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ORIGINAL ARTICLE

# ASSOCIATION BETWEEN OSTEOPROTEGERIN GENE POLYMORPHISMS AND RISK OF CORONARY ARTERY DISEASE: A SYSTEMATIC REVIEW AND META-ANALYSIS

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## ABSTRACT

Osteoprotegerin (OPG) has been demonstrated to be a novel biomarker for predicting prevalence and severity of coronary artery disease (CAD). Furthermore, recent studies have shown that *OPG* gene polymorphisms are associated with a susceptibility to CAD. However, published studies showed inconsistent results. Therefore, a meta-analysis of eligible studies reporting the association between *OPG* gene polymorphisms and CAD was carried out. A systematic search was conducted using PubMed, Web of Science, Cochrane Library, Chinese National Knowledge Infrastructure (CNKI) and Chinese Wan Fang databases. Odds ratio (OR) with corresponding 95% confidence interval (95% CI) were calculated. Overall, six eligible studies were included and four *OPG* gene polymorphisms (G209A, T245G, T950C and G1181C) were further evaluated for the association with susceptibility to CAD in this meta-analysis. Meta-analysis showed that G1181C and T950C polymorphisms were strongly associated with the risk of CAD, but no association existed between G209A and T245G polymorphisms and the risk of CAD. In conclusion, our meta-analysis is the first report to estimate the association between *OPG* gene polymorphisms and susceptibility to CAD. Further large scale case-control studies with rigorous design should be conducted to confirm the above conclusions in the future.

**Keywords:** Coronary artery disease (CAD); Gene polymorphism; Meta-analysis; Osteoprotegerin (OPG).

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## INTRODUCTION

Coronary artery disease (CAD), especially acute myocardial infarction, is becoming one of the major causes of morbidity and mortality worldwide [1]. Multiple factors, such as genetic variants, lifestyle and environmental factors, are believed to be involved in the occurrence and progression of CAD [2-4]. In recent years, more and more researchers carry out large scale genomewide association studies (GWAS) to elucidate the pathogenesis of CAD at the gene expression level and the results have demonstrated that gene polymorphisms are strongly associated with the susceptibility to CAD [5,6].

Osteoprotegerin (OPG), a new member of the tumor necrosis factor (TNF) receptor superfamily, has been identified as a soluble non-transmembrane glycoprotein secreted by osteoblasts (OCs) [7]. It plays a key role in the formation and resorption of bone through inhibiting differentiation and maturation of OC and inducing OC apoptosis [8]. In addition, OPG is also an important vascular modulator and strongly linked with the occurrence and progression of atherosclerosis and arteriosteogenesis [9]. More importantly, OPG has been associated with the presence and severity of CAD, as evidenced by elevated serum OPG concentrations in CAD patients [10,11].

The gene encoding OPG is located on the long arm of chromosome 8 at position 24. Some recent studies have shown that the T950C and G1181C polymorphisms of the *OPG* gene are associated with vulnerability of CAD [12-15], but the sample size was relatively small and the results were controversial [16]. Therefore, in the present study, we performed a meta-analysis to evaluate the association between *OPG* gene polymorphisms and the risk of CAD.

## MATERIALS AND METHODS

**Search Strategy.** A systematic search assembling the following terms: genetic polymorphism, single nucleotide polymorphism, gene mutation, genetic variants, coronary atherosclerosis, myocardial ischemia, acute coronary syndrome, coronary artery disease, myocardial infarction, ischemic heart disease, TNFRSF11B, osteoprotegerin, was conducted in PubMed, Web of Science, Cochrane Library, Chinese National Knowledge Infrastructure (CNKI) and Chinese Wan Fang databases up to May 1 2017 to identify all potentially relevant studies. Hand-searching was carried out to determine other potential eligible studies through scanning the references cited in the retrieved articles. The full-text articles were further reviewed to determine whether they were included in the final analysis strictly based on eligibility criteria. If two reviewers disagreed, all the authors critically evaluated the studies to judge of the inclusion or exclusion of a certain study.

