

# The Associations Between the Polymorphisms of Vitamin D Receptor and Coronary Artery Disease

## *A Systematic Review and Meta-Analysis*

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**Abstract:** Vitamin D receptor (VDR) polymorphisms were indicated to be associated with coronary artery disease (CAD); however, published studies reported inconsistent results.

The aim of this meta-analysis is to reach a more accurate estimation of the relationship between VDR genetic polymorphisms and CAD risk.

Eligible studies were retrieved by searching PubMed, Embase, VIP, Wanfang and China National Knowledge Infrastructure databases. Included and excluded criteria were formulated. The case group was patients with CAD, and the control group was healthy subjects. Summary odds ratios (ORs) and 95% confidence intervals (CIs) were used to evaluate VDR polymorphisms associations with CAD risk. Heterogeneity was evaluated by Q statistic and  $I^2$  statistic.

Seven studies of a total of 2306 CAD patients and 4151 control subjects met the inclusion criteria. The pooled results from Taq1 showed increased risk in allelic model (OR = 1.14, 95% CI = 1.02–1.28), dominant model (OR = 1.21, 95% CI = 1.02–1.43), heterozygote model (OR = 1.19, 95% CI = 1.00–1.42), and homozygote model (OR = 1.27, 95% CI = 1.01–1.61). Besides, Fok1 T > C showed decreased risk in allelic model (OR = 0.81, 95% CI = 0.65–1.00) and Fok1 A > G also showed decreased risk in allelic model (OR = 0.67, 95% CI = 0.45–1.00) and recessive model (OR = 0.55, 95% CI = 0.31–0.97). In Caucasian subgroup, Bsm1 showed increased risk in allelic model (OR = 1.23, 95% CI = 1.02–1.47), heterozygote model (OR = 1.20, 95% CI = 1.00–1.44), and homozygote model (OR = 1.22, 95% CI = 1.02–1.45). In CAD patients with type 2 diabetes mellitus (T2DM), Apa1 showed a decreased risk in heterozygote model (OR = 0.80, 95% CI = 0.66–0.98); however, increased risk in recessive model (OR = 5.00, 95% CI = 2.74–9.13) was discovered in CAD patients without T2DM.

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The Fok1 polymorphism may play a protective role in CAD, and the possible protective role in Apa1 CA genotype in CAD patients with T2DM needs further studies. The Taq1 polymorphism is found to be associated with a significant increase in CAD risk based on our analysis; moreover, increased risk in Apa1 polymorphism in CAD patients without T2DM and Bsm1 polymorphism in Caucasian group is also detected.

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**Abbreviations:** CAD = coronary artery disease, CI = confidence interval, HWE = Hardy–Weinberg equilibrium, OR = odd ratio, SNP = single-nucleotide polymorphism, T2DM = type 2 diabetes mellitus, VDR = vitamin D receptor.

## INTRODUCTION

Coronary artery disease (CAD) is the most common cause of death in developed countries and the second most common cause of death in developing country. The morbidity and mortality of CAD patients and the risk factors of CAD have substantially increased.<sup>1–3</sup> Many factors play important roles in the pathogenesis of CAD such as age,<sup>4</sup> gender,<sup>5</sup> region<sup>6</sup> and genetic factors also affect the occurrence of CAD.<sup>7–9</sup> The SNPs in vitamin D receptor (VDR) were found to be a potential risk factor of CAD, which might be associated with a low level of vitamin D in CAD patients, but the exact mechanism underlying the influence of VDR polymorphisms on the pathogenesis of CAD is still unknown.<sup>10–14</sup>

The vitamin D endocrine system is involved in a wide variety of biological processes including bone metabolism, regulation of cell proliferation, and differentiation and modulation of immune responses.<sup>15</sup> Both vitamin D and vitamin D receptor (VDR) play an important role in the skeletal metabolism. VDR gene belongs to the steroid hormone family of nuclear receptors which are responsible for the transcriptional regulation of a number of hormone responsive genes. The gene encoding the VDR is located at chromosome 12q and has 4 common polymorphisms. Four VDR polymorphisms Fok1, Bsm1, Apa1, and Taq1 were well characterized.<sup>16</sup> Polymorphisms within the VDR gene might potentially influence the expression of vitamin D receptor gene and the stability of VDR mRNA.<sup>15</sup>

More recent attention has been focused on the possible role of VDR gene polymorphisms in the development of a range of diseases, including osteoarthritis,<sup>17</sup> Parkinson disease,<sup>18</sup> diabetes,<sup>19</sup> as well as CAD.<sup>20,21</sup> However, the results of VDR gene polymorphisms in CAD are inconsistent. He and Wang<sup>10</sup> reported an association between VDR Fok1 and CAD in Chinese, but Pan et al<sup>12</sup> found opposite. Van Schooten et al<sup>14</sup> found an association between VDR Bsm1 polymorphism and coronary artery disease (CAD) in the Netherlands, while Ortlepp et al<sup>22</sup> reported no association on the CAD in Germany.

The small numbers and various populations of the published studies may partially account for the controversial results. This meta-analysis therefore aims to pool current evidence together for better understanding of the potential associations between the VDR gene polymorphisms and CAD.

## METHODS

The systematic review was written in adherence to the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-analyses) checklist.<sup>23</sup> Ethical approval was not necessary according to local legislation because of the type of study (meta-analysis).<sup>24</sup>

### Identification of the Related Studies

Embase, PubMed, VIP, Wanfang and China National Knowledge Infrastructure databases were thoroughly searched in August 2015 by the first 2 investigators to identify potential studies addressing the associations between the VDR polymorphisms and coronary artery disease. The terms “coronary artery disease,” “coronary heart disease,” “vitamin D receptor,” “VDR,” “polymorphism,” and “polymorphisms” were used. The missing data (the data that we failed to identify during the electronic search) were obtained by reviewing the citations of review articles and all eligible studies.

