Meta-analysis of *C242T* polymorphism in *CYBA* genes: risk of acute coronary syndrome is lower in Asians but not in Caucasians

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Abstract: Background: A lot of studies have demonstrated that *C242T* polymorphism in *CYBA* genes may play an important role in the pathological process of acute coronary syndrome (ACS). However, the results are not consistent. To further evaluate this debate, we performed a meta-analysis to determine the relationship between *C242T* polymorphism and ACS. Methods and results: We screened PubMed/MEDLINE, EBSCIO, and EMBASE research reports until Mar. 2014 and extracted data from 10 studies involving 6102 ACS patients and 8669 controls. Subgroup analysis by ethnicity documented a significant decreased risk of ACS for *C242T* polymorphism in the Asian population under allelic comparison (odd ratio (OR) 0.73; 95% confidence intervals (CI) 0.64–0.83), dominant model (OR 0.71; 95% CI 0.62–0.82), and homozygote comparison (OR 0.57; 95% CI 0.35–0.92). However, in the overall population and especially with Caucasians, no significant association was uncovered. Further meta-regression analysis revealed that the heterogeneity among studies was largely attributed to ethnicity. No publication bias was detected through a funnel plot and an Egger's linear regression test. Conclusions: Taken together, our results suggest that the *C242T* polymorphism might be a protective factor against developing ACS in the Asian population. Further researches will be needed to identify the confounding factors which modified the protective effect of T allele among Caucasians.

Key words: *CYBA*, *C242T* polymorphism, Acute coronary syndrome
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1 Introduction

With the extensive development of medication and interventional therapy, the mortality of coronary artery disease (CAD) in many countries has declined during the past decades (Nabel and Braunwald, 2012). However, a substantial number of patients still suffer from acute coronary syndrome (ACS) (Falk *et al.*, 2013). In the USA, approximately 1.2 million patients are hospitalized for ACS each year (Roger *et al.*, 2012). Rupture or erosion of an unstable atherosclerotic plaque and subsequent thrombosis are the chief pathological characteristics of ACS (Falk *et al.*, 2013; Thompson *et al.*, 2013).

Reactive oxygen species (ROS), including nitric oxide, superoxide, hydrogen peroxide, and peroxynitrite, play an important role in vascular pathophysiology and platelet aggregation (Griendling and Fitzgerald, 2003), which are also involved in the pathological process of ACS by activating matrix metalloproteinases (MMPs) (Galis *et al*, 1995), increasing smooth muscle cells apoptosis and thus leading to a plaque rupture (Bennett, 1999; Deshpande

et al., 2002). NAD(P)H oxidase is the predominant cellular source of ROS in the atherosclerotic lesions (Mueller *et al.*, 2005), which can be activated by p22phox (Sumimoto *et al.*, 1996). p22-phox is encoded by the *CYBA* gene, which is located in the human chromosome 16q24. The *C242T* polymorphism of the *CYBA* gene leads to a decreased production of ROS in the vasculature (Whitehead and Fitzgerald, 2001), indicating a protective role of *C242T* polymorphism in ACS. A lot of studies have been carried out to investigate the relationship between the *C242T* polymorphisms and ACS. However, the results are not consistent. Hence, we conducted this meta-analysis to evaluate the association between the *C242T* polymorphism and ACS.

2 Materials and methods

2.1 Literature search strategy

An extensive literature searching of PubMed/ MEDLINE, EBSCIO, and EMBASE reports was performed for relevant articles without restricting the language until Mar. 2014, using the combinations of the keywords "C242T", "p22phox", "CYBA", "NAD(P)H oxidase", "nicotinamide adenine dinucleotide phosphate oxidase", "rs4673", "polymorphism", "mut*", "varia*", "coronary", "myocardial infarction", "atherosclerosis", "acute coronary syndrome", and "unstable angina pectoris". Additional studies were identified by scanning the references of reviews and retrieved studies. We conducted the meta-analysis according to the guidelines of the 2009 Preferred Reporting Items for the Systematic Reviews and Meta-Analysis (PRISMA) statement (Checklist S1) (Moher *et al.*, 2009).

2.2 Inclusion criteria

Identified studies were screened according to the following inclusion criteria: (1) Case-control or cohort study assessing the association between *C242T* polymorphism and ACS risk as an original study. (2) Studies providing adequate information for calculating the genotypic odd ratio (OR) with 95% confidence interval (CI). (3) Published literatures of human genetics without ethnicity restriction. (4) If multiple articles originated from the same population, only the largest scale study was included. (5) The genotype frequencies amongst case and control must conform to the Hardy-Weinberg equilibrium (HWE). (6) ACS was defined as an acute myocardial infarction (MI) and unstable angina (Falk *et al.*, 2013).

2.3 Data extraction and quality assessment

All the data were extracted independently by two investigators following the inclusion criteria described above. The following was gathered from the eligible articles: name of the first author, publication year, ethnicity, endpoint, mean ages, sample sizes for case and control groups, genotype distributions and conformity to genotype frequencies with HWE, the proportion of male, hypertension, diabetes mellitus, and smoking. Two investigators used the Newcastle-Ottawa Scale (NOS) to assess the methodological quality of all eligible studies. Studies that were awarded 5 stars or more can be regarded to be of medium to high quality.

