

Association of miRNA-122-binding site polymorphism at the interleukin-1 α gene and its interaction with hepatitis B virus mutations with hepatocellular carcinoma risk

Yan Du^{1,*}, Xue Han^{2,*}, Rui Pu¹, Jiaxin Xie¹, Yuwei Zhang¹, Guangwen Cao (✉)¹

¹Department of Epidemiology, Second Military Medical University, Shanghai 200433, China; ²Division of Chronic Diseases, Center for Disease Control and Prevention of Yangpu District, Shanghai 200090, China

© Higher Education Press and Springer-Verlag Berlin Heidelberg 2014

Abstract This study was designed to investigate the contribution of miRNA-122-binding site polymorphism at the *IL-1A* gene and its multiplicative interactions with hepatitis B virus (HBV) mutations in the risk of hepatocellular carcinoma (HCC). A total of 1021 healthy controls, 302 HBV surface antigen (HBsAg) seroclearance subjects, and 2011 HBsAg-positive subjects (including 1021 HCC patients) were enrolled in this study. Quantitative PCR was used to genotype rs3783553. HBV mutations were determined by direct sequencing. Multivariate logistic regression analyses were performed to test the associations of rs3783553, mutations, and their interactions with the risk of HCC. No significant association was found between rs3783553 and the risk of HCC among healthy controls, HBsAg seroclearance subjects, HBsAg-positive subjects without HCC, and all controls. Additionally, rs3783553 was not significantly associated with chronic HBV infection, liver cirrhosis, HBV e antigen seroconversion, abnormal alanine aminotransferase, and high viral load ($> 10^4$ copies/ml). However, the TTCA insertion allele of rs3783553 was significantly associated with an increased frequency of HBV C7A mutation compared with homozygous TTCA deletion carriers [(del/ins + ins/ins) vs. del/del, adjusted odds ratio (OR)= 1.48, 95% confidence interval (CI)= 1.09–2.02, $P=0.013$]. Multiplicative interaction of rs3783553 with HBV preS deletion significantly reduced the risk of HCC in males, with an adjusted OR of 0.64 (95% CI= 0.42–0.98; $P=0.041$) after age and HBV genotype were adjusted. Although rs3783553 did not significantly affect genetic susceptibility to HBV-related HCC, its variant allele may predispose the host to selecting HBV C7A mutation during evolution and significantly reduce the risk of HCC caused by HBV preS deletion. This study provides an insight into the complex host-virus interaction in HBV-induced hepatocarcinogenesis and is helpful in determining HBsAg-positive subjects who are likely to develop HCC.

Keywords miRNA-122-binding site; *IL-1A*; rs3783553; hepatitis B virus (HBV) mutations; hepatocellular carcinoma (HCC); interaction

Introduction

Chronic infection with hepatitis B virus (HBV) or hepatitis C virus (HCV) is the leading cause of hepatocellular carcinoma (HCC) worldwide [1,2]. The mainland of China, a region with 94 million HBV surface antigen (HBsAg) carriers, alone accounts for half of the global HCC cases [3,4]. The cumulative lifetime (age: 30 to 75 years) incidence rates of

HCC for males and females who were only positive for HBsAg were 27.38% and 7.99%, whereas those without HBV and HCV infections were 1.55% and 1.03%, respectively [5]. In East Asia, where HBV genotypes B and C are endemic, active and persistent inflammation in the liver, high levels of circulating HBV DNA ($\geq 10^4$ vs. $< 10^4$ copies/ml), HBV genotype C (vs. genotype B), and viral mutations in the enhancer II/basal core promoter/precore (EnhII/BCP/PC) and the preS regions of HBV are significantly associated with increased risks of HCC [5–12].

The chronic inflammation caused by the interaction of HBV infection and subverted host immunity creates a microenvironment that facilitates hepatocarcinogenesis and

Received September 29, 2013; accepted January 7, 2014

Correspondence: gcao@smmu.edu.cn

*Yan Du and Xue Han contributed equally to this work.

HCC late recurrence [13–15]. Antiviral treatments with either class I interferon or nucleot(s)ide analogs not only prevent the occurrence of HCC but also improve postoperative survival [16–19]. HBV replication might promote active inflammation by dysregulating proinflammatory cytokines or chemokines. Of those, interleukin-1 (IL-1) family members have been associated with chronic inflammation, carcinogenesis, and metastasis [20]. MicroRNAs (miRNAs), a group of noncoding RNA molecules that are 18 to 25 nucleotides long, have post-transcriptional regulatory functions through binding to the 3' untranslated region (UTR), coding region, or 5'UTR of the target mRNAs; miRNAs are also involved in HBV-induced inflammatory diseases and hepatocarcinogenesis [21,22]. miRNAs regulate HCC-promoting inflammation possibly by regulating proinflammatory signaling pathways, such as nuclear factor- κ B [23]. Functional polymorphisms within miRNA binding sites may influence the susceptibility of an individual to cancer by altering the strength of miRNA binding and regulating target genes [24].

miRNA-122, a liver-specific miRNA, is related to viral immune escape and anti-viral defense [10,25]. rs3783553, a miRNA-122 binding site polymorphism at the *IL-1A* gene, has been reported to be associated with risk of HCC in Chinese population [26]. However, the effect of SNP and HBV mutation interactions on the risk of HCC was not investigated in this study. In the current study, we investigated the contribution of rs3783553 and its interactions with HBV mutations in HCC development. The results of our study might be helpful in understanding the complex host-virus interaction in the development of HCC and in identifying HBV-infected subjects who are more likely to develop HCC.

Materials and methods

Study population

The current study included six groups of previously described participants [27]. Briefly, Group A comprised 1012 healthy controls who were free of serological HBV and HCV parameters, including antibodies to HBc (anti-HBc) and HCV (anti-HCV), and without history of liver diseases. They were recruited from the Health Examination Center at Changhai Hospital of the Second Military Medical University from September 2009 to June 2010. Group B comprised 302 HBV natural clearance participants who reported no history of HBV vaccination and were seronegative for HBsAg, HBV DNA, and anti-HCV, but seropositive for anti-HCV and anti-HBc. Group C comprised 316 asymptomatic HBsAg carriers (ASCs) who were seropositive for HBsAg but free of any clinical liver disease and had normal alanine aminotransferase (ALT) level (< 40 U/L). Similar to group C, group B was free of clinical liver diseases. Both groups were initially recruited from our HBV-infected subjects cohort established in Yangpu District of Shanghai during the initial screening of

seropositivity for HBsAg in 2010. These groups were revisited during the follow-up from June to December 2011. We only enrolled subjects who yielded a 100% concordance with the previous results during follow-up examinations. Groups D [316 patients with chronic hepatitis B (CHB)], E [358 HBV-infected patients with liver cirrhosis (LC)], and F [1021 HBV-infected patients with HCC] were recruited from Changzheng Hospital (361 cases), Changhai Hospital (269 cases), and Eastern Hepatobiliary Surgery Hospital (321 cases) of this university, South-west Hospital in Chongqing (23 cases), and the 88th hospital in Taian City, Shandong (47 cases), China from October 2009 to September 2011. CHB, LC, and HCC were diagnosed according to previously described criteria [11]. Patients who were seropositive for antibodies to HCV, hepatitis δ virus (HDV), or human immunodeficiency virus were excluded. All participants were of Han Chinese ancestry. The study protocol is in accordance with the Declaration of Helsinki (2000) and was approved by the Ethics Committee of the Second Military Medical University. All participants provided written informed consent.

