Relationship between TNF-<i>&alpha;</i> Gene Promoter Polymorphisms and Outcomes of Hepatitis B Virus Infections: A Meta-Analysis

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Abstract

Background: The clearance of hepatitis B virus (HBV) is a complex process which may be influenced by many factors including polymorphisms in the tumor necrosis factor <i>&alpha;</i> (TNF-<i>&alpha;</i>) gene promoter. However, previous reports regarding the relationship between polymorphisms in the TNF-<i>&alpha;</i> promoter and HBV clearance have been inconsistent. Therefore, we performed a meta-analysis on a large population to address this inconsistency.

Methods: A meta-analysis was performed to examine the association between TNF-<i>&alpha;</i> promoter polymorphisms (-1031T/C, -863C/A, -857C/T, -308G/A and -238G/A) and chronic hepatitis B infection. Odds ratio (OR) and its 95% confidence interval (CI) were used.

Results: Twelve studies were chosen in our meta-analysis, involving 2,754 chronic HBV infection cases and 1,630 HBV clearance cases. The data showed that TNF-<i>&alpha;</i>-863 CC genotype was significantly associated with HBV clearance (-863 CC vs. AA: OR, 0.64; 95% CI, [0.42, 0.97]; p = 0.04) while patients carrying -308 GG genotype had a significantly increased risk of HBV persistence compared with those with GA or AA genotype (GG vs. GA+AA: OR, 1.35; 95% CI, [1.08, 1.70]; p = 0.01). For the other polymorphisms, no association with HBV infection outcome was found.

Conclusions: The data showed that polymorphisms -863 A and -308 G in the TNF-<i>&alpha;</i> gene promoter region might be risk factors for HBV persistence. Furthermore, ethnicity might play an important role in HBV infection outcome, leading to conflicting results. More studies on individuals from various ethnic groups will be necessary to determine the role of TNF-<i>&alpha;</i> promoter polymorphisms in the outcome of HBV infection.

Introduction

Approximately 5–10% of patients infected with hepatitis B virus (HBV) as adults are unable to clear the virus, ultimately developing chronic HBV infections[1]. The persistence of the virus is thought to be largely caused by a deficiency of the immune response to HBV[1]. The virus itself, environment factors, ethnic differences, and genetic susceptibility have also been reported to have some influence on the progression of this liver disease [2]. Recently, cytokine genetic polymorphisms have been found to be related factors that affect the progression of HBV infection [1].

Tumor necrosis factor <i>&alpha;</i>(TNF-<i>&alpha;</i>) is produced by macrophages, monocytes, neutrophils, T-cells and NK-cells after stimulation. In turn, TNF-<i>&alpha;</i> can stimulate cytokine secretion, increase the expression of adhesion molecules as well as activate neutrophils. Hence, it fulfills the role as a principal mediator of cellular immune response and inflammation, and may play an importance role in non-cytolytic and cytolytic clearance of HBV [3,4,5].

The TNF-<i>&alpha;</i> gene is located in the class III region of the major histocompatibility complex (MHC) on chromosome 6. The amount of cytokine production seems to be affected by the polymorphisms in the regulatory region. Therefore, there might be relationships between these single nucleotide polymorphisms (SNPs) and cytokine-mediated inflammation, which may affect the outcome of the disease.

There have been a number of studies on the association between chronic HBV infection (CHB), HBV clearance (HC), and TNF-<i>&alpha;</i> promoter polymorphisms -1031T/C, -863 C/A, -857C/T, -308G/A, and-238G/A. However, the results have been inconsistent. For instance, some studies indicated that patients carrying TNF-<i>&alpha;</i> genotypes (AA for -863, CC for -857, AA for -308, and AA for -283) have a higher risk of susceptibility to persistence of HBV [6,7,8,9] while other studies did not[10,11,12].

A single study may fail to completely demonstrate this complicated genetic relationship because of a small sample size.
which has low statistical power. Larger studies could overcome these disadvantages. Therefore, we performed a meta-analysis in an attempt to resolve this issue.

