Introduction

Coronary artery disease (CAD) and myocardial infarction (MI) are principal public health contributors to worldwide death and disability, and as such are primary causes of economic burden [1]. About 1 of every 6 deaths was attributed to CAD in the United States in 2008 and as rough estimate 785,000 Americans will have a new coronary attack and 470,000 will have a recurrent attack annually [2]. The multiple potential pathologic pathways of CAD have been widely investigated, ranging from cholesterol accumulation to plaque rupture with thrombus formation [3]. Among these pathways, oxidative stress is thought to play a pivotal role in the pathophysiology of atherosclerosis, which is manifested as increased availability of reactive oxygen species (ROS) and reactive nitrogen species because of an imbalanced redox state. ROS has been implicated in a variety of biological functions, including induction of gene expression and promotion of endothelial cell and smooth muscle cell (SMC) proliferation. Imbalance of ROS production is involved in oxidation of low density lipoprotein, which consequently promotes the progression of atherosclerosis [4,5].

NAD(P)H oxidase is the predominant cellular source of ROS in the context of atherosclerosis [6]. The phagocyte-specific cytochrome b, as an essential component of NAD(P)H oxidase, plays an important role in regulating NAD(P)H activity and ROS production. Phagocyte-specific cytochrome b is a membrane-associated heterodimer, consisting of 2 subunits (gp91phox and p22phox [CYBA]). [7]. P22phox is essential for vascular cell production of superoxide anion. Human p22phox is encoded by the CYBA gene which is located on 16q24. One of the most
common polymorphisms of CYBA gene is C242T(rs4673), resulting in a non-conservative substitution of histidine for tyrosine at codon 72 and an alteration of NAD(P)H activity by disrupting the heme binding site [8]. The C242T polymorphism has been demonstrated to be related to multiple inflammatory diseases [7] and metabolic disorders [9,10]. Recent epidemiological studies suggested that this polymorphism could be associated with CAD [11,12], but this could not be confirmed by other studies [13,14]. The current discordance in reported studies may result from real racial diversity, restrictive sample size and unadjusted environmental confounders. Large meta-analysis makes it possible to achieve a more precise evaluation of this issue. Although a previous meta-analysis of the C242T polymorphism indicated a significant protective effect among Asians, they had a relative small sample size and merely concerned the ethnic difference [15]. In addition, some studies they included were derived from Hardy-Weinberg equilibrium (HWE) [8,16]. None of these studies had comprehensively compared the effect of the C242T polymorphism on CAD risk in view of study differences. To maximize statistical power and robustly assess the relationship, we have incorporated the published studies to date and meta-analyzed the effect of the C242T polymorphism on CAD risk, while addressing heterogeneity and publication bias. We aimed to systematically estimate the CAD risk of the C242T polymorphism taking different study characteristics into account and go deeply into the origin of the heterogeneity.

Materials and Methods

Search strategy
We collected data by searching from PubMed/MEDLINE, Embase, CNKI (China Nation Knowledge Infrastructure Platform), Wanfang and CBM (China Biological Medicine Database) electronic databases up to January 2013. The search strategy followed the guidelines of the 2009 preferred reporting items for systematic reviews and meta-analyses (PRISMA) statement (Checklist S1) [17]. A combination of the following key words was used in the computer-based searches: ‘C242T’ or ‘rs4673’, ‘nicotinamide adenine dinucleotide phosphate oxidase’ or ‘NAD (P)H oxidases’, ‘cytochrome b’ or ‘CYBA’, ‘ph22phox’ and ‘coronary’ or ‘ischemic heart disease’ or ‘myocardial infarction’ (P)H oxidas’, ‘cytochrome b’ or ‘CYBA’, ‘ph22phox’ and ‘nicotinamide adenine dinucleotide phosphate oxidase’ or ‘NAD (P)H oxidases’, ‘cytochrome b’ or ‘CYBA’, ‘ph22phox’ and ‘coronary’ or ‘ischemic heart disease’ or ‘myocardial infarction’.

Selection criteria
All studies related to the association of the C242T polymorphism with CAD risk were potentially involved. The following criteria were adopted to the selection: (1) Articles published in English or Chinese journals or their supplements; (2) Studies providing adequate information on the C242T polymorphism by case-control status (studies without controls were excluded); (3) Published articles of human genetics (full texts or abstracts) without ethnicity restriction; (4) If articles involved more than one geographic or racial group, each group was considered as a separate study; (5) If multiple studies stemmed from the same population, only the largest scale study was included to avoid overlapping data; (6) The genotype frequency amongst control must conform to HWE.

Data Extraction
We undertook a standard data-collection protocol following the inclusion criteria described above. Two authors (Z.W. and Y. Lou) independently extracted the relevant characteristics from each selected study and entered the data into separate databases in duplicate. Moreover, the results of data extraction were compared and any encountered divergences were resolved at a consensus meeting. The following information was gathered on the C242T polymorphism on the basis of different cohort: First author’s name, publication year, ethnicity, country or area, study design, population source, disease outcomes, matching variables, clinical characteristics for case-patients and controls (such as age, body mass index [BMI], circulating lipid profiles and fibrinogen levels, the proportion of male, hypertension [HTN] , diabetes mellitus [DM] , smoking status and alcohol intake), the distribution of the C242T genotype both in patients and controls and conformity to genotype frequencies with HWE. Continuous variables manifested themselves as mean ± standard deviation (SD) or median (5th and 95th percentiles).

Quality Assessment
The Newcastle-Ottawa Scale (NOS) [22] was used to assess the methodological quality of all eligible studies. The NOS provides an easy and convenient tool for quality assessment of non-randomized studies based on three broad perspectives: Selection, Comparability, and Exposure for case-control studies. In our meta-analysis, age, gender, BMI and smoking were considered as important confounder factors. A study can be awarded a maximum of one star for each numbered item with the Selection and Exposure categories, and a maximum of two stars for Comparability. Studies can be awarded a maximum score of 9 stars with scores of 5 stars or more regarded to be of medium to high study quality. Quality assessment of each individual study was performed by three authors separately (Z.W., Y. Lou, and Q.C.). Authors W.J. and Y. Liu were consulted for any encountered disagreement.

