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*Review Article*

# Genetic variants of the paraoxonase 1 gene and risk of coronary heart disease and stroke in the Chinese population: a meta-analysis

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**Abstract:** Objective: Coronary heart disease (CHD) and ischemic stroke (IS) are the leading causes of disability and death worldwide. Two genetic variants of paraoxonase 1 (*PON1*) - Q192R and L55M - have been implicated as potential risk factors for these diseases; however, the results from individual studies are conflicting. This study aimed to investigate the associations of the two polymorphisms and the susceptibility to CHD and IS in the Chinese population using a systematic meta-analysis. Methods: Thirty-five eligible original publications including 44 separate studies were finally included in this meta-analysis. Both fixed- and random-effect models were applied to analyze the pooled odds ratio (OR) and its 95% confidence interval (CI). Publication bias and heterogeneity among studies were explored. Results: An association was found between the Q192R variant and CHD risk both under the dominant (pooled OR: 1.26, 95% CI: 1.04-1.54, $P = 0.021$) and recessive model (pooled OR: 1.20, 95% CI: 1.03-1.40, $P = 0.021$), but there is an indication of heterogeneity among the 17 studies. When the analyses were restricted to 3 large studies ($n \geq 500$ cases), the pooled OR was 1.27 (95% CI: 1.05-1.54). The Q192R variant was also associated with the risk of IS (pooled OR: 1.20, 95% CI: 1.06-1.35, $P = 0.003$, dominant model). The L55M variant did not show any association with the susceptibility to either CHD or IS. Conclusions: Our findings suggest that, in the Chinese population, the Q192R variant of *PON1* is associated with increased susceptibility to both CHD and IS, whereas there is no association between the L55M genetic variant and these diseases.

**Keywords:** Paraoxonase 1, genetics, coronary heart disease, ischemic stroke

## Introduction

Atherosclerotic cardiovascular diseases, such as coronary heart disease (CHD) and ischemic stroke (IS), are the leading causes of disability and death in developed countries and some developing countries, placing a heavy burden on the society [1, 2]. These diseases are recognized as heterogeneous multifactorial disorders which share much similar pathological mechanisms and can be caused by any or a combination of the traditional risk factors such as hypertension, hyperlipidemia, diabetes and smoking [3].

Accumulated evidences suggest that genetic factors contribute to the pathogenesis of cardio- and cerebro-vascular diseases. The shared genetic etiology of CHD and IS includes multiple genetic variants associated with atherosclerosis [4]. Of these potential candidates, variants of paraoxonase 1 (*PON1*) have been studied extensively. *PON1* is located in the paraoxonase gene cluster on 7q21.3-22 and encodes a 43-kDa calcium-dependent esterase. The liver is the major site of the expression of human PON1, and it releases PON1 into the circulation, where the enzyme binds to high-density lipoprotein particles. Functional studies have indicated that PON1 can protect low-density lipoprotein from peroxidation and preserve the function of high-density lipoprotein to prevent the development of atherosclerosis [5].

Two common genetic variants in the coding region of *PON1* have been investigated exten-

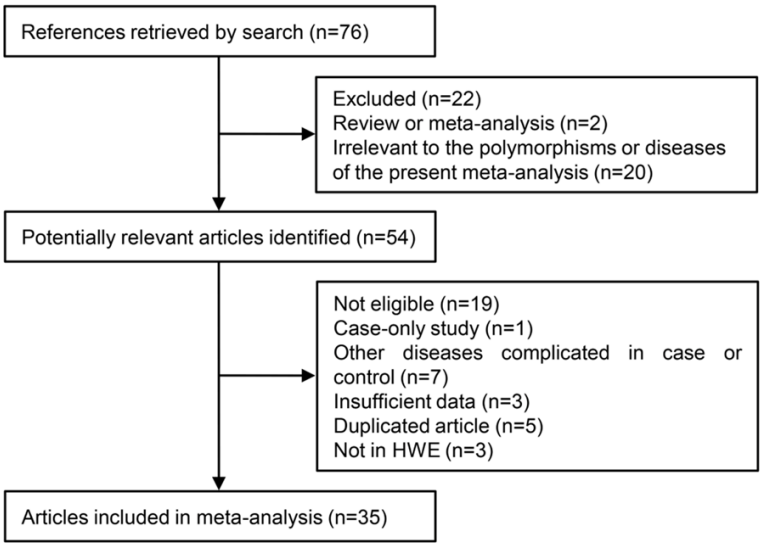


**Figure 1.** Flow chart summarizing the process of study selection.

sively - Q192R (rs662) which results in a glutamine (Q) to arginine (R) substitution at position 192 of *PON1* and L55M (rs854560) which results in a leucine (L) to methionine (M) substitution at position 55. These two variants influence the enzymatic activity as well as the serum concentration independently and contribute to the inter-individual variability in PON1 activity [6-8]. Multiple studies have demonstrated that both Q192R and L55M are associated with a genetic predisposition to CHD and IS; however, most of these studies involved a small sample size, and their results were inconclusive. In addition, ethnic influences on studies involving different populations further obscured the true nature of the association between these genetic variants and the susceptibility to CHD and IS. However, few studies have comprehensively investigated this issue in the Chinese population. Therefore, we conducted this meta-analysis to determine the relationship between Q192R and L55M genetic variants of *PON1* gene and the susceptibility to CHD and IS in the Chinese population.

## Materials and methods

### *Literature search*

We searched the PubMed, Embase, Web of Science, Wanfang database in China, and Chinese National Knowledge Infrastructure (CNKI) databases and reference lists of relevant papers to identify English and Chinese language studies (published before December 31, 2016) investigating the association between the *PON1* rs662 and/or rs854560 polymorphisms and the susceptibility to CHD and/or IS. Search strategies included different combinations of terms such as "paraoxonase", "PON1", "rs662","rs854560","Gln192-Arg", "Leu55Met", "Q192R", "L55M", "coronary artery disease", "coronary heart disease", "myocardial infarction", "ischemic heart disease", "ischemic stroke", "cerebral infarction", "atherosclerosis", "genetic variant", "polymorphism", "Chinese", "China" and their synonyms. This search was limited to human studies. Studies were included in the meta-analysis if they fulfilled the following criteria: (1) case-control studies; (2) cohort studies; (3) the studies that had complete data on genotype and allele frequencies and provide related clinical characteristics; (4) studies published as full-length articles in English or Chinese. The exclusion criteria were: (1) studies without the required raw data; (2) family-based studies; (3) case reports, narrative reviews, editorials, and letters to the editor or other manuscripts not reporting primary research results.

