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SUBJECT AREAS:
DIAGNOSTIC MARKERS
BIOMARKER RESEARCHReceived
27 January 2014Accepted
31 March 2014Published
22 April 2014Correspondence and
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Tumor necrosis factor-alpha 308G>A polymorphism and risk of rheumatic heart disease: a meta-analysis

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Rheumatic heart disease (RHD) remains a serious cardiovascular disorder across the world. Tumor necrosis factor alpha (TNF- α) codifies a potent immunomodulator and pro-inflammatory cytokine that mediates diverse pathological processes. A promoter 308G>A polymorphism in TNF- α has been implicated in RHD risk. However, the results remain controversial. Therefore, to evaluate more precise estimations of the relationship, a meta-analysis was performed. A total of 7 studies including 735 RHD cases and 926 controls were involved in this meta-analysis. Overall, our results revealed that there was a significant association with RHD risk in three genetic models (homozygous model: OR = 3.06, 95%CI = 1.22–10.60, P = 0.020; dominant model, OR = 2.03, 95%CI = 1.01–4.07, P = 0.048; and recessive model, OR = 4.26, 95%CI = 2.41–7.55, P < 0.001). Further ethnic population analysis found a significantly increased risk of RHD among Asians and Europeans. Interestingly, similar results were found among hospital-based studies. Begg's funnel plot and Egger's test did not reveal any publication bias. Taken together, this meta-analysis demonstrates that the TNF- α 308G>A polymorphism is associated with RHD susceptibility, and it contributes to the increased risk of RHD. However, additional well-designed studies with larger samples are warranted to confirm these findings.

Rheumatic heart disease (RHD) is an inflammatory disease of the heart tissue, which seriously affects the quality of life of patients, and causes a large economic burden on the national healthcare system^{1,2}. After recovery from the acute stage of carditis, patients developed RHD³. This makes RHD the major acquired heart disease in many developing countries. As a complex disease, the precise molecular mechanism of RHD is still unknown. However, it was widely accepted that the occurrence of RHD relied on the interaction of gene and environment^{4,5}.

Although the pathophysiology has not been fully understood, several lines of evidence suggest that inflammatory response is an essential part of the pathogenesis of RHD^{6,7}. Tumor necrosis factor-alpha (TNF- α) is a cytokine that mediates diverse pathological processes, such as shock during infection and inflammation during autoimmunity^{8,9}. Blood mononuclear cell from RHD patients produced more TNF- α than healthy controls¹⁰. A common 308G>A polymorphism (rs1800629) in the promoter region of TNF- α has been identified to elicit a regulatory effect on the TNF- α gene. The A allele is associated with increased levels of TNF in plasma compared with the G allele¹¹. Moreover, the 308G>A polymorphism has been shown to contribute to the susceptibility of several autoimmune diseases^{12–14}.

Although many studies on the relationship between TNF- α gene 308G>A polymorphism and RHD have been performed so far, the results were still inconsistent^{15–21}. These disparate findings may be partly due to limited sample size, false positive finding and publication bias. In addition, a previous meta-analysis on this issue also generated conflicting results, which had insufficient power in the meta-analysis²². In the present study, a meta-analysis on all published studies was performed to estimate the overall TNF- α gene 308G>A promoter polymorphism with RHD susceptibility.

Results

Characteristics of Studies. As shown in Figure 1, we identified 89 related articles, of which 7 studies were potentially appropriate. According to the inclusion criteria, a total of 735 cases and 926 controls were available for this analysis. Study characteristics are described in Table 1. Two main genotyping approaches were used, including polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) and PCR-sequence-specific primers (PCR-SSP). Population-based and hospital-based controls were involved in

