Int J Clin Exp Med 2015;8(6):8880-8892
www.ijcem.com /ISSN:1940-5901/IJCEM0009118

Original Article

Association between the TNF-α G-308A polymorphism and risk of ischemic heart disease: a meta-analysis

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Received April 14, 2015; Accepted June 10, 2015; Epub June 15, 2015; Published June 30, 2015

Abstract: Background: The role of the tumor necrosis factor-α (TNF-α) G-308A polymorphism in the risk of ischemic heart disease (IHD) has been controversial in recent decades. A substantial number of newly-published studies concerning the association between the TNF-α polymorphism and IHD risk have emerged after the publication of the latest meta-analysis. Therefore, we conducted an updated meta-analysis to further investigate the influence of this polymorphism on IHD. Methods: Electronic databases (PubMed, Embase, CNKI, and Wanfang) were systematically searched to identify all relevant papers published before September 25th, 2014. The quality of all eligible studies was assessed. Pooled odds ratios (ORs) with 95% confidence intervals (CIs) of all studies and high-quality studies were assessed by the fixed/random-effects model in Review Manager 5.0.25 and STATA 10.0. Heterogeneity and publication bias were detected; sensitivity analysis was conducted. Results: Data from 36 studies were recorded after study selection and exclusion. Under the dominant model, the results of pooled analysis of high-quality studies suggested that the G-308A polymorphism was associated with an increased risk of IHD in total population (P = 0.02, OR = 1.13, 95% CI = 1.02-1.24, $P_{heterogeneity}$ = 0.009, I^2 = 45%). No significant result was obtained in Asians, Caucasians, or Indians. Conclusion: The TNF-α -308A allele is probably associated with an increased risk of IHD in total population, but to further identify this association, more high quality studies in Indians and Africans are merited.

Keywords: TNF-α, polymorphism, ischemic heart disease

Introduction

Ischemic heart disease (IHD), the leading cause of death worldwide, has risen from the fourth to the top of the global disability-adjusted life years (DALYs) ranking for 291 diseases and injuries, accounting for 5.2% of global DALYs in 2010 [1]. IHD is caused by insufficient supply of oxygenated blood to cardiac myocytes or increased demand of oxygen by those myocytes. Most commonly, IHD occurs in oxygen supply failure [2] usually resulted from a chronic inflammatory condition [3] that converts into stable narrowing or sudden rupture or dissection of an atherosclerotic artery [2]. Coronary artery disease (CAD), myocardial infarction (MI), stable/unstable angina pectoris are the major cardiovascular phenotypes of IHD. The pathogenesis of IHD has been widely accepted to be multifactorial and polygenic, determined by both environmental components such as high-fat diet, smoking, low antioxidant levels, lack of exercise, and infectious agents [3], as well as genetic predisposition.

TNF-α, a pleiotropic pro-inflammatory cytokine with 233 amino-acids, is coded by the 4-exon TNF-α gene (6p21, 1585 bp), and generated by activated macrophages [4]. It has been considered to have a potent association with recruitment and infiltration of macrophages/monocytes into the sub-endothelium of arteries [5], the release of endothelial cytokines and reduced lipoprotein lipase activity [4]. All these processes were involved in the chronic inflammation course leading to atherosclerosis, the common pathogenic basis of IHD. Thus, altered expression of TNF-α might influence the patho-

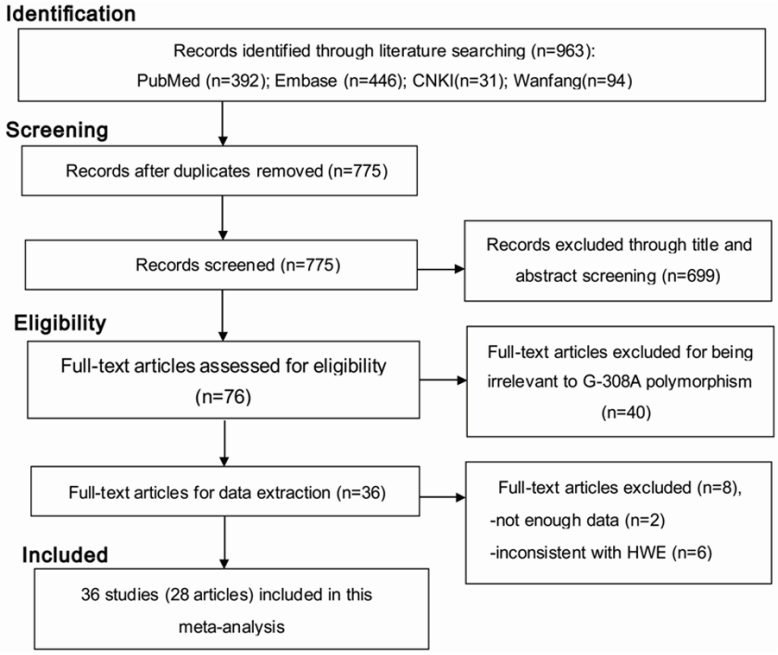

Figure 1. PRISMA flow chart for study selection. After comprehensive screening, 36 studies in 28 articles were eventually identified.

