European Review for Medical and Pharmacological Sciences 2021; 25: 287-292

# Changed expression of microRNAs may predict postoperative atrial fibrillation in patients with cardiac surgery

Z.-W. XU[1], Z.-L. JIANG[2], Z. FU[1], S. HUANG[1]

[1]Department of Cardiac Surgery, Huai'an First People's Hospital, The Affiliate[illegible]aian No. [illegible] People's Hospital of Nanjing Medical University, Huai'an, China
[2]Department of Cardiothoracic Surgery, Xinhua Hospital, Shanghai Jiao[illegible] Univ[illegible]y, School of Medicine, Shanghai, China



**Abstract. – OBJECTIVE: Changes of microRNAs (miRNAs) may contribute to the pathogenesis and progression of postoperative atrial fibrillation (POAF) in patients undergoing cardiac valve surgery. This study aimed to measure the expression levels of miRNAs in peripheral blood, as well as their target mRNAs, in POAF patients and normal controls (non-POAF), and to evaluate the potential of miRNAs as promising biomarkers to predict POAF.**

**PATIENTS AND METHODS: The express[illegible] of miRNAs in peripheral blood, including m[illegible] et miR-133a, miR-328, miR-499 and their [illegible] et mRNAs, was analyzed in 109 POAF patients [illegible] d 96 non-POAF patients via quantitative real-t[illegible] polymerase chain reaction (RT-PCR). We co[illegible] pared differences between th[illegible]oups an[illegible] also analyzed the treat reac[illegible]to a[illegible]darone.**

**RESULTS: All miRNAs i[illegible]AF pa[illegible]ts were significantly highly ex[illegible]ed. C[illegible]d to non-POAF, the expres[illegible]on [illegible]-[illegible]i[illegible], miR-328, miR-499 i[illegible]ased[illegible]n groups of POAF patients, a[illegible]iR-499 w[illegible]e only up-regulated miRN[illegible] the amiod[illegible] - group versus amio[illegible]roup and [illegible]n-POAF. Among the u[illegible]egula[illegible]iRNAs, miR-499 expression [illegible]ificantly ch[illegible]d in amiodarone + and ami[illegible]one - patients [illegible].005). The ROC curve[illegible]ysis r[illegible]ealed that [illegible]-499 might be a pot[illegible] thera[illegible]tic response biomarker. The mi[illegible]mRN[illegible]interact[illegible]ns revealed 10 mRNAs regul[illegible]iR-27b[illegible]-133a, and miR- 499.**

**CONC[illegible]ONS: [illegible] found an expression [illegible]iR-1[illegible]27b, miR-328, and miR-49[illegible]as sig[illegible]tly different between these gro[illegible], with a [illegible]gh expression being observed in [illegible]ients compared to non-POAF pa[illegible]ts. [illegible]er, the present results showed [illegible]at miR-499 was significantly upregulated in [illegible]odarone - patients, compared to non-POAF, a[illegible]miodarone + patients. This finding indicat[illegible] that miR-499 may be a potential biomarker for predicting the occurrence of POAF after cardiac valve surgery and treat the reaction to amiodarone.**

*Key Words:*
MicroRN[illegible], Posto[illegible]e atrial fibrillation, Cardiac surge[illegible]

## [illegible]roduction

A[illegible] fibri[illegible]n (AF) is one of the main causes [illegible], heart failure, sudden death, and cardiovascular disease and is considered to be an [illegible]dent factor that increases all-cause mor[illegible]ity. New-onset postoperative atrial fibrillation (POAF) is encountered after cardiac surgery with a high frequency, especially the valve surgery[1,2]. The incidence of POAF was reported from 20% to 50%. Many risk factors are related with POAF, including body mass index, age[3], gender, chronic obstructive disease, valvular heart disease[4], epicardial adipose tissue[5], and even obstructive sleep apnea[6]. But the exact mechanism remains unclear.

MicroRNAs (miRNAs) are short non-coding RNA and play an important role in many biological processes such as cellular proliferation, differentiation, inflammation, and apoptosis. The human genome encodes more than 1000 miRNAs, of which 30% were detected in the cardiovascular system. Many miRNAs are involved in the regulation of atrial contractility, ion channel performance, and automaticity[7]. MicroRNAs are also related to AF; however, the full range of the miRNAs functionality remains unknown.

