



Meta-analysis of TAFI polymorphisms and risk of cardiovascular and cerebrovascular diseases

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ABSTRACT. Cardiovascular and cerebrovascular diseases (CCVDs) are common and have high rates of morbidity, mortality, and recurrence. Thrombin-activatable fibrinolysis inhibitor (TAFI) is also known as carboxypeptidase B2 and is encoded by the *CPB2* gene; *CPB2* polymorphisms have been explored in a variety of studies, but their correlation to the risk of CCVDs remains ambiguous. We examined the hypothesized associations between *CPB2* mutations and CCVDs in a general population. We searched, Embase, the Cumulative Index to Nursing and Allied Health Literature, the Science Citation Index, and several Chinese databases without applying any language restrictions. Nine case-control studies were analyzed in the current meta-analysis, and odds ratios (ORs) with their 95% confidence intervals were calculated. The pooled ORs indicated that the *CPB2* rs3742264 G>A polymorphism was associated with an increased risk of CCVDs in the allele model (all P values < 0.05). A similar result for the *CPB2* rs1926447 C>T polymorphism and CCVDs risk was detected in the allele model (P < 0.05). Ethnicity subgroup analysis implied that the rs3742264 G>A polymorphism was more likely to

lead to the development of cerebrovascular disease in Asians (all P values < 0.05), whereas rs1926447 C>T was associated with cardiovascular disease among Africans (all P values < 0.05). These data suggest that the polymorphisms investigated, especially rs3742264 G>A and rs1926447 C>T, have a modest effect on susceptibility to CCVDs.

Key words: Thrombin-activatable fibrinolysis inhibitor; Carboxypeptidase B2; Single nucleotide polymorphism; Cardiovascular and cerebrovascular diseases; CCVD; Meta-analysis

INTRODUCTION

Cardiovascular and cerebrovascular diseases (CCVDs), also known as circulatory diseases, are regarded as the prime cause of death and disability globally, and are related to the organs and tissues that deliver blood, mainly the heart and the vascular system (Haaf et al., 2014). In the United States, it has been estimated that 83 million people have CCVDs - nearly one in three adults -, which accounts for 20% of all medical expenditure, equaling 298 billion dollars and leading to an excessive hospital admission and medical health burden (Montgomery and Brown, 2013). However, low- and middle-income countries may account for approximately 82% of mortalities due to CCVDs globally (Aslan and Dogan, 2011). Owing to the serious implications of CCVDs, more effective prognostic prediction by objectively measuring left atrial size and volume has been introduced, with the aim of preventing the progression of CCVDs (Russo et al., 2013). The most essential factors, such as hyperlipidemia, hypertension, and atherosclerosis, are conducive to ischemic and hemorrhagic symptoms, resulting in CCVDs (Kalaria, 2012). Moreover, an examination of the etiologic factors has revealed that a CCVD patient's level of fitness and obesity may be related to the development and prevention of CCVDs (Al-Hazzaa, 2002; Lee et al., 2012). In addition, large-scale research has shown that in developed countries patients with gout, possibly induced by alcohol consumption, immoderate sugar consumption, and consumption of purine- and protein-rich foods, are more likely to have CCVDs (Seminog and Goldacre, 2013). With regards to genetic factors, many studies have revealed that polymorphisms in the gene that encodes thrombin-activatable fibrinolysis inhibitor (TAFI) are associated with susceptibility to CCVDs (Kraft et al., 2010; Jood et al., 2012).

TAFI, which was discovered two or three decades ago, is an unstable carboxypeptidase and is recognized as one of the most common anti-fibrinolytic factors involved in the coagulation cascade (Colucci and Semeraro, 2012). It is thought that the physiological function of TAFI is to attenuate fibrinolysis by impeding the action of plasminogen, generally on the surface of fibrin clots; such action is widely blamed for intra-vascular thrombosis (Ammollo et al., 2010). TAFI was first isolated by Eaton et al. (1991), and is encoded by the carboxypeptidase B2 (*CPB2*) gene, which has the chromosomal locus 13q14.11, comprises 11 exons, and spans approximately 48 kb (Boffa et al., 1999). Obviously, any disturbance in the level of TAFI or its activity may lead to elevated rates of thrombotic complication in human cancer patients (Fidan et al., 2012). After an investigation of several publications on the relationship between genetic variations in *CPB2* and the occurrence of and susceptibility to human diseases and cancers, it was suggested that an increase in the level of TAFI may affect the formation of an arterial or venous thrombus, and may further contribute to the development of several CCVDs (Leebeek et al., 2005; Meltzer et al., 2009). Moreover, a different

