ORIGINAL RESEARCH

Predictive Value of Serum microRNA-29b-3p in Recurrence of Atrial Fibrillation After Radiofrequency Catheter Ablation

Junwei Zhan[1], Chengfei Peng[2], Yuxin Liu[1], Zhanhua Bi[1], Guoxiu Lu[1], Shanhu Hao[1], Yanan Tong[1], Guoxu Zhang [1]

[1]Department of Nuclear Medicine, General Hospital of Northern Theater Command, Shenyang, 110016, People's Republic of China; [2]Cardiovascular Research Institute and Department of Cardiology, General Hospital of Northern Theater Command, Shenyang, 110016, People's Republic of China

Correspondence: Guoxu Zhang; Yanan Tong, Department of Nuclear Medicine, General Hospital of Northern Theater Command, No. 83, Wenhua Road, Shenyang, 110016, People's Republic of China, Email zhangguoxu_502@163.com; tongyn1119@foxmail.com

Objective: Atrial fibrillation (AF) is a common arrhythmia. This study explored serum miR-29b-3p expression in AF patients and its value in predicting AF recurrence after radiofrequency catheter ablation (RFCA).

Methods: Totally 100 AF patients who underwent RFCA were enrolled, with 100 individuals without AF as controls. Serum miR-29b-3p expression in participants was determined using RT-qPCR. The correlation between miR-29b-3p and atrial fibrosis markers (FGF-21/FGF-23) was assessed by Pearson analysis. The diagnostic efficacy of serum miR-29b-3p and FGF-21/FGF-23 in predicting AF recurrence after RFCA was analyzed by the receiver operating characteristic (ROC) curves. The Kaplan-Meier method was adopted to evaluate the effect of miR-29b-3p expression on the incidence of AF recurrence after RFCA. The independent risk factors for AF recurrence after RFCA were analyzed by logistic regression analysis.

Results: Serum miR-29b-3p was poorly expressed in AF patients. After RFCA, AF patients showed elevated serum miR-29b-3p expression. Serum miR-29b-3p expression in AF patients negatively correlated with serum FGF-21 and FGF-23 concentrations. The cut-off values of serum miR-29b-3p, FGF-21, and FGF-23 in identifying AF recurrence were 0.860 (sensitivity: 100.00%, specificity: 39.71%), 222.2 pg/mL (sensitivity: 96.88%, specificity: 32.35%) and 216.3 ng/mL (sensitivity: 53.13%, specificity: 70.59%), respectively. Patients with low miR-29b-3p expression had a significantly higher incidence of AF recurrence than patients with high miR-29b-3p expression. Serum miR-29b-3p expression was one of the independent risk factors for AF recurrence after RFCA.

Conclusion: Low miR-29b-3p expression in AF patients has certain predictive values and is one of the independent risk factors for AF recurrence after RFCA.

Keywords: miR-29b-3p, atrial fibrillation, radiofrequency catheter ablation, recurrence, fibrosis marker, logistic regression analysis

Introduction

Atrial fibrillation (AF) is the most common persistent tachyarrhythmia in adults that involves a large number of health system resources.[1,2] With the aging of the global population, the incidence of AF is also on the rise.[3] In most cases, AF is the consequence of rheumatic valvular heart disease, and AF patients will experience significant atrial remodeling, which is typically manifested as atrial fibrosis.[4] Atrial fibrosis is a complex multifactorial and patient-specific process that is also implicated in the occurrence and maintenance of AF.[5] Furthermore, fibrosis is associated with the development and recurrence of AF.[6] Radiofrequency catheter ablation (RFCA) and antiarrhythmic drugs are the two main therapeutic methods for AF and can effectively reduce clinical symptoms and help maintain normal sinus rhythm, and RFCA is superior to drug therapy in terms of AF recurrence rate.[7] Even though RFCA is extensively used to treat AF patients who are refractory to drug therapy, 25% to 40% of patients will have late recurrence after intervention, and existing clinical indicators are of limited predictive values.[8] Therefore, it is urgent to find a feasible biomarker to identify AF recurrence after RFCA.

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microRNAs (miRNAs) are a class of small RNAs that are very stable in biological fluids and are considered promising non-invasive biomarkers for many pathological processes.[9] Changes in extracellular miRNAs in the circulating blood have been detected in a large number of human pathologies, AF included.[10] Dysregulated miRNAs can facilitate AF prevalence by deregulating transcription factors, modulating atrial excitability, and evoking atrial arrhythmias.[11,12] Multiple miRNAs have the potential to cause arrhythmias and participate in different types of AF.[13] miR-29b, as a regulator of cell proliferation, differentiation, and apoptosis in a variety of cancers, plays an essential role in the regulation of extracellular matrix during cardiac pathological remodeling.[14] Studies have found that the miR-29 family plays an important role in myocardial fibrosis and inflammation by regulating related target genes.[15,16] Meanwhile, miR-29 is down-regulated in atrial tissues of AF rats, and overexpression of miR-29 alleviates atrial fibrosis in AF rats.[17] Moreover, microRNA-29b-3p (miR-29b-3p) can reduce the degree of atrial fibrosis and the expression levels of fibrosis markers and exhibits weak expression in the serum of AF patients.[18,19] Besides, miR-29b-3p expression is reduced in AF patients, and lncRNA 19 drives cardiac fibroblast proliferation and collagen production by inhibiting the miR-29b-3p-VEGFA-TGF-β axis.[20] However, the clinical significance and values of serum miR-29b-3p expression in predicting AF recurrence after RFCA have not been reported so far. This study was intended to investigate the expression of miR-29b-3p in the serum of AF patients and its predictive value for AF recurrence after RFCA.

Materials and methods
Ethics statement
This study was approved by the Academic Ethics Committee of General Hospital of Northern Theater Command. All patients were fully informed of the purpose of this study and signed informed consent before sampling. Our study complied with the Declaration of Helsinki.

