Original Article
Polymorphism in miRNA-1 target site and circulating miRNA-1 phenotype are associated with the decreased risk and prognosis of coronary artery disease

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Abstract: MiRNA molecules have been identified to play key roles in a broad range of physiologic and pathologic processes. Polymorphisms in microRNA target sites (PolymiRTSs) can disturb or obstruct miRNA binding and consequentially influence regulation of the target genes. A two-step study design was used in this study. A case-control study was designed to assess the relationship between miRNA-1 target site rs9548934C→T polymorphism in target gene (Component of Oligomeric Golgi Complex 6, COG6) and risk of coronary artery disease (CAD) in 1013 patients and 610 normal controls. This genetic variant was also evaluated for the association with major adverse cardiovascular events (MACE) of CAD in a follow-up study, including 785 (785/1013) patients followed up for 42 months. The phenotypes of circulating miRNA-1 levels in 34 cases were slightly lower than that of 40 controls but not significantly different (P = 0.090). The CT and CT/TT genotypes were associated with a 34% and 26% decreased risk of CAD, and the TT and CT/TT genotypes were associated with a 76% and 49% decreased risk of MACE of CAD. Cox regression analysis showed that rs9548937 C/T variant was associated with a decreased risk of MACE, while age, diabetes mellitus, higher levels of CRP ($\geq$ 3.80 mg/L) and three pathological changes in the coronary artery were associated with an increased risk of MACE. Our findings implicate miRNA-1 target site rs9548934C→T genotypes, circulating miRNA-1 phenotype and CRP levels may modulate the occurrence and MACE of CAD.

Keywords: miRNAs, target gene, polymorphism, coronary artery disease, MACE

Introduction

Despite advances in the prevention, detection, and treatment in the last few decades, coronary artery disease (CAD) is the leading cause of morbidity and mortality in the world [1]. In China, more than 700,000 people die from CAD each year [2]. CAD is a complex disease caused by environmental and genetic factors [3]. Epidemiological studies show that smoking, diabetes, hypertension, obesity, physical inactivity, alcohol intake, high-fat diet and family history are associated with an increased risk of CAD [4, 5]. Genome-wide association studies (GWAS) conducted both in European ancestry and Chinese populations also reveal some important genetic variants related to occur-

rence risks of CAD, such as 9p21 [2]. Nonetheless, only a few of these studies have detected both the genotype and phenotype associated with the occurrence of major adverse cardiovascular events (MACE) of CAD.

MicroRNAs (miRNAs, miRs) are a class of small noncoding RNA with a length of ~22 nucleotides. MiRNAs regulate gene expression through binding the 3'untranslated region (3'UTR) of mRNAs of target genes, causing inhibition of translation or even promoting degradation of the mRNA. It is estimated that about 30% of human genes are regulated by miRNAs [6]. Increasing evidence demonstrates that polymorphisms in microRNA target sites (PolymiRTSs) can disturb or obstruct miRNAs bind-

Table 1. Distributions of selected variables in CAD cases and normal controls

Variants	Control group (N = 610)	CAD (N = 1013)	P value
Age (M ± SD)	65.55 ± 11.47	65.72 ± 9.95	0.759[a]
BMI (kg/m²)	23.97 ± 3.08	24.89 ± 3.06	< 0.001[a]
TG (mmol/L)[c] (M ± SD)	1.38 ± 0.72	1.60 ± 1.13	< 0.001[a]
TC (mmol/L)[c] (M ± SD)	4.67 ± 1.03	4.30 ± 1.04	< 0.001[a]
HDL-C (mmol/L)[c] (M ± SD)	1.35 ± 0.54	1.11 ± 0.41	0.014[a]
LDL-C (mmol/L)[c] (M ± SD)	2.53 ± 0.90	2.67 ± 1.27	< 0.001[a]
CRP (mmol/L) (Median [range])	1.20 [0.72-48.80]	3.80 [0.42-91.50]	< 0.001[a]
Sex (%)			
Male	407 (66.7)	719 (71.0)	0.075[b]
Female	203 (33.3)	294 (29.0)	
Diabetes Mellitus[d] (%)			
no	274 (88.1)	734 (72.6)	< 0.001[b]
yes	37 (11.9)	277 (27.4)	
Hypertension[e] (%)			
no	89 (28.8)	262 (26.0)	0.340[b]
yes	220 (71.2)	744 (74.0)	
Smoking status[f] (%)			
no	213 (72.0)	486 (49.6)	< 0.001[b]
yes	83 (28.0)	493 (50.4)	
Numbers of pathological changes in coronary arteries (n = 936) (%)			
1		402 (42.9)	
2		233 (24.9)	
3		301 (32.2)	
Pathological changes in coronary arteries (n = 936) (%)			
Left bole branch		109 (11.6)	
anterior descending branch		231 (24.7)	
Convolution branch		62 (6.6)	
Right corona branch		149 (15.9)	
More than one branches		385 (41.2)	