logical process of atherosclerosis. Different polymorphisms of the TNF-α gene might lead to variation in the plasma level of TNF-α, among which the -308G/A polymorphism has drawn most attention. Current evidence showed a mutation of G→A conducted more activated transcription in vitro [6], and in an atherogenic diet fed mice model, TNF-α deficiency was found to retard the formation of lipid lesion [7]. Accordingly, there might be putative association between -308G/A polymorphism and the etiology of IHD.

To date, a great many case-control studies have investigated the association of TNF-α-308G/A polymorphism and cardiovascular diseases, but the results remained inconclusive. A meta-analysis in 2007 found no association between the G-308A polymorphism and IHD in populations predominantly of European ancestry [8]. Nonetheless, another meta-analysis in 2011 demonstrated the TNF-α gene A variant conferred a 1.5-fold increased risk of developing CHD (AG + AA vs. GG, OR = 1.50, 95% CI: 1.23-1.77) in Caucasian population [9]. In recent years, a substantial number of new case-control studies were conducted to further assess this association in various ethnicities. Therefore, we presented the largest meta-analysis to date, with a good number of newly published original case-control studies, in hope of further evaluating the association between the TNF-α G-308A polymorphism and IHD.

Materials and methods

Search strategy and selection criteria

The association between TNF alpha G-308A polymorphism and IHD risk was identified by comprehensive searches in the following databases: PubMed, Embase, CNKI (China National Knowledge Infrastructure), and Wanfang up to September 25th, 2014 with language limitation in only English and Chinese. Terms used for searching were: ("TNF" or "TNF-α" or "TNF-alpha" or "tumor necrosis factor" or "tumor necrosis factor-α" or "tumor necrosis factor alpha") and ("polymorphism" or "mutation" or "variant" or "allele" or "genotype") ("CAD" or "coronary artery disease" or "coronary heart disease" or "ischemic heart disease" or "myocardial infarction" or "ischemic cardiovascular disease").

Study selection: inclusion/exclusion criteria

All studies were independently evaluated by two reviewers (Jiarong Wang and Yang Yang) for inclusion and exclusion. Studies were determined to be eligible if meeting criteria as follows: (1) Original articles assessed the association between TNF-α G-308A polymorphism and IHD risk; (2) The design had to be case-control or cohort that can provide sufficient data (genotype distributions were available) from both cases and controls for estimating an odds ratio (OR) with 95% confidence interval (CI); (3) cardiovascular phenotypes should meet the diagnostic criteria respectively. Coronary heart disease (CAD): stenosis confirmed by coronary angiography; myocardial infarction (MI): according to WHO criteria [10], based on diagnosis of clinical symptoms (especially chest pain), ele-

