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• BRIEF REPORTS •

Association of -238G/A polymorphism of tumor necrosis factor-alpha gene promoter region with outcomes of hepatitis B virus infection in Chinese Han population

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Abstract

AIM: To clarify whether -238G/A polymorphism of tumor necrosis factor-α (TNF-α) gene promoter region was associated with outcomes of hepatitis B virus (HBV) infection in Han population of northern China, and to analyze the gene-environment interaction between -238G/A polymorphism and cigarette smoking or alcohol consumption.

METHODS: A case-control study was conducted to analyze the association of TNF-α gene promoter polymorphism with HBV infection outcomes. A total of 207 patients with chronic hepatitis B (HB) and 148 cases of self-limited HBV infection from Ditan Hospital and Shunyi District Hospital in Beijing, respectively were recruited. History of smoking and alcohol drinking was inquired by a questionnaire. The -238G/A polymorphism of TNF-α gene promoter was genotyped by polymerase chain reaction-restricted fragment length polymorphism (PCR-RFLP).

RESULTS: The frequencies of GG and GA genotypes were 98.07% and 1.93% in chronic HB patients and 93.24% and 6.76% in self-limited HBV infection individuals, respectively (χ^2=5.30, *P*=0.02). The frequency of G allele was significantly higher in patients with chronic HB that in individuals with self-limited HBV infection (99.03% *vs* 96.62%, χ^2=5.20, *P*=0.02). Only modestly increased risk of onset of chronic HB was found in smokers (*OR*=1.40, *95% CI*: 0.87-2.28, *P*=0.14) and drinkers (*OR*=1.26, *95%CI*: 0.78-2.05, *P*=0.32). There was a positive interaction between genotype GG and cigarette smoking with an interaction index (II) of 2.95, or alcohol consumption with an II of 1.64.

CONCLUSION: The -238G/A polymorphism of TNF-α gene promoter region is independently associated with different outcomes of HBV infection.

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INTRODUCTION

Human beings are susceptible to HBV. HBV infection in adults is usually clinically inapparent, and the virus is cleared after infection. Only about 5-10% of them become persistently infected and develop chronic liver disease with varied severity[1], which could not be explained completely by the virus itself and environmental factors. Progress of HBV infection might be affected by host genetic susceptibility[2].

Since HBV is not cytolytic for hepatocytes, and hepatocellular injuries caused by HBV infection are predominantly immune-mediated[3-6]. Immune attacks by host against HBV are mainly mediated by a cellular reaction. Cytokines produced by immune cells, such as TNF-α, might play a role in immune pathogenesis of HBV infection.

TNF-α is secreted by macrophages, monocytes, neutrophils, T-cells and NK-cells following the stimulus by bacterial lipopolysaccharides and shows a broad spectrum of biological activities, causing cytolysis and cytostasis in many tumor cell lines *in vitro*. Several lines of evidence suggest the importance of TNF-α in HBV. Patients with acute and chronic hepatitis B have an elevated plasma concentration of TNF-α[7,8]. Some individual differences in cytokine production may be related to genetic components, and certain polymorphism alleles may be associated with higher or lower levels of TNF-α production, which has been ascribed to polymorphisms within the regulatory regions of cytokine genes[9-14].

There were some studies about the association of TNF-α gene promoter polymorphism with progress of the disease[13,15], but ethnic difference could lead to different results. The aim of the present study was to investigate whether the TNF-α promoter polymorphism at position -238 was associated with outcomes of HBV infection in Han people of northern China.

MATERIALS AND METHODS

Study design

Case-control study was used to analyze the association between the polymorphism at position -238 of TNF-α gene promoter and outcomes of HBV infection, as well as the interaction between the gene and smoking or alcohol drinking.

Subjects

The clinical diagnosis for all subjects in this study was based

on references[16,17]. Two hundred and seven patients with chronic HB from Ditan Hospital in Beijing, China during November 2001 to August 2002 were recruited, with inclusion criteria as follows: hepatitis B surface antigen (HBsAg) seropositive, anti-HBs antibodies (anti-HBs) seronegative, abnormally elevated serum alanine aminotransferase level, and duration of chronic HB ⩾2 years. One hundred and forty-eight subjects with self-limited HBV infection were from Shunyi District Hospital in Beijing, China during the same period, with inclusion criteria as positive for both anti-HBs and anti-HBc antibodies only, definitely negative for HBsAg, normal liver function tests, and no history of HBV vaccination. All subjects were Chinese Han people and they were recruited with their informed consent for genetic analysis. Venous blood was drawn from all subjects after an overnight fasting. Serum was separated immediately to detect ALT and blood corpuscles were stored at -70 ℃ to extract DNA and analyze genotypes.

