

Received: 2015.10.07  
Accepted: 2015.11.05  
Published: 2016.06.24

# Underexpression of CACNA1C Caused by Overexpression of microRNA-29a Underlies the Pathogenesis of Atrial Fibrillation

Authors' Contribution:  
Study Design A  
Data Collection B  
Statistical Analysis C  
Data Interpretation D  
Manuscript Preparation E  
Literature Search F  
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**Source of support:** Departmental sources

**Background:** The objective of this study was to investigate the molecular mechanism of atrial fibrillation (AF), as well as the negative regulatory relationship between miR-29a-3p and CACNA1C.

**Material/Methods:** We searched the online miRNA database ([www.mirdb.org](http://www.mirdb.org)) and identified the miR-29a-3p binding sequence within the 3'-UTR of the target gene, and then conducted luciferase assay to verify it. The cells were transfected with miR-29a-3p and  $I_{Ca,L}$  was determined in those cells.

**Results:** We validated CACNA1C to be the direct target gene of miR-29a-3p. We also established the negative regulatory relationship between miR-29a-3p and CACNA1C via studying the relative luciferase activity. We also conducted real-time PCR and Western blot analysis to study the mRNA and protein expression level of CACNA1C among different groups of cells treated with scramble control, 30nM miR-29a-3p mimics, and 60nM miR-29a-3p mimics, indicating a negative regulatory relationship between miR-29a-3p and CACNA1C. We next analyzed whether miR-29a-3p transfection in cardiomyocytes produced the effects on the  $I_{Ca,L}$  induced by electrical remodeling, and found a tonic inhibition of  $I_{Ba}$  by endogenous miR-29a-3p in atrial myocytes.

**Conclusions:** We validated the negative regulation between miR-29a-3p and CACNA1C, and found that miR-29a-3p might a potential therapeutic target in the treatment of AF.

**MeSH Keywords:** **Atrial Fibrillation • Caveolin 1 • MicroRNAs**

**Full-text PDF:** <http://www.medscimonit.com/abstract/index/idArt/896191>



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## Background

Atrial fibrillation (AF) is characterized by high incidence, prevalence, and mortality [1]. Increasing evidence has shown that uneven distribution of the vagus and sympathetic nerves, together with cardiac autonomic nerve remodeling (ANR), which is characterized by regeneration of nerve, are involved in the pathogenesis of both chronic and acute AF [2,3]. Structural remodeling, electricity, and ANR interact and promote each other, resulting in a vicious cycle that leads to AF [4,5]. The main risk factor related to myocardial-associated cerebrovascular events [6,7], especially fast atrial rates, results in progressive atrial structural and electrical changes (remodeling) that favor arrhythmia recurrence and maintenance. Fibrosis ranks among the most important structural changes associated with atrial remodeling [6,7]. Characterized by a marked shortening of the atrial action potential duration (APD) and refractoriness, electrical remodeling results from changes in Ca<sup>2+</sup> and K<sup>+</sup> channel densities [6–10]. Reduction of the density of the L-type Ca<sup>2+</sup> current (I<sub>Ca,L</sub>), which is generated by channels consisting of  $\alpha 2\delta$  (CACNA2D),  $\alpha 1c$ ,  $\beta 2$  (CACNB2) or Cav1.2 (encoded by CACNA1C) subunits, is a hallmark of the electrical remodeling [7,8]. Importantly, the molecular mechanisms underlying the upregulation of I<sub>Ca,L</sub> has not been completely elucidated yet. Some researchers consider that it is because of a reduction of expression of mRNA CACNB2 and CACNA1C,9,11 whereas others suggested post-transcriptional mechanisms, such as protein dephosphorylation [12].

MicroRNAs (miRNAs) are a class of small non-coding RNAs (ncRNAs) consisting of 21–24 nucleotides, which function in posttranscriptional regulation of gene expression and RNA silencing [13]. mRNA changes, driven by miRNAs, might be observed in AF patients. Some researchers carried out microarray experiments using samples from AF patients [14,15], while others have validated key roles for miRNAs, such as miR-1, miR-328, and miR-26, in AF pathogenesis [16–18]. These studies have added to our understanding of the factors involved in early AF development. MicroRNAs are important in a wide range of cardiac remodeling processes [19], and there is increasing evidence for participation in AF [20]; its upregulation contributes to AF stabilization because of electrical remodeling [21,22].

It was reported that dysregulation of CACNA1C is responsible for the development of AF [23]. In addition, miR-29a-3p and miR-3135b were shown to be differentially expressed in AF tissue samples [24], and we found the both miR-29a-3p and miR-3135b target CACNA1C. In this study, we aimed to validate the miRNA and mRNA regulatory relationship, and to explore the role of the miR-29a-3p and CACNA1C in the pathogenesis of AF.

