GST null polymorphisms may affect the risk of coronary artery disease: evidence from a meta-analysis

Hongling Su *, Yunshan Cao, Jing Li, Yan Zhu and Xuming Ma

Abstract

Background: Whether glutathione S-transferase (GST) null polymorphisms, namely GSTM1 null, GSTP1 null and GSTT1 null polymorphisms, influence the risk of coronary artery disease (CAD) or not remains unclear. Thus, the authors performed a meta-analysis to more robustly estimate associations between GST null polymorphisms and the risk of CAD by integrating the results of previous publications.

Methods: Medline, Embase, Wanfang, VIP and CNKI were searched comprehensively for eligible studies, and 45 genetic association studies were finally selected to be included in this meta-analysis.

Results: We found that GSTM1 null polymorphism was significantly associated with the risk of CAD in overall population (OR = 1.37, \( p = 0.003 \)) and mixed population (OR = 1.61, \( p = 0.004 \)), GSTP1 null polymorphism was significantly associated with the risk of CAD in overall population (OR = 1.23, \( p = 0.03 \)), whereas GSTT1 null polymorphism was significantly associated with the risk of CAD in overall population (OR = 1.23, \( p = 0.02 \)), Caucasians (OR = 1.23, \( p = 0.02 \)) and East Asians (OR = 1.38, \( p < 0.0001 \)).

Conclusions: This meta-analysis demonstrated that GSTM1 null, GSTP1 null and GSTT1 null polymorphisms were all significantly associated with an increased risk of CAD.

Keywords: Glutathione S-transferase (GST), Null polymorphisms, Coronary artery disease (CAD), Meta-analysis

Background

Coronary artery disease (CAD) is featured by stenosis or even occlusion of coronary arteries, and their associated myocardial ischemia or infarction [1, 2]. The exact cause and pathogenesis of CAD are still unclear despite extensive researches. Nevertheless, accumulating evidence supports that genetic factors play a crucial part in its development. First, family aggregation of CAD has been observed extensively, and past twin studies have demonstrated that the heredity grade of CHD can be as high as 50% [3, 4]. Second, numerous genetic polymorphisms have been found to be associated with an increased risk of CAD by previous genetic association studies, and screening of common causal mutations has also been demonstrated to be an efficient way to predict the individual risk of developing CAD [5, 6]. Overall, these findings jointly indicate that genetic architecture is important for the occurrence and development of CAD.

Oxidative stress, characterized by accumulation of free radicals, membrane lipid peroxidation and DNA damage, has been found to play a critical role in the pathogenesis of various atherothrombotic disorders including CAD [7, 8]. Glutathione-S-transferases (GSTs) are a group of enzymes that play vital roles in regulating cellular detoxification of various exogenous toxins [9]. Moreover, it has been shown that GSTs have anti-oxidation effects and they can protect cells against oxidative stress and its associated DNA damage.

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damage [10]. Previous experimental studies have demonstrated that GST null polymorphisms, which include null polymorphisms of GSTM1 (mu), GSTP1 (pi) and GSTT1 (theta) can result in a diminished gene expression level and a reduced enzymatic activity of GST [11, 12]. Consequently, it is biologically plausible that GST null polymorphisms may also affect the risk of CAD. Over the last decade, investigators across the world have repeatedly attempted to assess the associations between GST null polymorphisms and the risk of CAD, with inconsistent findings. So a meta-analysis was performed by us to more robustly estimate the associations between GST null polymorphisms and the risk of CAD by integrating the results of previous publications.

**Methods**

This meta-analysis was conducted in accordance with the PRISMA guideline [13].

**Literature search and inclusion criteria**

Medline, Embase, Wanfang, VIP and CNKI were comprehensively searched by the authors using the below keywords: (glutathione S-transferase OR GST) AND (polymorphism OR polymorphic OR variation OR variant OR mutant OR mutation OR SNP OR genotypic OR genotype OR allelic OR allele) AND (coronary atherosclerotic heart disease OR coronary heart disease OR coronary artery disease OR ischemic heart disease OR angina pectoris OR acute coronary syndrome OR myocardial infarction OR CHD OR CAD OR IHD OR ACS OR MI). Moreover, we also manually screened the references of retrieved publications to make up for the potential incompleteness of literature searching from electronic databases.