Serological viral marker examination, HBV genotyping, and viral mutation analysis

Serological testing for HBV markers, α -fetoprotein, ALT, and viral load was conducted as previously described [11,28,29]. HBV genotyping, PCR amplification of HBV EnhII/BCP/PC region and preS region, and viral mutation analysis were conducted according to previous protocols [11,12].

DNA extraction and SNP genotyping

QIAquick PCR purification kits (QIAGEN, Germany) were used to extract genomic DNA from blood samples. Genotyping was conducted using fluorescent-probe real-time quantitative PCR (qPCR) in a LightCyclerTM480 (Roche, Basel, Switzerland). Primers and probes (Minor Groove Binder [MGB]) were designed by GeneCore Bio Technologies Co. Ltd. (Shanghai, China). The primer sequences were 5'-TTTGACTCTTTTGCCATTAAACT-TACC-3' (forward) and 5'-TGGTCTCATGGTTGTCAAAG TTG-3' (reverse). The probes were FAM-TGTTTCATTCAA TTCC-MGB and HEX-TCTTGTTTCAATTCCACCTG-MGB. Genotyping was performed for all participants. Laboratory technicians were blinded to case-control status. For quality control, blind duplicates (10% of the samples) were included to assess laboratory reliability, and 100% concordance rate was achieved.

Statistical analysis

χ^2 -test was used to compare categorical variables among different groups. The levels of HBV DNA and ALT were transformed to normal distribution by logarithmic function.

Student's *t*-test or ANOVA was used to evaluate continuous variables. Hardy-Weinberg equilibrium (HWE) was tested with the exact test. Unconditional logistic regression was used to calculate the odds ratios (ORs) and 95% confidence intervals (CIs) after age and gender adjustments. Gender may be a possible confounder. Thus, we further stratified the study population into males and females and evaluated the association within each stratum. We assessed the dominant [(homozygotes of insertion allele + heterozygotes) vs. homozygotes of deletion allele] and recessive [homozygotes of insertion allele vs. (heterozygotes + homozygotes of deletion allele)] model. Multiplicative interaction of SNP rs3783553 and HCC-related HBV mutations in HCC risk was evaluated separately in females and males, using multivariate regression analyses after adjusting for age and HBV genotype. All significance tests were two-sided. $P < 0.05$ was considered statistically significant. All analyses were conducted using SPSS 16.0 for Windows (SPSS, Chicago, IL).

Results

Population characteristics

The demographic characteristics of the study participants are shown in Table 1. Briefly, HBV-infected participants (groups C, D, E, and F) were on average 10 years younger than

healthy controls (group A) and HBsAg seroclearance participants (group B). The HCC patients had a higher proportion (84.1%) of males compared with the healthy controls (75.4%), HBsAg seroclearance participants (56.0%), and the HBsAg-positive individuals without HCC. Infection with HBV genotype C and HBeAg seroconversion were more common in the patients with HCC than in the HBsAg-positive individuals without HCC.

Associations of rs3783553 with the risks of HCC and other HBV-related clinical features

The genotyping success rates were 99.7%, 99.7%, and 99.5% for the healthy controls, HBV natural clearance participants, and chronic HBV-infected participants, respectively. rs3783553 was in HWE in the healthy controls ($P = 0.42$), HBsAg seroclearance individuals ($P = 0.91$), HBsAg-positive individuals without HCC ($P = 0.54$), and HCC patients ($P = 0.80$). Table 2 presents the genotype distributions of this SNP among different groups and the associations of rs3783553 with the risk of HCC. In general, no significant results were detected in the overall population or after stratifying by gender, either in the dominant or recessive model. We further investigated the association of this SNP with HCC risk in the participants infected with HBV genotypes B and C, respectively. No significant associations were discovered in either group (data not shown). Table 3 shows the association

Table 1 Population characteristics

Characteristics	Group A (<i>n</i> = 1012)	Group B (<i>n</i> = 302)	HBV-infected subjects without HCC groups (<i>n</i> = 990)			Group F (<i>n</i> = 1021)	<i>P</i> value
			C (<i>n</i> = 316)	D (<i>n</i> = 316)	E (<i>n</i> = 358)		
Male, <i>n</i> (%)	763 (75.40)	169 (55.96)	186 (58.86)	230 (72.78)	264 (73.74)	864 (84.13)	<0.001 ^{a,b,c,e,f} 0.001 ^d
Age (mean±SD)	59.56±15.10	58.40±11.72	45.08±10.61	44.18±14.51	50.68±11.34	52.92±11.17	<0.001 ^{a,b,c,d,e,f}
HBV genotype, <i>n</i> (%)							
B	NA	NA	97 (34.28)	52 (25.00)	56 (22.86)	107 (16.39)	<0.001 ^{b,c}
C	NA	NA	186 (65.72)	156 (75.00)	189 (77.14)	546 (83.61)	
HBeAg, <i>n</i> (%)							
Positive	NA	NA	130 (41.14)	132 (45.36)	107 (35.55)	241 (25.08)	<0.001 ^{b, c}
Negative	NA	NA	186 (58.86)	159 (54.64)	194 (64.45)	720 (74.92)	
HBV DNA (log10 NA copies/ml) (mean±SD)	NA	NA	3.88±1.80	4.43±1.67	4.13±1.37	3.83±1.18	<0.001 ^c
ALT (log10 U/L) (mean±SD)	NA	NA	1.36±0.21	1.97±0.54	1.75±0.44	1.66±0.35	<0.001 ^{b,e}

ALT, alanine aminotransferase; ASC, asymptomatic hepatitis B surface antigen carrier; CHB, chronic hepatitis B; HBeAg, hepatitis B e antigen; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; LC, liver cirrhosis; NA, not applicable.

