

Association between caspase recruitment domain-containing protein 8 rs2043211 polymorphism and cardiovascular disease susceptibility: A systematic review and meta-analysis

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ABSTRACT

Objective: To verify the association between the caspase recruitment domain-containing protein 8 (*CARD8*) single nucleotide polymorphism rs2043211 in the *CARD8* gene and cardiovascular diseases.

Methods: A comprehensive search was conducted for related literature in the PubMed, Embase, Cochrane Library, Web of Science, Wanfang Data, and China National Knowledge Infrastructure databases. At last, Six eligible case-control studies were included in this meta-analysis. Crude odds ratios (ORs) and 95% confidence intervals (CI) were calculated to estimate the strength of the association between *CARD8* rs2043211 polymorphisms and the susceptibility of cardiovascular diseases.

Results: For the homozygous model, OR was 1.21 (1.08-1.36, $I^2=0.0\%$, $P_{\text{heterogeneity}}=0.542$). For the heterozygous model (AT vs. AA), OR was 1.20 (1.04-1.38, $I^2=57.2\%$, $P_{\text{heterogeneity}}=0.039$). For the dominant model, OR was 1.24 (1.14-1.34, $I^2=38.5\%$, $P_{\text{heterogeneity}}=0.149$). For the allele model, OR was 0.96 (0.77-1.20, $I^2=92.7\%$, $P_{\text{heterogeneity}}=0.000$). For the recessive model, OR was 1.00 (0.91-1.10, $I^2=48.4\%$, $P_{\text{heterogeneity}}=0.085$).

Conclusion: The present study showed that *CARD8* rs2043211 polymorphism is associated with cardiovascular diseases. (*Anatol J Cardiol* 2018; 20: 70-6)

Keywords: caspase recruitment domain-containing protein 8, cardiovascular diseases, polymorphism, meta-analysis

Introduction

The term cardiovascular diseases (CVDs) refers to diseases of the heart and/or blood vessels. CVDs are now recognized as the leading cause of death worldwide. In 2013, there were >54 million deaths (95% uncertainty interval=53.6–56.3 million) globally, and 32% of these deaths were attributable to CVDs (1). The three major causes of all CVD deaths are ischemic heart disease, stroke, and hypertensive heart disease (2). CVDs have been associated with lifestyle and environmental factors, as well as genetic markers that have been identified in large genome-wide association studies (GWAs). GWAs have been extensively used to study common complex diseases, such as coronary artery disease (CAD). They have revealed 153 CAD suggestive loci, of which at least 46 have been validated as having genome-wide significance (3). However, additional genetic factors for CVDs need to be elucidated. In recent years, it has been determined that inflammasomes are associated with CVDs; therefore, caspase recruitment

domain-containing protein 8 (*CARD8*) should more seriously considered as an inflammasome constituent (4).

The *CARD8* gene, which is also called the tumor-upregulated CARD-containing antagonist of caspase 9 (TUCAN), has been widely discovered in inflammatory diseases, including rheumatoid arthritis, gout, and aspirin-induced asthma (5-7). To date, *CARD8*-C10X (rs2043211) is the only *CARD8* single nucleotide polymorphism (SNP) that has been discussed as one of the components of the NLRP3 inflammasome. It is encoded by exon 13 in 19q13, and it changes cysteine to a premature termination codon at codon 10, thus influencing the protein's function in inflammasome-mediated processes and nuclear factor (NF)- κ B suppression (8, 9). Statistics have shown that *CARD8* polymorphisms are related to the CVD risk (10), but several studies have reported different conclusions (8, 11, 12). Here, we present the first meta-analysis verifying the association between SNP rs2043211 in the *CARD8* gene and CVDs.

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Methods

Search strategy

A comprehensive search was conducted for related literature in the PubMed, Embase, Cochrane Library, Web of Science, Wanfang Data, and China National Knowledge Infrastructure databases until March 2018 with the keywords “*CARD8*” and “Cardiovascular Diseases.” In addition, the references of the retrieved articles were also reviewed.