Selection criteria of this meta-analysis were listed below: 1. Studies of case-control or cohort design; 2. Give genotypic frequencies of GST null polymorphisms in cases with CAD and population-based controls; 3. The full manuscript with detailed genotypic frequencies of GST null polymorphisms is retrievable or buyable. Articles would be excluded if one of the following three criteria is satisfied: 1. Studies without complete genotypic data of GST null polymorphisms in cases with CAD and population-based controls; 2. Narrative or systematic reviews, meta-analysis or comments; 3. Case series of subjects with CAD only. If duplicate reports are retrieved, we would only include the most complete one for integrated analyses.

**Data extraction and quality assessment**

The authors extracted the following data items from eligible studies: 1. Last name of the leading author; 2. Year of publication; 3. Country and ethnicity of study population; 4. The number of cases with CAD and population-based controls; 5. Genotypic frequencies of GST null polymorphisms in cases with CAD and population-based controls. The quality of eligible publications was assessed by the Newcastle-Ottawa scale (NOS) [14], and these with a score of 7 - 9 were considered to be of good quality. Two authors extracted data and assessed quality of eligible literatures in parallel. A thorough discussion until a consensus is reached would be endorsed in case of any discrepancy between two authors.

**Statistical analyses**

All statistical analyses in this meta-analysis were performed with the Cochrane Review Manager software. Associations between GST null polymorphisms and the risk of CAD were explored by using odds ratio and its 95% confidence interval. The statistically significant p value was set at 0.05. The authors used I² statistics to estimate heterogeneities among included studies. The authors would use DerSimonian-Laird method, which is also known as the random effect model, to integrate the results of eligible studies if I² is larger than 50%. Otherwise, the authors would use Mantel-Haenszel method, which is also known as the fixed effect model, to integrate the results of eligible studies. Meanwhile, the authors also conduct subgroup analyses by ethnic groups. The overall population (with all study subjects of eligible studies for each polymorphism included) can be divided into Caucasians, Asians or the mixed populations. If the authors specify the ethnic origin of study subjects in their publications, then we would use these data to divide the publications into different subgroups. But if the authors failed to specify the ethnic origin of study subjects in their publications, then we would use the location of the authors’ affiliations to divide the publications into different subgroups. For the mixed population, since the authors failed to specify the ethnic origin of study subjects and we could not judge the ethnic origin of study subjects from authors’ affiliations neither, it may have several scenarios, which can be a mixture of Caucasians and Africans, a mixture of Caucasians and Asians, a mixture of Africans and Asians, or a mixture of Caucasians, Asians and Africans. Stabilities of integrated results were tested by deleting one study each time, and then integrating the results of the rest of eligible studies. Publication biases were evaluated by assessing symmetry of funnel plots.

**Results**

**Characteristics of included studies**

One hundred and eighty-four publications were retrieved by using our searching strategy. Among these publications, nine duplicate reports as well as one hundred and four unrelated publications (papers that were not about GST null polymorphisms and the risk of CAD) were omitted, and 71 publications were then selected to screen for eligibility. Seventeen reviews and
seven case series were further excluded, and another two publications without complete genotypic data were further excluded by the authors. Totally 45 studies met the inclusion criteria, and were finally enrolled for integrated analyses (Fig. 1). The eligible studies were published between 1996 and 2020. Data extracted from eligible studies were summarized in Table 1.

**GSTM1 null polymorphism and the risk of CAD**

Thirty-seven studies (17,054 cases and 36,630 controls) assessed relationship between *GSTM1* null polymorphism and the risk of CAD. The integrated analyses demonstrated that *GSTM1* null polymorphism was significantly associated with the risk of CAD in overall population (OR = 1.37, *p* = 0.003) and mixed population (OR = 1.61, *p* = 0.004) (see Table 2 and Fig. 2).

