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Association study between polymorphism of interleukin 12B 1188A/C and hepatitis B virus infection in a Chinese Han population

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Abstract

Background: *Several studies demonstrated that the genetic factor contributes a lot to the etiology of hepatitis B virus (HBV) infection. Interleukin 12 (IL-12) plays an important role in the immune response to many viruses. As a functional SNP locus, IL-12B 1188A/C polymorphism (rs3212227) showed an effect on IL-12 production, which may indicate that this polymorphism may influence immune response to HBV during a natural infection.*

Objective: *To investigate whether the IL-12B 1188A/C polymorphism is associated with a risk of HBV infection in the Chinese Han population.*

Material and methods: *Our study was performed on 594 HBV-infected persons and 535 controls. Genotyping procedures for identifying the rs3212227 polymorphism were effected by the polymerase chain reaction – restriction fragment length polymorphism (PCR-RFLP) method. Amplified ribosomal DNA restriction analysis was used to validate genotype results. χ^2 test was used to analyze association analysis.*

Results: *There was no statistically significant association between the IL-12B 1188 A/C polymorphism in HBV-infected persons and controls (for genotypes, $\chi^2 = 1.976$, $p = 0.372$, $df = 2$, and for alleles, $p = 0.623$, OR = 0.959, 95% CI: 0.811-1.134).*

Conclusions: *Our results indicated that the IL-12B1188 A/C polymorphism was not associated with the development of HBV infection in the Chinese Han population.*

Key words: *IL-12B 1188A/C, HBV, association study.*

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Introduction

Hepatitis B is a major global health problem. Over 2 billion people in the world are infected by hepatitis B virus (HBV) with 350-400 million people chronically infected. It may then develop to chronic liver diseases such as liver cirrhosis and hepatocellular carcinoma. And due to these complications of hepatitis B, about 600,000 people die annually. Although HBV infection is considered a polygenic and multifactorial disorder with viral, environmental, genetic, and immune elements, and host immunological and genetic factors may influence the course of the disease [1, 2], there are still several questions which have not been adequately answered about its exact pathogenetic mechanism. It is generally believed that HBV infection is a complex phenotype strongly influenced by both molecular genetic factors and the environmental factors. In recent years, a lot of research efforts have focused on identifying the molecular, genetic pathways and susceptibility genetic *loci* involved in the HBV infection. So far, researches have found two strong susceptibility *loci* for HBV infection including 8p12 [3] and 1p36.22 [4], both of which were unraveled by the genome-wide association study. Meanwhile, several other genes relevant to HLA-DP [5] HLA-DRB1*13 and TNF-α gene [6], cytotoxic T-lymphocyte antigen [7], IL-28B [8], IFN-γ [9] and so on have been suggested by a candidate gene approach. Recent studies have also demonstrated that cytokine genetic

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polymorphisms are related to the development of chronic HBV infection [6, 10-12].

It has been well documented that disturbed regulatory CD4 T helper (Th) cells account much for the pathogenesis of HBV infection. A weak or absent hepatitis B core antigen (HBcAg)-specific IFN-γ production will lead to a disturbed secretory function of Th1 cells, which plays a significant role in immunopathogenesis of HBV infection. At the same time, IL-12 stands for an acknowledged inducer of IFN-γ secretion by Th1 cells [13, 14]. Interleukin 12 is an effective immunostimulatory cytokine which was produced by antigen-presenting cells that not only can generate normal Th1 response, but also have the ability to induce IFN-γ secretion by T and natural killer cells) [15, 16]. Interleukin 12 plays an important role in the immune response to many viruses, including HBV [17]. The antiviral effect of IL-12 is mainly regulated through its ability to induce IFN-γ production. In addition, IL-12 inhibits HBV replication in transgenic mice [18]. As a functional single nucleotide polymorphism (A/C) of the 3′ untranslated region (3′UTR) at position 1188, IL-12B 1188 (rs3212227) is supposed to have a relationship to enhanced IL-12 production [19]. Therefore, we have the hypothesis that IL-12B 1188 A/C polymorphism may, through regulating IL-12 ability of inducing IFN-γ production, play a major role in the antiviral immune response to natural HBV infection. Here we analyzed the effect of the possible genetic association between IL-12B 1188A/C polymorphism and HBV infection patients in the Chinese Han population. Our results might identify the role of genetic susceptibility, which will not only help to explain the pathogenic mechanism of chronic hepatitis B, but also will provide basis for the treatment of HBV infection.