### *Data extraction*

Data were extracted independently by two investigators (Wang and Chen) from all eligible studies, and any disagreement was resolved by discussion. When a consensus could not be achieved, a third investigator (Zhai) was consulted to resolve the dispute. The following information was extracted from each study: name of the first author, year of publication, study design, geographic area, ethnicity, sample size, disease-diagnostic criteria, mean age of patients and controls, the percentage of males among patients and controls, genotyping method, frequency of genotypes, and Hardy-Weinberg equilibrium (HWE) in controls. For duplicate publications, only the most recent or the one with the largest sample size/most complete data was included.

**Table 1.** Characteristics of the studies of *PON1* gene polymorphisms and coronary heart diseases included in meta-analysis

| First author [Ref.] | Year | Geographic area | Disease | Polymorphism | Subjects, n (cases/controls) | Age (years), mean ± SD (cases/controls) | Gender component in case/control (% male) | Genotyping method | HWE |
|---|---|---|---|---|---|---|---|---|---|
| Sanghera DK [12] | 1997 | Singapore | CHD | Q192R | 490, 246/244 | 57.8±0.5/45.9±0.6*,† | 83.7/66.4 | PCR-RFLP | 0.589 |
| Ko YL [13] | 1998 | Taiwan, China | CAD | Q192R | 436, 218/218 | 61.50±0.60/61.29±0.60† | 77.1/77.1 | PCR-RFLP | 0.538 |
| Sanghera DK [28] | 1998 | Singapore | CHD | L55M | 300, 119/181 | 58.9±0.7/45.9±1.0*,† | 98.3/96.7 | PCR-RFLP | 0.616 |
| Liu R [14] | 2001 | Sichuan, China | CHD | Q192R | 246, 118/128 | 70±10/54±11.5* | 72.9/64.8 | PCR-RFLP | 0.075 |
| Chang ZW [15] | 2003 | North region, China | CHD | Q192R | 87, 49/38 | 71.7±8.0/70.8±10.8 | 68.4/76.3 | PCR-RFLP | 0.912 |
| Ma RX [16] | 2003 | Shandong, China | CHD | Q192R | 180, 76/104 | 63.6±7.8/63.8±6.9 | 52.6/52.9 | PCR-RFLP | 0.511 |
| Wang XD [17] | 2003 | Beijing, China | CHD | L55M, Q192R | 231, 93/138 | 54.05±7.12/52.88±8.40 | 52.7/51.4 | TaqMan | 0.403 |
| Su SY [18] | 2005 | Beijing, China | CHD | Q192R | 423, 184/239 | 56.55±7.96/55.84±8.77 | 0/0 | PCR-RFLP | 0.961 |
| Wang XL [19] | 2005 | Beijing, China | CHD | Q192R | 949, 474/475 | 54.13±8.92/53.83±10.20 | 100/100 | PCR-RFLP | 0.177 |
| Baum L [20] | 2006 | HongKong, China | MI | Q192R | 570, 234/336 | 58.3±9.6/71.0±5.9* | 81.6/45.2* | PCR-RFLP | 0.522 |
| Chi DS [29] | 2006 | Guangzhou, China | CHD | L55M | 362, 262/100 | 67.03±10.51/66.64±6.41 | 52.7/53 | PCR-RFLP | 0.960 |
| Su XM [21] | 2006 | Shaanxi, China | CHD | Q192R | 386, 222/164 | 58.22±8.10/57.70±8.50 | NA/NA | PCR-RFLP | 0.475 |
| Wei LY [22] | 2007 | Hebei, China | CHD | Q192R | 242, 151/91 | 55±7/53±8 | 69.5/47.3* | PCR-RFLP | 0.209 |
| Liu JR [23] | 2008 | Shandong, China | CHD | Q192R | 238, 128/110 | 65.25±9. 83/63.52±7.92 | 62.5/60.0 | PCR-RFLP | 0.947 |
| Liu SH [24] | 2010 | Shandong, China | AMI | Q192R | 135, 65/70 | 32~68/35~71 | 66.2/60.0 | PCR-RFLP | 0.233 |
| Kang YH [25] | 2013 | Guangzhou, China | CAD | Q192R | 1077, 538/539 | 63.4 (56-72)/53.5 (45-59)* | 83.46/69.57* | TaqMan | 0.003 |
| Liu T [26] | 2014 | Liaoning, China | CAD | L55M, Q192R | 1656, 792/864 | 54.4±9.35/52.3±8.57 | 69.9/67.2 | PCR-RFLP | 0.059 |
| | | Fujian, China | CAD | L55M, Q192R | 800, 400/400 | 54.5±10.3/53.1±10.4 | 66.0/66.0 | PCR-RFLP | 0.167 |
| Han Y [27] | 2015 | Singapore | CHD | Q192R | 1914, 688/1226 | 66.73±7.82/66.44±7.76 | 64.7/63.3 | Illumina | 0.252 |

*: P<0.05; †: mean±SE; CHD: coronary heart disease; CAD: coronary artery disease; MI: myocardial infarction; AMI: acute myocardial infarction; SD: standard deviation; NA: not available; PCR: polymerase chain reaction; RFLP: restriction fragment length polymorphisms; HWE: Hardy-Weinberg equilibrium.