study has revealed that *CPB2* gene variation is an independent variable in the development of ischemia stroke (Ladenvall et al., 2007). However, it may also be possible that a decrease in the level of TAFI suppresses or reduces the formation of a venous thrombus (Wang et al., 2007). In recent decades, associations between single nucleotide polymorphisms (SNPs) that affect TAFI expression levels and thrombotic events have often been explored (Tàssies et al., 2009; Akhter et al., 2010). It can be concluded from the results of this research that the common SNPs of the *CPB2* gene are strongly correlated to the constancy of its mRNA and the synthesis and stability of the TAFI protein, and therefore have an adverse influence on the plasma levels and activation of TAFI (de Bruijne et al., 2009; Kraft et al., 2010). Many SNPs have been identified in the *CPB2* gene. Ala147Thr (rs3742264) and Thr325Ile (rs1926447) polymorphisms in the coding region of the *CPB2* gene, which correspond to 505G>A and 1040C>T amino acid mutations, respectively, may result in a TAFI isoform with altered anti-fibrinolytic activity; they are the most common polymorphisms in the *CPB2* gene and are significantly associated with TAFI plasma antigen levels (Chengwei et al., 2013; Tokgoz et al., 2013). To the best of our knowledge, there are opposing opinions with regards to the relationship between *CPB2* gene polymorphisms and susceptibility to CCVDs (Sun et al., 2011; Tokgoz et al., 2013). In this meta-analysis, we investigated the association between alternations in TAFI levels arising from two common polymorphisms, rs3742264 G>A and rs1926447 C>T, and the risk of developing CCVDs.

MATERIAL AND METHODS

Literature search

We carefully searched, Embase, the Cumulative Index to Nursing and Allied Health Literature, the Science Citation Index, the Cochrane Library Database, the Current Contents Index, the Chinese Biomedical Database, the Chinese Journal Full-Text Database, and the Weipu Journal Database up to June 30, 2014. These computerized bibliographic databases were applied to identify relevant articles related to the association between the *CPB2* gene polymorphisms and susceptibility to CCVD. We used a combination of MeSH terms and various keywords in all fields as follows: ("Myocardial Infarction" OR "Coronary Artery Disease" OR "CAD" OR "MI" OR "myocardial infarct" OR "myocardiac infarction" OR "myocardium infarction" OR "cardiac infarction" OR "infarction myocardium" OR "myocardial infarcted" OR "heart infarction" OR "acute myocardial infarction" OR "Coronary Heart Disease" OR "CHD" OR "AMI") and ("Stroke" OR "Brain Infarction" OR "cerebral infarction" OR "cerebral ischemic stroke" OR "cerebral stroke" OR "ischemic stroke") and ("Carboxypeptidase U" OR "TAFI" OR "thrombin-activatable fibrinolysis inhibitor" OR "arginine carboxypeptidase" OR "carboxypeptidase R" OR "procarboxypeptidase U" OR "plasma procarboxypeptidase B") and ("Polymorphism, Genetic" OR "polymorphism" OR "polymorphisms" OR "variants" OR "SNP" OR "mutation" OR "genetic variants"). We also carried out manual searches for additional studies.

Study selection

We applied the following inclusion criteria when selecting the studies. First, we took into consideration the specific design of the various studies: only matched or unmatched case-control studies, cohort studies, or genome-wide association studies were included. Second, studies must

have been conducted within a human population exploring the relationship between *CPB2* gene polymorphisms and susceptibility to CCVDs. Most importantly, the diagnosis of CCVD in the patients must have been confirmed by the World Health Organization (1988) diagnostic criteria and imaging methods (computed tomography and magnetic resonance imaging of the brain). Sufficient information on the genotype frequencies of the polymorphisms within the *CPB2* genes must have been provided. Finally, the distributions of genotype frequencies in the *CPB2* genes of the controls must have complied with the Hardy-Weinberg equilibrium (HWE). Any studies that did not satisfy the inclusion criteria were excluded from this research. Furthermore, abstracts, reviews, case reports, letters, meta-analyses, proceedings, or publications and studies with duplicated or overlapping data were excluded from the current meta-analysis.

