Research Article

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Association of glutathione peroxidase-1 (GPx-1) rs1050450 Pro198Leu and Pro197Leu polymorphisms with cardiovascular risk: a meta-analysis of observational studies

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Abstract

Objective   To clarify the association between rs1050450 polymorphism in Glutathione peroxidase-1 (GPx-1) and the risk of cardiovascular diseases (CVD) by performing a meta-analysis of published studies. There is growing evidence from different study types for an association of the GPx-1 polymorphism and cardiovascular outcomes, but observational studies have so far shown inconsistent results. **Methods**   Relevant publications were searched through PubMed, Embase database databases and the Cochrane Library. We used odds ratios (ORs) with 95% confidence intervals (CIs) to assess the strength of association under the best genetic model. Both Q statistic and the I^2 were used to check heterogeneity. Meta-regression analysis was performed to explore heterogeneity source. Sensitivity analysis, cumulative meta-analysis analysis and publication bias were used to test the reliability of the results. **Results**   Data were available from two cohort studies and 8 case-control studies involving 1,430 cases and 3,767 controls. The pooled ORs for overall CVD risk was 1.36 with 95% CI: 1.08–1.70 under a co-dominant model, and that for East Asian subgroup was 1.84 (95% CI: 1.39–2.43). Substantial heterogeneity for ORs were detected among all the included studies, mainly caused by ethnic differences between East Asian and non-East Asian populations. Although Egger's regression test suggested no statistical significant publication bias, Begg's funnel plot exhibited obvious asymmetry. The statistical significance disappeared after adjusting for potential publication bias in the overall studies. However, no substantial publication bias was found in the East Asian subgroup. **Conclusions**   GPx-1 gene Pro198Leu and Pro197Leu polymorphisms considerably increased the risk of CVD in the East Asian population. Large-scale investigations are needed to confirm the results in different ethnicities.

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1   Introduction

Cardiovascular disease (CVD) is a complex clinical syndrome resulting from interactions of genetic and environment risk factors. Despite the development of staged, preventive strategies, CVD remains the leading cause of death and disability in the United States.[1] The identification of genetic risk factors which contribute to CVD is urgently needed for risk stratification and individualized treatment. In search for genetic risk factors, the glutathione peroxidase-1 (GPx-1) gene was investigated with contradictory results.

Redox homeostasis regulated by reactive oxygen species generating enzymes and antioxidant enzymes is linked to the onset and progression of chronic complex diseases, such as CVD, neurodegenerative diseases and cancers.[2] GPx is an endogenous selenium-dependent antioxidant enzyme in the cytoplasm and mitochondria. GPx-1, which detoxifies hydrogen peroxide to water and lipid peroxide to alcohols by utilizing glutathione, is a key antioxidant in defense against oxidative stress.[3,4] Recently, GPx-1 activity in whole blood was reported to be associated with severity and outcomes of CVD. A prospective study showed that GPx-1 activity, combined with homocysteine, could predict cardiovascular risk, even after adjustment for cardiovascular confounders.[5] Furthermore, the GPx-1 level was suggested to be a valuable marker for monitoring cardiovascular events. Cheng, *et al*.[6] reported that the incidence of acute myocardial infarction was higher in patients with impaired GPx-1 activity. Atherosclerotic burden analysis by Espinola-Klein, *et al*.[7] suggested that erythrocytic GPx-1 activity was lower in multi-vascular atherosclerosis patients,

and was inversely correlated with the event rate. Taken together, these results suggested GPx-1 played an important role in the etiology of CVD.

The human GPx-1 gene is located on chromosome 3p21.3,[8] which contains polymorphism of the cytosine-to-thymine (C > T) substitution (rs1050450) at codon 198 and 197, resulting in Pro198Leu and Pro197Leu variations. The Leu variant was reported to be associated with 40% reduction of GPx-1 activity and increased susceptibility of tumor.[9–13] Given that the accumulating evidence based on a relatively small sample size and limited statistical power also indicated GPx-1 was a possible candidate gene for CVD risk, the meta-analysis from published data was performed to determine the association of GPx-1 Pro198Leu and Pro197Leu variants with CVD risk.

2 Methods

2.1 Publication search

Relevant studies were identified by searching PubMed, Embase database and the Cochrane Library up to March 2013. The following search terms were used: (glutathione peroxidase or GPx) and (gene, polymorphism, genotype, mutation, genetic or variant) and (hypertension, diabetes, metabolic syndrome, heart disease, heart failure, coronary, cardiovascular, restenosis, ventricular hypertrophy, stroke). All the identified studies were retrieved, and their references as well as related articles were also checked. No publication date or language restrictions was applied.