[a]Two-sided *t* test. [b]Two-sided chi-square test. [c]Serum TC and TG levels were measured in 599 normal controls and 972 CAD cases. Serum HDL-C levels were measured in 421 normal controls and 945 CAD cases. Serum LDL-C levels were measured in 598 normal controls and 953 CAD cases. [d]Information on DM status was obtained for 311 controls and 1011 CAD cases. [e]Information on hypertension status was obtained for 309 controls and 1006 CAD case. [f]Information on smoking status was obtained for 296 controls and 979 CAD case.

ing [7, 8], through regulating their mRNA expression [9] and consequentially influence regulation of the target genes, which might be associated with disease susceptibility, including risk of cancer [10], myocardial infarction [11], Alzheimer's disease [12] and hypertension [13].

MiR-1 is a highly conserved miRNA and its expression is enriched specifically in cardiac and skeletal muscle. It is required for postnatal cardiac function and reinforces the striated muscle phenotype by regulating the smooth muscle gene expression network [14]. In mice, miR-1 regulates the transition from prenatal to neonatal stages by repressing the cardiac fetal gene program in the heart [15], and it also protects the heart from myocardial ischemia-reperfusion (IR) injury through regulating apoptosis-related genes [16]. However, very little is known about how genetic variation in miR-1 target sites might affect miR-1 biogenesis and function.

Recent studies have shown that some miRNAs are not only presented in tissues but also in systemic circulation, including serum and plasma [17]. Altered levels of circulating miRNAs have been found in patients with acute coronary syndrome (ACS) [18], acute myocardial infarction (AMI) [19], stable coronary artery disease [20], essential hypertension [21] and heart failure [22]. Ai, et al, found that miR-1 is over-expressed in ischemic myocardium of a rat model of AMI
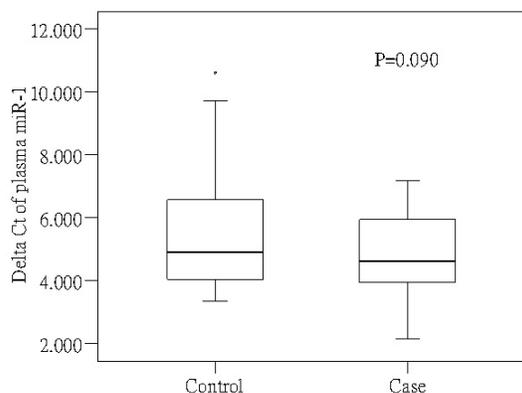
Figure 1. Circulating levels of miR-1 in plasma from CAD patients and normal controls.

and of individuals with CAD as well, which indicate that circulating miR-1 is a molecule with multiple roles in the process of the cardiovascular disease [23].

In this large-scale case-control and follow-up study, we obtained the miR-1 target site SNP of gene COG6 (rs9548934C→T) from PolymiRTS Database 2.0 [24], and analyzed the association between the target site SNP and the occurrence and MACE risks of CAD together with the circulating miRNA-1 phenotype.