**Eligibility Criteria.** All the eligible articles were supposed to meet the following major inclusion criteria: ***i)*** assessment of the association between *OPG* gene polymorphisms and CAD; ***ii)*** case-control or cohort studies; ***iii)*** data provided by articles about allele frequency should be sufficient for calculating genotypic odds ratio (OR) with corresponding to 95% confidence interval (95% CI) in cases and controls. Moreover, only when a single nucleotide polymorphism (SNP) in the *OPG* gene was reported by at least two articles would it be analyzed by meta-analysis. Studies were excluded when they were ***i)*** duplicated data; ***ii)*** case report, review articles and editorial comment. The diagnosis of the CAD case was based on the WHO criteria for CAD as previously described (stenosis ≥50.0% of the diameter in at least one major coronary artery based on computer-assisted assessments) [17]. All healthy control subjects were identified according to patient history, serum biochemistry examination and electrocardiogram (ECG) test.

**Data Extraction.** Data extraction was performed independently by two authors using a standardized data extraction form including following elements: ***1)*** author's name, year of publication; ***2)*** patient characteristics of each group; ***3)*** number of participants in case and control groups; ***4)*** study type; ***5)*** genotyping method; ***6)*** *p* value of Hardy-Weinberg equilibrium (HWE) test in the control; ***7)*** OR and 95% CI for association with CAD. Assessment of the quality of studies was performed using the Newcastle-Ottawa Scale (NOS) as previously described [18]. Briefly, two authors of this article separately evaluated the quali-

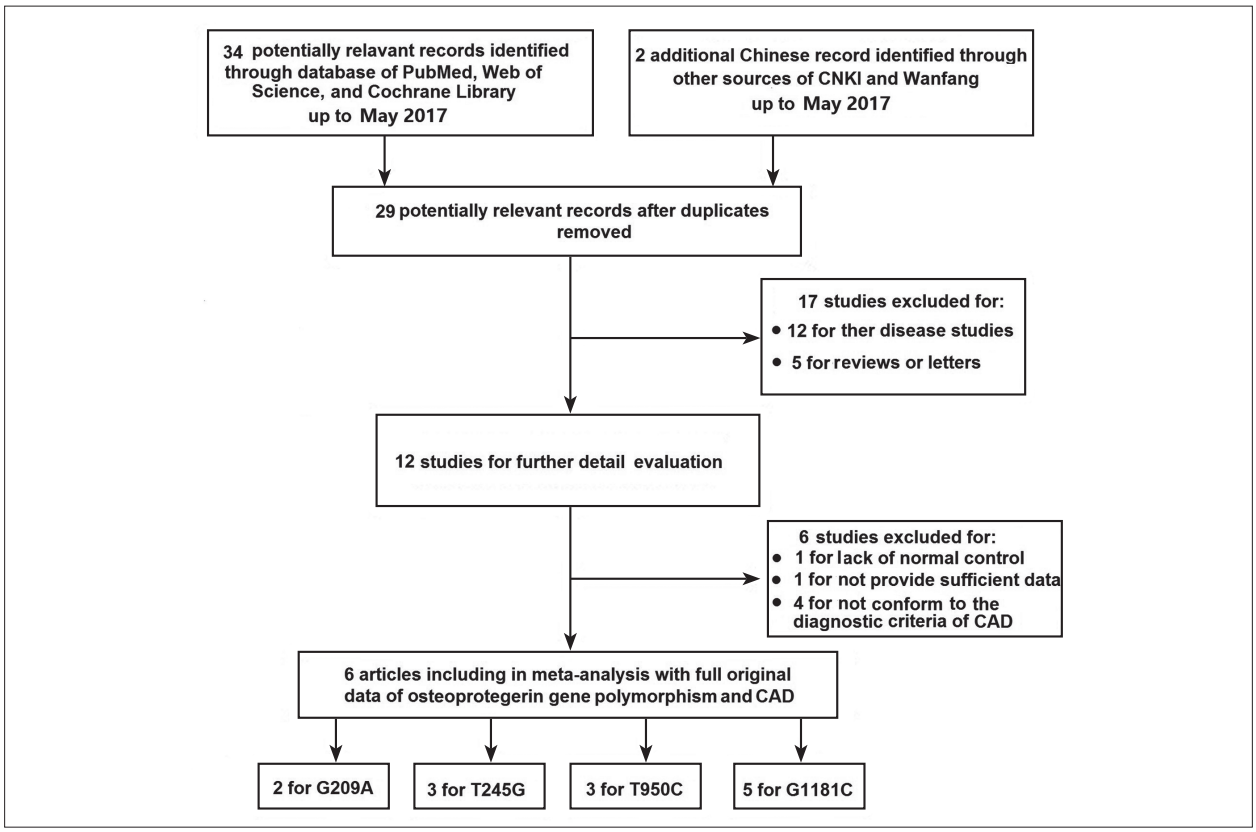


**Figure 1.** Flow diagram of the study selection process.

ties based on eight items and scored the studies from 0 to 9 points. Studies with a score not less than seven points were considered to be of high quality. Discrepancy was resolved as described above.

