

## Original Article

# The correlations of circulating microRNA-133a with the risk and severity of coronary heart disease

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**Abstract:** Objective: Coronary heart disease (CHD) is a very common health problem worldwide with high morbidity and mortality. MicroRNAs (miRNAs) are small single stranded non-coding RNA molecules and can modulate gene expression. The objective of this study was to evaluate the correlation of miRNAs expression with the risk and severity of CHD. Methods: Plasma samples were collected from patients with CHD (n = 79) and controls (n = 63) to measure expression of miRNA92a, miRNA21, miRNA125b, miRNA133a and miRNA133b by qRT-PCR. Correlation of miRNA-133a expression with risk and Gensini score of CHD was performed by statistical analysis. Results: MiRNA-133a level was highly increased in plasma samples from patients with CHD compared with controls (0.61 (0.28-1.02) vs. 0.45 (0.22-0.85),  $P = 0.047$ ). ROC analysis for miRNA-133a showed that the AUC was 0.597 (95% CI: 0.504~0.691) for miRNA-133a, with a sensitivity of 29.1% and specificity of 92.5% at best cut off. And univariable logistic regression revealed that circulating miRNA-133a level was a risk factor of CHD (OR: 2.565, 95% CI: 1.105-5.954,  $P = 0.028$ ). Moreover, there was a positive correlation between miR-133a expression and Gensini score in patients with CHD ( $r = 0.303$ ,  $P = 0.007$ ). Conclusion: Circulating miRNA-133a level was associated with the risk and severity of CHD, and might be used as a novel biomarker for CHD in the future.

**Keywords:** Coronary heart disease, miRNAs, miRNA-133a, Gensini score

## Introduction

Coronary heart disease (CHD) is the leading cause of morbidity and mortality around the world [1]. CHD is a typically polygenic and multifactorial disease. Recent advances have proposed the hypothesis that genetic factors in the presence of environmental factors could be involved in the pathogenesis of CHD [2]. Previous studies have demonstrated that gene susceptibility, smoking, hypertension, obesity, type 2 diabetes mellitus and high-fat diet could be identified as the risk factor for CHD [3]. However, the detailed pathogenesis of CHD is still obscure. Given the high morbidity and mortality of CHD, early diagnosis and treatment for CHD is of great importance at present time. Therefore, new diagnostic and prognostic biomarkers and novel treatment for CHD is a particularly urgent need.

MicroRNAs (miRNAs), derived from hairpin-structured precursors, are small single strand-

ed non-coding RNA molecules, which can directly bind to the potential target gene site in the 3' untranslated region of specific target mRNA [4, 5]. The main function of miRNAs is mRNA translation repression and target mRNAs degradation [6]. Recent studies have shown that miRNAs modulate the expression of genes, which are involved in metabolism and inflammation or DNA damage response [7-9]. Moreover, miRNAs participates in many biological processes in health and disease including cardiovascular diseases [10]. The finding that miRNA-processing enzyme Dicer depletion leads to the defects of angiogenesis, vessel formation, and cardiac development demonstrates the critical role of miRNAs in the cardiovascular system [10]. Previous studies have shown that the levels of miRNA-499, miRNA and miRNA-208 were shown to be increased in patients with acute myocardial infarction [11-13]. miRNA-423 was recently identified as a promising prognostic marker in patients with heart failure [14].

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However, the crucial role of miRNAs in the pathogenesis of CHD is still unclear.

In our current study, plasma samples were collected to determine expression of miRNAs, and we aimed to investigate the association of circulating miRNAs expression with the risk and severity of CHD.

