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Remodeling of $K_v1.5$ Channel in Right Atria from Han Chinese Patients with Atrial Fibrillation

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

A Xian-hong Ou*
D Miao-ling Li*
B Rui Liu*
C Xin-rong Fan
F Liang Mao
E Xue-hui Fan
D Yan Yang
G Xiao-rong Zeng

Institute of Cardiovascular Research, Luzhou Medical College, Luzhou, Sichuan, P.R. China

* These authors contributed equally to this paper

Corresponding Author: Xiaorong Zeng, e-mail: zxr8818@vip.sina.com

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Background: The incidence of atrial fibrillation (AF) in rheumatic heart diseases (RHD) is very high and increases with age. Occurrence and maintenance of AF are very complicated process accompanied by many different mechanisms. Ion-channel remodeling, including the voltage-gated potassium channel $K_v1.5$, plays an important role in the pathophysiology of AF. However, the changes of $K_v1.5$ channel expression in Han Chinese patients with RHD and AF remain poorly understood. The aim of the present study was to investigate whether the $K_v1.5$ channels of the right atria may be altered with RHD, age, and sex to contribute to AF.

Material/Methods: Right atrial appendages were obtained from 20 patients with normal cardiac functions who had undergone surgery, and 26 patients with AF. Subjects were picked from 4 groups: adult and aged patients in normal sinus rhythm (SR) and AF. Patients were divided into non-RHD and RHD groups or men and women groups in normal SR and AF, respectively. The expression of $K_v1.5$ protein and messenger RNA (mRNA) were measured using Western blotting and polymerase chain reaction (PCR) method, respectively.

Results: Compared with the SR group, the expression of $K_v1.5$ protein decreased significantly in the AF group. However, neither $K_v1.5$ protein nor *KCNA5* mRNA had significant differences in adult and aged groups, non-RHD and RHD group, and men and women group of AF.

Conclusions: The expression of $K_v1.5$ channel protein changes with AF but not with age, RHD, and sex in AF.

MeSH Keywords: Aging • Atrial Fibrillation • $K_v1.5$ Potassium Channel • Rheumatic Heart Disease • Sex

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Background

Atrial fibrillation (AF) is currently the most common sustained arrhythmia in humans and is associated with increased risks of stroke and heart failure. It can occur at any age but is very rare in children and becomes extremely common in the elderly, with a prevalence approaching 20% in patients >85 years of age [1]. Occurrence and maintenance of AF are very complicated process accompanied by atrial electrical, contractile, and structural remodeling [2,3]. The changes of the L-type Ca^{2+} channel and several potassium channels involved in atrial electrical remodeling [4], which result in shortening of the atrial effective refractory period (AERP) and slowing of exciting conduction velocity. These expression and functional changes of channel protein may cause AF or result in AF, which provide the substrate for occurrence and sustain of AF.

K_v1.5 channels play an important role in the pathophysiology of AF. K_v1.5 channels, encoded by *KCNA5*, carry ultra-rapid delayed rectified potassium current (I_{Kur}), which is much more abundant in atria than in ventricles. It has been shown that reduction of I_{Kur} prolongs the AERP and 90% of duration of action potential (APD_{90}) in remodeled atrial cells. However, the changes of K_v1.5 channel proteins in patients with RHD and AF remain poorly understood (no change [5,6], decreased [7,8]). Therefore, the purpose of this study was to analyze the expression of K_v1.5 channel protein and gene in Han Chinese patients with AF and its relationship with RHD, age, sex, and other echocardiographic parameters.

Material and Methods

Human atrial tissue collection

Human right atrial appendages were obtained from 20 patients with sinus rhythm undergoing open heart surgery for valve repair or coronary artery bypass grafting at Luzhou Hospital Affiliated to Luzhou Medical College (China) were enrolled. Among these, 26 cases presented with AF. AF was defined as duration of AF longer than 3 months as documented by electrocardiogram (ECG) characteristics include: (1) irregular R-R intervals (when atrioventricular [AV] conduction is present); (2) absence of distinct repeating P waves; and (3) irregular atrial activity, according to 2014 AHA/ACC/HRS Guideline for the Management of Patients With Atrial Fibrillation [9]. In addition, all tissue specimens were immediately frozen in liquid nitrogen and stored at -80°C. The human tissue collection protocol was approved by the Ethics Committee of Luzhou Medical College. Written informed patient consent was obtained from the patient's family. The characterization of the patients is shown in the table.

Echocardiography

Echocardiographic examination were performed with a Philips iE33 ultrasound system (Andover, Md., USA) with 1–5 MHz transducers, according to the guidelines of the American Society of Echocardiography[10]. Patients were examined in the left lateral position.

Western blots

Atrial samples were stored at –80°C before protein extraction and western blot analysis. Frozen tissue samples were extracted into extraction buffer, homogenates were centrifuged at 14,000 *g* for 10 min, the supernatant was decanted, and protein concentration was determined using the bicinchoninic acid assay. Extracted protein samples were denatured at 95°C for 5 min. Denatured proteins were separated on 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS/PAGE) and transferred on polyvinylidene fluoride (Immun-Blot® PVDF membrane; Bio-Rad, Hercules, CA, USA)). Membranes were blocked for 1 h in Tris Buffered Saline with Tween-20 (TBST) containing 5% nonfat dry milk at room temperature.