2.4 Statistical analysis

The HWE for the distributions of genotypes was assessed by the Pearson's chi-square test. The pooled effect for the relationship between the *C242T* polymorphism and ACS risk was calculated under different models, containing allele comparison, dominant genetic model, recessive genetic model and homozygote comparison. The Cochran's Q test and I^2 statistic were used to evaluate the between-study heterogeneity. The heterogeneity was qualified by I^2: I^2=0%–25%, low between-study heterogeneity; I^2=25%–50%, moderate heterogeneity; I^2=50%–100%, notable heterogeneity (Higgins *et al.*, 2003). The random effect model (REM) was adopted in the presence of notable heterogeneity (I^2>50%); otherwise, the fixed-effect model (FEM) was applied. A meta-regression was run to explore the origin of the genetic heterogeneity and then stratified analysis by subgroup was adopted. Sensitivity analysis was applied to identify the influence of each study, with successive omission of individual studies (Patsopoulos *et al.*, 2008). The Funnel plot and Egger's test were applied to evaluate the probability of publication bias. All statistical analyses were carried out with Stata Version 12.0 (Stata Co., College Station, TX, USA) and Review Manager Version 5.2 (RevMan, Cochrane Collaboration, Oxford, England). *P*<0.05 was considered statistically significant.

3 Results

3.1 Characteristics of the studies

The flow chart for the literature search is shown in Fig. 1. A total of 271 relevant references were included, with 257 publications excluded. One study (Katakami *et al.*, 2009) was excluded since it was overlapped by another study with a larger scale (Katakami *et al.*, 2010). Three publications did not meet the requirements of HWE ($P_{HWE}<0.05$) (Mata-Balaguer *et al.*, 2004; Morgan *et al.*, 2007; Hashad *et al.*, 2014). Finally, a total of 10 case-control studies were retrieved on ACS and the *CYBA C242T* polymorphism (Gardemann *et al.*, 1999; Stanger *et al.*, 2001; Yamada *et al.*, 2002; Murase *et al.*, 2004; Vasiliadou *et al.*, 2006; Macías-Reyes *et al.*, 2008; Katakami *et al.*, 2010; de Caterina *et al.*, 2011; Goliasch *et al.*, 2011; Narne *et al.*, 2012).

The basic characteristics and genotype frequencies of the included studies are listed in Tables 1 and 2. All the included studies were conducted from 1999 to 2012, with 6102 ACS patients and 8669 controls. MI was employed as the primary endpoint in five studies (Yamada *et al.*, 2002; Vasiliadou *et al.*, 2006; Katakami *et al.*, 2010; de Caterina *et al.*, 2011; Goliasch *et al.*, 2011), while MI and unstable angina were taken as the endpoint in two studies (Murase *et al.*, 2004; Macías-Reyes *et al.*, 2008). The endpoint of three studies was CAD, and MI was taken as the endpoint in subgroup analysis (Gardemann *et al.*, 1999; Stanger *et al.*, 2001; Narne *et al.*, 2012). There were six studies based on the Caucasian population, and four studies conducted on the Asian population. The genotype distributions among patients and controls conformed to HWE. All the 10 studies were assessed according to the NOS scale and most studies
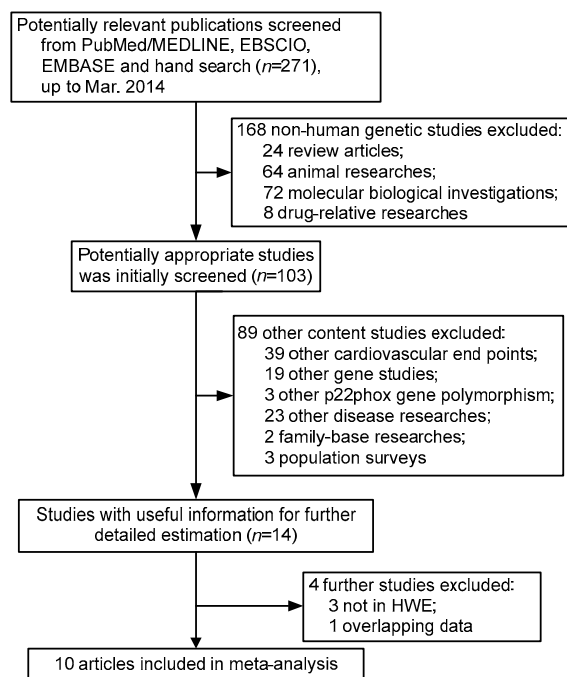
(80%) scored 5 stars or more, suggesting a moderate to good quality (Table 3).

3.2 Quantitative synthesis

The combined results of the *C242T* polymorphisms with ACS are summarized in Table 4. There was no significant association observed under any of the four genetic models (allele comparison: $P=0.18$, OR=0.91, 95% CI 0.78–1.05; dominant model: $P=0.22$, OR=0.90, 95% CI 0.75–1.07; recessive model: $P=0.58$, OR=1.04, 95% CI 0.91–1.18; homozygote comparison: $P=0.51$, OR=1.05, 95% CI 0.91–1.21) (Figs. 2–5). We also found notable heterogeneity in the allele comparison ($I^2=74$) and dominant model ($I^2=71$).

