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Research Paper

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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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[1]. So far, factors that determine the variable outcomes of HBV infection are little known. Besides pathogenesis of virus, environment factors, ethnic differences and genetic susceptibility have also been reported to have an effect on the progression of this liver disease[2]. Recently, a number of studies have shown that genetic polymorphisms of cytokines have a correlation with the outcomes of HBV infection[3,4]. 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[5]. During viral infections, the expression pattern of cytokines is changed and *IFN-*γ level is increased[6]. Interestingly, a single nucleotide polymorphism (SNP) located in the *IFN-*γ gene intron (at position +874) was involved in transcriptional regulation of *IFN-*γ [7] and HBV susceptibility[8]. 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[9–11], while Cheong et al.[12] observed no significant difference in susceptibility risk.

Interleukin 28B (*IL28B*, interferon-λ3 [IFN-λ3]) 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.[13] 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[14,15].

It was reported that estrogen could directly activate the promoter of *IFN-*γ and this effect was mediated by estrogen receptors (ERs, *ER*α and ERβ)[16]. 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[17,18]. Previous studies[19–21] reported an association of *ER* polymorphisms with susceptibility to chronic HBV infection and other chronic hepatic diseases. Deng et al.[19] found that, as a haplotype-tagging SNP, *ER*α 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[22]. Therefore, it is rational to consider that *ER*α 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 *ER*α 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 *ER*α rs2077647 T/C polymorphisms and clearance and/or susceptibility of persistent HBV infection; (4) the study provided sufficient data for examining an odds ratio

