Association of interleukin-18 gene polymorphisms with the outcomes of hepatitis C virus infection in high-risk Chinese Han population

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ABSTRACT

Interleukin 18 (IL-18) gene polymorphisms have been reported to be associated with the outcomes of hepatitis C virus (HCV) infection in Americans, Indians and Europeans. We aimed to investigate whether the association can be replicated in Chinese Han population. Three IL-18 variants, −656G>T, −137G>C and +105A>C, were genotyped in three independent Han cohorts consisting of 552 cases and 784 controls. By using logistic regression analysis and multiple testing, haplotype GCC were associated with a protection from susceptibility to HCV. After stratified analysis, both the carriage of −137C allele in the older or hemodialysis subgroup and the carriage of +105C allele in the younger subgroup were found to be significantly associated with a decreased risk of HCV susceptibility. By using logistic regression analysis and multiple testing for the resolution of HCV infection, our study showed that +105C allele and haplotype GCC displayed a negative effect on HCV persistence. After stratified analysis, a significantly decreased risk for HCV persistence was found in +105C allele in the subgroups of young, male or female, drug user or hemodialysis and HCV-1 or HCV mixed genotype. No significant association was observed between −656G>T and the outcomes of HCV infection. Our results demonstrated that the carriage of −137C allele, +105C allele, haplotype −656G/−137C+/+105C and haplotype −656G/−137C/+105C of IL-18 gene may contribute to better outcomes of HCV infection in high-risk Chinese Han population.

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1. Introduction

Hepatitis C virus (HCV)-infected 170 million individuals spread worldwide [1]. HCV infection has a high rate of chronic development, with more than 50% HCV-infected patients progressing from acute to persistent infection, among whom 10–20% patients are likely to develop cirrhosis and hepatocellular carcinoma (HCC) [2,3]. Unfortunately, no effective HCV vaccine is available. In China, the prevalence of HCV infection is about 3.2% [4], and transfusion, injection drug use, organ transplantation, and haemodialysis have been the important transmission routes of HCV [5].

As known, the immune response and host genetic factors are the important determinators for the prognosis of HCV infection [6]. And cytokines are assumed to play a critical role in the pathogenesis of HCV infection through influencing the innate and adaptive immune response and immune system activation, which are implicated in the diverse clinical outcomes of HCV infection [7], including the susceptibility, spontaneous clearance and viral persistence. Yet, the mechanisms underlying HCV infection are not fully enunciated.

The human interleukin-18 (IL-18) is mainly secreted by activated macrophages and Kupffer cells and can promote IFN-γ production [8]. IL-18 was reported to have a variety of biological activities, including mediating inflammatory reaction [9], inducing target cell apoptosis [10]. The combination of interleukin-18
receptor (IL-18R) and IL-18, a cytokine structurally related to interleukin-1β (IL-1β) [11], activates myeloid differentiation factor 88 (MyD88) and interleukin 1 receptor-associated kinase 4 (IRAK-4) adapter molecules [12,13], followed by the transcriptional activation of transcription factor nuclear factor-κB (NF-κB) and nuclear factor of activated T cells (NFAT), and results in the regulation of the innate immunity signal transduction [14]. Additionally, IL-18 can induce a host Th1 immune response with the presence of IL-12, and induce Th2 immune response without IL-12 [15]. IL-18 influences T cell immunity by bridging the innate and adaptive immunity through IFN-γ and the CD134 costimulatory pathway [16], which has been associated with the outcomes of HCV infection.

