Polymorphisms of C242T and A640G in CYBA Gene and the Risk of Coronary Artery Disease: A Meta-Analysis

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Abstract

Background: Coronary artery disease (CAD) is a leading cause of mortality in many countries. Considerable studies have been carried out to investigate the relationship between the C242T and A640G polymorphisms of CYBA gene and CAD, but the results were still inconsistent. Hence we conducted a meta-analysis to clarify the association.

Methods and Results: A total of 21 eligible literatures were included in the meta-analysis. We observed a significant decreased risk of CAD for C242T polymorphism in Asian population under an allelic model (OR 0.75; 95% CI 0.67–0.84) and a dominant model (OR 0.69; 95% CI 0.61–0.79), however, in overall population and other population no significant association was revealed. We also found A640G polymorphism may contribute to reducing CAD risk under an allelic model (OR 0.84; 95% CI 0.75–0.93), dominant model (OR0.77; 95% CI 0.64–0.92) and recessive model (OR0.82; 95% CI 0.69–0.97). No publication bias was found.

Conclusion: Our meta-analysis confirmed a protective effect of C242Tpolymorphism on CAD in Asian population and indicated that A640G polymorphism was significantly associated with decreased risk of CAD.

Introduction

Coronary artery disease (CAD), also namely ischemic heart disease (IHD) or coronary heart disease (CHD), mainly including angina and myocardial infarction (MI), is still a leading cause of mortality in many countries with three-fourths of global deaths due to CAD in the low- and middle-income countries [1]. According to a report issued by the World Health Organization, the CAD death toll was expected to account for 13.4% of the total population death by the year 2030 [2]. The death caused by CAD in men and women aged ≥60 years, is surpassed only by human immunodeficiency virus/acquired immune deficiency syndrome in persons aged 15 to 59 years [3]. Oxidative stress in the vasculature and even the whole body induced by superoxide anion via the NADH/NADPH pathway has been implicated in atherosclerosis which is the basic and initial pathogenesis of CAD [4–6]. Besides, evidence has also shown that oxidative stress contributes to the development of cardiovascular diseases by vascular wall remodeling and endothelial dysfunction [7].

Evidence over recent years has indicated that the predominant cellular source of superoxide anion in the context of cardiovascular diseases is the NADPH oxidase family [8,9], which is a class of membrane-associated enzymes that catalyzes the one electron reduction of oxygen to produce reactive oxygen species (ROS) using NADH or NADPH as the electron donor [10]. Among the components of NADPH oxidase, the p22phox protein, an essential subunit for the activation of the NADPH oxidase [11], is expressed in various cells such as human endothelial cells and vascular smooth muscle cells [12]. In addition, the higher expression of p22phox was found in human atherosclerotic coronary arteries than in non atherosclerotic arteries [13].

P22-phox is encoded by the CYBA gene, which is located on chromosome 16q24 and consists of six exons and five introns [14] with a length of 8.5 kb. A large number of genetic variations of this gene, such as C242T, A640G,-930A/G, -675A/T and C549T polymorphism, have been reported. Among these polymorphisms, the two polymorphisms of C242T and A640G have been extensively studied and considered the most interesting. The C242T polymorphism is located in exon 4 and results in an amino acid substitution (histidine to tyrosine) [14], which leads to a loss of oxidative function and a decreased production of ROS and oxidative stress in the vasculature [15]. An *in-vivo* study [16] also showed the C242T allele was associated with reduced NAD(P)H oxidase activity in human blood vessels. While the A640G polymorphism is located in 3′ untranslated region (3′UTR) [17] of CYBA gene with no amino acid substitution and has also been found an effect on the ROS generation [18,19]. It has been assumed that A640G modified the stability of mRNA and translational activity of CYBA through the interaction with other regions of mRNA [18].