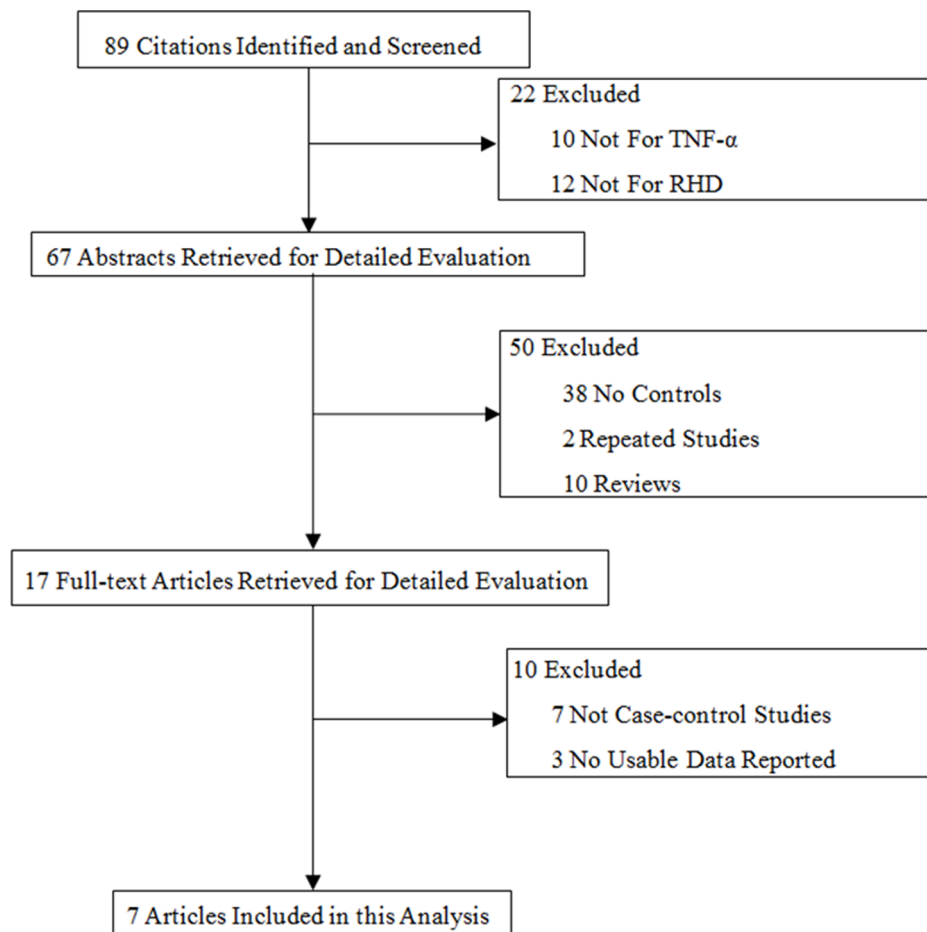


Figure 1 | Study identification, inclusion, and exclusion for the meta-analysis.

different studies. We also assessed deviation from HWE in controls, and the results demonstrated that most genotype distributions among controls were well goodness of fit except for one study¹⁸. Of these case-control studies, five reported on Europeans, while two reports were on Asians.

Quantitative synthesis. The A allele frequency of the TNF- α 308G>A polymorphism in Asians and Europeans were 0.055 and 0.156, respectively. The pooled analysis showed that the TNF- α 308G>A polymorphism was significantly associated with RHD risk (homozygous model: OR = 3.06, 95%CI = 1.22–10.60; dominant model, OR = 2.03, 95%CI = 1.01–4.07, Figure 2; and recessive model, OR = 4.26, 95%CI = 2.41–7.55). In the ethnicity-stratified analysis, the significant risk was observed to be associated

with recessive model among Asians and Europeans (OR = 7.11, 95%CI = 1.26–40.07 for Asians, and OR = 3.92, 95%CI = 2.13–7.21 for Europeans). Moreover, in the stratification of source of controls, significant main effects were found in the hospital-based studies (homozygous model: OR = 6.34, 95%CI = 2.91–31.84; heterozygous model, OR = 1.79, 95%CI = 1.37–2.34; dominant model, OR = 2.29, 95%CI = 1.66–3.16; and recessive model, OR = 4.49, 95%CI = 2.29–10.44) (Table 2).

