The association between TNF-α 238A/G and 308A/G polymorphisms and juvenile idiopathic arthritis

An updated PRISMA-compliant meta-analysis

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Abstract

Objective: A previous meta-analysis concluded that TNF-α 238A/G and TNF-α 308A/G polymorphisms were not associated with the risk of juvenile idiopathic arthritis (JIA) in the overall population or Caucasian subjects. With the publication of a fair number of studies on the association between TNF-α polymorphisms and JIA in recent years, we conducted this updated meta-analysis to make a more accurate evaluation of such relationship.

Methods: We adopted PubMed, EMBASE, ISI Web of Science and CNKI to identify observational studies that addressed the association between TNF-α polymorphisms and risk for JIA. The allelic effect of variant A for the risk of JIA was expressed as odds ratio (OR) along with the associated 95% confidence interval (95% CI). Meta-analyses were performed by pooling ORs and 95%CI from included studies using RevMan 5.3 software. The stratified-analysis based on ethnicity was performed to confirm the ethnicity-dependent effect on the relationship.

Results: A total of 15 case-control studies including 2845 patients in JIA groups and 4771 patients in control groups were included in our study. The findings indicated a statistically significant association between the A allele of the TNF-alpha 238A/G polymorphism and the decreased JIA risk in Caucasians ($P = .0002$). The study in Iranian showed similar results ($P = .0002$) whereas the studies in other ethnicities failed to replicate this finding: Han ($P = .29$), Mexican ($P = .64$) and Turkish population ($P = .32$). TNF-α 308A/G was not statistically associated with JIA in overall subjects or Caucasians.

Conclusion: Our study confirmed the protective role of the A allele in TNF-α 238A/G but not TNF-α 308A/G against the occurrence of JIA in the Caucasian population. To exactly validate the correlation between TNF-α polymorphisms and JIA in other ethnic backgrounds, additional studies are required.

Abbreviations: 95% CI = 95% confidence interval, JIA = juvenile idiopathic arthritis, NOS = the Newcastle–Ottawa Scale, OR = odds ratio, SNP = single nucleotide polymorphism.

Keywords: juvenile idiopathic arthritis, meta-analysis, polymorphism, TNF-α

1. Introduction

Juvenile idiopathic arthritis (JIA) is 1 of the most common childhood rheumatic diseases, which is characterized by arthritis for more than 6 weeks with onset before age of 16 years, and in severe cases may be debilitating, even life-threatening. According to the International League of Associations for Rheumatology (ILAR), JIA is a term which encompasses all forms of arthritis, including systemic arthritis, oligo-arthritis, polyarthritis rheumatoid factor (RF) negative, polyarthritis RF positive, psoriatic arthritis, enthesitis-related arthritis (ERA), and undifferentiated arthritis, but not a single disease.[1,2] One of the most intriguing questions of JIA is what triggers the disease and decides its pathogenesis. Despite the heterogeneity of JIA, genetic susceptibility, balancing tolerance and inflammation are all considered to be closely related to the subtypes of JIA.[3–5]

TNF-α is a multifunctional pro-inflammatory cytokine that belongs to the TNF superfamily, which is mainly secreted by macrophages.[6] This pro-inflammatory cytokine is involved in the regulation of a wide spectrum of biological processes including cell proliferation, differentiation, apoptosis, lipid metabolism, and coagulation, through binding to its receptors TNFRSF1A/TNFR1 and TNFRSF1B/TNFBR.[7] As 1 of the members of the TNF superfamily, it has been implicated in a variety of pathological processes including autoimmune diseases,[8] insulin resistance,[9] inflammatory diseases[10] and cancer.[11] Currently, the combination of methotrexate and tumor necrosis factor α blockade is the primary treatment program for JIA,[12] in spite of the exception of a possible small increased risk of lymphoma, other types of cancer and serious infections in children.[13,14] Additionally, a large number of

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studies have confirmed elevated levels of TNF-α in the serum and synovial fluid of JIA patients, which indicates the potential relationship between elevated level of TNF-α and occurrence of JIA.[15]

Single nucleotide polymorphisms (SNPs) are single nucleotide variations that can occur at any site in the DNA, which distribute widely across the human genome and may influence transcription of protein. Despite lots of research that has been conducted to study the association of SNPs of TNF promoter −308A/G and −238A/G and JIA susceptibility, the outcomes have been inconsistent. As far as we know, only 1 meta-analysis has been carried out to assess the correlation between TNF-α polymorphisms and JIA, the authors concluded that there was no association between the A allele (the variant allele) of TNF-α 238A/G or TNF-α 308A/G and JIA in European populations.[16] However, the sample size of that meta-analysis was not large enough, and they included an article that did not fit the inclusion criteria. Therefore, with the publication of a fair number of studies on the association between TNF-α polymorphisms and JIA in recent years, we conducted this updated meta-analysis to critically evaluate all the currently available evidence and to determine whether there is any association between SNPs of TNF-α promoter and risk of JIA.

2. Methods

This meta-analysis was performed in accordance with the PRISMA guideline.[17] This study does not need an ethical approval because it is a meta-analysis and does not include any data directly linked to individual patient information.

2.1. Literature search strategy

A comprehensive literature search was performed in PubMed, EMBASE, ISI Web of Science, and CNKI to identify potentially eligible studies that were published before July 1, 2017. No language restriction was imposed. We used a structural literature search strategy to retrieve observational studies that analyzed the association between TNF-α polymorphisms and JIA, the literature search strategy used in English databases was as follow: (SNP or SNPs or "Polymorphism, Single Nucleotide" [Mesh]) and ("Arthritis, Juvenile"[Mesh] or juvenile arthritis or JIA or juvenile rheumatoid arthritis or juvenile chronic arthritis or JIA or JRA) and ("Tumor Necrosis Factor-α"[Mesh] or TNF-α or Tumor Necrosis Factor-α). In CNKI, we searched relevant Chinese publications using "TNF-α" and "Duo Tai Xing" and "Guan Jie Yan". We used "Guan Jie Yan" which means "arthritis" to increase the sensitivity of our literature search in CNKI. Additionally, we screened the reference lists of relevant reviews and meta-analyses to avoid missing eligible studies.

2.2. Inclusion criteria and exclusion criteria

Available data were extracted from published articles, thus abstracts in meetings and conferences were not included in our study. The PICOS principle was used to establish the inclusion and exclusion criteria:

(1) subjects in JIA groups should be diagnosed as JIA according to well-established criteria such as American College of Rheumatology criteria,[18] ILAR classification [19] or Durban criteria;[20]
(2) subjects enrolled in control groups should be healthy individuals without a history of JIA and other autoimmune diseases;

(3) the type of included studies was required to be observational studies (case–control or cohort studies) that featured an assessment of the association between TNF-α polymorphisms and risk of JIA;
(4) the association between TNF-α SNP and JIA was expressed as OR and 95% confidence interval (95% CI), consequently all included studies should report the OR along with the 95% CI, or the OR and 95% CI could be calculated based upon the genotype frequencies. In the cases in which several studies reported an overlapping population, the most complete 1 was deemed eligible for inclusion.

2.3. Data extraction

Two independent reviewers (X Li and C Liang) did the literature search separately and were blinded to the results of the other investigator. A stringent screening according to the predetermined eligibility criteria was performed to determine potentially eligible article, the results of the literature search were compared afterwards. Data was extracted independently by 2 reviewers from these eligible articles using a standardized data collection sheet, which included first author, country, year of the publication, ethnicity, sample sizes, genotyping method, diagnostic criteria for JIA, frequencies of A allele in JIA groups and control groups, main results of each included study and HWE of control groups. Any discrepancy between the 2 investigators was resolved through discussion until a consensus was reached. The third review author (Z Lv) was consulted if a consensus could not be reached.

2.4. Quality assessment

The Newcastle–Ottawa Scale (NOS) for the assessment of non-randomized studies was adopted to assess the risk of bias in case–control studies and cohort studies.[21] Three broad perspectives including selection of cases and controls, comparability of the groups and ascertainment of outcome of interest were assessed using the Star system (http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp). Two reviewers (X Li and C Liang) independently evaluated the methodological quality of included studies. The results of risk of bias assessment were compared afterwards. In cases of discrepancies between 2 reviewers, the third reviewer (Z Lv) was consulted.