*Statistical analysis*

Deviations from HWE in the controls were tested by using the chi-squared test, and a threshold of $P < 0.001$ was considered to indicate deviation from HWE. For the two variants, both the dominant (risk (or minor) allele carriers vs. homozygotes for the common (or major) allele) and recessive (homozygotes for the risk allele vs. all others) genetic models were used to calculate the pooled odds ratios (ORs). A fixed or random effect model was employed based on the heterogeneity assumption, which was examined by the chi-squared-based Q test and $I^2$ statistics. The random effect model (DerSimonian and Laird method) was used as the pooling method in the presence of substantial heterogeneity [9, 10]; otherwise, the fixed effect model (Mantel-Haenszel method) was employed to assess the pooled OR. Subgroup analyses were performed to investigate the probable source of heterogeneity according to sample size (≥ 500, 200-499, and < 200 cases), CHD end-points (coronary stenosis or myocardial infarction), genotyping method (restriction fragment length polymorphism or others), and geographic area (north region, south region or others). Publication bias was evaluated with the Begg's test and Egger's test [11], where $P < 0.05$ was regarded as representative of statistically significant publication bias. If the number of studies was more than ten, bias was also assessed by inverted funnel plot. In addition, one-way sensitivity analyses were used to estimate the stability of the me ta-analysis results. All statistical tests were performed with STATA version 11.0 (Stata Corporation, College station, TX, USA), and all *P*-values tested were two-tailed.

## Results

*Characteristics of the included studies*

Thirty-five eligible original articles including 44 separate studies were finally included in the meta-analysis (**Figure 1**). The main characteristics of the included studies for the analysis of CHD and IS risk are summarized in **Tables 1** and **2**, respectively. A total of eighteen publications including 22 studies were about association between these two genetic variants and the risk of CHD. For the Q192R variant and CHD, 17 studies [12-27] were available, including a total of 4,676 patients with CHD and 5,384 controls; for the L55M variant and CHD, 5 studies [17, 26-29] were available, including a total of 1,666 patients with CHD and 1,683 controls. A total of eighteen publications including 22 studies were about association between these two genetic variants and the risk of IS. For the Q192R variant and IS, 18 studies [20, 30-46] were available, including a total of 4,006 patients with IS and 7,373 controls; and for the L55M variant and IS, 4 studies [35, 37, 44, 45] were available, including a total of 883 patients with IS and 876 controls. Thirteen of these publications were published in English language and the others were in Chinese.

*Association between the PON1 Q192R variant and the susceptibility to CHD and IS*

Under the dominant model, an association was found between the Q192R variant and CHD risk using the random-effects model, and the pooled OR was 1.26 (95% CI: 1.04-1.54, $P = 0.021$; **Figure 2A**). Significant heterogeneity was found among the studies ($X^2 = 41.98$, $I^2 = 61.9\%$, $P < 0.001$). No clear evidence for publication bias was observed considering all studies (Begg's test $P = 0.537$, Egger's test $P = 0.385$; Funnel plots refer to **Figure 3A**). The recessive model of inheritance (random-effects model) suggested similar qualitative results, with a pooled OR of 1.20 (95% CI: 1.03-1.40, $P = 0.021$; **Figure 2B**); significant heterogeneity ($X^2 = 44.52$, $I^2 = 64.1\%$, $P < 0.001$) was detected, and no significant publication bias was observed (Begg's test $P = 0.343$, Egger's test $P = 0.120$; Funnel plots refer to **Figure 3B**).

To clarify the heterogeneity, subgroup analyses were performed. Results indicated that sample size, CHD end-points, genotyping method, and geographic area contributed to the heterogeneity, and under the dominant model, when studies were restricted to those with sample ≥ 500 cases, the heterogeneity was significantly reduced ($I^2 = 15.4\%$, $P = 0.307$) and the association was still significant (pooled OR = 1.27, 95% CI: 1.05-1.54, $P = 0.013$) (**Table 3**).

In the case of IS, under the dominant model, an association between the Q192R variant and IS risk was detected (pooled OR = 1.20, 95% CI: 1.06-1.35, $P = 0.003$; fixed-effects model; **Figure 2C**), with moderate heterogeneity ($\chi^2 = 33.02$, $I^2 = 48.5\%$, $P = 0.011$). No publication bias was detected (Begg's test $P = 0.363$,

**Table 2.** Characteristics of the studies of *PON1* gene polymorphisms and stroke included in meta-analysis

| First author [Ref.] | Year | Geographic Area | Disease | Polymorphism | Subjects, n (cases/controls) | Age (years), mean ± SD (cases/controls) | Gender component in case/control (% male) | Genotyping method | HWE |
|---|---|---|---|---|---|---|---|---|---|
| Chen JH [30] | 2003 | Shanxi, China | ACI | Q192R | 100, 52/48 | 66±11/59±7 | 63.5/62.5 | PCR-RFLP | 0.555 |
| Song Y [31] | 2005 | Tianjin, China | ATCI | Q192R | 103, 48/55 | 63.2±11.3/59.8±8.7 | 58.3/61.8 | PCR-RFLP | 0.566 |
| Wu J [32] | 2005 | Hunan, China | CI | Q192R | 470, 131/339 | 63.0±10.6/61.6±7.1 | 55.7/56.3 | PCR-RFLP | 0.237 |
| Yu LT [33] | 2005 | North region, China | IS | Q192R | 2006, 1046/960 | 61.9±10.6/62.1±10.1 | 59.9/58.8 | TaqMan | 1.000 |
| Baum L [20] | 2006 | Hong Kong, China | IS | Q192R | 582, 246/336 | 70.7±12/71.0±5.9 | 54.5/45.2* | PCR-RFLP | 0.052 |
| Chen WR [34] | 2006 | Hunan, China | LI | Q192R | 448, 109/339 | 63.0±9.6/61.6±7.1 | 60.6/56.3 | PCR-RFLP | 0.237 |
| Huang Q [35] | 2006 | Hunan, China | CI | L55M, Q192R | 306, 153/153 | 59.8±12.0/57.7±8.8 | 60.1/58.2 | PCR-RFLP | 0.676 |
| Liu JY [36] | 2006 | Hebei, China | IS | Q192R | 108, 53/55 | 57.3±13.9/52.8±11.7 | 71.7/67.3 | PCR-RFLP | 0.992 |
| Qian JQ [37] | 2006 | Fujian, China | CI | L55M, Q192R | 255, 127/128 | 64.1±1.02/62.7±0.91† | 53.5/48.4 | PCR-RFLP | 0.949 |
| Lu Y [38] | 2008 | Ningxia, China | ATCI | Q192R | 119, 74/65 | 61.4±10.2/60.6±9.3 | 62.2/58.5 | PCR-RFLP | 0.449 |
| Xiao ZJ [39] | 2009 | Hunan, China | CI | Q192R | 714, 375/339 | 62.7±11.3/61.6±7.1 | 59.2/56.3 | PCR-RFLP | 0.237 |
| Liu JL [40] | 2010 | Guangxi, China | IS | Q192R | 266, 131/135 | 39±5/42±3 | 64.9/57.8 | PCR-RFLP | 0.563 |
| Man BL [41] | 2010 | Hong Kong, China | IS | Q192R | 358, 191/167 | 70.1±11.7/71.8±6.8 | 54/48 | PCR-RFLP | 0.436 |
| Leu HB [42] | 2011 | Taiwan, China | IS | Q192R | 3300, 80/3250 | 59.1±8.3/50.0±12.3* | 52.5/45.3 | MALDI-TOF | 0.753 |
| Yang ZH [43] | 2011 | Shanghai, China | CI | Q192R | 412, 295/117 | 63.89±9.93/63.26±9.41 | NA/NA | PCR-RFLP | 0.450 |
| Wang YF [44] | 2012 | Shanxi, China | ACI | L55M, Q192R | 205, 105/100 | NA | NA | PCR-RFLP | 0.009 |
| Zhang GJ [45] | 2013 | Beijing, China | IS | L55M, Q192R | 823, 328/495 | 60.45±14.27/56.48±4.55* | 28/28 | Massarray | 0.778 |
| Hou JJ [46] | 2015 | Nanjing, China | CI | Q192R | 584, 292/292 | 69.2±12.5/67.9±9.4 | 60.3/65.1 | Massarray | 0.106 |

