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Original Article

TNFSF4 rs3850641 A>G polymorphism is associated with the risk of coronary heart disease: a meta-analysis involving 16,942 subjects

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Abstract: To address the important role of the TNFSF4 rs3850641 A>G polymorphism in the etiology of coronary heart disease (CHD), we conducted a comprehensive meta-analysis, which enrolled 13 eligible studies with 8,394 CHD cases and 8,548 controls published up to March 25, 2016. The pooled odds ratios (ORs) with their 95% confidence intervals (95% CIs) were conducted for four genetic models: allele model (G vs. A), homozygous model (GG vs. AA), dominant model (AG+GG vs. AA) and recessive comparison (GG vs. AA+AG). Overall, *TNFSF4* rs3850641 A>G polymorphism conferred increased susceptibility to CHD in one genetic model (GG vs. AA+AG: OR, 1.36; 95% CI, 1.08-1.73; P = 0.010). In subgroup analyses by ethnicity, individuals with *TNFSF4* rs3850641 G allele had a higher CHD susceptibility among Asians (GG vs. AA+AG: OR, 1.40; 95% CI, 1.09-1.80; P = 0.009). Since literature bias was found in dominant genetic model, the nonparametric "trim-and-fill" method was performed. The results indicated the adjusted ORs and CIs were not substantively changed (GG+AG vs. AA: adjusted pooled OR = 0.91, 95% CI: 0.73-1.14, P = 0.416). In summary, our findings suggest that *TNFSF4* rs3850641 A>G polymorphism may be a risk factor for CHD, especially among Asians.

Keywords: Polymorphism, *TNFSF4,* coronary heart disease, susceptibility, meta-analysis

Introduction

Cardiovascular disease is the foremost and first cause of preventable death globally. As one kind of cardiovascular disease, coronary heart disease (CHD) still remains the leading fatal reason worldwide, although its mortality has decreased slightly because of improvements in health care. According to 2010 statistics, in the United States an estimated 379,559 deaths were caused by CHD, one in every six deaths [1]. In Britain, this number is over 65,000, more than any other disease [2]. In many developing countries, morbidity and mortality of CHD have risen exponentially.

CHD is a life-long chronic progressive disease. It is defined as myocardial ischemic symptoms related to angina, myocardial infarct (MI) or evidence of 50% or more stenosis of one major coronary artery at least according to coronary angiography [3]. Most clinical manifestations of the disease are as results of severe stenosis of arterial lumen or sudden occlusion of thrombus caused by the rupture of coronary artery plaques. Chronic activation of inflammatory signal system can aggravate the senescent phenotypes [4] and then contribute to vascular dysfunction and CHD [5]. Arterial wall inflammation is a vital hallmark of atherosclerosis and contributes to adverse clinical events including stroke and CHD [6-8]. Besides innate immunity, acquired immunity involving T-cell mediated pathogenic immunoreactions plays a vital role in the inflammation process during atherogen-

Table 1. Characteristics of the candidate studies in the meta-analysis

Study	Year	Country	Ethnicity	Case/ Control	Type	Genotype method	Adjusted factors	Published language	Source of control
Cheng et al. [46]	2015	China	Asians	285/645	MI	PCR-LDR	Age, ethnic background, and geographic origin	English	Hospital-based
Chen et al. [25]	2011	China	Asians	235/220	ACS	PCR-RFLP	Age, gender, smoking, hypertension, diabetes and lipids level	English	Hospital-based
Cheng et al. [44]	2011	China	Asians	682/713	CHD	PCR-RFLP	Age, gender, ethnic background, and geographic origin	English	Hospital-based
Cheng et al. [44]	2011	China	Asians	377/407	CHD	PCR-RFLP	Age, gender, ethnic background, and geographic origin	English	Hospital-based
Wang et al. [47]	2010	China	Asians	241/212	CHD	TaqMan	Gender, ethnic background, and geographic origin	Chinese	Hospital-based
zhao et al. [45]	2010	China	Asians	243/138	ACS	TaqMan	Age, gender, ethnic background, and geographic origin	Chinese	Hospital-based
zhao et al. [45]	2010	China	Asians	209/138	CHD	TaqMan	Age, gender, ethnic background, and geographic origin	Chinese	Hospital-based
Ria et al. [32]	2010	Sweden	Caucasians	387/387	MI	RT-PCR	Age, gender, ethnic background, and geographic origin	English	Population-based
Li et al. [48]	2008	China	Asians	369/360	CHD	PCR-RFLP	Ethnic background, and geographic origin	Chinese	Hospital-based
Malarstig et al. [30]	2008	USA	Caucasians	344/2386	CHD	Fluorescence-based discrimination method	Age, ethnic background, and geographic origin	English	Population-based
Koch et al. [49]	2008	Geman	Caucasians	3657/1211	MI	PCR-RFLP	Ethnic background, and geographic origin	English	Hospital-based
Wang et al. [29]	2005	Sweden	Caucasians	766/784	MI	TaqMan	Age, HDL, LDL, triglyceride, BMI, smoking and geographic origin	English	Population-based
Wang et al. [29]	2005	Sweden	Caucasians	674/964	MI	TaqMan	Age, HDL, LDL, triglyceride, BMI, smoking and geographic origin	English	Population-based

