BRIEF COMMUNICATION

IL28B rs12979860 polymorphism does not influence outcomes of hepatitis B virus infection

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Abstract
rs12979860 in interleukin 28B (IL28B) gene is associated with response to interferon-α therapy and natural viral clearance in hepatitis C. The role of this polymorphism is less known in hepatitis B virus (HBV) infection. We evaluated whether rs12979860 was associated with outcomes of HBV infection. There were 651 individuals with persistent infection (387 with liver cirrhosis, 264 without cirrhosis) and 226 healthy individuals who recovered from HBV infection. The genotypic distributions were compared between different phenotypes pertaining to disease progression and HBV markers. The polymorphism had no association with clearance of hepatitis B surface antigen and hepatitis B e antigen, HBV-DNA level, apparent hepatitis onset and liver cirrhosis (P > 0.05). These results suggest that rs12979860 does not have such a strong effect in hepatitis B compared to hepatitis C.

Recent genome-wide association studies have linked a single nucleotide polymorphism (SNP), rs12979860 in the upstream of interleukin 28B (IL28B) gene on chromosome 19 with response to pegylated interferon (IFN) therapy and spontaneous viral clearance in hepatitis C (1–5). rs12979860 CC genotype was associated with a greater chance of spontaneous resolution of hepatitis C virus (HCV) infection and sustained virological response than CT and TT genotypes. Some studies reported that patients with rs12979860 CC genotype had more likely elevated liver enzyme levels (6, 7) and higher HCV-RNA levels (1, 8). rs12979860 lies at 3 kb upstream of IL28B gene. IL28B gene encodes interferon-lamda 3 (IFN-λ3) which belongs to type III IFNs family including IFN-λ1, IFN-λ2 and IFN-λ3 (9). IFN-λ3 interacts with a transmembrane receptor to induce potent antiviral responses, which are mediated through the activation of the JAK-STAT and MAPK pathways (10). Although the mechanism of how the SNP affects IL28B function has not been elucidated, effect of this SNP on the outcomes of other chronic viral infections such as hepatitis B virus (HBV) infection could give us more evidence about its role. HBV infection is endemic highly in China with a hepatitis B surface antigen (HBsAg) seropositivity rate as high as 5%–12% (11). The natural history of HBV infection varies from spontaneous recovery post infection, chronic asymptomatic carrier to decompensated cirrhosis and hepatocellular carcinoma, which confer a high mortality.
rate and heavy economic burden (12, 13). In this study, we evaluated whether the SNP was associated with persistence of infection, apparent hepatitis onset and liver cirrhosis in a Chinese population infected with HBV.

A total of 877 participants were enrolled from patients referred to clinics in this observational study. They were all Chinese Han participants from an epidemic region of HBV in Eastern China. Serum HBV markers including HBsAg, antibody to HBsAg (anti-HBs), hepatitis B e antigen (HBeAg), antibody to HBeAg (anti-HBe) and antibody to hepatitis B core antigen (anti-HBc) of immunoglobulin (Ig) G type were measured with chemiluminescent assays (Roche Diagnostics, Basel, Switzerland). The copy numbers per milliliter of plasma HBV-DNA were measured with Roche Lightcycler system. Persistent HBV infection denoted an HBsAg positivity status for at least 10 years according to disease history. Spontaneous clearance was defined as HBsAg negative, HBeAg negative, anti-HBc positive, anti-HBs positive and HBV-DNA negative in healthy people. Cirrhosis in persistent HBV infection persons was determined based on the clinical manifestations (ascites, varices hemorrhage, hepatic encephalopathy and spontaneous bacterial peritonitis), typical radiological findings (hepatatrophia, ascites, varices, portal vein dilation, splenomegaly), cirrhotic pathology in liver biopsy or laboratory features (low platelets count, low white blood cell count, hyperbilirubinemia, hypoalbuminemia and prothrombin time prolongation) pertaining to portal hypertension and impaired liver function. The persistent infection group without cirrhosis did not display any of the above signs. Phenotypes of hepatitis onset were defined in persistent HBV infection persons irrespective of cirrhosis status. Apparent hepatitis onset indicated elevation of liver enzymes in this population and the rest belonged to the control group. HBeAg phenotypes were classified as positivity and negativity in persistent infection group. Patients with evidence of chronic hepatitis C, autoimmune liver diseases, non-alcoholic fatty liver disease, alcoholic liver disease, drug-induced liver disease and Wilson’s disease were excluded. Patients with previous anti-HBV therapy were also excluded. The polymorphism was genotyped using matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF MS) with Sequenom’s MassARRAY system and iPLEX technology (Sequenom, San Diego, CA; www.sequenom.com). The t-test and Pearson’s chi-squared test were used for univariate analysis. The chi-squared test was also used for evaluating Hardy–Weinberg equilibrium. HBV-DNA level was reported using a logarithmic scale to the base 10. Logistic regression analysis was performed to determine the adjusted significance, odds ratio and 95% confidence interval where age and sex were co-variables.

