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# Association of Plasma MiR-17-92 With Dyslipidemia in Patients With Coronary Artery Disease

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**Abstract:** Circulating microRNAs (miRNAs) have already been proposed as sensitive and informative biomarkers for the diagnosis of multiple diseases. We investigated the miRNA expression patterns in plasma samples of patients with coronary artery disease (CAD) and explored the potential functions of certain miRNAs.

Deep sequencing analysis was performed to determine the miRNA expression profiles using RNA samples isolated from 20 healthy subjects and 20 patients with CAD. Quantitative reverse transcription polymerase chain reaction was applied to confirm the differential expression of the miR-17-92 cluster in 81 patients and 50 healthy volunteers. The association between the miR-17-92 cluster and clinical characteristics of patients with CAD were analyzed using SPSS16.0, SPSS Inc, Chicago, IL.

Hundreds of miRNAs were detected and most members from the miR-17-92 cluster and its paralogs, including miR-18a, miR-92a, miR-106b, and miR-17, exhibited differential expression in the plasma of patients with CAD compared with controls. Moreover, these miRNAs were found widely related to the blood lipids in the patients with CAD, as miR-17 was positively correlated with total cholesterol, low-density lipoprotein cholesterol, and apolipoprotein B, while miR-92a was found positively related to high-density lipoprotein cholesterol (HDL-C) but negatively related to lipoprotein-a. Additionally, miR-106b was positively related to HDL-C and apolipoprotein A-I.

Taken together with existing evidence from mechanistic studies, the current results of our study support a relationship between the miR-17-92 family and lipid metabolism, which merits further study.

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**Abbreviations:** ACE-I = angiotensin-converting enzyme inhibitors, ApoA-I = apolipoprotein A-I, ApoB = apolipoprotein B, BMI = body mass index, CAD = coronary artery disease, HDL-C = high-density lipoprotein cholesterol, LDL-C = low-density lipoprotein cholesterol, Lpa = lipoprotein-a, TC = total cholesterol, TG = triglyceride.

## INTRODUCTION

MicroRNAs (miRNAs) are a class of small noncoding RNAs that function as translational repressors. They bind through canonical base pairing to a complementary site and can thus direct the degradation or translational repression of these transcripts.[1] MiRNAs have been shown to play important roles in development, stress responses, angiogenesis, and oncogenesis.[2] Accumulating evidence also point to an important role of miRNAs in the cardiovascular system.[3,4] The crucial role of miRNAs in the cardiovascular system is supported by the finding that depletion of the miRNA-processing enzyme Dicer leads to defects in angiogenesis, vessel formation, and cardiac development in mouse.[5]

Plasma miRNAs have been found to display unique disease-specific expression patterns, supported by the fact that specific tumor miRNAs was identified in plasma of cancer patients and can serve as useful biomarkers for the diagnosis and prediction of cancer[6]; also it was reported that tissue-specific miRNAs were released into plasma and may serve as diagnostically sensitive plasma biomarkers of tissue injury.[7,8] Recent studies have also suggested that circulating myocardia-derived miRNAs might be useful as potential biomarkers for infarction.[9,10] Although the stimuli that trigger the secretion of miRNA into circulation are unclear, it was reported that circulating miRNAs can be incorporated into microvesicles or apoptotic bodies and delivered into recipient cells.[11,12] Whether the endogenous levels of circulating miRNAs can be delivered into target cells and regulate systemic gene expression needs to be further elucidated.

Coronary artery disease (CAD) is a multifactorial disease driven, in part, by chronic inflammation in response to cholesterol accumulation in the arterial wall followed by proliferation and differentiation of smooth muscle cell.[13,14] Several risk factors, such as hypercholesterolemia, are known to promote atherosclerosis,[15–17] and various biomarkers including miRNAs have been shown to identify patients at risk of CAD.[18] However, the effects of atherosclerogenesis on circulating miRNAs expression and the potential relation between circulating miRNAs and CAD risk factors is unknown. Therefore, we determined the levels of circulating miRNAs in patients with CAD and found that miR-17-92 exhibited differential expression between patients with CAD and healthy controls.

MiR-17-92 is one of the most extensively studied miRNA clusters. This cluster contains 6 pairs of mature miRNAs,

including miR-17-5p/miR-17-3p, miR-18a/miR-18a*, miR-19a/miR-19a*, miR-20a/miR-20a*, miR-19b-1/miR-19b-1*, and miR-92a-1/miR-92a-1*, and have 2 paralogs, miR-106b-25 and miR-106a-363, for sequence and function similarity.[19] The miR-17-92 cluster has already been reported to play important roles in regulating diverse cell activities including angiogenesis and cancer.[20,21] Recent studies also imply that this cluster is closely related to cardiovascular system. Hereby, we further explored the correlation between miR-17-92 cluster and known risk factors of CAD.