**GSTP1 null polymorphism and the risk of CAD**

Eleven studies (4595 cases and 4390 controls) assessed relationship between *GSTP1* null polymorphism and the risk of CAD. The integrated analyses demonstrated that *GSTP1* null polymorphism was significantly associated with the risk of CAD in overall population (OR = 1.23, *p* = 0.03) (see Table 2 and Fig. 2).

**GSTT1 null polymorphism and the risk of CAD**

Thirty-nine studies (17,120 cases and 38,115 controls) assessed relationship between *GSTT1* null polymorphism...
Table 1: The characteristics of included studies in this meta-analysis

| First author, year | Country          | Ethnicity | Type of disease            | Sample size Case/Control | Null genotype [n(%)] Cases/Controls | NOS score |
|--------------------|------------------|-----------|-----------------------------|--------------------------|-------------------------------------|-----------|
| **GSTM1 null**     |                  |           |                             |                          |                                     |           |
| Abu-Amero 2006     | Saudi Arabia     | Mixed     | Coronary artery disease (CAD) | 1054/762                | 655 (62.1%) 117 (15.3%)             | 7         |
| Bazo 2011          | Brazil           | Mixed     | Coronary artery disease (CAD) | 297/96                  | 160 (53.8%) 40 (41.7%)              | 7         |
| Bhat 2016          | India            | Mixed     | Coronary artery disease (CAD) | 200/200                 | 62 (31.0%) 36 (18.0%)               | 8         |
| Bhatti 2018        | India            | Mixed     | Coronary artery disease (CAD) | 562/564                 | 217 (38.6%) 127 (22.5%)             | 7         |
| Cora 2013          | Turkey           | Caucasian | Myocardial infarction (MI)   | 324/296                 | 182 (56.1%) 143 (48.3%)             | 8         |
| Cornelis 2007      | Canada           | Caucasian | Myocardial infarction (MI)   | 2042/2042               | 980 (48.0%) 1041 (51.0%)            | 7         |
| Evans 1996         | Saudi Arabia     | Mixed     | Coronary artery disease (CAD) | 90/884                  | 57 (63.3%) 484 (54.8%)              | 7         |
| Girisha 2004       | India            | Mixed     | Coronary artery disease (CAD) | 197/198                 | 46 (23.4%) 41 (20.7%)               | 7         |
| Hayek 2006         | Israel           | Mixed     | Coronary artery disease (CAD) | 193/2399                | 88 (45.6%) 1142 (47.6%)             | 8         |
| Kadićli 2016       | Turkey           | Caucasian | Coronary artery disease (CAD) | 29/30                   | 17 (58.6%) 14 (46.7%)               | 7         |
| Kariž 2012         | Slovenia         | Caucasian | Myocardial infarction (MI)   | 206/257                 | 142 (69.0%) 166 (64.6%)             | 7         |
| Kim 2008           | Korea            | East Asian| Coronary artery disease (CAD) | 356/336                 | 198 (55.6%) 191 (56.8%)             | 7         |
| Li 2000            | USA              | Mixed     | Coronary artery disease (CAD) | 400/790                 | 178 (44.5%) 354 (44.8%)             | 7         |
| Macie 2009         | Brazil           | Mixed     | Coronary artery disease (CAD) | 869/1573                | 557 (64.1%) 789 (50.2%)             | 7         |
| Manfredi 2007      | Italy            | Caucasian | Coronary artery disease (CAD) | 169/53                  | 99 (58.6%) 24 (45.3%)               | 7         |
| Manfredi 2009      | Italy            | Caucasian | Coronary artery disease (CAD) | 184/47                  | 108 (58.7%) 18 (38.3%)              | 7         |
| Martin 2009        | USA              | Mixed     | Coronary artery disease (CAD) | 67/63                   | 41 (61.2%) 19 (30.2%)               | 7         |
| Masetti 2003       | Italy            | Caucasian | Coronary artery disease (CAD) | 308/122                 | 163 (52.9%) 66 (54.1%)              | 8         |
