Association between *TNF-α* rs1799724 and rs1800629 polymorphisms and the risk of Crohn's disease

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ABSTRACT. We investigated the associations between 2 major tumor necrosis factor-α (*TNF-α*) polymorphisms, rs1799724 C>T and rs1800629 G>A, and the susceptibility to Crohn's disease (CD) using a meta-analysis framework. The PubMed, EBSCO, Ovid, Wiley, Web of Science, WANFANG, and VIP databases (last updated search in October 2014) were comprehensively searched for relevant published studies. The studies retrieved from database searches were filtered based on inclusion and exclusion criteria, and the resultant data extracted from the selected studies were analyzed using the Comprehensive Meta-analysis 2.0 software. Eleven case-control studies, containing 2000 CD patients and 3499 healthy controls, were identified as relevant to this meta-analysis. Data extracted from these 11 studies were analyzed to understand the role of the 2 *TNF-α* polymorphisms in CD. We found that the *TNF-α* rs1799724 C>T

polymorphism increased the susceptibility to CD (allele model: OR = 1.293, 95%CI = 1.090-1.534, P = 0.003; dominant model: OR = 1.258, 95%CI = 1.031-1.534, P = 0.024). In contrast, we found no significant association between the *TNF-α* rs1800629 G>A polymorphism and CD susceptibility (allele model: OR = 1.005, 95%CI = 0.864-1.170, P = 0.945; dominant model: OR = 0.962, 95%CI = 0.809-1.145, P = 0.667). This meta-analysis showed that the *TNF-α* rs1799724 C>T polymorphism is associated with CD susceptibility, while the *TNF-α* rs1800629 G>A polymorphism appeared to have no correlation with the susceptibility to CD.

Key words: Case-control study; Crohn's disease; Meta-analysis; Polymorphism; Tumor necrosis factor-α

INTRODUCTION

Crohn's disease (CD), along with ulcerative colitis, is one of the main subphenotypes of inflammatory bowel disease (IBD) that principally affects young adults and causes considerable morbidity (Lee et al., 2011). As a multifactorial disease with an unclear pathogenesis, CD is a chronic unremitting immune-mediated inflammation involving the gastrointestinal tract, characterized by intestinal inflammation and progressive bowel damage (Peyrin-Biroulet et al., 2010). The prevalence of CD is more common in western countries, observed at rates of 3.2 per 1000 people in Europe and North America, while it is less common in Asia and Africa (Molodecky et al., 2012). Generally, symptoms of CD include chronic diarrhea, abdominal pain, rectal bleeding, and weight loss, as well as growth failure in children (Lee et al., 2011; Baumgart and Sandborn, 2012). CD can occur in any part of the gastrointestinal tract, but most commonly occurs in the terminal ileum or the perianal region; CD, unlike ulcerative colitis, is associated with complications such as strictures (narrowing of the bowel), fistulas (creation of abnormal passageways between the bowel and other structures), and perianal disease (comprised of fissures, fistulas, and abscesses) (Dretzke et al., 2011; Khor et al., 2011). Although the exact etiology of CD remains unclear, both genetic and environmental factors are implicated (Cucchiara et al., 2012). With respect to genetic factors, genome-wide association studies found 30 genetic loci related to the risk of CD, accounting for 20% of the total genetic risk for this disease (Fransen et al., 2010; Baumgart and Sandborn, 2012).

The human tumor necrosis factor-α (TNF)-α gene, located on chromosome 6p21.3, is approximately 3 kb in length and contains 4 exons (Old, 1985). TNF-α is secreted chiefly by activated macrophages, although it is also produced by many other cell types, including CD4+ lymphocytes, natural killer cells, neutrophils, mast cells, eosinophils, and neurons (Wynn et al., 2013). Particularly, TNF-α stimulates acute phase responses, resulting in elevated levels of C-reactive protein, interleukin-1 (IL-1), and prostaglandin E2 production (Fan et al., 2011). Abnormal TNF-α expression and secretion is observed in several human diseases, including Alzheimer's disease, cancers, major depressions, and IBD (Locksley et al., 2001). It is well known that polymorphisms in the *TNF-α* promoter region affect its gene expression, particularly leading to TNF-α overproduction, and is implicated in several autoimmune disorders, including IBD, especially in CD (Maeda, 1990; Bradley, 2008). Two single nucleotide polymorphisms located at *TNF-α* rs1800629 G>A and *TNF-α* rs1799724 C>T have been extensively investigated for their ability to confer increased susceptibility to a variety of autoimmune disorders, including rheumatoid