## METHODS

### Study Population

Twenty patients with angiographic documentation of CAD were included in the initial study cohort for RNA sequencing. Twenty healthy volunteers without any evidence of CAD or dyslipidemia, hypertension, diabetes, leukopenia, thrombocytopenia, severe hepatic or renal dysfunction, as well as inflammatory or malignant disease were served as the control group, and general exclusion of criteria included a history of using lipid lowering or antihypertensive, antidiabetic, and anti-inflammatory drugs. The results obtained in the initial cohort were then tested in a validation cohort of 50 controls with angiographic exclusion of CAD and 81 patients with angiographically documented CAD, which included the 20 controls and 20 patients with CAD in the initial cohorts. All patients were recruited from the Department of Cardiology, Guangzhou Military Region General Hospital, Guangdong, China, between January 2009 and May 2010, and all healthy controls were simultaneously recruited from the Medical Examination Center of the same hospital to avoid potential bias of participant selection between groups. The study size was determined according to the related studies that have already published. The 2 study groups were matched in terms of age, gender, and the prevalence of smoking and adipositas. This study was approved by the Ethics committee of Sun Yat-Sen University, Guangdong, and informed consent was obtained from each individual.

### Sample Progressing and RNA Extraction

Whole blood samples were collected from healthy donors and patients with CAD in the Main Hospital of Guangzhou Military Region. To harvest cell-free plasma, blood samples were centrifuged twice at 4°C. After the first centrifugation at 1600*g* for 10 minutes, the supernatant was centrifuged at 16,000*g* for 10 minutes to thoroughly remove blood cells and cell fragments. Total RNA including small RNAs were extracted using Trizol LS (Invitrogen; Life Technologies, CA) and the mirVana miRNA isolation kit (AM1650, Ambion; Life Technologies) according to manufacturers' instructions. For Solexa sequencing, total RNA was extracted using Trizol LS, 250 μL plasma was homogenized in 750 μL Trizol LS, followed by adding 200 μL chloroform and centrifugation at 13,000*g* for 10 minutes. After an additional chloroform extraction and precipitation with isopropanol, the RNA sample was suspended in 30 μL of nuclease-free water.

### Expression Pattern of Plasma MiRNAs in Healthy Subjects and Patients With CAD

The plasma from 20 healthy subjects and 20 patients with CAD were pooled separately, and total RNA of 2 pooled plasma sample were extracted using Trizol LS (Invitrogen) for sequencing; Solexa technology was employed to sequence all small RNAs (<30 nt) found in the plasma of both healthy subjects and patients with CAD. Briefly, the 18- to 30-nt fraction from 250 ng of plasma total RNA was isolated by polyacrylamide gel electrophoresis. Purified miRNAs were then 3′ and 5′ ligated to single-stranded oligonucleotide that contained universal primer sequences for reverse transcription and PCR. Reverse transcription and PCR generated a library of complementary DNA (cDNA) derived from small RNA. The cDNA is then amplified clusterly on a solid phase using the Cluster Generation Kits, followed by sequencing using the Illumina genome analyzer. Sequence reads obtained were compared with a reference database of known miRNA sequences (miRBase Release v.14.0) and GenBank.

### Quantification of MiRNA by Real-Time RT-PCR Analysis

We performed quantitative reverse transcription polymerase chain reaction (qRT-PCR) analysis using the TaqMan MicroRNA Assay (Applied Biosystems) for the detection of miR-17 (Assay ID 000393), miR-18a (Assay ID 000431), miR-106b (Assay ID 002422), miR-92a (Assay ID 000442), and RNU6B (Assay ID 001093). PCR was performed according to the manufacturer's recommendation. All samples were repeated in triplicate and all miRNAs were normalized to U6, which has previously been used for qRT-PCR normalization. There was no significant difference in U6 Ct values between control and plasma samples of the patients with CAD, suggesting that the expression of U6 is stable in our plasma sample. The ΔCt method was used for analysis [ΔCt = mean Ct (miRNA of interest) − mean Ct (U6)], and all the data were expressed as $2^{-\Delta Ct}$.

### Plasma Lipid and Lipoprotein Determination

Total cholesterol (TC) and triglyceride (TG) concentrations were measured by using the cholesterol oxidase phenol 4-aminoantipyrine peroxidase method (CH201, Randox) and glycerol phosphate oxidase-p-amino phenazone methods (TR1697, Randox). High-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) concentrations were measured by using the clearance method (CH2652 and CH2657, Randox). Apoprotein A-1 (ApoA-I) and apoprotein B (ApoB) concentrations were measured by immunoturbidimetry (LP2116 and LP2117, Randox). Lipoprotein-a (Lpa) concentrations were measured by using enzyme-linked immunosorbent assay (LP3403, Randox).

