Genetics and Molecular Research

VKORC1 rs2359612 and rs9923231 polymorphisms correlate with high risks of cardiovascular and cerebrovascular diseases

Y. Li[1]*, J. Zhu[2]* and J.Q. Ding[3]

[1]Department of Pharmacy, Ministry of Health Beijing Hospital, Beijing, China
[2]Medical Department, Henan Provincial Corps Hospital of the Chinese People Armed Police Force, Zhengzhou, Henan, China
[3]Department of Pharmacy, Henan Provincial Corps Hospital of the Chinese People Armed Police Force, Zhengzhou, Henan, China

*These authors contributed equally to this study.
Corresponding author: J.Q. Ding
E-mail: dingjianqiang222@163.com

Genet. Mol. Res. 14 (4): 14731-14744 (2015)
Received June 2, 2015
Accepted September 9, 2015
Published November 18, 2015
DOI http://dx.doi.org/10.4238/2015.November.18.38

ABSTRACT. We aimed to confirm the correlations between rs2359612 and rs9923231 single nucleotide polymorphisms (SNPs) in the vitamin K epoxide reductase complex subunit 1 (*VKORC1*) gene and the risk of cardiovascular and cerebrovascular diseases (CCVDs) using meta-analysis. Electronic databases were exhaustively searched for relevant case-control studies by employing stringent inclusion and exclusion criteria. Manual retrieval was also conducted to obtain additional pertinent literature. The STATA statistical software was employed for the process of evidence synthesis. The initial literature search broadly identified 225 studies relevant to our topic of interest, and after multiple rounds of screening, 10 clinical case-control studies met the final inclusion criteria and were selected for this meta-analysis. The selected studies represented a combined total of 7329 patients with CCVD and 7951 healthy controls. Our meta-analysis demonstrated that the

VKORC1 rs2359612 and rs9923231 SNPs were closely associated with high risk for CCVD (rs2359612: allelic: OR = 1.23, 95%CI = 1.00-1.50, P = 0.047; dominant: OR = 1.32, 95%CI = 1.19-1.46, P < 0.001; rs9923231: allelic: OR = 0.74, 95%CI = 0.63-0.87, P < 0.001; dominant: OR = 0.67, 95%CI = 0.55-0.82, P < 0.001). Our meta-analysis provides strong evidence that two SNPs in the *VKORC1* gene, rs2359612 and rs9923231, contribute to the risk of CCVD.

Key words: *VKORC1*; Polymorphisms; rs2359612; rs9923231; Cardiovascular and cerebrovascular disease; Meta-analysis

INTRODUCTION

Cardiovascular and cerebrovascular diseases (CCVD) together are major killers worldwide, and affect multiple organ systems causing dysfunction of the heart, brain, or blood vessels as a result of high blood pressure, atherosclerosis, heart enlargement, and easy blood clotting (Fuster and Bansilal, 2010; Tao et al., 2013). CCVD is a collective term to include cardiovascular diseases (CVD) and cerebrovascular diseases (CBVD); furthermore, CVD itself has a wide disease range including coronary artery disease, peripheral vascular disease, dyslipidemias, heart failure, arrhythmia, and myocardial infarction as well as hypertension (Lee and Kim, 2014). On the other hand, CBVD triggers neurological deficit, including brain disorders related to cerebral vascularization such as ischemic stroke, cerebrovascular anomalies, transient ischemic stroke, and hemorrhagic stroke (Patyar et al., 2011). CCVD claims more than 17.3 million lives every year worldwide, and its incidence has seen a sharp 3-fold increase within the last decade in the United States (Fuster and Bansilal, 2010; Tao et al., 2013). Multiple-recognized environmental factors including dyslipidemia, hypertension, diabetes mellitus, obesity, alcohol use, lack of physical activity, poor diet, tobacco use, and air pollution are linked to a high risk of CCVD (Kim and Johnston, 2011; Turin et al., 2012; Belanger et al., 2014). In light of the high incidence and mortality of CCVD, we are interested in the underlying mechanisms with an aim of identifying causative factors; thus, we noted recent studies that suggested the vitamin K epoxide reductase complex subunit 1 (*VKORC1*) gene as a leading candidate in the genetic predisposition to CCVD risk (Kim and Johnston, 2011; Owens, 2011; Holden et al., 2014).

VKOR is a 163-amino acid polytopic membrane protein essential for the reductive branch of vitamin K required for modification of vitamin K-dependent protein in blood coagulation (Schulman et al., 2010; Tie et al., 2012). Furthermore, VKOR is a multisubunit enzyme, and the single major peptide, VKORC1, is largely responsible for its reductase activity (Aomori et al., 2009). VKORC1 is a polytopic membrane protein of the endoplasmic reticulum, and plays a prominent role in gamma-carboxylation of vitamin K-dependent coagulation factors and vitamin K-dependent matrix protein Gla, and reduces vitamin K to vitamin K hydroquinone (Wang et al., 2008; Tie et al., 2014). Recently, an increasing number of studies have shown particular interest in two single nucleotide polymorphisms (SNPs) in the *VKORC1* gene, rs2359612 and rs9923231, for their strong association with multiple-human disease conditions (Yang et al., 2010; Ragia et al., 2013). These SNPs have since been found to be of great value in predicting the risk of CCVD (Ragia et al., 2013; Wang et al., 2013). On the other hand, there are also studies reporting the lack of any correlation between *VKORC1* SNPs and

CCVD (Arnold et al., 2008; Lemmens et al., 2008). Therefore, we performed the present meta-analysis with the purpose of evaluating whether *VKORC1* SNPs can be risk factors in CCVD.

