

# Association of Genetic Variants in miR-217 Gene with Risk of Coronary Artery Disease: A Case–Control Study

Xia Han<sup>1,\*</sup>  
Xiaotang Liang<sup>2,\*</sup>  
Menghai Wu<sup>1</sup>  
Lijun Zhang<sup>1</sup>  
Honglei Jiang<sup>2</sup>

<sup>1</sup>Department of Cardiology, Jinan People's Hospital Affiliated to Shandong First Medical University, Laiwu, 271199, People's Republic of China; <sup>2</sup>Shandong Second Provincial General Hospital, Jinan, Shandong Province, 250000, People's Republic of China

\*These authors contributed equally to this work

**Objective:** To evaluate the associations of genetic variants of the miR-217 gene with coronary artery disease (CAD) risk, as well as plasma level of vascular endothelial growth factor (VEGF).

**Methods:** A case–control study with 498 CAD patients and 499 frequency-matched healthy controls was conducted to evaluate the associations of four tagSNPs of the miR-217 gene, including rs6724872, rs4999828, rs10206823, and rs41291177, with CAD risk and plasma level of VEGF.

**Results:** SNP rs6724872 and rs4999828 were significantly associated with increased risk of CAD (P value was smaller than 0.05 even after Bonferroni multiple adjustment). Compared with the G allele, C allele of rs6724872 was significantly associated with 1.73-fold increased risk of CAD (95% CI: 1.25–2.39; P = 0.001). While C allele of rs4999828 was significantly associated with 1.75-fold increased risk of CAD, compared with T allele (95% CI: 1.34–2.29; P =  $4 \times 10^{-5}$ ). Meanwhile, rs6724872 and rs4999828 were also significantly associated with higher level of VEGF (P < 0.001).

**Conclusion:** These findings highlighted the important role of genetic variants of the miR-217 gene in the pathogenesis of CAD and potential targets for intervention.

**Keywords:** coronary artery disease, miR-217, VEGF, genetic

## Introduction

Coronary artery disease (CAD) is the major cause of death throughout the world.<sup>1</sup> As reported by GBD 2017 Causes of Death Collaborators, the estimated years of life lost (YLLs) increased for CAD (ranked first in 2017).<sup>2</sup> During the past decade, a marked rising trend of atherosclerosis-related burden (especially for CAD) in Eastern Asia was observed.<sup>3</sup> Although endothelial dysfunction contributes essentially to the atherosclerosis, the molecular pathways underlying disease occurrence are not fully understood.

MicroRNAs (miRNAs) play important roles in the pathophysiology of cardiovascular diseases through the posttranscriptional control of gene networks.<sup>4,5</sup> Among them, miR-217 was reported to aggravate atherosclerosis and promote cardiovascular dysfunction through downregulating a network of endothelial NO synthase (eNOS) activators, including vascular endothelial growth factor (VEGF).<sup>6</sup> VEGF, a signal protein stimulating the formation of blood vessels, acted as a potential biomarker to predict the occurrence of CAD, and increased VEGF

Correspondence: Honglei Jiang  
Shandong Second Provincial General Hospital, No. 4, Xingxi Street, Jinan, Shandong Province, 250000, People's Republic of China  
Email jianghl\_sd@21cn.com

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level was associated with poor coronary collateralization in patients with stable CAD.<sup>7</sup> Besides, inhibition of miR-217 could protect against myocardial ischemia-reperfusion injury through inactivating NF-kappaB and MAPK pathways by targeting DUSP14.<sup>8</sup> These findings highlighted a potential role of miR-217 in pathogenesis of CAD. Whether genetic variants of the miR-217 gene contributed to the occurrence of CAD was still undetermined and worthy to be explored. Thus, we aimed to conduct a case-control study among Chinese population to evaluate the associations of genetic variants of the miR-217 gene with CAD risk, as well as plasma level of VEGF.

## Patients and Methods

### Study Subjects

In the current case-control study, we totally recruited 498 CAD patients and 499 healthy controls (frequency-matched by age, gender, and living areas). CAD diagnosis of any major coronary artery with diameter stenosis of more than 50%, or previous angioplasty, coronary bypass surgery, or myocardial infarction (MI) history verified by electrocardiogram (ECG) changes was evaluated by two cardiologists.<sup>9</sup> This study has been approved by the institute committee of Jinan people's Hospital. All participants in the study received informed consent and followed the guidelines set out in the Helsinki declaration.