**Methods**

**Search strategy**

We searched the PubMed, EMBASE, ISI Web of Science, Google Scholar, Chinese National Knowledge Infrastructure Database and China Biological Medicine Database to collect all papers associated with TNF-<alpha> polymorphism and HBV (last search update: 31st July 2010). The following key words were used: “hepatitis B”, “HBV”, “Tumor necrosis factor <alpha>”, “TNF-<alpha>”, “polymorphism” and “SNP”. We also combined these key words to maximize the sample size in our analysis. TNF-<alpha> promoter polymorphisms -1031T/C, -863 C/A, -857C/T, -308G/A and -238G/A were investigated. The electronic searching was supplemented by checking reference lists from identified articles and reviews for additional original reports. The language of the reviewed articles was limited to Chinese and English. Data were extracted by two authors independently and a consensus was achieved for all data. We excluded studies that were not full-length publications, and those that included no more than 10 participants. When study recruitment overlapped by more than 30% in two or more articles by the same author(s), the one with the largest population of participants or the most recent one was selected. We used Chi-square test to evaluate whether the observed frequencies of genotypes conformed to Hardy-Weinberg Equilibrium (HWE). CHB was defined as a condition in which serum HBsAg was positive for at least 6 months. HBV clearance (HC) was defined as a condition in which HBsAg was negative, but both HBV core antibody (anti-HBc) and HBV surface antibody (anti-HBs) were positive. None of the patients included in our study had any other type of liver disease such as hepatitis C or alcoholic liver disease.

**Data extraction**

The following information was extracted by two authors independently, and a consensus was achieved: first author’s name, year of publication, country, mean age of the study subjects, gender, genotype, genotyping method, cases of CHB and HC with various genotypes, polymorphisms of TNF-<alpha> promoter.

**Statistical analysis**

Odds ratios (ORs) with their confidence intervals (CIs) were calculated for each study. Heterogeneity was tested by chi-square-based Q test and I² = 100%×(Q-df)/Q [13,14]. The fixed effects model (Mantel-Haenszel method) [15] was used for calculating Egger’s linear regression test. A P value <0.10 was considered to indicate statistically significant publication bias.

**Results**

**Extraction process and characteristics of the studies**

One hundred and six studies were identified after searching the databases. Seventy-six studies that did not focused on chronic HBV (e.g., Severe Hepatitis B infection, Hepatitis C or D infection, liver fibrosis, hepatocellular carcinoma, intrauterine infection, etc.) were excluded after title review. Twelve studies were excluded after abstract review, three of them were not focusing on chronic HBV and eight lacked HC cases and one was a review article. After full text review, six studies were excluded, two of them were not focused on chronic HBV and two were lacking HC cases and the other two were previously written by the same authors (Cheong, J.Y. et al [16] and Lü, H. Q. et al [17]) of the rest studies, we selected the latest ones [10,18]. The extraction process was showed in Fig. 1.

Finally a total of 12 relevant studies were selected [6,8,9,10, 11,12,18,19,20,21,22,23] involving 2754 chronic HBV infection cases and 1630 HBV clearance cases. All studies included that the distribution of genotypes in the controls or recovered was consistent with Hardy-Weinberg equilibrium, except for two studies for -857C/T [Chen, D.Q et al [6] and -308G/A; Xing, P.X. et al [24]] .The included studies had been conducted on Chinese, Korean, Thai, Italian, Iranian, Brazilian and German participants. A summary of characteristics of these 12 included studies was showed in Table 1.

**Meta-analysis**

Table 2 lists the results of the meta-analysis and heterogeneity test. Because the study by Kummee et al [20] contained a large number of individuals with HCC, subjects with or without HCC were included in our studies.

For TNF-<alpha>-1031T/C and -857C/T, we found no association between the polymorphisms and HBV clearance. For TNF-<alpha>-863, overall, we found that compared to -863 AA, TNF-<alpha>-863 CC genotype was associated with chronic HBV clearance (-863 CC vs. AA: OR, 0.64; 95% CI, [0.42, 0.97]; p = 0.04) (Fig 2). When we excluded the subjects with HCC, the significance seemed to be weaken (OR, 0.66; 95% CI, [0.43, 1.00]; p = 0.05), implying that -863 CC might play a relatively different role in HCC patients. In a subgroup analysis of TNF-<alpha>-863 CC vs. AA by ethnicity, the pooled OR was significant in Asians (OR, 0.64; 95% CI, [0.11, 0.99]; p = 0.04). In addition, the result of AA vs. GA+CC model showed that -863 AA maybe a risk factor for HBV persistence (overall: OR, 1.60; 95% CI, [1.06, 2.41]; p = 0.02); Asian: OR, 1.60; 95% CI, [1.05, 2.45]; p = 0.02) which implied the counteractive function between -863 CC and -863 AA in virus clearance. A European subgroup analysis was not conducted because only one study [8] was available.