Inclusion and exclusion criteria

All the studies included in the meta-analysis were case-control studies that focused on the association between SNPs or haplotypes in the *CARD8* gene and CVDs. The studies were excluded if the genotype frequencies in the cases and controls were unavailable, despite contacting the authors via e-mail. In addition, case reports, reviews, and unpublished data were excluded. The inclusion and exclusion criteria were independently screened by two investigators. Flow diagram of studies included in this meta-analysis is shown in Figure 1.

Data extraction

Three reviewers independently extracted the following information from all the identified studies using a standardized data collection form: author name, publication year, ethnicity, location, number of cases and controls, disease type, source of control, the Hardy–Weinberg equilibrium (HWE) in the controls, genotyping method, and odds ratio (OR) with corresponding 95% confidence intervals (CIs). Any disagreements were resolved by a group discussion. The data was merged in cases with the same author in the same experimental conditions but with different disease subgroups.

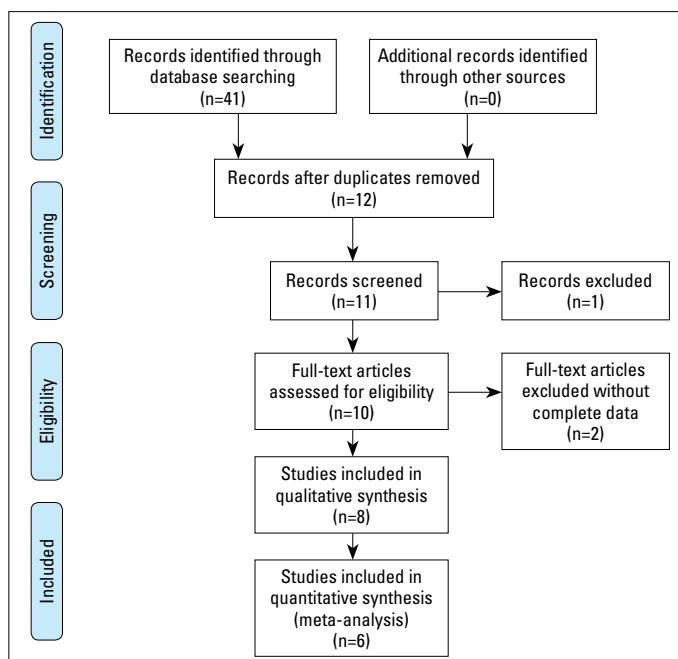


Figure 1. Flow diagram of studies included in this meta-analysis*

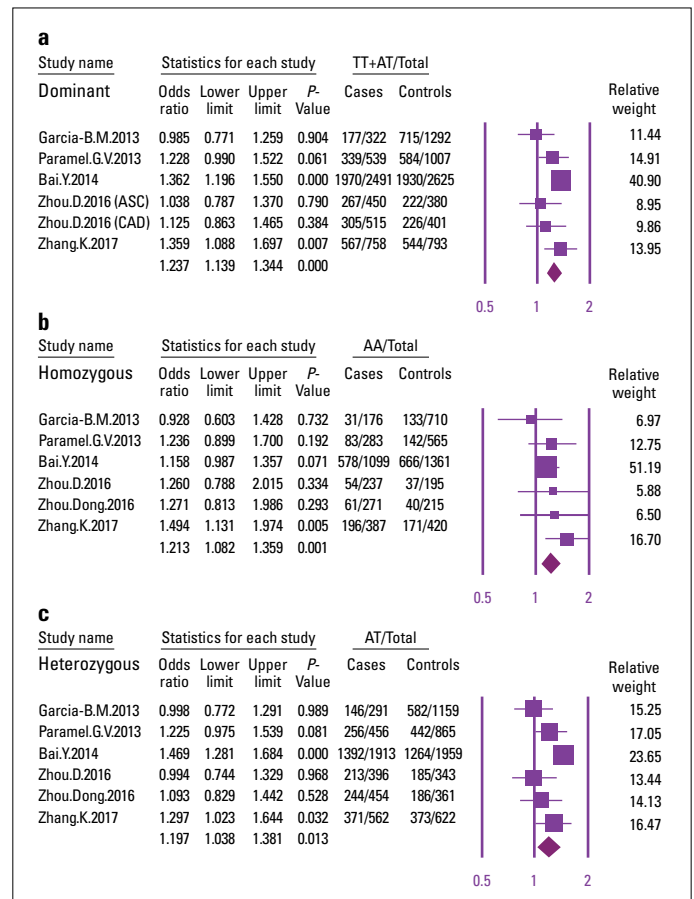


Figure 2. The association of *CARD8* rs2043211 (T>A) with CVD risk in different genetic models. a) Dominant model (TT+AT vs. AA). b) Homozygous model (TT vs. AA). c) Heterozygous model (AT vs. AA)

