Associations of IFN-γ rs2430561 T/A, IL28B rs12979860 C/T and ERα rs2077647 T/C polymorphisms with outcomes of hepatitis B virus infection: a meta-analysis

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Abstract

Several studies investigated associations of IFN-γ rs2430561 T/A, IL28B rs12979860 C/T and ERα rs2077647 T/C gene polymorphisms with outcomes of hepatitis B virus (HBV) infection, but the results were controversial. Therefore, we performed a meta-analysis of all published observational studies to address this inconsistency. Literature was searched in online database and a systematic review was conducted based on the search results. A total of 24 studies were included and dichotomous data were presented as odds ratio (OR) with a 95% confidence interval (CI). The rs2430561 T allele was associated with reduced persistent HBV infection risk (T vs. A: OR, 0.690; 95% CI, [0.490, 0.971]), while the rs2077647 T allele significantly increased the risk of persistent HBV infection (T vs. C: OR, 1.678; 95% CI, [1.212, 2.323]). Rs 2077647 CC might play a role in protecting individuals against HBV persistence (TT vs. CC: OR, 4.109; 95% CI, [2.609, 6.473]). Furthermore, carriers of the rs2430561 TT genotype were more likely to clear HBV spontaneously compared with those of the AA genotype (TT vs. AA: OR, 0.555; 95% CI, [0.359, 0.856]). For rs12979860 C/T polymorphism, no significant correlation with HBV infection outcomes was found. In subgroup analyses, the results were similar to those of overall analysis. However, for rs2077647 TT vs. TC+CC, significantly increased risks were observed in the Asian and hospital-based population, but not in the overall analysis. IFN-γ rs2430561 T/A and ERα rs2077647 T/C genetic polymorphisms were associated with outcomes of HBV infection, but no association was found between IL28B rs12979860 C/T and HBV infection.

Keywords: meta-analysis, single nucleotide polymorphism, IFN-γ rs2430561 T/A, IL28B rs12979860 C/T, ERα rs2077647 T/C, hepatitis B virus

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Association of host SNPs and outcomes of HBV infection

INTRODUCTION

Infection by hepatitis B virus (HBV) appears under different forms of evolution, ranging from the asymptomatic and self-limited infection to the chronic state, which can develop into chronic hepatitis, cirrhosis, and hepatocellular carcinoma. So far, factors that determine the variable outcomes of HBV infection are little known. Besides pathogenesis of virus, environmental factors, ethnic differences and genetic susceptibility have also been reported to have an effect on the progression of this liver disease. Recently, a number of studies have shown that genetic polymorphisms of cytokines have a correlation with the outcomes of HBV infection. However, controversies exist among similar studies.

Interferon-γ (IFN-γ) is known as a Th1 cytokine, and plays a pivotal role in defending against the invasion of intracellular pathogens and the induction of an immune-mediated inflammatory response. During viral infections, the expression pattern of cytokines is changed and IFN-γ level is increased. Interestingly, a single nucleotide polymorphism (SNP) located in the IFN-γ gene intron (at position +874) was involved in transcriptional regulation of IFN-γ and HBV susceptibility. Some studies demonstrated that patients carrying rs2430561 AA genotype in the IFN-γ gene had a high risk of susceptibility to chronic infection of HBV, while Cheong et al. observed no significant difference in susceptibility risk.

Interleukin 28B (IL28B, interferon-ω [IFN-ω]) is another cytokine involved in the host immune response to virus infection, such as hepatitis C virus (HCV) and HBV. There also had been several studies on the relationship between chronic HBV infection, HBV clearance and genetic polymorphism of the IL28B gene rs12979860 C/T. However, the results are inconsistent. For instance, Ren et al. found that the frequency of CC homozygosity was significantly higher in healthy controls than that in chronic hepatitis B patients, but other studies did not find such difference.

