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Original Article

Glutathione S-transferase M1 (GSTM1) null genotype and coronary artery disease risk: a meta-analysis

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Abstract: Background: The Glutathione S-Transferase M1 (*GSTM1*) null genotype has been indicated to be correlated with coronary artery disease (CAD) susceptibility, but study results are still debatable. Thus, a meta-analysis was conducted. Materials and methods: Databases including PubMed, Embase, Web of Science, and Chinese National Knowledge Infrastructure (CNKI) were searched. Data were extracted and pooled odds ratios (OR) with 95% confidence intervals (CI) were calculated. Results: Twenty-six studies with 10595 cases and 13782 controls were included in this meta-analysis. The association between *GSTM1* null genotype and CAD risk was significant (OR = 1.35; 95% CI, 1.09 - 1.67; $P < 0.01$). When stratified by ethnicity, the significantly elevated risk were observed in Caucasians (OR = 1.39; 95% CI, 1.07 - 1.81; $P = 0.01$) but not in Asians (OR = 1.27; 95% CI, 0.87 - 1.86; $P = 0.22$). No significantly increased myocardial infarction risk was observed (OR = 0.96; 95% CI, 0.78 - 1.18; $P = 0.68$). Subgroup analysis on the smoking status showed that the increased risk was found in smokers (OR = 1.66; 95% CI, 1.14 - 2.42; $P < 0.01$) but not in non-smokers (OR = 1.30; 95% CI, 1.74 - 2.28; $P = 0.37$). Conclusion: In conclusion, this meta-analysis suggested that *GSTM1* null genotype was a risk factor for CAD, especially in Caucasians and smokers.

Keywords: Coronary artery disease, GSTM1, meta-analysis, genetic

Introduction

Coronary artery disease (CAD) is a leading cause of morbidity and mortality worldwide [1]. Despite it is well established that a poor diet, advanced age, smoking, hypertension, diabetes, and dyslipidemia are associated with increased risk of CAD, a detailed etiology underlying CAD is still obscure. In the past three decades, genetic association studies have revealed a considerable number of candidate loci and genes for CAD [2].

Glutathione S-transferases (GSTs) are phase II enzymes involved in the detoxification of a broad range of toxic compounds and carcinogens. The *GSTM1* gene, located on chromosome 1p13.3, codes for cytosolic GST μ class enzyme, and has a deletion polymorphism that results in the complete absence of a functional gene product [3]. Many investigators have investigated the association between the *GSTM1* null genotype and CAD risk [4-28]. But the results were conflicted and inconclusive. As a single study may lack the power to provide reliable conclusion, we performed this meta-analysis.

Methods

Publication search

We searched all published studies (up to March 2, 2014) in databases of PubMed, Embase, Web of Science, and Chinese National Knowledge Infrastructure (CNKI). The following keywords were used for searching: “glutathione S-transferase” OR “GSTM1” AND “polymorphism” OR “mutation” OR “variant” OR “variation” OR “genotype” AND “coronary artery disease” OR “CAD” OR “coronary heart disease” OR “CHD” OR “ischemic cardiovascular disease” OR “atherosclerosis”. We also perused the reference lists of selected research papers and reviews to identify additional relevant studies. No language restrictions were imposed.

Inclusion and exclusion criteria

The inclusion criteria for identified articles were as follows: (1) studies on the relationship

