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Association Between IL-4 Polymorphisms and Risk of Liver Disease

An Updated Meta-Analysis

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Abstract: Interleukin-4 (IL-4) polymorphisms have been reported to influence an individual's susceptibility to liver disease as it is a central anti-inflammatory Th2 cytokine; however, these results remain controversial.

A comprehensive meta-analysis of the relevant literature was thus performed to better estimate the relationship between IL-4 polymorphisms and liver disease.

Systematic searches of various databases (PubMed, Embase, Cochrane Library, and China National Knowledge Infrastructure) for studies published before July 5, 2015 were performed. Odds ratios (ORs) with 95% confidence intervals (CIs) calculated in fixed or random-effects models were used to estimate the strength of the association. Subgroup analyses, meta-regression, Galbraith plots, and sensitivity analyses were also performed.

A total of 16 case–control studies, of which 15 involved the -590C/T polymorphism and 3 involved the -33T/C polymorphism, were included in the study. With respect to the -590C/T polymorphism, a significantly increased risk of liver diseases was found in the overall population (TT + CT vs CC: OR = 1.25, 95% CI = 1.06–1.49, $P = 0.009$ and CT vs CC: OR = 1.22, 95% CI = 1.00–1.48, $P = 0.048$) and the Asian population (TT + CT vs CC: OR = 1.28, 95% CI = 1.04–1.57, $P = 0.020$). Further subgroup analyses also showed significant associations between the -590C > T polymorphism and the risk of hepatitis C infection and hepatocellular carcinoma. However, no association was found between the -33T/C polymorphism and risk of liver diseases in all comparison models.

This meta-analysis suggested that the *IL-4* -590C > T polymorphism is associated with an increased risk of hepatitis C infection and hepatocellular carcinoma, especially among the Asian population.

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Abbreviations: CIs = confidence intervals, HBV = hepatitis B virus, HCC = hepatocellular carcinoma, HCV = hepatitis C virus, HWE = Hardy–Weinberg equilibrium, IL-4 = interleukin-4, LC = liver cirrhosis, ORs = odds ratios, SNP = single nucleotide polymorphism.

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INTRODUCTION

Interleukin-4 (IL-4), a multifunctional pleiotropic cytokine discovered in the mid-1980s, is one of the most frequently studied cytokines in inflammation-mediated diseases.[1] This mediator is mainly produced by activated T helper 2 (Th2) cells, although mast cells, basophils, and eosinophils are also known to secrete IL-4.[2] As a central anti-inflammatory Th2 cytokine, IL-4 is best known for defining the Th2 phenotype of lymphocytes and regulating cell proliferation and apoptosis and the gene expression of many cell types, including lymphocytes, macrophages, and fibroblasts.[3,4] Therefore, IL-4 plays important roles in both humoral and cell-mediated immunity.[1] It has been suggested that factors that could influence the expression and function of IL-4 may lead to a weakened cell-mediated immune response, thus making the host vulnerable to infections and inflammation-related diseases.[5] Such a hypothesis has been previously proved in certain *IL-4* gene polymorphisms and our recent study.[6–9]

The human *IL-4* gene is located on chromosome 5q31, within 25 kbp of the proximal portion.[10] So far, more than 50 single nucleotide polymorphisms (SNPs) of *IL-4* have been elucidated (http://www.ncbi.nlm.nih.gov/SNP/), and several epidemiological studies have proved *IL-4* gene polymorphisms to be associated with various diseases.[6–8] Among them, a change from the C to T allele at loci -590 (rs2243250) and a change from the T to C allele at loci -33 (rs2070874) have been the most studied. Significant associations between these changes and various diseases have been found, including asthma,[11] respiratory syncytial virus infection,[12] gastric cancer,[13] and nonsmall cell lung cancer,[14] among others. Further, the specific relationship between these 2 polymorphisms and liver diseases has also been investigated; the *IL-4* (-590) CT and CC genotype frequencies have been shown to be significantly higher in chronic hepatitis B virus (HBV) infected patients with abnormal alanine aminotransferase levels.[15] However, controversially, some studies have suggested that *IL-4* polymorphisms are actually not associated with the risk of liver diseases such as HBV, hepatitis C virus (HCV) infection, liver cirrhosis (LC), or hepatocellular carcinoma (HCC).[16–21]

This issue has been discussed in 2 meta-analyses published in May 2013 and August 2013,[22,23] although the results remain controversial. Cui et al[23] demonstrated no correlation between the -590C/T polymorphism and susceptibility to HBV infection. On the other hand, Zheng et al[22] suggested that the -590C > T polymorphism might increase the risk of HBV and HCV infections. At the same time, both meta-analyses failed to include all eligible studies—a study by Naslednikova et al[24]

in 2007 concerning HBV was not included in Cui et al's study,[23] while a study published in 2005[25] regarding HCC was not included in that of Zheng et al.[22] Additionally, a further 5 papers linking the -590C/T SNP and the risk of liver diseases have been published since the last meta-analysis was conducted.[9,26–29] Moreover, we also found several studies investigating the relationship between the -33T/C SNP and liver diseases in which the results were also inconsistent.[9,27,30] Thus, we performed a meta-analysis pooling all eligible studies published to date to derive a more precise estimation of the association between the -590C/T SNP and liver diseases. We also explored the potential role that the -33T/C SNP plays in liver diseases by performing the first such meta-analysis.

METHODS

This study was performed according to the Meta-Analysis of Observational Studies in Epidemiology (MOOSE) guidelines for reporting[31] and no ethics approval was needed.

Search Strategy

To identify all publications relevant to the association between *IL-4* polymorphisms and liver disease, 2 investigators (WQ and JZ) performed a comprehensive literature search of electronic databases, including PubMed, Embase, Cochrane Library, and China National Knowledge Infrastructure (CNKI), using the MeSH term ''IL-4'' in combination with the following terms: (''liver diseases'' or ''hepatitis'' or ''LC'' or ''HCC'' or ''liver injury'' or ''fatty liver'') and (''polymorphism'' or ''mutation'' or ''variant''). The articles selected were limited to studies in humans, but without restriction on time period, sample size, population, or language of the published paper. A further manual search of bibliographies cited in published articles was also carried out for potential relevant studies. The entire literature search was conducted independently by 2 researchers and the last search was updated to July 5, 2015.

Study Selection

The studies included had to meet the following criteria: evaluating the association between *IL-4* -590C/T or -33T > C polymorphisms and liver diseases; with a case–control design; with sufficient data available to estimate the odds ratios (ORs) with their 95% confidence intervals (95% CIs); and controls were a healthy population. Exclusion criteria were: assessing the association between *IL-4* and liver diseases in other SNPs; conference abstracts, case reports, editorials, review articles, meta-analyses, and letters. For articles without sufficient information for data extraction, letters would be written to contact study authors to request missing data, if no further information was obtain, the article would be excluded from our study. In the situation of dual or multiple studies were reported, the most recent or highest quality published work was chosen according to the quality assessment described below.