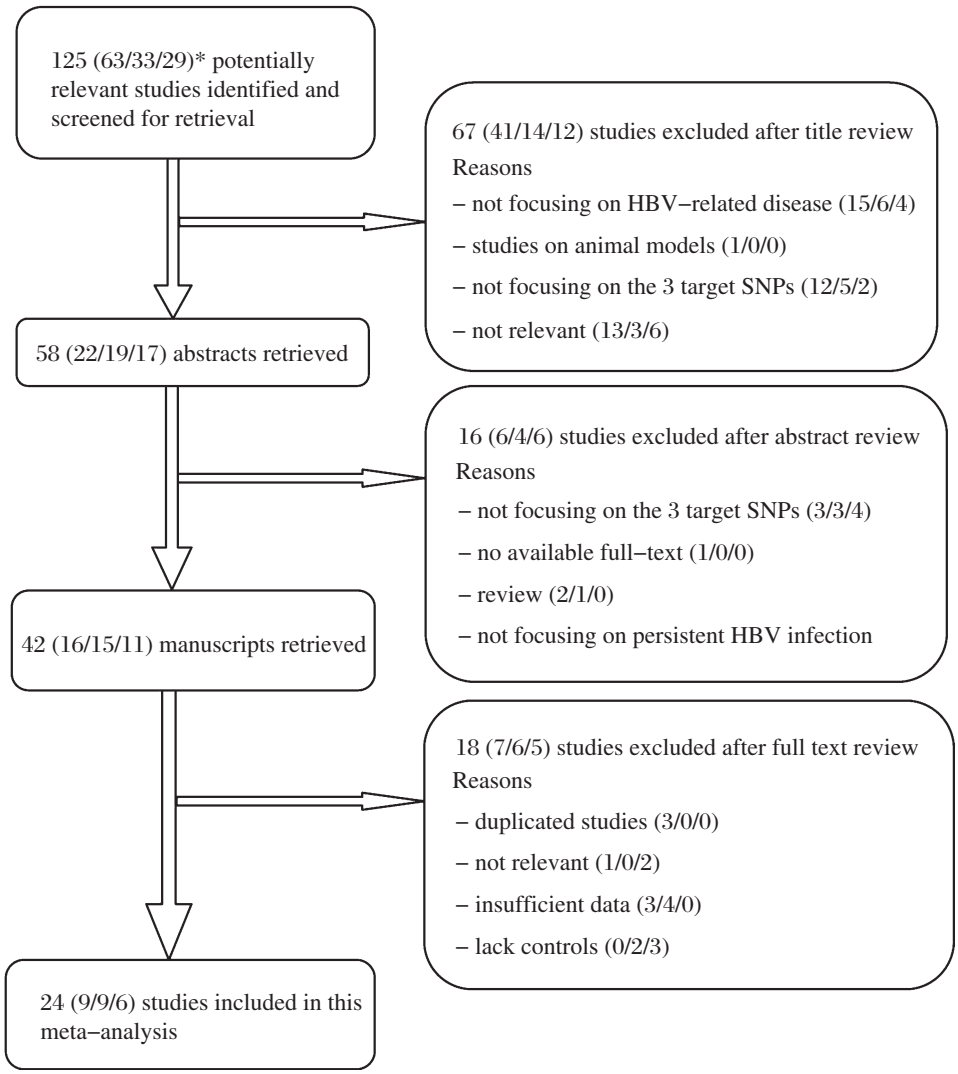

***Fig. 1* Flow diagram of studies included in the meta-analysis.** [a](x/y/z) represents the number of studies of *IFN-γ* rs2430561T/A, *IL28B* rs12979860 C/T and *ERα* rs2077647 T/C, respectively.

(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-γ* rs2430561, *IL28B* rs12979860 or *ERα* 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-γ* rs2430561, *IL28B* rs12979860 and *ERα* 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, sen–sitivity analysis was performed to assess the stability

of results while omitting the study not in HWE, one at a time. Q-statistic and I^2 statistic were performed to evaluate statistical heterogeneity. When $P > 0.10$ for Q-statistic or $I^2 \leq 50\%$, 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[10,11,13,19].

Association of individual polymorphisms with susceptibility to persistent HBV infection

We compared persistent HBV infection cases with healthy controls to discover the relation of *IFN-γ* rs2430561 T/A, *IL28B* rs12979860 C/T and *ERα* rs2077647 T/C SNPs to persistent 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[19] 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[10,11,13,19] 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.[11] was omitted, the result of *IFN-γ* rs2430561 T *vs.* A 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
						HC	SLC	PI	
Zhang PA[11]	2006/China	Asian	Hospital-based	*IFN-γ* rs2430561	TT/TA/AA T vs A	17/31/87 65 vs 205	23/39/103 85 vs 245	21/28/182 70 vs 392	0.00 [a]
Gao QJ[10]	2009/China	Asian	Population-based	*IFN-γ* rs2430561	TT/TA/AA T vs A	7/53/14 67 vs 81	-	9/35/25 53 vs 85	0.00
Cheong JY[12]	2006/South Korea	Asian	Hospital-based	*IFN-γ* rs2430561	TT/TA/AA T vs A	-	3/47/151 53 vs 347	5/94/314 104 vs 722	0.76
Ribeiro CSS[33]	2007/Brasil	European, African and Amerindians	Hospital-based	*IFN-γ* rs2430561	TT/TA/AA T vs A	3/23/14 29 vs 51	-	6/12/12 24 vs 36	0.12
Arababadi MK[34]	2011/ Iran	Caucasian	Population-based	*IFN-γ* rs2430561	TT/TA/AA T vs A	25/47/28 97 vs 103	-	14/25/18 53 vs 61	0.55
Wu JM[35]	2008/China	Asian	Hospital-based	*IFN-γ* rs2430561	TT/TA/AA T vs A	-	23/30/7 76 vs 44	32/50/36 114 vs 122	0.55
Zhi LT[36]	2006/China	Asian	Hospital-based	*IFN-γ* rs2430561	TT/TA/AA T vs A	-	7/108/351 122 vs 810	6/87/193 99 vs 473	0.69
Peng XM[37]	2007/China	Asian	Hospital-based	*IFN-γ* rs2430561	TT/TA/AA T vs A	-	2/33/65 37 vs 163	4/89/247 97 vs 583	0.35
Liu MQ[8]	2006/China	Asian	Hospital-based	*IFN-γ* rs2430561	T vs A	253 vs 291	-	101/261	>0.05[b]
Luz MC[14]	2012/Spain	Caucasian	Hospital-based	*IL28B* rs12979860	CC/CT/TT C vs T	-	22/21/6 65 vs 33	29/17/3 75 vs 23	0.78
Martin MP[15]	2010/United States	Caucasian	Population-based	*IL28B* rs12979860	CC/CT/TT C vs T	-	157/175/52 489 vs 279	99/94/33 292 vs 160	0.77
Shi XD[32]	2011/China	Asian	Hospital-based	*IL28B* rs12979860	CC/CT/TT C vs T	19/0/0 38 vs 0	-	114/23/0 251 vs 23	>0.05[b]
Ren S[13]	2012/China	Asian	Hospital-based	*IL28B* rs12979860	CC/CT/TT C vs T	43/4/0 90 vs 4	33/7/3 73 vs 13	177/46/16 400 vs 78	0.01 [a]
Fabris C[38]	2010/Italy	Caucasian	Population-based	*IL28B* rs12979860	CC/CT/TT C vs T	164/145/35 473 vs 215	-	36/35/4 107 vs 43	0.72
Chen J[39]	2012/China	Asian	Population-based	*IL28B* rs12979860	CC/CT/TT C vs T	213/29/2 455 vs 33	-	1043/152/10 2238 vs 172	0.37
Peng LJ[40]	2011/China	Asian	Population-based	*IL28B* rs12979860	CC/CT+TT	-	206/20	574/77	>0.05[b]
Li WY[28]	2011/China	Asian	Hospital-based	*IL28B* rs12979860	CC/CT+TT	179/24	180/23	178/25	>0.05[b]
Lee DH[41]	2013/Korea	Asian	Hospital-based	*IL28B* rs12979860	C vs T	381 vs 23	973 vs 62	-	>0.05[b]
Deng GH[19]	2004/China	Asian	Hospital-based	*ERα* rs2077647	TT/TC/CC T vs C	-	246/388/108 880 vs 604	528/595/148 1651 vs 891	0.02
Zhou N[42]	2009/China	Asian	Hospital-based	*ERα* rs2077647	TT/TC/CC T vs C	23/35/22 81 vs 79	-	96/57/19 249 vs 95	0.26
Li ZX[43]	2007/China	Asian	Hospital-based	*ERα* rs2077647	TT/TC/CC T vs C	21/33/16 75 vs 65	-	73/49/15 195 vs 79	0.66
Shan KR[44]	2010/China	Asian	Population-based	*ERα* rs2077647	TT/TC/CC T vs C	26/26/8 78 vs 42	-	20/23/2 63 vs 27	0.71
Long L[45]	2009/China	Asian	Population-based	*ERα* rs2077647	TT/TC/CC T vs C	71/79/36 221 vs 151	-	70/94/7 234 vs 108	0.10
Anghel A[46]	2010/Romania	Caucasian	Hospital-based	*ERα* rs2077647	TT/TC/CC T vs C	48/57/9 153 vs 75	-	4/7/1 15 vs 9	0.16