Irregular expression of miRNAs is associated with POAF and may play a key role in this disease[8]. In addition, the presence of miRNAs in biological fluids, such as semen, saliva and blood, makes them more attractive as diagnostic and prognostic biomarkers of diseases[9]. The

*Corresponding Author:* Su Huang, MD; e-mail: aslleng588@126.com

purpose of this study was to measure and compare the expression levels of peripheral blood miRNAs, including miR-27b, miR-133a, miR-328 and miR-499, as well as their target mRNAs in POAF patients and normal controls (non-POAF) after valve surgery. The efficacy of miRNAs as biomarkers to monitor the response of POAF patients to amiodarone was also evaluated.

## Patients and Methods

### Patients

This study was approved by institutional Review Board at Huai'an First People's Hospital, Jiangsu, China, and informed consent was obtained from all patients recruited in this study.

From January 2019 to July 2019, one hundred and nine POAF patients (40 males and 69 females) undergoing valve surgeries, with the mean age of 62.6 ± 7.7 years, were recruited in our study. A total of 96 non-POAF patients were selected for the study by matching the most relevant clinic variables such as age, sex and ethnicity, as well as possible clinical characteristics such as types of su[illegible] hypertension, body mass index, myocardial[illegible] tion, smoking, left ventricular ejection fra[illegible]n, and cardiopulmonary bypass. POAF was defi[illegible] as multiple AF lasting >30 seconds, recorded electrocardiogram monitor from [illegible]iately aft[illegible] heart surgery to discharge, rec[illegible]g a[illegible]F treatment (usually intravenous [illegible]iodaron[illegible] All patients were screened to e[illegible] that th[illegible] experienced AF preop[illegible]ive [illegible] quest[illegible]g and 12-lead electroc[illegible]ogram [illegible]nation before surgery. Other ex[illegible]n criteria i[illegible]d a history of severe r[illegible] disease, ner[illegible]s system disease, coag[illegible]ion di[illegible] renal or hepatic insufficienc[illegible]rrent treatm[illegible]th anti-arrhythmic drugs [illegible]t b-blockers, left [illegible]ricular ejection fracti[illegible]55%, [illegible]ergency procedure surgery.

[illegible]AF [illegible]p, amiodarone was used to treat new-[illegible]AF wit[illegible]rmal protocol. It was [illegible]ned [illegible]miod[illegible]e positive (amiodarone [illegible]en si[illegible]m came back before discha[illegible] Other[illegible], it was amiodarone negative (a[illegible]e -). Administration of amiodarone [illegible]mended dose (300 mg intravenous [illegible]ading dose followed by 600 mg orally twice [illegible] for 5 days) shown to be safe and effective[11].

### Samples Collections

Total RNA, including miRNA, was extracted using the miRNeasy Serum/Plasma kit and spike-in control (Qiagen, Redwood City, CA, USA), according to the manufacturer's protocols. For quality control of the RNA isolation, three synthetic RNA spike-ins (UniSp2, UniSp4[illegible] UniSp5) from the RNA Spike-in Kit f[illegible] gen, Redwood City, CA, USA) were[illegible]ed to the samples prior to purification at[illegible]centrations recommended by the manufacture[illegible] cDNA was synthesized from purif[illegible] miR[illegible]cord-ing to the protocol suppli[illegible]th the mi[illegible] LNA RT Kit (Qiagen, R[illegible]ood C[illegible] CA, U[illegible] with the addition of[illegible] spike-[illegible] (UniSp6 and cel-miR-39-3p) int[illegible]DNA s[illegible]hesis control.

The target[illegible]es were p[illegible]ct[illegible]by miRWalk2.0, w[illegible] online co[illegible]entary prediction to[illegible] $p < 0$[illegible]UM score > 5). The miRTar Base was used as[illegible]perimentally validated tool[illegible] rotein-prote[illegible]teractions of target g[illegible] were evaluated base[illegible] on STRING11.0 da-[illegible]se. Moreover[illegible]e bimolecular network model [illegible] plotted to d[illegible]ver the interactions of miRN[illegible] with targ[illegible]enes, visualized on Cytoscap[illegible] Ov[illegible], the constructed miRNA-gene networ[illegible]es information about genes involved in POAF.