### Inclusion and Exclusion Criteria

Studies in the meta-analysis must meet the following inclusion criteria: evaluation of the association between VDR polymorphisms and coronary artery disease; case-control study or cohort design; detailed genotype data could be acquired to calculate odds ratios (ORs) and 95% confidence intervals (CIs); exclusion criteria: duplication of previous publications; comment, review and editorial; study with no detailed genotype data. The selection of the studies was achieved by 2 investigators independently, according to the inclusion and exclusion criteria by screening the title, abstract and full-text. Any dispute was solved by discussion.

### Data Extraction

From each study, the following data were independently extracted by the first 2 investigators using a standardized form: first author's last name, year of publication, study country, ethnicity, genotyping methods, the criteria of CAD, Hardy-Weinberg equilibrium, number of cases and controls, genotype frequency in cases and controls for VDR. Different ethnicity descents were classified as Caucasian and Asian. Disagreements were resolved through discussion with a 3rd investigator (FH).

### Statistics Analysis

Hardy-Weinberg equilibrium (HWE) was evaluated for each study by  $\chi^2$  test in control groups, and  $P < 0.05$  was considered a significant departure from HWE. Odds ratio (OR) and 95% confidence intervals (CIs) were calculated to evaluate the strength of the association between VDR polymorphisms and CAD. Pooled ORs were performed for allelic model (Fok1: C versus T and G versus A; Bsm1: A versus G; Apa1: C versus A; Taq1: C versus T), heterozygote model (Fok1: CT versus TT and GA versus AA; Bsm1: AG versus GG; Apa1: CA versus AA; Taq1: CT versus TT), homozygote model (Fok1: CC versus TT and GG versus AA; Bsm1: AA versus GG; Apa1: CC versus AA; Taq1: CC versus TT), dominant model (Fok1: CC + CT versus TT and GG + GA versus AA; Bsm1: AA + AG versus

GG; Apa1: CC + CA versus AA; Taq1: CC + CT versus TT), recessive model (Fok1: CC versus CT + TT and GG versus GA + AA; Bsm1: AA versus AG + GG; Apa1: CC versus CA + AA; Taq1: CC versus CT + TT), respectively. The statistically significant level was determined by Z-test with a  $P$  value less than 0.05. Heterogeneity was evaluated by Q statistic (significance level of  $P < 0.1$ ) and  $I^2$  statistic (greater than 50% as evidence of significant inconsistency). Heterogeneity between studies was evaluated with the  $I^2$  test, and a higher  $I^2$  values means higher levels of heterogeneity ( $I^2 > 90\%$ : extreme heterogeneity;  $I^2 = 70\%$  to  $90\%$ : large heterogeneity;  $I^2 = 50\%$  to  $70\%$ : moderate heterogeneity;  $I^2 < 50\%$ : no heterogeneity). In heterogeneity evaluation, when the  $I^2 < 50\%$ , the fixed-effects model would be used; if the  $I^2 = 50\%$  to  $90\%$ , a random-effects model was used; if the  $I^2 > 90\%$ , the studies would not be pooled. Whenever heterogeneity was significant, sensitivity analysis was performed to detect the heterogeneity by omitting each study in each turn. Besides, subgroup analyses were stratified by ethnicity (Caucasian, Asian) and medical complication (with or without type 2 diabetes mellitus). Publication bias was not detected because the number of studies included was less than 10.<sup>25</sup> Review Manager, Version 5.3 (The Nordic Cochrane Centre, The Cochrane Collaboration, Copenhagen, Denmark) was used for all analyses.

## RESULTS

### Characteristics of the Included Studies

One hundred fifty-eight articles were obtained by online and manual search. After removing duplicates and screening title and abstract, 8 independent studies that contained detailed genotype distribution data were included, among which 1 study<sup>32</sup> was excluded because the genotype distributions of the control group departed from HWE. Finally, a total of 7 studies from 6 published articles,<sup>10–13,26,27</sup> involving 2306 cases and 4151 controls were included in this meta-analysis (Table 1) (seen in the flow chart).

### Meta-Analysis Results

Table 2 shows the main results of this meta-analysis and the heterogeneity of the VDR Apa1, Bsm1, Fok1 and Taq1 polymorphisms and CAD. For the 4 polymorphisms, Fok1 and Taq1 showed significant associations between the polymorphisms and CAD based on combined results from all studies, but Apa1 and Bsm1 did not show any significance. The Fok1 SNP has 2 kinds of polymorphisms, which are T > C and A > G. Our results showed that Fok1 T > C polymorphism was associated with decreased risk of CAD in allelic model (C vs. T: OR = 0.81, 95% CI = 0.65–1.00,  $P_h = 0.29$ ) (Figure 1), and Fok1 A > G polymorphism was also associated with decreased risk of CAD in allelic model (G vs. A: OR = 0.67, 95% CI = 0.45–1.00, heterogeneity: not applicable) and recessive model (GG vs. GA + AA: OR = 0.55, 95% CI = 0.31–0.97, heterogeneity: not applicable). The Taq1 T > C polymorphism was associated with increased risk of CAD in allelic model (C vs. T: OR = 1.14, 95% CI = 1.02–1.28,  $P_h = 0.79$ ), dominant model (CC + CT vs. TT: OR = 1.21, 95% CI = 1.02–1.43,  $P_h = 0.87$ ), heterozygote model (CT vs. TT: OR = 1.19, 95% CI = 1.00–1.1.42,  $P_h = 0.89$ ) and homozygote model (CC vs. TT: OR = 1.27, 95% CI = 1.01–1.61,  $P_h = 0.80$ ) (Figure 2). As stratified by ethnicity, our results showed that Bsm1 polymorphism was associated with increased risk of CAD in allelic model (A vs. G: OR = 1.23, 95% CI = 1.02–1.47,  $P_h = 0.15$ ),