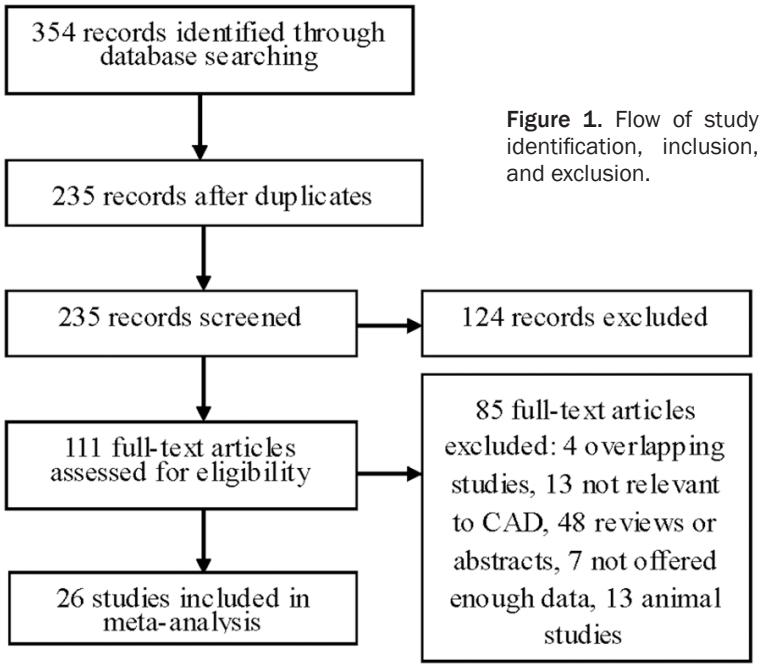

Figure 1. Flow of study identification, inclusion, and exclusion.

between GSTM1 null genotype and CAD risk; (2) case-control studies; (3) studies with full text articles; (4) sufficient data for estimating an odds ratio (OR) with 95% confidence interval (CI). Studies were excluded if one of the following existed: (1) not relevant to CAD or GSTM1, (2) not designed as case-control studies, (3) genotype frequencies or number not offered, (4) animal studies, (5) editorials, reviews and abstracts, and (6) overlapping studies.

Data extraction

Data were extracted independently and entered into separate databases from each qualified study: first author's last name, publication date, population ethnicity, gender, sample size, endpoint, smoking status, *GSTT1* interaction.

Statistical analysis

The strength of the associations between the *GSTM1* null genotype and CAD risk was measured by ORs and 95% CIs. The random-effects model was used. The statistical significance of summary OR was determined with *Z* test. Between-study heterogeneity was assessed by Chi-square test, and was quantified using the I^2 statistic (ranging from 0 to 100%), which was defined as the percentage of the observed between-study variability that is due to heterogeneity rather than chance. To evaluate the ethnic-specific and smoke-specific effects, subgroup analyses were performed. We also investigated the association between *GSTM1* null genotype and myocardial infarction (MI) risk. To explore the source of the heterogeneity, Galbraith plots were used. To access the stability of the meta-analysis, one-way sensitivity analysis was carried out. Publication bias was assessed by visual inspection of funnel plots, in which the standard error of log (OR) of each study was plotted against its log (OR). The Egger's test was used to assess publication bias statistically [29]. All statistical tests were performed by using STATA 11.0 software (Stata Corporation, College Station, TX). A P value of < 0.05 was considered significant. All the P values were two-sided.

Results

Study characteristics

The result of study selection process was shown in **Figure 1**. The initial search produced 354 studies from PubMed, Embase, Web of Science, and CNKI. After exclusion of duplicates, 235 potentially eligible studies were selected. After detailed evaluations, 26 studies were selected for final meta-analysis [4-28]. There were 9 studies on Asians and 17 studies on Caucasians. Three studies were performed in female patients and other studies were conducted in female and male patients. Six studies provided the information of MI, while other studies reported information of CAD. Smoking status can be extracted from 16 studies. The characteristics of each study included in this meta-analysis are shown in **Table 1**.

Quantitative data synthesis

The association between *GSTM1* null genotype and CAD risk was investigated in 26 case-control studies with a total of 10595 cases and 13782 controls (**Table 2**). The null genotype of *GSTM1* was associated with a significantly increased risk of CAD when compared with present genotype (OR = 1.35; 95% CI, 1.09 -

Table 1. The baseline characteristics of all eligible studies in the meta-analysis

