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BRIEF ARTICLE

Relationship between interleukin 18 polymorphisms and susceptibility to chronic hepatitis B virus infection

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Abstract

AIM: To identify the relationship between the tagging single nucleotide polymorphism sites (tagSNPs) of the Interleukin-18 (IL-18) gene and genetic susceptibility to chronic hepatitis B virus infection in Chinese patients.

METHODS: Five hundred and one cases of chronic hepatitis B virus (HBV) infection and 301 HBV natural clearance controls were studied. Two tagSNPs in the IL-18 gene (rs1946518A/C and rs574424C/G) were genotyped by the Multiplex Snapshot technique. The genotype and allele frequencies were calculated and analyzed.

RESULTS: In the genotypes of rs1946518, the AA type was present at a higher frequency in the patients compared to those in the controls. Odds ratio (OR) of the AA genotype for the comparison with that of the AC and the CC genotype was 1.537 (95% confidence intervals (CI): 1.116-2.218, $P = 0.009 < 0.025$). In phenotypes, the allele C at rs1946518 was of a significantly lower frequency in the patients with chronic hepatitis B than that in the controls ($P = 0.017 < 0.025$). OR of the allele A for the comparison with that of the allele C was 1.279 (95% CI: 1.045-1.567). As for the rs574424 genotypes, no significant difference in this genotype distribution or in this allele frequency between the patients and the control subjects was observed. No significant difference in the haplotype frequencies between the patients with chronic hepatitis B and HBV natural clearance individuals was displayed.

CONCLUSION: The data suggest that genotype AA and the allele A of the IL-18 at position rs1946518 are closely associated with the resistance to chronic hepatitis B and may be the dangerous gene. However, no statistical association was found between polymorphisms of rs574424 for IL-18 and hepatitis B.

Key words: Hepatitis B virus; Interleukin 18; tagSNP; Genetic susceptibility

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INTRODUCTION

The outcome of HBV infection is mainly influenced by the virus, immune response and genetic diversity[1-3]. The clinical course of chronic hepatitis B virus (HBV) infection varies from spontaneous recovery after acute hepatitis to a chronic persistent infection that may progress to chronic hepatitis B virus carrier, chronic hepatitis B, cirrhosis or hepatocellular carcinoma. Many studies strongly support the role of host genetic components in determining the outcome of HBV infection[4-6]. Genetic susceptibility of HBV infection is considered to be determined at different functional levels, such as cytokine production, antigen presentation and receptor recognition, and the single nucleotide polymorphisms (SNPs) of cytokine genes involved in the immune response after HBV infection become the emphasis of gene susceptibility, which may highlight the genetic background of HBV infection[7].

Interleukin-18 (IL-18), which was first described as an interferon-γ (IFN-γ) producing factor, has multiple functions, including the activation of cytotoxic T lymphocytes, natural killer cells and the promotion of T-helper type 1 (Th1) immune responses[8]. IL-18 leads to activities against pathogens, activates effector cells involved in the cellular interactions that occur during inflammation, is part of the acute and chronic stages of viral hepatitis and induces target-cell apoptosis. Migita *et al*[9] reported that a strong virus-specific CD4+ and CD8+ T lymphocyte response to hepatitis B virus was associated with IL-18's production decided by the IL-18 gene.

IL-18 gene polymorphisms have been reported to be implicated in susceptibility to chronic hepatitis B, in its pathogenesis[10] or in disease evolvement[11]. Many SNPs in the IL-18 gene region were predicted to be involved in clearing hepatitis B virus, such as -607C/A and -137G/C in the IL-18 promoter regions[9], 148G/C[12] and 105A/C[13] in regulatory gene sequences, and so on. Even although the role of the IL-18 polymorphism in the outcome of HBV infection has been examined in many different nations, no firm conclusion has been reached for the Chinese Han population. The purpose of this report was to investigate the association between the tagSNPs of the IL-18 gene and the genetic susceptibility to HBV infection.

