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Original Article

VKORC1 and *CD-14* genetic polymorphisms associate with susceptibility to cardiovascular and cerebrovascular diseases

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Abstract: Objective: To investigate the associations of *VKORC1* rs2359612 and rs9923231 and *CD-14* rs2569190 with susceptibility to cardiovascular and cerebrovascular diseases (CCVD). Methods: A case-control study was conducted with 614 cases of CCVD patients selected at our hospital between January 2011 and June 2012 as case group and 590 healthy individuals participating physical examination during the same period as control group. Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) assay was used to detect genotypes of *VKORC1* and *CD-14* genetic polymorphisms. SHEsis software was used to conduct haplotype analysis and logistic regression analysis was used to identify risk factors for CCVD. Results: The genotype and allele frequencies of *VKORC1* rs2359612 and rs9923231 and *CD-14* rs2569190 between the case and control groups were statistically different (all P<0.05). Haplotype analysis showed that the frequencies of CAT and TAT haplotypes were significantly higher while the frequencies of TAC and TGC haplotypes were significantly lower in the case group than those in the control group (P = 0.013, 0.029, 0.019 and 0.042, respectively). Logistic regression analysis showed that age, systolic pressure, smoking history and *VKORC1* rs2359612 maybe risk factors for CCVD; and body mass index (BMI), diastolic pressure and *VKORC1* rs9923231 may be protective factors for CCVD (all P<0.05). Conclusion: *VKORC1* rs2359612 and rs9923231, and *CD-14* rs2569190 might associate with susceptibility to CCVD. CAT and TAT haplotypes may be risk factors while TAC and TGC haplotype may be protective factors for CCVD.

Keywords: *VKORC1* gene polymorphism, *CD-14* gene polymorphism, cardiovascular and cerebrovascular diseases, haplotype analysis, Logistic regression analysis, rs2359612, rs9923231, rs2569190

Introduction

Cardiovascular and cerebrovascular diseases (CCVD) are the leading causes of death worldwide, with coronary artery disease and cerebral hemorrhage are still the first and second causes of death of human beings till 2020 [1, 2]. As the leading cause of sustained neurological disability in the world, the incidence of cerebrovascular diseases has increased by 100% in developing nations and has become the second leading cause of death in middle-income countries [3]. Cardiovascular diseases cause more than half of all deaths and one-third of disability, mainly due to uncompensated cardiovascular diseases, such as strokes and heart attacks [4]. Hypertension is a major risk factor for stroke, myocardial infarction and heart failure [5]. Characterized by premature onset and high mortality, coronary heart disease (CHD) has been reported in more than 70% of coronary deaths occur in subjects older than 70 in North America and Western Europe with the mortality of the young increasing in recent years [6]. Chronic heart failure (CHF) is a chronic progressive disease and a cardiac dysfunction syndrome, and the condition of CHF patients remain stable after treatment, but will also significantly worsen under the influence of various factors, such as infection and rapid ventricular rate arrhythmia [7]. As the leading cause of permanent disability and the third most common cause of death in well-developed nations, almost 80% of stroke cases are caused by ischemia [8]. Many factors have been reported to contribute to the development of CCVD, including age, gender, race, lifestyle such as obesity and level of physical activity, and genetic factors [9-11].

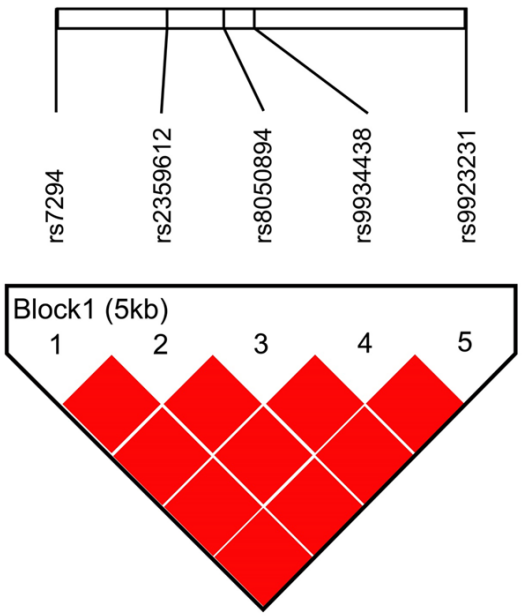

Figure 1. Haploview screening *VKORC1* gene site diagram (Red colors represent a very strong LD pattern). Note: VKORC1, vitamin K epoxide reductase complex subunit 1; LD, linkage disequilibrium.