arthritis and IBD (Vasilopoulos et al., 2011). Some studies suggest that the *TNF-α* rs1800629 G>A and *TNF-α* rs1799724 C>T polymorphisms are not involved in CD susceptibility (Xie et al., 2012; López-Hernández et al., 2014). However, another study found a strong association between the *TNF-α* rs1800629 G>A polymorphism and susceptibility to CD (Fan et al., 2011). In order to resolve these conflicting results, we conducted the present meta-analysis to investigate the role of the *TNF-α* rs1800629 G>A and *TNF-α* rs1799724 C>T polymorphisms in CD susceptibility.

MATERIAL AND METHODS

Literature retrieval

Literature retrieval was conducted using databases PubMed, EBSCO, Cochrane Library database, Ovid, Springerlink, Embase, Web of Science, WANFANG, China National Knowledge Infrastructure, and VIP to identify all relevant studies. The search strategy involved a combination of key words related to *TNF-α*, polymorphism, and CD. Database searching was carried out to retrieve studies published prior to October 2014 with language restricted to Chinese and English. The search terms included: ("tumor necrosis factor-alpha" or "tumor necrosis factor alpha" or "TNF alpha" or "tumor necrosis factor" or "tumor necrosis factor ligand superfamily member 2" or "cachectin" or "TNF superfamily, member 2"), ("Crohn disease" or "Crohn's enteritis" or " regional enteritis" or "Crohn's disease" or "inflammatory bowel disease 1" or "granulomatous enteritis" or "enteritis, regional" or "ileocolitis" or "granulomatous colitis" or "terminal ileitis" or "regional ileitis"), and ("polymorphism, genetic" or "genetic polymorphism" or "polymorphism genetics"). Manual searching was also conducted to identify additional relevant papers.

Inclusion and exclusion criteria

Selected studies were evaluated for their eligibility based on the following inclusion criteria: 1) study type: case-control studies; 2) research topic: association between *TNF-α* rs1800629 G>A and rs1799724 C>T polymorphisms and CD susceptibility; and 3) subject investigated: CD patients and healthy controls. The exclusion criteria were as follows: 1) literature showing an inconsistent diagnosis for CD; 2) duplicate literature; and 3) incomplete original data.

Data collection

Literature screening was performed by 2 independent reviewers. Relevant information including first author, published year, country, ethnicity, language, genotype method, number of patients and controls, gender, and gene frequency were extracted from all eligible studies. Disagreements with data collection were resolved by consultation with a third investigator.

Statistical analysis

Comprehensive Meta-Analysis 2.0 (Biostatic Inc., Englewood, NJ, USA) was used for data analysis in the present meta-analysis. Differences in allele and genotype frequencies of the *TNF-α* rs1800629 G>A and rs1799724 C>T polymorphisms were compared by odds ratio (OR) with 95% confidence intervals (95%CI). *Z* test was carried out to evaluate the significance of the overall effect

values (Chen et al., 2012). Forest plots were applied to reflect the comparisons of ORs and 95%CI between case and control groups. Heterogeneity among all the studies was evaluated using the Cochran's Q-statistic ($P < 0.05$ was considered to be significant) and the I^2 test (0%, no heterogeneity; 100%, maximal heterogeneity) (Peters et al., 2006; Chen et al., 2012). In order to calculate the pooled ORs, fixed-/random-effect models were used; when heterogeneity was observed ($P < 0.05$ or $I^2 > 50\%$), a random-effect model was used, and otherwise the fixed-effect model was employed (Zintzaras and Ioannidis, 2005). Univariate and multivariate meta-regression analysis were utilized to identify potential sources of heterogeneity and Monte Carlo simulation was employed for further confirmation (Ferrenberg and Swendsen, 1988). Sensitivity analysis was performed to evaluate whether the removal of a single study would influence the overall outcomes. The funnel plot, classic fail-safe N, and Egger linear regression tests were adopted to assess publication bias to ensure the reliability of the results (Egger et al., 1997; Sterne and Egger, 2001; Wikstrom et al., 2009). A bilateral test was conducted with $P < 0.05$ indicating significance.