When we performed analysis on TNF-<alpha>-308 G/A we found we two models had statistic significance (G allele vs. A allele: OR, 1.30; 95% CI, [0.104, 1.63]; p = 0.02; G vs. GA+AA: OR, 1.35; 95% CI, [1.08, 1.70]; p = 0.01) (Fig 3) indicating that -308 G might be an unfavorable factor for the elimination of HBV. In subgroup analysis, the result of Asian group was similar (G allele vs. A allele:OR, 1.41; 95% CI, [1.08, 1.83]; p = 0.01; G vs. GA+AA: OR, 1.47; 95% CI, [1.13, 1.91]; p = 0.004) while the European group lacked such an association. We also performed an analysis for East Asian individuals by removing a study conducting on Iraqi (Somi, M. H [12]) from the rest studies, we selected a model Polymorphisms and HBV Infection Outcome
I² = 79%) (Table 2). A random-effects model was employed in these studies.

**Publication bias**

Begg’s funnel plot and Egger’s test were performed to access the publication bias of the studies. No evidence of publication bias showed in -308 G allele vs. A allele or -308 GG vs. AA model (funnel plot data not show, Egger’s test p = 0.470 and 0.556, respectively). However, funnel plot showed some asymmetry in -863 CC vs. AA and -863 AA vs. CA+CC models (Fig 4) and Egger’s proved the existence of publication in these two models (p = 0.041 and 0.038, respectively). We excluded one study from the meta-analysis to see whether the publication bias still presented. The results showed that after the exclusion of study Chen, D. Q. et al [6], the publication bias was eliminated (funnel plot showed in Fig 4, Egger’s test -863 CC vs. AA; p = 0.785; -863 AA vs. CA+CC: p = 0.541), and the conclusion still stable (-863 CC vs. AA: OR, 0.42; 95% CI, [0.23, 0.77]; p = 0.005; -863 AA vs. CA+CC: OR, 2.44; 95% CI, [1.34, 4.45]; p = 0.003).

**Discussion**

The mechanism of effective clearance of HBV from the human body is likely related to both environmental and host genetic factors. Several studies have reported that TNF-<alpha> plays an important role in HBV clearance. In an *in vitro* study, TNF-<alpha> was reported to be able to accelerate HBV mRNA destruction, and inhibit the replication of HBV[25]. An *in vivo* study also found that TNF limited chronic infection by destabilizing HBV nucleocapsids and reducing the cccDNA[26]. Furthermore, a clinical study showed that elevation of TNF-<alpha> levels in IFN-<alpha>-treated patients led to HBV elimination[27].

In the current study, we performed a meta-analysis to examine the association between the SNPs in the promoter region of TNF-<alpha> and the outcome of HBV infection. According to our findings, the presence of G at the position -308 of TNF-<alpha> promoter gene polymorphisms increased the risk of HBV persistent infection significantly while -308 A may have a positive effect in virus clearance. Similar conclusion was drew by Zheng, M. H. et al [28] who also performed a meta-analysis focus on healthy individual (including spontaneously recovered case) and Chronic hepatitis B patient, they found that -308 A allele was a protective factor for CHB infection, especially in Mongoloid populations. Our analysis was restricted in CHB and HC case, thus our case size was smaller, but higher specificity would provide us more reliable evidence to our conclusion. Taken together, -308 A may play a crucial role in anti-virus mechanism in human body; it can not only protect healthy people from HBV infection but also enhance the scavenging of virus while being infected. This virus clearing function of TNF-<alpha>-308A allele was associated with the enhancement of TNF-<alpha> transcriptional activation as well as production [29,30].