### Materials and methods

#### *Patients*

142 subjects who underwent coronary angiography at the Department of Cardiology, from the Second Affiliated Hospital of Zhejiang University School of Medicine between December 2014 and May 2016 were enrolled in this study. Among them, 79 cases (57 males and 22 females, aged  $58 \pm 12$  years) were diagnosed as CHD, and 63 cases (39 males and 24 females, aged  $55 \pm 11$  years) were excluded from CHD and served as controls. The diagnostic criteria of CHD included the following: older than 18 years, unrelated individuals, and coronary angiography showing at least one vessel disease ( $\geq 50\%$  narrowing of luminal diameter). The exclusion criteria for CHD were as follows: vasospastic angina, acute infection, heart failure, history of malignancy, hepatic failure, renal failure and history of bone marrow transplantation [14]. The study was approved by the Ethical Committee of the Second Affiliated Hospital of Zhejiang University School of Medicine. Written informed consent was also obtained from all subjects before initiating the study protocol.

#### *Biochemical examination*

Venous blood (3-5 mL) was drawn from all the subjects after 12 hours of fasting on the second day of hospitalization. Biochemical assays included triglyceride (TG), total cholesterol (TC), fasting high-density lipoprotein cholesterol (HDL-C), fasting low-density lipoprotein cholesterol (LDL-C), fasting blood glucose (FBG), creatinine (CR).

#### *Gensini score*

All the subjects were received coronary angiography, and CHD was defined as at least one major epicardial vessel with  $>50\%$  stenosis; the control was defined as all of the major epicardial vessels with  $<50\%$  stenosis. Disease

severity was based on the Gensini scoring system. The Gensini score was calculated according to reduction in the lumen diameter and the roentgenographic appearance of concentric lesions and eccentric plaques [15]. Reductions in lumen diameter of 25%, 50%, 75%, 90%, 99% and complete occlusion were scored as 1, 2, 4, 8, 16 and 32, respectively. Moreover, the scores were multiplied by each principal vascular segment according to the functional significance of the myocardial area supplied by that segment: the left main coronary artery,  $\times 5$ ; the proximal segment of left anterior descending coronary artery (LAD),  $\times 2.5$ ; the proximal segment of the circumflex artery,  $\times 2.5$ ; the mid-segment of the LAD,  $\times 1.5$ ; the right coronary artery, the distal segment of the LAD, the posterolateral artery, and the obtuse marginal artery,  $\times 1$ ; and all others,  $\times 0.5$ .

#### *Plasma samples*

Blood samples (5 ml) were collected from each patient in EDTA plasma tubes on the day before coronary angiography. Blood samples were processed within 6 hours and stored at  $4^\circ\text{C}$ . After centrifugation at 1200 rpm for 15 minutes, plasma in the supernatants were collected and stored at  $-80^\circ\text{C}$  to further analysis.

#### *RNA isolation and qRT-PCR analysis*

Total RNA was extracted from the plasma with the miRNeasy Mini Kit (Qiagen) according to manufacturer's instructions, and was then reversed transcribed to complementary DNA (cDNA). For the determination of miRNAs, the qRT-PCR assay was performed using a TaqMan PCR kit according to the manufacturer's instructions ( $95^\circ\text{C}$  for 5 min, followed by 40 cycles of  $95^\circ\text{C}$  for 15 sec and  $60^\circ\text{C}$  for 60 sec). U6 was used as a housekeeping gene. The relative expression of each miRNAs was calculated based on the threshold cycle (CT), and data were analyzed by  $2^{-\Delta\text{Ct}\Delta\text{Ct}}$  method.