*: P<0.05; †: mean±SE; IS: Ischemic stroke; ACI: atherosclerosis cerebral infarction; ATCI: atherothrombotic cerebral infarction; CI: cerebral infarction; LI: lacunar infarction; SD: standard deviation; NA: not available; PCR: polymerase chain reaction; RFLP: restriction fragment length polymorphisms; MALDI-TOF: matrix-assisted laser desorption ionization-time of flight; HWE: Hardy-Weinberg equilibrium.

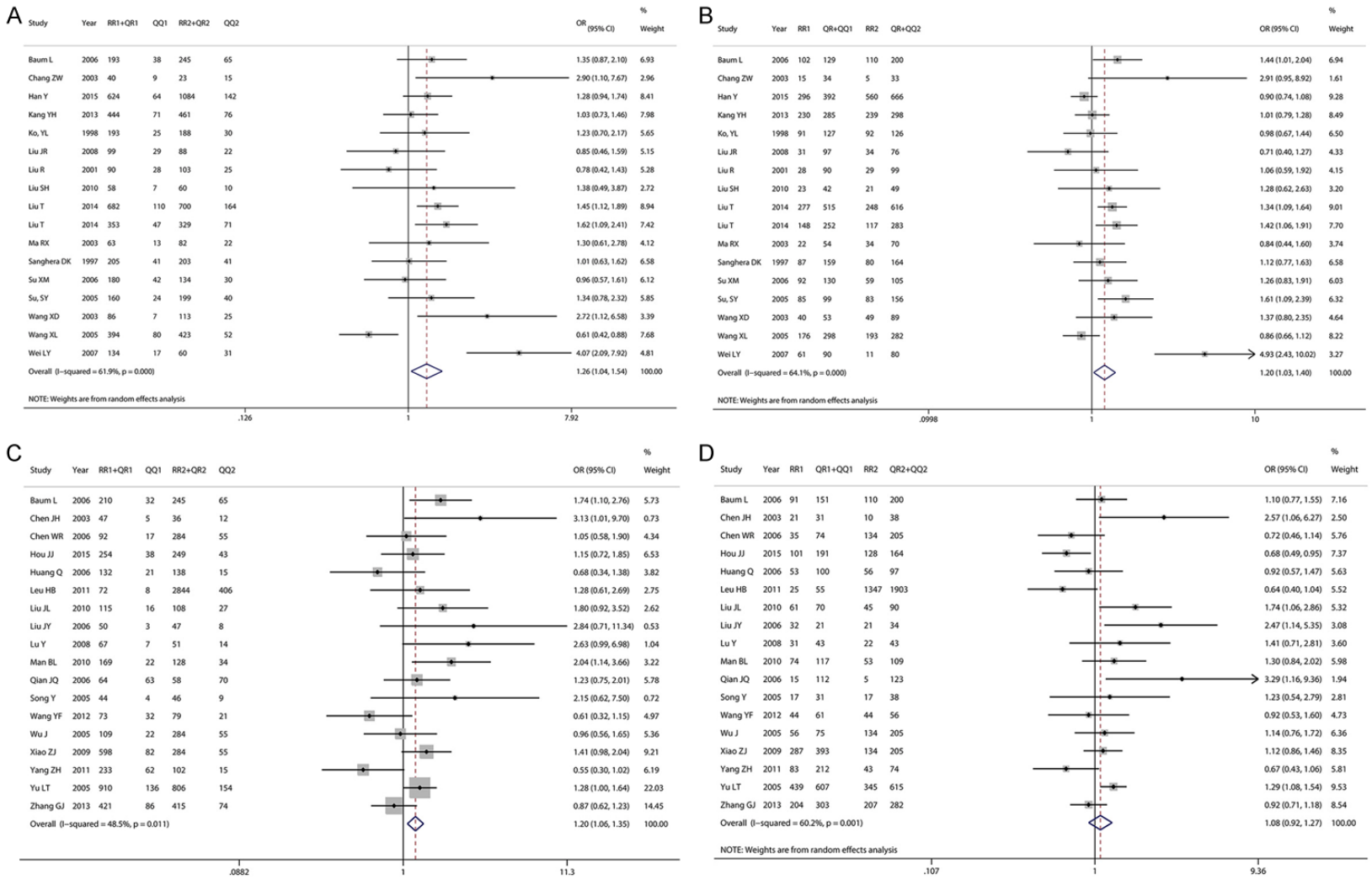


**Figure 2.** Pooled results of Q192R variant and the susceptibility of cardiovascular disease and stroke. A. Association between the Q192R variant and cardiovascular disease risk using dominant model. B. Association between the Q192R variant and cardiovascular disease risk using recessive model. C. Association between the Q192R variant and stroke risk using dominant model. D. Association between the Q192R variant and stroke risk using recessive model.