Fig. 1 Flow chart for the literature search strategy

Table 1 Characteristics of the included studies

Fisrt author	Year	Ethnicity	End point	Mean age (year)	Male (%)	Sample size	Smoking (%)	HTN (%)	DM (%)
Gardemann	1999	Caucasian	MI*	62.2/61.4	100/100	1031/499		62.0/54.0	19.0/11.0
Stanger	2001	Caucasian	MI*	52.9/54.2	100/100	71/45			
Yamada	2002	Asian	MI	62.1/62.0	100/100	1784/1074	57.8/55.2	47.0/57.3	34.7/16.2
Murase	2004	Asian	UA/MI	58.0/61.0	100/100	453/762	84.3/51.8	64.2/63.9	15.0/18.9
Vasiliadou	2006	Caucasian	MI	46.9/		197/204			
Macías-Reyes	2008	Caucasian	UA/MI	56.0/54.5	78/74	304/315	50.0/27.3		33.9/12.1
Katakami	2010	Asian	MI	62.2/59.5	64/61	226/3593	45.1/43.2	82.3/72.9	100/100
de Caterina	2011	Caucasian	MI	39.6/39.6	89/89	1864/1864	87.2/48.9	27.42/9.18	7.6/0.8
Goliasch	2011	Caucasian	MI	37.3/34.7	87/90	99/192	78.0/43.1	42.0/17.5	30.0/12.5
Narne	2012	Asian	MI*			73/121			100/100

Data are expressed as values for case/control. MI: myocardial infarction; UA: unstable angina; HTN: hypertension; DM: diabetes mellitus.
* Subgroup analysis

Table 2 *CYBA* gene *C242T* polymorphism genotype and allele distributions between ACS patients and controls, and *P*-value of HWE in controls and cases

First author	Genotype (TT/TC/CC)		Allele (C/T) (%)		P_{HWE}	
	Case	Control	Case	Control	Control	Case
Gardemann	127/457/447	65/208/226	65.5/34.5	66.1/33.9	0.12	0.54
Stanger	11/30/30	6/24/15	63.4/36.6	60.0/40.0	0.46	0.45
Yamada	25/319/1440	16/242/816	89.7/10.3	87.2/12.8	0.69	0.13
Murase	2/67/384	5/170/587	92.2/7.8	88.2/11.8	0.61	0.61
Vasiliadou	34/98/65	21/95/88	57.9/42.1	66.4/33.6	0.53	0.78
Macías-Reyes	48/137/119	47/145/123	61.7/38.3	62.1/37.9	0.69	0.42
Katakami	0/36/190	33/618/2942	92.0/8.0	90.5/9.5	0.93	0.19
de Caterina	322/888/654	296/889/679	58.9/41.2	60.3/39.7	0.86	0.49
Goliasch	11/47/41	31/79/82	65.2/34.8	63.3/36.7	0.11	0.65
Narne	5/27/41	22/54/45	74.7/25.3	59.5/40.5	0.42	0.85

HWE: Hardy-Weinberg equilibrium

Table 3 Quality assessment conducted according to the Newcastle-Ottawa criteria for all the included studies

First author	Year	Quality indicators		
		Selection	Comparability	Exposure
Gardemann	1999	**	*	**
Stanger	2001	**	**	*
Yamada	2002	****	*	*
Murase	2004	***		**
Vasiliadou	2007		**	
Macías-Reyes	2008	****	**	**
Katakami	2010	**	*	*
de Caterina	2011	***	*	*
Goliasch	2011	***	**	*
Narne	2012	***		**

Table 4 Results of meta-analysis for *CYBA* gene *C242T* polymorphism and acute coronary syndrome

Genotype contrast	Population	Study number (case/control)	Model for analysis	OR (95% CI)	$P_{heterogeneity}$	I^2 (%)	*P*-value
Allele comparison	Total	10 (6102/8669)	REM	0.91 (0.78, 1.05)	<0.001	74	0.18
(T vs. C)	Asian	4 (2536/5550)	FEM	0.73 (0.64, 0.83)	0.18	39	<0.001
	Caucasian	6 (3566/3119)	FEM	1.06 (0.99, 1.14)	0.33	13	0.11
Dominant model	Total	10 (6102/8669)	REM	0.90 (0.75, 1.07)	<0.001	71	0.22
(TT+CT vs. CC)	Asian	4 (2536/5550)	FEM	0.71 (0.62, 0.82)	0.21	34	<0.001
	Caucasian	6 (3566/3119)	FEM	1.07 (0.97, 1.19)	0.47	0	0.16
Recessive model	Total	10 (6102/8669)	FEM	1.04 (0.91, 1.18)	0.19	27	0.58
(TT vs. CT+CC)	Asian	4 (2536/5550)	FEM	0.63 (0.39, 1.02)	0.31	16	0.06
	Caucasian	6 (3566/3119)	FEM	1.08 (0.94, 1.24)	0.33	13	0.26
Homozygote comparison	Total	10 (6102/8669)	FEM	1.05 (0.91, 1.21)	0.07	43	0.51
(TT vs. CC)	Asian	4 (2536/5550)	FEM	0.57 (0.35, 0.92)	0.20	35	0.02
	Caucasian	6 (3566/3119)	FEM	1.11 (0.96, 1.29)	0.27	21	0.16