Since Inoue first found T allele of the C242T polymorphism might have a protective effect against CAD [20], the association of the CYBA gene C242T polymorphism with CAD has been extensively studied over the last decade, however, the results have been conflicting. Nasti even stated the opposite effect with T allele as a risk factor for CAD [21]. As to the association of CYBA gene

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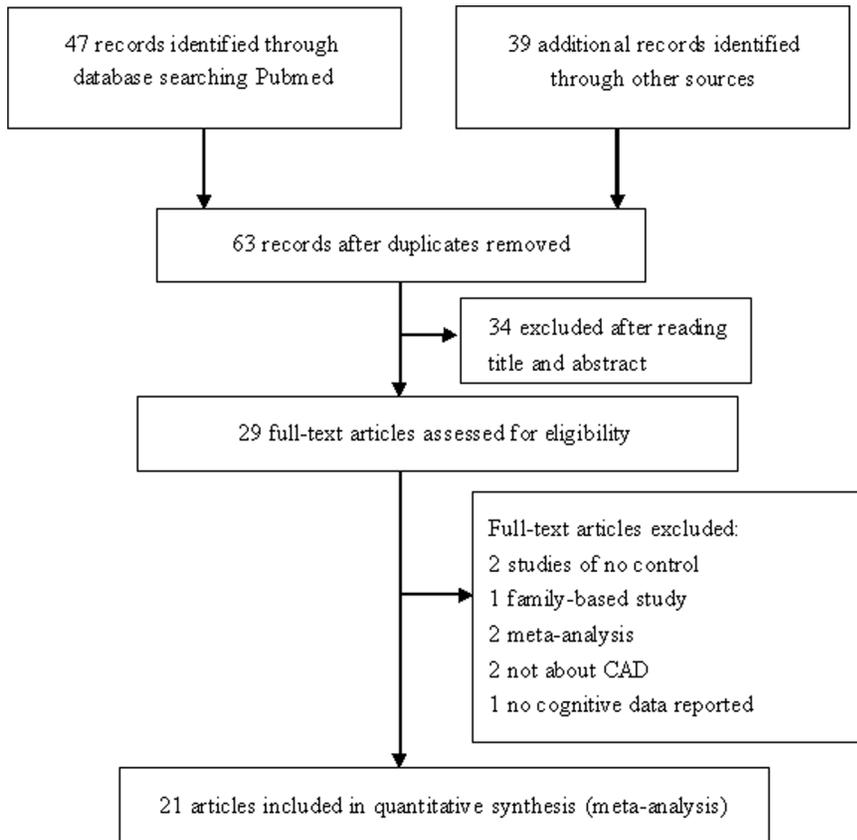
Figure 1. Flow chart of the literature search. CAD, coronary artery disease.
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A640G polymorphism with CAD, the related studies are relatively fewer, but controversy still exists. For example, Inoue found no association between A640G polymorphism and CAD [20], whereas, Gardemann's study suggested a protective effect on CAD exerted by the G allele [22]. So we conducted this meta-analysis integrating previous publications to study the association between the two polymorphisms of CYBA gene and CAD.

Materials and Methods

Search Strategy and Identification of Relevant Studies

An extensive literature searching of PubMed, EMBASE, ISI Web of Science and Chinese Wan Fang Data was performed for relevant articles without restricting language from June 1996 to May 2012, using the combinations of the keywords "Coronary artery disease", "Coronary heart disease", "ischemic heart disease", "angina pectoris", "myocardial infarction", "Polymorphism", "CYBA", "P22phox", "C242T", "A640G", "NADPH oxidase". References of reviews and retrieved studies were also scanned and request to the author to access to the paper has been also tried. We conducted the meta-analysis and reported its results according to the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) statement (Checklist S1).

The following inclusion criteria had to be fulfilled: (1) case-control study or large-scale replication study assessing the association between C242T polymorphism, A640G polymorphism and CAD risk as an original study. (2) Numbers of case and control groups or available data for calculating genotypic OR with 95% CI. (3)The CAD cases defined as coronary heart disease (CHD), coronary artery disease (CAD), myocardial infarction (MI), acute myocardial infarction (AMI), or unstable angina (UA).

Data Extraction

All the data were extracted independently by two reviewers who reached a consensus on all of the items. Following information was extracted from the eligible literature: first author's last name, year of publication, country, ethnic origin of the studied population, definition of cases, number in case and control groups, genotype distributions, male percentage and mean ages in case and control groups.

Statistical Analysis

Pooled effect was calculated for the allelic model, dominant model and recessive model in both C242T polymorphism and A640G polymorphism respectively.