Our study showed that -863 CC and -863 AA might be another important factors in HBV clearance. Previous study reported that the -863 C/A performed their protective or deteriorative function for CHB infection through a different way that -863 A allele can lower TNF-<alpha> promoter gene transcription and plasma levels by weakening the affinity between specific protein and the segment of TNF-<alpha> promoter spanning from position -876 to position -845 [31].

Although the association between TNF-<alpha> and HCC patient has not been clearly understood, our studies gave some indirect evidence on a different response to the TNF-<alpha> in CHB and HCC individuals, for the discard of HCC cases from the analysis could affect the results, especially in -863 CC vs. AA.
| Studies (Year/Countries) | Age (mean±SD) | Case of CHB (Males/%) | Case of HC (Males/%) | Methods                          | Studied Polymorphisms | Findings                                                                                                                                 |
|-------------------------|---------------|-----------------------|---------------------|----------------------------------|-----------------------|------------------------------------------------------------------------------------------------------------------------------------------|
| Hohler, T.[9] (1998/ Germany) | NA            | NA                    | 71(NA)              | 32(NA)                           | PCR                   | −238, −308 The TNF-<alpha>−238 GG statistically significant differences between the CHB and recovered individuals                          |
| Kim, Y. J.[7] (2003/Korea)  | Male 49.9±10.4; Female 50.8±10.6 | Male 54.7±10.9; Female 53.6±11.0 | 1109(74.4)          | 291(65.5)                       | Single base primer extension assay | −163, −238, −308, −857, −863, 1311 The TNF-<alpha>−308 AA or GA or −863 CC were strongly associated with the resolution of HBV infection                                    |
| Zhang, P.A [23] (2004/China) | 54.7±14.8      | 56.4±14.0             | 131(74.4)           | 291(65.5)                       | Single base primer extension assay | −238, −308, −857 −863 The TNF-<alpha>−863 A allele may have a favorable effect in the clearance of HBV.                                      |
| Li, H. Q.[17] (2006/China)  | 33.37±12.67    | 34.68±11.34           | 433(81.8)           | 291(55.8)                       | PCR                   | −238, −857 The frequency of -238GG genotype in self-limited group was significantly higher than chronic HBV group.                           |
| Nino, G. A.[8] (2005/Italy) | 52±12          | 46.3±7.4              | 184(81.5)           | 96(79.2)                        | DNA sequencing        | −238, −308, −863 The TNF-<alpha>−308GG was associated with an unfavorable prognosis in patients with chronic HBV infection.                     |
| Cheong, J. Y.[10] (2006/South Korea) | 37.74±8.90     | 47.96±8.77            | 261(75.1)           | 204(72.5)                       | Single base primer extension assay | −238, −308 The TNF-<alpha>−308 and −238 were not different between the clearance and the persistence group                                    |
| Somi, M. H.[12] (2006/Iran) | NA             | NA                    | 100(NA)             | 91(NA)                          | PCR                   | −308 The TNF-<alpha>−308 A had no association with development of chronic HBV infection.                                                |
| Kummee, P.[20] (2007/Thailand) With HCC: 50.8±13.9 | 51.0±12.3      | With HCC: 100(68.0)   | 100(48.0)           | PCR-RFLP                       | −238, −308, −863     | The TNF-<alpha>−863 A/A or A/C genotype were associated with increased the TNF-<alpha>−863 CC in chronic HBV group and induced hepatocyte damage. |
| Without HCC: 57.5±14.2   | NA             | NA                    | 252(60.0)           | 109(55.0)                       | PCR-RFLP             | −308 The TNF-<alpha>−308 G/A had no association between chronic patients and self-limited infection                                     |
| Ribeiro, C. S.[11] (2007/Brazil) | 37±11.65       | 39.5±10.2             | 30(60.0)            | 41(48.8)                        | PCR                   | −308 The TNF-<alpha>−308 G/A had no association between chronic patients and self-limited infection                                     |
| Xing, P.[22] (2007/China)  | 39.65±19.3     | 38.5±18.2             | 111(85.6)           | 100(66.0)                       | Gene Chips            | −238, −308 The TNF-<alpha>−308 GG and G allele were higher in chronic HBV infection group than control group, but no association between TNF-<alpha>−238 and HBV infection was found. |
| Chen, D. Q.[6] (2010/China) | 40.9±10.9      | NA                    | 252(60.7)           | 109(55.0)                       | PCR-RFLP             | −238, −308, −857 −863 The TNF-<alpha>−308 G/G genotype was more frequent in patients than controls, while the frequencies of TNF-<alpha>−308 A/G genotype was higher in controls than in the patient group                         |
| Wan, P.[21] (2010/China)  | 54.7±14.8      | 56.4±14.0             | 74(59.5)            | 64(62.5)                        | PCR-RFLP             | −857, −863 In Guangxi population, the TNF-<alpha>−863 AA might go against the clearance of HBV.                                      |