### [illegible]ne Polymerase Chain Reaction (RT-PCR)

Expression levels of miRNAs were further determined by RT-PCR. RT-PCR experiments were performed as usual. Briefly, RT-PCR was performed according to the manufacturer's protocol (Bioteke, Beijing, China). A twenty-microliter reaction contained 2.5 µl reverse transcription products diluted five-fold, 600 nM of each primer, 10 µl 2×9 SYBR Green PCR master mix, 0.4 µl ROX, and 4.1 µl water. The reactions were prepared in a 96-well plate, and the thermal cycler program was as follows: 95°C for 2 min, followed by 40 cycles at 95°C for 15 s, and then 62°C for 32 s, on an ABI 7500 Real-Time PCR System. The thermal denaturation protocol was run at the end of the PCR to determine the number of products that were present in the reaction. All reactions were run in triplicate.

### Statistical Analysis

All statistical analyses were performed using the Statistical Package for the Social Science v. 20.0 (IBM, Armonk, NY, USA). Parametric variables were analyzed using Student's *t*-test and ANOVA followed by the Tukey's test. Nonpara-

**Table I.** Comparison of baseline characteristics between the groups of patients with POAF or non-POAF.

| Variables | POAF (n = 109) | non-POAF (n = 96) | *p* |
|---|---|---|---|
| Male sex | 40 (36.7%) | 39 (40.6%) | 0. |
| Age (y) | 62.6 ± 7.7 | 60.3 ± 5.1 | |
| BMI | 25.9 ± 3.1 | 26.2 ±2.3 | .329 |
| Smoking | 32 (29.4%) | 28 (29.2%) | 0.655 |
| Hypertension | 77 (70.6%) | 71 (74.0%) | .202 |
| PCI | 13 (11.9%) | 11 (11.5%) | 3 |
| GLU (mmol/L) | 6.4 ± 2.7 | 6.1 ± 1.7 | |
| LVEF (%) | 54.6 ± 8.5 | 57.2 ± 7.9 | 0. |
| LVDd (mm) | 50.5 ± 6.3 | 48.2 ± | 0.106 |
| LADs (mm) | 39.3 ± 4.4 | 37.5 | 0.096 |
| NYHA heart function before surgery | 2.5 ± 0.7 | 2.3 ± | 0. |
| Operation | | | |
| MVR (%) | 67 (61.5%) | (60.4%) | 72 |
| AVR (%) | 26 (23.8%) | 5 (26.0%) | .259 |
| DVR (%) | 16 (14.7%) | (13.6%) | 0.316 |
| CPB (min) | 97.7 ± 16.2 | 21.4 | 0.074 |
| Ventilation time (h) | 17.5 ± 2.1 | 15 | 0.117 |
| β-blocker | 82 (81.7%) | 73 (7 | 0.081 |

Values are presented as n (%), mean ± standard deviation, or median ge). POAF, Postoperative a ial fibrillation; BMI, body massive index; PCI, percutaneous coronary intervention; GLU, fas blood-glucose; LVEF, left ventricular ejection fraction; LVDd, left ventricular diastolic diameter; LADs, left atrial end sys diameter; CPB diopulmonary bypass; NYHA, New York Heart Association; MVR, mitral valve replacement; AVR, aort ve replacemen VR, double valves replacement.

metric variables were analyzed using the skal-Wallis tests. To investigate the pote miR-499 as a therapeutic biomarker, the re er operating characteristic (ROC) curve was g ated, and the area under the curve (AUC) was culated by computing sensitivit pecificit All *p*-values <0.05 were co red istically significant.

## sults

### Clinical Dat tudy Popu ion

One hund and POAF patients (40 males an females), the mean age of 62.6 ± years, as well sex and age-matc non-P F (39 males and 57 females), wi mea e of 60.3 ± 5.1 years, were recruite arison inical and paraclinical mete twee e two groups of patients ted i cant differences in terms of y mass dex, smoking, hypertension, m infarction, left ventricular ejection ation and cardiopulmonary bypass able I). The patients in POAF group were subded into two groups, based on the electrocar gram at discharge: amiodarone + (n=76) and amiodarone - (n=33). And no adverse effect caused by amiodarone was observed at the recommended dose.