2.5. Statistical analysis

To test the association between TNF-α 238A/G or TNF-α 308A/G and JIA, the allelic effect of A (the variant allele) versus G (the common allele) was expressed as OR and 95% CI using the genotype frequency data derived from the included studies. Heterogeneity among included studies was estimated using a Q test and the Higgins I^2 test, where $P > .1$ and $I^2 < 50\%$ indicate acceptable heterogeneity. I^2 values of 0%, 25%, 50%, and 75% were defined as no, low, moderate and high heterogeneity, respectively.[22] We combined the OR of each study using the fixed-effect model if there was no high between-study heterogeneity across the eligible comparisons. Otherwise, a random-effect model was employed.[23,24]

As indicated by previous meta-analysis, there was a strong ethnicity-specific effect on the association between TNF-α polymorphisms and JIA risk, thus we performed subgroup analysis by ethnicity. The leave-1-out sensitivity analysis was conducted by removing each included study at a time and

reevaluating the resulting effect on pooled results. Begg rank correlation test and Egger linear regression test using Stata version 12.0 (Stata Corp LP) were combined to assess the publication bias if the number of included studies were larger than 5.[25] Publication bias was considered present with $P < .05$. The forest plots and funnel plots were generated via RevMan 5.3 software (Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2014).

3. Results

3.1. Literature search

A total of 200 and 42 studies were identified through literature searches including 59 from PubMed, 90 from EMBASE, 92 from ISI Web of Science, and 1 from CNKI (Fig. 1). One hundred eighty-four records were identified after the removal of 58 duplicates for titles and abstracts screen. One hundred sixty-four records were removed because of irrelevance, the remaining 20 records were screened through a full-page browse, and 2 records

were deleted for its unavailable data, 3 records were removed because they were not relative to the association between SNPs of TNF and JIA. Finally, 15 studies [26–40] were included in our meta-analysis according to our strict inclusion criteria.

3.2. Main characteristics of included studies

A total of 15 case-control studies published from 2002 to 2016 were identified, including 2845 patients in JIA groups and 4771 patients in control groups. These studies focused on different populations, 12 studies [26–34,36,38,39] were conducted in Caucasian populations, and the remaining 4 studies were performed in Han,[40] Iranian,[35] Mexican [37] and Turkish populations [31] respectively. The sample size of identified records varied from 114 to 1958. All JIA patients were diagnosed according to well-established diagnostic criteria including American College of Rheumatology (ACR) criteria, International League of Associations for Rheumatology (ILAR) classification criteria or Durban classification criteria. The majority of included studies failed to report the HWE of the genotypes distribution of
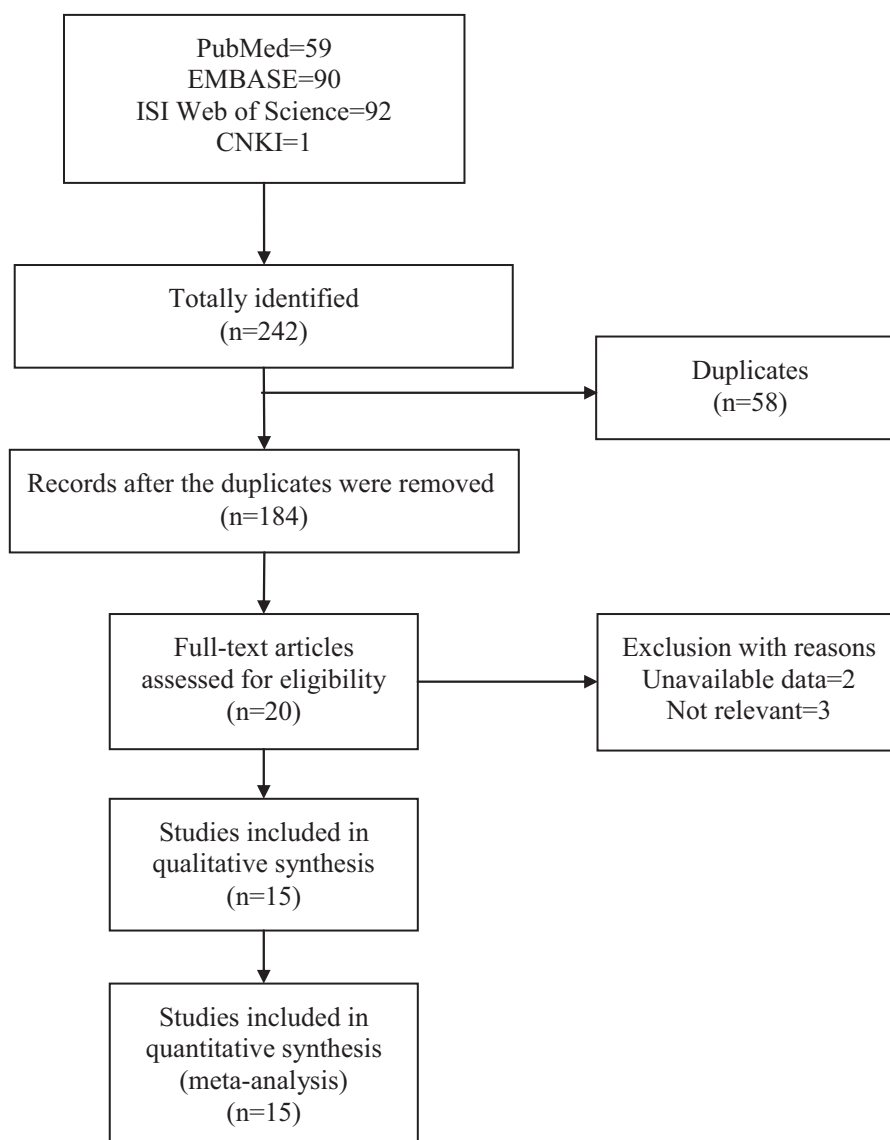

Figure 1. Flow diagram of literature search process.

Table 1

Main characteristics of included studies about the association between TNF-alpha-238A allele and JIA.

Study	Country	Ethnicity	Sample size (case/control)	Diagnostic criteria for JIA	A allele (%)		Association		Genotyping method	HWE
					JIA	Control	OR	95% CI		
Cimaz, 2007	Italy	Caucasian	107/630	ILAR	4.8	6.6	0.686	0.265–1.776	TaqMan	NR
Jimenez-Morales, 2009	Mexico	Mexican	171/400	ACR	6.1	4.1	1.155	0.627–2.128	TaqMan	0.44
Kaalla, 2013	USA	Caucasian	647/750	ILAR	4.0	6.0	0.660	0.460–0.950	TaqMan	>0.0125
Maddah, 2016	Iran	Iranian	53/137	ILAR	1.9	20.8	0.070	0.017–0.292	PCR-RFLP	NR
Miterski, 2004	Germany	Caucasian	130/375	ACR	17.2	15.5	0.714	0.289–1.768	PCR-RFLP	NR
Modesto, 2005	Spain	Caucasian	55/59	Durban	7.3	9.3	0.763	0.295–1.973	PCR-RFLP	NR
Ozen, 2002a	Czech	Caucasian	159/100	Durban	23.3	17.0	1.481	0.943–2.326	PCR-RFLP	NR
Ozen, 2002b	Turkey	Turkish	51/93	Durban	21.6	26.9	0.748	0.422–1.326	PCR-RFLP	NR
Reinards, 2015	Netherland	Caucasian	639/1319	ILAR	3.0	5.0	0.560	0.370–0.830	iPLEX MassARRAY	NR
Scardapane, 2015	Italy	Caucasian	74/77	ILAR	4.4	5.0	0.887	0.291–2.705	ABI-PRISM	0.59
Schmeling, 2006	Germany	Caucasian	228/196	ILAR	3.3	2.6	1.299	0.577–2.926	ABI-PRISM	NR
Zeggini, 2002	UK	Caucasian	137/76	ILAR	3.7	8.6	0.405	0.173–0.947	PCR-RFLP	NR
Zhen, 2008	China	Han	127/106	ILAR	7.1	4.7	1.541	0.695–3.414	PCR-RFLP	<0.01

ACR = American College of Rheumatology; HWE = Hardy–Weinberg equilibrium; ILAR = International League of Associations for Rheumatology; JIA = juvenile idiopathic arthritis; NR = not reported; PCR-RFLP = polymerase chain reaction-restriction fragment-length polymorphism.