Table 1. Baseline characteristics of eligible studies

First Author	#,[a]	Year of Publication	Country	Ethnicity	Endpoints	Source of Controls	Matching Criteria for Controls	Control Health	Quality Control	Genotyping Method	Study Score[b]	Number		HWE (χ2)
												case	control	
Allen		2001	UK	Caucasian	CAD	PB	Sex	Healthy	NA	PCR-RFLP	9	180	329	0.22
Babu		2012	India	Indian	ACS	PB	Age, sex	Healthy	NA	PCR-ARMS	11	651	432	0.42
Banerjee		2009	India	Indian	ACS	HB	Age, sex, area	Healthy	NA	PCR-RFLP	10	210	232	1.19
Bennet		2006	Sweden	Caucasian	MI	PB	Age, sex, area	Healthy	NA	DASH	13	1167	1497	0.81
Bernard	1	2003	France	Caucasian	SA	PB	NA	Healthy	NA	PCR-RFLP	9	95	80	0.07
Bernard	2	2003	France	Caucasian	ACS	PB	NA	Healthy	NA	PCR-RFLP	9	204	80	0.07
Bhanushali		2013	India	Indian	CAD	NA	NA	NA	Yes	PCR	7	100	150	0.04
Chen		2001	China	Asian	CAD	HB	NA	Healthy	NA	PCR	8	40	30	0.05
Chen		2014	China	Asian	CAD	HB	NA	UnHealthy	Yes	MALDI-TOF MS	10	433	477	0.39
Chu	1	2012	China	Asian	CAD	PB	Area	Healthy	Yes	TaqMan	11	535	1020	2.41
Chu	2	2012	China	Asian	MI	PB	Area	Healthy	Yes	TaqMan	11	420	1020	2.41
Dedoussis		2005	Greece	Caucasian	ACS	HB	Age, area, sex	UnHealthy	Yes	PCR	13	199	200	0.06
Elahi		2008	UK	Caucasian	CAD	NA	Ethnicity	Healthy	NA	PCR	6	97	95	0.67
Garg		2013	India	Indian	CAD	NA	Area, ethnicity	Healthy	NA	PCR-RFLP	6	137	185	0.79
Georges		2003	France	Caucasian	CAD	PB	NA	Healthy	NA	PCR-RFLP	11	849	314	1.91
Ghazouani		2010	Tunisia	African	IHD	PB	Area	Healthy	NA	PCR-RFLP	10	418	406	0.02
Giacconi		2006	Italy	Caucasian	SA	PB	Age, sex	Healthy	NA	PCR	12	105	190	0.38
Herrmann	1	1998	France	Caucasian	CAD	PB	Age, area	Healthy	NA	PCR-SSCP	11	445	534	1.10
Herrmann	2	1998	Ireland	Caucasian	CAD	PB	Age, area	Healthy	NA	PCR-SSCP	11	196	176	3.08
Hou	1	2009	China	Asian	CAD	PB	Age, sex	Healthy	NA	PCR-RFLP	12	804	905	0.40
Hou	2	2009	China	Asian	MI	PB	Age, sex	Healthy	NA	PCR-RFLP	12	504	905	0.40
Koch	1	2001	Germany	Caucasian	CAD	NA	Age, sex	NA	NA	PCR	12	998	340	2.95
Koch	2	2001	Germany	Caucasian	MI	NA	Age, sex	NA	NA	PCR	12	793	340	2.95
Mishra	1	2012	India	Indian	IHD	NA	Age, sex, ethnicity	Healthy	Yes	PCR	10	290	230	1.37
Mishra	2	2013	India	Indian	IHD	NA	Age, sex, ethnicity	Healthy	Yes	PCR	10	310	230	1.37
Padovani		2000	Brazil	Caucasian	MI	PB	Age, sex, ethnicity	Healthy	NA	PCR	12	148	148	0.71
Qi		2014	China	Asian	CAD	PB	Area	Healthy	Yes	PCR	10	206	274	1.13
Rodriguez		2011	Spain	Caucasian	IHD	HB	NA	UnHealthy	NA	TaqMan	8	93	494	0.26
Sbarsi		2007	Italy	Caucasian	CAD	PB	Age, sex, area	Healthy	NA	PCR-RFLP	11	248	241	0.13
Sun	1	2009	China	Asian	CAD	HB/PB	Area	Healthy	Yes	PCR	10	73	138	0.84
Sun	2	2009	China	Asian	CAD	HB/PB	Area	Healthy	Yes	PCR	10	114	138	0.84
Tobin		2004	UK	Caucasian	MI	PB	Area	Healthy	NA	PCR-RFLP	9	547	505	1.45
Tulyakova	1	2004	Russia	Caucasian	MI	PB	NA	Healthy	NA	PCR-RFLP	9	306	246	0.08
Tulyakova	2	2004	Russia	Caucasian	SCD	PB	NA	Healthy	NA	PCR-RFLP	9	149	246	0.08
Vendrell		2003	Spain	Caucasian	CAD	PB	NA	Healthy	NA	PCR	10	341	207	0.45
Xiang		2004	China	Asian	IHD	NA	Age, area	Healthy	NA	PCR	6	162	182	0.55

[Notes] #,[a]: number of case-control studies separately reported by articles; Study Score; [b]: quality of studies scored by the scale of Thakkinstian *et al* [14]; NA: not available; CAD: coronary heart disease; ACS: acute coronary syndrome; MI: myocardial infarction; SA: stable angina; IHD: ischemic heart disease; SCD: sudden cardiac death; PB: population-based study; HB: hospital-based study; NA: not available; HWE: Hardy-Weinberg equilibrium; PCR-RFLP: polymerase chain reaction-restriction fragment length polymorphism; PCR: polymerase chain reaction; DASH: dynamic allele specific hybridization; MALDI-TOF MS: matrix-assisted laser desorption/ionization time-of-flight mass spectrometry; PCR-SSCP: single strand conformation polymorphism analysis of polymerase chain reaction.

vation in cardiac enzymes, or ECG changes; stable angina (SA): the presence of chest discomfort brought on exertion, relieved with nitrite therapy, and had not changed in its characteristics (frequency, duration, severity, time of appearance, and precipitating factors) in the past 60 days; unstable angina (UA): the presence of typical angina at rest associated with acute or transient ST segment or T wave changes, with or without progressive exercise-induced angina without enzyme elevation; (4) genotype distributions in the control groups had to be consistent with HWE. Studies were excluded if they were: (1) duplicate publications of data from the same study; (2) meta-analyses, reviews, conference abstracts or editorial articles; (3) studies containing overlapping case sources. If overlapping data in different papers were discovered, only the study with the largest sample size was selected.