| Mir 2016           | India            | Mixed     | Coronary artery disease (CAD) | 100/100                 | 42 (42.0%) 26 (26.0%)               | 8         |
| Nomaní 2011        | Iran             | Mixed     | Coronary artery disease (CAD) | 209/108                 | 100 (47.8%) 57 (52.8%)              | 8         |
| Norskov 2011       | Denmark          | Caucasian | Coronary artery disease (CAD) | 4930/21684              | 2052 (41.6%) 11362 (52.4%)          | 7         |
| Olshanski 2003     | USA              | Mixed     | Coronary artery disease (CAD) | 526/868                 | 252 (47.9%) 352 (40.6%)             | 8         |
| Pašalić 2017       | Croatia          | Caucasian | Coronary artery disease (CAD) | 71/174                  | 29 (40.8%) 69 (39.7%)               | 7         |
| Phulukdaree 2012   | India            | Mixed     | Coronary artery disease (CAD) | 102/100                 | 37 (36.3%) 18 (18.0%)               | 7         |
| Poureramati 2020   | Iran             | Mixed     | Coronary artery disease (CAD) | 244/281                 | 128 (52.5%) 138 (49.1%)             | 8         |
| Ramprasath 2011    | India            | Mixed     | Coronary artery disease (CAD) | 290/270                 | 128 (44.1%) 56 (20.7%)              | 7         |
| Salama 2002        | USA              | Mixed     | Coronary artery disease (CAD) | 130/90                  | 45 (34.6%) 33 (36.7%)               | 7         |
| Singh 2011         | India            | Mixed     | Myocardial infarction (MI)   | 230/300                 | 56 (24.3%) 65 (21.7%)               | 8         |
| Tamir 2004         | Turkey           | Caucasian | Coronary artery disease (CAD) | 148/247                 | 67 (45.3%) 103 (41.7%)              | 7         |
| Tang 2009          | China            | East Asian| Coronary artery disease (CAD) | 277/277                 | 89 (32.1%) 59 (21.3%)               | 7         |
| Taspinar 2012      | Turkey           | Caucasian | Coronary artery disease (CAD) | 122/142                 | 51 (41.8%) 66 (46.5%)               | 7         |
| Wang 2002          | Australia        | Caucasian | Coronary artery disease (CAD) | 612/256                 | 343 (56.0%) 153 (59.8%)             | 7         |
| Wang 2008          | China            | East Asian| Coronary artery disease (CAD) | 277/277                 | 89 (32.1%) 59 (21.3%)               | 7         |
| Wilson 2000        | UK               | Caucasian | Myocardial infarction (MI)   | 356/187                 | 191 (53.7%) 107 (57.2%)             | 8         |
| Wilson 2003        | UK               | Mixed     | Coronary artery disease (CAD) | 170/203                 | 70 (41.2%) 107 (52.7%)              | 7         |
| Yeh 2013           | Taiwan           | East Asian| Coronary artery disease (CAD) | 458/209                 | 253 (55.2%) 121 (57.9%)             | 8         |
| Zhang 2011         | China            | East Asian| Coronary artery disease (CAD) | 255/145                 | 120 (47.1%) 46 (31.7%)              | 7         |
| **GSTP1 null**     |                  |           |                             |                          |                                     |           |
| Bhat 2016          | India            | Mixed     | Coronary artery disease (CAD) | 200/200                 | 132 (66.0%) 104 (52.0%)             | 8         |
| Bhatti 2018        | India            | Mixed     | Coronary artery disease (CAD) | 560/545                 | 366 (65.4%) 307 (56.3%)             | 7         |
| Cornelis 2007      | Canada           | Caucasian | Myocardial infarction (MI)   | 2042/2042               | 817 (40.0%) 817 (40.0%)             | 7         |
| Kariž 2012         | Slovenia         | Caucasian | Myocardial infarction (MI)   | 206/257                 | 135 (65.5%) 140 (54.5%)             | 7         |
Table 1 The characteristics of included studies in this meta-analysis (Continued)