## Material and Methods

### Human atrial samples

The tip of the right atrial appendage was removed as part of the general surgical procedure in patients undergoing valve replacement, including 25 patients with AF and 25 without AF, open-heart surgery for bypass grafting, or a combination of both after written informed consent was obtained. The diagnosis of AF was reached by evaluating medical records and 12-lead electrocardiogram findings. Those who had other cardiovascular diseases, such as heart failure, mitral valve stenosis/regurgitation, or cardiomyopathy, were excluded from this study. All biopsies were performed at the same site, which is at the tip of the right atrial appendage. The samples were flash frozen in liquid nitrogen after collection in accordance with protocols approved by the ethics committee of the No. 7 People's Hospital in Zhengzhou City.

### Quantitative real-time PCR

iScript™ cDNA Synthesis Kit (Bio-Rad, Hercules, CA) was applied to perform reverse transcription in order to measure levels of miR-29a-3p and mRNA of CACNA1C. To measure levels of miRNAs, reverse transcription and quantitative real-time (qRT)-PCR were carried out in accordance with the instructions provided by the manufacturer. Mastercycler ep realplex (Eppendorf, Hamburg, Germany), PerfeCTa® SYBR® Green FastMix® (Quanta Biosciences, Gaithersburg, MD) were employed to perform qRT-PCR reactions in 96-well plates in triplicate. The 2<sup>-ΔΔCT</sup> method was applied to calculate the relative expression levels after normalization to U6.

### Western blot

The culture cells and tissue samples were homogenized and lysed by lysis buffer, and the lysates were loaded onto the 10% PAGE gel. The separated proteins were transferred onto a PVDF membrane (Millipore), and the blots were incubated with the primary antibodies. Primary antibodies against  $\beta$ -actin (Cell Signaling Technology, MA, USA) at a final concentration of 1: 1000 and against CACNA1C (Abcam, Cambridge, UK) at a final concentration of 1:250 were used to carry out Western blot analysis. Blots were washed 3 times and incubated with secondary antibody (Cell Signaling Technology, MA, USA). We then utilized Quantity One software (Bio-Rad, Hercules, CA, USA) to measure the band intensity (area  $\times$  OD) of each group in order to quantify the Western blot bands. Data were normalized to the control values.

### Cell transfection and luciferase assays

We purchased pGL3 with luciferase and inserted amplified CACNA1C full-length 3'UTR with a miR-29a-3p binding site

deletion (pGL3-CACNA1C-UTR) or mutant CACNA1C full-length 3'UTR (pGL3-CACNA1C-UTR-mutant) from Shanghai Biobuy Biotech Co., Ltd. (Shanghai, China). Sequencing was performed to confirm the plasmids. The constructed vectors transfected the cells with the help of lipofectamine 2000 (Invitrogen, CA, USA). We added 50 ng of pRL-TK (Promega, Madison, WI, USA) to correct the efficiency of transfection in each group. Dual-Luciferase Reporter Assay System (Promega) was used to measure luciferase activity. We expressed the activity of promoter as the ratio of Firefly luciferase activity to Renilla luciferase activity.

### MiRNA target prediction

Computational prediction of putative targets for miR-29a-3p was performed by searching [www.mirdb.org](http://www.mirdb.org), using the target prediction algorithms.

### Transfection studies

We transfected the HL-1 cells with miR-29a-3p mimics (Genepharma, Shanghai, China) in accordance with the manufactures' protocol and took fluorescein-conjugated scrambled siRNA as control. We transfected them in accordance with the manufacturer's protocol. MiR-29a-3p mimics were transfected into HL-1 with the help of lipofectamine 2000 (Invitrogen).

### Calcium current recordings

With the whole-cell patch-clamp technique (micropipette resistance <3.5 MΩ), we recorded the currents in human HL-1 cells and atrial myocytes at room temperature. We compensated for series resistance when ≥80% compensation was achieved. No significant voltage errors (<5 mV) were caused by rising series resistance with the micropipettes used under our experimental conditions.

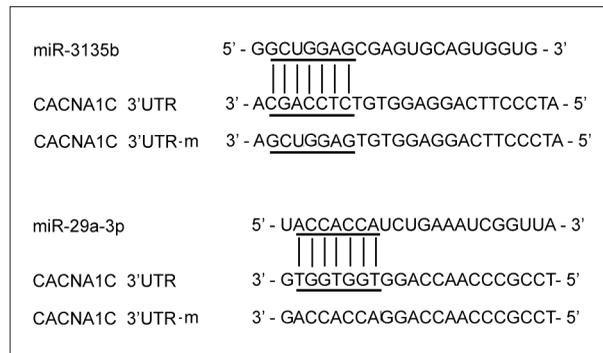
### Statistical analysis

Fisher's exact test and 2-tailed Student's t-test were used where appropriate. Data are presented as mean ±SEM and p<0.05 was considered statistically significant, except for the miRNA microarray data analyzed by LC Sciences (Houston, TX) using a validated method.

## Results

### CACNA1C was a target of miR-29a-3p

Voltage-operated L-type Ca<sup>2+</sup> channels (LCCs) play a critical role in the electrical and mechanical functions of the heart, and disorders of LCCs have been linked to atrial fibrillation (AF).



