

## Original Article

# Association of COX-2 -765G>C genetic polymorphism with coronary artery disease: a meta-analysis

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**Abstract:** Background: Previous studies suggested the single nucleotide polymorphism (SNP) of COX-2 -765G>C (rs20417) is associated with coronary artery disease (CAD), but the results were conflicting. In order to derive a more precise estimation of the associations, we performed a meta-analysis of the relationship between rs20417 and CAD in all published studies. Method: Databases including PubMed, Web of Science, Wanfang, SinoMed and CNKI were systematically searched. Data were extracted using standardized methods. The association was assessed by odds ratio (OR) with 95% confidence intervals (CIs). The statistical tests were performed using Review Manager 5.3.3 and Stata 12.0 software. Results: We identified a total of 14 studies involving a total of 18227 subjects. The pooled odds ratio (OR) for the association between COX-2 -765G>C and CAD and its corresponding 95% confidence interval (95% CI) were evaluated by random or fixed effect model. A significant statistical association between COX-2 -765G>C and CAD was observed in an allelic model (P=0.02, OR=0.64, 95% CI: 0.43-0.94), dominant model (P=0.04, OR=0.74, 95% CI: 0.56-0.99), and recessive model (P=0.02, OR=0.46, 95% CI: 0.23-0.90). Conclusion: This meta-analysis suggested that COX-2 -765G>C is a protective for CAD.

**Keywords:** PTGS2, coronary heart disease, polymorphism, meta-analysis

## Introduction

In recent years, more and more studies showed that genetic polymorphism is an important risk factor for coronary artery disease (CAD). CAD is a complex multifactorial and polygenic disorder in which multiple environmental and genetic factors are involved simultaneously. Therefore, to study the possible mechanism of CAD in molecular level can provide a scientific basis for early diagnosis and prevention of CAD. For the past few years, there were many of case-control studies suggested that -765G>C polymorphism in COX-2 gene was associated with the risk of CAD [1-8]. Prostacyclin synthase (prostacyclin synthase, PGIS) is one of the cytochrome P450 superfamily members to catalyze prostaglandin H<sub>2</sub> (PGH<sub>2</sub>) to generate PGI<sub>2</sub>. The cyclooxygenase (Cyclooxygenase; COX) is a prostaglandin biosynthesis rate-limiting enzyme which has COX-1, COX-2 and COX-3 subtypes. and there is a close relationship between COX-2 and cardiovascular disease [9-13]. COX-2 -765G>C genetic variation can affect the

level of mRNA expression of the COX-2 protein, thus affecting the generation of the participation of PGI<sub>2</sub> in the occurrence of CAD [14-16]. Previous studies suggested that COX-2 is up-regulated in atherosclerotic plaques [17], and the COX-2 polymorphisms were associated with ischemic heart disease and stroke [18, 19]. However, some studies [4, 11] indicated that there are no association between COX-2 genetic polymorphism and CAD. In order to investigate the correlation between COX-2 and CAD, we conducted a meta-analysis of the published studies.

## Methods

### Literature search

All studies that reported the association between the COX-2 -765G>C polymorphism and CAD were identified by comprehensive computer-based searches of PubMed, Web of Science, Wanfang, China Biological Medicine Database (SinoMed) and China National Knowledge

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Infrastructure (CNKI). These computer searches were limited to English and Chinese language articles before June 2014. The following keywords were used for searching: "PTGS2" OR "COX-2" OR "cyclooxygenase" OR "rs20417" AND "polymorphism" OR "mutation" OR "genotype" OR "variant" AND "coronary heart disease" OR "CHD" OR "coronary artery disease" OR "CAD" OR "myocardial infarction" OR "MI" OR "Acute coronary syndrome" OR "ACS".

### *Inclusion criteria*

The diagnosis of CAD meets to the examination results of coronary arteriography, clinical symptoms combined with echocardiography, treadmill exercise test, electrocardiogram and myocardial perfusion imaging in Emission Computed Tomography (ECT).

The inclusion criteria were as follows: (1) studies are limited to COX-2 polymorphism and CAD; (2) Independent case-control studies using either a hospital-based or a population-based design; (3) complete data with genotype and allele frequencies. (4) The literature has a comprehensive statistical index, sufficient data for estimating an odds ratio (OR) with 95% confidence interval (CI); (5) the genotype frequency of cases and controls was within Hardy-Weinberg equilibrium.

### *Excluded studies*

(1) Studies which were not possible to extract data from the published results; (2) studies that did not report appropriate outcomes; (3) Duplicated studies were also excluded.