Study subjects
This study prospectively enrolled 134 AF patients who were hospitalized in the General Hospital of Northern Theater Command and underwent RFCA intervention for the first time between September 2018 and August 2020 as the experimental group (AF group). After excluding 34 patients who dropped out of the experiment and were lost to follow-up, 100 patients were finally included, with an average age of (61.3 ± 6.1) years, 53 males and 47 females. AF patients were allocated to the recurrent group (N = 32) and non-recurrent group (N = 68) according to whether AF recurred within 12 months after RFCA intervention. A total of 100 individuals without AF during the same period were enrolled as controls, with an average age of (60.7 ± 6.1) years, 58 males and 42 females. The diagnosis of AF patients was made based on the diagnostic criteria of European Society of Cardiology (ESC) AF management guidelines issued in 2016.[21]

Inclusion and Exclusion Criteria
Inclusion criteria: aged 18–75 years old with non-valvular AF; treated with RFCA for the first time; without associated sinus rhythm and severe surgical complications; with complete electrocardiogram (ECG) and preoperative examination of disease-related indicators.

Exclusion criteria: < 18 years old; left ventricular ejection fraction (LVEF) < 50%; left atrial thrombus; severe valve stenosis or incomplete heart disease; severe hepatic and renal insufficiency or heart failure; history of coronary atherosclerotic heart disease; chronic cor pulmonale; autoimmune diseases; concomitant malignant tumor; acute infection or intolerance to surgical treatment; history of RFCA intervention.

RFCA
Cardiac RFCA was performed under the guidance of the cardiac three-dimensional mapping system. Transesophageal echocardiography was performed one day before intervention to exclude left atrial appendage thrombosis. All patients received radiofrequency circumferential pulmonary vein isolation (PVI). Other procedures (including linear ablation and fragmentation potential ablation) were dependent on the assessment made by the operator. Successful intervention was defined as efferent block in all pulmonary veins and afferent block in all left atria, which was reconfirmed 30 minutes after the last PVI.

Data Collection

We recorded the clinical data of all study populations at enrollment, including age, sex, body mass index (BMI), hypertension, diabetes, smoking history, drinking history, systolic blood pressure (SBP), and diastolic blood pressure (DBP). Left atrial mean diameter (LAD) was measured using echocardiography (LOGIQ E9, Hanfei Medical Instrument, Shanghai, China). Left atrial volume index (LAVI) and LVEF were calculated using the following formulas: LAVI = left atrial volume (LAV)/body surface area, LAV = double plane area − length, and body surface area = 0.0061 × height + 0.0128 × body mass − 0.1529; LVEF = (EDV-ES) × 100%/EDV. EDV = end-diastolic volume, ES = end-systolic volume.

Enzyme-Linked Immunosorbent Assay (ELISA)

First, 5 mL of peripheral venous blood was collected from all subjects on an empty stomach on the morning of the day before RFCA and the day of the first follow-up after RFCA[22] and placed in a vacuum blood collection tube without anticoagulant, followed by centrifugation at $1500 \times g$ and 4°C for 10 minutes. Then the upper serum was isolated and stored in the refrigerator at −80°C until determination within 1 week. The expression levels of fibroblast growth factor-21 (FGF-21; 1:4, JL19322-96T, Jianglai Biotech, Shanghai, China),[23] fibroblast growth factor-23 (FGF-23; 1:4, ab267652, Abcam, Cambridge, UK),[24] hyper-sensitive C-reactive protein (hs-CRP; TW14171, Tongwei Biotechnology, Shanghai, China), and N-terminal B-type natriuretic peptide precursor (NT-proBNP; JL13111-48T, Jianglai Biotech, Shanghai, China) in serum of all subjects were determined using Human ELISA kits. The samples were incubated with coating solution in ELISA plates at 37°C for 2 hours and then incubated overnight at 4°C. Then the samples were sealed overnight at 4°C with 10% fetal bovine serum, washed, and incubated with primary antibody at 37°C for 2 hours and with secondary antibody at the same temperature for 1 hour. After the chromogenic reaction, the termination solution was added to end the reaction, and the absorbance of each well at 450 nm was measured.

Treatment and Follow-Up

After discharge, patients receiving RFCA were followed up by telephone or outpatient visits for 12 months, once every 3 months, and the 12-lead ECG and 24-h dynamic ECG were performed once a week and once a month, respectively. Antiarrhythmic drugs and anticoagulants were administered during the first three months, followed by treatment based on AF recurrence. Patients with suspected recurrent arrhythmia symptoms such as palpitations, dizziness, or chest tightness during the follow-up period should be immediately treated in local hospitals and examined by the 12-lead ECG or 24-h dynamic ECG. AF recurrence was defined as atrial tachycardia, atrial flutter, and AF of any atrial arrhythmia duration ≥ 30s recorded on the 12-lead ECG or 24-h dynamic ECG during follow-up;[21] and patients who maintained sinus rhythm for 12 months after intervention were defined as having no recurrence of AF.

Reverse Transcription Quantitative Polymerase Chain Reaction (RT-qPCR)

The relative serum levels of miR-29b-3p were determined by RT-qPCR. Total RNA was extracted from the serum by TRIzol reagent (SBJ-L0055, SenBeiJia Biotech, Nanjing, China), and the concentration and purity of RNA were detected by a spectrophotometer (QuickDrop, Molecular Devices Corporation, Shanghai, China). cDNA was synthesized using a reverse transcription kit (TR102-01/02, Vazyme Biotech, Nanjing, China). U6 was used as an internal reference gene of miR-29b-3p, and primer sequences (Table 1) were obtained from Daixuan Biotech (Shanghai, China). Real-time fluorescence qPCR instrument (StepOnePlus, Sicaen Biotech, Beijing, China) was used for PCR amplification. Reaction conditions were as follows: 95°C for 30 seconds, 95°C for 10 seconds, and 60°C for 30 seconds, a total of 40 cycles. The relative expression level of miR-29b-3p standardized to that of the internal reference U6 was calculated by the $2^{-\Delta\Delta Ct}$ method.