MATERIAL AND METHODS

Publication search

Electronic databases [PubMed, Embase, EBSCO, CINAHL, Web of Science, Springerlink, Cochrane Library, China BioMedicine (CBM), VIP, and China National Knowledge Infrastructure (CNKI)] since inception to October 2014 were searched, combined with a manual search using free words and key words. Our search terms included "VKORC1 protein, human" or "VKORC1" or "vitamin K epoxide reductase complex 1" or "vitamin K epoxide reductase" and "coronary disease" or "myocardial infarction" or "myocardial infarction" or "hypertension" or "arrhythmias, cardiac" or "blood pressure, high" or "CHD" or "arrhythmia" or "stroke" or "infarction" or "cerebral infarction" or "brain infarction" or "intracranial embolism" or "ischemic attack, transient" or "ITA".

Inclusion and exclusion criteria

The predefined inclusion criteria during the process of study selection in our meta-analysis were: 1) study design: clinical case-control studies regarding the correlation between SNPs of the *VKORC1* gene and CCVD; 2) study subjects: patients with CCVD together with healthy controls; 3) detection methods: polymerase chain reaction-ligase detection reaction (PCR-LDR), PCR-restriction fragment length polymorphism (PCR-RFLP), TaqMan assay, and MassARRAY; and 4) outcome indicators: allele and dominant gene frequencies of patients and controls. The exclusion criteria were: 1) reviews, abstracts, letters, non-human studies, or duplicates; 2) studies unrelated to the research topic; 3) studies without complete data; and 4) non-Chinese or non-English publications.

Data extraction

Y. Li and J. Zhu independently undertook data extraction for meta-analysis based on a predefined form, and any disagreement was resolved after reaching a consensus among several investigators. The main information collected was as follows: first author, publication time, country, ethnicity, language, age, gender, study design, number of patients, detection method, disease, sample source, sample size, and SNP.

Statistical analysis

All statistical analyses of data were performed using the STATA version 12.0 software (Stata Corp., College Station, TX, USA). ORs with 95%CIs among study groups and *Z*-tests were employed to confirm the significance of the overall effect size (Chen et al., 2012). The Cochran's Q-statistic ($P < 0.05$ was considered to be significant) and I^2 test (0%, no heterogeneity; 100%, maximal heterogeneity) were applied to reflect heterogeneity among studies (Peters et al., 2006; Jackson et al., 2012). Additionally, the evidence of significant heterogeneity indicated the utilization of the random-effect model in the process of statistical analyses ($P <$

0.05 or I^2 test exhibited >50%), and if heterogeneity was absent, a fixed-effect model was employed (Higgins and Thompson, 2002; Zintzaras and Ioannidis, 2005b). We utilized univariate meta-regression and multivariate-meta-regression analyses to evaluate possible sources of heterogeneity; in addition, multiple-calibration tests were conducted using the Monte Carlo method (Huizenga et al., 2011; Jackson et al., 2012). Subsequently, sensitivity analyses were utilized to evaluate whether removal of each single study influenced the overall outcomes. Furthermore, the existence of publication bias was detected by funnel plot and contour-enhanced and Egger's linear regression tests (Zintzaras and Ioannidis, 2005a). All tests were two-sided and $P < 0.05$ was considered to be significant.

RESULTS

Baseline characteristics of the studies included

A total of 225 published studies were initially retrieved by our search criteria and subsequently 215 studies were excluded for the following reasons: 55 studies for duplication; 12 for letters, reviews, and meta-analyses; 26 for non-human studies; 96 for no relation to the research topic; 8 for lack of data; and 18 for absence of relevant data. Finally, 10 clinical case-control studies (Watzka et al., 2007; Arnold et al., 2008; Lemmens et al., 2008; Shyu et al., 2010; Zhang and Zhang, 2012; Li, 2013; Ragia et al., 2013; Wang et al., 2006, 2013; Niu and Wei, 2014), published from 2006 to 2014 and representing 7329 patients with CCVD and 7951 healthy controls, were selected for this meta-analysis. Genotype distribution in the meta-analysis was consistent with Hardy-Weinberg equilibrium (HWE) (all $P > 0.05$). Six studies were performed in Asians and four in Caucasians. The present meta-analysis involves the SNPs of the *VKORC1* gene, rs2359612, and rs9923231 (Figure 1). The SNP detection methods included PCR-LDR, PCR-RFLP, TaqMan, and MassARRAY. The baseline characteristics of the enrolled studies are listed in Table 1.

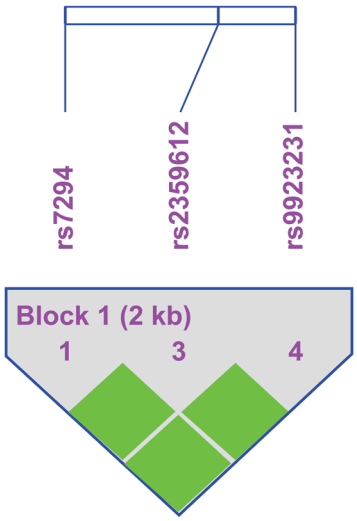

Figure 1. Schematic of the two common polymorphisms of the *VKORC1* gene, rs2359612 and rs9923231, examined in this study.