Data extraction

Two independent investigators (Jiarong Wang and Yazhou He) extracted data independently in accordance with the inclusion criteria. When conflicting evaluation occurred, a third investigator was consulted until consensus was reached after discussion. In the current meta-analysis, the following variables were collected from each study: (1) author, country, year of publication; (2) sample size of the study; (3) source and matching criteria for the control population; (4) characteristics of patients and controls--ethnicity, source of controls (including population based--PB and hospital based--HB), and endpoints of IHD (including CAD, MI, stable/unstable angina, acute coronary syndrome); (4) genotyping methods and quality control of genotyping; (5) HWE of the control group.

Quality score assessment

Based on the STREGA guidelines of reporting genetic association studies, an extension of STROBE statements, it is fundamental to assess the strength and weakness of included studies so as to develop more objective and unbiased conclusion [11]. The quality of included studies was assessed by the scale of Thakkinstian et al [12]. Total scores ranged from 0 (worst) to 16 (best). The quality score assessment was implemented independently by two reviewers (Jiarong Wang and Yazhou He). A study was considered as of good quality with a score of 10 or higher, and of poor quality with a score less than 10.

Data analysis

Crude odds ratios and 95% CI were calculated to assess the strength of the association between the TNF alpha G-308A polymorphism and IHD risk in dominant genetic model (GA + AA vs. GG), recessive model (AA vs. GA + GG), additive model (A vs. G), homozygous model (AA vs. GG) and heterozygous model (GA vs. GG). To derive more stable estimation, further pooled analysis was carried out based on ORs of high-quality studies with a score ≥ 10. In subgroup analyses, we evaluated the specific ORs according to the ethnicity of participants, the endpoints of IHD [CAD and MI], the source of controls, and the quality control of genotyping. The statistical significance of pooled ORs was determined by Z-test and $P < 0.05$ was considered significant. Hardy-Weinberg equilibrium (HWE) was examined in the control group using the chi-square test [13].

The Cochran's Q test and the I^2 statistic were performed to assess heterogeneity across included studies [14]. A *P*-value greater than 0.10 in the Q-test, suggests no heterogeneity among included studies, while in I^2 statistics $I^2 > 30\%$ indicated that heterogeneity among studies existed [15]. To pool the results from included articles, Random-effects model using the DerSimonian and Laird method [16] was utilized where significant heterogeneity was found ($P < 0.10$), otherwise fixed-effects model applying Mantel-Haenszel method [17] were adopted. To evaluate evidence for publication bias, a funnel plot and Egger's test were applied [18]. Besides, sensitivity analysis was performed to assess the stability of the results by excluding individual study sequentially. All the statistical analysis was performed using Review Manager 5.0.25 (Oxford, England) and STATA10.0 (Stata Corporation, College Station, Texas, USA).

Results

Study characteristics

The process of study selection was described in **Figure 1**. According to our search criteria, a total of 775 items were retrieved after dupli-

Table 2. Summary estimates for ORs and 95% CI in five models from analysis of participants in all the included studies, in the high quality studies and in different subgroups

Variables	Sample Size	Dominant Model	Recessive Model	Additive Model	Homozygous Model	Heterozygous Model
	study number, (Case/control), n (n/n)	GA + AA vs. GG	AA vs. GA + GG	A vs. G	AA vs. GG	GA vs. GG
All	36, (12567/13216)	1.09 [0.99, 1.19]	1.11 [0.85, 1.46]	1.08 [0.99, 1.18]	1.15 [0.86, 1.54]	1.08 [0.99, 1.17]
High Quality Study[a]	24, (10457/10594)	1.13 [1.02, 1.24]	1.21 [0.88, 1.65]	1.12 [1.02, 1.24]	1.27 [0.91, 1.78]	1.11 [1.01, 1.21]
Ethnicity						
Asian	10, (3291/5089)	1.06 [0.94, 1.20]	1.60 [0.86, 2.97]	1.09 [0.96, 1.24]	1.60 [0.86, 2.97]	1.04 [0.92, 1.18]
Caucasian	19, (7160/6262)	1.04 [0.92, 1.18]	1.02 [0.71, 1.47]	1.04 [0.92, 1.18]	1.03 [0.71, 1.48]	1.04 [0.93, 1.16]
Indian	6, (1698/1459)	1.24 [0.86, 1.78]	1.53 [0.98, 2.39]	1.20 [0.88, 1.65]	1.94 [1.23, 3.07]	1.22 [0.86, 1.73]
African	1, (418/406)	1.11 [0.83, 1.48]	0.70 [0.32, 1.55]	1.04 [0.82, 1.33]	0.74 [0.33, 1.64]	1.15 [0.86, 1.55]
Endpoints						
CAD	21, (6166/6283)	1.05 [0.92, 1.19]	1.34 [0.87, 2.07]	1.08 [0.94, 1.23]	1.33 [0.85, 2.09]	1.02 [0.91, 1.13]
MI	14, (5726/6311)	1.12 [0.96, 1.30]	0.94 [0.63, 1.40]	1.12 [0.96, 1.30]	1.01 [0.64, 1.60]	1.12 [0.97, 1.29]
Population Based Study	21, (8518/9755)	1.08 [0.97, 1.20]	1.18 [0.90, 1.57]	1.08 [0.98, 1.18]	1.24 [0.92, 1.67]	1.06 [0.96, 1.17]
Quality Control	10, (2680/3877)	1.25 [1.00, 1.56]	1.64 [0.81, 3.31]	1.29 [1.03, 1.62]	1.66 [0.82, 3.35]	1.18 [0.97, 1.44]