### Statistics

All statistical analyses were performed by using SPSS (version 16.0; SPSS Inc, Chicago, IL). Results for continuous variables were expressed as means ± standard deviation. Mann–Whitney test or Student *t* test were used for groupwise comparison of continuous variables. For categorical variables, Fisher exact test or $\chi^2$ test were used. Correlations between continuous variables were calculated using Spearman rank correlation coefficients. Associations between categorical variables were calculated using $\chi^2$ test. Multiple linear regression analysis was used to adjust potential confounders for multivariable analysis. All tests were considered significant at $P$ value $<0.05$.

## RESULTS

### Baseline Characteristics of Study Subjects

Characteristics of the initial cohort for sequencing are listed in supplementary Table 1 (Ling_SDC1.doc; http://links.lww.com/MD/A55) and that of the validation cohort are summarized in Table 1. The ages and gender of selected subjects from both groups are similar. Also there is no statistic significance with regard to the prevalence of smoking and adipositas. Some of the CAD cases are with complications symptoms such as dyslipidemia, hypertension, and diabetes.

**TABLE 1.** Characteristics of All Participants

| | Healthy Volunteers (n = 50) | Patients With CAD (n = 81) | *P* Value |
|---|---|---|---|
| Age, y | 54.66 ± 6.94 | 56.60 ± 8.45 | 0.17 |
| Gender (male) | 30 (60%) | 51 (63%) | 0.73 |
| Hypertension | 0 | 60 (73.2%) | <0.001 |
| Active smoker | 16 (32%) | 30 (37%) | 0.55 |
| Adipositas (BMI > 25) | 12 (24%) | 23 (28.9%) | 0.58 |
| Hyperlipidemia | 0 | 36 (44.4%) | <0.001 |
| Diabetes mellitus | 0 | 19 (23.2%) | <0.001 |
| Number of vessels | | | |
| I | 0 | 28 (34.5%) | — |
| II | 0 | 22 (27.2%) | — |
| III | 0 | 31 (38.3%) | — |
| Concurrent medication | | | |
| Aspirin | 0 | 67 (81.2%) | <0.001 |
| ACE-I | 0 | 36 (45%) | <0.001 |
| Statins | 0 | 61 (79.1%) | <0.001 |

ACE-I = angiotensin-converting enzyme inhibitor, BMI = body mass index, CAD = coronary artery disease.

### MiRNA Expression Pattern in Patients Versus Healthy Volunteers

To determine the effects of CAD on the levels of circulating miRNAs, we performed miRNA profile analysis by deep sequencing using RNA isolated from 20 patients with CAD and 20 healthy controls. Two hundred ninety-six miRNAs were detected in the plasma of healthy individuals and 196 in patients with CAD. Among all the miRNAs detected, 173 miRNAs were found in both groups, only 123 miRNAs were detected in healthy individuals, while only 23 miRNAs were detected in patients with CAD.

The levels of circulating miRNA profoundly differed between patients and healthy controls, as demonstrated in the scatter (Figure 1). There were 53 miRNAs that showed more than 2-fold differential expression between groups. Detailed information of these miRNAs is listed in supplementary Table 2 (Ling_SDC2.doc; http://links.lww.com/MD/A56). Interestingly, we found most members of