**TABLE 1.** Characteristics of CAD Studies Included in the Meta-Analysis

First Author/ Publication Year	Study Country	Ethnicity	CAD (Case/Control)	Method	HWE <i>P</i> Value
<b>Apa1</b>					
Ferrarezil (DIABHYCAR) 2013	France	Caucasian	483/2654	PCR	0.091
Ferrarezil (NCH) 2013	France	Caucasian	230/483	PCR	0.283
He 2015	China	Asian	215/67	PCR	0.955
Maaty 2014	Egypt	Caucasian	137/58	PCR	0.084
<b>Bsm1</b>					
Orelepp 2001	Germany	Caucasian	217/76	PCR	0.136
Orelepp 2003	Germany	Caucasian	2087/1354	PCR	0.002
Pan 2009	China	Asian	152/212	PCR-RFLP	0.285
Ferrarezil (DIABHYCAR)2013	France	Caucasian	483/2654	PCR	0.649
Ferrarezil (NCH) 2013	France	Caucasian	230/483	PCR	0.176
<b>Fok1</b>					
Pan 2009	China	Asian	152/212	PCR-RFLP	0.270
Nezhad 2014	Iran	Asian	642/118	PCR-RFLP	0.136
He 2015	China	Asian	215/67	PCR	0.234
<b>Taq1</b>					
Ferrarezil (DIABHYCAR)2013	France	Caucasian	483/2654	PCR	0.953
Ferrarezil (NCH) 2013	France	Caucasian	230/483	PCR	0.184
He 2015	China	Asian	215/67	PCR	0.801
Maaty 2014	Egypt	Caucasian	137/58	PCR	0.637

CAD = coronary artery disease, HWE = Hardy–Weinberg equilibrium, PCR = polymerase chain reaction, PCR-RFLP = polymerase chain reaction-restriction fragment length polymorphism.

heterozygote model (AG vs. GG: OR = 1.20, 95% CI = 1.00–1.44,  $P_h = 0.87$ ) and homozygote model (AA vs. GG: OR = 1.22, 95% CI = 1.02–1.45,  $P_h = 0.84$ ) among Caucasian (Figure 3). As stratified by medical complications, our results showed that Apa1 polymorphism was associated with a decreasing risk in heterozygote model (CA vs. AA: OR = 0.80, 95% CI = 0.66–0.98,  $P_h = 0.31$ ) from the CAD with T2DM subgroup, compared with CAD without T2DM group, there was an increased risk in recessive model (CC vs. CA + AA: OR = 5.00, 95% CI = 2.74–9.13,  $P_h = 0.13$ ).

### Test of Heterogeneity and Subgroup Analysis

The heterogeneity test showed that heterogeneity was significant in Apa1, Bsm1, and Taq1 but not in Fok1 polymorphism (as shown in Table 2). To explore the potential sources of heterogeneity among studies, we evaluated the pooled ORs under all comparisons via subgroup. In the subgroup analysis by ethnicity, the heterogeneity of Bsm1 was reduced in allelic model (A vs. G:  $P = 0.150$ ,  $I^2 = 47\%$ ) and dominant model (AA + AG vs. GG:  $P = 0.540$ ,  $I^2 = 0\%$ ), but the heterogeneity of Apa1 was significant in both Asian and Caucasian studies. When stratified by source of medical complications, heterogeneity of Apa1 was reduced in CAD with T2DM studies, especially in CAD without T2DM studies. The heterogeneity of recessive model in Taq1 polymorphism was significant (CC vs. CT + TT:  $P = 0.000$ ,  $I^2 = 97\%$ ). No matter stratified by source of ethnicity or medical complications, the heterogeneity was still significant (shown in Table 2). When we analyzed the original manuscripts repeatedly, we found something related. In the study of He et al,<sup>10</sup> the frequencies of Taq1-CC genotypes are low in Chinese, which resulted in no CC genotypes in patients and controls and may cause remarkable

heterogeneity when pooled with other studies. In the study of Ferrarezi et al,<sup>27</sup> 2 different T2DM cohorts were included and the degree of CAD was assessed in different manner which would cause the difference in CC-genotypes patients and controls. In another study of Abu El Maaty et al,<sup>11</sup> females were not included in the cohort, which would lead to the change in CC-genotype distribution.

### Sensitivity Analysis

Sensitivity analysis was performed by sequentially omitting 1 individual study at a time; in order to reflect the influence of each study on the overall meta-analysis. For only 2 studies about Fok1 T > C polymorphism and 1 study about Fok1 A > G polymorphism, no heterogeneity was observed in the Fok1 polymorphism (data not shown). As shown in Figures 4 to 6, sensitivity tests suggested that no single study greatly influenced the estimates of overall risk for VDR Apa1, Bsm1, and Taq1 polymorphisms, thus the results of our meta-analysis were stable.

### DISCUSSION

CAD is a major type of heart disease and a global health problem bedeviling an increasing number of people.<sup>28</sup> Vitamin D's relationship with CAD has been a highly investigated theme in the past decade. Whether on an epidemiological or a molecular level, vitamin D has been repeatedly demonstrated to positively influence cardiovascular health.<sup>17,29</sup> VDR is an important regulator of vitamin D pathway, which involves the conversion of serum 25-hydroxyvitamin D into the active hormone, 1,25-dihydroxyvitamin D. VDR is required for the functions of vitamin D.<sup>30</sup> VDR harbors 4 known functional