Group A = healthy controls; group B = HBsAg seroclearance subjects; group C = ASCs; group D = CHB patients; group E = LC patients; Group F = HBV-HCC patients.

^a Group F versus group A.

^b Group F versus group C.

^c Group F versus groups (C + D + E).

^d Groups (C + D + E) versus group A.

^e Group F versus group B.

^f Groups (C + D + E) versus group B.

Table 2 Associations of rs3783553 genotype with the risk of HCC

Genotype	Group A	Group B	Groups (C+D+E)	Group F	Adjusted OR (95% CI)			
					Group F versus group A	Group F versus group B	Group F versus groups (C+D+E)	Group F versus groups (A+B+C+D+E)
Total								
del/del	431	132	439	434	1.00	1.00	1.00	1.00
del/ins	463	134	430	451	0.98 (0.88–1.08)	1.01 (0.86–1.18)	1.03 (0.94–1.14)	1.01 (0.93–1.10)
ins/ins	111	33	115	113	1.09 (0.79–1.49)	1.01 (0.63–1.62)	1.08 (0.79–1.48)	1.04 (0.81–1.34)
Dominant ^a					0.98 (0.81–1.18)	1.01 (0.76–1.35)	1.07 (0.89–1.29)	1.03 (0.88–1.20)
Recessive ^b					1.11 (0.83–1.52)	1.01 (0.65–1.56)	1.03 (0.78–1.39)	0.97 (0.76–1.23)
HWE <i>P</i>	0.42	0.91	0.54	0.80				
Females								
del/del	102	59	135	59	1.00	1.00	1.00	1.00
del/ins	115	59	130	74	1.02 (0.81–1.28)	1.08 (0.84–1.40)	1.17 (0.94–1.45)	1.10 (0.91–1.33)
ins/ins	29	12	44	25	1.34 (0.69–2.57)	1.89 (0.86–4.12)	1.59 (0.86–2.96)	1.44 (0.84–2.46)
Dominant ^a					1.10 (0.71–1.70)	1.29 (0.79–2.09)	1.40 (0.93–2.11)	1.25 (0.87–1.79)
Recessive ^b					1.30 (0.70–2.38)	1.69 (0.81–3.57)	1.27 (0.72–2.17)	0.78 (0.48–1.27)
HWE <i>P</i>	0.69	0.61	0.17	0.82				
Males								
del/del	329	73	304	375	1.00	1.00	1.00	1.00
del/ins	348	75	300	377	0.97 (0.87–1.08)	0.97 (0.80–1.18)	1.00 (0.90–1.12)	0.99 (0.91–1.09)
ins/ins	82	21	71	88	1.04 (0.72–1.48)	0.72 (0.40–1.27)	0.96 (0.67–1.38)	0.96 (0.72–1.28)
Dominant ^a					0.95 (0.77–1.18)	0.89 (0.61–1.28)	1.00 (0.81–1.23)	0.98 (0.83–1.16)
Recessive ^b					1.08 (0.76–1.52)	0.75 (0.44–1.27)	0.96 (0.68–1.35)	1.03 (0.78–1.35)
HWE <i>P</i>	0.48	0.80	0.81	0.64				

ASC, asymptomatic hepatitis B surface antigen carrier; CHB, chronic hepatitis B; CI, confidence interval; del, deletion; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HWE, Hardy-Weinberg equilibrium; ins, insertion; LC, liver cirrhosis; OR, odds ratio (adjusted for age and gender in the whole population; adjusted for age after stratification by gender).

Group A = healthy controls; group B = HBsAg seroclearance subjects; group C = ASCs; group D = CHB patients; group E = LC patients; group F = HCC patients.

^a (ins/ins + del/ins) vs. (del/del).

^b (ins/ins) vs. (del/ins + del/del).

of rs3783553 with other HBV-related clinical features. Under the recessive model, the homozygotes of the TTCA insertion allele were associated with an increased risk of developing LC compared with the heterozygotes and homozygotes of the deletion allele combined. However, this association was not significant after stratifying by gender.

Associations of rs3783553 with HCC-related HBV mutations

HBV EnhII/BCP/PC region and the preS region were successfully sequenced from 57.7% and 47.1% of the HBsAg-positive subjects, respectively. Our previous study has identified the HBV mutations that are associated with risk of HCC [28,29]. In the current study, stepwise multivariate logistic regression analyses were used to assess the factors, including age, gender, rs3783553 genotype [(ins/ins + del/ins) versus (del/del), indicating dominant model], and selected HBV mutations with risk of HCC. The HBV mutations T1674C/G, A1762T/G1764A, G1896A, and T1753V in the EnhII/BCP/PC region and preS deletion,

preS2 start codon mutation, C2875A, C76A, and C7A in the preS region, rather than the rs3783553 genotype, were significantly associated with an increased risk of HCC (Table 4). We then evaluated the associations of SNP rs3783553 with the frequencies of these HBV mutations using the data of HBsAg-positive participants, including the HCC patients. The TTCA insertion allele was significantly associated with an increased frequency of HBV C7A mutation compared with homozygous TTCA deletion carriers [(del/ins + ins/ins) versus del/del, adjusted OR = 1.48, 95% CI = 1.09–2.02, *P* = 0.013].

Multiplicative interactions of rs3783553 with the HBV mutations and their associations with HCC

We tested the multiplicative interactions of rs3783553 with all the significant HBV mutations (Table 4) in males and females separately. The interaction of rs3783553 with T1674C/G increased risk of HCC in females (OR = 2.23, 95% CI = 1.10–4.53). However, the association was not significant after age and HBV genotype were adjusted (Table 5). The

Table 3 Associations of rs3783553 genotype with HCC-free chronic HBV infection, LC, HBeAg seroconversion, abnormal ALT, and high viral load