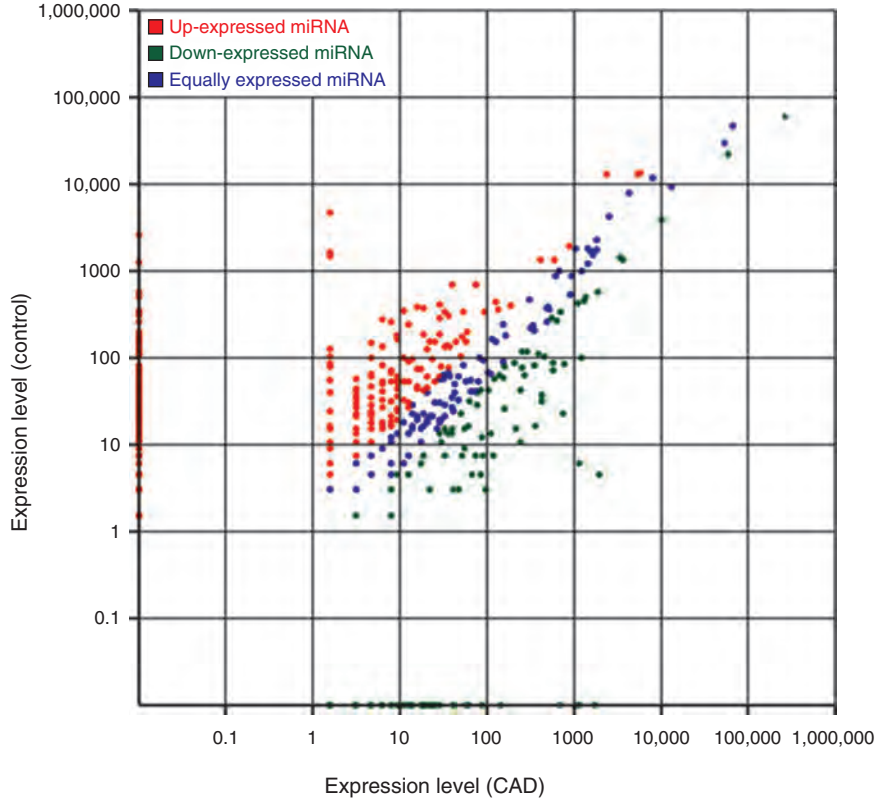


**FIGURE 1.** MiRNAs differentially expressed between healthy control and patient with CAD. Each point represent a miRNA, the *x* axis and *y* axis show expression of miRNAs in 2 groups, respectively, red points represent miRNAs up-regulated in patients with CAD, blue points represent miRNAs equally expressed while green points represent miRNAs down-regulated in patients with CAD. CAD = coronary artery disease.

the miR-17-92 cluster and its 2 paralogs, miR-106b-25 and miR-106a-363, also showed differential expression. Expression levels of miRNAs from the 3 clusters are listed in Table 2.

To confirm the findings obtained from sequencing, we measured the expression of 4 selected miRNAs from miR-17-92 family as well as its paralog miR-106a-363 in an entire cohort of 131 subjects by using TaqMan PCR. As shown in Figure 2, miR-92a, miR-106b, and miR-18a were decreased whereas miR-17 was increased in patients with CAD compared with healthy controls.

## Association of Circulating MiRNAs With Risk Factors in Patients With CAD

As we found that miRNAs from the miR-17-92 family were dysregulated in patients with CAD, we further explored the association of those miRNAs with known risk factors such as age, gender, diabetes, hypertension, dyslipidemia, obesity, as well as leukocyte count. It was reported that medications may affect miRNAs levels, so we also analyzed the association of miRNAs with statins, angiotensin-converting enzyme inhibitors (ACE-I), and aspirin. For continuous variables such as age, body mass index (BMI), and leukocyte counts, we analyzed the correlation of miRNAs with those variables using Spearman rank correlation coefficients. For categorical variables, we classified 81 patients into 2 subgroups according to their gender or diabetes, hypertension, hyperlipidemia, and medication status, respectively, and divided miRNAs level into low and high groups according to their median value; then association was analyzed by $\chi^2$ test and association coefficients were calculated. According to the results shown in Table 3, we found that none of the miRNAs were significantly associated with gender, diabetes, and hypertension, and none of them was correlated to age and leukocyte counts. miR-18a was found to be related to BMI. Among all the 4 miRNAs detected, miR-17 and miR-106b showed differential expression according to hyperlipidemia status. As regard to medication status, none of the miRNAs was influenced by statins or ACE-I, only miR-92a showed association with aspirin therapy. Multiple linear regression analysis, in which all the risk factors were included, was also used to further explore the potential association between miRNAs and risk factors. In the multiple model, the association of miR-17 and miR-106b with hyperlipidemia status as well as association of miR-18a with BMI sustained, while the association of miR-92a with aspirin was no longer significant after adjustment for other factors. The results are shown in Table 4.

## Correlation of Circulating MiRNAs With Blood Lipids in Patients With CAD

MiR-17 and miR-106b levels varied with dyslipidemia status in patients with CAD, indicating a potential relationship between miR-17 and lipid metabolism; therefore, we further analyzed the potential correlation between miR-17, miR-18a, miR-92a, miR-106b, and circulating TC, TG, HDL-C, LDL-C, ApoA-I, ApoB, and Lpa. As the results listed in Table 5, we found that miR-17 was positively correlated with TC, LDL-C, and ApoB, while miR-92a was found positively related to HDL-C but negatively related to Lpa. Moreover, miR-106b from the miR-106a-363 family was also found positively related to HDL-C and ApoA-I. Multiple linear regression model was also used to confirm that the correlation between blood lipids and miRNAs is independent from other risk