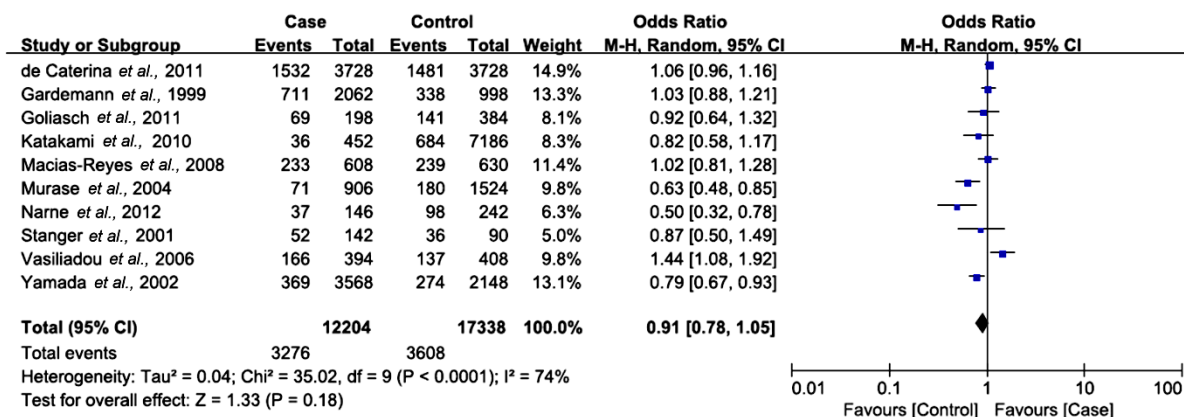OR: odds ratio; 95% CI: 95% confidence interval; REM: random-effects model; FEM: fix-effects model

Study or Subgroup	Case		Control		Weight	Odds Ratio M-H, Random, 95% CI	Odds Ratio M-H, Random, 95% CI
	Events	Total	Events	Total			
de Caterina *et al.*, 2011	1532	3728	1481	3728	14.9%	1.06 [0.96, 1.16]	
Gardemann *et al.*, 1999	711	2062	338	998	13.3%	1.03 [0.88, 1.21]	
Goliasch *et al.*, 2011	69	198	141	384	8.1%	0.92 [0.64, 1.32]	
Katakami *et al.*, 2010	36	452	684	7186	8.3%	0.82 [0.58, 1.17]	
Macias-Reyes *et al.*, 2008	233	608	239	630	11.4%	1.02 [0.81, 1.28]	
Murase *et al.*, 2004	71	906	180	1524	9.8%	0.63 [0.48, 0.85]	
Narne *et al.*, 2012	37	146	98	242	6.3%	0.50 [0.32, 0.78]	
Stanger *et al.*, 2001	52	142	36	90	5.0%	0.87 [0.50, 1.49]	
Vasiliadou *et al.*, 2006	166	394	137	408	9.8%	1.44 [1.08, 1.92]	
Yamada *et al.*, 2002	369	3568	274	2148	13.1%	0.79 [0.67, 0.93]	
Total (95% CI)		12204		17338	100.0%	0.91 [0.78, 1.05]	
Total events	3276		3608				

Heterogeneity: Tau² = 0.04; Chi² = 35.02, df = 9 (P < 0.0001); I² = 74%
Test for overall effect: Z = 1.33 (P = 0.18)

0.01 0.1 1 10 100
Favours [Control] Favours [Case]

Fig. 2 Forest plot for the overall association between the *C242T* polymorphism and ACS under the allele comparison (T vs. C)

Study or Subgroup	Case		Control		Weight	Odds Ratio M-H, Random, 95% CI	Odds Ratio M-H, Random, 95% CI
	Events	Total	Events	Total			
de Caterina *et al.*, 2011	1210	1864	1185	1864	15.2%	1.06 [0.93, 1.21]	
Gardemann *et al.*, 1999	584	1031	273	499	13.4%	1.08 [0.87, 1.34]	
Goliasch *et al.*, 2011	58	99	110	192	7.2%	1.05 [0.64, 1.72]	
Katakami *et al.*, 2010	36	226	651	3593	9.7%	0.86 [0.59, 1.23]	
Macias-Reyes *et al.*, 2008	185	304	192	315	10.7%	1.00 [0.72, 1.38]	
Murase *et al.*, 2004	69	453	175	762	11.1%	0.60 [0.44, 0.82]	
Narne *et al.*, 2012	32	73	76	121	5.8%	0.46 [0.26, 0.83]	
Stanger *et al.*, 2001	41	71	30	45	3.9%	0.68 [0.31, 1.49]	
Vasiliadou *et al.*, 2006	132	197	116	204	8.8%	1.54 [1.03, 2.31]	
Yamada *et al.*, 2002	344	1784	258	1074	14.2%	0.76 [0.63, 0.91]	
Total (95% CI)		6102		8669	100.0%	0.90 [0.75, 1.07]	
Total events	2691		3066				

