Association between RANTES -403A/G polymorphism and susceptibility to hepatitis B virus infection: A meta-analysis

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ABSTRACT

The relationship between RANTES (regulated on activation, normal T cell expressed and secreted) -403A/G polymorphism and susceptibility to hepatitis B virus (HBV) infection was still controversial and unclear. This meta-analysis was performed to resolve this issue. After literature search, eight studies, comprising 2749 cases and 2753 controls, were collected and determined eligible for analysis. No significant association was found between the 403A/G A allele and persistent HBV infection (OR = 1.04, 95% CI = 0.96–1.13, P = 0.34). By using subgroup analysis divided by ethnicity, significant association was found between the RANTES -403A/G gene polymorphism and susceptibility to HBV infection in the allele model for the Caucasian group (OR = 1.68, 95% CI = 1.38–2.04, P < 0.001). In the genotype model, there was also a significant association between -403A/G and the risk of HBV infection (AG versus GG: OR = 1.85, 95% CI = 1.42–2.41, P < 0.001; AG+AA versus GG: OR = 1.75, 95% CI = 1.35–2.26, P < 0.001) among Caucasians. When using healthy individuals as controls, there were significant associations between the SNP and HBV infection (A versus G: OR = 1.22, 95% CI = 1.08–1.39, P = 0.002; AG+AA versus GG: OR = 1.32, 95% CI = 1.12–1.56, P = 0.001). When comparing the Han Chinese (CHB) and spontaneously recovered people, there was also a significant association (A versus G: OR = 1.22, 95% CI = 1.08–1.37, P = 0.002). In conclusion, in the Caucasian population, the RANTES -403A allele is a risk factor of susceptibility to persistent HBV infection. The RANTES -403A/G polymorphism is likely to associate with the persistent infection of hepatitis B virus, regardless of virus clearance. More studies are needed to validate this relationship.

INTRODUCTION

Hepatitis B virus (HBV) is still a severe challenge to the global public health, leading to over 400 million infections all over the world [1]. Approximately, 5–10% of adult patients and 29–40% of child patients may not be able to clear HBV and will eventually progress into chronic hepatitis B (CHB) without elucidated mechanisms [2]. Many factors can lead to chronic HBV infection and further progress into cirrhosis and liver cancer, including host immune, viral replication level, genetics, HBV variants and others [3, 4].

RANTES (regulated on activation, normal T cell expressed and secreted) promotes the accumulation and activation of CD4+ T cells, CD8+ T cells, natural killer cells and dendritic cells (DCs), it is a powerful immune-regulatory chemokine in inflammatory disorders [5–7]. It acts as a ligand for CC receptor 5 (CCR5) that were observed to be significantly decreased in CHB patients compared to healthy people [8]. Some studies have also suggested that the serum RANTES level in CHB patients was
significantly elevated, suggesting a correlation with the progress of disease, but the specific mechanisms were not clear [9, 10]. Recently, several polymorphisms of RANTES have been reported to associate with the risk of HBV infection, including -403A/G, -28C/G and In1.1T [11]. In Koreans, RANTES polymorphism at position -403 did not show any effect on increased HBV infection risk [12]. However, a significant association was observed between RANTES -403A/G and the risk of HBV infection in Saudi population [13] as well as in North Americans [14].

The relationship between RANTES -403A/G polymorphism and the risk of HBV infection are still controversial. The aim of this meta-analysis is to explore their relationship in a larger cohort.

RESULTS

A total of 35 relevant studies were selected for possible inclusion. Two of them were not performed in humans; seven studies were not focused on RANTES; four studies were vaccine related; eight were not case-control studies; one of them used the same dataset with a previous study; six studies did not analyze RANTES -403 polymorphism. According to our search strategy, eight case-control studies were eventually included in this meta-analysis [11–14, 17–20]. Specific selection process is displayed in the flow chart in Figure 1.

A total of 5502 individuals were curated in this meta-analysis of eight studies, including 2749 (49.96%) patients with persistent HBV infection and 2753 (50.04%) controls. Among the controls, 1256 were healthy individuals and 1497 were spontaneously recoveries (SRs) from HBV infection. All control individuals were in accordance with Hardy-Weinberg equilibrium. Two studies were of Caucasians [13, 14]; the others were focused on Asians [11, 12, 17–20]. However, two studies only presented the allelic data, our attempts of obtaining genotypic data from these two studies have failed. Therefore, we analyzed the allelic association between cases and controls from all the eight studies, and the genotypic association from six studies. The basic characteristics of eight studies are listed in Table 1.