**TABLE 2.** Expression Level of MiR-17-92 Cluster and Its Paralogs by Sequencing

| MiRNA | Control (Copy Number) | CAD (Copy Number) | Fold Change | Sig-Label |
|---|---|---|---|---|
| hsa-miR-106a | 0.01 | 78.8051 | 12.94407328 | ** |
| hsa-miR-106a* | 0.1209 | 74.3633 | 9.26463274 | ** |
| hsa-miR-106b | 98.7567 | 0.01 | −13.26964799 | ** |
| hsa-miR-106b* | 113.2620 | 37.3966 | −1.59868489 | ** |
| hsa-miR-17 | 378.264 | 791.090 | 1.063502942 | ** |
| hsa-miR-17* | 47.3839 | 0.01 | −12.21019591 | ** |
| hsa-miR-18a | 279.226 | 0.1 | −11.44722900 | ** |
| hsa-miR-18a* | 89.449 | 0.1 | −9.80491571 | ** |
| hsa-miR-19b | 24.7798 | 24.0714 | −0.04184449 | |
| hsa-miR-20a | 23.0876 | 102.0167 | 2.14361522 | ** |
| hsa-miR-20b | 10.5163 | 0.01 | −10.03841875 | ** |
| hsa-miR-20b* | 541.0466 | 321.3813 | −0.75146686 | |
| hsa-miR-25 | 155.6899 | 103.0197 | −0.59575511 | |
| hsa-miR-25* | 633.6386 | 638.7508 | 0.01159297 | |
| hsa-miR-363 | 156.7778 | 82.5304 | −0.92572374 | |
| hsa-miR-363* | 83.6471 | 138.1238 | 0.72357450 | |
| hsa-miR-92a | 9498.0512 | 4489.2378 | −1.08926733 | ** |
| hsa-miR-92a-1* | 598.3424 | 591.4677 | −0.01667192 | |
| hsa-miR-92a-2* | 71.0758 | 66.9127 | −0.08707837 | |
| hsa-miR-92b | 2052.7375 | 1284.6656 | −0.67615629 | |
| hsa-miR-92b* | 845.8990 | 441.8814 | −0.93682622 | |
| hsa-miR-93 | 305.0942 | 297.1667 | −0.03798230 | |

CAD, coronary artery disease.

All the data are shown as absolute copy numbers derived from sequencing. Fold change was calculated by $\log_2$ CAD/Control. Sig-label stands for statistic significance. ** stands for $P < 0.001$.

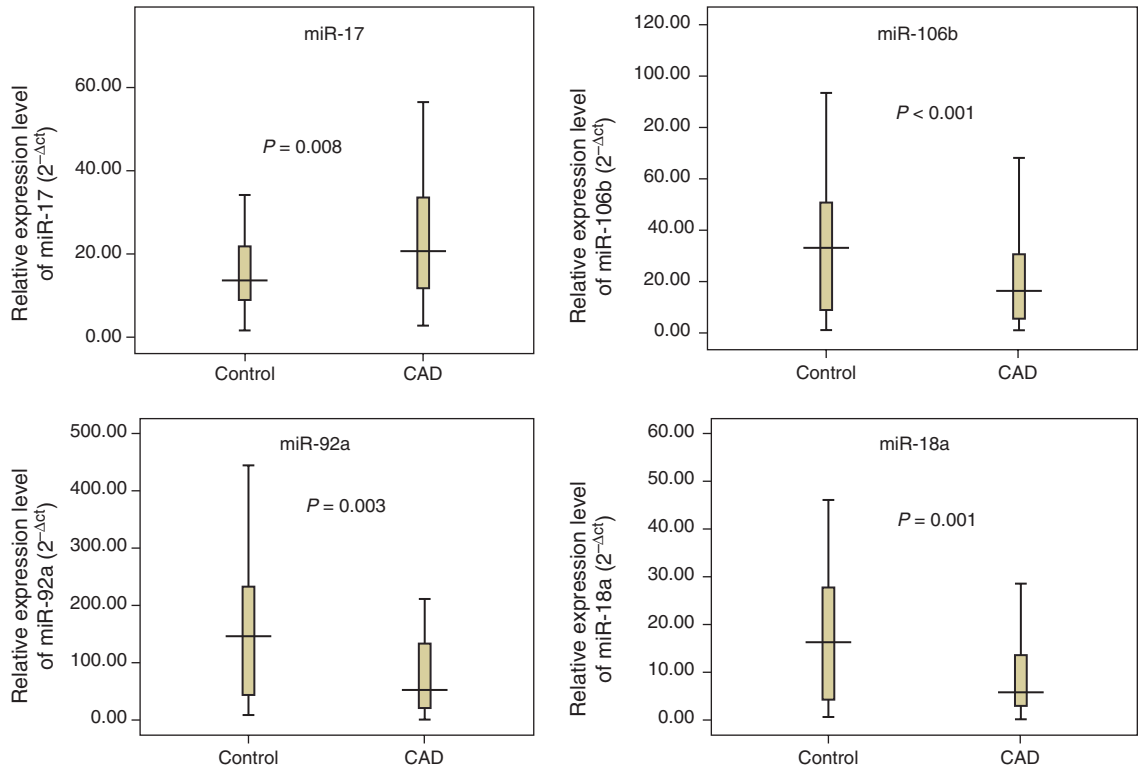


**FIGURE 2.** Differential expression of selected miRNAs in all participants using qRT-PCR. Expression of miR-17, miR-18a, miR-92a, and miR-106b in plasma obtained from patients with CAD (n = 81) and healthy controls (n = 50), as determined by TaqMan PCR. Values were quantified by normalizing to U6 and the final data were expressed as $2^{-\Delta Ct}$; probability values were calculated by Mann–Whitney test compared with controls. CAD = coronary artery disease, qRT-PCR = quantitative reverse transcription polymerase chain reaction.

factors, as the results shown in Table 6. In the multiple model, after adjustment for all the potential cofounders, the correlation between miRNAs and blood lipids were mostly not altered, except for the correlation between miR-17 and LDL-C.