Test of Heterogeneity, publication bias and sensitivity analysis. There was moderate heterogeneity among all the genetic models except the recessive model ($P = 0.626$, and $I^2 = 0.0\%$ in the recessive model). We used funnel plot and Egger's test to assess the publication bias of literatures. As shown in Figure 3, the shape

Table 1 | Main characteristics of included studies in the meta-analysis

Author	Publication year	Country	Ethnicity	Sample size (cases/controls)	Quality scores	Source of controls	Genotyping method	P^a
Hernandez-Pacheco	2003	Mexico	Caucasian	82/101	8	Population-based	PCR-RFLP	0.880
Sallakci	2005	Turkey	Caucasian	63/89	7	NA	PCR-RFLP	0.615
Chou	2006	China	Asian	115/103	6	Population-based	PCR-RFLP	0.459
Setfin	2007	Egypt	Caucasian	46/98	5	NA	PCR-SSP	<0.001
Ramasawmy	2007	Brazil	Caucasian	199/281	7	Hospital-based	PCR-RFLP	0.295
Mohamed	2010	Egypt	Caucasian	80/50	6	Hospital-based	PCR-RFLP	0.649
Rehman	2013	Pakistan	Asian	150/204	7	Hospital-based	PCR-SSP	0.444

^a P value of Hardy-Weinberg equilibrium.

PCR-RFLP, PCR-restriction fragment length polymorphism; PCR-SSP, PCR-sequence-specific primers; NA, not available.

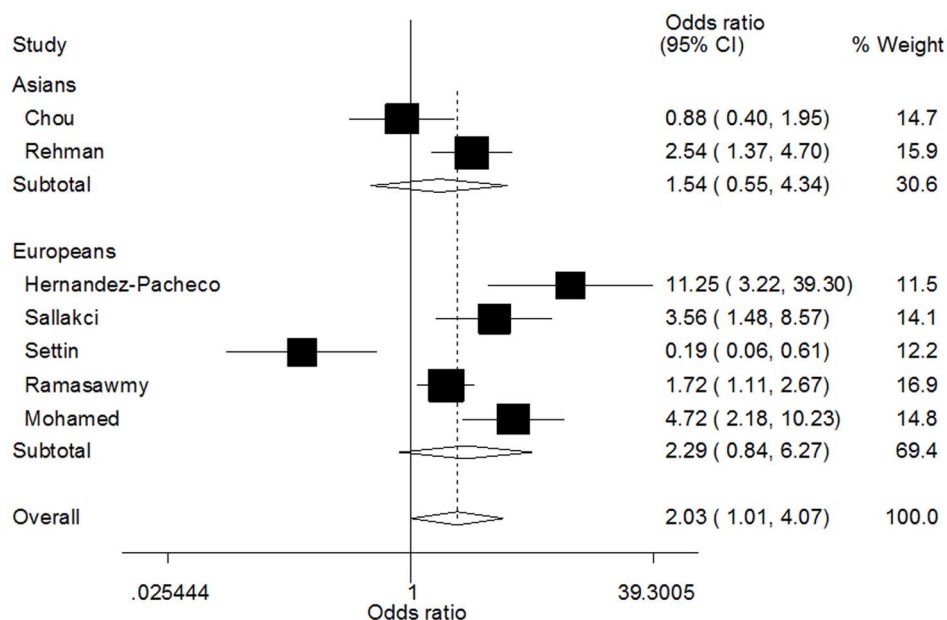


Figure 2 | Forest plot of RHD risk associated with the TNF- α 308G>A (GA/AA vs. GG) among Asians and Europeans. The squares and horizontal lines correspond to the study-specific OR and 95% CI.

of the funnel plots did not show asymmetrical in the dominant model, suggesting absence of publication bias. Moreover, we did not find significant publication bias in the Egger's test ($t = 0.07$, $P = 0.945$ in the dominant model). Sensitivity analysis was carried out by removing each study. The pooled ORs were not materially altered (data not shown), implying that the results were robust.

Discussion

TNF- α is produced by macrophages, monocytes, neutrophils, T-cells and NK-cells after stimulation. In turn, TNF- α can stimulate cytokine secretion, increase the expression of adhesion molecules as well as activate neutrophils^{23,24}. TNF- α has been shown to be relevant for the physiopathology of various inflammatory conditions like rheumatic fever, rheumatoid arthritis, and RHD²⁵. Furthermore, the TNF- α 308G>A promoter polymorphism has been reported to be associated with high TNF production and this has been associated with increased susceptibility for many inflammation diseases^{26,27}. Therefore, it is biologically plausible that the TNF- α 308G>A polymorphism is associated with RHD risk. Compared with the literature by Shen et al.²², we also performed a stratification of ethnic and source of controls in our study, which could explain the difference results among Asians and Europeans. As a result, in the stratified analysis, we found that the main association was pronounced among Asians and Europeans, suggesting that there was no different genetic background

contributing to the risk of RHD. However, there was no reported study on African populations. Therefore, additional studies are needed to elucidate the possible ethnic differences in the effect of TNF- α 308G>A polymorphism on RHD risk. Importantly, when stratifying by source of controls, a significantly elevated risk was observed among hospital-based studies but not among population-based studies. This may be due to that the hospital-based case-control studies have some selection biases because such controls might not be a representative of the general population²⁸. Thus, the selection bias and matching criteria should be considered in the design of case-control study.