Heterogeneity among studies was assessed using Q test [23] and Higgins I^2 [24], and was considered significant when $P < 0.05$ for Q statistic. Then heterogeneity was qualified by I^2: $I^2 = 0\%–30\%$, no or marginal between-study heterogeneity; $I^2 = 30\%–75\%$, mild heterogeneity; $I^2 = 75\%–100\%$, notable heterogeneity [25]. A fixed effect model (Mantel-Haenszel method) was applied when there is no heterogeneity ($P < 0.05$), otherwise a random effect model (DerSimonian and Laird method) was adopted [26]. A meta-regression model was employed to explore the sources of the heterogeneity [27] and then we carried out stratified analysis by subgroup. Sensitivity analysis was conducted to assess the influence of each study on overall pooled OR, with sequential omission of individual study [28]. Funnel plot and Egger's test described by

Table 1. Basic characteristics of the included studies.

Study	Year	Country	Ethnicity	Disease	Genotype						Sample size	% of male (case/control)	Age mean (case/control)
					Case			Control					
C242T					TT	TC	CC	TT	TC	CC			
Inoue	1998	Japan	Asian	CAD	2	26	173	0	53	148	201/201	0.70/0.70	59.8/54.9
Gardemann	1999	German	Caucasian	CAD	207	748	751	65	208	226	1706/499	1.00/1.00	62.2/61.4
Li	1999	America	Other	CAD	27	70	52	14	42	47	149/103	0.77/0.48	60/57
Cai	1999	Australia	Caucasian	CAD	65	250	235	13	62	64	550/139	0.80/0.47	56.9/54.5
Saha	1999	India	Asian	CHD	17	66	43	20	77	57	126/154	0.88/0.93	55.2/53.9
Saha	1999	China	Asian	CHD	3	23	125	3	25	139	151/167	1.00/1.00	55.3/54.5
Lee	2001	Korean	Asian	CAD	4	40	261	1	49	165	305/215	1.00/1.00	56.1/50.2
Stanger	2001	Australia	Caucasian	CAD	15	50	43	6	24	15	108/45	1.00/1.00	52.9/54.2
Yamada	2002	Japan	Asian	MI	25	319	1440	16	242	816	1784/1074	1.00/1.00	62.1/62.0
Zafari	2002	America	Other	CAD	21	70	58	6	26	18	164/52	0.99/0.94	61.7/57.1
Balague	2004	Spain	Caucasian	AMI	2	41	61	2	47	57	104/106	0.88/0.89	50.2/50.4
Murase	2004	Japan	Asian	MI/UA	2	67	384	5	170	587	593/1595	0.76/0.48	50.0/60.6
Fan	2006	Finland	Caucasian	CAD	12	66	172	10	56	86	250/152	0.77/0.58	62.0/55.0
Nasti	2006	Italy	Caucasian	CAD	37	147	92	25	95	98	276/218	0.83/0.68	65.5/66.2
He MA	2007	China	Asian	CAD/MI	2	32	531	2	64	543	565/609	0.57/0.52	61.9/64.7
Niemiec	2007	Poland	Caucasian	CAD	25	80	67	18	85	66	172/169	0.67/0.70	43.8/34.2
Reyes	2008	Spain	Other	MI/UA	48	137	119	47	145	123	304/315	0.78/0.74	56.0/54.5
Alexey	2010	Russia	Caucasian	CAD	34	91	188	4	41	87	313/132	0.54/0.57	59.2/55.5
Goliasch	2011	Austria	Caucasian	AMI	11	47	41	31	79	82	99/192	0.87/0.90	37.3/34.7
Najafi	2012	Italy	Caucasian	CAD	23	47	44	12	32	24	114/68	0.68/0.32	62.8/55.9
A640G					GG	AG	AA	GG	AG	AA			
Inoue	1998	Japan	Asian	CAD	82	83	36	80	79	42	201/201	0.70/0.70	59.8/54.9
Gardemann	1999	German	Caucasian	CAD	313	735	438	141	251	107	1486/499	1.00/1.00	62.2/61.4
Zafari	2002	America	Other	CAD	37	75	37	13	25	12	149/50	0.99/0.94	61.7/57.1
Reyes	2008	Spain	Other	MI/UA	104	155	46	48	165	96	305/309	0.78/0.74	56.0/54.5
Goliasch	2011	Austria	Caucasian	AMI	23	47	28	39	103	50	98/192	0.87/0.90	37.3/34.7
Niemiec	2011	Poland	Caucasian	CAD	39	68	53	40	75	45	160/160	0.68/0.76	43.8/59.3

Abbreviations: N, Number of studies OR, Odds ratio; 95% CI, 95% confidence interval; REM, random-effects model; FEM, fix-effects model.
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Egger et al [29] for funnel plot asymmetry were applied to evaluate the evidence for publication bias. All statistical analyses were carried out with *R* software (version 15.3) and a probability value of $P<0.05$ was considered statistically significant.