[a]HWE was calculated by the merged data of healthy controls and self-limiting controls. [b] *P* value for HWE was extracted from original publication.
Abbreviations: SNPs, single nucleotide polymorphisms; HC, healthy control; SLC, self-limiting 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**

			OR (95% CI)			Homogeneity			Publication bias	
SNPs	Comparison	n	OR	CI	*P*	Q	*P*	I^2 (%)	*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	0.806	0.079
rs2430561	TT/AA	4	0.779	(0.487,1.245)	0.296	2.54	0.468	0.0	1.000	0.798
	TT/(TA+AA)	4	0.854	(0.554,1.318)	0.477	1.57	0.665	0.0	1.000	0.032
	AA/(TA+TT)	4	1.048	(0.392,2.801)	0.926	24.80	0.000	87.9	1.000	0.256
IL28B	C/T	4	0.698	(0.373,1.305)	0.260	10.26	0.016	70.8	1.000	0.316
rs12979860	CC/TT [a]	3	0.980	(0.476,2.018)	0.956	3.50	0.174	42.9	1.000	-[b]
	CC/(CT+TT)	5	0.821	(0.630,1.069)	0.143	7.33	0.120	45.4	1.000	0.293
	TT/(CC+CT) [a]	3	0.921	(0.453,1.873)	0.821	3.27	0.195	38.9	1.000	-[b]
ERα	T/C	5	1.678	(1.212,2.323)	0.002*	9.80	0.044	59.2	0.806	0.125
rs2077647	TT/ CC	5	4.109	(2.609,6.473)	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	0.806	0.968
	CC/(TT+TC)	5	0.301	(0.199,0.454)	0.000*	3.54	0.472	0.0	0.806	0.911

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

[b]*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[113].

