Int J Clin Exp Med 2021;14(3):1468-1477
www.ijcem.com /ISSN:1940-5901/IJCEM0114497

Original Article

Abnormal expressions of plasma DNMT3A in elderly patients with recurrent atrial fibrillation

Huan Zeng[1], Xinzong Liu[2], Ying Li[3], Chang Zhou[4]

[1]Department of Cardiac Function, The First College of Clinical Medical Science, China Three Gorges University & Yichang Central People's Hospital, Yichang, China; [2]Institute of Orthopedics and Traumatology, The People's Hospital of Three Gorges University, The First People's Hospital of Yichang, Yichang, China; [3]Department of Cardiac Function, The First College of Clinical Medical Science, China Three Gorges University & Yichang Central People's Hospital, Yichang, China; [4]Department of Echocardiography, The First College of Clinical Medical Science, China Three Gorges University & Yichang Central People's Hospital, Yichang, China

Received May 18, 2020; Accepted October 19, 2020; Epub March 15, 2021; Published March 30, 2021

Abstract: Abnormal expressions of miR-200b play an important role in the occurrence and development of atrial fibrillation (AF). DNA methyltransferase 3A (DNMT3A) is a key molecule that catalyzes the methylation of the promoter-specific sites of multiple microRNAs. This study aims to investigate the effect of plasma DNMT3A activity on miR-200b expressions and the activation of the PI3K-Akt signaling pathway in elderly patients with recurrent AF, providing a reference for the molecular targeted therapy of AF. 84 elderly patients with non-valvular AF were recruited as the study cohort. Their mean age was 65.5±7.7 years, and 50 had primary AF (the initial group) and 34 had recurrent AF (the recurrent group). 45 healthy, elderly patients were selected as the control group, and their mean age was 66.5±7.9. The results showed that the left atrial volumes in the recurrent group were larger than they were in the initial and control groups ($P<0.05$), but the left ventricular volumes, LVEF values, and serum pro-BNP levels were not significantly different ($P>0.05$). The serum protein DNMT3A and PI3K-Akt levels in the recurrent group were higher than they were in the initial and control groups, but the miR-200b levels were decreased ($P<0.05$). A Pearson test found that the serum protein DNMT3A and PI3K-Akt levels were positively correlated with the left atrial volumes, and the miR-200b was negatively correlated with the left atrial volume and the DNMT3A and PI3K-Akt protein levels (the *P* values were all <0.05). Therefore, DNMT3A may be involved in recurrent AF in the elderly by down-regulating miR-200b and activating PI3K-Akt.

Keywords: Recurrent AF, DNMT3A, PI3K-Akt signaling pathway, miR-200b, myocardial fibrosis

Introduction

Atrial fibrillation (AF) is the most common arrhythmic disease, and old age, hypertension, myocardial ischemia, and heart valve disease are independent risk factors for AF. AF requires long-term medication, easily recurs, and has progressive development characteristics. Complications such as cardiogenic thrombosis and stroke, pulmonary embolism, and peripheral arterial embolism often have high rates of disability and mortality [1]. However, the mechanism of AF remains unclear. Recurrent AF in elderly patients is more complex and more severe, and recurrent AF patients have more complications and worse survival outcomes [2]. The animal model of AF [3] is often accompanied by myocardial fibrosis, which promotes the development of AF and ultimately leads to thrombosis and impaired cardiac function. Cardiomyocyte fibrosis is regulated by some related genes, including the DNA methylation shadows of multiple microRNAs. The abnormal expression of miR-200b plays an important role in the development of AF [4]. It was noted that miR-200b further improves the transforming ability of precancerous oral submucosal myofibroblasts into fibrosis by targeting transcription factor zeb2 [5]. TGF-β1 induces human Tenon's fibroblasts (HTFs) fibrosis through miR-200b and inhibits the signal transduction of PTEN [6]. Studies indicate that TGF-β1 promotes the expression of α-smooth muscle actin (α-SMA), fibronectin, and COL1A1 in HTFs by up-regulat-

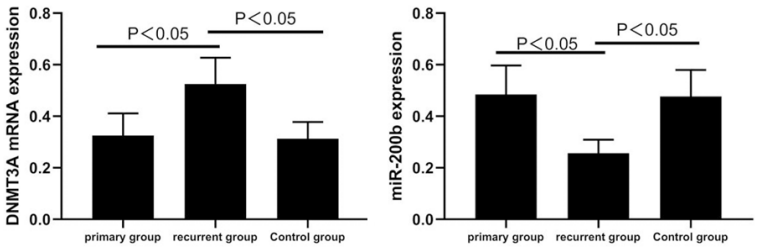


Figure 1. The serum DNMT3A and miR-200b mRNA expression levels were measured using RT-PCR in each group (the serum DNMT3A mRNA expression levels were significantly increased in the recurrent group compared with the initial group or the control group, respectively, and the miR-200b expression level was significantly decreased, P<0.05).

