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The association between *PIN1* genetic polymorphisms and the risk of chronic hepatitis B and hepatitis B virus-related liver cirrhosis
A case-control study

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Abstract

Peptidyl-prolyl cis/trans isomerase NIMA-interacting 1 (PIN1) reportedly plays a crucial role in tissue inflammation and tumourigenesis. Our previous studies have demonstrated that *PIN1* gene polymorphisms are significantly related to the pathogenesis of hepatitis B virus (HBV)-related liver cancer in a Guangxi population. As chronic hepatitis B (CHB), liver cirrhosis (LC), and liver cancer are development processes, we further investigated whether any relationship exists between *PIN1* gene polymorphisms and the risk of CHB and HBV-related LC. We used the polymerase chain reaction restriction fragment length polymorphism and the deoxyribonucleic acid sequencing method to analyze 3 common single-nucleotide polymorphisms (SNPs) (rs2233678, rs2233679, and rs2233682) of the *PIN1* gene in 192 CHB patients, 171 HBV-related LC patients, and 201 healthy controls in this research. The results revealed that carriers of the rs2233682 A allele had a significantly decreased risk of HBV-related LC (LC vs. controls: odds ratio [OR] = 0.262, 95% confidence interval [CI] = 0.071–0.959, P = .043; LC vs. CHB: OR = 0.198, 95% CI = 0.049–0.803, P = .023). Similar relationships were observed for the *PIN1* rs2233682 GA genotype among the groups (LC vs. controls: OR = 0.248, 95% CI = 0.067–0.919, P = .037; LC vs. CHB: OR = 0.184, 95% CI = 0.044–0.773, P = .021). This reduced risk was more obvious in older CHB patients (age ≥50 years). No such correlations were found for *PIN1* rs2233678 and rs2233679. However, the haplotypes constructed from these SNP (GCA for controls and CCG for CHB) were associated with a significantly decreased risk of HBV-related LC. In summary, the findings of this study suggest that the *PIN1* rs2233682 A allele might be related with a decreased risk of HBV-related LC in a Guangxi population.

Abbreviations: AD= Alzheimer's disease, BMI= body mass index, CHB= chronic hepatitis B, CI= confidence interval, CTGF= connective tissue growth factor, HBV= hepatitis B virus, HBx = HBV X protein, HCC= hepatocellular carcinoma, HSCs= hepatic stellate cells, LC= liver cirrhosis, NASH= nonalcoholic steatohepatitis, OR= odds ratio, p53= tumour suppressor p53, PCR-RFLP= polymerase chain reaction restriction fragment length polymorphism, *PIN1= Peptidyl-prolyl cis/trans isomerase NIMA-interacting 1*, SNP= single-nucleotide polymorphism, TGFβ1= transforming growth factor.

Keywords: chronic hepatitis b, liver cirrhosis, *peptidyl-prolyl cis/trans isomerase nima-interacting 1*, single-nucleotide polymorphisms

1. Introduction

Hepatitis B virus (HBV) infection and its related conditions remain a challenging primary global public health issue.

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Approximately two billion people have been infected with HBV worldwide, and 780,000 people with chronic HBV infections die of liver cirrhosis (LC) and liver cancer each year.[1] Previous studies have confirmed that some individuals with persistent HBV became asymptomatic carriers, whereas others develop LC during the chronic phase, which finally develops into hepatocellular carcinoma (HCC).[2,3] However, the pathogenesis of HBV-related LC is still not completely clear. With the growing prevalence of HBV-related LC worldwide, it is becoming increasingly crucial to identify potentially modifiable factors that may contribute to this disease burden.

Peptidyl-prolyl cis/trans isomerase NIMA-interacting 1 (PIN1) is an isomerase that induces conformational changes in proteins and influences their phosphorylation status, protein stability, protein–protein interactions, subcellular location, and enzymatic activity through the phosphorylation of isomerizing specific serine or threonine residues preceding proline motifs. Previous studies have confirmed that PIN1 protein overexpression in a multitude of diseases, including in some inflammatory and fibrosis diseases.[4–7] In a study of the involvement of PIN1 in the pathogenesis of nonalcoholic steatohepatitis (NASH) in mice, Nakatsu et al [7] found that the PIN1 expression was elevated markedly in NASH mice livers, and the *PIN1* knock-out (KO) mice were highly resistant to the development of NASH;