Data Extraction

Information from each eligible paper including the first author, year of publication, country, ethnicity, type of liver disease, genotyping method, number of cases and controls, source of control, genotype distribution in cases and controls, and *P* value for the control population in the Hardy–Weinberg equilibrium (HWE) were extracted and tabulated by 2 independent reviewers (CH and YL). In the event of differing results, a discussion with a third reviewer (ZW) was conducted to solve the discrepancies. When a study reported the results on both the -590C/T and -33T > C polymorphisms, these were treated as separate in the meta-analysis.

Quality Assessment

To evaluate the quality of studies fulfilling the inclusion criteria, a set of predetermined criteria initially derived by Thakkinstian et al[32] was used. The predetermined criteria, which cover the representativeness of cases, the credibility of controls, specimens of cases when determining genotypes, HWE in controls, and total sample size, have been previously structured as a 16-item list with scores ranging from 0 to 15 by Qin et al[33] (Table S1). This list has been widely used in the quality assessment of studies included in various meta-analyses.[34,35] As in previous meta-analyses, a study score $\geq$10 was considered to be high quality, while <10 was considered low quality.

Statistical Analysis

The strength of association between each *IL-4* polymorphism (-590C/T and -33T/C) and risk of liver diseases was estimated by calculating the pooled ORs and 95% CIs under different comparison models, including an allele model (T vs C for -590C/T and C vs T for -33T/C), a dominant model (TT + CT vs CC for -590C/T and CC + CT vs TT for -33T/C), a recessive model (TT vs CC + CT for -590C/T and CC vs TT + CT for -33T/C), and a co-dominant model (TT vs CC, CT vs CC for -590C/T and CC vs TT, CT vs TT for -33T/C). Subgroup analyses were also conducted to further evaluate the effect of the -590C/T polymorphism on the susceptibility to liver diseases in different types of liver diseases (HBV, HCV, HBV–HCV, LC, HCC, drug-induced liver injury, and biliary atresia), in different populations (Asian and Caucasian), and through different genotyping methods (eg, polymerase chain reaction–restriction fragment length polymorphism [PCR-RFLP]). For the -33T/C polymorphism, subgroup analyses were performed for different types of liver diseases (HBV and other) and in different populations (Asian and Caucasian) due to the limited number of studies included. Given that most of original studies have not took environment factors into consideration, adjustment for environmental effects were not carried out in the present analyses.

The heterogeneity between the studies was assessed by the Q test and I^2 statistics. According to the presence ($P_Q < 0.1$ or $I^2 \geq 50\%$) or absence ($P_Q \geq 0.1$ and $I^2 < 50\%$) of heterogeneity, either the Der Simonian–Laird random-effects model or the Mantel–Haenszel fixed-effects model were used to calculate the pooled ORs. If heterogeneity was detected, logistic meta-regression was performed to explore the sources of heterogeneity among studies, with the following characteristics included as covariates in the analysis: ethnicity, genotyping method, type of liver diseases, source of control, and quality score. Further, Galbraith plot analyses were also conducted to detect whether outliers were the potential major sources of heterogeneity.

To assess the stability of the results, a sensitivity analysis was performed by sequential omission of individual studies, especially those whose genotype frequencies in the control populations were deviated from the HWE, as they may generate possible bias. The HWE in the control group population was tested by using a goodness-of-fit χ^2 test. For each polymorphism, funnel plots and Egger's linear regression were used to test the publication bias ($P < 0.05$ indicated a significant publication bias). All analyses were performed with Stata software (version

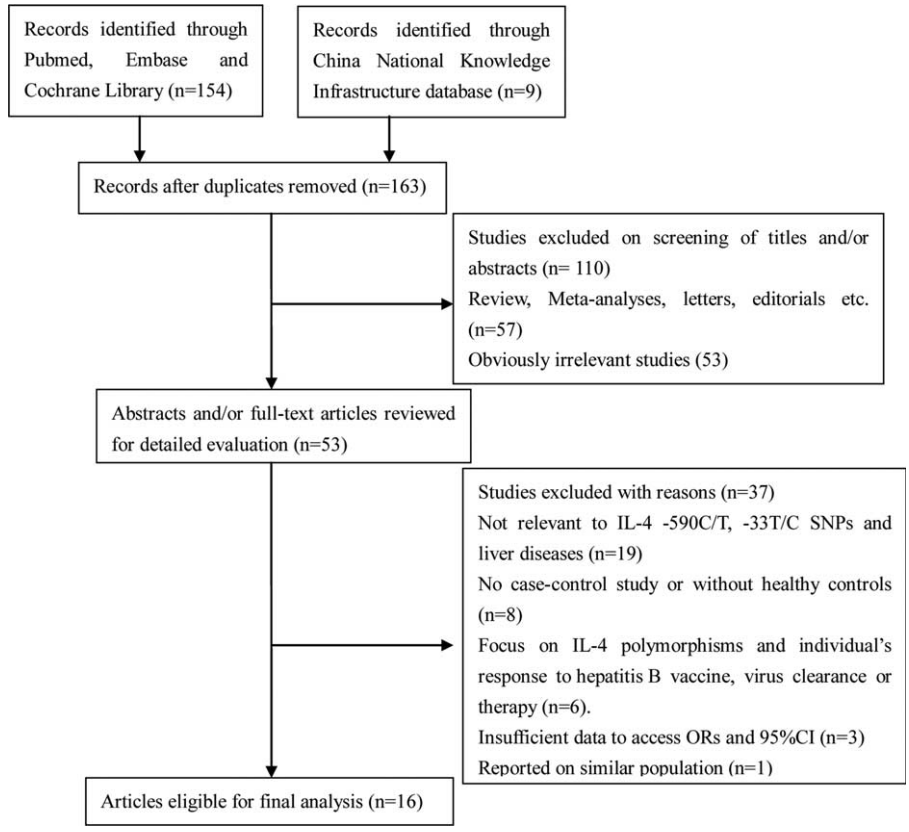

FIGURE 1. Flow diagram for the selection of articles for inclusion in the meta-analysis.

12.0, Stata Corp, College Station, TX) and all *P* values were 2-sided.