Mounting evidence pointed that IL-18 gene polymorphisms affect the IL-18 transcription activity and patients' sera levels of IL-18 [11] and suggest that the genetic variants of IL-18 gene may influence the host susceptibility, viral clearance or persistence, tissue injury and antiviral treatment response of HCV infection. Besides, a previous study has demonstrated that −607C/A and −656G>T, single nucleotide polymorphisms (SNPs) in the promoter region of IL-18, were significantly associated with serum levels of IL-18 in patients with sarcoidosis and the haplotype −137G/−607C−656G may account for the significantly higher promoter activity of IL-18 and serum IL-18 level among Japanese population [17]. Similarly, +105A>C, a synonymous SNP in exon 4 of IL-18 [9], has been found to be associated with the altered IL18 transcriptional activity in vitro [18]. Additionally, the polymorphisms at the position −607 and −137 were found to be associated with susceptibility to chronic hepatitis B in Chinese Han population [19]. Until recent years, several similar studies showed that the mutation from C to A at site −607, the mutation from G to C at site −137 and some haplotypes were related to viral clearance, the protection of severe liver disease or treatment response in American [20], Indian [21] and European [22] HCV patients. IL-18 gene polymorphisms have been implicated in many diseases with abnormal, dysregulated immune response, such as cardiovascular disease [23], type I diabetes [24], asthma [25], rheumatoid arthritis [26], multiple sclerosis [27], and so on. Nevertheless, the results of previous studies on these SNPs are limited and inconsistent, and the exact functions of these SNPs and the influence mechanisms on IL-18 level have been less well understood. However, so far, there are no reports on the relationship with the polymorphism of −137G>C and the outcomes of HCV infection in Chinese HCV patients and no associations between two SNPs (−656G>T and +105A>C) and the outcomes of HCV infection were recorded in the world.

In light of the potential immune regulating effect of IL-18 polymorphisms in HCV infection, we examined the relationships of the IL-18 gene polymorphisms and HCV infection outcomes in Chinese Han population.

2. Materials and methods

2.1. Ethic statement

Written informed consents were obtained from all participants and the study protocol, which conforms to the ethical guidelines of the 1975 Declaration of Helsinki (6th revision, 2008), was approved by the institutional review board of Nanjing Medical University.

2.2. Patients and controls

A total of 552 anti-HCV positive patients were collected, including 362 drug users (173 persistent infection and 189 spontaneous clearance) enrolled from Nanjing compulsory drug rehabilitation center (Nanjing, Jiangsu, China) from May to December 2006 and 190 anti-HCV positive hemodialysis (HD) patients (86 persistent infection and 104 spontaneous clearance) enrolled from nine hospital hemodialysis centers in southern China from Oct 2008 to Jan 2010. Cases were subdivided as follows: (1) the persistent HCV infection group consisted of 259 HCV sero-positive and HCV-RNA positive cases, with persistently normal or elevated ALT levels; (2) the spontaneous viral clearance group consisted of 293 HCV sero-positive and HCV-RNA negative cases, with persistently normal ALT levels (<40 U/L); (3) uninfected control group consisted of 784 HCV sero-negative and HCV-RNA negative persons, including 513 drug users came from Nanjing compulsory drug rehabilitation center (Nanjing, Jiangsu, China) and 271 HD patients came from nine hospital hemodialysis centers in southern China during the same period as the case collected, respectively. The control subjects were matched to the persistent HCV infection subjects or the spontaneous viral clearance subjects for age (5-year interval), sex and geographic location (town and country). Subjects co-infected with any other virus or used any antiviral medications during the trial were excluded. All results of serology confirmed by at least three separated serological tests within consecutive 6 months during the follow-up. All the subjects were diagnosed by experienced doctors on the basis of clinical and laboratory findings and internationally accepted criteria [28].

All study participants were interviewed using structural and standardized questionnaires. The information of main risk factors of HCV infection, as previously reported in literature [29], including gender, age, region, history of HCV infection, environmental risk exposure histories of hepatitis C, were collected. The route of infection was determined according to the subjects’ written medical histories and the questionnaires obtained in the study. Quality assurance procedures of epidemiological investigation were established to ensure the quality of the data used in the study. All the subjects’ demographic and clinical characteristics are summarized in Table 1.