### *Data extraction*

Two authors (Ming-Ming Zhang and Xiang Xie) independently extracted data. Disagreement was resolved by consensus. If these two authors could not reach a consensus, the result was reviewed by a third author (Yi-Tong Ma). The extracted data were consisted of the follow items: the first author's name, publication year, population (Ethnicity), number of genotypes, Methods, genotyping, study design, matching criteria, sex, total number of cases and controls, age (years).

### *Quality assessment*

To determine the methodological quality of each study, we used the Newcastle-Ottawa

scale (NOS), which uses a "star" rating system to judge the quality of all observational studies. The NOS ranges between zero (worst) up to nine stars (best). Studies with a score equal to or higher than seven were considered to be of high quality. Two investigators (Ming-Ming Zhang and Xiang Xie) independently assessed the quality of included studies, and the result was reviewed by a third investigator (Yi-Tong Ma). Disagreement was resolved by discussion.

### *Statistical analysis*

The associations between -765G>C polymorphism in the COX-2 gene and CAD were compared by using the odds ratio (OR) corresponding to 95% confidence interval (CI) by using Review Manager 5.33 (Cochrane Collaboration, The Nordic Cochrane Centre, Copenhagen). Heterogeneity between studies was assessed by  $I^2$  statistic,  $P < 0.10$  and  $I^2 > 50\%$  indicated evidence of heterogeneity. If heterogeneity existed among the studies, the random effects model was used to estimate the pooled OR (the DerSimonian and Laird method). Otherwise, the fixed effects model was adopted (the Mantel-Haenszel method). For the -765G>C polymorphism, we investigated associations between the genetic variant and CAD risk in a recessive, dominant genetic model and allelic contrast. Z test was used to determine the pooled OR and significance was set at  $< 0.05$ . Hardy-Weinberg equilibrium (HWE) for each single nucleotide polymorphism was assessed for the controls in each study using  $\chi^2$  test at a significant level of  $P < 0.05$ . The potential publication bias was investigated by using funnel plot. Egger's test ( $P < 0.05$ ) was also considered representative of statistically significant publication bias, which was conducted by using Stata12.0.

## **Results**

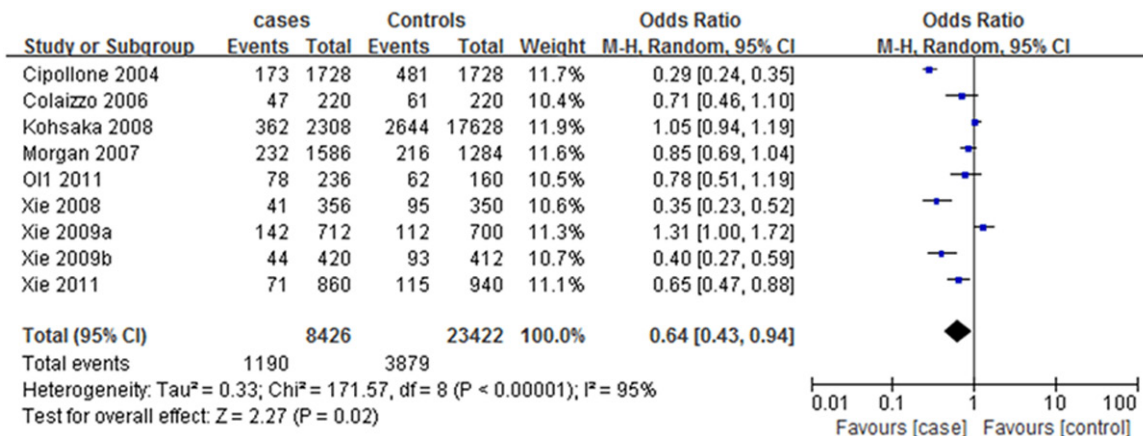
### *Characteristics of included studies*

A total of 14 case-control studies and cohort studies were included in the final meta-analysis according to the inclusion criteria. One study being not in line with Hardy-Weinberg equilibrium was excluded from the final analysis. 14 case-control studies were divided into two parts, 9 studies can be retrieved intact GG/GC/CC genotype number, but 5 studies can be retrieved intact GG/GC+CC genotype number.