MATERIALS AND METHODS

Patients

Eight hundred and two irrelevant Han Chinese with HBV infection were enrolled in this study. They were recruited with their informed consent for genetic analysis. They had no abnormalities, based on physical examination, chest radiography, electrocardiogram, urinalysis and routine laboratory blood testing. Liver, renal, endocrine and cardiovascular disorders were excluded. Five hundred and one were chronic HBV infection patients (221 males and 280 females). The remaining 301 were HBV natural clearance individuals and served as the control group (143 females and 158 males). The average age was 44.2 years for HBV chronic carriers and 44.9 years for controls. All patients with chronic HBV infection fulfilled the diagnostic criteria of the Proposal of Prevention and Treatment of Viral Hepatitis, 2005, issued by the Chinese Society of Infectious Diseases and Parasitology and the Chinese Society of Hepatology of the Chinese Medical Association[14]. Clinical criteria of self-limiting HBV infection patients were positive for HBsAb and HbcAb but negative for HBsAg, plus without a history of HBV vaccination. Controls were age and sex-matched subjects with cases ($P > 0.1$). All cases and controls were followed for more than 6 mo. No anti-HBV therapy had been given to the patients.

Isolation of DNA from whole blood

Genomic DNA was isolated from whole blood of all the subjects, using phenol/chloroform with MaXtract high-density tubes. Genomic DNA was extracted from the peripheral blood leucocytes pellet using a DNA extraction kit (Yuan Ping-Hao Biotechnology Co., Ltd. Tianjin, China), according to the manufacturer's instructions. The DNA samples were stored at -80 °C with a concentration of 100 ng/μL.

tag SNP selection

We selected SNPs on the basis of the following principal criteria: tag SNPs (tagSNP) were identified using genotype data from the panel (Han Chinese in Beijing) of the phase II HapMap Project. The criteria for tagSNPs were $r^2 > 0.8$, minor allele frequency MAF > 0.1, functional relevance and importance, and SNPs significantly associated with diseases in previous studies. A total of two tagSNPs in IL-18 gene (rs1946518A/C and rs574424C/G, $r^2 = 0.981$) were selected, which captured 100% of common SNPs (minor allele frequency > 0.1) in the HapMap Chinese database at $r^2 > 0.8$.

Determination of the IL-18 genotypes

The two SNPs of IL-18 were genotyped using the Multiplex Snapshot technique. The primers and probes used were (5'to3'): for the rs1946518: forward primer: 5'-CCCTCTCCCCAAGCTTACTTTC-3', reverse primer: 5'-CCCCTTCCTCCCAAGCTCAATA-3', and extended primer: 5'-TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTCTGTTGCAGAAAGTGTAAAAATTATTA-3'; and those for the rs5744247: forward primer: 5'-CACCCTGCCTGTACCCTCAGAT-3', reverse primer: 5'-CACCTGAGGATGCCATAAACACA-3', and extended primer: 5'-TTTTTTTTTTTTTTTTTTTTTTTTTTCGTGCCCTTTAGGAAGGACT-3'.

The PCR amplification conditions were: a 15-μL final volume containing 10 μL × 1.5 μL buffer, 0.3 μL dNTPs (10 mmol/L), 0.9 μL $MgCl_2$ (25 mmol/L), 0.1 μL HotstarR Taq DNA polymerase, 0.5 μL each primer (10 pmol/L) and 1μL DNA template (20 mg/L). Conditions for the multiplex PCR reaction using touch-down PCR response procedures included initial denaturation at 95 °C for 15 min, denaturation at 94 °C for 40 s, annealing at 63 °C for 1 min, and recursive-descent 0.5 °C, followed

Table 1 Comparison of IL-18 gene promoter polymorphism between patients with chronic hepatitis B and controls

