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Plasma microRNAs are associated with atrial fibrillation and change after catheter-ablation (the miRhythm Study)

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Abstract

Background—MicroRNAs (miRNAs) are associated with cardiovascular disease (CVD), control gene expression, and are detectable in the circulation.

Objective—To test the hypothesis that circulating miRNAs would be associated with atrial fibrillation (AF).

Methods—Using a prospective study design powered to detect subtle differences in miRNAs, we quantified plasma expression of 86 miRNAs by high-throughput quantitative reverse-transcriptase

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polymerase chain reaction (qRT-PCR) in 112 participants with AF and 99 without AF. To examine parallels between cardiac and plasma miRNA profiles, we quantified atrial tissue and plasma miRNA expression using qRT-PCR in 31 participants undergoing surgery. We also explored the hypothesis that lower AF burden after ablation would be reflected in the circulating blood pool by examining change in plasma miRNAs after AF ablation (n=47).

Results—The mean age of the cohort was 59 years. 58% of participants were men. Plasma miRs-21 and 150 were 2-fold lower in participants with AF than in those without AF after adjustment ($p = 0.0006$). Plasma levels of miRs-21 and 150 were also lower in participants with paroxysmal AF than in those with persistent AF ($p < 0.05$). Expression of miR-21, but not miR-150, was lower in atrial tissue from patients with AF compared to no AF ($p < 0.05$). Plasma levels of miRs-21 and 150 increased 3-fold after AF ablation ($p = 0.0006$).

Conclusions—Cardiac miRs-21 and 150 are known to regulate genes implicated in atrial remodeling. Our findings show associations between plasma miRs-21 and 150 and AF, suggesting that circulating miRNAs provide insights into cardiac gene regulation.

Keywords

atrial fibrillation; epidemiology; circulation; microRNA; risk factor

Abbreviations

microRNA (miRNA); atrial fibrillation (AF)

Introduction

Atrial fibrillation (AF) is an important cardiovascular health problem with major adverse health consequences.¹ The prevalence of AF is 5.2 million in the US today and is predicted to rise to 12.1 million by 2030.² Clinical risk scores show modest predictive ability at identifying individuals at risk for AF and few robust AF biomarkers exist.³

MicroRNAs (miRNAs) are a class of RNA species with gene regulatory functions. Tissue levels of miRNAs have been associated with processes underlying cardiovascular diseases,^{4–6} including cardiac electrical remodeling (miR-328),^{7–10} and structural remodeling (miR-21).^{11–13} miRNAs can be exported by cardiomyocytes¹⁴ and fibroblasts and are present in the circulation,^{15,16} but little is known about the source and significance of circulating miRNAs, particularly as it relates to arrhythmias.¹⁷

Despite reports implicating miRNAs as determinants of susceptibility to AF under experimental conditions,^{18–20} few studies have examined the relations between circulating miRNAs and AF in humans.^{9,19} In a previous study, we showed that whole blood levels of miR-328, a miRNA associated with L-type Ca^{2+} channel density and AF susceptibility,²¹ was associated with prevalent AF.^{9,22,23} We hypothesized that plasma miRNAs would provide a clear window into cardiac gene regulation in AF and after ablation. We sought to explore these hypotheses by profiling expression of 86 miRNAs in plasma and atrial tissue in a prospectively recruited cohort.

Methods

Study Sample

The miRhythm Study is an ongoing, prospective study of AF and its gene regulatory mechanisms. Study participants include individuals with and without AF who presented to the University of Massachusetts Medical Center (UMMC) for evaluation or treatment between 4/2011 and 1/2014. To date, investigators have enrolled 453 individuals presenting to the UMMC, including 227 presenting for an ablation procedure, 114 presenting for surgery, and 112 presenting for cardiovascular evaluation. For this investigation, the sample size is 211. For each of the miRNAs, the study required $n=92$ to have 80% power to detect a 0.47 cycle threshold difference in miRNA expression between groups ($\alpha=0.00026$).

