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Association of-238G/A and -857C/T Polymorphisms of Tumor Necrosis Factor-Alpha Gene Promoter Region With Outcomes of Hepatitis B Virus Infection[1]

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Objectives To determine whether -238G/A and -857C/T polymorphisms of tumor necrosis factor-alpha (TNF-α) gene promoter were associated with outcomes of hepatitis B virus infection. **Methods** A total of 246 HBV self-limited infected subjects and 443 chronic hepatitis B (HB) patients were recruited in this case-control study. TNF-α-238G/A and -857C/T gene promoter polymorphisms were examined by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). **Results** The frequency of TNF-α-238 GG (90.7%) in chronic HB group was significantly lower than that (95.1%) in self-limited group (P=0.041). The frequency of TNF-α-857 CC (79.7%) in chronic HB patients was significantly higher than that (70.9%) in self-limited infected subjects (P=0.021). Multiple logistic regression analysis revealed that both TNF-α-238GA and -857CC were independently associated with chronic HB. **Conclusions** TNF-α promoter variants are likely to play a substantial role in influencing the outcomes of HBV infection.

Key words: Hepatitis B; TNF-α gene; Single nucleotide polymorphism (SNP); Haplotype

INTRODUCTION

It is estimated that there are more than 350 million HBV-infected people worldwide. HBV infection causes various clinical outcomes in patients, 90%-95% of adults infected with HBV might successfully eliminate the virus through self-limiting hepatitis and only 5%-10% of them become chronic HBV carriers, 20%-30% of the chronic infections lead to liver cirrhosis and 5% develop hepatocellular carcinoma in a long run of disease course. Hepatitis B is becoming one of the severe public health problems that should be coped with urgently[1].

Outcomes of HBV infection are associated with a number of variables in the host genetic factors. Cytokines play an important role in defense against viral infection, indirectly through determination of the predominant pattern of the host response, and directly through inhibition of viral replication[2]. TNF-α is one of the important cytokines involved in noncytotoxic antiviral mechanism, and participates in viral clearance and host immune response to HBV[3]. It is found that individuals with acute HB have higher TNF-α plasma levels than controls[4]. Cytokine production in individuals largely depends on promoter genetic polymorphisms [5]. Accordingly, the study of TNF-α promoter polymorphisms may be very important to reveal the potential factors in influencing outcomes of HBV infection.

The present case-control study was to analyze the TNF-α-238G/A, -857C/T promoter genetic polymorphisms and to elucidate whether host genetic polymorphisms were associated with outcomes of hepatitis B virus infection.

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MATERIALS AND METHODS

Subjects

From November 2002 to March 2004, 246 HBV self-limited infected subjects and 443 chronic hepatitis B patients were recruited at the Beijing You-an Hospital in this case-control study. The criteria of HBV self-limited infection were as follows: positive for anti-HBs or for both anti-HBs and anti-HBc, definitely negative for hepatitis B surface antigen (HBsAg), normal liver function tests, and no history of acute/chronic hepatitis B and HBV vaccination. All chronic hepatitis B patients were hepatitis B surface antigen (HBsAg) seropositive, HBs antibodies (anti-HBs) seronegative, with abnormally elevated serum alanine aminotransferase level, and duration of chronic HB $\geqslant$0.5 years. All subjects gave informed consent for genetic analysis in the study. All subjects were Chinese Han people not infected with other viral hepatitis.

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).

Measurement of TNF-α Gene Promoter Polymorphisms

Genomic DNA was extracted from peripheral blood leucocytes collected in EDTA by standard phenol-chloroform extraction. Allelic polymorphisms in the TNF-α gene promoter at positions -238G/A[6-7] and -857C/T[8-9] were 152- and 131-bp fragments amplified by PCR with the primers (for 152-bp fragment, sense 5′ AGAAGACCCCCCTCGGAACC 3′ and antisense 5′ ATCTGGAGGAAGCGGTAGTG 3′; For 131-bp fragment, sense 5′ AAGTCGAGTATGGGGACCCCCCGTTAA 3′ and antisense 5′ CCCCAGTGTGTGGCCATATCTTCTT 3′). Amplification of the -238 and -857 fragments was performed in a volume of 25 μL containing 50 ng of genomic DNA, 20 pmol/L of each primer, 200 μmol/L dNTP,1.5 mmol/L $MgCl_2$, buffer and 1U Taq polymerase (Shanghai Biorolor). PCR procedure of -238 was as follows: predenaturation at 94℃ for 2 min, 30 cycles of denaturing at 94℃ for 30 s, annealing at 60℃ for 30 s, extension at 72℃ for 30 s, and a final extension at 72℃ for 10min in Perkin Elmer thermocycler (2700, applied biosystems, Foster City, CA). For amplification of -857 polymorphism, the annealing temperature was 64℃ and other thermal cycling parameters were the same as those of -238.