#### *Selection of miRNAs*

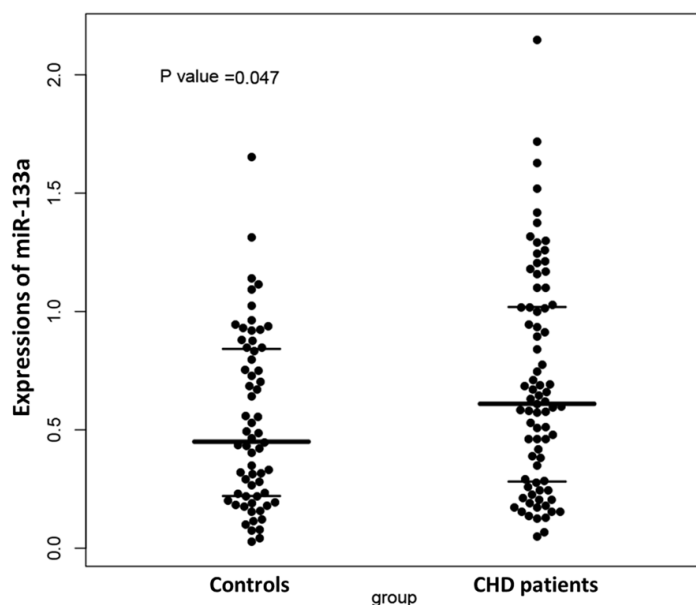
Previous studies revealed that miRNA-92a, miRNA-21, miRNA-125b, miRNA-133a and miRNA-133b might be involved in the pathogenesis of cardiovascular diseases [10, 15-17]. Therefore, we chose to measure the expression of these miRNAs in patients with CHD in Chinese population.

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**Table 1.** Characteristics of coronary heart disease (CHD) patients and controls

Parameters	CHD patients (n = 79)	Controls (n = 63)	p Value
Age (years)	58±12	55±11	0.127
Male (%)	57 (72%)	39 (62%)	0.195
BMI (kg/m <sup>2</sup> )	24.8±4.12	24.0±3.7	0.231
Hypertension (%)	39 (49%)	28 (44%)	0.559
Hyperglycemia (%)	18 (23%)	12 (19%)	0.775
TG	1.67±0.71	1.51±0.62	0.161
TC	3.93±1.15	3.68±1.06	0.185
HDL-C	1.17±0.29	1.35±0.31	0.001
LDL-C	2.81±0.88	2.49±0.82	0.028
FBG	5.78±1.95	5.27±1.74	0.107
CR	81.4±19.5	75.1±14.5	0.048
Gensini score	47.50 (19.50-72.50)	2.00 (1.00-4.00)	<0.001

Data are presented as Mean values ± SD, median and 25<sup>th</sup>-75<sup>th</sup> quartile or percentages. Significance of the comparison is determined by the Student t test, the Mann-Whitney test and the  $\chi^2$  test. BMI, body mass index; TG, triglyceride; TC, total cholesterol; HDL-C, fasting high-density lipoprotein cholesterol; LDL-C, fasting low-density lipoprotein cholesterol; FBG, fasting blood glucose; CR, creatinine.



**Figure 1.** The expression level of miR-133a in coronary heart disease (CHD) patients and controls.

### Statistical analysis

Statistical analysis was performed using SPSS V.20.0 (SPSS, Chicago, Illinois, USA). Data for age, gender, BMI, hypertension, hyperglycemia, TG, TC, HDL-C, LDL-C, FBG, CR and Gensini score were presented as Mean values ± SD, median and 25<sup>th</sup>-75<sup>th</sup> quartile or percentages and

significance of the comparison is determined by the Student t test, the Mann-Whitney test or the  $\chi^2$  test. Data for circulating miRNA levels were presented as median and 25<sup>th</sup>-75<sup>th</sup> quartile and significance of the comparison is determined by Mann-Whitney test. Univariate logistic regression was performed to evaluate plasma miRNA-133a level for the risk of CHD; multivariable logistic regression was performed to identify whether miRNA-133a level was independent risk factor of CHD. The Spearman two-way test was used to assess the relationship between Gensini scores with miRNA-133a expression. The susceptibility and sensitivity of miRNA-133a expression were determined using the area under the receiver operating characteristic (ROC) curve. *p* Values of <0.05 were considered to be statistically significant.

### Results

#### *Clinical data and biochemical criterion in patients with CHD and controls*

The baseline characteristics of all the subjects enrolled in this study were listed in **Table 1**. There was no significant difference in age, gender, BMI, hypertension, hyperglycemia, TG, TC and FBG between CHD patients and controls. Moreover, the level of HDL-C in patients with CHD was significantly lower than that in control ( $P = 0.001$ ). However, levels of LDL-C, CR and Gensini score were highly increased in CHD patients compared with that in controls ( $P < 0.05$ ), which indicated a correlation between these characteristics and CHD.