Material and methods

Study subjects

All patients with HBV-infected relative disease were consecutively selected from the Affiliated Hospital of the Qingdao University Medical College between January 2011 and July 2012. The control subjects underwent a routine medical check-up in the Regular Physical Examination Center of the Affiliated Hospital of the Qingdao University Medical College during the same period. 594 HBV infection patients and 535 ethnically matched healthy controls were genotyped for the IL-12 1188 A/C. The mean age of these patients was 36.54 ±12.76 (mean ± SD) years at HBV infection onset, and 383 (64.5%) were male, while 211 (35.5%) were female. And the age of the healthy controls was 34.32 ±10.58 (mean ± SD) years, and 236 (44.1%) were male, while 299 (55.9%) were female. The diagnosis of HBV infection was established in accordance with the Proposal of Prevention and Treatment of Viral Hepatitis, 2005, issued by the Chinese Society of Infectious Diseases and Parasitology and the Society of Hepatology of the Chinese Medical Association.

The study examined 535 consecutive healthy persons (236 males and 299 females), who had no history of HBV vaccination infection, as a control group. They were matched to the patients on the basis of age, gender, smoking and drinking status and they were all negative for HBV infection markers and were HBsAg-/HBV-DNA-/anti-HBc- with normal liver function tests. All HBV markers except anti-HBs were seronegative for these subjects. No study participant was coinfected with the delta virus or human immunodeficiency virus. All patients were positive for HbsAg and negative for HbsAb, without any history of liver diseases. HBV markers, including HBsAg and its antibody (anti-HBs), HbeAg and its antibody (anti-HBe), and hepatitis B core anti-body (anti-HBc), were detected using radioimmunoassay. The serum HBV DNA was quantitated with a detection limit of 1.7 IU/ml by a liquid hybridization assay and detected by the Pharmacia luminometer and the hybrid capture system with chemiluminescence readout (HBV DNA Assay, Digene). HBeAg seroconversion was defined as serum HBeAg clearance and the presence of anti-HBe antibody for at least 6 months. Exclusion criteria included the presence of other type liver diseases (toxic hepatitis, autoimmune hepatitis, alcoholic liver disease and primary biliary cirrhosis), positive for antibodies to hepatitis C virus, hepatitis D virus and HIV infection. They were previously untreated with antiviral or immunosuppressive therapy within 6 months. All human rights of the subjects in this research were protected and any necessary approval was obtained from the ethics committee. The study was performed with the approval of the ethics committee of the Affiliated Hospital of the Qingdao University Medical College. All participants signed the informed written consent.

Genomic DNA from peripheral blood cells was extracted using TIANamp Blood DNA Kit (Tiangen Biotect (Beijing) Co., China). DNA concentration and purity of each sample were measured by ultraviolet spectrophotometer. The segment of this polymorphism was replicated using the following the primer: 5' CCCATGGCAACTTGAGAGCTGG-3'and 5'CCCATGGCAACTTGAGAGCTGG 3'. Polymerase chain reaction was carried out in a final volume of 20 µl containing 2 × PCR MasterMix, 100 ng of genomic DNA and 0.4 mmol/l of each primer. The reaction for IL-12B 1188A/C was carried out as follows: 94°C for 4 min, followed by 35 cycles of 94°C for 30 s, 55°C for 1 min, 72°C for 30 s and 72°C for 5 min. After cycling, 6 µl of the PCR product was digested with the restriction enzyme TaqI (10 U per reaction) at 65°C overnight and then was electrophoresed in 3.0% agarose gels. TaqI identifies the sequences 5'-T/CGA-3' and 3'-AGC/T-5'. The C allele sequence was digested into two fragments of 156 and 71 bp, while the A allele sequence remained intact. In order to confirm the reliability of the genotype result, we

randomly selected more than 40 subjects to be genotyped by DNA sequencing techniques.

Statistical analysis

All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS version 17.0, SPSS Inc., Chicago, IL, USA). The allelic and genotypic frequencies were calculated using direct counts. The allelic and genotypic frequencies for the patients and controls were compared using a Pearson χ^2 test. (If expected values were below 5, the Fisher's exact test was necessarily used). Hardy-Weinberg equilibrium was checked of observed-versus-predicted genotype distributions by χ^2 test or Fisher's exact test. We also calculated the odds ratio (OR) and 95% confidence intervals (95% CI) to measure the strength of relationships between the patients and controls in the genotype distribution and allele frequencies. Statistical significance was set at the $P < 0.05$ level.

Results

The control population was in Hardy-Weinberg equilibrium for IL-12B 1188A/C. Table 1 shows the association of polymorphism IL-12B 1188 A/C between patients and controls in the genotype and allele. Evaluation of polymorphisms within the 1188A/C region of the IL-12 gene by TaqI restriction digestion demonstrated that IL-12 1188A/C distribution of C/C, C/A, and A/A genotype frequencies was 105 (17.7%), 294 (49.5%), and 195 (32.8%), respectively, in HBV patients. In turn, in controls, the frequencies of C/C, C/A, and A/A genotype were 82 (15.3%), 279 (54.2%) and 174 (32.5%). The difference of distribution of two alleles was not statistically significant) ($\chi^2 = 1.976$, $p = 0.372$, df = 2), indicating that there may be no correlation between variant genotypes and HBV infection. The frequency of the C allele of rs3212227 in HBV patients and healthy controls was 504 (42.4%) and 443 (41.4%), respectively. In HBV patients, six hundred and eighty four (57.6%) A alleles were detected, while the frequency in the control group was 627 (58.6%). The difference between the two groups was not statistically significant ($p = 0.623$, OR = 0.959, 95% CI: 0.811-1.134). There is no association with allele frequency and the incidence of HBV (Table 1).