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**Table 1.** Characteristics of included studies

Studies	Year	Ethnicity	Case						Control					
			N	Genotype (n)			allele		N	Genotype (n)			allele	
				GG	GC	CC	G	C		GG	GC	CC	G	C
Ol et al.	2011	USA	118	48	62	8	158	78	80	27	44	9	98	62
Kohsaka et al	2008	USA	1154	825	296	33	1946	362	8814	6391	2202	221	14984	2644
Morgan et al	2007	USA	793	576	202	15	1354	232	642	447	174	21	1068	216
Cipollone et al	2004	Italy	864	700	155	9	1555	173	864	437	373	54	1247	481
Colaizzo et al	2006	Helsinki	110	65	43	2	173	47	110	60	39	11	159	61
Xie et al	2008	China	178	140	35	3	315	41	175	97	61	17	255	95
Xie et al	2009a	China	356	234	102	20	570	142	350	245	98	7	588	112
Xie et al	2009b	China	210	170	36	4	376	44	206	128	63	15	319	93
Xie et al	2011	China	430	364	61	5	789	71	470	368	89	13	825	115
Studies	Year	Ethnicity	N	GG	GC+CC				N	GG	GC+CC			
Lee et al	2008a	USA	776	546	230				607	425	182			
Lee et al	2008b	USA	224	113	111				291	147	144			
Huuskonen et al	2008	Helsinki	300	223	77				228	172	56			
Hegener et al	2006	USA	600	474	126				600	463	137			
McGettigan et al	2011	Canada	460	336	124				640	478	162			



**Figure 1.** Forest plot of CAD and COX-2 genetic polymorphism in an allelic comparison, the horizontal lines correspond to the study-specific OR and 95% CI, respectively. The area of the squares reflects the study-specific weight. The diamond represents the pooled results of OR and 95% CI.

**Table 1** showed the studies identified and their characteristics.

### Main results, heterogeneity and sensitivity analyses

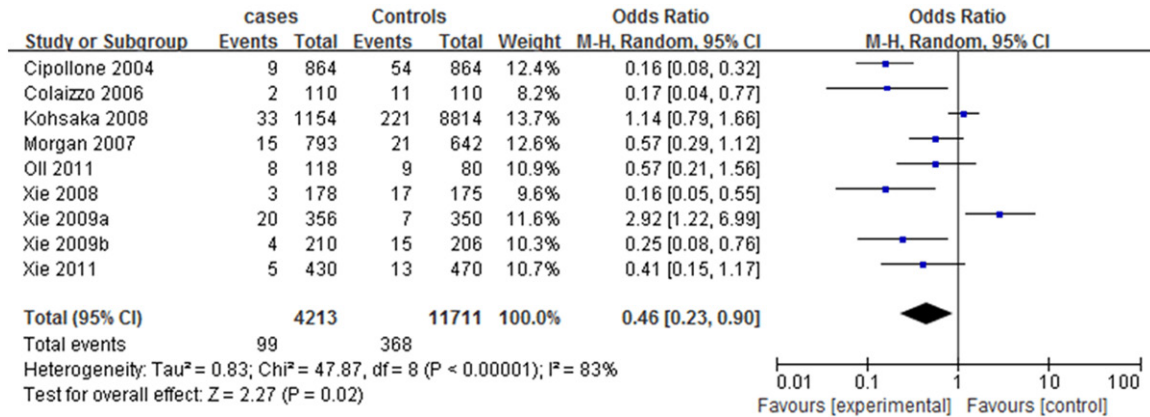
A significant heterogeneity was found in an allelic contrast ( $I^2=95\%$ ,  $P=0.02$ ), a recessive genetic model ( $I^2=83\%$ ,  $P=0.02$ ), a dominant genetic model ( $I^2=95\%$ ,  $P=0.03$ ), and a co-dominant genetic model ( $I^2=93\%$ ,  $P=0.04$ ). Therefore, the random-effects model (DerSimonian and Laird) was applied to merge the OR. Significant statistical association was found

between COX-2 -765G>C and CAD in the allelic contrast (C vs. G,  $P=0.02$ ,  $OR=0.64$ , 95% CI: 0.43-0.94) (**Figure 1**). Both the recessive genetic model (CC vs. GC+GG,  $P=0.02$ ,  $OR=0.46$ , 95% CI: 0.23-0.90, **Figure 2**) and the dominant genetic model (GC+CC vs. GG,  $P=0.04$ ,  $OR=0.74$ , 95% CI=0.56-0.99; **Figure 3**) were also obtained similar results.