**Statistical Analyses.** First, the genotype frequencies of the *OPG* gene polymorphism among the controls of all included studies were assessed under HWE using a $\chi^2$ goodness-of-fit test. Odds ratios with corresponding 95% CIs were used to estimate the strength of association between *OPG* gene polymorphisms and CAD. The between-study heterogeneity across all eligible comparisons was tested by the $\chi^2$-based Q statistic. Heterogeneity was considered significant when the *p* value was less than 0.10. When heterogeneity existed, the random effects model was performed to calculate the pooled OR of each eligible study, otherwise, the fixed effect model was used. Generally, we assessed the association between the *OPG* gene polymorphism and CAD under five genetic models: allele, homozygote, heterozygote, dominant and recessive models. Sensitivity analysis was conducted through omitting one study at a time to examine its influence on the overall estimate to evaluate the stability of the meta-analysis. Publication bias was also analyzed using the Egger's linear regression test and funnel plots and publication bias was considered present when the

**Table 1.** Main characteristics of studies included in the meta-analyses.

| Studies [references] | [15] | [14] | [19] | [12] | [13] | [16] |
|---|---|---|---|---|---|---|
| Country | Poland | China | China | Germany | Japan | China |
| Ethnicity | Caucasian | Asian | Asian | Caucasian | Asian | Asian |
| Number (case/control) | 31/30 | 184/68 | 178/312 | 522/468 | 405/126 | 222/146 |
| Age, year (case/control) | 65.6/70.5 | 62.6/60.79 | 58.3/57.2 | NA | 63.0/59.0 | 70.1/63.7 |
| Male% (case/control) | 0.0/0.0 | 79.3/57.4 | 64.0/62.5 | 100.0/100.0 | NA | 72.5/59.6 |
| Hypertension% (case/control) | NA | NA | 68.5/56.7 | NA | 63.0/51.0 | 77.0/65.8 |
| Study type | case-control | case-control | case-control | case-control | case-control | case-control |
| Primary outcome | CAD | ACS | CAD | CAD | CAD | CAD |
| Genotyping method | PCR-RFLP | Sanger sequencing | PCR-RFLP | PCR-RFLP | PCR-RFLP | PCR-RFLP |
| NOS score | 9 | 9 | 9 | 9 | 9 | 9 |
| HWE test (control) | yes | yes | yes | yes | yes | yes |

NA: not available; CAD: coronary artery disease; ACS: acute coronary syndrome; PCR-RFLP: polymerase chain reaction-restriction fragment length polymorphism; HWE: Hardy-Weinberg equilibrium.