Individuals with a history of AF hospitalized at the UMMC were defined as having AF.²³ A history of AF was defined on the basis of a clinical history of AF documented in the UMMC AF Treatment Registry and/or hospital electronic records. When possible, 12-lead ECGs were reviewed to validate the diagnosis of AF. The reference group comprised individuals presenting for evaluation of chest discomfort or elective cardiac surgery at the UMMC, who were in normal sinus rhythm, had no evidence of an acute coronary syndrome or heart failure, and no history of AF.

Baseline venous blood samples were obtained within 3 hours of hospital presentation (K₂-ethylenediaminetetraacetic acid tubes) in all participants and were stored using methods proven to maintain miRNA stability.⁹ Participants with AF undergoing ablation were contacted at a routine 1-month follow-up visit and venous blood was obtained and stored using the same methods. All participants consented on hospital admission to transcriptomic research and had plasma miRNA expression quantified. Study protocols were approved by the UMMC Review Board (IRB 14875,14586).

Data abstraction

All miRhythm study participants underwent a clinically indicated history, examination, and laboratory evaluation as part of their evaluation. Information about each participant was abstracted from the UMMC's electronic medical record system, including information about patients' age, sex, race, comorbidities (e.g., myocardial infarction), as well as laboratory characteristics and treatment.

Selection of miRNAs

The 19 most abundant miRNAs in the heart account for more than 90% of all cardiac miRNAs.²⁵ Several prior investigations in dogs, mice and humans have identified several circulating and tissue miRNAs associated with atrial electrical remodeling, structural remodeling, or AF.^{15,16} For hypothesis testing and to minimize multiple testing concerns, we focused on 86 miRNAs expressed in the heart or associated with processes implicated in the pathogenesis of atrial remodeling or AF (www.miRTarBase.org, Supplemental Table 1).²⁶

Atrial Tissue Sampling

Atrial tissue and plasma samples were obtained from a subgroup of patients (n=31) who underwent cardiac surgery. Plasma from all miRhythm study participants undergoing cardiac surgery was included in the primary study analysis. Right atrial tissue was obtained in all but one case. Tissue was immediately flash frozen, labeled, and stored at -80°C until miRNA quantification.

MiRNA expression profiling

We isolated RNA and profiled 86 candidate miRNAs and 3 negative controls (SNORD61; SNORD95, RNU6-2 RNA), a Reverse Transcription Control, and a PCR Positive Control in miRhythm participants using Qiagen chemistry-based assays from tissue or venous blood samples. The BioMark System used to quantify miRNA levels has been validated extensively²⁷ and detects single miRNA copies at 26–27 Ct. Standard methods for cDNA conversion, pre-amplification, and qRT-PCR with Qiagen miScript Assays were used (Supplemental Tables 2–7, Supplemental Methods). Since circulating miRNA levels can change within hours after organ injury,²⁸ we assessed miRNA levels within 3 hours of hospital presentation in participants with AF as well as in hospital controls.

Statistical analyses

For dichotomous variables, descriptive statistics are reported as number (%), whereas for continuous variables, we report mean $\pm$ SD. As has been recommended in prior studies and to account for potential expression bias, we performed global mean normalization prior to analyses.²⁹ We used logistic regression to examine associations between plasma miRNAs and AF. MiRNA was considered the exposure and AF was used as the dependent variable. We adjusted for known factors associated with AF,³ including age, sex, smoking, diabetes mellitus, hypertension, heart failure, and myocardial infarction. We did not adjust for additional covariates to avoid over-fitting. Due to the high degree of co-linearity between antiarrhythmic and anticoagulant drug use and AF status, we did not adjust for medication use. Due to their associations with atrial remodeling and/or AF, we examined crude associations between miRNA expression and commonly prescribed medications, C-reactive protein levels, and left atrial volume index. Based on Bonferroni corrections for multiple testing, we used a two-sided p value cutoff of 0.0006 (0.05/86 miRNAs) to define the level of statistical significance for the primary analysis and used a cutoff of 2-fold change to identify highly variable miRNAs associated with AF.