It was reported that estrogen could directly activate the promoter of IFN-γ and this effect was mediated by estrogen receptors (ERs, ERα and ERβ). The expression and function of ER might be influenced by its own variation and thus modulate diverse pathologies correlated with prognosis and survival of chronic hepatopathy. Previous studies reported an association of ER polymorphisms with susceptibility to chronic HBV infection and other chronic hepatic diseases. Deng et al. found that, as a haplotype-tagging SNP, ERα rs2077647 T/C genotype (previous reported c.30T > C, exon 1) had an influence on susceptibility to persistent HBV infection and HBV-related hepatocellular carcinoma. It was also observed that the relative messenger RNA levels of the at-risk C allele of rs2077647 were consistently higher than those of the T allele in the heterozygous cells. Therefore, it is rational to consider that ERα may be a biological candidate susceptibility gene for chronic HBV infection.

To our knowledge, the recent results about the associations of IFN-γ rs2430561 T/A, IL28B rs12979860 C/T and ERα rs2077647 T/C gene polymorphisms with the outcomes of HBV infection in many studies are inconsistent. Therefore, to assess the associations between these SNPs and the outcomes of HBV infection, we performed a meta-analysis of all the published observational studies.

MATERIALS AND METHODS

Publication search

PubMed (http://www.ncbi.nlm.nih.gov/pubmed), web of science (http://www.thomsonscientific.com.cn/), CNKI (China National Knowledge Infrastructure) (http://epub.cnki.net/kns/default.htm) and Chinese Biomedicine databases (http://www.sinomed.ac.cn) were searched (the last search was updated in July 2013) using the search terms: ‘hepatitis B’ or ‘HBV’, ‘polymorphism’ or ‘mutation’ or ‘variant’, ‘interferon-gamma’ or ‘interferon γ’ or ‘interleukin 28B’ or ‘estrogen receptor alpha’ or ‘estrogen receptor α’. The results were supplemented with manual searches of references of final published articles. Review articles, editorials or conference abstracts were excluded. When more than one of the same patient population was included in several publications, only the most recent or complete study was used in this meta-analysis. A flow diagram of the study selection process is shown in Fig. 1.

Inclusion and exclusion criteria

Inclusion criteria were as follows: (1) patients with no detectable HBV infection were defined as healthy controls (HCs); patients whose serum HBV surface antigen (HBsAg) was negative but HBV surface antibody (anti-HBs) and/or HBV core antibody (anti-HBc) were positive were defined as self-limiting infection controls (SLCs); patients who had been positive for HBsAg or HBV DNA for at least 6 months were included as persistent HBV infection cases (PIs); (2) design type of the study was a case-control study; (3) the study aimed to examine the relationship between the IFN-γ rs2430561 or IL28B rs12979860 or ERα rs2077647 T/C polymorphisms and clearance and/or susceptibility of persistent HBV infection; (4) the study provided sufficient data for examining an odds ratio
(OR) with 95% confidence interval (CI); (5) the patients recruited in the studies had not received prior HBV-related treatment. Exclusion criteria were as follows: (1) the study fitted no diagnosis criteria; (2) the study was not a case-control study; (3) the study reported no usable data.

Data extraction

Two investigators (ST and JW) extracted information from all eligible publications independently according to the inclusion criteria listed above. Disagreements were resolved by discussion. The following information was extracted from each publication, including name of the first author, year of publication, country/region of the first or corresponding author, ethnicity, number of cases and controls, genotyping methods, and polymorphisms of \( IFN-\gamma \) rs2430561, \( IL28B \) rs12979860 or \( ER\alpha \) rs2077647. If the information mentioned in this section and above section was unavailable in relevant articles, a request was sent to corresponding author for additional data.