Table 1 Primer Sequences of RT-qPCR

Gene	Forward 5'-3'	Reverse 5'-3'
miR-29b-3p	GCTCTAGATCAGTTACAGAAAGACCACGA	GCTCTAGATAGTGTCCATGCACGGACC
U6	CTCGCTTCGGCAGCACA	AACGCTTCACGAATTTGCGT

Statistical Analysis

SPSS21.0 statistical software (IBM Corp. Armonk, NY, USA) and GraphPad Prism 6.0 Software (GraphPad Software Inc., San Diego, CA, USA) were used for statistical analysis and plotting. The prior estimation of sample size was conducted using the Gpower software (Supplementary Figure 1). The Shapiro–Wilk was used to verify the normal distribution of data. Measurement data were expressed as mean ± standard error of mean (SEM) and analyzed by the *t*-test for data comparisons between groups. Fisher's exact test was used for comparative analysis of categorical variables. The receiver operating characteristic (ROC) curve was used to analyze the diagnostic value of miR-29b-3p in AF and its efficacy in predicting AF recurrence after RFCA. Pearson's coefficient was used to analyze the correlation between miR-29b-3p and the levels of fibrosis markers. The Kaplan-Meier curve was adopted to analyze the effect of miR-29b-3p on the incidence of AF recurrence after RFCA. Logistic multivariate regression analysis was used to evaluate the influencing factors of the recurrence of AF after RFCA in AF patients. Differences were considered statistically significant at $P < 0.05$.

Results

Comparison of General Clinical Baseline Data Between the Control Group and AF Group Before RFCA

There were no statistical differences in age, sex, BMI, hypertension, diabetes, smoking history, drinking history, SBP, DBP, LVEF, and hs-CRP between the AF group and control group (all $P > 0.05$), but statistical differences were noticed in the levels of LAD, LAVI, FGF-21, FGF-23, and NT-proBNP between groups (all $P < 0.05$) (Table 2).

miR-29b-3p Was Poorly Expressed in the Serum of AF Patients

The serum expression of miR-29b-3p in the AF group and control group was compared. The results manifested that the serum miR-29b-3p level was 1.0 ± 0.2 in the control group and 0.7 ± 0.2 in the AF group, which reflected that the level of miR-29b-3p in the AF group was lower than that in the control group ($P < 0.01$, Figure 1), indicating low expression of miR-29b-3p in the serum of AF patients.

Table 2 Clinical Baseline Characteristics of the Control Group and AF Group

Factors	Control (N=100)	AF (N=100)	P
Average age (years)	60.7 ± 6.1	61.3 ± 6.1	0.496
Sex (Male/female)	58/42	53/47	0.477
BMI (kg/m^2)	24.1 ± 2.6	23.8 ± 2.1	0.464
Hypertension (Yes/No)	37/63	48/52	0.116
Diabetes (Yes/No)	34/66	42/58	0.244
Smoking history (Yes/No)	54/46	51/49	0.671
Drinking history (Yes/No)	51/49	57/43	0.395
SBP (mmHg)	120.1 ± 10.2	123.2 ± 12.9	0.056
DBP (mmH)	75.2 ± 7.5	76.3±7.6	0.272
LVEF (%)	59.3 ± 5.3	58.5 ± 5.4	0.291
LAD (mm)	37.9 ± 3.2	39.8 ± 3.3	< 0.001
LAVI (mL/m2)	21.6 ± 4.7	37.1 ± 10.6	< 0.001
FGF-21 (pg/mL)	175.4 ± 18.2	250.5 ± 30.0	< 0.001
FGF-23 (ng/mL)	109.5 ± 10.1	208.2 ± 19.6	< 0.001
hs-CRP (mg/L)	4.2 ± 0.2	4.3 ± 0.2	0.072
NT-proBNP (pg/mL)	24.9 ± 5.4	105.5 ± 25.7	< 0.001

Notes: Measurement data were expressed as mean ± standard deviation or number of cases. The comparison between two groups was analyzed by *t*-test, and the classification variables were analyzed by Fisher's exact test. Differences were considered statistically significant at $P < 0.05$.
Abbreviations: BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; LVEF, left ventricular ejection fraction; LAD, left atrial mean diameter; LAVI, left atrium volume index; FGF-21, fibroblast growth factor-21; FGF-23, fibroblast growth factor-23; hs-CRP, hypersensitive C-reactive protein; NT-proBNP, N-terminal B-type natriuretic peptide precursor; AF, Atrial fibrillation.
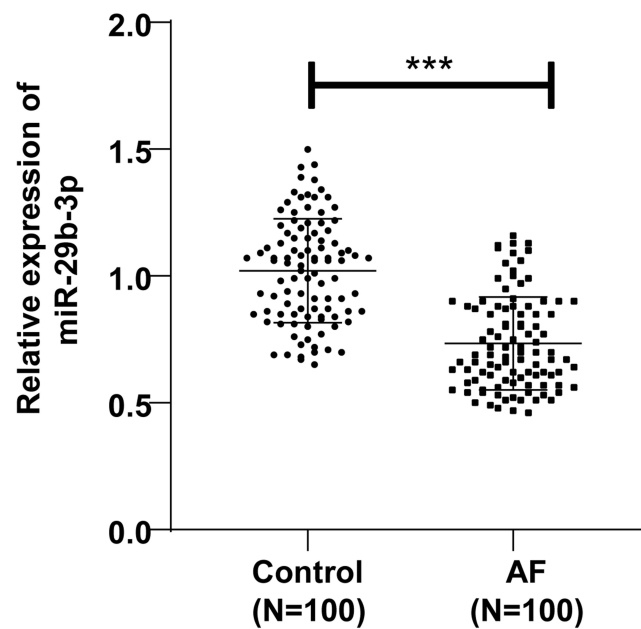
Figure 1 Expression levels of miR-29b-3p in the serum of AF patients. The serum expression of miR-29b-3p was determined by RT-qPCR. The *t*-test was used for data comparisons between two groups. ***P < 0.001.

Differential Expression of Serum miR-29b-3p in AF Patients Before and After RFCA

As a major modality to treat AF, RFCA can effectively ameliorate the clinical symptoms of patients.[25] RT-qPCR revealed that the expression levels of serum miR-29b-3p in AF patients before and after RFCA were 0.8 ± 0.2 and 0.7 ± 0.2, respectively, and it's evident that serum miR-29b-3p expression level was raised after RFCA, and the difference was of statistical significance (P < 0.01, Figure 2).