Discussion

Interleukin 12, through its ability to induce IFN-γ, probably plays an important role in the antiviral immune response to HBV during natural infection. Interleukin 12 family cytokines have a major role in cell-mediated immunity by stimulating cytotoxic lymphocyte and natural killer cell activity and antigen processing and presentation. Interleukin 12 could direct the HBV-specific T cell response to a favorable Th1-type response [20, 21]. A previous study showed that IL-12 levels correlate with hepatitis B antigen during viral infections. Rossol *et al.* reported that the serum levels of IL-12 were increased in HBV infection patients [17] while some research showed opposite results [22, 23]. As an important functional SNP local, the 1188 polymorphism of IL-12B is believed to be involved in regulation of IL-12 expression [24]. Seegers *et al.* [19] reported a correlation between this polymorphism and increased IL-12 secretion and showed individuals with heterozygous AC and homozygous AA genotypes produced a higher amount of IL-12 than those with the CC genotype. While Morahan *et al.* demonstrated that this polymorphism was associated with a decreased IL-12B mRNA expression by EBV transformed cell lines [25]. Arababadi *et al.* suggested that the polymorphisms in 1188 region of IL-12 and +874 region of IFN-γ would not affect the expression of both cytokines at a serum level in Occult HBV Infected Patients [26]. In addition, previous research provided several reports about the association between the polymorphism of IL-12A with HBV infection. Liu L's findings indicated that the polymorphism of rs568408 in IL-12A may be related to the risk of HCC and suggest a HCC risk associated with HBV infection, while Park *et al.* showed that SNPs and haplotype of IL-12A are not associated with HBV persistence and development of HCC in Korean patients with HBV infection [24].

Our results showed that there was no statistically significant association of the IL-12B 1188 A/C polymorphism between HBV-infected persons and controls. In contrast

Table 1. The association of polymorphism IL-12B 1188 A/C in genotypes and alleles between cases and controls

	Polymorphism IL-12B 1188A/C			
Genotypes	**Patients**	**Controls**	***p* value**	**95% CI**
AA	195 (0.328)	174 (0.325)		
CC	105 (0.177)	82 (0.153)	0.372	
AC	294 (0.495)	279 (0.542)	$\chi^2 = 1.976$	
Alleles				
A	684 (0.576)	627 (0.586)	0.623	
C	504 (0.424)	443 (0.414)	$\chi^2 = 0.242$	0.811-1.134

with our own results, Wang *et al.* reported that the C/C and C/ A genotypes of IL-12B 1188 were related to a decreased susceptibility to HBV infection in Chinese Han haemodialysis patients [27]. We speculated that this disagreement was caused by following reasons which cause this polymorphism of IL-12B 1188 A/C and HBV infection was not found. The most important factor is a relatively small sample size. As we all know, small sample size studies cannot have enough statistical power to show us real accuracy of the expected effects. Data from other populations and ethnic groups are still needed to be evaluated. Heterogeneity is at the second level. It has been suggested that the contradictory results are due to possible differences in IL-12 regulation in different cell types. The third reason could be a possible relationship between IL-12 alleles and IL-12 gene expression and protein production, which was previously examined in different experimental settings. Interleukin 12 gene expression is complex and firmly regulated at the transcriptional, posttranscriptional, and translational levels. It is a major limitation not detecting plasma levels of IL-12. Finally, there is no explicit functional relationship between the 1188A/C polymorphism and its exact role in regulating IL-12 expression.

There are many limitations in the above studies which make our research difficult to give expectant effects of IL-12 1188 polymorphisms on morbidity of HBV infection. However, since human susceptibility to HBV is a complicated polygenic trait, there must be some other uncovered mechanisms that involve the susceptibility to HBV infection. We still need to evaluate interactive effects between the polymorphism studied and other SNPs in human genes (including those in CXCR1, IL-1, IL-17, IL-22 and IL-28 which also influence the process of the inflammation). Therefore, our future research will be focused on exploring polymorphisms of related cytokines (and even further – their receptors) and their expression levels among HBV infection patients. Further work will give a clearer picture about the role of the IL-12 gene expression in HBV infection and its understanding at the molecular level. Further studies with a large sample size should be conducted to validate this association.

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The authors declare no conflict of interest.

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