RESULTS

Study Characteristics

As shown in Figure 1, the literature search identified 163 publications (154 from PubMed, Embase, and Cochrane Library; 9 from CNKI). One duplicate was removed and, following the screening of titles and abstracts, 110 articles were rejected. After a careful abstract and/or full-text review of the remaining 53 studies, another 37 studies were excluded on the basis of the exclusion criteria mentioned previously and a list of full-text excluded articles were showed in the supporting information (Table S2). No additional eligible studies were found through manual search of the reference lists. Consequently, a total of 16 relevant studies, of which 14 were in English and 2 in Chinese, were finally included in the meta-analysis.[9,15–21,24–30,36] Of the selected studies, 2 contained data on both the -590C/T and -33T/C polymorphisms[9,27] and 4 evaluated the association in different types of liver diseases[9,15,24,26]; therefore, these were treated independently. Thus, 15 studies consisting of 3206 controls and 2441 cases (including 1106 HBV patients, 184 HCV patients, 98 HBV–HCV patients, 248 LC patients, 583 HCC patients, 169 drug-induced liver injury patients, and 53 biliary atresia patients) assessed the association in the -590C/T SNP,[9,15–21,24–29,36] and 3 studies consisting of 413 controls and 734 cases (including 260 HBV patients, 62 LC patients, 154 HCC patients, and 258 alcoholic liver disease patients) assessed the association in the -33T/C SNP.[9,26,30] Among the 16 studies, 5 were conducted in a Caucasian population and 11 in an Asian population. Only 4 studies were population-based and the rest were hospital-based studies. All included studies used blood samples for genotyping, with the majority of them using the PCR–RFLP method. The genotype distributions of the controls in 3 studies were not consistent with the HWE,[16,17,26] and such results were inconsistent with those reported by Zheng et al[22] and Saxena et al.[26] All of the studies included met the quality criteria with scores ranging from 7 to 13, 13 studies were considered as high quality and 3 were low quality. Detailed characteristics of all of the studies included in this meta-analysis are presented in Table 1.

Meta-Analysis Results

The pooled analysis suggested that the -590C/T polymorphism was significantly associated with an increased risk of liver diseases in both the dominant (TT + CT vs CC; OR = 1.25, 95% CI = 1.06–1.49, $P = 0.009$) and co-dominant models (CT vs CC; OR = 1.22, 95% CI = 1.00–1.48, $P = 0.048$). A similar situation was also found in subgroup analysis stratified by ethnicity, where in the dominant model, the -590C/T polymorphism showed a significant contribution to the risk of liver diseases in the Asian population (OR = 1.28, 95% CI = 1.04–1.57, $P = 0.020$), while a null result was noted in the Caucasian population in all genetic models. When stratified by different types of liver disease, a significantly

TABLE 1. Characteristics of Studies Included in This Meta-Analysis

Author, year	Country	Ethnicity	Liver Disease	Genotyping Method	Source of Control	Cases	Controls	Cases			Controls			HWE of Control	QS
								TT	CT	CC	TT	CT	CC		
−590C/T (rs2243250)															
Gao, 2009	China	Asian	HBV	PCR–RFLP	P-B	69	74	35	33	1	38	31	5	0.693	13
			HCV			55		28	26	1					
			HBV–HCV			79		39	38	2					
			Liver cirrhosis			24		11	13	0					
Ognjanovic, 2009	The USA	Caucasian	HCC	TaqMan assay	P-B	120	230			71			147	NR	10
Pachkoria, 2008	Spain	Caucasian	Drug-induced liver injury	TaqMan assay	P-B	145	94	4	45	96	3	23	68	0.546	12
Liu, 2007	China	Asian	Liver cirrhosis	PCR–RFLP	H-B	100	124	56	36	8	64	48	12	0.500	10
Chen, 2007	Taiwan	Asian	HCV	PCR–RFLP	H-B	72	180	43	28	1	131	45	4	0.953	12
Naslednikova, 2007	Russia	Caucasian	HBV	PCR–RFLP	H-B	24	48	7	9	8	6	28	14	0.166	10
			HCV			57		6	43	8					
			HBV–HCV			19		3	6	10					
Wang, 2006	China	Asian	HBV	PCR–RFLP	H-B	313	203	28	59	226	15	29	159	<0.001	9
Zhu, 2005	China	Asian	HBV	TaqMan assay	H-B	36	120	25	8	3	79	25	16	<0.001	7
Aithal, 2004	The UK	Caucasian	Drug-induced liver injury	PCR–RFLP	H-B	24	321	16	8	0	270	46	5	0.073	11
Add: Nieters, 2005	China	Asian	HCC	PCR-RFLP	H-B	250	250			6			12	>0.200	11
Lee, 2012	Taiwan	Asian	Biliary atresia	TaqMan assay	H-B	53	904	34	18	1	611	255	38	0.088	10
Sodsai, 2013	Thai	Asian	HBV	LIFECODES Cytokine SNP Typing kit	P-B	131	142	83	38	9	75	56	11	0.903	12
Saxena, 2014	India	Asian	HBV	PCR–RFLP	H-B	124	153	4	70	50	7	88	58	<0.001*	9
			Liver cirrhosis			62		5	39	18					
			HCC			59		3	40	16					
Lu, 2014	China	Asian	HBV	PCR–RFLP	H-B	129	170	77	43	9	115	51	4	0.550	11
			Liver cirrhosis			62		37	22	3					
			HCC			154		111	39	4					
Wu, 2015	Taiwan	Asian	HBV	Multiplex PCRs	H-B	280	193	189	81	10	124	63	6	0.554	12
−33T > C (rs2070874)															
Marcos, 2009	Spanish	Caucasian	Alcoholic liver disease	TaqMan assay	H-B	258	101	4	67	182	2	28	71	0.690	11
Sodsai, 2013	Thai	Asian	HBV	LIFECODES Cytokine SNP Typing kit	P-B	131	142	80	42	9	75	56	11	0.903	12
Lu, 2014	China	Asian	HBV	PCR–RFLP	H-B	129	170	77	42	10	113	52	5	0.737	11
			Liver cirrhosis			62		36	22	4					
			HCC			154		97	49	8					

HB = hospital-based; HBV = hepatitis B virus; HCC = hepatocellular carcinoma; HCV = hepatitis C virus; HWE = Hardy–Weinberg equilibrium in control population; NR = not report; PCR–RFLP = polymerase chain reaction–restriction fragment length polymorphism; PB = population-based; SNP = single nucleotide polymorphism; QS = quality score.

*The result of P value for HWE calculated by us were inconsistent with the original result reported by Saxena et al, but they did not provided a detailed *P* value for HWE.