2.2 Inclusion and exclusion criteria

Studies fulfilling the following selection criteria were included in this meta-analysis: (1) they evaluated the association between GPx-1 polymorphism and CVD; (2) they were case-control or cohort studies; and (3) they should report GPx-1 Pro198Leu or Pro197Leu genotype distribution. Studies were excluded if any of the following criteria existed, (1) the studies were not relevant to GPx-1 polymorphism, or CVD; (2) they were not involving humans; and (3) they were reviews or comments. For overlapping studies, only the one with the largest sample size was included.

2.3 Data extraction

The following variables were extracted from each study if available: name of first author, publication year, ethnicity of study participants, study design, numbers of cases and controls, case ascertainment, source of control and genotyping methods. Information was carefully entered into predesigned data collection forms, independently, by two of the investigators (Zhang and Wang). The accuracy of the

data was verified by comparing collection forms from each investigator. Any discrepancy was resolved by discussion.

2.4 Qualitative assessment

Two authors (Zhang and Wang) independently assessed the quality of the selected articles by using Newcastle-Ottawa scale (NOS).[14] Any disagreement was resolved by consensus. Total scores ranged from 0 (worst) to 9 (best) for cohort studies and 0 (worst) to 10 (best) for case-control studies.

2.5 Statistical analysis

Deviation from Hardy-Weinberg Equilibrium (HWE) was measured by Pearson or Fisher's exact chi-square test for controls with case-control design and all participants with cohort design.[15] If deviation from HWE presented, sensitivity analysis or subgroup analysis would be performed to test the robustness of the findings. The strength of association between the GPx-1 Pro198Leu and Pro197Leu polymorphisms with CVD risk was calculated by odds ratio (OR) and 95% confidence interval (95% CI). The Q statistic and the inconsistency index (I^2) were used to assess the degree of heterogeneity. Provided there was heterogeneity among studies, univariate meta-regression was used to explore the source of heterogeneity and the random-effect model was selected to pool the ORs; otherwise, a fixed-effect model was adopted.

To determine the overall gene effect, the putative risk T allele was compared to the common C allele. If the overall gene effect was statistically significant, genotype results would be pooled under the appropriate genetic model. The best genetic model was selected according to the recommended criteria:[15] OR1 (TT *vs*. CC), OR2 (TC *vs*. CC) and OR3 (TT *vs*. TC) were calculated with T as the risk allele. If OR1 = OR2 ≠ 1 and OR3 = 1, the dominant model is selected; if OR1 = OR3 ≠ 1 and OR2 = 1, the recessive model is adopted; if OR2 = 1/OR3 ≠ 1 and OR1 = 1, the overdominant model is recommended; and if OR1 > OR2 > 1 and OR1 > OR3 > 1 (or OR1 < OR2 < 1 and OR1 < OR3 < 1), the co-dominant model is suggested.

Subgroup analyses were carried out with regard to the source of heterogeneity. Sensitivity analysis and cumulative analysis were performed to assess the influence of an individual study. The potential publication bias was inspected visually in Begg's funnel plot of log against its standard error (SE) tested with Egger's regression. The 'Trim and Fill' method was used to estimate theoretically missing studies and adjust publication bias if obvious asymmetry existed. In that case, the robustness of pooled ORs was retested again after adjustment of publication bias.

All statistical tests were performed using STATA 12.0 software (Stata Corporation, College Station, TX, USA). $P < 0.05$ was considered statistically significant, except for tests of heterogeneity, where a level of 0.10 was used.

3 Results

3.1 Literature search

We initially retrieved 451 unique citations from the PubMed, Embase databases and the Cochrane Library. Of these, the majority were excluded after the first screening based on abstracts or titles mainly because they were reviews, investigated irrelevant outcomes, irrelevant genes, carried out *in vitro*, or on animals. After full-text review of 20 papers, three studies focused on GPx-3 variants to the risk of cerebral venous thrombosis or arterial ischemic stroke,[16–18] and one study related to GPx-4 and stroke risk were excluded.[19] Two studies were excluded because the ALA6[20] and 599 C/T variants[21] of GPx-1 were related. Four studies concerned with GPx-1 polymorphism and the risk of thoracic aortic aneurysm,[22] Keshan disease,[23] diabetic peripheral neuropathy,[24] and aerobic power of CVD patients were also excluded.[25] Finally, two cohort studies [26,27] and eight case-control studies[28–35] were included in our meta-analysis. A flow chart summarizing the study selection is presented in Figure 1.
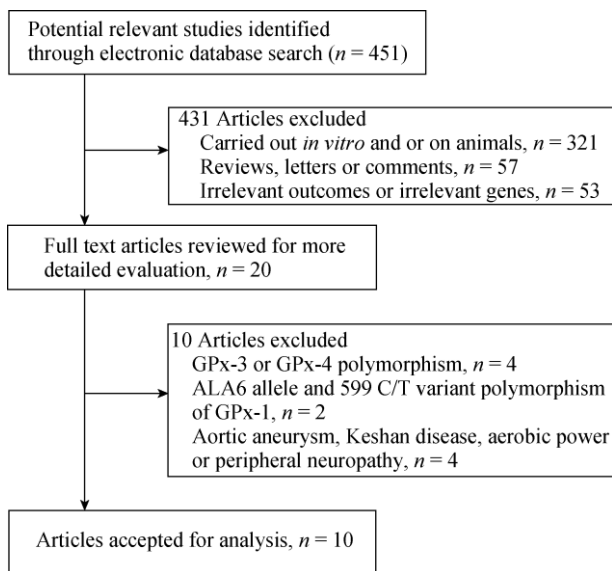