moreover, they found that PIN1 in the hematopoietic is crucial for the progression of NASH from simple fat accumulation to fibrosis. NASH is characterized by the coexistence of fat accumulation and inflammation, and eventually leads to LC and HCC. In another study of the correlations between NASH and serum PIN1, the researchers found that the serum PIN1 levels were increased obviously in NASH patients, and the over-expression of PIN1 in patients with advanced fibrosis was even more obvious; therefore, they suggested that the serum PIN1 level may be used as a potential independent marker of NASH and the stages of histopathological liver fibrosis.[8] Recent studies about the pathophysiological role of PIN1 in liver fibrogenesis revealed that tumor suppressor p53 may stimulate the expression of connective tissue growth factor (CTGF, p53/CTGF pathway), eventually leading to hepatocyte apoptosis and hepatic fibrosis,[9] and the PIN1 plays a crucial part in the p53/CTGF pathway by regulating the activity of p53 have been well demonstrated.[10] Moreover, the transforming growth factor-β1 (TGF-β1), a key fibrogenic mediator, which is mainly produced by activated hepatic stellate cells (HSCs) in the liver, plays an essential role in the progression of liver fibrogenesis; and PIN1 induction during hepatic fibrosis by regulating TGF-β1 expression and Smad2/3 signaling in HSCs have been confirmed.[11] These above investigations indicated that PIN1 is of vital importance in liver inflammation and fibrosis. However, the data of molecular and protein mechanisms of PIN1 in chronic hepatitis B (CHB) and HBV-related LC are limited. Taking the increased expression of PIN1 in inflammation and fibrosis into account, we can reasonably speculate that PIN1 polymorphisms may be correlated with risk of HBV-related live diseases.

Human PIN1 gene, which spans >14 kb on chromosome 19p13, encodes a protein of 163-amino acid, contains 4 exons and has a promoter region of 1.5 kb.[12] Several putative functional polymorphisms have been identified in the coding and promoter regions of PIN1, including 2 variants in the promoter region (842G>C, rs2233678 and 667T>C, rs2233679) and 1 synonymous change (Gln33Gln, G>A, rs2233682) in the exon 2 coding region (http://www.ncbi.nlm.nih.gov/SNP/). Recently, many studies have confirmed that PIN1 polymorphisms are closely related to a large number of diseases.[12–15] Interestingly, we recently found that PIN1 genetic polymorphisms might affect the occurrence and development progress of HBV-related HCC in Guangxi.[13] Nonetheless, so far, no research has been carried out on the role of PIN1 genetic polymorphisms in CHB and LC susceptibility. Therefore, we further examined whether the PIN1 promoter single-nucleotide polymorphisms (SNPs) (842G>C, rs2233678 and 667T>C, rs2233679) and the exon 2 synonymous SNP (Gln33Gln, G>A, rs2233682) influence one's susceptibility to CHB and HBV-

related LC in a Guangxi population. A better understanding of the role of PIN1 in the pathogenesis of HBV-related diseases may lead to the identification of molecular targets both for prevention and therapeutic intervention.

2. Materials and methods

2.1. Study population

We used a retrospective case–control study. The participants were all selected from the First Affiliated Hospital of Guangxi Medical University in Guangxi, China, from April 2014 to March 2016. In total, 363 HBV-infected patients (192 with CHB and 171 with HBV-LC) and 201 healthy participants were enrolled in this research. This study was approved by the hospital's ethics committee, and all participants provided written informed consent. The diagnostic criteria for CHB were as follows: the persistence of hepatitis B surface antigens for >6 months; serum HBV-DNA levels ≥1000 copies/mL; and serum aspartate aminotransferase or alanine aminotransferase levels >40 IU/mL. HBV-LC was diagnosed by using a combination of the patient's CHB history, pathological examination, and typical morphology upon ultrasonography or computed tomography (CT) imaging.[16,17] Patients who had any of the following conditions were excluded from the study: concomitant with other liver disease or mixed etiologies, such as hepatitis A/C/D/E virus-related liver disease, NASH, autoimmune hepatitis, alcoholic liver cirrhosis, among others; had a history of autoimmune or inflammatory diseases such as rheumatoid arthritis, systemic lupus erythematosus, or inflammatory bowel disease, among others. We randomly recruited 201 volunteers from the general health check-up division of the same hospital during the same time period to serve as the controls; all of them tested negative for HBV markers and were also without any clinical evidence of liver disease or cancer. The sex, ethnicity, body mass index (BMI), age, alcohol consumption, and smoking habits of all included participants were also collected.

2.2. Genotyping

We used the standard phenol-chloroform method to extract genomic DNA from leukocytes, and then detected PIN1 gene SNPs using the polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) assay described by You et al.[18] The primer sequences and the reaction condition for genotyping PIN1 polymorphisms are presented in Table 1. The results of RFLP for genotyping were described as Figure 1. To validate the genotyping results, we randomly selected 10% of the samples to be directly sequenced with an ABI Prism 3100. The

Table 1

Primer sequence and the reaction condition for genotyping PIN1 polymorphisms.

