REVIEW ARTICLE

Tumour necrosis factor alpha (*TNF-α*) genetic polymorphisms and the risk of autoimmune liver disease: a meta-analysis

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Abstract

Epidemiological studies have evaluated the association between tumour necrosis factor alpha (*TNF-α*)-308G/A and (*TNF-α*)-238G/A polymorphisms, and the risk of autoimmune liver disease (AILD), yet the results are conflicting. To derive a more precise estimation of the relationship, we performed this meta-analysis. A systematic review was conducted to identify all eligible studies of *TNF-α* polymorphisms and AILD risk. We used odds ratios (ORs) with 95% confidence intervals (CIs) to assess the strength of the association between the two *TNF-α* polymorphisms and AILD risk. A total of 15 eligible studies were identified. Overall, positive associations of -308G/A polymorphism with AILD risk were found (A vs G allele: OR = 1.45, 95%CI = 1.13–1.86; AA vs GG: OR = 2.74, 95%CI = 1.51–4.96; GA vs GG: OR = 1.46, 95%CI = 1.11–1.92; dominant model: OR = 1.57, 95%CI = 1.18–2.10; recessive model: OR = 2.22, 95%CI = 1.31–3.76). In subgroup analysis by ethnicity, a significantly higher risk was found in Caucasians. In subgroup analysis by AILD category, significant association was observed in autoimmune hepatitis and primary sclerosing cholangitis, especially in Caucasians. Patients carrying *TNF-α*-238A allele had a slightly decreased risk of developing AILD (OR = 0.65, 95%CI = 0.48–0.87). However, we found both *TNF-α* polymorphisms were not associated with primary biliary cirrhosis risk, even in subgroup analysis. Our meta-analysis suggests that the *TNF-α*-308G/A and -238G/A polymorphisms may contribute to AILD susceptibility in Caucasians, especially for autoimmune hepatitis and primary sclerosing cholangitis. Nevertheless, we found both *TNF-α* polymorphisms were unlikely to be associated with the risk of primary biliary cirrhosis.

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Introduction

Autoimmune liver disease (AILD) is a series of immune-mediated liver injury disease, giving rise to varied clinical presentations, which are characterized by positive circulating autoantibodies, high level of immunoglobulin G (IgG), necroinflammatory histology on liver biopsy, and an excellent response to immunosuppressive agents (Washington 2007; Della *et al.* 2012; Kumari *et al.* 2013). AILD includes three major categories: autoimmune hepatitis (AIH), primary biliary cirrhosis (PBC), and primary sclerosing cholangitis (PSC) (Trivedi and Hirschfield 2012). These diseases are assumed to be associated with the interaction between multiple genetic predisposition and environmental factors, although the details have not been fully understood yet (Gershwin *et al.* 2005; Czaja 2008; Lleo 2008; Yoshizawa *et al.* 2011; Karlsen 2012). Susceptibility could be partially explained by genetic variations like single nucleotide polymorphisms (SNPs) in susceptibility genes.

Cytokines and chemokines play critical roles in pathogenesis of autoimmune diseases. Amongst these, tumour necrosis factor alpha (TNF-α), derived from monocytes or lymphocytes, is a key cytokine with pleiotropic biological effects that is primarily focussed on the promotion of a strong inflammatory response (Beutler 1999; Commins *et al.* 2010). Overexpression of TNF-α in AILD sera was reported, and the level of the TNF-α expression correlated directly to the degree of progression of AILD, which indicated that TNF-α plays an important role in the pathogenesis of AILD (Neuman *et al.* 2002; Barak *et al.* 2009). The *TNF-α* gene

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encoding TNF-α is located on chromosome 6p21.3 band, within the HLA class III region, belonging to the TNF superfamily (Yahagi *et al.* 2007). Two polymorphisms, in particular at positions -308 and -238 of *TNF-α* gene promoter region (-308G/A, rs1800629 and -238G/A, rs361525), have been shown to alter the expression of *TNF-α* gene (Wilson *et al.* 1997; Louis *et al.* 1998). Recently, two polymorphisms have been widely investigated for involvement in susceptibility to a range of autoimmune disease, including systemic lupus erythematosus (Lin *et al.* 2009; Farid *et al.* 2011), rheumatoid arthritis (Mosaad *et al.* 2011; Rodriguez-Rodriguez *et al.* 2011), multiple sclerosis (Ristic *et al.* 2007; Akcali *et al.* 2010). The hypothesis that *TNF-α* polymorphisms may have important roles in the risk of AILD was also debated. Many studies have been done to investigate the potential relationship between *TNF-α* gene polymorphisms and AILD (Bernal *et al.* 1999; Cookson *et al.* 1999; Czaja *et al.* 1999; Gordon *et al.* 1999; Jones *et al.* 1999; Tanaka *et al.* 1999; Bathgate *et al.* 2000; Bittencourt *et al.* 2001; Mitchell *et al.* 2001; Bittencourt *et al.* 2003; Fan *et al.* 2004; Ma and Qiu 2004; Poupon *et al.* 2008; Niro *et al.* 2009; Juran *et al.* 2010). However, conflicting results have also been reported. Therefore, we performed a meta-analysis of all eligible studies to evaluate the association between the two *TNF-α* polymorphisms and the risk of AILD. To our knowledge, this is the most comprehensive meta-analysis on the association between AILD susceptibility and the *TNF-α*-308G/A and *TNF-α*-238G/A polymorphisms.

Methods

Identification and eligibility of relevant studies

We performed a comprehensive systematic electronic search of the EMBASE, PubMed and Web of Science databases for original articles (last search update: Feb 2013), using the following combinations of keywords and search terms: 'TNF', 'tumour necrosis factor', 'polymorphism', 'variant', 'AILD', 'autoimmune liver disease', 'AIH', 'autoimmune hepatitis', 'PBC', 'primary biliary cirrhosis', 'PSC' and 'primary sclerosing cholangitis'. All searches were limited to human subjects without language, study design and publication status restrictions. Reference lists of all retrieved primary articles were reviewed manually for any additional relevant studies. Studies included in our meta-analysis had to meet the following inclusion criteria: (i) case–control studies; (ii) evaluation of the *TNF-α*-308G/A or *TNF-α*-238G/A polymorphism with the susceptibility to AILD; (iii) sufficient data about genotype frequency of cases and controls or could be calculated from the article text. Any letters, case reports, reviews and conference abstracts were excluded because of limited data. Publications identified as duplicate reports were eliminated so that each report was unique. Studies without controls, or studies that did not show genotypes frequency, or family-based design studies were also excluded from this meta-analysis.