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

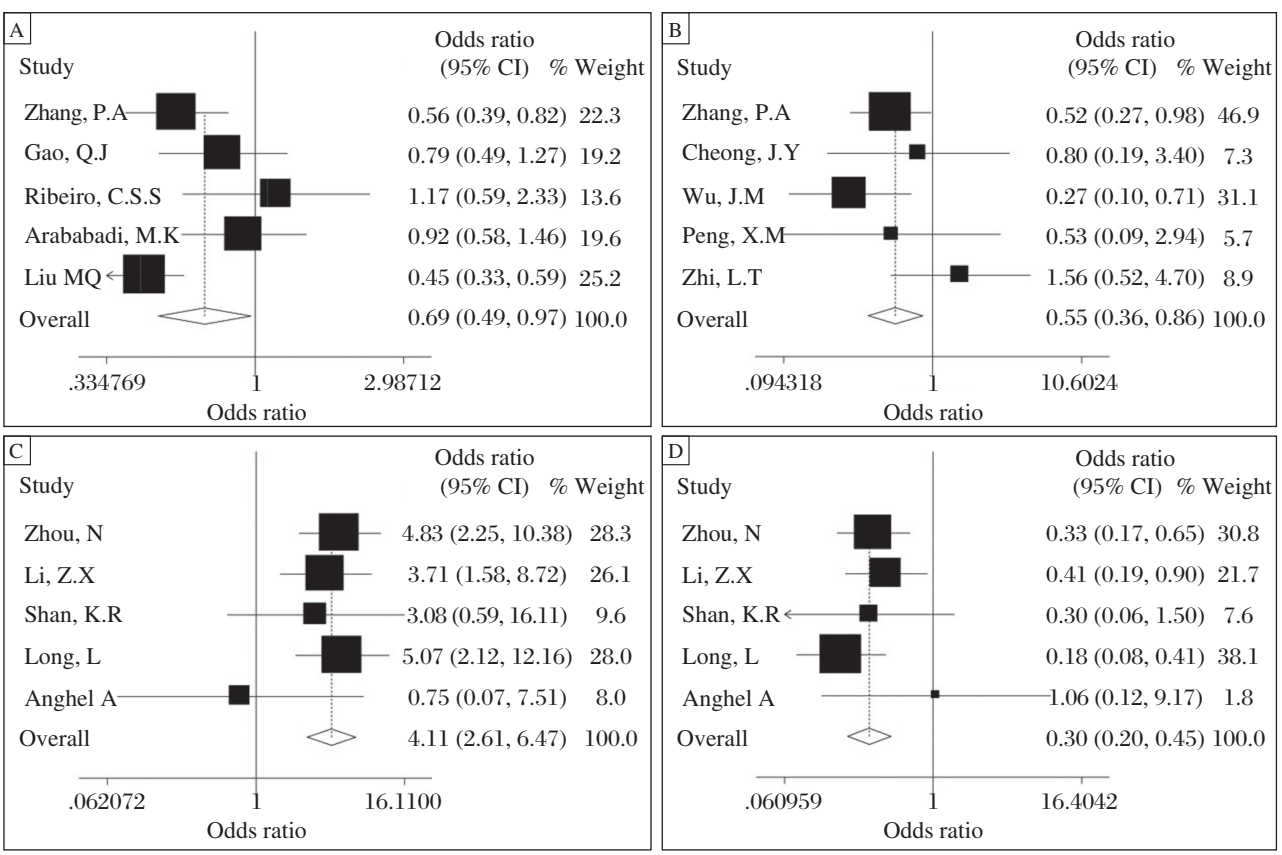

Fig. 2 **Forest plots of associations between polymorphisms and outcomes of HBV infection.** A: *IFN* rs2430561 T *vs.* A in comparison of PI and HC; B: *IFN* rs2430561 TT *vs.* AA in comparison of PI and SLC; C: *ERα* rs2077647 TT *vs.* CC in comparison of PI and HC; D: *ERα* rs2077647 CC *vs.* (TT+TC) in comparison of PI and HC. HC: healthy control; SLC: self-limiting control; PI: persistent infection.