Correlation Between miR-29b-3p and Atrial Fibrosis Indicators

Atrial fibrosis is a marker of atrial remodeling in AF.[5] As members of the FGF superfamily, FGF-21 is positively correlated with atrial fibrosis in patients with rheumatic heart fibrillation, and FGF-23 is closely associated with the occurrence of cardiovascular diseases.[23,26,27] Moreover, FGF-21 and FGF-23 are up-regulated in the serum of AF patients and play an essential role in the prognosis of AF.[23,26] To further explore the relationship between miR-29b-3p and atrial fibrosis indicators, we analyzed the correlation of miR-29b-3p with FGF-21 and FGF-23 by the Pearson method, which revealed the miR-29b-3p serum levels in the AF group were negatively correlated with serum concentrations of FGF-21 (r = −0.797) and FGF-23 (r = −0.692) (all P < 0.001, Figure 3A and B).

Comparison of Clinical Baseline Data Between the Non-Recurrent Group and the Recurrent Group Before RFCA

AF patients were assigned to the non-recurrent AF group (N = 68) and recurrent AF group (N = 32) according to whether AF recurred within 12 months after RFCA. We compared the clinical indicators between the recurrent group and non-recurrent group and discovered that there was no significant difference in age, sex, BMI, hypertension, diabetes, smoking history, drinking history, and levels of LVEF, LAD, hs-CRP, and NT-proBNP between the recurrent group and non-recurrent group (all P > 0.05), while there were statistical differences in LAVI, FGF-21, and FGF-23 levels (all P < 0.05). The specific results are shown in Table 3.

Poor Expression of miR-29b-3p Predicted a High Risk of AF Recurrence After RFCA

To ascertain whether the expression level of miR-29b-3p is related to the recurrence of AF, we measured miR-29b-3p expression level in the serum of AF patients in the non-recurrent and recurrent groups by RT-qPCR. The results
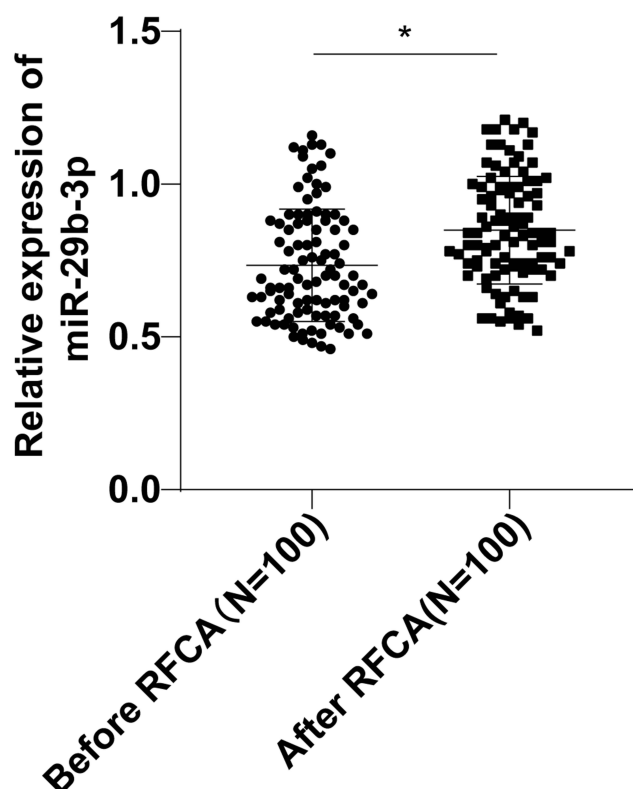
Figure 2 Differential expression of serum miR-29b-3p in AF patients before and after RFCA. The expression levels of serum miR-29b-3p in AF patients before and after RFCA were measured by RT-qPCR. Data comparisons between groups were analyzed by the *t*-test. *$P < 0.05$.
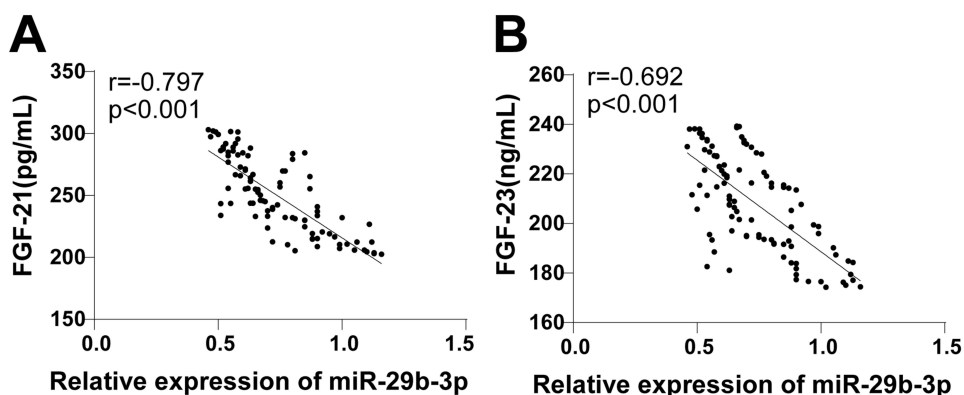

Figure 3 Correlation analysis between miR-29b-3p and markers of atrial fibrosis. The Pearson method was used to analyze the correlation between the expression level of miR-29b-3p and serum (**A**) FGF-21 and (**B**) FGF-23 levels in the AF group. (**A** and **B**) were analyzed by Pearson's coefficient.

uncovered that the relative level of miR-29b-3p in the serum of patients in the recurrent group was visibly lower than that of patients in the non-recurrent group ($P < 0.01$) (Figure 4A). We further plotted the ROC curve of serum miR-29b-3p for the evaluation of recurrence in AF patients after RFCA (Figure 4B) and found that the area under the curve (AUC) was 0.716, with a cut-off value of 0.860 (sensitivity: 100.00%, specificity: 39.71%). Briefly, the serum miR-29b-3p level < 0.860 can assist in predicting recurrence after RFCA in AF patients. What's more, the AUC of serum FGF-21 and FGF-23 for the prediction of AF recurrence was 0.637 (cut-off value: 222.2 pg/mL, sensitivity: 96.88%, specificity 32.35%) and 0.625 (cut-off value: 216.3 ng/mL, sensitivity: 53.13%, specificity: 70.59%) (Figure 4C and D).