Heterogeneity: Tau² = 0.05; Chi² = 30.71, df = 9 (P = 0.0003); I² = 71%
Test for overall effect: Z = 1.22 (P = 0.22)
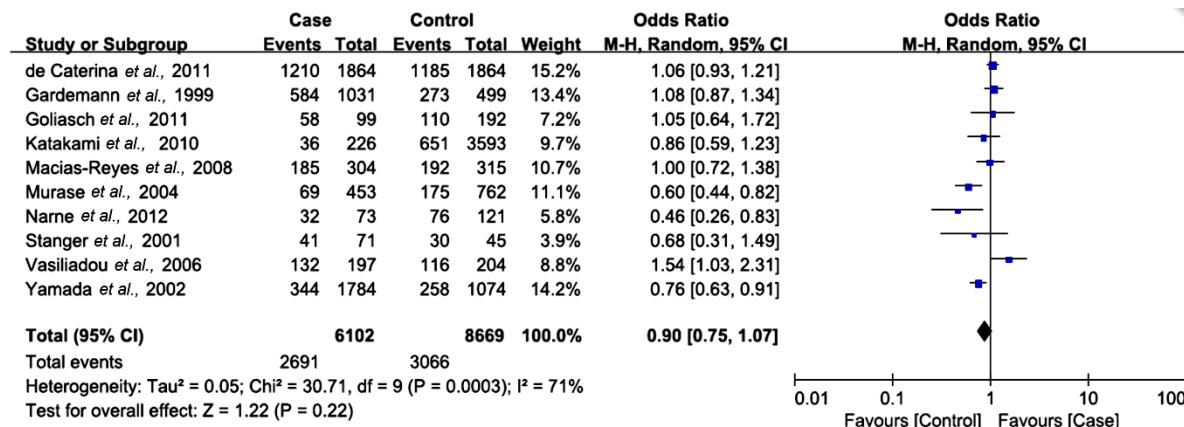
0.01 0.1 1 10 100
Favours [Control] Favours [Case]

Fig. 3 Forest plot for the overall association between the *C242T* polymorphism and ACS under the dominant model (TT+CT vs. CC)

Study or Subgroup	Experimental		Control		Weight	Odds Ratio M-H, Fixed, 95% CI	Odds Ratio M-H, Fixed, 95% CI
	Events	Total	Events	Total			
de Caterina *et al.*, 2011	322	1864	296	1864	55.0%	1.11 [0.93, 1.31]	
Gardemann *et al.*, 1999	127	1031	65	499	17.2%	0.94 [0.68, 1.29]	
Goliasch *et al.*, 2011	11	99	31	192	4.2%	0.65 [0.31, 1.35]	
Katakami *et al.*, 2010	0	226	33	3593	0.9%	0.23 [0.01, 3.84]	
Macias-Reyes *et al.*, 2008	48	304	47	315	8.7%	1.07 [0.69, 1.66]	
Murase *et al.*, 2004	2	453	5	762	0.8%	0.67 [0.13, 3.47]	
Narne *et al.*, 2012	5	73	22	121	3.5%	0.33 [0.12, 0.92]	
Stanger *et al.*, 2001	11	71	6	45	1.4%	1.19 [0.41, 3.49]	
Vasiliadou *et al.*, 2006	34	197	21	204	3.8%	1.82 [1.01, 3.26]	
Yamada *et al.*, 2002	25	1784	16	1074	4.4%	0.94 [0.50, 1.77]	
Total (95% CI)		6102		8669	100.0%	1.04 [0.91, 1.18]	
Total events	585		542				

Heterogeneity: Chi² = 12.38, df = 9 (P = 0.19); I² = 27%
Test for overall effect: Z = 0.56 (P = 0.58)
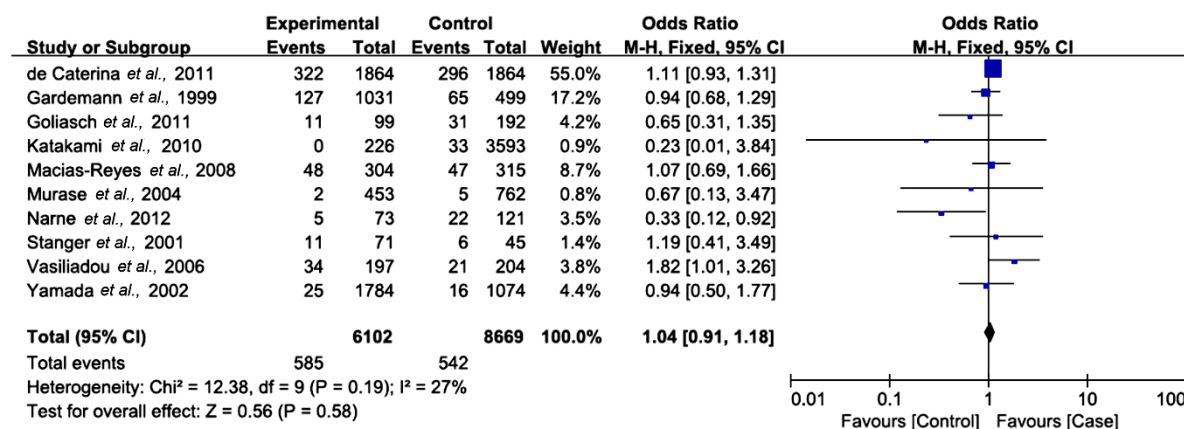
0.01 0.1 1 10 100
Favours [Control] Favours [Case]