Materials and methods

Study population

This study included 1013 consecutive inpatients with newly-diagnosed CAD and 610 CAD-free controls. All patients and controls were recruited between July 2005 and December 2010 in ZhongDa Hospital Affiliated to Southeast University, Nanjing, China. All CAD patients received quantitative coronary angiography (QCA) with a cardiovascular measurement system (QCA-CMS: Angiostar plus Siemens German) shortly after being admitted to the hospital, and coronary angiograms were analyzed by two experienced interventional cardiologists. CAD patients were defined as having angiographic coronary stenosis of at least 50% lumen reduction in at least one major epicardial coronary artery.

610 CAD-free controls were randomly selected from individuals with no history of cardiovascular events or signs of dysfunction of the cardiovascular system as evidenced by physical examination. Electrocardiogram (ECG) and ultrasonic echocardiogram (UCG) examination were conducted to exclude those with myocardial ischemia, segmental ventricular wall motion dysfunction, or cardiac dysfunction from controls.

After informed consent was obtained, each subject was interviewed using a structured questionnaire administered by interviewers at enrollment to collect information on demographic data, risk factors related to CAD, and personal history of vascular events. The complete clinical history acquisition of each subject was collected, including sex, age, body mass index (BMI), hypertension, hyperglycemia, diabetes mellitus (DM), smoking, and family history of coronary disease. Individuals who smoked once a day for over one year were categorized as smokers. The study was approved by the Ethical Committee of Clinic Medical College, Southeast University, and Nanjing, China.

Follow-up and outcomes measure

A total of 785 (785/1013) patients had completed follow-ups for 3.5 years (42 months). MACE was defined as unstable angina pectoris, myocardial infarction, cardiac insufficiency, cerebral infarction, and death due to CAD. Outcomes were determined from patient interviews, hospital chart reviews, and telephone interviews with the patient, a close relative, or a referring physician.

Among the remaining 228 (22.51%) patients with incomplete follow-up, 61 (6.02%) had incorrect telephone numbers, 87 (8.59%) refused to participate, and 80 (7.89%) moved or were unavailable for unknown reasons. However, there was no significant difference in the distribution of demographic characters (e.g., age and sex), or smoking status between the CAD cases with and without follow-up/clinical information.

Biochemical variables and genotype analyses

Five milliliter venous blood samples were collected from subjects after fasting for at least 12 h. Plasma total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) levels were determined by kit procedures supplied by Merck Diagnostica (Merck KGaA, Darmstadt, Germany).

Table 2. Logistic regression analysis on the association between the rs9548934 C>T polymorphism in hsa-miR-1 binding site in the 3'UTR of COG6 and risk of CAD

Genotypes	Controls[a] (n = 610)		CAD (n = 1013)		Adjusted OR[b] (95% CI)
	N	%	N	%	
hsa-miR-1 binding site in COG6 rs9548934 C→T					
CC	200	32.8	451	44.5	1.00
CT	286	46.9	391	38.6	0.66 (0.49-0.89)
TT	124	20.3	171	16.9	0.98 (0.65-1.49)
CT/TT	410	67.2	562	55.5	0.74 (0.56-0.97)
C allele		43.8		36.2	

[a]The observed genotype frequency among the control subjects was in agreement with the Hardy-Weinberg equilibrium (x^2 = 1.377, *P* = 0.240). [b]Adjusted for age, sex, BMI and smoking status.

Table 3. Genotypes of hsa-miR-1 binding site rs9548934 C>T polymorphisms and the HR of the cardiovascular events on the patients with CAD

Genotypes	Cases N	Events N	Log-rank *P*	Adjusted HR[e] (95% CI)	*P*
hsa-miR-1 binding site in COG6 rs9548934 C→T[a]					
CC	288	116		1.00	
CT	242	103	< 0.001	0.78 (0.58-1.04)	0.085
TT	134	33		0.24 (0.16-0.36)	< 0.001
CT/TT	376	136	< 0.001	0.51 (0.39-0.68)	< 0.001
CRP levels cut off by the median of the follow-up patients (mg/L)[b]					
< 3.80	321	65	< 0.001	1.00	
≥ 3.80	322	181		3.44 (2.55-4.64)	< 0.001
Delta Ct of plasma miRNA-1 cut off by the median of the follow-up patients[c]					
< 4.63	18	7	0.582	1.00	0.826
≥ 4.63	18	5		0.82 (0.14-4.77)	
Numbers of pathological changes in coronary arteries[d,f]					
1	291	85		1.00	
2	147	57	< 0.001	1.24 (0.87-1.75)	0.235
3	177	91		1.84 (1.36-2.50)	< 0.001