RESULTS

Database searches and manual searching initially retrieved 214 relevant articles. After excluding duplicates (N = 28), non-human studies (N = 23), letters, reviews, meta-analyses (N = 22), and unrelated topics (N = 54), 87 full-text articles remained. Eleven studies met the inclusion criteria after we eliminated studies that were not case-control studies (N = 15), not relevant to *TNF-α* (N = 32), not relevant to CD (N = 9), not relevant to polymorphisms (N = 13), and without correlative data (N = 7). These 11 studies contained a combined total of 2000 CD patients and 3499 healthy controls. Sample sizes in the 11 studies varied from 150-846 and publication years were from 1999-2014 (Negoro et al., 1999; González et al., 2003; Balding et al., 2004; Fowler et al., 2005; Song et al., 2005; Zipperlen et al., 2005; Fidder et al., 2006; Yang et al., 2006; Mittal et al., 2007; Hradsky et al., 2008; López-Hernández et al., 2014). Of these, 7 studies were conducted in Caucasians and the other 4 studies were in Asians. Genotyping methods in these 11 studies included: polymerase chain reaction-restriction fragment length polymorphism, TaqMan assay, and polymerase chain reaction sequence-specific oligonucleotide probes. The genotype distributions of the studies included were in accordance with Hardy-Weinberg equilibrium (all $P > 0.05$), except for *TNF-α* rs1800629 G>A in Negoro et al. (1999), Mittal et al. (2007), and López-Hernández et al. (2014), and all $P < 0.05$. The detailed characteristics of the 11 eligible studies are summarized in Table 1.

Table 1. Baseline characteristics of 11 eligible studies in the present meta-analysis.

First author	Country	Ethnicity	Language	Disease	Case group	Control group	Gender (M/F)		Gene	SNP	Genotype method
							CD	Control			
López-Hernández (2014)	Spain	Caucasian	English	CD	54	135	24/30	-	TNF-α	rs1800629 G/A	PCR-SSOP
Hradsky (2008)	Czech	Caucasian	English	CD	345	501	154/191	-	TNF-α	rs1800629 G/A	TaqMan
Mittal (2007)	India	Asian	English	CD	22	164	-	-	TNF-α	rs1800629 G/A	PCR-RFLP
Yang (2006)	Korea	Asian	English	CD	462	668	-	-	TNF-α	rs1799724 C/T	PCR-RFLP
Fidder (2006)	Netherland	Caucasian	English	CD	243	240	85/68		TNF-α	rs1799724 C/T	PCR-RFLP
Zipperlen (2005)	Canada	Caucasian	English	CD	128	103	-	-	TNF-α	rs1800629 G/A	PCR-RFLP
Fowler (2005)	Australia	Caucasian	English	CD	501	404	192/112		TNF-α	rs1799724 C/T	PCR-RFLP
Song (2005)	China	Asian	Chinese	CD	28	220	-	-	TNF-α	rs1800629 G/A	PCR-RFLP
Balding (2004)	Ireland	Caucasian	English	CD	64	389	-	-	TNF-α	rs1800629 G/A	PCR-RFLP
González (2003)	Spain	Caucasian	English	CD	50	100	30/20	45/55	TNF-α	rs1800629 G/A	PCR-RFLP
Negoro (1999)	Japan	Asian	English	CD	103	575	-	-	TNF-α	rs1799724 C/T	PCR-RFLP

CD = Crohn's disease; M = male; F = female; PCR-RFLP = polymerase chain reaction-restriction fragment length polymorphism; TaqMan = TaqMan assay; PCR SSOP = PCR sequence-specific oligonucleotide probes.