2.3. Serologic testing and HCV genotyping

Peripheral venous blood (5 ml) obtained from each participant was collected in EDTA tubes. Plasma was isolated by centrifugation and stored at −80 °C until assayed. Antibody to HCV was assayed by third-generation enzyme-linked immunosorbent assay (ELISA) (Architect Anti-HCV assay; Abbott Laboratories, Abbott Park, IL, USA). The Sera HCV RNA load was quantified using commercially available kits (Cobas TaqMan HCV Test, Roche Diagnostics, Mannheim, Germany) according to the manufacturer’s instructions. The Murex HCV Serotyping 1–6 Assay ELISA Kit (Abbott, Wiesbaden, Germany) was used to determine the type-specific antibodies to various HCV genotypes as described previously [30].

2.4. SNPs selection

The IL-18 gene is located on human chromosome 11q22.2–q22.3 and consists of six exons and five introns [31]. Based on the pertinent literatures, we searched the SNPs from the database of CHB population of HapMap (http://www.hapmap.org), and selected SNPs with minor allele frequency (MAF)>5%. SNPs at position −656G>T (dbSNP accession number rs1946519), −137G>C (dbSNP accession number rs187238) and +105A>C (dbSNP accession number rs549908) were selected as candidates. −656G>T and −137G>C are separately located in promoter [9], which were reported to be associated with transcriptional activity of IL-18 gene HCV patients among other ethnicities [20–22], while +105A>C is located in exon 4 without amino acid change of IL-18 [9], which was reported to be associated with other diseases in Asians [17,25,32].
2.5. Genotyping assays

Genomic DNA was extracted from leukocytes using the phenol-chloroform method and stored at −20 °C. The SNPs were genotyped by TaqMan assay and specific forward/reverse PCR primers and TaqMan minor groove-binding (TaqMan-MGB) probes designed and synthesized by Applied Biosystems (Foster, CA, USA) (Table 2).

PCR was performed in a final volume of 5 μl consisting of 2.5 μl TaqMan Master Mix (Applied Biosystems, Foster, CA, USA), 0.225 μl forward and reverse primer (20 pmol/μl) with a final concentration of 900 mM, 0.125 μl VIC and FAM (10 pmol/μl) with a final concentration of 250 nM, 1 μl genomic DNA (10 ng/μl) and 0.8 μl sterile double distilled H2O (ddH2O). Each determination was conducted with three blank controls to identify the agent or system contaminations. The detection was carried out by Sequence Detection System 7900 (SDS; Applied Biosystems, Foster, USA). The PCR cycling conditions were one hold at 50 °C for 5 min, followed by one hold at 95 °C for 10 min, and 35 cycles at 95 °C for 30 sec and 60 °C for 30 s. After measuring the fluorescence intensity at the endpoint, allelic discrimination was analyzed by allele-calling specialized software (SDS, version 2.3; Applied Biosystems, Foster, USA).

The two operators for the interpretation of genotyping results were blinded to the clinical data. Those samples testing with inconsistent or unsuccessful genotyping results were repeated until agreements were reached. Repeated experiments were performed in 5% random samples and the agreements were 100%. The genotyping success rates of three polymorphism variants were approximately equal to 98%.

2.6. Statistical analysis

Data were checked and verified and then entered into a database using EpiData 3.1 [33] by two different researchers for further analysis. Quantitative variables were presented as mean ± standard deviations (SD). The differences of the demographic characteristics, HCV biological indicators and the distributions of IL-18 allele frequencies between the case and the control groups were analyzed by χ2 test or Student’s t-test. Hardy–Weinberg equilibrium test was conducted by using the χ2 test for goodness of fit. Haplotypes were estimated with the PHASE software, version 2.1 [34]. Both single and multiple factors logistic regression analysis were performed to calculate the odds ratios (ORs) and 95% confidence intervals (CIs). A two-sided P value < 0.05 was considered statistically significant. To correct for multiple comparisons, we applied the false discovery rate approach (FDR), less conservative than the commonly Bonferroni correction. All calculations were performed with SPSS software (version17. 0; SPSS Institute, Chicago, IL, USA) and SAS software (version 9.1.3; SAS Institute, Cary, NC, USA).