Data extraction and quality assessment

We used a standard reporting form to extract data from each included study, and the following descriptive information was collected: surname and initials of the first author, year of submission, country, racial descent, study design, number of cases and controls, demographic variables, disease types, SNP information, method used for genotype detection, genotype frequencies, allele frequencies, results of HWE test, and confirmation of diagnosis.

The quality of the studies was also independently assessed by two reviewers using guidelines proposed by the NCI-NHGRI Working Group on Replication in Association Studies (the NCI is the National Cancer Institute and the NHGRI is the National Human Genome Research Institute) (Zhang et al., 2012). A checklist of 53 conditions from the guidelines was supplied for article authors, journal editors, and referees allowing an unambiguous and comprehensive explanation of the original data and results of genome-wide and other genotype-phenotype-associated research. Notably, in our meta-analysis, the first 34 conditions devised were regarded as applicable and suitable for the quality assessment of every single included study. Each condition was assigned a score. When a requirement was met, a score of 1 was awarded; otherwise, a score of 0 was given. The total score was considered the final quality score for each study.

Statistical analysis

Adjusted odds ratios (ORs) and 95% confidence intervals (95% CIs) calculated by the Z-test were used to compare cases and controls in the following five different genotype models: the allele model (W allele vs M allele), the dominant model (WW + WM vs MM), the recessive model (WW vs WM + MM), the homozygous model (WW vs MM), and the heterozygous model (WW vs WM) (W = wild; M = mutant). Within the secondary analyses, specific ORs were calculated according to the racial descent of the subjects (divided into Caucasians and Asians) and disease types (cerebrovascular diseases and cardiovascular diseases). Between-study heterogeneity was assessed using Cochran's Q-test ($P < 0.05$ was considered significant) and I^2 tests ($I^2 > 50\%$) (Zintzaras and Ioannidis, 2005a). The random-effect model was applied for significant heterogeneity, whereas ORs were pooled based on the fixed-effect model (Zintzaras and Ioannidis, 2005b). In addition, sensitivity analyses evaluated whether one single study had the weight to influence the overall estimate. Publication bias was detected by the Egger linear regression test ($P < 0.05$ was considered significant), and an evaluation of funnel plot asymmetry was used to assess publication bias (Peters et al., 2006). For all statistical analyses performed herein, the STATA statistical software (Version 12.0, Stata Corporation, College Station, TX, USA) was used.

RESULTS

Selection of eligible studies

After the literature search, 229 potential articles were collected, of which two were obtained by manual searching. One study duplicated other data and was therefore removed, and 141 irrelevant studies were subsequently excluded after screening of the title and abstract. Next, 87 studies were excluded after more detailed full text assessment, and 10 studies were selected for qualitative analysis. An additional study was removed owing to a lack of data integrity. Ultimately, we obtained nine case-control research papers published between 2003 and 2013 that were of moderate-to-high quality (Zorio et al., 2003; Leebeek et al., 2005; Xu et al., 2008; Biswas et al., 2009; Si, 2010; Kamal et al., 2011; Shi et al., 2011; Sun et al., 2011; Tokgoz et al., 2013). The demographic information, baseline characteristics, and other features, and the methodological quality of the extracted studies are presented in Tables 1 and 2.

Table 1. Main characteristics and methodological quality of eligible studies.

Study	Country	Sample size		Gender (M/F)		Age (years)		Genotyping method	SNP	Disease
		Case	Control	Case	Control	Case	Control			
Tokgoz (2013)	Turkey	59	100	18/41	40/60	38.0 ± 13.7	38.0 ± 13.3	PCR-RFLP	rs3742264 G>A rs1926447 C>T	CVT
Sun (2011)	China	136	85	89/47	52/33	33-76	52.9 ± 9.6	PCR-RFLP	rs3742264 G>A rs1926447 C>T	CHD
Shi (2011)	China	98	100	79/19	67/33	61.1 ± 7.9	62.3 ± 8.3	PCR-RFLP	rs3742264 G>A rs1926447 C>T	CI
Kamal (2011)	Egypt	46	54	33/13	39/15	55.7 ± 8.1	-	TaqMan assay	rs1926447 C>T	MI
Si (2010)	China	100	90	78/22	71/19	59.8 ± 8.5	58.6 ± 8.2	PCR-RFLP	rs3742264 G>A rs1926447 C>T	MI
Biswas (2009)	India	58	58	-	-	-	-	PCR-RFLP	rs3742264 G>A	Stroke
Xu (2008)	China	210	190	154/56	142/48	-	57.8 ± 8.9	PCR-RFLP	rs3742264 G>A rs1926447 C>T	CHD
		100	190	78/22	142/48	59.8 ± 8.5	57.8 ± 8.9	PCR-RFLP	rs3742264 G>A rs1926447 C>T	MI
		110	190	76/34	142/48	56.2 ± 9.6	57.8 ± 8.9	PCR-RFLP	rs3742264 G>A rs1926447 C>T	Angina
Leebeek (2005)	Netherlands	124	125	66/58	66/59	56.0 ± 12.0	56.0 ± 12.0	PCR-RFLP	rs3742264 G>A rs1926447 C>T	IS
Zorio (2003)	Spain	127	99	114/13	90/9	44.0 ± 5.5	42.0 ± 8.9	AS-PCR	rs1926447 C>T	MI