Vitamin K epoxide reductase subunit complex subunit 1 (*VKORC1*) gene encodes vitamin K epoxide reductase which responsible for the recycling of vitamin K, and the reduced vitamin K is essential for the gamma carboxylation of vitamin K dependent proteins such as the procoagulant clotting factors II, VII, IX, X and the anticoagulant proteins C and S [12, 13]. Besides its hemostatic effect, *VKORC1* plays a pivotal role in bone mineralization and is associated with deep venous thrombosis [14, 15]. Furthermore, *VKORC1* rs2359612 is associated with a higher risk of coronary artery disease in the presence of coronary artery calcification and a higher incidence of cardiovascular events [16]. Cluster of differentiation 14 (CD14), a component of the innate immune system, exists in two forms, one anchored to membranes (mCD14), the other a soluble form (sCD14) [17]. CD14 mediates the inflammatory response via recognition of lipopolysaccharide, implicating in Helicobacter pylori infections and resulting in gastric carcinoma [18]. *CD14*-159C/T genome was reported to be associated with decreased TH1 function and increased intensity of atopy [19, 20]. Moreover, *CD14* variants may contribute to stimulation of atherogenic responses in vascular smooth muscle cell, and *CD14* C-260T polymorphism is a risk factor of CHD [21].

However, the exact associations between VKORC1 and CD-14 and CCVD are not clearly stated. In this study, we aim to investigate the associations of VKORC1 rs2359612 and rs9923231 and CD-14 rs2569190 with susceptibility to CCVD, and analyze the risk factors, both environmental and genetic factors, for the development of CCVD.

Materials and methods

Participants and ethnic statement

Between January 2011 and January 2012, 614 cases with hypertension or CHD or CHF or ischemic stroke enrolled at the Department of Geriatrics, The First Hospital of Jilin University were selected as case group. Criteria for the diagnosis of CHD were based on the "Naming and diagnostic criteria of ischemic heart disease" made by a joint thematic group of International Society of Cardiology and World Health Organization (WHO) clinical naming standardization, and the coronary artery diameter stenos is greater than or equal to 50% was diagnosed as CHD [22]. The diagnostic standard of CHF was based on the Framingham heart failure diagnostic criteria [23]. Diagnostic criteria of hypertension referred to the seventh session of the WHO/international hypertension alliance conference held in September 1998 in Japan [24] and hypertension was diagnosed with systolic pressure greater than or equal to 18.7 kPa (140 mmHg) and diastolic pressure greater than or equal to 12.0 kPa (90 mmHg) when detected for 3 times in different days. Diagnostic criteria of ischemic stroke were according to the revised diagnostic criteria published in China's Fourth International Conference on Cerebrovascular Disease [18] and ischemic stroke patients with large artery atherosclerosis were collected. Exclusion criteria: patients with secondary hypertension, cardiomyopathy, valvular heart disease, congenital heart disease, diabetes and renal failure; ischemic stroke patients of cardiogenic embolism, small artery occlusion, other unexplained etiology types; and patients with non-primary CCVD. Inclusion criteria: new cases of CCVD diagnosed at our hospital; and CCVD patients without preoperative radiotherapy or chemotherapy. In the same period, 590 healthy individuals enrolled at the medical examination center of our hospital were selected as control group. Inclusion criteria: individuals of same ethnicity, similar gender proportion, no symptoms, signs

Table 1. Primer design for *VKORC1* and *CD-14* gene polymorphisms

	Primers	Location	Length
rs2359612	Forward 5'-TCTGAACCATGTGTCAGCCAGGACC-3'	Second intron	290 bps
	Reverse 5'-GAACAGAGAGAGGAACCAAGGGAGTGGA-3'		
rs9923231	Forward 5'-GGTAGGTGCAACAGTAAGGGA-3'	Promoter	276 bps
	Reverse 5'-AAATGCTAGGATTATAGGCGTGA-3'		
rs2569190	Forward 5'-AAGTCTTCCGAACCTCTGAGC-3'	Promoter	162 bps
	Reverse 5'-GGTGGCAGGAGATCAACATAA-3'		

Note: VKORC1, vitamin K epoxide reductase complex subunit 1; CD-14, cluster of differentiation 14; bp, base pairs.