[a]664 CAD patients were followed up with the detected genotype of rs9548934 and 252 patients obtained the CAD events. [b]643 CAD patients were followed up with the detected CRP levels and 246 patients obtained the CAD events. [c]36 CAD Patients were followed up with the detected plasma miRNA-1 levels and 12 patients obtained the CAD events. [d]615 CAD Patients were followed up with the detected plasma miRNA-1 levels and 233 patients obtained the CAD events. [e]Adjusted for age, sex, smoking status, TD, hypertension and numbers of pathological changes in coronary arteries. [f]Adjusted for age, sex, smoking status, TD, hypertension.

Genomic DNA was extracted from the leukocyte pellet obtained from the buffy coat of each blood sample. The miR-1 target gene COG6 rs9548934 C→T polymorphisms were detected by employing the polymerase chain reaction (PCR) using mismatch primer design method. Briefly, the primers were: 5'-TGTCACTACG-TTCAATTCTTTGCCTT-3' (forward), 5'-AGTCAG-GGTGGAGCAGGTGACT-3' (reverse) for hsa-miR-1 target gene COG6 rs9548934 C→T. The PCR products were digested with *FspB*I (New England BioLabs, Beverly, MA, USA). Genotyping was performed without knowing the subjects' status and the same number of cases and controls were assayed in each 96-well PCR plate with a positive control of a DNA sample with known heterozygous genotype. Two research assistants independently read the gel pictures and performed the repeated assays in the cases when they did not reach a consensus on the tested genotype.

