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Association of interleukin-12 *p40* gene 3'-untranslated region polymorphism and outcome of HCV infection

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Abstract

AIM: To investigate the effect of interleukin-12 *p40* gene (*IL12B*) 3'-untranslated region polymorphism on the outcome of HCV infection.

METHODS: A total of 133 patients who had been infected with HCV for 12-25 (18.2±3.8) years, were enrolled in this study. Liver biochemical tests were performed with an automated analyzer and HCV RNA was detected by fluorogenic quantitative polymerase chain reaction. B-mode ultrasound was used for liver examination. Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) was used for the detection of *IL12B* (1188A/C) polymorphism.

RESULTS: Self-limited infection was associated with AC genotype (OR = 3.48; $P = 0.001$) and persistent infection was associated with AA genotype (OR = 0.34; $P = 0.014$) at site 1188 of *IL12B*. In patients with persistent HCV infection, no significant differences were found regarding the age, gender, duration of infection and biochemical characteristics ($P>0.05$). According to B-mode ultrasound imaging and clinical diagnosis, patients with persistent infection were divided into groups based on the severity of infection. No significant differences were found in the frequency of IL-12 genotype (1188A/C) between different groups ($P>0.05$).

CONCLUSION: The polymorphism of *IL12B* (1188A/C) appears to have some influence on the outcome of HCV infection.

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INTRODUCTION

Hepatitis C virus (HCV) is the major cause of post-transfusion hepatitis. As is estimated by WHO, approximately 170 million people globally may have been infected with HCV[1]. Although chronic HCV infections are often clinically silent for decades, an estimated 85% of individuals infected with HCV develop persistent infection, and these patients are likely to end up with cirrhosis and liver cancer[1-3]. HCV infection persists despite the presence of specific humoral and cellular immune responses, and the factors leading to viral clearance or persistence are poorly understood. But some researches showed that the outcome might already be determined at an early time point following infection[4-7]. Patients with acute HCV infection presenting a self-limited acute hepatitis develop a strong Th1 response. In contrast, patients developing a chronic infection show a predominant Th2 response, but a weak Th1 response. These findings suggest that the imbalance or skewness between responses of Th1 and Th2 cells is involved in disease progression and in the incapability to eradicate HCV[8-10]. Interleukin 12 (IL-12) is a key cytokine presented with the initiation of immune response, which is one of the most clearly defined factors determining Th1 and Th2 differentiation[11,12]. IL-12 might, therefore, play an important role in the pathogenesis of HCV infection by affecting the Th1/Th2 balance. Single nucleotide polymorphism (SNP) (1188A/C) was identified at position 1188 in the 3'-untranslated region (UTR) of IL-12 *p40* gene (*IL12B*) and was found to correlate with many diseases[13-15].

In this study, we proposed that some genotypes of SNP (1188A/C) might associate with either disease outcome or the state of illness in chronic HCV infection. To test this hypothesis, we determined the frequency of genotypes at the SNP site using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) in HCV infected patients, as well as the relationship between *IL12B* and outcome of HCV infection.

MATERIALS AND METHODS

Subjects

A total of 133 patients with confirmed diagnoses of HCV infection in Gu'an County, Hebei Province, China, who had been infected with HCV for 12-25 (18.2±3.8) years, were enrolled in this study. All the patients were investigated in January 2002. There were 61 (45.9%) male and 72 (54.1%) female patients ranging from 30 to 69 years old (mean age, 46.5±8.3 years). All subjects had no access to antivirus treatment.

Laboratory examination

Venous blood was drawn from each individual and genomic DNA was extracted from clotted blood with a protocol by using silica (Sigma, S-5631). Genotyping of SNP (1188A/C) was carried out by PCR-RFLP according to Hall *et al*.[13]. Liver biochemistry tests including alanine aminotransferase (ALT), aspartate aminotransferase (AST), γ-glutamyltransferase (γ-GT), alkaline phosphatase (ALP), total bilirubin (TBil), direct bilirubin (DBil), total protein (Tp), albumin (ALB) and serum albumin/globulin ratio (A/G) were measured with HITACHI 7170 automatic

biochemistry analyzer. The B-mode ultrasound was performed for liver examination. HCV RNA was detected with quantitative fluorogenic PCR.

Statistical analysis

Data were expressed as mean±SD. Student's *t*-test, one-way analysis of variance and Chi-square tests were used for statistical analysis according to the data obtained. Logistic regression was used to assess the impact of variables on the odds of the outcome of HCV infection. Multivariate analysis of variance was used to analyze the difference of clinical characteristics among patients with persistent infection. All univariate and multivariable calculations, including odds ratios (OR), 95% confidence intervals (95% CI), and *P* values were calculated using the SPSS (version 10).