Serological tests

Enzyme-linked immunoadsorbent assay (ELISA) was used for detection of serum HBsAg, anti-HBs, and anti-HBc (IMX; Abbott Diagnostics, North Chicago, IL).

Analysis of TNF-α gene promoter polymorphism

Genomic DNAs were obtained from peripheral blood leukocytes by standard phenol-chloroform extraction[18]. The -238G/A polymorphism in the promoter region of TNF-α gene was detected by PCR-RFLP as described by Miyazoe *et al*[19]. A 152-bp fragment was amplified using primers (5' : 5' AGAAG ACCCCCCTCGGAACC3' and 3' : 5' ATCTGGAGGAAGCG GTAGTG3'). Amplification was performed in a Perkin Elmer thermocycler (2700; Applied Biosystems, Foster City, CA) with 50 ng of genomic DNA, 20 pmol/L of each primer, 200 μmol/L each dNTP, 1.5 mmol/L $MgCl_2$, standard polymerase chain reaction (PCR) buffer and 1U Taq polymerase (Shanghai Biocolor) to 25 μL reaction system. PCR procedure was as follows: predenaturation at 94 ℃ for 2 min, followed by 30 cycles of denaturation at 94 ℃ for 1 min, annealing at 59 ℃ for 1 min and extension at 72 ℃ for 1 min, with a final extension at 72 ℃ for 5 min to terminate the reaction. After amplification, 10 μL PCR product was digested with restriction endonuclease (Msp-I 3U, Takara Bio Cor Dalian) at 37 ℃ for 5 h after addition of appropriate incubation buffer and ddH_2O to 20 μL. The digestion products were separated on 3% agarose gel and visualized directly under UV light with ethidium bromide staining. One base-exchange substitution from A to G position at position -238 created the Msp-I restriction site, resulting in 20- and 132-bp fragments with Msp-I digestion, where -238A allele could not create the Msp-I restriction site, which resulted in an 152-bp fragment.

Cigarette smoking and alcohol consumption

Cigarette smoking and alcohol consumption of the subjects were assessed by their self-report in a questionnaire.

Evaluation of the interaction

The gene-environment interaction was defined according to Rothman *et al*[20], with a formula to estimate interaction index $(II)=OR_{11}/OR_{01}\times OR_{10}$. II>1 was defined as positive, and II<1 as negative.

Statistical analysis

The frequencies of TNF-α promoter region alleles and genotypes were estimated. The Hardy-Weinberg equilibrium and frequencies of the alleles and genotypes between two groups were compared by χ^2 tests with two-tailed *P* values[21]. Odds ratios and their 95% confidence intervals were also calculated as measures of association of the polymorphism with outcomes of HBV infection. All the statistical procedures were performed with SAS version 6.12.

RESULTS

Characteristics of subjects

The main characteristics of study subjects are summarized in Table 1. The average age of the patients with chronic HB and subjects with self-limited HBV infection was 40.06(40.06±14.55) and 37.75(37.75±13.35) years, respectively, without significant difference (t=1.53, P=0.40). The number of men was more in the group of patients with chronic HB that in the group of subjects with self-limited HBV infection (χ^2=36.54, P<0.01). The proportion of married subjects was also different between the two groups (χ^2=6.29, P=0.01), whereas their education level was not statistically different (χ^2=5.66, P=0.06).

Table 1 Main characteristics of study groups

Variable	Chronic HB n=207(%)	Self-limited HBV infection n=148 (%)	P
Age (mean±SD)	40.06±14.55	37.75±13.35	0.40
Male / Female	162 / 45	70 / 78	<0.01
Marital status			0.01
Married	162 (78.26)	131 (88.51)	
Unmarried	45 (21.74)	17 (11.49)	
Education level			0.06
Lower than high school	13 (6.28)	18 (12.16)	
High school	126 (60.87)	94 (63.51)	
Above high school	68 (32.85)	36 (24.32)	

Association of -238G/A polymorphism of TNF-α promoter and behavior factors with outcomes of HBV infection

The distribution of genotype frequencies in patients with chronic HB and subjects with self-limited HBV infection was coincident with Hardy-Weinberg equilibrium (χ^2=0.02, P=0.89; χ^2=0.02, P=0.89).