SNP TNF-α-238A/G and TNF-α-308A/G in control groups. The main characteristics and genotypes distribution of TNF-α polymorphisms of included studies were listed in Tables 1 and 2.

3.3. Quality of included studies

The NOS was used to assess the methodological quality of included case control studies and the results were summarized in Table 3. Our included studies achieved an average of 5.9 stars, according to the definition and explanation given in each column of the NOS.

3.4. Meta-analyses results

The association between A allele of TNF-α-238A/G and risk of JIA was presented in Figure 2. The pooled OR was calculated for the JIA patients in different ethnic subgroups. There is significant association between the A allele of the TNF-α 238A/G

polymorphism and the decreased JIA risk in Caucasian patients (OR 0.77, 95% CI 0.63, 0.94; P = .009). The study in Iranian showed similar results (OR 0.07, 95% CI 0.02, 0.29; P = .0002) whereas the studies in other ethnicities failed to replicate this finding: Han (OR 1.54, 95% CI 0.70, 3.42; P = .29), Mexican (OR 1.15, 95% CI 0.63, 2.13; P = .64), and Turkish populations (OR 0.75, 95% CI 0.42, 1.33; P = .32).

The polymorphism of TNF-α 308A/G was found not to be related to the risk of JIA in all populations (OR 1.11, 95% CI 0.90, 1.38; P = .34). When stratified by ethnicity, the pooled ORs in Caucasian populations revealed that A allele of TNF-α 308A/G was not associated with risk of JIA (OR 1.13, 95% CI 0.91, 1.41; P = .26). Only the Iranian study indicated a statistically significant association between A allele of TNF-α 308A/G polymorphism and decreased risk of JIA (OR 0.12, 95% CI 0.03, 0.48; P = .003), whereas the Mexican population showed a significant association between the A allele of TNF-α 308A/G polymorphism and

Table 2

Main characteristics of included studies about the association between TNF-alpha-308A allele and JIA.

Study	Country	Ethnicity	Sample size (case/control)	Diagnostic criteria for JIA	A allele (%)		Association		Genotyping method	HWE
					JIA	Control	OR	95% CI		
Basic, 2010	Serbia	Caucasian	66/65	ILAR	39.4	30.0	1.835	1.086–3.101	PCR-RFLP	0.87
Cimaz, 2007	Italy	Caucasian	107/630	ILAR	9.7	13.7	0.652	0.327–1.300	TaqMan	NR
Jimenez-Morales, 2009	Mexico	Mexican	171/400	ACR	6.0	2.9	2.210	1.206–4.050	TaqMan	0.68
Kaalla, 2013	USA	Caucasian	647/750	ILAR	14.0	17.0	0.820	0.660–1.010	TaqMan	>0.0125
Maddah, 2016	Iran	Iranian	53/137	ILAR	1.8	14.2	0.116	0.028–0.489	PCR-RFLP	NR
Miterski, 2004	Germany	Caucasian	130/375	ACR	2.3	3.2	1.130	0.760–1.680	PCR-RFLP	NR
Modesto, 2005	Spain	Caucasian	55/59	Durban	12.7	11.9	1.083	0.491–2.390	PCR-RFLP	NR
Mourao, 2009	Portugal	Caucasian	115/118	Durban	12.2	11.4	1.073	0.611–1.884	PCR-RFLP	NR
Ozen, 2002a	Czech	Caucasian	159/100	Durban	27.0	15.5	2.021	1.281–3.188	PCR-RFLP	NR
Ozen, 2002b	Turkey	Turkish	51/93	Durban	25.5	31.2	0.755	0.439–1.299	PCR-RFLP	NR
Reinards, 2015	Netherland	Caucasian	639/1319	ILAR	15.0	17.0	0.870	0.710–1.060	iPLEX MassARRAY	NR
Scardapane, 2015	Italy	Caucasian	74/77	ILAR	11.5	5.8	2.091	0.901–4.852	ABI-PRISM	0.68
Schmeling, 2006	Germany	Caucasian	228/196	ILAR	13.8	17.1	0.778	0.535–1.130	ABI-PRISM	NR
Schubert, 2006	Germany	Caucasian	86/270	ILAR	14.5	14.6	0.992	0.610–1.614	PCR-RFLP	>0.05
Zeggini, 2002	UK	Caucasian	138/75	ILAR	23.6	12.7	2.124	1.218–3.702	PCR-RFLP	NR
Zhen, 2008	China	Han	127/106	ILAR	5.9	4.7	1.268	0.557–2.884	PCR-RFLP	0.89

ACR = American College of Rheumatology; HWE = Hardy-Weinberg equilibrium; ILAR = International League of Associations for Rheumatology; JIA = juvenile idiopathic arthritis; NR = not reported; PCR-RFLP = polymerase chain reaction-restriction fragment-length polymorphism.

Table 3

Quality assessment of included case-control studies.

Item	1	2	3	4	5	6	7	8
Basic, 2010	*	—	—	*	**	*	*	*
Cimaz, 2007	*	—	—	*		*	*	*
Jimenez-Morales, 2009	*	—	—	*	*	*	*	*
Kaalla, 2013	*	—	—	*	*	*	*	*
Maddah, 2016	*	*	*	*	—	*	*	*
Miterski, 2004	*	—	—	*	—	*	*	*
Modesto, 2005	*	—	—	*		*	*	*
Mourao, 2009	*	–	—	*	**	*	*	*
Ozen, 2002	*	—	—	*	—	*	*	*
Reinards, 2015	*	—	—	*		*	*	*
Scardapane, 2015	*	—	—	*	**	*	*	*
Schmeling, 2006	*	—	—	*	**	*	*	*
Schubert, 2006	*	—	—	*	*	*	*	*
Zeggini, 2002	*	—	—	*		*	*	*
Zhen, 2008	*	—	—	*	—	*	*	*

A study could be awarded a maximum of one star for each item except for the item Control for important factor or additional factor. The definition/explanation of each column of the Newcastle–Ottawa Scale is available from http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp; Item 1: Adequate definition of cases; Item 2: Representativeness of cases; Item 3: Selection of control subjects; Item 4: Definition of control subjects; Item 5: Control for important factor or additional factor; Item 6: Exposure assessment; Item 7: Same method of ascertainment for all subjects; Item 8: Non-response rate.
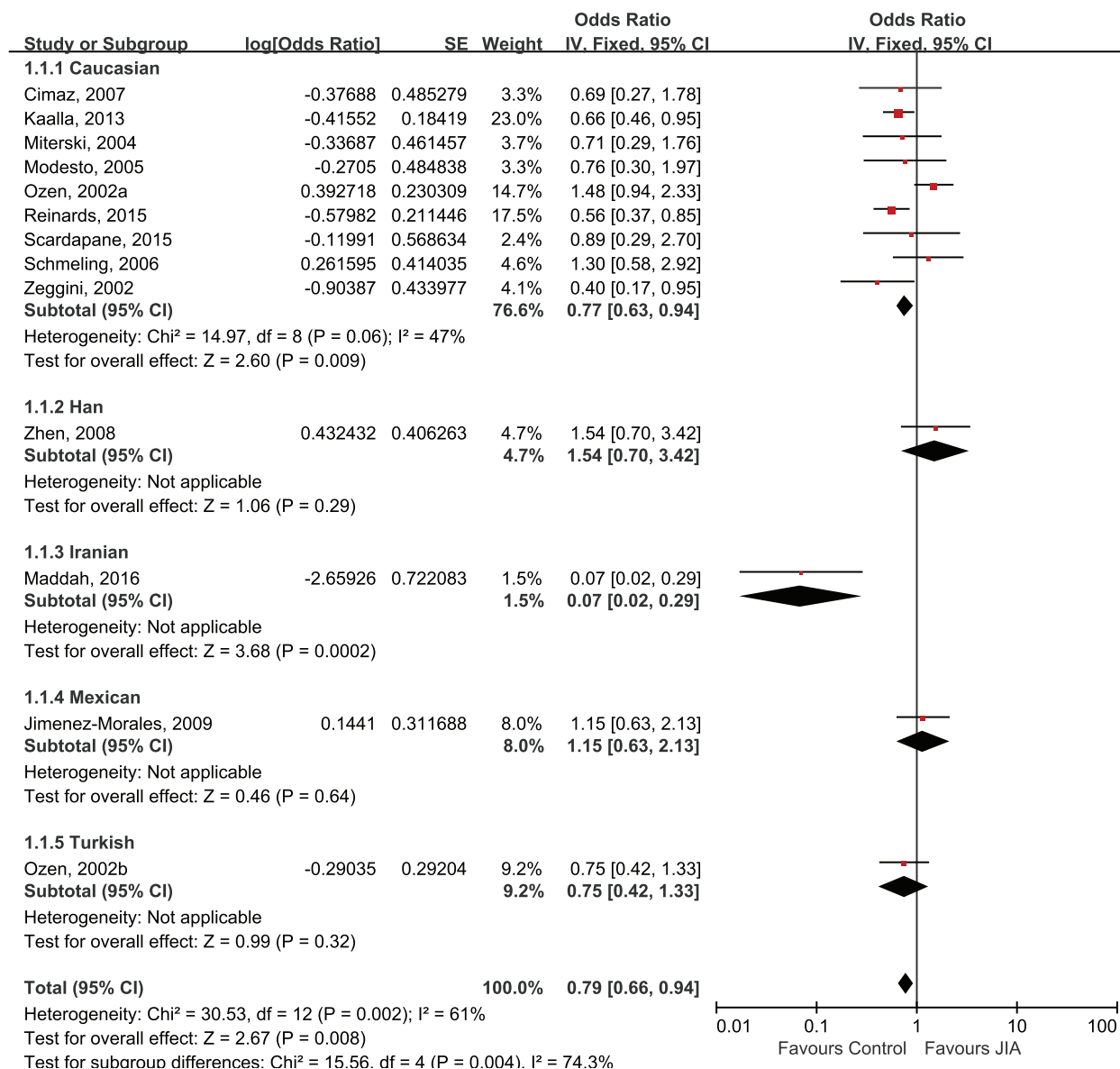