RESULTS

Of 133 cases investigated in this study, 91 (68.4%) were HCV RNA positive and 42 (31.6%) were HCV RNA negative. SNP typing of DNA samples from all the subjects is shown in Table 1. The proportions of male subjects were 31.0% in self-limited infection and 52.7% in persistent infection. Female gender was closely related to self-limited infection ($P<0.05$). All patients were HCV infected as a consequence of plasma donation. The mean durations of infection were 17.76 and 18.44 years for patients with self-limited infection and matched patients with persistent infection, respectively. HCV genotype 1b was found in all the patients except two. In addition, the two groups were indistinguishable with respect to age, source of infection, duration of infection and HCV genotype ($P>0.05$).

Table 1 Features of subjects enrolled in the study

Group	*n*	Gender (male/female)	Age (yr)	Duration of infection (yr)
Self-limited infection	42	13/29	45.71±7.68	17.76±3.82
Persistent infection	91	48/43	46.85±8.56	18.44±3.82

Agarose gel electrophoresis result of three genotypes of SNP (1188A/C) is shown in Figure 1. Genotype frequencies at SNP (1188A/C) are listed in Table 2. There was significant difference in genotype distribution between subjects with self-limited infection and subjects with persistent infection ($P<0.01$). The distribution of genotype showed a good fit to Hardy-Weinberg equilibrium. At SNP (1188A/C) locus, the AC homozygous genotype was found more frequently in subjects with self-limited infection compared to those with persistent infection: 64.3% *vs* 34.1% (OR = 3.48; 95% CI: 1.52-8.10; $P = 0.001$). The AA genotype was more frequent in individuals with persistent infection compared to those with self-limited infection: 40.7% *vs* 19.0% (OR = 0.34; 95% CI: 0.12-0.87; $P = 0.014$).

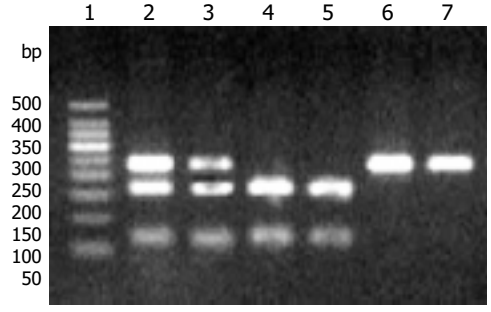

Figure 1 2% agarose gel electrophoresis of restriction enzyme digested products. Lane 1: 50 bp DNA ladder; lanes 2 and 3: AC genotype; lanes 4 and 5: CC genotype; lanes 6 and 7: AA genotype.

Table 2 Genotype frequencies of 1188A/C SNP of *IL12B* in patients

Genotype	Self-limited infection (%) *n* = 42	Persistent infection (%) *n* = 91	OR	95% CI	*P*
AA	8 (19.0)	37 (40.7)	0.34	0.12-0.87	0.014
CC	7 (16.7)	23 (25.3)	0.59	0.20-1.61	0.270
AC	27 (64.3)	31 (34.1)	3.48	1.52-8.10	0.001

Comparisons of genotype distribution using chi square test showed significant difference between self-limited infection and persistent infection ($P = 0.004$).

Effects of variables on the outcome of HCV infection were investigated by means of binary logistic regression analysis (Table 3). Both genotypes of SNP (1188A/C) and gender were independently associated with the outcome of HCV infection (OR 0.43, $P = 0.001$; OR 0.41, $P = 0.029$, respectively).

Table 3 Multivariate analysis of the effects of variables on the outcome of HCV infection

Variable	*P*	Multivariate odds ratio (95% CI)
Genotype	0.001	0.43 (0.27-0.70)
Gender	0.029	0.41 (0.18-0.91)
Duration of infection	NS	-

The general features, biochemical characteristics and HCV RNA levels in patients with persistent infection grouped by the genotype of SNP (1188A/C) of *IL12B* were analyzed (Table 4). No significant differences were found in age, gender or the duration of infection between three groups. And no significant differences were found in ALT, AST, γ-GT, ALP, TBil, DBil, Tp, ALB, A/G or HCV RNA levels between three groups ($P>0.05$). According to the result from B-mode ultrasound and clinical diagnosis, patients with persistent infection were divided into groups based on severity. No significant differences were found in genotype frequencies between different groups ($P>0.05$). Multivariate analysis of variance was used to analyze the different biochemical characteristics between three groups, and no difference was found ($P>0.05$) (data not shown).

Table 4 Characteristics of patients with different SNP (1188A/C) genotypes during persistent infection

Characteristics	AA *n* = 38	CC *n* = 23	AC *n* = 30
Age (yr)	46.9±8.2	46.7±9.7	46.8±8.4
Gender (male/female)	21/17	12/11	15/15
Duration of disease (yr)	18.0±3.8	18.8±4.0	18.1±3.8
ALT (U/L)	51±44	51±60	44±26
AST (U/L)	50±30	51±34	47±20
γ-GT (U/L)	24±26	35±33	28±34
ALP (U/L)	88±26	92±28	93±22
Tbil (μmol/L)	29±11	8.95±2.59	10.1±4.2
Dbil (μmol/L)	5.8±2.0	5.3±2.2	5.4±2.4
Tp (g/L)	75.3±3.9	72.24±5.95	75.0±5.8
ALB (g/L)	44.4±2.3	43.8±2.3	44.5±2.6
A/G	1.46±0.21	1.59±0.32	1.51±0.30
HCV RNA[1]	5.07±1.49	4.95±1.52	5.58±1.24

[1]Values are expressed as $\log_{10}$ RNA copies per mL.