PCR-LDR: Polymerase chain reaction-ligase detection reaction; PCR-RFLP: Polymerase chain reaction-restriction fragment length polymorphism; RT-PCR: Real-time polymerase chain reaction.

Table 2. Distribution of *TNFSF4* rs3850641 A>G polymorphism genotypes and alleles

Study	Year	Case			Control			Case		Control		HWE
		AA	GA	GG	AA	GA	GG	G	A	G	A	
Cheng et al. 43]	2015	178	88	19	399	215	31	126	444	277	1013	Yes
Chen et al. [25]	2011	179	53	3	162	51	7	59	411	65	375	Yes
Cheng et al. [44]	2011	504	157	21	489	209	16	199	1165	241	1187	Yes
Cheng et al. [44]	2011	269	97	11	295	105	7	119	635	119	695	Yes
Wang et al. [47]	2011	170	53	18	148	44	20	89	393	84	340	No
zhao et al. [45]	2010	80	110	53	71	50	17	216	270	84	192	Yes
zhao et al. [45]	2010	91	80	38	71	50	17	156	262	84	192	Yes
Ria et al. [32]	2010	N/A	N/A	N/A	N/A	N/A	N/A	111	607	92	672	Yes
Li et al. [48]	2008	195	64	6	280	65	2	76	454	69	625	Yes
Malarstig et al. [30]	2008	241	92	11	1622	697	67	117	571	811	3961	Yes
Koch et al. [49]	2008	N/A	N/A	N/A	N/A	N/A	N/A	1207	6107	415	2007	Yes
Wang et al. [29]	2005	N/A	N/A	N/A	N/A	N/A	N/A	238	1294	190	1378	Yes
Wang et al. [29]	2005	N/A	N/A	N/A	N/A	N/A	N/A	228	1234	258	1670	Yes

HWE: Hardy-Weinberg equilibrium; N/A: Not available.

esis in mice and humans [9, 10]. Recent research has illustrated that CHD results from an uncontrolled immunoreaction and T lymphocytes have central roles in the genesis and development of the disease [7, 11].

Costimulation is central to T lymphocytes activation, survival, and effector differentiation [12]. TNFRSF4, also called as CD134 or OX40, is a member of the tumor necrosis factors (TNFs) costimulatory receptor family and is expressed in the cell surface of activated T cells, especially CD4+ T cells [13, 14]. The ligand of TNFRSF4, TNFSF4, belongs to the TNF superfamily and was first identified as glycoprotein 34 on human T Cell leukemia virus-1 transformed cells. It is a type II glycoprotein consisting of a 23-amino acid cytoplasmic tail and a 133-amino acid extracellular domain. It is in the form of a trimer and possesses a TNF homology domain; thus, its structure is similar to other members of the TNF superfamily and has some homologous sequence. The TNFSF4 expression level is actually low, but can be induced preferentially in the professional antigen presenting cells (APCs), including macrophages [15], B cells [16], and dendritic cells [17], for T-cell priming via TNFRSF4 engagement after antigen recognition [14, 18]. The number of the cells which can be induced to express TNFSF4, is more than for TNFRSF4. TNFSF4 is expressed on Langerhans cells [19], vascular endothelial cells [20], mast cells [21], malignancies [22], and bronchial smooth muscle [23]. The crystallized complex of human TNFRSF4/TNFSF4 is a trimeric configuration of three TNFRSF4 monomers and one TNFSF4 molecule [24].

The *TNFSF4* gene locates on chromosome 1 in human, clustered with two genes, *FasL* and *GITRL*, which code two other TNF family members. TNFRSF4/TNFSF4 pairs were suggested to be associated with acute coronary syndrome (ACS) [25]. Recently it was also suggested that single nucleotide polymorphisms (SNPs) in the *TNFSF4* gene were associated with myocardial infarction (MI) and the severity of CHD in humans [26]. A recent study illustrated that interruption of the TNFSF4/TNFRSF4 pathway reduced the severity of atherogenesis in low density lipoprotein receptor-deficient mice [27]. Furthermore, *TNFRSF4* gene polymorphisms were associated to essential hypertension [28]. Current evidence showed that *TNFSF4* was the gene underlying the susceptibility to atherosclerosis locus 1 in mice, and that the polymorphisms of *TNFSF4* gene were associated with the severity of CHD and MI in humans [29]. Malarstig et al. suggested that *TNFSF4* polymorphisms were associated with the incident atherothrombosis and venous thromboembolism risk in Caucasions [30].