Table 1. Baseline characteristics the of studies included.

First author	Year	Country	Race	Number		Age (years)		Disease	Genotyping method	SNP
				Patients	Controls	Patients	Control			
Niu GP	2014	China	Asian	200	100	49.5	47	Cardiovascular disease	PCR-LDR	rs9923231
Li J	2013	China	Asian	200	200	67.2 ± 12.2	68.4 ± 10.4	Cardiovascular disease	PCR-RFLP	rs9923231
Wang Y	2013	China	Asian	1026	1013	58.6 ± 8.2	59.0 ± 7.7	Cardiovascular disease	MassARRAY	rs2359612
Ragia G	2013	Greece	Caucasian	145	145	67 ± 12	67 ± 13	Cerebrovascular disease	PCR-RFLP	rs9923231
Zhang YL	2012	China	Asian	362	362	42.7 ± 12.1	43.3 ± 11.5	Cardiovascular disease	PCR-RFLP	rs9923231
Shyu HY	2010	China	Asian	117	115	72.7 ± 11.3	72.8 ± 5.5	Cerebrovascular disease	PCR-RFLP	rs9923231
Lemmens R-a	2008	Belgium	Caucasian	907	1309	56.4 ± 9.2	64.3 ± 12.9	Cardiovascular disease	TaqMan	rs2359612
Lemmens R-b	2008	Belgium	Caucasian	627	1309	63.4 ± 13.1	64.3 ± 12.10	Cerebrovascular disease	TaqMan	rs2359612
Arnold ML	2008	Germany	Caucasian	293	326	50.2 ± 8.9	44.0 ± 8.2	Cerebrovascular disease	PCR-RFLP	rs2359612
Watzka M	2007	Germany	Caucasian	901	521	53.5 (33-64)		Cardiovascular disease	TaqMan	rs2359612
Wang Y-a	2006	China	Asian	740	740	56.6 ± 10.7	58.5 ± 9.2	Cardiovascular disease	PCR-RFLP	rs2359612
Wang Y-b	2006	China	Asian	1811	1811	60.3 ± 9.4	59.6 ± 8.5	Cerebrovascular disease	PCR-RFLP	rs2359612

PCR-LDR = polymerase chain reaction-ligase detection reaction; PCR-RFLP = polymerase chain reaction-restriction fragment length polymorphism; SNP = single nucleotide polymorphism.

Correlation between rs2359612 and CCVD

Five of ten studies reporting the correlation between the rs2359612 SNP in the *VKORC1* gene and CCVD showed significant heterogeneity (allelic: I^2 = 86.1%, P < 0.001; dominant: I^2 = 80.8%, P < 0.001), thus the random-effect model was used. Meta-analysis demonstrated a significant correlation between rs2359612 and the risk of CCVD (allelic: OR = 1.23, 95%CI = 1.00-1.50, P = 0.047; dominant: OR = 1.32, 95%CI = 1.19-1.46, P < 0.001) (Figure 2A and B). A subgroup analysis based on ethnicity found that, in Asians, rs2359612 closely correlated with the risk of CCVD (allelic: OR = 1.54, 95%CI = 1.24-1.92, P < 0.001; dominant: OR = 1.63, 95%CI = 1.43-1.86, P < 0.001). However, in Caucasians, there was no notable correlation between rs2359612 and the risk of CCVD (allelic: OR = 1.01, 95%CI = 0.93-1.09, P = 0.879; dominant: OR = 1.01, 95%CI = 0.87-1.17, P = 0.918) (Figure 3A and B and Table 2). Another subgroup analysis based on disease type suggested that, in the allelic model, there were no correlations between rs2359612 in patients with CVD and CBVD and the risk of CCVD (CVD: OR = 1.17, 95%CI = 0.99-1.39, P = 0.062; CBVD: OR = 1.28, 95%CI = 0.72-2.28, P = 0.409), while in the dominant model, rs2359612 in patients with CVD and CBVD notably correlated with the risk of CCVD (CVD: OR = 1.23, 95%CI = 1.07-1.40, P = 0.003; CBVD: OR = 1.43, 95%CI = 1.24-1.66, P < 0.001) (Figure 3C and D).