### Blood Sampling and Measurement

Fasting venous blood was collected into plasma tubes containing 0.1% ethylenediaminetetraacetic acid (EDTA) and stored at  $-80^{\circ}\text{C}$  prior to analysis. Total RNAs were isolated using the miRNeasy kit (Qiagen) according to the manufacturer's protocol. TaqMan miRNA assay kits (Applied Biosystems) were used for miRNA amplification, and real-time polymerase chain reaction (RT-PCR) was performed to detect miR-217, while cel-miRNA-39 was added as a spike-in control. Plasma VEGF level among the healthy controls was determined by multiplex analysis using Bioplex suspension arrays (Bio-Rad, Veenendaal, The Netherlands) according to the manufacturer's specifications. All samples were thawed only once and measured three times.

### TagSNP Selection, DNA Extraction and Genotyping

TagSNPs were selected among the 1kb flanking region of the miR-217 gene according to 1000 genome CHB data (phase

3, minor allele frequency  $\geq 5\%$ , pairwise  $r^2 \geq 0.8$ ) using the Haploview 4.2 software.<sup>10</sup> Finally, four tagSNPs, including rs6724872, rs4999828, rs10206823, and rs41291177, were determined. Genomic DNA was extracted from peripheral blood samples using QIAamp DNA blood Mini Kit (Qiagen, Hilden, Germany). Genotyping was performed by TaqMan analysis (Applied Biosystems [ABI], Foster City, CA) according to the manufacturer's instructions. A randomly selected group of 10% of the samples was tested twice by different individuals with 100% concordance of results.

### Statistical Analysis

Statistical analyses were carried out using IBM SPSS Statistics version 22.0, while two-tailed P-values  $< 0.05$  were considered significant. All the demographic data were presented as proportions. Deviation of candidate SNPs from Hardy-Weinberg equilibrium in the control group was assessed by the goodness-of-fit  $\chi^2$  test. Allele frequencies and demographic variables between the two groups were assessed with chi-square tests. Odds ratios (ORs), 95% confidence levels (CIs), and corresponding P values were calculated for each SNP using logistic regression analysis, adjusted for age, gender, smoking status, drinking status, diabetes, and hypertension.

## Results

### Characteristics of Study Subjects

Table 1 lists the comparison of clinical features between 498 CAD cases and 499 controls. The results showed that there was no significant difference in age, gender, drinking status, diabetes and hypertension ( $P > 0.05$ ). However, compared with the control group, the patients have higher percentage of smokers (controls vs cases: 26.7% vs 42.4%;  $P < 0.001$ ).

### Plasma Level of miR-217 and CAD Risk

We first evaluated the association between plasma level of miR-217 and CAD risk to validate the role of miR-217 in CAD development. As shown in Figure 1, plasma level of miR-217 was analyzed in 50 randomly selected patients with CAD and controls. We found plasma level of miR-217 in CAD cases was significantly higher than that in controls ( $P < 0.001$ ).

### Genetic Associations of Candidate tagSNPs with CAD Risk

As shown in Table 2, all four tagSNPs (rs6724872, rs4999828, rs10206823, and rs41291177) were in Hardy-

**Table I** Clinical Characteristics of CAD Cases and Controls

Variables	Cases (n=498)	Controls (n=499)	P value
Age			
≥60	231 (46.4%)	242 (48.5%)	0.692
<60	258 (53.6%)	257 (51.5%)	
Gender			
Male	347 (69.7%)	345 (69.1%)	0.853
Female	151 (30.3%)	154 (30.9%)	
Smoking status			
Smokers	211 (42.4%)	133 (26.7%)	<0.001
Non-Smokers	287 (57.6%)	366 (73.3%)	
Drinking status			
Drinkers	123 (24.7%)	101 (20.2%)	0.092
Non-drinkers	375 (75.3%)	398 (79.8%)	
Diabetes			
Yes	100 (20.0%)	79 (15.8%)	0.081
No	398 (80.0%)	420 (84.2%)	
Hypertension			
Yes	276 (55.4%)	251 (50.3%)	0.105
No	222 (44.6%)	248 (49.7%)	

Weinberg equilibrium in healthy controls, which indicated that the sampled subjects were representative of the population without any deviation of genotype frequencies ( $P > 0.05$ ). Of the four tagSNPs in the miR-217 gene region, rs6724872 and rs4999828 were significantly associated with increased risk of CAD ( $P$  value was smaller than 0.05 even after Bonferroni multiple adjustment).