| Polymorphism	Primer sequence (5' →3')	Annealing temperature (°C)	Restriction enzyme	Product size, bp
rs2233678	Forward: AGACTCTATTTTAAGTTGGC	55°C	BanII	GG: 114+31
	Reverse: TTACTTCCTTTATTCCCTCGCAG		65°C for 4 h	GC: 145+114+31
				CC: 145
rs2233679	Forward: CGGGCTCTGCAGACTCTATT	55°C	SacI	CC: 296
	Reverse: AAATTTGGCTCCTCCATCCT		37°C for 4 h	CT: 296+213+83
				TT: 213+83
rs2233682	Forward: TTTGAGTCACTCCCTGTCCC	50°C	BsrI(BseNI)	GG: 372+99+53
	Reverse: CTTGGTCCGGGTGATCTTCT		37°C for 4 h	GA: 524+372+99+53
				AA: 471+53
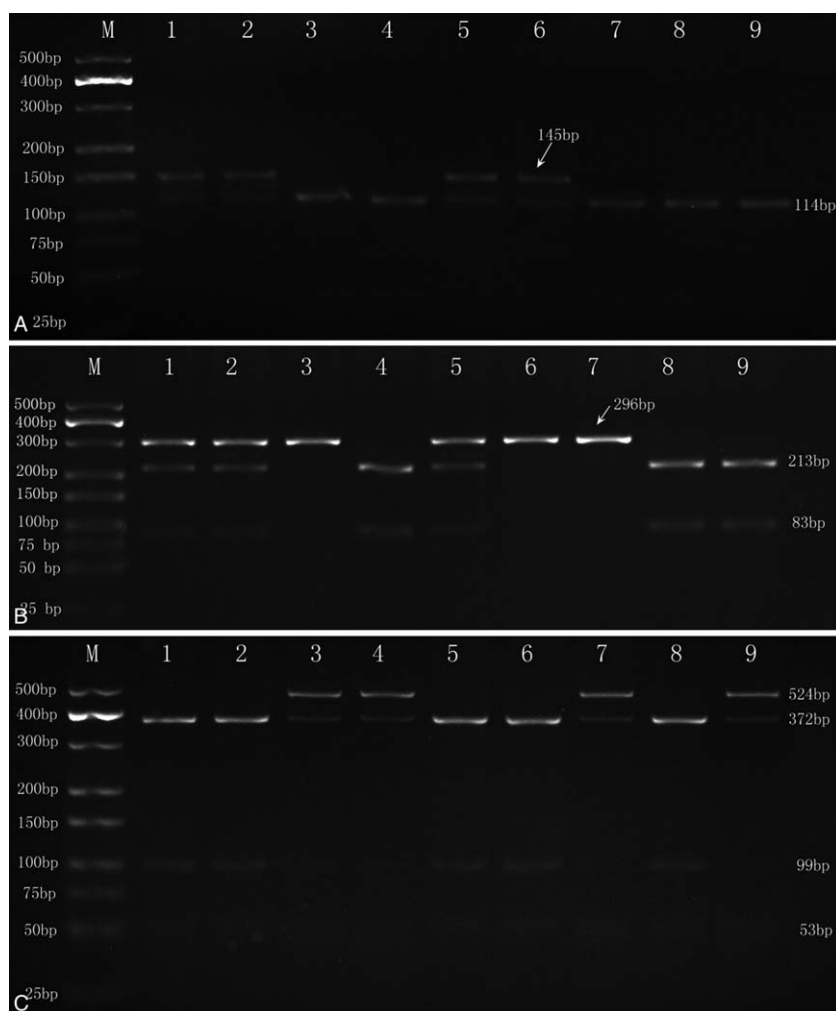
Figure 1. Polymerase chain reaction-restriction fragment length polymorphism assay for analyzing the rs2233678, rs2233679, and rs2233682 polymorphisms in *PIN1* gene. Polymerase chain reaction products were digested by restriction enzyme and digested fragments were separated by electrophoresis in 2% agarose gel. (A) rs2233678-lanes M: DNA marker; lanes 1, 2, 5, and 6 show GC genotype; lanes 3, 4, 7, 8, and 9 show GG genotype. (B) rs2233679-lanes M: DNA marker; lanes 3, 6, and 7 show CC genotype; lanes 1, 2, and 5 show CT genotype; lanes 4, 8, and 9 shows TT genotypes. (C) rs2233682-lanes M: DNA marker; lanes 1, 2, 5, 6, and 8 show GG genotype; lanes 3, 4, 7, and 9 show GA genotype.

genotyping results were in accordance with the results of the sequencing (Figs. 2–4).

2.3. Statistical analysis

A 1-way analysis of variance (ANOVA) test and 2-sided χ^2 tests were used to analyze the general demographic information among both the cases and controls. Hardy-Weinberg equilibrium (HWE) for each SNP was performed using a χ^2 goodness of fit test. The relative risk that a particular genotype and allele conferred was assessed with the corresponding 95% confidence interval (CI) and the odds ratios (ORs), which were calculated by a binary logistic regression model after adjustments were made for some confounding factors (age, sex, BMI, ethnicity, alcohol drinking, and tobacco smoking). SHEsis software was adopted for the haplotype analysis,[19] and all other statistical analyses were carried out using the Statistical Package for the Social Sciences (SPSS) software (version 16.0). P values <.05 were considered statistically significant.

3. Results

3.1. Demographic characteristics

The characteristics of the study population are summarized in Table 2. The data reveal that CHB patients were significantly younger than both patients with LC and the controls ($P < .001$). Similarly, patients with CHB or LC were more likely to be drinkers ($P < .001$) and/or smokers ($P = .004$). However, there was no significant difference in these three groups regarding gender, BMI or ethnicity ($P = 0.098$, $P = 0.096$ and $P = 0.708$, respectively).