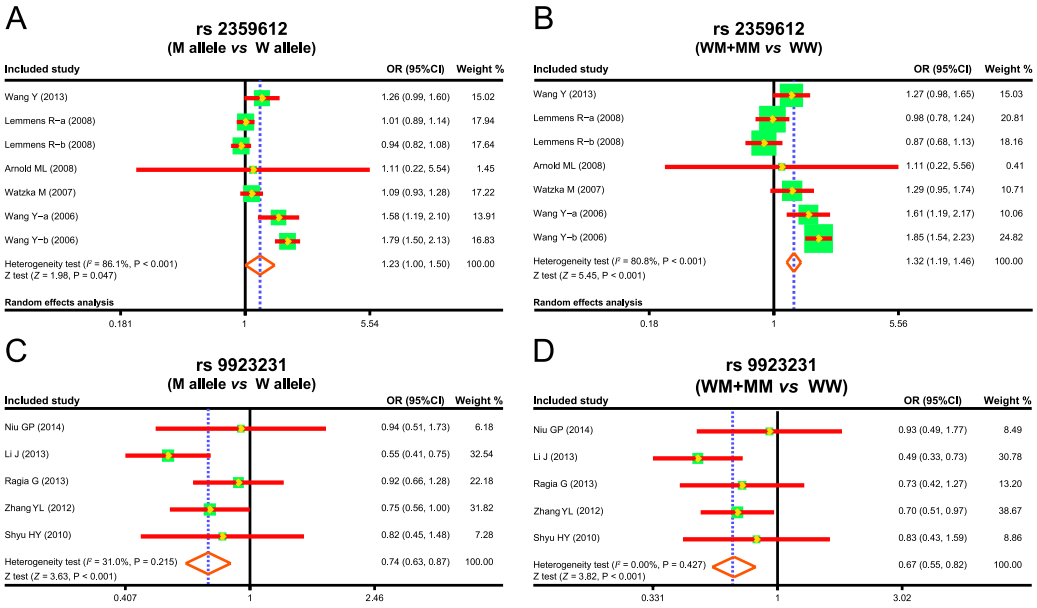

Figure 2. Forest plots showing the correlations between rs2359612 and rs9923231 in the *VKORC1* gene and the risks of cardiovascular and cerebrovascular diseases.

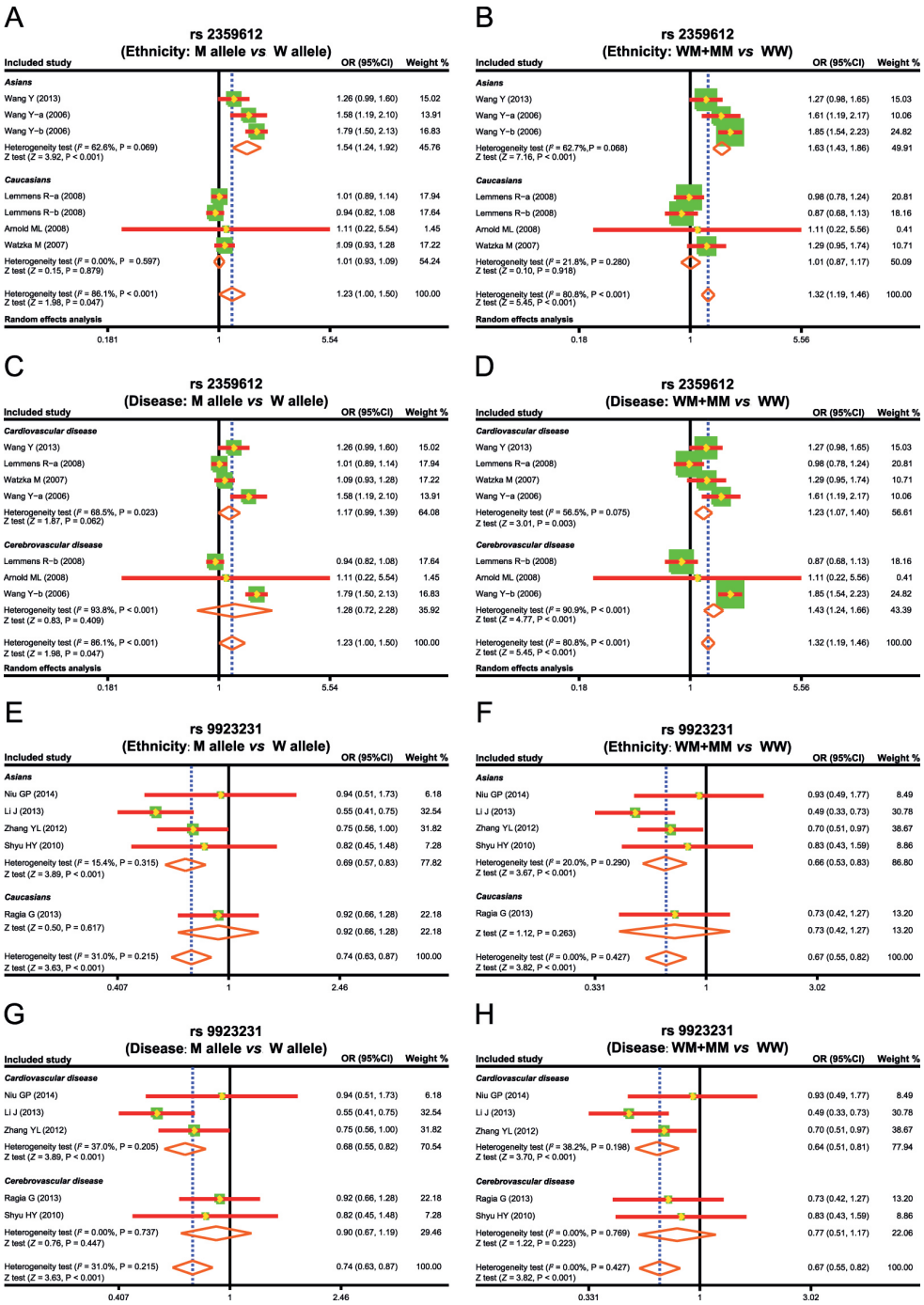

Figure 3. Forest plots showing the correlations between rs2359612 and rs9923231 in the *VKORC1* gene and the risks of cardiovascular and cerebrovascular diseases based on subgroups of ethnicity and disease type.