Notes: High Quality Study[a]: Studies with score ≥ 10 in the Quality Score Assessment; CAD: coronary heart disease; MI: myocardial infarction.

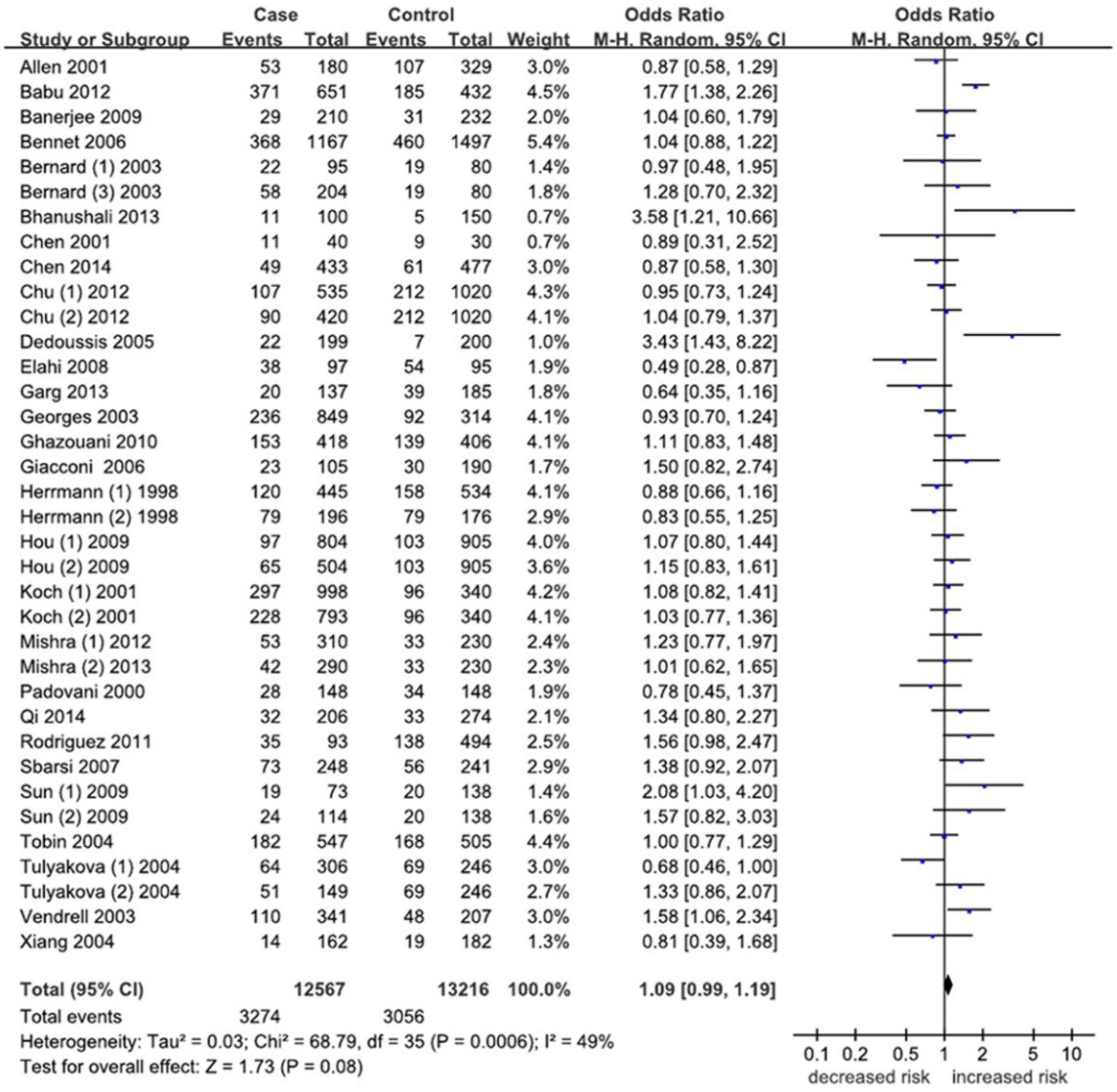

Figure 2. Forest plot for the association between the TNF-α G-308A polymorphism and risk of Ischemic heart disease (IHD) in pooled analysis of all included studies. No significant association was observed between the G-308A variant and risk of IHD.