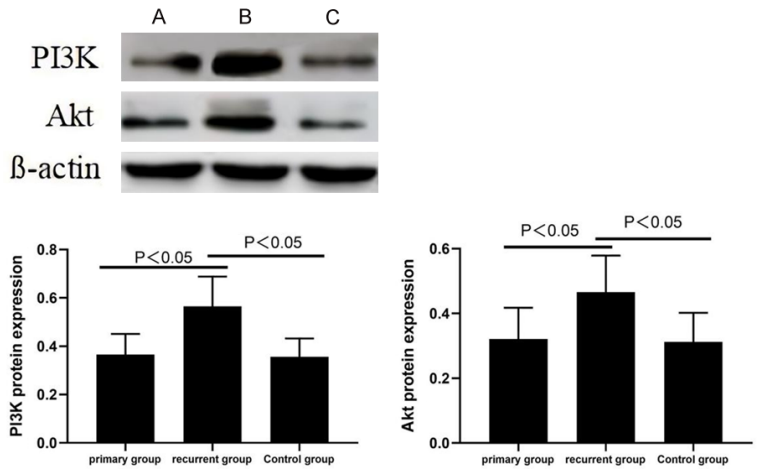


Figure 2. Western blot assays of the serum PI3K-Akt protein levels in each group (A. Initial group; B. Recurrent group; C. Control group. The serum PI3K-Akt protein expression levels were significantly increased in the recurrent group compared with the corresponding levels in the initial group or the control group, respectively, P<0.05).

ing the expression of miR-200b, and the addition of miR-200b mimics or inhibitors can enhance or inhibit the TGF-β1-induced α-SMA, fibronectin, and COL1A1 expression in HTFs. The above cited studies indicate that miR-200b may be closely related to the fibrosis process of multiple cells.

DNA methyltransferase 3A (DNMT3A) is a key functional enzyme that catalyzes promoter methylation on a variety of microRNAs and is an important participant in the regulation of the epigenetic silencing of genes [7]. Studies have shown that DNMT3A and miR-200b play important roles in the autophagy and myocardial fibrosis of cardiac myofibroblasts [8]. The autophagous behavior of myocardial fibroblasts may be an important myocardial fibrosis mechanism, and the up-regulation of DNMT3A is negatively correlated with the expression of miR-200b in fibrotic tissues and myocardial fibroblasts. It was also found that the miR-200b inhibitor can activate the autophagy of rat myocardial fibroblasts, which are inactivated by miR-200b mimics. DNMT3A knockdown is able to significantly increase the expression of miR-200b. It was also found that the interaction of miR-200b-3p with DNMT3A has an important effect on the proliferation and apoptosis of chondrocytes in patients with osteoarthritis [9]. DNMT3A might be one of the important molecules affecting the functional expression of miR-200b.

Considering this, the effects of plasma DNMT3A activity on the mir-200b expression and the PI3K-Akt signaling pathway activation in elderly patients with recurrent AF were investigated to provide a potential molecular targeted therapy for AF.

Materials and methods

Target information

In this study, a total of 84 elderly patients with non-valvular AF diagnosed in our hospital from January 2019 to January 2020 were recruited for the study, and their average age was 65.5±7.7 years. They were divided into the initial group (n=50, patients with primary AF) and the recurrent group (n=34, patients with recurrent AF). In addition, 45 healthy, elderly patients with the same genders and ages were recruited as the control group (their average age was 66.5±7.9 years). The design of the inclusion and exclusion of atrial fibrillation (AF) and the control group was shown in **Figure 4**.

Inclusion criteria: 1. Age greater than 60. 2. Each patient's AF diagnosis conforms with the typical ECG manifestations. 3. Primary AF: upon the first found atrial fibrillation, the patient may have symptoms or no symptoms, so the occurrence of atrial fibrillation can be manifested as paroxysmal atrial fibrillation, persistent atrial

fibrillation, or permanent atrial fibrillation. 4. Recurrent AF should be considered when the patient has two or more episodes, 5. Signed informed consent in accordance with medical ethics. Exclusion criteria: 1. Congenital heart disease, valvular heart disease. 2. Severe liver or kidney dysfunction or autoimmune disease.

Ethical statement

The study design was reviewed and approved by the ethics committee. All subjects had to sign an informed consent in written form to undergo the diagnostic and therapeutic procedures at the time of hospitalization. All the procedures in this study were carried out in compliance with the Declaration of Helsinki.