3.2. PIN1 polymorphisms and the risk of HBV-related patients

The distributions of the *PIN1* gene genotypes and alleles among the groups are presented in Table 3. The genotype frequencies of these 3 SNPs in the healthy controls all agreed with the HWE ($P = .745$, $P = .139$, and $P = .636$, respectively). When the
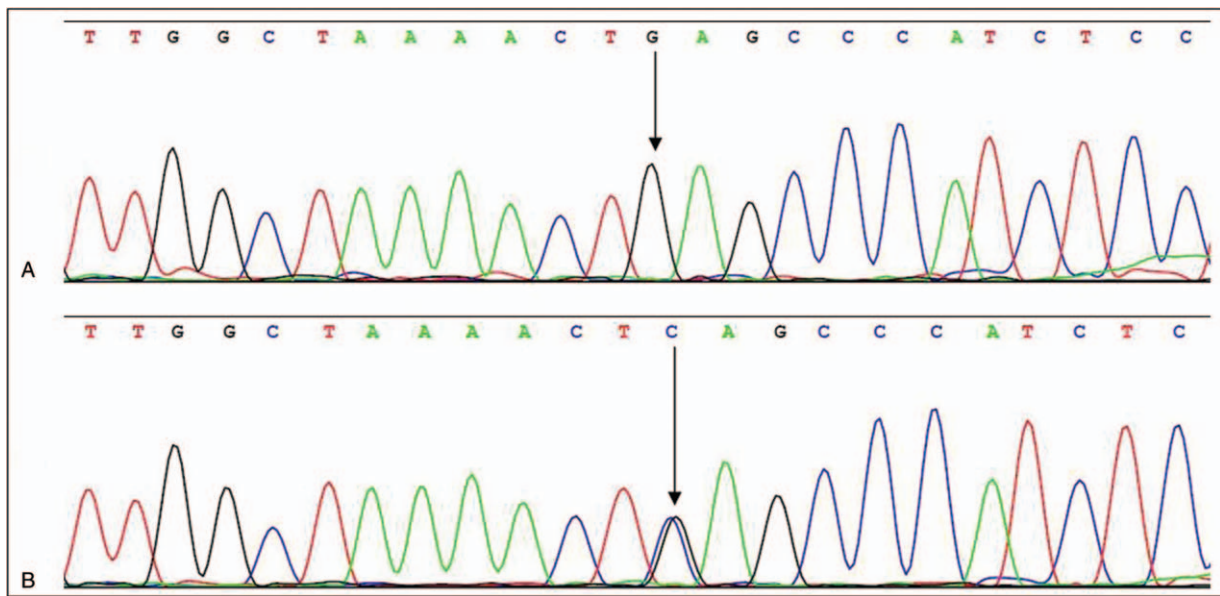
Figure 2. Sequencing map for genotypes of *PIN1* gene rs2233678 polymorphism in genotyping by direct sequencing. The arrows in A–B map show GG and GC genotypes.
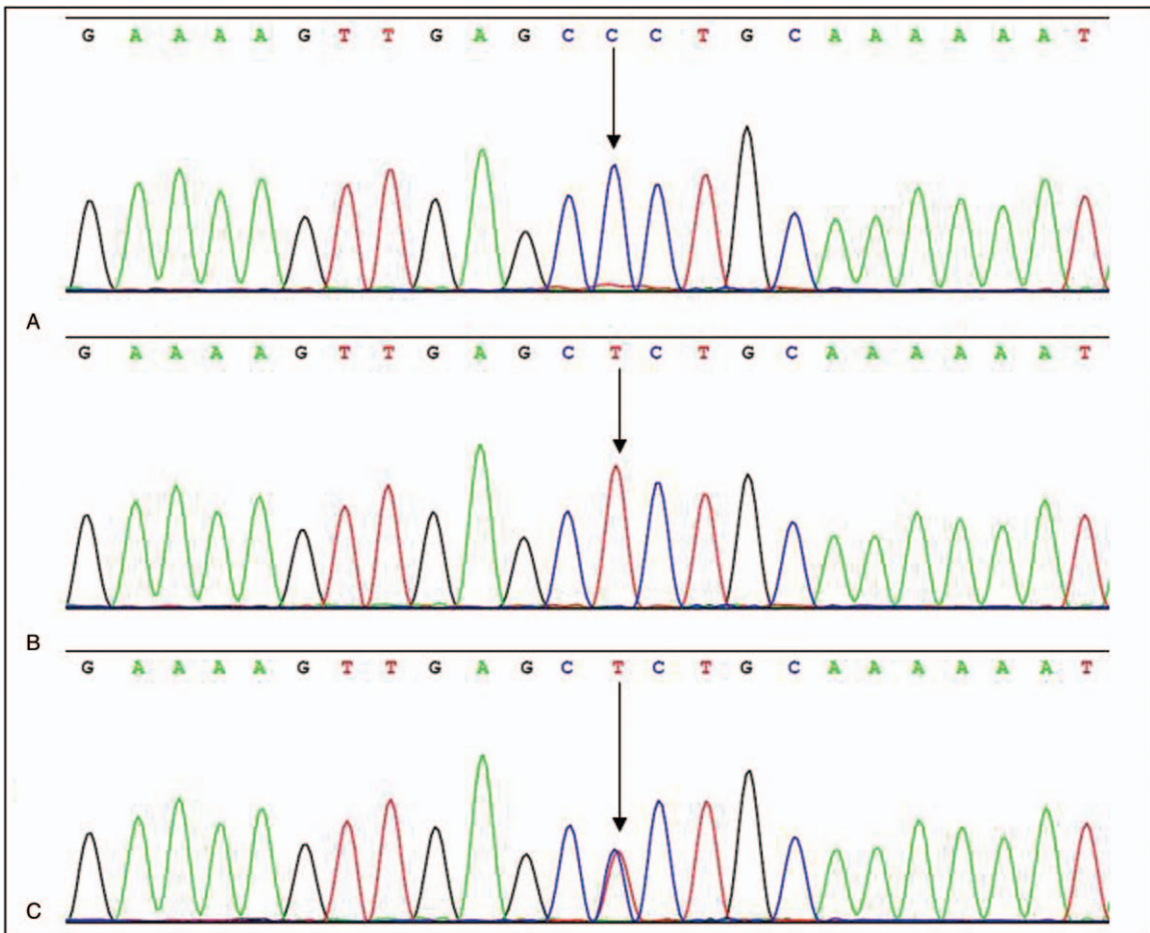