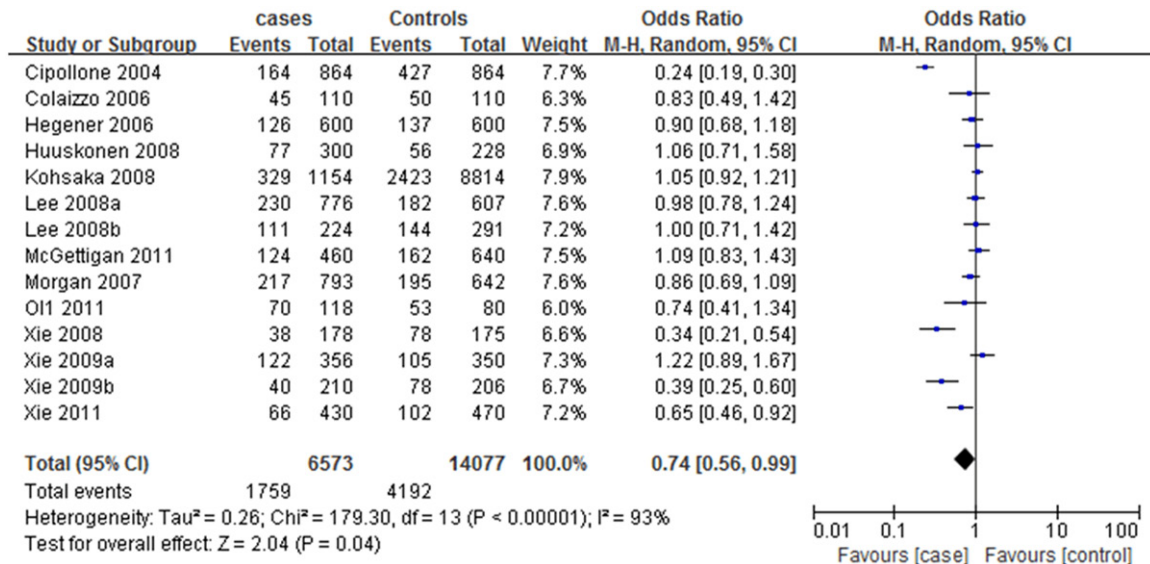
### Publication bias

The publication bias of the individual studies was evaluated by using funnel plot and Egger's test. No visual publication bias was found in the

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**Figure 2.** Forest plot of CAD and COX-2 genetic polymorphism in a recessive model, the horizontal lines correspond to the study-specific OR and 95% CI, respectively. The area of the squares reflects the study-specific weight. The diamond represents the pooled results of OR and 95% CI.



**Figure 3.** Forest plot of CAD and COX-2 genetic polymorphism in a dominant model, the horizontal lines correspond to the study-specific OR and 95% CI, respectively. The area of the squares reflects the study-specific weight. The diamond represents the pooled results of OR and 95% CI.

funnel plots (**Figure 4**). No statistically significant difference was detected in the Egger's test which indicated that the publication bias was low in the current meta-analysis by using the allelic contrast (T=-0.9, P=0.399), dominant genetic model (T=-0.92, P=0.387), recessive genetic model (T=-1.82, P=0.112).