Fig. 4 Forest plot for the overall association between the *C242T* polymorphism and ACS under the recessive model (TT vs. CT+CC)

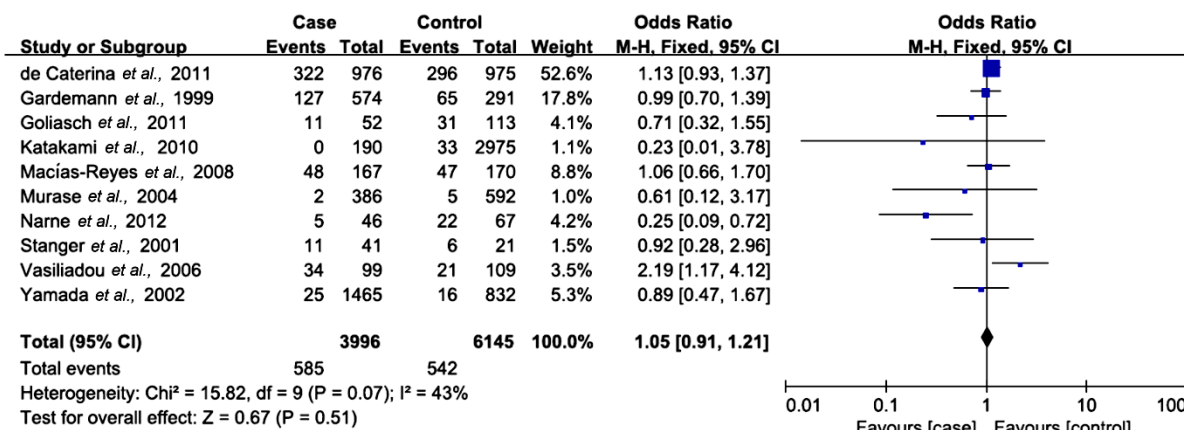| Study or Subgroup	Case Events	Total	Control Events	Total	Weight	Odds Ratio M-H, Fixed, 95% CI
de Caterina *et al.*, 2011	322	976	296	975	52.6%	1.13 [0.93, 1.37]
Gardemann *et al.*, 1999	127	574	65	291	17.8%	0.99 [0.70, 1.39]
Goliasch *et al.*, 2011	11	52	31	113	4.1%	0.71 [0.32, 1.55]
Katakami *et al.*, 2010	0	190	33	2975	1.1%	0.23 [0.01, 3.78]
Macías-Reyes *et al.*, 2008	48	167	47	170	8.8%	1.06 [0.66, 1.70]
Murase *et al.*, 2004	2	386	5	592	1.0%	0.61 [0.12, 3.17]
Narne *et al.*, 2012	5	46	22	67	4.2%	0.25 [0.09, 0.72]
Stanger *et al.*, 2001	11	41	6	21	1.5%	0.92 [0.28, 2.96]
Vasiliadou *et al.*, 2006	34	99	21	109	3.5%	2.19 [1.17, 4.12]
Yamada *et al.*, 2002	25	1465	16	832	5.3%	0.89 [0.47, 1.67]
Total (95% CI)		3996		6145	100.0%	1.05 [0.91, 1.21]
Total events	585		542			

Heterogeneity: Chi² = 15.82, df = 9 (P = 0.07); I² = 43%
Test for overall effect: Z = 0.67 (P = 0.51)

Fig. 5 Forest plot for the overall association between the *C242T* polymorphism and ACS under the homozygote comparison (TT vs. CC)

Table 5 Sensitivity analysis of pooled OR

Study omitted	OR (95% CI)	I^2 (%)	Study omitted	OR (95% CI)	I^2 (%)
Allele comparison			Dominant model		
Gardemann	0.88 (0.74, 1.05)	77	Gardemann	0.87 (0.71, 1.06)	72
Stanger	0.91 (0.78, 1.06)	77	Stanger	0.91 (0.76, 1.09)	73
Yamada	0.92 (0.79, 1.08)	72	Yamada	0.92 (0.76, 1.11)	67
Murase	0.95 (0.82, 1.09)	70	Murase	0.95 (0.80, 1.12)	64
Vasiliadou	0.87 (0.75, 1.00)	71	Vasiliadou	0.85 (0.72, 1.01)	68
Macías-Reyes	0.89 (0.75, 1.05)	77	Macías-Reyes	0.88 (0.73, 1.07)	74
Katakami	0.91 (0.78, 1.07)	77	Katakami	0.90 (0.74, 1.09)	74
de Caterina	0.88 (0.74, 1.04)	72	de Caterina	0.87 (0.71, 1.07)	68
Goliasch	0.90 (0.77, 1.06)	77	Goliasch	0.88 (0.73, 1.07)	74
Narne	0.94 (0.82, 1.08)	70	Narne	0.93 (0.79, 1.11)	68

3.3 Sensitivity analysis

A sensitivity analysis was run to look for studies making the largest contributions to the notable heterogeneity in allele comparisons and dominant models. The results showed that no single study dramatically affected the heterogeneity, ORs and 95% CIs (Table 5).