Figure 3. Sequencing map for genotypes of *PIN1* gene rs2233679 polymorphism in genotyping by direct sequencing. The arrows in A–C map show CC, TT, and CT genotypes, respectively.
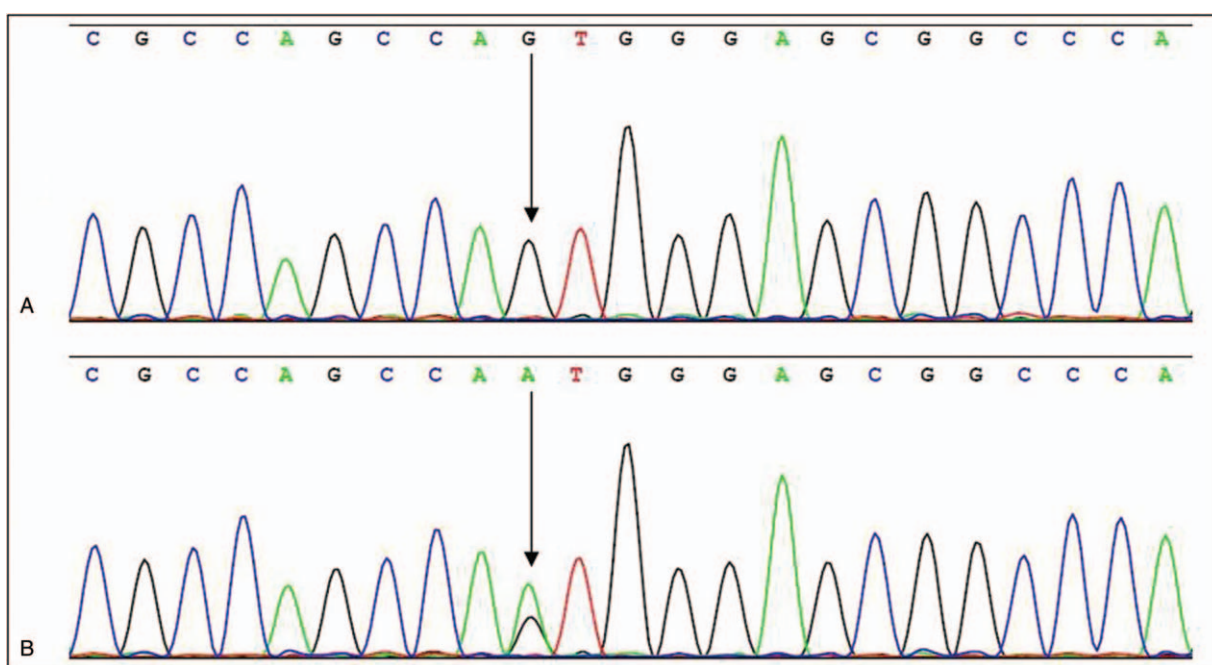
Figure 4. Sequencing map for genotypes of *PIN1* gene rs2233682 polymorphism in genotyping by reverse sequencing. The arrows in A–B map show GG and GA genotypes.