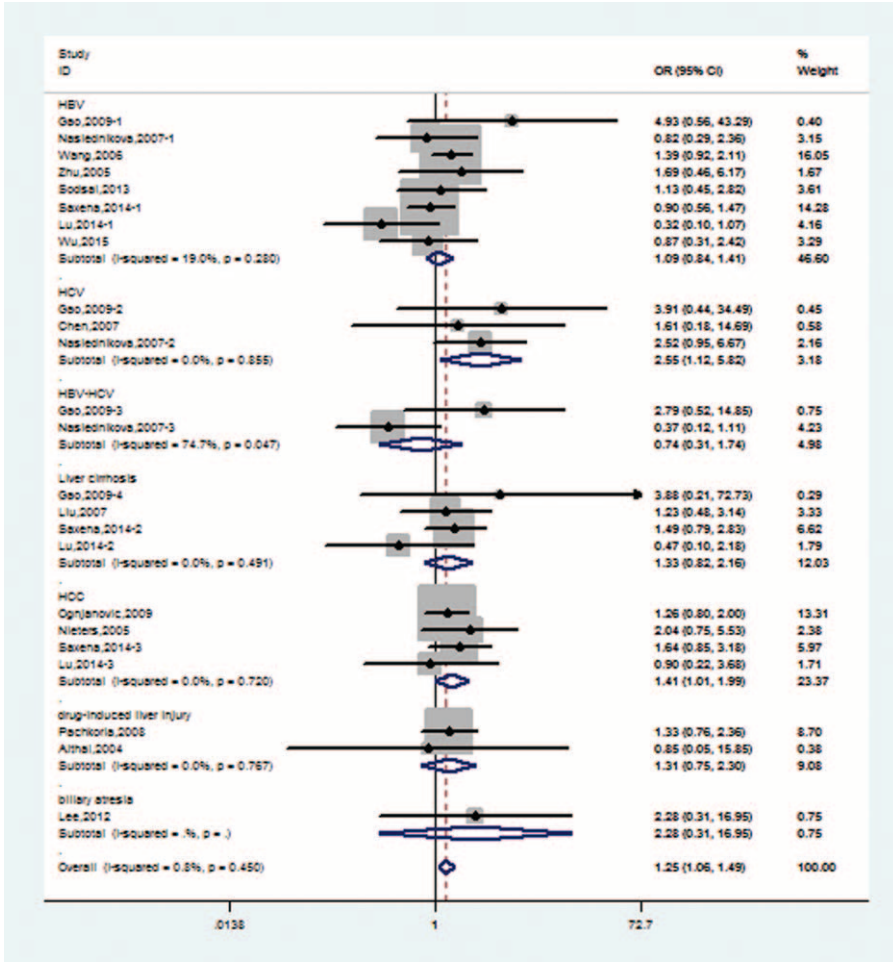

FIGURE 2. Forest plots of interleukin-4 -590C/T polymorphisms and liver disease's risk in dominant model TT + CT versus CC (fixed-effects model, stratified by liver diseases).

increased risk was also found in HCV in the same 2 models (OR = 2.55, 95% CI = 1.12–5.82, $P = 0.026$ and OR = 2.87, 95% CI = 1.24–6.63, $P = 0.014$, respectively) and in HCC in the dominant model only (OR = 1.41, 95% CI = 1.01–1.99, $P = 0.046$) (Figures 2 and 3). Finally, a significantly increased risk was found only in the PCR–RFLP group in the dominant model when stratified by genotyping methods. The details are presented in Table 2.

With respect to the -33T/C polymorphism, the results of pooling all studies showed that this polymorphism was not associated with liver disease risk in all genetic models. In the subgroup analysis stratified by different types of liver diseases and different populations, similar insignificant results were also noted in all comparison models. Details are presented in Table 3.

Heterogeneity Analysis

For both -590C/T and -33T/C polymorphisms, there was no between-study heterogeneity when all eligible studies were pooled into meta-analysis in all comparison models (with all $I^2 < 50\%$ and $P_Q \geq 0.1$); thus, the fixed-effects model was used to pool the overall results. However, an obvious significant between-study heterogeneity was observed when subgroup analysis was conducted in the T versus C, TT + CT versus CC, and CT versus CC models in the -590C/T polymorphism, as well as in the C versus T, CC + CT versus TT, and CC versus TT models in the -33T/C polymorphism. We therefore performed meta-regression analyses and Galbraith plot analyses in these comparison models.

For the -590C/T SNP, a meta-regression analysis of the data showed that ethnicity, genotyping method, type of liver disease, source of control, and quality score were not effective modifiers in TT + CT versus CC and CT versus CC models, but quality score might be a effective modifier in T versus C model ($P = 0.041$). Galbraith plot analysis indicated that, in the T versus C model, the study by Lu et al[9] on HBV was the outlier and main contributor to heterogeneity in this comparison model (Figure 4A). A forest plot omitting the outlier study was conducted; however, the insignificance of the ORs was not altered and heterogeneity in the subgroup of drug-induced liver injury remained the same ($I^2 = 73.9\%$, $P_Q = 0.05$). For the TT + CT versus CC model, the Galbraith plot analysis indicated that the study of Lu et al[9] on HBV and that of Nasledn kova et al[24] on HBV–HCV were the outliers (Figure 4B). After the forest plot omitting the outlier studies was conducted, the significance of the ORs was not altered and there was no

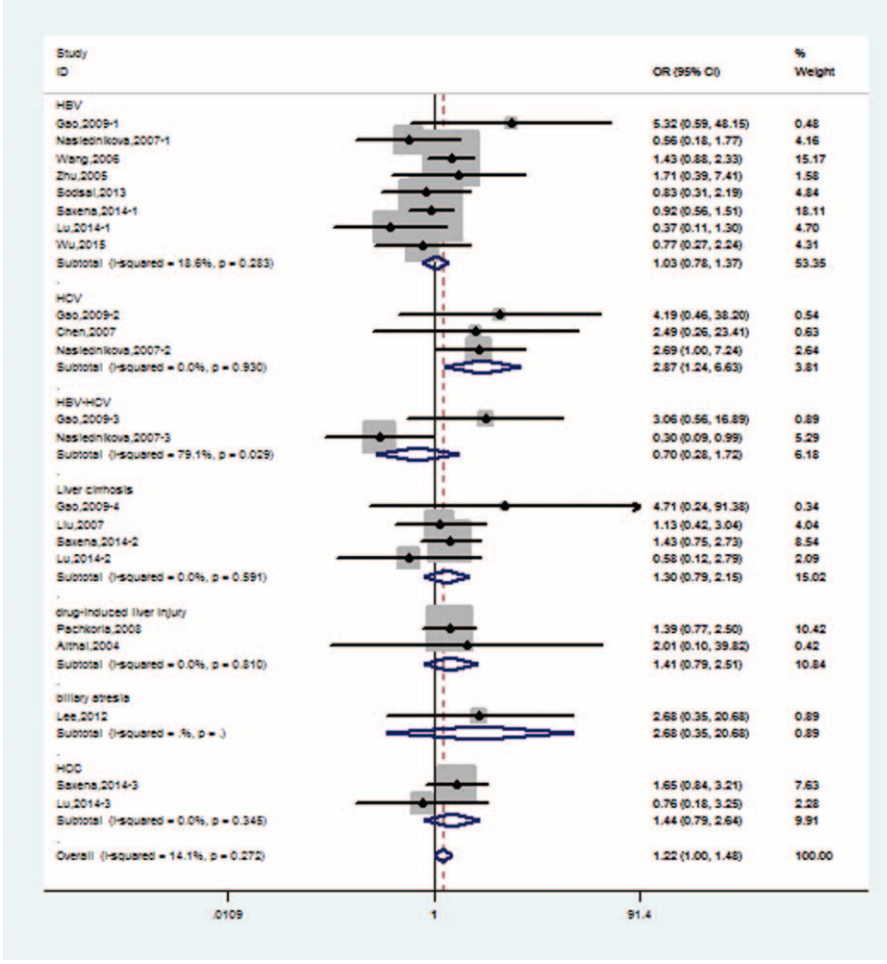

FIGURE 3. Forest plots of interleukin-4 -590C/T polymorphisms and liver disease's risk in co-dominant model CT versus CC (fixed-effects model, stratified by liver diseases).