The association between RANTES -403A/G alleles and the risk of persistent HBV infection was shown in Figure 2. There was strong heterogeneity ($I^2 = 86.5\%$, $p = 0.000$ at -403A/G), therefore we applied the random-effects models. No significant association was found between RANTES -403A/G gene polymorphism and HBV infection in allelic model (OR = 1.04, 95% CI = 0.96–1.13, $p = 0.34$). The different genotypes : (a) AA versus GA: OR = 1.15, 95% CI = 0.93–1.42, $p = 0.20$; (b) AA versus GG: OR = 1.13, 95% CI = 0.91–1.41, $p = 0.26$; (c) GA versus GG: OR = 1.15, 95% CI = 1.00–1.32, $p = 0.05$; (d) GA+AA versus GG: OR = 1.17, 95% CI = 1.03–1.34, $p = 0.02$, were shown in Figure 3. The GG genotype was potentially related to the decreased risk of HBV infection.

Subgroup analyses based on ethnicity was also performed. In the Caucasian population, the A allele was significantly associated with a higher risk of HBV infection (OR = 1.68, 95% CI = 1.38–2.04, $P < 0.001$). Similarly, we also observed significant associations in different genotypic models (AG versus GG: OR = 1.85, 95% CI = 1.42–2.41, $P < 0.001$; AG+AA versus GG: OR = 1.75, 95% CI = 1.35–2.26, $P < 0.001$). However, in Asians, no significant association between the RANTES -403A/G allele polymorphism and HBV infection were observed (OR = 1.05; 95% CI = 0.96–1.16, $p = 0.27$) (Table 2).

Figure 1: Flowchart of study selection in this meta-analysis.
We then stratified the controls into two types (healthy individuals [11–13, 18, 20] and SRs [12, 14, 17–19]). We found that RANTES-403A allele was a risk factor of persistent HBV infection when compared to healthy controls (A versus G: OR = 1.22, 95% CI = 1.08–1.39, \(p = 0.002\); AG versus GG: OR = 1.29, 95% CI = 1.09–1.54, \(P = 0.004\); AG+AA versus GG: OR = 1.32, 95% CI = 1.12–1.56, \(P = 0.001\) (Table 3). Interestingly, when individuals with HBV infection were combined with SR and compared with controls [11–13, 18, 20], we and found there was also a significant difference between the new case group and healthy controls (A versus G: OR = 1.22, 95% CI = 1.08–1.37, \(p = 0.002\)).

Sensitivity analysis was performed by removing individual study at one time. This analysis showed there was no influence of each study on ORs. Publication bias was assessed by Egger’s linear regression test and funnel plot for the included studies. Figure 4 revealed that there

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### Table 1: The basic characteristics of eight articles in this meta-analysis about the relationship between RANTES-403 polymorphism and chronic HBV infection

<table>
<thead>
<tr>
<th>Author</th>
<th>Year</th>
<th>Ethnicity</th>
<th>Cases</th>
<th>Controls</th>
<th>Control type</th>
<th>Genotypes of cases</th>
<th>Genotypes of controls</th>
<th>Quality (stars)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Al-Qahtani</td>
<td>2012</td>
<td>Caucasian</td>
<td>484</td>
<td>473</td>
<td>Healthy</td>
<td>G/A: 710/258</td>
<td>G/A: 299/193</td>
<td>5</td>
</tr>
<tr>
<td>DuanZP</td>
<td>2005</td>
<td>Asian</td>
<td>152</td>
<td>139</td>
<td>Healthy</td>
<td>G/A: 198/106</td>
<td>G/A: 64/18</td>
<td>5</td>
</tr>
<tr>
<td>Hsiu-Ting Tsai</td>
<td>2012</td>
<td>Asian</td>
<td>102</td>
<td>347</td>
<td>Healthy</td>
<td>G/A: 126/78</td>
<td>G/A: 42/18</td>
<td>6</td>
</tr>
</tbody>
</table>

SR: spontaneously recovered; NR: not reported. Quality: quality assessment according to the Newcastle-Ottawa Quality Assessment Scale.

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Figure 2: Forest plot of the risk of chronic HBV infection related to A allele versus G allele about RANTES-403A/G polymorphism in all studies. Black square means value of OR, and the size of the square means inversely proportional to its variance. Horizontal line means 95% confidence interval (CI) of OR. Black diamond means pooled results.
is no evidence of obvious asymmetry of the funnel plots, indicating no significant publication bias existed in this meta-analysis (Egger’s test $p = 0.679$).