First author	Year	Ethnicity	Man (%)	No. of case	No. of control	Endpoint	Smoking status	*GSTT1* interaction
Evans	1996	Caucasian	100	90	884	CAD	NA	No
Li	2000	Caucasian	76	400	890	CAD	Mixed*	No
Wilson	2000	Caucasian	68	356	187	MI	Mixed*	No
Wang	2002	Caucasian	72	609	259	CAD	Mixed	No
Masetti	2003	Caucasian	85	308	122	CAD	Mixed*	Yes
Olshan	2003	Caucasian	66	526	868	CAD	Mixed	Yes
Wilson	2003	Asian	85	170	203	CAD/MI	Mixed*	No
Girisha	2004	Asian	85	59	132	CAD	Mixed*	Yes
Tamer	2004	Caucasian	72	148	247	CAD	Mixed*	Yes
Abu-Amero	2006	Caucasian	61	1054	762	CAD	Mixed*	Yes
Hayek	2006	Caucasian	75	403	2962	CAD	Mixed	No
Cornelis	2007	Caucasian	74	2042	2042	MI	Mixed	No
Manfredi	2007	Caucasian	94	165	53	CAD	Smoker	Yes
Kim	2008	Asian	40	582	110	CAD	Mixed*	No
Wang	2008	Asian	59	277	277	CAD	Mixed*	Yes
Maciel	2009	Caucasian	60	871	1577	CAD	Mixed	No
Martin	2009	Caucasian	84	67	63	CAD	Mixed*	Yes
Ramprasath	2011	Asian	74	290	492	CAD	Non-smoker	No
Bazo	2011	Caucasian	69	297	100	CAD	Mixed	Yes
Nomani	2011	Caucasian	55	209	108	CAD	Mixed*	Yes
Singh	2011	Asian	85	230	300	MI	Mixed*	No
Karič	2012	Caucasian	56	206	257	MI	Mixed	Yes
Lakshmi	2012	Asian	NA	352	282	CAD	Mixed	No
Phulukdaree	2012	Asian	NA	102	100	CAD	Mixed*	No
Cora	2013	Caucasian	61	324	296	MI	Mixed*	No
Yeh	2013	Asian	74	458	209	CAD	Mixed	No

*More information can be extracted. CAD, coronary artery disease; MI, myocardial infarction; NA, not available.

Table 2. Main results of this meta-analysis

	Test of association		Heterogeneity	
	OR (95% CI)	*P* Value	χ^2	I^2 (%)
Overall	1.35 (1.09-1.67)	< 0.01	242.87	90.0
Caucasian	1.39 (1.07-1.81)	0.01	192.07	92.0
Asian	1.27 (0.87-1.86)	0.22	50.78	84.0
MI	0.96 (0.78-1.18)	0.68	10.56	53.0
Smoker	1.66 (1.14-2.42)	< 0.01	65.43	82.0
Non-smoker	1.30 (0.74-2.28)	0.37	172.27	94.0
GSTT1 interaction	2.41 (1.09 5.34)	0.03	138.59	93.0

MI, myocardial infarction.

1.67; $P < 0.01$; $I^2 = 90\%$; **Figure 2**). When stratified by ethnicity, the significantly elevated risk were observed in Caucasians (OR = 1.39; 95% CI, 1.07 - 1.81; $P = 0.01$; $I^2 = 92\%$) but not in Asians (OR = 1.27; 95% CI, 0.87 - 1.86; $P = 0.22$; $I^2 = 84\%$). In the MI subgroup, no significantly increased MI risk was observed (OR = 0.96; 95% CI, 0.78 - 1.18; $P = 0.68$; $I^2 = 53\%$). Subgroup analysis on the smoking status showed that the increased risk was found in smokers (OR = 1.66; 95% CI, 1.14 - 2.42; $P < 0.01$; $I^2 = 82\%$) and non-smokers (OR = 1.30; 95% CI, 1.74 - 2.28; $P = 0.37$; $I^2 = 94\%$).

We also assessed the interaction between *GSTM1* null and *GSTT1* null genotypes. We found that both null genotypes of *GSTM1* and *GSTT1* carriers had an increased CAD risk (OR = 2.41; 95% CI, 1.09 - 5.34; $P = 0.03$; $I^2 = 93\%$). A single study involved in the meta-analysis was deleted each time to reflect the influence of the individual data set to the pooled ORs, and the corresponding pooled ORs were not materially altered (**Figure 3**).