evidence of heterogeneity in the overall populations or in any of the subgroup analyses (OR = 1.34, 95% CI = 1.12–1.59, $P = 0.001$, $I^2 = 0.0\%$, $P_Q = 0.900$ for the overall analysis; data for other subgroups is not shown). However, for the HBV–HCV subgroup analysis, heterogeneity could not be assessed since only 1 study remained following the exclusion of that by Naslednikova et al.[24] With respect to the CT versus CC model, the result of Galbraith plot analysis indicated that the study by Naslednikova et al on HBV–HCV was the major source of heterogeneity (Figure 4C). The I^2 values decreased significantly and P_Q values were >0.10 following exclusion of the study from the subgroup analysis of the Caucasian population ($I^2 = 27.6\%$, $P_Q = 0.246$). Further, the significance of the ORs for this compared model in the overall population and subgroup analyses did not change through the omission of this study. Similarly, heterogeneity in the HBV–HCV subgroup analysis could not be calculated as only 1 study remained.

For the -33T/C polymorphism, meta-regression analysis of data showed that none of the covariates listed above were effect modifiers in any of the compared models. Galbraith plot analysis also showed no outliers in the -33T/C C versus T, CC + CT versus TT, and CC versus TT models (Figure 5A–C). The main cause of heterogeneity in the subgroup analysis of these models may be attributed to the limited number of studies included.

Sensitivity Analysis

As the genotype frequencies of the control group in 3 studies deviated significantly from HWE in the -590C/T SNP,[16,17,26] the influence of each individual study in the meta-analysis of the pooled ORs was examined by rerunning the meta-analysis with the exclusion of 1 study each time. The significance of ORs was not influenced excessively through the omission of any single study in all compared models (data not shown), except for the CT versus CC model, in which, following the individual exclusion of the Wang Kun et al,[17] Zhu et al,[16] and Saxena et al[26] studies, the overall OR became insignificant (OR = 1.18, 95% CI = 0.95–1.46, $P = 0.128$; OR = 1.21, 95% CI = 0.99–1.48, $P = 0.058$ and OR = 1.22, 95% CI = 0.96–1.56, $P = 0.102$, respectively). For the -33T/C polymorphism, there were no studies whose control populations deviated from HWE; however, a sensitivity analysis of sequential omission of individual studies was also performed. Nevertheless, no individual studies were seen to significantly affect the pooled ORs.

TABLE 2. Meta-Analysis and Heterogeneity Test of the Interleukin-4 −590C/T Polymorphism and Liver Disease Risk

	Allele Model (T vs C)			Dominant Model (TT + CT vs CC)			Recessive Model (TT vs CC + CT)			Co-Dominant Model (TT vs CC)			Co-Dominant Model (CT vs CC)		
Subgroups	**OR (95% CI)**	***P***	P_Q	**OR (95% CI)**	***P***	P_Q	**OR (95% CI)**	***P***	P_Q	**OR (95% CI)**	***P***	P_Q	**OR (95% CI)**	***P***	P_Q
Liver diseases															
HBV	1.09 (0.94–1.26)	0.265	0.228	1.09 (0.84–1.41)	0.503	0.280	1.12 (0.91–1.37)	0.299	0.284	1.16 (0.78–1.72)	0.523	0.219	1.03 (0.78–1.37)	0.816	0.283
HCV	0.97 (0.71–1.31)	0.821	0.152	**2.55 (1.12–5.82)**	**0.026**	0.855	0.72 (0.47–1.09)	0.115	0.449	2.01 (0.71–5.72)	0.190	0.791	**2.87 (1.24–6.63)**	**0.014**	0.930
HBV–HCV	0.92 (0.60–1.40)	0.684	0.304	0.92 (0.13–6.62)*	0.932*	0.047	0.97 (0.54–1.75)	0.926	0.673	1.32 (0.43–4.01)	0.624	0.276	0.88 (0.09–8.61) *	0.915*	0.029
Liver cirrhosis	1.07 (0.84–1.36)	0.594	0.341	1.33 (0.82–2.16)	0.255	0.491	0.98 (0.69–1.39)	0.907	0.407	1.34 (0.69–2.60)	0.394	0.369	1.30 (0.79–2.15)	0.302	0.591
HCC	1.22 (0.90–1.65)	0.204	0.768	**1.41 (1.01–1.99)**	**0.046**	0.720	1.22 (0.78–1.92)	0.383	0.894	1.20 (0.43–3.35)	0.723	0.646	1.44 (0.79–2.64)	0.231	0.345
Drug-induced liver injury	0.81 (0.32–2.03) *	0.652*	0.050	1.31 (0.75–2.30)	0.337	0.767	0.48 (0.22–1.06)	0.069	0.359	0.89 (0.23–3.48)	0.862	0.839	1.41 (0.79–2.51)	0.244	0.810
Biliary atresia	0.96 (0.58–1.59)	0.885	–	2.28 (0.31–16.95)	0.420	–	0.86 (0.48–1.53)	0.604	–	2.11 (0.28–15.87)	0.466	–	2.68 (0.35–20.68)	0.344	–
Ethnicity															
Caucasian	1.05 (0.79–1.39)	0.732	0.171	1.20 (0.89–1.63)	0.234	0.193	0.89 (0.52–1.53)	0.668	0.122	1.25 (0.61–2.56)	0.538	0.828	1.01 (0.47–2.20) *	0.971*	0.047
Asian	1.06 (0.96–1.18)	0.255	0.376	**1.28 (1.04–1.57)**	**0.020**	0.543	1.01 (0.87–1.16)	0.943	0.534	1.23 (0.92–1.66)	0.165	0.636	1.24 (0.99–1.55)	0.058	0.543
Genotype method															
PCR–RFLP	1.02 (0.91–1.15)	0.681	0.195	**1.24 (1.01–1.53)**	**0.038**	0.184	0.92 (0.77–1.09)	0.340	0.381	1.22 (0.89–1.68)	0.212	0.598	1.22 (0.98–1.52)	0.080	0.137
Other	1.18 (0.97–1.43)	0.105	0.824	1.27 (0.94–1.72)	0.116	0.950	1.18 (0.92–1.52)	0.192	0.607	1.27 (0.74–2.17)	0.385	0.914	1.22 (0.80–1.85)	0.353	0.687
Overall	1.06 (0.96–1.17)	0.236	0.314	**1.25 (1.06–1.49)**	**0.009**	0.450	1.00 (0.86–1.15)	0.965	0.372	1.24 (0.94–1.63)	0.129	0.824	**1.22 (1.00–1.48)**	**0.048**	0.272

CI = confidence interval; HBV = hepatitis B virus; HCC = hepatocellular carcinoma; HCV = hepatitis C virus; OR, odds ratio; PCR–RFLP = polymerase chain reaction–restriction fragment length polymorphism; P_Q, *P* value of heterogeneity test.