### Targe and Network Establishment

The miRNA–mRNA interactions showed 14 regulated by miR-27b, miR-133, and (Figure 1). Ingenuity Pathway Analysis (IPA) detected no target or role for miR-328 in regulating POAF mechanisms.

### Expression Levels of MiRNAs in POAF Patients Reacted to Amiodarone

To evaluate the expression levels of miRNAs in the peripheral blood of POAF patients

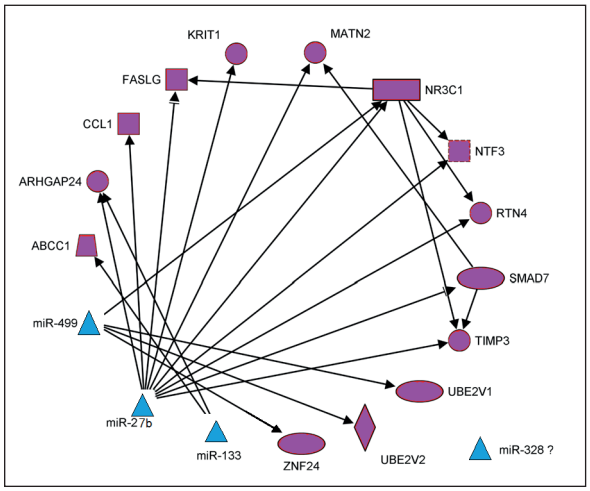


**Figure 1.** Interaction of miRNAs with mRNAs involved in heart disease. Blue - miRNA; Purple – mRNAs.

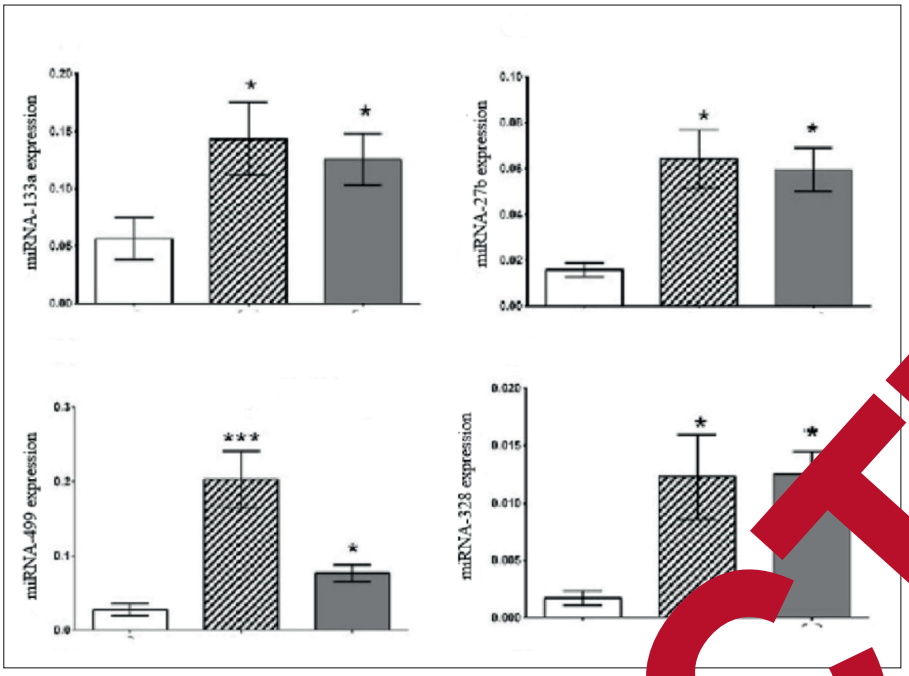


**Figure 2.** The high expression of miRNAs in the peripheral blood of POAF patients compared to the non-POAF. The results are pr... mean ± SEM (* ..., vs. * $p < 0.05$).



with regard to the treatment of amiodarone, four miRNAs (miR-27b, miR-133a, miR-32... miR-499) were analyzed. As indicated in ... 2, all selected miRNAs were significantl... regulated in POAF patients, compared to ... POAF.