To explore whether or not key cardiac miRNAs had a dose-response relationship with duration and intensity of AF, we also compared expression of miR-21 and miR-150 in patients with paroxysmal AF (n=73) vs. persistent AF (n=37). To examine changes in miRNAs between baseline and 1-month postablation, we used logistic regression models adjusting for age, sex, and time-to-sample-acquisition and a two-sided p value cutoff of 0.0006 to define statistical significance. Due to the limited follow-up period and loss to follow-up, we were under-powered to examine long-term freedom from AF after ablation in relation to miRNA expression.

We also compared atrial tissue miRNA expression in surgical patients with AF to those without AF using logistic regression models adjusting for age and sex. To better understand the potential usefulness of miRNAs associated with AF as predictors of incident AF, we also examined associations between plasma miR-21 and 150 levels and CHA2DS2-VASc risk score³⁰ in participants without AF.

Results

The characteristics of the miRhythm participants who comprised our study sample are shown in Table 1. The clinical and surgical characteristics of the subsample of miRhythm participants who donated atrial tissue and plasma are shown in Table 2.

The overall sample included middle age and older adults with a modest burden of cardiovascular risk factors. Participants with AF were more likely to be men and have higher body mass as well as echocardiographic left atrial volume indices. Study participants with AF were less likely to have a history of myocardial infarction and diabetes mellitus, but were more likely to have hypertension and heart failure than participants without AF. Despite these differences, overall CHA2DS2-VASc risk scores were similar between participants with AF and those without AF. Among participants with AF (n=112), the majority had paroxysmal AF (65%), with the remainder having persistent (29%) or long-standing persistent AF (5%).

A list of the 86 plasma miRNAs with potential mechanistic associations with cardiac remodeling or AF that were quantified in all miRhythm participants and their cycle threshold (Ct) values are shown among participants with and without AF in Table 3. After adjustment for age, sex, hypertension, smoking, diabetes mellitus, heart failure, myocardial infarction, and smoking, 21 plasma miRNAs were associated with AF (Supplemental Table 8). After multivariable adjustment for the same factors and using the same cutoff for statistical significance, 33 were up or down-regulated after AF ablation (Supplemental Table 9).

Plasma levels of two miRNAs known to influence atrial fibrosis and pro-fibrillatory potential under experimental conditions, miRs-21 and 150,¹¹ were 2-fold lower among patients with AF as compared to those without AF (Figure 1). Notably, expression of miR-21 (−6.37 vs. −5.54, $p=0.03$) and miR-150 (−3.81 vs. −3.26, $p=0.04$) were lower in patients with persistent or longstanding persistent AF than in those with paroxysmal AF, even after adjustment for age and sex (Figure 2). All of the plasma miRNAs that were examined in our study were detectable in atrial tissue, but only two of the plasma miRNAs associated with AF, miRs-21 and 411, met the predefined cutoff for statistical significance ($p < 0.05$) when levels of miRNAs in atrial tissue were compared between 19 participants with AF and 12 participants without preoperative AF (Figure 1). Levels of miR-21 and 150 increased 3-fold when pre-procedure plasma miRNA expression was compared to plasma miRNA expression 1 month after AF ablation (Figure 1).

Among participants with no AF, we also noted an inverse correlation between plasma levels of miRs-21 and 150 and CHA2DS2-VASc score, but these associations did not remain

statistically significant after adjustment for age and sex. None of the top AF-related miRNAs were associated with commonly prescribed medications, including beta-blockers, aspirin, statin drugs, or warfarin (Supplemental Table 10, no participants were on novel anticoagulants). We observed that miR-155 and miR-206 were associated with C-reactive protein in models adjusting for age and sex (Supplemental Table 11). No miRNAs were associated with left atrial volume index (Supplemental Table 11).