Research methods

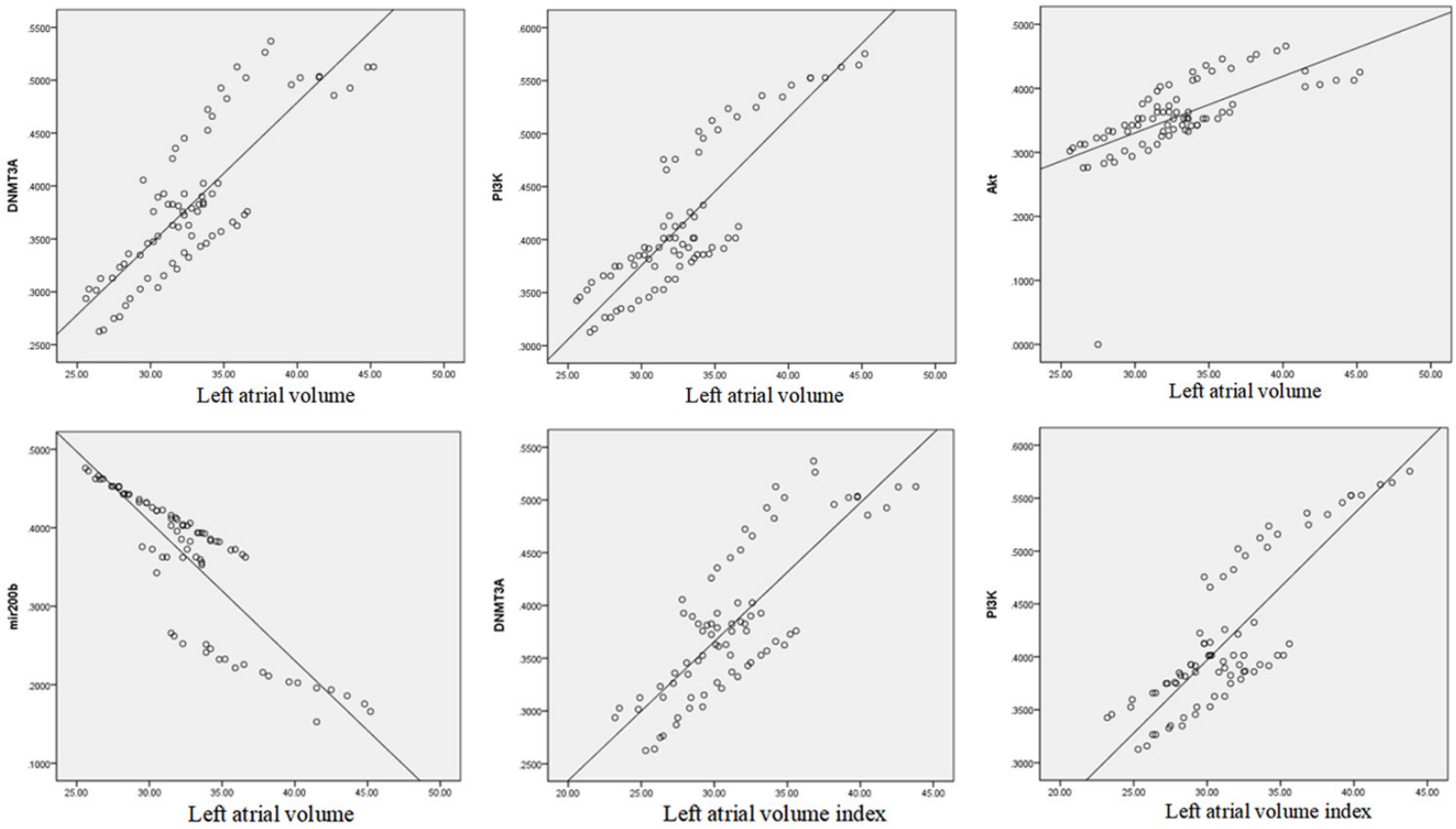
The AF patients were admitted to the hospital within 24 to 48 hours without any drug intervention before the completion of their blood sampling and ultrasound examination. The left atrium and left ventricular volume and left ventricular ejection fraction (LVEF) values were determined using color doppler ultrasound. The serum pro-BNP levels were measured using radio-immunoassays. The serum miR-200b levels were measured using real-time quantitative PCR (RT-PCR), and the DNMT3A and PI3K-Akt protein levels were determined using Western blot.

Testing methods

Ultrasonography to measure cardiac volume and LVEF: We instructed the patient to lie on the examination bed, relax, and breathe normally. We used a Philips IE Elite ultrasonic diagnostic instrument with an S5-1 heart probe at a frequency of 1.0-5.0 MHz, a transesophageal three-dimensional X7-2t probe with a frequency of 2.0-7.0 MHz. The left anterior and posterior atrium diameters were measured from the vertical left atria at the aorta roots, and the left atrial maximum volume was measured using the area-length method [10]. The modified two-planar Simpson method was used to measure the left ventricular end-diastolic volumes (LVEDV) and end-systolic volumes (LVSDV) on the cut surfaces of the apical four-compartment and apical two-compartment hearts. The mean left ventricular ejection fraction (LVEF) was calculated using the formula LVEF=(LVEDV-LVSDV)/LVEDVLVEF=100% [11]. The patients had to fast from water for 8 h, their mouths contained 2% lidocaine glue after the local anesthesia, they were then asked to assume a right supine position, connect the body surface to the electrocardiogram, adjust the radian of the probe and put the probe into the esophagus through the occluder, then we asked the patient to relax and breathe deeply, and we placed the probe smoothly through the oropharynx, so the probe was about 30-40 cm from the incisor tooth to clearly show the four-cavity heart section, and the handle was operated for the transesophageal ultrasound examination, and the structures of the left atrial appendage and the left atrial appendage from about 0-180° were observed continuously by rotating the probe to observe whether there was a strong echo of thrombus [12].

Measuring the serum pro-BNP levels using radioimmunoassays: We drew 810 ml of peripheral venous blood from the patients who had fasted overnight and transferred it to anticoagulant tubes. After centrifugation at 2000 g for 15 min, the supernatant was retained and preserved at -80°C. The reagents were purchased from Sigma in the United States. The reagent inter-batch and intra-batch variabilities were <15%. Each patient was measured three times to determine the mean according to the instructions.