Results

Characteristics of Included Studies

The study selection process is shown in Figure 1. A comprehensive search identified 86 references. After removing the duplicate literature and reports, a total of 21 publications [20–22,30–47] preliminarily fit the inclusion criteria. However, after further examination, we removed Morgan's study that was a large-scale application study with 1461 participants and 85 genetic variants indicating that none of the variants was unequivocally validated. Since 1 article included two populations, both of them were considered as an independent study. There were totally 21 studies included in the meta-analysis. The characteristics of these studies were listed in Table 1. There were 7 studies based on Asian population, 10 studies conducted in Caucasian population

and 4 studies from other population, such as Spanish, American. The diagnoses in the included articles were CHD, UA, MI, AMI and CAD. For C242T polymorphism, 20 studies were available with a total of 8845 cases and 6855 controls, while for A640G polymorphism; only 6 studies covered a total of 2399 cases and 1411 controls.

Results of the Overall Meta-analysis

Figure 2 and Figure 3 summarizes the ORs with corresponding 95% CIs for the association between C242T and A640G polymorphisms in the p22phox gene and the risk for CAD in the allelic, dominant and recessive models. A random-effect model was applied to the allelic model and dominant model in the study of C242T polymorphism and to all the genetic models in the study of A640G polymorphism, while a fixed-effect model was chosen for the recessive model in C242T polymorphism according to the *P* values for heterogeneity. For both C242T polymorphism and A640G polymorphism, no significant association was observed under all the three genetic models. The results are presented in Table 2.
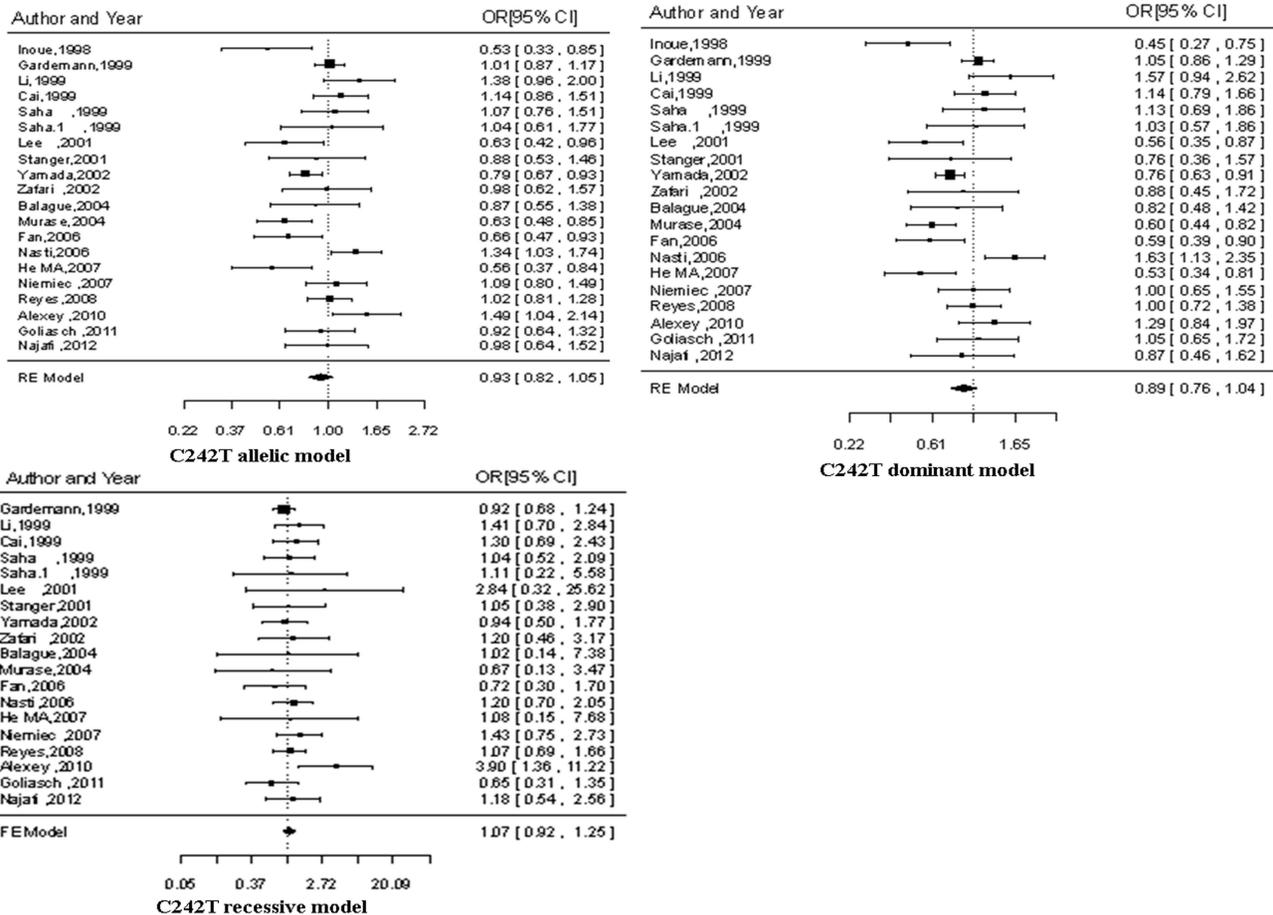
Figure 2. The forest plots of OR with 95% CI for C242T polymorphism and CAD risk. OR, Odds ratio; 95% CI, 95% confidence interval; RE, random-effects model; FE, fix-effects model; CAD, coronary artery disease.
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Meta-regression Analysis and Stratified Analysis