The *TNFSF4* rs3850641 A>G polymorphism is located in the first intron of the *TNFRSF4* gene. Numerous genetic studies have indicated this *TNFSF4* polymorphism was related to car-

Table 3. Meta-analysis of the *TNFSF4* rs3850641 A>G polymorphism and coronary heart disease

	No. of study	G vs. A			GG vs. AA			GG+AG vs. AA			GG vs. AA+AG		
		OR (95% CI)	*P*	*P* (Q-test)	OR (95% CI)	*P*	*P* (Q-test)	OR (95% CI)	*P*	*P* (Q-test)	OR (95% CI)	*P*	*P* (Q-test)
Overall	13	1.04 (0.92-1.19)	0.506	< 0.001	1.40 (1.00-1.96)	0.051	0.088	1.09 (0.89-1.32)	0.413	0.002	1.36 (1.08-1.73)	0.010	0.267
Overall in HWE	12	1.05 (0.92-1.21)	0.439	< 0.001	1.55 (1.20-2.01)	0.001	0.167	1.10 (0.89-1.37)	0.377	0.001	1.48 (1.15-1.91)	0.002	0.446
Type													
MI	5	0.97 (0.82-1.15)	0.700	0.006	1.37 (0.76-2.50)	0.298	-	0.97 (0.73-1.30)	0.863	-	1.41 (0.79-2.55)	0.248	-
Mixed	2	1.24 (0.57-2.69)	0.587	0.002	1.14 (0.17-7.77)	0.892	0.011	1.37 (0.57-3.33)	0.483	0.003	1.00 (0.21-4.79)	0.998	0.033
CHD	6	1.06 (0.89-1.26)	0.531	0.027	1.31 (0.97-1.76)	0.082	0.341	1.03 (0.84-1.26)	0.792	0.035	1.31 (0.97-1.76)	0.078	0.404
Ethnicity													
Asians	8	1.13 (0.93-1.37)	0.234	0.001	1.45 (0.99-2.12)	0.058	0.069	1.12 (0.89-1.41)	0.328	0.001	1.40 (1.09-1.80)	0.009	0.209
Caucasians	2	0.95 (0.80-1.12)	0.538	0.007	1.10 (0.58-2.12)	0.764	-	0.91 (0.71-1.16)	0.439	-	1.14 (0.60-2.19)	0.685	-
Source of control													
Hospital-based	9	1.11 (0.95-1.29)	0.182	0.001	1.45 (0.99-2.12)	0.058	0.069	1.12 (0.89-1.41)	0.328	0.001	1.40 (1.09-1.80)	0.009	0.209
Population-based	4	0.92 (0.75-1.19)	0.450	0.015	1.10 (0.58-2.12)	0.764	-	0.91 (0.71-1.16)	0.439	-	1.14 (0.60-2.19)	0.685	-
Quqlity score													
High	8	1.05 (0.87-1.28)	0.603	< 0.001	1.66 (1.23-2.24)	0.001	0.326	1.13 (0.82-1.54)	0.457	< 0.001	1.55 (1.16-2.08)	0.003	0.795
Low	5	1.01 (0.95-1.07)	0.347	0.168	1.07 (0.58-2.12)	0.844	0.091	1.05 (0.88-1.14)	0.593	0.212	1.07 (0.55-2.10)	0.845	0.093

HWE: Hardy-Weinberg equilibrium; CHD: Coronary heart disease; MI: Myocardial infarct.

Table 4. Quality score of the included studies

Study	Year	Selection				Comparability of the cases and controls	Exposure			Total stars
		Adequate case definition	Representativeness of the cases	Selection of the controls	Definition of controls		Ascertainment of exposure	Same ascertainment method for cases and controls	Non-response rate	
Cheng et al. [46]	2015	★	★	-	★	★	★	★	-	6
Chen et al. [25]	2011	★	★	-	★	-	★	-	-	4
Cheng et al. [44]	2011	★	★	-	★	★★	★★	-	-	7
Cheng et al. [44]	2011	★	★	-	★	★★	★★	-	-	7
Wang et al. [47]	2011	★	★	-	★	-	★	★	-	5
zhao et al. [45]	2010	★	★	-	★	★★	★	★	-	7
zhao et al. [45]	2010	★	★	-	★	★★	★	★	-	7
Ria et al. [32]	2010	★	★	★	★	★★	★	-	★	8
Li et al. [48]	2008	★	★	-	★	-	★	★	-	5
Malarstig et al. [30]	2008	★	★	★	★	★★	★	-	-	7
Koch et al. [49]	2008	★	★	-	★	-	★	★	★	6
Wang et al. [29]	2005	★	★	-	★	★★	★★	-	-	7
Wang et al. [29]	2005	★	★	★	★	★	★	★	-	7