Statistical analysis

The statistical analysis was conducted using Stata 10.0 (StataCorp, College Station, TX, USA). At the beginning of the analysis, we assessed Hardy-Weinberg equilibrium (HWE) in the controls for each study using Chi-square test (\( P \leq 0.05 \) was considered as a deviation from HWE). Then, the risks (ORs, and 95% CIs) of persistent HBV infection associated with \( IFN-\gamma \) rs2430561, \( IL28B \) rs12979860 and \( ER\alpha \) rs2077647 were estimated for each study based on extracted genotype data. We also carried out stratified analyses by ethnicity (Asian and Caucasian) and sources (hospital-based and population-based). Moreover, sensitivity analysis was performed to assess the stability

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**Fig. 1 Flow diagram of studies included in the meta-analysis.** (x/y/z) represents the number of studies of \( IFN-\gamma \) rs2430561T/A, \( IL28B \) rs12979860 C/T and \( ER\alpha \) rs2077647 T/C, respectively.
of results while omitting the study not in HWE, one at a time. Q-statistic and I² statistic were performed to evaluate statistical heterogeneity. When $P > 0.10$ for Q-statistic or I² $\leq 50\%$, heterogeneity was considered to be absent and a fixed effects model was used; otherwise, a random effects model was used. Several methods were used to assess the potential for publication bias, including visual inspection of asymmetry in funnel plots, Begg’s test and Egger’s test. $P \leq 0.05$ was considered to be representative of significant publication bias.

**RESULTS**

**Characteristics of studies**

A total of 24 relevant studies (Table 1) evaluating IFN-γ rs2430561 T/A, IL28B rs12979860 C/T and ERα rs2077647 T/C SNPs were found in our search, and the characteristics of each study are summarized according to the polymorphisms in Table 1. The countries in which these studies were carried out include China, South Korea, Brazil, Iran, Spain, United States, Italy and Romania. Eighteen studies were on Asians, 5 on Caucasians and 1 on Brazilians, which included mixed ancestry of Caucasians (European), African and “other” (Amerindian). Sixteen of the 24 eligible studies were hospital-based case-control studies while the remaining 8 were population-based. The distribution of genotypes in the controls of all studies was in agreement with HWE except for 4 studies.

**Association of individual polymorphisms with susceptibility to persistent HBV infection**

We compared persistent HBV infection cases with healthy controls to discover the relationship between the three target SNPs and HBV infection susceptibility. As shown in Table 2, the IFN-γ rs2430561 T allele was associated with significantly reduced persistent HBV infection risk (T vs. A: OR, 0.690; 95% CI [0.490, 0.971]; $P = 0.033$) (Fig. 2A), while the ERα rs2077647 T allele significantly increased the risk of persistent HBV infection (T vs. C: OR, 1.678; 95% CI [1.212, 2.323]; $P = 0.002$). In addition, the results of TT vs. CC and CC vs. CT+TT implied that ERα rs2077647 CC might play a role in protecting individuals against HBV persistence (TT vs. CC: OR, 4.109; 95% CI [2.609, 6.473]; $P < 0.001$; CC vs. CT+TT: OR, 0.301; 95% CI [0.199, 0.454]; $P < 0.001$) (Fig. 2C and D, respectively). No evidence of a relationship between IL28B rs12979860 C/T and persistent HBV infection risk was observed in all comparison models.

We also performed stratified analyses for Asian and hospital-based individuals, and the results were still stable (Table 3). Caucasian and population-based subgroup analyses were not conducted because only 1 or 2 studies were available. Interestingly, for the comparison of ERα rs2077647 TT vs. TC+CC, significantly increased risks were observed in the Asian and hospital-based population (Asian subgroup, TT vs. TC+CC: OR, 1.778; 95% CI, [1.004, 3.149]; $P = 0.048$; hospital-based subgroup, TT vs. TC+CC: OR, 2.204; 95% CI, [1.140, 4.264]; $P = 0.019$), but not in the overall analysis.

**Association of individual polymorphisms with HBV clearance**

We also compared persistent HBV infection cases with self-limiting infection controls to discover the relationship between the three target SNPs and HBV infection clearance. As shown in Table 4, the meta-analysis provided estimated odds ratios and $P$-value of all comparison models, but only IFN-γ rs2430561 TT vs. AA showed significant difference (TT vs. AA: OR, 0.555; 95% CI, [0.359, 0.856]; $P = 0.008$) (Fig. 2B), indicating that carriers of IFN-γ rs2430561 TT genotype were more likely to clear HBV spontaneously compared with those carrying AA genotype, and there was no significant correlation of IL28B rs12979860 C/T polymorphism with HBV clearance. We did not conduct meta-analysis of ERα rs2077647 T/C because only 1 study was available.