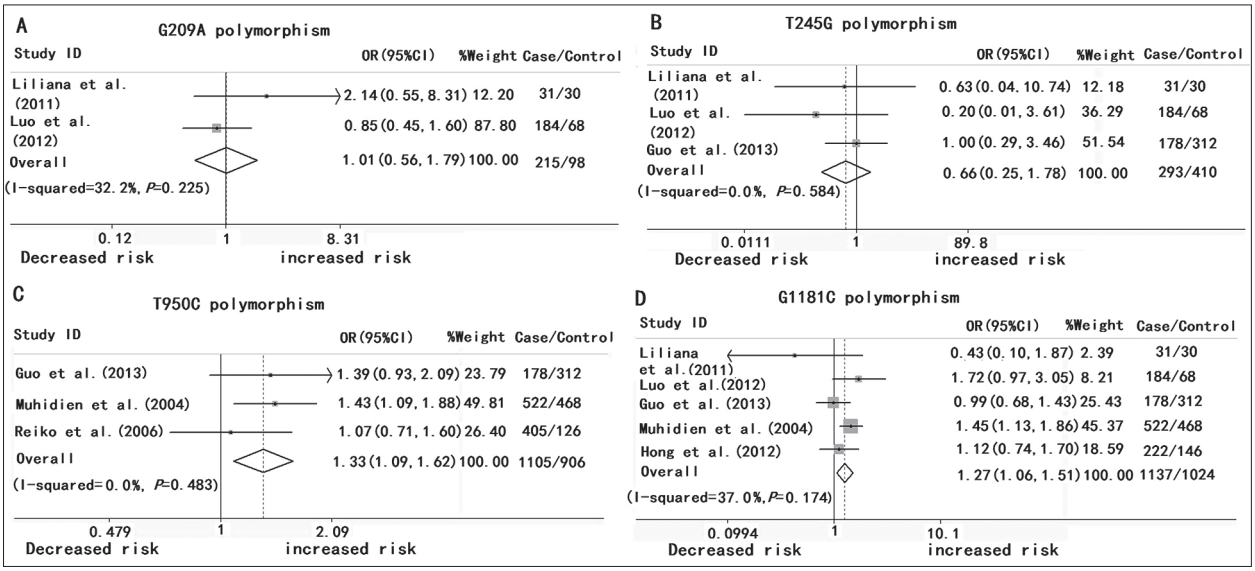


**Figure 2.** Forest plots for *OPG* gene polymorphisms and the risk of CAD. (A) Forest plot for G209A polymorphism and the risk of CAD under dominant (AA/AG *vs.* GG) model. (B) Forest plot for T245G polymorphism and the risk of CAD under dominant (TT/TG *vs.* GG) model. (C) Forest plot for T950C polymorphism and the risk of CAD under dominant (CC/CT *vs.* TT) model. (D) Forest plot for G1181C polymorphism and the risk of CAD under dominant (CC/CG *vs.* GG) model.

*p* value was less than 0.05. All statistical analyses were done using STATA version 11.0 (STATA Corporation, College Station, TX, USA). All *p* values were two-tailed.

## RESULTS

**Results of the Literature Search.** As shown in Figure.1, 36 potentially eligible records were initially identified through literature search. Thirty articles were excluded, including seven articles that were duplicated, five articles that were reviews, 12 articles that did not involve CAD, four articles that did not conform to the diagnostic criteria of CAD, one article that lacked normal controls, and one article that did not provide sufficient data for the distribution of the genotype. Finally, six articles in accordance with the inclusion criteria were included in this meta-analysis [12-16,19]. To be specific, two studies involved the G209A polymorphism, three studies with T245G polymorphism, three studies with T950C polymorphism, and five studies with G1181C polymorphism.

**Table 2.** Main results of the meta-analyses of the pooled odds ratios.

| Variable | Cases/Controls (*n*) | ORb (95% CI) Ph Value | | | | |
|---|---|---|---|---|---|---|
| | | 1 *vs.* 2 | 11 *vs.* 22 | 12 *vs.* 22 | (11 or 12) *vs.* 22 | 11 *vs.* (12 or 22) |
| G209A (1=A; 2=G) | 215/98 | 1.128 (0.664-1.916) 0.655a | NA | 0.898 (0.500-1.613) 0.718a | 1.005 (0.564-1.792) 0.986a | NA |
| T245G (1=T; 2=G) | 393/410 | 0.940 (0.678-1.302) 0.708a | 0.679 (0.236-1.957) 0.474a | 0.628 (0.209-1.886) 0.407a | 0.664 (0.247-1.785) 0.417a | 0.979 (0.682-1.405) 0.909a |
| T950C (1=C; 2=T) | 1137/1024 | 1.264 (1.108-1.442) 0.000a | 1.615 (1.242-2.100) 0.000a | 1.227 (0.994-1.515) 0.057a | 1.327 (1.090-1.617) 0.005a | 1.376 (1.097-1.726) 0.006a |
| G1181C (1=C; 2=G) | 1105/906 | 1.203 (0.957-1.514) 0.114 | 1.139 (0.677-1.917) 0.623 | 1.243 (1.027-1.504) 0.026a | 1.268 (1.064-1.511) 0.008a | 1.227 (0.943-1.595) 0.127a |

ORb: crude odds ratio; 95% CI: 95% confidence interval; Ph: *p* value for Chochran's Q test between study heterogeneity in each genetic comparison model; a: fixed effects model was used for all of the above; NA: not available.