**TABLE 2. Results of Meta-Analysis for VDR Polymorphisms and CAD**

Study Group	Study*	Case/Control	C vs. A OR (95% CI)	$P_H/I^2$	CC+CA vs. AA OR (95% CI)	$P_H/I^2$	CC vs. CA+AA OR (95% CI)	$P_H/I^2$	CA vs. AA OR (95% CI)	$P_H/I^2$	CC vs. AA OR (95% CI)	$P_H/I^2$
Apa1A > C												
Total	4	1065/3262	0.94 [0.76, 1.16]	0.060/59%	0.91 [0.66, 1.26]	0.080/56%	1.23 [0.25, 6.03]	0.000/98%	0.90 [0.67, 1.22]	0.150/43%	0.89 [0.60, 1.32]	0.090/54%
Ethnicity												
Caucasian	3	850/3195	0.85 [0.65, 1.10]	0.050/75%	0.88 [0.61, 1.27]	0.050/67%	0.69 [0.20, 2.36]	0.000/96%	0.85 [0.70, 1.02]	0.310/2%	0.84 [0.67, 1.05]	0.060/65%
Asian†	1	215/67	1.10 [0.73, 1.67]	—	1.28 [0.51, 3.20]	—	<b>6.50 [4.09, 10.31]</b>	—	1.25 [0.47, 3.28]	—	1.30 [0.50, 3.41]	—
Medical complication												
CAD with T2DM	2	713/3123	0.85 [0.65, 1.10]	0.050/75%	0.77 [0.58, 1.02]	0.150/51%	0.32 [0.18, 0.56]	0.010/84%	<b>0.80 [0.66, 0.98]</b>	<b>0.310/2%</b>	0.74 [0.45, 1.21]	0.060/73%
CAD without T2DM	2	352/125	1.18 [0.88, 1.60]	0.620/0%	1.48 [0.87, 2.51]	0.070/0%	<b>5.00 [2.74, 9.13]</b>	1.50 [0.85, 2.66]	1.41 [0.76, 2.62]	0.640/0%	1.41 [0.76, 2.62]	0.840/0%
Bsm1G > A	Study*		A vs. G		AA+AG vs. GG		AA vs. AG+GG		AG vs. GG		AA vs. GG	
Total	5	1082/3425	1.17 [0.95, 1.44]	0.070/57%	<b>1.19 [1.01, 1.40]</b>	<b>0.200/36%</b>	0.97 [0.23, 4.06]	0.000/97%	1.15 [0.97, 1.36]	0.410/0%	1.48 [0.99, 2.22]	0.140/46%
Ethnicity												
Caucasian	4	930/3213	<b>1.23 [1.02, 1.47]</b>	<b>0.150/47%</b>	<b>1.25 [1.06, 1.48]</b>	<b>0.540/0%</b>	0.51 [0.42, 0.62]	0.000/98%	<b>1.20 [1.00, 1.44]</b>	<b>0.870/0%</b>	<b>1.56 [1.01, 2.42]</b>	<b>0.090/58%</b>
Asian†	1	152/212	0.74 [0.44, 1.23]	—	0.72 [0.41, 1.26]	—	0.51 [0.09, 2.81]	—	0.73 [0.41, 1.30]	—	0.66 [0.12, 3.65]	—
Fok1T > C	Study*		C vs. T		CC+CT vs. TT		CC vs. CT+TT		CT vs. TT		CC vs. TT	
Total	2	794/330	<b>0.81 [0.65, 1.00]</b>	<b>0.290/11%</b>	0.82 [0.54, 1.23]	0.870/0%	0.75 [0.55, 1.01]	0.160/49%	0.89 [0.57, 1.39]	0.400/0%	0.77 [0.49, 1.22]	0.490/0%
Fok1A > G	Study*		G vs. A		GG+GA vs. TT		GG vs. GA+AA		GA vs. AA		GG vs. AA	
Total†	1	215/67	<b>0.67 [0.45, 1.00]</b>	—	0.70 [0.35, 1.41]	—	<b>0.55 [0.31, 0.97]</b>	—	0.90 [0.42, 1.92]	—	0.51 [0.24, 1.11]	—
Taq1T > C	Study*		C vs. T		CC+CT vs. TT		CC vs. CT+TT		CT vs. TT		CC vs. TT	
Total	4	1065/3262	<b>1.14 [1.02, 1.28]</b>	<b>0.790/0%</b>	<b>1.21 [1.02, 1.43]</b>	<b>0.870/0%</b>	0.44 [0.36, 0.54]	0.000/97%	<b>1.19 [1.00, 1.42]</b>	<b>0.890/0%</b>	<b>1.27 [1.01, 1.61]</b>	<b>0.800/0%</b>
Ethnicity												
Caucasian	3	850/3195	<b>1.14 [1.02, 1.28]</b>	<b>0.710/0%</b>	<b>1.20 [1.01, 1.42]</b>	<b>0.790/0%</b>	0.87 [0.20, 3.71]	0.000/97%	<b>1.18 [0.99, 1.41]</b>	<b>0.860/0%</b>	<b>1.27 [1.01, 1.61]</b>	<b>0.800/0%</b>
Asian†	1	215/67	1.59 [0.53, 4.72]	—	1.62 [0.53, 4.90]	—	—†	—	1.62 [0.53, 4.90]	—	—†	—
Medical complication												
CAD with T2DM	2	713/3137	<b>1.13 [1.00, 1.27]</b>	<b>0.530/0%</b>	<b>1.20 [1.01, 1.41]</b>	<b>0.510/0%</b>	0.39 [0.14, 1.07]	0.000/93%	1.19 [0.99, 1.42]	<b>0.590/0%</b>	1.25 [0.98, 1.60]	<b>0.680/0%</b>
CAD without T2DM	2	352/125	1.32 [0.88, 1.98]	0.720/0%	1.36 [0.77, 2.41]	0.710/0%	<b>4.50 [2.29, 8.85]</b>	—‡	<b>1.26 [0.69, 2.29]</b>	<b>0.580/0%</b>	1.58 [0.68, 3.66]	—‡