Association between *TNF-α* rs1799724 C>T and CD susceptibility

A total of 5 studies investigated the association between the *TNF-α* rs1799724 C>T polymorphism and CD susceptibility. No heterogeneity was detected in the allele model and dominant model, and thus the fixed-effect model was applied (both P > 0.05). The main result of our meta-analysis was that the *TNF-α* rs1799724 C>T polymorphism increased the susceptibility to CD in both the allele model (OR = 1.293, 95%CI = 1.090-1.534, P = 0.003) and dominant model (OR = 1.258, 95%CI = 1.031-1.534, P = 0.024) (Figure 1 and Table 2). Subgroup analysis based on ethnicity demonstrated that the *TNF-α* rs1799724 C>T polymorphism increased the susceptibility to CD in Asians (allele model: OR = 1.493, 95%CI = 1.202-1.854, P < 0.001; dominant model: OR = 1.536, 95%CI = 1.187-1.986, P = 0.001), but not in Caucasians (allele model: OR = 1.019, 95%CI = 0.772-1.346, P = 0.893; dominant model: OR = 0.938, 95%CI = 0.686-1.282, P = 0.687) (Figure 2 and Table 2).

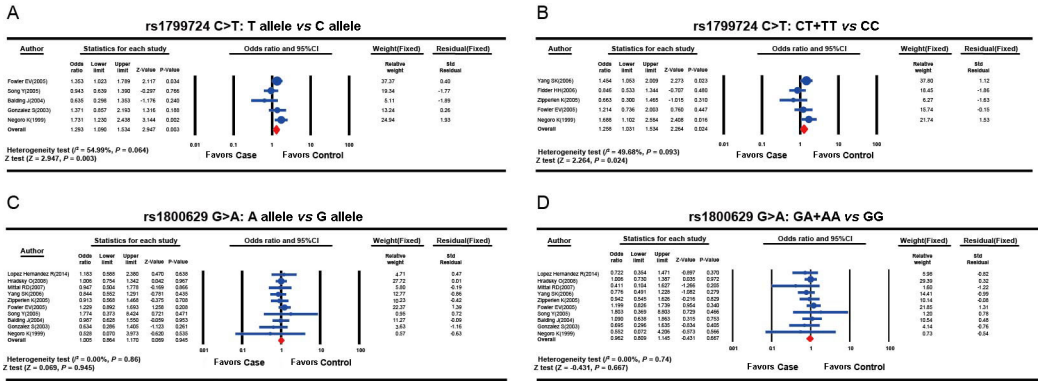

Figure 1. Forest plots for the differences of genotype frequencies in the present meta-analysis to investigate the association between the *TNFα* rs1800629 G>A and *TNF-α* rs1799724 C>T polymorphisms and the susceptibility to Crohn's disease.

Table 2. Comparisons of genotype and allele frequencies between case and control groups in the present meta-analysis investigating the association between the *TNF-α* rs1800629 G>A and *TNF-α* rs1799724 C>T polymorphisms and susceptibility to Crohn's disease.

SNP	Ethnicity	rs1799724 C>T			rs1800629 G>A		
		OR	95%CI	P	OR	95%CI	P
M allele *vs* W allele (Allele model)	Asians	1.493	1.202-1.854	<0.001	0.892	0.636-1.251	0.508
	Caucasians	1.019	0.772-1.346	0.893	1.036	0.874-1.228	0.683
	Overall	1.293	1.090-1.534	0.003	1.005	0.864-1.170	0.945
WM + MM *vs* WW (Dominant model)	Asians	1.536	1.187-1.986	0.001	0.766	0.508-1.154	0.202
	Caucasians	0.938	0.686-1.282	0.687	1.012	0.835-1.226	0.904
	Overall	1.258	1.031-1.534	0.024	0.962	0.809-1.145	0.667
MM *vs* WW (Homozygous model)	Overall	2.176	1.288-3.677	0.004	1.040	1.754-0.617	0.883
MM *vs* WM (Heterozygous model)	Overall	0.575	0.335-0.987	0.045	0.892	0.526-1.512	0.671
MM *vs* WW + WM (Recessive model)	Overall	2.035	1.212-3.414	0.007	1.117	0.670-1.863	0.671

OR = odds ratio; 95%CI = 95% confidence intervals.