Among miRNAs with incre... levels, mi... 499 was significantly upregu... in ...odarone - patients versus non-P... ($p$ < ...5), and its expression level wa... erent ... the amiodarone + and a...oda... oups ($p$ = 0.005). Moreover, ... ROC ... analysis revealed that miR-... might be a p...tial therapeutic respons...r (Figure 3). The ROC curve analysis ...owed ...timal cut-off point of 0.3 for th...ative expres... of miR-499 to discrimin...etween amiodaro... and amiodarone - gro..., with ...ificity of 62% and sensitivity of ...(AUC ...72; $p$ = 0.006). Analysis of the expre... of oth...iRNAs showed no sig...ant ...ence...ween amiodarone + and a...arone ...

## Discussion

...icroRNAs have been shown to be highly st...in biological fluids, such as plasma, which provides a non-invasive and accessible method to obtain samples[12]. In addition, studies on the expression of miRNA in human plasma provide uni...ta th...ay help in monitoring and diagnosi...s diseases, such as AF. Besides, ...atients with paroxysmal and persistent AF ex...ifferent miRNA expression profiles[13].

...present study, we evaluated miRNA expression patterns in the peripheral blood of POAF patients in terms of amiodarone + or amiodarone - and compared the results with non-POAF. The selected miRNAs showed significantly high expression in both groups of POAF patients, com-

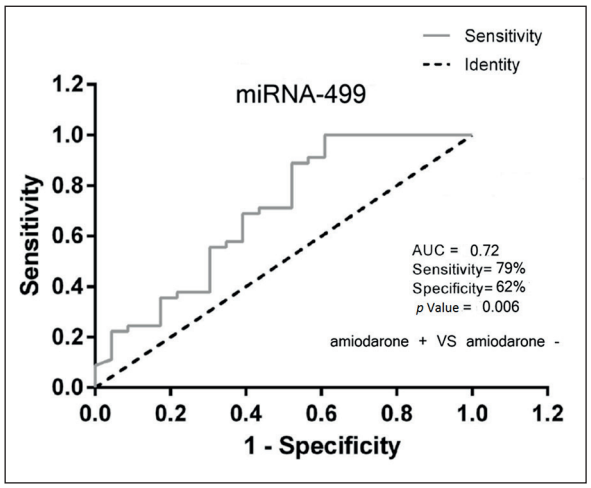


**Figure 3.** The receiver operating characteristic (ROC) curve shows that potential capacity of miRNA-499 in predicting the treat reaction to amiodarone of POAF patients ($p$ = 0.006).

pared to non-POAF. Compared to amiodarone + patients, miR-499 was the only upregulated miRNAs in the amiodarone - group with significantly high expression.

Cardiac electrophysiology deterioration can promote atrial remodeling by partial losing of the function of ion channels, which were basis for maintaining atrial fibrillation. MiR-499 functionally control the ion regulating the activity of $Ca^{2+}$ and $K^{+}$ channels[14,15]. In this study, we found that the expression level of miR-499 elevated in both amiodarone + and amiodarone - groups of patients versus non-POAF, and the amiodarone + group showed a lower expression of miR-499, compared to the amiodarone - group ($p = 0.005$). Moreover, the ROC curve analysis showed the potential capacity of miR-499 in predicting the treat reaction to amiodarone with specificity of 62% and sensitivity of 79% (AUC = 0.72).

The bioinformatic analysis conducted in this study showed that the relationship between miR-27b, miR-133, and miR-499, and mechanisms closely related to POAF, such as fibrosis, hypertrophy and cardiogenesis. Additionally, the 10 mRNAs (NR3C1, RTN4, TIMP3, SM… NTF3, UBE2V2, ABCC1, FASLG, MAT… UB2V1) that were the targets of the can…te four miRNAs in this study are known to b… volved in heart disease. In previous literatu… the mRNAs SMAD7 and FASL… associate… with atrial fibrillation[16]. SM… ssion is shown to be related to th… ration … AF and FAS/FASLG expression … ed to … of apoptosis and atrial fi… sis … perim… l results showed the … of m… 2 in the AF process, but the … nship was … tected in our study. In … h, we first … ied that miR-499 can … edict t… t reaction of POAF to amioda… e.

## Conclusions

Briefly, … found … t expression of miR-133a, … 7b, … and miR-499 was significan… differen… etween these groups, with a hi… ssion being observed in POAF patients … pa… non-POAF patients. Also, the pres… t results showed that miR-499 was remarkably … gulated in amiodarone - patients, compared to … -POAF, and amiodarone + patients. This finding first indicates that miR-499 may be a potential biomarker for predicting the treat reaction to amiodarone in POAF patients.