Table 3 Comparison of Preoperative General Clinical Data Between Non-Recurrent and Recurrent AF Groups

Characteristics	Non-Recurrent Group (N=68)	Recurrent Group (N=32)	*P* value
Age (years)	61.4 ± 6.6	61.0 ± 5.2	0.762
Gender (Male/female)	38/30	15/17	0.399
BMI (kg/m^2)	23.8 ± 2.2	23.9 ± 2.2	0.711
Hypertension (Yes/No)	34/34	14/18	0.560
Diabetes (Yes/No)	27/41	15/17	0.498
Smoking history (Yes/No)	32/36	19/13	0.250
Drinking history (Yes/No)	37/31	20/12	0.446
LVEF (%)	58.4 ± 5.5	58.7 ± 5.4	0.800
LAD (mm)	39.8 ± 3.4	39.8 ± 3.1	0.939
LAVI (mL/m^2)	34.4 ± 10.6	42.9 ± 8.3	< 0.001
FGF-21 (pg/mL)	245.9 ± 31.0	260.2 ± 25.7	0.026
FGF-23 (ng/mL)	205.5 ± 19.5	214.1 ± 18.7	0.039
hs-CRP (mg/L)	4.3 ± 0.2	4.3 ± 0.3	0.668
NT-proBNP (pg/mL)	102.7 ± 26.2	111.3 ± 24.0	0.121

Notes: Measurement data were expressed as mean ± standard deviation or number of cases. The comparison between two groups was analyzed by *t*-test, and the classification variables were analyzed by Fisher's exact test. Differences were considered statistically significant at *P* < 0.05.
Abbreviations: BMI, body mass index; LVEF, left ventricular ejection fraction; LAD, left atrial mean diameter; LAVI, left atrium volume index; FGF-21, fibroblast growth factor-21; FGF-23, fibroblast growth factor-23; hs-CRP, hypersensitive C-reactive protein; NT-proBNP, N-terminal B-type natriuretic peptide precursor.

Additionally, AF patients were divided into the low expression group (miR-29b-3p < 0.691, N=50) and the high expression group (miR-29b-3p > 0.691, N=50) according to the median expression level of serum miR-29b-3p (0.691) in 100 AF patients, and the incidence of AF recurrence after RFCA was compared between the two groups. There were differences in the prognosis between the two groups (χ^2 = 3.869, *P* < 0.05), and the incidence of recurrence of AF after RFCA in the low expression group was 38.00%, higher than that in the high expression group (26.00%) (Table 4). In addition, Kaplan-Meier analysis showed that the curve shifted left in the low expression group (*P* < 0.05, Figure 4E), indicating that the cumulative incidence of AF recurrence after RFCA was higher in the low expression group during the same follow-up period. Taken together, the low expression of serum miR-29b-3p predicted a high risk of AF recurrence after RFCA.

Low Expression of miR-29b-3p Was One of the Independent Risk Factors for AF Recurrence After RFCA

To accurately evaluate the effects of miR-29b-3p on AF recurrence after RFCA, we analyzed the independent correlation between miR-29b-3p expression level and AF. With AF recurrence as the dependent variable, the independent variables (including LAVI, FGF-21, FGF-23, and NT-proBNP) (*P* ≤ 0.1) and miR-29b-3p were included in the binary multivariate logistics regression analysis model according to the analysis results in Table 3. The logistics regression model showed that after adjusting the indicators LAVI, low expression of miR-29b-3p resulted in an increased risk of AF after RFCA and was one of the independent risk factors for AF recurrence after RFCA [*P* = 0.027, odds ratios = 0.002, 95% confidence interval (CI): 0.000~0.494] (Table 5).

Discussion

AF is a multifactorial disease that normally occurs in response to underlying cardiac abnormalities and is supported by changes in the electrophysiological, anatomical, and structural properties, generally referred to as atrial remodeling.[28] Recently, miRNAs have been confirmed to play an essential role in the pathophysiology of AF by regulating the
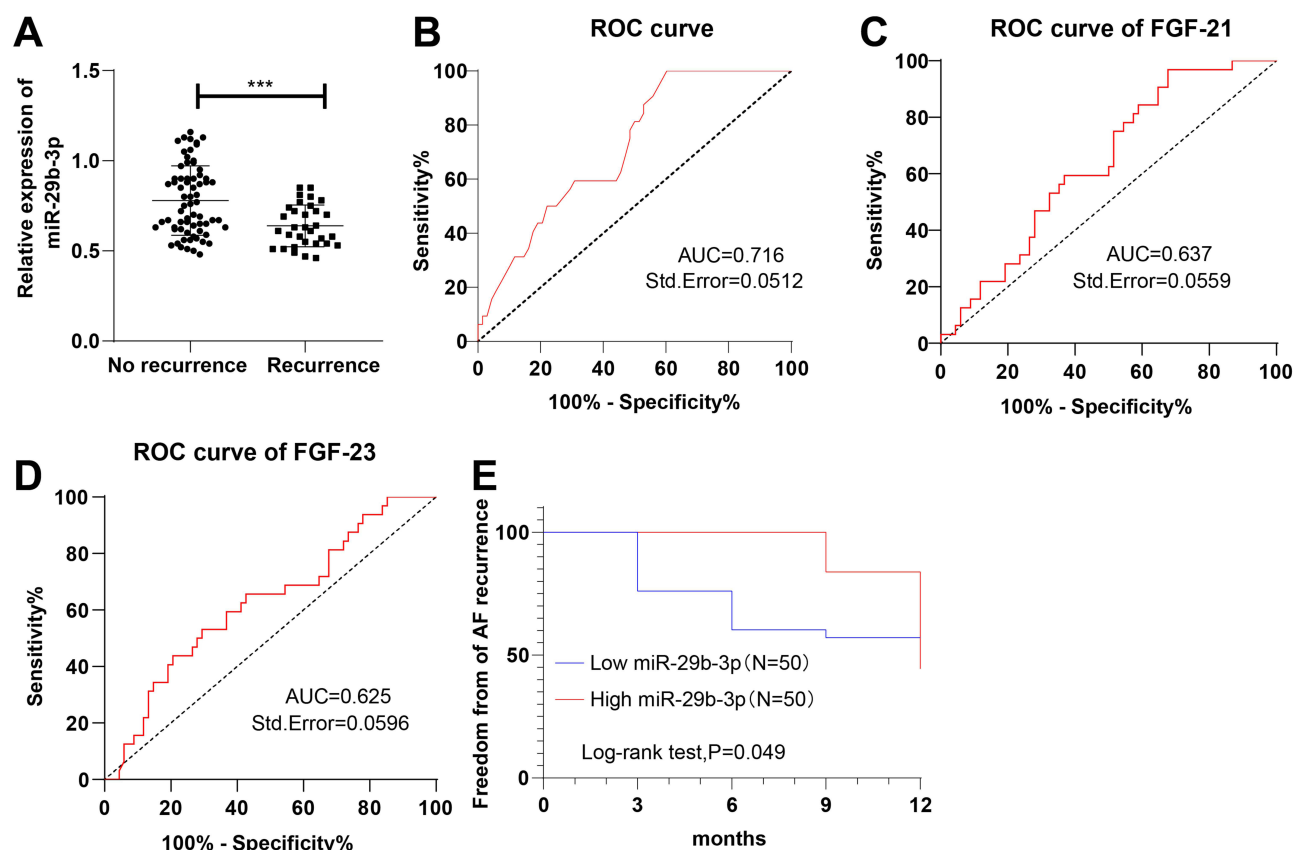
Figure 4 Low expression of serum miR-29b-3p predicted a high risk of recurrence of AF after RFCA. (**A**) RT-qPCR was used to determine the differential expression of serum miR-29b-3p between the AF non-recurrent and recurrent groups; (**B**–**E**) The ROC curves of serum miR-29b-3p, FGF-21, and FGF-23 levels for the identification of AF recurrence after RFCA; (**E**) Kaplan-Meier curve analysis. (**A**) was analyzed using the *t*-test. ***$P < 0.001$.