Data extraction

Two reviewers independently extracted the data from all eligible studies on the basis of the inclusion and exclusion criteria. If there was any conflicting evaluation between the two reviewers, they would check the data again and have a discussion to make an agreement. If they could not reach an agreement, a third investigator was invited to the discussion. For each study, the following data were extracted if available: first author, year of publication, country of origin, ethnicity, total number of cases and controls, the genotypic distribution of *TNF-α*-308G/A and *TNF-α*-238G/A polymorphisms in cases and controls, genotyping method and *P* value for control population in Hardy–Weinberg equilibrium (HWE).

Statistical analysis

Statistical analysis was performed using the software STATA (ver. 10.1, Stata Corporation, USA). The risk of AILD associated with the -308G/A and -238G/A polymorphisms of *TNF-α* gene was measured by overall odds ratio (OR) and 95% confidence interval (95% CI). In primary analysis, we mainly examined the overall effect for the polymorphisms. Two-sided *P* values less than 0.05 were considered statistically significant. The statistical significance of the summary OR was evaluated with the *Z*-test. For the -308G/A polymorphism, we first estimated the risks of the variant alleles with wild allele (A vs G allele), and then accessed the comparison of variant homozygote and heterozygote compared with the wild-type homozygote (AA vs GG; AG vs GG). Subsequently, we compared the variant homozygote with variant heterozygote (AA vs AG). After that, according to the ORs of the three comparisons, we chose the dominant model (AA + AG vs GG) or the recessive model (AA vs AG + GG) as the main approach of our analysis. For the -238G/A polymorphism, we evaluated the same effects in AILD.

Heterogeneity among included studies was checked using the chi-square-based *Q*-statistics and was regarded to indicate statistical significance for $P < 0.1$ (Zintzaras and Ioannidis 2005). If $P > 0.1$, it indicated that heterogeneity between studies was not significant, the summary ORs of included studies were calculated by the fixed-effects model (the Mantel-Haenszel method; Mantel and Haenszel 1959). Otherwise, we used a random effects model (the DerSimonian and Laird method; DerSimonian and Laird 1986) to calculate the summary ORs. We also measured the effect of heterogeneity using a quantitative measure, $I^2 = 100\% \times (Q-df)/Q$ (Higgins and Thompson 2002). I^2 ranges between 0 and 100%, and represents the percentage of variability among summary indices that was caused by heterogeneity rather than chance (Higgins *et al.* 2003). Heterogeneity was deemed apparent if I^2 was greater than 50%.

HWE in control group population was tested by using a professional web-based program (http://ihg2.helmholtz-muenchen.de/cgi-bin/hw/hwa1.pl). Subgroup analysis based on ethnicity and categories of autoimmune liver disease was

used to explore and explain the diversity among the results of different studies. Sensitivity analysis was performed by omission of every individual study in turn. In addition, funnel plots, as well as Begg's and Egger's tests were used to evaluate the potential presence of publication bias (Song *et al.* 2002). Possible publication bias was suggested when the *P* value was less than 0.05.

Results

Characteristics of the included studies

The systematic literature search generated a total of 184 references based on the search strategy in PubMed, EMBASE and Web of Science (83 in PubMed, 62 in EMBASE and 39 in Web of Science). We excluded the unsatisfactory studies by screening the titles and abstracts, and assessing the full text articles. The flow chart of study selection is shown in figure 1. Finally, a total of 15 studies (Bernal *et al.* 1999; Cookson *et al.* 1999; Czaja *et al.* 1999; Gordon *et al.* 1999; Jones *et al.* 1999; Tanaka *et al.* 1999; Bathgate *et al.* 2000; Bittencourt *et al.* 2001; Mitchell *et al.* 2001; Bittencourt *et al.* 2003; Fan *et al.* 2004; Ma and Qiu 2004; Poupon *et al.* 2008; Niro *et al.* 2009; Juran *et al.* 2010) were included in our meta-analysis based on the inclusion criteria for AILD susceptibility related to the *TNF-α*-308G/A and *TNF-α*-238G/A polymorphisms. The baseline characteristics and the genotype distribution of each included study are summarized in table 1. Among them, three of the eligible studies contained data on two different ethnic groups, and we treated them independently (Cookson *et al.* 1999; Mitchell *et al.* 2001; Juran *et al.* 2010). Therefore, for the *TNF-α*-308G/A polymorphism, there were totally 18 studies with 2058 cases and 2722 controls, including 14 Caucasian, two Asian and two mixed populations. Of the total studies we considered, seven, ten and four studies evaluated the association between the polymorphism of *TNF-α*-308G/A and AIH, PBC and PSC, respectively. For the *TNF-α*-238G/A polymorphism, there were nine studies with 906 AILD patients and 1196 controls, which consisted of seven Caucasian populations and two Asian populations. Among them, five, four and one studies examined the relationship between *TNF-α*-238G/A polymorphism and susceptibility to AIH, PBC and PSC, respectively. Only one study (Poupon *et al.* 2008) provided A allele frequency, therefore we included it only in evaluating A allele vs G allele for PBC susceptibility. All included studies were case–control studies and used polymerase chain reaction (PCR) or PCR-restriction fragment length polymorphism (PCR-RFLP) to detect genotypes. The genotype distributions among the controls of all studies were consistent with HWE except one of ethnic population groups of Bathgate *et al.* (2000) and Juran *et al.* (2010) studies for