Statistical analysis
The synthetic studies’ odd ratios (ORs) corresponding to 95% confidence intervals (CIs) for the relationship between the C242T polymorphism and CAD risk were calculated under different inheritance models, containing allele comparison (T versus C), dominant genetic model (CT+TT versus CC), recessive genetic model (TT versus CT+CC) and homozygote comparison (TT versus CC). We appraised the individual effect size together and modulated the study weights based on in-study variance by exploiting the DerSimonian & Laird method in a random-effects model. The possibility of heterogeneity was also assessed by using the Mantel-Haenszel model [23]. The between-study heterogeneity was estimated using Cochran’s chi-square based Q statistic test [24] and P<0.1 was considered significantly heterogeneous among studies. The degree of consistency was estimated via index I² statistic, which determines the percentage of the between-study
variability caused by heterogeneity rather than chance. Large values of $I^2$ strongly implied the presence of between-study heterogeneity [25,26]. The significance of the pooled OR was evaluated by the Z test, which was considered to be significant for $P < 0.05$. Additionally, we carried out a meta-regression with restricted maximum likelihood estimation to investigate the extent to which covariates explained genetic heterogeneity among the individual ORs. To further assess the origins of inter-study heterogeneity, we examined pre-specified groupings of study characteristics with homogeneous effects, such as ethnicity (Caucasian, Asian and others), study design (matched and not mentioned), population source (hospital-based [H-B] and population-based [P-B]) and endpoints (CAD, ACS and MI).

We undertook a sensitivity analysis by successive one-study removal approach to identify the most influential studies, likely introducing bias to the overall estimation [27]. The visual funnel plot and Egger’s test were used to appraise the probability of publication bias. The standard error of log (OR) of CAD risk under the allele comparison was plotted against its OR for each study. The publication bias could be visual by an asymmetric plot and be confirmed by Egger’s linear regression test [28]. Egger’s test was considered significant for $P < 0.05$. A cumulative meta-analysis might indicate whether initial published research had an impact on subsequent publications and the evolution of the pooled estimates with ascending date of publication.

Consistency of the C242T polymorphism to HWE was examined via the chi-square test or Fisher’s exact test based on a Web program [http://ihg2.helmholtz-muenchen.de/cgi-bin/hw/hwa1.pl]. Data processing and statistical analyses were performed using Review Manager software release 5.0 (Oxford,
Table 1. The baseline characteristics of all qualified studies included in the meta-analysis.

| First Author | Year | Ethnicity | geographic location | Source | Endpoint | Study design | Status | Age, year | Gender, M(%) | HTN,% | DM,% | Smoke,% | BMI, kg/m² |
|-------------|------|-----------|---------------------|--------|----------|--------------|--------|-----------|-------------|-------|------|---------|------------|
| Cai H       | 1999 | Caucasian | Australia           | H-B    | CAD      | not mentioned | cases  | 56.9 ±7.0 | 80          | 46.3  | 13.6 | 71.2    | 28.2 ±4.7  |
| controls    |      |           |                     |        |          |              |        | 54.5 ±8.3 | 46.8        | 35.8  | 5.9  | 52.6    | 27.6 ±4.7  |
| Fan M       | 2006 | Caucasian | Finland             | P-B    | CAD      | not mentioned | cases  | 62 ±10   | 77          | 95    | 29   | 59      | 27.3 ±4.1  |
| controls    |      |           |                     |        |          |              |        | 55 ±11   | 58          | 95    | 16   | 52      | 27.2 ±4.6  |
| Fang SX     | 2012 | Asian     | China               | H-B    | CAD      | matched      | cases  | 50.6 ±7.4 | 68.6        | 46.5  | –   | –       | 39.5        |
| controls    |      |           |                     |        |          |              |        | 68.5 ±7.4| –           | –     | –   | –       | –           |
|              |      |           |                     |        |          |              |        | 496 ±8.7 | 73          | 36.2  | –   | –       | 46.7        |
| Gardemann A | 1999 | Caucasian | Germany             | H-B    | CAD      | not mentioned | cases  | 62.7 ±9.3| –           | 65    | 20   | –       | 26.9 ±3.3  |
| controls    |      |           |                     |        |          |              |        | 58.5 ±10.5| –           | 54    | 11   | –       | 26.9 ±3.5  |
| Goliach G   | 2011 | Caucasian | Vienna              | H-B    | MI      | matched      | cases  | 37.3(33.8–39) | 87.1   | 42   | 30   | 78      | 27.5(24.3–29.8) |
| controls    |      |           |                     |        |          |              |        | 34.7(30.6–38.3)| 90     | 17.5 | 12.5 | 43.1    | 24.6(22.4–27.8) |
| He MA       | 2007 | Asian     | China               | H-B    | CAD      | not mentioned | cases  | 64.7 ±103| 57          | 64.6  | 21.59| 49.2    | 24.2 ±3.5  |
| controls    |      |           |                     |        |          |              |        | 61.9 ±7.3| 51.6         | 34.6  | 6.57 | 37.4    | 24.6 ±3.4  |
| Katakami N  | 2010 | Asian     | Japan               | H-B    | MI      | not mentioned | cases  | 62.2 ±9.4| 64.2         | 82.3  | 100  | 45.1    | 24.0 ±3.5  |
| controls    |      |           |                     |        |          |              |        | 59.5 ±10.5| 60.6         | 72.9  | 100  | 43.2    | 24.2 ±3.8  |
| Lee WH      | 2001 | Asian     | Korea               | P-B    | CAD      | not mentioned | cases  | 56.1 ±10.7| 100         | 37.1  | 19   | 79      | 24.4 ±3.2  |
| controls    |      |           |                     |        |          |              |        | 50.2 ±10.1| 100         | 7     | 5.1  | 75.8    | 23.9 ±2.7  |
| Li A        | 1999 | Other     | US                  | H-B    | CAD      | not mentioned | cases  | 60.8     | 76.5         | 47.7  | 27.5 | 12.1    | –           |
| controls    |      |           |                     |        |          |              |        | 57 ±1.1  | 47.6         | 40.8  | 8.7  | 14.6    | –           |
| Macias-Reyes A | 2008 | Caucasian | Spain               | P-B    | ACS     | matched      | cases  | 56 ±10  | 78          | –     | 33.9 | 50      | 27.2 ±3.7  |
| controls    |      |           |                     |        |          |              |        | 54.5 ±11 | 73.7         | –     | 12.1 | 27.3    | 27.3 ±3.8  |
| Morgan TM   | 2007 | Caucasian | US                  | H-B    | ACS     | matched      | cases  | 60.7 ±1.2(M) | 67.8   | 60.1 | 23.8 | 33.1    | 29.1 ±5.5(M) |
| controls    |      |           |                     |        |          |              |        | 63.1 ±13.2(M)| –     | –   | –       | 28.9 ±6.9(F) |
|             |      |           |                     |        |          |              |        | 61.8 ±12.8(F)| –     | –   | –       | 27.7 ±6.9(F) |
| Najafi M    | 2012 | Caucasian | Iran                | H-B    | CAD      | matched      | cases  | 62.8 ±11 | 67.5         | –     | –   | 24.6    | 25.5 ±4.3  |
| controls    |      |           |                     |        |          |              |        | 55.9 ±13.9| 32.4         | –     | –   | 17.7    | 26.2 ±6.2  |
| Narn K      | 2012 | Others    | India               | H-B    | CAD      | not mentioned | cases  | –        | –           | –     | 100 | –       | –           |
| controls    |      |           |                     |        |          |              |        | –        | –           | –     | 100 | –       | –           |
| Nasti S     | 2006 | Caucasian | Italia              | H-B    | CAD      | not mentioned | cases  | 65.5 ±100| 83.2         | 69.1  | 26.2 | 71.6    | 26.2 ±3.3  |
| controls    |      |           |                     |        |          |              |        | 62.2 ±14.8| 67.9         | 56.3  | 13.5 | 77.2    | 26.0 ±4.4  |
| Niemiec P   | 2007 | Caucasian | Poland              | P-B    | CAD      | matched      | cases  | 43.8 ±5.9| 66.9         | 59.3  | 5.8  | 57.6    | 27.0 ±3.3  |
| controls    |      |           |                     |        |          |              |        | 34.2 ±10.4| 69.2         | 4.1   | 0    | 32      | 24.6 ±3.9  |
| Nikitin AG  | 2009 | Caucasian | Russia              | H-B    | CAD      | matched      | cases  | 59.2 ±7.9| 54.3         | 29.3  | 0    | 21.6    | 28.4 ±5.1  |
Table 1. Cont.