Position	Polymorphism	Control n = 301 (%)	Patient n = 501 (%)	χ^2	P value	OR (95% CI)
rs1946518	AA	60 (0.199)	141 (0.281)	6.742	0.009	1.573 (1.116-2.218)
	AC	156 (0.518)	239 (0.477)	1.279	0.258	0.848 (0.637-1.129)
	CC	85 (0.269)	121 (0.242)	1.437	0.200	0.809 (0.585-1.119)
	A	276 (0.279)	521 (0.619)	10.84	0.017	1.279 (1.045-1.567)
	C	326 (0.622)	481 (0.381)			
rs574424	CC	127 (0.422)	198 (0.395)	0.557	0.456	0.895 (0.670-1.197)
	GC	134 (0.445)	232 (0.463)	0.243	0.622	1.075 (0.806-1.433)
	GG	40 (0.133)	71 (0.142)	0.123	0.751	1.070 (0.705-1.623)
	C	388 (0.645)	628 (0.627)	0.512	0.475	0.926 (0.750-1.149)
	G	214 (0.355)	374 (0.373)			

by extension at 72 °C for 1.5 min for a total of 15 cycles. This was followed by 25 cycles of denaturation at 94 °C for 40 s, annealing at 56 °C for 40 s, extension at 72 °C for 1.5 min, with a final extension at 72 °C for 8 min. Amplified samples were stored at 4 °C. After amplification, 1.5 μL PCR product was examined on an agarose gel to test for successful amplification.

SNaPshot reaction: Take the purified PCR product, each concentration of 0.2 μmol/L SnaPshot primer mixture, SNaPshot fluorescent mixtures (containing Ampli Taq DNA polymerase and different fluorescently labeled ddNTP) consisting of an PCR reaction system. SNaPshot response procedures: (1) initial denaturation at 96 °C for 10 s; (2) denaturation at 96 °C for 10 s; (3) annealing at 53 °C for 5 s; (4) extension at 60 °C for 30 s; and (5) for a total of 25 cycles. Finally, keep extension at 60 °C for 30 s. Amplified samples were stored at 4 °C. SNaPshot PCR products using SAP purification in 10 μL the SNaPshot PCR product with 1 U SAP or 1 U CIP, mixed, insulated at 37 °C for 1 h, 75 ℃ for 15 min to inactivate the enzyme. The samples can be stored at 4 °C for 24 h or -20 °C for long term.

DNA sequencing: The Snapshot product was diluted 20-fold. In a total volume of 10 μL, we mixed 8.6 μL HiDiFormamide (high-purity formamide), 0.9 μL GeneScan-120 LIZ Size Standard and 0.5 μL Snapshot purification product. Samples were incubated at 95 °C for 5 min, chilled quickly for 4 min and then loaded on an ABI 3730XL DNA sequence detector for capillary electrophoresis, running GeneMapper4.0 software analysis of experimental results.

Statistical analyses

Allele and genotype frequencies were obtained by direct counting and the χ^2 test was used to compare allele and genotype distributions. We assessed the quality of the genotype data by testing for Hardy-Weinberg equilibrium in the case and control samples using Fisher's exact test ($P > 0.05$). We adjusted the significant threshold to $P < 0.05/2 = 0.025$ after Bonferroni's correction. Odds ratio (OR) and 95% confidence intervals (CI) were calculated according to Woolf's method.

RESULTS

Polymorphisms at the position rs1946518 and rs574424 in the IL-18 gene were analyzed by SNaPSHOT reaction. In every polymorphic site, a common reverse primer and two sequence-specific forward primers were used and two SNaPSHOT reactions were performed for every individual DNA. In total, 802 Chinese subjects were studied for IL-18 polymorphisms. All the genotypes of IL-18 gene polymorphisms were in Hardy-Weinberg equilibrium in both the case and control subjects. As shown in Table 1, there were AA, AC and CC genotypes at position rs1946518, and CC, GC and GG genotypes at position rs574424.