relationships between *PIN1* SNPs and HBV-related disease risk (CHB or LC) were analyzed via binary logistic regression analyses after adjusting for confounding factors, we found that the *PIN1* Gln33Gln genotype and allele frequencies were significantly different between HBV-related LC and controls ($P=.037$ and $P=.043$, respectively). Carriers of the rs2233682 GA genotype had a significantly decreased risk of HBV-related LC when compared with the GG genotype carriers with an adjusted OR of 0.248 and a 95% CI of 0.067 to 0.919. Similar relationships were observed for the *PIN1* Gln33Gln A allele between HBV-related LC and controls (OR=0.262, 95% CI= 0.071–0.959, $P=.043$). In addition, when we used the CHB group as the control and compared it with Gln33Gln GG genotype, participants who carried the Gln33Gln GA genotype

were at significantly decreased risk of HBV-related LC (OR= 0.184, 95% CI=0.044–0.773, $P=.021$). Similar relationships were observed for the *PIN1* Gln33Gln A allele between HBV-related LC and CHB (OR=0.198, 95% CI=0.049–0.803, $P=.023$). However, no such significant relationships were noted between the *PIN1* promoter SNPs (rs2233678 and rs2233679) and the risk of HBV-related diseases (Table 3).

3.3. Stratified analysis

We stratified our population according to sex and age to determine the effect that these potentially confounding variables had on *PIN1* polymorphisms and the risk of HBV-related liver diseases. For the *PIN1* gene rs2233682, a decreased risk of LC

Table 2

Characteristics of the study population.

Variables	Controls	CHB	LC	*P*
Total number	201	192	171	
Age, y (mean±SD)	47.13±6.78	37.72±11.75	50.14±11.72	**<.001**
Sex (%)				
Male	131 (65.2)	139 (72.4)	128 (74.9)	.098
Female	70 (34.8)	53 (27.6)	43 (25.1)	
BMI (mean±SD)	22.44±3.40	21.83±4.03	22.70±4.35	.096
Ethnicity, n (%)				
Han	106 (52.7)	113 (58.9)	101 (59.1)	.708
Zhuang	84 (41.8)	70 (36.5)	63 (36.8)	
Others	11 (5.5)	9 (4.7)	7 (4.1)	
Alcohol drinking, n (%)				
Yes	60 (29.9)	92 (47.9)	65 (38.0)	**<.001**
No	141 (70.1)	100 (52.1)	106 (62.0)	
Tobacco smoking, n (%)				
Yes	66 (32.8)	71 (37.0)	84 (49.1)	**.004**
No	135 (67.2)	121 (63.0)	87 (50.9)	

BMI=body mass index, CHB=chronic hepatitis B, LC=liver cirrhosis, SD=standard deviation. Bold values indicate a significant difference ($P < .05$).

Table 3

Genotype and allele frequencies of *PIN1* polymorphisms in controls and patients and their association with HBV-related diseases.

SNPs		Controls N=201 (%)	CHB N=192 (%)	LC N=171 (%)	CHB vs. controls OR* (95% CI)	P*	LC vs. controls OR* (95% CI)	P*	LC vs. CHB OR* (95% CI)	P*
rs2233678	GG	192 (95.5)	172 (89.6)	163 (95.3)	1.00ref	–	1.00ref	—	1.00ref	—
	GC	9 (4.5)	20 (10.4)	8 (4.7)	1.467 (0.570–3.778)	.427	1.147 (0.421–3.126)	.789	0.702 (0.259–1.907)	.488
	CC	0 (0.0)	0 (0.0)	0 (0.0)	—	–	—	—	—	—
	G allele	393 (97.8)	364 (94.8)	334 (97.7)	1.00ref	–	1.00ref	—	1.00ref	—
	C allele	9 (2.2)	20 (5.2)	8 (2.3)	1.398 (0.559–3.499)	.474	1.174 (0.438–3.151)	.750	0.718 (0.272–1.892)	.503
rs2233679	CC	66 (32.8)	61 (31.8)	62 (36.3)	1.00ref	–	1.00ref	—	1.00ref	—
	CT	107 (53.2)	103 (53.6)	88 (51.5)	1.207 (0.724–2.011)	.471	0.845 (0.532–1.344)	.477	0.740 (0.434–1.261)	.268
	TT	28 (13.9)	28 (14.6)	21 (12.3)	1.072 (0.513–2.240)	.853	0.808 (0.406–1.608)	.544	0.951 (0.430–2.101)	.901
	C allele	239 (59.5)	225 (58.6)	212 (62.0)	1.00ref	–	1.00ref	—	1.00ref	—
	T allele	163 (40.5)	159 (41.4)	130 (38.0)	1.063 (0.765–1.476)	.715	0.896 (0.660–1.216)	.481	0.915 (0.645–1.297)	.617
rs2233682	GG	188 (93.5)	180 (93.8)	168 (98.2)	1.00ref	–	1.00ref	—	1.00ref	—
	GA	13 (6.5)	12 (6.3)	3 (1.8)	1.150 (0.463–2.855)	.763	**0.248 (0.067–0.919)**	**.037**	**0.184 (0.044–0.773)**	**.021**
	AA	0 (0.0)	0 (0.0)	0 (0.0)	—	–	—	—	—	—
	G allele	389 (96.8)	372 (96.9)	339 (99.1)	1.00ref	–	1.00ref	—	1.00ref	—
	A allele	13 (3.2)	12 (3.1)	3 (0.9)	1.091 (0.448–2.659)	.848	**0.262 (0.071–0.959)**	**.043**	**0.198 (0.049–0.803)**	**.023**