Figure 2. Forest plot of A allele of TNF-α 238A/G and risk of juvenile idiopathic arthritis.

Study or Subgroup	log[Odds Ratio]	SE	Weight	Odds Ratio IV. Random, 95% CI	Odds Ratio IV. Random, 95% CI
2.1.1 Caucasian					
Basic, 2010	0.607044	0.267624	6.5%	1.83 [1.09, 3.10]	
Cimaz, 2007	-0.42771	0.352084	5.1%	0.65 [0.33, 1.30]	
Kaalla, 2013	-0.19845	0.110747	9.6%	0.82 [0.66, 1.02]	
Miterski, 2004	0.122218	0.202375	7.8%	1.13 [0.76, 1.68]	
Modesto, 2005	0.079735	0.403595	4.4%	1.08 [0.49, 2.39]	
Mourao, 2009	0.070458	0.287304	6.2%	1.07 [0.61, 1.88]	
Ozen, 2002a	0.703592	0.232628	7.2%	2.02 [1.28, 3.19]	
Reinards, 2015	-0.13926	0.103688	9.7%	0.87 [0.71, 1.07]	
Scardapane, 2015	0.737642	0.429537	4.1%	2.09 [0.90, 4.85]	
Schmeling, 2006	-0.25103	0.191051	8.0%	0.78 [0.54, 1.13]	
Schubert, 2006	-0.00803	0.248094	6.9%	0.99 [0.61, 1.61]	
Zeggini, 2002	0.753301	0.28372	6.2%	2.12 [1.22, 3.70]	
Subtotal (95% CI)			81.7%	1.13 [0.91, 1.41]	

Heterogeneity: Tau² = 0.08; Chi² = 32.97, df = 11 (P = 0.0005); I² = 67%
Test for overall effect: Z = 1.14 (P = 0.26)

2.1.2 Han					
Zhen, 2008	0.237441	0.41971	4.2%	1.27 [0.56, 2.89]	
Subtotal (95% CI)			4.2%	1.27 [0.56, 2.89]	

Heterogeneity: Not applicable
Test for overall effect: Z = 0.57 (P = 0.57)

2.1.3 Iranian					
Maddah, 2016	-2.15417	0.725197	1.9%	0.12 [0.03, 0.48]	
Subtotal (95% CI)			1.9%	0.12 [0.03, 0.48]	

Heterogeneity: Not applicable
Test for overall effect: Z = 2.97 (P = 0.003)

2.1.4 Mexican					
Jimenez-Morales, 2009	0.792993	0.309022	5.8%	2.21 [1.21, 4.05]	
Subtotal (95% CI)			5.8%	2.21 [1.21, 4.05]	

Heterogeneity: Not applicable
Test for overall effect: Z = 2.57 (P = 0.01)

2.1.5 Turkish					
Ozen, 2002b	-0.28104	0.276642	6.4%	0.75 [0.44, 1.30]	
Subtotal (95% CI)			6.4%	0.75 [0.44, 1.30]	

Heterogeneity: Not applicable
Test for overall effect: Z = 1.02 (P = 0.31)

Total (95% CI)			100.0%	1.11 [0.90, 1.38]	

Heterogeneity: Tau² = 0.12; Chi² = 49.73, df = 15 (P < 0.0001); I² = 70%
Test for overall effect: Z = 0.96 (P = 0.34)
Test for subgroup differences: Chi² = 16.74, df = 4 (P = 0.002), I² = 76.1%

0.01   0.1   1   10   100
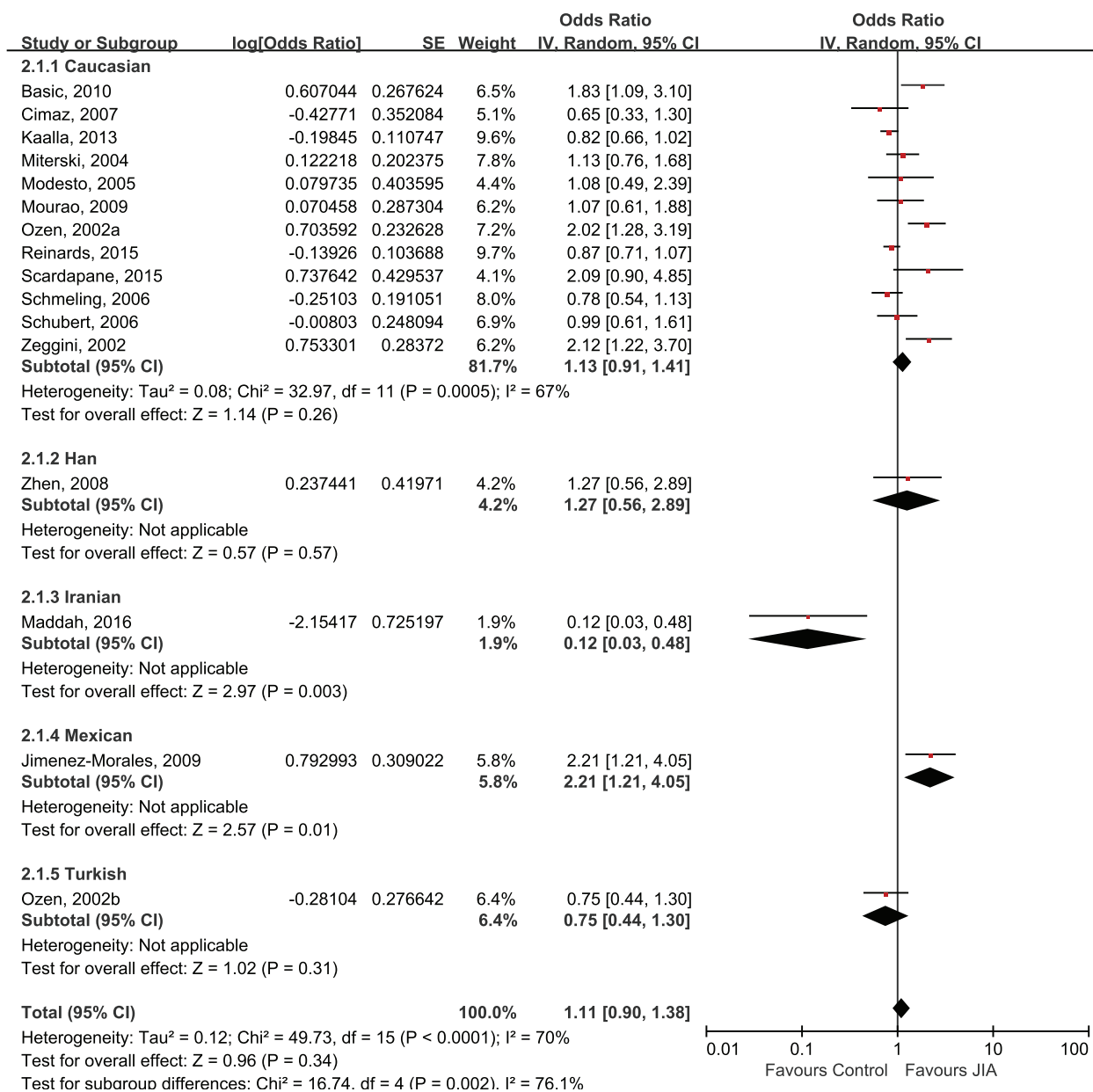Favours Control   Favours JIA

Figure 3. Forest plot of A allele of TNF-α 308A/G and risk of juvenile idiopathic arthritis.

increased risk of JIA (OR 2.21, 95% CI 1.21, 4.05; $P = .01$) (Fig. 3).