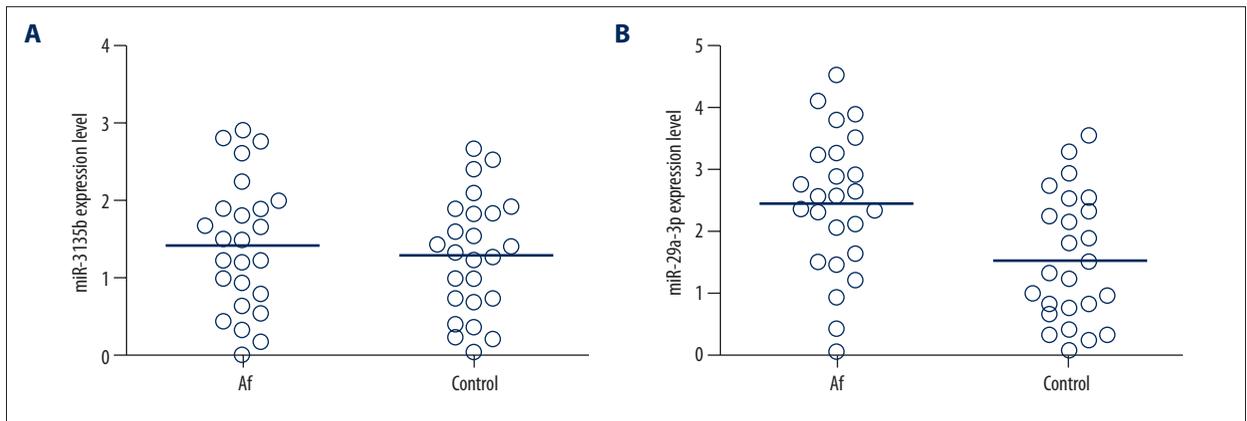
**Figure 1.** CACNA1C as the candidate target gene of miR-3135b and miR-29a-3p with the 'seed sequence' in the 3'UTR.

A characteristic of AF is atrial electric remodeling associated with profound reduction of L-type Ca<sup>2+</sup> current and shortening of the action potential duration. Atrial LCC is a multi-subunit complex consisting of the performing α1 subunit (encoded by CACNA1C) and auxiliary subunits, such as Cavβ2 and α2 δ, in which the function of CACNA1C would affect the atrial fibrillation. As shown in Figure 1, we therefore used online miRNA target prediction tools to search the regulatory miRNA of CACNA1C, and consequently identified CACNA1C as the candidate target gene of miR-3135b and miR-29a-3p in HL-1 cells with the 'seed sequence' in the 3'UTR of CACNA1C. To confirm expression of miR-3135b and miR-29a-3p in AF tissues, we evaluated the expression of miR-3135b and miR-29a-3p in 80 AF and normal frozen heart tissues using quantitative reverse transcriptase PCR (qRT-PCR). As shown in Figure 2, the expression level of miR-3135b was comparable in the 2 groups, while the expression level of miR-29a-3p in AF tissues was significantly higher than in controls.

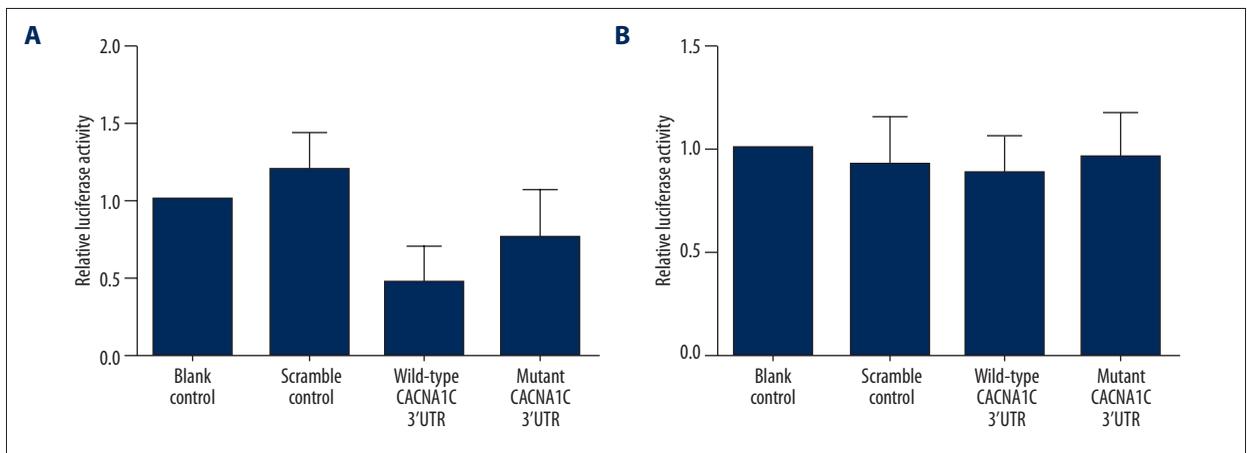
To validate the regulatory relationship between miR-29a-3p and miR-3135b and CACNA1C, we also conducted luciferase activity reporter assay in HEK293 cells. We found that only the luciferase activity from the cells co-transfected with miR-29a-3p and CACNA1C 3'UTR decreased significantly (Figure 3A), while cells co-transfected with miR-3135b and CACNA1C 3'UTR were comparable (Figure 3B). The results confirmed that CACNA1C was a validated target of miR-29a-3p.