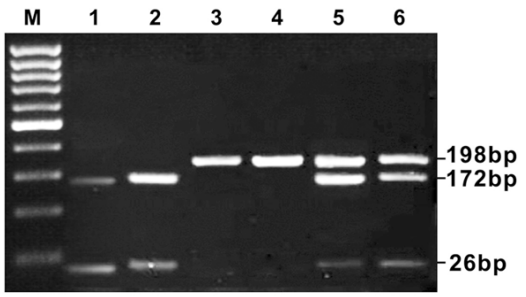

Figure 2. *VKORC1* gene rs2359612PCR digested product electrophoresis diagram. Note: M represents DNA molecular weight marker (Marker); 1 and 2 represent TT genotype; 3 and 4 represent CC genotype; 5 and 6 represent CT genotype; VKORC1, vitamin K epoxide reductase complex subunit 1; PCR, polymerase chain reaction.

and medical history of CCVD, no kidney, cancer, endocrine, blood and digestive system diseases, and no blood relationship with the included CCVD patients. Exclusion criteria: individuals with chronic diseases such as liver, kidney, thyroid, diabetes, and other chronic diseases, or with heart disease, stroke, and chronic congestive heart failure. The research program was reviewed and approved by the ethics committee of the First Hospital of Jilin University, and all the research subjects were informed and agreed to the study and signed informed consent. Ethical approval for this study conformed to the standards of the Declaration of Helsinki [25].

Genotyping and PCR-RFLP

Peripheral blood (5 ml) was collected from subjects of all the case and control groups who had been fasted overnight, and 2% EDTA-K was used as an anticoagulant and the blood samples were stored at a temperature of -20°C in a refrigerator. The experiment was conducted according to the instruction of DNA kit (Qiagen Inc., Germany). We found *VKORC1* rs2359612 and rs9923231, and *CD-14* rs2569190 associated with CCVD. Haploview software was used to screen *VKORC1* gene sites and *VKORC1* rs2359612 and rs9923231 were found strong linkage disequilibrium (LD), thus *VKORC1* rs2359612 and rs9923231, and *CD-14* rs2569190 were selected (**Figure 1**). Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method was used to identify gene polymorphism and Primer 5.0 software was used to design PCR amplification primers for *VKORC1* and *CD-14* sites and the primers were designed and synthesized by Beijing Bioko biological Ltd., and the primer sequences were shown in **Table 1**.

VKORC1 reaction system (20 μL): 3 μL 10 × Buffer, 0.5 μL 0.5 U Taq DNA polymerase, 3 μL 2.5 mmol/L $MgCl_2$, 3 μL 0.1 mmol/L dNTP, 1 μL 10 μmol/L for each upstream and downstream primers, 2 μL 100 ng/μ LDNA templates and 6.5 μL sterilized deionized water. Reaction conditions: pre-denaturation at 95°C for 5 min, 35 cycles of denaturation at 94°C for 30 s, annealing at 62°C for 30 s and extension at 72°C for 60 s, followed by extension at 72°C for 7 min after the final cycle. The PCR reaction was performed with Gene Amp PCR System 9700 (Shanghai Tanon company, Shanghai, China). PCR amplification products (5 μL) were digested by 8 U restriction enzyme MspI (Dalian Takara company, Dalian, China) at 37°C for 3 h.

CD-14 reaction system (25 μL): 2.5 μL 10 × Buffer, 2 μL 0.5 U Taq DNA polymerase, 2 μL 2.5 mmol/L $MgCl_2$, 2 μL dNTP, 1 μL 10 μmol/L for each upstream and downstream primers, 10 μL DNA templates and 4.5 μL sterilized deionized water. Reaction conditions: pre-denaturation at 95°C for 7 min, 35 cycles of denaturation at 94°C for 30 s, annealing at 56°C for 30 s and extension at 72°C for 30 s,

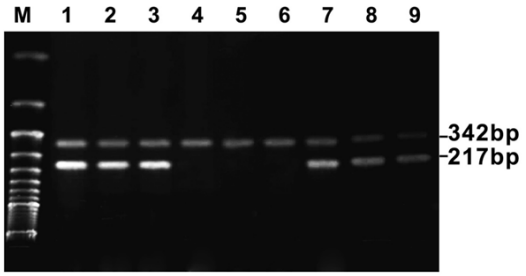

Figure 3. *VKORC1* gene rs9923231 PCR digested product electrophoresis diagram. Note: M represents DNA molecular weight marker (Marker); 1 and 2 represent GA genotype; 3 and 4 represent AA genotype; 5 and 6 represent GG genotype; VKORC1, vitamin K epoxide reductase complex subunit 1; PCR, polymerase chain reaction.