Figure 1. Flow of study identification, inclusion, and exclusion. GPx-1: Glutathione peroxidase-1.

3.2 Study characteristics

The characteristics of the 10 studies are presented in Table 1. The sample sizes for cases and controls were 1,430 and 3,767, respectively. These studies were published between 2000 and 2012. Four studies were conducted in Japan, two in Russia and East Slavic, two in China, one in India and one in Sweden. In the included studies, eight studies observed coronary heart disease, myocardial infarction, peripheral vascular disease, cerebrovascular disease or stroke; one observed metabolic syndrome and one observed in-stent restenosis. Among the case-control studies, four studies used population based controls. The ascertainment of increased CVD risk included medical history, angiographic criteria or coronary artery calcium score.

3.3 GPx-1 polymorphism and risk of CVD

All included studies were consistent with HWE. Allele T frequency was 8.57% in East Asian and 29.56% in non-East Asian. The layout of genotype distributions or allele frequencies is shown in Table 2. The combined ORs of allele T versus allele C were obtained from nine studies because Kuzuya's study[27] did not provide raw genotype data of cases and controls, respectively, in Figure 2. Allele T was found to confer 1.3 fold higher risk of CVD when compared to allele C, 95% CI: 1.07–1.59. From the nine studies, the best genetic model was determined by six studies because three studies from Asia were lacking the minor TT genotype. The co-dominant model (TT *vs*. CC and TC *vs*. CC) was adopted according to the criteria aforementioned, in Table 3.

Substantial heterogeneity was observed in the co-dominant model, $Q = 21.37$ and $I^2 = 57.9\%$. Univariate meta-regression suggested ethnicity was the major source of the between-study variance ($P = 0.007$), while sample size, study design, source of control (diabetes or non-diabetes) and NOS scores did not elicit heterogeneity, in Table 4. When dividing the studied population according to ethnicity, the heterogeneity effectively decreased, $I^2 = 15.3\%$ in East Asian group ($P = 0.316$) and no heterogeneity was found in non-east Asian group ($I^2 = 0$, $P = 0.512$).

The pooled risk of GPx-1 variants with CVD was statistical significant (OR = 1.36, 95% CI: 1.08–1.70), in Figure 3. Subgroup analysis indicated that GPx-1 variants had a higher risk of CVD in the East Asian population (OR: 1.84, 95% CI: 1.39–2.43), in Figure 4. However, the association of GPx-1 variants with CVD was inconclusive in the non-East Asian group, OR = 1.08 with 95% CI: 0.95–1.23, in Figure 5. Sensitivity analysis by sequentially excluding individual studies showed no single study significantly influenced the heterogeneity and the risk strength of GPx-1 variants, in Supplement 1. In the cumulative analysis, the strength of GPx-1 risk increased by adding studies in the sequence of study year, in Supplement 2.

Table 1.   Basic characteristics of the included studies in the meta-analysis.