To explore the source of heterogeneity, a meta-regression analysis of C242T under the allelic model and the dominant model respectively was performed. We conducted a series univariate model by adding single covariates including ethnicity, publication year, disease, sex and age. In the univariate analysis, only the ethnicity can explain the between-study heterogeneity in allelic model ($P = 0.0154$, tau^2 reducing from 0.0506 to 0.0335) and dominant model ($P = 0.0140$, tau^2 reducing from 0.0807 to 0.0518).The meta-regression results were presented at Table S1. After stratified by ethnicity, heterogeneity in the subgroup decreased and a decreased CAD risk was conferred in Asian population both in the allelic model (OR = 0.75, 95% CI = 0.67–0.84) and the dominant model (OR = 0.69, 95% CI = 0.61–0.79). Nonetheless, in Caucasian population and other population, no significant association between C242T and CAD was still found (Table 2).

Sensitivity Analysis

The results for C242T polymorphism showed that none of the studies dramatically affected the combined results under the allelic model and the recessive model, while under the dominant model the overall result changed significantly after removing Nasti's study [21] with OR(95% CI) changing from 0.89(0.76–1.04) to 0.85(0.73–0.99), though the heterogeneity did not decrease sharply

($I^2 = 62.53\%$) (Table S2).This may be because of the marginal statistical significance and moderate effect of the polymorphism. In the sensitivity analysis on A640G polymorphism, in view of the considerable heterogeneity caused by Reyes's study [43] which probably resulted from the population admixture in the study, we removed it and found a significant association between A640G and CAD under an allelic model (OR 0.84; 95% CI 0.75–0.93), dominant model (OR 0.77; 95% CI 0.64–0.92) and recessive model (OR 0.82;95% CI 0.69–0.97)) and no statistically significant heterogeneity existed (Table S2). We will have a more detailed discussion in the following part. The adjusted results were showed in Table 3.

Publication Bias

As demonstrated by the funnel plot and the Egger's test, there was no significant publication bias in any overall meta-analysis with all P for Egger's test>0.05 (Table 2, Figure 4).