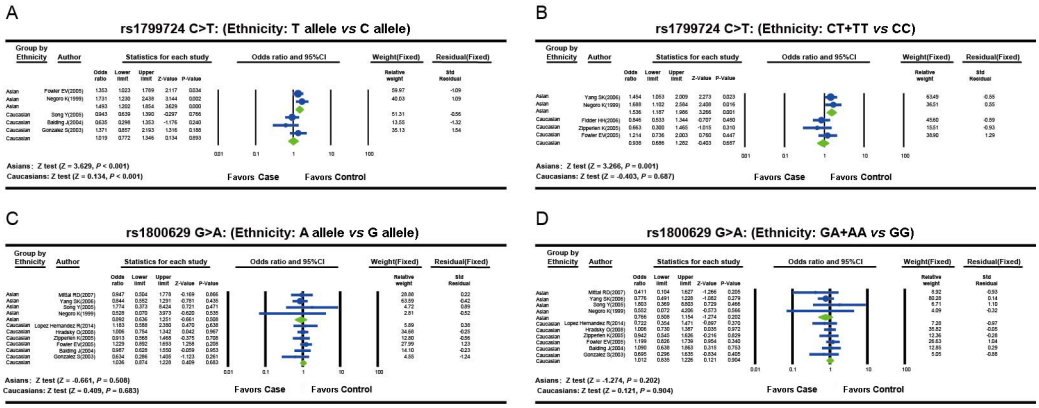

Figure 2. Subgroup analysis based on ethnicity in the present meta-analysis to investigate the association between the *TNF-α* rs1800629 G>A and *TNF-α* rs1799724 C>T polymorphisms and the susceptibility to Crohn's disease.

Association between *TNF-α* rs1800629 G>A and CD susceptibility

Ten studies reported an association between *TNF-α* rs1800629 G>A and CD susceptibility. Because of the lack of heterogeneity among studies, the fixed-effect model was used in the allele model and dominant model (both P > 0.05). The results of the present meta-analysis showed no significant association between the *TNF-α* rs1800629 G>A polymorphism and CD susceptibility in both the allele model (OR = 1.005, 95%CI = 0.864-1.170, P = 0.945) and the dominant model (OR = 0.962, 95%CI = 0.809-1.145, P = 0.667) (Figure 1 and Table 2). Subgroup analysis based on ethnicity also showed no significant association between the *TNF-α* rs1800629 G>A polymorphism and CD susceptibility in both Asians and Caucasians (all P > 0.05) (Figure 2 and Table 2).

Potential sources of heterogeneity

Univariate meta-regression analysis showed that publication year and country were the main sources of heterogeneity and key factors in the overall effect size (both P < 0.05) and that ethnicity, single nucleotide polymorphisms, genotyping methods, and sample size were not the potential sources of heterogeneity and key factors in the overall effect size (all P > 0.05). Multivariate meta-regression analysis showed that publication year, country, ethnicity, single nucleotide polymorphisms, genotyping methods, and sample size were not potential sources of heterogeneity (Figure 3 and Table 3). Sensitivity analysis demonstrated that no single study had a significant effect on pooled ORs for the association between the *TNF-α* rs1800629 G>A and *TNF-α* rs1799724 C>T polymorphisms and the susceptibility to CD, except for the study by Negoro et al. (1999) in the allele model of *TNF-α* rs1799724 C>T, Yang et al. (2006) in the dominant model of *TNF-α* rs1799724 C>T, and Negoro et al. (1999) in the dominant model of *TNF-α* rs1799724 C>T (Figure 4) (Negoro et al., 1999; Yang et al., 2006). The shape of funnel plots revealed no evidence of funnel plot asymmetry and the statistical results showed no publication bias. Classic fail-safe N and Egger linear regression tests further confirmed no significant publication bias (both P > 0.05) (Figure 5).