3.4 Meta-regression analysis and subgroup analysis

In view of a notable heterogeneity, we performed a series of univariate meta-regression analysis under the allelic model and the dominant model by adding single covariates including publication year, age, ethnicity, the proportion of male, hypertension, diabetes mellitus, and smoking status. In the univariate analysis, a large proportion of the between-study heterogeneity was significantly attributed to ethnicity (Table 6). Hence, we undertook subgroup analyses on

Table 6 Meta-regression analysis for heterogeneity under the allelic model and dominant model of *p22phox* gene *C242T* polymorphism

Variable	Coefficient	*P*-value	tau^2-value	R^2 (%)
Allelic model				
Ethnicity	−0.4427	0.035	0.0506	45.68
Year	0.0006	0.981	0.1079	−15.92
Age	−0.0064	0.608	0.0925	−12.55
Male (%)	−0.7707	0.391	0.0736	1.56
DM (%)	−0.4094	0.273	0.0875	5.48
Hypertension (%)	−0.3943	0.211	0.0065	38.22
Smoking (%)	−0.1693	0.238	0.0853	18.15
Dominant model				
Ethnicity	−0.3109	0.015	0	100
Year	0.0178	0.372	0.0407	−11.90
Age	−0.0125	0.177	0.0303	20.22
Male (%)	−0.3030	0.632	0.0218	−16.66
DM (%)	0.0149	0.953	0.0236	−25.00
Hypertension (%)	−0.4886	0.294	0.0213	11.73
Smoking (%)	0.0892	0.922	0.0286	−42.73

ethnicity and the heterogeneity significantly decreased both in the allelic model and the dominant model. The risk of ACS decreased in the Asian population (allelic comparison: $P<0.01$, OR=0.73, 95% CI 0.64–0.83; dominant model: $P<0.01$, OR=0.71, 95% CI 0.62–0.82; homozygote comparison: $P=0.02$, OR=0.57, 95% CI 0.35–0.92).

However, no significant association was observed in the Caucasian population (allelic comparison: $P=0.11$, OR=1.06, 95% CI 0.99–1.14; dominant model: $P=0.16$, OR=1.07, 95% CI 0.97–1.19; recessive model: $P=0.26$, OR=1.08, 95% CI 0.94–1.24; homozygote comparison: $P=0.16$, OR=1.11, 95% CI 0.96–1.29) (Table 4).

3.5 Publication bias

The Funnel plot (Fig. 6) and Egger's regression were applied to evaluate the probability of publication bias. No significant publication bias was found in the overall estimates ($t=-0.88$, $P=0.40$ for allele comparison).
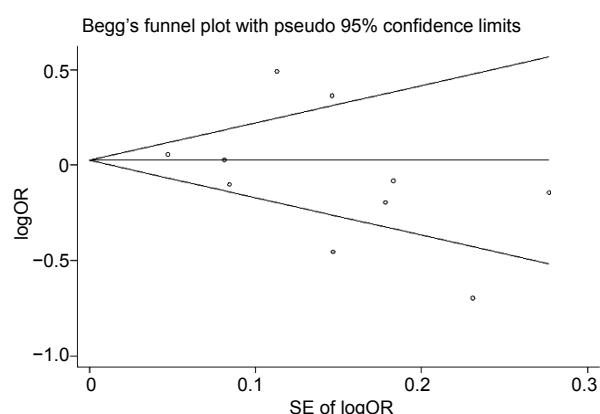
Fig. 6 Funnel plot analysis to evaluate publication bias for the allele comparison of the *C242T* polymorphism

4 Discussion

Due to the conflicting results about the relationship between *C242T* polymorphisms and ACS, we conducted this meta-analysis. A total of 10 articles with 6102 ACS patients and 8669 controls were included in our study. Finally, in our meta-analysis, no significant association was observed under different genetic models with notable heterogeneity. This unexpected result may be due to the presence of notable heterogeneity, which confounded the possible effect.

Further meta-regression identified that a large proportion of between-study heterogeneity was explained by ethnicity. Subgroup analysis by ethnicity documented a significant decreased risk of ACS under allelic comparison, dominant model, and homozygote comparison in the Asian population, while no significant association was observed among Caucasians. ROS were involved in plaque rupture and platelet aggregation (Galis *et al.*, 1995; Bennett, 1999; Deshpande *et al.*, 2002; Griendling and Fitzgerald, 2003), suggesting the important role of ROS in the pathological process of ACS. NAD(P)H oxidase is the predominant cellular source of ROS in the context of atherosclerosis (Mueller *et al.*, 2005), which can be activated by p22phox (Sumimoto *et al.*, 1996). The *C242T* polymorphism results in a substitution of Tyr for His at residue 72 of p22phox, and significantly reduced vascular NAD(P)H oxidase activity (Guzik *et al.*, 2000). This might be expected to reduce the generation of ROS, indicating a protective role of T allele in ACS, which is consistent with the results for the Asian population as suggested by our meta-analysis.