Discussion

In our prospective study of 211 participants (Table 1) powered to detect subtle differences in plasma miRNA expression, we observed that miRs-21 and 150 were differentially expressed in the plasma of patients with pre-existing AF and that associations persisted after adjustment for factors known to influence circulating miRNA profiles and susceptibility to AF. We also observed that miR-21 expression was lower in atrial tissue isolated from participants with AF and that miRs-21 and 150 increased after AF ablation (Figure 1).

Although few prior studies have described the circulating transcriptomic profile of patients with AF,^{9,19} circulating miRNAs have been associated with other forms of heart disease, including heart failure and coronary artery disease.³¹ miRNAs can be exported by cardiomyocytes and fibroblasts, for example in the context of a myocardial infarction,³¹ and are stable in the circulation. Mouse and dog models have shown that manipulation of atrial miRNAs (e.g., through the use of miR-transgenic animals or antagomiRs), including miR-21, alters cardiac gene expression profiles, can contribute to pathological atrial remodeling, and enhance AF vulnerability.^{11, 21} Despite strong signals that miRNAs provide insights into different forms of cardiovascular disease, our study is the first to examine plasma expression of key cardiac miRNAs in relation to AF using contemporary high-throughput PCR technologies, to compare plasma with atrial miRNA profiles associated with AF, and to examine changes over time in plasma miRNA expression after AF ablation.

Catheter ablation can promote “reverse” remodeling,³² a phenomenon characterized by structural and electrophysiological changes, and this has been linked to freedom from AF. In light of recent data showing that circulating miRNAs are released after an acute myocardial infarction and ventricular septal ablation,³³ we hypothesized that plasma miRNA associated with AF would show dynamic regulation after AF ablation and provide insights into gene regulatory pathways important to atrial remodeling. Although within individual variability of circulating miRNAs is not well established, several chronic disease states are associated with persistent abnormalities in certain plasma miRNAs, including heart failure, validating our experimental design. Unfortunately, we were under-powered to examine long-term freedom from AF after ablation in relation to miRNA expression.

In contrast to our prior study associating whole-blood miR-328 levels with prevalent AF, in the present investigation we did not observe an association between plasma miR-328 expression and AF. Since most miRNAs isolated from whole blood preparations are intracellular in origin, we hypothesize that miR-328 expression in circulating cells, as opposed to plasma, may differ between participants with and without AF, thus explaining

the distinct study findings. This hypothesis is supported by prior work showing that miR-328 is platelet enriched.²⁴

Two particular miRNAs associated with AF warrant further discussion on the basis of our findings and in light of prior literature associating them with AF or atrial remodeling. Levels of the first of these miRNAs, miR-150, were 2.3-fold lower in participants with AF compared to controls and levels increased over 3-fold after catheter ablation (Figure 1). MiRNA-150 regulates cellular proliferation, cardiac fibrosis, and has previously been associated with myocardial infarction and heart failure (Supplemental Table 1).²² MiRNA-150 levels were associated with AF in the only other published study to examine the associations between AF and plasma miRNAs.¹⁹ In this investigation, 4 candidate miRNAs were identified and profiled in a total of 90 participants. Despite the smaller sample and fewer miRNAs profiled, the authors similarly observed that plasma miR-150 levels were lower in participants AF as compared to healthy controls without AF (p=0.001). Our findings validate these results in a much larger, better-characterized sample of patients with AF and using contemporary PCR-based methods. We also demonstrate a dose-response relationship between miR-150 and AF intensity and duration by showing that miR-150 levels are lower in patients with persistent AF compared to paroxysmal AF and show that miR-150 levels in atrial tissue do not relate to AF status, suggesting that plasma miR-150 levels in AF may not relate to cardiac miR-150 expression. Last, we significantly extend prior work by showing that miR-150 levels show dynamic change after catheter-ablation, perhaps reflecting reverse remodeling.¹⁹