M = male; F = female; PCR-RFLP = polymerase chain reaction-restriction fragment length polymorphism; SNP = single nucleotide polymorphism; CVT = cerebral venous thrombosis; CHD = coronary heart disease; MI = myocardial infarction; CI = cerebral infarction; IS = ischemic stroke.

Table 2. Methodologic quality of studies included in the final analysis based on the Newcastle-Ottawa scale for assessing the quality of case-control studies.

Study	Selection (score)				Comparability (score)	Exposure (score)			Total score**
	Adequate definition of patient case	Representativeness of patient cases	Selection of controls	Definition of controls		Control for important factor or additional factor	Ascertainment of exposure (blinding)	Same method of ascertainment for participants	
Tokgoz	1	1	1	1	1	0	1	1	7
Sun	1	1	1	1	1	1	1	1	8
Shi	1	1	1	1	0	1	1	1	7
Kamal	1	0	1	1	1	0	1	1	6
Si	1	1	1	1	1	0	1	1	7
Biswas	1	0	1	1	0	1	1	1	6
Xu	1	1	1	1	1	1	1	1	8
Leebeek	1	0	1	1	1	1	1	1	7
Zorio	1	1	1	1	0	1	1	1	7

*When there was no significant difference in the response rate between both groups using the χ^2 test ($P > 0.05$), 1 point was awarded. **Total score could range from 0 to 9 points.

Baseline information of the included studies

Of the nine eligible studies, seven correlated the *CPB2* rs3742264 G>A genetic polymorphism with susceptibility to CCVD; and eight studies focused on the relationship between the *CPB2* rs1926447 C>T genetic polymorphism and susceptibility to CCVD. Of the nine included studies, two were conducted on Caucasians, one on Africans, and the remaining six on Asians. With respect to the genotyping methods, the detection of the *CPB2* gene polymorphisms (rs3742264 G>A and rs1926447 C>T) was accomplished by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP), TaqMan assay, and allele-specific PCR.

Meta-analysis of rs3742264 G>A and rs1926447 C>T polymorphisms

The nine included studies exploring the relationship between rs3742264 G>A and rs1926447 C>T polymorphisms and CCVDs contained information about 1168 cases and 901 healthy controls. The results showed that carriers of the *CPB2* rs3742264 G>A polymorphism faced a higher risk of CCVDs in both the allele and dominant models, based on the ORs from the combined results of all the included studies (allele model: OR = 1.19, 95%CI = 1.05-1.35, P = 0.008; dominant model: OR = 1.25, 95%CI = 1.05-1.50, P = 0.014) (Figure 1). Carriers of the *CPB2* rs1926447 C>T polymorphism were more likely to develop CCVDs than those without it in the allele model (P = 0.006), which was significantly different from the dominant model (OR = 1.17, 95%CI = 0.98-1.40, P = 0.058).