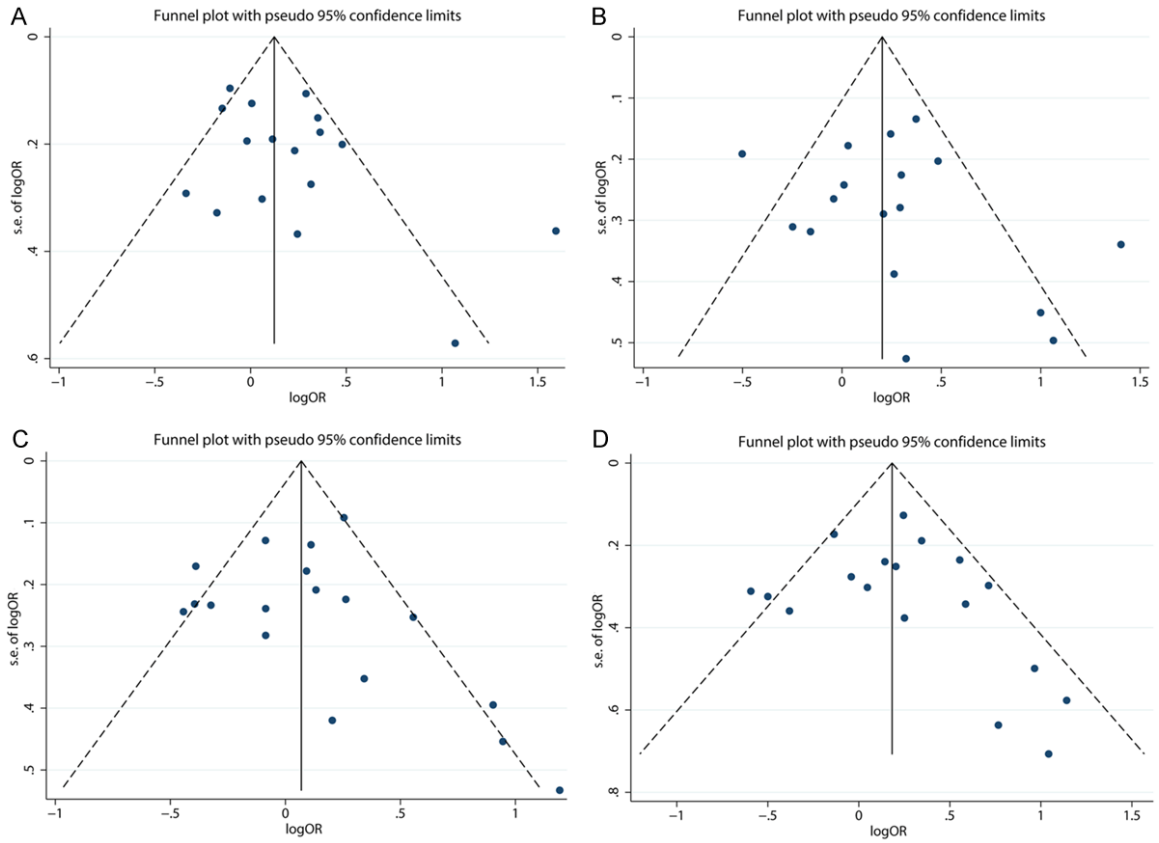


**Figure 3.** Funnel plot for Q192R variant and the susceptibility of coronary heart disease and ischemic stroke. A. Funnel plot for the Q192R variant and CHD risk using dominant model. B. Funnel plot for the Q192R variant and CHD risk using recessive model. C. Funnel plot for the Q192R variant and IS risk using dominant model. D. Funnel plot for the Q192R variant and IS risk using recessive model.

Egger's test $P$ = 0.400; Funnel plots refer to **Figure 3C**). However, under the recessive model, no association was found between the Q192R variant and IS risk (pooled OR = 1.08, 95% CI: 0.92-1.27, $P$ = 0.357; random-effects model; **Figure 2D**), with significant heterogeneity ($\chi^2$ = 42.71, $I^2$ = 60.2%, $P$ = 0.001) and no publication bias (Begg's test $P$ = 0.173, Egger's test $P$ = 0.528; Funnel plots refer to **Figure 3D**).

*Association between the PON1 L55M variant and the susceptibility to CHD and IS*

No significant association was detected between the L55M variant and CHD or IS risk. Under the dominant model, the pooled OR of L55M for CHD was 1.13 (95% CI: 0.67-1.92, $P$ = 0.649; random-effects model; **Figure 4A**) with high heterogeneity ($\chi^2$ = 12.88, $I^2$ = 68.9%, $P$ = 0.012); under the recessive model, the corresponding pooled OR was 0.72 (95% CI: 0.28-1.84, $P$ = 0.492; fixed-effects model; **Figure 4B**), with no significant heterogeneity ($\chi^2$ = 1.85, $I^2$ = 0.0%, $P$ = 0.396). And in both models, no publication bias was detected (Begg's test $P$ = 0.086 and Egger's test $P$ = 0.119 in dominant model; and Begg's test $P$ = 1.000 and Egger's test $P$ = 0.764 in recessive model, respectively).

Regarding the risk of IS, under the dominant model, the pooled OR of L55M was 1.08 (95% CI: 0.66-1.79, $P$ = 0.756; random-effects model; **Figure 4C**) with high heterogeneity ($\chi^2$ = 6.23, $I^2$ = 51.8%, $P$ = 0.101); under the recessive model, the corresponding pooled OR was 1.62 (95% CI: 0.61-4.29, $P$ = 0.331; fixed-effects model; **Figure 4D**), with no significant heterogeneity ($\chi^2$ = 0.14, $I^2$ = 0.0%, $P$ = 0.706). And no publication bias was detected (Begg's test $P$ = 0.734 and Egger's test $P$ = 0.819 in dominant model; and Begg's test $P$ = 1.000 and Egger's test unavailable as the study number is only two in recessive model).