## DISCUSSION

Generating miRNA profiles by deep sequencing is a direct and efficient method of obtaining maximum and accurate miRNA information.[22] In our study, we collected and pooled plasma samples of 20 patients with CAD and 20 healthy individuals for sequencing analysis. The results showed that hundreds of miRNAs were detectable in these blood samples. Most of those miRNAs exhibited overlapping expression between patients and control, but some were only detected in controls while others were specifically expressed in patients. For miRNAs that were expressed in both groups, a large number were differentially expressed in the patient with CAD group as compared with the control. CAD is a metabolic-related disorder that involves a complex interplay between many pathologic processes. The abundance of miRNAs that were found to be dysregulated in the plasma of patients with CAD suggested that miRNA can be detected in circulating blood and that these circulating miRNAs might be useful biomarkers for CAD.

More interestingly, we found that most miRNAs from the miR-17-92 family were detected in plasma samples and exhibited differential expression between the 2 groups. Previous research reported that the miR-17-92 cluster was highly expressed in human endothelial cell and in a mouse model of limb ischemia as well as myocardial infarction. Systemic administration of an antagomir designed to inhibit miR-92a led to enhanced blood vessel growth and functional recovery of damaged tissue.[20,23] These data suggest that miR-17-92 may play important roles in the process of angiogenesis following acute myocardial infarction, thus exhibiting higher or lower expression in plasma of patients with CAD. We selected four miRNAs from miR-17-92 and its paralogs according to sequence similarity and conducted association analysis between miRNAs and known CAD risk factors. The results showed that plasma miR-17-92 expression levels were widely related to blood lipid abnormalities. TC, LDL-C, and ApoB are reported to be detrimental whereas HDL-C and ApoA-1 are thought to be protective in patients with CAD. In our study, miR-17 expression was higher in patients with CAD and was positively correlated with TC, LDL-C, and ApoB. Expression of miR-92a and miR-106b were lower in patients with CAD and were positively related to HDL-C and ApoA-1. The results indicate that although these miRNAs may be all related to lipid metabolism, individual miRNAs can exert independent and differential effect.

In fact, previous studies have already shown involvement of miRNAs in the expression of genes that regulate lipid transport and metabolism, and that miRNAs may be protected against degradation by being packaged in lipid vesicles or associated with protein or lipoprotein complexes, and HDL can transport miRNAs into cells.[24] MiR-122, the

**TABLE 3.** Association of Circulating MiRNAs With Clinical Characteristics in Patients With CAD

| | MiR-17 | MiR-18a | MiR-92a | MiR-106b |
|---|---|---|---|---|
| Age | R = −0.038 | R = 0.066 | R = −0.026 | R = −0.023 |
| | *P* = 0.73 | *P* = 0.65 | *P* = 0.89 | *P* = 0.90 |
| | N = 81 | N = 81 | N = 81 | N = 81 |
| Gender | R = 0.021 | R = 0.189 | R = 0.099 | R = 0.042 |
| | *P* = 0.88 | *P* = 0.12 | *P* = 0.75 | *P* = 0.71 |
| | N = 81 | N = 81 | N = 81 | N = 81 |
| Diabetes | R = 0.003 | R = 0.213 | R = 0.036 | R = 0.097 |
| | *P* = 0.95 | *P* = 0.09 | *P* = 0.54 | *P* = 0.38 |
| | N = 81 | N = 81 | N = 81 | N = 81 |
| Hypertension | R = 0.032 | R = 0.001 | R = 0.128 | R = 0.005 |
| | *P* = 0.57 | *P* = 0.98 | *P* = 0.36 | *P* = 0.94 |
| | N = 81 | N = 81 | N = 81 | N = 81 |
| Hyperlipidemia | R = 0.384 | R = 0.266 | R = 0.143 | R = 0.305 |
| | *P* = 0.02 | *P* = 0.10 | *P* = 0.21 | *P* = 0.03 |
| | N = 81 | N = 81 | N = 81 | N = 81 |
| BMI | R = 0.110 | R = −0.223 | R = 0.013 | R = −0.025 |
| | *P* = 0.32 | *P* = 0.04 | *P* = 0.91 | *P* = 0.82 |
| | N = 81 | N = 81 | N = 81 | N = 81 |
| Leukocyte | R = 0.065 | R = −0.072 | R = 0.191 | R = 0.109 |
| | *P* = 0.56 | *P* = 0.62 | *P* = 0.31 | *P* = 0.55 |
| | N = 81 | N = 81 | N = 81 | N = 81 |
| Statins | R = 0.139 | R = 0.089 | R = 0.164 | R = 0.172 |
| | *P* = 0.21 | *P* = 0.76 | *P* = 0.13 | *P* = 0.11 |
| | N = 81 | N = 81 | N = 81 | N = 81 |
| ACE-I | R = 0.149 | R = 0.182 | R = 0.126 | R = 0.002 |
| | *P* = 0.17 | *P* = 0.11 | *P* = 0.25 | *P* = 0.98 |
| | N = 81 | N = 81 | N = 81 | N = 81 |
| Aspirin | R = 0.044 | R = 0.006 | R = 0.216 | R = 0.040 |
| | *P* = 0.69 | *P* = 0.94 | *P* = 0.04 | *P* = 0.74 |
| | N = 81 | N = 81 | N = 81 | N = 81 |