Table 2. Comparisons of genotype and allele frequencies between patient and control groups.

SNP			VKORC1 (rs2359612)			VKORC1 (rs9923231)		
Genetic model			OR	95%CI	P	OR	95%CI	P
M *vs* W allele (Allelic model)		Overall	1.23	1.00-1.50	0.047	0.74	0.63-0.87	<0.001
	Race	Asian	1.54	1.24-1.92	<0.001	0.69	0.57-0.83	<0.001
		Caucasian	1.01	0.93-1.09	0.879	0.92	0.66-1.28	0.617
	Disease	Cardiovascular disease	1.17	0.99-1.39	0.062	0.68	0.55-0.82	<0.001
		Cerebrovascular disease	1.28	0.72-2.28	0.409	0.90	0.67-1.19	0.447
WM + MM *vs* WW (Dominant model)		Overall	1.32	1.19-1.46	<0.001	0.67	0.55-0.82	<0.001
	Race	Asian	1.63	1.43-1.86	<0.001	0.66	0.53-0.83	<0.001
		Caucasian	1.01	0.87-1.17	0.918	0.73	0.42-1.27	0.263
	Disease	Cardiovascular disease	1.23	1.07-1.40	0.003	0.64	0.51-0.81	<0.001
		Cerebrovascular disease	1.43	1.24-1.66	<0.001	0.77	0.51-1.17	0.223
MM *vs* WW (Homozygous model)		Overall	1.07	0.91-1.25	0.399	0.59	0.39-0.89	0.13
MM *vs* WM (Heterozygous model)		Overall	0.98	0.87-1.11	0.778	1.04	0.71-1.53	0.849
MM *vs* WW + WM (Recessive model)		Overall	1.03	0.93-1.15	0.557	0.79	0.55-1.13	0.197

SNP = single nucleotide polymorphism.

Correlation between rs9923231 and CCVD

For rs9923231, five of ten studies reported correlations between this *VKORC1* gene SNP and CCVD. rs9923231 showed absence of heterogeneity; therefore fixed-effect models were utilized (allelic: I^2 = 31.0%, P = 0.215; dominant: I^2 = 0.0%, P = 0.427). Meta-analysis results demonstrated that rs9923231 was strongly relevant to the risk of CCVD (allelic: OR = 0.74, 95%CI = 0.63-0.87, P < 0.001; dominant: OR = 0.67, 95%CI = 0.55-0.82, P < 0.001) (Figure 2C and D and Table 2). Ethnicity subgroup analysis uncovered that, in Asians, rs9923231 was strongly relevant to the risk of CCVD (allelic: OR = 0.69, 95%CI = 0.57-0.83, P < 0.001; dominant: OR = 0.66, 95%CI = 0.53-0.83, P < 0.001). However, in Caucasians, there was no obvious correlation between rs9923231 and the risk of CCVD (allelic: OR = 0.92, 95%CI = 0.66-1.28, P = 0.617; dominant: OR = 0.73, 95%CI = 0.42-1.27, P = 0.263) (Figure 3E and F). Another subgroup analysis on the basis of disease type showed that, in CVD, rs2359612 were significantly correlated with the risk of CCVD (allelic: OR = 0.68, 95%CI = 0.55-0.82, P < 0.001; dominant: OR = 0.64, 95%CI = 0.51-0.81, P < 0.001), while in CBVD, there was no obvious correlation between rs2359612 and the risk of CCVD (allelic: OR = 0.90, 95%CI = 0.67-1.19, P = 0.447; dominant: OR = 0.77, 95%CI = 0.51-1.17, P = 0.223) (Figure 3G and H).

Sensitivity analysis and publication bias

Sensitivity analyses confirmed that no single study in our meta-analysis exerted significant influence on pooled ORs. For the correlation between the *VKORC1* gene and the risk of CCVD, contour-enhanced funnel plots implied that the literature included were barely interspersed among P > 0.05, and further use of the Egger test demonstrated that publication bias was absent in the literature included (P > 0.05; Figure 4).

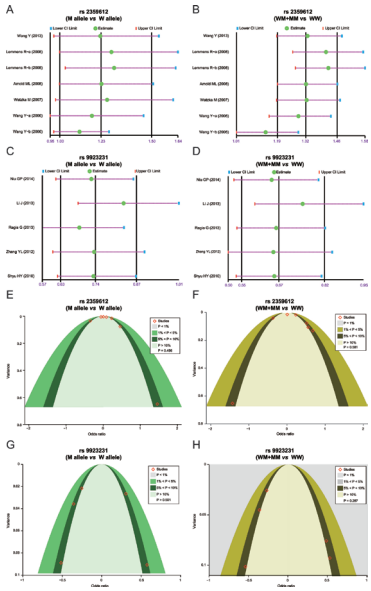

Figure 4. Sensitivity analyses of the correlations between rs2359612 and rs9923231 in the *VKORC1* gene and the risks of cardiovascular and cerebrovascular diseases.