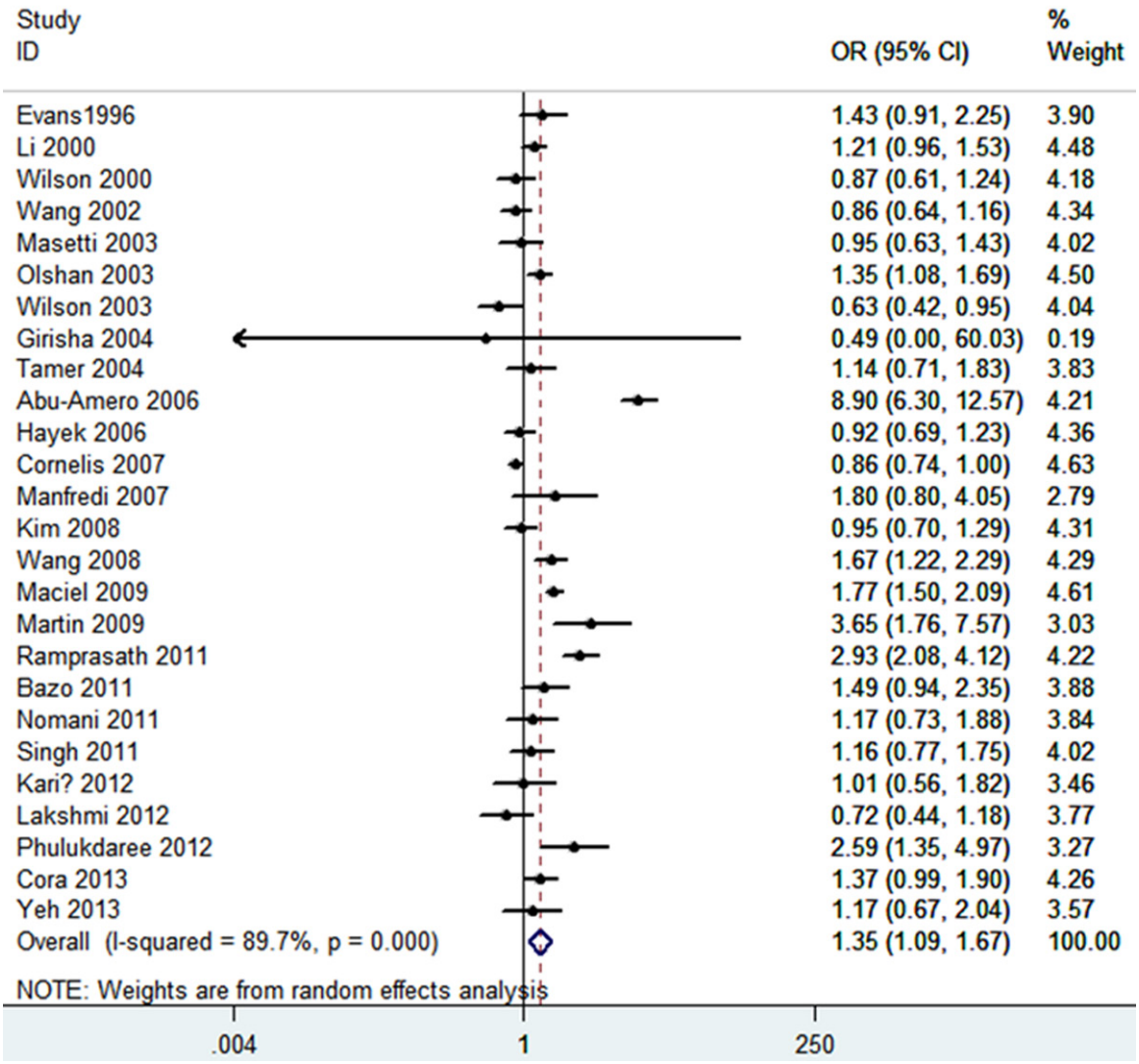

Figure 2. Meta-analysis of the association between the *GSTM1* null genotype and CAD risk.

The Galbraith plot was used to find the source of the heterogeneity. Eleven studies were the outliers (data not shown). After excluding these studies, the between-study heterogeneity effectively decreased and there was no obvious heterogeneity (I^2 = 0%, P = 0.63). Besides, the result was still statistically significant (OR = 1.25; 95% CI, 1.14 - 1.38; P < 0.01).

Publication bias was assessed by funnel plot. The shape of the funnel plot showed symmetric (data not shown). Furthermore, no significant publication bias was detected by Egger's test (P = 0.57).

Discussion

This meta-analysis of 26 case-control studies systematically evaluated the associations between *GSTM1* null genotype and CAD risk. We found that *GSTM1* null genotype was significantly associated with CAD risk. This result suggested that individuals with *GSTM1* null genotype had increased CAD risk. In the subgroup analysis, we found that *GSTM1* null genotype carriers had increased CAD risk in Caucasians but not in Asians, suggesting a possible influence among environmental exposures and different genetic backgrounds. When subgroup analysis was performed according to smoking status, the significant association was showed in smokers. This result suggested that smoking status changed the role of *GSTM1* null genotype on CAD. In the MI subgroup, however, we did not detect a significant association between *GSTM1* null genotype and MI. This result indicated that *GSTM1* null genotype may play no