ACE-I = angiotensin-converting enzyme inhibitor, BMI = body mass index, CAD = coronary artery disease.

For variables of age, BMI, and leukocyte, "R" stands for correlation coefficients from Spearman rank correlation analysis. For variables of gender, diabetes, hypertension, hyperlipidemia, statins, ACE-I, and aspirin, "R" stands for association coefficients from $\chi^2$ test. "*P*" stands for statistic significance and "N" stands for number of cases included in the analysis.

**TABLE 4.** Multiple Linear Regression Analysis of the Association Between Clinical Characteristics and Circulating MiRNAs in Patients With CAD

| | MiR-17 | | MiR-18a | | MiR-92a | | MiR-106b | |
|---|---|---|---|---|---|---|---|---|
| | *β* | ***P*** | *β* | ***P*** | *β* | ***P*** | *β* | ***P*** |
| Age | −0.047 | 0.72 | −0.087 | 0.51 | −0.183 | 0.31 | −0.208 | 0.11 |
| Gender | −0.003 | 0.98 | −0.011 | 0.93 | −0.134 | 0.17 | −0.169 | 0.18 |
| Diabetes | 0.038 | 0.76 | 0.12 | 0.34 | 0.166 | 0.06 | 0.053 | 0.66 |
| Hypertension | −0.052 | 0.68 | 0.109 | 0.41 | 0.007 | 0.93 | −0.01 | 0.93 |
| Hyperlipidemia | 0.37 | 0.01 | 0.054 | 0.66 | −0.001 | 0.98 | −0.296 | 0.01 |
| BMI | 0.027 | 0.82 | −0.283 | 0.02 | 0.007 | 0.95 | −0.021 | 0.86 |
| Leukocyte | 0.08 | 0.51 | −0.071 | 0.56 | −0.083 | 0.50 | −0.094 | 0.43 |
| Statins | −0.125 | 0.31 | −0.08 | 0.51 | −0.173 | 0.16 | −0.143 | 0.07 |
| ACE-I | −0.04 | 0.74 | −0.108 | 0.38 | −0.162 | 0.19 | 0.001 | 0.98 |
| Aspirin | −0.01 | 0.93 | 0.151 | 0.22 | 0.204 | 0.09 | 0.142 | 0.23 |

ACE-I = angiotensin-converting enzyme inhibitor, BMI = body mass index, CAD = coronary artery disease.

In the multiple linear regression model, miRNA was considered as a dependent variable while age, gender, diabetes, hypertension, hyperlipidemia, BMI, leukocyte, statins, ACE-I, and aspirin were considered as independent variables. "*β*" means standardized regression coefficients; "*P*" stands for significance.

**TABLE 5.** Correlation Between MiRNAs and Blood Lipids in Patients With CAD

| | MiRNA-17 | | MiRNA-18a | | MiRNA-106b | | MiRNA-92a | |
|---|---|---|---|---|---|---|---|---|
| | R | *P* | R | *P* | R | *P* | R | *P* |
| TG | 0.09 | 0.42 | −0.088 | 0.54 | 0.02 | 0.91 | 0.025 | 0.89 |
| TC | 0.226 | 0.04 | 0.003 | 0.98 | 0.12 | 0.51 | 0.342 | 0.06 |
| LDL-C | 0.247 | 0.001 | −0.083 | 0.57 | −0.062 | 0.74 | 0.005 | 0.97 |
| HDL-C | −0.036 | 0.63 | 0.017 | 0.91 | 0.332 | 0.03 | 0.209 | 0.04 |
| ApoA-1 | 0.132 | 0.41 | 0.015 | 0.92 | 0.352 | 0.02 | −0.001 | 0.99 |
| ApoB | 0.322 | 0.005 | −0.044 | 0.77 | −0.065 | 0.73 | 0.046 | 0.81 |
| Lpa | −0.064 | 0.58 | −0.038 | 0.81 | 0.047 | 0.81 | −0.339 | 0.01 |