Regression analysis

The results of univariate meta-regression analysis indicated that for rs2359612, detection methods, country, and ethnicity could represent possible sources for the heterogeneity in published reports regarding the correlation between the *VKORC1* gene and patients with CCVD (detection methods: P = 0.003; country: P = 0.049; ethnicity: P = 0.007), while publication time and sample size were shown to be insignificant factors (all P > 0.05). For rs9923231, publication time, sample size, detection methods, country, and ethnicity were not related to the heterogeneity (all P > 0.05; Figure 5). Furthermore, multivariate meta-regression analysis results showed that publication time, sample size, detection methods, country, and ethnicity were not the main sources of heterogeneity (Tables 3 and 4).

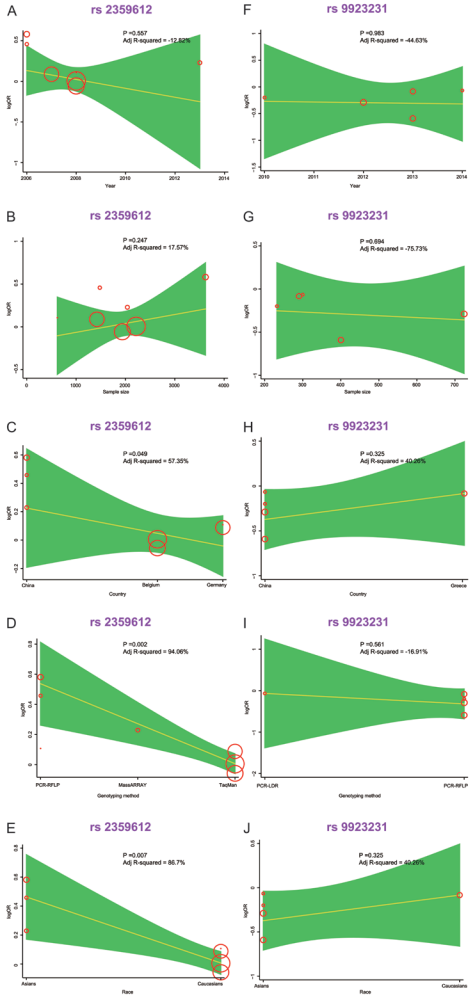

Figure 5. Meta-regression analyses of potential sources of heterogeneity for the correlations between rs2359612 and rs9923231 in the *VKORC1* gene and the risks of cardiovascular and cerebrovascular diseases. PCR-RFLP, polymerase chain reaction-restriction fragment length polymorphism; PCR-LDR, PCR-ligase detection reaction.

Table 3. Meta-regression analyses of potential sources of heterogeneity (rs2359612).

Heterogeneity factors	Coefficient	SE	*t*	P (adjusted)	95%CI	
					LL	UL
Year	-0.024	0.059	-0.41	0.966	-0.769	0.72
Sample size	<0.001	<0.001	1.37	0.653	-0.001	0.001
Country	0.147	0.089	1.65	0.598	-0.982	1.276
Genotyping method	-0.058	0.381	-0.15	1	-4.901	4.785
Race	-0.613	0.561	-1.09	0.728	-7.734	6.513

SE = standard error; LL = lower limit; UL = upper limit.

Table 4. Meta-regression analyses of potential sources of heterogeneity (rs9923231).

Heterogeneity factors	Coefficient	SE	*t*	P (adjusted)	95%CI	
					LL	UL
Year	-0.018	0.144	-0.13	1	-1.845	1.808
Sample Size	<0.001	0.001	-0.14	1	-0.013	0.012
Country	0.228	0.442	0.52	0.911	-5.384	5.841
Genotyping method	-0.333	0.355	-0.94	0.597	-1.862	1.196
Race	0.316	0.236	1.34	0.498	-0.701	1.332

SE = standard error; LL = lower limit; UL = upper limit.

DISCUSSION

In this study, we investigated whether published studies could reveal a definitive link between two important SNPs in the *VKORC1* gene and the risk of CCVD. The overall result in our meta-analysis is that rs2359612 and rs9923231 SNPs are associated with an increased risk of CCVD. VKORC1 regulates gamma-carboxylation of vitamin K-dependent proteins such as Gas6, matrix-GLA protein (MGP), osteocalcin, hemostatic proteins C, S, and Z, and coagulation factors II, VII, IX, and X (Smadja et al., 2008). The rs2359612 and rs9923231 SNPs in the *VKORC1* gene promote elevated expression of VKORC1, resulting in higher γ-carboxylation of vitamin K-dependent proteins and vitamin K-dependent proteins involved in calcification, thus promoting vascular calcification through multiple mechanisms (Wang et al., 2013). Vascular calcification is an important marker that correlates with enhanced CCVD morbidity and mortality and MGP is an inhibitor of vascular calcification (Rennenberg et al., 2010b). Rennenberg et al. (2010a) showed that more calcification is associated with higher serum undercarboxylated MGP levels. On the other hand, blood flow, coagulation factors, endothelial cells and platelets are components of the coagulation system and tissue factor is a potent initiator for coagulation cascade, which are associated with the pathophysiology of atherothrombosis and induceincluding platelet aggregation along with the activation of a coagulation cascade in patients with CCVD (Suh et al., 2009). The fact that the coagulation factors II (prothrombin), VII, IX, and X, which have procoagulant activities, are regulated by VKORC1 suggests that SNPs that increase VKORC1 activity would have a strong impact on the coagulation pathway (Teichert et al., 2008). Consistent with this, Pérez-Andreu et al. (2012) and Watzka et al. (2007) also concluded that VKORC1 is a relevant molecule that could be used to assess the risk of CCVD.