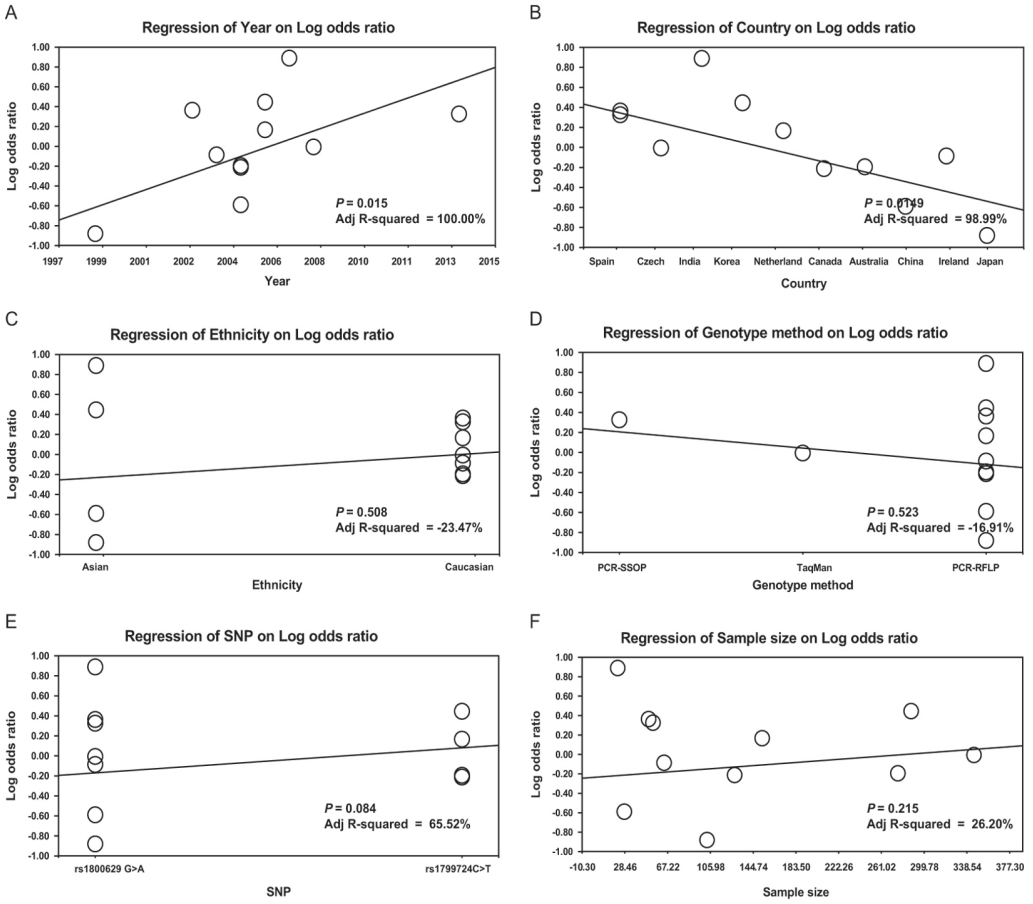

Figure 3. Meta-regression analysis in the present meta-analysis investigate the association between the *TNF-α* rs1800629 G>A and *TNF-α* rs1799724 C>T polymorphisms and the susceptibility to Crohn's disease.

Table 3. Meta-regression analyses of potential sources of heterogeneity in the present meta-analysis investigating the association between the *TNF-α* rs1800629 G>A and *TNF-α* rs1799724 C>T polymorphisms and the susceptibility to Crohn's disease.

Heterogeneity factors	Coefficient	SE	*t*	P	95%CI	
				(Adjusted)	LL	UL
Year	-0.024	0.042	-0.57	0.982	-0.141	0.932
Country	0.031	0.036	0.86	0.903	-0.068	0.13
Ethnicity	-0.084	0.117	-0.72	0.942	-0.408	0.24
Method	-0.16	0.179	-0.89	0.895	-0.656	0.337
SNP	0.051	0.138	0.37	0.997	-0.331	0.433
Sample	0	0	0.23	1	0	0

SE = standard error; LL = lower limit; UL = upper limit; SNP = single nucleotide polymorphism.