RNA preparation and circulating (plasma) miRNA-1 level detection

Blood samples for circulating (plasma) miRNA detection were collected from the 34 CAD patients and 40 controls and were processed within 1 h of collection by two-step centrifugation. The supernatant was transferred to RNase/DNase-free tubes and stored at -80°C. Total RNA in plasma were isolated by using mir-Vana™ miRNA (Ambion, Austin, TX, USA) following the instructions from the manufacturer with
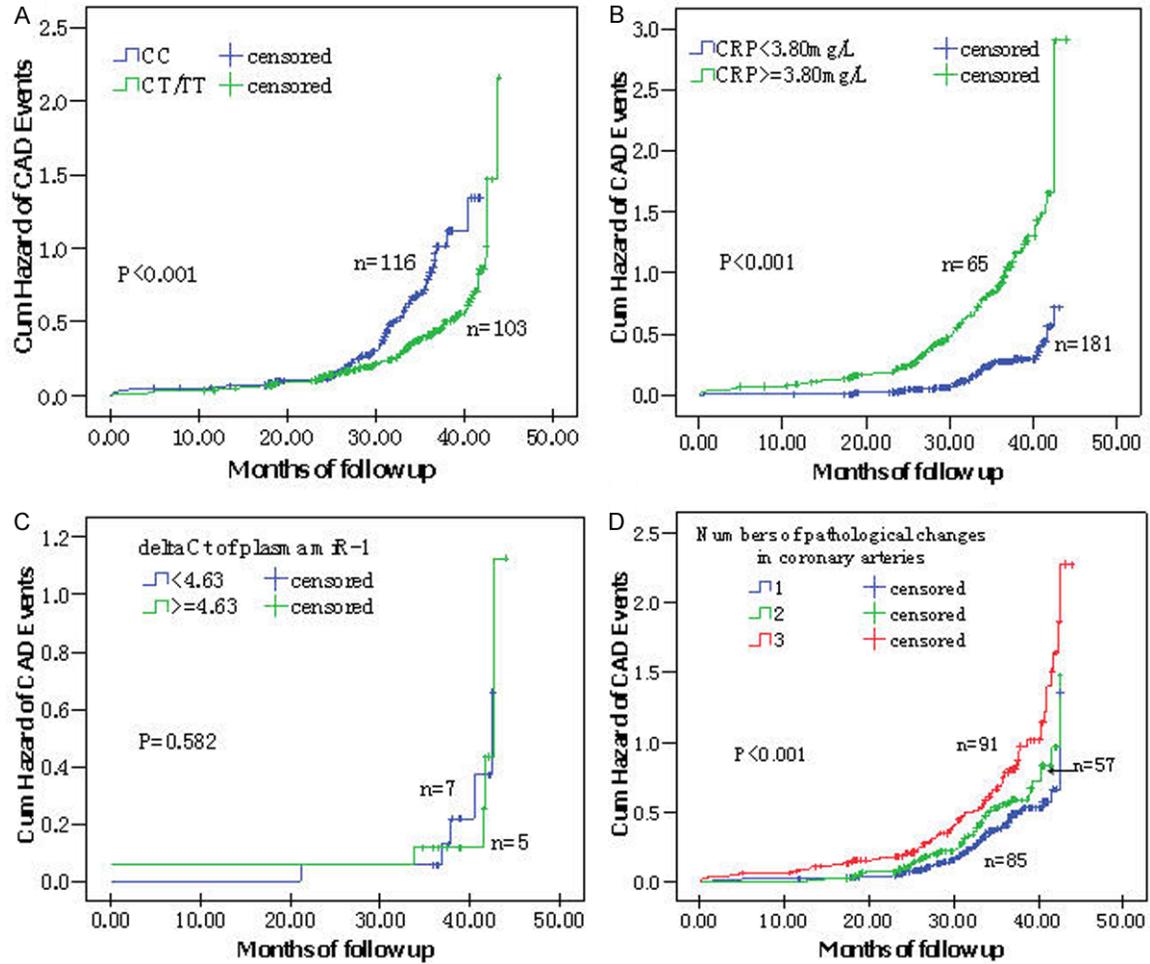
Figure 2. Kaplan-Meier survival curves of cardiovascular events in CAD patients with different genotypes, CRP levels, circulating miR-1 levels and numbers of pathological changes in coronary arteries. A: rs9548934 C→T, CC *vs.* CT/TT; B: CRP < 3.80 mg/L *vs.* ≥ 3.80 mg/L; C: Delta Ct of plasma miR-1 < 4.63 *vs* ≥ 4.63; D: Numbers of pathological changes in coronary arteries: 1 *vs.* 2 *vs.* 3.

appropriate modification. RNA concentration was determined by the NanoDrop® ND-1000 spectrophotometer (NanoDrop, USA), and the concentration of RNA (100-200 ng/μl), purity (ultraviolet 260 OD and 280 OD ratio range of 1.6-2.0) and gross (≥ 400 ng) all met the research requirement.

cDNA synthesis was performed according to the manufacturer's instructions (Reverse Transcription System, Cat. #A3500, Promega) as described previously [25]. The miScript SYBR Green PCR kit (Qiagen) was used in real-time PCR for relative quantification of miRNAs in our study with U6 used as an internal control. qRT - PCR was performed on 7500 FAST Real-Time PCR System (Applied Biosystems). The data were analyzed with automatic setting for

assigning baseline; the threshold cycle (Ct) was defined as the fractional cycle number at which the fluorescence exceeds the given threshold. The plasma levels of miRNA were detected and analyzed by two investigators who were blinded to the clinical data of patients. The relative circulating level for miR-1 was computed using the comparative Ct method.

$$\Delta Ct = Ct \, (miRNA) - Ct \, (RNU6B)$$

Statistical analyses

Differences in demographic characteristics, selected variables, frequencies and serum lipids levels between the cases and controls were evaluated using the χ^2 or student's *t* test. The associations between the rs9548934 C→T genotypes and risk of CAD were estimated by