3. Results

3.1. Basic characteristics

The distribution of all subjects’ characteristics is shown in Table 1. HCV-uninfected control group included 784 subjects (517 males, 267 females; mean age 42.59 ± 15.42 years). The persistent HCV infection group consisted of 259 cases (184 males, 75 females; mean age 42.96 ± 12.39 years). The spontaneous viral clearance
3.2. Association analysis of IL-18 gene polymorphisms with susceptibility to HCV infection

Compared with reference +105A>C AA genotype, the carriage of C allele was associated with a decreased risk of the susceptibility to HCV (OR [95% CI] = 0.712 [0.532–0.953], P = 0.022), while it did not remain significant after multiple comparisons (P = 0.066). However, no significant association was observed between −656G>T and −137G>C with HCV susceptibility (Table 3).

Further stratification analysis indicated that, compared with the GG genotype, a significant decreased risk was found in −137C allele in the older subgroup (>40 years old, OR [95% CI] = 0.501 [0.313–0.801], P = 0.004), which is stratified according to the previous literature [35], and HD subgroup (OR [95% CI] = 0.454 [0.276–0.749], P = 0.002). Position +105A>C carriage of the C allele seemed to be more liable to spontaneous viral clearance in the younger subgroup (≤40 years old, OR [95% CI] = 0.665 [0.445–0.982], P = 0.045), compared with the AA genotype (Table 4). Nevertheless, no significant effect of −656G>T on the HCV infection risk was found in different strata (all P > 0.05).

3.3. Association analysis of IL-18 gene polymorphisms with the resolution of HCV infection

Compared with +105 AA wild-type genotype, logistic regression analysis showed that the carriage of C allele seemed to be associated with the protection from persistent HCV infection (OR [95% CI] = 0.500 [0.330–0.759], P = 0.001), and this remained significant after accounting for multiple comparisons (P = 0.003). No statistical difference was found for the genotypes of −656G>T and −137G>C between the two HCV infected groups (Table 3).

The stratified analysis showed that +105A>C carriage of C allele might favor spontaneous viral clearance in the terms of young (≤40 years old, OR[95% CI] = 0.387 [0.212–0.708], P = 0.002), male (OR [95% CI] = 0.521 [0.317–0.857], P = 0.010) or female (OR [95% CI] = 0.431 [0.194–0.959], P = 0.039), drug user (OR [95% CI] = 0.516 [0.311–0.858], P = 0.011) or HD (OR [95% CI] = 0.462 [0.219–0.974], P = 0.042) and HCV-1 (OR [95% CI] = 0.568 [0.336–0.960], P = 0.035) or HCV mixed genotype-infected (OR[95% CI] = 0.316 [0.113–0.884], P = 0.028) subgroups (Table 4). No significant difference was observed in −656G>T and −137G>C genotypes between spontaneous clearance and persistent HCV infection in different strata (all P > 0.05).

3.4. Haplotype analysis among the three groups

The three-locus haplotypes were consisted of −656G>T, −137G>C and +105A>C variant alleles. Compared with the most frequent TGA haplotype, GCC haplotype was significantly associated with the decreased risk of the susceptibility to HCV infection (OR [95% CI] = 0.586 [0.408–0.842], P = 0.004), whereas GGC haplotype was significantly associated with the protection
Table 4
Stratified analysis of −137G > C, +105A > C genotypes among persistent infection, spontaneous clearance and control group.