	Year	Ethnicity	Study design	Sample Size Case	Sample Size Control	Case ascertainment	Scource of control	Confounders	Genotyping methods	NOS scores
Forsberg, *et al*.[29]	2000	Swedish	PCC	101	214	First ever stroke individuals from the MONICA/CASTRO study population	Age matched participants free of CVD	NA	PCR-RFLP	6
Sergeeva, *et al*.[33]	2001	Russian	PCC	103	52	Patients with complications: MI or stroke	T2DM, EH	NA	PCR-RFLP	3
Hamanishi, *et al*.[26]	2004	Japanese	Cohort	53	131	CHD and peripheral vascular disease	T2DM	Matched	PCR direct sequencing	8
Oguri, *et al*.[31]	2007	Japanese	HCC	107	354	In-stent restenosis by angiography	CHD	Diabetes, stent diameter, prestenting RD, poststenting RD and MLD	PCR and suspension array	7
Nemoto, *et al*.[30]	2007	Japanese	HCC	11	80	Coronary artery calcium score ≥ 1000 or 0-999	T2DM	Matched	PCR-RFLP	5
Tang, *et al*.[34]	2008	Chinese	HCC	265	265	CHD by angiography	Age and sex matched patients without symptoms or signs of CVD	BMI, EH, dyslipidemia, rate of smoking and glucose	PCR-RFLP	6
Kuzuya, *et al*.[27]	2008	Japanese	Cohort	1105	1087	Metabolic syndrome by IDF criteria	Normal population	Men: waist-hip ratio, triglycerides, IRIHOMA-beta, SBP, DBP Women: body fat mass, IRI	Allele-specific PCR	5
Ramprasath, *et al*.[32]	2011	Indian	HCC	241	285	CHD by angiography	T2DM	BMI, HbA1c, total cholesterol, LDL, HDL, TG	PCR-RFLP	5
Chen, *et al*.[28]	2012	Chinese	HCC	85	83	A history of ischemic CVD, such as previous MI, angina, or CABG	T2DM	Age, hypertension, age at diagnosis of DM, DM duration, fasting c-peptide, 2-h peptide, 2h insulin, TG, smoking	PCR-RFLP or PCR direct sequencing	5
Zeikova, *et al*.[35]	2012	East Slavic	PCC	412	197	CVD with WHO criteria; death, including CVD and cerebrovascular disease	Normal population (Tomsk)	NA	PCR-RFLP	2

BMI: body mass index; CHD: coronary heart disease; CVD: cardiovascular diseases; DBP: diastolic blood pressure; DM: diabetes mellitus; EH: elementary hypertension; HbA1c: hemoglobin A1c; HCC: hospital based case-control study; HDL: high-density lipoprotein; IDF: International diabetes federation; IRI: insulin resistance index; LDL: low-density lipoprotein; MLD: minimal luminal diameter; NA: not available; NOS: Newcastle-Ottawa scale; PCC: population based case-control study; PCR-RFLP: polymerase chain reaction-restriction fragment length polymorphism; RD: reference vessel diameter; SBP: systolic blood pressure; TG: triglycerides; T2DM: type 2 diabetes mellitus.

Table 2.   Distribution of GPx-1 Pro198Leu and Pro197Leu polymorphisms among cases and controls, and *P*-value of HWE.

	Ethnicity	Case CC	Case CT	Case TT	Control CC	Control CT	Control TT	Case T	Case C	Control T	Control C	OR (95%CI)	*P*-HWE
Forsberg, *et al*.[29]	Non-East Asian	56	38	7	113	85	16	52	150	117	311	0.90 (0.45–1.35)	1.00
Sergeeva, *et al*.[33]	Non-East Asian	56	41	6	27	19	6	53	153	31	73	0.78 (0.13–1.43)	0.97
Hamanishi, *et al*.[26]	East Asian	35	18	0	116	15	0	18	88	15	247	3.98 (1.68–9.38)	0.78
Oguri, *et al*.[31]	East Asian	84	21	2	315	37	2	25	189	41	667	2.13 (0.72–3.55)	0.69
Nemoto, *et al*.[30]	East Asian	6	5	0	65	15	0	5	17	15	145	3.61 (0.75–16.16)	0.57
Tang, *et al*.[34]	East Asian	197	65	3	222	43	0	71	459	43	487	1.70 (1.08–2.69)	0.54
Kuzuya, *et al*.[27]	East Asian	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	1.34 (0.90–1.78)	0.27
Ramprasath, *et al*.[32]	Non-East Asian	101	118	22	137	128	20	162	320	168	402	1.29 (0.84–1.73)	0.35
Chen, *et al*.[28]	East Asian	62	23	0	63	20	0	23	147	20	146	1.17 (0.55–2.49)	0.18
Zeikova, *et al*.[35]	Non-East Asian	79	71	22	206	176	30	115	229	236	588	1.13 (0.73–1.54)	0.99