Significant results are marked in bold.  
 CAD = coronary artery disease, CI = confidence interval,  $I^2$  = the proportion of the total variation across studies due to heterogeneity, OR = odds ratio,  $P_H$  =  $P$  value for heterogeneity from Q test, T2DM = type 2 diabetes mellitus.  
 \*The number of studies.  
 †No heterogeneity was observed for only 1 study.  
 ‡No CC genotypes in patients or controls in the study by He et al.<sup>10</sup>

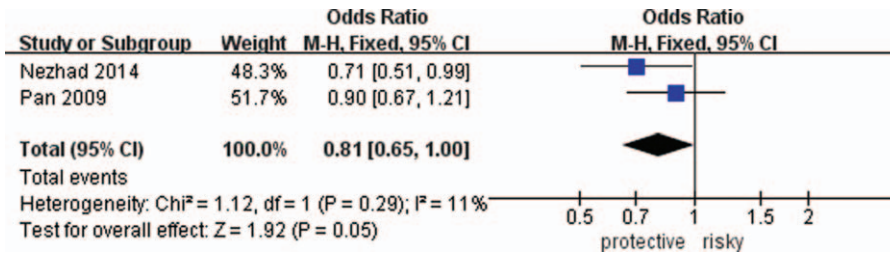


FIGURE 1. Forest plot of CAD risk associated with the C allele compared with the T allele in VDR Fok1 polymorphism. CI = confidence interval, OR = odd ratio.

polymorphisms and these polymorphisms have been intensively investigated.<sup>15</sup>

On the basis of 2306 patients with CAD and 4151 control subjects, we analyzed the associations between VDR polymorphisms and CAD risk by means of meta-analysis, a widely used quantitative approach that could provide strong evidence for SNP associations. Four common allelic polymorphisms were analyzed in our study, which were polymorphisms of Apa1, Bsm1, Fok1, and Taq1. The result shows significant associations between the VDR polymorphisms and CAD risk.

The meta-analysis of Bsm1 polymorphism showed a 19% increase in CAD risk in dominant model in pooled studies. In the stratified analysis by ethnicity, increased risk of CAD was suggested in Caucasian populations, the A-allele had a 23% increased risk of CAD against G-allele, the AA and AG genotypes increased CAD risk by 56% and 20% versus GG genotype, respectively. A 25% increased risk of CAD was also observed in dominant model, but no effect modification was indicated in Asians. The allele of the Bsm1 polymorphism located between intron 8 of the VDR gene and exon 9, which has the linkage disequilibrium (LD) among other genes in different ethnic population groups. This special feature may be the reason for no associations in Asians. For the Taq1 polymorphism, the result showed increased risk with CAD ranging from 14% to 27% under allelic model, dominant model, heterozygote model, and homozygote model. The heterogeneity of recessive model was so high that it could not be pooled for meta-analysis. For the Apa1 polymorphism, a 20% decreased risk was discovered in heterozygote model from CAD with T2DM subgroup, which suggested that Apa1 CA genotype might have a protective effect in CAD with T2DM and needed to be confirmed by studies with larger sample sizes. Besides, an obvious increased risk of CAD was observed in recessive model from CAD without T2DM subgroup. The polymorphism of Fok1 has 2 different gene polymorphisms. For the Fok1 T > C polymorphism, a 19% decreased risk was found in allelic model. A 33% and 45% decreased risk of CAD were discovered

in allelic model and recessive model in the Fok1 A > G polymorphism respectively, which suggested that Fok1 allele-C, allele-G, and GG genotype may contribute to a protective effect in CAD.

Our results showed a protective effect both in Apa1 and Fok1 polymorphisms. For the polymorphism of Apa1, the protection from CAD was discovered in CA genotype in the subgroup of CAD with T2DM, which was adverse to the results reported by Ferrarezi et al.<sup>27</sup> The same protective effect of Apa1 polymorphism was observed in follicular carcinoma from the results of Penna-Martinez et al.<sup>31</sup> In his study, the association between 25(OH) D (3) and 1, 25(OH)(2)D(3) plasma levels and Apa1 polymorphism was analyzed. Although no association was found, the study provided a possible way to decipher the protective role of Apa1 polymorphism. However, the relationship between the level of 1, 25-dihydroxyvitamin D and the polymorphism of Apa1 was not estimated in the study of Ferrarezi et al. Since VDR plays a role in the vitamin D signaling pathway, it could be hypothesized that Apa1 polymorphism may modify the risk for coronary artery disease, either by direct vitamin D effects or through gene-gene or gene-environment interactions. So we need more studies to investigate the potential mechanism of the protective role of Apa1 polymorphism in CAD. As for the Fok1 polymorphisms, the protective effect was observed in different alleles and genotypes, which indicated a strong protective effect of Fok1 polymorphism in CAD. Fok1 in exon 2 consists of a T to C change, which seems to cause gene-specific and cell type-specific effects. Some genes and some cell types will be more sensitive to the effect of the polymorphism than others.<sup>12</sup> Several studies have demonstrated that a wide variety of cells express VDR, suggesting that vitamin D may be involved in regulating different cellular processes, including proliferation, differentiation, and migration<sup>32-34</sup> that are involved in the progression of CAD.<sup>35</sup> Thus, it is likely the Fok1 polymorphism has a functional role in CAD indirectly via vitamin D deficiency. The result from Hossein-Nezhad et al showed

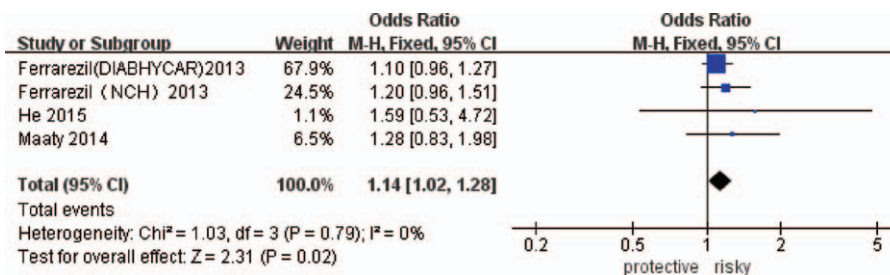


FIGURE 2. Forest plot of CAD risk associated with the C allele compared with the T allele in VDR Taq1 polymorphism. CI = confidence interval, OR = odd ratio.