Participants with AF had 2.1-fold lower plasma levels of miR-21 compared to controls (Figure 1), miR-21 levels were 2-fold lower in atrial tissue, and miR-21 expression increased by 3.4-fold after catheter-ablation. As with miR-150, miR-21 levels were lower in patients with persistent AF as compared with paroxysmal AF (Figure 2), suggesting that intensity and duration of AF influences plasma miR-21 expression. Most miR-21 gene targets are tumor suppressor genes,³⁴ but miR-21 also has important links to cardiac remodeling and disease and regulates *collagen-1*, *collagen-3*, and *TGF- β* .^{11, 35} In a murine model of myocardial infarction, levels of miR-21 were lower in infarcted myocardium and over-expression of miR-21 had a protective effect against myocyte damage and cellular apoptosis.¹⁵ However, another recent study showed higher miR-21 expression in left atrial tissue from patients with permanent AF (n=5) undergoing mitral valve surgery compared to controls, pointing more towards a pro-fibrotic effect of higher cardiac miR-21, perhaps via inhibition of Sprouty 1.²⁰ A pro-fibrillatory effect of miR-21 is also supported by the fact that miR-21 antagonism suppressed atrial fibrosis and AF vulnerability in a murine heart failure model.¹¹ We hypothesize that differential miR-21 expression in right vs. left atria may explain why we observed lower, as opposed to higher, levels of miR-21 in AF, since we analyzed primarily right atrial tissue, whereas Adam et al. analyzed miR-21 expression in the left atrial appendage.²⁰ In sum, our data adds to an emerging literature suggesting that miR-21 is a key transcriptional regulator of atrial structural remodeling and AF. Our findings build significantly on prior work by showing that not only cardiac but also circulating miR-21 relates to AF status and that miR-21 expression correlates with severity of AF (paroxysmal vs. persistent).

Study Strengths and Limitations

The strengths of the present investigation include the large number of miRNAs quantified using validated high-throughput qRT-PCR methods, the relatively large number of participants with intensive AF characterization and clinical phenotyping, the comparison of atrial tissue and plasma miRNA profiles associated with AF, and the description of change in miRNAs after AF ablation. Our study has several limitations that should be considered. In an effort to focus on miRNAs that might serve as clinical biomarkers, our study design focused on highly variant miRNAs. Nevertheless, small changes in miRNA expression may have relevance in pathologic disease states. We cannot exclude residual confounding or the possibility that differences between referents and individuals with AF contributed to our findings. Our cohort was comprised largely of white individuals, thereby limiting generalizability of our primary findings to other racial and ethnic groups.

Conclusion

In summary, we observed strong associations between plasma levels of miRs-21 and 150, two miRNAs expressed in cardiac tissue and linked to atrial remodeling, with AF. Our findings support the hypothesis that circulating miRNAs provide key insights into cardiac gene regulation and implicate miRs-21 and 150 as markers of susceptibility to AF.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Clinical Perspectives

AF is highly prevalent and the burden of AF is increasing. New avenues are needed to identify cellular and developmental pathways for AF that incorporate transcriptomic data and provide a mechanism-based framework for identifying novel AF biomarkers. To accomplish this end, we used a novel transcriptional regulator, miRNA, to assess regulatory mechanisms underlying AF. We investigated the associations between circulating levels of cardiac-specific or enriched miRNAs with AF using a high-throughput polymerase chain reaction technology. We found that plasma levels of miRs-21 and 150, two miRNAs expressed in atrial tissue and linked to atrial remodeling, relate to AF status and type (paroxysmal vs. persistent). Our findings support the hypothesis that circulating miRNAs may provide insights into cardiac gene regulation and be useful biomarkers of susceptibility to AF. Replication of our findings in other well-characterized, large samples with standardized miRNA assessment and contemporaneous ascertainment of echo traits will strengthen our findings and provide further justification for the use of circulating miRNAs as AF biomarkers.

Figure 1.

Plasma levels of miR-21 and 150 are lower in plasma of patients with AF compared to controls (A). Plasma levels of miR-21 and 150 increase after ablation (B). Tissue levels of miR-21 are lower in patients with AF compared to controls (C).