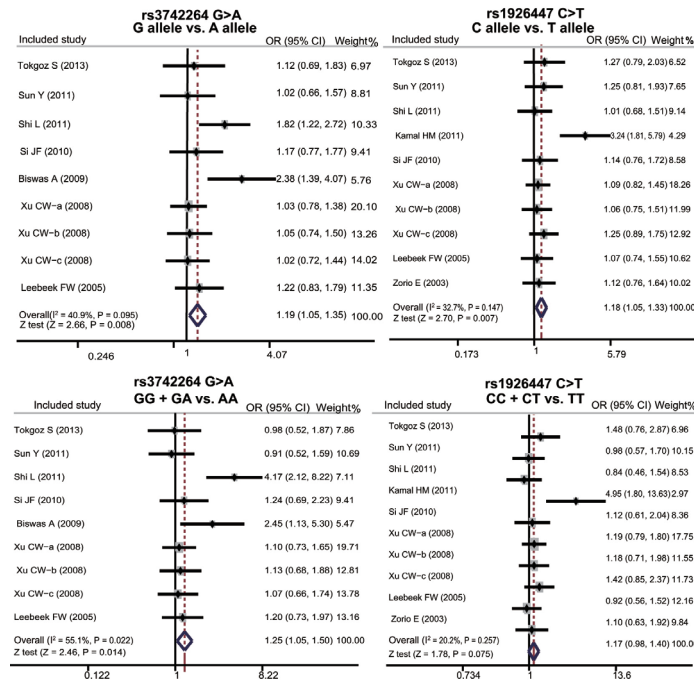


Figure 1. Forest plots of the relationships between *CPB2* rs3742264G>A and rs1926447C>T polymorphisms and cardiovascular and cerebrovascular risk in the allele and dominant models.

Subgroup analysis

Since obvious heterogeneity was found, subgroup analyses were continued with respect to ethnicity and diseases. Firstly, within the allele model, *CPB2* rs3742264 G>A occurred more frequently in CCVDs patients compared with the controls in the Asian subgroup (OR = 1.19, 95%CI = 1.04-1.36, P = 0.013), but not in the Caucasian subgroup (OR = 1.22, 95%CI = 0.83-1.79, P = 0.302); a similar association was observed with *CPB2* rs3742264 G>A in the dominant model (Asian subgroup: OR = 1.28, 95%CI = 1.06-1.55, P = 0.012; Caucasian subgroup: OR = 1.27, 95%CI = 0.73-1.97, P = 0.483, respectively). With regards to *CPB2* rs1926447 C>T, we observed an obvious association between *CPB2* rs1926447 C>T and an increased risk of developing CCVDs in the African subgroup in both the allele and dominant models (all P < 0.05), whereas a similar association was not found in the Asian or Caucasian subgroups (all P > 0.05) (Figure 2). In addition, disease-stratified subgroup analysis demonstrated that *CPB2* rs3742264 G>A carriers had significantly higher susceptibility to cerebrovascular disease (P < 0.05) than cardiovascular disease (allele model: OR = 1.05, 95%CI = 0.90-1.23, P = 0.547; dominant model: OR = 1.08, 95%CI = 0.87-1.35, P = 0.473). As shown in Figure 3, *CPB2* rs1926447 C>T carriers had an increased risk of developing cardiovascular disease (allele model: OR = 1.22, 95%CI = 1.06-1.40, P = 0.006; dominant model: OR = 1.25, 95%CI = 1.02-1.54, P = 0.029), than cerebrovascular disease (all P > 0.05).

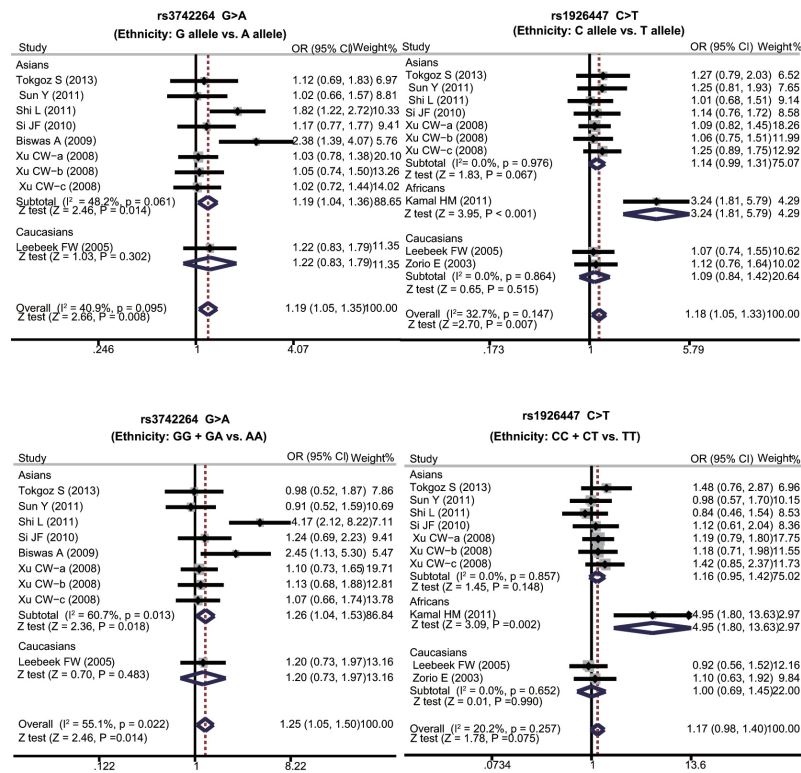


Figure 2. Subgroup analysis based on ethnicity of the relationships between *CPB2* rs3742264G>A and rs1926447C>T polymorphisms and cardiovascular and cerebrovascular risk in the allele and dominant models.