3.5. Sensitivity analysis and publication bias

The sensitivity analysis was only performed in Caucasian subjects due to the limited number of studies in other ethnicities. The association between A allele of TNF-α 238A/G polymorphism and JIA remained statistically significant after the removal of any included study (data not shown). However, the heterogeneity across studies was absent after the removal of Ozens' study ($I^2 = 0\%$).[31] In comparison to other included studies, Ozen et al reported a much higher A allele frequency in both JIA group (Ozen: 23.3% vs mean: 8.3%) and control groups (Ozen: 17.0% vs 10.2%). As a result, Ozens' study was excluded in our meta-analysis (Fig. 4). The sensitivity analysis also indicated that the association between TNF-

α 308A/G was stable because the negative finding remained unchanged after the exclusion of each study. Since the Ozens' study reported much higher A allele frequency in their study, we excluded this study in our repeated meta-analysis, resulting in a decline of heterogeneity declined to $I^2 = 57\%$ (Fig. 5).

The funnel plots were symmetric and thus suggesting no obvious publication bias (Figs. 6 and 7). The Begg's test (TNF-α 238A/G: z = 0.87, $P = .386$; TNF-α 308A/G: z = 1.40, $P = .161$) and Egger test (TNF-α 238A/G: t = 0.88, $P = 0.415$; TNF-α 308A/G: t = 2.20, $P = .056$) also reveal no statistically significant publication bias.

4. Discussion

In our meta-analysis, a total of 15 studies (2845 patients in JIA groups and 4771 patients in control groups) were identified and
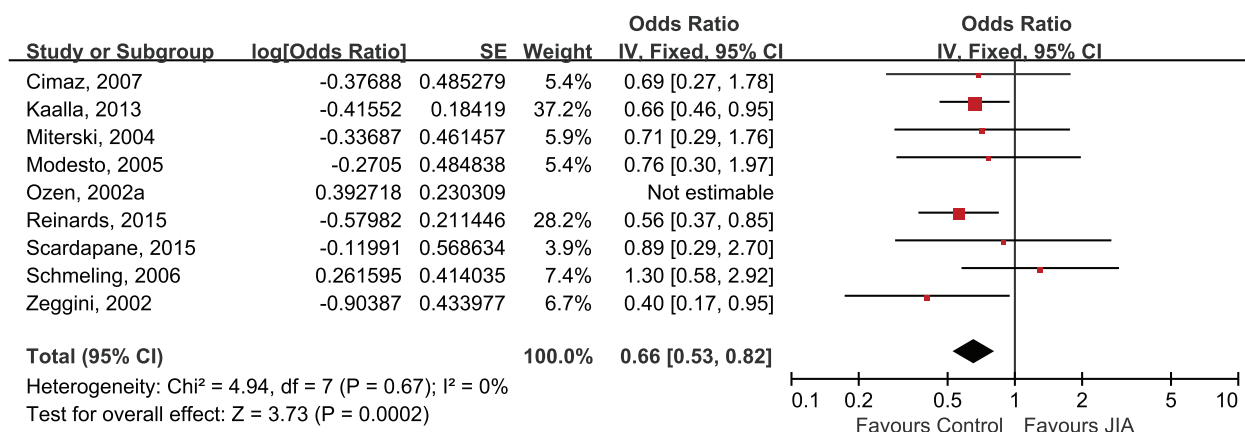
Figure 4. Sensitivity analysis for TNF-α 238A/G and risk of juvenile idiopathic arthritis in Caucasians.
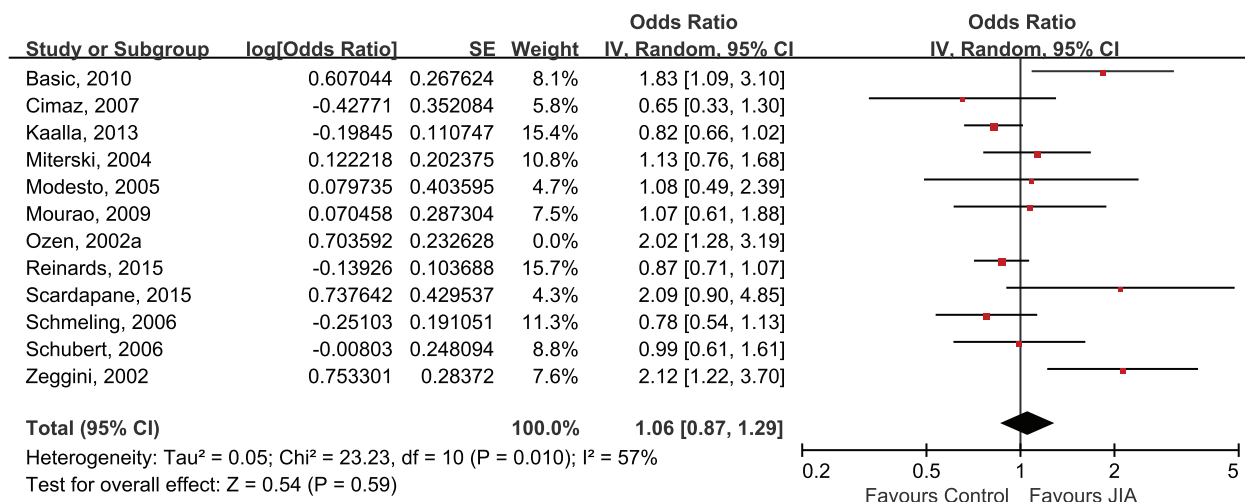

Figure 5. Sensitivity analysis for TNF-α 308A/G and risk of juvenile idiopathic arthritis in Caucasians.

included in our present study, which were composed of 5 ethnicities from 12 countries. Meanwhile, the stratification by ethnicity was performed to confirm the ethnicity-dependent effect on the relationship between the SNP of TNF-α and the risk of JIA. The results of the meta-analyses indicated that there was a significant association between the A allele of the TNF-α 238A/G but not TNF-α 308A/G polymorphism and a decreased JIA risk in Caucasian patients. The pooled ORs for A allele of the TNF-α 238A/G polymorphism indicated that it conferred a protective role against JIA in Caucasian groups. In terms of polymorphism of TNF-α 308A/G, no significant association for the risk of JIA was found in Caucasians. However, significant findings in the Mexican populations and Iranian were hampered by the limited number of included studies.