*Estimates for random-effects model.

$P < 0.05$ was considered a statistical significance.

TABLE 3. Meta-Analysis and Heterogeneity Test of the Interleukin-4 −33T/C Polymorphism and Liver Disease Risk

	Allele Model (C vs T)			Dominant Model (CC + CT vs TT)			Recessive Model (CC vs TT + CT)			Co-Dominant Model (CC vs TT)			Co-Dominant Model (CT vs TT)		
Subgroups	**OR (95% CI)**	***P***	**P_Q**	**OR (95% CI)**	***P***	**P_Q**	**OR (95% CI)**	***P***	**P_Q**	**OR (95% CI)**	***P***	**P_Q**	**OR (95% CI)**	***P***	**P_Q**
Liver diseases															
HBV	1.05 (0.59–1.88)*	0.861*	0.037	0.98 (0.53–1.81)*	0.946*	0.068	1.42 (0.72–2.82)	0.310	0.115	1.45 (0.39–5.39)*	0.580*	0.070	0.92 (0.64–1.31)	0.632	0.151
Other	1.21 (0.94–1.56)	0.132	0.712	1.26 (0.88–1.79)	0.208	0.865	1.26 (0.81–1.95)	0.300	0.480	1.89 (0.86–4.12)	0.111	0.834	1.18 (0.81–1.70)	0.388	0.893
Ethnicity															
Caucasian	1.08 (0.69–1.70)	0.732	–	1.26 (0.23–6.98)	0.793	–	1.08 (0.65–1.80)	0.758	–	1.28 (0.23–7.15)	0.777	–	1.20 (0.21–6.91)	0.841	–
Asian	1.15 (0.93–1.41)	0.195	0.133	1.10 (0.86–1.41)	0.451	0.206	1.61 (0.94–2.76)	0.083	0.405	1.59 (0.92–2.75)	0.094	0.261	1.03 (0.80–1.33)	0.823	0.366
Overall	1.13 (0.94–1.37)	0.186	0.227	1.10 (0.86–1.41)	0.434	0.332	1.31 (0.90–1.88)	0.154	0.404	1.56 (0.93–2.63)	0.091	0.398	1.03 (0.80–1.33)	0.802	0.525

CI = confidence interval; HBV = hepatitis B virus; OR = odds ratios; P_Q = *P* value of heterogeneity test.
*Estimates for random-effects model.

Publication Bias

To assess possible publication bias, Begg's funnel plots and Egger's tests were performed. The funnel plots for both -590C/T and -33T/C SNPs were symmetrical, indicating no significant publication bias in any of the genetic models (Figure 6). Egger's test, with *P* values >0.05, also revealed no evidence of publication bias in the meta-analysis (Figure 6).

DISCUSSION

Nowadays, liver diseases are increasingly common, in particular HBV and HCV infections,[37] the high prevalence of which is considered the most frequent cause of liver disease. Further, toxins, such as alcohol and drugs, and genetic, vascular, or biliary disorders which may cause liver cell damage can also lead to acute or chronic liver diseases.[38] Although the precise pathogenic mechanisms responsible for these diseases are not fully elucidated, epidemiological and clinical evidence has suggested a link between the immune response and various liver diseases, including HBV infection, HCC, chronic HCV, and immune-mediated drug-induced liver injury.[36,39–42] Since the normal function of the immune system depends on a genetically determined balance between Th1 and Th2 lymphocytes,[24] the role of IL-4, as a critical mediator of this balance, is of major importance. Previous studies have shown that the -590C > T polymorphism affects IL-4 secretion[43,44] and that the mutant T allele of -33T/C can alter IL-4 expression in both patients with bronchial asthma and healthy controls.[45] Taken together, these results led to the speculation that *IL-4* genetic mutations may alter the susceptibility to liver diseases by influencing the expression and function of IL-4.

Such potential association between the *IL-4* polymorphisms and the risk of liver diseases has aroused great attention, leading to many studies being devoted to this topic, albeit with inconsistent results. This meta-analysis was therefore performed to elucidate and provide a quantitative reassessment of the association. To our knowledge, this is the most comprehensive meta-analysis to date investigating the association between -590C > T and -33T/C polymorphisms and the risk of various liver diseases. The results presented herein suggest that the -590C/T polymorphism might increase the risks of liver disease, especially in the Asian population. Further subgroup analyses also showed significant associations between the -590C > T polymorphism and increased risk of HCV infection and HCC. However, these findings were partially inconsistent with those of Zheng et al,[22] who indicated that the -590C > T polymorphism may increase the risk of HBV and HCV infections, yet their subgroup analyses showed this polymorphism to be associated with an increased risk among Caucasian populations.

The possible factors leading to such controversial results may include the relatively small sample sizes used in previous studies and errors in the data reported. As mentioned above, both meta-analyses failed to include all eligible studies,[22,23] which may have led to a database and publication bias and may have further distorted the results of the meta-analyses. Further, several novel case–control studies focusing on the association of *IL-4* polymorphisms with liver disease susceptibility have emerged since the publication of the studies by Zheng et al[22] and Cui et al[23]; these have been included into the present study. Additionally, during the process of data extraction, we found that the data presented by Zheng et al[22] for the study by Naslednikova et al[24] was not accurately reported. The number of patients actually indicated to have shown an association