#### *miRNA-133a level was highly increased in patients with CHD*

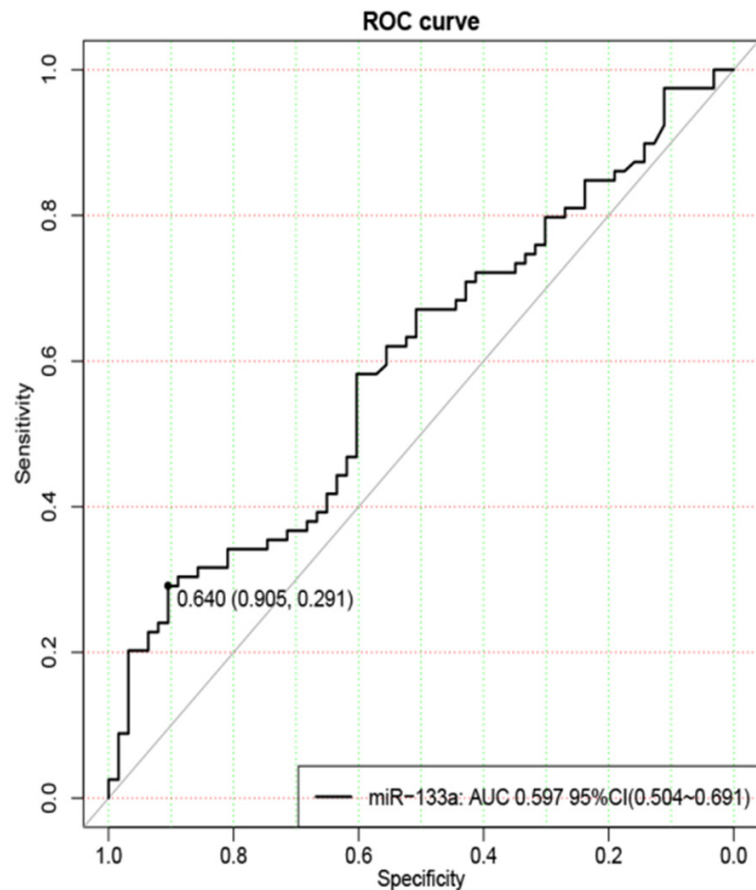
To determine the role of miRNAs in the pathogenesis of CHD, qRT-PCR analysis was performed to examine miRNAs expression in plasma samples from patients with CHD and controls. Interestingly, the level of miRNA-133a

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**Table 2.** Circulating micro-RNAs levels in coronary heart disease (CHD) patients and controls

Micro-RNAs	CHD patients (n = 79)	Controls (n = 63)	p Value
miRNA-21	0.72 (0.39-1.03)	0.78 (0.44-1.09)	0.525
miRNA-92a	0.31 (0.12-0.84)	0.29 (0.11-0.78)	0.723
miRNA-125b	0.17 (0.07-0.32)	0.21 (0.10-0.38)	0.163
miRNA-133a	0.61 (0.28-1.02)	0.45 (0.22-0.85)	0.047
miRNA-133b	0.24 (0.15-0.42)	0.28 (0.18-0.44)	0.268

Data are presented as median and 25<sup>th</sup>-75<sup>th</sup> quartile. Significance of the comparison is determined by Mann-Whitney test.



**Figure 2.** ROC curve of miR-133a for coronary heart disease (CHD).

was observed to be significantly increased in patients with CHD compared with control (0.61 (0.28-1.02) vs 0.45 (0.22-0.85),  $P = 0.047$ , **Figure 1**). However, there was no significant difference of the levels of miRNA-21, miRNA-92a, miRNA-125b and miRNA-133b between CHD patients and controls ( $P > 0.05$ ) (**Table 2**). Collectively, these data indicated that miRNA-133a may be involved in the pathogenesis of CHD, however, the potential role was still unclear.