<table>
<thead>
<tr>
<th>SNPs</th>
<th>Allele (1/2)</th>
<th>Subgroups</th>
<th>Group A n (%)</th>
<th>Group B n (%)</th>
<th>Group C n (%)</th>
<th>OR (95% CI)</th>
<th>P&lt;sub&gt;a&lt;/sub&gt;</th>
<th>OR (95% CI)</th>
<th>P&lt;sub&gt;a&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>−137G &gt; C</td>
<td>G/C</td>
<td>Age</td>
<td>11/12/22</td>
<td>11/12/22</td>
<td>11/12/22</td>
<td>1.134 (0.748–1.719)</td>
<td>0.555</td>
<td>1.174 (0.670–2.055)</td>
<td>0.575</td>
</tr>
<tr>
<td></td>
<td></td>
<td>≤40</td>
<td>75 (55.4)</td>
<td>87 (56.3)</td>
<td>108 (50.9)</td>
<td>0.501 (0.313–0.801)</td>
<td>0.004</td>
<td>0.974 (0.519–1.829)</td>
<td>0.935</td>
</tr>
<tr>
<td></td>
<td></td>
<td>&gt;40</td>
<td>70 (44.6)</td>
<td>82 (43.7)</td>
<td>108 (50.9)</td>
<td>0.501 (0.313–0.801)</td>
<td>0.004</td>
<td>0.974 (0.519–1.829)</td>
<td>0.935</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Route of infection</td>
<td>11/12/22</td>
<td>11/12/22</td>
<td>11/12/22</td>
<td>1.134 (0.748–1.719)</td>
<td>0.555</td>
<td>1.174 (0.670–2.055)</td>
<td>0.575</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Drug use HD</td>
<td>77 (59.7)</td>
<td>70 (50.0)</td>
<td>90 (45.1)</td>
<td>0.501 (0.313–0.801)</td>
<td>0.004</td>
<td>0.974 (0.519–1.829)</td>
<td>0.935</td>
</tr>
<tr>
<td>+105A &gt; C</td>
<td>A/C</td>
<td>Age</td>
<td>11/12/22</td>
<td>11/12/22</td>
<td>11/12/22</td>
<td>1.134 (0.748–1.719)</td>
<td>0.555</td>
<td>1.174 (0.670–2.055)</td>
<td>0.575</td>
</tr>
<tr>
<td></td>
<td></td>
<td>≤40</td>
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<td>108 (50.9)</td>
<td>0.501 (0.313–0.801)</td>
<td>0.004</td>
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<td>0.935</td>
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<td>70 (44.6)</td>
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<td>108 (50.9)</td>
<td>0.501 (0.313–0.801)</td>
<td>0.004</td>
<td>0.974 (0.519–1.829)</td>
<td>0.935</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Gender</td>
<td>Male</td>
<td>11/12/22</td>
<td>11/12/22</td>
<td>11/12/22</td>
<td>1.134 (0.748–1.719)</td>
<td>0.555</td>
<td>1.174 (0.670–2.055)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Female</td>
<td>65 (47.8)</td>
<td>70 (50.0)</td>
<td>90 (45.1)</td>
<td>0.501 (0.313–0.801)</td>
<td>0.004</td>
<td>0.974 (0.519–1.829)</td>
<td>0.935</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Route of infection</td>
<td>11/12/22</td>
<td>11/12/22</td>
<td>11/12/22</td>
<td>1.134 (0.748–1.719)</td>
<td>0.555</td>
<td>1.174 (0.670–2.055)</td>
<td>0.575</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Drug use HD</td>
<td>77 (59.7)</td>
<td>70 (50.0)</td>
<td>90 (45.1)</td>
<td>0.501 (0.313–0.801)</td>
<td>0.004</td>
<td>0.974 (0.519–1.829)</td>
<td>0.935</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Viral genotype Genotype-1</td>
<td>11/12/22</td>
<td>11/12/22</td>
<td>11/12/22</td>
<td>1.134 (0.748–1.719)</td>
<td>0.555</td>
<td>1.174 (0.670–2.055)</td>
<td>0.575</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Non-1</td>
<td>65 (47.8)</td>
<td>70 (50.0)</td>
<td>90 (45.1)</td>
<td>0.501 (0.313–0.801)</td>
<td>0.004</td>
<td>0.974 (0.519–1.829)</td>
<td>0.935</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mixed</td>
<td>65 (47.8)</td>
<td>70 (50.0)</td>
<td>90 (45.1)</td>
<td>0.501 (0.313–0.801)</td>
<td>0.004</td>
<td>0.974 (0.519–1.829)</td>
<td>0.935</td>
</tr>
</tbody>
</table>