Genotype	HCC-free chronic HBV infection ^a	LC	HBeAg seroconversion ^b	Abnormal ALT (U/L) ^b	Viral load (copies/ml) ^b
	HBV-infected ones versus healthy controls Adjusted OR (95% CI)	LC patients versus (ASCs + CHB patients) Adjusted OR (95% CI)	Negative versus positive Adjusted OR (95% CI)	<40 vs. ≥40 Adjusted OR (95% CI)	<10 ⁴ vs. ≥10 ⁴ Adjusted OR(95% CI)
Total					
del\del	1.00	1.00	1.00	1.00	1.00
del\ins	0.99 (0.89–1.10)	0.91 (0.79–1.05)	0.97 (0.87–1.08)	0.95 (0.85–1.07)	1.03 (0.92–1.15)
ins\ins	1.11 (0.79–1.57)	1.42 (0.91–2.19)	0.84 (0.61–1.17)	0.84 (0.59–1.19)	1.04 (0.73–1.49)
Dominant ^c	1.01 (0.82–1.23)	0.93 (0.71–1.22)	0.91 (0.75–1.12)	0.89 (0.72–1.11)	1.06 (0.85–1.31)
Recessive ^d	1.11 (0.81–1.54)	1.54 (1.03–2.33)	0.86 (0.63–1.16)	0.88 (0.64–1.22)	1.01 (0.73–1.41)
Females					
del\del	1.00	1.00	1.00	1.00	1.00
del\ins	0.94 (0.76–1.15)	0.83 (0.62–1.11)	1.09 (0.88–1.36)	0.97 (0.75–1.26)	1.02 (0.79–1.32)
ins\ins	0.99 (0.53–1.84)	1.55 (0.73–3.32)	0.72 (0.40–1.29)	0.68 (0.34–1.36)	1.01 (0.49–2.05)
Dominant ^c	0.91 (0.61–1.35)	0.87 (0.51–1.47)	1.04 (0.70–1.55)	0.86 (0.53–1.38)	1.03 (0.64–1.66)
Recessive ^d	1.06 (0.60–1.89)	1.82 (0.90–3.70)	0.66 (0.38–1.12)	0.68 (0.36–1.28)	0.99 (0.51–1.92)
Males					
del\del	1.00	1.00	1.00	1.00	1.00
del\ins	1.01 (0.89–1.14)	0.94 (0.79–1.11)	0.93 (0.82–1.05)	0.95 (0.83–1.08)	1.03 (0.91–1.17)
ins\ins	1.12 (0.74–1.70)	1.41 (0.82–2.41)	0.90 (0.60–1.34)	0.93 (0.62–1.40)	1.09 (0.73–1.64)
Dominant ^c	1.04 (0.82–1.31)	0.96 (0.70–1.32)	0.87 (0.69–1.10)	0.90 (0.71–1.15)	1.07 (0.84–1.37)
Recessive ^d	1.10 (0.75–1.61)	1.52 (0.92–2.50)	0.97 (0.67–1.41)	0.99 (0.68–1.45)	1.05 (0.71–1.56)

ALT, alanine aminotransferase; ASC, asymptomatic hepatitis B surface antigen carrier; CHB, chronic hepatitis B; CI, confidence interval; del, deletion; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; ins, insertion; LC, liver cirrhosis; OR, odds ratio.

Adjusted OR: adjusted for age and gender in the total population; adjusted for age after stratification by gender.

^aHBV-infected ones: ASCs, CHB patients, and LC patients.

^bAll HBV-infected subjects including HCC patients.

^c(ins/ins + del/ins) versus (del/del).

^d(ins/ins) versus (del/ins + del/del).

Table 4 Stepwise multivariate logistic regression analyses of factors independently associated with the risk of HCC

Variables	Adjusted OR (95% CI)	P value
HBV EnhII/BCP/PC region		
Age (year)	1.04 (1.03–1.06)	<0.0001
Gender (male)	1.74 (1.20–2.54)	0.004
T1674C/G	2.18 (1.46–3.24)	<0.000
A1762T/G1764A	2.38 (1.65–3.43)	<0.0001
G1896A	2.07 (1.49–2.86)	<0.0001
T1753V	1.55 (1.03–2.33)	0.037
HBV preS region		
Age (year)	1.05 (1.04–1.07)	<0.0001
Gender (male)	2.62 (1.73–3.99)	<0.0001
HBV Genotype C	4.19 (2.11–8.34)	<0.0001
preS deletion	1.92 (1.24–2.96)	0.004
preS2 start codon mutation	1.97 (1.23–3.15)	0.005
C2875A	2.22 (1.34–3.70)	0.002
C76A	9.51 (4.22–21.44)	<0.0001
C7A	3.92 (2.25–6.82)	0.000

CI, confidence interval; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; EnhII/BCP/PC, enhancer II/basal core promoter/precore of HBV; OR, odds ratio.

interaction of rs3783553 with preS deletion significantly decreased risk of HCC in males after age and HBV genotype were adjusted (OR = 0.64, 95% CI = 0.42–0.98; $P = 0.041$) (Table 6).

Discussion

In this study, we did not find a significant association between rs3783553 and risk of HCC among the healthy controls, HBsAg seroclearance subjects, or HBsAg-positive subjects with or without liver diseases (Table 2). These data were inconsistent with the previous report that the rs3783553 variant was significantly associated with a decreased risk of HCC [26]. This inconsistency might have been caused by different controls in the two studies. Identifying the status of the controls is important to explore genetic susceptibility to

HBV-related HCC in case-control studies. Recent genome-wide association studies have also reported contrasting results in genetic susceptibility to HBV-induced HCC [30–33]. The reasons may be complex, but we believe that HBV viral factors are the major confounders that affect the discovery of HCC genetic susceptible loci. The sizes of the effects (e.g., OR values in case-control studies) of genetic polymorphisms on HCC risk are usually smaller than those of the HBV mutations or genotypes [27,28,34,35]. The effect of genetic polymorphisms on risk of HCC can be greatly masked by the HBV mutations, whereas the effect of HBV mutations on risk of HCC can be solely significant in subjects with certain genetic background [27,28].

In the present study, the rs3783553 TTCA insertion allele was significantly associated with an increased frequency of HBV C7A mutation. HBV C7A mutation is extremely higher in HBV-infected subjects with liver diseases (CHB, LC, and HCC) than in those with ASCs [12]. C7A was more frequent

Table 5 Associations of the HBV mutations in the EnhII/BCP/PC region and their interactions with rs3783553 polymorphism with the risk of HCC