To determine whether the expression of CACNA1C is controlled by miR-29a-3p, we collected tissue samples from the patients with or without AF, we found that miR-29a-3p levels were dramatically increased (Figure 2B), and also found a significant decrease in CACNA1C mRNA (Figure 4A) and protein (Figure 4B) in atrial tissues with AF compared to those without AF. These findings indicate the negative regulatory relationship between miR-29a-3p and CACNA1C.

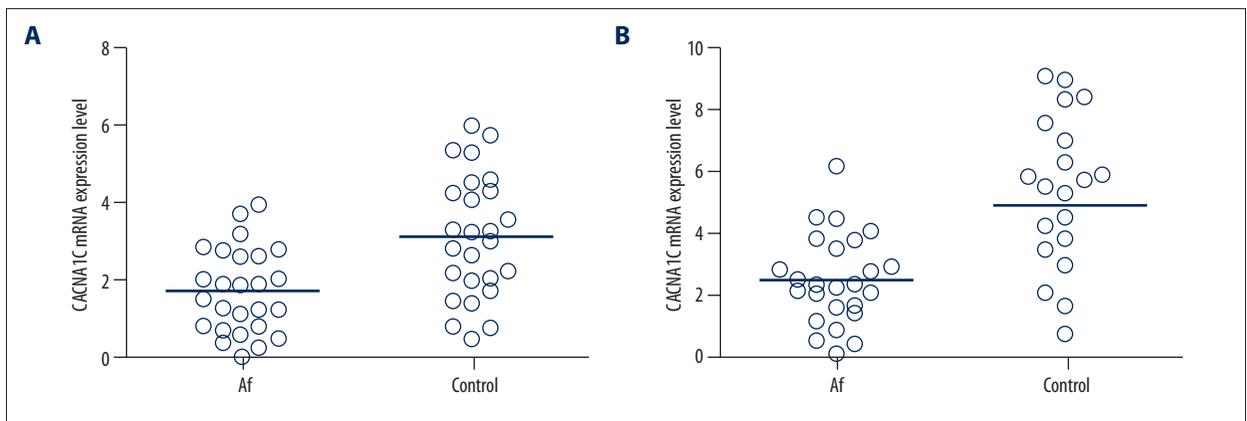
To further validate the hypothesis of the negative regulatory relationship between miR-29a-3p and CACNA1C, we investigated



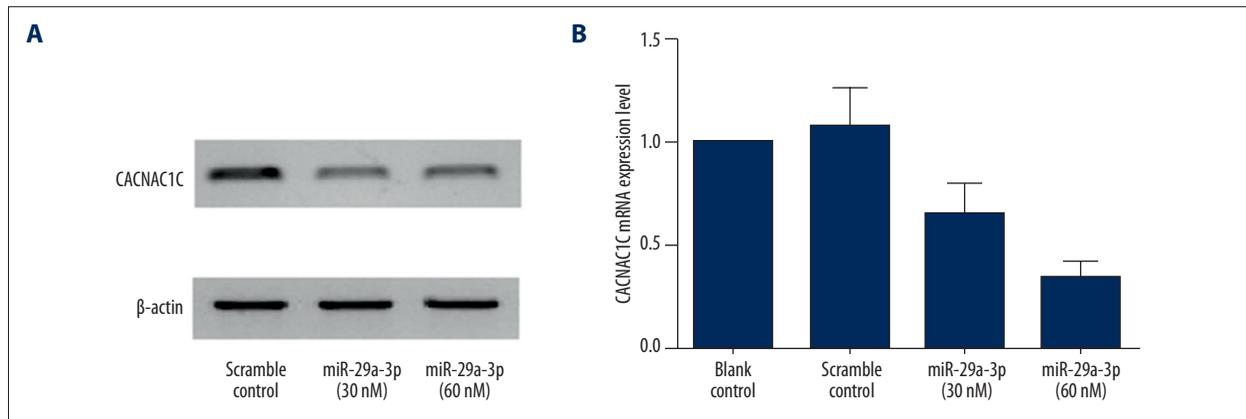
**Figure 2.** The expression level of miR-3135b (A) was comparable in the 2 groups, while the expression level of miR-29a-3p (B) in AF tissues was statistically higher than in controls.