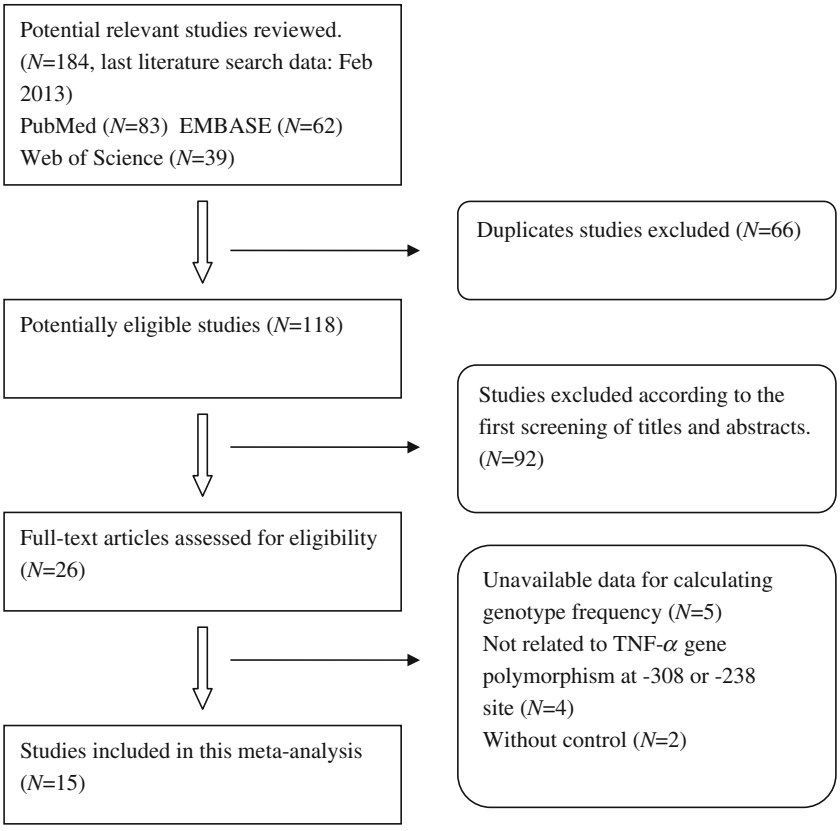

Figure 1. The flow chart of study selection.

Table 1. General characteristics of the included studies in the meta-analysis.

First author	Year	Country	Ethnicity	Disease	Genotyping method	Number of cases					Number of controls					HWE (control) *P*
						GG	GA	AA	Total	A allele[d]	GG	GA	AA	Total	A allele[f]	
TNF-α-308G/A																
Bernal	1999	England	Caucasian	PSC	PCR	46	44	20	110	0.382	89	35	2	126	0.155	0.735
Cookson[a]	1999	England	Caucasian	AIH-1	PCR	33	24	8	65	0.308	61	31	1	93	0.177	0.171
Cookson[b]	1999	America	Caucasian	AIH-1	PCR	37	38	10	85	0.342	75	25	2	102	0.142	0.960
Czaja	1999	USA	Caucasian	AIH-1	PCR	67	68	17	152	0.336	75	25	2	102	0.142	0.960
Gordon	1999	England	Caucasian	PBC	PCR-RFLP	66	20	5	91	0.165	122	78	9	209	0.23	0.459
Jones	1999	England	Caucasian	PBC	PCR-RFLP	111	53	4	169	0.361	87	49	9	145	0.231	0.556
Tanaka	1999	Italy	Caucasian	PBC	PCR-RFLP	56	14	1	71	0.113	109	24	0	133	0.09	0.253
Bathgate	2000	England	Caucasian	AIH/PBC/PSC	PCR	40	32	14	86	0.349	65	24	17	106	0.274	0.001
Bittencourt	2001	Brazil	Mixed	AIH-1	PCR	59	31	3	93	0.199	63	19	1	83	0.127	0.736
Mitchell[a]	2001	Norway	Caucasian	PSC	PCR	47	45	18	110	0.368	76	31	3	110	0.168	0.939
Mitchell[b]	2001	England	Caucasian	PSC	PCR	16	28	6	50	0.4	23	10	2	35	0.2	0.593
Bittencourt	2003	Brazil	Mixed	PBC	PCR-RFLP	48	9	0	57	0.079	63	19	1	83	0.127	0.744
Fan	2004	China	Asian	AIH/PBC	PCR	94	13	0	107	0.071	133	27	0	160	0.084	0.605
Ma	2004	China	Asian	AIH-1	PCR-RFLP	15	14	3	32	0.313	34	10	3	47	0.17	0.395
Poupon	2008	France	Caucasian	PBC	PCR	…[c]	…[c]	…[c]	258	0.126	…[c]	…[c]	…[c]	286	0.315	NR
Niro	2009	Italy	Caucasian	PBC	PCR-RFLP	90	16	1	107	0.084	115	25	1	141	0.096	0.776
Juran[a]	2010	America	Caucasian	PBC	PCR-RFLP	236	114	10	360	0.186	289	105	10	404	0.155	0.900
Juran[b]	2010	Canada	Caucasian	PBC	PCR-RFLP	360	121	24	505	0.167	269	76	12	357	0.14	0.044
TNF-α-238G/A																
Czaja	1999	England	Caucasian	AIH-1	PCR	145	9	0	154	0.029	97	7	0	104	0.034	0.722
Cookson[a]	1999	America	Caucasian	AIH-1	PCR	64	3	0	67	0.022	80	11	2	93	0.081	0.098
Cookson[b]	1999	England	Caucasian	AIH-1	PCR	80	5	0	85	0.029	94	7	0	101	0.035	0.718
Gordon	1999	England	Caucasian	PBC	PCR-RFLP	79	11	1	91	0.071	182	22	1	205	0.059	0.706
Jones	1999	England	Caucasian	PBC	PCR-RFLP	154	12	2	168	0.048	135	10	0	145	0.034	0.667
Bernal	1999	England	Caucasian	PSC	PCR	93	4	1	98	0.031	80	11	2	93	0.081	0.098
Fan	2004	China	Asian	AIH/PBC	PCR	93	11	0	104	0.053	139	20	1	160	0.069	0.763
Ma	2004	China	Asian	AIH-1	PCR-RFLP	30	2	0	32	0.031	44	4	0	48	0.042	0.763
Niro	2009	Italy	Caucasian	PBC	PCR-RFLP	101	6	0	107	0.028	125	16	0	141	0.057	0.475

AILD, autoimmune liver disease; AIH, autoimmune hepatitis; PBC, primary biliary cirrhosis; PSC, primary sclerosing cholangitis; PCR-RFLP, PCR-restriction fragment length polymorphism; NR, not reported; HWE, Hardy–Weinberg equilibrium; $P < 0.05$ was considered to be statistically significant.

[a,b]Different populations in one study.

[c]An absence of data for the study.

[d]A allele frequency.

Table 2. Meta-analysis of the *TNF-α*-308G/A polymorphism on AILD susceptibility.