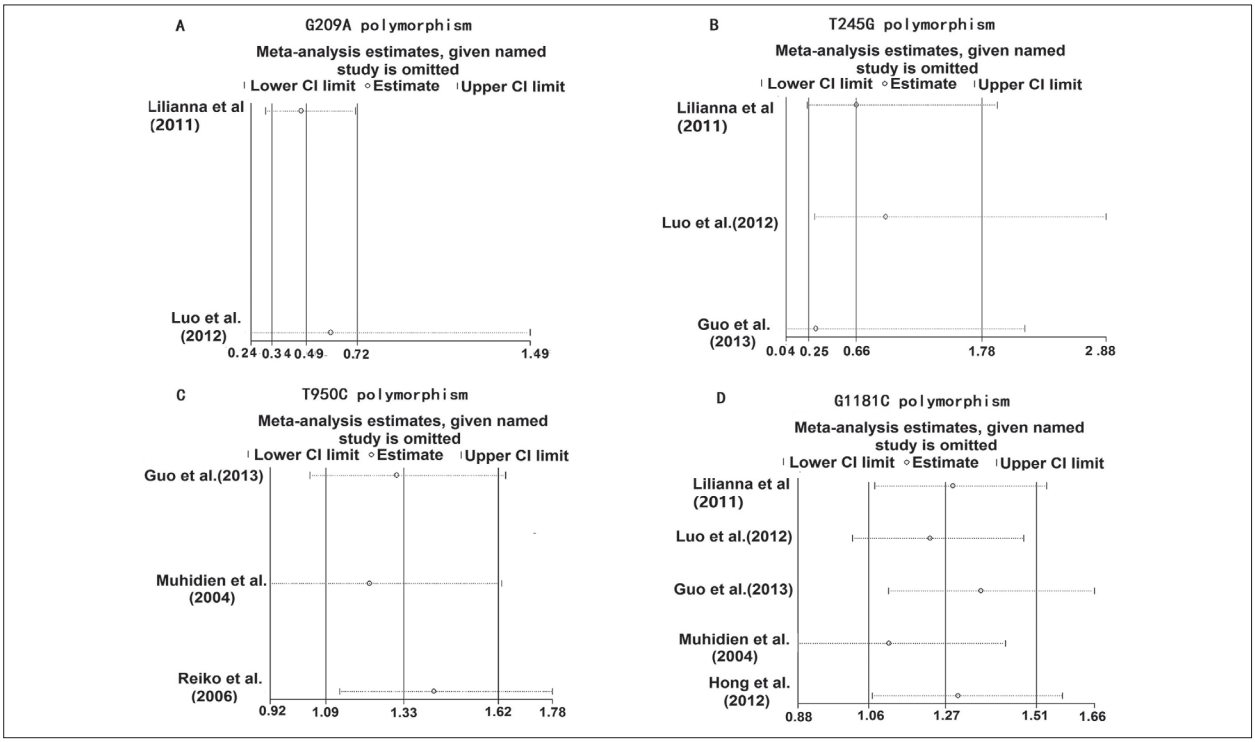


**Figure 3.** Sensitivity analysis results. (A) Sensitivity analysis for G209A polymorphism and the risk of CAD under dominant (AA/AG *vs.* GG) model. (B) Sensitivity analysis for T245G polymorphism and the risk of CAD under dominant (TT/TG *vs.* GG) model. (C) Sensitivity analysis for T950C polymorphism and the risk of CAD under dominant (CC/CT *vs.* TT) model. (D) Sensitivity analysis for G1181C polymorphism and the risk of CAD under dominant (CC/CG *vs.* GG) model.

**Characteristics of Included Studies.** The characteristics of the included studies are summarized in Table 1. Overall, four studies were conducted in Asians, and the other two studies were carried out in Caucasians. The polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method was used to detect the gene polymorphisms in five out of six studies. The average score of NOS was at 9. The genotype distribution of the controls in all studies was consistent with HWE.