| First Author | Year | Ethnicity | geographic location | Source | Endpoint | Study design | Status | Age, year | Gender, M(%) | HTN, % | DM, % | Smoke, % | BMI, kg/m² |
|--------------|------|-----------|---------------------|--------|----------|-------------|--------|-----------|-------------|--------|--------|----------|------------|
| Saha N[Chinese] | 1999 | Asian | Singapore | P-B | CAD | matched | cases | 55.3±9.8 | 100 | – | 28.5 | 61.6 | 23.9±3.7 |
| Saha N[Indian] | 1999 | Others | Singapore | P-B | CAD | matched | cases | 55.2±7.9 | 88.1 | – | 44.4 | 43.7 | 24.9±3.3 |
| Stanger O | 2001 | Caucasian | Austria | H-B | CAD | matched | cases | 52.9±5.8 | 100 | 40.7 | 0 | 60.2 | 26.6±2.9 |
| Vasiliadou C | 2007 | Caucasian | Greece | H-B | MI | matched | cases | 46.9±1.1 | – | – | – | – | – |
| Yamada Y | 2002 | Asian | Japan | H-B | MI | not mentioned | cases | 62.1±10.1 | 100 | 47 | 34.7 | 57.8 | 23.6±2.9 |
| Zafari AM | 2002 | Caucasian | US | H-B | CAD | not mentioned | cases | 56.4±17.4d | 98.8 | 70.1 | 39 | 79.9 | – |
|             |      |           |        |      |        |        | controls | 57.1±9.3 | 94.2 | 63.5 | 28.8 | 63.5 | – |

P-B: population-based study; H-B: hospital-based study; CAD: coronary artery disease; MI: myocardial infarction; ACS: acute coronary syndrome; M(%): male(percent); F: female; HTN: hypertension; DM: diabetes mellitus; BMI: body mass index;

a: early-onset coronary artery disease;
b: late-onset coronary artery disease;
c: data not available;
d: single-vessel disease;
e: multi-vessel disease; Age and BMI are expressed as mean ± SD (standard deviation) or median (5th and 95th percentiles).

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England) and Stata 11.0 (Stata Corporation, College Station, Texas, USA). All P values were 2-sided.

**Results**

Flow of included studies

The flow diagram schematizing the selection process of included studies is shown in Figure 1. A total of 150 relevant publications were retrieved in accordance with our search strategy and inclusion criteria. After applying selection criteria, 124 studies were removed. The data from 26 studies depicting the association between the C242T polymorphism and CAD risk were pooled into the provisional data set. Among these studies, 2 data sets conducted in the Chinese [29] and Japanese population [10] were overlapped by other studies with larger cohort [11,30]. Moreover, the genotyping frequency in the control population of Mata-Balaguer et al. [16] and Inoue et al. [8] did not conform to HWE (PHWE = 0.034 & 0.029); these two studies were precluded. Because the study by Saha et al. [31] contained data from two different racial descents (Chinese & Indian), we treated them as two independent studies when estimating the individual effect sizes. The Indian population was classified into the “others” group as its lineage was miscellaneous and cannot simply be grouped as Asian or Caucasian [32–34]. Because the study population used in Li et al. [14] was of mixed background (82.9% of Caucasian), this study together with 2 Indian studies [31,35], were collapsed into the “others” group.

In total, 21 publications involving 22 studies (20 articles written in English [11,13,14,30,31,35–49] and 1 in Chinese) with sufficient information fulfilled our search strategy. Of the 22 qualifying studies, 13 used a Caucasian population [13,36–39,41–48], 6 used an Asian population [11,30,31,40,49] and 3 performed in others [14,31,35]. All studies were published between 1999 and 2012 and utilized a retrospective case-control design. P-B controls were adopted in 6 studies [31,37,41,45,49] and H-B in 16 studies [11,13,14,30,35,36,38–40,42–44,46–48]. CAD patients and controls were matched for age and gender in 11 studies [13,31,39,41–43,45–47], while the other 11 failed to mention this in their study design [11,14,30,35–38,40,44,48,49]. CAD was employed as the primary outcome in 16 of the studies [13,14,30,31,35–38,43–46,48,49], while MI and ACS were taken as endpoints in 2 studies [41,42], respectively.