Comparison	Population	Sample size		Association test		Heterogeneity test		Effects model
		Case	Control	OR (95%CI)	*P*	I^2 (%)	*P*	
A vs G Allele	Overall	2508	2722	1.45(1.13–1.86)	0.001	80.1	<0.001	Random
	Caucasian	2219	2349	1.53(1.16–2.02)	0.003	82.8	<0.001	Random
	AIH	484	693	2.34(1.63–3.36)	<0.001	55.9	0.034	Random
	PBC	1737	2024	0.95(0.79–1.13)	0.544	37.7	0.107	Fixed
	PSC	287	377	2.80(2.14–3.65)	<0.001	0.0	0.997	Fixed
AA vs GG	Overall	2459	2934	2.74(1.51–4.96)	0.001	63.9	<0.001	Random
	Caucasian	2219	2349	2.95(1.51–5.77)	0.002	70.4	<0.001	Random
	AIH	435	533	7.41(3.45–15.89)	<0.001	0.0	0.539	Fixed
	PBC	1737	2024	0.96(0.61–1.50)	0.856	7.6	0.371	Fixed
	PSC	287	377	7.39(3.52–15.52)	<0.001	10.0	0.343	Fixed
GA vs GG	Overall	2250	2436	1.46(1.11–1.92)	0.007	72.3	<0.001	Random
	Caucasian	1961	2063	1.53(1.13–2.08)	0.006	74.8	<0.001	Random
	AIH	484	693	2.14(1.39–3.28)	0.001	49.8	0.063	Random
	PBC	1479	1738	0.96(0.73–1.25)	0.739	52.2	0.033	Random
	PSC	287	377	2.56(1.79–3.67)	<0.001	0.0	0.801	Fixed
AA vs GA	Overall	2459	2934	1.53(0.97–2.40)	0.066	38.6	0.049	Random
	Caucasian	2219	2349	1.63(0.98–2.70)	0.02	47.9	0.059	Random
	AIH	435	533	2.28(1.12–4.65)	0.024	0.0	0.498	Fixed
	PBC	1737	2024	0.88(0.50–1.56)	0.671	29.4	0.193	Fixed
	PSC	287	377	2.91(1.26–6.70)	0.012	23.4	0.27	Fixed
AA+GA vs GG	Overall	2250	2436	1.57(1.18–2.10)	0.002	79.0	<0.001	Random
	Caucasian	1961	2063	1.68(1.21–2.35)	0.002	81.4	<0.001	Random
	AIH	484	693	2.39(1.54–3.70)	<0.001	54.8	0.039	Random
	PBC	1479	1738	0.95(0.74–1.21)	0.654	47.1	0.057	Random
	PSC	287	377	3.25(2.32–4.56)	<0.001	0.0	0.947	Fixed
AA vs GA+GG	Overall	2459	2934	2.22(1.31–3.76)	0.003	55.2	0.004	Random
	Caucasian	2219	2349	2.38(1.31–4.32)	0.004	63.3	0.001	Random
	AIH	435	533	4.85(2.44–9.67)	<0.001	0.0	0.689	Fixed
	PBC	1737	2024	0.93(0.58–1.49)	0.748	15.0	0.312	Fixed
	PSC	287	377	4.94(2.24–10.90)	<0.001	27.1	0.249	Fixed

AILD, autoimmune liver disease; AIH, autoimmune hepatitis; PBC, primary biliary cirrhosis; PSC, primary sclerosing cholangitis; OR, odds ratio; CI, confidence interval; random effects model was used when $I^2 > 50\%$ and/or *P* value of heterogeneity test <0.1; otherwise, fixed-effects model was used.

TNF-α-308G/A, and another one (Poupon *et al.* 2008) whose HWE could not be calculated because of unsatisfactory data.

TNF-α-308G/A polymorphism and the risk of AILD

The evaluation of the association between -308G/A polymorphism and the risk of AILD is presented in table 2. In the overall analysis, we found that -308G/A polymorphism was associated with the risk of AILD in the comparison A vs G allele under a random effects model (OR = 1.45, 95%CI = 1.13–1.86, $P = 0.001$). We conducted the three comparisons in turn: AA vs GG (OR = 2.74, 95%CI = 1.51–4.96, $P = 0.001$), GA vs GG (OR = 1.46, 95%CI = 1.11–1.92, $P = 0.007$), AA vs GA (OR = 1.53, 95%CI = 0.97–2.40, $P = 0.066$); The results suggested that *TNF-α*-308A allele plays a dominant role on AILD risk, so we chose the dominant model (AA + GA vs GG: OR = 1.57, 95%CI = 1.18–2.10, $P = 0.002$) as the main approach of our analysis. Interestingly, a positive association was also found in recessive model (AA vs GA + GG: OR = 2.22, 95%CI = 1.31–3.76, $P = 0.003$). In overall analysis, random effects model was used when $I^2 > 50\%$ and/or P value of heterogeneity test <0.1; otherwise, fixed-effects model was used.

Considering that the small number of studies of Asian and mixed populations may have insufficient power in subgroup analysis, we only evaluated the studies on Caucasian populations. In subgroup analysis stratified by ethnicity, we found that *TNF-α*-308G/A polymorphism was significantly associated with AILD susceptibility only in Caucasians: A vs G allele (OR = 1.53, 95%CI = 1.16–2.02, $P = 0.003$); AA vs GG (OR = 2.95, 95%CI = 1.51–5.77, $P = 0.002$); GA vs GG (OR = 1.53, 95%CI = 1.13–2.08, $P = 0.006$); AA