In subgroup analysis of SNP IFN-γ rs2430561, since all the studies were from Asian and hospital-based, and the results were the same as in Table 3. Moreover, for IL28B rs12979860 C/T polymorphism, the results of subgroup analyses were similar to those of overall analyses. Namely, no significant association of IL28B rs12979860 C/T polymorphism with HBV clearance was observed in both overall analysis and stratified analysis.

**Sensitivity analysis**

There were 4 studies not in HWE within our included studies. In the sensitivity analysis, the influence of these 4 studies on the pooled OR was examined by repeating the meta-analysis while omitting each of them, one at a time. When excluded the 4 studies mentioned above, most of the estimated pooled OR did not change at all. However, when the study by Zhang et al. was omitted, the result of IFN-γ rs2430561 T vs. AA model showed no significant association with susceptibility to persistent HBV infection. Furthermore, for IFN-γ rs2430561 TT vs. AA model,
Table 1 Characteristics and IFN-γ rs2430561, IL28B rs12979860 and ERα rs2077647 polymorphism genotype distributions in studies included in the meta-analysis

| Author | Year/countries | Ethnicity | Sources | SNPs       | Genotypes | Sample size | HWE in control |
|--------|----------------|-----------|---------|------------|------------|-------------|----------------|
| Zhang PA | 2009/China    | Asian     | Hospital-based | IFN-γ rs2430561 | TT/TA/AA T vs A | 17/31/87 65 vs 205 | 71 vs 392 | 0.00 * |
| Gao QF | 2009/China    | Asian     | Population-based | IFN-γ rs2430561 | TT/TA/AA T vs A | 7/53/14 67 vs 81 | 53 vs 85 | 0.00 |
| Cheong JY | 2006/South Korea | Asian  | Hospital-based | IFN-γ rs2430561 | TT/TA/AA T vs A | - 3/47/151 53 vs 347 | 104 vs 722 | 0.76 |
| Ribeiro CSS | 2007/Brasil | European, African and Amerindians | Hospital-based | IFN-γ rs2430561 | TT/TA/AA T vs A | 323/14 29 vs 51 | 6/13/12 | 0.12 |
| Arababadi MK | 2011/Iran | Caucasian | Population-based | IFN-γ rs2430561 | TT/TA/AA T vs A | 25/47/28 97 vs 103 | 14/25/18 | 0.55 |
| Wu JM | 2008/China    | Asian     | Hospital-based | IFN-γ rs2430561 | TT/TA/AA T vs A | - 23/30/56 76 vs 44 | 114 vs 122 | 0.55 |
| Zhi LT | 2006/China    | Asian     | Hospital-based | IFN-γ rs2430561 | TT/TA/AA T vs A | - 7/10/8511 122 vs 810 | 99 vs 473 | 0.69 |
| Peng XM | 2007/China    | Asian     | Hospital-based | IFN-γ rs2430561 | TT/TA/AA T vs A | - 2/34/65 37 vs 163 | 489/2/7 | 0.35 |
| Liu MQ | 2006/China    | Asian     | Hospital-based | IFN-γ rs2430561 | TT/TA/AA T vs A | 253 vs 291 | - | 101/26 | >0.05 |
| Luz MC | 2012/Spain    | Caucasian | Hospital-based | IL28B rs12979860 | CC/CT/TT C vs T | - 22/21/06 65 vs 33 | 75 vs 23 | 0.78 |
| Martin MP | 2010/United States | Caucasian | Population-based | IL28B rs12979860 | CC/CT/TT C vs T | - 157/175/52 489 vs 279 | 292 vs 160 | 0.77 |
| Shi XD | 2011/China    | Asian     | Hospital-based | IL28B rs12979860 | CC/CT/TT C vs T | 19/00/38 38 vs 0 | - | 114/230 | >0.05 |
| Ren S | 2012/China    | Asian     | Hospital-based | IL28B rs12979860 | CC/CT/TT C vs T | 43/40/90 90 vs 4 | 33/73/13 73 vs 13 | 177/46/16 | 0.01 * |
| Fabris C | 2010/Italy    | Caucasian | Population-based | IL28B rs12979860 | CC/CT/TT C vs T | 164/145/35 473 vs 215 | - | 36/35/4 | 0.72 |
| Chen JF | 2012/China    | Asian     | Population-based | IL28B rs12979860 | CC/CT/TT C vs T | 12/32/28 455 vs 33 | - | 104/35/20 | 0.37 |
| Peng LJ | 2011/China    | Asian     | Population-based | IL28B rs12979860 | CC/CT+TT C vs T | - 206/200 | 57/47 | 223/172 | >0.05 |
| Li W | 2011/China    | Asian     | Hospital-based | IL28B rs12979860 | CC/CT+TT | 179/24 189/23 | 1782 vs 85 | 0.05 |
| Lee DH | 2013/Korea    | Asian     | Hospital-based | ERα rs2077647 | TT/TC/CC C vs T | 381 vs 23 973 vs 62 | - | >0.05 |
| Deng GH | 2004/China    | Asian     | Hospital-based | ERα rs2077647 | TT/TC/CC T vs C | - 246/38/108 800 vs 604 | 528/95/148 | 0.02 |
| Zhou N | 2009/China    | Asian     | Hospital-based | ERα rs2077647 | TT/TC/CC T vs C | 23/35/22 81 vs 79 | - | 965/71 | 0.26 |
| Li ZX | 2007/China    | Asian     | Hospital-based | ERα rs2077647 | TT/TC/CC T vs C | 21/33/16 75 vs 65 | - | 738/15 | 0.66 |
| Shan KR | 2010/China    | Asian     | Population-based | ERα rs2077647 | TT/TC/CC T vs C | 26/26/81 78 vs 42 | - | 20/32 | 0.71 |
| Long L | 2009/China    | Asian     | Population-based | ERα rs2077647 | TT/TC/CC T vs C | 71/19/36 221 vs 151 | - | 70/84 | 0.10 |
| Anghel A | 2010/Romania  | Caucasian | Hospital-based | ERα rs2077647 | TT/TC/CC T vs C | 48/57/9 153 vs 75 | - | 4/7 | 0.16 |