Table 3 **Stratified analyses of *IFN-γ* rs2430561, *IL28B* rs12979860 and *ERα* rs2077647 polymorphisms on the outcomes of HBV infection**

					OR (95% CI)			Homogeneity		
	SNPs	Subgroups	Comparison	n	OR	CI	*P*	Q	P	I^2 (%)
Susceptibility to persistent infection of HBV (persistent HBV infection cases *vs.* healthy controls)	*IFN-γ* rs2430561	Asian	T/A	3	0.558	(0.407,0.764)	0.000*	4.28	0.117	53.3
		Hospital	T/A	3	0.603	(0.388,0.937)	0.024*	6.64	0.036	69.9
	IL28B rs12979860	Asian	C/T	3	0.416	(0.114,1.516)	0.184	8.31	0.016	75.9
			CC/(CT+TT)	4	0.672	(0.364,1.240)	0.204	6.87	0.076	56.3
		Hospital	CC/(CT+TT)	3	0.447	(0.137,1.462)	0.183	5.90	0.052	66.1
	ERα rs2077647	Asian	T/C	4	1.822	(1.338,2.480)	0.000*	6.74	0.081	55.5
		Asian	TT/ CC	4	4.402	(2.775,6.984)	0.000*	0.49	0.921	0.0
		Asian	TT/(TC+CC)	4	1.778	(1.004,3.149)	0.048*	11.63	0.009	74.2
		Asian	CC/(TT+TC)	4	0.287	(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	TT/ CC	3	3.838	(2.192,6.721)	0.000*	2.28	0.319	12.5
		Hospital	TT/(TC+CC)	3	2.204	(1.140,4.264)	0.019*	4.69	0.096	57.4
		Hospital	CC/(TT+TC)	3	0.387	(0.234,0.640)	0.000*	1.10	0.577	0.0
Clearance of HBV (persistent HBV infection cases *vs.* self-limiting controls) [a]	*IFN-γ* rs2430561	Asian	Since all the studies were from Asian and hospital-based, the results were the same as ***Table 3***.							
		Hospital								
	IL28B rs12979860	Asian	CC/(CT+TT)	3	0.809	(0.571,1.145)	0.231	0.35	0.838	0.0
		Hospital	CC/(CT+TT)	3	1.067	(0.713,1.596)	0.754	2.12	0.346	5.9

[a]Analysis of *ERα* 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.

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 4 **Quantitative data synthesis of individual polymorphisms, persistent hepatitis B virus infection cases versus self-limiting infection controls[a]**

			OR (95% CI)			Homogeneity			Publication bias	
SNPs	Comparison	n	OR	CI	*P*	Q	*P*	I^2 (%)	*P* for Begg's test	*P* for Egger's test
IFN-γ	T/A	5	0.779	(0.524,1.159)	0.218	23.53	0.000	83.0	0.806	0.390
rs2430561	TT/AA	5	0.555	(0.359,0.856)	0.008*	5.76	0.218	30.6	1.000	0.717
	TT/(TA+AA)	5	0.691	(0.466,1.024)	0.066	1.99	0.738	0.0	1.000	0.944
	AA/(TA+TT)	5	1.401	(0.839,2.339)	0.197	25.21	0.000	84.1	0.221	0.389
IL28B	C/T	4	1.060	(0.873,1.288)	0.557	2.37	0.499	0.0	0.734	0.633
rs12979860	CC/TT	3	1.092	(0.700,1.704)	0.697	1.49	0.475	0.0	1.000	0.855
	CC/(CT+TT)	5	1.009	(0.804,1.267)	0.937	4.21	0.378	5.0	0.462	0.346
	TT/(CC+CT)	3	0.996	(0.653,1.518)	0.984	1.20	0.549	0.0	1.000	0.395

[a]Analysis of *ERα* 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* [23]. On the other hand, cell clones from persons with chronic HBV infection produce a predominantly type 2 response[24]. *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[25]. It had been demonstrated that possession of rs2430561T and A alleles should be associated with high and low *IFN-γ* expression, respectively[7,25]. 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[26,27]. These conflicting results suggested that although HBV and HCV shared a similar natural history, pathogenesis and transmission modality[28], 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[29]. In addition, when used as a vaccine adjuvant, *IL28B* could significantly decrease splenic regulatory T cells, increase splenic and peripheral blood CD8+ T cells, and lead to increased antigen-specific perforin induction and degranulation[30]. These adaptive immune responses might be more important in HCV infection[15]. 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[31]. This sexual dimorphism might be due to lower expression of estrogen and a reduced response to the action of estrogen[32]. 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 ER*β*)[16]. The production and function of ER might be influenced by its own variation, and thus influence the diverse pathologies of chronic hepatopathy[17,18]. 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.[22] 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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