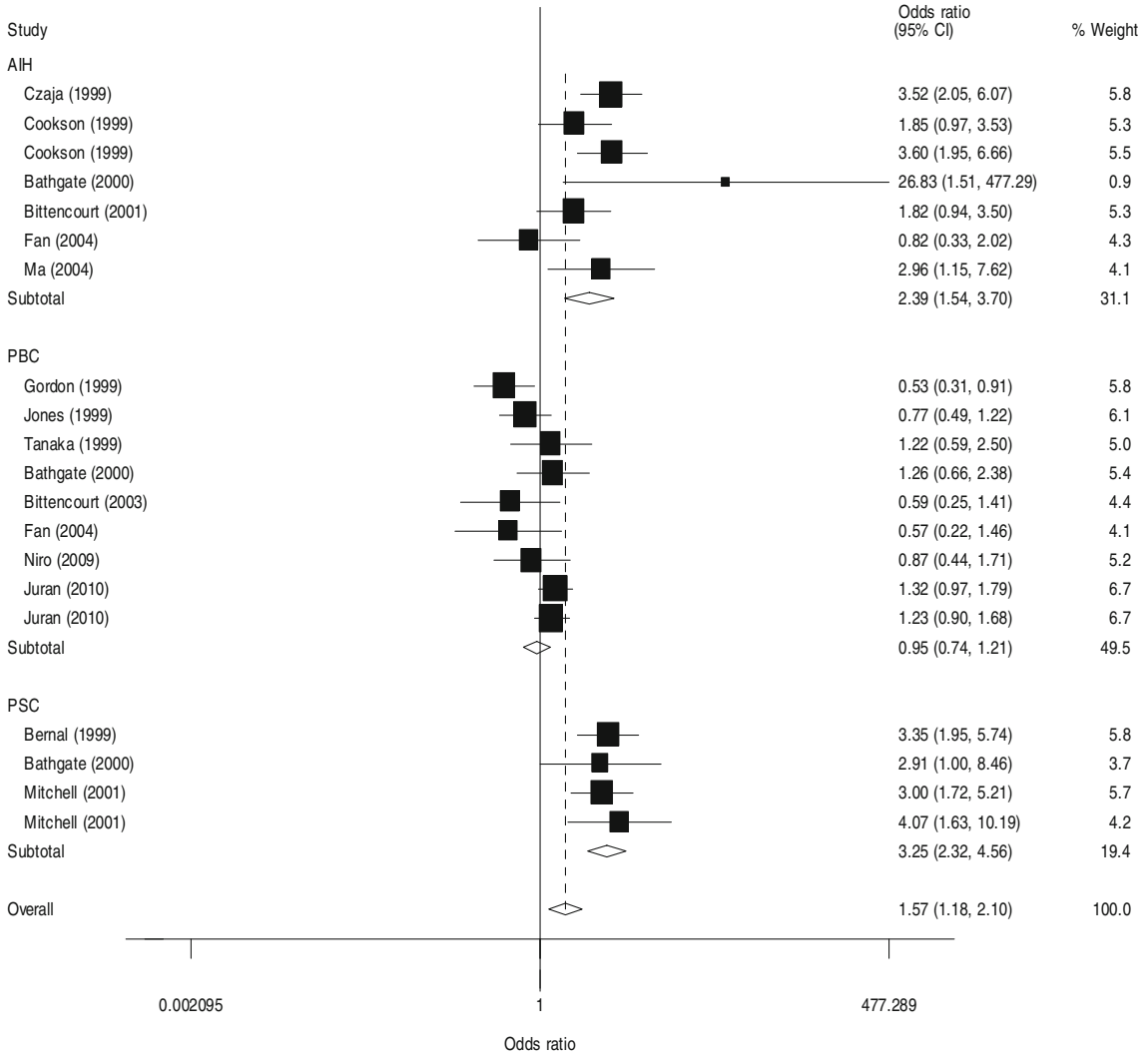

Figure 2. Subgroup analysis by the categories of AILD in the meta-analysis of studies on the association between *TNF-α*-308G/A polymorphism and AILD risk using a random effects model (dominant model AA+ GA vs GG).

vs GA (OR = 1.63, 95%CI = 0.98–2.70, $P = 0.059$); AA + GA vs GG (OR = 1.68, 95%CI = 1.21–2.35, $P = 0.002$); AA vs GA + GG (OR = 2.38, 95%CI = 1.31–4.32, $P = 0.004$) (table 2).

We also performed a subgroup analysis stratified by categories of AILD to evaluate the association between -308G/A polymorphism with susceptibility to any kind of AILD (figure 2). We found that -308A can significantly increase the risk of AIH in all genetic models: A vs G allele (OR = 2.34, 95%CI = 1.63–3.36, $P < 0.001$); AA vs GG (OR = 7.41, 95%CI = 3.45–15.89, $P < 0.001$); GA vs GG (OR = 2.14, 95%CI = 1.39–3.28, $P = 0.001$); AA vs GA (OR = 2.28, 95%CI = 1.12–4.65, $P = 0.024$); AA + GA vs GG (OR = 2.39, 95%CI = 1.54–3.70, $P < 0.001$); AA vs GA + GG (OR = 4.85, 95%CI = 2.44–9.67, $P < 0.001$). The same effect was observed in PSC patients, all of whom were from Caucasian populations: A vs G allele (OR = 2.80, 95%CI = 2.14–3.65, $P < 0.001$); AA vs GG (OR = 7.39, 95%CI = 3.52–15.52, $P < 0.001$); GA vs GG (OR = 2.56, 95%CI = 1.79–3.67, $P < 0.001$); AA vs GA (OR = 2.91, 95%CI = 1.26–6.70, $P = 0.012$); AA + GA vs GG (OR = 3.25, 95%CI = 2.32–4.56, $P < 0.001$); AA vs GA + GG (OR = 4.94, 95%CI = 2.24–10.90, $P < 0.001$). Nevertheless, the overall ORs and 95%CIs implied that individuals carrying the *TNF-α*-308A allele had no increased risk of developing PBC (table 2).

A previous meta-analysis (Qin *et al.* 2012) about the *TNF-α* polymorphism and PBC risk showed negative results, even in subgroup analysis stratified by ethnicity. Therefore, we tried to explore the main factor in ethnicity for AIH and PBC, respectively, in our meta-analysis. It came out that -308G/A polymorphism was not significantly associated with risk of PBC in Caucasian population (OR = 1.01, 95%CI = 0.78–1.30, $P = 0.943$, figure 1 in electronic supplementary material at http://www.ias.ac.in/jgenet/), which was similar to the results of the previous meta-analysis. On the other hand, we found that the patients who carried -308A allele had an increasing risk of developing AIH in Caucasians, but not Asians (OR = 3.09, 95%CI = 1.90–5.03, $P < 0.001$, figure 3).