*HWE was calculated by the merged data of healthy controls and self-liming controls. * P value for HWE was extracted from original publication.

Abbreviations: SNPs, single nucleotide polymorphisms; HC, healthy control; SLC, self-liming control; PI, persistent infection; HWE, Hardy-Weinberg equilibrium.
Table 2 Quantitative data synthesis of individual polymorphisms, persistent hepatitis B virus infection cases versus healthy controls

| SNPs     | Comparison | n | OR (95% CI) | Homogeneity | Publication bias |
|----------|------------|---|-------------|-------------|-----------------|
|          |            |   |             | Q | P  | F (%) | P for Begg's test | P for Egger's test |
| IFN-γ    | T/A        | 5 | 0.690 (0.490,0.971) | 0.033* | 12.58 | 0.014 | 68.2 | 0.096 | 0.079 |
| rs2430561| TT/AA      | 4 | 0.779 (0.487,1.245) | 0.296 | 2.54 | 0.468 | 0.0 | 1.000 | 0.798 |
|          | AA/(TA+TT) | 4 | 0.854 (0.554,1.318) | 0.477 | 1.57 | 0.665 | 0.0 | 1.000 | 0.032 |
| IL28B    | CT         | 4 | 0.698 (0.373,1.305) | 0.260 | 10.26 | 0.016 | 70.8 | 1.000 | 0.316 |
| rs12979860| CC/TT      | 3 | 0.980 (0.476,2.018) | 0.956 | 3.50 | 0.174 | 42.9 | 1.000 | 0.032 |
|          | TT/(CT+TT) | 5 | 0.821 (0.630,1.069) | 0.143 | 7.33 | 0.120 | 45.4 | 1.000 | 0.293 |
| ERα      | T/C        | 5 | 1.678 (1.212,2.323) | 0.002* | 9.80 | 0.044 | 59.2 | 0.096 | 0.125 |
| rs2077647 | TT/CC      | 5 | 4.109 (2.699,6.473) | 0.000* | 2.66 | 0.616 | 0.0 | 0.462 | 0.079 |
|          | TT/(TC+CC) | 5 | 1.595 (0.929,2.738) | 0.091 | 13.53 | 0.009 | 70.4 | 0.096 | 0.968 |
|          | CC/(TT+TC) | 5 | 0.301 (0.199,0.454) | 0.000* | 3.54 | 0.472 | 0.0 | 0.096 | 0.911 |