remodeling process.[29] This study demonstrated that the low expression of miR-29b-3p in AF patients has certain predictive values in AF recurrence after RFCA, and is an independent risk factor for the recurrence of AF after RFCA.

Mounting evidence indicates that miRNA expression level is correlated with the occurrence and severity of AF.[12] A previous study has shown that miR-29b is down-regulated in the plasma of AF patients.[30] Our study also showed the same expression trend of miR-29b-3p in the enrolled AF patients as the findings of our peers. RFCA may cure AF for some symptomatic patients.[31] Our results presented that serum miR-29b-3p expression was elevated in AF patients after RFCA. Atrial fibrosis is a marker of atrial remodeling and is involved in the occurrence and maintenance of AF.[5] Meanwhile, miRNAs play an important role in the pathophysiology of fibrosis development at the ventricular and atrial levels.[12,32] In particular, miR-29b-3p is considered to be an anti-fibrotic factor, and overexpression of miR-29b-3p may inhibit cardiac fibrosis and systemic sclerosis.[33] Moreover, changes in serum expression levels of FGF-21 and FGF-23, biomarkers of atrial fibrosis, are of great significance in predicting the occurrence of AF.[34] The serum expression levels of FGF-21 and FGF-23 in AF patients are positively correlated with the degree of atrial fibrosis.[27,35] In our present study,

Table 4 Comparison of Postoperative Recurrence of AF in Patients with Different miR-29b Levels

	Recurrence	No Recurrence	Total
miR-29b-3p low expression group	19	31	50
miR-29b-3p high expression group	13	37	50
Total	32	68	100

Table 5 Logistic Regression Analysis of Factors Influencing Postoperative AF Recurrence

Factors	*P* value	OR	95% CI
LAVI	0.035	1.074	1.005~1.147
FGF-21	0.288	0.985	0.958~1.013
FGF-23	0.256	0.981	0.948~1.014
NT-proBNP	0.078	1.019	0.998~1.041
miR-29b-3p	0.027	0.002	0.000~0.494

Notes: Binary logistic multivariate regression analysis was conducted, and a significance level of *P* < 0.05 was considered statistically significant.
Abbreviations: LAVI, left atrial volume index; FGF-21, fibroblast growth factor-21; FGF-23, fibroblast growth factor-23; NT-proBNP, N-terminal B-type natriuretic peptide precursor; miR-29b-3p, microRNA-29b-3p; OR, odds ratios; CI, confidence interval.

the serum expression level of miR-29b-3p in AF patients was negatively correlated with the concentration of FGF-21 and FGF-23. In brief, miR-29b-3p was correlated with atrial fibrosis.

RFCA is an effective method for AF treatment, but the relatively high rate of AF recurrence after RFCA is a long-standing problem.[36] miRNAs play a regulatory role in the recurrence of AF after RFCA.[37,38] As expected, serum miR-29b-3p was poorly expressed in patients with AF recurrence, along with diminished LAVI and FGF-23 levels. Circulating miRNAs are identified as potential biomarkers for AF[13] since they can be detected in the plasma or serum with high sensitivity and specificity.[39] Furthermore, we plotted the ROC curves of miR-29b-3p in diagnosing AF patients to evaluate its diagnostic efficacy. Interestingly, the AUC of miR-29b-3p was 0.716 (the cut-off value: 0.860, 100.00% sensitivity, 39.71% specificity), higher than that of FGF-21 and FGF-23. Previously, miR-409-3p, miR-630, miR-146b-5p, and miR-367 alone have potential diagnostic values in differentiating AF patients from healthy controls.[40] Our study initially highlighted that serum miR-29b-3p expression levels < 0.860 can assist in predicting the recurrence of AF after RFCA.

In addition, the incidence of AF recurrence after RFCA was higher in patients with low miR-29b-3p expression than that in patients with high miR-29b-3p expression, and the cumulative incidence of recurrence after RFCA was higher in patients with low miR-29b-3p expression. Although many studies have shown down-regulation of miR-29b in AF,[18,20] there are little data on the association between miR-29b-3p and AF ablation outcomes. Circulating miR-21 is associated with ablation success in patients with persistent AF who underwent catheter ablation.[32] Our study illustrated that poor expression of miR-29b-3p predicted a high risk of AF recurrence after RFCA. Furthermore, after adjusting LAVI, poor expression of miR-29b-3p led to an increased risk of AF after RFCA and was one of the independent risk factors for AF after RFCA. These results were suggestive of the functionality of miR-29b-3p on AF recurrence after RFCA.