Abbreviations: CHB, chronic HBV infection; HC, HBV clearance; HCC, hepatocellular carcinoma; PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism; NA, Not Applicable.

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investigations are demanded to interpret the relationship among model, thus, caution should be paid in this result and more TNF-\(\alpha\) promoter polymorphisms on the risk of HBV persistence.

Table 2. Meta-analysis of effect of TNF-\(\alpha\) promoter polymorphisms on the risk of HBV persistence.

| Genotype comparisons | NO. of CHB | NO. of HC | OR [95% CI] | Z (p value) | Heterogeneity of study design |
|----------------------|------------|-----------|-------------|-------------|-----------------------------|
| 1031 T allele vs. C allele | Overall 1617 | 495 | 0.86[0.68,1.08] | 1.27(0.21) | 0.01 1(0.90) 0% |
| TT vs. CC | Overall 827 | 263 | 0.54[0.29,1.02] | 1.89(0.06) | 0.21 1(0.65) 0% |
| TT vs. TC +CC | Overall 1222 | 379 | 0.82[0.65,1.05] | 1.55(0.12) | 0.06 1(0.81) 0% |
| CC vs. TC +TT | Overall 1222 | 379 | 1.77[0.94,3.31] | 1.78(0.08) | 0.26 1(0.61) 0% |
| 863 C allele vs. A allele | Overall 2450 | 1387 | 0.91[0.78,1.07] | 1.11(0.27) | 8.40 5(0.14) 40% |
| Asian | Overall 2208 | 1262 | 0.91[0.77,1.08] | 1.06(0.29) | 8.40 4(0.08) 52% |
| CC vs. AA | Overall 1312 | 743 | 0.64[0.42,0.97] | 2.09(0.04) | 5.11 5(0.40) 2% |
| Asian | Overall 1107 | 676 | 0.64[0.42,0.99] | 2.01(0.04) | 5.10 4(0.28) 22% |
| CC vs. CA+AA | Overall 1881 | 1065 | 0.95[0.68,1.33] | 0.29(0.77) | 17.06 5(0.004) 71% |
| Asian | Overall 1697 | 969 | 0.97[0.65,1.44] | 0.16(0.87) | 17.06 4(0.002) 77% |
| AA vs. CA+CC | Overall 1868 | 1065 | 1.60[1.06,2.41] | 2.25(0.02) | 6.21 5(0.29) 19% |
| Asian | Overall 1682 | 969 | 1.60[1.05,2.45] | 2.18(0.03) | 6.21 4(0.18) 36% |
| −857 C allele vs. T allele | Overall 1993 | 851 | 1.04[0.85,1.28] | 0.38(0.70) | 5.55 2(0.06) 64% |
| CC vs. TT | Asian 1215 | 527 | 0.90[0.51,1.59] | 0.35(0.73) | 2.20 2(0.33) 9% |
| CC vs. CT +TT | Asian 1604 | 689 | 1.13[0.69,1.85] | 0.47(0.64) | 9.47 2(0.009) 79% |
| TT vs. CT+CC | Asian 1604 | 689 | 1.15[0.65,2.01] | 0.47(0.64) | 2.08 2(0.35) 4% |
| −308 G allele vs. A allele | Overall 2175 | 1171 | 1.30[1.04,1.63] | 2.30(0.02) | 7.50 7(0.38) 7% |
| Asian | Overall 1832 | 964 | 1.41[1.08,1.83] | 2.56(0.01) | 4.89 4(0.30) 18% |