RT-PCR quantification of the serum mir-200b mRNA levels: Total RNA was extracted from the human serum samples using Trizol reagent, and the concentration and purity of the RNA solution were determined using the ultraviolet absorption method (the spectrophotometer was purchased from Invitrogen, USA). The RNA was then reverse transcribed into cDNAs using a reverse transcription kit (Santa Cruz, USA). The upstream sequence of the target gene cDNA, miR-200b was: 5'-GGTTATTCCAGAGAGAGAGAGAGAGAGAG-3', the downstream sequence was: 5'-ACAGATAGGTGCGCC-3'; the Ginseng GAPDH upstream sequence was: 5'-CGCGAGAGAGATGACCCAGAT-3', the downstream sequence was: 5'-GCACTGTT GGCGTACAGAGGG3'. Then the reaction system was configured according to the PCR Kit's instructions (TaKaRa Company, Japan): SYBR Green 1 dye 10 μl + primers 1 μl + Taq Polymerase 2 μl + cDNA 5 μl + dH_2o 30 μl, for a total volume of 50 μl. The PCR amplification parameters were set to 93°C for 2 min pre-denaturation, followed by a total


DNMT3A
Left atrial volume
PI3K
Left atrial volume
Akt
Left atrial volume
mir200b
Left atrial volume
DNMT3A
Left atrial volume index
PI3K
Left atrial volume index

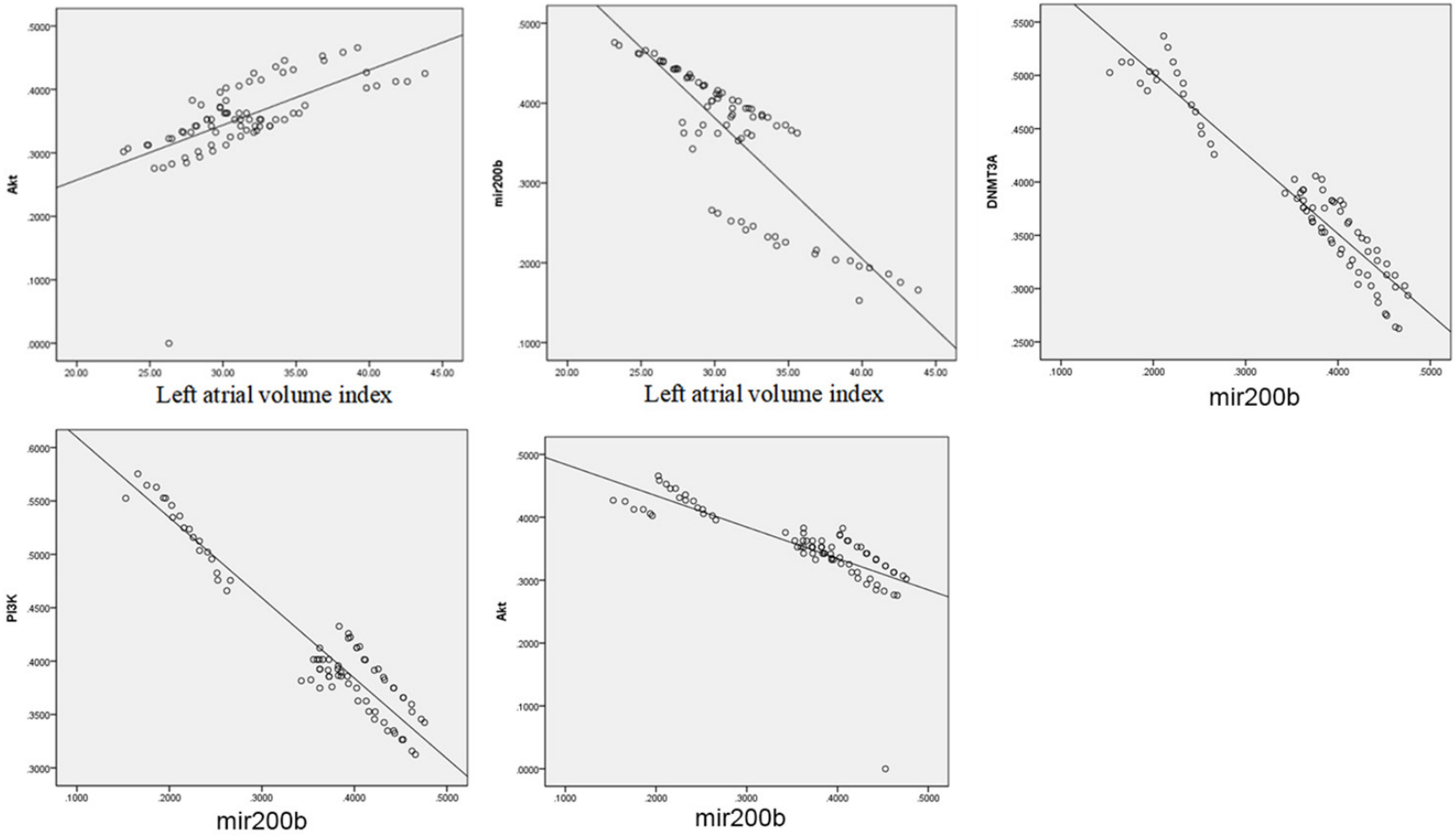


Figure 3. The correlations between the serum DNMT3A, miR-200b, and PI3K-Akt protein levels and the left atrial volumes and volume indexes as analyzed using Pearson tests.