rs3783553	HBV mutation	Females				Males			
		Non-HCC ^a	HCC ^b	OR (95% CI)	Adjusted OR (95% CI) ^c	Non-HCC ^a	HCC ^b	OR (95% CI)	Adjusted OR (95% CI) ^c
	T1674C/G								
del/del	T	54	21	Ref	Ref	136	118	Ref	Ref
del/del	C/G	13	11	2.18 (0.84–5.62)	3.27 (1.06–10.09)	24	66	3.17 (1.87–5.38)	2.83 (1.56–5.13)
ins/del	T	51	18	0.91 (0.43–1.90)	0.93 (0.62–1.38)	140	118	0.97 (0.69–1.38)	0.97 (0.80–1.17)
ins/del	C/G	6	23	9.86 (3.52–27.62)	11.06 (3.57–34.28)	27	53	2.26 (1.34–3.82)	1.89 (1.04–3.45)
For interaction				2.23 (1.10–4.53)	2.06 (0.96–4.40)			0.86 (0.59–1.24)	0.85 (0.57–1.28)
	A1762T/G1764A								
del/del	AG/AA/TG	48	9	Ref	Ref	88	37	Ref	Ref
del/del	TA	34	23	3.61 (1.49–8.76)	4.28 (1.49–12.29)	88	142	3.84 (2.41–6.12)	3.48 (2.07–5.85)
ins/del	AG/AA/TG	57	8	0.75 (0.27–2.09)	0.78 (0.46–1.35)	85	33	0.92 (0.53–1.61)	0.98 (0.73–1.33)
ins/del	TA	22	35	8.48 (3.49–20.65)	10.94 (3.56–33.61)	92	130	3.36 (2.11–5.37)	2.89 (1.76–4.74)
For interaction				1.77 (0.94–3.35)	1.96 (0.99–3.88)			0.97 (0.70–1.36)	0.91 (0.64–1.30)
	G1896A								
del/del	G	64	20	Ref	Ref	123	73	Ref	Ref
del/del	A	18	13	2.31 (0.97–5.53)	2.22 (0.83–5.98)	59	89	2.54 (1.64–3.94)	2.31 (1.40–3.79)
ins/del	G	59	19	1.03 (0.50–2.12)	0.94 (0.61–1.43)	125	78	1.05 (0.70–1.58)	0.98 (0.79–1.23)
ins/del	A	22	22	3.20 (1.47–6.95)	2.94 (1.29–6.71)	54	76	2.37 (1.51–3.73)	2.01 (1.22–3.31)
For interaction				1.16 (0.65–2.08)	1.22 (0.63–2.36)			0.94 (0.69–1.29)	0.94 (0.67–1.32)
	T1753V								
del/del	T	70	19	Ref	Ref	156	118	Ref	Ref
del/del	V	14	13	3.42 (1.38–8.49)	4.74 (1.65–13.60)	26	61	3.10 (1.85–5.21)	3.24 (1.80–5.81)
ins/del	T	73	29	1.46 (0.75–2.85)	1.21 (0.85–1.73)	157	110	0.93 (0.66–1.30)	1.00 (0.83–1.21)
ins/del	V	7	12	6.32 (2.19–18.25)	7.40 (2.14–25.61)	28	53	2.50 (1.49–4.20)	1.79 (0.99–3.21)
OR for interaction				1.12 (0.57–2.23)	1.05 (0.50–2.20)			0.93 (0.65–1.35)	0.77 (0.51–1.14)

CI, confidence interval; EnhII/BCP/PC, enhancer II/basal core promoter/precure of HBV; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; OR, odds ratio.

^a HBsAg-positive subjects without HCC.

^b HBsAg-positive patients with HCC.

^c Adjusted for age and HBV genotype.

Table 6 Associations of the HBV mutations in the preS region and their interactions with rs3783553 polymorphism with the risk of HCC

rs3783553	HBV mutation	Females				Males			
		Non-HCC ^a	HCC ^b	OR (95%CI)	Adjusted OR (95% CI) ^c	Non-HCC ^a	HCC ^b	OR (95%CI)	Adjusted OR (95% CI) ^c
	preS deletion								
	del/del	54	11	Ref	Ref	102	96	Ref	Ref
	del/del	5	14	13.75 (4.10–46.08)	11.40 (3.20–40.60)	18	48	2.83 (1.54–5.21)	2.54 (1.33–4.84)
	ins/del	51	25	2.41(1.08–5.39)	1.48 (0.97–2.26)	83	97	1.24 (0.83–1.86)	1.18 (0.95–1.47)
	ins/del	8	7	4.30 (1.29–14.32)	4.43 (1.27–15.46)	31	50	1.72 (1.01–2.91)	1.49 (0.85–2.61)
	For interaction			0.36 (0.16–0.82)	0.46 (0.19–1.11)			0.70 (0.47–1.05)	0.64 (0.42–0.98)
	preS2 start codon mutation								
	del/del	52	19	Ref	Ref	102	102	Ref	Ref
	del/del	7	7	2.74 (0.85–8.84)	2.55 (0.73–8.90)	18	49	2.72 (1.49–4.99)	2.48 (1.31–4.70)
	ins/del	53	27	1.39 (0.69–2.81)	1.19 (0.82–1.73)	95	123	1.30 (0.88–1.90)	1.16 (0.94–1.42)
	ins/del	6	8	3.65 (1.12–11.90)	2.86 (0.84–9.75)	19	31	1.63 (0.87–3.07)	1.67 (0.85–3.28)
	For interaction			0.98 (0.43–2.23)	0.89 (0.37–2.14)			0.68 (0.44–1.05)	0.70 (0.44–1.11)
	C2875A								
	del/del	43	17	Ref	Ref	99	113	Ref	Ref
	del/del	19	11	1.46 (0.58–3.72)	2.22 (0.70–7.06)	42	52	1.09 (0.67–1.77)	2.12 (1.10–4.12)
	ins/del	46	23	1.27 (0.60–2.68)	1.14 (0.76–1.72)	94	109	1.02 (0.69–1.49)	1.00 (0.81–1.22)
	ins/del	17	10	1.49 (0.57–3.89)	5.18 (1.37–19.58)	47	54	1.01 (0.63–1.62)	1.83 (0.96–3.48)
	For interaction			0.90 (0.47–1.73)	0.77 (0.37–1.60)			0.96 (0.68–1.35)	1.02 (0.70–1.48)
	C76A								
	del/del	55	19	Ref	Ref	105	117	Ref	Ref
	del/del	3	6	5.79 (1.32–25.45)	36.94 (2.75–497.51)	11	31	2.53 (1.21–5.28)	5.62 (1.98–15.98)
	ins/del	52	33	1.84 (0.93–3.63)	1.46 (1.00–2.12)	101	123	1.09 (0.75–1.59)	1.04 (0.85–1.27)
	ins/del	4	2	1.45 (0.25–8.55)	1.79 (0.21–15.15)	12	31	2.32 (1.13–4.75)	7.94 (2.56–24.59)
	For interaction			0.37 (0.12–1.16)	0.14 (0.04–0.54)			0.92 (0.55–1.53)	1.26 (0.72–2.23)
	C7A								
	del/del	30	6	Ref	Ref	50	35	Ref	Ref
	del/del	27	18	3.33 (1.16–9.62)	2.90 (0.78–10.83)	62	102	2.35 (1.38–4.01)	2.06 (1.09–3.87)
	ins/del	25	3	0.60 (0.14–2.65)	0.69 (0.32–1.51)	36	38	1.51 (0.81–2.83)	1.34 (0.93–1.93)
	ins/del	31	31	5.00 (1.83–13.70)	4.30 (1.31–14.14)	70	109	2.22 (1.32–3.76)	1.94 (1.03–3.64)
	OR for interaction			1.58 (0.68–3.65)	1.76 (0.74–4.15)			0.79 (0.54–1.16)	0.73 (0.48–1.10)

CI, confidence interval; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; OR, odds ratio.