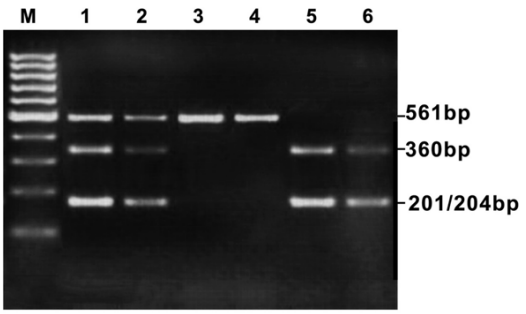

Figure 4. *CD-14* gene rs2569190 PCR digested product electrophoresis diagram. Note: M represents DNA molecular weight marker (Marker); 1 and 2 represent CT genotype; 3 and 4 represent TT genotype; 5 and 6 represent CC genotype; CD-14, Cluster of differentiation 14; PCR, polymerase chain reaction.

followed by extension at 72°C for 7 min after the final cycle. PCR amplification products (5 μL) were digested by 8 U restriction enzyme Hae III (Dalian Takara company, Dalian, China) at 37°C for 3 h. All of the above enzyme digestion products were with separated by 20% agarose electrophoresis, stained with ethidium bromide and observed under ultraviolet (UV) lamps to take photos and record (**Figures 2**-**4**).

Statistical analysis

SPSS 19 (Inc, Chicago, IL, USA) statistical software was used for data processing. Enumeration data were expressed as percentage or rate. Chi square test was used to compare the difference of genotype and allele frequencies of each group. Whether the genotype frequencies were consistent with the Hardy-Weinberg equilibrium law was tested. Odds ratio (OR) and 95% confidence interval (CI) indicated the risks of CCVD. Measurement data were presented as mean ± standard deviation (SD) and were analyzed by *t* test (Homogeneity of variance test was conducted before *t* test). SHEsis software was used to select TagSNPs and conduct SNP analysis ($r^2 > 0.75$ was as selection reference), and the corresponding haplotype analysis was conducted based on and based on LD between TagSNPs. $P < 0.05$ was regarded as statistically significant.

Results

Baseline characteristics

As shown in **Table 2**, the mean ages, diastolic pressure and systolic pressure of the case group were significantly higher than those of the control group (mean ages: 61.5 ± 8.0 vs. 44.0 ± 9.0, $t = 35.69$, $P < 0.001$; diastolic pressure: 83.5 + 12.0 mmHg vs. 76.0 + 8.0 mmHg, $t = 12.71$, $P < 0.001$; 136.5 + 20.0 mmHg vs. 122.0 + 9.0 mmHg; $t = 16.11$, $P < 0.001$). The body mass index (BMI) of the case group were significantly lower than those of the control group (BMI: 22.8 ± 2.5 vs. 23.2 ± 3.3, $t = 2.377$, $P = 0.018$). There were significant differences in family history and smoking history between the case group and the control group (family history: $P < 0.001$, OR = 1.792, 95% CI = 1.376-2.335; smoking history: $P < 0.001$, OR = 1.589, 95% CI = 1.240-2.037). However, there were no significant differences in gender ratio and drinking history between the two groups (both $P > 0.05$).

Associations between VKORC1 and CD-14 gene polymorphisms and risk of CCVD

In both the case group and the control group, the genotype distributions were tested by Hardy-Weinberg method and all the genotypes and allele frequencies were genetic equilibrium (all $P > 0.05$), suggesting the representativeness of group. Genotype distribution frequencies of *VKORC1* rs2359612 were 1.14%, 19.06% and 76.8% for CC, CT and TT genotypes respectively in the case group, while were 0.51%, 12.03% and 87.46% in the control group, suggesting statistical significance ($P = 0.002$). The risk of CCVD in individuals carrying C allele (CC+CT) were 1.765 times higher than individuals with TT genotype (OR = 1.765, 95% CI = 1.290-2.414); the risk of CCVD of C allele