In order to assess other influencing factors affecting the validity of our overall results, our meta-analysis employed two subgroup analyses. The analysis based on ethnicity showed that significant correlations between the rs2359612 and rs9923231 SNPs and the risks

of CCVD were found in an Asian population. To our surprise, given the importance of these two SNPs, no correlation of rs2359612 and rs9923231 with any significant risk of CCVD was identified in Caucasians. We suspect that genetic polymorphisms at other loci such as protein disulfide isomerase or in the loci for other members of the multi-subunit VKORC1 complex, geographical location of the study population, or limitations in existing detection methods could account for these observations, and we intend to follow up with further studies to address ethnic differences in the association of *VKORC1* SNPs and CCVD risk. Another subgroup analysis based on disease type suggested the following results: in an allelic model, no correlations were found between rs2359612 SNPs in patients with CVD and CBVD and the risk of CCVD; in a dominant model, the rs2359612 SNP was significantly related to the risk of CCVD; and in patients with CVD, the rs2359612 SNP was significantly correlated with the risk of CCVD, whereas in CBVD, there was no obvious correlation between the rs2359612 SNP and the risk of CCVD.

As with all meta-analyses, some limitations to the interpretation of our data should be mentioned. First, diverse protein detection methods were used among the studies included, which might negatively influence our overall results. Second, the quantity of literature included was comparatively small, which might result in a lack of confidence in the pooled results. Finally, the inclusion and exclusion criteria might not be complete, which, together with manual selection, might lead to a reduction in the accuracy of the results by increasing the likelihood of having missed more detailed studies.

Collectively, our meta-analysis demonstrated that the rs2359612 and rs9923231 SNPs in the *VKORC1* gene might contribute to the risk of CCVD. Considering the above-mentioned limitations, further detailed studies are warranted to understand the roles of *VKORC1* SNPs in the risk of CCVD as a prelude to their clinical application.

Conflicts of interest

The authors declare no conflict of interest.

ACKNOWLEDGMENTS

We are specifically grateful to the reviewers for their profound comments on this paper.

REFERENCES

Aomori T, Yamamoto K, Oguchi-Katayama A, Kawai Y, et al. (2009). Rapid single-nucleotide polymorphism detection of cytochrome P450 (*CYP2C9*) and vitamin K epoxide reductase (*VKORC1*) genes for the warfarin dose adjustment by the SMart-amplification process version 2. *Clin. Chem*. 55: 804-812.

Arnold ML, Lichy C, Werner I, Radbruch A, et al. (2008). Single nucleotide polymorphisms in the *VKORC1* gene and the risk of stroke in the Southern German population. *Thromb. Haemost*. 100: 614-617.

Belanger M, Poirier M, Jbilou J and Scarborough P (2014). Modelling the impact of compliance with dietary recommendations on cancer and cardiovascular disease mortality in Canada. *Public Health* 128: 222-230.

Chen H, Manning AK and Dupuis J (2012). A method of moments estimator for random effect multivariate meta-analysis. *Biometrics* 68: 1278-1284.

Fuster V and Bansilal S (2010). Promoting cardiovascular and cerebrovascular health. *Stroke* 41: 1079-1083.

Higgins JP and Thompson SG (2002). Quantifying heterogeneity in a meta-analysis. *Stat. Med*. 21: 1539-1558.

Holden RM, Booth SL, Tuttle A, James PD, et al. (2014). Sequence variation in vitamin K epoxide reductase gene is associated with survival and progressive coronary calcification in chronic kidney disease. *Arterioscler. Thromb. Vasc. Biol*. 34: 1591-1596.

Huizenga HM, Visser I and Dolan CV (2011). Testing overall and moderator effects in random effects meta-regression. *Br. J. Math. Stat. Psychol*. 64: 1-19.

Jackson D, White IR and Riley RD (2012). Quantifying the impact of between-study heterogeneity in multivariate meta-analyses. *Stat. Med*. 31: 3805-3820.

Kim AS and Johnston SC (2011). Global variation in the relative burden of stroke and ischemic heart disease. *Circulation* 124: 314-323.

Lee CH and Kim JH (2014). A review on the medicinal potentials of ginseng and ginsenosides on cardiovascular diseases. *J. Ginseng Res*. 38: 161-166.

Lemmens R, Abboud S, Vanhees L, Goris A, et al. (2008). Lack of association between variants in the *VKORC1* gene and cerebrovascular or coronary heart disease. *J. Thromb. Haemost*. 6: 2220-2223.

Li J (2013). The correlation of *VKORC1* gene 1639 G>A polymorphism and atrial fibrillation patients in Xinjiang Kazakh and Han nationality. *J. Xinjiang Med. Univ.* 4: 444-449.

Niu GP and Wei YY (2014). Gene polymorphism of *CYP2C9* and *VKORC1* and their relationships to the dosage of warfarin. *Lab. Med.* 29: 635-639.

Owens WB (2011). Blood pressure control in acute cerebrovascular disease. *J. Clin. Hypertens.* 13: 205-211.

Patyar S, Prakash A, Modi M and Medhi B (2011). Role of vinpocetine in cerebrovascular diseases. *Pharmacol. Rep*. 63: 618-628.