Table 4. Cox regression analysis on the risk factors of the prognosis of cardiovascular events on the patients with CAD

Variables	β	SE	HR	95% CI	P
Sex [1 = male, 2 = female]	0.129	0.198	1.14	0.77-1.68	0.517
Age	0.017	0.008	1.02	1.00-1.03	0.034
BMI (kg/m^2)	0.017	0.022	1.02	0.98-1.06	0.421
TG (mmol/L)	-0.053	0.085	0.95	0.80-1.12	0.534
TC (mmol/L)	-0.008	0.117	0.982	0.79-1.25	0.944
HDL-C (mmol/L)	0.225	0.222	1.25	0.81-1.93	0.310
LDL-C (mmol/L)	-0.027	0.119	0.97	0.77-1.23	0.823
Smoking status [0 = no, 1 = yes]	0.146	0.171	1.16	0.83-1.62	0.396
Diabetes Mellitus [0 = no, 1 = yes]	0.446	0.143	1.56	1.18-2.07	0.002
Hypertension [0 = no, 1 = yes]	-0.205	0.163	0.82	0.59-1.12	0.211
rs9548934 CT/TT *vs* CC	-0.671	0.145	0.51	0.38-0.68	<0.001
CRP [lower than 3.80 mg/L = 0, more than 3.80 mg/L = 1]	1.008	0.179	2.74	1.93-3.90	<0.001
Numbers of pathological changes in coronary arteries (1) [n = 2]	0.234	0.181	1.26	0.89-1.80	0.197
Numbers of pathological changes in coronary arteries (2) [n = 3]	0.535	0.161	1.71	1.25-2.34	0.001

β: regression coefficient.

estimating adjusted ORs and 95% CIs from logistic regression analyses, with adjustment for age, sex, BMI and cigarette smoking. The Hardy-Weinberg equilibrium was tested by a goodness-of-fit χ^2 test to compare the observed genotype frequencies with the expected ones among control subjects.

Follow-up time was calculated from the date of CAD diagnosis to the date of the last follow-up. The associations between overall cardiovascular events and demographic characteristics, serum lipids levels and miR-1 target site SNP were estimated using the Kaplan-Meier method and log-rank test. Univariate or multivariate Cox proportional hazards regression models were performed to estimate the crude HRs or adjusted HRs and their 95% confidence intervals (CI). The Cox stepwise regression model was also used to determine factors predictive of cardiovascular events, with a significance level of $P < 0.05$ for entering and $P > 0.10$ for removal of the respective explanatory variables.

All of the statistical analyses were performed with Statistical Analysis System software (v.9.1.3e; SAS Institute, Cary, NC).

Results

Characteristics of the study population

The distribution of demographic characteristics and clinical items of the 1013 CAD cases and

610 controls are shown in **Table 1**. There were no significant differences in the distribution of the age, sex, and hypertension between the cases and controls ($P > 0.05$), suggesting that our frequency-matching on age and sex was satisfactory. The average body mass index (BMI) of CAD cases was significantly higher than that of the controls ($P < 0.001$). In addition, the serum TG, TC and LDL-C levels were significantly higher in cases, and levels of HDL-C were significantly lower in cases as compared with the controls ($P < 0.001$). With regards to disease history, 277 (27.4%) patients had a history of DM, significantly more than amongst controls (11.9%) ($P < 0.001$), but no significant difference in hypertension was shown between cases and controls ($P = 0.340$). There were more smokers in the case group (50.4%) than in controls (28.0%) ($P < 0.001$). Among the cases, 936 (92.4%) had information regarding pathological changes in coronary arteries: 551 (58.9%) cases had one lesion, and 385 (41.1%) had more than one coronary artery lesion.

Circulating levels of miR-1 in cases and controls

Circulating levels of miR-1 in plasma from 34 CAD patients and 40 normal controls are shown in **Figure 1**. The mean ΔCt of circulating miR-1 in cases was 4.948 (± 1.315), and that of controls was 5.618 (± 1.918). The median of miR-1

in plasma of CAD patients was slightly lower than that of normal controls; however, there were no statistical significant differences in the circulating levels of miR-1 in plasma between CAD patients and normal controls (*P* = 0.090).