The PCR products of the -238 and -857 were digested with Msp Ⅰ and Hinc Ⅱ restriction enzymes respectively. TNF-α-238 allele 1 was identified by 20- and 132-bp fragments and allele 2 by a single 152 bp fragment (Fig.1). TNF-α-857 allele 1 was identified by 25- and 106-bp fragments and allele 2 by a single 131 bp fragment (Fig. 2).

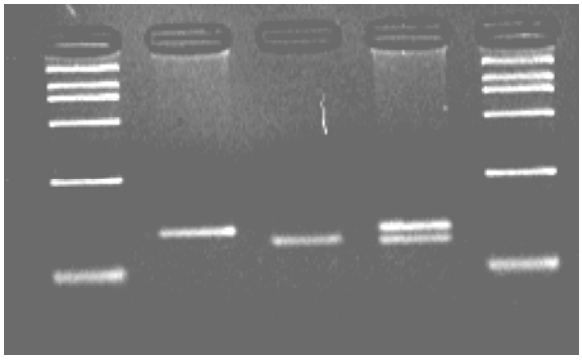

FIG. 1. Msp Ⅰ digestion for TNF-α-238 polymorphism.

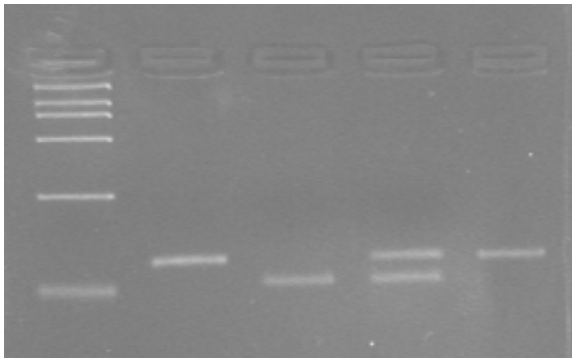

FIG. 2. *Hinc* Ⅱ digestion for TNF-α-857 polymorphism.

Statistical Analysis

Comparisons were made using Student's *t* test and Chi square test. SAS version 6.12 software package was used to analyze the data. All *P* values were two-tailed, and $P<0.05$ was considered statistically significant. Haplotypic analysis was performed using EH2.0 program.

RESULTS

Characteristics of Subjects

The average age of the patients with chronic HB and subjects with self-limited HBV infection was 35.34 and 34.68 years, respectively. There was no significant difference in age between the two groups ($P>0.05$).

The ratio of male to female patients (354/89) in chronic HB group was significantly higher than that

(138/108) in self-limited group (P<0.01).

Association of TNF-α–238G/A and –857C/T Genotypes With Outcomes of HBV Infection

The genotype distribution and allele frequencies of TNF-α-238 and-857 promoter are summarized in Tables 1 and 2.

The frequency of the -238GG genotype (90.7%) in chronic HB group was significantly lower than that (95.1%) in self-limited group (χ^2= 4.16, P=0.041). The frequency of the -238 G allele (94.5%) in chronic HB group was significantly lower than that (97.5%) in self-limited group (χ^2= 3.99, P=0.046). There was no significant difference in Hardy-Weinberg between the two groups (χ^2=0.155, P=0.69).

TABLE 1

Comparison of TNF-α-238 Polymorphism Between Chronic HB Group and Self-limited Group

Group	*n*	Genotype (%)[a]			Alleles (%)[b]	
		GG	GA	AA	G	A
Chronic HB	443	402 (90.7)	41 (9.3)	0 (0.00)	845 (94.5)	41 (4.6)
Self-limited HBV Infection	244	232 (95.1)	12 (4.9)	0 (0.00)	476 (97.5)	12 (2.5)

Note. [a]χ^2= 4.16, P=0.041, [b]χ^2= 3.99, P=0.046. Hardy-Weinberg χ^2=0.155, P=0.69.