| First author, year | Country        | Ethnicity | Type of disease               | Sample size Case/Control | Null genotype [n(%)] Cases Controls | NOS score |
|---------------------|----------------|-----------|-------------------------------|---------------------------|-------------------------------------|-----------|
| Kovacs 2014         | Hungary        | Caucasian | Myocardial infarction (MI)    | 54/78                     | 27 (50.0%) 26 (33.3%)               | 7         |
| Nomani 2011         | Iran           | Mixed     | Coronary artery disease (CAD) | 209/108                   | 118 (56.4%) 60 (55.5%)              | 8         |
| Phulukdaree 2012    | India          | Mixed     | Coronary artery disease (CAD) | 102/100                   | 36 (35.3%) 52 (52.0%)               | 7         |
| Pourkeramati 2020   | Iran           | Mixed     | Coronary artery disease (CAD) | 244/281                   | 64 (26.2%) 56 (19.9%)               | 8         |
| Ramprasad 2011      | India          | Mixed     | Coronary artery disease (CAD) | 290/270                   | 196 (67.6%) 152 (56.3%)             | 7         |
| Singh 2011          | India          | Mixed     | Myocardial infarction (MI)    | 230/300                   | 90 (39.1%) 117 (39.0%)              | 8         |
| Yeh 2013            | Taiwan         | East Asian| Coronary artery disease (CAD) | 458/209                   | 125 (27.3%) 59 (28.2%)              | 8         |
| GSTT1 null          |                |           |                               |                           |                                     |           |
| Abu-Amero 2006      | Saudi Arabia   | Mixed     | Coronary artery disease (CAD) | 1054/762                  | 463 (43.9%) 66 (8.7%)               | 7         |
| Bazo 2011           | Brazil         | Mixed     | Coronary artery disease (CAD) | 297/100                   | 69 (23.2%) 19 (19.0%)               | 7         |
| Bhat 2016           | India          | Mixed     | Coronary artery disease (CAD) | 200/200                   | 12 (6.0%) 25 (12.5%)                | 8         |
| Bhatti 2018         | India          | Mixed     | Coronary artery disease (CAD) | 562/564                   | 86 (15.3%) 129 (22.9%)              | 7         |
| Cosa 2013           | Turkey         | Caucasian | Myocardial infarction (MI)    | 324/296                   | 106 (32.7%) 63 (21.3%)              | 8         |
| Cornelis 2007       | Canada         | Caucasian | Myocardial infarction (MI)    | 2042/2042                 | 388 (19.0%) 408 (20.0%)             | 7         |
| Decharatchakul 2020 | Thailand       | East Asian| Coronary artery disease (CAD) | 279/735                   | 115 (41.9%) 242 (32.9%)             | 8         |
| Garcia 2018         | Mexico         | Mixed     | Coronary artery disease (CAD) | 79/101                    | 15 (19.0%) 8 (7.9%)                 | 7         |
| Girlisha 2004       | India          | Mixed     | Coronary artery disease (CAD) | 197/198                   | 15 (7.6%) 36 (18.2%)                | 7         |
| Hayek 2006          | Israel         | Mixed     | Coronary artery disease (CAD) | 193/2399                  | 30 (15.5%) 392 (16.3%)              | 8         |
| Kadiçoğlu 2016      | Turkey         | Caucasian | Coronary artery disease (CAD) | 29/30                     | 6 (20.7%) 5 (16.7%)                 | 7         |
| Kariž 2012          | Slovenia       | Caucasian | Myocardial infarction (MI)    | 206/257                   | 77 (37.4%) 108 (42.0%)              | 7         |
| Kim 2008            | Korea          | East Asian| Coronary artery disease (CAD) | 356/336                   | 196 (55.0%) 187 (55.7%)             | 7         |