Some limitations of this meta-analysis should be addressed. First, the ORs extracted from each eligible study were based on unadjusted estimates, because not all studies reported adjusted ORs. Second, the number of each ethnic subgroup was relatively small, which limited the statistical power to detect the association between the TNF- α 308G>A polymorphism and RHD risk among Asians and Europeans. Third, there was significant between-study heterogeneity from studies of the TNF- α 308G>A polymorphism, and the genotype distribution also showed deviation from HWE in one study¹⁸.

In summary, this meta-analysis of seven case-control studies indicated that the TNF- α 308G>A polymorphism was associated with a significantly increased risk of RHD. These results provided the important role of the TNF- α polymorphisms in the RHD susceptibility,

Table 2 | Main results of pooled ORs of the TNF- α 308G>A polymorphism on RHD risk

Variable	n ^a	AA vs. GG		GA vs. GG		GA/AA vs. GG (dominant)		AA vs. GG/GA (recessive)	
		OR (95% CI)	P _h ^b	OR (95% CI)	P _h ^b	OR (95% CI)	P _h ^b	OR (95% CI)	P _h ^b
Overall	7	3.60 (1.22–10.60)	0.045	1.73 (0.87–3.44)	<0.001	2.03 (1.01–4.07)	<0.001	4.26 (2.41–7.55)	0.626
Ethnicities									
Asian	5	7.52 (1.34–42.34)	0.469	1.43 (0.87–2.36)	0.079	1.54 (0.55–4.34)	0.039	7.11 (1.26–40.07)	0.506
European	3	3.10 (0.79–12.08)	0.021	1.93 (0.71–5.25)	<0.001	2.29 (0.84–6.27)	<0.001	3.92 (2.13–7.21)	0.479
Case-control									
Population-based	2	4.89 (0.55–43.34)	0.624	2.75 (0.23–33.71)	<0.001	3.01 (0.24–37.98)	0.001	4.35 (0.49–38.81)	0.708
Hospital-based	3	6.34 (2.91–13.84)	0.071	1.79 (1.37–2.34)	0.549	2.29 (1.66–3.16)	0.079	4.89 (2.29–10.44)	0.140

^aNumber of reports.

^bP value of Q-test for heterogeneity test.

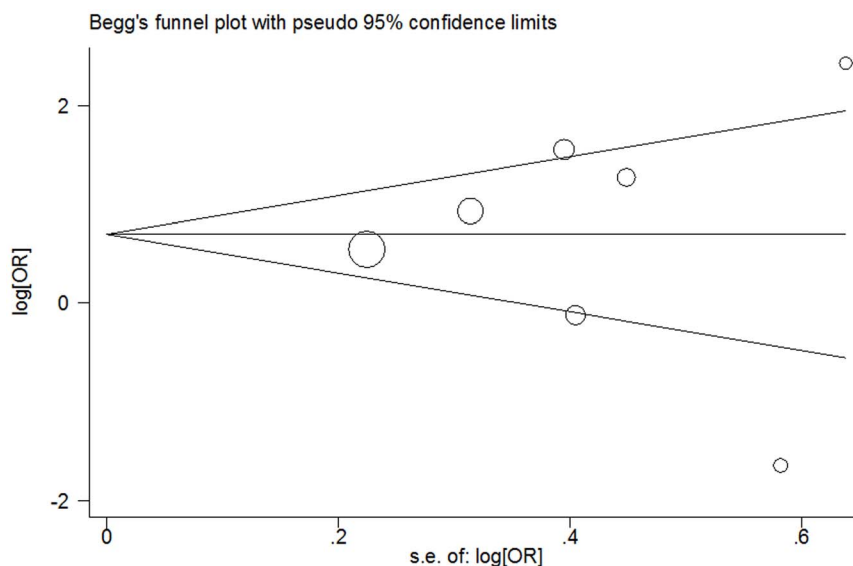


Figure 3 | Publication bias on the TNF- α 308G>A polymorphism (GA/AA vs. GG) and RHD risk.

and our findings need to be validated by future well-designed large studies.