cates removal. Through screening the abstract and title, 76 potential articles were left for full-text review. Further screening of these articles, 40 were excluded for not relevant to the G-308A polymorphism. Thus, 36 articles were remained for data extraction, and 2 were excluded for not reporting enough data [19, 20] and 6 were discarded for not consistent with HWE [21-26]. Besides, 8 articles presented data including more than one characteristic of the subjects and each subgroup was taken as a separate study [27-34]. Thus, a total of 36 studies in 28 articles were finally identified [19-54]. The characteristics of eligible studies were summarized in **Table 1**. The quality score of each study was also shown in **Table 1**. Among these studies, 10 were conducted in Asian population [28, 30, 33, 40, 41, 49, 54], 19 were conducted in Caucasians [27, 29, 31, 34, 35, 38, 42, 43, 45, 47, 48, 50-53], 6 performed in Indians [32, 36, 37, 39, 44] and 1 in Africans [46]. Population-based controls were selected in 21 studies [27-30, 34-36, 38, 45-49, 51-53]. Patients and controls were matched for age and sex only in 11 studies [31, 32, 36-38, 42, 47, 48, 51], while others only matched for area or ethnicity, or failed to mention matching criteria in their study design. CAD was chosen to be the end-

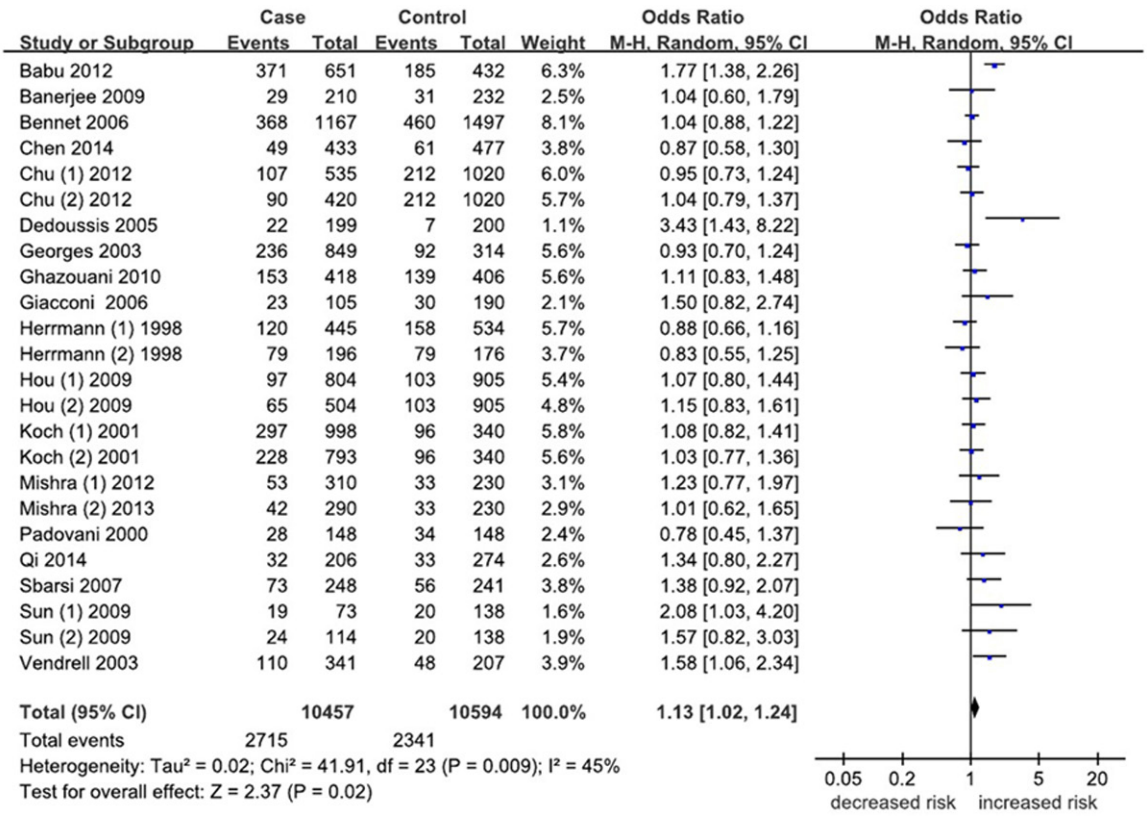

Figure 3. Forest plot for the association between the TNF-α G-308A polymorphism and risk of Ischemic heart disease (IHD) in pooled analysis of all high quality studies. Significant association was observed between the G-308A variant and risk of IHD.

point in 21 studies [27-33, 35, 39-41, 43-45, 47, 49, 51, 53] and MI and ACS were taken as the endpoints in 14 studies [27, 28, 30-32, 34, 36-38, 42, 48, 52].