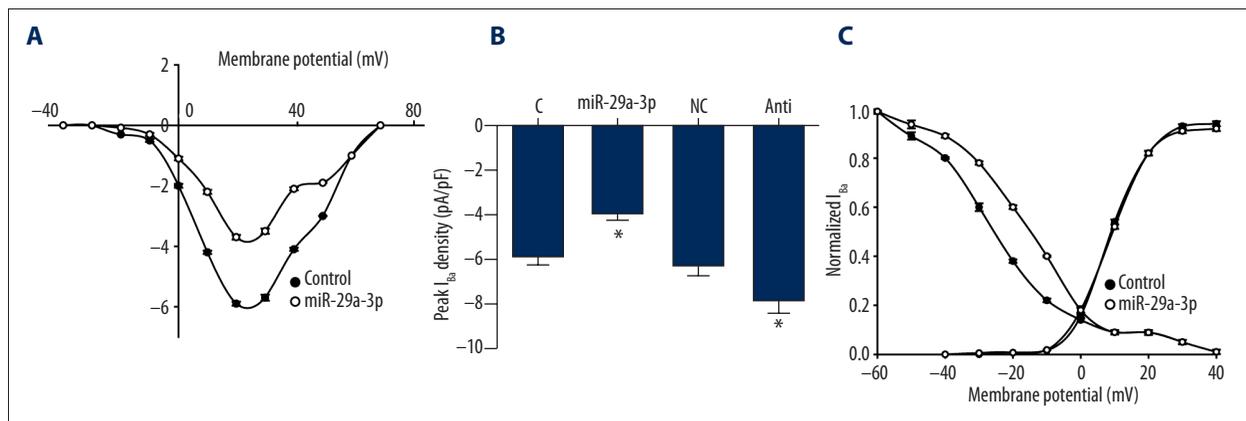
**Figure 3.** In luciferase activity reporter assay in HEK293 cells, we can see only that the luciferase activity from the cells co-transfected with miR-29a-3p and CACNA1C 3'UTR was decreased significantly (A), while cells co-transfected with miR-3135 b and CACNA1C 3'UTR were comparable (B). The results confirmed that CACNA1C was a validated target of miR-29a-3p in HEK293 cells.



**Figure 4.** A significant decrease in CACNA1C mRNA (A) and protein (B) in atrial tissues of mice with AF.



**Figure 5.** We investigated the mRNA/protein expression level of CACNA1C of atrial myocytes treated with scramble control, 30nM miR-29a-3p mimics, and 60nM miR-29a-3p mimics. The CACNA1C protein (A) and mRNA expression level (B) of atrial myocytes treated with 30nM miR-29a-3p mimics were clearly lower than in the scramble control, while those of the sample group treated with 60nM miR-29a-3p mimics were even lower than in the 30nM treatment group, validating the negative regulatory relationship between miR-499a and PFGFRB.



**Figure 6.** Current density reached its maximum value at +20 mV (n=30) and transfection of miR-29a-3p mimic significantly reduced I<sub>Ba</sub> density (n=33; P=0.02) and slowed the time course of inactivation (P=0.005), without modifying activation kinetics (A, B). I<sub>Ba</sub> density was not modified by the negative control miRNA (30 nmol/L; n=15), but was significantly increased by anti-miR-29a-3p (30 nmol/L, n=15; C), demonstrating a tonic inhibition of I<sub>Ba</sub> by endogenous miR-29a-3p in atrial myocytes.

the mRNA/protein expression level of CACNA1C of atrial myocytes treated with scramble control 30 nM or 60nM miR-29a-3p mimics. The CACNA1C protein (Figure 5A) and mRNA expression level (Figure 5B) of atrial myocytes treated with 30nM miR-29a-3p mimics were clearly lower than the scramble control, while those of the cells treated with 60nM miR-29a-3p mimics were even lower than in the 30nM treatment group, validating the negative regulatory relationship between the miRNA and mRNA.

### miR-29a-3p reduces the density of L-type calcium currents

qPCR experiments demonstrated that AF myocytes displayed greater miR-29a3p expression than non-AF myocytes and that there was a correlation between I<sub>Ca,L</sub> density and miR-29a-3p expression. We next analyzed whether miR-29a-3p transfection

in atrial myocytes cells produced the effects on the I<sub>Ca,L</sub> induced by electrical remodeling.

Currents through L-type Ca<sup>2+</sup> channels were recorded in I<sub>Ca,L</sub>-predominant cells using Ba<sup>2+</sup> as the charge carrier (I<sub>Ba</sub>). I<sub>Ba</sub> traces were recorded by applying the above protocol in cells transfected or not with miR-29a-3p mimic (30 nmol/L). As can be observed in Figure 6A, current density reached its maximum value at +20 mV (n=30) and transfection of miR-29a-3p mimic significantly reduced I<sub>Ba</sub> density (n=33; P=0.02) and slowed the time course of inactivation (P<0.01), without modifying activation kinetics (Figure 6A, 6B). I<sub>Ba</sub> density was not modified by the negative control miRNA (30 nmol/L; n=15), but was significantly increased by anti-miR-29a-3p (30 nmol/L, n=15; Figure 6C), demonstrating a tonic inhibition of I<sub>Ba</sub> by endogenous miR-29a-3p in atrial myocytes.