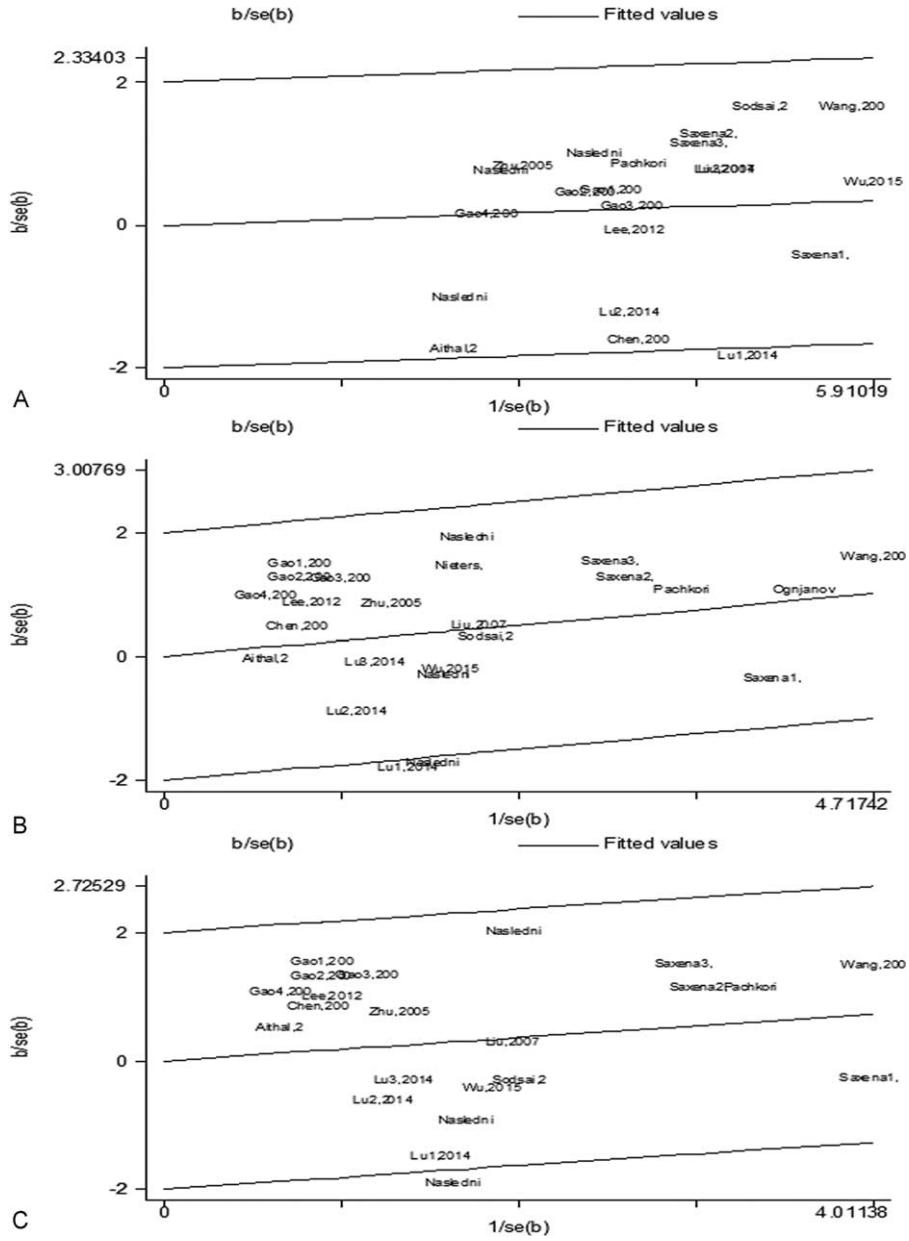

FIGURE 4. Galbraith plots of interleukin-4 -590C/T polymorphisms and risk of liver disease in different contrast models. (A) The studies of Lu et al on HBV was outlier in the contrast T versus C; (B) The study of Lu et al on HBV and that of Naslednikova et al on HBV–HCV were the outliers in the contrast TT + CT versus CC; (C) The studies of Naslednikova et al on HBV–HCV was the outlier in the contrast CT versus CC. HBV = hepatitis B virus; HCV = hepatitis C virus.

between the -590C > T polymorphism and risk of HBV, HCV, and HBV–HCV co-infection was 24, 57, and 19, respectively. However, Zheng et al[22] extracted these as 25, 56, and 17, which in fact corresponded to the number of patients showing an association between the *IL-2* T330G polymorphism and risk of these chronic viral hepatitides in the same study. The last but not the least, the different Chinese database we searched may lead to a selection bias,[46] of which might further responsible for our significance findings with Asian populations. However, when considering both 2 Chinese literatures enrolled in the present study were also included in studies by Zheng et al[22] and Cui et al,[23] we exclude such a possibility.

Increasing evidence has suggested that IL-4 may be associated with HCC as well as HCV. As is well known, HCC is an example of an inflammation-related cancer, and M2—the alternatively activated macrophage—has been proved to play a pivotal role in the tumor progression of such inflammation-related cancers.[47] Since IL-4 and IL-13 are the archetypal inducers of M2,[1] it is not surprising that IL-4 might participate in this carcinogenetic process. In addition, IL-4 can also promote the development of specific immune cell subtypes, such as macrophages, B cells, CD4 + Th2 cells, and CD8+ T cells, which produce more IL-4.[48] IL-4 then induces macrophages to polarize into M2, thus further contributing to

