Catheter ablation restores decreased plasma miR-409-3p and miR-432 in atrial fibrillation patients

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Aims
Despite numerous studies identifying specific microRNA (miRNA) expression profiles associated with atrial fibrillation (AF), changes in plasma miRNA expression in pre- and post-operative AF patients who have received catheter ablation, remain poorly characterized. This study aimed to reveal disease-related biomarkers by detecting plasma miRNA expression in AF patients, and examining the levels of AF-specific miRNAs in patients after catheter ablation, in order to help gauge therapeutic effects and assess prognosis.

Methods and results
A total of 100 Han Chinese patients with AF who had received catheter ablation, and 100 healthy individuals, were sequentially recruited to the study. Atrial fibrillation-specific plasma miRNAs were detected by Solexa sequencing and quantitative reverse transcription polymerase chain reaction. The expression levels of AF-specific miRNAs were also investigated in 40 post-operative patients (24–48 h) and 20 patients followed up (58.52 ± 36.00 days) after catheter ablation, to explore changes in miRNA expression. The expressions of miR-409-3p and miR-432 in the plasma of AF patients were lower than healthy individuals. In binary logistic regression analyses, reduced miR-409-3p and miR-432 levels were independently associated with AF (95% confidence interval: 1.02–2.22 and 1.09–2.43, P = 0.040 and 0.018, respectively). The levels of miR-409-3p and miR-432 showed no significant difference between post-operative patients and healthy individuals (P = 0.411 and 0.681, respectively), or between followed-up patients and healthy individuals (P = 0.720 and 0.073, respectively).

Conclusion
We suggest that plasma miR-409-3p and miR-432 are potential markers of AF, and catheter ablation restores their decreased levels in AF patients.

Keywords
Plasma • MiRNA • Atrial fibrillation • Differential expression • Catheter ablation

Introduction
Atrial fibrillation (AF) is one of the commonest clinical arrhythmias, with increasing incidence. Atrial fibrillation affects more than 1% of the general population and up to 10% of individuals over 80. Atrial fibrillation can lead to stroke, heart failure, and even death, with a high rate of fatality and disability. Aside from its impact on health and survival, AF imposes an enormous economic burden. Radiofrequency catheter ablation has emerged as an important treatment option for patients with AF. The latest consensus provides a class 1, level of evidence A recommendation for catheter ablation of AF in patients with paroxysmal AF, who have not responded to treatment with at least one antiarrhythmic medication. The assessment of procedural AF termination after catheter ablation is still a subject of debate in the medical community.

MicroRNA (miRNA) is a small non-coding RNA molecule that plays an important role in regulating transcription in different cells during development, by modulating the expression of protein-coding genes. MicroRNAs have recently been identified in several types of body fluid, from both healthy individuals and patients with various types of diseases, and may have the potential to serve as novel non-invasive biomarkers. Circulating miRNAs are stable and abundant...
in body fluids, and undergo characteristic changes in disease states, and can therefore be used as non-traumatic biomarkers for the prediction, diagnosis, and prognosis of a disease. This study suggests that miRNAs play a critical role in regulating gene expression changes that contribute to AF, and constitute novel therapeutic targets in AF. Numerous studies have identified specific miRNA expression profiles associated with different histological features of AF, mainly in animal models, and in atrial tissue obtained from patients with valvular AF. For example, Xiao et al. reported the dysregulation of miR-1202 in mitral stenosis patients with AF. Nishi et al. demonstrated that up-regulation of miRNA-21 in human atrial tissue was related to atrial fibrosis. Dawson et al. reported that miR-29b expression was also reduced in the atria of dogs and chronic AF patients. Important progress has been made recently in the study of circulating miRNA in coronary heart diseases, heart failure, and other cardiovascular diseases. However, there are few published researches concerning plasma miRNA and AF.