**Table 3.** Stratified meta-analysis of the effect of Q192R polymorphism of the *PON1* gene on risk for CHD

| Variables | No.of studies | No.of subjects | | Dominant model | | | | Recessive model | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Per allele risk | | Test forhet-erogeneity within group | | Per allele risk | | Test for heterogeneity within group | |
| | | Cases | Controls | OR (95% CI) | P | $I^2$ | P | OR (95% CI) | P | $I^2$ | P |
| End point | | | | | | | | | | | |
| Coronary stenosis | 15 | 4377 | 4978 | 1.26 [1.01, 1.56] | 0.039 | 66.5% | <0.001 | 1.18 [1.00, 1.40] | 0.050 | 67.0% | <0.001 |
| Myocardial infarction | 2 | 299 | 406 | 1.35 [0.90, 2.03] | 0.145 | 0.0% | 0.966 | 1.41 [1.03, 1.92] | 0.033 | 0.0% | 0.773 |
| Genotyping method | | | | | | | | | | | |
| PCR-RFLP | 14 | 3357 | 3481 | 1.25 [0.99, 1.59] | 0.066 | 65.6% | <0.001 | 1.26 [1.05, 1.51] | 0.015 | 62.7% | 0.001 |
| Others | 3 | 1319 | 1903 | 1.29 [0.90, 1.86] | 0.164 | 51.7% | 0.126 | 0.97 [0.83, 1.14] | 0.735 | 14.0% | 0.312 |
| Sample size for patients | | | | | | | | | | | |
| ≥500 cases | 3 | 2018 | 2629 | 1.27 [1.05, 1.54] | 0.013 | 15.4% | 0.307 | 1.06 [0.83, 1.36] | 0.613 | 74.7% | 0.019 |
| 200-499 cases | 6 | 1794 | 1837 | 1.07 [0.78, 1.47] | 0.668 | 65.5% | 0.013 | 1.15 [0.95, 1.38] | 0.141 | 44.5% | 0.109 |
| <200 cases | 8 | 864 | 918 | 1.58 [1.02, 2.44] | 0.041 | 65.1% | 0.005 | 1.43 [0.96, 2.12] | 0.078 | 69.5% | 0.002 |
| Geographic Area | | | | | | | | | | | |
| North region | 10 | 2234 | 2293 | 1.40 [0.98, 2.02] | 0.068 | 75.1% | <0.001 | 1.32 [1.01, 1.73] | 0.044 | 71.5% | <0.001 |
| Sorth region | 5 | 1508 | 1621 | 1.20 [0.96, 1.51] | 0.111 | 21.7% | 0.276 | 1.17 [0.99, 1.40] | 0.073 | 24.8% | 0.256 |
| Others | 2 | 934 | 1470 | 1.19 [0.92, 1.54] | 0.190 | 0.0% | 0.417 | 0.94 [0.79, 1.13] | 0.537 | 7.8% | 0.298 |
| Overall | 17 | 4676 | 5384 | 1.26 [1.04, 1.54] | 0.021 | 61.9% | <0.001 | 1.20 [1.03, 1.40] | 0.021 | 64.0% | <0.001 |

*Sensitivity analysis*

Sensitivity analyses were performed by omitting one study each time. In the analysis of the association of the Q192R variant and CHD under the dominant model, when the study by Wei et al. [22] was omitted, the significance disappeared (pooled OR: 1.26, 95% CI: 0.99-1.40, $P > 0.05$). Nevertheless, all other sensitivity analyses showed that the overall results remained similar for the risk of CHD or IS in both genetic models (Supplementary Figures 1, 2).

## Discussion

The atheroprotective properties of *PON1* have been validated by experiments involving *PON1*-deficient mice [47, 48] and *PON1*-transgenic mice models [49, 50]; clinical studies have also confirmed the anti-atherogenic role of *PON1*, which showed an inverse correlation between serum PON1 concentration and atherogenic risk [51, 52].

In the coding region of human *PON1*, there are two well-studied functional variants, Q192R and L55M, and each of these variants shows an independent association with enzymatic activity and contributes to the inter-individual variability in PON1 activity [6-8]. The present meta-analysis for the first time provides the most comprehensive assessment of the two functional variants of *PON1* and the risk of CHD or IS in the Chinese population. The data pooled from the available published studies suggests that the L55M polymorphism is not associated with the susceptibility to either CHD or IS, while the Q192R variants might increase the risk of both CHD and IS in the Chinese population.

Several previous studies have demonstrated that the Q192R and L55M variants in *PON1* could contribute to the risk of CHD, possibly through the exacerbation of atherosclerotic events [12, 53, 54]. However, discordant results were reported from later studies, and a meta-analysis on Q192R polymorphism failed to show any strong evidence that the polymorphism is associated with CHD risk in the Caucasian population [55]. Studies evaluating the association between *PON1* gene variants and stroke are also apparently conflicting. While earlier studies suggested that the variants were risk factors for stroke, the results could not be confirmed [56].

Differences in ethnicity, diet, gene-environment interaction and sample size may be responsible for the discrepancies in the results of previous studies. Therefore, when considerable numbers of reports are available on ethnically distinct populations, separate meta-analysis based on population groups is necessary in