| European | Overall 304 | 159 | 1.15[0.70,1.99] | 0.56(0.57) | 0.58 1(0.45) 0% |
| GG vs. AA | Overall 2012 | 1178 | 0.62[0.23,1.67] | 0.94(0.35) | 0.98 6(0.98) 0% |
| Asian | Overall 1785 | 1047 | 0.67[0.19,3.23] | 0.64(0.52) | 0.44 3(0.93) 0% |
| European | Overall 206 | 97 | 0.35[0.04,2.95] | 0.97(0.33) | 0.02 1(0.89) 0% |
| GG vs. GA+AA | Overall 1967 | 1012 | 1.35[1.08,1.70] | 2.59(0.01) | 9.20 7(0.24) 24% |
| Asian | Overall 1682 | 843 | 1.47[1.13,1.91] | 2.85(0.004) | 5.58 4(0.23) 28% |
| European | Overall 384 | 258 | 1.31[0.90,1.91] | 1.41(0.16) | 0.54 2(0.76) 0% |
| AA vs. GA +GG | Overall 2271 | 1373 | 1.57[0.94,2.61] | 0.90(0.37) | 1.33 6(0.97)* 0% |
| Asian | Overall 1836 | 1113 | 1.65[0.40,6.77] | 0.69(0.49) | 0.24 2(0.89) 0% |
| European | Overall 384 | 258 | 1.49[0.47,4.71] | 0.68(0.50) | 0.26 2(0.88) 0% |
| −238 G allele vs. A allele | Overall 2715 | 1588 | 0.92[0.72,1.18] | 0.65(0.52) | 12.25 8(0.14) 35% |
| Asian | Overall 2421 | 1451 | 1.01[0.77,1.33] | 0.10(0.92) | 8.36 6(0.21) 28% |
| European | Overall 294 | 137 | 0.50[0.77,1.33] | 1.87(0.06) | 1.22 10(0.27) 18% |
| GG vs. AA | Overall 2281 | 1384 | 0.88[0.32,2.45] | 0.52(0.70) | 0.52 4(0.97)* 0% |
| Asian | Overall 2065 | 1265 | 0.83[0.25,2.70] | 0.23(0.75) | 0.23 2(0.89) 0% |
| European | Overall 216 | 119 | 1.07[0.14,8.28] | 0.24(0.95) | 0.24 1(0.62) 0% |
| GG vs. GA+AA | Overall 2498 | 1486 | 0.90[0.70,1.16] | 0.42(0.42) | 14.68 8(0.07) 46% |
| Asian | Overall 2243 | 1358 | 1.01[0.77,1.32] | 0.07(0.95) | 9.52 6(0.15) 37% |
| European | Overall 255 | 128 | 0.45[0.22,0.93] | 2.15(0.03) | 1.77 10(0.18) 44% |
| AA vs. GA +GG | Overall 2498 | 1486 | 1.08[0.39,2.98] | 0.14(0.89) | 0.66 4(0.96) 0% |
| Asian | Overall 2243 | 1358 | 1.18[0.36,3.86] | 0.27(0.79) | 0.20 2(0.90) 0% |
| European | Overall 255 | 128 | 0.81[0.11,5.16] | 0.20(0.84) | 0.34 1(0.56) 0% |

*−308 A/A and −238 A/A were rare genotypes. Therefore, some studies had to be excluded because they contained no individuals carrying these genotypes. In the study on −308 AA vs. GA +GG, the report by Kumme et al [20] was excluded; in −238 GG vs. AA and −238 AA vs. GA +GG, Cheong et al [10] and Li et al [17] were excluded.

Abbreviations: CHB, chronic HBV infection; HC, HBV clearance; OR, odds ratio; CI, confidence interval; df, degree of freedom.