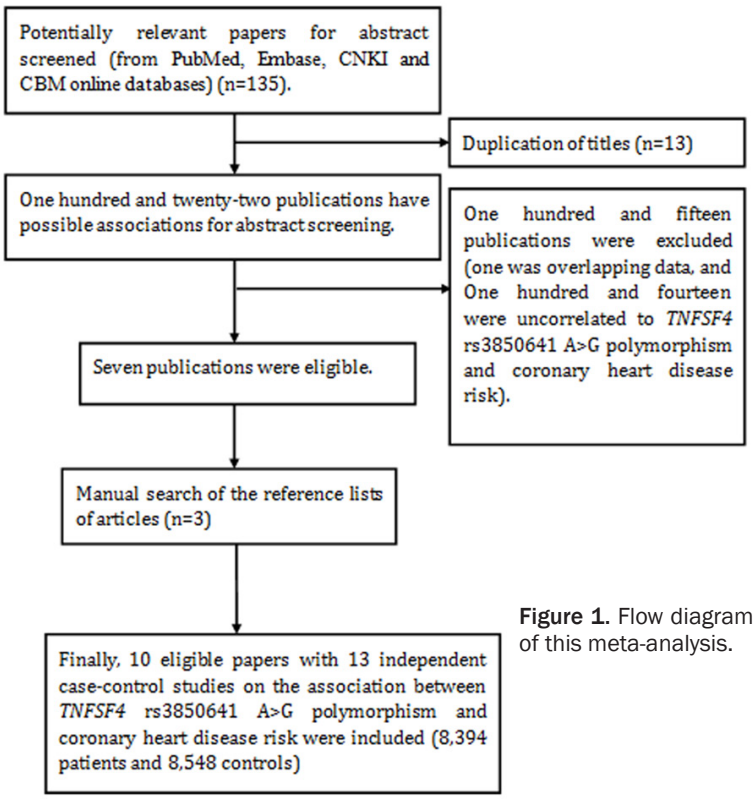

Figure 1. Flow diagram of this meta-analysis.

diovascular diseases [30], stroke in diabetic patients [31], and MI [32]. A meta-analysis concluded that the *TNFSF4* rs3850641 A>G polymorphism has not any association with risk of CHD [33]. Up to now, 13 studies focus on the association between the *TNFSF4* rs3850641 A>G polymorphism and CHD risk; however, the observed results remain conflicting. In our present study, we conducted an updated meta-analysis of the eligible studies to further probe the association of the *TNFSF4* rs3850641 A>G polymorphism with the risk of CHD.

Materials and methods

Search strategy

PubMed, EMBASE, China National Knowledge Infrastructure (CNKI) and China Biology Medicine (CBM) databases (published up to March 25, 2016) were searched using the following terms: 'tumor necrosis factor superfamily member 4' or 'TNFSF4' or 'OX40L' and 'SNP' or 'polymorphism' or 'variant' or 'mutation' and 'coronary artery disease' or 'CAD' or 'coronary heart disease' or 'CHD' or 'myocardial infarction' or 'MI'. The literature search was limited to English or Chinese articles. References of eligible publications and reviews were manually searched for additional studies.

Inclusion and exclusion criteria

Studies included in our analysis had to meet the following criteria: (1) they focused on the association between the *TNFSF4* rs3850641 A>G polymorphism and CHD; (2) they were case-control or cohort studies; (3) they supplied the available frequencies of alleles or genotypes. The major exclusion criteria were: (1) incomplete data; (2) overlapping data; (3) reviews, (4) comments, editorials, meta-analyses or letters.

Data extraction

In a standardized form of data extraction, three researchers (F. Gong, B. Chen and H. Qiu) extracted the data independently. The following items were extracted from the eligible literatures: first author, year of publication, country, ethnicity, CHD type, alleles or genotype frequencies, sample size (total cases and controls), genotyping method and the source of control. When meeting conflicting assessments, all disagreements were settled through a discussion among all authors.

Quality score

We harnessed the Newcastle-Ottawa Scale (http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp) to evaluate the quality score of the eligible studies [34]. Each included studies were assessed by 8 items of 3 aspects. When quality score was ≥ 7 stars, it was considered as high-quality study. The results are shown in **Table 4**.