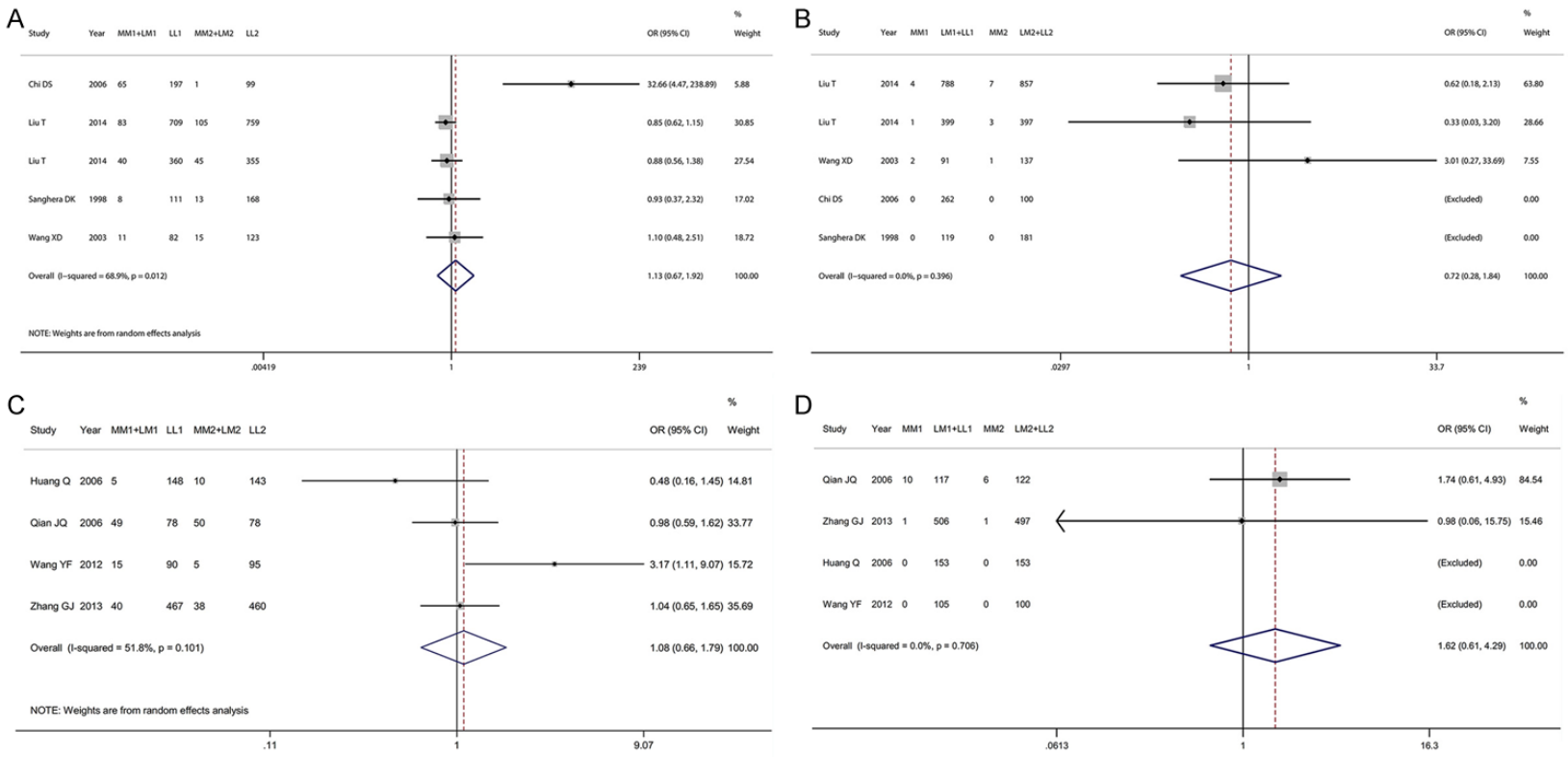


**Figure 4.** Pooled results of L55M variant and the susceptibility of cardiovascular disease and stroke. A. Association between the L55M variant and cardiovascular disease risk using dominant model. B. Association between the L55M variant and cardiovascular disease risk using recessive model. C. Association between the L55M variant and stroke risk using dominant model. D. Association between the L55M variant and stroke risk using recessive model.

order to provide results that are more convincing. Focusing on the Chinese population, the present comprehensive meta-analysis on the association between the two *PON1* gene polymorphisms and the predisposition to CHD and IS suggests a weak association between the Q192R polymorphism and the susceptibility to CHD and IS, indicating that the QQ homozygote genotype could be a protective factor; however, no statistically significant association was found between the L55M and CHD or IS risks. Considering that the source data may be skewed owing to publication bias, this effect was also evaluated by Begg's and Egger's tests and no substantial publication bias was detected. Furthermore, sensitivity analysis omitting specific studies was applied to examine the reliability and stability of the results. It is noteworthy that under the dominant model, the association between Q192R and CHD disappeared when a study that involved a small-sample size [22] was omitted. Adequate sample size is crucial in genetic case-control studies and underpowered studies may skew the genetic effects in individual studies on cardiovascular risk [57]. Nevertheless, when only studies with sample ≥ 500 cases were enrolled in the subgroup analysis, the pooled OR was 1.27 (95% CI: 1.05-1.54, $P$ = 0.013; without significant heterogeneity). Meanwhile, all other sensitivity analyses showed that the overall results remained similar for the risk of CHD or IS in both genetic models.

The current study has some limitations. First, the results of the selected studies could inevitably be influenced by confounding factors - such as age, gender, obesity, cigarette smoking, or ethnic admixture - either between studies or between cases and controls within each study, and the lack or incompleteness of all individual raw data restricted further adjustments of the results and attenuated the power of the meta-analysis. However, the odds ratios adjusted for those conventional risk factors such as age, gender, body mass index and smoking, were extracted from each study in this meta-analysis, which may minimize the confounding effects. Second, variations in the clinical classification of CHD or IS patients and controls, as well as the differences in the methodologies among the selected studies could have influenced the overall outcome. Third, the results might have been distorted by potential weakness and biases of genetic association studies, such as genotyping error, phenotype misclassification, and gene-gene or gene-environment interactive effect. Finally, we only retrieved studies published in English and Chinese, which might introduce language bias. Therefore, results of the present meta-analysis should be interpreted cautiously.

## Conclusions

In conclusion, accumulated evidence suggests that *PON1* Q192R variant in the Chinese population is associated with an increased risk for both CHD and IS, whereas there is no association between the *PON1* L55M variant and these two diseases. Further, analyses on sub-types as well as gene-gene and gene-environment interactions were necessary because of the heterogeneity of CHD and IS. Genetic variation in the *PON1* gene cluster merits further investigation to elucidate the molecular basis of the observed genetic effects.

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## Disclosure of conflict of interest

None.