A previous meta-analysis for the association between TNF polymorphisms and JIA has been published, which showed no significant relationship between either TNF-α 308A/G or TNF-α 238A/G polymorphisms and JIA in European population.[16] However, the sample size of that meta-analysis was not large enough (7 studies for TNF-α 238A/G and 9 studies for TNF-α

308A/G), and they included an article that did not fit the inclusion criteria for the diagnosis of JIA. In Hohlers' study, subjects in JIA and control groups were all adults and the "juvenile onset" was defined as onset before age of 40 in their study.[41] In our study, we incorporated sufficient European samples assessing the association between TNF-α 238A/G or TNF-α 308A/G polymorphisms and JIA with the consideration of between-study heterogeneity. But our results did not support the findings of the previous meta-analysis, a statistically significant association between TNF-α 238A/G and decreased risk of JIA was found in Caucasian subjects (OR 0.66, 95% CI 0.53, 0.82; $P=.0002$). We then tried to determine whether the exclusion of Hohlers' study and the addition of newly identified studies led to the positive association between TNF-α 238A/G and JIA. Based on the forest plot of previous meta-analysis (combined OR 1.25, 95% CI 0.60, 2.57; $P=.56$), the conclusion remained statistically non-significant even after the removal of Hohlers' study (OR 1.08, 95% CI 0.77, 1.50; $P=.67$), suggesting that the inclusion of more additional studies contributed to the positive finding in our present study. The positive finding was further confirmed by
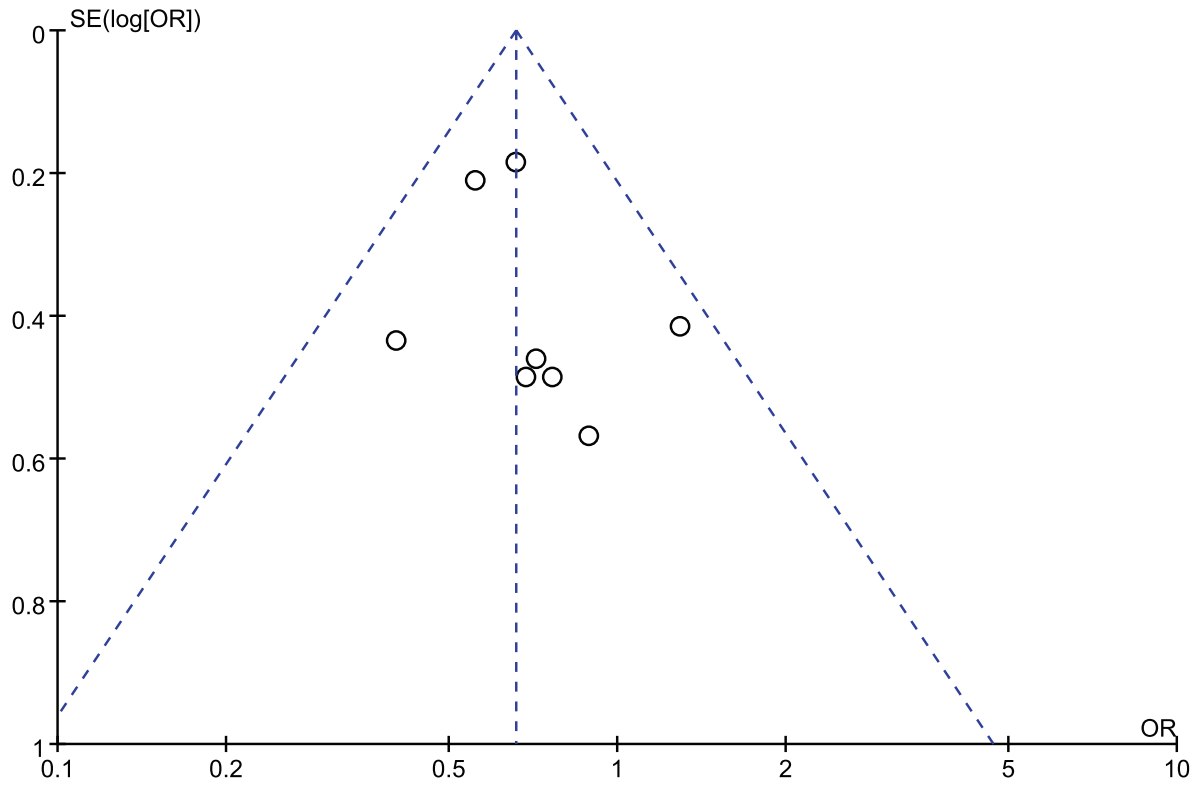
7

Figure 6. Funnel plot of TNF-α 238A/G and risk of juvenile idiopathic arthritis in Caucasians.
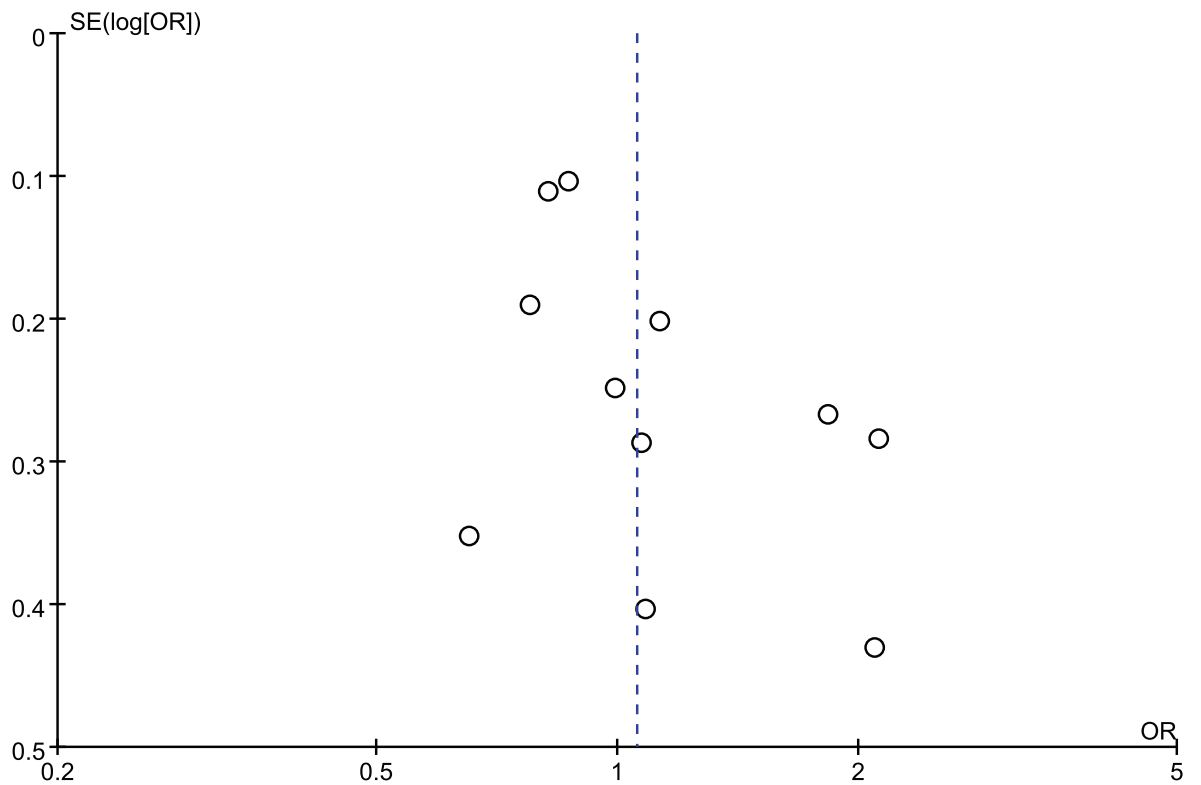

Figure 7. Funnel plot of TNF-α 308A/G and risk of juvenile idiopathic arthritis in Caucasians.

sensitivity analysis and statistically low between-study heterogeneity ($I^2 = 0\%$). In terms of the association between TNF-α 308A/G and JIA, despite the heterogeneity across studies of the European sample was up to 67%, the stability and reliability of conclusion was validated by sensitivity analysis.