Statistical analysis

Crude odds ratios (ORs) and 95% confidence intervals (95% CIs) were used to evaluate the strength of correlation between the *TNFSF4* rs3850641 A>G polymorphism and CHD. The pooled ORs were conducted for four genetic models: allele model (G vs. A), homozygous model (GG vs. AA), dominant model (AG+GG vs.

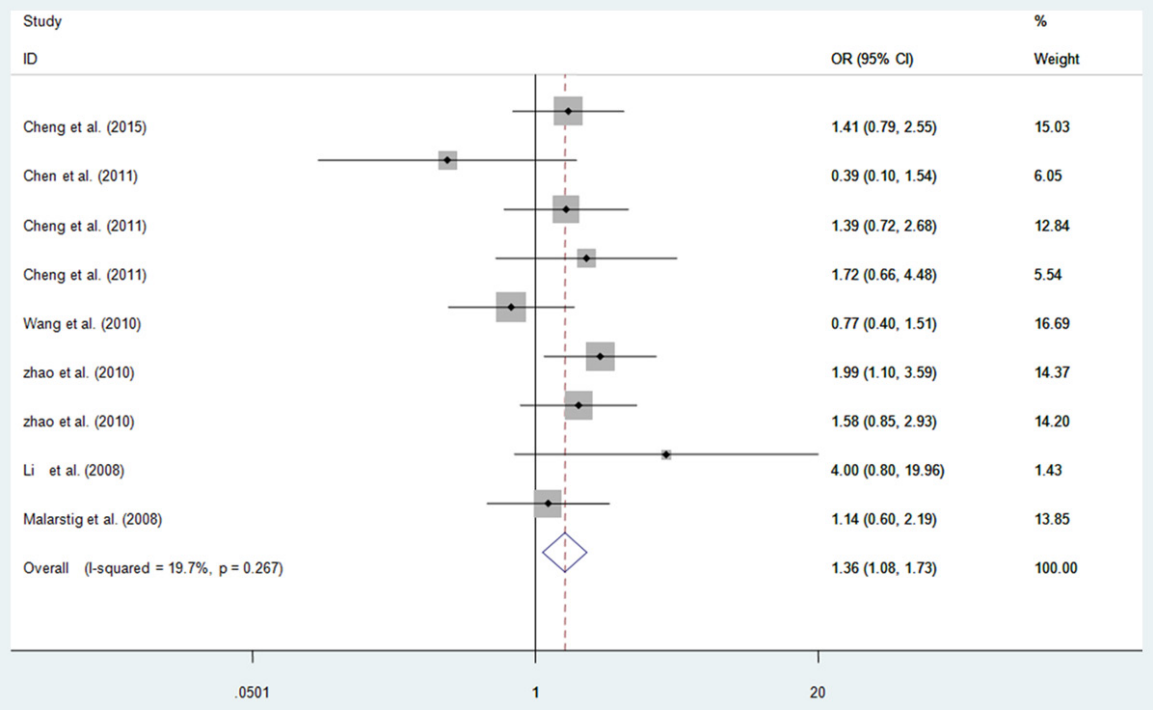

Figure 2. Meta-analysis for the association between *TNFSF4* rs3850641 A>G polymorphism and coronary heart disease risk in the different ethnicity (fixed-effects model, GG vs. AA+AG genetic comparison).

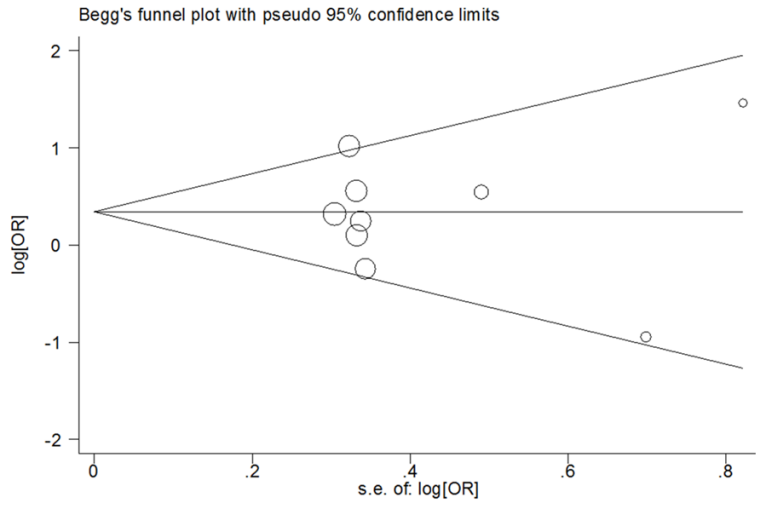

Figure 3. Begg's funnel plot of meta-analysis of the association between the *TNFSF4* rs3850641 A>G polymorphism and risk of coronary heart disease (GG vs. AA genetic model).