Associations between miR-1 target gene COG6 rs9548934 C→T polymorphism and risk of CAD

The distribution of MiR-1 target gene COG6 binding site rs9548934 C→T genotypes in the CAD and controls are shown in **Table 2**. The observed genotype frequency among the control subjects was in agreement with the Hardy-Weinberg equilibrium (χ^2 = 1.377, *P* = 0.240). After adjusting for age, sex, BMI and smoking status, we found that heterozygote CT and CT/TT genotypes were associated with a significantly reduced risk of CAD, compared with the wide genotype CC (adjusted OR = 0.66, 95% CI = 0.49-0.89 for CT, adjusted OR = 0.74, 95% CI = 0.56-0.97 for CT/TT, respectively), but homozygote TT in dominant model did not show a significant association with reduced CAD risk (adjusted OR = 0.98, 95% CI = 0.65-1.49).

MiR-1 target gene COG6 rs9548934 C→T polymorphism and the hazard of MACE on the patients with CAD

We obtained genotypes and follow-up information for 664 CAD patients. Survival analyses showed that the differences of MACE hazards between carriers with rs9548934 TT, CT/TT and CC genotypes were statistically different (*P* < 0.001), but not for CT and CC genotypes (*P* = 0.085). Multivariate Cox proportional hazard regression analysis also showed that rs9548934 TT and CT/TT were significantly associated with MACE risks compared with CC wide genotype (adjusted HR = 0.24, 95% CI = 0.16-0.36 for TT; adjusted HR = 0.51, 95% CI = 0.39-0.68 for CT/TT) (**Table 3**).

When the median of CRP levels and Delta Ct of plasma miRNA-1 in the follow-up patients were considered as the cut-off value, there was a significant increased risk of MACE in the patients with higher CRP levels (≥ 3.8 mg/L) (adjusted HR = 3.44, 95% CI = 2.55-4.64), but there was no significantly decreased risk of serious prognosis of CAD in patients with higher Delta Ct of plasma miRNA-1 (≥ 4.63) (adjusted HR = 0.82, 95% CI = 0.14-4.77). Patients with three patho-

logical changes in coronary arteries had an 84% increased risk of MACE, compared with those with one pathological change (adjusted HR = 1.84, 95% CI = 1.36-2.50).

As shown in **Figure 2**, cumulative hazard curves also showed that CAD patients carrying the COG6 rs9548934 CC, higher CRP level, and three pathological changes in coronary arteries had higher HR of MACE than others (**Figure 2A**, **2B**, **2D**). However, a higher delta Ct of plasma miR-1 variant was not associated with a significantly decreased hazard of MACE in CAD patients (**Figure 2C**).

Cox proportional hazard regression analysis on the cardiovascular events hazard of CAD patients

Both the univariate and multivariate Cox proportional hazard regression models were used to evaluate the correlation between variables including selected demographic characteristics, clinical features, and the miRNA SNPs and HR of MACE. Five variables (age, diabetes mellitus, COG6 rs9548934 C→T, CRP and numbers of pathological changes in coronary arteries,) were included in the both univariate and multivariate models, with a significance level of *P* < 0.05 for entering and *P* = 0.10 for removal from the model (**Table 4**). CAD patients with elder age, diabetes mellitus, rs9548934 CC genotype, higher CRP level and three pathological changes in coronary arteries had a higher HR of MACE.

Discussion

Herein, we found that the genetic variant of miRNA-1 target gene COG6 binding site rs9548934C→T was associated with a significantly decreased risk and MACE of CAD, while the circulating miRNA-1 phenotype in CAD cases was slightly lower than that of controls but not statistically significant. In addition, age, diabetes mellitus, higher level of CRP and three pathological changes in coronary arteries were associated with the MACE of CAD.