Quantitative data synthesis

Our meta-analysis encompassed 22 studies with 9,279 CAD patients and 9,349 controls. The selected characteristics of these eligible studies were shown in Table 1. The allele and genotype frequencies of the C242T polymorphism of each individual study which corresponded to HWE (PHWE>0.05) were also listed in Table 2. The combined overall frequency of the T allele was 23.8% in cases and 18.0% in controls. The frequency of the T

### Table 2. Sample size, the distribution of the C242T allele frequencies and genotypes among CAD patients and controls, and P value of HWE in controls.

| First Author | Sample size cases | T allele, % | C allele, % | TT genotype cases | CT genotype cases | CC genotype cases | HWE, P value |
|--------------|-------------------|------------|------------|------------------|------------------|------------------|-------------|
| Cai H        | 550               | 34.5       | 31.7       | 65.5             | 68.3             | 65               | 0.72        |
| Fan M        | 250               | 18.0       | 25.0       | 82.0             | 75.0             | 0.10             | 0.12        |
| Fang SX      | 746               | 9.5        | 6.5        | 90.5             | 93.5             | 0.04             | 0.13        |
| Gardemann A  | 1706              | 34.1       | 33.9       | 65.9             | 66.1             | 207              | 0.12        |
| Gollasch G   | 102               | 34.8       | 36.7       | 65.2             | 63.3             | 11               | 0.11        |
| He MA        | 565               | 3.2        | 5.6        | 96.8             | 94.4             | 2                | 0.04        |
| Katakan N    | 226               | 8.0        | 9.5        | 92.0             | 90.5             | 0                | 0.93        |
| Lee WH       | 305               | 7.9        | 11.9       | 92.1             | 88.1             | 4                | 0.19        |
| Li A         | 149               | 41.6       | 34.0       | 58.4             | 66.0             | 27               | 0.36        |
| Macías-Reyes A | 304        | 38.5       | 37.8       | 61.5             | 62.2             | 48               | 0.12        |
| Morgan TM    | 811               | 34.7       | 33.7       | 65.3             | 66.3             | 121              | 0.97        |
| Najafi M     | 114               | 40.8       | 41.2       | 59.2             | 58.8             | 23               | 0.81        |
| Narne P      | 160               | 27.5       | 40.5       | 72.5             | 59.5             | 13               | 0.42        |
| Nasti S      | 276               | 40.0       | 33.3       | 60.0             | 66.7             | 37               | 0.79        |
| Niemiec P    | 172               | 37.8       | 35.8       | 62.2             | 64.2             | 25               | 0.22        |
| Nikitin AG   | 313               | 25.4       | 18.6       | 74.6             | 81.4             | 34               | 0.75        |
| Saha N[Chinese] | 151          | 9.6        | 9.3        | 90.4             | 90.7             | 3                | 0.15        |
| Saha N[Indian] | 126          | 39.7       | 38.0       | 60.3             | 62.0             | 17               | 0.45        |
| Stanger O    | 108               | 37.0       | 40.0       | 63.0             | 60.0             | 15               | 0.46        |
| Vasiiladou C | 197               | 42.1       | 31.6       | 57.9             | 66.4             | 34               | 0.53        |
| Yamada Y     | 1784              | 37.6       | 38.0       | 62.4             | 60.2             | 21               | 0.46        |
| Zafari AM    | 164               | 37.6       | 38.0       | 62.4             | 60.2             | 21               | 0.58        |
| Total        | 9279              | 23.8       | 18.0       | 76.2             | 82.0             | 748              | 0.64        |

HWE: Hardy–Weinberg equilibrium. The P-value of HWE determined by the χ² test or Fisher’s exact test among controls. doi:10.1371/journal.pone.0070885.t002
Table 3. Quality assessment for all the included studies according to the Newcastle-Ottawa Scale.

| First Author | Year | Selection | Comparability | Exposure |
|--------------|------|-----------|---------------|----------|
| Cai H        | 1999 | ★★☆☆☆    | ★             | ★☆       |
| Fan M        | 2006 | ★☆☆☆☆     | ★             | ★☆       |
| Fang SX      | 2012 | ★☆☆☆☆     | ★             | ★☆       |
| Gardemann A  | 1999 | ★☆☆☆☆     | ★             | ★☆       |
| Gollasch G   | 2011 | ★☆☆☆☆     | ★             | ★☆       |
| He MA        | 2007 | ★☆☆☆☆     | ★             | ☆         |
| Katakami N   | 2010 | ★☆☆☆☆     | ★             | ★☆       |
| Lee WH       | 2001 | ★☆☆☆☆     | ★             | ★☆       |
| Li A         | 1999 | ★☆☆☆☆     | ★             | ☆         |
| Macias-Reyes A | 2008 | ★☆☆☆☆☆   | ★             | ★☆       |
| Morgan TM    | 2007 | ★☆☆☆☆☆   | ★             | ★☆       |
| Najafi M     | 2012 | ★☆☆☆☆☆   | ★             | ☆         |
| Nanne P      | 2012 | ★☆☆☆☆☆   | ★             | ★☆       |
| Nasti S      | 2006 | ★☆☆☆☆☆   | ★             | ★☆       |
| Niemiec P    | 2007 | ★☆☆☆☆☆   | ★             | ★☆       |
| Nikitin AG   | 2009 | ★☆☆☆☆☆   | ★             | ★☆       |
| Saha [Chinese]| 1999 | ★☆☆☆☆☆   | ★             | ★☆       |
| Saha [Indian]| 1999 | ★☆☆☆☆☆   | ★             | ★☆       |
| Stanger O    | 2001 | ★☆☆☆☆☆   | ★             | ★☆       |
| Vasiliadou C | 2007 | ★☆☆☆☆☆   | ★             | ★☆       |
| Yamada Y     | 2002 | ★☆☆☆☆☆   | ★             | ☆         |
| Zafari AM    | 2002 | ★☆☆☆☆☆   | ★             | ☆         |