^aHBsAg-positive subjects without HCC.

^bHBsAg-positive patients with HCC.

^cAdjusted for age and HBV genotype.

in the subjects with rs3783553 TTCA insertion allele than those with the deletion allele. This result indicates that the rs3783553 genetic variant facilitates immuno-selection of this HBV mutation during the evolutionary process. The effect of viral factors and host proinflammatory molecules on tumor-promoting inflammation and anti-tumor immunity is complex. The interaction of viral mutations with genetic polymorphisms of some inflammatory molecules may contribute to the risk of HCC.

We found that multiplicative interaction of rs3783553 and preS deletion reduced the risk of HCC in males after age and HBV genotype were adjusted (Table 6). The interaction of rs3783553 with T1674C/G increased the risk of HCC in females (Table 5), and the interaction of rs3783553 with preS deletion reduced the risk of HCC in females (Table 6). However, the sample sizes became very small after the stratification, and the statistical power was low. We hypothesize that the variant genotype (TTCA insertion) of rs3783553 significantly decreases the influence of HBV preS deletion in promoting hepatocarcinogenesis. HBV preS deletion is a strong HCC-risk HBV mutation [9,10,12]. rs3783553 is an insertion/deletion (insertion or deletion of TTCA bases) polymorphism that is located at the miR-122 binding site in the 3'UTR of *IL-1A*. The TTCA insertion allele of rs3783553, which is associated with high IL-1 α expression *in situ* or in serum, has also been associated with reduced risks of HCC and nasopharyngeal carcinoma in Chinese populations [26,36]. IL-1 is a pleiotropic cytokine that primarily affects inflammatory and immune responses as well as induces the expression of several important pro-inflammatory genes [37]. IL-1 α , which is locally expressed in macrophages, has a mediating function in chronic liver damage and inflammation to HCC and expression of IL-1 α on malignant cells stimulates anti-tumor immunity [20,37]. MiR-122 is a liver-specific miRNA that may influence HCV replication by binding RNA folding or RNA accumulation into replication complexes. Therefore, miR-122 serves as a therapeutic target of HCV infection [21,38]. Recently, miR-122 has been linked to HBV infection. miR-122 levels, which may facilitate viral replication and persistence, are significantly decreased in HBV-infected patients [39]. HBV mRNA inhibits miR-122 production and promotes HCC by upregulating miR-122-targeted molecule pituitary tumor-transforming gene 1 binding factor [40]. HBV X protein binds peroxisome proliferator-activated receptor- γ and inhibits miR-122 transcription [41]. miR-122 inhibits IL-1 α expression in a dose-dependent manner, especially in cells with rs3783553 TTCA deletion [26]. Downregulation of miR-122 in HBV-infected subjects also facilitates IL-1 α expression. Thus, the variant genotype of rs3783553 might predispose the host to express IL-1 α in resident macrophages in the liver upon HBV infection, upregulate immune response to HBV, and attenuate the carcinogenic effect of HBV preS deletion. Further functional studies are warranted to clarify the preventive

effect of rs3783553 TTCA insertion allele on HBV preS deletion-promoted hepatocarcinogenesis.

The present study has several limitations that need to be addressed. First, the two HBV regions were not amplified from a fraction of the HBsAg-positive participants, so the sample size for subsequent analyses was reduced. Second, although we did not find any statistically significant association between rs3783553 and risk of HBV-related HCC, other genetic polymorphisms at the miR-122 binding-sites should be evaluated for predicting the risk of this inflammation-related malignancy. Third, our study design is cross-sectional case-control study in nature.

In summary, our study suggested that the miRNA-122 binding site polymorphism at the *IL-1A* gene (rs3783553) did not significantly affect the risk of HBV-related HCC. However, the variant allele of rs3783553 significantly decreased the cancer-promoting effect of HBV preS deletion in the HBV-infected subjects. Future prospective studies should be conducted to evaluate the interaction of *IL-1A* gene polymorphisms, such as rs3783553, with HBV mutations in HBV-induced hepatocarcinogenesis.

Acknowledgements

This study was funded by the National Outstanding Youth Fund (81025015) and Creative Research Group (30921006) from National Natural Scientific Foundation of China, Science and Technology Commission of Shanghai Municipality Fund (12ZR1453600, 12ZR1429300), and Shanghai Health Bureau Fund (20114066).

Abbreviations

ALT	alanine aminotransferase
ANOVA	analysis of variance
anti-HBc	antibody to HBc
anti-HCV	antibody to HCV
ASC	asymptomatic HBsAg carriers
CHB	chronic hepatitis B
CI	confidence interval
HBsAg	HBV surface antigen
HBV	hepatitis B virus
HCC	hepatocellular carcinoma
HCV	hepatitis C virus
HDV	hepatitis δ virus
HWE	Hardy-Weinberg equilibrium
IL-1	interleukin-1
LC	liver cirrhosis
miRNA	mircoRNA
mRNA	messenger RNA
OR	odds ratio
qPCR	quantitative PCR
3'UTR	3' untranslated region

Compliance with ethics guidelines

Yan Du, Xue Han, Rui Pu, Jiabin Xie, Yuwei Zhang, and Guangwen Cao declare that they have no conflict of interest. This study was approved by the ethics committee of the Second Military Medical University, and the study protocol is in accordance with the Declaration of Helsinki [revised in 2000 (5)]. All participants provided written informed consents.