**DISCUSSION**

This is the first meta-analysis to investigate the relationship between the *RANTES*-403A/G polymorphism and risk for HBV infection. Eight studies were collected following stringent inclusion criteria in order to validate the association between *RANTES*-403A/G polymorphisms and persistent HBV infection. We demonstrated the importance of the *RANTES*-403A/G polymorphism in the HBV infection.

In the process of HBV infection, the immune system have a direct impact on the disease manifestation and outcome, which may induce hepatocellular damage [21]. The majority of adults with HBV infection may recover due to efficient immune response. The differences of immune mediators at the genetic level can influence the
host’s immune ability to counter HBV infection [22–25]. Recently, chemokines and their receptors that were proved to play an important biological role in immune responses should arise more attention [26, 27].

Previous studies have suggested RANTES as one of the candidate genes in the development of persistent HBV infection. The RANTES-CCR5 pathway have shed some light on viral clearance mechanisms, such as with hepatitis C virus [28, 29] and West Nile virus [30]. Studies have demonstrated that it plays an important role in sustaining CD8 T cell responses during the chronic infection [31, 32]. The mechanism of virus clearance is largely unknown and
needs to be solved. To date, there were a series of RANTES polymorphisms been reported, including -403A/G, -28C/G, intronic variant INT1.1T/C and 524T/C [11–14, 17–20]. They were involved in viral and non-viral diseases, such as AIDS, tuberculosis, type-1 diabetes, coronary artery disease, systemic lupus [33–37]. Different RANTES polymorphisms have different effects on the risk of HBV infection. The conclusions were inconsistent throughout different populations and studies [11–14, 17–20]. Relevant meta-analyses reported that the RANTES -28G allele may be beneficial to the resistance to HIV-1 infection among Asians [38, 39].

Eight studies were included in this meta-analysis based on standardized search strategy, which demonstrated that RANTES -403A/G polymorphism has significant association with the susceptibility to persistent HBV infection among Caucasians in allelic and genotypic models. Subgroup analysis by control types showed that RANTES -403A allele might be the risk factor of HBV infection when compared to healthy controls but not to the SR controls. When combined the cases and SRs and compared them with the healthy controls we found there was also a significant difference in allelic model. Subgroup analysis by ethnicity revealed a significant association between the RANTES gene -403 A/G polymorphism and HBV infection in the Caucasian population but not in the Asian population. The controversial results might come from the variations of ethnicity, sample size, and haplotypes. When stratified by different control groups, the allele was a risk factor compared to the healthy group.

### Table 2: Subgroup analyses by ethnicity about association between RANTES gene -403 A/G polymorphism and susceptibility to chronic HBV infection

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Ethnicity</th>
<th>Cases</th>
<th>Controls</th>
<th>OR (95%CI)</th>
<th>P</th>
<th>Heterogeneity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>df (P-value)</td>
</tr>
<tr>
<td>A / G</td>
<td>Caucasian</td>
<td>304/1001</td>
<td>383/1267</td>
<td>1.68 (1.38,2.04)</td>
<td>&lt;0.001</td>
<td>1 (0.96)</td>
</tr>
<tr>
<td></td>
<td>Asian</td>
<td>1661/2507</td>
<td>1465/2463</td>
<td>1.05 (0.96,1.16)</td>
<td>0.27</td>
<td>5 (0.002)</td>
</tr>
<tr>
<td>AG/ GG</td>
<td>Caucasian</td>
<td>230/240</td>
<td>155/299</td>
<td>1.85 (1.42,2.41)</td>
<td>&lt;0.001</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Asian</td>
<td>666/543</td>
<td>711/622</td>
<td>0.96 (0.81,1.13)</td>
<td>0.59</td>
<td>4 (0.24)</td>
</tr>
<tr>
<td>AA / GG</td>
<td>Caucasian</td>
<td>14/240</td>
<td>19/299</td>
<td>0.92 (0.45,1.87)</td>
<td>0.81</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Asian</td>
<td>230/543</td>
<td>204/622</td>
<td>1.16 (0.92,1.46)</td>
<td>0.21</td>
<td>4 (0.0003)</td>
</tr>
<tr>
<td>AG+AA/ GG</td>
<td>Caucasian</td>
<td>244/240</td>
<td>174/299</td>
<td>1.75 (1.35,2.26)</td>
<td>&lt;0.001</td>
<td>81</td>
</tr>
<tr>
<td></td>
<td>Asian</td>
<td>896/543</td>
<td>915/622</td>
<td>1.01 (0.87,1.18)</td>
<td>0.87</td>
<td>4 (0.02)</td>
</tr>
</tbody>
</table>