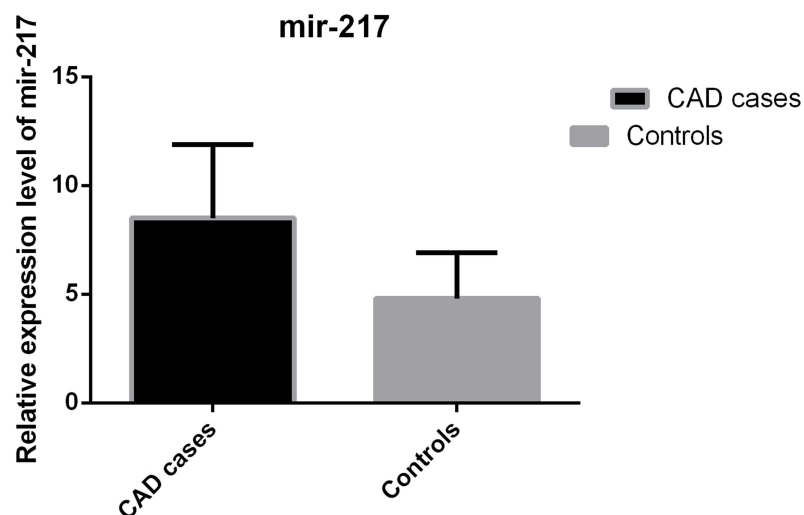
Compared with the G allele, C allele of rs6724872 was significantly associated with 1.73-fold increased risk of CAD (95% CI: 1.25–2.39;  $P=0.001$ ). While C allele of rs4999828 was significantly associated with 1.75-fold increased risk of CAD, compared with T allele (95% CI: 1.34–2.29;  $P=4 \times 10^{-5}$ ).

## Plasma VEGF Level with Different Genotypes of rs6724872 and rs4999828

To further evaluate the influence of susceptibility SNPs upon plasma level of VEGF, we compared the VEGF level among healthy controls with different genotypes of rs6724872 and rs4999828. As shown in Figure 2, with the increase in number of minor alleles, the plasma level of VEGF increased significantly for both rs6724872 and rs4999828 ( $P < 0.001$ ). This means rs6724872 and rs4999828 were significantly associated with higher level of VEGF.

## Discussion

Coronary heart disease is a common and frequent disease, which brings serious trouble to people's quality of life.<sup>2,11</sup> The exploration of the etiology of CAD is a complex and systematic project, and researchers have explored multiple aspects and perspectives.<sup>12,13</sup> The current study explored associations between the associations of genetic variants of the miR-217 gene with CAD risk, as well as plasma level of VEGF, using a case-control study design. We found plasma level of miR-217, rs6724872 and rs4999828 were significantly associated with increased risk of CAD, as well as

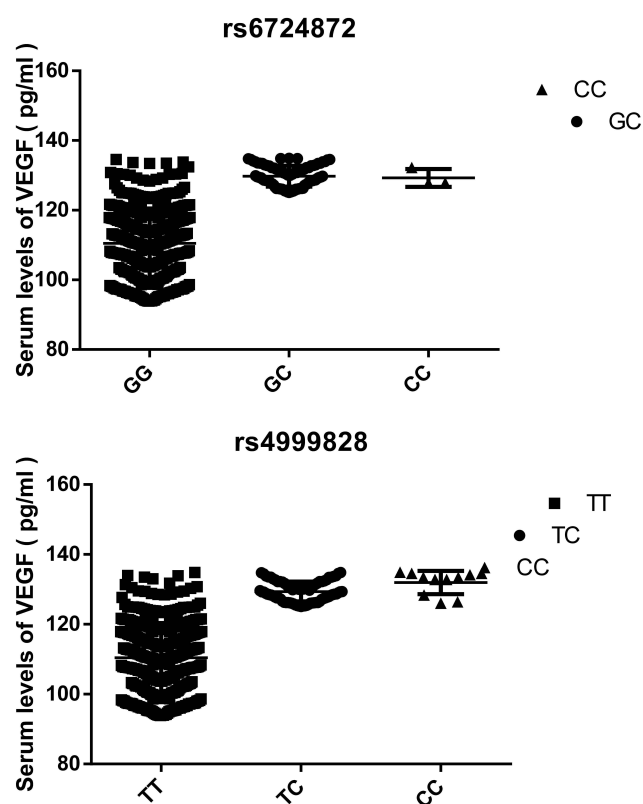


**Figure 1** Plasma level of miR-217 and CAD risk. Plasma level of miR-217 was analyzed in 50 randomly selected patients with CAD and controls, and plasma level of miR-217 in CAD cases was significantly higher than that in controls ( $P < 0.001$ ).