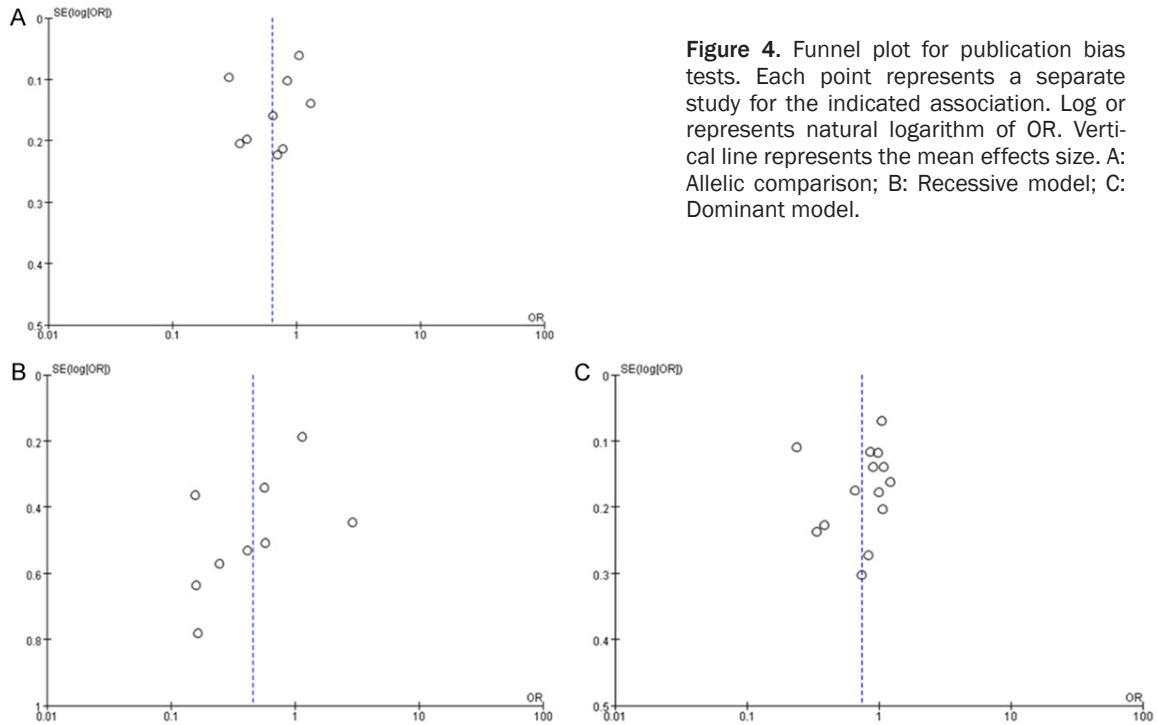
### Discussion

The association between -765G>C polymorphism and CAD has been intensively studied, but the results remain inconclusive. We found COX-2-765G>C (rs20417) is a protective factor

for CAD by conducting a meta-analysis in the present study.

Previously, several studies reported that COX-2 genetic polymorphism play an important role in the pathogenesis of atherosclerosis and the COX-2 gene was significantly associated with cardiovascular disease, including CAD and stroke [20, 21]. Papafili et al [11], suggested that -765G>C polymorphism in the promoter region of the COX-2 gene disrupts the Sp1 binding site that may alter the susceptibility to develop CAD. Papafili et al [11] and Corella D et al. [22] also found that the -765C allele is

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**Figure 4.** Funnel plot for publication bias tests. Each point represents a separate study for the indicated association. Log or represents natural logarithm of OR. Vertical line represents the mean effects size. A: Allelic comparison; B: Recessive model; C: Dominant model.

associated with decreased carotid intima-media thickness and lower levels of inflammatory cytokines. Cipollone et al. [4] found that the -765C allele is a protective factor for CAD and stroke. Sowers et al. [23] also suggested that the C allele may be associated with lower levels of inflammatory markers such as C-reactive protein and interleukin-6 in cerebrovascular and hypercholesterolemic patients. However, Kohsaka et al. [3] reported that the COX-2 -765G>C polymorphism was not a significant predictor of CAD in either White or African-Americans, although the -765C allele was a significant predictor of incident stroke in African-Americans. Lee et al [6] and Hegener et al. [19] reported that there is no significant association between -765C and decrease of incident CAD.

In the present meta-analysis, a total of 14 case-control studies and cohort studies were included in the final meta-analysis according to the inclusion criteria.

All the studies checked genotypes for quality control and be in line with Hardy-Weinberg equilibrium, although there is significant heterogeneity between all the studies. The funnel plot did not reveal obvious asymmetry, and the Egger test further witnessed no considerable published bias in the present meta-analysis. Most importantly, the systematic review of the

association COX-2 G-765C of polymorphisms with CAD risk has the benefit to overcome most of the limitation of too small sample size of the study populations by increasing the sample size and may generate more robust data. Therefore, our results are more credible and accurate to some extent.

However, some limitations of the present meta-analysis should be acknowledged. Firstly, the genotyping testing methods were not the same. There are TaqMan, Chips, Sequenom MALDI-TOF, and PCR-RFLP methods. Secondly, other clinical factors such as race, age, gender and different chemotherapies in each study might have led to bias. Thirdly, only full text articles published in English and Chinese were included in this meta-analysis, missing some eligible studies which were unpublished or reported in other languages. Thus, some inevitable publication bias may exist in the results. Finally, coronary artery disease is a complex multifactorial and polygenic disorder in which multiple environmental and genetic factors are simultaneously involved, involving potential interactions among gene-gene and gene-environment. However, many eligible studies included in this meta-analysis didn't consider the environmental factors. This might be a certain influence on the present meta-analysis.

In conclusion, our meta-analysis suggests that the COX-2 -765G>C (rs20417) is a protective factor for coronary artery disease. G>C mutation, in a certain extent, decreased the risk of coronary artery disease.

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**Disclosure of conflict of interest**

None.

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