TNF-α-238G/A polymorphism and the risk of AILD

The evaluation of the association between -238G/A polymorphism and the risk of AILD is shown in table 3. In the overall analysis, OR and 95% CI showed that patients carrying the *TNF-α*-238A allele had a slightly decreased risk of developing AILD (OR = 0.65, 95%CI = 0.48–0.87, $I^2 = 0.0$ and $P = 0.435$ for heterogeneity). In the other genetic model comparisons, we found no relationship between *TNF-α*-238G/A polymorphism and AILD risk. And we found the same results when the analysis was stratified by ethnicity in Caucasian population (OR = 0.68, 95%CI = 0.48–0.95, $I^2 = 18.4$ and $P = 0.29$ for heterogeneity). Moreover, subgroup analysis stratified by categories of AILD was also

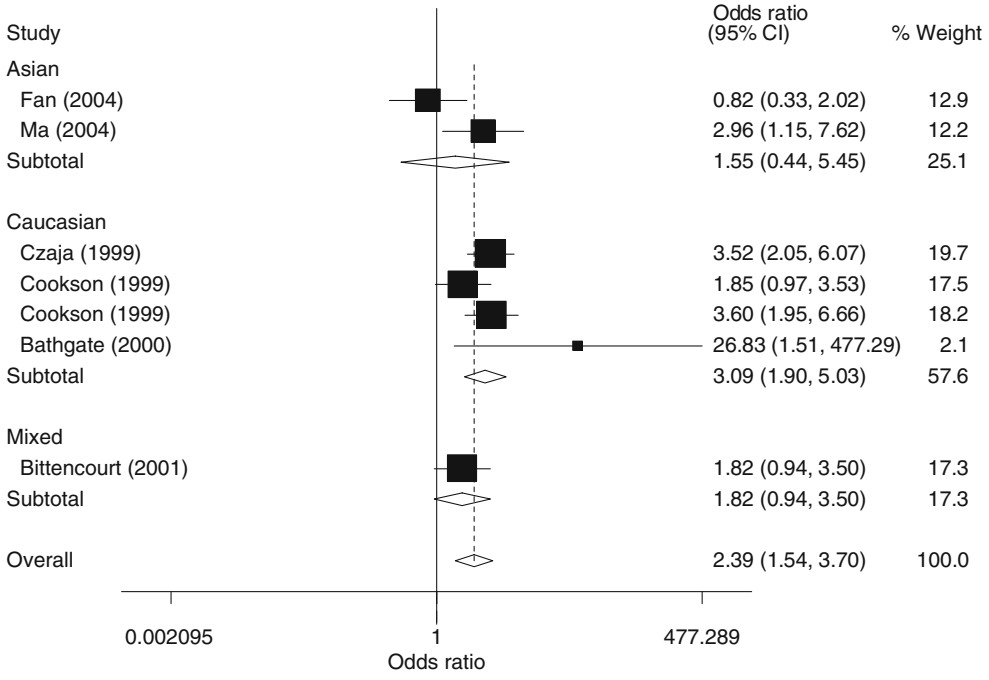

Figure 3. Subgroup analysis by ethnicity in the meta-analysis of studies on the association between AIH risk and *TNF-α*-308G/A polymorphism using a random effects model (dominant model AA+ GA vs GG).

Table 3. Meta-analysis of the *TNF-α*-238G/A polymorphism on AILD susceptibility.

Comparison	Population	Sample size		Association test		Heterogeneity test		Effects model
		Case	Control	OR (95%CI)	*P*	I^2 (%)	*P*	
A vs G allele	Overall	906	1196	0.65(0.48–0.87)	0.005	0.0	0.435	Fixed
	Caucasian	770	882	0.68(0.48–0.95)	0.024	18.4	0.29	Fixed
	AIH	385	452	0.54(0.32–0.90)	0.019	0.0	0.540	Fixed
	PBC	423	651	0.79(0.53–1.17)	0.231	0.0	0.449	Fixed
AA vs GG	Overall	540	835	0.87(0.28–2.66)	0.805	0.0	0.620	Fixed
	Caucasian	424	536	0.95(0.28–3.18)	0.934	0.0	0.474	Fixed
	AIH	114	199	0.45(0.05–3.96)	0.472	0.0	0.517	Fixed
	PBC	316	510	2.31(0.45–11.60)	0.31	0.0	0.785	Fixed
GA vs GG	Overall	906	1196	0.73(0.52–1.01)	0.056	0.0	0.607	Fixed
	Caucasian	770	882	0.71(0.49–1.02)	0.065	4.5	0.392	Fixed
	AIH	385	452	0.66(0.38–1.13)	0.126	0.0	0.845	Fixed
	PBC	423	651	0.89(0.58–1.37)	0.591	0.0	0.508	Fixed
AA vs GA	Overall	540	835	1.56(0.48–6.89)	0.462	0.0	0.978	Fixed
	Caucasian	424	536	1.73(0.43–6.89)	0.437	0.0	0.877	Fixed
	AIH	114	199	0.95(0.09–9.74)	0.965	0.0	0.725	Fixed
	PBC	316	510	2.13(0.40–11.34)	0.376	0.0	0.805	Fixed
AA+GA vs GG	Overall	906	1196	0.72(0.53–0.99)	0.046	0.0	0.451	Fixed
	Caucasian	770	882	0.71(0.50–1.01)	0.06	23.2	0.252	Fixed
	AIH	385	452	0.62(0.37–1.06)	0.08	0.0	0.733	Fixed
	PBC	423	651	0.91(0.60–1.39)	0.673	0.0	0.457	Fixed
AA vs GA+GG	Overall	540	835	0.92(0.30–2.82)	0.88	0.0	0.633	Fixed
	Caucasian	424	536	1.01(0.30–3.39)	0.987	0.0	0.491	Fixed
	AIH	114	199	0.48(0.06–4.23)	0.509	0.0	0.527	Fixed
	PBC	316	510	2.38(0.47–12.01)	0.294	0.0	0.784	Fixed

AIH, autoimmune hepatitis; PBC, primary biliary cirrhosis; PSC, primary sclerosing cholangitis; OR, odds ratio; CI, confidence interval; random effects model was used when $I^2 > 50\%$ and/or *P* value of heterogeneity test < 0.1; otherwise, fixed-effects model was used.