It is accepted that the analysis of AF-specific miRNAs should be more informative with respect to the mechanism and diagnosis of AF, whereas the analysis of plasma miRNAs following catheter ablation might be more effective in predicting the progression and outcomes of treatment. However, few studies have compared the differential expressions of circulating miRNAs between the pre- and post-operative plasma of AF patients. Investigating the specificity of plasma miRNA in AF may provide a new target in the pathogenesis of the disease. Measuring the expression levels of AF-specific plasma miRNAs after catheter ablation could reveal its impact on miRNA, and contribute significantly towards gauging therapeutic effects, and assessing prognosis.

Methods

Study subjects

A total of 100 AF patients received radiofrequency catheter ablation and were recruited as the study group from December 2010 to September 2011 in our centre. One hundred gender- and age-matched healthy individuals without a history of AF were selected as the control group. Medical history of each AF patient was recorded, along with a physical examination, a thyroid function test, a chest X-ray, and a transoesophageal echocardiogram. The echocardiogram estimated the left ventricular ejection fraction, left atrial diameter, and left ventricular diastolic diameter. Exclusion criteria were as follows: aged below 18 years, structural heart disease, valvular disease, left atrial or left atrial appendage thrombosis, acute coronary syndrome within 6 months, hyperthyroidism, electrolyte disorders, major surgical procedure or trauma within 60 days, malignancy, acute or chronic inflammatory diseases, immune system diseases, serious liver or renal dysfunctions, cerebrovascular accident, or nervous system diseases.

This study was approved by the Medical Ethical Review Committee of Guangdong General Hospital. All patients who participated in the study provided written informed consent for genetic and blood biomarker analysis. All of the procedures were done in accordance with the Declaration of Helsinki and relevant policies in China.

Definition of types of atrial fibrillation

Paroxysmal AF is self-terminating within 7 days. Persistent AF is present when an AF episode lasts longer than 7 days, or requires termination by cardioversion, either with drugs or by direct current cardioversion. The decision to adopt a rhythm control strategy is taken once long-standing persistent AF has lasted for 1 year or more.

CHA$_2$DS$_2$-VASc score system

The risk factors for CHA$_2$DS$_2$-VASc score system include heart failure, hypertension, age ≥75 years (2 points), diabetes, stroke, or transient ischaemic attack (2 points), and vascular disease, age 65–74 years, and female (up to 9 points). We classified a CHA$_2$DS$_2$-VASc score ≥2 as ‘high-risk’ of developing stroke and thromboembolism.

Technological process

Both the study group and the control group were each randomly divided into three subgroups of 30, 30, and 40 individuals before catheter ablation.

The study included three steps. First, screening step: the plasma from the first subgroup (study—control: 30–30) was pooled, and pooled plasma miRNA levels were detected by a Solexa sequencing method. Second, technique validation step: differentially expressed plasma miRNAs were verified using poly(A)-tail quantitative reverse transcription polymerase chain reaction (qRT-PCR) in the first pair of pooled samples and the second pair of pooled samples from the second subgroup (study—control: 30–30). Third, study population validation step: differentially expressed plasma miRNAs detected by different methods and different pools of plasma, were further verified in individuals from the third subgroup (study—control: 40–40) by stem-loop qRT-PCR.

Dynamic changes in AF-specific plasma miRNAs were also investigated in individuals from the third subgroup: pre-operation, post-operation, and at 1–3 months’ follow-up. Every patient underwent radiofrequency catheter ablation, and the blood samples of 40 AF patients in the third subgroup were collected 24–48 h post-operation. Twenty patients in the third subgroup were followed up for 1–3 months.

Finally, the relationship between AF-specific plasma miRNA levels and the CHA$_2$DS$_2$-VASc score of stroke for AF patients was assessed. A technological process flow chart is shown in Figure 1.
Patients management

Antiarrhythmic drugs were discontinued at least 3 days before ablation. Oral anticoagulation was stopped 3 days before the intervention and replaced by subcutaneous low-molecularweight heparin.