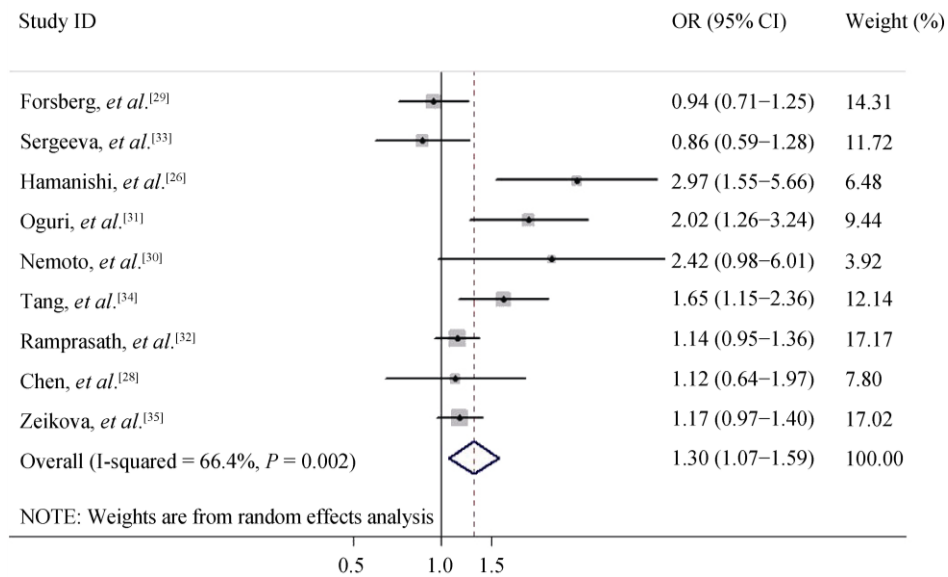HWE: Hardy-Weinberg Equilibrium, NA: not available.

Figure 2. Forest plot describing the meta-analysis with a random-effect for the association of allele T versus allele C with CVD risk, accompanied by the respective 95% confidence intervals (CIs). Values of OR and CIs > 1 implied an increased risk for cardiovascular diseases with the allele T.

Table 3. Calculation of OR1, OR2 and OR3 to determine the best genetic model.

	OR1 = 1.32				OR2 = 1.153				OR3 = 1.238			
	Case		**Control**		**Case**		**Control**		**Case**		**Control**	
	TT	CC	TT	CC	CT	CC	CT	CC	TT	CT	TT	CT
Forsberg, *et al*.[29]	7	56	16	113	38	56	85	113	7	38	16	85
Sergeeva, *et al*.[33]	6	56	6	27	41	56	19	27	6	41	6	19
Oguri, *et al*.[31]	2	84	2	315	21	84	37	315	2	21	2	37
Tang, *et al*.[34]	3	197	O	222	65	197	43	222	3	65	O	43
Ramprasath, *et al*.[32]	22	101	20	137	118	101	128	137	22	118	20	128
Zeikova, *et al*.[35]	22	79	30	206	71	79	176	206	22	71	30	176

Table 4. The univariate meta-regression analysis for heterogeneity of polymorphism.

Variable	Coefficient	T value	P value	Tau2 value	I^2% (residual)	Adj R^2 %
Ethnicity	−0.52	−3.58	0.01	0	2.48	100.00
Sample size	0.00	0.17	0.87	0.09	61.20	−22.48
Study design	−0.33	−1.87	0.1	0.04	47.92	42.76
Quality assessment	0.13	1.77	0.12	0.07	55.57	10.46
Source of control	−0.01	−0.05	0.96	0.10	61.88	−32.99

Adj-R^2: proportion of between-study variance explained; I^2% (residual): percents of residual variation due to heterogeneity; Tau2: estimate of between-study variance.

Although the funnel plot exhibited obvious asymmetry, the *P* value of publication bias tested by Egger's regression was 0.066, in Figure 6. After the 'Trim and Fill' adjustment, two estimated studies were added to attenuate the publication

bias, in Figure 7. Filled meta-analysis was performed after including the estimated effect of missing studies. As the result, the statistical power of overall effect of GPx-1 variants with CVD risk was lost in the random effect models, OR = 1.26, 95% CI: 0.98–1.6. However, there was no obvious publication bias as for the East Asian subgroup, $P = 0.379$, in Figure 8.

Study ID	OR (95% CI)	Weight (%)
Forsberg, *et al*.[29]	0.94 (0.71–1.24)	16.03
Sergeeva, *et al*.[33]	0.88 (0.52–1.51)	9.61
Hamanishi, *et al*.[26]	3.98 (1.68–9.38)	5.21
Oguri, *et al*.[31]	1.98 (1.23–3.18)	10.93
Nemoto, *et al*.[30]	3.61 (0.75–16.16)	1.96
Tang, *et al*.[34]	1.70 (1.08–2.69)	11.39
Kuzuya, *et al*.[27]	1.51 (0.86–2.65)	9.17
Ramprasath, *et al*.[32]	1.14 (0.96–1.35)	18.94
Chen, *et al*.[28]	1.17 (0.55–2.49)	6.30
Zeikova, *et al*.[35]	1.26 (0.77–2.07)	10.45
Overall (I-squared = 57.9%, P = 0.011)	1.36 (1.08–1.70)	100.00