Methods

Search strategy. A comprehensive literature search was performed using the PubMed database for relevant articles published. The following terms were used in this search: ‘TNF- α or tumor necrosis factor-alpha’ and ‘rheumatic heart disease or RHD’ and ‘polymorphism or polymorphisms’. Additional studies on this topic were identified by contacting the corresponding authors or a hand search of references of related articles. Studies included in the current meta-analysis should meet the following requests: (a) only the case-control studies were considered; (b) evaluated the TNF- α gene 308G>A promoter polymorphism and RHD risk; and (c) had usable reported data on the genotypes among cases and controls.

Data extraction. Information was carefully extracted from all eligible studies independently by two investigators according to the inclusion criteria listed above

and the result was reviewed by a third investigator. The following information was extracted from each study: the first author, publication year, country, ethnicity, number of cases and controls, source of controls, genotyping methods, and evidence of Hardy-Weinberg equilibrium (HWE). Different ethnicity descents were categorized as European and Asian. Quality of studies was assessed according to the predefined criteria based on previous observational studies^{29,30} (Table 3).

Statistical Analysis. Odds ratios (ORs) were used as a measure of the association between the TNF- α 308G>A polymorphism and RHD risk. We evaluated the risk of the AA or GA genotype on RHD compared with the GG genotypes, and then calculated the ORs of GA/AA versus GG and AA versus GG/GA, using dominant and recessive genetic models of the A allele, respectively. Pooled estimates of the OR were obtained by calculating a weighted average of OR from eligible study. The between-study heterogeneity I^2 was assessed with the Q-test³¹. Heterogeneity was considered significant if the $P < 0.05$ among studies. The pooled OR was calculated using the fixed-effects model. Otherwise, the random-effects model was used. The HWE was assessed by Fisher’s exact test with significance set at $P < 0.05$ level. The potential publication bias was constructed by a funnel plot. The funnel plot symmetry was evaluated by using Egger’s linear regression test on the natural logarithm scale of the OR and significance was set at $P < 0.05$ level. All analyses were performed using the Stata software (version 10.0 StataCorp LP, College Station, TX).

Table 3 | Methodological quality of included studies

Criteria	Score
A. Representativeness of cases	
Selected from population or hospital	2
Selected from any cardiovascular diseases service	1
Selected without clearly defined inclusion/exclusion criteria	0
B. Credibility of controls	
Population-based	3
Blood donors or volunteers	2
Hospital-based	1
Not described	0
C. Ascertainment of rheumatic heart disease	
Two doctors confirmed	2
Diagnosis of rheumatic heart disease by patient medical record	1
Not described	0
D. Genotyping examination	
Genotyping done under blinded condition	1
Not mentioned	0
E. Hardy-Weinberg equilibrium	
Equilibrium in controls	2
Disequilibrium in controls	1
No checked	0
F. Association assessment	
Assess association between genotypes and rheumatic heart disease with appropriate statistics	2
Assess association between genotypes and rheumatic heart disease with logistic regression	1
Inappropriate statistics used	0

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Author contributions

Conceived and designed the experiments: J.W., Z.R. Performed the experiments: J.W., Z.H., Z.R. Analyzed the data: J.W., Z.H. Contributed reagents/material/analysis tools: J.W., Z.H., Z.R. Wrote the main manuscript text: J.W., Z.H., and Z.R. Reference collection and data management: J.W., Z.R. Statistical analyses and paper writing: Z.R., J.W. Study design: J.W., J.W., Z.H. Prepared figures 1–3: Z.R. All authors reviewed the manuscript.

Additional information

Competing financial interests: The authors declare no competing financial interests.

How to cite this article: Zheng, R.-L., Zhang, H. & Jiang, W.-L. Tumor necrosis factor-alpha 308G>A polymorphism and risk of rheumatic heart disease: a meta-analysis. *Sci. Rep.* **4**, 4731; DOI:10.1038/srep04731 (2014).



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