Antiarrhythmic agents were initiated during the first 24 h after ablation. Each patients received amiodarone and warfarin after ablation in our center. The daily dose of amiodarone was 200–600 mg, according to the rhythm of the patients. Warfarin was administered and adjusted locally to an international normalized ratio of 2.0–3.0. During follow-up, patients had their clinical symptoms recorded, a physical examination, 24 h Holter, and their blood samples collected. Each patient, in the case of any palpitations, was instructed to have
Plasma collection

All blood samples were collected and anti-coagulated with ethylenediamine tetraacetic acid dipotassium salt in the morning. The blood samples of AF patients in the third subgroup were collected pre-operation and 24–48 h post-operation. The blood samples of 20 follow-up patients were collected 1–3 months after catheter ablation. Plasma was isolated within one hour by centrifugation at 2000 × g for 10 min at 4°C, and then stored at −80°C until further analysis.

MicroRNA extraction

Total RNA, which contained small RNA, was extracted from plasma using an miRNA extraction kit (BioTeke) according to the manufacturer’s instructions. MicroRNA was collected by further centrifugation and stored in RNase-free water at −80°C.

Solexa sequencing and quantitative reverse transcription polymerase chain reaction

Solexa sequencing was carried out by the BGI Company to analyse plasma miRNA expression from the first mixed pool. Small RNA digitalization analysis was performed based on HiSeq high-throughput sequencing by synthesis. The sequencing result was analysed to screen for plasma miRNAs with differences in expression. We selected miRNAs that fulfilled the following two criteria: at least 30 copies in either group and showing at least a two-fold difference in expression between the two pools.

Target plasma miRNAs were determined by qRT-PCR. Two micrograms of total RNA was reverse transcribed using M-MLV reverse transcriptase (Invitrogen) in accordance with the manufacturer’s protocol. In short, the 10-μL reactions were incubated for 1 h at 42°C, 1 min at 85°C, and then stored at −20°C.

Quantitative reverse transcription polymerase chain reaction was performed on the MJ Opticon II Quantitative PCR System (MJ Research, Waltham, MA, USA). Reactions (10 μL) contained 2 μL of cDNA, 0.5 μM of each forward and reverse primer, and Maxima SYBR Green/Fluorescein qPCR Master Mix. The reactions were incubated at 95°C for 5 min, followed by 40 cycles of 95°C for 15 s and 58°C for 45 s. Triplicate samples, validated endogenous controls, and inter-assay controls, were used throughout. Hsa-miR-16 18 was used as an internal parameter and the relative expression level of each miRNA was calculated from the equation 2^(-ΔΔCt), where ΔΔCt = mean Ct miRNA—mean Ct miR16. The sequences of primers used in the qRT-PCR reaction are listed in Table 1.

Statistical analysis

Demographic and clinical characteristics were summarized using counts (%) for categorical variables and mean ± standard deviation if data were normally distributed, or the median (inter-quartile range, Q1–Q3) for continuous variables. The normality of the distribution of continuous variables was assessed by the Kolmogorov–Smirnov test, and the homogeneity of variances were assessed by the Levene test. Group comparisons for the levels of miRNAs were performed with two independent samples by the Wilcoxon rank sum test. The relationships between plasma miRNA level and age, gender, hypertension, and the presence of AF, were assessed by Binary logistic regression analyses, with the presence of AF as the dependent variable. The miRNA levels in pre-operative, post-operative, and follow-up samples, were compared with each other by randomized block design, the Friedman M-test, because these levels were abnormally distributed. Results
were considered to be statistically significant if $P < 0.05$. Data analyses were performed with SPSS 17.0 (SPSS Inc.).

Results

Baseline characteristics

The characteristics of the study group and control group are given in Table 2. The course of AF was from 1 month to 20 years (mean $4.0 \pm 5.3$ years) in the study group. Our study included 88 patients with paroxysmal AF, 8 patients with persistent AF, and 4 patients with long-standing persistent AF.