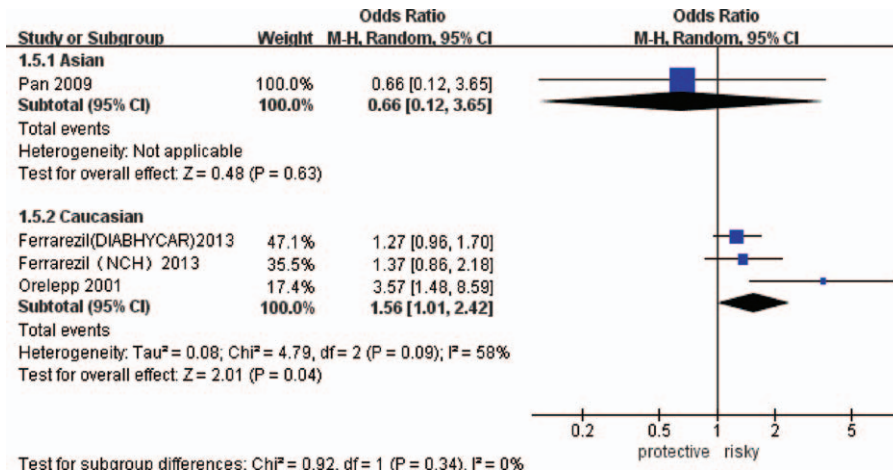


FIGURE 3. Forest plot of CAD risk associated with the AA genotypes compared with the GG genotype in VDR Bsm1 polymorphism in subgroup analysis of ethnicity. CI= confidence interval, OR= odd ratio.

vitamin D deficiency was less common in patients with CC genotype, which implied a protective role of C allele in CAD. As for the polymorphism of Fok1 A > G, there was only 1 study investigating the relationship between Fok1 polymorphism and

CAD risk, which showed patients with Fok1-GG genotype had a higher HDL as compared with those with Fok1 AA and AG genotype and supported the hypotheses that VDR Fok1-GG genotype may predict a low risk for CAD.<sup>10</sup> Although the