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allele among Caucasians (34.3% cases, 33.5% controls) was markedly higher than that among Asians (8.7% cases, 9.5% controls). The T allele proportion in the “other” group (35.9% cases, 64.1% controls) was similar to that in the Caucasian group. All the 22 studies were assessed according to the NOS scale and the results of the quality assessment are summarized in Table 3. Most studies (82%) scored 5 stars or more, suggesting a moderate to good quality. Furthermore, we assessed the association of the C242T polymorphism with CAD risk under different genetic models. The summary results of the meta-analysis were shown in Table 4. There was no predominant association between the C242T polymorphism and CAD produced by comparing the T allele with C allele overall (allele comparison: P = 0.87, OR = 0.99, 95% CI 0.89–1.11; dominant model: P = 0.58, OR = 0.96, 95% CI 0.84–1.10; recessive model: P = 0.15, OR = 1.14, 95% CI 0.95–1.36; homozygote comparison: P = 0.20, OR = 1.14, 95% CI 0.93–1.40) (Figure 2). We identified a large between-study heterogeneity across all genotypic models (allele comparison: I² = 67.8%, P_heterogeneity < 0.0001; dominant model: I² = 65.7%, P_heterogeneity < 0.0001; homozygote comparison: I² = 35.7%, P_heterogeneity = 0.05), with the exception of the recessive model (I² = 27.9%, P_heterogeneity = 0.11). Because every subject contributes two observations under the allele comparison and we cannot a priori hypothesis that these two observations are independent, we computed log ORs with standard errors in each study and then used them in our meta-analysis. As a result, the risk estimates did not change if the clustering of the alleles in each subject was taken into account.

Sensitivity analysis
The striking heterogeneity identified across studies could influence the total outcome of the meta-analysis. Therefore, we performed a sensitivity analysis to look for the study or studies making the greatest contribution to heterogeneity. No single study or group of studies could be confirmed as disproportionately influencing the heterogeneity and ORs in total and subgroup analysis.

Cumulative analysis and publication bias
We did not find any clear evidence to suggest that the first published study affected and brought about the ensuing replication via cumulative meta-analysis (data not shown). In addition, funnel plot analysis suggested that the overall results were not asymmetric and Egger’s regression asymmetry test further verified an absence of publication bias (t = −0.25, P = 0.81 for allele comparison) in the overall estimates (Figure 3).

Subgroup analysis
In view of a conspicuous heterogeneity present in total analysis, we undertook a panel of subgroup analyses on ethnicity, population source, study design and endpoint type. The initial aim was to evaluate the possible variability of overall estimates due to ethnicity and data was classified in accordance with the 3 racial descent groups: Caucasian (13 studies recruited 5,067 cases and 2,843 controls), Asian (6 studies recruited 3,777 cases and 6128 controls), and others (3 study recruited 435 cases and 378 controls). The ORs of the C242T polymorphism appeared to decrease in the Asian group (allele comparison: P = 0.24, OR = 0.85, 95% CI 0.64–1.12, P_heterogeneity = 0.001, I² = 75.6%; dominant model: P = 0.21, OR = 0.82, 95% CI 0.61–1.12, P_heterogeneity < 0.0001, I² = 77.4%) and in others group (allele comparison: P = 0.81, OR = 0.94, 95% CI 0.56–1.59, P_heterogeneity = 0.001, I² = 84.8%; dominant model: P = 0.93, OR = 0.97, 95% CI 0.51–1.94, P_heterogeneity = 0.01, I² = 80.1%) without significance. Interestingly, the risk estimates of the C242T polymorphism with CAD differed between Caucasians and Asians, with an increased OR of CAD for the former (allele comparison: P = 0.19, OR = 1.07, 95% CI 0.97–1.19, P_heterogeneity = 0.06, I² = 41%; dominant model: P = 0.58, OR = 1.04, 95% CI 0.90–1.20, P_heterogeneity = 0.05, I² = 43.5%). In particularly, we observed a marginal increased risk of CAD under the recessive genetic model (P = 0.05, OR = 1.21, 95% CI 1.00–1.46, P_heterogeneity = 0.15, I² = 29.1%) and in homozygote comparison (P = 0.06, OR = 1.22, 95% CI 0.99–1.49, P_heterogeneity = 0.16, I² = 28.3%) among Caucasians without distinct heterogeneity (Figure 4).