Genotype and allele frequencies for IL-18 polymorphisms are summarized in Table 1. The genotype frequencies were in agreement with the Hardy-Weinberg ($P > 0.05$ for all analyses). As the rs1946518 genotypes, of 501 patients with chronic hepatitis B, 141 had the AA type (28.1%), 239 the AC type (47.7%) and 121 the CC type (24.2%). Of the 301 control subjects, 60 had the AA type (19.9%), 156 the AC type (51.8%) and 85 the CC (28.2%). In genotypes, the AA type at position rs1946518 was present at a higher frequency in patients with chronic hepatitis B compared to those in the controls. OR of the AA genotype for the comparison with that of the AC and the CC genotype was 1.573 (95% CI: 1.116-2.218, $P = 0.009 < 0.025$). In phenotypes, the allele C at rs1946518 was of a significantly lower frequency in patients with chronic hepatitis B than that in the controls ($\chi^2 = 10.84$, $P = 0.017 < 0.025$). As for the rs574424 genotypes, 198 of the 301 patients with chronic hepatitis B had the CC type (39.5%), 232 the GC type (46.3%) and 71 the GG type (14.2%). 127 of the 301 control subjects were type CC (42.2%), 134 were GC (44.5%) and 40 were CC (13.3%). No significant difference in the genotype distribution or in the allele frequency between the patients with chronic hepatitis B and the control subjects was observed.

Haplotype analysis

We also estimated the IL-18 haplotype frequencies and evaluated the association among these variants and HBV infection. We observed three haplotype combinations, but non-significant association was found in the distribu-

Table 2 Haplotype frequencies of two interleukin-18 bi-allelic polymorphisms in chronic hepatitis B and healthy controls

Haplotypes	rs1946518	rs574424	Controls (%)	Patients (%)	χ^2	*P* value
Ⅰ	A	C	62 (10.3)	147 (14.7)	2.02	0.155
Ⅱ	A	G	214 (35.5)	374 (37.3)	0.511	0.475
Ⅲ	C	C	326 (54.2)	481 (48.0)	2.623	0.101

tion of the haplotype frequencies between cases and controls ($P > 0.025$). Haplotype frequency less than 0.03 will be ignored in analysis (Table 2).

Three haplotypes of the IL-18 at position rs1946518 and rs574424 were present in both patients and controls (haplotypes Ⅰ, Ⅱ and Ⅲ in Table 2). The frequencies of haplotype I, Ⅱ and Ⅲ in the controls were 10.3%, 35.5% and 54.2%, respectively. The frequencies of haplotype Ⅰ, Ⅱ and Ⅲ in the patients with chronic hepatitis B were 14.7%, 37.3% and 48.0% respectively. The frequencies of haplotype Ⅰ and Ⅱ, which bear A at rs1946518, in the patients were little higher than that in the healthy control subjects, but no significant difference in the haplotype frequencies between the patients and HBV natural clearance individuals was displayed.

DISCUSSION

The human IL-18 gene is located on chromosome 11q22.2-q22.3 and is composed of six exons and five introns[15]. Sugiura *et al*[16]described that there were some SNPs at position -607C/A, -137G/C, -656G/T and 105A/C within IL-18 gene exons. Cloning and gene expression analysis showed that the SNPs of the promoter of IL-18 gene at position -607 and -137 were suggested to cause the differences in transcription factor binding and have a critical impact on IL-18 gene activity and potentially also to IFN-gamma[17]. Further studies showed that the people with allele C at position -137 in the promoter of IL-18 gene may be protected against HBV infection; moreover, AA genotype at position -607 may be closely linked to inhibiting HBV-DNA replication. Meanwhile, haplotype frequencies' distributions suggested that the frequencies of -607C/-137C and -607A/-137C haplotypes in the chronic hepatitis B groups were significantly lower than that in normal controls[18]. But a recent study found that the polymorphisms at position -148, +8925 and +13925 could play a main role in the expression of IL-18 and had a clear correlation between IL-18 and IFN-gamma mRNA expression[19]. Because IFN-gamma, mainly mediated by IL-18, could limit the hepatitis B virus by activating the immune cells, high levels of expression of IL-18 caused by the above genotypes might explain the mechanism of viral clearance in HBV infection. But not all studies had the same view and with scientific and technological progress, the susceptibility genes in IL-18 will be discovered more and more.