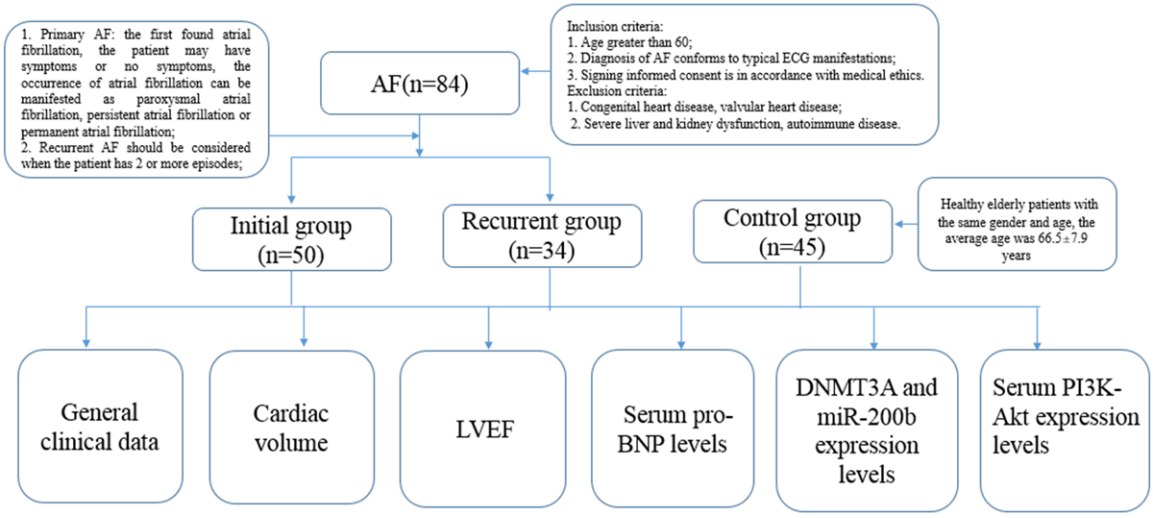


Figure 4. The above flow chart shows the design of the inclusion and exclusion of atrial fibrillation (AF) and the control group. To study the relationship between DNMT3A and recurrent atrial fibrillation, the cardiac volumes, the LVEF, the pro-BNP levels, the DNMT3A levels, and the miR-200b and PI3K-Akt levels in the groups were respectively compared.

of 40 cycles at 93°C for 1 min, 55°C for 1 min, 72°C for 1 min, and finally 72°C for 7 min. The PCR products and DNA ladder were electrophoretic and stained with 2% agarose gel. The comparative quantification was determined using the $2^{-\Delta\Delta Ct}$ method.

Western blot assays for determining the DNMT3A and PI3K-Akt protein levels: The serum samples were centrifuged at 2500 g for 20 min to take the supernatant, and the protein concentration and purity were determined using BCA protein quantitative kits (R & D, USA), and the internal reference β-actin antibody was used for the dose standard. Protein lysates were separated on 8% SDS-PAGE and transferred to polyvinylidene difluoride (PVDF) membranes, after being blocked in 5% fat-free milk overnight at 4°C, and then the membranes were then incubated with the following primary antibodies (β-actin plus mouse anti-human DNMT3A, PI3K-Akt and β-actin, dilution concentration: 1:2000, American Sigma Corporation), the second antibody (1:500, American Sigma Corporation) of rabbit anti-mouse antibody was added dropwise after PBS washing and incubated at room temperature for 4 h, ECL coloration. The results were scanned and saved, and Lab Works 4.5 gel imaging software (Invitrogen, USA) was used to perform a semi-quantitative analysis, and the results were expressed as integral optical density (IOD), which calculated the ratio of the band gray value of the target protein to the internal reference protein.

Statistical analysis

The statistical analyses were performed using SPSS 20.0 statistical software. One-way ANOVA was used for the comparisons between the three groups, LSD-T tests were used for the comparisons between two groups, and χ was used for the comparisons of the count data (%)[2] tests, and the correlation analysis was carried out using Pearson tests. $P<0.05$ was considered statistically significant.

Results

The general patient clinical data

The patients' baseline characteristics are shown in **Table 1**. There were no significant differences in gender, age, body mass index (BMI), or the percentages with hypertension, diabetes, or smoking in the two groups ($P>0.05$). In addition, there was no difference in the type or course of AF between the primary and recurrent AF groups ($P>0.05$).