| Li 2000             | USA            | Mixed     | Coronary artery disease (CAD) | 400/890                   | 74 (18.5%) 166 (18.7%)              | 7         |
| Lakshmi 2012        | India          | Mixed     | Coronary artery disease (CAD) | 352/282                   | 81 (23.0%) 39 (13.8%)               | 7         |
| Levinson 2014       | Sweden         | Caucasian | Coronary artery disease (CAD) | 112/1221                  | 11 (9.8%) 168 (13.8)                | 7         |
| Macie 2009          | Brazil         | Mixed     | Coronary artery disease (CAD) | 869/1573                  | 209 (24.1%) 337 (21.4%)             | 7         |
| Manfredi 2007       | Italy          | Caucasian | Coronary artery disease (CAD) | 169/53                    | 95 (56.2%) 13 (24.5%)               | 7         |
| Manfredi 2009       | Italy          | Caucasian | Coronary artery disease (CAD) | 184/47                    | 84 (45.7%) 13 (27.7%)               | 7         |
| Martin 2009         | USA            | Mixed     | Coronary artery disease (CAD) | 67/63                     | 12 (17.9%) 12 (19.7%)               | 7         |
| Masetti 2003        | Italy          | Caucasian | Coronary artery disease (CAD) | 308/122                   | 117 (38.0%) 40 (32.8%)              | 8         |
| Mir 2016            | India          | Mixed     | Coronary artery disease (CAD) | 100/100                   | 23 (23.0%) 16 (16.0%)               | 8         |
| Nomani 2011         | Iran           | Mixed     | Coronary artery disease (CAD) | 209/108                   | 16 (7.7%) 17 (15.7%)                | 8         |
| Norskov 2011        | Denmark        | Caucasian | Coronary artery disease (CAD) | 4930/21684                | 740 (15.0%) 3161 (14.6%)            | 7         |
| Olshan 2003         | USA            | Mixed     | Coronary artery disease (CAD) | 526/868                   | 75 (14.3%) 165 (19.0%)              | 8         |
| Palmer 2003         | UK             | Caucasian | Coronary artery disease (CAD) | 51/57                     | 40 (78.4%) 35 (61.4%)               | 7         |
| Pašalić 2017        | Croatia        | Caucasian | Coronary artery disease (CAD) | 68/177                    | 17 (25.0%) 54 (30.5%)               | 7         |
| Pourkeramati 2020   | Iran           | Mixed     | Coronary artery disease (CAD) | 244/281                   | 129 (52.9%) 143 (50.8%)             | 8         |
| Ramprasad 2011      | India          | Mixed     | Coronary artery disease (CAD) | 290/492                   | 136 (46.9%) 118 (24.0%)             | 7         |
| Salama 2002         | USA            | Mixed     | Coronary artery disease (CAD) | 130/90                    | 32 (26.7%) 14 (15.6%)               | 7         |
| Singh 2011          | India          | Mixed     | Myocardial infarction (MI)    | 230/300                   | 23 (10.0%) 61 (20.3%)               | 8         |
| Tamer 2004          | Turkey         | Caucasian | Coronary artery disease (CAD) | 148/247                   | 48 (32.4%) 70 (28.3%)               | 7         |
| Tang 2009           | China          | East Asian| Coronary artery disease (CAD) | 277/277                   | 77 (27.8%) 53 (19.1%)               | 7         |
| Taspinar 2012       | Turkey         | Caucasian | Coronary artery disease (CAD) | 122/142                   | 28 (23.0%) 25 (17.6%)               | 7         |
| Wang 2008           | China          | East Asian| Coronary artery disease (CAD) | 277/277                   | 77 (27.8%) 53 (19.1%)               | 8         |
and the risk of CAD. The integrated analyses demonstrated that GSTT1 null polymorphism was significantly associated with the risk of CAD in overall population (OR = 1.23, \( p = 0.02 \)), Caucasians (OR = 1.23, \( p = 0.02 \)) and East Asians (OR = 1.38, \( p < 0.0001 \)) (see Table 2 and Fig. 2).