To address study design, stratification by the matched information for age and gender between cases and controls was performed, as a result, the T allele carriers showed a remarkable increased risk of CAD under most genetic models (allele comparison: P = 0.02, OR = 1.13, 95% CI 1.02–1.26, P_heterogeneity = 0.24, I² = 21.6%; recessive model: P = 0.02, OR = 1.32, 95% CI 1.06–1.66, P_heterogeneity = 0.26, I² = 19.1%; homozygote comparison: P = 0.03, OR = 1.31, 95% CI 1.03–1.66, P_heterogeneity = 0.29, I² = 16%) but not under the dominant model (P = 0.22, OR = 1.09, 95% CI 0.95–1.26, P_heterogeneity = 0.23, I² = 22.9%) in matched studies (Figure 5). Although the ORs of the C242T polymorphism seem to be reversed in studies which did not specify whether the matched case-control study design was used (allele comparison: P = 0.11, OR = 0.87, 95% CI 0.73–1.03, P_heterogeneity < 0.0001, I² = 75%; dominant model: P = 0.15, OR = 0.85,
| Genotype contrasts | study population | study number, (case/control),n(n/n) | $P_{\text{heterogeneity}}$ | $I^2,\%$ | $P$ value* | OR | 95% CI |
|--------------------|------------------|-------------------------------------|-----------------------------|-----------|------------|-----|--------|
| **Total studies**  |                  |                                     |                             |           |            |     |        |
| Allele comparison  |                  | 22(9279/9349)                       | 0.00                        | 0.00      | 0.87       | 0.99| 0.89–1.11 |
| (T versus C)       |                  |                                     |                             |           |            |     |        |
| Dominant model     |                  | 22(9279/9349)                       | 0.00                        | 0.00      | 0.58       | 0.96| 0.84–1.10 |
| (CT+TT versus CC)  |                  |                                     |                             |           |            |     |        |
| Recessive model    |                  | 22(9279/9349)                       | 0.11                        | 0.11      | 0.15       | 1.14| 0.95–1.36 |
| (TT versus CT+CC)  |                  |                                     |                             |           |            |     |        |
| Homozygote comparison |              | 22(9279/9349)                       | 0.05                        | 0.05      | 0.20       | 1.14| 0.93–1.40 |
| (TT versus CC)     |                  |                                     |                             |           |            |     |        |
| **Ethnicity**      |                  |                                     |                             |           |            |     |        |
| Allele comparison  | Caucasian        | 13(5067/2843)                       | 0.06                        | 0.06      | 0.19       | 1.07| 0.97–1.19 |
|                    | Asian            | 6(3777/6128)                        | 0.00                        | 0.00      | 0.75       | 0.24| 0.85–1.12 |
|                    | Others           | 3(435/378)                          | 0.00                        | 0.00      | 0.84       | 0.81| 0.94–1.59 |
| Dominant model     | Caucasian        | 13(5067/2843)                       | 0.05                        | 0.05      | 0.43       | 0.58| 1.04–1.20 |
|                    | Asian            | 6(3777/6128)                        | 0.00                        | 0.00      | 0.77       | 0.21| 0.82–1.12 |
|                    | Others           | 3(435/378)                          | 0.01                        | 0.01      | 0.80       | 0.93| 0.97–1.59 |
| Recessive model    | Caucasian        | 13(5067/2843)                       | 0.15                        | 0.15      | 0.29       | 0.05| 1.21–1.46 |
|                    | Asian            | 6(3777/6128)                        | 0.65                        | 0.65      | 0.00       | 0.89| 1.04–1.20 |
|                    | Others           | 3(435/378)                          | 0.04                        | 0.04      | 0.69       | 0.65| 0.84–1.76 |
| Homozygote comparison | Caucasian  | 13(5067/2843)                       | 0.16                        | 0.16      | 0.28       | 0.06| 1.22–1.49 |
|                    | Asian            | 6(3777/6128)                        | 0.65                        | 0.65      | 0.00       | 0.96| 0.99–1.58 |
|                    | Others           | 3(435/378)                          | 0.01                        | 0.01      | 0.80       | 0.76| 0.85–1.33 |
| **Study design**   |                  |                                     |                             |           |            |     |        |
| Allele comparison  | matched          | 11(3144/2574)                       | 0.24                        | 0.24      | 21.6       | 0.02| 1.13–1.26 |
|                    | not mentioned    | 11(6135/6775)                       | 0.00                        | 0.00      | 75.0       | 0.11| 0.87–1.03 |
| Dominant model     | matched          | 11(3144/2574)                       | 0.23                        | 0.23      | 22.9       | 0.22| 1.09–1.26 |
|                    | not mentioned    | 11(6135/6775)                       | 0.00                        | 0.00      | 75.5       | 0.15| 0.85–1.06 |
| Recessive model    | matched          | 11(3144/2574)                       | 0.26                        | 0.26      | 19.1       | 0.02| 1.32–1.66 |
|                    | not mentioned    | 11(6135/6775)                       | 0.37                        | 0.37      | 8.1        | 0.75| 0.97–1.20 |
| Homozygote comparison | matched          | 11(3144/2574)                       | 0.29                        | 0.29      | 16.0       | 0.03| 1.31–1.66 |
|                    | not mentioned    | 11(6135/6775)                       | 0.07                        | 0.07      | 41.8       | 0.89| 0.98–1.34 |
| **Population source** |               |                                     |                             |           |            |     |        |
| Allele comparison  | P-B              | 6(1308/1172)                        | 0.08                        | 0.08      | 48.4       | 0.35| 0.91–1.11 |
|                    | H-B              | 16(7971/8177)                       | 0.00                        | 0.00      | 72.4       | 0.77| 1.02–1.17 |
| Dominant model     | P-B              | 6(1308/1172)                        | 0.09                        | 0.09      | 47.7       | 0.19| 0.85–1.08 |
|                    | H-B              | 16(7971/8177)                       | 0.00                        | 0.00      | 69.8       | 0.95| 1.00–1.19 |
| Recessive model    | P-B              | 6(1308/1172)                        | 0.80                        | 0.80      | 0.0        | 0.53| 1.10–1.47 |
|                    | H-B              | 16(7971/8177)                       | 0.03                        | 0.03      | 43.8       | 0.25| 1.15–1.45 |
| Homozygote comparison | P-B              | 6(1308/1172)                        | 0.74                        | 0.74      | 0.0        | 0.68| 1.07–1.46 |
|                    | H-B              | 16(7971/8177)                       | 0.01                        | 0.01      | 49.5       | 0.26| 1.16–1.51 |
| **Endpoint**       |                  |                                     |                             |           |            |     |        |
| Allele comparison  | CAD              | 16(5855/3313)                       | 0.00                        | 0.00      | 69.5       | 0.88| 0.99–1.15 |
|                    | MI               | 4(2309/5071)                        | 0.01                        | 0.01      | 77.0       | 0.78| 0.96–1.28 |
| Dominant model     | CAD              | 16(5855/3313)                       | 0.00                        | 0.00      | 68.5       | 0.65| 0.96–1.16 |
|                    | MI               | 4(2309/5071)                        | 0.02                        | 0.02      | 71.5       | 0.90| 0.98–1.15 |
| Recessive model    | CAD              | 16(5855/3313)                       | 0.23                        | 0.23      | 19.6       | 0.38| 1.10–1.37 |
|                    | MI               | 4(2309/5071)                        | 0.10                        | 0.10      | 52.1       | 0.99| 1.00–1.80 |
95% CI 0.69–1.06, $P_{\text{heterogeneity}} < 0.0001$, $I^2 = 75.5\%$; recessive model: $P = 0.75$, OR = 0.97, 95% CI 0.78–1.20, $P_{\text{heterogeneity}} = 0.37$, $I^2 = 8.1\%$, homozygote comparison: $P = 0.89$, OR = 0.98, 95% CI 0.71–1.34, $P_{\text{heterogeneity}} = 0.07$, $I^2 = 41.8\%$), this was not statistically significant. In further subgroup analysis, a lack of dramatic association of the C242T polymorphism with CAD risk was returned when using any genetic model in which data were categorized according to population sources and disease outcomes (Table 4).