References

- Lok AS. Does antiviral therapy for hepatitis B and C prevent hepatocellular carcinoma? *J Gastroenterol Hepatol* 2011; 26(2): 221–227
- Tan YJ. Hepatitis B virus infection and the risk of hepatocellular carcinoma. *World J Gastroenterol* 2011; 17(44): 4853–4857
- Yin J, Zhang H, He Y, Xie J, Liu S, Chang W, Tan X, Gu C, Lu W, Wang H, Bi S, Cui F, Liang X, Schaefer S, Cao G. Distribution and hepatocellular carcinoma-related viral properties of hepatitis B virus genotypes in Mainland China: a community-based study. *Cancer Epidemiol Biomarkers Prev* 2010; 19(3): 777–786
- Siegel R, Naishadham D, Jemal A. Cancer statistics, 2012. *CA Cancer J Clin* 2012; 62(1): 10–29
- Huang YT, Jen CL, Yang HI, Lee MH, Su J, Lu SN, Iloeje UH, Chen CJ. Lifetime risk and sex difference of hepatocellular carcinoma among patients with chronic hepatitis B and C. *J Clin Oncol* 2011; 29(27): 3643–3650
- Fang ZL, Sabin CA, Dong BQ, Ge LY, Wei SC, Chen QY, Fang KX, Yang JY, Wang XY, Harrison TJ. HBV A1762T, G1764A mutations are a valuable biomarker for identifying a subset of male HBsAg carriers at extremely high risk of hepatocellular carcinoma: a prospective study. *Am J Gastroenterol* 2008; 103(9): 2254–2262
- Yang HI, Yeh SH, Chen PJ, Iloeje UH, Jen CL, Su J, Wang LY, Lu SN, You SL, Chen DS, Liaw YF, Chen CJ; REVEAL-HBV Study Group. Associations between hepatitis B virus genotype and mutants and the risk of hepatocellular carcinoma. *J Natl Cancer Inst* 2008; 100(16): 1134–1143
- Chou YC, Yu MW, Wu CF, Yang SY, Lin CL, Liu CJ, Shih WL, Chen PJ, Liaw YF, Chen CJ. Temporal relationship between hepatitis B virus enhancer II/basal core promoter sequence variation and risk of hepatocellular carcinoma. *Gut* 2008; 57(1): 91–97
- Liu S, Zhang H, Gu C, Yin J, He Y, Xie J, Cao G. Associations between hepatitis B virus mutations and the risk of hepatocellular carcinoma: a meta-analysis. *J Natl Cancer Inst* 2009; 101(15): 1066–1082
- Zhang Q, Cao G. Genotypes, mutations, and viral load of hepatitis B virus and the risk of hepatocellular carcinoma: HBV properties and hepatocarcinogenesis. *Hepat Mon* 2011; 11(2): 86–91
- Yin J, Xie J, Liu S, Zhang H, Han L, Lu W, Shen Q, Xu G, Dong H, Shen J, Zhang J, Han J, Wang L, Liu Y, Wang F, Zhao J, Zhang Q, Ni W, Wang H, Cao G. Association between the various mutations in viral core promoter region to different stages of hepatitis B, ranging of asymptomatic carrier state to hepatocellular carcinoma. *Am J Gastroenterol* 2011; 106(1): 81–92
- Yin J, Xie J, Zhang H, Shen Q, Han L, Lu W, Han Y, Li C, Ni W, Wang H, Cao G. Significant association of different preS mutations with hepatitis B-related cirrhosis or hepatocellular carcinoma. *J Gastroenterol* 2010; 45(10): 1063–1071
- Han YF, Zhao J, Ma LY, Yin JH, Chang WJ, Zhang HW, Cao GW. Factors predicting occurrence and prognosis of hepatitis-B-virus-related hepatocellular carcinoma. *World J Gastroenterol* 2011; 17(38): 4258–4270
- Chemin I, Zoulim F. Hepatitis B virus induced hepatocellular carcinoma. *Cancer Lett* 2009; 286(1): 52–59
- Chen L, Zhang Q, Chang W, Du Y, Zhang H, Cao G. Viral and host inflammation-related factors that can predict the prognosis of hepatocellular carcinoma. *Eur J Cancer* 2012; 48(13): 1977–1987
- Lim SG, Mohammed R, Yuen MF, Kao JH. Prevention of hepatocellular carcinoma in hepatitis B virus infection. *J Gastroenterol Hepatol* 2009; 24(8): 1352–1357
- Cao GW. Clinical relevance and public health significance of hepatitis B virus genomic variations. *World J Gastroenterol* 2009; 15(46): 5761–5769
- Wu CY, Chen YJ, Ho HJ, Hsu YC, Kuo KN, Wu MS, Lin JT. Association between nucleoside analogues and risk of hepatitis B virus-related hepatocellular carcinoma recurrence following liver resection. *JAMA* 2012; 308(18): 1906–1914
- Yin J, Li N, Han Y, Xue J, Deng Y, Shi J, Guo W, Zhang H, Wang H, Cheng S, Cao G. Effect of antiviral treatment with nucleotide/nucleoside analogs on postoperative prognosis of hepatitis B virus-related hepatocellular carcinoma: a two-stage longitudinal clinical study. *J Clin Oncol* 2013; 31(29): 3647–3655
- Apte RN, Dotan S, Elkabets M, White MR, Reich E, Carmi Y, Song X, Dvozkin T, Krelin Y, Voronov E. The involvement of IL-1 in tumorigenesis, tumor invasiveness, metastasis and tumor-host interactions. *Cancer Metastasis Rev* 2006; 25(3): 387–408
- Zhang Q, Pu R, Du Y, Han Y, Su T, Wang H, Cao G. Non-coding RNAs in hepatitis B or C-associated hepatocellular carcinoma: potential diagnostic and prognostic markers and therapeutic targets. *Cancer Lett* 2012; 321(1): 1–12
- Wang W, Zhao LJ, Tan YX, Ren H, Qi ZT. Identification of deregulated miRNAs and their targets in hepatitis B virus-associated hepatocellular carcinoma. *World J Gastroenterol* 2012; 18(38): 5442–5453
- Ding J, Huang S, Wang Y, Tian Q, Zha R, Shi H, Wang Q, Ge C, Chen T, Zhao Y, Liang L, Li J, He X. Genome-wide screening reveals that miR-195 targets the TNF- α /NF- κ B pathway by down-regulating I κ B kinase α and TAB3 in hepatocellular carcinoma. *Hepatology* 2013; 58(2): 654–666
- Yu Z, Li Z, Jolicoeur N, Zhang L, Fortin Y, Wang E, Wu M, Shen SH. Aberrant allele frequencies of the SNPs located in microRNA target sites are potentially associated with human cancers. *Nucleic Acids Res* 2007; 35(13): 4535–4541
- Sonkoly E, Stähle M, Pivarcsi A. MicroRNAs and immunity: novel players in the regulation of normal immune function and inflammation. *Semin Cancer Biol* 2008; 18(2): 131–140
- Gao Y, He Y, Ding J, Wu K, Hu B, Liu Y, Wu Y, Guo B, Shen Y, Landi D, Landi S, Zhou Y, Liu H. An insertion/deletion polymorphism at miRNA-122-binding site in the interleukin-1 α 3' untranslated region confers risk for hepatocellular carcinoma. *Carcinogenesis* 2009; 30(12): 2064–2069