To identify the relationship between the SNPs of IL-18 gene and genetic susceptibility to chronic hepatitis B virus infection in Chinese patients, we selected two SNPs in IL-18 (rs1946518and rs574424) using genotype data from the panel (Han Chinese in Beijing) of the phase II HapMap Project. The two tagSNPs captured 100% of common SNPs (minor allele frequency > 0.1) in the HapMap Chinese database at $r^2 = 0.981$. We analyzed the associations of the two SNP alleles with HBV-infected patients compared to spontaneously cleared HBV controls.

In the present study, we found that the allele frequencies of rs1946518A in the chronic hepatitis B group were markedly higher than those in the control group and there was a significant correlation between them (Table 1). These findings suggest that rs1946518 (A > C) is closely associated with the susceptibility to chronic hepatitis B and may be the susceptible gene. We analyzed the rs574424C/G genotype in a series of patients with chronic hepatitis B and acute hepatitis B and it was not associated with HBV infection. But Zhang[18] confirmed that rs574424C alleles were associated with the clearance of HBV infection and protected people against chronic hepatitis B. Those conclusions were rather contradictory. It is likely that the contrary results may be related to the size of samples or the criteria of inclusion.

With regard to the haplotypes, Giedraitis V[20] found that patients with acute hepatitis B carrying haplotype of rs1946518A/ rs187238C had a more vigorous CD8+ T cell response to HBV core than patients not carrying rs1946518A/rs187238C, suggesting that rs1946518A/ rs187238C was associated with a self-limited course of HBV infection. Unfortunately, no haplotype (rs1946518A/574424C or G) was detected to have association with HBV infection in our study. The reason for different results is likely due to different gene, races and sample sizes.

The results of the present study suggest that the genotypes rs1946518AA (OR = 1.573) and the allele A (OR = 1.279) are closely associated with chronic hepatitis B and may be the dangerous gene. IL-18 gene is an important factor that determines the outcome of HBV infection, which will give some new clues in the study of the pathogenesis of chronic hepatitis B. In fact, responses to HBV infection and HBV antigens (vaccines) and treatment are connected with genetic traits. Such correlations are still not clear, especially with regard to different populations, age and course of disease. These investigations should be continued, especially in patients treated with interferon, still the most important means of treatment. This could be useful in typing patients for this very expensive therapy.

COMMENTS

Background

Persistent hepatitis B virus (HBV) infection is considered a multifactorial and polygenic disorder with viral, environmental and genetic components, as well as contribu-

tions from HBV genomic variability, host age, gender, concurrent infection with the hepatitis C virus, hepatitis D virus and human immune deficiency virus. Interleukin-18 (IL-18) plays an important role in the response of the innate immune system to viral infection. High levels of expression of IL-18 caused by the above genotypes might affect induction of IFN-γ expression.

Research frontiers

This study is the first to investigate the association between two tagSNPs (rs1946518/ rs574424) of IL-18 and the genetic susceptibility to chronic HBV infection in Chinese patients using the Multiplex Snapshot technique.

Innovations and breakthroughs

The genotype AA and the allele A of the IL-18 at position rs1946518 are closely associated with the susceptibility to chronic hepatitis B and may be the dangerous gene. But the tagSNPs of IL-18 at rs574424 position are not associated with HBV infection in Chinese patients.

Applications

Based on the results of our study, further genetic studies are needed to examine the roles of other IL-18 SNPs and their association with disease progress in chronic HBV infection.

Terminology

The human IL-18 gene is located on chromosome 11q22.2-q22.3 and is composed of six exons and five introns. Interleukin-18 (IL-18) was first described as an interferon-g (IFN-g)–producing factor and has multiple functions, including the activation of cytotoxic T lymphocytes or natural killer cells and the promotion of T-helper type 1 (Th1)-type immune responses, which limits virus infection.

Peer review

This manuscript investigates the association between 2 SNPs claimed to be TaqSNP for common haplotype in the Chinese population and chronic hepatitis B. It is well written and interesting.

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