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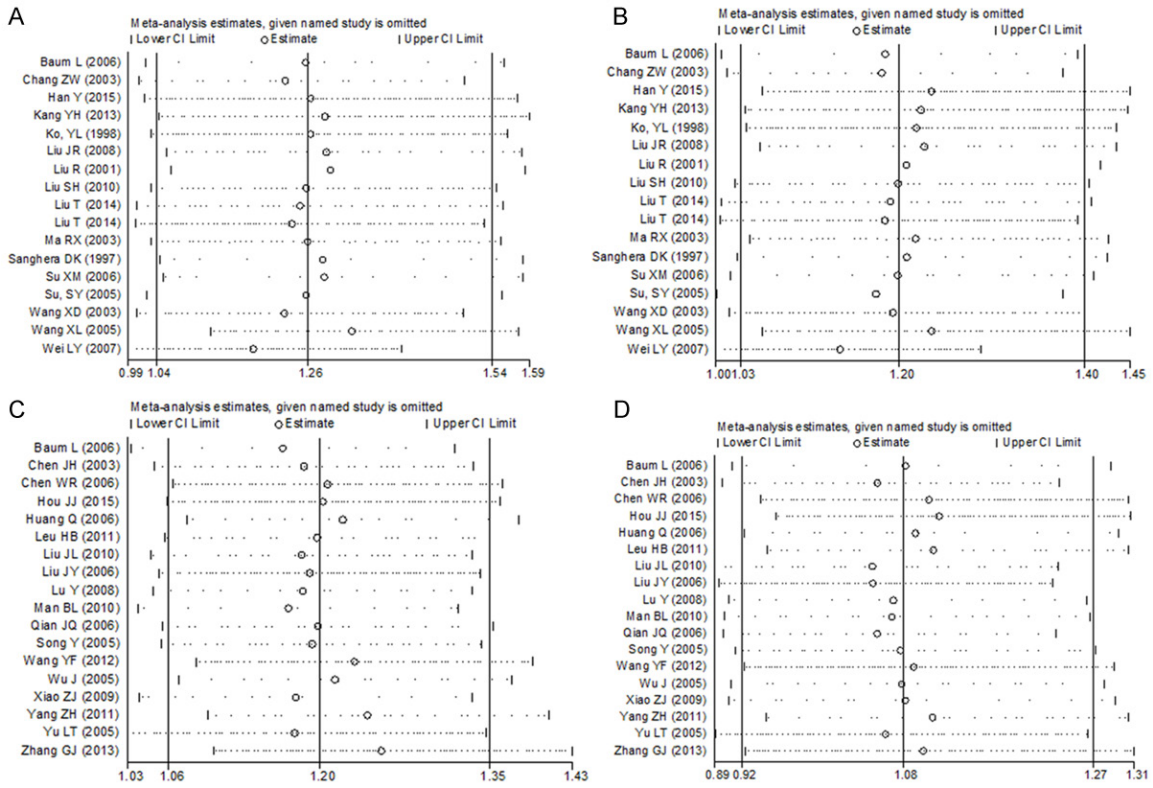
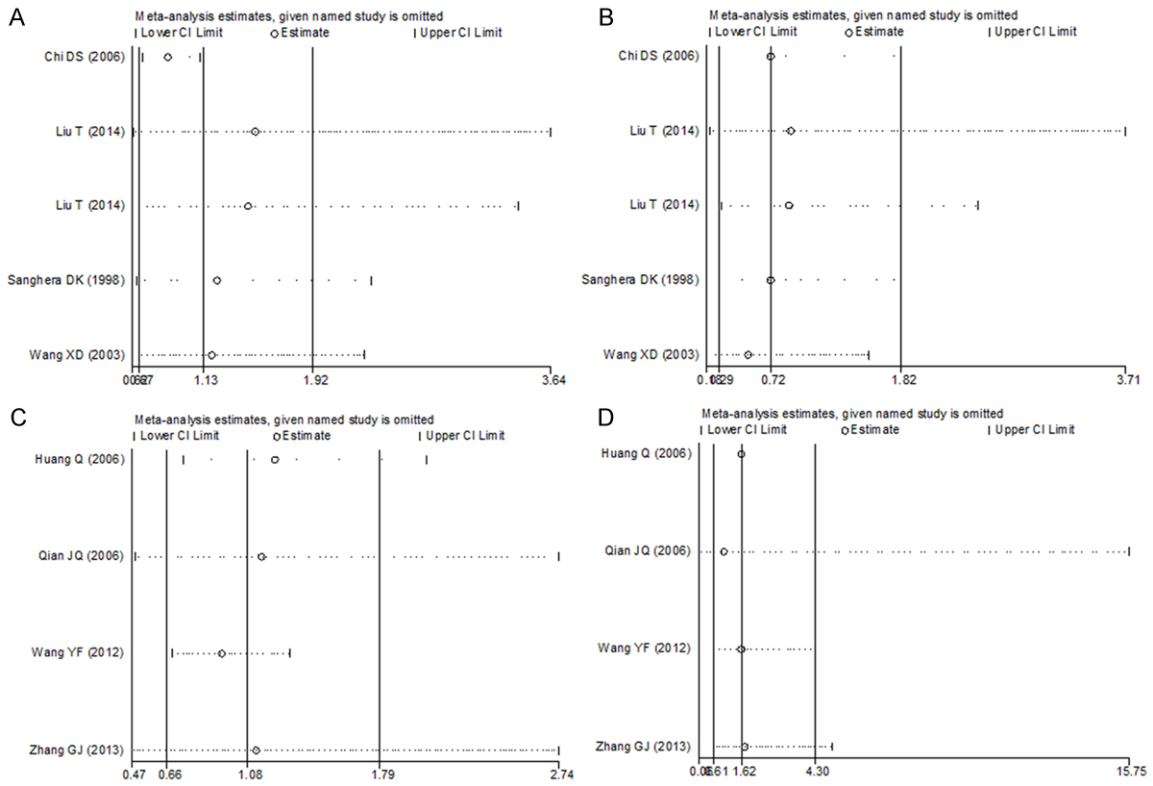



**Supplementary Figure 1.** Sensitive analysis for Q192R variant and the susceptibility of coronary heart disease and ischemic stroke. A. Association between the Q192R variant and CHD risk using dominant model. B. Association between the Q192R variant and CHD risk using recessive model. C. Association between the Q192R variant and IS risk using dominant model. D. Association between the Q192R variant and IS risk using recessive model.




**Supplementary Figure 2.** Sensitive analysis L55M variant and the susceptibility of coronary heart disease and ischemic stroke. A. Association between the L55M variant and CHD risk using dominant model. B. Association between the L55M variant and CHD risk using recessive model. C. Association between the L55M variant and IS risk using dominant model. D. Association between the L55M variant and IS risk using recessive model.