*The study of Shi [32] had to be excluded because it contained no individuals carrying IL28B rs12979860 TT genotype.

**P** for Egger’s test could not be evaluated since there was no healthy controls carrying IL28B rs12979860 TT genotype in the study of Ren S [13].

Abbreviations: SNPs, single nucleotide polymorphisms. *P<0.05.

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**Fig. 2** Forest plots of associations between polymorphisms and outcomes of HBV infection. A: IFN rs2430561 T vs. A in comparison of PI and HC; B: IFN rs2430561 TT vs. AA in comparison of PI and SLC; C: ERα rs2077647 TT vs. CC in comparison of PI and HC; D: ERα rs2077647 CC vs. (TT+TC) in comparison of PI and HC. HC: healthy control; SLC: self-limiting control; PI: persistent infection.
no significance was observed on the clearance of HBV when the study was excluded\(^{10}\).

Tests of heterogeneity and publication bias

Q-statistic and I\(^2\) statistic were used to evaluate the statistical heterogeneity and several comparison models were found to have heterogeneities (Table 2 and Table 4). Thus, a random-effects model was employed in these studies. Begg’s funnel plot and Egger’s test were performed to assess the publication bias of the studies (Table 2 and Table 4). As a result, no evidence of publication bias was found in all comparison models.

Discussion

It is well known that the elimination of HBV is attributed to a coordinated innate and adaptive humoral and cell-mediated immune response. During this immune process, cytokines play a crucial role in modulating almost all phases of host immune response. Genetic polymorphisms of the cytokines and factors regulating cytokines may influence the expression of

Table 3 Stratified analyses of IFN-\(\gamma\) rs2430561, IL28B rs12979860 and ER\(\alpha\) rs2077647 polymorphisms on the outcomes of HBV infection

| SNPs                  | Subgroups | Comparison | n   | OR (95% CI) | Homogeneity |
|-----------------------|-----------|------------|-----|-------------|-------------|
|                       |           |            |     | OR          | CI          | P         | Q   | P  | I\(^2\) (%) | P for Begg’s test | P for Egger’s test |
| IFN-\(\gamma\) rs2430561 | Asian     | T/A        | 3   | 0.558 (0.407,1.764) | 0.000* | 4.28 | 0.117 | 53.3 |
|                       | Hospital  | T/A        | 3   | 0.603 (0.383,0.937) | 0.024* | 6.64 | 0.036 | 69.9 |
| IL28B rs12979860      | Asian     | C/T        | 3   | 0.416 (0.111,1.516) | 0.184 | 8.31 | 0.016 | 75.9 |
|                       | Hospital  | C/C/(C+T+T)| 4   | 0.672 (0.364,1.240) | 0.204 | 6.87 | 0.076 | 56.3 |
| ER\(\alpha\) rs2077647 | Asian     | T/C        | 4   | 1.822 (1.382,2.480) | 0.000* | 6.74 | 0.081 | 55.5 |
|                       | Asian     | T/T/CC     | 4   | 4.402 (2.775,6.984) | 0.000* | 0.39 | 0.921 | 0.0  |
|                       | Asian     | T/T/(T+CC) | 4   | 1.773 (1.004,3.149) | 0.048* | 11.63 | 0.009 | 74.2 |
|                       | Asian     | C/C/(T+T)  | 4   | 0.297 (0.189,0.435) | 0.000* | 2.27 | 0.519 | 0.0  |
|                       | Hospital  | T/C        | 3   | 1.894 (1.156,3.102) | 0.011* | 5.49 | 0.064 | 63.6 |
|                       | Hospital  | T/T/CC     | 3   | 3.383 (2.192,6.721) | 0.000* | 2.28 | 0.319 | 12.5 |
|                       | Hospital  | T/T/(T+CC) | 3   | 2.204 (1.140,4.264) | 0.019* | 4.69 | 0.096 | 57.4 |
|                       | Hospital  | C/C/(T+T)  | 3   | 0.387 (0.234,0.640) | 0.000* | 1.10 | 0.577 | 0.0  |