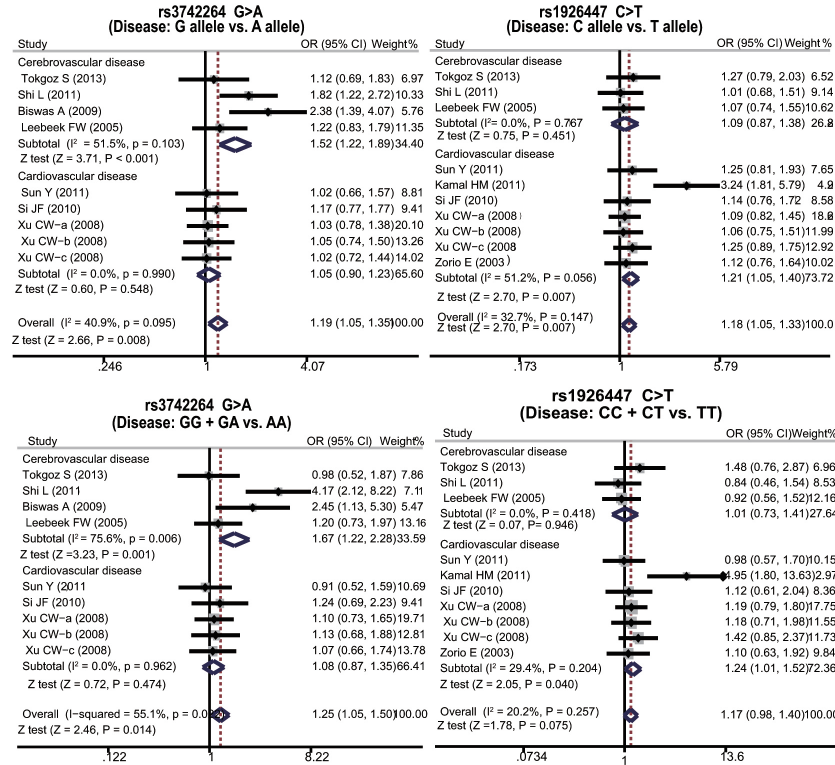


Figure 3. Subgroup analysis based on diseases of the relationships between *CPB2* rs3742264G>A and rs1926447C>T polymorphisms and cardiovascular and cerebrovascular risk in the allele and dominant models.

From the univariate and multivariate meta-regression analyses presented in Table 3, the potential source of heterogeneity may be derived from the disease types through the evidence for the association between *CPB2* rs3742264 and CCVD risk (univariate: $P = 0.008$; multivariate: $P = 0.003$), but *CPB2* rs1926447 showed no statistical significance.

Table 3. Univariate and multivariate meta-regression analyses of potential source of heterogeneity.

Heterogeneity factors		rs3742264 G>A						rs1926447 C>T					
		Coefficient	SE	z	P	95%CI		Coefficient	SE	z	P	95%CI	
						LL	UL					LL	UL
Year	Univariate	0.019	0.041	0.46	0.648	-0.062	0.100	0.024	0.033	0.75	0.452	-0.039	0.088
	Multivariate	-0.062	0.056	-1.10	0.272	-0.172	0.048	0.013	0.044	0.30	0.766	-0.073	0.100
Ethnicity	Univariate	0.004	0.285	0.01	0.989	-0.555	0.563	0.246	0.178	1.39	0.166	-1.019	0.595
	Multivariate	-0.697	0.418	-1.67	0.095	-1.516	0.122	0.153	0.282	0.54	0.587	-0.340	0.706
Disease	Univariate	-0.367	0.138	-2.65	0.008	-0.638	-0.096	0.204	0.200	1.02	0.307	-0.187	0.594
	Multivariate	-0.622	0.209	-2.97	0.003	-1.031	-0.212	0.122	0.278	0.44	0.659	-0.421	0.666

SE = standard error; 95%CI = 95% confidence interval; UL = upper limit; LL = lower limit.