Twenty AF patients in the third subgroup after catheter ablation were followed up for 58.52 $\pm$ 36.00 days. Among these, 15 patients were in sinus rhythm and had accidental atrial premature beats, 5 had frequent atrial premature beats and paroxysmal atrial tachycardia, and none had AF.

MicroRNA sequencing analysis

A total of 389 and 517 miRNAs were detected in the study group and the control group, respectively, by Solexa sequencing. According to the screening criteria, 5 miRNAs (miR-454, miR-374a, miR-9, miR-152, and miR-664) were found to be up-regulated in the study group, while 11 miRNAs (miR-874, miR-486-5p, miR-328, miR-338-5p, miR-766, miR-409-3p, miR-16-2*, miR-487b, miR-493, miR-432, and miR-4732-3p) were down-regulated.

Comparing quantitative reverse transcription polymerase chain reaction and sequencing results

The 16 candidate miRNAs were detected by qRT-PCR in the two pool pairs, revealing that miR-432, miR-409-3p, and miR-328 were down-regulated in the study group, while 11 miRNAs (miR-874, miR-486-5p, miR-328, miR-338-5p, miR-766, miR-409-3p, miR-16-2*, miR-487b, miR-493, miR-432, and miR-4732-3p) were down-regulated.

Verification of microRNA

The expression of miR-409-3p, miR-328, and miR-432 were detected in individuals of the third subgroup before catheter ablation. The values of miR-409-3p, miR-432, and miR-328 were showed in Table 3. The levels of miR-409-3p and miR-432 declined in the study group.

### Table 2 Characteristics of the study participants

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>AF ($n = 100$)</th>
<th>Control group ($n = 100$)</th>
<th>$P$ values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>53.9 $\pm$ 13.4</td>
<td>52.3 $\pm$ 11.0</td>
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</tr>
<tr>
<td>Female sex ($n$)</td>
<td>28</td>
<td>28</td>
<td>1.000</td>
</tr>
<tr>
<td>Hypertension ($n$)</td>
<td>28</td>
<td>28</td>
<td>1.000</td>
</tr>
<tr>
<td>Diabetes mellitus ($n$)</td>
<td>6</td>
<td>8</td>
<td>0.783</td>
</tr>
<tr>
<td>Coronary artery disease ($n$)</td>
<td>5</td>
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<tr>
<td>Hyperlipidemia ($n$)</td>
<td>12</td>
<td>16</td>
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<tr>
<td>Smoking ($n$)</td>
<td>26</td>
<td>29</td>
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</tr>
<tr>
<td>Alcohol drinking ($n$)</td>
<td>7</td>
<td>6</td>
<td>1.000</td>
</tr>
<tr>
<td>Medications in use at baseline ($n$)</td>
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<td></td>
</tr>
<tr>
<td>Antihypertensive drug</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACEI/ARB</td>
<td>16</td>
<td>11</td>
<td>0.408</td>
</tr>
<tr>
<td>Calcium ion antagonist</td>
<td>20</td>
<td>21</td>
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<tr>
<td>Beta-blocker</td>
<td>22</td>
<td>9</td>
<td>0.018</td>
</tr>
<tr>
<td>Lipid-lowering agents</td>
<td>6</td>
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<td>1.000</td>
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<td>Anticoagulant</td>
<td>38</td>
<td>3</td>
<td>&lt;0.001</td>
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<tr>
<td>Class I antiarrhythmic drugs</td>
<td>5</td>
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<td>&lt;0.001</td>
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<tr>
<td>Class II antiarrhythmic drugs</td>
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<td>&lt;0.001</td>
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<tr>
<td>Class III antiarrhythmic drugs</td>
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<td>&lt;0.001</td>
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<tr>
<td>Amiodarone</td>
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<td>&lt;0.001</td>
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<tr>
<td>Digoxin</td>
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<tr>
<td>NYHA ($n$)</td>
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</tr>
<tr>
<td>I</td>
<td>89</td>
<td>95</td>
<td>0.191</td>
</tr>
<tr>
<td>II</td>
<td>11</td>
<td>5</td>
<td>0.191</td>
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<tr>
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<td>0</td>
<td></td>
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<tr>
<td>Echocardiogram</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>LA (mm)</td>
<td>35.9 $\pm$ 5.1</td>
<td>N</td>
<td></td>
</tr>
<tr>
<td>LVDd (mm)</td>
<td>48.8 $\pm$ 2.3</td>
<td>N</td>
<td></td>
</tr>
<tr>
<td>LVEF (%)</td>
<td>65.5 $\pm$ 4.6</td>
<td>N</td>
<td></td>
</tr>
</tbody>
</table>