Among the study population, there were 651 individuals with HBV persistence and 226 with spontaneous HBV clearance. Evident cirrhosis was diagnosed in 387 of 651 individuals with persistent HBV infection. There were no significant differences between the cirrhosis group and non-cirrhosis group and between the persistent infection group and spontaneous clearance group in mean age and sex ratio ($P > 0.05$) (Table 1). Compared to the non-cirrhosis group, higher serum bilirubin and lower albumin levels were present in the cirrhosis group, which suggested apparent liver injury (both $P < 0.05$). There were 141 participants with positive HBeAg in persistent infection group. HBV level was higher in the cirrhosis group than in the non-cirrhosis group ($P < 0.05$). The distribution of rs12979860 genotypes (CC 88.9%, CT 10.4%, TT 0.7%) was in Hardy–Weinberg equilibrium ($P > 0.05$). The C allele had a dominantly higher frequency (94.1%) in this Chinese population than that in Caucasians and Africans (14). So, the genotypes with the minor allele T (CT and TT) were combined for association analysis in comparison to the most protective CC genotype. The genotype frequencies in different phenotypic groups are shown in Table 2. In both univariate analysis and regression analysis, rs12979860 showed no significant association with spontaneous HBV clearance, liver cirrhosis, apparent hepatitis onset, clearance of HBeAg and HBV-DNA level ($P > 0.05$) (Table 2).

### Table 1 Characteristics of the study population

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Persistent infection (c)</th>
<th>Spontaneous clearance (d)</th>
<th>$P$ value$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cirrhosis (a)</td>
<td>Non-cirrhosis (b)</td>
<td>(a), (b)</td>
</tr>
<tr>
<td>Total number $= 877$</td>
<td>387</td>
<td>264</td>
<td>226</td>
</tr>
<tr>
<td>Age (years)</td>
<td>49.3 ± 11.5</td>
<td>47.5 ± 13.5</td>
<td>48.4 ± 12.1</td>
</tr>
<tr>
<td>Male, n (%)</td>
<td>305 (78.8)</td>
<td>194 (73.5)</td>
<td>173 (75.4)</td>
</tr>
<tr>
<td>Platelet (10$^9$/l)</td>
<td>96.1 ± 59.6</td>
<td>195.3 ± 62.1</td>
<td>208.9 ± 69.9</td>
</tr>
<tr>
<td>Total bilirubin (µmol/l)</td>
<td>33.1 ± 64.2</td>
<td>12.9 ± 11.0</td>
<td>11.6 ± 5.5</td>
</tr>
<tr>
<td>ALT (IU/l)</td>
<td>102.4 ± 253.1</td>
<td>77.4 ± 169.0</td>
<td>21.6 ± 15.9</td>
</tr>
<tr>
<td>Albumin (g/l)</td>
<td>35.1 ± 6.8</td>
<td>41.1 ± 4.2</td>
<td>40.2 ± 3.8</td>
</tr>
<tr>
<td>HBeAg positive, n (%)</td>
<td>141 (36.4)</td>
<td>61 (23.1)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Log$_{10}$(HBV-DNA (copies/ml))</td>
<td>4.09 ± 1.86</td>
<td>2.75 ± 2.69</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

ALT, alanine aminotransferase; HBeAg, hepatitis B virus e antigen; Log$_{10}$, logarithmic value to the base 10.

$^a$Age, platelet, total bilirubin, ALT and Log$_{10}$(HBV-DNA) were expressed as mean ± standard deviation.

$^b$t-test for continuous variables and chi-squared test for categorical variables.
Recent studies have shown that the rs12979860 polymorphism is strongly associated with spontaneous and treatment-induced clearance of HCV (1–5). In this study, there were no significant associations of rs12979860 with spontaneous HBV clearance and HBV-related disease progression. Some recent studies also reported that the SNP was not associated with spontaneous HBV recovery, HBV-related liver cirrhosis or HBV treatment outcomes with pegylated IFN-α plus adefovir (15–17). Although IFN-λ3 stimulates IFN-stimulated genes (ISGs) expression (10), which is important in both HBV and HCV infection, rs12979860 CC genotype had higher levels of liver enzymes and were less likely to be asymptomatic than patients with CT and TT genotypes during HCV infection. The CC genotype was also reported to be correlated with higher HCV viral load (1, 8).

As both IFN-α and IFN-λ3 signal through the JAK-STAT pathway to upregulate ISGs that are vital to control HBV and HCV, there are supposed to be differences or other ways independent of ISGs stimulation for IFN-λ3 activity, which may be important for controlling HCV infection. IFN-λ3 use the same heterodimeric receptor consisting of interleukin 10 receptor beta (IL10Rβ) and IFN-λ3 receptor alpha (IL28Rα), which are different from IFN-α (10, 18). IFN-λ may be a distinct receptor that may upregulate a different set of ISGs from IFN-α (19). IFN-λ can induce expression of ISGs in a steady increasing way whereas IFN-α induces the same genes with more rapid and transient kinetics (19). Basic research showed that IFN-λ could not suppress HBV replication well in human cells (20, 21). The interactions between the viruses and the SNP need to be investigated to explain the different effects. This could lead to improved understanding of the pathogenesis and help to determine whether the SNP could be used as a predictor of disease progression and response to antiviral therapy.

Our study can not completely rule out the possible association of the SNP with outcomes of HBV infection because of several limitations. Because of ethnic differences between Caucasians and Chinese, the minor allele T had a low frequency in Chinese, and thus the sample size is relatively small. Specific pathogenetic differences between HBV and HCV may also account for the controversial results. Considering that clinical outcome of chronic hepatitis B is influenced by viral and host factors, it might be useful to determine the HBV serotype/genotype and perform a subanalysis in relation to rs12979860. One finding suggested that SNPs of HBV antigens could influence clinical outcomes of hepatitis B (22). This makes evaluation of host polymorphism more challenging in hepatitis B than hepatitis C.

In conclusion, IL28B rs12979860 polymorphism has no association with spontaneous HBV clearance and HBV-related disease progression. The polymorphism does not have such a strong effect in hepatitis B compared to hepatitis C. The interactions between IL28B polymorphism and the two viral infections need to be further elucidated.

Acknowledgments

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Conflict of interest

The authors have declared no conflicting interests.

References