## Conflict of Interest

The Authors declare that they have no conflict of i…

## Declaration

This study was funded by 1. Research Fo… n of Nanjing Medical University: NMUP… 019352; … search Foundation of The Affiliated H… No. 1 Peop… pi… tal of Nanjing Medical Unive… YGRX 201914.

## …fer…

1) Nair SG, … fibrillation a… ac surgery. Ann Ca… 2010; 13: 15… 5.
2) Girerd … Piba… Fournier D, Daleau P, Voisine P, O'Hara G. M… ged men with increased … cumference a… vated C-reactive protein level are at higher r… for postoperative atrial fibrillation following coronary artery bypass grafting surg… Eur Heart J 2009; 30: 1270-1278.
3) …nki SF, … w DP, Adams DH, Rizzo RJ, … nderVliet M. Predictors of atrial fibri… ter coronary artery surgery current trends and impact on hospital resources. Circulation 1997; 94: 2084-2085.
4) …ew JP, Fontes ML, Tudor IC, Ramsay J, Duke P, Mazer CD. A multi-center risk index for atrial fifibrillation after cardiac surgery. JAMA 2004; 13: 57-58.
5) Opolski MP, Staruch AD, Kusmierczyk M, Witkowski A, Kwiecinska S, Kosek M. Computed tomography angiography for prediction of atrial fifibrillation after coronary artery bypass grafting: proof of concept. J Cardiol 2015; 65: 285-292.
6) Qaddoura A and Baranchuk A. Risk factors for post coronary artery bypass graft atrial fifibrillation: role of obstructive sleep apnea. Medwave 2016; 16: e6810.
7) Duong E, Xiao J, Qi XY and Nattel S. MicroRNA-135a regulates sodium-calcium exchanger gene expression and cardiac electrical activity. Heart Rhythm 2017; 14: 739-748.
8) Galenko O, Jacobs V, Knight S, Taylor M, Cutler MJ, Muhlestein JB. The role of microRNAs in the development, regulation, and treatment of atrial fibrillation. J Interv Card Electrophysiol 2019; 55: 33-36.
9) K. Murata, H. Yoshitomi, S. Tanida, M. Ishikawa, K. Nishitani, H. Ito. Plasma and synovial fluid microRNAs as potential biomarkers of rheumatoid arthritis and osteoarthritis. Arthritis Res. Ther 2010; 12: R86-89.
10) Frendl G, Sodickson AC, Chung MK, Waldo AL, Gersh BJ and Tisdale JE. 2014 AATS guidelines for the prevention and management of perioperative atrial fibrillation and flutter for thoracic surgi-

cal procedures. J Thorac Cardiovasc Surg 2014; 148: e153-193.

11) Riber LP, Christensen TD, Jensen HK, Hoejsgaard A and Pilegaard HK. Amiodarone significantly decreases atrial fibrillation in patients undergoing surgery for lung cancer. Ann Thorac Surg 2012; 94: 339–344.

12) McManus DD, Lin H, Tanriverdi K. Relations between circulating microRNAs and atrial fibrillation: Data from the Framingham Offspring Study. Hear Rhythm 2014; 11: 663-669.

13) Liu Z, Zhou C, Liu Y. The expression levels of plasma microRNAs in atrial fibrillation patients. PLoS One 2012; 7: e44906.

14) Tian-You Ling, Xiao-Li Wang, Qiang Chai. Regulation of the SK3 channel by MicroRNA-499 - potential role in atrial fibrillation. Heart Rhythm 2013; 10: 1001–1009.

15) Lu Y, Zhang Y, Wang N. MicroRNA-328 contributes to adverse electrical remodeling in atrial fibrillation. Circulation 2010; 122: 2378-

16) He X, Gao X, Peng L. Atrial fibrilla induce myocardial fibrosis through ang sin II type 1 receptor-specific Arkadia- ted down-regulation of Smad7. Circ Res 2 08: 164-175.

17) Müller P, Deneke T, Sc at F. Increa operative serum ap is ma er fas correlates with his thologi d new-on of atrial fibrillation tient er cardiac surgery. J Cardi asc ysiol , 24: 1110-1115.