Table 2. Baseline characteristics of case group and control group

	Case group (n = 614)	Control group (n = 590)	P
Sex (male/female)	344/270	357/233	0.115
Age	61.5 ± 8.0	44.0 ± 9.0	< 0.001
Family history	190 (30.95%)	118 (20.00%)	< 0.001
BMI (kg/m^2)	22.8 ± 2.5	23.2 ± 3.3	0.018
Systolic pressure (mmHg)	136.5 ± 20.0	122.0 ± 9.0	< 0.001
Diastolic pressure (mmHg)	83.5 ± 12.0	76.0 ± 8.0	< 0.001
Smoking history	217 (35.34%)	151 (25.59%)	< 0.001
Drinking history	144 (23.45%)	138 (23.39%)	0.979
P*	all > 0.05	all > 0.05	

Note: BMI, body mass index; P*, presented the Hardy-Weiberg test analyzing values of genotype distributions of rs2359612, rs9923231, and rs2569190.

were 1.711 times higher than T allele (OR = 1.711, 95% CI = 1.275-2.294). The AA, AG and GG genotype distribution frequencies of *VKORC1* rs9923231 in the case group were 75.08%, 22.48% and 2.44%, while were 67.29%, 30.17% and 2.54% in the control group, showing the statistical significance (P = 0.009). The risk of CCVD in individuals carrying G allele (GG+AG) was 0.631 times than AA genotype individuals (OR = 0.683, 95% CI = 0.531-0.878) and the risk of CCVD in G allele was 1.350 times than A allele (OR = 1.350, 95% CI = 1.082-1.684). The *CD-14* rs2569190 CC, CT and TT genotype distribution frequencies were 16.45%, 38.27% and 45.28% respectively in the case group and were 21.18%, 44.75% and 34.07% respectively in the control group, indicating statistical significance between the two groups (P = 0.001). Individuals carrying C allele (CC+CT) had 0.625 times of CCVD risk than individuals TT genotype (OR = 0.625, 95% CI = 0.495-0.788) and C allele had 0.716 times of CCVD risk than T allele (OR = 0.716, 95% CI = 0.608-0.843) (**Table 3**).

Haplotype analysis of VKORC1 rs2359612 and rs9923231, and CD-14 rs2569190

Haplotypes of *VKORC1* rs2359612 and rs9923231, and *CD-14* rs2569190 were shown in **Table 4**. SHEsis software was used to analyze the haplotypes of different sites of *VKORC1* and *CD-14* gene, the frequencies of haplotypes less than 3% were neglected, and thus CGC (0.52%) and CGT (0.94%) haplotypes were excluded. The frequencies of CAT and TAT haplotypes were significantly higher while the frequencies of TAC and TGC haplotypes were significantly lower in the case group than those in the control group (CAT: OR = 2.038, 95% CI = 1.146-3.622, P = 0.013; TAT: OR = 1.288, 95% CI = 1.026-1.616, P = 0.029; TAC: OR = 0.746, 95% CI = 0.583-0.954; P = 0.019; TGC: OR = 0.600, 95% CI = 0.365-0.987; P = 0.042). The results indicated that CAT and TAT haplotypes may be risk factors for CCVD, while TAC and TGC haplotype may be protective factors for CCVD.

Logistic regression analysis of risk factors of CCVD

Based on age, BMI, systolic pressure, diastolic pressure, family history, smoking history, *VKORC1* rs2359612 and rs9923231, *CD-14* rs2569190 as independent variables and whether individuals suffering from CCVD as the dependent variable, binary logistic regression analysis were conducted. Logistic regression analysis showed that age, systolic pressure, smoking history, and *VKORC1* rs2359612 mayberisk factors for CCVD (age: OR (95% CI) = 2.094 (1.839-2.384); systolic pressure: OR (95% CI) = 0.321 (1.236-1.413); smoking history: OR (95% CI) = 1.361 (0.692-2.677); *VKORC1* rs2359612: OR (95% CI) = 4.374 (2.042-9.370); all P < 0.05) and BMI, diastolic pressure and *VKORC1* rs9923231 may be protective factors for CCVD (BMI: OR (95% CI) = 0.313 (0.245-0.400); diastolic pressure: OR (95% CI) = 0.644 (0.577-0.718); *VKORC1* rs9923231: OR (95% CI) = 0.362 (0.154-0.690); all P < 0.05) (**Table 5**).