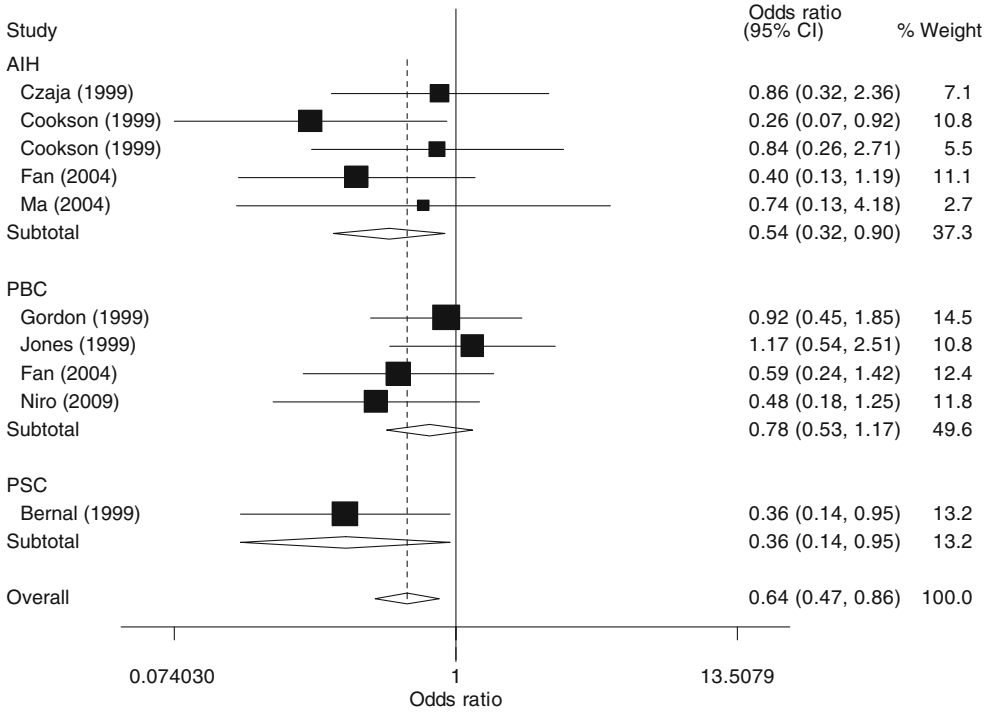

Figure 4. Subgroup analysis by the categories of AILD in the meta-analysis of studies on the association between *TNF-α*-238G/A polymorphism and AILD risk using a fixed-effects model (A vs G allele).

performed (figure 4). For evaluation of statistically significant association was found with the TNF-238A allele polymorphism and the AIH susceptibility (OR = 0.54, 95%CI = 0.32–0.90, $I^2 = 0.0$ and $P = 0.540$ for heterogeneity). For evaluation of the PBC susceptibility, we also did not find any association with *TNF-α*-238G/A polymorphism in any comparison. Because of limited numbers of eligible studies for AIH and PBC, we did not evaluate stratification analysis by ethnicity for AIH and PBC. There was no statistically significant heterogeneity in all comparison models, therefore we used fixed-effects model to assess the pooled OR.

Sensitivity analysis

Sensitivity analysis was carried out to identify potentially influential studies by sequential omission of each individual study. We found the results in all individual analysis and subgroup analyses were similar before and after the deletion of each included study, suggesting high stability of the meta-analysis results (-308A/G, figure 2 in electronic supplementary material). In addition, when excluding two studies whose genotype frequencies in controls were not in accordance with HWE and one study that did not report the result of HWE, the significance of the estimated pooled ORs were not influenced excessively, indicating that our results were robust.

Population bias

Funnel plots, as well as Begg's and Egger's tests were used to estimate publication bias of literatures. The publication bias of the meta-analysis on the association between *TNF-α*-308G/A and *TNF-α*-238G/A polymorphism and AILD risk was detected on the comparison of G vs A allele. The shapes of the funnel plots did not reveal any evidence of significant asymmetry (figure 5). Moreover, the Begg's and Egger's tests were used to provide statistical evidence of funnel plot symmetry, and the statistical results did not suggest any evidence of publication bias (for -308G/A polymorphism: Begg's test $P = 0.910$, Egger's test $P = 0.538$; for -238G/A polymorphism: Begg's test $P = 0.297$, Egger's test $P = 0.371$).

Discussion

TNF-α is a potent proinflammatory and immunoregulatory cytokine, playing a key role in the immune response (Strieter *et al.* 1993; Elahi *et al.* 2009). It can inhibit B-cell activation and antibody production, support delayed hypersensitivity reactions and simultaneously enhance cellular cytotoxicity (Peters 1996). Moreover, an increased TNF-α level correlates with the severity of hepatic inflammation, fibrosis and tissue injury (Kamal *et al.* 2006). Up to now, a

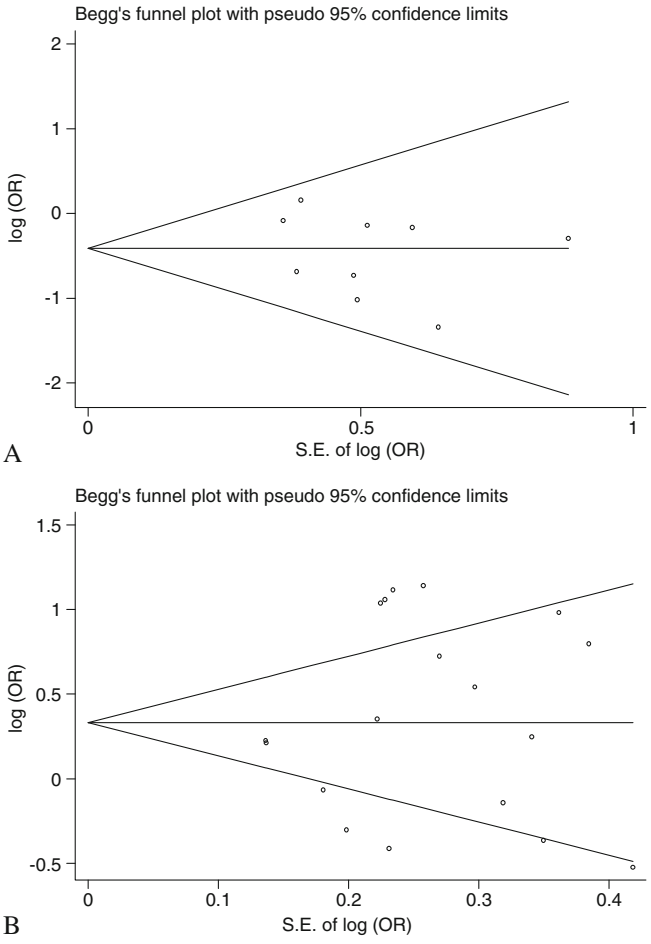

Figure 5. Funnel plots for publication bias in the studies of the meta-analysis on the association between TNF-α polymorphisms and AILD risk of the overall populations and (A allele vs G allele, (A) for *TNF*-α-308G/A polymorphism and (B) for *TNF*-α-238G/A polymorphism).