### Table 3: Subgroup analyses by controls types about association between RANTES gene -40 A/G polymorphism and susceptibility to chronic HBV infection

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Control types</th>
<th>Cases</th>
<th>Controls</th>
<th>OR (95%CI)</th>
<th>P</th>
<th>Heterogeneity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>df (P-value)</td>
</tr>
<tr>
<td>A/G</td>
<td>SR</td>
<td>1523/2474</td>
<td>1142/1924</td>
<td>0.93 (0.84,1.03)</td>
<td>0.15</td>
<td>4 (0.0003)</td>
</tr>
<tr>
<td></td>
<td>HC</td>
<td>920/1712</td>
<td>706/1806</td>
<td>1.22 (1.08,1.39)</td>
<td>0.002</td>
<td>4 (0.0004)</td>
</tr>
<tr>
<td></td>
<td>Overall</td>
<td>1965/3508</td>
<td>1848/3730</td>
<td>1.04 (0.96,1.13)</td>
<td>0.34</td>
<td>7 (&lt;0.001)</td>
</tr>
<tr>
<td>AG/GG</td>
<td>SR</td>
<td>554/437</td>
<td>370/266</td>
<td>0.91 (0.74,1.11)</td>
<td>0.36</td>
<td>2 (0.96)</td>
</tr>
<tr>
<td></td>
<td>HC</td>
<td>600/556</td>
<td>496/655</td>
<td>1.29 (1.09,1.54)</td>
<td>0.004</td>
<td>4 (0.001)</td>
</tr>
<tr>
<td></td>
<td>Overall</td>
<td>896/783</td>
<td>866/921</td>
<td>1.15 (1.00,1.32)</td>
<td>0.05</td>
<td>5 (0.0004)</td>
</tr>
<tr>
<td>AA/GG</td>
<td>SR</td>
<td>194/437</td>
<td>118/266</td>
<td>1.02 (0.77,1.34)</td>
<td>0.89</td>
<td>2 (0.87)</td>
</tr>
<tr>
<td></td>
<td>HC</td>
<td>160/556</td>
<td>105/655</td>
<td>1.24 (0.92,1.66)</td>
<td>0.16</td>
<td>4 (0.0004)</td>
</tr>
<tr>
<td></td>
<td>Overall</td>
<td>244/783</td>
<td>223/921</td>
<td>1.13 (0.91,1.41)</td>
<td>0.26</td>
<td>5 (0.0007)</td>
</tr>
<tr>
<td>AG+AA/GG</td>
<td>SR</td>
<td>748/437</td>
<td>488/266</td>
<td>0.94 (0.77,1.13)</td>
<td>0.5</td>
<td>2 (0.94)</td>
</tr>
<tr>
<td></td>
<td>HC</td>
<td>760/556</td>
<td>601/655</td>
<td>1.32 (1.12,1.56)</td>
<td>0.001</td>
<td>4 (0.0009)</td>
</tr>
<tr>
<td></td>
<td>Overall</td>
<td>1089/921</td>
<td>1140/783</td>
<td>0.85 (0.75,0.97)</td>
<td>0.02</td>
<td>5 (0.0002)</td>
</tr>
</tbody>
</table>

SR: spontaneously recovered; HC: healthy; OR: odds ratio; CI: confidence interval; df: degrees of freedom.
instead of the SR control group. We inferred that RANTES -403A/G gene polymorphism may be associated to HBV infection, instead of virus clearance. It was demonstrated that RANTES -403A was associated with the susceptibility to HBV infection, but in the case of clearance progression, other factors need to be explored.

Although meta-analysis is a predominant statistical method, there are still some limitations. Firstly, there was a significant heterogeneity in our study. Secondly, only eight studies were collected and as few as six had specific genotypic data. Insufficient sample size may cause some deviation in data analysis. Therefore, additional studies are needed for further investigation. Thirdly, some unpublished negative results, have not been included. Lastly, we only analyzed the association between -403A/G and HBV infection without taking account of relevant environmental factors, such as alcohol consumption and smoking. Gene-environment interaction may be an important risk factor for the HBV infection, therefore to analyze the interaction may be essential. In the present study, we were unable to perform such analysis due to insufficient data regarding environmental factors in all included studies.