Quantitative synthesis

The results of meta-analysis in all genetic models were summarized in **Table 2**. Due to significant heterogeneity under the dominant model (I^2 = 49%, *P* = 0.0006), we used random effects model to pool the results of all included studies. As shown in **Figure 2**, we did not observe a significant association between the TNF-α G-308A polymorphism and IHD risk in total population under the dominant model (*P* = 0.08, OR = 1.09, 95% CI = 0.99-1.19). The results of overall analysis in other genetic models are listed in **Table 2**.

The credibility of observational studies depends on a critical assessment of the strengths and weaknesses in study design, conduct, and analysis [55]. According to the results of quality score assessment, we carried out pooled analysis of high-quality studies. Under the dominant model, the heterogeneity diminished compared with that observed in overall analysis, though still existed (I^2 = 45%, P = 0.009). As shown in **Figure 3**, there was significant association between the TNF alpha G-308A polymorphism and IHD risk in total population (*P* = 0.02, OR = 1.13, 95% CI = 1.02-1.24). Summary of the results under other genetic models are listed in **Table 2**.

Subgroup analyses were performed after stratifications of the data by ethnicity and endpoints of IHD. In the subgroup analysis by ethnicity, no significant association was found in Asians (dominant model: *P* = 0.32, OR = 1.06, 95% CI = 0.94-1.20, I^2 = 0%, *P* = 0.52) and Caucasians (dominant model: *P* = 0.52, OR = 1.04, 95% CI = 0.92-1.18, I^2 = 49%, *P* = 0.008), neither in Indians (dominant model: *P* = 0.26, OR = 1.24, 95% CI = 0.86-1.78, I^2 = 69%, *P* = 0.006). When stratified by endpoints of IHD, we failed to observe a significant association between

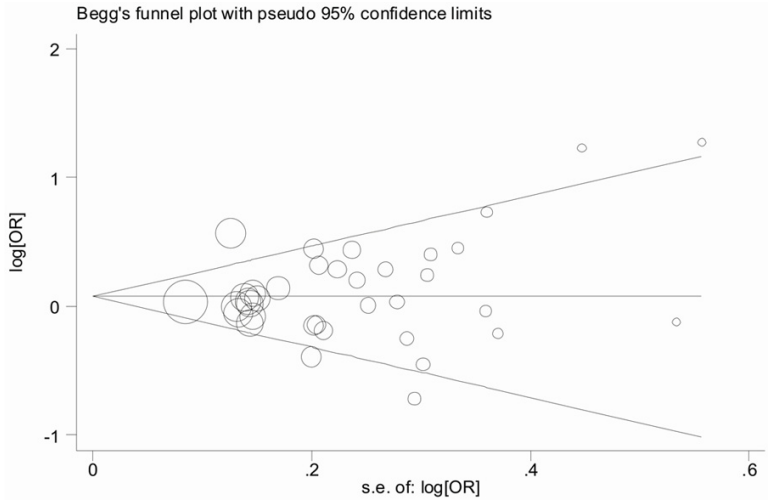

Figure 4. Funnel plot on publication bias for eligible studies (GA + AA vs. GG). The funnel plot seemed symmetrical, suggesting low probability of publication bias.

G-308A polymorphism and the risk of CAD (dominant model: $P = 0.46$, OR = 1.05, 95% CI = 0.92-1.19, $I^2 = 40\%$, $P = 0.03$), or MI (dominant model: $P = 0.15$, OR = 1.12, 95% CI = 0.96-1.30, $I^2 = 58\%$, $P = 0.003$).

Publication bias

A funnel plot and Egger's test were applied to evaluate the publication bias of the literatures. The shape of the funnel plots seemed approximately symmetrical in the dominant model (**Figure 4**), which was further confirmed by Egger's test (t = 0.82 and $P = 0.418$).

Sensitivity analysis

In order to examine the sensitivity of the present meta-analysis, we carried out a Leave-one-out sensitivity analysis by omitting one study at a time for all studies. In the overall analysis, the result changed significantly after removing Elahi's study [43] with OR (95% CI) changing from 1.09 (0.99-1.19) to 1.10 (1.01-1.20). However, in the pooled analysis of high-quality studies, the corresponding synthetic results were not significantly altered by deleting each study, which suggested that the results of pooled analysis of high-quality studies were more stable and robust.