Discussion

Our results demonstrated that *VKORC1* rs2359612 and rs9923231, and *CD-14* rs2569190 associated with susceptibility to CCVD. CAT and TAT haplotypes may be risk factors while TAC and TGC haplotype may be protective factors for CCVD. To the best of our knowledge, this is the first study systematically study the roles of *VKORC1* and *CD-14* genetic polymorphisms in the development of CCVD. Located in the promoter of *VKORC1* gene, *VKORC1* -1639 G > A polymorphism (rs9923231) could lower mRNA and protein expressions, and activity of VKORC1 in individu-

Table 3. Genotype and allele distributions of *VKORC1* and *CD-14* gene polymorphisms in case group and control group

SNP	Case group (n = 614)	Control group (n = 590)	χ^2	P	OR	95% CI
rs2359612						
CC	7 (1.14%)	3 (0.51%)		0.002		
CT	117 (19.06%)	71 (12.03%)	13.052			
TT	490 (79.8%)	516 (87.46%)				
CC+CT	124 (20.20%)	74 (12.54%)	12.821	0.001	1.765	1.290-2.414
TT	490 (79.80%)	516 (87.46%)				
C	131 (10.67%)	77 (6.53%)	13.081	0.001	1.711	1.275-2.294
T	1097 (89.33%)	1103 (93.47%)				
rs9923231						
AA	461 (75.08%)	397 (67.29%)		0.009		
AG	138 (22.48%)	178 (30.17%)	9.362			
GG	15 (2.44%)	15 (2.54%)				
AG+GG	153 (24.92%)	193 (32.71%)	8.923	0.003	0.683	0.531-0.878
AA	461 (75.08%)	397 (67.29%)				
A	1060 (86.32%)	972 (82.37%)	7.112	0.001	1.35	1.082-1.684
G	168 (13.68%)	208 (17.63%)				
rs2569190						
CC	101 (16.45%)	125 (21.18%)		0.001		
CT	235 (38.27%)	264 (44.75%)	16.142			
TT	278 (45.28%)	201 (34.07%)				
CC+CT	336 (54.72%)	389 (65.93%)	15.781	< 0.001	0.625	0.495-0.788
TT	278 (45.28%)	201 (34.07%)				
C	437 (35.59%)	514 (43.56%)	16.011	< 0.001	0.716	0.608-0.843
T	791 (64.41%)	666 (56.44%)				

Note: VKORC1, vitamin K epoxide reductase complex subunit 1; CD-14, cluster of differentiation 14; SNP, single nucleotide polymorphism; OR, odd ratio; 95% CI, 95% confidence interval.

Table 4. Haplotype analysis of *VKORC1* gene polymorphisms rs2359612 and rs9923231 and CD-14 gene polymorphism rs2569190

Haplotype			Case group (n = 614)	Control group (n = 590)	χ^2	P	OR (95% CI)
VKORC1 rs2359612	*VKORC1* rs9923231	*CD-14* rs2569190					
C	A	C	20 (3.28%)	14 (2.34%)	0.858	0.354	1.385 (0.693-2.769)
C	A	T	37 (5.93%)	18 (3.04%)	6.109	0.013	2.038 (1.146-3.622)
T	A	C	168 (27.44%)	198 (33.54%)	5.462	0.019	0.746 (0.583-0.954)
T	A	T	305 (49.67%)	256 (43.45%)	4.775	0.029	1.288 (1.026-1.616)
T	G	C	27 (4.35%)	42 (7.18%)	4.124	0.042	0.600 (0.365-0.987)
T	G	T	48 (7.87%)	55 (9.30%)	0.871	0.351	0.825 (0.550-1.237)

Note: VKORC1, vitamin K epoxide reductase complex subunit 1; CD-14, cluster of differentiation 14; OR, odd ratio; 95% CI, 95% confidence interval.

als with AA genotype and *VKORC1* -1639 G > A polymorphism has been reported to be associated with maximum carotid intima-media thickness in type 2 diabetes mellitus patients due to atherosclerosis characterized by increased calcification [26]. *VKORC1* genotype is associated with cardiovascular patients with drug-eluting stent implantation and the presence of the C allele of the *VKORC1* +2255 (rs2359612) conferred twice the risk of arterial vascular disease