**Table 2** Associations Between Genetic Variations and Risk of CAD

	CAD Cases	Controls	OR (95% CIs)*	P value
rs6724872				
GG	400	434	1.00 (Reference)	
GC	88	62	1.60 (1.11–2.31)	0.011
CC	10	3	3.76 (1.14–12.41)	0.030
C vs G			1.73 (1.25–2.39)	0.001
rs4999828				
TT	356	406	1.00 (Reference)	
TC	121	82	1.75 (1.27–2.41)	0.001
CC	21	11	2.26 (1.09–4.69)	0.028
C vs T			1.75 (1.34–2.29)	4×10 <sup>-5</sup>
rs10206823				
AA	337	341	1.00 (Reference)	
AT	139	141	1.04 (0.02–52.43)	0.985
TT	22	17	1.36 (0.67–2.77)	0.393
T vs A			1.10 (0.76–1.59)	0.609
rs41291177				
AA	360	377	1.00 (Reference)	
AG	121	110	1.20 (0.84–1.72)	0.326
GG	17	12	1.54 (0.70–3.38)	0.279
G vs A			1.24 (0.92–1.66)	0.155

**Notes:** \*Adjusted for age, gender, smoking status, drinking status, diabetes, and hypertension.



**Figure 2** Circulating level of VEGF in subjects with different miR-217 genotypes. Plasma VEGF level among the healthy controls were determined by multiplex analysis using Bioplex suspension arrays. With the increasement of number of minor alleles, the plasma level of VEGF increased significantly for both rs6724872 and rs4999828 ( $P < 0.001$ ).

higher level of VEGF. These findings highlighted the important role of miR-217 in the pathogenesis of CAD and potential targets for intervention.

MiRNAs are implicated in the regulation of proliferation and apoptosis of endothelial cells, induction of immune responses and different stages of plaque formation, which finally results atherosclerosis and CAD.<sup>5,14,15</sup> A previous meta-analysis identified that a total of 48 dysregulated miRNAs were confirmed for their role in CAD development, while MiR-122-5p and miR-133a-3p may be valuable biomarkers for CAD.<sup>16</sup> Another two studies confirmed that predictive value of miRNA-21 and miRNA-126 on coronary restenosis after percutaneous coronary intervention (PCI) in patients with CAD.<sup>17,18</sup> Previously, miR-217 was most studied in the field of cancer biology.<sup>19–23</sup> Zhao et al reported that downregulated miR-217 could regulate KRAS and function as a tumor suppressor in pancreatic ductal adenocarcinoma (PDAC).<sup>19</sup> Further, Menghini et al pinpointed miR-217 as an endogenous inhibitor of SirT1 was potentially amenable to the prevention of endothelial

dysfunction.<sup>24</sup> Recently, Yebenes then reported that miR-217 could aggravate atherosclerosis and promote cardiovascular dysfunction.<sup>6</sup> Taking the findings above together, it is important to extensively explore the role of miR-217 in the pathogenesis of CAD and to investigate the association of its genetic variants with the risk of disease development.

Genetic variants in miRNAs have been widely explored for their functions among pathophysiological mechanism of cardiovascular diseases, and offer new insight into the causal role of microRNAs in CAD.<sup>25–31</sup> Glinsky et al revealed identifies a consensus disease phenocode through a SNP-guided microRNA map of fifteen common human disorders.<sup>31</sup> Ghanbari et al systematically evaluated 230 variants located within miRNA-binding sites in the 3'-untranslated region of 155 cardiometabolic genes, and 37 were functional in their corresponding genomic loci.<sup>28</sup> In the current study, rs6724872 and rs4999828 were significantly associated with increased risk of CAD, as well as higher level of VEGF, which means the important role in CAD

development. Using RegulomeDB 2.0, we found both rs6724872 and rs4999828 were located in the TF binding and DNase peak region.<sup>32</sup> The findings of HaploReg v4.1 also validated their functions in gene regulation.<sup>33</sup>

Conclusively, We found rs6724872 and rs4999828 were significantly associated with increased risk of CAD, as well as higher level of VEGF. Although these findings need further validation in larger cohorts for definitive results, they reveal new mechanisms by which genetic variations in miR-217 gene may coordinate the development of CAD. The gathered evidence could be further exploited in prevention strategies or screening protocols for CAD.

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## Disclosure

The authors declare that they have no conflict of interest.

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