Discussion

Due to the key role of p22phox in CAD and the conflicting results about the relationship of its polymorphisms and CAD, this meta-analysis was conducted and the following conclusions were drawn: (1) The C242T polymorphism of CYBA gene provided significantly protective effect on CAD in Asian populations under
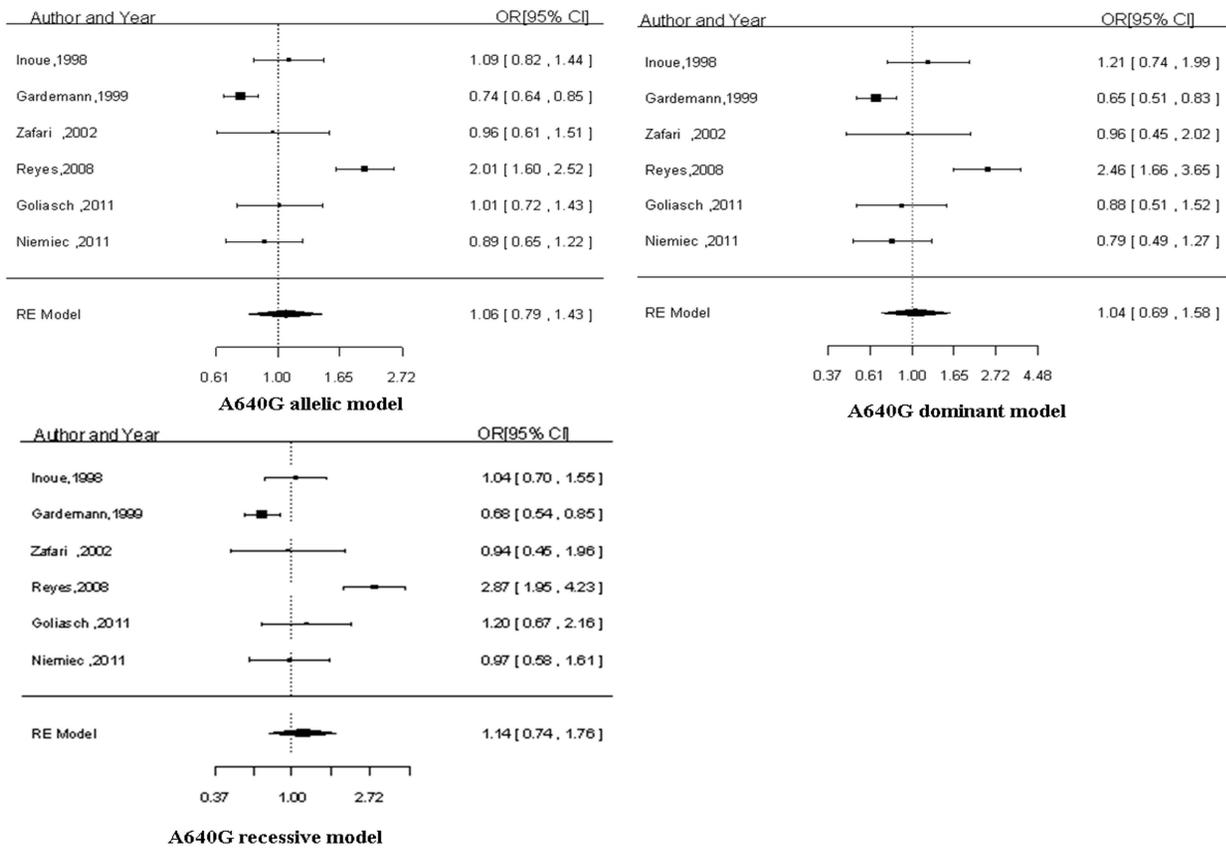
Figure 3. The forest plots of OR with 95% CI for A640G polymorphism and CAD risk. OR, Odds ratio; 95% CI, 95% confidence interval; RE, random-effects model; FE, fix-effects model; CAD, coronary artery disease.
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Table 2. Initial pooled estimates and stratified analysis for the association between polymorphisms and CAD.

Genetic Model	N	Model for analysis	OR (95% CI)	P for heterogeneity	I^2 (%)	P for Egger's test
C242T						
Allelic model	20	REM	0.93(0.82,1.05)	<0.0001	67.88	0.6768
Asian	**7**	**FEM**	**0.75(0.67,0.84)**	**0.0618**	**52.16**	**0.6371**
Caucasian	10	FEM	1.04(0.95,1.14)	0.0599	48.31	0.7246
Other	3	FEM	1.09(0.91,1.30)	0.3400	10.75	0.7679
Dominant model	20	REM	0.89(0.76,1.04)	<0.0001	68.15	0.9351
Asian	**7**	**FEM**	**0.69(0.61,0.79)**	**0.0522**	**52.30**	**0.7497**
Caucasian	10	FEM	1.04(0.93,1.18)	0.0685	47.86	0.4538
Other	3	FEM	1.09(0.85,1.40)	0.2730	21.63	0.8526
Recessive model	19	FEM	1.07(0.92,1.25)	0.8181	0.00	0.2440
A640G						
Allele contrast	6	REM	1.06(0.79,1.43)	<0.0001	86.73	0.5087
Dominant model	6	REM	1.04(0.69,1.58)	<0.0001	80.75	0.4531
Recessive model	6	REM	1.14(0.74,1.76)	<0.0001	83.45	0.4144

Abbreviations: N, Number of studies OR, Odds ratio; 95% CI, 95% confidence interval; REM, random-effects model; FEM, fix-effects model.
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Table 3. Adjusted pooled measure on the relation of A640G polymorphism to CAD after sensitivity analysis.