Table 5. Logistic regression analysis of risk factors for cardiovascular and cerebrovascular diseases

Risk factors	B	S.E.	Wald	P	Exp (B)	95% CI
Age	0.739	0.660	124.623	< 0.001	2.094	1.839-2.384
BMI	-1.161	0.125	86.37	< 0.001	0.313	0.245-0.400
Systolic pressure	0.279	0.340	66.956	< 0.001	1.321	1.236-1.413
Diastolic pressure	-0.441	0.560	62.43	< 0.001	0.644	0.577-0.718
Family history	0.270	0.364	0.550	0.458	1.310	0.642-2.673
Smoking history	0.308	0.345	0.799	0.037	1.361	0.692-2.677
VKORC1 rs2359612	1.476	0.389	14.414	< 0.001	4.374	2.042-9.370
VKORC1 rs9923231	-1.122	0.383	8.582	0.003	0.362	0.154-0.690
CD-14 rs2569190	0.011	0.329	0.001	0.975	1.011	0.530-1.927

Note: VKORC1, vitamin K epoxide reductase complex subunit 1; CD-14, cluster of differentiation 14; BMI, body mass index; S.E., standard error; 95% CI, 95% confidence interval.

such as aortic dissection, stroke and coronary heart disease in general population through vascular calcification and coagulation cascade regulations [27]. *VKORC1* genes alters vitamin K cycle dynamics which play important roles in the synthesis of blood coagulation factors, thus may influence individual susceptibility to stroke disease development [28]. Zhang et al. found that individuals carrying the 1639G or 1173C allele might have an increased risk for ischemic cerebrovascular disease [29]. Wang et al. demonstrated that the prevalence of the haplotype G-C-G-C-A of *VKORC1* was significantly more frequent in vascular disease patients than in controls, thus the haplotype may serve as an important genetic marker for risks of CHD, stroke and aortic dissection [30].

CD14 gene, consisting of two exons and one short intron, has been mapped to chromosome 5 at bands q23-q31, and *CD14* C-260 T (rs2569190) located in the *CD14* promoter has been reported to be associated with enhanced transcriptional activity and decreased affinity of specificity protein binding [31]. Both unstimulated and after lipopolysaccharide stimulation, CD14 expression and monocyte cytokine production are increased in moderate-severe CHF compared to mild CHF, suggesting that circulating monocytes might increase CD14 expression, which may play an important role in the immunologic imbalance in advanced CHF [32]. *CD14* C-260 T polymorphism has been reported to regulate the density of CD14 expression on monocytes and T variants of the SNP can promote the gene transcription and cause higher expression of CD14 on the monocytes, leading to an enhanced inflammatory response [33]. *CD*14 C-260 T polymorphism TT genotype was associated with cerebral ischemia due to microangiopathy or large artery atherosclerosis in Middle European subjects [34].

Besides, our logistic regression analysis showed that age, systolic pressure, smoking history and *VKORC1* rs2359612 mayberisk factors for CCVD, and BMI, diastolic pressure and *VKORC1* rs9923231 may be protective factors for CCVD, which further verified that *VKORC1* rs2359612 and rs9923231 associated with the development of CCVD, and the development of CCVD is a multi-factor process with both environmental and genetic factors. CCVD disease is one of the most common diseases related with age and also one of the most common death causing diseases in the general population [35]. Individuals with isolated systolic hypertension have increased risk for cardiovascular disease and wide pulse pressure is an important risk modifier for the adverse effect of low diastolic pressure [36]. Moreover, after adjustment for multiple risk factors and excluding of addressing passive smoking, a strong relationship between smoking and heart disease and coronary heart disease mortality among young adults are also showed [37]. Furthermore, overweight and obesity (high BMI) were associated with increased risk of coronary heart disease and stroke, and interventions to control blood pressure, cholesterol, and glucose can largely or fully present the excess risk of coronary heart disease and stroke in obesity individuals [38].

In conclusion, our study for the first time systematically demonstrated the associations between *VKORC1* rs2359612 and rs9923231, and *CD-14* rs2569190, and susceptibility to CCVD. Furthermore, we also showed both environmental and genetic factors in the development of CCVD, thus our study could provide valuable information for study on the pathogen-

esis of CCVD. However, our results lacked to include other genes or other environmental factors, such as physical activity, air pollution, etc., in the development of CCVD. Further investigated are needed to enrich our results.

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