MiRNA regulates gene expression by binding and modulating the translation of specific miRNAs [26], and it has been increasingly implicated in the control of various biological processes, including cell differentiation, cell proliferation, development and apoptosis, and

many pathological processes such as cancer, diabetes, and cardiovascular disease [27-29]. Recently, miRNAs have been detected in serum and plasma, and circulating miRNA profiles have now been associated with a range of different tumor types [30, 31], stroke and heart disease [32]. Several studies suggest that plasma/serum concentrations of miR-1 may be a useful indicator for AMI [33, 34]. In this study, we detected the circulating expressions of miR-1 in CAD patients and controls, that level of miR-1 was lower in patients than in controls. Although it is not a significant finding ($P = 0.090$), the trend of miR-1 declines CAD patients was consisted with AMI [34]. Meanwhile, miR-1 expression level was significantly reduced in tumor tissues [35].

The presence of SNP within the 3'untranslated regions of target DNA gene could alter the binding with specific miRNAs, modulating gene expression, and ultimately could affect disease susceptibility. Evidence suggests that polymorphisms in miRNA-binding sites of genes are associated with increased risks of cancer, but few have demonstrated this effect on heart diseases. Lin et al, demonstrated that T allele genotypes (rs12537 CT and TT) of MTMR3, which was in miR-181a binding sites, were associated with significantly increased gastric risk and poor overall survival [36]. Naccarati et al, found that variants of miRNA-binding sites of nucleotide excision repair (NER) genes RPA2 rs7356 and GTF2H1 rs4596 were associated with colorectal cancer risk [10]. In this present study, we found that in the miR-1 target gene COG6 rs9548934 C→T variant, CT and CT/TT were associated with 34% and 26% significantly decreased risks of CAD, compared with the wide genotype CC, which suggests that polymorphism in microRNA target sites (PolymiRTSs) may be modulated in miR-1 targeting, whereas affected the mediate functions of miR-1.

C-reactive protein (CRP) is an acute phase protein which reflects a measure of the acute phase response. CRP is used as one of the markers of choice in monitoring the acute phase response because it increases to a relatively high concentration compared to basal concentration. Elevated plasma CRP has been suggested as a risk factor for ischemic stroke (IS) and coronary ischemic disease [37]. Huang et al, revealed that elevated plasma high sensi-

tive CRP (> 3.0 mg/L) independently predicted risk of all-cause death within three months after acute IS in Chinese patients [38], while in this study, there was a significantly increased risk of cardiovascular events hazards in the Chinese patients with the higher CRP level (≥ 3.8 mg/L).

MiR-1 is being used in the oncology field to characterize tumors and predict the survival of cancer patients [39], while few studies were conducted on the prognosis of CAD. In this study, age, DM, higher level of CRP and three pathological changes in coronary arteries were associated with the increased risk of MACE, while the miR-1 target gene COG6 rs9548934 C→T variant was associated with decreased risk of MACE. This variant might be modulated the expression level of miR-1, which was associated with the decreased HR of MACE among CAD patients, but the biological mechanism of this regulation should be further studied.

Because small variation in the miRNA-binding sites of genes may have an effect on thousands of target mRNAs and result in diverse functional consequences, the most common genetic variation, SNPs, in miRNA sequences may also be functional and therefore may represent ideal candidate biomarkers for diseases prognosis. This is the first study to our knowledge to describe miR-1-binding site of gene COG6 rs9548934 C→T and CAD risk and prognosis outcome with a relatively large study population size and a high statistical power (despite the number of controls was less than that of cases--if we set α=0.05, based on our data set for COG6 rs9548934 TT, we have an 80% power to detect an OR of 0.72). Nevertheless, some limitations should be taken into consideration. First, the follow-up period was relatively short for some patients. Second, the mechanisms by which the miR-1 and its target mRNAs regulate CAD progression are unknown. This subject warrants further in vitro and in vivo studies, and further characterization of SNPs of miRNAs would improve our understanding of miRNA biogenesis and the potential contribution of these SNPs to disease development and prognosis, which may also favor therapeutic interventions.

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Disclosure of conflict of interest

None.

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