TNF-α, a gene located in the HLA class 3 region, plays an essential role in the pathology process of inflammation and autoimmune disorders.[8,42] A large number of literatures have demonstrated the fluctuating TNF-α concentration in both the plasma and synovial fluid of juvenile with chronic arthritis.[43–45] The fluctuation in levels of TNF-α would affect the levels of multiple cytokines, including IL-1β, IL-6, IL-8, MCP-1, GM-CSF, and CCL5 [46] as well as several matrix metalloproteinase.[47] These cytokines were further involved in the progression of joint destruction in JIA. Miyamaet al[48] reported that IL-6 suppressed early differentiation of ATDC5 chondrocyte, which may result in the growth impairment of systemic JIA patients. Meanwhile, MacRae VE and his team demonstrated that the multiple suppressive effects would be exerted on chondrogenic progenitor cells when they were exposed to IL-1β, including the retardation of chondrocyte proliferation and secretion of Aggrecan and Collagen types II and X.[49] TNF-α can also promote the apoptosis of the chondrocyte population and restrain the formation of cartilaginous nodule and the secretion of cartilage-specific proteoglycan.[50,51] It has been shown that TNF-α regulated the cartilage metabolism by complicated signaling pathways, involving the induction of IGF-1 resistance, suppressor of cytokine signaling proteins (SOCS)-3, activation of the intracellular tyrosine kinase JAK2, as well as several cellular proteins.[52–55] For the promotor of TNF-α, several polymorphisms have been studied to explore whether there was any relationship between the polymorphisms and the function of TNF-α.[56,57] Among the polymorphisms, 2 of the most important were SNP TNF-α 238A/G and TNF-α 308A/G, which are associated with enhanced TNF-α production and TNF- α secretion.[58,59] And it has been demonstrated that the TNF-238 polymorphism could induce the CpG methylation site to regulate the gene transcriptional rate.[60]

Zeggini and coworkers for the first time identified 4 haplotypes (−1031, −863, −308, +489) that could be associated with risk of JIA.[26] Haplotype-tagging SNPs were determined for transmitted and non-transmitted haplotype groups separately since findings of non-transmitted haplotypes could represent SNP patterns observed in the normal population whereas data derived from the tests of transmitted haplotypes reflect findings in patients with JIA. Maddah et al [35] observed a significant positive association for TNF-α GG haplotypes (positions −308, −238) in patients with JIA compared to healthy volunteers ($P < .01$), a notable negative haplotypic association was found for TNF-α AG and GA haplotypes at the same positions in case group when comparing with control group. However, only 2 of our included studies evaluated the association between TNF-α polymorphisms and risk of JIA at the haplotypic level, definite conclusion could not be drawn owing to limited number of included studies.

Several potential limitations of this meta-analysis should be acknowledged. First and the most important is the insufficient number of sample sizes. Though the sample size of European is plenty, the number of other ethnicity is not large enough, which may lead to the inaccurate assessment for the association between TNF-α polymorphisms and susceptibility to JIA in other ethnic backgrounds. Although we found a protective effect of the A allele of TNF-α 238A/G against the occurrence of JIA, this conclusion could not be generalized amongst other ethnicities.

Secondly, the disease categories of JIA were classified into 7 subtypes according to the classification of ILAR.[19] However, a meta-analysis based on JIA subtype could not be conducted because of the limited quantity of literature. Third, despite of complex genetic diseases, the phenotype and organ specificity of JIA were associate with other confounding factors, involving in age at disease onset, gender, the level of ANA auto-antibodies and so on.[61,62] Therefore, more clinical materials are required to exactly validate the correlation between TNF-α polymorphisms and JIA subtypes.

In conclusion, our study illuminates the association between TNF-α 238A/G and TNF-α 308A/G polymorphisms and JIA in different ethnicities. The findings indicate that the A allele of the TNF-α 238A/G polymorphism would decrease JIA risk in Caucasian subjects, but A allele of TNF-α 308A/G cannot confer a protective role for JIA. The potential role of TNF-α polymorphisms in JIA could not be confirmed in other ethnicities due to insufficiency of available data. Despite potential limitations, we provided a high-quality and reliable result about the role of TNF-α polymorphisms in the occurrence and progression of JIA.

Author contributions

Zheng-tao Lv produced the idea of this study and was responsible for making the final version of this paper. Xing-yan Li and Chun-hua Liang did the literature search, screened the potentially eligible studies and evaluated the data from each included study, they both contributed equally to this work. Virginia Parkman was involved in revising the manuscript, including some important intellectual content and English editing. Zheng-tao Lv had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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References

[1] Prakken B, Albani S, Martini A. Juvenile idiopathic arthritis. Lancet 2011;377:2138–49.
[2] Martini A, Lovell DJ. Juvenile idiopathic arthritis: state of the art and future perspectives. Ann Rheum Dis 2010;69:1260–3.
[3] Woo P, Colbert RA. An overview of genetics of paediatric rheumatic diseases. Best practice & research. Clin Rheumatol 2009; 23:589–97.
[4] Glass DN, Giannini EH. Juvenile rheumatoid arthritis as a complex genetic trait. Arthritis Rheum 1999;42:2261–8.
[5] Wedderburn LR, Patel A, Varsani H, et al. Divergence in the degree of clonal expansions in inflammatory T cell subpopulations mirrors HLA-

associated risk alleles in genetically and clinically distinct subtypes of childhood arthritis. Int Immunol 2001;13:1541–50.

[6] Riches DW, Chan ED, Winston BW. TNF-alpha-induced regulation and signalling in macrophages. Immunobiology 1996;195:477–90.

[7] Horiuchi T, Mitoma H, Harashima S, et al. Transmembrane TNF-alpha: structure, function and interaction with anti-TNF agents. Rheumatology (Oxford, England) 2010;49:1215–28.

[8] Doss GP, Agoramoorthy G, Chakraborty C. TNF/TNFR: drug target for autoimmune diseases and immune-mediated inflammatory diseases. Front Biosci (Landmark edition) 2014;19:1028–40.

[9] Hotamisligil GS, Shargill NS, Spiegelman BM. Adipose expression of tumor necrosis factor-alpha: direct role in obesity-linked insulin resistance. Science 1993;259:87–91.

[10] Esposito E, Cuzzocrea S. TNF-alpha as a therapeutic target in inflammatory diseases, ischemia-reperfusion injury and trauma. Curr Med Chem 2009;16:3152–67.

[11] Wu Y, Zhou BP. TNF-alpha/NF-kappaB/Snail pathway in cancer cell migration and invasion. Br J Cancer 2010;102:639–44.

[12] Goldzweig O, Hashkes PJ. Abatacept in the treatment of polyarticular JIA: development, clinical utility, and place in therapy. Drug Des Devel Ther 2011;5:61–70.

[13] Ruperto N, Martini A. Juvenile idiopathic arthritis and malignancy. Rheumatology (Oxf Engl) 2014;53:968–74.

[14] Toussi SS, Pan N, Walters HM, et al. Infections in children and adolescents with juvenile idiopathic arthritis and inflammatory bowel disease treated with tumor necrosis factor-alpha inhibitors: systematic review of the literature. Clin Infect Dis 2013;57:1318–30.

[15] Kaminiarczyk-Pyzalka D, Adamczak K, Mikos H, et al. Serum TNF-alpha levels and indicators of disease activity in children with oligoarticular juvenile idiopathic arthritis (oJIA) in the first year of the disease. Clin Lab 2014;60:799–807.

[16] Lee YH, Bae SC, Song GG. TNF promoter −308A/G and −238A/G polymorphisms and juvenile idiopathic arthritis: a meta-analysis. Mol Biol Rep 2012;39:8497–503.

[17] Moher D, Liberati A, Tetzlaff J, et al. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. PLoS medicine 2009;6:e1000097.

[18] Arnett FC, Edworthy SM, Bloch DA, et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum 1988;31:315–24.

[19] Petty RE, Southwood TR, Manners P, et al. International league of associations for rheumatology classification of juvenile idiopathic arthritis: second revision, Edmonton, 2001. J Rheumatol 2004;31: 390–2.

[20] Petty RE, Southwood TR, Baum J, et al. Revision of the proposed classification criteria for juvenile idiopathic arthritis: Durban, 1997. J Rheumatol 1998;25:1991–4.

[21] Wells GA, Shea B, O'Connell D, et al. The Newcastle-Ottawa Scale (NOS) for assessing the quality of nonrandomised studies in meta-analyses. Available at: http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp. Accessed 1 July, 2017.

[22] Higgins JP, Thompson SG, Deeks JJ, et al. Measuring inconsistency in meta-analyses. BMJ 2003;327:557–60.

[23] Mantel N, Haenszel W. Statistical aspects of the analysis of data from retrospective studies of disease. J Ntl Cancer Inst 1959;22:719–48.

[24] DerSimonian R, Laird N. Meta-analysis in clinical trials. Control ClinTrials 1986;7:177–88.

[25] Egger M, Davey Smith G, Schneider M, et al. Bias in meta-analysis detected by a simple, graphical test. BMJ 1997;315:629–34.

[26] Zeggini E, Thomson W, Kwiatkowski D, et al. Linkage and association studies of single-nucleotide polymorphism-tagged tumor necrosis factor haplotypes in juvenile oligoarthritis. Arthritis Rheum 2002;46:3304–11.

[27] Schubert K, Von Bonnsdorf H, Burke M, et al. A comprehensive candidate gene study on bronchial asthma and juvenile idiopathic arthritis. Dis Markers 2006;22:127–32.