AA) and recessive model (GG vs. AA+AG). $P < 0.05$ (two tailed) was defined as statistical significance. Heterogeneity was determined by a chi square-based Q statistical test. When $P < 0.1$, the random effect model was used [35], otherwise, the fixed-effect model was applied [36]. We tested HWE in controls by a Pearson's χ^2 test (available in: http://ihg.gsf.de/cgi-bin/hw/hwa1.pl) [37-42]. Subgroup analyses were conducted by CHD type, HWE, ethnicity, quality score and source of control. We performed one-way sensitivity analysis to determine the stability of our findings. The evidence of publication bias was determined by the Begg's test and Egger's test [43]. If publication bias was found, nonparametric "trim-and-fill" method was performed. The data analyses were conducted with STATA 12.0 software package (Stata Corp LP, College Station, Texas).

Results

Characteristics

After the initial search, we retrieved one hundred and thirty-five papers relevant to the topic from PubMed, Embase, CNKI and CBM online databases. With our detailed selections and filters, one hundred and twenty-six

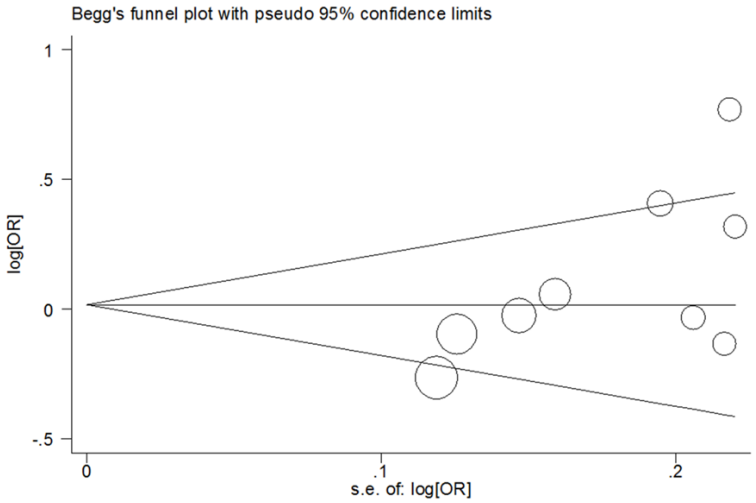

Figure 4. Begg's funnel plot of meta-analysis of the association between the *TNFSF4* rs3850641 A>G polymorphism and risk of coronary heart disease (GG+AG vs. AA genetic model).

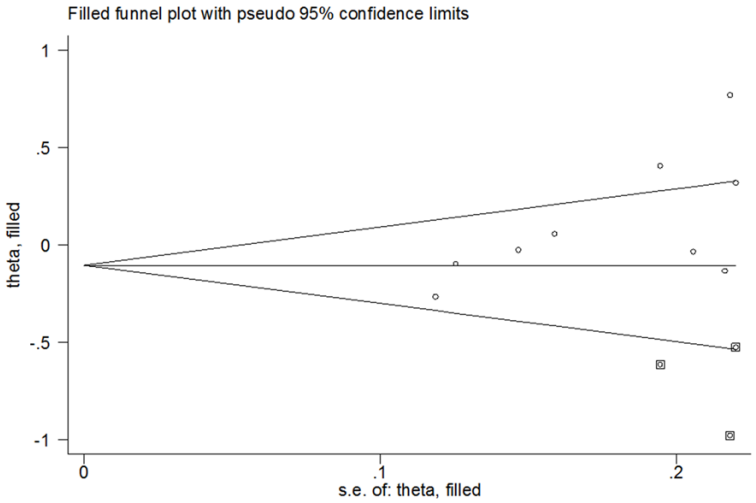

Figure 5. Filled funnel plot of meta-analysis of between the *TNFSF4* rs3850641 A>G polymorphism and risk of coronary heart disease (GG+AG vs. AA genetic model).

of these papers were excluded (one was overlapping data, and one hundred and fourteen were uncorrelated to *TNFSF4* rs3850641 A>G polymorphism and coronary heart disease risk). There were some subgroups in eligible articles, and we treated them separately [29, 44, 45]. After this step, as shown in **Figure 1**, thirteen qualified case-control studies fit the major inclusion criteria. Overall, thirteen independent case-control studies with 8,394 patients and 8,548 controls on the association between the *TNFSF4* rs3850641 A>G polymorphism and CHD susceptibility were recruited in our study [25, 29, 30, 32, 44-49]. In one study, the genotype distribution in controls was not in agreement with HWE [47]. As for subjects, eight were Asians [25, 44-48] and five were Caucasians [29, 30, 32, 49]. Among the thirteen applicable studies, four studies focused on MI, six studies focused on CHD, and two studies focused on mixed CHD. **Table 1** shows the characteristics of the included studies [25, 29, 30, 32, 44-49]. The distribution of the *TNFSF4* rs3850641 A>G polymorphism and allele among CHD patients and controls is summarized in **Table 2**.