TABLE 2

Comparison of TNF-α-857 Polymorphism Between Chronic HB Group and Self-limited Group

Group	*n*	Genotype (%)[a]			Alleles (%)[b]	
		CC	CT	TT	C	T
Chronic HB	433	345 (79.7)	69 (15.9)	19 (4.4)	759 (87.6)	107 (12.4)
Self-limited HBV Infection	244	173 (70.9)	60 (24.6)	11 (4.5)	406 (83.2)	82 (16.8)

Note. [a]χ^2= 7.71, P=0.021, [b]χ^2= 5.14, P=0.023. Hardy-Weinberg χ^2=3.54, P=0.059.

The frequency of TNF-α-857 promoter genotypes CC (79.7%) in chronic HB group was significantly higher than that (70.9%) in self-limited group (χ^2= 7.71, P=0.021). The frequency of –857C allele (87.6%) in chronic HB group was significantly higher than that (83.2%) in self-limited group (χ^2= 5.14, P=0.023). There is no significant difference in Hardy-Weinberg between the two groups (χ^2=3.54, P=0.059).

The results of haplotype analysis are summarized in Table 3. No significant difference was found in all haplotypes between the two groups.

Multivariate Logistic Regression Analysis

Multivariate unconditional logistic regression model was used to analyze the association of outcomes of HBV infection with sex, age, TNFα promoter polymorphisms. The results showed that both -238GA and -857CC were independently associated with chronic HB after confounding effects of gender and age were adjusted (Table 4).

TABLE 3

Comparison of TNF-238/857 Haplotype Between Chronic HB Group and Self-limited Group

No.	Haplotype	Self-limited Infected Group (%)	Patient (%)	*P* value
1	GC	0.81151	0.8278	0.652
2	AC	0.02046	0.04777	0.066
3	GT	0.1639	0.1246	0.169
4	AT	0.00413	0.00035	0.184

TABLE 4

Multivariate Logistic Regression Analyses for Determination of Chronic HB

Variable	β	χ^2	*P*	OR
Intercept	-0.9002	15.468	0.0001	-
Sex (Male =1, Female=0)	1.373	52.8847	0.0001	3.947
Age (≤40 years=1, >40 years=0)	0.662	10.7502	0.001	1.939
-238GA (GA=1, GG=0)	0.7738	4.2976	0.0382	2.168
-857CC (CC=1, TT+CT=0)	0.5226	6.5844	0.0103	1.686

DISCUSSION

Our findings indicate that both genotypes TNF-α-238GA and -857CC are positively associated with chronic HB. The frequency of -238GA genotype in chronic HB group was significantly higher than that in self-limited group, which is in agreement with the result studied in Caucasian[7] and Korea[10] population. The frequency of TNF-α-857 CC in chronic hepatitis B group was significantly higher than that in self-limited group, which is consistent with that reporteded by Jung MC[3]. It was reported that TNF-α production varies among individuals and correlates with SNP in the promoter region, and TNF-α can suppress the expression and replication of HBV. *In vitro* recombinant TNF-α inhibits HBV replication through α post-translational mechanism that accelerates the degradation of HBV messenger RNA[11]. Furthermore, the core promoter element is sensitive to TNF-α[12].

The result of multiple logistic regression analysis indicated TNF-α-238GA and-857CC were associated with chronic HB in males under the age of 40 years. The males seem more likely to develop chronic HB. The mechanism underlying the association remains unclear, however, viral replication is sensitive to sex hormones[13], suggesting that age of the subjects might be correlated with age at the time of infection, which appears to have a remarkable effect on outcomes of HBV infection[14]. In addition, both TNF-α-238GA and -857CC have been found to be independently associated with chronic HB after adjustment for sex and age.

In summary, the findings of this study show that genetic factors are important in the pathogenesis of HBV infection. TNF-α promoter genetic polymorphisms may play an important role in the development of HBV infection.

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