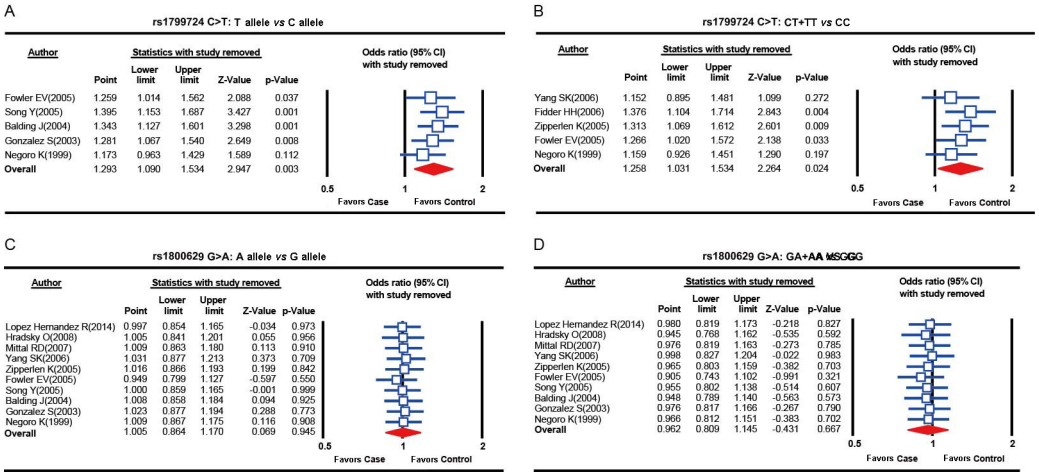

Figure 4. Sensitivity analyses of the summary odds ratio coefficients for the differences of genotype frequencies in the present meta-analysis investigate the association between the *TNF-α* rs1800629 G>A and *TNF-α* rs1799724 C>T polymorphisms and the susceptibility to Crohn's disease.

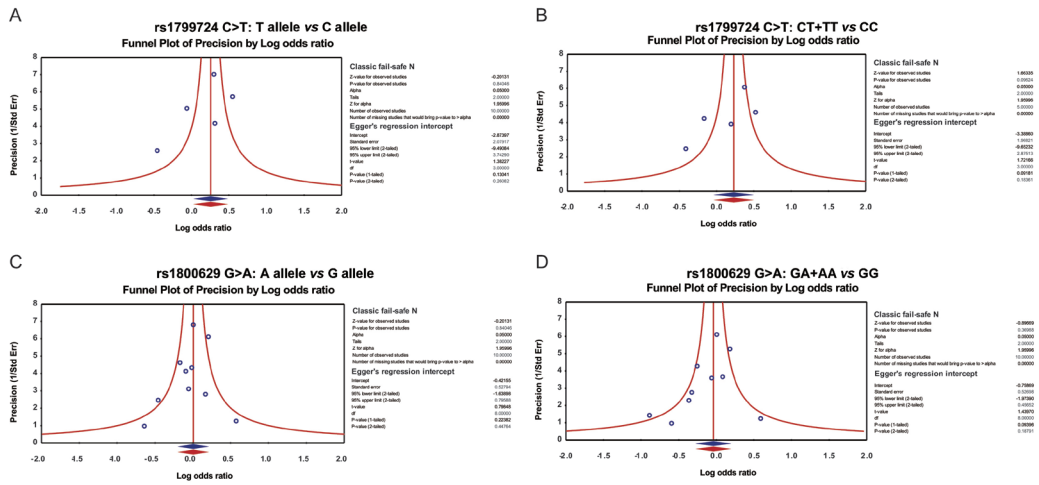

Figure 5. Publication biases for genotype frequencies in the present meta-analysis investigate the association between the *TNF-α* rs1800629 G>A and *TNF-α* rs1799724 C>T polymorphisms and the susceptibility to Crohn's disease.