Quantitative synthesis

The association of *TNFSF4* rs3850641 A>G polymorphism with CHD susceptibility is presented in **Table 3**. Overall, *TNFSF4* rs3850641 A>G polymorphism conferred increased susceptibility to CHD in one genetic model (GG vs. AA+AG: OR, 1.36; 95% CI, 1.08-1.73; $P = 0.010$; **Table 3** and **Figure 2**). In subgroup analyses by ethnicity, individuals with *TNFSF4* rs3850641 G allele had a higher CHD susceptibility in one comparison model among Asians (GG vs. AA+AG: OR, 1.40; 95% CI, 1.09-1.80; $P = 0.009$; **Table 3** and **Figure 2**). When restricting the analysis to the type of CHD, *TNFSF4* rs3850641 G allele was not associated with the susceptibility of CHD (**Table 3**).

Tests for publication bias, sensitivity analyses, and heterogeneity

The potential publication bias was determined by Begg's funnel plot and Egger's regression test [43]. Begg's funnel plot seemed symmetrical (G vs. A: Begg's test $P = 0.127$; GG vs. AA:

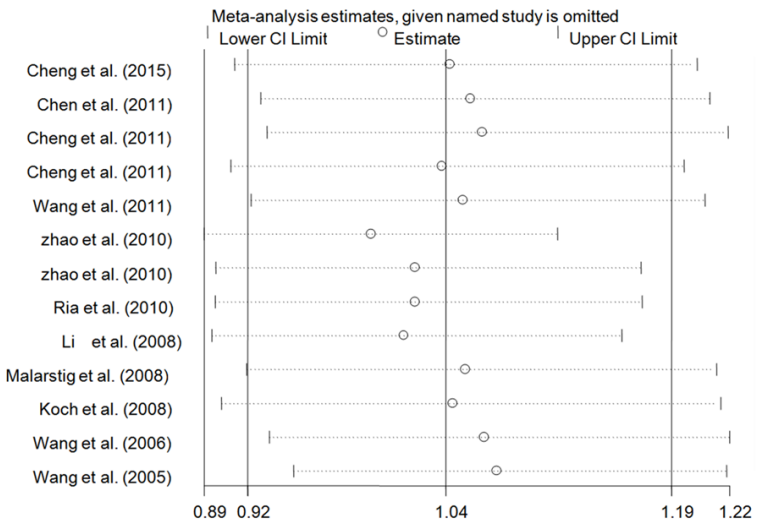

Figure 6. Sensitivity analysis of the influence of G vs. A genetic model (random-effects estimates).

Begg's test P = 0.466; GG+AG vs. AA: Begg's test P = 0.048 and GG vs. AG+AA: Begg's test P = 0.466; **Figure 3** and **Figure 4**). Then, the Egger's regression test was performed to detect publication bias for statistical test (G vs. A: Egger's test P = 0.247; GG vs. AA: Egger's test P = 0.907; GG+AG vs. AA: Egger's test P = 0.032 and GG vs. AG+AA: Egger's test P = 0.856). Since publication bias was found in dominant genetic model, the nonparametric "trim-and-fill" method was performed. The results indicated that the adjusted ORs and CIs were not substantively changed, suggesting the robustness of our findings (GG+AG vs. AA: adjusted pooled OR = 0.91, 95% CI: 0.73-1.14, P = 0.416) (**Figure 5**).

A one-way sensitivity analysis was used to evaluate the influence of each study on the overall findings, with each particular data set omitted in turn and re-calculating the results for overall estimates. Stability of odds ratio assessments was found for the relationship of *TNFSF4* rs3850641 A>G polymorphism with CHD risk (**Figure 6**). The genotype distribution in controls was not in agreement with HWE in one study [47]. Meanwhile, after the dropping of this study departure from HWE, the increased risk of CHD was also found in two genetic models (GG vs. AA: OR, 1.55; 95% CI, 1.20-2.01; P = 0.001; GG vs. AA+AG: OR, 1.48; 95% CI, 1.15-1.91; P = 0.002; **Table 3**).