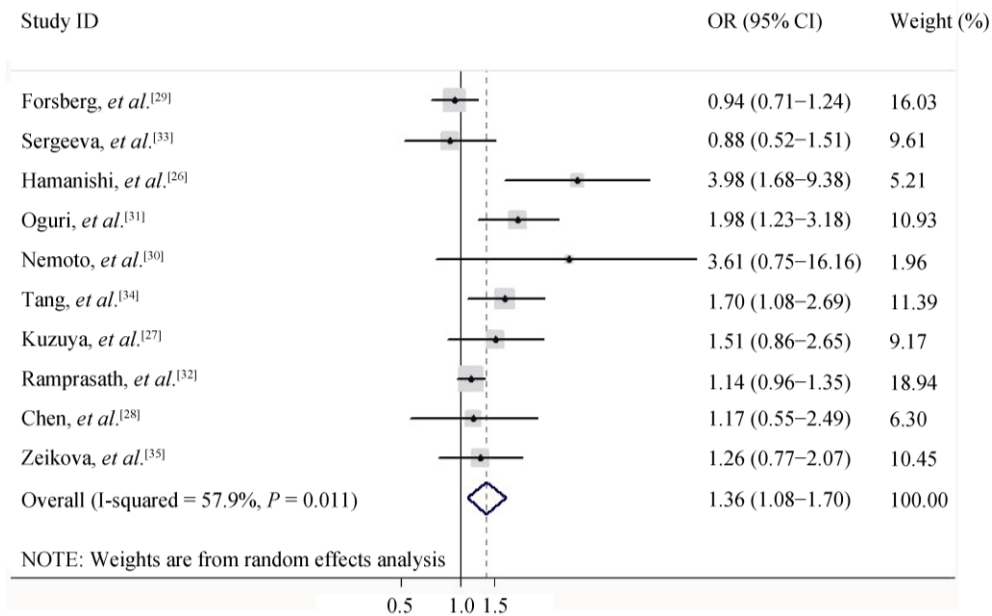NOTE: Weights are from random effects analysis

0.5   1.0 1.5

Figure 3. Forest plot describing the meta-analysis with a random-effect for the association of GPx-1 Pro198Leu and Pro197Leu polymorphisms with cardiovascular diseases risk, accompanied by the respective 95% confidence intervals (CIs). Values of OR and CIs > 1 implied an increased risk for cardiovascular diseases under the co-dominant model.

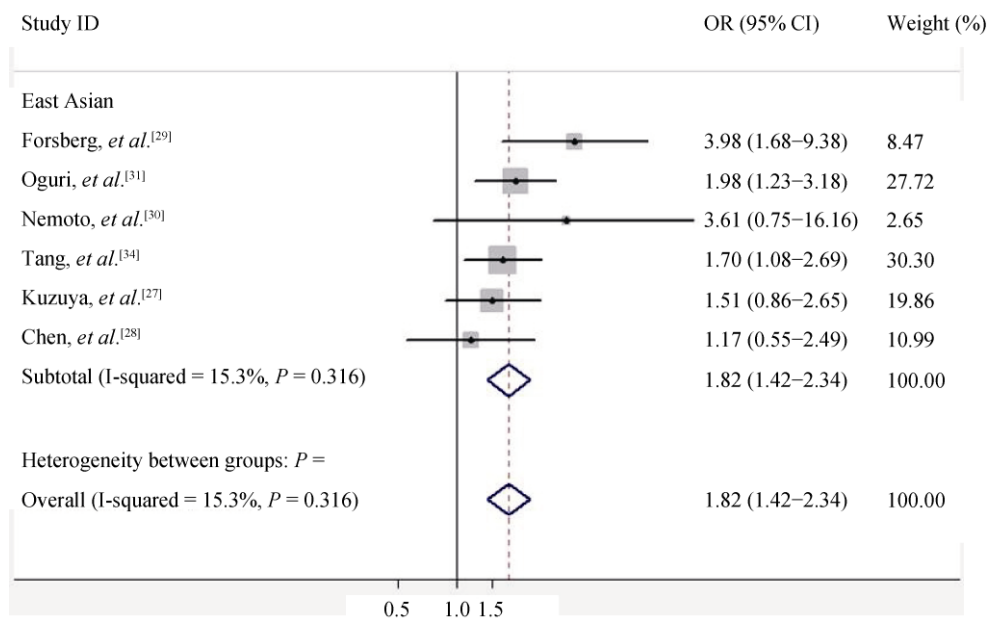| Study ID	OR (95% CI)	Weight (%)
East Asian		
Forsberg, *et al*.[29]	3.98 (1.68–9.38)	8.47
Oguri, *et al*.[31]	1.98 (1.23–3.18)	27.72
Nemoto, *et al*.[30]	3.61 (0.75–16.16)	2.65
Tang, *et al*.[34]	1.70 (1.08–2.69)	30.30
Kuzuya, *et al*.[27]	1.51 (0.86–2.65)	19.86
Chen, *et al*.[28]	1.17 (0.55–2.49)	10.99
Subtotal (I-squared = 15.3%, P = 0.316)	1.82 (1.42–2.34)	100.00
Heterogeneity between groups: P =		
Overall (I-squared = 15.3%, P = 0.316)	1.82 (1.42–2.34)	100.00