In conclusion, this meta-analysis demonstrated that there was no significant difference between RANTES -403A/G gene polymorphism and the outcome of persistent HBV infection. But according to the subgroup analyses, the A allele plays a risk role in HBV infected patients in the Caucasian population. Further studies are needed to verify this association.

**MATERIALS AND METHODS**

**Search strategy**

Literature search was performed by two independent researchers in PubMed/MEDLINE (last updated on 1 January 2017), CNKI (last updated on 1 January 2017) using the following keywords: (‘CCL5’ or ‘chemokine ligand 5’ or ‘RANTES’ or ‘regulated upon activation normal T cell expressed and secreted’) and (‘HBV’ or ‘hepatitis B’ or ‘liver disease’ or ‘chronic hepatitis B virus’) and (‘polymorphism’ or ‘gene polymorphisms’ or ‘gene mutation’ or ‘gene variants’). No language restrictions were used in the search. Consensuses on controversial issues were reached through consultation between two researchers, or with our supervisor (Zheng Zeng).

**Criteria of inclusion and exclusion**

The inclusion criteria were as follows: (a) evaluating the relationship between RANTES -403A/G gene polymorphisms and HBV infection; (b) case-control studies; (c) containing abundant genotype data to calculate the odds ratios (ORs) with 95% confidence intervals (CIs); (d) full-text; (e) human studies; (f) using the following diagnostic criteria: patients with the serum hepatitis B surface antigen (HBsAg) positive for ≥6 months were diagnosed as persistent HBV infection; individuals with HBsAg(–), anti-HBc(–) and anti-HBs(–) were defined as...

**Figure 4: Funnel plot association between RANTES -403A/G polymorphism and chronic HBV infection.** Log OR means nature logarithm of OR (odds ratio). Horizontal line means the summary estimate, while the sloping lines mean the expected 95% confidence interval. Egger’s test p = 0.679.
health controls (HC); individuals with HBsAg(−), anti-
HBc(+), and anti-HBs(+) were defined spontaneously
recovered (SR) controls. The criteria of excluding studies
were: (a) the data of the studies were overlapped; (b) no
full-text; (c) not a case-control study; (d) not in line with
the diagnostic criteria.

In the meantime, we applied Newcastle-Ottawa Quality Assessment Scale (NOS) to assess the quality
of all the included case-control studies. Studies with low
quality (<5 stars) were excluded.

Data extraction

The following characteristics were selected from
each study: first author’s name, year of publication,
country and ethnicity of the study population, clinical
subtype, numbers of the cases and controls, genotyping
methods, sex of the cases and controls, mean age of the
cases and controls and the HWE test results. The control
groups included healthy individuals (HC) and spontaneous
recovered individuals (SR). The case group included
all clinical subtypes, such as asymptomatic patients,
patients with chronic hepatitis B, liver cirrhosis (LC), or
hepatocellular carcinoma (HCC).

Statistical analysis

The odds ratios (ORs) and the 95% confidence intervals (CIs) were calculated to assess the relationship
between RANTES -403 gene polymorphism and chronic
hepatitis B according to gene allele and genotype: the
allelic model (A allele versus G allele), the homozygous
model (AA versus GG), the heterozygous model (AA
versus GA), the dominant model (GA+AA versus GG).
Subgroup analyses were also performed based on clinical
control group types and ethnicities. Heterogeneity was
estimated by Cochrane’s Q-test and I² measurement,
which means the proportion of variability across
studies is due to heterogeneity rather than by chance.
I² > 50% indicated a significant heterogeneity [15] and
the random-effects model would be applied. Otherwise,
the fixed-effects model would be applied. The quality
and reliability of this meta-analysis were evaluated by
sensitivity analysis through excluding one study each
time. The publication bias was assessed by the Egger’s
test and funnel plots [16]. All Quantitative analysis was
performed using Stata 11.0 software (StataCorp LP,
College Station, TX, USA).

Abbreviations

CCR5: CC receptor 5; CI: confidence interval; DC:
dendritic cell; HC: healthy control; HCC: hepatocellular
carcinoma; HBsAg: hepatitis B surface antigen; HBV:
hepatitis B virus; HWE: Hardy-Weinberg equilibrium; LC:
liver cirrhosis; OR: Odds ratio; SR: spontaneous recovered.

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CONFLICTS OF INTEREST

The authors have no conflicts of interest relevant to
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