ACEI/ARB, angiotension-converting enzyme inhibitor/angiotensin-receptor blocker; NYHA, New York Heart Function Assessment; LVEF, left ventricular ejection fraction; LA, left atrial diameter; LVDd, left ventricular diastolic diameter.
Catheter ablation restores decreased plasma miR-409-3p and miR-432

Table 3 The values (median (inter-quartile range, Q1-Q3)) of miR-409-3P, miR-432, miR-328 in the control group (n = 40) and pre-operative (n = 40), post-operative (n = 40), and follow-up (n = 20) patients

<table>
<thead>
<tr>
<th></th>
<th>miR-409-3P</th>
<th>miR-432</th>
<th>miR-328</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group (n = 40)</td>
<td>0.97 (0.39–2.40)</td>
<td>0.94 (0.38–2.35)</td>
<td>1.06 (0.46–2.50)</td>
</tr>
<tr>
<td>Pre-operative (n = 40)</td>
<td>0.45 (0.21–0.99)</td>
<td>0.30 (0.12–0.85)</td>
<td>0.67 (0.16–2.00)</td>
</tr>
<tr>
<td>Post-operative (n = 40)</td>
<td>1.25 (0.51–3.64)</td>
<td>0.95 (0.30–2.13)</td>
<td>0.82 (0.17–2.40)</td>
</tr>
<tr>
<td>Follow-up (n = 20)</td>
<td>1.52 (0.42–6.10)</td>
<td>0.92 (0.44–2.86)</td>
<td>1.79 (0.76–5.25)</td>
</tr>
</tbody>
</table>

Figure 2 Expression levels of miR-409-3P and miR-432 in the control group (n = 40) and pre-operative (n = 40), post-operative (n = 40), and follow-up (n = 20) patients.

Discussion

Our study showed that there were differences in miRNA expression in the plasma of AF patients, with lower expression of miR-409-3P and miR-432. In the binary logistic regression analyses, reduced miR-409-3p and miR-432 levels were independently associated with AF, while age, gender, and hypertension were not associated with AF.

Comparison of plasma microRNA expression in pre-operative, post-operative (24–48 h), and follow-up samples

The miRNA levels of 40 post-operative patients (24–48 h) and 20 follow-up patients (58.52 ± 36.00 d) were compared with the control group. The relative expression level of miR-409-3p increased in the post-operative samples (the median and inter-quartile ranges of pre-operative vs. post-operative samples were 0.45:0.81 and 0.78:1.98, respectively), along with the level of miR-432 (median and inter-quartile ranges of pre-operative vs. post-operative of 0.30:0.95 and 0.73:1.83, respectively). The levels of miR-409-3p and miR-432 showed no significant difference (P = 0.411 and 0.681, respectively) between post-operative samples and the control group, no difference (P = 0.720 and 0.073, respectively) between follow-up samples and the control group (Figure 2).