Genetic model	N	Model for analysis	OR (95% CI)	P for heterogeneity	I^2(%)	P for Egger's test
A640G						
Allele contrast	5	FEM	0.84(0.75,0.93)	0.0923	49.08	0.0741
Dominant model	5	FEM	0.77(0.64,0.92)	0.2346	35.92	0.1073
Recessive model	5	FEM	0.82(0.69,0.97)	0.1851	40.69	0.0755

Abbreviations: N, Number of studies OR, Odds ratio; 95% CI, 95% confidence interval; FEM, fix-effects model.
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an allelic model (OR 0.75; 95% CI 0.67–0.84) and a dominant model (OR 0.69; 95% CI 0.61–0.79), while no protective effect was found in Caucasian population and other population, just consistent with the results in Di Castelnuovo A, et al's meta-analysis [48] which provided great reference for our study. (2) A significant association between p22phox gene A640G polymorphism and CAD was found under an allelic model (OR 0.84; 95% CI 0.75–0.93), dominant model (OR 0.77; 95% CI 0.64–0.92) and recessive model (OR 0.82; 95% CI 0.69–0.97).

In a Japanese population Inoue [20] firstly found 242T as a decreased risk of CAD. Subsequently, Lee [35] and He [40]

confirmed that T variant allele was significantly associated with reduced CAD risk both in Korean and Chinese populations. However, Zafari [31] and Cai [33] negated this conclusion in American and Australian populations respectively. Our meta-analysis, of 19 articles with 20 populations from different ethnic origin containing 8845 cases and 6855 controls, afforded us a much higher possibility to reach the reasonable conclusions, which were just consistent with the results in Fang's meta-analysis [49]. The T allele had contributed to the reduced NADH-stimulated superoxide production and decreased NAD(P)H oxidase reactivity, with reduced activity (by about 30%) of the phagocytic enzyme
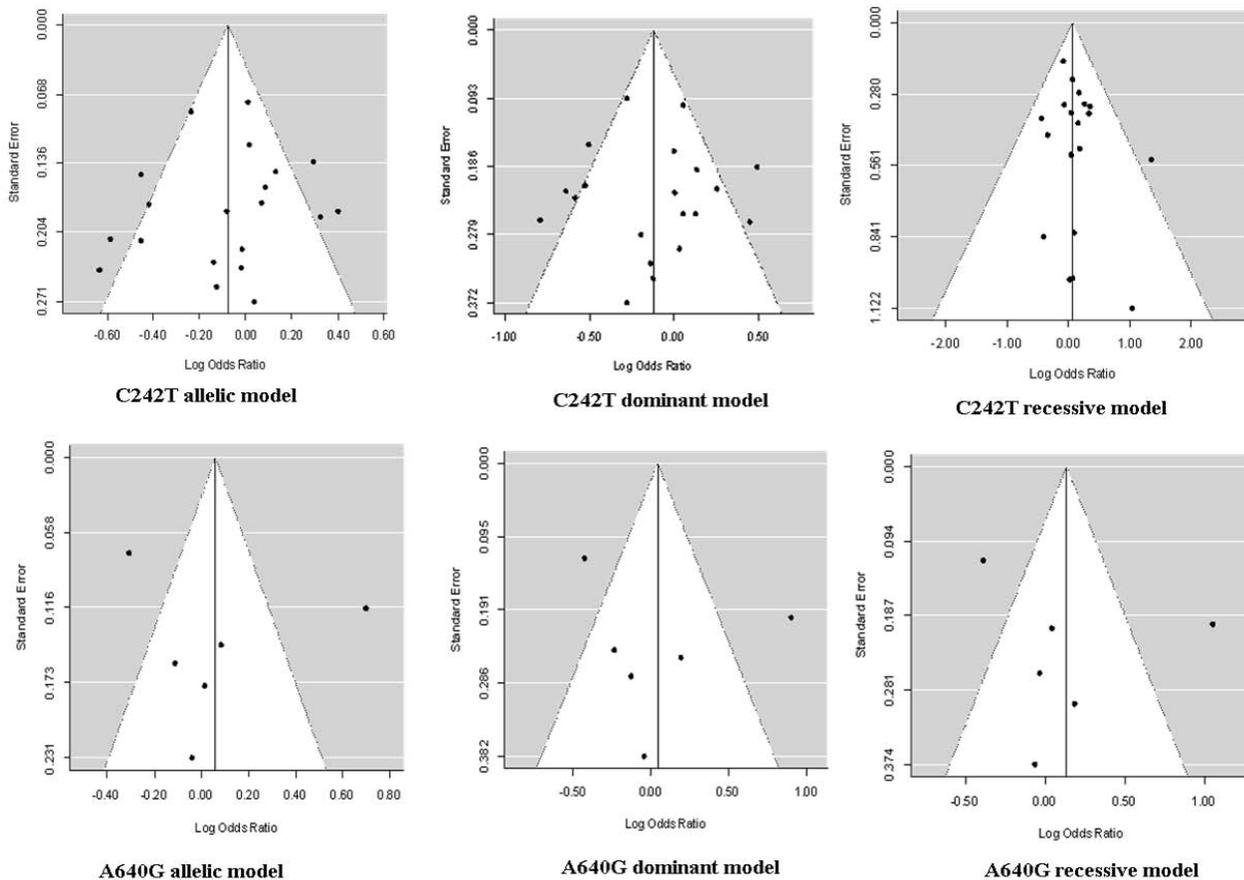
Figure 4. The funnel plots of natural logarithm of OR against inverse standard error in each study.
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after stimulation with phorbol 12-myristate 13-acetate (PMA) [16,50], indicating a weak protective role, which may account for the Asian population as suggested by our findings. Such conflicting results in Asian populations and non-Asian populations are likely to exist because multiple long-standing risk factors confound the possible effect of this polymorphism on CAD such as diabetes, smoke, hypertension and hypercholesterolaemia. Nevertheless, caution is needed in conclusion drawing due to our limited subgroup samples and attention should be paid to the heterogeneity, which still existed though decreased after a subgroup analysis because of the relatively large meta-analysis and the inevitable between-study heterogeneity.