Sensitivity analyses

Sensitivity analyses were performed and demonstrated that no single study had the weight to affect the overall estimate of the association between the *CPB2* gene polymorphisms and risk of CCVDs (Figure 4).

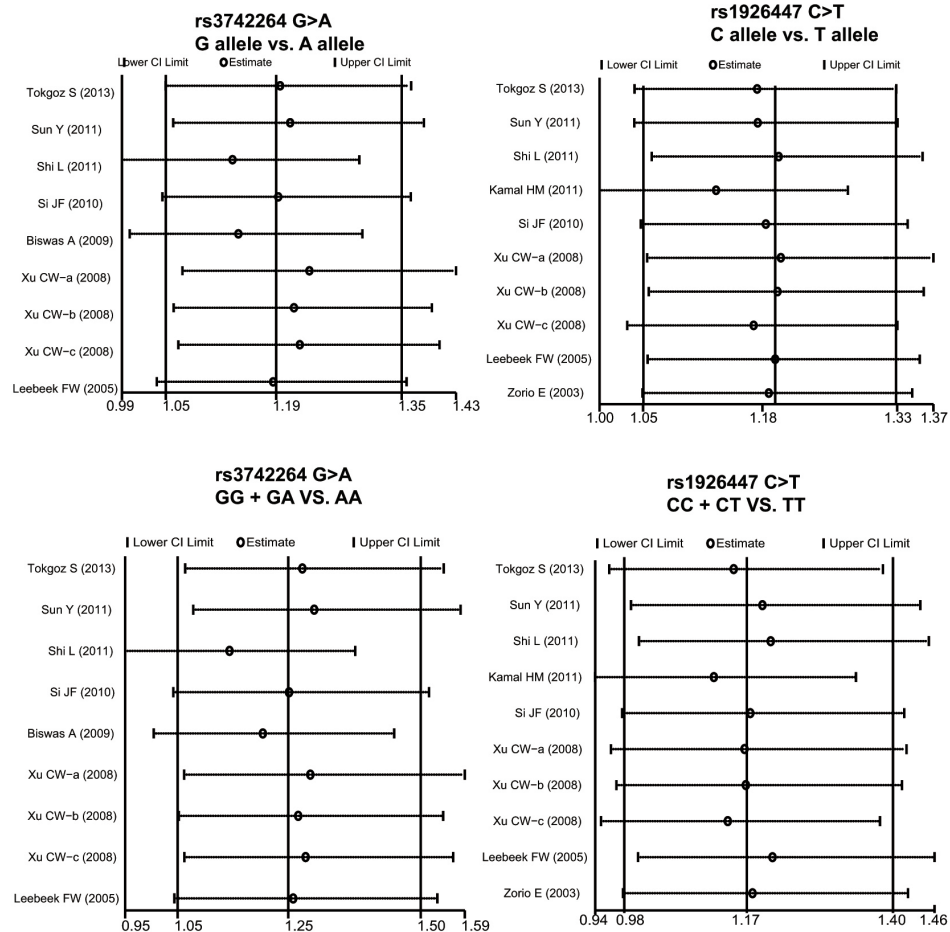


Figure 4. Sensitivity analysis of the summary odds ratios for the relationships between *CPB2* rs3742264G>A and rs1926447C>T polymorphisms and cardiovascular and cerebrovascular risk in the allele and dominant models.

DISCUSSION

This meta-analysis was conducted to study the relationship between rs3742264 G>A and rs1926447 C>T polymorphisms in the *CPB2* gene and susceptibility to CCVDs. Collectively, the results of our present meta-analysis revealed a significant relationship between rs3742264 G>A and rs1926447 C>T polymorphisms and the pathogenesis of CCVDs. Comprehensively speaking, TAFI, also known as carboxypeptidase B2 or carboxypeptidase U, is a zymogen synthesized by the liver and present in the plasma of humans that plays an essential role in fibrinolysis and the activation of plasmin (Chengwei et al., 2013). In general, TAFI can be activated by trypsin-like enzymes such as plasmin, thrombin, or the complex of thrombin/thrombin-thrombomodulin, leading to exposure of the TAFI active site (Ladenvall et al., 2007). Activated TAFI (TAFIa) can inhibit fibrinolysis by the removal of the C-terminal lysine residues of partially degraded, plasmin-modified