### Sensitivity analyses

The authors examined stabilities of integrated analyses results by deleting one study each time, and then integrating the results of the rest of studies. The trends of associations were not significantly altered in sensitivity analyses, which indicated that from statistical perspective, our integrated analyses results were reliable and stable (Relevant datasets can be found at https://osf.io, username: suhonglingxxx@163.com, password: suhonglingxxx@).

### Publication biases

The authors examined potential publication biases in this meta-analysis by assessing symmetry of funnel plots. Funnel plots were found to be generally symmetrical, which indicated that our integrated analyses results were not likely to be severely deteriorated by publication biases (see Fig. 3).

### Discussion

To our knowledge, this is so far the very first meta-analysis regarding associations of GSTM1 and GSTP1 null polymorphisms with the risk of CAD, and this is also so far the most complete meta-analysis regarding GSTT1 null polymorphism and the risk of CAD. The integrated analyses showed that GSTM1 null, GSTP1 null and GSTT1 null polymorphisms were all significantly associated with an increased risk of CAD. Sensitivity analyses suggested that the positive associations observed were quite statistically robust, and no publication bias was detected.

The following points are worth noting when interpreting our integrated findings. Firstly, based on the findings of previous observational studies, we speculated that the investigated GST null polymorphisms may lead to a diminished gene expression level of GST, which may subsequently affect biological functions of GST, result in excessive oxidative stress and ultimately increase the risk of CAD [11, 12]. Secondly, considering that the functional significances of investigated GST null polymorphisms are well established. Our pooled analyses may be still statistically inadequate to detect the actual associations between GST null polymorphisms and CAD in certain ethnic subgroups. Therefore, further studies with larger sample sizes in different populations still

### Table 1

The characteristics of included studies in this meta-analysis (Continued)

| First author, year | Country | Ethnicity | Type of disease | Sample size (Case/Control) | Null genotype [n(%)] Cases Controls | NOS score |
|-------------------|---------|-----------|-----------------|---------------------------|-----------------------------------|-----------|
| Wilson 2000       | UK      | Caucasian | Myocardial infarction (MI) | 356/187 | 90 (25.3%) 36 (19.3%) | 8         |
| Wilson 2003       | UK      | Mixed     | Coronary artery disease (CAD) | 170/203 | 34 (20.0%) 44 (21.7%) | 7         |
| Yeh 2013          | Taiwan  | East Asian| Coronary artery disease (CAD) | 458/209 | 276 (60.3%) 110 (52.6%) | 8         |
| Zhang 2011        | China   | East Asian| Coronary artery disease (CAD) | 255/145 | 141 (55.3%) 60 (41.4%) | 7         |

Abbreviations: HWE Hardy-Weinberg equilibrium, NOS Newcastle-Ottawa scale, NA Not available

### Table 2

Integrated analyses for GST null polymorphisms and CAD

| Polymorphisms | Population | Sample size (Cases/controls) | Null genotype vs. Present genotype | \( P \) value | OR (95%CI) | \( I^2 \) statistic |
|---------------|------------|------------------------------|-----------------------------------|--------------|-----------|-------------------|
| GSTM1 null    | CAD        | 17054/36630                 | 0.003                             | 1.37 (1.11-1.70) | 95%       |
|               | Caucasian  | 9501/25537                  | 0.72                              | 1.04 (0.85-1.26) | 84%       |
|               | East Asian | 1623/1244                   | 0.07                              | 1.35 (0.97-1.88) | 76%       |
|               | Mixed population | 5930/9849              | 0.004                             | 1.61 (1.16-2.22) | 94%       |
| GSTP1 null    | CAD        | 4595/4390                   | 0.03                              | 1.23 (1.02-1.48) | 70%       |
|               | Caucasian  | 2302/2377                   | 0.17                              | 1.35 (0.88-2.07) | 76%       |
|               | East Asian | 1835/1804                   | 0.11                              | 1.23 (0.96-1.50) | 68%       |
|               | Mixed population | 17120/38115            | 0.02                              | 1.23 (1.03-1.46) | 89%       |
| GSTT1 null    | CAD        | 17120/38115                 | 0.02                              | 1.23 (1.03-1.47) | 67%       |
|               | Caucasian  | 9049/26562                  | 0.02                              | 1.23 (1.03-1.47) | 67%       |
|               | East Asian | 1902/1979                   | < 0.0001                          | 1.38 (1.20-1.59) | 36%       |
|               | Mixed population | 6169/9574              | 0.61                              | 1.11 (0.76-1.62) | 94%       |

Abbreviations: OR Odds ratio, CI Confidence interval, NA Not available, CAD Coronary artery disease

The values in bold represent there is statistically significant differences between cases and controls.
**Fig. 2** Forest plots for this meta-analysis