0.5   1.0 1.5

Figure 4. The forest plot describing the meta-analysis with a fixed-effect for the association of GPx-1 Pro198Leu and Pro197Leu polymorphisms with cardiovascular diseases risk in East Asian population, accompanied by the respective 95% confidence intervals (CIs). Values of OR and CIs > 1, implied an increased risk for cardiovascular diseases under the co-dominant model.
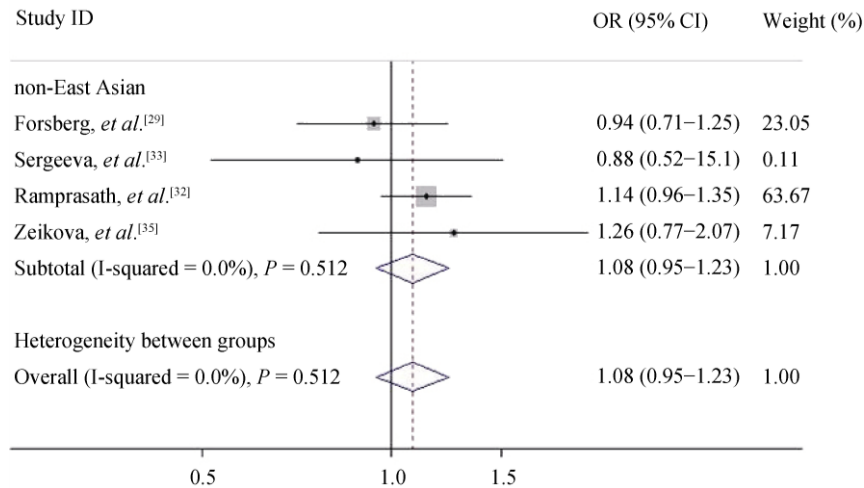
Figure 5. Forest plot describing the meta-analysis with a fixed-effect for the association of GPx-1 Pro198Leu and Pro197Leu polymorphisms with cardiovascular diseases risk in non-East Asian population, accompanied by the respective 95% confidence intervals (CIs). Values of OR > 1, while the lower limit of CI < 1, implied an indefinite risk for cardiovascular diseases under the co-dominant model.
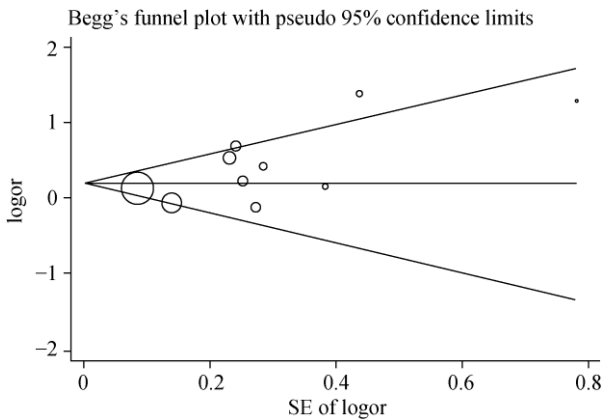

Figure 6. Begg's funnel plot with pseudo 95% confidence intervals (CIs) of GPx-1 Pro198Leu and Pro197Leu polymorphisms. The size of the circle is proportional to the weight of the study.
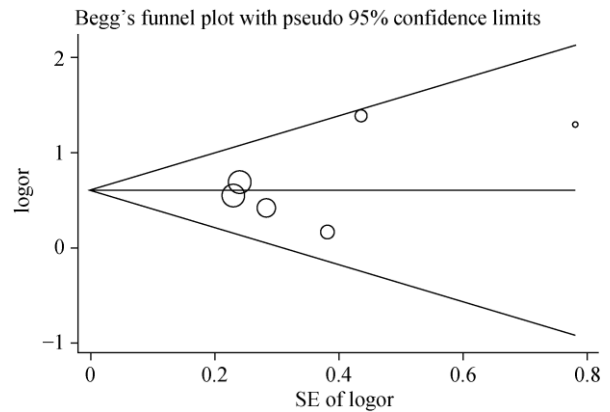

Figure 8. Begg's funnel plot with pseudo 95% confidence intervals (CIs) of GPx-1 Pro198Leu and Pro197Leu polymorphisms in East Asian population. The size of the circle is proportional to the weight of the study.

Figure 7. Filled funnel plot with pseudo 95% confidence intervals (CIs) after 'Trim and Fill' adjustment. The filled data are indicated by the addition of a square placed around the circle.