In conclusion, as a prospective study, this study measured the serum expression levels of miR-29b-3p in AF patients and explored the predictive effect of miR-29b-3p expression level on AF recurrence after RFCA, providing a new entry point for clinical condition judgment and recurrence prediction of AF. However, the number of cases and events included in this study was limited, and the regulatory mechanism of miR-29b-3p in AF was not thoroughly discussed. In the future, a multi-center prospective study will be conducted to expand the sample size with matched controls to increase the credibility of the results and clarify the diagnostic and prognostic evaluation capabilities of miR-29b-3p. In addition, the expression levels of miR-29b-3p in cells of AF patients could be determined to study their effects on cell proliferation, apoptosis, and cell cycle.

Data Sharing Statement

All the data generated or analyzed during this study are included in this published article.

Ethics Approval and Consent to Participate

This study was approved by the Academic Ethics Committee of General Hospital of Northern Theater Command. All patients were fully informed of the purpose of this study and signed informed consent before sampling. Our study complied with the Declaration of Helsinki.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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The authors report no conflicts of interest in this work.

References

1. Cao F, Li Z, Ding W-M, Yan L, Zhao Q-Y. LncRNA PVT1 regulates atrial fibrosis via miR-128-3p-SP1-TGF-β1-Smad axis in atrial fibrillation. *Mol Med.* 2019;25(1):7. doi:10.1186/s10020-019-0074-5

2. Steinberg BA, Holmes DN, Pieper K, et al. Factors associated with large improvements in health-related quality of life in patients with atrial fibrillation: results from ORBIT-AF. *Circ Arrhythm Electrophysiol.* 2020;13(5):e007775. doi:10.1161/CIRCEP.119.007775

3. Magnani JW, Wang N, Benjamin EJ, et al. Atrial fibrillation and declining physical performance in older adults: the health, aging, and body composition study. *Circ Arrhythm Electrophysiol.* 2016;9(5):e003525. doi:10.1161/CIRCEP.115.003525

4. Guan Z, Zhang YJ, Liu L, et al. The association of preoperative atrial fibrillation with post-cardiopulmonary bypass hyperfibrinolysis in rheumatic valvular heart disease patients. *Heart Lung.* 2019;48(6):515–518. doi:10.1016/j.hrtlng.2019.05.014

5. Li CY, Zhang JR, Hu WN, Li SN. Atrial fibrosis underlying atrial fibrillation (Review). *Int J Mol Med.* 2021;47:3. doi:10.3892/ijmm.2020.4842

6. Begg GA, Swoboda PP, Karim R, et al. Imaging, biomarker and invasive assessment of diffuse left ventricular myocardial fibrosis in atrial fibrillation. *J Cardiovasc Magn Reson.* 2020;22(1):13. doi:10.1186/s12968-020-0603-y

7. Yi F, Hou W, Zhou C, et al. Radiofrequency ablation versus antiarrhythmic drug therapy for atrial fibrillation: meta-analysis of safety and efficacy. *J Cardiovasc Pharmacol.* 2019;73(4):241–247. doi:10.1097/FJC.0000000000000654

8. Shi J, Chen L, Chen S, Wu B, Yang K, Hu X. Circulating long noncoding RNA, GAS5, as a novel biomarker for patients with atrial fibrillation. *J Clin Lab Anal.* 2021;35(1):e23572. doi:10.1002/jcla.23572

9. Wang J, Song S, Xie C, et al. MicroRNA profiling in the left atrium in patients with non-valvular paroxysmal atrial fibrillation. *BMC Cardiovasc Disord.* 2015;15:97. doi:10.1186/s12872-015-0085-2

10. Zhelankin AV, Vasiliev SV, Stonogina DA, et al. Elevated plasma levels of circulating extracellular miR-320a-3p in patients with paroxysmal atrial fibrillation. *Int J Mol Sci.* 2020;21:10. doi:10.3390/ijms21103485

11. Torrado M, Franco D, Lozano-Velasco E, et al. A MicroRNA-transcription factor blueprint for early atrial arrhythmogenic remodeling. *Biomed Res Int.* 2015;2015:263151. doi:10.1155/2015/263151

12. van den Berg NWE, Kawasaki M, Berger WR, et al. MicroRNAs in atrial fibrillation: from expression signatures to functional implications. *Cardiovasc Drugs Ther.* 2017;31(3):345–365. doi:10.1007/s10557-017-6736-z

13. da Silva AM, de Araujo JN, de Freitas RC, Silbiger VN. Circulating MicroRNAs as potential biomarkers of atrial fibrillation. *Biomed Res Int.* 2017;2017:7804763. doi:10.1155/2017/7804763

14. Yang Q, Wu F, Mi Y, et al. Aberrant expression of miR-29b-3p influences heart development and cardiomyocyte proliferation by targeting NOTCH2. *Cell Prolif.* 2020;53(3):e12764. doi:10.1111/cpr.12764

15. Yang J, Chen Y, Jiang K, et al. MicroRNA-182 supplies negative feedback regulation to ameliorate lipopolysaccharide-induced ALI in mice by targeting TLR4. *J Cell Physiol.* 2020;235(9):5925–5937. doi:10.1002/jcp.29504

16. Xue Y, Fan X, Yang R, Jiao Y, Li Y. miR-29b-3p inhibits post-infarct cardiac fibrosis by targeting FOS. *Biosci Rep.* 2020;40:9. doi:10.1042/BSR20201227

17. Han X, Wang S, Yong Z, Zhang X, Wang X. miR-29b ameliorates atrial fibrosis in rats with atrial fibrillation by targeting TGFbetaRIota and inhibiting the activation of Smad-2/3 pathway. *J Bioenerg Biomembr*. 2022;54(2):81–91. doi:10.1007/s10863-022-09934-7