**Meta-regression analysis**

We performed a univariate meta-regression analysis for pre-defined underlying sources of heterogeneity, including ethnicity, population source, case definition, study-design, the baseline characteristics of total population (such as age, gender, BMI, the proportion of HTN, DM and smoking status). The adjusted $R^2$ statistic indicates the proportion of between-study variance explained by the covariates. Accordingly, a large proportion of heterogeneity was significantly attributed to BMI ($P = 0.03$) and study design ($P = 0.03$) respectively, with CAD risk of the T allele being increasing in high BMI population (OR = 1.07, 95% CI 1.01–1.15) compared to low BMI population and in matched studies (OR = 1.30, 95% CI 1.02–1.64) compared to studies which did not mention whether the matched case-control study design was used (Table 5).

Furthermore, we compared the descriptive factors between Caucasian and non-Caucasian studies to investigate underlying confounders which may influence the results (Table 6). We found the distribution in study design and BMI was distinctly different between the two groups and could be a likely source of potential confounders. The proportion of Caucasian studies using matched study design (61.5%) was almost double that of non-Caucasian studies (33.3%). The out point of the BMI (25.8 kg/m$^2$) was chosen as the median. Interestingly, most Caucasian studies (81.8%) had BMI $\geq 25.8$ kg/m$^2$, while none of non-Caucasian studies had BMI $\geq 25.8$ kg/m$^2$.

| Genotype contrasts | study population | study number, (case/control), n(m/n) | $P_{\text{heterogeneity}}$ | $I^2$,% | $P$ value | OR | 95% CI |
|-------------------|------------------|-------------------------------------|-----------------------------|--------|-----------|-----|--------|
| Homozygote comparison |
| ACS              | 2(1115/965)      | 0.18                                 | 43.2                        | 0.11   | 1.33      | 0.94–1.88 |
| CAD              | 16(5855/3313)    | 0.07                                 | 37.4                        | 0.36   | 1.13      | 0.87–1.49 |
| MI               | 4(2309/5071)     | 0.06                                 | 59.6                        | 0.90   | 1.05      | 0.53–2.06 |
| ACS              | 2(1115/965)      | 0.39                                 | 0.0                         | 0.10   | 1.25      | 0.96–1.64 |

*Test for overall effect:

P:B-population-based; H:B: hospital-based; CAD: coronary artery disease; MI: myocardial infarction; ACS: acute coronary syndrome.

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Figure 2. Meta-analysis for the overall association between the CYBA C242T polymorphism and CAD under the allele comparison (T versus C).

*Events* indicates the total number of T allele. *Total* indicates the total number of T allele plus C allele.

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We took ethnicity and BMI into account by separately adding them into the multivariable regression. There was a decrease in the OR increase of population source, study design and BMI and a slight increase in OR increase of disease endpoint after adjustment for Caucasian/non-Caucasian, which was similar to that after adjustment for BMI. There were no substantial changes observed in the effect estimates of age, gender, HTN, DM and smoking status after adjusting for ethnicity and BMI. Nevertheless, the adjusted effects did not attain significance. The frequent reduction by adjusting may imply that confounding of the potential factors by ethnicity and BMI was likely to overestimate the influence of the study factors on the CAD risk estimates [50]. We also found that the adjusted R² value significantly increased after adjustment for BMI.

**Discussion**

The correlation of the C242T polymorphism with CAD risk has yet to be clarified. Reckoning the CAD risk of the minor allele in independent case-control studies generated a conspicuous divergence, which was attributed to such causes as racial diversity, research strategy and limited sample size [51]. To address these issues, we commenced a meta-analysis and provided a comprehensive assessment of the publically available studies [52]. The current meta-analysis, including a total of 9,279 cases and 9,349 controls, is one of the largest systematic reviews addressing the association between the C242T polymorphism and CAD risk. Consequently, the overall comparison of T allele with C allele yielded a non-significant risk for CAD.
Figure 5. Meta-analysis for the association between the C242T polymorphism and CAD risk in matched studies. The C242T polymorphism shows a significant increased risk of CAD under the allele comparison (T versus C). ‘Events’ indicates the total number of T allele. ‘Total’ indicates the total number of T allele plus C allele.

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under different genetic models incorporating distinguishable heterogeneity.

Given that distinct heterogeneity was present and could not be completely eliminated [53], we conducted a subgroup analysis to investigate the potential effect of the C242T polymorphism on different groups with homogeneous characteristics. As a result, we verified that ethnicity is an underlying source of between-study heterogeneity. In fact, the risk estimates of the C242T polymorphism on CAD among Caucasians were dramatically different to that among Asians. We observed a borderline increased risk of CAD under the recessive model and the homozygote comparison in the Caucasian group, while the opposite trend was observed among Asians. This discrepancy could be attributed to the high variation in C242T allele frequency across different ethnic populations. The T allele frequency among Caucasians was approximately fourfold higher than that among Asians. In addition, we speculate that the pleiotropic effect of the C242T polymorphism due to various genetic ancestral backgrounds could be another explanation. Our finding are inconsistent with a previous meta-analysis involving 6,273 cases and 5,045 controls [15]. The previous meta-analysis by Fang et al. [15] showed that the T allele carriers had a significant protective effect among Asians but not among Caucasians. This discrepancy is probably due to their relative small sample size and inadequate statistical power. Another explanation could be that the Fang et al. [15] meta-analysis included the studies by Mata-Balaguer et al. [16] and Inoue et al. [8], which significantly violated HWE. Deviations of HWE in control group are likely owing to genotyping errors, population classification and selection bias in the choice of control population and environmental exposures, resulting in reduced precision and raising doubt about the validity in estimation of effect sizes [54]. Hence, we excluded these two studies in order to avoid biasing the overall estimates in our meta-analysis. It is essential to set up an optimized databank of the C242T polymorphism concerning CAD in defined racial groupings to ascertain the reliability of our results.