[28] Schmeling H, Horneff G. Tumour necrosis factor alpha promoter polymorphisms and etanercept therapy in juvenile idiopathic arthritis. Rheumatol Int 2007;27:383–6.

[29] Scardapane A, Ferrante R, Nozzi M, et al. TNF-α gene polymorphisms and juvenile idiopathic arthritis: Influence on disease outcome and therapeutic response. SeminArthritis Rheum 2015;45:35–41.

[30] Reinards THCM, Albers HM, Brinkman DMC, et al. CD226 (DNAM-1) is associated with susceptibility to juvenile idiopathic arthritis. Ann Rheum Dis 2015;74:2193–8.

[31] Ozen S, Alikasifoglu M, Bakkaloglu A, et al. Tumour necrosis factor alpha G−>A -238 and G−>A -308 polymorphisms in juvenile idiopathic arthritis. Rheumatology (Oxf, Engl) 2002;41:223–7.

[32] Mourao AF, Caetano-Lopes J, Costa P, et al. Tumor necrosis factor-α -308 genotypes influence inflammatory activity and TNF-α serum concentrations in children with juvenile idiopathic arthritis. JRheumatol 2009;36:837–42.

[33] Modesto C, Patiño-García A, Sotillo-Piñeiro E, et al. TNF-α promoter gene polymorphisms in Spanish children with persistent oligoarticular and systemic-onset juvenile idiopathic arthritis. Scandinavian J Rheumatol 2005;34:451–4.

[34] Miterski B, Drynda S, Boschow G, et al. Complex genetic predisposition in adult and juvenile rheumatoid arthritis. BMC genetics 2004;5:2.

[35] Maddah M, Harsini S, Ziaee V, et al. Association of tumour necrosis factor-alpha G/A -238 and G/A -308 single nucleotide polymorphisms with juvenile idiopathic arthritis. Int J Immunogenet 2016;43:391–6.

[36] Kaalla MJ, Broadaway KA, Rohani-Pichavant M, et al. Meta-analysis confirms association between TNFA-G238A variant and JIA, and between PTPN22-C1858T variant and oligoarticular, RF-polyarticular and RF-positive polyarticular JIA. Pediatr Rheumatol 2013;11:

[37] Jiménez-Morales S, Velázquez-Cruz R, Ramírez-Bello J, et al. Tumor necrosis factor-α is a common genetic risk factor for asthma, juvenile rheumatoid arthritis, and systemic lupus erythematosus in a Mexican pediatric population. Human Immunol 2009;70:251–6.

[38] Cimaz R, Cazalis MA, Reynaud C, et al. IL1 and TNF gene polymorphisms in patients with juvenile idiopathic arthritis treated with TNF inhibitors. Ann Rheum Dis 2007;66:900–4.

[39] Basic J, Pavlovic D, Jevtovic-Stoimenov T, et al. Etanercept reduces matrix metalloproteinase-9 level in children with polyarticular juvenile idiopathic arthritis and TNF-α-308GG genotype. J Physiol Biochem 2010;66:173–80.

[40] Zhen XG, Peng S, Cao YJ. Correlation between single nucleotide of polymorphisms of -238, -308G/A in tumor necrosis factor-a gene promoter and juvenile idiopathic arthritis. J Appl Clin Pediatr 2008; 23:677–9.

[41] Hohler T, Kruger A, Schneider PM, et al. A TNF-alpha promoter polymorphism is associated with juvenile onset psoriasis and psoriatic arthritis. J Invest Dermatol 1997;109:562–5.

[42] Brenner D, Blaser H, Mak TW. Regulation of tumour necrosis factor signalling: live or let die. Nature reviews. Immunology 2015;15:362–74.

[43] Kutukculer N, Caglayan S, Aydogdu F. Study of pro-inflammatory (TNF-alpha, IL-1alpha, IL-6) and T-cell-derived (IL-2, IL-4) cytokines in plasma and synovial fluid of patients with juvenile chronic arthritis: correlations with clinical and laboratory parameters. Clin Rheumatol 1998;17:288–92.

[44] Mangge H, Kenzian H, Gallistl S, et al. Serum cytokines in juvenile rheumatoid arthritis. Correlation with conventional inflammation parameters and clinical subtypes. Arthritis Rheum 1995;38:211–20.

[45] Lepore L, Pennesi M, Saletta S, et al. Study of IL-2, IL-6, TNF alpha, IFN gamma and beta in the serum and synovial fluid of patients with juvenile chronic arthritis. Clin Exp Rheumatol 1994;12:561–5.

[46] Kapoor M, Martel-Pelletier J, Lajeunesse D, et al. Role of proinflammatory cytokines in the pathophysiology of osteoarthritis. Nature reviews. Rheumatology 2011;7:33–42.

[47] Basic J, Pavlovic D, Jevtovic-Stoimenov T, et al. Etanercept reduces matrix metalloproteinase-9 level in children with polyarticular juvenile idiopathic arthritis and TNF-alpha-308GG genotype. J Physiol Biochem 2010;66:173–80.

[48] Gaspari S, Marcovecchio ML, Breda L, et al. Growth in juvenile idiopathic arthritis: the role of inflammation. Clin Exp Rheumatol 2011;29:104–10.

[49] MacRae VE, Farquharson C, Ahmed SF. The restricted potential for recovery of growth plate chondrogenesis and longitudinal bone growth following exposure to pro-inflammatory cytokines. J Endocrinol 2006;189:319–28.

[50] Aizawa T, Kon T, Einhorn TA, et al. Induction of apoptosis in chondrocytes by tumor necrosis factor-alpha. J Orthop Res 2001; 19:785–96.

[51] Horiguchi M, Akiyama H, Ito H, et al. Tumour necrosis factor-alpha up-regulates the expression of BMP-4 mRNA but inhibits chondrogenesis in mouse clonal chondrogenic EC cells, ATDC5. Cytokine 2000;12: 526–30.

[52] MacRae VE, Farquharson C, Ahmed SF. The pathophysiology of the growth plate in juvenile idiopathic arthritis. Rheumatology (Oxf, Engl) 2006;45:11–9.

[53] Wojcik J, Postel-Vinay MC. Signal transduction of the growth hormone (GH) receptor, and GH-binding protein. Growth Horm IGF Res 1999;9 (suppl A):51–5.

[54] Yoshizato H, Tanaka M, Nakai N, et al. Growth hormone (GH)-stimulated insulin-like growth factor I gene expression is mediated by a

tyrosine phosphorylation pathway depending on C-terminal region of human GH receptor in human GH receptor-expressing Ba/F3 cells. Endocrinology 2004;145:214–20.

[55] Shi H, Tzameli I, Bjorbaek C, et al. Suppressor of cytokine signaling 3 is a physiological regulator of adipocyte insulin signaling. J Biol Chem 2004;279:34733–40.

[56] Field M. Tumour necrosis factor polymorphisms in rheumatic diseases. QJM Monthly J Assoc Phys 2001;94:237–46.

[57] Zhu F, Zhao H, Tian X, et al. Association between tumor necrosis factor-alpha rs1800629 polymorphism and risk of gastric cancer: a meta-analysis. Tumour Biol 2014;35:1799–803.

[58] Mira JP, Cariou A, Grall F, et al. Association of TNF2, a TNF-alpha promoter polymorphism, with septic shock susceptibility and mortality: a multicenter study. JAMA 1999;282:561–8.

[59] Louis E, Franchimont D, Piron A, et al. Tumour necrosis factor (TNF) gene polymorphism influences TNF-alpha production in lipopolysaccharide (LPS)-stimulated whole blood cell culture in healthy humans. Clin Exp Immunol 1998;113:401–6.

[60] Gao J, Shan G, Sun B, et al. Association between polymorphism of tumour necrosis factor alpha-308 gene promoter and asthma: a meta-analysis. Thorax 2006;61:466–71.

[61] Saurenmann RK, Levin AV, Feldman BM, et al. Risk factors for development of uveitis differ between girls and boys with juvenile idiopathic arthritis. Arthritis Rrheum 2010;62:1824–8.

[62] Saurenmann RK, Levin AV, Feldman BM, et al. Risk of new-onset uveitis in patients with juvenile idiopathic arthritis treated with anti-TNFalpha agents. J Pediatr 2006;149:833–6.