## Discussion

In this study we validated CACNA1C as a direct gene of miR-29a-3p, and established the negative regulatory relationship between miR-29a-3p and CACNA1C by using luciferase activity. We confirmed the negative regulatory relationship between miR-29a-3p and CACNA1C by treating the cells with miR-29a-3p mimics. We also found a tonic inhibition of  $I_{Ba}$  by endogenous miR-29a-3p in atrial myocytes.

Recent evidence indicated that miRNAs are involved in  $I_{Ca,L}$  downregulation. In this line, miR-1 and miR-328 decrease  $I_{Ca,L}$  expression by binding to 3'UTR of CACNA1C [17,25]. In contrast to patients in sinus rhythm (SR), expression of miR-29a-3p increased in the samples from chronic AF (CAF) patients [26]. Adam et al. [27] found a 2.5-fold upregulation of miR-29a-3p expression in left atrial appendages from patients with AF compared with samples from patients with SR. They believed that miR-29a-3p took part in atrial fibrosis formation and contributed to the structural remodeling induced by AF [27,28]. Indeed, cardiac fibroblasts have a higher expression level of miR-29a-3p, where it is implicated in atrial profibrillatory remodeling and activation of profibrotic pathways [29] in experimental models of heart failure [30]. However, miR-29a-3p expression in cardiac myocytes has not been consistently observed, and how it affects the electrical remodeling is currently unknown. We hypothesized that miR-29a-3p is upregulated in myocytes and is expressed in human atrial myocytes from patients with AF compared with those in SR, playing a role in the AF-related  $I_{Ca,L}$  decrease. In this study we used online miRNA target prediction tools to search for the regulatory miRNA of CACNA1C, and consequently identified CACNA1C as the candidate target gene of miR-3135b and miR-29a-3p in HEK293 cells with the 'seed sequence' in the 3'UTR of CACNA1C. To confirm expression of miR-3135b and miR-29a-3p in AF tissues, we evaluated the expression of miR-3135b and miR-29a-3p in 80 AF and normal frozen heart tissues using quantitative reverse transcriptase PCR (qRT-PCR). We showed that the expression level of miR-3135b was comparable in the 2 groups, while the expression level of miR-29a-3p in AF tissues was significantly higher than in controls. Furthermore, we performed luciferase assay to confirm that CACNA1C was a validated target of miR-29a-3p.

Encoded by CACNA1C gene, protein CaV1.2 is  $\alpha$ 1-subunits of cardiac L-type calcium channel (LTCC) and comprises the ion permeating subunit, which determines the main pharmacological and biophysical properties of the channel [31]. A consistent reduction of  $I_{Ca,L}$  density has been observed in AF experimental models as well as in CAF patients [32], and it is believed that the process was triggered by  $Ca^{2+}$  overload secondary to the rapid atrial rates [33]. The molecular mechanisms underlying  $I_{Ca,L}$  downregulation are complex and may include impaired

Cav1.2 protein trafficking induced by impaired Src kinase activity, ankyrin-B dysfunction, enhanced Cav1.2  $\alpha$ -subunit S-nitrosylation,  $Ca^{2+}$  channel dephosphorylation because of serine/threonine protein phosphatase activation,  $Ca^{2+}$  channel dephosphorylation because of serine/threonine protein phosphatase activation, activation of the  $Ca^{2+}$ /calmodulin/calcineurin/nuclear factor of activated T cells system, causing transcriptional downregulation of the Cav1.2  $\alpha$ -subunit or a zinc-binding protein [7,34–36]. In addition to all these mechanisms, miRNAs have been recently proposed to be involved in AF-related  $I_{Ca,L}$  downregulation [17,37,38]. Only miR-328 has been shown to regulate  $Ca^{2+}$  channel  $\alpha$ 1c and  $\beta$  subunits expression among all the miRNAs that are upregulated in CAF patients [17]. MiR-328 is increased in right atrial appendages from CAF patients but not in those with SR. Overexpression of miR-328 through transgenic manipulation in mice and adenoviral infection in canine atria enhanced AF susceptibility, decreased  $I_{Ca,L}$  density, and shortened atrial APD as a result of the repression of CACNA1C expression. Other miRNAs, such as miR-26 and miR-1, have been reported to play important roles in AF-related electrical remodeling, mainly by targeting Kir2.1 channels [16,18,39]. Both miRNAs take part in the shortening of the APD as well as the AF-related increase of the inward rectifier. Consequently, we consider that all these miRNAs (miR-1, miR-29a-3p, miR-26, and miR-328) may contribute to AF-related electrical remodeling and eventually to the perpetuation of the arrhythmia. In this study, we investigated the mRNA/protein expression level of CACNA1C of atrial myocytes treated with scramble control, 30nM miR-29a-3p mimics, and 60nM miR-29a-3p mimics. The CACNA1C protein (Figure 5A) and mRNA expression level (Figure 5B) of atrial myocytes treated with 30nM miR-29a-3p mimics were clearly lower than in the scramble control, while those of the sample group treated with 60nM miR-29a-3p mimics were even lower than in the 30nM treatment group, validating the negative regulatory relationship between the miRNA and mRNA. In addition, we observed that current density reached its maximum value at +20 mV ( $n=30$ ) and transfection of miR-29a-3p mimic significantly reduced  $I_{Ba}$  density ( $n=33$ ;  $P=0.02$ ) and slowed the time course of inactivation ( $P=0.005$ ), without modifying activation kinetics (Figure 6A, 6B).  $I_{Ba}$  density was not modified by the negative control miRNA (30 nmol/L;  $n=15$ ), but was significantly increased by anti-miR-29a-3p (30 nmol/L;  $n=15$ ; Figure 6C), demonstrating a tonic inhibition of  $I_{Ba}$  by endogenous miR-29a-3p in atrial myocytes.