Group A: Persistent infection patients; Group B: Spontaneous clearance patients; Group C: Controls; Group (A + B): Infected individuals, including persistent infection group and spontaneous clearance group. Non-1: genotype 2, 3 and unknown; Mixed: co-infected with genotype 1, 2 and 3; HD: Hemodialysis.

The P value, odds ratio (OR), 95% confidence intervals (CI) were calculated on the basis of the binary logistic regression model, adjusted by gender, age, route of infection, and/or viral genotype in dominant model (GG vs. [GC + CC] for −137G > C, AA vs. [AC + CC] for +105A > C). The actual P value was not shown for lack of space. Bold type indicates statistically significant results.
Table 5
Haplotypes frequencies constituted with polymorphisms of three SNPs among persistent infection, spontaneous clearance and control group.

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>Group A (%)</th>
<th>Group B (%)</th>
<th>Group C (%)</th>
<th>Group A (B) / Group C</th>
<th>OR (95% CI)</th>
<th>P</th>
<th>OR (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>TGA</td>
<td>252 (48.6)</td>
<td>250 (42.7)</td>
<td>716 (45.7)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>GGA</td>
<td>177 (34.2)</td>
<td>212 (36.1)</td>
<td>523 (33.3)</td>
<td>1.046 (0.846-1.293)</td>
<td>0.677</td>
<td>-</td>
<td>0.827 (0.628-1.088)</td>
<td>0.175</td>
</tr>
<tr>
<td>GCC</td>
<td>32 (6.2)</td>
<td>38 (6.5)</td>
<td>162 (10.3)</td>
<td>0.586 (0.408-0.842)</td>
<td>0.004</td>
<td>-</td>
<td>0.810 (0.486-1.351)</td>
<td>0.420</td>
</tr>
<tr>
<td>GCC</td>
<td>12 (2.3)</td>
<td>41 (7.0)</td>
<td>77 (4.9)</td>
<td>0.991 (0.638-1.538)</td>
<td>0.967</td>
<td>-</td>
<td>0.276 (0.137-0.537)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Others</td>
<td>45 (8.7)</td>
<td>45 (7.7)</td>
<td>90 (5.7)</td>
<td>1.181 (0.803-1.736)</td>
<td>0.397</td>
<td>-</td>
<td>1.022 (0.645-1.620)</td>
<td>0.926</td>
</tr>
</tbody>
</table>

*Group A: persistent infection patients; Group B: spontaneous clearance patients; Group C: controls; Group A (B): Infected individuals, including persistent infection group and spontaneous clearance group.*

*Order of single nucleotide polymorphisms comprising the IL-18 haplotypes: −656G>T, −137C>G, and +105A>C.*

*Logistic regression model, adjusted by gender, age, route of infection, and/or viral genotype.*

*All the haplotypes with a frequency of less than 5% were combined with others. Bold type indicates statistically significant results.*