The degree of heterogeneity was measured using the following criteria: if the heterogeneity analysis results were $I^2 \geq 50\%$, a random-effects or fixed-effects model was used. Therefore, in this paper, heterozygous and alleles models were used with a random-effects model. Additionally, the publication bias was confirmed by a funnel plot and Egger’s test with a p-value of < 0.05 ; otherwise, it was considered to have no publication bias.

Statistical analysis

To evaluate the effect of an individual study on the overall CVD risk, a leave-one-out sensitivity analysis with a recomputed pooled OR was adopted. HWE was calculated using a chi-squared test for each study in the control groups. We used the random-effects model when $I^2 \geq 50\%$, which suggested that the results of this meta-analysis were stable and robust. The entire statistical analysis involved in this meta-analysis was performed using Comprehensive Meta-Analysis version 2 software (Biostat, NJ, USA; <https://www.meta-analysis.com>).

Trial sequential analysis

To reduce the systematic errors (bias) and random errors (chance), to adjust the threshold for statistical significance, and

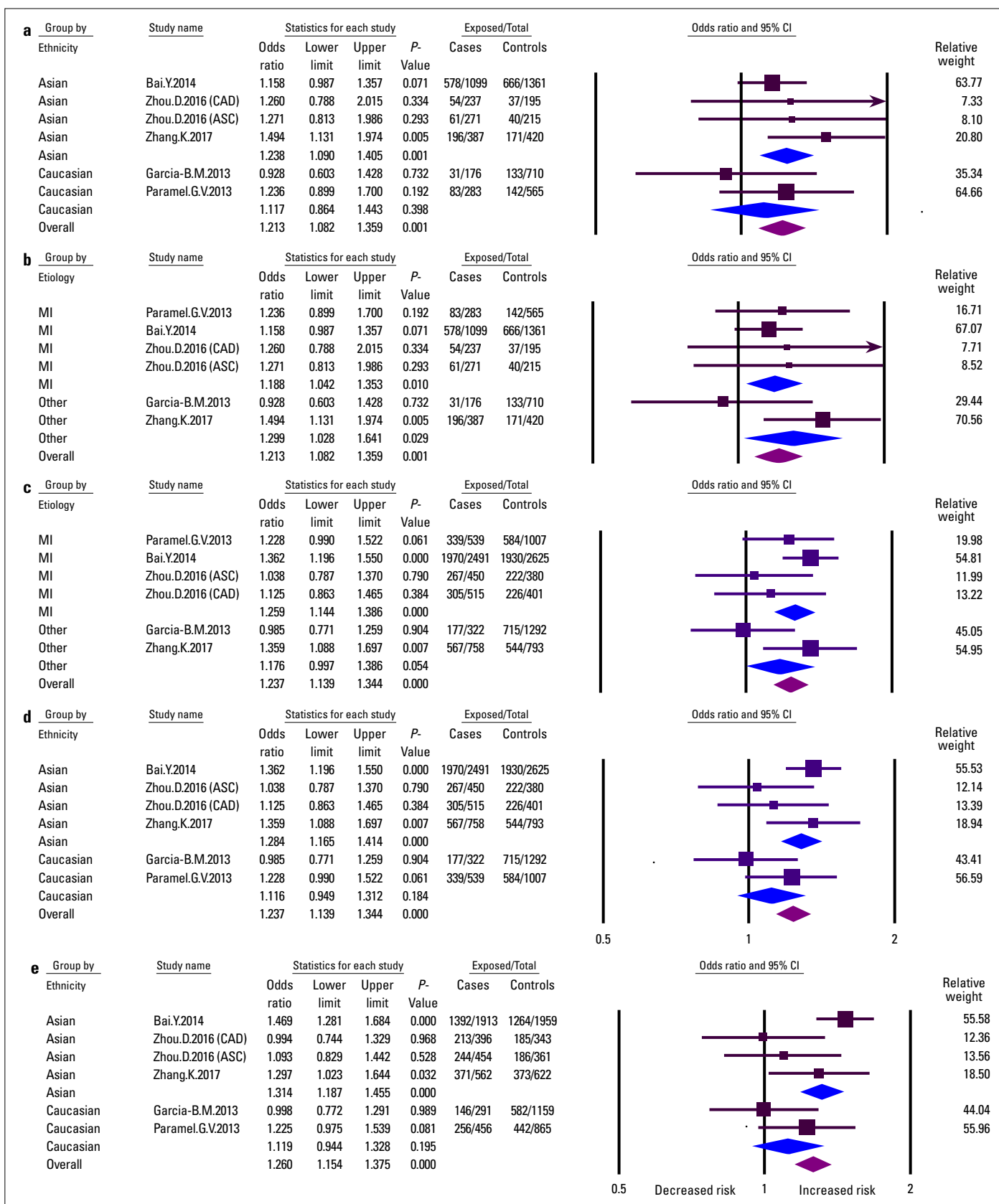


Figure 3. The associations of rs2043211 with CVDs in different genetic models by subgroup. a) Homozygous model (TT vs. AA) by ethnicity, b) Homozygous model (TT vs. AA) by etiology, c) Dominant model (TT+AT vs. AA) by etiology, (D) Dominant model (TT+AT vs. AA) by ethnicity, e) Heterozygous model (AT vs. AA) by ethnicity

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

Trial	Country	Ethnicity	Disease	Control source	Methods	AA/AT/TT			Case Control			NOS Scale			Score			
						AA	AT	TT	Case	Control	1	2	3					
Garcia-B, M. 2013	Spain	Caucasian	CVDs	HB	RT-PCR†	145	146	31	577	582	133	582	133	0.44	***	*	***	7
Paramel, G. V. 2013	China	Caucasian	MI	HB	RT-PCR	200	256	83	423	442	142	442	142	0.09	****	**	***	9
Bai, Y. 2014	Sweden	Asian	CAD+IS	HB	RT-PCR	521	1392	578	695	1264	666	1264	666	0.06	***	**	***	8
Zhou, D. 2016	China	Asian	ASC	PB	PCR	183	213	54	158	185	37	185	37	0.1	**		***	5
Zhou, D. 2016	China	Asian	CAD	PB	PCR	210	244	61	175	186	40	186	40	0.35	**		***	5
Zhang, K. 2017	China	Asian	AO	PB	RT-PCR	191	371	196	249	373	171	373	171	0.16	**	*	***	6