Figure 2.

Plasma levels of miR-21 and 150 are lower in patients with persistent as compared to paroxysmal AF.

Table 1

Characteristics of miRhythm Participants according to Atrial Fibrillation Status

Variable	No AF* (n=99)	AF (n=112)
Age, y	57 ± 13	60 ± 11
Men, n (%)	50 (51)	73 (65)
Race, white (%)	67 (68)	108 (96)
<i>Physiologic characteristics</i>		
Body mass index, kg/m ²	31 ± 8	33 ± 17
Systolic blood pressure, mm Hg	138 ± 21	125 ± 19
Diastolic blood pressure, mm Hg	79 ± 16	74 ± 12
Heart rate, bpm	76 ± 15.5	75 ± 22
<i>Medical History, n (%)</i>		
CHA2DS2VASc Risk Score	2.2 ± 1.7	2.4 ± 1.0
Current smoking	23 (23)	12 (11)
Myocardial infarction	27 (27)	23 (21)
Hypertension	64 (65)	84 (75)
Heart failure	4 (4)	20 (18)
Diabetes mellitus	33 (33)	24 (21)
<i>Laboratory characteristics</i>		
Creatinine, mg/dL	0.9 ± 0.3	1.0 ± 0.3
Left atrial volume index **, ml/m ²	35 ± 12	40.3 (12.1)
C-reactive protein ***, ng/dL	4.7 ± 3.5	4.5 ± 7.2
<i>Treatment characteristics, n (%)</i>		
Aspirin	36 (36)	58 (52)
Warfarin	0 (0)	58 (52)
ACEI or ARB ****	35 (35)	55 (49)
Statin drug	38 (38)	55 (49)
Beta-blocker	34 (34)	64 (57)

Data are presented as means ± standard deviation or number (percentage).

AF = atrial fibrillation. Values reported were measured at enrollment.

* No AF indicates absence of diagnosed AF at baseline

** Left atrial volume index available in 107 participants (88 with AF)

*** C-reactive protein available in 83 participants (76 with AF)

**** ACEI or ARB denotes angiotensin converting enzyme inhibitor or angiotensin receptor blocker use

Table 2

Characteristics of miRhythm Participants Included in the Atrial Tissue Sub-study According to AF Status

Variable	No Atrial Fibrillation (n=19)	Atrial Fibrillation (n=12)
Age, y	61 ± 11	68 ± 14
Men, n (%)	9 (80)	13 (68)
Race, white (%)	11 (92)	19 (100)
<i>Medical History, n (%)</i>		
Myocardial infarction	5 (42)	10 (53)
Hypertension	11 (92)	16 (84)
Heart failure	2 (17)	9 (47)
Diabetes mellitus	5 (42)	6 (32)
<i>Laboratory characteristics</i>		
Creatinine, mg/dL	1.2 ± 0.4	1.2 ± 0.5
<i>Surgical characteristics, n (%)</i>		
Coronary Artery Bypass Graft *	6 (50)	5 (26)
Aortic valve replacement	2 (17)	1 (5)
Mitral valve replacement	2 (17)	6 (32)
Multi-valve replacement	1 (8)	4 (21)
Other valve surgery **	1 (8)	3 (16)

Data are presented as means standard deviation or number (percentage).

AF = atrial fibrillation. Values reported were measured at enrollment.