Pérez-Andreu V, Teruel R, Corral J, Roldán V, et al. (2012). miR-133a regulates vitamin K 2,3-epoxide reductase complex subunit 1 (*VKORC1*), a key protein in the vitamin K cycle. *Mol. Med*. 18: 1466-1472.

Peters JL, Sutton AJ, Jones DR, Abrams KR, et al. (2006). Comparison of two methods to detect publication bias in meta-analysis. *JAMA* 295: 676-680.

Ragia G, Marousi S, Ellul J, Manolopoulos VG, et al. (2013). Association of functional *VKORC1* promoter polymorphism with occurrence and clinical aspects of ischemic stroke in a Greek population. *Dis. Markers* 35: 641-646.

Rennenberg RJ, de Leeuw PW, Kessels AG, Schurgers LJ, et al. (2010a). Calcium scores and matrix Gla protein levels: association with vitamin K status. *Eur. J. Clin. Invest*. 40: 344-349.

Rennenberg RJ, van Varik BJ, Schurgers LJ, Hamulyak K, et al. (2010b). Chronic coumarin treatment is associated with increased extracoronary arterial calcification in humans. *Blood* 115: 5121-5123.

Schulman S, Wang B, Li W and Rapoport TA (2010). Vitamin K epoxide reductase prefers ER membrane-anchored thioredoxin-like redox partners. *Proc. Natl. Acad. Sci. U. S. A*. 107: 15027-15032.

Shyu HY, Fong CS, Fu YP, Shieh JC, et al. (2010). Genotype polymorphisms of *GGCX*, *NQO1*, and *VKORC1* genes associated with risk susceptibility in patients with large-artery atherosclerotic stroke. *Clin. Chim. Acta* 411: 840-845.

Smadja DM, Loriot MA, Hindorff LA, Mellottee L, et al. (2008). No clear link between *VKORC1* genetic polymorphism and the risk of venous thrombosis or peripheral arterial disease. *Thromb. Haemost*. 99: 970-972.

Suh JW, Baek SH, Park JS, Kang HJ, et al. (2009). Vitamin K epoxide reductase complex subunit 1 gene polymorphism is associated with atherothrombotic complication after drug-eluting stent implantation: 2 - Center prospective cohort study. *Am. Heart J*. 157: 908-912.

Tao W, Xu X, Wang X, Li B, et al. (2013). Network pharmacology-based prediction of the active ingredients and potential targets of Chinese herbal Radix Curcumae formula for application to cardiovascular disease. *J. Ethnopharmacol*. 145: 1-10.

Teichert M, Visser LE, van Schaik RH, Hofman A, et al. (2008). Vitamin K epoxide reductase complex subunit 1 (*VKORC1*) polymorphism and aortic calcification: the Rotterdam Study. *Arterioscler. Thromb. Vasc. Biol*. 28: 771-776.

Tie JK, Jin DY and Stafford DW (2012). Human vitamin K epoxide reductase and its bacterial homologue have different membrane topologies and reaction mechanisms. *J. Biol. Chem*. 287: 33945-33955.

Tie JK, Jin DY and Stafford DW (2014). Conserved loop cysteines of vitamin K epoxide reductase complex subunit 1-like 1 (VKORC1L1) are involved in its active site regeneration. *J. Biol. Chem*. 289: 9396-9407.

Turin TC, Kita Y, Rumana N, Nakamura Y, et al. (2012). Ambient air pollutants and acute case-fatality of cerebro-cardiovascular events: Takashima Stroke and AMI Registry, Japan (1988-2004). *Cerebrovasc. Dis*. 34: 130-139.

Wang D, Chen H, Momary KM, Cavallari LH, et al. (2008). Regulatory polymorphism in vitamin K epoxide reductase complex subunit 1 (*VKORC1*) affects gene expression and warfarin dose requirement. *Blood* 112: 1013-1021.

Wang Y, Zhang W, Zhang Y, Yang Y, et al. (2006). *VKORC1* haplotypes are associated with arterial vascular diseases (stroke, coronary heart disease, and aortic dissection). *Circulation* 113: 1615-1621.

Wang Y, Chen J, Zhang Y, Bin L, et al. (2013). *VKORC1* rs2359612C allele is associated with increased risk of coronary artery disease in the presence of coronary calcification. *Hum. Genet*. 132: 29-37.

Watzka M, Nebel A, El Mokhtari NE, Ivandic B, et al. (2007). Functional promoter polymorphism in the *VKORC1* gene is no major genetic determinant for coronary heart disease in Northern Germans. *Thromb. Haemost*. 97: 998-1002.

Yang L, Ge W, Yu F and Zhu H (2010). Impact of *VKORC1* gene polymorphism on interindividual and interethnic warfarin dosage requirement - a systematic review and meta analysis. *Thromb. Res*. 125: e159-166.

Zhang YL and Zhang YF (2012). Effects of gene polymorphisms on individual warfarin dose. *Pract. Pharm. Clin. Rem.* 15: 196-198.

Zintzaras E and Ioannidis JP (2005a). HEGESMA: genome search meta-analysis and heterogeneity testing. *Bioinformatics* 21: 3672-3673.

Zintzaras E and Ioannidis JP (2005b). Heterogeneity testing in meta-analysis of genome searches. *Genet. Epidemiol*. 28: 123-137.