* Data were calculated by binary logistic regression analysis with adjusted for age, sex, BMI, ethnicity, alcohol drinking, and tobacco smoking.
CI = confidence interval, CHB = chronic hepatitis B, *PIN1* = peptidyl-prolyl cis/trans isomerase NIMA-interacting 1, LC = liver cirrhosis, OR = odds ratio. Bold values indicate a significant difference ($P < .05$).

relative to the GA genotype was more evident among older (≥50 years) people than it was in younger (<50 years) CHB patients (OR = 0.090, 95% CI = 0.009–0.997, P = .042). Similarly, rs2233682 A allele carriers had a 0.104-fold decreased risk of LC (95% CI = 0.011–0.997, P = .050). However, no such differences were found in the stratified analysis of the *PIN1* rs2233678 and rs2233679 SNPs and for the risk of HBV-related LC (data not shown).

3.4. Haplotype analysis

Five haplotypes were structured from the observed genotypes in this study (Table 4). We found that the most frequent haplotype in all groups was GCG. The CCG haplotype in the CHB patients (OR = 0.273, 95% CI = 0.078–0.958, P = .030) and the GCA haplotype in the controls (OR = 0.088, 95% CI = 0.011–0.679, P = .003) were associated with a significantly decreased risk of HBV-related LC.

4. Discussion

The current case–control study investigated the relationship between *PIN1* SNPs and the risk of CHB and HBV-related LC in a Guangxi population. Our results confirmed that one *PIN1*

synonymous change(Gln33Gln;G>A,rs2233682) on exon 2 was significantly correlated with the risk of HBV-related LC. We verified that rs2233682 GA genotype or A allele carriers had a reduced risk of HBV-related LC. However, no correlations were found between the genetic variants of the *PIN1* promoter (rs2233678 and rs2233679) and the risk of CHB and HBV-related LC.

A multitude of studies have demonstrated that PIN1 plays a paramount role in inflammation and endothelial dysfunction.[8,14,20–23] For example, Jeong et al have proved that PIN1 could induct and stimulate the expression of proinflammatory protein during rheumatoid arthritis progress.[4] In addition, Cengiz et al[8] have revealed that the serum level of PIN1 was remarkably increased in NASH and related to the histopathological liver fibrosis stages. It is well known that CHB is an inflammatory disease, and researchers have confirmed that the positive expression ratio and intensity of PIN1 were stronger in hepatitis B than that in normal liver tissue.[24] In view of the facts that PIN1 was related to the disease process of CHB, we deduced that the genetic variants of *PIN1* may be associated with CHB. However, the results of this study were not consistent with the results of previous researches, and we did not find any correlation between the 3 common *PIN1* gene SNP polymorphisms and the risk of CHB. These different results may be

Table 4

Associations between *PIN1* haplotypes frequencies and risk of CHB and LC.

Haplotypes*	Controls N=402	CHB N=384	LC N=342	CHB vs. controls OR (95% CI)	P	LC vs. controls OR (95% CI)	P	LC vs. CHB OR (95% CI)	P
CCG	8 (0.019)	12 (0.032)	3 (0.009)	1.732 (0.694–4.320)	.234	0.476 (0.126–1.799)	.263	**0.273 (0.078–0.958)**	**.030**
CTG	1 (0.003)	7 (0.019)	5 (0.014)	5.427 (0.881–33.441)	.041	4.199 (0.638–27.640)	.105	0.769 (0.241–2.450)	.656
GCA	13 (0.032)	5 (0.012)	1 (0.003)	0.372 (0.128–1.078)	.058	**0.088 (0.011–0.679)**	**.003**	0.236 (0.027–2.053)	.155
GCG	218 (0.543)	208 (0.541)	208 (0.608)	0.995 (0.751–1.318)	.972	1.323 (0.987–1.774)	.061	1.310 (0.975–1.761)	.073
GTG	162 (0.402)	146 (0.379)	123 (0.360)	0.910 (0.683–1.213)	.521	0.844 (0.627–1.137)	.265	0.919 (0.679–1.243)	.583

* A total of 8 possible haplotypes (CCG, CTG, GCA, GCG, GTA, GTG, CCA, CTA) were derived from the observed genotypes, and the number of haplotype CCA, CTA and GTA in groups was 0 (or too small) and we fail to obtain the value of OR and CI (or the value of OR and CI is too big to convince); therefore, we did not show these data in the table.
Bold faced values indicate a significant difference. CI = confidence interval, CHB = chronic hepatitis B, LC = liver cirrhosis, OR = odds ratio, *PIN1* = Peptidyl-prolyl cis/trans isomerase NIMA-interacting 1.