The genotype distribution and allele frequencies of the -238 polymorphism in both groups are shown in Table 2. The homozygous AA genotype was not found in the study. Two hundred and three (98.07%) patients with chronic HB had GG genotype, significantly increased as compared with subjects with self-limited HBV infection (χ^2=5.30, P=0.02). The frequency of G allele in patients with chronic HB was significantly higher than that in subjects with self-limited HBV infection (99.03% *vs* 96.62%, χ^2=5.20, P=0.02).

The frequency of exposure to cigarette smoking or alcohol consumption in patients with chronic HB was significantly higher than that in subjects with self-limited HBV infection (OR>1), but there was no significant difference between the two groups (χ^2=2.13, P=0.14 and χ^2=0.99, P=0.32).

Multivariate unconditional logistic regression model was used to analyze the association of outcomes of HBV infection with age, sex, cigarette smoking, alcohol consumption and genotypes. It indicated that genotype GG was independently associated with chronic HB after the other factors were controlled (Table 4).

Gene-environmental interaction

As GG genotype was defined as positive exposure in this study, the results of gene-environmental interaction analysis between GG genotype and smoking or alcohol drinking are shown in Tables 5, 6. The odds ratios for smoking exposure alone and GG genotype alone were 0.50 (P=1.00) and 2.60 (P=0.19), respectively, whereas the odds ratio for combination of smoking and GG genotype was 3.84 (P=0.07) in a synergic pattern

Table 2 Genotype and allele frequencies in subjects with chronic HB and self-limited HBV infection

Group	*n*	Genotype (%)[1]			Allele (%)[2]	
		A / A	A / G	G / G	A	G
Chronic HB	207	0 (0.0)	4 (1.93)	203 (98.07)	4 (0.97)	410 (99.03)
Self-limited HBV infection	148	0 (0.0)	10 (6.76)	138 (93.24)	10 (3.38)	286 (96.62)

[1]χ^2=5.30, *P*=0.02, [2]χ^2=5.20, *P*=0.02.

Table 3 Association of behavior factors with risk of chronic HB

Characteristics	Chronic HB (%)	Self-limited HBV infection (%)	OR (95%CI)	*P*
Cigarette smoking			1.40 (0.87, 2.28)	0.14
Yes	74 (35.75)	42 (28.38)		
No	133 (64.25)	106 (71.62)		
Alcohol consumption			1.26 (0.78, 2.05)	0.32
Yes	69 (33.33)	42 (28.38)		
No	138 (66.67)	106 (71.62)		

Table 4 Multivariate logistic regression analysis for determinants of chronic HB

Variable	β	χ^2	*P*	*OR*	*95%CI*
Intercept	-0.5076	7.2203	0.0072	–	–
Sex (Male=1, Female=0)	1.4088	35.1138	<0.0001	4.091	2.567–6.519
-238G / A (GG=1, GA=0)	1.4183	5.1467	0.0233	4.132	1.212–14.085

(II=3.84/ 0.50×2.60=2.95). The odds ratio was 0.78 (*P*=1.00) for alcohol consumption alone and 3.18 (*P*=0.11) for GG genotype alone, respectively, and their combined odds ratio was 4.07 (*P*=0.05), indicating an effect of interaction between them (II=4.07/0.78×3.18=1.64).

Table 5 Case-control analysis for interaction between cigarette smoking and GG genotype

Cigarette smoking	GG genotype	Case[1]	Control[2]	OR (*95%CI*)	*P*
-	-	3	6	1	–
-	+	130	100	2.60 (0.56-13.49)	0.19
+	-	1	4	0.50 (0.01-10.45)	1.00
+	+	73	38	3.84 (0.79-20.73)	0.07

[1]Chronic HB patients, [2]self-limited HBV infection individuals.

Table 6 Case-control analysis for interaction between alcohol consumption and GG genotype

Alcohol consumption	GG genotype	Case[1]	Control[2]	OR (*95%CI*)	*P*
-	-	3	7	1	–
-	+	135	99	3.18 (0.72-15.96)	0.11
+	-	1	3	0.78 (0.02-18.21)	1.00
+	+	68	39	4.07 (0.08-21.25)	0.05

[1]Chronic HB patients, [2]self-limited HBV infection individuals.