DISCUSSION

For the first time, we investigated here the polymorphism of

SNP (1188A/C) of *IL12B* in patients with HCV infection and demonstrated that AA genotype decreased and AC genotype increased in self-limited infection. Although there was no significant difference in allele distribution between patients with self-limited infection and patients with persistent infection, A allele tended to be decreased and C genotype to be increased in self-limited infection. In this study, however, there was no association between genotype of SNP (1188A/C) of *IL12B* and biochemical characteristics of subjects with persistent infection. And no association was found between genotype of SNP (1188A/C) and the severity of subjects with persistent infection.

HCV infection is characterized by a broad spectrum of possible outcomes. Infection is self-limited in a fortunate minority, while the majority of patients develop persistent infection[16,17]. Patients with acute HCV infection presenting a self-limited acute hepatitis and with eradication of the virus develop a strong Th1 response but a weak or absent Th2 response. In contrast, patients developing a chronic infection show a predominant Th2 response, but a weak Th1 response. These observations suggest that Th1 cytokine effect is essential for protection against HCV infection, while the production of Th2 cytokine seems to have an inhibiting effect on the patient's immunological system[8,10]. IL-12 is a key regulator of the polarization of immune responses to T helper 1 or 2 categories and plays a role in autoimmune and infectious diseases[11,12,18]. IL-12 might, therefore, have effects on the pathogenesis of HCV infection by affecting Th1/Th2 balance.

IL-12 is comprised of two disulphide-linked protein subunits designated p35 and p40, which are encoded by two different genes, *IL12A* and *IL12B*[19,20]. IL-12 *p40* expression is restricted to the production of *p70* because *p35* is expressed ubiquitously and constitutively at low levels. Thus, secretion of the biologically active p70 heterodimer appears to be predominantly regulated at the level of *IL12B*[12]. IL-12 is secreted mainly by antigen-presenting cells and plays a key role in innate resistance and adaptive immunity. IL-12 acts on T cells and NK cells by enhancing generation and activity of cytotoxic lymphocytes and inducing proliferation and production of cytokines, especially IFN-γ. IL-12 is also the major cytokine responsible for differentiation of T helper 1 cells, which are potent producers of IFN-γ[12,19]. And IFN-γ plays important roles in the immune response to HCV infection. Some studies have been reported that the deletions of IL-12 *p40* lead to serious impairment of immunity to intracellular bacteria[21]. The SNP at position 1188 in the untranslated region of IL-12 *p40* gene mapped to chromosome 5q31-33. Although SNP is located in UTR and does not alter the coding sequence, some researches showed that this SNP site was associated with immune-mediated disease[13,22].

Many studies showed that there was close relationship between IL-12 and hepatitis C. Nelson *et al*. reported that patients with chronic HCV infection had elevated IL-12, which correlated with serum ALT and intrahepatic HCV-specific CTL activity. These data indicate that IL-12 is involved in the immunopathogenesis of chronic HCV infection, especially in cell-mediated immunity, which might be important in spontaneous or interferon mediated viral clearance[23,24]. Another study reported the impairment in allostimulatory capacity of peripheral blood dendritic cells in HCV- infected individuals, which is likely due to low IL-12 expression and restored by endogenous IL-12[25]. Some studies have found that IL-12 can produce lower HCV-RNA level[26-31].

Studies on functional characteristics of this polymorphism suggest that they influence the secretion of cytokines. Morahan *et al*. reported the 3'-UTR alleles showed different levels of *IL12B* mRNA expression in cell lines. They identified EBV-transformed cell lines homozygous for each allele. Expression of *IL12B* mRNA was significantly reduced in the C/C genotype cell line in contrast to the A/A line[32]. But Seegers *et al*. found that a TaqI polymorphism (C/C) in IL12 *p40* 3'-UTR correlated with increased IL-12 *p70 in vitro*[33]. The results were complicated and the functional characteristics of this polymorphism still need to be evaluated. Currently our group is working on the IL-12 mRNA and protein levels of three different genotypes to clarify how SNP (1188A/C) influences the course of hepatitis C.

In summary, we have reported that the frequency of A/A genotype of *IL12B* 3'-UTR SNP was decreased in self-limited HCV infection. It suggests that this SNP is associated with different outcomes of HCV infection, presumably by affecting Th1/Th2 balance. Nevertheless, since the outcome of HCV infection is a complicated polygenic trait, the interactive effects between SNP (1188A/C) of *IL12B* and other factors involved in the outcome of hepatitis C still need to be evaluated.

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