The present study has certain limitations. Firstly, the sample size was relative small and further studies with larger populations and different ethnic backgrounds are warranted. Secondly, no animal model was used in this study, which makes the study less persuasive. Further animal experiments, especially in transgenic animal models, is need to confirm the conclusions drawn in this study.

## Conclusions

Our results indicate that miR-29a-3p is substantially overexpressed in myocytes isolated from AF patients. In addition, the upregulation of miR-29a-3p downregulated Ca<sup>2+</sup> channel subunits expression, and thereby decreased I<sub>Ca,L</sub> density. These

findings indicate that that miR-29a-3p can take part in the I<sub>Ca,L</sub> downregulation in the development of AF.

## Conflict of interest

No conflict of interest is declared.

## References:

1. Murphy NF, Simpson CR, Jhund PS et al: A national survey of the prevalence, incidence, primary care burden and treatment of atrial fibrillation in Scotland. *Heart*, 2007; 93(5): 606–12
2. Chang CM, Wu TJ, Zhou S et al: Nerve sprouting and sympathetic hyperinnervation in a canine model of atrial fibrillation produced by prolonged right atrial pacing. *Circulation*, 2001; 103(1): 22–25
3. Yu FS, Zhang Y, Feng Y et al: [Nerve remodeling in a canine model of atrial fibrillation induced by 48 hours right atrial pacing]. *Zhonghua Xin Xue Guan Bing Za Zhi*, 2010; 38(7): 644–47
4. Yu L, Scherlag BJ, Sha Y et al: Interactions between atrial electrical remodeling and autonomic remodeling: How to break the vicious cycle. *Heart Rhythm*, 2012; 9(5): 804–9
5. Wijffels MC, Kirchhof CJ, Dorland R, Allesie MA: Atrial fibrillation begets atrial fibrillation. A study in awake chronically instrumented goats. *Circulation*, 1995; 92(7): 1954–68
6. Schotten U, Verheule S, Kirchhof P, Goette A et al: Pathophysiological mechanisms of atrial fibrillation: a translational appraisal. *Physiol Rev*, 2011; 91(1): 265–325
7. Wakili R, Voigt N, Kääh S et al: Recent advances in the molecular pathophysiology of atrial fibrillation. *J Clin Invest*, 2011; 121(8): 2955–68
8. Van Wagoner DR, Pond AL, Lamorgese M et al: Atrial L-type Ca<sup>2+</sup> currents and human atrial fibrillation. *Circ Res*, 1999; 85(5): 428–36
9. Yue L, Melnyk P, Gaspo R et al: Molecular mechanisms underlying ionic remodeling in a dog model of atrial fibrillation. *Circ Res*, 1999; 84(7): 776–84
10. Caballero R, de la Fuente MG, Gómez R et al: In humans, chronic atrial fibrillation decreases the transient outward current and ultrarapid component of the delayed rectifier current differentially on each atria and increases the slow component of the delayed rectifier current in both. *J Am Coll Cardiol*, 2010; 55(21): 2346–54
11. Brundel BJ, Van Gelder IC, Henning RH et al: Ion channel remodeling is related to intraoperative atrial effective refractory periods in patients with paroxysmal and persistent atrial fibrillation. *Circulation*, 2001; 103(5): 684–90
12. Christ T, Boknik P, Wöhr S et al: L-type Ca<sup>2+</sup> current downregulation in chronic human atrial fibrillation is associated with increased activity of protein phosphatases. *Circulation*, 2004; 110(17): 2651–57
13. He L, Hannon GJ: MicroRNAs: small RNAs with a big role in gene regulation. *Nat Rev Genet*, 2004; 5(7): 522–31
14. Cooley N, Cowley MJ, Lin RC et al: Influence of atrial fibrillation on microRNA expression profiles in left and right atria from patients with valvular heart disease. *Physiol Genomics*, 2012; 44(3): 211–19
15. Liu H, Chen GX, Liang MY et al: Atrial fibrillation alters the microRNA expression profiles of the left atria of patients with mitral stenosis. *BMC Cardiovasc Disord*, 2014; 14: 10
16. Girmatsion Z, Biliczki P, Bonauer A et al: Changes in microRNA-1 expression and IK1 up-regulation in human atrial fibrillation. *Heart Rhythm*, 2009; 6(12): 1802–9