**Quantitative Data Synthesis.** Meta-analysis of the G209A polymorphism was involved with two studies consisting of 215 CAD cases and 98 controls. There was no association between the G209A polymorphism and the risk of CAD when pooling all the data in the meta-analysis (AA/AG *vs.* GG: OR = 1.005, 95% CI = 0.564-1.792, $p$ = 0.986) (Figure 2A) (Table 2). For the T245G polymorphism, three studies with 393 CAD cases and 410 controls were included for final meta-analysis, but the results showed no relationship between the T245G polymorphism and the risk of CAD either (TT/TG *vs.* GG: OR = 0.664, 95% CI = 0.247-1.785, $p$ = 0.417) (Figure 2B) (Table 2). For the T950C polymorphism, 1105 CAD cases and 906 controls were included in the meta-analysis. A significant association was found between the T950C polymorphism and risk of CAD under the dominant model (CC/CT *vs.* TT: OR = 1.327, 95% CI = 1.090-1.617, $p$ = 0.005) (Figure 2C), allele model (C *vs.* T: OR = 1.264, 95% CI = 1.108-1.442, $p$ <0.001), homozygote model (CC *vs.* TT: OR = 1.615, 95% CI = 1.242-2.100, $p$ <0.001), recessive model (CC *vs.* CT/TT: OR = 1.376, 95% CI = 1.097-1.726, $p$ = 0.006) (Table 2). Meta-analysis of the G1181C polymorphism was involved with five studies consisting of 1137 CAD cases and 1024 controls and the results indicated that the G1181C polymorphism was significantly associated with the risk of CAD under the dominant model (CC/CG *vs.* GG: OR = 1.268, 95% CI = 1.064-1.511, $p$ = 0.008) (Figure 4D). In addition, a statistically significant association also existed between the G1181C polymorphism and risk of CAD under the heterozygote model (CG *vs.* GG: OR = 1.243, 95% CI = 1.027-1.504, $p$ = 0.026) (Table 2).

**Sensitivity Analysis and Publication Bias.** The result of the sensitivity analysis showed that the pooled ORs of the G209A, T245G, T950C and G1181C polymorphisms were not considerably affected by eliminating any