fibrin and reduction of plasmin formation. Therefore, TAFI levels and activity might be linked to fibrinolysis and coagulation (Miah and Boffa, 2009; Wu et al., 2009). In this regard, TAFIa could be considered to participate in the development of autoimmune diseases with a tendency to thrombus, such as systemic lupus erythematosus and Behçet disease, and endocrine disorders with hypofibrinolysis, such as diabetes mellitus and obesity (Martinez-Zamora et al., 2009; Ermantas et al., 2010). Additionally, TAFI may also help the growth and spread of tumor cells owing to the accumulation of intra-tumor fibrin and activation of the blood coagulation system (Fidan et al., 2012). TAFI is important in CCVDs, such as coronary heart disease, angina pectoris, ischemic stroke, myocardial infarction, and venous thrombosis, mainly owing to its role in thrombosis treatment (Zorio et al., 2003; Leebeek et al., 2005; Tokgoz et al., 2013). Polymorphisms of the *CPB2* gene result in changes in the amino acid sequence and function of TAFI, especially in the molecular stability of TAFIa and its anti-fibrinolytic activity in arterial thrombosis. Among those polymorphisms, rs3742264 (505G/A, Ala147Thr) in exon 6 and rs1926447 (1040C/T, Thr325Ile) in exon 10 are associated with changeable expression levels of TAFI (de Bruijne et al., 2009). It has been reported that TAFI levels and activity in rs3742264 G>A and rs1926447 C>T polymorphisms are higher than in the wild type, and might be closely linked to cerebral infarction (Shi et al., 2011). From the above analysis, we conclude that the rs3742264 G>A and rs1926447 C>T polymorphisms can affect the expression and activation of TAFI, which inhibits fibrinolysis and promotes thrombus formation, thereby participating in the progression of CCVDs. In accordance with our analysis, Kamal et al. (2011) reported that the Thr/Ile and Ile/Ile genotypes of rs1926447 were more frequent in myocardial infarction patients than in the control group, indicating the association between the *CPB2* gene polymorphism and the risk of myocardial infarction. Moreover, Biswas et al. (2009) revealed a strong connection between the rs3742264 polymorphism and the development of pediatric stroke.

In view of the many other factors that might influence the connection between rs3742264 G>A and rs1926447 C>T polymorphisms and disease development, we performed a stratified analysis on the basis of ethnicity and disease. Subgroup analysis on ethnicity showed that the rs3742264 G>A polymorphism is related to diseases in Asians but not in Caucasians, while the rs1926447 C>T polymorphism is related to diseases in Africans but not in Asians or Caucasians, which might be explained by differences in living environments and genetic backgrounds. In addition, from the stratified analysis based on different kinds of diseases, that is CCVDs, we determined that the rs3742264 G>A polymorphism is closely linked with cerebrovascular diseases but not cardiovascular diseases, while rs1926447 C>T is more closely linked to cardiovascular diseases than cerebrovascular diseases, which could be due to differences in the pathogenesis of the different kinds of diseases. In summary, our results are partly in agreement with previous studies that the polymorphisms of rs3742264 G>A and rs1926447 C>T in the *CPB2* gene have an intimate relationship with CCVDs, indicating that *CPB2* gene polymorphisms might be an important genetic marker in the diagnosis and prognosis of CCVDs.

Finally, because CCVDs are complex, the available number of patients may have had a major influence on the applicability of the results to clinical usage. In addition, there was a lack of unified consensus on the lower mean age of the CCVDs patients included, which may have minimized the whole effects of the diseases because hypertension, diabetes, and coronary artery disease are among the causes of thrombosis in older people. Most importantly, our research did not take into consideration the measurement of TAFI antigen levels. Therefore, the effects of genetic polymorphisms of *CPB2* on the expression levels of TAFI may not be entirely clear. Finally,

differences in ethnic distribution, disease type, controls, genotyping and detection methods, and SNP types could have influenced the heterogeneity of this meta-analysis.

In summary, this is the first study to examine the relationship between the rs3742264 G>A and rs1926447 C>T *CPB2* gene polymorphisms and CCVDs. Our results do support the prospective independent association of rs3742264 G>A and rs1926447 C>T polymorphisms with the extent and severity of CCVDs, especially among Asian populations. The modest associations between *CPB2* polymorphisms and CCVDs may contribute to our understanding of the process underlying susceptibility to CCVDs, and any positive findings in the future could help identify people at risk of CCVDs, thereby allowing preventive measures.

Conflicts of interest

The authors declare no conflict of interest.

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