4　Discussion

The main finding of this meta-analysis is that the GPx-1 Pro198Leu and Pro197Leu polymorphisms are associated modestly, but significantly, to increased risks of CVD, especially in East Asian populations. To the best of our knowledge, it is the first meta-analysis for the relationship of GPx-1 variants and CVD risk.

GPx-1 acts as part of imperative peroxidases in the regulation of reactive oxygen species. A rise in GPx-1 expression was found to be an adaptive mechanism through which the endothelial cells maintained their anti-atherosclerotic properties.[36] Deficiency of GPx-1 accelerates the progression of atherosclerosis in apolipoprotein E-deficient mice on a Western-type diet.[37] Lewis, *et al.*[38] reported that in mice

the lack of functional GPx-1 accelerated diabetes-associated atherosclerosis via up-regulation of proinflammatory and profibrotic pathways, suggesting GPx-1 as an important anti-atherogenic therapeutic target of diabetic macrovascular disease. A growing body of clinical evidence also demonstrated a decreased GPx-1 level in whole blood was linked to higher risk of CVD.[5–7] Considering GPx-1 with T allele (proline to leucine substitution) was less responsive to the stimulation of selenium supplementation[39] and a reverse dose effect response in the T allele with GPx-1 activity was observed in breast cancer patients,[40] it is plausible to hypothesize that GPx-1Pro198Leu and Pro197Leu polymorphisms are associated with CVD risk. However, previous studies on GPx-1 variants with CVD risk found conflicting results. Some studies reported an increased CVD risk in GPx-1 variants.[26,31,34] Nevertheless, most studies did not find a significant association of GPx-1 variants with CVD risk.[27–30,32,33,35] However, in this meta-analysis, GPx-1 Pro198Leu and Pro197Leu polymorphisms were the modest risk factor in the development of CVD in the overall study population. The negative results of the previous reports might be due to small sample size of individual studies which led to less statistical power and underestimates of risk.

In this meta-analysis of genetic association study, genetic models in most studies are not identified [26,28–30,32,33,35] except that in Oguri's study,[31] the dominant and the additive one models were discussed; in Kuzuya's study,[27] a dominant model (TT and TC combined) was adopted simply because TT homozygosity was a minor genotype (0.3%); and in Tang's study,[34] TT and TC were combined using a dominant model because none of the TT homozygosity was genotyped in the control group. Nonetheless, genotype information available from six included studies was supportive of co-dominant model, which is consistent with the finding that the dose of T allele was related to the GPx-1 activity.[40] Considering the existence of clinical confounders, such as diversity of CVD endpoints, source of control, as well as difference of genotyping methods of each included study, the co-dominant model of GPx-1 polymorphisms merits further identification.

Ethnic difference is a vital factor to produce heterogeneity in genetic studies, and thus to confound factual genetic effects. Previous investigations found that the frequency distribution of T allele significantly varied in different ethnicities (36% in Caucasians, 33% in Africans and 5% in Japaneses),[41,42] similar to our meta-analysis (8.57% in East Asian and 29.56% in non-East Asian). In the present meta-analysis, evident heterogeneity reduction in respective ethnic subgroups suggested the existence of strong ethnic divergence of GPx-1 variants with CVD risk. The ethnic

divergence was also reported in the cancer risk of TC/TT (Pro/Leu and Leu/Leu) genotypes, in which Asian populations with the variants had higher risk.[13] However, given only four studies in non-East Asians in this meta-analysis, more studies with larger sample size from non-East Asian population are needed to investigate the relationship of GPx-1 Pro198Leu and Pro197Leu polymorphisms with CVD risk in different ethnicities.

In addition, gene-environment interaction was not taken into consideration in this meta-analysis. The correlation between GPx-1 variants and selenium intake had been documented in endemic heart failure in the Keshan region in China, which might influence the effect of polymorphic variants.[23] Alcohol consumption is another environmental exposure to influence GPx-1 activity.[40] Furthermore, little is known about the gene-gene interaction because of no information available from the original data. Adjusted risks of GPx-1 variants including the gene-gene interaction and environmental status are warranted in ongoing studies.

Moreover, the potential publication bias may attenuate the validity of the CVD risk of GPx-1 variants in this meta-analysis because statistical significance disappeared after adjustment for publication bias in overall populations. However, no substantial publication bias was found in the East Asian subgroup, suggesting the robustness of the association in East Asian population.

In conclusion, GPx-1 Pro198Leu and Pro197Leu polymorphisms are related to increased risks of CVD, especially in East Asian populations. However, the conclusion should be interpreted with caution. Our suggestions for the future studies including the detailed analysis of genetic models, inclusion of a larger non-East Asian population and comprehensive study design related to gene-gene and gene-environment interactions.

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