For the first time this meta-analysis was conducted to explore the association between p22phox gene A640G polymorphism and CAD. And interestingly, we found a significant association between A640G and CAD under three genetic models after removing Reyes's study [43]. In view of the considerable heterogeneity caused by this study and to obtain a stable result, we removed this study for reanalysis which hardly affected the power of our meta-analysis (the sample size dropped from 2399 cases and 1411 controls to 2094 cases and 1102 controls) and thus, the final results can be acceptable. In a functional study, Wyche et al. [50] indeed did not confirm any changes in superoxide production by the A640G polymorphism. However, Gardemann et al. [22] found that the A640G SNP might modify processing or stability of p22phox mRNA or alternatively act as a neutral marker. Schirmer et al. [19] observed a significantly reduced ROS formation with increasing numbers of 640G variant alleles in a study of the functional significance of A640G, suggesting a protective effect of the A640G polymorphism on CAD. All these evidence supported a protective effect of 640G on CAD.

Nevertheless, notation should be paid to the results because of the limited samples.

In conclusion, this meta-analysis was a renewed and confirmed study to assess the association between C242T polymorphism and CAD. It's the first time that a meta-analysis was conducted to summarize the relationship of A640G polymorphism and CAD. Our combined results implicated the p22phox gene C242T and A640G SNPs played a vital role in coronary artery disease, collectively confirming a genetic involvement of the two polymorphisms in CAD. Further large and well-designed studies will be needed to clarify the association of the polymorphisms and CAD risk. Additional meta-analysis based on GWAS data will also be essential in the future.

Supporting Information

Table S1 The meta-regression analysis for heterogeneity under the allelic model and dominant model of p22phox gene C242T polymorphism.
(DOC)

Table S2 Sensitive analysis of pooled OR.
(DOCX)

Checklist S1 PRISMA 2009 Checklist.
(DOCX)

Author Contributions

Conceived and designed the experiments: QMX JW BC. Performed the experiments: HW WL XMS. Analyzed the data: FFY. Contributed reagents/materials/analysis tools: JW. Wrote the paper: QMX FFY.

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