The miRNA levels of 20 pre-operative, post-operative, and follow-up samples were compared with the Friedman M-test. The relative expression levels of miR-409-3p and miR-432 in pre-operative, post-operative, and follow-up samples were different (P = 0.013 and 0.004, respectively). The difference in miR-409-3p levels between pre-operative and post-operative samples, and between pre-operative and follow-up samples, was statistically significant (P = 0.007 and 0.002, respectively), but was no difference between post-operative and follow-up samples (P = 0.590). The difference in miR-432 expression levels between post-operative and post-operative samples, and between pre-operative and follow-up samples, was statistically significant (P = 0.006 and ≤0.001, respectively), but was not statistically significant between post-operative and follow-up samples (P = 0.848).

CHA₂DS₂-VASc score for grouping

The CHA₂DS₂-VASc score system was used to carry out stroke risk assessment for AF patients. The number of AF patients with a CHA₂DS₂-VASc score ≥2 was 16 (mean score: 2.38 ± 0.50), while 24 cases demonstrated a CHA₂DS₂-VASc score <2 (mean score: 0.50 ± 0.51). The levels of miR-409-3p and miR-432 declined in patients with a score ≥2, but without statistical significance (z = -0.645 and -0.645, P = 0.519 and 0.519, respectively) (Figure 3).
miR-432 expression with cardiopathy. The expression levels of miR-409-3P and miR-432 were significantly downregulated in AF patients. Reduced miR-409-3p and miR-432 levels were associated with AF, but not with age, gender, or hypertension. This study suggested that miR-409-3p and miR-432 may serve as biomarkers for AF. The lower levels of miR-409-3P and miRNA-432 expression observed in this study may also provide an experimental basis for, and new targets in, the pathogenesis of AF.

Radiofrequency catheter ablation is increasingly applied in paroxysmal AF. We showed that the levels of miR-409-3P and miRNA-432 increased in post-operative and follow-up samples, compared with pre-operative ones, and were no different from those in the control group, suggesting that they could be applied in the assessment of procedural AF termination after catheter ablation. Catheter ablation restored decreased miR-409-3p and miR-432 levels in AF patients.

An electrocardiogram is the only currently available method for assessing AF termination or recurrence after catheter ablation. However, this method is unsatisfactory, particularly for the early detection of recurrence and prediction of future recurrence. Moreover, whether the levels of miR-409-3P and miR-432 can be used to predict disease recurrence after catheter ablation remains in question. The latest consensus underlines that a blanking period of three months should be employed after ablation when reporting efficacy outcomes. Thus, early recurrences of AF, atrial flutter, atrial tachycardia within the first 3 months should not be classified as treatment failure. Because the follow-up period was short, and none of these patients had early recurrence; therefore, future analysis might extended follow-up period and focus on patients with operation failure or AF recurrence, to clearly define the relationship between miRNA expression and AF. Such analysis might determine whether miR-409-3P and miR-432 could be used as biomarkers for disease recurrence after catheter ablation, or be used to predict recurrence and disease progression.

The levels of miR-409-3p and miR-432 declined in patients with a CHA2DS2-VASc score ≥ 2, compared with those score < 2, but with no statistical significance. There was no relationship between miRNA expression and the risk of stroke, but the AF patients in our study did not have a history of stroke. Fort et al. reported that miR-409-3p could directly target the fibrinogen ββ mRNA 3′-untranslated region, leading to lower β-Fibrinogen gene mRNA levels, and thus reduced total fibrinogen protein production. An increase in fibrinogen levels can cause increased blood viscosity, platelet aggregation, thrombophilia, and proliferation of vascular endothelial and smooth muscle cells. The increase in fibrinogen levels plays an important role in platelet aggregation. In AF patients, blood flow congestion in the atrium and irregular atrial wall motion abnormalities can increase platelet aggregation, and is associated with a higher risk of thrombosis. We speculate that the lower expression of miR-409-3P observed in AF patients might increase fibrinogen levels, and subsequently increase the risk of thrombosis, but this needs to be confirmed by further experiments in patients with stroke.