### Correlation of miRNA-133a level with the risk of CHD

To further explore whether circulating miRNA-133a level could be used a potential prognostic biomarkers of CHD, ROC analyses were performed. As shown in **Figure 2**, the AUC was 0.597 (95% CI: 0.504~0.691) for miRNA-133a, with a sensitivity of 29.1% and specificity of 92.5% at best cut off (**Figure 2**). Moreover, univariable logistic regression and multivariable logistic regression were performed to explore whether plasma miRNA-133a level was the risk of CHD. Interestingly, univariable logistic regression showed that circulating miRNA-133a level was a risk factor of CHD (OR: 2.565, 95% CI: 1.105-5.954,  $P = 0.028$ , **Table 3**). However, multivariable logistic regression revealed that miRNA-133a level was not an independent risk factor of CHD (OR: 2.052, 95% CI: 0.862-4.882,  $P = 0.104$ , **Table 3**), adjusted by age, gender, triglyceride, total cholesterol, fasting high-density lipoprotein cholesterol, fasting low-density lipoprotein cholesterol, fasting blood glucose and creatinine. Taken together, these data demonstrated that plasma circulating miRNA-133a level might be a risk factor of CHD.

### Correlation of miRNA-133a level with the severity of CHD

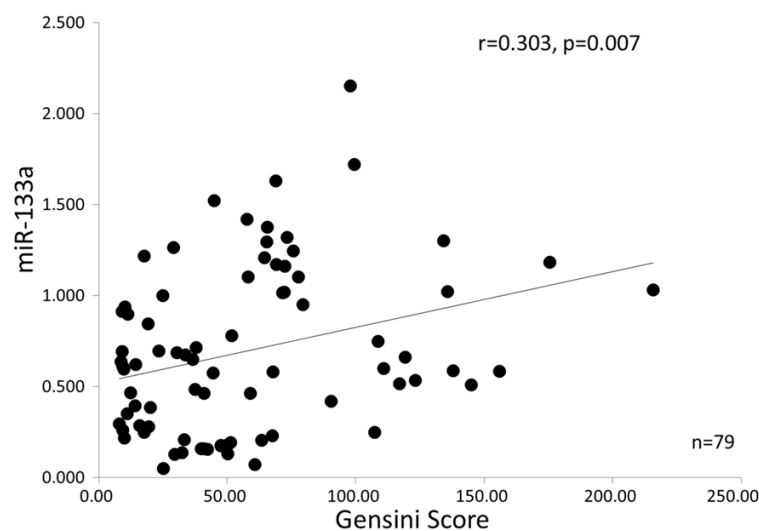
Given that Gensini score was significantly high in CHD patients and can reflected the severity of CHD, we then analyzed the correlation between miR-133a level and Gensini score. Interestingly, we observed that highly expression of miR-133a was positively correlated with high Gensini score in patients with CHD ( $r = 0.303$ ,  $P = 0.007$ ) (**Figure 3**), suggesting that

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**Table 3.** Circulating miRNA-133a level might increase the risk of coronary heart disease (CHD)

	Model	Odds Ratio	95% CI		p-Value
			Lower	Higher	
miRNA-133a	Univariable	2.565	1.105	5.954	0.028
	Multivariable	2.052	0.862	4.882	0.104

Data are presented as odds ratio with 95% CI and *p* value. Univariable logistic regression was performed to evaluate plasma miRNA-133a level for the risk of CHD; multivariable logistic regression was performed to identify whether miRNA-133a level was independent risk factor of CHD (miRNA-133a level, age, gender, triglyceride, total cholesterol, fasting high-density lipoprotein cholesterol, fasting low-density lipoprotein cholesterol, fasting blood glucose and creatinine were included in the analysis).



**Figure 3.** The correlation between miR-133a level and Gensini score in coronary heart disease (CHD).

plasma miR-133a levels was associated with the degree of coronary artery stenosis.