As well as the obvious association to ethnicity, other environment exposures have confounding potential for the interpretation of between-study heterogeneity. When taking multiple study-level covariates from clinical characteristics into account, we identified study design and BMI as exhibiting significance and positivity in univariate meta-regression analysis, and as such should be cautiously deciphered. Moreover, we found that there was a larger proportion of the Caucasian studies using matched study design compared to non-Caucasian studies. The studies among Caucasians were also more often performed in high BMI populations than those among non-Caucasians. Then accounting for the effects of ethnicity and BMI in the multivariable meta-regression, study design was no longer detected as a significant source of heterogeneity. Therefore, study design and BMI are likely confounders. The estimated advantage of Caucasians over non-Caucasians may be partially accounted for by the effect of study design and BMI and therefore, the effect of ethnicity on the variation cannot be regarded as an independent factor. Although meta-regression suggests an ecological correlation rather than a causal inference, our result implies underlying confounding of the environmental factors with genotype. Large amounts of observational studies have verified that increased BMI contributes causally to CAD [55–57]. Considering a relative wide range of confidence intervals in our results, further carefully designed genetic association studies involving the interaction of BMI are needed to more accurately estimate the risk evaluation of CAD.

Although the relatively large sample size and conformation to HWE strengthen our meta-analysis [58], there are some methodology limitations [59]. First, large positive results are more likely to be accepted for publication than small negative results. Therefore, it was likely that the unpublished studies and “grey” literature (articles in languages other than English and Chinese) were not included. Although the Egger’s test and funnel plots revealed that an absence of publication bias in our meta-analysis, this could not be ruled out completely. Second, some H-B studies recruited controls from an ill-defined background, which could not represent the true exposure experience of the source population and probably resulted in a biased estimate of the ORs. Third, our meta-analysis was specific to a single polymorphism on the CYBA gene and did not account for possible synergistic effects of other candidate genes or polymorphisms. Genetic susceptibility to CAD is most likely based on the interaction of polygenic and environmental factors [60,61] and optimized models concerning these aspects are required.
Table 5. Results from the meta-regression analysing the association of potential factors with the CAD risk.

| Univariate meta-regression | OR     | 95% CI  | Pcov a | R²%  |
|----------------------------|---------|---------|--------|------|
| Ethnicity                  | 1.215   | 0.939–1.572 | 0.130 | 12.46|
| Population source          | 1.126   | 0.835–1.519 | 0.416 | –2.66|
| Study design               | 1.296   | 1.024–1.640 | 0.033 | 25.13|
| Endpoint                   | 1.001   | 0.750–1.335 | 0.996 | –8.99|
| Male, %                    | 0.997   | 0.989–1.004 | 0.366 | 2.24 |
| Age, year                  | 0.999   | 0.981–1.016 | 0.861 | –10.45|
| Smoking, %                 | 0.995   | 0.986–1.002 | 0.170 | 5.28 |
| HTN, %                     | 0.996   | 0.988–1.005 | 0.401 | –1.25|
| DM, %                      | 0.995   | 0.991–1.000 | 0.050 | 33.83|
| BMI, kg/m²                 | 1.074   | 1.008–1.145 | 0.029 | 50.82|

The pooled effect estimate after adjustment for ethnicity

| Univariate meta-regression | OR     | 95% CI  | Pcov a | R²%  |
|----------------------------|---------|---------|--------|------|
| Population source          | 1.114   | 0.833–1.490 | 0.244 | 9.94 |
| Study design               | 1.250   | 1.978–1.597 | 0.061 | 27.69|
| Endpoint                   | 1.018   | 0.770–1.345 | 0.327 | 3.71 |
| Male, %                    | 0.997   | 0.989–1.005 | 0.485 | –0.41|
| Age, year                  | 1.000   | 0.982–1.018 | 0.677 | –11.40|
| Smoking, %                 | 0.995   | 0.988–1.002 | 0.146 | 6.72 |
| HTN, %                     | 0.996   | 0.987–1.005 | 0.396 | 3.04 |
| DM, %                      | 0.997   | 0.992–1.002 | 0.064 | 45.53|
| BMI, kg/m²                 | 1.030   | 0.916–1.158 | 0.067 | 56.16|

The pooled effect estimate after adjustment for BMI

| Ethnicity                  | 1.201   | 0.781–1.847 | 0.067 | 56.16|
| Population source          | 1.039   | 0.812–1.329 | 0.099 | 39.17|
| Study design               | 1.133   | 0.903–1.422 | 0.054 | 38.47|
| Endpoint                   | 1.056   | 0.839–1.329 | 0.091 | 43.04|
| Male, %                    | 1.001   | 0.994–1.009 | 0.101 | 37.61|
| Age, year                  | 0.997   | 0.982–1.012 | 0.094 | 40.22|
| Smoking, %                 | 0.999   | 0.991–1.001 | 0.120 | 26.94|
| HTN, %                     | 0.997   | 0.989–1.004 | 0.065 | 46.93|
| DM, %                      | 0.999   | 0.993–1.005 | 0.114 | 40.79|

HTN: hypertension; DM: diabetes mellitus; BMI: body mass index; Age and BMI were considered as continuous variables.

P values for significance of covariates in the pooled genetic effect.
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Experimental studies have suggested the C242T polymorphism affects the heme-binding site and alters electron flow and superoxide production [62], which results in decreased basal and NAD(P)H-stimulated ROS production. Therefore, reduced ROS levels in the atherosclerotic vessels might be a sensitive signal of declining SMC survival. SMC-depleted plaques tend to be more vulnerable which is characterized by erosion or disruption of their caps [63,64]. Our results complement the previous evidences suggesting that a decrease in NAD(P)H activity, and thus decreased ROS production, could also accelerate the progression of atherosclerosis. Further confirmation of our results is needed before it is possibly to link p22phox to the development of atherosclerosis.

Taken together, our meta-analysis, comprising 18,628 participants, implied that the T allele might carry an increased risk of CAD. However, it is conceivable that the C242T polymorphism has a heterogeneous effect on CAD across different ethnicities, being moderate among Caucasians but lacking significance among Asians. Our meta-analysis also highlights that it is necessary to consider potential gene-gene and gene-environment interactions when trying to interpret and combine observed data. For now, it remains unclear whether the T allele carriers have positive or negative effects on CAD and further population surveys and functional researches will be required to elucidate the true relationship.

Supporting Information

Checklist S1 The PRISMA checklist for this meta-analysis.

(DOC)

Author Contributions

Conceived and designed the experiments: ZW. Lou Y. Lou YX GL. Performed the experiments: ZW. Lou WJ Y. Liu. Analyzed the data: ZW. Lou WJ Y. Liu LL QC YX GL. Contributed reagents/materials/analysis tools: ZW Y. Lou WJ Y. Liu LL QC YX GL. Wrote the paper: ZW Y. Lou.

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