18. Guo Y, Sun Z, Chen M, Lun J. LncRNA TUG1 regulates proliferation of cardiac fibroblast via the miR-29b-3p/TGF-beta1 Axis. *Front Cardiovasc Med*. 2021;8:646806. doi:10.3389/fcvm.2021.646806

19. Lv X, Lu P, Hu Y, Xu T. Overexpression of MiR-29b-3p inhibits atrial remodeling in rats by targeting PDGF-B signaling pathway. *Oxid Med Cell Longev*. 2021;2021:3763529. doi:10.1155/2021/3763529

20. Guo F, Tang C, Huang B, et al. LncRNA H19 drives proliferation of cardiac fibroblasts and collagen production via suppression of the miR-29a-3p/miR-29b-3p-VEGFA/TGF-beta axis. *Mol Cells*. 2022;45(3):122–133. doi:10.14348/molcells.2021.0066

21. Kirchhof P, Benussi S, Kotecha D, et al. 2016 ESC Guidelines for the management of atrial fibrillation developed in collaboration with EACTS. *Eur J Cardiothorac Surg*. 2016;50(5):e1–e88. doi:10.1093/ejcts/ezw313

22. Wang G, Cheng B, Jia R, Tan B, Liu W. Altered expression of microRNA-92b-3p predicts survival outcomes of patients with prostate cancer and functions as an oncogene in tumor progression. *Oncol Lett*. 2021;21(1):4. doi:10.3892/ol.2020.12265

23. Han X, Chen C, Cheng G, et al. Serum fibroblast growth factor 21 levels are increased in atrial fibrillation patients. *Cytokine*. 2015;73(1):176–180. doi:10.1016/j.cyto.2015.02.019

24. Liu L, Su J, Li R, Luo F. Changes in intestinal flora structure and metabolites are associated with myocardial fibrosis in patients with persistent atrial fibrillation. *Front Nutr*. 2021;8:702085. doi:10.3389/fnut.2021.702085

25. Popa MA, Kottmaier M, Risse E, et al. Early arrhythmia recurrence after catheter ablation for persistent atrial fibrillation: is it predictive for late recurrence? *Clin Res Cardiol*. 2022;111(1):85–95. doi:10.1007/s00392-021-01934-8

26. Chen J, Liu M, Luo X, et al. Exosomal miRNA-486-5p derived from rheumatoid arthritis fibroblast-like synoviocytes induces osteoblast differentiation through the Tob1/BMP/Smad pathway. *Biomater Sci*. 2020;8(12):3430–3442. doi:10.1039/c9bm01761e

27. Wang R, Yi X, Li X, Jiang X. Fibroblast growth factor-21 is positively associated with atrial fibrosis in atrial fibrillation patients with rheumatic heart disease. *Int J Clin Exp Pathol*. 2015;8(11):14901–14908.

28. Schotten U, Verheule S, Kirchhof P, Goette A. Pathophysiological mechanisms of atrial fibrillation: a translational appraisal. *Physiol Rev*. 2011;91(1):265–325. doi:10.1152/physrev.00031.2009

29. Clauss S, Wakili R, Hildebrand B, et al. MicroRNAs as biomarkers for acute atrial remodeling in marathon runners (the miRathon study--A sub-study of the Munich marathon study). *PLoS One*. 2016;11(2):e0148599. doi:10.1371/journal.pone.0148599

30. Dawson K, Wakili R, Ordog B, et al. MicroRNA29: a mechanistic contributor and potential biomarker in atrial fibrillation. *Circulation*. 2013;127(14):1466–1475. doi:10.1161/CIRCULATIONAHA.112.001207

31. Dorn L, Kranzburg A, Saumell A, Gregory T, Reich S. Radiofrequency catheter ablation for atrial fibrillation. *JAAPA*. 2015;28(5):40–45. doi:10.1097/01.JAA.0000464275.36493.2d

32. Zhou Q, Maleck C, von Ungern-Sternberg SNI, et al. Circulating MicroRNA-21 correlates with left atrial low-voltage areas and is associated with procedure outcome in patients undergoing atrial fibrillation ablation. *Circ Arrhythm Electrophysiol*. 2018;11(6):e006242. doi:10.1161/CIRCEP.118.006242

33. Li J, Cen B, Chen S, He Y. MicroRNA-29b inhibits TGF-beta1-induced fibrosis via regulation of the TGF-beta1/Smad pathway in primary human endometrial stromal cells. *Mol Med Rep*. 2016;13(5):4229–4237. doi:10.3892/mmr.2016.5062

34. Coteli C, Hazirolan T, Aytemir K, et al. Evaluation of atrial fibrosis in atrial fibrillation patients with three different methods. *Turk J Med Sci*. 2021. doi:10.3906/sag-2103-194

35. Chua W, Purmah Y, Cardoso VR, et al. Data-driven discovery and validation of circulating blood-based biomarkers associated with prevalent atrial fibrillation. *Eur Heart J*. 2019;40(16):1268–1276. doi:10.1093/eurheartj/ehy815

36. Jiang N, Liu HX, Liang HY, Feng XH, Liu BY, Zhou YY. Osteogenic differentiation characteristics of Hip joint capsule fibroblasts obtained from patients with ankylosing spondylitis. *Ann Transl Med*. 2021;9(4):331. doi:10.21037/atm-20-7817

37. Kiliszek M, Maciak K, Maciejak A, et al. Serum microRNA in patients undergoing atrial fibrillation ablation. *Sci Rep*. 2020;10(1):4424. doi:10.1038/s41598-020-61322-6

38. Santulli G, Iaccarino G, De Luca N, Trimarco B, Condorelli G. Atrial fibrillation and microRNAs. *Front Physiol*. 2014;5:15. doi:10.3389/fphys.2014.00015

39. Weber JA, Baxter DH, Zhang S, et al. The microRNA spectrum in 12 body fluids. *Clin Chem*. 2010;56(11):1733–1741. doi:10.1373/clinchem.2010.147405

40. Cao Y, Cui L. Identifying the key microRNAs implicated in atrial fibrillation. *Anatol J Cardiol*. 2021;25(6):429–436. doi:10.14744/AnatolJCardiol.2020.41625