34, 95 % CI = 1.00–1.78; AA vs. AC + CC: OR = 1.61, 95 % CI = 1.09–2.39; AA vs. CC: OR = 1.74, 95 % CI = 1.05–2.89). Nevertheless, there was no apparent correlation detected between cancer susceptibility and the polymorphisms of rs2280883 and rs3761549.

To date, only a study by Chen et al., in 2013 [24], which focused on 344 CHB patients, 392 HCC patients, and 372 matched controls, has explored the influence of the FOXP3 rs2280883 and rs3761549 polymorphisms on HCC risk in a population of Chinese. Their data displayed that the FOXP3 rs2280883 and rs3761549 polymorphisms may be associated with HCC. Thus far, the connection between the FOXP3 polymorphisms at rs3761547, rs3761548, and rs2232365 and HCC risk has not been investigated. Here, both the analyses of the overall group and the subgroup stratified by age, gender, smoking, drinking, and ethnicity proposed no significant correlation between the rs3761547, rs3761548, and rs2232365 polymorphisms and HCC risk in Chinese subjects.

Table 3
Association analysis of FOXP3 polymorphisms between cases and controls.

	Controls	CHB	LC	HCC	CHB vs. Controls		LC vs. Controls		HCC vs. Controls	
SNPs	N = 188	N = 125	N = 109	N = 156	OR (95 % CI)[a]	P^a	OR (95 % CI)[a]	P^a	OR (95%CI)[a]	P^a
rs3761547										
Genotype (F)										
AA	63	12	14	8						
AG	25	14	3	6						
GG	8	3	7	2						
Allele (M)										
A	71	79	73	110						
G	21	17	12	30						
Allele (F + M)										
A	222	117	104	132	1^{ref}		1^{ref}		1^{ref}	
G	62	37	29	40	1.735 (0.985–3.057)	0.056	1.018 (0.590–1.757)	0.949	1.146 (0.679–1.934)	0.610
rs3761548										
Genotype (F)										
CC	77	16	19	12						
CA	15	7	1	2						
AA	4	6	4	2						
Allele (M)										
C	78	81	76	121						
A	14	15	9	19						
Allele (F + M)										
C	247	120	115	147	1^{ref}		1^{ref}		1^{ref}	
A	37	34	18	25	1.573 (0.843–2.937)	0.155	0.968 (0.494–1.894)	0.924	1.141 (0.604–2.154)	0.684
rs2232365										
Genotype (F)										
AA	22	1	7	1						
AG	68	20	9	14						
GG	6	8	8	1						
Allele (M)										
A	55	64	58	94						
G	37	32	27	46						
Allele (F + M)										
A	167	86	81	110	1^{ref}		1^{ref}		1^{ref}	
G	117	68	52	62	1.272 (0.787–2.058)	0.326	0.967 (0.610–1.531)	0.885	0.926(0.590–1.453)	0.739

F, female; M, male.
[a] Adjusting for age, BMI, gender, smoking, drinking, and ethnicity by logistic regression.

Table 4
Distribution frequency of FOXP3 polymorphisms in HCC patients and controls stratified by age.

	Controls	HCC	≤50 years		Controls	HCC	>50 years	
Allele	N = 129	N = 93	OR(95 % CI)[a]	P^a	N = 59	N = 63	OR(95 % CI)[a]	P^a
rs3761547								
A	164	80	1^{ref}		58	52	1^{ref}	
G	37	22	1.272 (0.621–2.604)	0.511	25	18	1.02 (0.454–2.290)	0.961
rs3761548								
C	174	86	1^{ref}		73	61	1^{ref}	
A	27	16	1.08 (0.485–2.402)	0.851	10	9	1.369 (0.439–4.271)	0.588
rs2232365								
A	123	66	1^{ref}		44	44	1^{ref}	
G	78	36	0.949 (0.522–1.725)	0.864	39	26	0.869 (0.412–1.833)	0.713

[a] Adjusting for BMI, gender, smoking, drinking, and ethnicity by logistic regression.

There are several shortcomings that should be considered in our study. First, the small sample size of the overall group and subgroup may have contributed to the false-negative results. Second, selection bias was inevitable in this retrospective study. Therefore, the findings may not be indicative of other populations. Third, only three FOXP3 SNPs were explored, and the functional significance of other SNPs was unknown. Fourth, it is not clear whether the SNPS at rs3761547, rs3761548, and rs2232365 affect the protein levels of FOXP3, which may be a possible mechanism for the present non-significant results. Fifth, the relationships between the rs3761547, rs3761548, and rs2232365 polymorphisms and the histological grade, tumor size, TNM stage, distant metastasis, as well as lymph node metastasis of HCC were not investigated. Further study is needed to fully address these issues. Therefore, given the purpose and limitations of this study, the results of this study must be interpreted with caution.