number of studies have demonstrated a significant association of *TNF*-α polymorphisms with susceptibility to chronic hepatitis B (Niro *et al.* 2005; Cheong *et al.* 2006; Wang *et al.* 2010) as well as autoimmune diseases (Cookson *et al.* 1999; Ristic *et al.* 2007; Lin *et al.* 2009; Juran *et al.* 2010). However, the results from the genetic studies about the relationship of *TNF*-α polymorphisms and AILD risk are controversial. Cookson *et al.* (1999) reported that both *TNF*-α-308G/A and -238G/A were associated with an increased susceptibility to type 1 AIH, while Czaja *et al.* (1999) and Ma and Qiu (2004) suggested that only *TNF*-α-308G/A polymorphism was associated with type 1 AIH, and no significant association was found in other studies (Bittencourt *et al.* 2001; Fan *et al.* 2004). For PBC risk and *TNF*-α polymorphisms, Gordon *et al.* (1999) found that TNF-α-308A allele was a protective factor for PBC, while Juran *et al.* (2010) reported that *TNF*-α-308A allele significantly increased the risk of PBC. Therefore, we performed this meta-analysis to investigate if there is any association of the two potentially polymorphisms of the *TNF*-α gene and AILD risk.

The overall results of this meta-analysis suggested that the *TNF*-α-308G/A all A genotypes (A allele, AA, GA, GA/AA genotypes) are the risk factors of AILD, while -238A allele was associated with a decreased risk. As for subgroup analysis, similar correlation was observed in Caucasians and AIH, as well as PSC for both *TNF*-α polymorphisms. In PSC group, all the cases and controls were Caucasians, and the overall ORs suggested that for *TNF*-α-308G/A all A genotypes were associated with PSC risk. Therefore, we tried to access the AIH group stratified by ethnicity, and we found all statistically significant ORs for *TNF*-α-308G/A polymorphism were increased in the Caucasians, compared with the overall populations. And most of the included AIH patients were type 1 AIH (AIH-1). This finding suggests that Caucasians with *TNF*-α-308A allele may be under more AIH-1 risk. Moreover, we found neither *TNF*-α-308G/A nor *TNF*-α-238G/A polymorphism was associated with the susceptibility to PBC, even in the subgroup analysis of Caucasian populations. The results were consistent with the previous analysis (Qin *et al.* 2012). Compared to the previous meta-analysis, our study had greater advantage. On one hand, Qin *et al.* (2012) only included eight studies with 2508 patients to evaluate the association between *TNF*-α-308G/A or *TNF*-α-238G/A polymorphism with PBC risk, while we assayed totally 15 studies with 5230 individuals about the association with the two polymorphisms and three kinds of AILD (AIH, PBC and PSC). On the other hand, we tried all the three comparison patterns to find out the most suitable method for data pooling, while the previous meta-analysis only showed results of dominant and recessive models. Besides, we comprehensively assessed publication bias using several methods such as Egger's and Begg's tests, as well as funnel plot tests, while the previous meta-analysis used only Begg's test.

Large heterogeneity among studies was observed in all comparisons for *TNF*-α-308G/A polymorphism. We placed more emphasis on exploring the potential sources of heterogeneity via stratification and sensitivity analysis. The main reason for heterogeneity in the gene polymorphism association meta-analysis may be the different populations used and the different kinds of AILD. Subsequent subgroup analysis of Caucasian populations identified large heterogeneity, indicating that ethnicity may contribute little to the existence of overall heterogeneity. However, when the analysis was stratified by the categories of AILD, we found I^2 of the heterogeneity test decreased in all genetic comparisons. And, expect GA vs GG model, and the dominant mode in AIH and PBC, other modes all had no heterogeneity, which suggested that the overall heterogeneity may be due to different categories of AILD. Sensitivity analysis showed that our meta-analysis results were robust, indicating the potential studies which were not in HWE or without HWE did not affect the overall ORs.

Some limitations of the present meta-analysis should be acknowledged. First, our results were based on unadjusted estimates, whereas a more precise evaluation should be adjusted by other covariants such as age, gender, body mass

index, race, drinking status and other lifestyle factors. Second, lack of the original data of the reviewed studies limited our precise estimation of the relationship. Considering the absence of genotype frequency in one study, we only included it to evaluate *TNF-α*-308 A allele vs G allele comparison model. The evaluations of other genetic models did not include this study. Third, studies on the association of *TNF-α* polymorphisms with AILD were predominantly conducted on Caucasian population; only a few were conducted in Asian countries, and none in African population. This made results from the subgroup analysis of Asian less reliable. Therefore, we only investigated the Caucasian population in the subgroup analysis. Further, not all the controls included in the studies were in HWE (Juran *et al.* 2010). However, the results of sensitivity analysis showed that their studies had no influence on the overall ORs. Finally, the numbers of published studies were not sufficiently large for a comprehensive analysis, although we had tried our best to find as many publications as we could by means of various searching approaches. Some studies with small size may not have enough statistical power to explore the real association. With these limitations in mind, the association of the *TNF-α* gene polymorphisms with AILD risk needs further confirmation of more additional studies in different population. The results mentioned above should be interpreted with caution.

Despite these limitations, a systematic review of the association of *TNF-α* polymorphisms with AILD risk is statistically more powerful than any single study. In conclusion, our study suggests that the *TNF-α*-308G/A polymorphism played an important role in the development of AILD. And *TNF-α*-238 A allele may be protective against AILD in Caucasians. Subgroup analysis demonstrated that *TNF-α*-308G/A polymorphism was associated with AIH or PSC risk in Caucasian population, while there was no association between both *TNF-α* polymorphisms and PBC risk. Further cases–control studies with large numbers of worldwide researches in various ethnicities, especially in Asian and African populations, are expected to be performed to validate this conclusion.

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