*Analysis of ER\(\alpha\) rs2077647 T/C was not conducted to explore the association with HBV clearance because there was only one study\(^{19}\) available. \(*P<0.05.\)

SNPs: single nucleotide polymorphisms.

Table 4 Quantitative data synthesis of individual polymorphisms, persistent hepatitis B virus infection cases versus self-limiting infection controls

| SNPs                  | Comparison | OR (95% CI) | Homogeneity |
|-----------------------|------------|-------------|-------------|
|                       |            | OR          | CI          | P         | Q   | P  | I\(^2\) (%) | P for Begg’s test | P for Egger’s test |
| IFN-\(\gamma\)       | T/A        | 0.779 (0.524,1.159) | 0.218 | 23.53 | 0.000 | 83.0 | 0.086 | 0.090 |
| rs2430561             | T/T/AA     | 0.555 (0.359,0.856) | 0.008* | 5.76 | 0.218 | 30.6 | 1.000 | 0.717 |
|                       | T/T/(T+AA) | 0.691 (0.466,1.024) | 0.066 | 1.99 | 0.738 | 0.0  | 1.000 | 0.944 |
|                       | A/A/(A+T)  | 1.401 (0.839,2.339) | 0.197 | 25.21 | 0.000 | 84.1 | 0.221 | 0.389 |
| IL28B                 | C/T        | 1.060 (0.673,1.288) | 0.557 | 2.37 | 0.499 | 0.0  | 1.000 | 0.633 |
| rs12979860            | C/C/(C+T)  | 1.092 (0.700,1.704) | 0.067 | 1.49 | 0.475 | 0.0  | 1.000 | 0.655 |
|                       | C/C/(C+T)  | 1.009 (0.804,1.267) | 0.937 | 4.21 | 0.378 | 5.0  | 0.462 | 0.346 |
|                       | T/T/(C+C)  | 0.996 (0.653,1.518) | 0.984 | 1.20 | 0.549 | 0.0  | 1.000 | 0.395 |

*Analysis of ER\(\alpha\) rs2077647 T/C was not conducted in this table because there was only one study\(^{19}\) available. \(*P<0.05.\)

SNPs: single nucleotide polymorphisms.
cytokines, thus determining the various clinical outcomes of HBV infection.

As one of the most representative Th1 cytokines, IFN-γ plays an important role in the clearance of HBV. On one hand, in acute self-limited HBV infection, the antigen-specific fraction of T cells selectively secrete Th1-type cytokines, with a predominance of IFN-γ. On the other hand, cell clones from persons with chronic HBV infection produce a predominantly type 2 response. IFN-γ rs2430561T/A is a SNP located in the first intron of the IFN-γ gene, which is confirmed to coincide with the nuclear factor-κB (NF-κB) binding region. It had been demonstrated that possession of rs2430561T and A alleles should be associated with high and low IFN-γ expression, respectively. In this study, we performed a meta-analysis and concluded that IFN-γ rs2430561 T allele was associated with significantly reduced risk of persistent HBV infection and the carriers with the IFN-γ rs2430561 TT genotype were more likely to clear HBV spontaneously compared with those carrying the IFN-γ rs2430561 AA genotype. In stratified analyses, the results did not change in Asian and hospital-based subgroups. However, when studies not in HWE were excluded, the results showed no significance. Nevertheless, it was still meaningful that IFN-γ rs2430561 T allele and TT genotype could up-regulate the expression of IFN-γ, rendering these subjects less prone to persistent HBV infection. Most importantly, more functional analyses should be conducted to demonstrate it.