because of the following explanations. First, the limitation of *PIN1* gene SNPs in this study may be the main reason, and this research only studied the relationship between 3 SNPs of *PIN1* gene and susceptibility to CHB; nevertheless, the PIN1 protein level in liver may be regulated by some other unknown SNPs in *PIN1* gene. Second, the different sample size and ethnic variation in different study groups even may lead to contradictory results. For example, in a study of relationship between the genetic variants in the promoter region of the *PIN1* gene (positions -842G/C and -667T/C) and Alzheimer disease (AD) risk in an Italian population, Segat et al [25,26] confirmed that the -842 CC genotype or the CC haplotype carriers can significantly raise the risk of AD in a group of 111AD patients. However, in contrast to previous observations of AD, a meta-analysis of which data were available from a total of 7 case-control studies with 2322 controls and 2504 AD patients in different ethnicity revealed that *PIN1* gene polymorphisms -842G/C and -667T/C were unlikely to contribute to AD susceptibility.[26] Taken together, we deduced that the *PIN1* SNPs in this study may have no relation with the inflammatory process of CHB and future prospective multicenter study should be validated.

For the role of the PIN1 in liver cirrhosis, quite a few studies have been revealed that PIN1 over-expression in fibrotic liver tissue in both humans and mice, and its pathological mediator has been confirmed to involve in the activation of hepatic stellate cells.[8,11] However, the data on the *PIN1* gene polymorphisms and susceptibility to LC are limited. In this study, we verified that rs2233682 GA genotype or A allele carriers had a reduced risk of HBV-related LC, and it can play a protective role in the progress of hepatitis B to HBV-related LC. Interestingly, the results of this present study were not completely consistent with our previous results, which showed that the TT genotype of *PIN1* promoter rs2233679, along with the GA genotype and A alleles of the synonymous SNP (rs2233682) in the coding region, may be significantly related to HBV-HCC.[13] Synonymous mutations are commonly assumed to have no function for not changing the amino acid composition of the encoded proteins during evolution. However, recent studies have revealed that those variants can affect splicing accuracy, translation fidelity, mRNA structure and protein folding, and therefore associated with diseases.[27] For example, synonymous mutations located in microRNA-binding or transcription factors-binding region would have effect on the binding ability of microRNAs and transcription factors, and result in functional consequences and diseases.[27–29] However, the molecular mechanisms of **PIN1** rs2233682 synonymous has not been possible to experimentally demonstrated to causes a disease because of technical limitations.[27] Furthermore, Pang et al have confirmed that *PIN1* could bond with its Ser41-Pro motif during HCC progression to enhance the stability of the HBV X protein (HBx), which participates in hepatocarcinogenesis and is expressed in the progressive stage of CHB-LC-HCC.[30] Taken together, we speculated that individuals carrying the *PIN1* gene rs2233682 A allele could cause changes in PIN1 protein expression, conformation, and function owing to some unknown mechanism and then synergize with the HBx to influence the process of HBV-related hepatic fibrosis.[13,27]

A haplotype is a set of closely linked genetic markers on the same chromosome, known as inherited genetic markers, which tend to occur more frequently than expected. Therefore, compared to each gene polymorphism, the risk alleles can be better identified using a haplotype.[31] Therefore, the haplotype analysis of this investigation revealed that the CCG haplotype in

the CHB patients and the GCA haplotype in the controls were significantly associated with a decreased risk of HBV-related LC. Genetic variants among individuals may also influence the expression of the protein; consequently, we can speculate that *PIN1* gene promoter genotypes and allele variants might alter PIN1 production levels and play a potential role in HBV-related diseases, perhaps it can provide new ideas for the treatment of LC.

Certain potential limitations of the current research must be considered when interpreting our results. First, the participants were all recruited from a single-centre study; thus, there may be selection bias, and the results may not be representative of other populations. We also should not ignore the fact that the capacity of the sample in this research was considerably smaller. Therefore, the study's statistical power is limited. Second, although the HBeAg status and baseline HBV DNA levels of the HBV carrier are the risk predictors of developing liver cirrhosis, we failed to collect these data to the stratified analyses due to the relatively small sample size in our study. Moreover, considering the antiviral therapy can reduce the risk of cirrhosis in CHB, and we cannot precisely obtain the accurate time that the patients had been infected with HBV, and the stratified analyses according to the severity of the related disease could not be implemented in this study. Therefore, to obtain a better understanding of the role of *PIN1* in the pathogenesis of HBV-related diseases, more sample size and more detailed inclusion criteria for HBV-patients should be performed in further research. Finally, this study only investigated the functional role of 3 *PIN1* SNPs in HBV-related disease; it would be interesting to examine more *PIN1* gene SNPs and identify their relationships with HBV-liver diseases.

In conclusion, we confirmed that *PIN1* rs2233682 polymorphisms might be associated with a decreased risk of HBV-related LC. Therefore, *PIN1* gene synonymous SNP (Gln33Gln, G>A) might act as a candidate molecular targets for HBV-related disease prevention and therapeutic intervention. Further prospective studies with a large sample size and more detailed inclusion criteria for HBV-patients should be performed in various ethnic groups to validate the relationships between *PIN1* genetic polymorphisms and the risk of liver disease.

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