ApoB = apolipoprotein B, ApoA-I = apolipoprotein A-I, CAD = coronary artery disease, HDL-C = high-density lipoprotein cholesterol, LDL-C = low-density lipoprotein cholesterol, Lpa = lipoprotein a, TC = total cholesterol, TG = triglyceride.
"R" stands for correlation coefficient from Spearman rank correlation analysis.

most abundantly expressed miRNA in the liver, likely modulates the hepatic cholesterol network by directly regulating cholesterol synthesis genes or indirectly modulating another molecular pathway. Consequences of miR-122 loss include a significant drop in mRNA related to cholesterol synthesis, a reduction of plasma and hepatic cholesterol content, as well as an observed decrease in fatty acid synthesis.[25,26] It was also reported that the antagonism of miR-33 in mice promoted both reverse cholesterol transport and regression of atherosclerosis by targeting ABCA1.[27] Another study revealed that inhibiting miR-33a/b in non-human primates raised plasma HDL-C and lowered very low-density lipoprotein cholesterol (VLDL-C) and TGs.[28] miR-26 is another miRNA that has been reported to regulate cellular cholesterol levels through the modulation of ABCA1 and Arl7 gene.[29] Additionally, miR-10b has been reported to regulate ABCA1/ABCG1-mediated reverse cholesterol transport.[30] Bioinformatics analysis using miRNA targeting prediction tool (Targetscan and Pictar) found that most members for miR-17-92 and its paralogs potentially target a wide range of genes such as ABCA1, LDL-R, VLDL-R, peroxisome proliferator-activated receptor (PPAR)-α, and PPAR-γ,[31,32] all of which are critical in lipid and lipoprotein metabolism. In an ongoing study, we have successfully demonstrated that PPAR-α is a direct target of miR-17 and high levels of miR-17 can promote steatosis and fatty liver. Thus, we hypothesized that besides its potential role in angiogenesis and recovery of acute injury, miR-17-92 might also be closely related to lipid homeostasis. This was partially reflected by the differential plasma miRNA expression profiles, since dyslipidemia is prevalent in patients with CAD. This finding indicated another new potential function of the miR-17-92 family in a wide range of dyslipidemia-related diseases.

Although certain questions regarding their function and regulation still remain to be further elucidated, miRNAs have been considered to be promising pharmacological targets, because of the fact that miRNA can target different genes within 1 biological process plus previous existing antisense technology, and gene therapy approaches have greatly facilitated the development of therapies to modulate miRNA levels *in vivo*.[33] If the relationship between miR-17-92 family and lipid metabolism will be confirmed by mechanistic study, miR-17-92 family and its paralogs might serve as a potential therapeutic target in the treatment of dyslipidemia and related diseases, thus providing a new perspective for the clinical treatment of complex diseases.

**TABLE 6.** Correlation Between Blood Lipids and MiRNAs in Multiple Linear Regression Analysis

| | MiR-17 | | MiR-18a | | MiR-92a | | MiR-106b | |
|---|---|---|---|---|---|---|---|---|
| | *β* | *P* | *β* | *P* | *β* | *P* | *β* | *P* |
| TG | 0.139 | 0.26 | 0.047 | 0.69 | 0.009 | 0.94 | −0.075 | 0.53 |
| TC | 0.234 | 0.03 | 0.051 | 0.65 | 0.103 | 0.37 | 0.033 | 0.78 |
| LDL-C | 0.204 | 0.07 | 0.067 | 0.56 | 0.106 | 0.35 | 0.025 | 0.83 |
| HDL-C | 0.042 | 0.73 | 0.12 | 0.30 | 0.25 | 0.04 | 0.297 | 0.01 |
| ApoA-I | −0.156 | 0.22 | −0.107 | 0.38 | −0.076 | 0.54 | 0.285 | 0.01 |
| ApoB | 0.261 | 0.04 | −0.202 | 0.11 | 0.031 | 0.71 | −0.031 | 0.80 |
| Lpa | 0.039 | 0.75 | −0.02 | 0.87 | −0.284 | 0.01 | −0.024 | 0.84 |

ApoA-I = apolipoprotein A-I, ApoB = apolipoprotein B, HDL-C = high-density lipoprotein cholesterol, LDL-C = low-density lipoprotein cholesterol, Lpa = lipoprotein a, TC = total cholesterol, TG = triglyceride.
In the multiple linear regression model, miRNA was considered as dependent variable, and age, gender, diabetes, hypertension, BMI, leukocyte, statins, ACE-I, and aspirin were all adjusted as confounders. "*β*" means standardized regression coefficients; "*P*" stands for significance.

The relatively small sample size limits the significance of this study. Cohort studies should be carried out to confirm the observational results we found and further experimental studies are needed to illuminate whether this correlation that we found is truly causal and of pathologic significance.

## CONCLUSIONS

Our study applied miRNA profile analysis using next-generation sequencing to find that miR-17-92 and its paralogs were dysregulated in atherosclerosis. We are the first to report that this cluster might be implicated in lipid metabolism disturbances in patients with CAD.

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