It is really puzzling to us that no significant association was observed among Caucasians. The ethnicity-related discrepancy in the effect of the *C242T* polymorphism could be attributed to multiple cardiovascular risk factors, which confound the possible effects. Fan *et al.* (2007) reported that body adiposity may modify the genetic effect of *C242T* polymorphism. The prevalence of adult obesity in the United States (2011–2012) is 34.9% (Ogden *et al.*, 2014), while it is only 12.9% in China (2010) (Li *et al.*, 2012). Obesity may be one of the confounding factors, which modified the effect of T allele among Caucasians, which is in accordance with the suggestion of previous meta-analysis studies (Wu *et al.*, 2013). Recently, some investigators reported that increased body mass index (BMI) in adults is associated with increased methylation at the HIF3A (Dick *et al.*, 2014). Hence, we forward the hypothesis that obesity regulated the methylation level of the *CYBA* gene, and then modified the protective effect of *C242T* polymorphism among Caucasians. Further researches will be needed to confirm our hypothesis, and the important effects of other confounding factors should also be studied. It has been reported that physical activity could modify this genetic effect of the *CYBA* gene (Zhu *et al.*, 2012). The c.-930A>G promoter polymorphism and the

640A>G polymorphism in the *CYBA* gene have also been implicated in the development of cardiovascular disease (Goliasch *et al.*, 2011; Xu *et al.*, 2014). Further researches will be needed to clarify the possible correlation of the three haplotypes of the *CYBA* gene.

Three previous meta-analyses had evaluated the effect of the *C242T* polymorphism on CAD risk, the results are in conformity with notable heterogeneity. Fang *et al.* (2010) first undertook a meta-analysis suggesting that *C242T* polymorphism had a significant protective effect among Asians but not among Caucasians, which is consistent with the results as suggested by Xu *et al.* (2014). However, Wu *et al.* (2013) reported that the T allele had a marginal risk increase of CAD among Caucasians (recessive model: OR=1.21, 95% CI 1.00–1.46) and no significant association was observed in the Asian population. The typical pathology of ACS and stable angina are significantly different (Falk *et al.*, 2013; Thompson *et al.*, 2013). ROS play an important role in the pathological process of ACS rather than stable angina. The stable angina cases included in the three meta-analyses may be contributed to the notable heterogeneity. Analyzing the effect of the *C242T* polymorphism on ACS may be more convincing. Fang *et al.* (2010) and Xu *et al.* (2014) had not discussed the relationship between the *C242T* polymorphisms and ACS. Although Wu *et al.* (2013) had carried out tests to investigate the relationship between the *C242T* polymorphisms and ACS, the subgroup analysis included only six ACS studies with notable heterogeneity, and the genotype frequencies of one study did not conform to HWE (Morgan *et al.*, 2007). Hence, our meta-analysis excluded patients with stable angina and conformed to HWE in both the controls and patients with ACS. A large proportion of between-study heterogeneity was explained by ethnicity in our meta-analysis. The heterogeneity of subgroup analysis by ethnicity was low or moderate. The reason for the similar results between our paper and two of the three meta-analyses may be the population source (Fang *et al.*, 2010; Xu *et al.*, 2014). A large proportion of the eligible articles, which are included in the two meta-analyses, were hospital-based, and most of the patients were hospitalized for ACS.

Although we have tried our best to improve this manuscript, there are still some limitations. First, the number of eligible articles in our meta-analysis is small. Second, the data of BMI in some articles are missing, which makes it difficult to confirm our hypothesis. Third, our meta-analysis did not account for the possible synergistic effects of the three common polymorphisms in the *CYBA* gene. Finally, the observed association between *C242T* polymorphism and ACS does not necessarily imply causality.

In conclusion, this meta-analysis suggests that *C242T* polymorphism is related to ACS risk reduction only in the Asian population. We forward the hypothesis that obesity may modify the protective effect of *C242T* polymorphism among Caucasians. Our observations support the need for further investigation into the modifying effect of possible confounding factors. We suggest that the "good" or "bad" effects of the *CYBA* gene are related to different haplotypes and confounding factors, not just *C242T* polymorphism.

Compliance with ethics guidelines

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中文概要

题　目：***CYBA* 基因 *C242T* 基因多态性的荟萃分析：亚洲人群急性冠脉综合征风险降低而高加索人群没有**

目　的：探讨 *CYBA* 基因 *C242T* 基因多态性与急性冠脉综合征的关系。

创新点：提出了 *C242T* 基因多态性对急性冠脉综合征的影响存在种族差异。

方　法：荟萃分析了 *C242T* 基因多态性与急性冠脉综合征的关系。

结　论：对于亚洲人群而言，*C242T* 基因多态性为急性冠脉综合征的保护因素。

关键词：*CYBA*；*C242T* 基因多态性；急性冠脉综合征