### Discussion

CHD, a common cardio-vascular disease, is affecting millions of people in both developed and developing countries. Every year, it may account for about seven million deaths and 129 million loss of disability-adjusted life years [18]. Therefore, pursuing an effective biomarker for early diagnosis and treatment of CHD is urgent. MiRNAs, noncoding genes, are small molecules and comprised of approximately 22 nucleotides in length [19]. They modulate other gene expression on a posttranscriptional level as intracellular RNAs and have been reported that they can be used as potential therapeutic targets [20]. Previous studies have demon-

strated that miRNAs participate in the pathogenesis of various cardiovascular conditions, including heart failure, hypertension and CHD [21, 22]. However, the role of miRNAs in CHD is not very clear until now. In this current study, our data reveal that miRNA-133a level was elevated in patients with CHD and is positively correlated with the risk and severity of CHD.

MiRNAs can be detected in serum or plasma, and are considered as circulating miRNAs [23]. Studies have shown that miRNAs are secreted in microvesicles or exosomes from different cell types, which are also referred as source of circulating miRNAs [19]. MiRNA-133, enriched in muscle, is almost the most abundant of the miRNAs present in the normal heart [24, 25]. MiRNA-133a and miRNA-133b are highly conserved genes in the musculatures of flies, mice and humans, characterized as muscle specific miRNAs [26], and regulated by myocyte enhancer factor 2 and serum response factor

[27]. Studies have reported that miRNA-133 is involved in heart development and some cardiovascular diseases, including myocardial infarction, myocardial injury after operation and cardiomyopathy [28, 29]. Previous studies have reported that miRNA-92a, miRNA-21, miRNA-125b are involved in the pathophysiological process of cardiovascular diseases [10, 15, 16]. Therefore, we detected expression of these miRNAs by qRT-PCR in our study. And we observed that plasma miRNA-133a levels were highly increased in patients with CHD, which suggested that miRNA-133a participated in the pathophysiological process of CHD and consistent with the results in acute myocardial infarction [19]. However, no significant levels of miRNA-21, miRNA-92a, miRNA-125b and miRNA-133b were observed, the possible reasons for which might be that some of these miRNAs

did not participate in the process of CHD or the number of enrolled cases were too little, and the role of these miRNA need to be further studied.

Rapid and correct diagnosis is crucial to treatment and prognosis of CHD. Up to date, coronary angiography is the gold standard for diagnose CHD, and age, gender, triglyceride, total cholesterol, fasting high-density lipoprotein cholesterol, fasting low-density lipoprotein cholesterol, fasting blood glucose and creatinine are the main risk factors of CHD [2]. However, their clinical value is limited in many cases, and it is not enough for rapid and correct diagnosis of CHD. Circulating miRNAs, secreted by cardiac cells and accumulated in blood, can reflect cardiac injury and some pathological conditions to some extent [19]. Studies have reported that circulating miRNAs (such as miRNA-208, miRNA-125b, miRNA-1291, miRNA-663b) could provide unique biomarkers for diagnostic and therapeutic interventions of cardiovascular diseases [15, 19, 30, 31]. In our study, univariable logistic regression showed that circulating miRNA-133a level was a risk factor of CHD, and its highly expression was positively correlated with the degree of coronary artery stenosis, which indicated that high levels of miRNA-133a in plasma can be used as a useful biomarker to evaluate the risk and severity of CHD. However, the detailed mechanisms are still obscure and need to be further investigated.

In summary, in our current study, circulating miRNA-133a level was enhanced in patients with CHD. MiRNA-133a might be a risk factor of CHD and positively correlated with Gensini score in patients with CHD. Our present study provides the first insights into the interaction between miRNA-133a levels with risk and severity of CHD. Therefore, circulating miRNA-133a can be used as biomarker in CHD in the future. Moreover, advanced well-designed studies with larger cohort and long follow-up studies are still needed.

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### Disclosure of conflict of interest

None.

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