Comparison of the cardiac volumes in the two groups

An increase in the left atrial diameter is a risk factor for poor prognosis in patients with AF. As shown in **Table 2**, the left atrial volumes and volume indexes of the patients in the recurrent group were both larger than the corresponding

Table 1. General patient clinical data

Group	Initial group (n=50)	Recurrent group (n=34)	Control group (n=45)	F/χ^2	P
Male/female	30/20	20/14	24/21	0.471	0.790
Age (Year)	65.1±7.3	68.6±9.2	66.5±7.9	0.865	0.231
BMI $(kg/m)^2$	25.3±2.2	25.4±2.1	25.2±1.9	0.326	0.614
Hypertension	18 (36.0)	14 (41.2)	17 (37.8)	0.231	0.891
Diabetes	7 (14.0)	5 (14.7)	6 (13.3)	0.030	0.985
Smoking	12 (24.0)	9 (26.5)	10 (22.2)	0.192	0.909
Type of AF				4.130	0.127
Paroxysm	13 (26.0)	6 (17.6)	-		
Continuity	31 (62.0)	18 (52.9)	-		
Permanent	6 (12.0)	10 (29.4)	-		
Course of AF (month)	18.5±4.6	19.3±5.1	-	0.852*	0.196

Note: *, Test.

Table 2. Comparison of the cardiac volumes in the groups

Group	Number of cases	Left atrium volume (ml)	Volume index (ml/m^2)	LVEDV (ml)	LVSDV (ml)
Initial Unit	50	32.2±4.3	30.8±3.8	143.8±10.2	61.2±5.3
Recurrent group	34	39.5±5.2#	36.4±3.5#	145.2±9.6	59.6±5.4
Control group	45	31.8±4.1	28.9±3.6	142.8±8.7	58.2±5.2
F		8.635	7.532	0.569	0.342
P		<0.001	<0.001	0.632	0.724

Note: LVEDV, left ventricular end-diastolic volume; LVSDV, end-systolic volume; the recurrent group was compared with the initial group or the control group, #P<0.05.

Table 3. Comparison of LVEF and serum pro-BNP levels in the groups

Group	Number of cases	LVEF (%)	pro-BNP (pg/ml)
Initial Unit	50	53.2±4.3	153.6±23.2
Recurrent group	34	54.6±4.5	158.4±32.5
Control group	45	55.8±5.9	150.8±25.4
F		0.865	1.032
P		0.189	0.135

values in the initial and control groups (P<0.05), but there were no significant differences in the left ventricular volumes among the three groups (P>0.05). This indicates that a change in the left atrial structure is closely related to the occurrence of AF, and especially to the recurrence of AF.

Comparison of the LVEF and serum pro-BNP levels in the groups

Pro-BNP plays an important role in the occurrence and development of cardiac insufficiency, which is a peptide substance with neurovascular activity secreted by the ventricles. In addition, LVEF reflects cardiac function. Therefore, the correlations among the LVEF levels, the serum pro-BNP levels, and AF were analyzed. As shown in **Table 3**, there were no significant differences in the LVEF or serum pro-BNP levels in the three groups (P>0.05).

Comparison of the serum DNMT3A and miR-200b levels among the groups

The serum DNMT3A and miR-200b mRNA expression levels were measured using RT-PCR in each group. As shown in **Figure 1**, the serum DNMT3A mRNA expression levels were significantly increased in the recurrent group compared with both the initial and control groups (P<0.05), respectively, and the miR-200b expression levels were significantly decreased in the recurrent group compared with both the initial and control groups (P<0.05), suggesting that recurrent AF is closely associated with the down-regulation of serum miR-200b expressions and the up-regulation of DNMT3A activity.

Table 4. Pearson analysis of the correlations between the indicators

Indicators	DNMT3A	PI3K	Akt	Left atrium volume	Volume index
miR-200b	R=-0.962, P=0.000	R=-0.964, P=0.000	R=-0.777, P=0.000	R=-0.862, P=0.000	R=-0.852, P=0.000
DNMT3A	/	/	/	R=0.829, P=0.000	R=0.813, P=0.000
PI3K	/	/	/	R=0.868, P=0.000	R=0.856, P=0.000
Akt	/	/	/	R=0.667, P=0.000	R=0.653, P=0.000

Comparison of the serum PI3K-Akt protein levels among the groups

To further investigate whether PI3K-Akt is involved in recurrent AF, the serum PI3K-Akt protein levels in each group was analyzed using Western blot. As shown in **Figure 2**, the serum PI3K-Akt protein expression levels were significantly increased in the recurrent group compared with the corresponding levels in the initial and control groups, respectively (P<0.05).

Related analysis

A Pearson test found that the serum DNMT3A and PI3K-Akt protein levels were positively correlated with the left atrium volume and the volume index, but the miR-200b was negatively correlated with the left atrium volumes, the volume indexes, and the DNMT3A and PI3K-Akt protein levels (the *P* values were all <0.05) (**Table 4** and **Figure 3**).

Discussion

With the ongoing study of the pathogenesis of AF, the development of AF from simple arrhythmia to arrhythmia-based cardiomyopathy becomes more complicated [13]. Advanced gadolinium-enhanced cardiac magnetic resonance imaging (MRI), which shows atrial fibrosis, is a powerful outcome predictor in interventional AF therapy. However, individualized and fibrosis-oriented treatment strategies for AF appear promising, but the results of prospective, multicenter trials have yet to be confirmed [14]. We also believe that patients with AF have significant myocardial fibrosis, and AF is closely related to the structural, electrical, and contractile remodeling of the atrium [15]. The occurrence and development of atrial fibrosis is a hallmark of AF structural remodeling and is considered the reason for the persistence of AF. Meanwhile, ventricular fibrosis appears to contribute to cardiac diastolic and systolic abnormalities and the development of heart failure, which is common in AF. Given that AF and heart failure often co-exist and that both conditions affect a patient's prognosis, a better understanding of the interaction between AF fibrosis and heart failure is particularly important [16].