DISCUSSION

IBD is comprised of ulcerative colitis and CD, 2 chronic conditions characterized by intestinal inflammation and progressive bowel damage (Khor et al., 2011). CD is a multifactorial disease influenced by environmental risk factors and genetic susceptibilities (Mondot et al., 2011). Genome-wide association studies recently identified more than 30 distinct loci implicated in CD pathogenesis, with most genes involved in the autophagy pathway and innate immune system

(Fransen et al., 2010; Baumgart and Sandborn, 2012). However, host genetic predisposition is likely not the only explanation for CD occurrence (Cucchiara et al., 2012). Environmental factors including smoking, appendectomy, or endogenous microbiota have also been implicated in the onset of CD (Mondot et al., 2011). Recent studies have shown inconsistent results regarding the role of the *TNF-α* rs1800629 G>A polymorphism in the pathophysiology of CD (Fan et al., 2011; López-Hernández et al., 2014). Therefore, this meta-analysis was conducted to examine the associations between the *TNF-α* rs1800629 G>A and *TNF-α* rs1799724 C>T polymorphisms and CD susceptibility. Based on the results of this meta-analysis, the *TNF-α* rs1799724 C>T polymorphism significantly increased the susceptibility to CD, while no significant association between the *TNF-α* rs1800629 G>A polymorphism and CD susceptibility was evident. Our results confirmed those of previous studies that found no association between the *TNF-α* rs1800629 G>A gene polymorphism and susceptibility to CD (Fan et al., 2011; López-Hernández et al., 2014). Our study also showed that the *TNF-α* rs1799724 C>T polymorphism increased the susceptibility to CD, which was in agreement with the results of 2 previous studies (Yang et al., 2006). Dysregulation of TNF-α is associated with the susceptibility to CD (Mikocka-Walus et al., 2007). It is well-known that *TNF-α* promoter polymorphisms affect gene expression (Mishra and Arankalle, 2011). Susceptibility to CD may be stimulated by a complex signaling network of cytokines, and increased TNF-α secretion may stimulate the secretion of IL-1A, IL-6, IL-17, CXCL-5, and IL-8, thereby differentially modulating the chemoattraction and function of immune effectors (Lee et al., 2008). Consistent with this, TNF-α and IL-1A cooperate to promote increased epithelial antigen uptake in the ileum in CD patients and in the release of T-helper cytokines, which is important for the onset and progression of CD (Fan et al., 2011). TNF-α controls multiple cellular processes, such as the production of inflammatory mediators, cell proliferation, and cell death, which are intricately associated with the epithelial response to injury (Leppkes et al., 2014). Furthermore, an indirect effect of TNF-α on epithelial homeostasis, mediated by mesenchymal cells, was proposed as an underlying mechanism of TNF-α-driven CD pathogenesis (Khor et al., 2011; Abdallah Hajj Hussein et al., 2012).

Based on ethnicity, study subjects in the present meta-analysis were classified into 2 subgroups, including Caucasians and Asians. This meta-analysis also revealed no significant association between the *TNF-α* rs1800629 G>A polymorphism and susceptibility to CD, which in accordance with our main results. A significant association between the *TNF-α* rs1799724 C>T polymorphism and susceptibility to CD was found in Asians, but not in Caucasians. Although CD was once considered as "Western" disease, mainly affecting North America and Western Europe, this disease is quickly increasing in other parts of the world, particularly in India, Japan, China, and the Middle East (Lee et al., 2011). However, the different susceptibilities between Caucasians and Asians found in this study could not be because of geographic differences alone.

There were some limitations to our meta-analysis. First, our lack of access to the original data from the included studies limited further assessment of potential interactions between other factors and susceptibility to CD, such as environment and genetic influences. Second, most of the 11 eligible studies were performed in Caucasians and Asians, which may have led to bias. Moreover, studies published in languages other than English and Chinese were not included in our meta-analysis.

In summary, our meta-analysis results showed that the *TNF-α* rs1799724 C>T polymorphism is associated with CD susceptibility. We found no significant association between *TNF-α* rs1800629 G>A and susceptibility to CD, which requires further investigation. Future studies including a larger sample size are required to better understand CD pathology.

Conflicts of interest

The authors declare no conflict of interest.

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