Lu et al. analysed left atrial samples from dogs with AF and human atrial samples from AF patients with rheumatic heart disease, and found that the miR-328 level was elevated in these situations. However, the expression of miR-328 decreased in 40 AF patients without statistical significance in our study. The subjects of the Lu et al. study were AF patients with rheumatic heart disease, but the subjects in our study were patients with non-valvular AF. Luo et al. suggested that miR-664 was up-regulated in the atrial tissue of canine models of tachypacing-induced AF. Xiao et al. showed that miR-874 was down-regulated in the right atrial appendages of patients with mitral stenosis and AF. We also found that miR-664 was up-regulated and miR-874 down-regulated in AF patients by Solexa sequencing, but without statistically significant difference by qRT-PCR. With the exception of miR-664 and miR-874, the miRNA levels observed in our study were not consistent with previous studies. Shan et al. reported that over-expression of miR-133 and miR-590 resulted in the post-transcriptional repression of TGF-b1 and TGF-bRII, respectively, which reduced collagen production in cultured canine atrial fibroblasts. Girmatsion et al. showed that miR-1 levels were reduced in the left atrial of patients with persistent AF, leading to increased Ik1. The subjects in these studies were canine models or patients with valvular AF, most of which had a longer disease course or even persistent AF. The subjects in our study were patients with non-valvular AF, mainly paroxysmal AF (88 patients with paroxysmal AF), New York Heart Association class I/II, which were different to initial studies. This contradictory finding may be related to sample selection. Liu et al. demonstrated that plasma miR-150 levels in AF patients were substantially lower than those in healthy people, but in the logistic regression model they did not observe significant associations between paroxysmal AF and miRNA-150. The subject of our study was mainly paroxysmal AF, and miR-150 was not detected in the first pool-pair by sequencing, which was consistent with Liu et al. Different types of AF have distinct underlying mechanisms, and may be regulated by different miRNAs.

MicroRNAs are thought to play a critical role in regulating the expression of a variety of genes and signaling pathways, and constitute a
regulatory network. MicroRNA target gene prediction softwares, such as TargetScan, DIANA-miRPath, miRanda, miRDB, and pictar, were used comprehensively to predict the signal pathways and binding sites of target genes. With this software, we predicted that miR-409-3P works by interaction with the TGF-β signaling pathway, ECM receptors, gap junction channels, and the signaling pathway target genes, SMAD2, ITGB3, CD44, and PKG. MiR-432 relates to the TGF-β signaling pathway, the renin–angiotensin system, gap junction channels, the MAPK signaling pathway, and the signaling pathway target genes, ACE, CDKN2B, PKA, and PKA. Signaling pathway and target genes constitute a regulatory network involving in AF occurrence and maintenance.

**Limitations**

Our study is restricted by the small number of samples used in our analysis, which limits the ability to confirm the diagnostic power of miRNA signatures. Because some of the patients resided in other provinces, we therefore notified these patients to visit the hospitals nearby, in clinical practice. So their follow-up data, including clinical symptoms and 24 h Holter data, were collected by telephone or fax; however, their blood samples could not be collected. For the patients who would revisit our hospital, we notified the ablated patients to come back to follow-up at 3 months, but some of the patients came back during 3 months for some reasons, in clinical practice. So, there was a floating range of the follow-up time. Future trials need to be performed with cytological experiments in vitro to directly identify signaling pathway and target genes, and provide more detailed information at the molecular level, such as the identification of specific genes that are directly affected by miRNA modifications.

**Conclusions**

In summary, we have identified plasma miR-409-3P and miR-432 as potential markers of AF. The miRNA expression signature described in this study may contribute to the understanding of AF biology, and also provide potential targets for future clinical applications.

**Conflict of interest**: none declared.

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**References**