The aberrant expression of microRNA-221 shows myocardial protective and anti-fibrotic effects in a rat model of myocardial infarction [17]. It was also found that melatonin attenuates myocardial fibers in patients with diabetic cardiomyopathy by inhibiting the LncRNA-malat1/miR-141-mediated inflammatory response and the TGF-β1/Smads signaling collateral activation [18]. In this study, we examined the relationship between the atrial structure and the serum miR-200b and DNMT3A levels in elderly patients with recurrent AF. Our results show that the left atrial volumes and the volume indexes of the patients in the recurrent group were higher than they were in the initial and control groups, but there were no significant differences among the left ventricular volumes, the LVEF values, or the serum pro-BNP levels, indicating that the changes in the left atrial structure are closely related to the occurrence of AF, and especially the recurrence of AF [19]. The serum protein DNMT3A and PI3K-Akt levels in the recurrent group were higher than they were in the initial and control groups, while the miR-200b was decreased, indicating that recurrent AF is closely associated with the down-regulation of serum miR-200b, the up-regulation of DNMT3A, and the activation of the PI3K-Akt signaling pathway. It has been reported that myc and DNMT3A-mediated DNA methylation inhibit miR-200b in triple negative breast cancer [20]. Tao et al. [21] also confirmed that DNMT3A silencing Ras associated domain family 1 subtype a (RASSF1A) promotes myocardial fibers by up-regulating ERK1/2. Our results are consistent with the findings of the previous studies.

Next, a Pearson test showed that the serum DNMT3A and PI3K-Akt protein levels were positively correlated with the left atrium volumes

and volume indexes, and that miR-200b was negatively correlated with the left atrium volume, volume indexes, and the DNMT3A and PI3K-Akt protein levels, indicating that the alteration of left atrium structure in elderly patients with recurrent AF is closely related to the down-regulation of the miR-200b expression, the up-regulation of the DNMT3A activity, and the activation of the PI3K-Akt signaling pathway. In addition to altering the left atrium structure which was considered an important factor in triggering and maintaining AF, the abnormal expression of miR-200b and DNMT3A may be involved in cardiomyocyte fibrosis through the PI3K-Akt signaling pathway [22].

Above all, DNMT3A may be involved in the occurrence of recurrent AF in the elderly by down-regulating miR-200b and activating the PI3K-Akt signaling pathway. The innovation of this study is that we found the miR-200b, DNMT3A, and PI3K-Akt signaling pathways may play an important role in the occurrence of recurrent AF and myocardial fibrosis in the elderly. The deficiency lies in the fact that the fibrosis of atrial myocytes in patients with AF cannot be observed, and the abnormal expressions of miR-200b, DNMT3A, and PI3K-Akt molecules in the circulating serum may have a variety of interfering factors and cannot be directly related to AF. In addition, whether the abnormal expression of miR-200b is directly related to DNMT3A needs further study. Therefore, the specific mechanism needs to be further verified using in vitro and in vivo experiments.

Disclosure of conflict of interest

None.

Address correspondence to: Huan Zeng, Department of Cardiac Function, The First College of Clinical Medical Science, China Three Gorges University & Yichang Central People's Hospital, No. 183 Yiling Avenue, Yichang 443003, Hubei, China. E-mail: zenghuan311@126.com

References

[1] January CT, Wann LS, Alpert JS, Calkins H, Cigarroa JE, Cleveland JC Jr, Conti JB, Ellinor PT, Ezekowitz MD, Field ME, Murray KT, Sacco RL, Stevenson WG, Tchou PJ, Tracy CM and Yancy CW; American College of Cardiology/American Heart Association Task Force on Practice Guidelines. 2014 AHA/ACC/HRS guideline for the management of patients with atrial fibrillation: a report of the American College of Cardiology/American Heart Association Task Force on practice guidelines and the Heart Rhythm Society. Circulation 2014; 130: e199-267.

[2] Dretzke J, Chuchu N, Chua W, Fabritz L, Bayliss S, Kotecha D, Deeks JJ, Kirchhof P and Takwoingi Y. Prognostic models for predicting incident or recurrent atrial fibrillation: protocol for a systematic review. Syst Rev 2019; 8: 221.

[3] Sun J, Wugeti N and Mahemuti A. Reversal effect of Zhigancao decoction on myocardial fibrosis in a rapid pacing-induced atrial fibrillation model in New Zealand rabbits. J Int Med Res 2019; 47: 884-892.

[4] Jiang S, Guo C, Zhang W, Che W, Zhang J, Zhuang S, Wang Y, Zhang Y and Liu B. The integrative regulatory network of circRNA, microRNA, and mRNA in atrial fibrillation. Front Genet 2019; 10: 526.