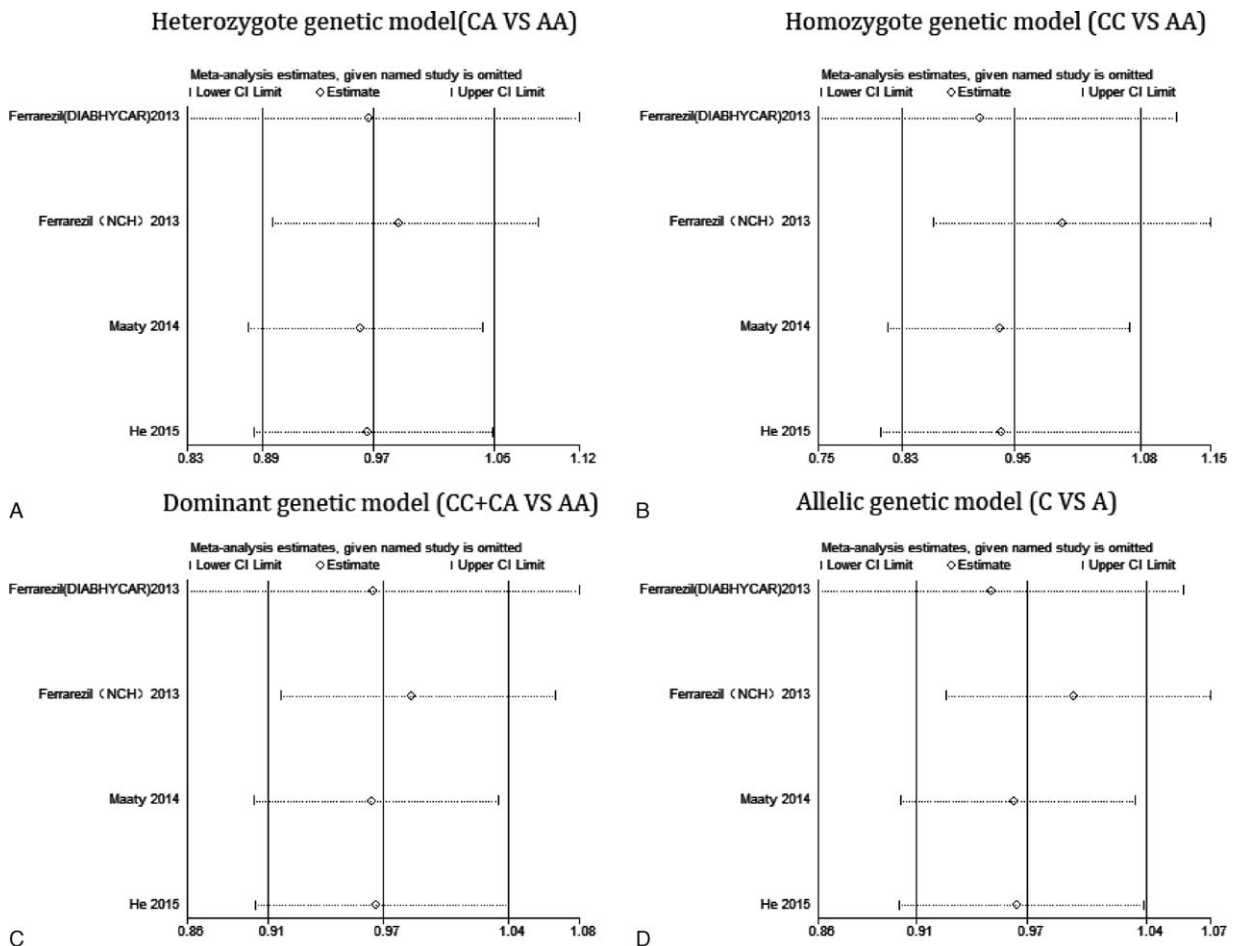
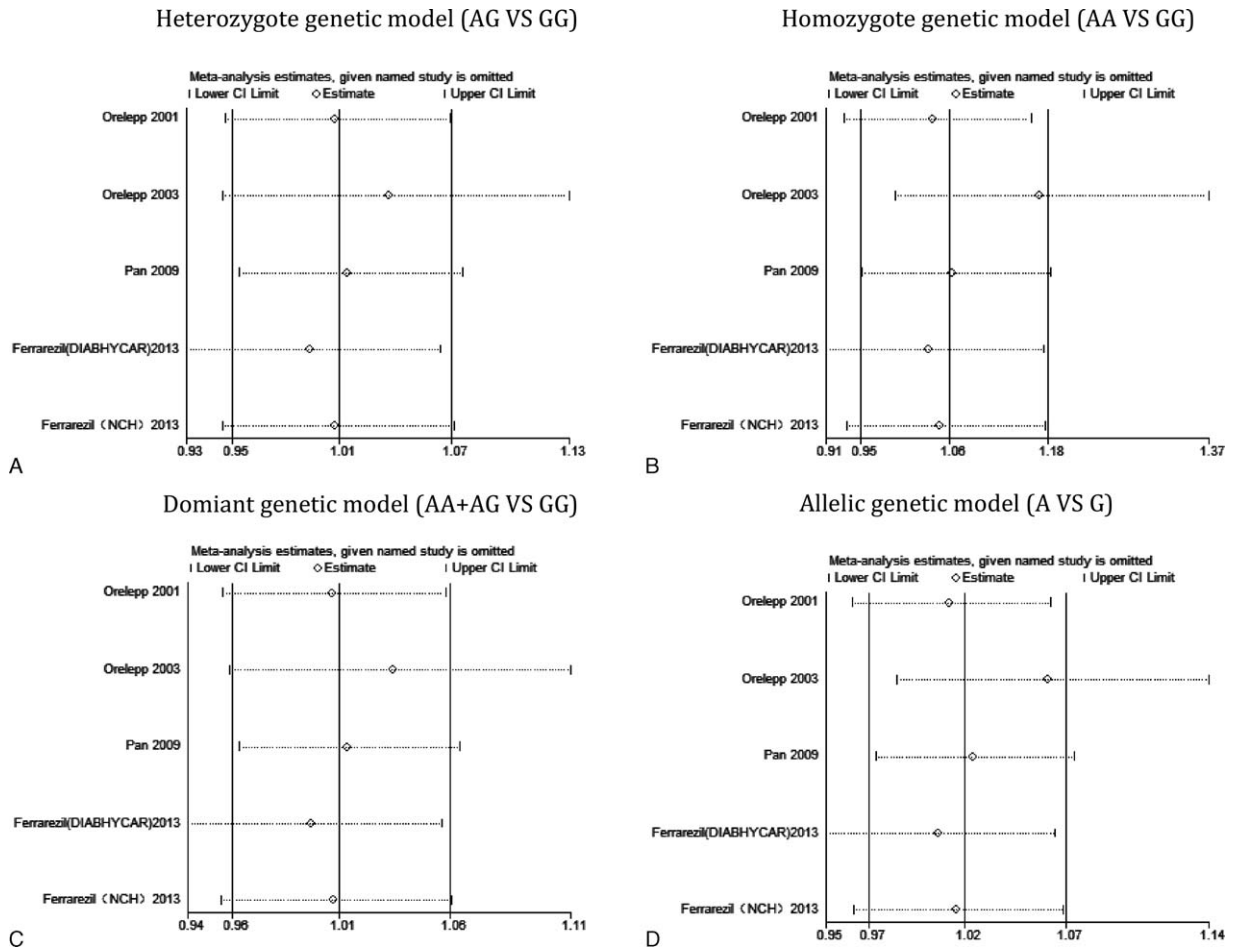


FIGURE 4. Influence analysis of Apa1 polymorphism and CAD risk. A, Heterozygote genetic model analysis. B, Homozygote genetic model analysis. C, Dominant genetic model analysis. D, Allelic genetic model analysis.



**FIGURE 5.** Influence analysis of Bsm1 polymorphism and CAD risk. A, Heterozygote genetic model analysis. B, Homozygote genetic model analysis. C, Dominant genetic model analysis. D, Allelic genetic model analysis.