This meta-analysis documented that the SNP 4 kilobases upstream of IL28B (rs12979860) was not associated with outcomes of HBV infection. However, prior studies had shown that this SNP correlated with both spontaneous and anti-viral treatment-induced HCV clearance. These conflicting results suggested that although HBV and HCV shared a similar natural history, pathogenesis and transmission modality, rs12979860 might alter the immune responses against HCV but not HBV. Potentially, this could be explained by the characteristics of IL28B. By triggering a cascade through the JAK-STAT (Janus kinase-signal transducer and activator of transcription, JAK-STAT) pathway, IL28B up-regulates the IFN-stimulated genes (ISGs) and produces an antiviral state. In addition, when used as a vaccine adjuvant, IL28B could significantly decrease splenic and peripheral blood CD8+ T cells, increase splenic and peripheral blood CD8+ T cells, and lead to increased antigen-specific perforin induction and degranulation. These adaptive immune responses might be more important in HCV infection. Therefore, IL28B plays a more important role in the infection of HCV than that of HBV.

Chronic hepatitis B progresses at unequal rates between males and females, being more frequent in men than in women. This sexual dimorphism might be due to lower expression of estrogen and a reduced response to the action of estrogen. Furthermore, it was reported that estrogen could directly activate the promoter of IFN-γ and this effect was mediated by estrogen receptors (ERs, ERα and ERβ). The production and function of ER might be influenced by its own variation, and thus influence the diverse pathologies of chronic hepatopathy. According to our findings, compared to CT+TT, ERα rs2077647 CC genotype might play a role in protecting individuals against HBV persistence, since the risk of persistent HBV infection was reduced to 0.289 (95% CI, [0.187, 0.446]; P < 0.001). However, ERα rs2077647 T/C is a synonymous polymorphism located in exon 1, and Zhai et al. investigated that such effect could be caused by some other functional polymorphisms (such as a [TA]n repeat and a PvuII RFLP) in LD with rs2077647 T/C. Thus, to date, the potential function of rs2077647 T/C could not be confirmed, and further investigation was required to explore the exact mechanism and association of ERα polymorphisms with outcomes of HBV infection.

It should be noted that there were certain limitations to our study. Firstly, due to the limited availability of published results, the number of studies included in each meta-analysis was small. We expected that as more studies become available, an accurate estimation of the relationship of the 3 SNPs with susceptibility and clearance to persistence HBV infection would be obtained. Secondly, several comparison models were found to have heterogeneities, and the genotype distribution also showed deviation from HWE in four studies. These could be attributed to ethnic differences (Asian versus non-Asian descent), potential selection bias (in-patients versus outpatients) or other factors. The third limitation of this analysis is that we did not have original data for all studies to account for other factors, like presence of co-infection with HIV or immune deficiency, genotypes of HBV and transmission modality that may modify the risk estimates. In spite of these, meta-analysis is a powerful statistical tool to summarize inconsistent results from different studies, so our meta-analysis provided more convincing conclusions.

In summary, this meta-analysis suggested that IFN-γ rs2430561 T/A and ERα rs2077647 T/C genetic polymorphisms were associated with the outcomes of HBV infection, but association between IL28B rs12979860 C/T and HBV infection was not found. Since limitations were present in our study, it is critical that lager...
and well-designed multicenter studies should be performed to re-evaluate the associations. Moreover, further studies incorporating diverse populations and functional assays are warranted to validate and extend our findings.

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