**a. Forest plot of GSTM1 null polymorphism and CAD**

**b. Forest plot of GSTP1 null polymorphism and CAD**

**c. Forest plot of GSTT1 null polymorphism and CAD**
Fig. 3 Funnel plots for this meta-analysis
need to confirm our findings. Thirdly, we want to study all polymorphic loci of the GST gene initially. Nevertheless, our comprehensive literature searching did not reveal sufficient eligible studies to support integrated analyses for any other polymorphic loci of the GST gene, so we only explored associations with the risk of CAD for three most commonly investigated polymorphisms of the GST gene in this meta-analysis. Fourthly, it is worth noting that previously, Song et al. [15] also tried to investigate associations between GSTT1 null polymorphism and the risk of CAD through a meta-analysis. Nevertheless, this previous meta-analysis only covered relevant genetic association studies that were published before 2014. Since our literature searching revealed that many related studies were published after 2014, an updated meta-analysis like ours is warranted to get more reliable findings. Consistent with the previous meta-analysis, a similar significant finding for GSTT1 null polymorphism was observed in our integrated analyses. Considering that our updated analyses were derived from more eligible studies, our observations should be considered as a valuable confirmation for pre-existing literatures. Fifthly, GST null polymorphisms have also been found to be closely associated with the risk of diabetes, essential hypertension and other types of atherothrombotic disorders such as ischemic stroke or peripheral artery disease [16–20]. Considering that the above mentioned diseases are either considered to be conventional risk factors of CAD or usually manifest as co-morbid conditions of CAD, it would be interesting to perform some stratified analyses accordingly. Nevertheless, due to the fact that the vast majority of eligible studies failed to report genotypic data according to co-morbid conditions, it is impossible for us to conduct such analyses, and we highly recommend future genetic association studies to carry out stratified analyses according to the co-morbid status of these diseases.

The major limitations of our integrated analyses were listed below. Firstly, our integrated analyses results were derived from unadjusted pooling of previous studies. Without access to raw data of eligible studies, we can only assess associations between GST null polymorphisms and the risk of CAD based on re-calculations of raw genotypic frequencies provided by eligible studies, and we need to admit that lack of further adjustment for baseline characteristics such as age, gender or co-morbid conditions may possibly influence reliability of our findings [21]. Secondly, environmental factors such as smoking status, eating habits or exercise levels may also influence associations between polymorphisms in GST null polymorphisms and the risk of CAD. However, since most of previous studies only paid attention to genetic associations, it is almost impossible for us to explore genetic-environmental interactions in a meta-analysis based on these previous literatures [22]. Thirdly, we did not select ‘grey literatures’ that were not formally published in peer-reviewed scientific journals for integrated analyses because these literatures are generally considered to be incomplete and it is almost impossible for us to extract all necessary data items from these literatures or assess their quality through the NOS scale. Nevertheless, since we did not select ‘grey literatures’ for integrated analyses, despite that funnel plots were found to be overall symmetrical, it should be acknowledged that publication biases still may influence reliability of our integrated analyses results [23].

Conclusion
In conclusion, this meta-analysis demonstrated that GSTM1 null, GSTP1 null and GSTT1 null polymorphisms were all significantly associated with an increased risk of CAD. These findings suggested that GSTM1 null, GSTP1 null and GSTT1 null polymorphisms may have the potential to serve as genetic biomarkers of CAD and they may be used to identify subjects at higher risk of developing CAD. Further studies with larger sample sizes in different populations are still needed to confirm our findings. Moreover, experimental studies are also warranted to reveal the exact underlying mechanisms of the positive associations observed between above mentioned GST null polymorphisms and the risk of CAD in the future.

Abbreviations
GST: Glutathione S-transferase; CAD: Coronary artery disease; HWE: Hardy-Weinberg equilibrium; NOS: Newcastle-Ottawa scale; OR: Odds ratios; CI: Confidence intervals

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Authors' contributions
HS and YC conceived and designed this meta-analysis. YC and JL searched literatures. YZ and XM analyzed data. HS and YC wrote the manuscript. All authors have approved the final manuscript as submitted.

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