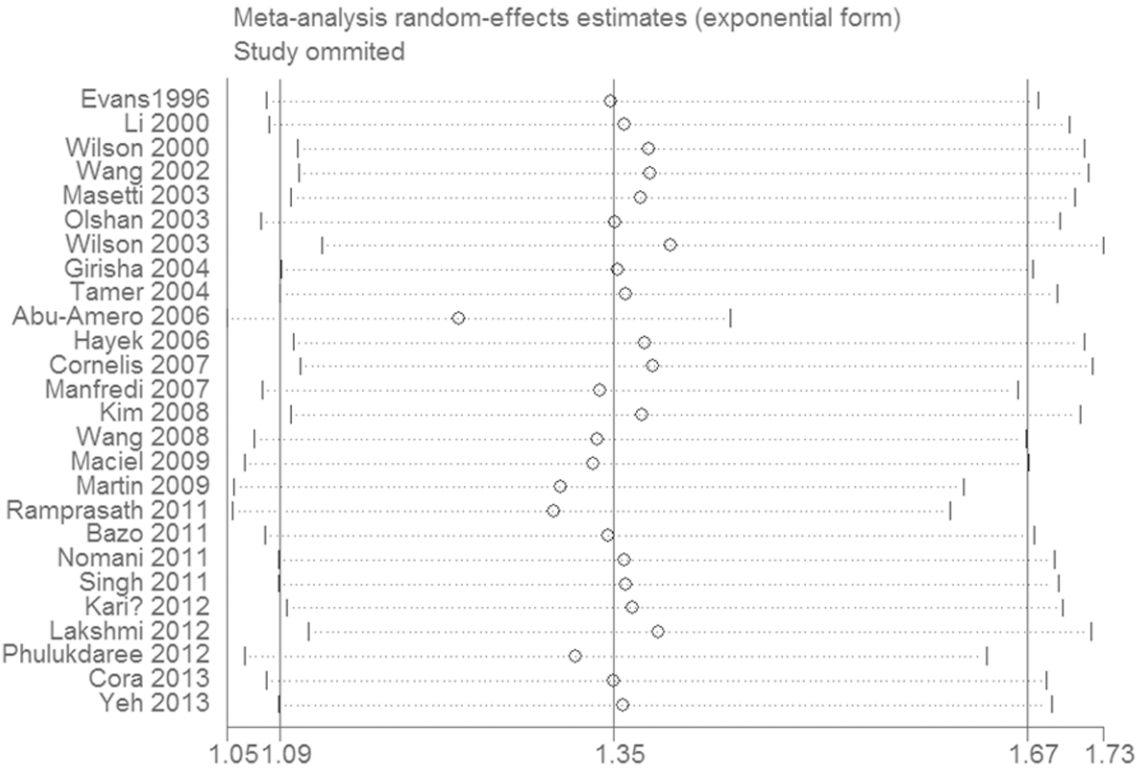

Figure 3. Sensitivity analysis for the *GSTM1* null genotype and CAD risk.

role in the development of MI. Because of the complex nature of CAD, it is unlikely that a polymorphism in one single gene would be obviously associated with an increased CAD risk, without consideration of any other genes. Thus, the interaction between *GSTM1* null and *GSTT1* null genotypes was investigated. Both null genotypes carriers had increased CAD risk. This result indicated that these polymorphisms had the same effect on the pathogenesis of CAD.

Heterogeneity is a potential problem that may affect the interpretation of the results. Significant heterogeneity existed in this meta-analysis. Galbraith plots were used to find the sources of heterogeneity. We found that I^2 value was decreased after excluding the outliers. The result suggested that the outlying studies may be the major source of the heterogeneity. Moreover, heterogeneity did not influence the results, because the significance of the result was not altered after excluding the outliers. Results from one-way sensitivity analysis suggested stability of these results. Additionally, funnel plots and Egger's tests did not find potential publication bias. All together, these results suggested that results of this meta-analysis were reliable.

However, some limitations should be noted. First, subgroup analyses were not performed by the factors such as gender and age because insufficient data could be extracted from the primary articles. Second, because small negative studies are less likely to published, the possibility of publication bias cannot be ruled out completely, even though the Egger's test and funnel plots did not provide any evidence of publication bias in this meta-analysis. Third, a lack of original data from the eligible studies limited evaluation of the effects of the gene-environment interactions during CAD development.

In conclusion, this meta-analysis of 26 studies suggested that *GSTM1* null genotype was associated with increased CAD risk. Further studies with large sample size were needed to confirm our findings.

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Disclosure of conflict of interest

None.

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