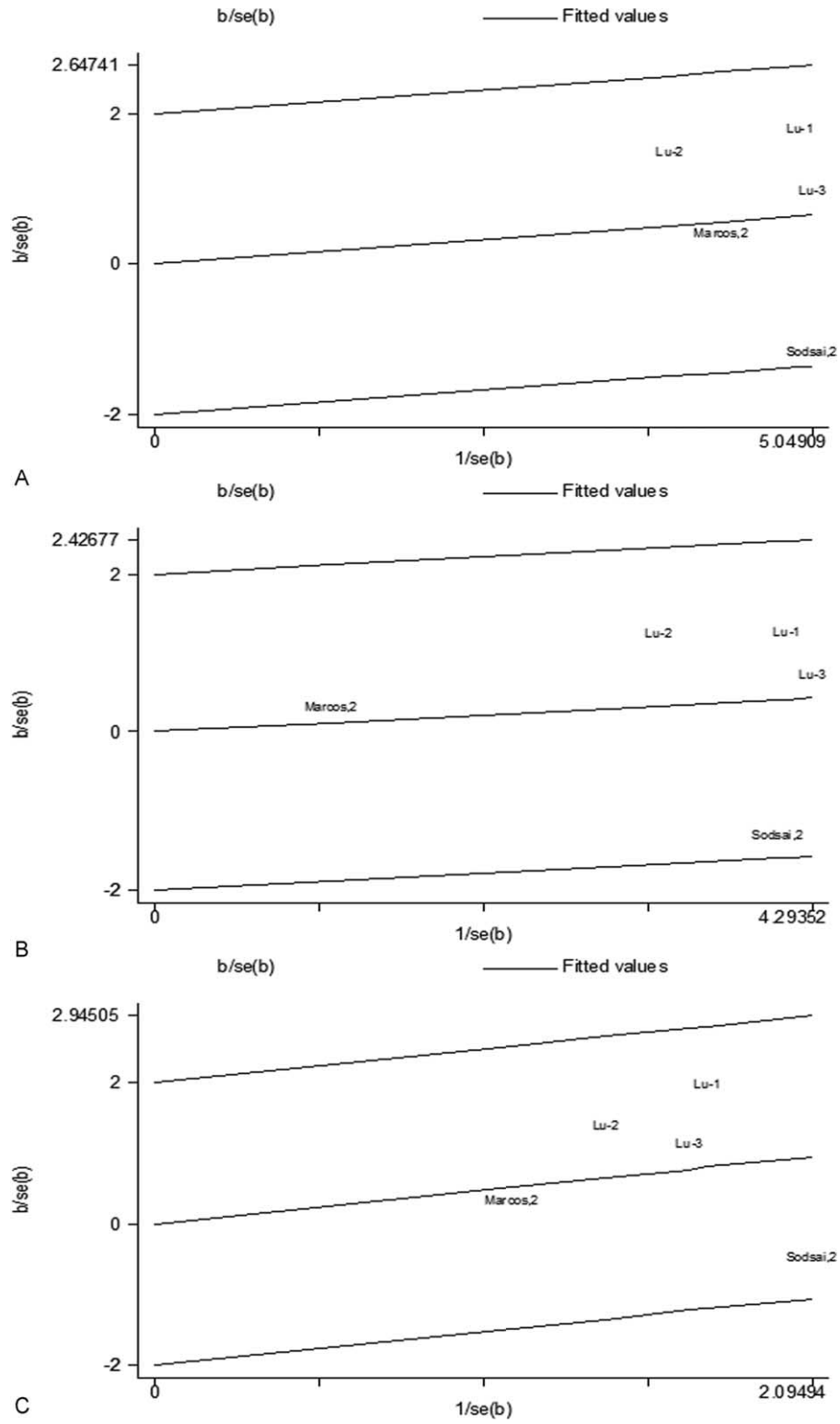

FIGURE 5. Galbraith plots of interleukin-4 -33T/C polymorphisms and risk of river disease in different contrast models. No outlier was found in: (A) C versus T model; (B) CC + CT versus TT model; and (C) CC versus TT model.

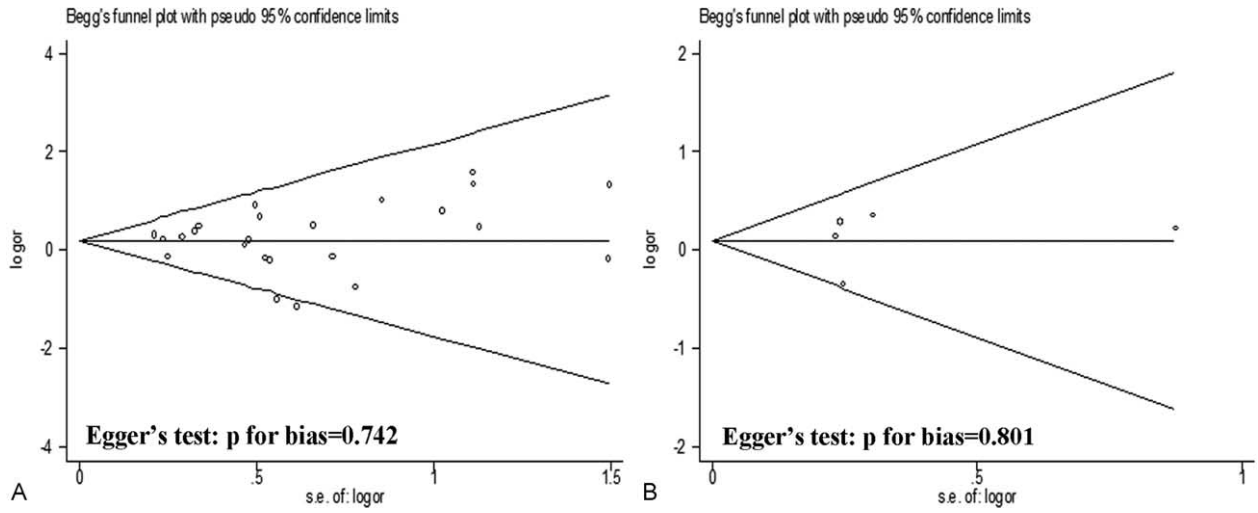

FIGURE 6. Funnel plot analysis and Egger's test to detect publication bias. Each point represents a separate study for the indicated association. (A) Funnel plot for contrast TT + CT versus CC of -590C/T polymorphism in overall analysis and (B) Funnel plot for allele contrast CC + CT versus TT of -33T/C polymorphism in overall analysis.

the development of HCC. Additionally, IL-4 is a key Th2 cytokine. Th1/Th2 imbalances have long been shown to play an important role in the establishment of chronic viral infections in humans,[49] and excess Th2 production has been shown to counteract the Th1 effect, leading to a reduced antiviral state,[50] and thus increasing the susceptibility to HCV infection. Consequently, it is not surprising that *IL-4* polymorphisms might be associated with risks of HCC and HCV, since polymorphisms of this gene have been shown to alter its expression.[43–45] However, since HBV infection also a classical viral infections, but no association was found between the -590C/T polymorphism and the disease risk, such result was in agreement with Cui et al.[23] Furthermore, no significant relationships between the -33T/C polymorphism and risk of various liver diseases were found, which may be attributed to the limited number of studies included or to the fact that the -33T/C polymorphism may not play any facilitative role in the development of liver disease. Another explanation is that, since the -33T/C SNP has been found in linkage disequilibrium with -590C/T SNP,[51] its real role may be masked by the -590C/T SNP.

Similar to previous meta-analyses, our study had some limitations. The most noteworthy point—language bias, may exist as we only included studies in English and Chinese. According to Pan et al,[46] the impact of language biases on meta-analyses of observational studies may be as large as or even larger than its impact on randomized evidence, the lack of appropriately captured for a global, inclusive outlook in genetic epidemiologic studies may result in the meta-analyses assessing evidence for variant implication an opportunistic results. Second, only 3 studies assessed the association in the -33T/C SNP, and therefore the sample size was relatively small and may not have provided sufficient statistical power. Third, the overall results of our study were based on crude ORs as researchers do not always make the same decisions concerning confounding factors, although a more precise evaluation should adjust for possible risk factors such as age, sex, smoking, and drinking status. Fourth, because no attempts were made to obtain unpublished studies, publication bias may exist, although Begg's funnel plots and Egger's test results did not reveal any. Fifth, as most studies were conducted in Asian and Caucasian populations, the relative lack of ethnic diversity demands further studies.

Aside from its limitations and its very preliminary nature, this meta-analysis suggests that the *IL-4* -590C > T polymorphism was associated with an increased risk of liver diseases, especially in the Asian population. A similar association was also found in subgroup analyses for HCV infection and HCC. However, no association was found between the -33T/C polymorphism and risk of liver diseases. Considering the limitations mentioned above, we believe that further investigations remain to be done to confirm our results.

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