As shown in **Table 3**, the significant heterogeneity was found in some comparison models. Subgroup analyses were used to detect the influence of different population, different type of CHD, quality score and source of control. Interestingly, results indicate that mixed CHD, Asians subgroups, high quality studies (≥ 7 stars) and hospital-based studies may contribute to the heterogeneity across the included studies.

Discussion

Atherosclerosis is the pathological basis for the development of CHD, one of the leading cause of death worldwide. CHD is a complex trait modified by the interactions of many genes and environmental factors. Paigen et al. found that susceptibility gene, such as peroxiredoxin 6, Fasl and TNFSF4, may affect atherosclerosis based on a mouse diet-induced atherosclerosis model [29, 50]. Of late, a crowd of epidemiologic case-control investigations have paid attention to the potential role of polymorphism in susceptibility gene for the development of CHD [51]. The functional SNPs in susceptibility genes, which may alter the expression of these genes, could influence the risk of CHD [51]. TNFSF4 (also known as OX40L, CD134L and gp34, GenBank accession no. NM_003326), the ligand of the OX40, is a vital member of the tumor necrosis factor superfamily (TNFSF). TNFSF4 is a T-cell activating factor that seems to facilitate the survival and/or promote anti-CD3-induced CD4+ T cells proliferation at the time of inflammation [52]. T-cells may play an important role in the development of atherosclerosis [53]. It is reported that TNFSF4 is expressed in activated vascular endothelial cells, CD4+ and CD8+ T cells and B cells [54]. Recently, several case-control studies focused on the association between *TNFSF4* rs3850641 A>G polymorphism and CHD risk [25, 29, 30, 32, 44-49]. However, results of these studies remain controversial rather than conclusive. To address the potential correlations between *TNFSF4* rs3850641 A>G polymorphism and CHD susceptibility, we performed this comprehensive analysis by pooling the sufficient published data to evalu-

ate influence of *TNFSF4* rs3850641 A>G polymorphism on CHD risk.

In this meta-analysis, ten qualified publications [25, 29, 30, 32, 44-49] with 8,394 patients and 8,548 controls fit the major inclusion criteria for pooled analysis. We demonstrated that *TNFSF4* rs3850641 A>G polymorphism was associated with an increased risk of CHD. The similar associations were also identified among Asians. With the boom of epidemiologic case-control studies, it is necessary to pool available data to overcome difficulties in acquiring robust, replicable results. Nevertheless, a common SNP may make a small contribution to complex human disease susceptibility, the present meta-analysis urges the necessity of relatively large sample sizes to determine precise assessments of *TNFSF4* rs3850641 A>G polymorphism with the risk of CHD. Some prior studies reported the positive signals of *TNFSF4* rs3850641 A>G polymorphism with CHD risk [44, 45, 47, 48]; however, other studies reported null associations [25, 30, 32, 46, 47]. A recent meta-analysis reported that *TNFSF4* rs3850641 A>G polymorphism was not associated with the risk of CHD [33]. In the present study, we included the more publications. And we concluded that *TNFSF4* rs3850641 A>G polymorphism conferred an increased risk to the development of CHD. Interestingly, in one study, the genotype distribution in controls was not in agreement with HWE [47]. When we dropped this study, the increased risk of CHD was also found, suggesting the robustness of our findings.

We have to consider the influence of publication bias, which may alter the final decision of meta-analyses. Since an obvious publication bias was found in dominant genetic model, the nonparametric "trim-and-fill" method was harnessed to determine the stability of our results. The results indicated that adjusted ORs and CIs for final decision were not substantively changed, suggesting the robustness of our findings (GG+AG vs. AA: adjusted pooled OR = 0.91, 95% CI: 0.73-1.14, P = 0.416).

The present meta-analysis has some limitations. Firstly, the vital limitation to publication based pooled-analysis is that of reporting bias. Studies with 'negative' results, in all probability, may be unpublished. In the present study, only published literature was enrolled. This may lead to a certain bias in some ways. Secondly, for lack of sufficient data of co-variates (e.g. family history, body mass index, life style and so on), the overall findings were based on crude ORs, while a more precise determination should be adjusted by other relative gene-environment factors. Finally, it is worth noting that significant heterogeneity between the included studies was found in some comparison models, these findings should be interpreted with caution. Considering all of the enrolled studies were conducted in Asians and Caucasians, there were possible associations, reinforcing more studies to warrant our finding.

In summary, results of the meta-analysis indicate that *TNFSF4* rs3850641 A>G polymorphism is associated with the increased risk of CHD among Asians. Therefore, for practical reasons, further large sample prospective studies with an adequate methodological quality are warranted to verify the important role of rs3850641 A>G polymorphism of *TNFSF4* gene in CHD.

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Disclosure of conflict of interest

None.

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