27. Han Y, Pu R, Han X, Zhao J, Zhang Y, Zhang Q, Yin J, Xie J, Shen Q, Deng Y, Ding Y, Li W, Li J, Zhang H, Cao G. Associations of pri-miR-34b/c and pre-miR-196a2 polymorphisms and their multiplicative interactions with hepatitis B virus mutations with hepatocellular carcinoma risk. *PLoS ONE* 2013; 8(3): e58564
28. Xie J, Zhang Y, Zhang Q, Han Y, Yin J, Pu R, Shen Q, Lu W, Du Y, Zhao J, Han X, Zhang H, Cao G. Interaction of signal transducer and activator of transcription 3 polymorphisms with hepatitis B virus mutations in hepatocellular carcinoma. *Hepatology* 2013; 57(6): 2369–2377
29. Xie JX, Zhao J, Yin JH, Zhang Q, Pu R, Lu WY, Zhang HW, Wang HY, Cao GW. Association of novel mutations and haplotypes in the preS region of hepatitis B virus with hepatocellular carcinoma. *Front Med China* 2010; 4(4): 419–429
30. Zhang H, Zhai Y, Hu Z, Wu C, Qian J, Jia W, Ma F, Huang W, Yu L, Yue W, Wang Z, Li P, Zhang Y, Liang R, Wei Z, Cui Y, Xie W, Cai M, Yu X, Yuan Y, Xia X, Zhang X, Yang H, Qiu W, Yang J, Gong F, Chen M, Shen H, Lin D, Zeng YX, He F, Zhou G. Genome-wide association study identifies 1p36.22 as a new susceptibility locus for hepatocellular carcinoma in chronic hepatitis B virus carriers. *Nat Genet* 2010; 42(9): 755–758
31. Li S, Qian J, Yang Y, Zhao W, Dai J, Bei JX, Foo JN, McLaren PJ, Li Z, Yang J, Shen F, Liu L, Yang J, Li S, Pan S, Wang Y, Li W, Zhai X, Zhou B, Shi L, Chen X, Chu M, Yan Y, Wang J, Cheng S, Shen J, Jia W, Liu J, Yang J, Wen Z, Li A, Zhang Y, Zhang G, Luo X, Qin H, Chen M, Wang H, Jin L, Lin D, Shen H, He L, de Bakker PI, Wang H, Zeng YX, Wu M, Hu Z, Shi Y, Liu J, Zhou W. GWAS identifies novel susceptibility loci on 6p21.32 and 21q21.3 for hepatocellular carcinoma in chronic hepatitis B virus carriers. *PLoS Genet* 2012; 8(7): e1002791
32. Clifford RJ, Zhang J, Meerzaman DM, Lyu MS, Hu Y, Cultraro CM, Finney RP, Kelley JM, Efroni S, Greenblum SI, Nguyen CV, Rowe WL, Sharma S, Wu G, Yan C, Zhang H, Chung YH, Kim JA, Park NH, Song IH, Buetow KH. Genetic variations at loci involved in the immune response are risk factors for hepatocellular carcinoma. *Hepatology* 2010; 52(6): 2034–2043
33. Jiang DK, Sun J, Cao G, Liu Y, Lin D, Gao YZ, Ren WH, Long XD, Zhang H, Ma XP, Wang Z, Jiang W, Chen TY, Gao Y, Sun LD, Long JR, Huang HX, Wang D, Yu H, Zhang P, Tang LS, Peng B, Cai H, Liu TT, Zhou P, Liu F, Lin X, Tao S, Wan B, Sai-Yin HX, Qin LX, Yin J, Liu L, Wu C, Pei Y, Zhou YF, Zhai Y, Lu PX, Tan A, Zuo XB, Fan J, Chang J, Gu X, Wang NJ, Li Y, Liu YK, Zhai K, Zhang H, Hu Z, Liu J, Yi Q, Xiang Y, Shi R, Ding Q, Zheng W, Shu XO, Mo Z, Shugart YY, Zhang XJ, Zhou G, Shen H, Zheng SL, Xu J, Yu L. Genetic variants in STAT4 and HLA-DQ genes confer risk of hepatitis B virus-related hepatocellular carcinoma. *Nat Genet* 2013; 45(1): 72–75
34. Du Y, Xie J, Chang W, Han Y, Cao G. Genome-wide association studies: inherent limitations and future challenges. *Front Med* 2012; 6(4): 444–450
35. He Y, Zhang H, Yin J, Xie J, Tan X, Liu S, Zhang Q, Li C, Zhao J, Wang H, Cao G. IkappaBalpha gene promoter polymorphisms are associated with hepatocarcinogenesis in patients infected with hepatitis B virus genotype C. *Carcinogenesis* 2009; 30(11): 1916–1922
36. Yang ZH, Dai Q, Zhong L, Zhang X, Guo QX, Li SN. Association of IL-1 polymorphisms and IL-1 serum levels with susceptibility to nasopharyngeal carcinoma. *Mol Carcinog* 2011; 50(3): 208–214
37. Nicklin MJ, Weith A, Duff GW. A physical map of the region encompassing the human interleukin-1 alpha, interleukin-1 beta, and interleukin-1 receptor antagonist genes. *Genomics* 1994; 19(2): 382–384
38. Pan QW, Henry SD, Scholte BJ, Tilanus HW, Janssen HL, van der Laan LJ. New therapeutic opportunities for hepatitis C based on small RNA. *World J Gastroenterol* 2007; 13(33): 4431–4436
39. Wang S, Qiu L, Yan X, Jin W, Wang Y, Chen L, Wu E, Ye X, Gao GF, Wang F, Chen Y, Duan Z, Meng S. Loss of microRNA 122 expression in patients with hepatitis B enhances hepatitis B virus replication through cyclin G(1)-modulated P53 activity. *Hepatology* 2012; 55(3): 730–741
40. Li C, Wang Y, Wang S, Wu B, Hao J, Fan H, Ju Y, Ding Y, Chen L, Chu X, Liu W, Ye X, Meng S. Hepatitis B virus mRNA-mediated miR-122 inhibition upregulates PTTG1-binding protein, which promotes hepatocellular carcinoma tumor growth and cell invasion. *J Virol* 2013; 87(4): 2193–2205
41. Song K, Han C, Zhang J, Lu D, Dash S, Feitelson M, Lim K, Wu T. Epigenetic regulation of MicroRNA-122 by peroxisome proliferator activated receptor-gamma and hepatitis b virus X protein in hepatocellular carcinoma cells. *Hepatology* 2013; 58(5): 1681–1692