Overall, a correlation between the rs3761547, rs3761548, and rs2232365 polymorphisms in the FOXP3 gene and HBV-HCC in Guangxi Chinese subjects was not observed. Thus, multi-center researches with larger sample sizes should be further developed to

Table 5
Distribution frequency of FOXP3 polymorphisms in HCC patients and controls stratified by gender.

	Controls	HCC	Females		Controls	HCC	Males	
Allele	N = 96	N = 16	OR(95 % CI)[a]	P^a	N = 92	N = 140	OR(95 % CI)[a]	P^a
rs3761547								
A	151	22	1^{ref}		71	110	1^{ref}	
G	41	10	1.964 (0.784–4.925)	0.150	21	30	0.935 (0.492–1.775)	0.837
rs3761548								
C	169	26	1^{ref}		78	121	1^{ref}	
A	23	6	1.500 (0.516–4.358)	0.456	14	19	0.901 (0.421–1.927)	0.787
rs2232365								
A	112	16	1^{ref}		55	94	1^{ref}	
G	80	16	1.363 (0.604–3.076)	0.456	37	46	0.745 (0.429–1.293)	0.296

[a]Adjusting for age, BMI, smoking, drinking, and ethnicity by logistic regression.

Table 6
Distribution frequency of FOXP3 polymorphisms in HCC patients and controls stratified by smoking.

	Controls	HCC	Nonsmokers		Controls	HCC	Smokers	
Allele	N = 128	N = 107	OR(95 % CI)[a]	P^a	N = 60	N = 49	OR(95 % CI)[a]	P^a
rs3761547								
A	155	92	1^{ref}		67	40	1^{ref}	
G	39	31	1.412 (0.771–2.587)	0.264	23	9	0.661 (0.210–2.081)	0.480
rs3761548								
C	168	104	1^{ref}		79	43	1^{ref}	
A	26	19	1.213 (0.597–2.464)	0.593	11	6	0.787 (0.180–3.432)	0.750
rs2232365								
A	116	75	1^{ref}		51	35	1^{ref}	
G	78	48	1.053 (0.628–1.766)	0.844	39	14	0.580 (0.201–1.677)	0.315

[a] Adjusting for age, BMI, gender, drinking, and ethnicity by logistic regression.

Table 7
Distribution frequency of FOXP3 polymorphisms in HCC patients and controls stratified by drinking.

	Controls	HCC	Nondrinkers		Controls	HCC	Drinkers	
Allele	N = 141	N = 108	OR(95 % CI)[a]	P^a	N = 47	N = 48	OR(95 % CI)[a]	P^a
rs3761547								
A	168	94	1^{ref}		54	38	1^{ref}	
G	46	30	1.227 (0.675–2.230)	0.502	16	10	1.163 (0.322–4.193)	0.818
rs3761548								
C	186	106	1^{ref}		61	41	1^{ref}	
A	28	18	1.288 (0.628–2.644)	0.490	9	7	0.965 (0.225–4.146)	0.962
rs2232365								
A	126	78	1^{ref}		41	32	1^{ref}	
G	88	46	0.980 (0.585–1.639)	0.938	29	16	1.007 (0.334–3.040)	0.990

[a] Adjusting for age, BMI, gender, smoking, and ethnicity by logistic regression.

Table 8
Distribution frequency of FOXP3 polymorphisms in HCC patients and controls stratified by ethnicity.

	Controls	HCC	Han		Controls	HCC	Zhuang	
Allele	N = 87	N = 88	OR(95 % CI)[a]	P^a	N = 90	N = 64	OR(95 % CI)[a]	P^a
rs3761547								
A	90	77	1^{ref}		117	51	1^{ref}	
G	33	17	0.643 (0.303–1.363)	0.249	27	23	2.110 (0.996–4.471)	0.051
rs3761548								
C	105	79	1^{ref}		127	64	1^{ref}	
A	18	15	1.211 (0.514–2.854)	0.661	17	10	1.427 (0.543–3.751)	0.471
rs2232365								
A	65	62	1^{ref}		91	44	1^{ref}	
G	58	32	0.717 (0.379–1.355)	0.306	53	30	1.402 (0.713–2.756)	0.327

[a] Adjusting for age, BMI, gender, smoking, and drinking by logistic regression.

confirm this finding.

Funding

The authors declare that no funds, grants, *or other* support *were received during the preparation of this manuscript.*

5. Ethical conduct of research

This study was approved by the Ethics Committee of the First Affiliated Hospital of Guangxi Medical University. All subjects included in this study previously provided informed consent.

Data availability

Data will be made available on request.

CRediT authorship contribution statement

Xiaolian Zhang: Writing – review & editing, Writing – original draft, Visualization, Validation, Investigation, Conceptualization. **Jinwan Li:** Supervision, Software, Methodology, Formal analysis, Data curation, Conceptualization. **Xue Qin:** Software, Investigation, Formal analysis, Data curation, Conceptualization. **Shan Li:** Supervision, Resources, Project administration, Conceptualization. **Dong Liang:** Supervision, Resources, Project administration, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

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

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