* Includes 4 patients who underwent coronary artery bypass graft + valve replacement

** Includes aortic aneurysm repair and aortic valve replacement as well as tricuspid valve replacement.

Table 3

Complete list of miRNAs in plasma of participants in miRhythm study and mean expression.

	miRNA	Mean Expression (CT) AF (n=112)	Mean Expression (CT) No AF (n=99)
1	miR-1	23.15	21.58
2	miR-7-5p	20.27	19.21
3	let-7b-5p	15.2	14.31
4	let-7c-5p	16.81	15.92
5	let-7f-5p	18.52	17.17
6	let-7g-5p	17.79	16.75
7	miR-10b-5p	21.2	21.06
8	miR-19a-3p	15.44	14.22
9	miR-19a-5p	31.48	N/A
10	miR-20a-5p	15.17	13.57
11	miR-21-5p	15.09	14.19
12	miR-24-3p	17.53	16.91
13	miR-25-3p	14.75	13.65
14	miR-26a-5p	17.6	16.34
15	miR-29a-3p	17.96	17.13
16	miR-29b-3p	19.79	18.5
17	miR-30a-3p	25.22	22.32
18	miR-30a-5p	17.18	15.95
19	miR-30c-5p	20.12	19.39
20	miR-31-3p	30.86	23.07
21	miR-92a-3p	13.78	12.7
22	miR-99b-3p	23.48	18.56
23	miR-99b-5p	22.05	21.52
24	miR-100-3p	25.23	21.78
25	miR-100-5p	19.31	19.38
26	miR-106b-5p	16.21	14.62
27	miR-122-5p	16.09	15.86
28	miR-125a-5p	18.3	18.77
29	miR-125b-5p	18.65	18.45
30	miR-126-3p	15.85	15.15
31	miR-133a-3p	31.13	22.84
32	miR-133b	29.28	23.16
33	miR-141-3p	30.01	22.8
34	miR-144-3p	18.09	16.45
35	miR-146a-3p	29.1	23.08
36	miR-146a-5p	18.75	18.47

	miRNA	Mean Expression (CT) AF (n=112)	Mean Expression (CT) No AF (n=99)
37	miR-146b-5p	21.09	20.06
38	miR-148b-3p	19.58	18.73
39	miR-148b-5p	29.67	22.92
40	miR-150-5p	17.68	17
41	miR-155-5p	26.52	22.57
42	miR-182-5p	24.67	21.89
43	miR-184	31.02	23.81
44	miR-192-3p	31.81	22.62
45	miR-196b-5p	25.93	21.89
46	miR-199a-5p	23.2	21.94
47	miR-199b-5p	22.67	21.14
48	miR-206	24.73	22.08
49	miR-208a-3p	32.32	23.31
50	miR-208b-3p	31.41	22.89
51	miR-218-5p	26.68	22.71
52	miR-221-3p	17.63	16.75
53	miR-221-5p	30.06	22.43
54	miR-223-3p	15.05	14.34
55	miR-302a-3p	30.86	23.45
56	miR-302b-3p	31.43	22.93
57	miR-320a	17.78	16.71
58	miR-320b	17.47	14.36
59	miR-328-3p	15.01	11.7
60	miR-342-3p	19.03	18.36
61	miR-367-3p	31.67	23.47
62	miR-374a-5p	21.9	19.81
63	miR-375	21.38	20.54
64	miR-409-3p	25.22	22.47
65	miR-410-3p	20.91	18.16
66	miR-411-3p	32.19	22.7
67	miR-411-5p	21.73	21.36
68	miR-421	15.15	11.97
69	miR-451a	9.65	8.03
70	miR-483-5p	25.99	22.15
71	miR-491-3p	30.88	23.05
72	miR-499a-5p	25.87	22.32
73	miR-510-5p	31.03	23
74	miR-525-3p	32.08	N/A
75	miR-525-5p	25.15	22.08

	miRNA	Mean Expression (CT) AF (n=112)	Mean Expression (CT) No AF (n=99)
76	miR-539-5p	29.18	22.9
77	miR-576-3p	29.34	23.16
78	miR-576-5p	22.75	20.95
79	miR-589-3p	27.11	22.42
80	miR-589-5p	30.93	22.68
81	miR-590-3p	29.01	22.13
82	miR-590-5p	21.76	19.39
83	miR-598-3p	24.49	21.93
84	miR-601	25.99	22.34
85	miR-941	26.01	21.87
86	miR-922	33.1	23.8

CT = cycle threshold; AF = atrial fibrillation.