DISCUSSION

It is estimated that HBV is present in about 130 million chronic carriers, accounting for 10% of Chinese population[22]. HBV infection can result in acute hepatitis, HBV carriage, chronic hepatitis, liver cirrhosis, even primary hepatocellular carcinoma. One reason of broad spectrum of HBV infection could be attributed to the interaction of genetic and environmental factors. The majority of human genetic studies on HBV infection focused on human leucocyte antigen (HLA) in recent years[23-27]. Several pro-inflammatory cytokines such as interleukin-2 and interferon-γ and TNF-α, have been identified to participate in the process of viral clearance and host immune response to HBV[28,29]. In addition, TNF-α/TNF-α receptor system has an important role in the pathogenesis of liver damage and viral clearance[30].

TNF-α is a principal mediator of inflammation and cellular immune response regulated both transcriptionally and posttranscriptionally[31]. In the past years -238G/A polymorphism in a putative regulation box of the TNF-α gene promoter region has been identified. Genetic polymorphisms in the regulatory regions of various cytokine genes could influence the amount of cytokines produced in response to inflammatory stimuli.

In our study, chronic HB patients and self-limited HBV infection individuals (the same as the patients recovered from HB in other studies) were recruited to examine the TNF-α promoter polymorphism at position –238. The results demonstrated that 98.07% of the patients carried genotype GG, significantly higher than the frequency in those with self-limited infection, suggesting that genotype GG could increase the risk of chronic HB and was different from the report of Hohler[32]. Fifty-three (75%) of 71 subjects with chronic HB were homozygous in TNF-α G/G, lower than the frequency of those with self-limited HBV infection (94%). Ethnic difference could play a certain role in these conflicting results, because the results from several studies suggested that the distribution of TNF promoter polymorphisms in the study subjects was different from those with other racial origins[7,32,33].

The difference in genotype and allele frequency between patients with chronic HB and subjects with self-limited HB infection in our study suggested that GG genotype might have no advantage to antigen presenting, but further study would be needed to demonstrate its significance as a susceptible gene. This difference may be due to the fact that the TNF-α promoter polymorphism at position -238, likely serving as a marker, was in linkage disequilibrium with neighboring genes encoding HLA or other undefined genes, thus possibly influencing the

outcomes of diseases.

Some studies suggested that the TNFA-A allele falling within a putative Y regulation box of the TNF-α promoter, was associated with increased TNF-α expression[34-36], which was inconsistent with other studies[37-40]. It is necessary to carry out the experimental study to confirm the causality between the -238G/A polymorphism of TNF-α promoter gene and the outcome of HBV infection, based on the population study.

The gene of TNF-α is located in the HLA class III region in the short arm of chromosome 6. Some single nucleotide polymorphism (SNP) loci have been found in the promoter region of TNF-α gene. It is speculated that these loci would be in linkage disequilibrium with other unknown mutations or HLA genes. It is important to further demonstrate the association of their constructed haplotypes with outcomes of HBV infection.

Epidemiological findings indicated that alcohol consumption and viral hepatitis could act synergistically to promote the development and progression of liver disease. Patients with viral hepatitis and alcohol consumption accelerated their liver injury with a higher risk of liver cirrhosis and primary hepatocellular carcinoma than those with viral hepatitis alone or alcohol consumption alone[41,42]. Wang[43] reported cigarette smoking and alcohol consumption were independently associated with elevated ALT levels among anti-HCV-seropositive individuals. Our study showed cigarette smoking and alcohol consumption might be risk factors of chronic HB (OR>1), but further study is needed due to lack of evidence that could reveal statistically significant differences between groups of chronic HB and self-limited HBV infection.

The analysis of gene-environmental interaction in this study showed there was a synergic effect between GG genotype and cigarette smoking or alcohol consumption. The very wide confidence interval was due to only one subject who smoked or drank without GG genotype in patient group, which needs a larger sample size to be confirmed.

In summary, different outcomes of HBV infection are independently associated with TNF-α promoter polymorphism at position –238, and there might be a synergic effect between TNF-α promoter gene and cigarette smoking or alcohol consumption in the development of chronic HB.

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