17. Lu Y, Zhang Y, Wang N et al: MicroRNA-328 contributes to adverse electrical remodeling in atrial fibrillation. *Circulation*, 2010; 122(23): 2378–87
18. Luo X, Pan Z, Shan H et al: MicroRNA-26 governs profibrillatory inward-rectifier potassium current changes in atrial fibrillation. *J Clin Invest*, 2013; 123(5): 1939–51
19. Divakaran V, Mann DL: The emerging role of microRNAs in cardiac remodeling and heart failure. *Circ Res*, 2008; 103(10): 1072–83
20. Wang Z, Lu Y, Yang B: MicroRNAs and atrial fibrillation: new fundamentals. *Cardiovasc Res*, 2011; 89(4): 710–21
21. Nattel S, Burstein B, Dobrev D: Atrial remodeling and atrial fibrillation: mechanisms and implications. *Circ Arrhythm Electrophysiol*, 2008; 1(1): 62–73
22. Tanaka K, Zlochiver S, Vikstrom KL et al: Spatial distribution of fibrosis governs fibrillation wave dynamics in the posterior left atrium during heart failure. *Circ Res*, 2007; 101(8): 839–47
23. Barana A, Matamoros M, Dolz-Gaitón P et al: Chronic atrial fibrillation increases microRNA-21 in human atrial myocytes decreasing L-type calcium current. *Circ Arrhythm Electrophysiol*, 2014; 7(5): 861–68
24. Zhang Y, Zheng S, Geng Y et al: MicroRNA profiling of atrial fibrillation in canines: miR-206 modulates intrinsic cardiac autonomic nerve remodeling by regulating SOD1. *PLoS One*, 2015; 10(3): e0122674
25. Rau F, Freyermuth F, Fugier C et al: Misregulation of miR-1 processing is associated with heart defects in myotonic dystrophy. *Nat Struct Mol Biol*, 2011; 18(7): 840–45
26. Liu Z, Zhou C, Liu Y et al: The expression levels of plasma microRNAs in atrial fibrillation patients. *PLoS One*, 2012; 7(9): e44906
27. Adam O, Löhfelm B, Thum T et al: Role of miR-21 in the pathogenesis of atrial fibrosis. *Basic Res Cardiol*, 2012; 107(5): 278
28. Dobrev D: Is altered atrial microRNA-ome a critical contributor to the pathophysiology of atrial fibrillation? *Basic Res Cardiol*, 2012; 107(5): 284
29. Thum T, Gross C, Fiedler J et al: MicroRNA-21 contributes to myocardial disease by stimulating MAP kinase signalling in fibroblasts. *Nature*, 2008; 456(7224): 980–84
30. Cardin S, Guasch E, Luo X et al: Role for MicroRNA-21 in atrial profibrillatory fibrotic remodeling associated with experimental postinfarction heart failure. *Circ Arrhythm Electrophysiol*, 2012; 5(5): 1027–35
31. Catterall WA: Structure and function of voltage-gated sodium and calcium channels. *Curr Opin Neurobiol*, 1991; 1(1): 5–13
32. Sabashnikov A, Weymann A, Haldar S et al: Position of totally thoracoscopic surgical ablation in the treatment of atrial fibrillation: An alternative method of conduction testing. *Med Sci Monit Basic Res*, 2015; 21: 76–80
33. Goette A, Honeycutt C, Langberg JJ: Electrical remodeling in atrial fibrillation. Time course and mechanisms. *Circulation*, 1996; 94(11): 2968–74
34. Doshi D, Jayawardana R: Amiodarone-induced life-threatening refractory hypotension. *Am J Case Rep*, 2015; 16: 617–20
35. Cunha SR, Hund TJ, Hashemi S et al: Defects in ankyrin-based membrane protein targeting pathways underlie atrial fibrillation. *Circulation*, 2011; 124(11): 1212–22
36. Greiser M, Halaszovich CR, Frechen D et al: Pharmacological evidence for altered src kinase regulation of I<sub>(Ca,L)</sub> in patients with chronic atrial fibrillation. *Naunyn Schmiedebergs Arch Pharmacol*, 2007; 375(6): 383–92
37. Castillo JM, Afanador HF, Manjarrez E, Morales XA: Non-traumatic spontaneous spinal subdural hematoma in a patient with non-valvular atrial fibrillation during treatment with rivaroxaban. *Am J Case Rep*, 2015; 16: 377–81
38. Zhao Z, Liu T, Wang X, Li G: MicroRNAs as novel antiarrhythmic targets for atrial fibrillation. *Int J Cardiol*, 2013; 168(5): e135–37
39. Kathrotia A, Hindupur MR: Massive purulent pericardial effusion presenting as atrial fibrillation with rapid rate: Case report and review of the literature. *Am J Case Rep*, 2014; 15: 504–7