†RT-PCR - real time polymerase chain reaction

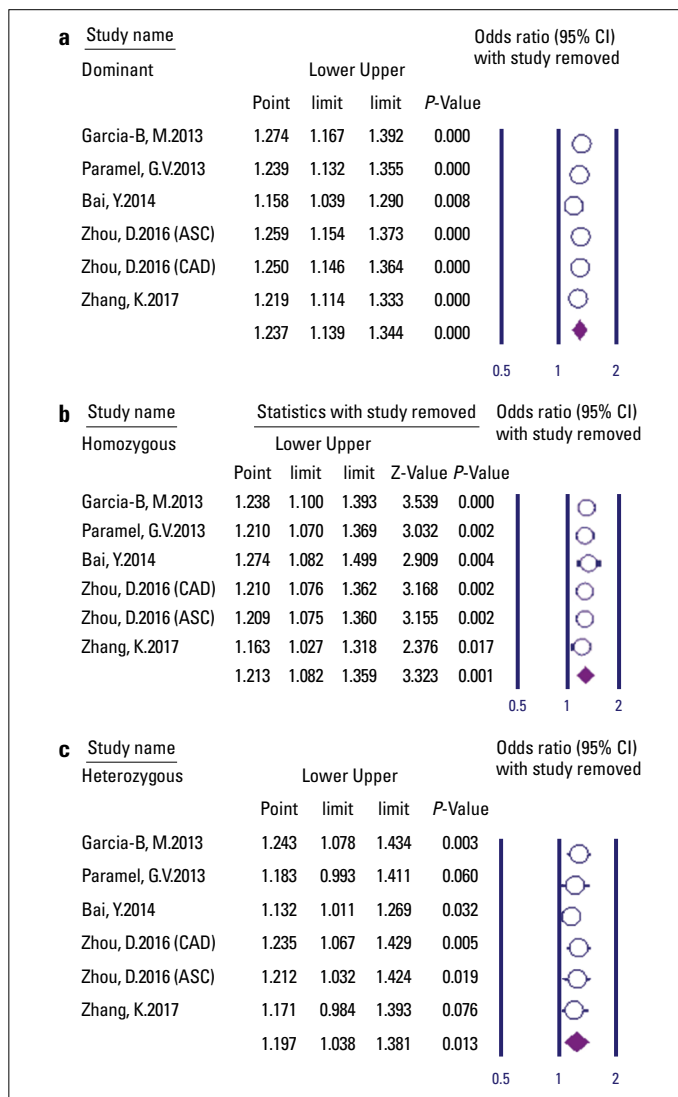


Figure 4. Sensitivity analysis for the models of *CARD8* rs2043211 with CVD risk in different genetic models. a) Dominant model (TT+AT vs. AA). b) Homozygous model (TT vs. AA). c) Heterozygous model (AT vs. AA)

to estimate the power of the current conclusion (13, 14) the Trial Sequential Analysis (TSA) tool (Copenhagen Trial Unit, Centre for Clinical Intervention Research, Denmark) was used. TSA version

3.0 (<http://www.ctu.dk/tsa/>) was used while performing the present investigation.

Results

Literature search characteristics

Six case-control articles were included in this meta-analysis, consisting of 11,573 subjects, 5,075 cases, and 6,498 controls (8, 10-12, 15, 16) after removing the duplicate articles, unrelated articles, and articles lacking a complete genotype distribution. The characteristics of all the included articles are summarized in Table 1. Among these included studies, two were performed in Caucasian populations and four in Asian populations. These studies included many CVDs, such as myocardial infarctions, CADs, acute coronary syndrome, and arteriosclerosis obliterans.

Meta-analysis results

All the studies described the genotype distributions for the AA, AT, and TT allele combinations and were divided into five models. For the homozygous model, OR was 1.21 (1.08–1.36, $I^2=0.0\%$, $P_{\text{heterogeneity}}=0.542$). For the heterozygous model (AT vs. AA), OR was 1.20 (1.04–1.38, $I^2=57.2\%$, $P_{\text{heterogeneity}}=0.039$). For the dominant model, OR was 1.24 (1.14–1.34, $I^2=38.5\%$, $P_{\text{heterogeneity}}=0.149$). For the allele model, OR was 0.96 (0.77–1.20, $I^2=92.7\%$, $P_{\text{heterogeneity}}=0.00$). For the recessive model, OR was 1.00 (0.91–1.10, $I^2=48.4\%$, $P_{\text{heterogeneity}}=0.085$) (Fig. 2).

Regarding the ethnicity subgroup, in the homozygous, heterozygous, and dominant models, rs2043211 indicated a CVD risk in the Asian population (Fig. 3). As stratified by etiology for rs2043211, significant associations were found in the myocardial infarction group, a increased risk of CVDs was observed in the homozygous and dominant models.

Sensitivity analysis

To detect the influence of each study and the stability of the results, a sensitivity analysis was conducted by omitting one individual study. Heterogeneity was not found in any of the gene models (Fig. 4).

Publication bias

A bias analysis was performed by generating funnel plots for each polymorphism of the dominant and heterozygous models (Fig. 5). Obviously, the allelic ($p=0.60$), recessive ($p=0.14$), and codominant homozygous ($p=0.88$) genetic models exhibited no publication bias. We observed that the codominant heterozygous ($p=0.007$) and allelic ($p=0.04$) models may have exhibited bias, so the trim and fill method was used (17). By removing and supplementing part of the study, the results showed no statistical difference in the conclusion, so we believe that the results were stable.