[5] Liao YW, Yu CC, Hsieh PL and Chang YC. miR-200b ameliorates myofibroblast transdifferentiation in precancerous oral submucous fibrosis through targeting ZEB2. J Cell Mol Med 2018; 22: 4130-4138.

[6] Tong J, Chen F, Du W, Zhu J and Xie Z. TGF-beta1 induces human tenon's fibroblasts fibrosis via miR-200b and its suppression of PTEN signaling. Curr Eye Res 2019; 44: 360-367.

[7] Wang JY, Cheng H, Zhang HY, Ye YQ, Feng Q, Chen ZM, Zheng YL, Wu ZG, Wang B and Yao J. Suppressing microRNA-29c promotes biliary atresia-related fibrosis by targeting DNMT3A and DNMT3B. Cell Mol Biol Lett 2019; 24: 10.

[8] Zhao XD, Qin RH, Yang JJ, Xu SS, Tao H, Ding XS and Shi KH. DNMT3A controls miR-200b in cardiac fibroblast autophagy and cardiac fibrosis. Inflamm Res 2018; 67: 681-690.

[9] Wu J, Tao Y, Shang A, Wang W, Zhang Y, Hu L, Wang J, Wang Y and Guo N. Effect of the interaction between MiR-200b-3p and DNMT3A on cartilage cells of osteoarthritis patients. J Cell Mol Med 2017; 21: 2308-2316.

[10] Jordan K, Yaghi S, Poppas A, Chang AD, Mac Grory B, Cutting S, Burton T, Jayaraman M, Tsivgoulis G, Sabeh MK, Merkler AE, Kamel H, Elkind MSV, Furie K and Song C. Left Atrial volume index is associated with cardioembolic stroke and atrial fibrillation detection after embolic stroke of undetermined source. Stroke 2019; 50: 1997-2001.

[11] Potter E and Marwick TH. Assessment of left ventricular function by echocardiography: the case for routinely adding global longitudinal strain to ejection fraction. JACC Cardiovasc Imaging 2018; 11: 260-274.

[12] He Y, Zhang B, Zhu F, Hu Z, Zhong J and Zhu W. Transesophageal echocardiography measures left atrial appendage volume and function and

predicts recurrence of paroxysmal atrial fibrillation after radiofrequency catheter ablation. Echocardiography 2018; 35: 985-990.
[13] Sohns C and Marrouche NF. Atrial fibrillation and cardiac fibrosis. Eur Heart J 2020; 41: 1123-1131.
[14] Korodi S, Toganel R, Benedek T, Hodas R, Chitu M, Ratiu M, Kovacs I, Mester A and Benedek I. Impact of inflammation-mediated myocardial fibrosis on the risk of recurrence after successful ablation of atrial fibrillation - the FIBRO-RISK study: protocol for a non-randomized clinical trial. Medicine (Baltimore) 2019; 98: e14504.
[15] Dzeshka MS, Lip GY, Snezhitskiy V and Shantsila E. Cardiac fibrosis in patients with atrial fibrillation: mechanisms and clinical implications. J Am Coll Cardiol 2015; 66: 943-959.
[16] Prabhu S, Voskoboinik A, Kaye DM and Kistler PM. Atrial fibrillation and heart failure - cause or effect? Heart Lung Circ 2017; 26: 967-974.
[17] Zhou Y, Richards AM and Wang P. MicroRNA-221 is cardioprotective and anti-fibrotic in a rat model of myocardial infarction. Mol Ther Nucleic Acids 2019; 17: 185-197.
[18] Che H, Wang Y, Li H, Li Y, Sahil A, Lv J, Liu Y, Yang Z, Dong R, Xue H and Wang L. Melatonin alleviates cardiac fibrosis via inhibiting lncRNA MALAT1/miR-141-mediated NLRP3 inflammasome and TGF-beta1/Smads signaling in diabetic cardiomyopathy. FASEB J 2020; 34: 5282-5298.
[19] Kurzawski J, Janion-Sadowska A, Zandecki L, Piatek L, Koziel D and Sadowski M. Global peak left atrial longitudinal strain assessed by transthoracic echocardiography is a good predictor of left atrial appendage thrombus in patients in sinus rhythm with heart failure and very low ejection fraction - an observational study. Cardiovasc Ultrasound 2020; 18: 7.
[20] Pang Y, Liu J, Li X, Xiao G, Wang H, Yang G, Li Y, Tang SC, Qin S, Du N, Zhang H, Liu D, Sun X and Ren H. MYC and DNMT3A-mediated DNA methylation represses microRNA-200b in triple negative breast cancer. J Cell Mol Med 2018; 22: 6262-6274.
[21] Tao H, Yang JJ, Chen ZW, Xu SS, Zhou X, Zhan HY and Shi KH. DNMT3A silencing RASSF1A promotes cardiac fibrosis through upregulation of ERK1/2. Toxicology 2014; 323: 42-50.
[22] Ambale-Venkatesh B, Liu CY, Liu YC, Donekal S, Ohyama Y, Sharma RK, Wu CO, Post WS, Hundley GW, Bluemke DA and Lima JAC. Association of myocardial fibrosis and cardiovascular events: the multi-ethnic study of atherosclerosis. Eur Heart J Cardiovasc Imaging 2019; 20: 168-176.