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Figure 2. OR of HBV infection associated with $\text{TNF-\alpha} \rightarrow -863$ C/A for the CC genotype compared with the AA genotypes and AA compared to the CA+AA genotype.
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| Study or Subgroup | Weight | M-H, Fixed, 95% CI | Odds Ratio | M-H, Fixed, 95% CI |
|-------------------|--------|-------------------|------------|-------------------|
| Chen, D. Q.       | 32.6%  | 1.09 [0.59, 2.03] |            |                   |
| Kim, Y. J.        | 28.2%  | 0.44 [0.17, 1.14] |            |                   |
| Kummee, P.        | 10.2%  | 0.35 [0.07, 1.72] |            |                   |
| Niro, G. A.       | 6.5%   | 0.82 [0.12, 3.14] |            |                   |
| Wan, P. Q.        | 11.6%  | 0.29 [0.06, 1.43] |            |                   |
| Zhang, P. A.      | 10.1%  | 0.47 [0.11, 1.93] |            |                   |
| Total (95% CI)    | 100.0% | 0.64 [0.42, 0.97] |            |                   |
| Total events      |        |                   |            |                   |

Heterogeneity: Chi$^2$ = 5.11, df = 5 ($P = 0.40$); I$^2$ = 22%
Test for overall effect: Z = 2.09 ($P = 0.04$)

Figure 3. OR of HBV infection associated with $\text{TNF-\alpha} \rightarrow -308$ G/A for the G allele compared with the A allele and the GG genotype compared with the GA+AA genotypes.
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| Study or Subgroup | Weight | M-H, Fixed, 95% CI | Odds Ratio | M-H, Fixed, 95% CI |
|-------------------|--------|-------------------|------------|-------------------|
| Cheong, J. Y.     | 20.3%  | 1.09 [0.64, 1.66] |            |                   |
| Hohler, T.        | 8.9%   | 0.99 [0.38, 2.07] |            |                   |
| Kim, Y. J.        | 21.2%  | 1.70 [1.10, 2.64] |            |                   |
| Kummee, P.        | 11.7%  | 1.23 [0.63, 2.40] |            |                   |
| Niro, G. A.       | 13.5%  | 1.33 [0.72, 2.44] |            |                   |
| Ribeiro, C. S.    | 6.4%   | 0.67 [0.23, 1.93] |            |                   |
| Sonni, M. H.      | 13.2%  | 1.05 [0.54, 2.04] |            |                   |
| Zhang, P. A.      | 4.7%   | 2.91 [1.15, 7.39] |            |                   |
| Total (95% CI)    | 100.0% | 1.30 [1.04, 1.63] |            |                   |
| Total events      |        |                   |            |                   |

Heterogeneity: Chi$^2$ = 7.50, df = 7 ($P = 0.38$); I$^2$ = 7%
Test for overall effect: Z = 2.30 ($P = 0.02$)
research is needed to demonstrate the underlying cause for this inconsistency.

In our study, other polymorphisms in the TNF-\(<alpha>\) promoter (-1031T/C,-857C/T) did not show any association with HBV outcome, which is different from some previous reports [7,9,17]. Possible explanations for this difference are a lack of data, and ethnicity diversity.

Our study provided a more believable result due to a larger size sample, and provides explanations for the inconsistencies observed in previous studies. Meta-analysis is a powerful statistical tool that provides a consensus by combining the data from diverse studies that reveal inconsistent results on the same problem. Some results of our study did not show any statistical significance although we combined relatively large numbers of studies. However, subgroup analysis by ethnicity showed a statistical significant result. Hence, a meaningful outcome can be produce only when it is correctly used. On the other hand, the more studies included, the more accurate the results would be.

In conclusion, this study provides evidence of a positive association between HBV clearance and TNF-\(<alpha>\) promoter -863 CC. Conversely, -308 GG/GG+GA genotypes increased the risk of chronic infection. These genotypes might affect the outcome of HBV infection through regulation of TNF-\(<alpha>\) transcriptional activation and production. Ethnic diversity may complicate the outcome of infection. More studies of individuals of diverse ethnicities will be necessary to determine the effects of TNF-\(<alpha>\) promoter polymorphisms on the outcome of HBV infection.

Author Contributions
Conceived and designed the experiments: QX ZC FC. Performed the experiments: QX LFZ DCL. Analyzed the data: QX LFZ FC. Contributed reagents/materials/analysis tools: QX LFZ DCL. Wrote the paper: QX FC.

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Figure 4. Funnel plot analysis to examine publication bias. Some asymmetry in -863 CC vs. AA (A) and -863 AA vs. CA+CC (A). After one study removed, the publication bias was eliminated. See -863 CC vs. AA (B) and -863 AA vs. CA+CC (B).
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