Trial sequential analysis

We performed TSAs for the allelic and heterozygous models (AT vs. AA) of SNP rs2043211 (Fig. 6). The results of both showed that the blue line of the cumulative z-curve crossed the TSA monitoring boundary. Moreover, the heterozygous model touched the required sample size. Therefore, based on the heterozygous model, the results suggested that no further studies were necessary to confirm the association. However, the allelic

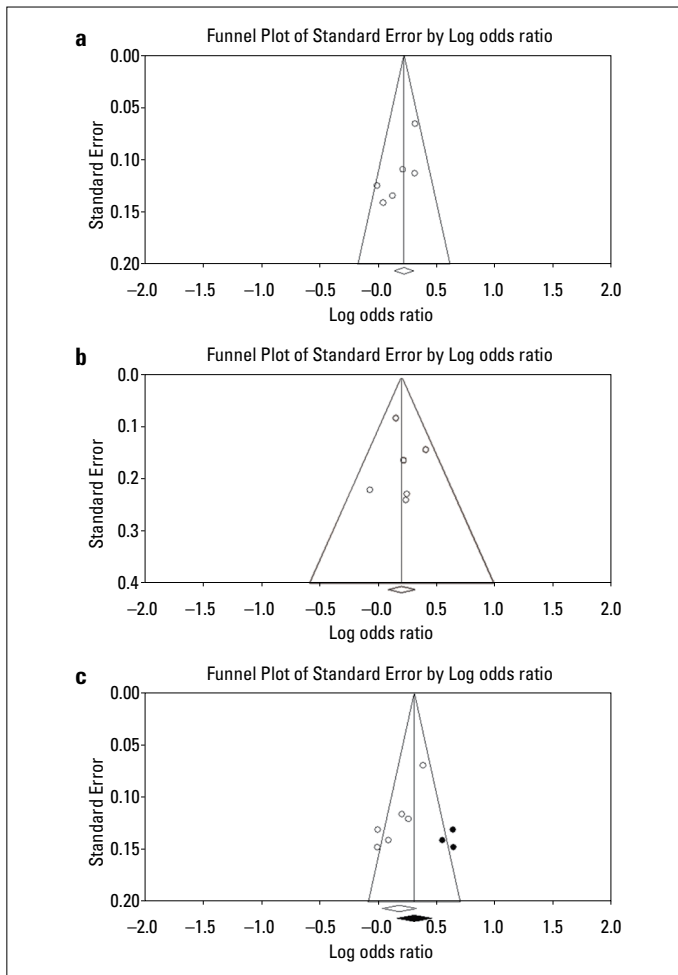


Figure 5. Funnel plots for the models of *CARD8* rs2043211 with CVD risk in different genetic models. a) Dominant model (TT+AT vs. AA). b) Homozygous model (TT vs. AA). c) Heterozygous model (AT vs. AA)

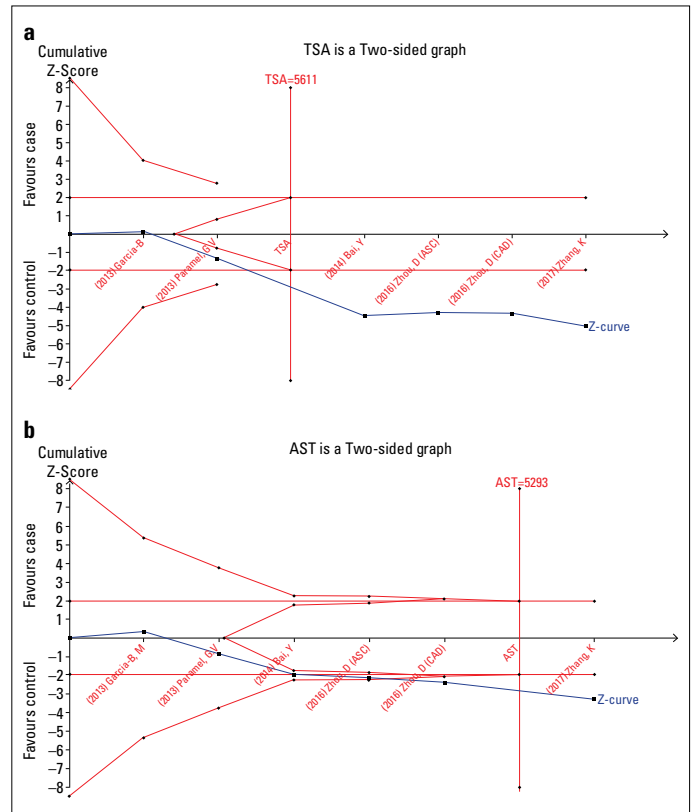


Figure 6. Trial sequential analysis for the models of *CARD8* rs2043211 with CVD risk in different genetic models. a) Dominant model (TT+AT vs. AA). b) Homozygous model (TT vs. AA)

model did not reach either the TSA required or the cumulative sample size, intimating that there may be a false positive.