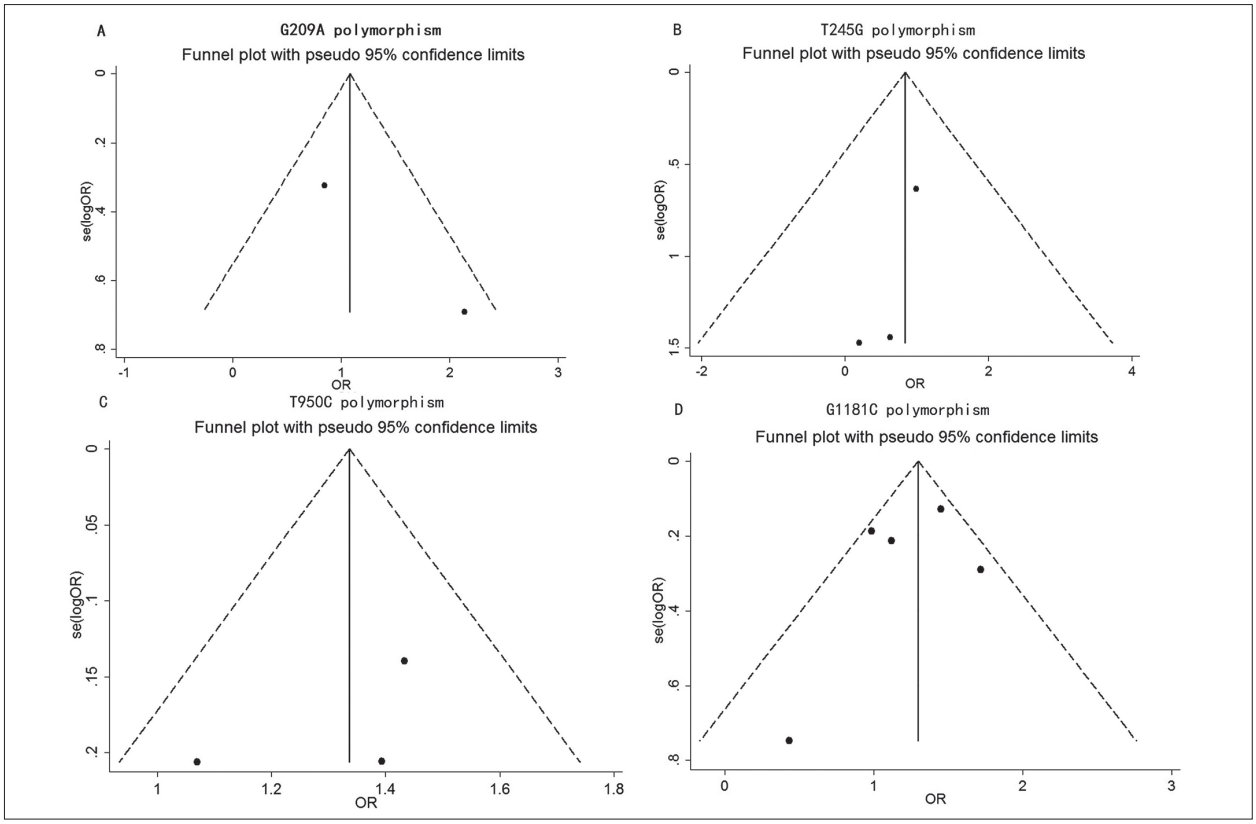


**Figure 4.** Evaluation for publication bias. (A) Funnel plot for G209A polymorphism and the risk of CAD under dominant (AA/AG *vs.* GG) model. (B) Funnel plot for T245G polymorphism and the risk of CAD under dominant (TT/TG *vs.* GG) model. (C) Funnel plot for T950C polymorphism and the risk of CAD under dominant (CC/CT *vs.* TT) model. (D) Funnel plot for G1181C polymorphism and the risk of CAD under dominant (CC/CG *vs.* GG) model.

individual study (Figure 3). The funnel plots were symmetrical by visual inspection (Figure 4) and Egger's test also suggested no publication bias ($p > 0.05$). These results confirmed that this meta-analysis was robust.

## DISCUSSION

Osteoprotegerin has been considered as a novel biomarker for predicting prevalence and severity of CAD [20]. Jono *et al.* [10] and Schoppet *et al.* [11] have demonstrated that serum OPG levels are associated with the presence and severity of CAD. Ren *et al.* [21] have further confirmed that increased plasma OPG levels are associated with the presence and severity of acute coronary syndrome. These studies suggest the potential prognostic utility of OPG as a biomarker in a clinical practice. In addition, some recent studies have found that several polymorphisms of the *OPG* gene, such as T950C and G1181C, are associated with the risk of CAD, but the results are still controversial [12-16]. Considering these inconsistent results, in this study, we performed meta-analysis to analyze the association between the G209A, T245G, T950C and G1181C polymorphisms of the *OPG* gene and the risk of CAD.

G1181C is the most controversial in all polymorphisms of the *OPG* gene. Even in populations from the same country, the results are diverse [14,16]. The results of meta-analysis showed that the G1181C polymorphism was strongly associated with the risk of CAD with little heterogeneity across studies. In addition, the results also showed that the CC or CG genotypes may increase susceptibility to CAD, which agreed with previous findings [12].

The T950C polymorphism is located 233 bp upstream from the translation initiation site in the promoter region, and it could derive its functional significance by altering the promoter activity [13]. Previous research has demonstrated that T950C is the only polymorphism that is associated with serum OPG levels [12]. Our meta-analysis indicated that the T950C polymorphism was remarkably linked with the risk of CAD, which was consistent with previous results [12]. Moreover, we also reported that no association existed between G209A and T245G polymorphisms and the risk of CAD, which was in line with previous studies [14,15,19].

Similar to other meta-analyses, several limitations existed in our meta-analysis. First, the sample size is still relatively small and may not provide sufficient statistical power to estimate the correlation between the *OPG* gene polymorphisms and the susceptibility to CAD. More studies with larger sample size are still needed to accurately provide a more representative statistical analysis. Secondly, we did not evaluate the potential publication bias that may influence the result. Finally, although little heterogeneity exists, subgroup analysis should be performed to assess the association between the *OPG* gene polymorphisms and the susceptibility to CAD in different populations or countries.

In conclusion, to the best of our knowledge, this meta-analysis is the first report to pool published studies to estimate the association between the *OPG* gene polymorphisms and the susceptibility to CAD. This study demonstrated that G1181C and T950C polymorphisms were strongly associated with the risk of CAD, but no association existed between G209A and T245G polymorphisms and the risk of CAD. Further large scale case-control studies with a rigorous design should be conducted to confirm the above conclusions in the future.

## ACKNOWLEDGMENTS

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**Declaration of Interest.** The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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