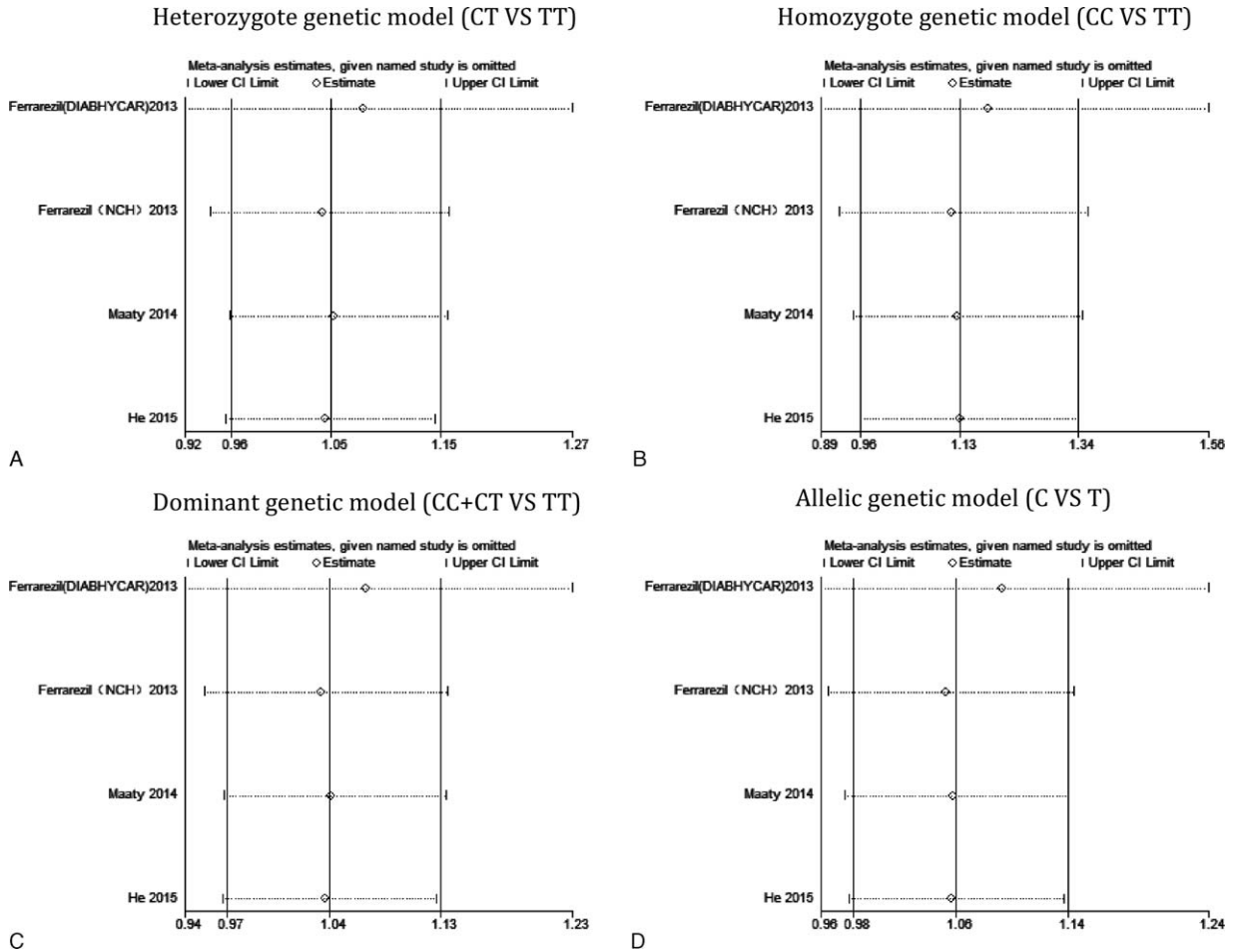
protective role in Fok1 polymorphism was reported in some studies, more researches are required to confirm it.

Increased risk was discovered in Bsm1, Apa1, and Taq1 polymorphisms in our results. A low vitamin D level is associated with increased cardiovascular morbidity and mortality in the general population,<sup>33</sup> and vitamin D deficiency is more prevalent in patients with CAD.<sup>26</sup> Endothelial dysfunction is a key factor in the pathogenesis of coronary artery disease. Although the precise mechanism of how a low vitamin D level influences CAD has not been fully delineated, vitamin D deficiency may play an important role in endothelial dysfunction due to vitamin D's functional roles in cellular proliferation including different stages of the cell cycle, differentiation, and apoptosis that could affect normal angiogenesis and endothelial cell differentiation.<sup>26,32,36,37</sup> However, vitamin D cannot act as its physiological role without VDR, which plays an important role in the conversion of serum 25-hydroxyvitamin D into the active hormone, 1, 25-dihydrovitamin D. The 3 SNPs in VDR gene may have an influence on the expression of VDR mRNA and contribute to a low level of vitamin D in CAD patients. Although genetic factors contribute substantially to the variability of circulating levels of 25(OH) D, with heritability estimated to be 30% to 40%,<sup>38,39</sup> whether Bsm1, Apa1, Taq1 polymorphisms have a functional role in increasing CAD risk indirectly via vitamin D deficiency needs further investigations.

Furthermore, linkage disequilibrium was observed strongly in the polymorphisms of Bsm1, Apa1, and Taq1, and they were in the same haploid domain with 3'UTR, besides, the haploid domain includes 4–9 exons and 3'UTR, which is related to the regulation of gene expression, especially the regulation of mRNA stability.<sup>40</sup> The structure specialty may affect the VDR mRNA and contribute to vitamin D deficiency.

There were several limitations in this meta-analysis. First, only English and Chinese articles were included in our study, which thus may bias the results. Second, patient heterogeneity and confounding factors might have distorted the analysis. Third, there was significant heterogeneity in some of the pooled analyses, which may have affected the meta-analysis results, even though we adopted the random effects model. Fourth, the number of included studies was relatively small in some subgroups; thus results should be interpreted with caution. In addition, the potential influence on genotype-CAD associations by environment factors is worthy of consideration.

In conclusion, our meta-analysis suggests that the Fok1 polymorphism may play a protective role in CAD, and the possible protective role in Apa1 CA genotype in CAD patients with T2DM still needed more studies to support. The Taq1 polymorphism is found to be associated with a significant increase in CAD risk based on our analysis. Moreover, increased risk in Apa1 variation in CAD patients without



**FIGURE 6.** Influence analysis of Taq1 polymorphism and CAD risk. A, Heterozygote genetic model analysis. B, Homozygote genetic model analysis. C, Dominant genetic model analysis. D, Allelic genetic model analysis.

T2DM and Bsm1 variation in Caucasian group is also detected. Further studies are needed to confirm the prediagnostic effect of VDR gene polymorphisms in CAD risk.

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