Discussion

Subclinical chronic inflammation due to endothelial vessel wall damage has long been hypothesized as part of the pathophysiology of CVDs (18). Extensive clinical and pathophysiological research has confirmed that therapeutic intervention targeted against inflammatory mediators is effective for the treatment of myocardial infarction (19). For example, Luo reported that NLRP3 gene silencing therapy ameliorated cardiac inflammation, pyroptosis, fibrosis, and cardiac function (20). Genetic variations leading to the altered production of inflammatory cytokines or altered inflammasome function have been linked to various inflammatory disease; the most frequently studied genetic variations are NLRP3 and CARD. The NLRP3 inflammasome is composed of the NLRP3 scaffold protein, CARD-containing adaptor protein, and caspase-1 (21).

CARD8 contains a homotypic interaction motif called the caspase recruitment domain, and its polymorphism can introduce a translation stop codon at codon 10 (known as Cys10Stop or C10X), thereby expressing a premature *CARD8* protein with almost no function. Regarding the C10X polymorphism in the *CARD8* gene, associations were seen between rs2043211 and different dis-

ease susceptibilities. It has been reported that a *CARD8* mutation causes Crohn's disease (22) and that *CARD8* polymorphisms influence higher disease activity in aspirin-induced asthma and rheumatoid arthritis (22). In addition, ankylosing spondylitis, gout, and Alzheimer's disease have been found to have relationships with the *CARD8* variant rs2043211, as well as CVDs (6, 7, 23).

CARD8 rs2043211 showed no association with the risks of myocardial infarction or CAD or the risk of developing cardiovascular events in patients with rheumatoid arthritis. However, it was found to be associated with a lower expression of *CARD8* in the plaque as well as with lower C-reactive protein and monocyte chemoattractant protein-1 levels in the plasma (8, 15, 16). However, in one Chinese cohort, rs2043211 was associated with ischemic stroke (10), and it is probably associated with the development of arteriosclerosis obliterans in the Chinese Han male population (11). Moreover, it has shown a modest protective effect against abdominal aortic aneurysms (24). Therefore, the CVDs risk of *CARD8* rs2043211 is worth assessing.

To our knowledge, this is the first meta-analysis involving the relationship between *CARD8* and the CVD risk. We found that rs2043211 indicated a CVD risk in the homozygous, heterozygous, and dominant models, particularly in the Asian population. In the etiology subgroup, in the homozygous and dominant models, there was also a risk of myocardial ischemia. Moreover, the TSA results showed that based on the heterozygous model, no further studies were necessary to confirm the association, and that ischemic heart disease is one of the leading causes of premature death worldwide (2).

This meta-analysis has several limitations. First, we were unable to analyze the potential gene-environment and gene-gene interactions. Second, significant between-study heterogeneity was detected in some of the comparisons, which might have affected the results. Finally, different genotype methods and disease statuses may have influenced the data explanation of the included studies.

Conclusion

In conclusion, this meta-analysis showed that the dominant model, heterozygous model and homozygote model of the *CARD8* rs2043211 polymorphism may be associated with CVDs and that the association seems to be population-dependent. However, the exact mechanism by which the *CARD8* rs2043211 gene polymorphism influences CVDs susceptibility remains to be elucidated. Further studies based on a larger sample size and case-control design in different populations are needed to clarify this association.

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Peer-review: Externally peer-reviewed.

Authorship contributions: Concept – H.H., Y.H.; Design – H.H., Y.H.; Supervision – Q.B., H.W.; Fundings – Y.H.; Materials – Q.B., H.W.; Data collection &/or processing – Q.B., B.L.; Analysis &/or interpretation – H.H., H.W.; Literature search – H.H., Q.B.; Writing – H.H.; Critical review – H.H., Y.H.

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