4. Discussion

This is the first report that the gene polymorphisms of IL-18 were associated with the outcomes of HCV infection in high-risk Chinese HCV patients. In this study, the overall and stratified analysis of the susceptibility or resolution of HCV infection showed that +105A allele and −137C allele were found to confer a partly protective effect on HCV infection, correspond to the previous works very well. As an existed research, +105A>C, a synonymous SNP, was not observed to be associated with differences in plasma IL-18 levels [25]. However, it was demonstrated that +105A allele may be associated with increased IL-18 production in vitro which may liable to develop HCV infection or persistence [18]. Although the polymorphism of +105A>C was found not to cause amino acid exchange, due to the linkage disequilibrium between the polymorphism of +105A allele and −137G allele of IL-18 gene [18,25], it was plausible that +105C allele may associated with the decreased transcription capacity and expression of IL-18 through −137G allele. Previous report suggested that HCV-infected patients had significantly higher levels of IL-18 correlated with disease severity of hepatitis C [36]. In addition, it was documented that the low production of IL-18 contributes to influenza A virus clearance and enhanced activation of CD4+ T cells [37]. Therefore, it may be reasonable that the mutation from A to C at position +105A>C was associated with the decreased IL-18 level or enhanced activation of CD4+ T cells in this study, so exhibit a partly protective effect on HCV infection. Although the variation of +105A>C would not cause amino acid change, previous study indicated that the alteration of synonymous SNP in other gene play a role in the modulation of transcription of the gene or RNA stabilization [38]. There also might be unknown strong linkage disequilibrium between +105A>C and other SNPs of IL-18 gene, which alters the functional property of IL-18 or its gene regulation and results in various outcomes of HCV infection. Such modulations or unknown linkage relationships may exert more effective protection for HCV infection. In addition, the different protection exhibited between the resolution of HCV infection and the susceptibility to HCV may due to the different influence of this SNP infection. Further research of differential effects of +105A>C on the balance of Th1 and Th2 during different period of HCV will help to clarify various outcomes of HCV infection.

Previous studies reported that a mutation from G to C at −137G>C interfered the H4TF-1 nuclear factor binding site and reduced the transcription factor binding and decrease IL-18 gene expression [27,39]. Our findings further proved the association between −137G allele and the protective effect of HCV infection. Other evidence from population also demonstrated that IL18 −137C was significantly associated with the protection of HCV clearance and treatment response [20,22] as well as protection HBV infection in Chinese Han population [19].

Some studies had discovered that most of drug users and HD patients had typical maladjustment of microecology and low immune function [40,41] which was correlated with high susceptibility to HCV infection. Furthermore, HD patients may be exposed to larger HCV inoculum by transfusion, compared with drug users assumedly received small HCV inoculum through sharing needles. This could result in the ineffective immune response to HCV in HD patients, compared with drug users. This finding also agrees well with previous studies [20]. The different degree of immune dysfunction and the different initial HCV inoculum between HD patients and drug users was able to account for the significantly increased risk of the carriage of the C allele at position −137 in HD patients, compared with drug users with the same genotype. Meanwhile, no significant protective effect exhibited in HCV-non-1 genotype-infected patients may be due to a relatively less liable to develop viral persistence in this subgroup.

The haplotype analysis showed that haplotype GCC (−656G/−137C/+105C) and haplotype GCC (−656G/−137G/+105C) displayed significant protection against HCV infection, and persistent HCV infection, respectively, which suggest that these three loci might have joint effect on the HCV infection. Further functional research on these SNPs should be warranted.

Due to the occult infection of HCV, a potential limitation of this study was that it is difficult to estimate the acquisition age or initial viral inoculum of HCV infection in our cohort, which was closely related the activation level and status of host immune response and may play a role in various outcomes of HCV infection. Besides, in view of high-risk (drug user and HD) subjects enrolled in this study, the selection bias was inevitable and the cohorts may not be representative of the general Chinese HCV population. However, we have minimized these potential confounding factors by matching gender, age and region. Further studies based on large sample and general HCV patients are needed to confirm our findings.

5. Conclusions

Our findings indicate that the genetic variants of IL-18 gene were associated with the outcomes of HCV infection in high-risk Chinese population and that suggest that host genetic factors, like IL-18, may be partly associated with the pathogenesis, prevention and control of hepatitis C.

Competing interest

All authors declare that they have no competing interests.
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