Discussion

TNF-α is a pro-inflammatory mediator repeatedly found in the pathological mechanism of cardiovascular disease, thus the alteration of its expression level might contribute to the etiology of IHD. In recent years, a great many case-control studies assessed the association between the TNF-α G-308A polymorphism and IHD risk, but the results remained inconclusive. To pool all these existing statistics, our meta-analysis, based on 36 studies with 12567 cases and 13216 controls, was conducted to provide a more robust and precise estimation of the association between the TNF-α G-308A polymorphism and IHD risk. In the analysis of high quality studies, we found a significant association between the G-308A polymorphism and susceptibility of IHD in total population.

Compared with previous meta-analyses [8, 28, 56], our meta-analysis further strengthened the statistical power of the pooled results to a large degree. First of all, the included studies were confined to original studies with adequate genotype distributions strictly consistent with HWE. Moreover, our meta-analysis substantially enlarged the number of included studies and added subgroup analysis on Indian population. Furthermore, we performed a pooled analysis on good-quality studies with a score of 10 or higher, so as to provide a more reliable estimation on the association between the TNF-α G-308A polymorphism and IHD risk. Notably, the results stratified by high-score studies showed a reverse trend to that recorded in the overall analysis, indicating that the TNF-α G-308A polymorphism was a risk factor for IHD.

Non-randomized studies, including case-control studies, are considered to be challenging to implement and conduct [57]. Thus, assessment of the quality of such studies is essential for a proper and acute understanding of non-randomized studies, especially when pooling the results together [57]. Our meta-analysis revealed that the TNF-α G-308A polymorphism was significantly associated with an increased risk of IHD only when excluding the poor-quality studies. The distinct quality of each included study may shed light on this discrepancy.

As for genetic association studies, the choices made for study design, selection of study groups and data analysis can potentially affect the magnitude and orientation of the results. The assessment of transparent reporting on those fields helps address gaps in empirical evidence [58], such as the effects of non-matched control group and genotyping errors. Besides, it will also assist to evaluate the impact of currently controversial issues such as population stratification, departure from HWE and multiple testing on effect estimates in different studies [11]. Therefore, evaluating the quality of each study is essential for developing a better evidence basis during meta-analysis. In the light of this statement, the pooled analysis on high-quality studies can develop more transparent and unbiased genetic association results and avoid implicit subjectivity compared with overall analysis. On the other hand, the operation of sensitivity analysis revealed that the results of pooled analysis concerning high-quality studies did not change by removing the study sequentially, which was much more consolidated than the results of crude analysis of all studies. In summary, the results yielded from the pooled analysis of high-quality studies were, thus, convincible.

Provided that distinct heterogeneity was present and could not be completely eliminated [58], we performed subgroup analyses to examine the underlying effect of the G-308A polymorphism on groups with homogenous characteristics. In the subgroup analysis on ethnicity, we observed no heterogeneity among studies in Asian population, but significant ones in Caucasian and Indian population. Though the pooled results revealed that the -308A allele might not be a risk factor for IHD in all the three populations, the results of the latter two should be interpreted with caution for their significant heterogeneity. The probable explanations may lie in insufficient statistical power due to limited sample size and various endpoints of IHD which might contribute to the heterogeneity of the results. To further investigate this problem, more high-quality case-control studies with large sample size were required in the future.

Despite the relatively large sample size and analysis of high-quality studies strengthen our meta-analysis, there are still some limitations needed to be elucidated. Firstly, there was wide heterogeneity among all included studies, even in the group of high-quality. Heterogeneity might be probably caused by the distinct endpoints of IHD and different racial discrepancy, for heterogeneity diminished greatly in the subgroups stratified by endpoints and ethnicity. The results should be interpreted with caution and merited further investigation in the future. Secondly, due to language restriction, articles in languages other than English and Chinese were not included, which might contribute to publication bias. Thirdly, the number of studies in some subgroup analyses was inadequate; hence some subgroup analyses might not have enough statistical power to provide an accurate evaluation of the association between the G-308A polymorphism and risk of IHD. Finally, possible synergistic effects of other candidate genes were important in the elucidation of the genetic susceptibility to IHD. However, since detailed individual information was limited, we were not able to carry out haplotype analysis.

In conclusion, our meta-analysis indicates that the TNF-α G-308A polymorphism may carry an increased risk of IHD. However, the exact roles of the -308A allele in different ethnicities still remain unclear due to insufficient number of high-quality studies. To further interpret and combine genetic association data, more large-scale studies with high-quality evaluation on the effects of gene-gene and gene-environment interactions are warranted in the future.

Disclosure of conflict of interest

None.

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