The membranes were incubated with rabbit polyclonal antibodies recognizing K_v1.5 (1/200 dilution, BlueGene Biotech CO., LTD., Shanghai, China) and GAPDH (1/5,000, Santa Cruz Biotechnology, Texas, USA)) at 4°C over night (approximately 14 h). After washing in TBST, the blots were incubated with a biotin (1: 10,000, Millipore, Bedford, MA, USA) for 1 h and anti-horseradish peroxidase (HRP)-conjugated antibodies (1:50,000, Santa Cruz) for 30 min at room temperature. The blots were washed again in TBST, and the bands were detected using ECL western blotting substrate (Pierce Biotechnology Inc, Rockford, IL. USA) and quantified using Quantity One software version 4.2.6 (Bio-Rad). The immunoblot experiments were repeated at least 3 times. The GAPDH protein served as a loading control. The mean normalized optical density (OD) of detected K_v1.5 protein bands relative to the OD of the GAPDH band from the same individual was calculated and subjected to statistical analyses.

Real-time reverse transcription polymerase chain reaction

Gene expression was validated by real-time PCR using by SYBR Green Real-Time Q-PCR (CFD-3220, MJ, USA). Open-chest cardiac surgery was performed and 50-100 mg samples of right atrial appendages were rapidly collected with sterilized Eppendorf tubes including free-RNA liquid. Total cellular RNA was extracted from 100-200 mg human right atrial appendages using the TRIzol reagent (DP419, TIANGEN, Beijing, China), followed by chloroform extraction and isopropanol precipitation, DNAse treatment, and quality control with polyacrylamide gel electrophoresis. RNA concentration and purity were evaluated using

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Table 1. Clinical and echocardiographic parameters.

Parameter	SR (*n*=20)	AF (*n*=26)	*P* value
Age, yr	32±19	50±9	<0.05
Male/Female	8:12	19:7	<0.05
Underlying heart disease (rheumatic: non-rheumatic)	3:8	17:9	<0.05
Hypertension,%	1	4	
SBP, mmHg	102±9	132±20	
DBP, mmHg	64±10	82±19	
LA diameter, mm	26±12	53±8	<0.05
RV diameter, mm	16±6	35±19	<0.05
LVESD, mm	36±12	53±6	<0.05
LVEDD, mm	25±10	38±6	<0.05
LVPW, mm	7±3	9±1	
LVEF,%	53±8	49±7	
RVOT, mm	20±6	26±4	
AOD, mm	22±5	27±4	
AAO, mm	23±7	36±5	<0.05
IVS, mm	7±3	9±1	
MPA,mm	17±6	25±4	
mPG	59±32	33±3	<0.05

a spectrophotometer. Aliquots of RNA were stored at -80°C until use. First-strand cDNA was synthesized from 2 μg of total RNA with High Capacity cDNA Archive Kits. The sequences of *KCNA5* and β-actin were provided by Sangon Biotech (Shanghai, China). *KCNA5* (F: 5'-GACGAGATACGCTTCAAC-3'; R: 5'-ATGATGGAGATGAGGATAAC-3') and β-actin (F: 5'-TGGCACCCAGCACAA TGAA-3'; R: 5'-CTAAGTCATAGTCCGCC TAGAAGCA-3'). PCR reaction mixtures contained cDNA template, PCR primers, free-ddH_2O and 1×ABsolute QPCR SYBR Green Mix in a final volume of 20 μL. Real-time PCR conditions included enzyme activation at 95°C for 15 min, followed by 40 cycles (94°C for 30 sec, 58°C for 30 sec, 72°C for 30 sec and 78°C for 1 sec), plate read 72°C for 10 min, melting curve from 65°C for 98°C, read every 1°C, hold 1 sec, 72°C 10 min. Size of amplicons and absence of nonspecific products were controlled by melting curves and gel electrophoresis.

Starting quantities of target cDNAs were calculated by comparing threshold cycle (C_T) values of each sample with C_T values of the respective standard curve with the use of Mastercycler Ep Realplex software version 2.0 (Eppendorf, Hamburg, Germany). Expression levels of target genes were normalized to β-actin transcript levels. Data are given as mean ±SD of normalized gene expression levels from 3 differentiation experiments.

Statistical analysis

Data are presented as means ±SD. The term *n* represented the number of patients. Paired and/or unpaired Student's *t*-test was used as appropriate to evaluate the statistical significance of differences between 2 group means and ANOVA was used for multiple groups. Linear regression was used for correlation between the protein or mRNA amount and age, sex, and RHD. $P<0.05$ was considered statistically significant. SPSS version 19.0.0 for Windows (SPSS Inc., Chicago, IL, USA) was used for data analysis.

Results

Clinical parameters of the enrolled patients

Parameters such as age, sex, underlying heart disease, atrial diameter, ventricular diameter, left ventricular end-systolic diameter (LVESD), left ventricular end-diastolic diameter (LVEDD), aorta ascendens (AAO), and mean pressure gradient (mPG) were significantly different between the SR and AF groups (Table 1). Briefly, aged patients, male patients, and patients with RHD might be more likely to suffer from AF. AF

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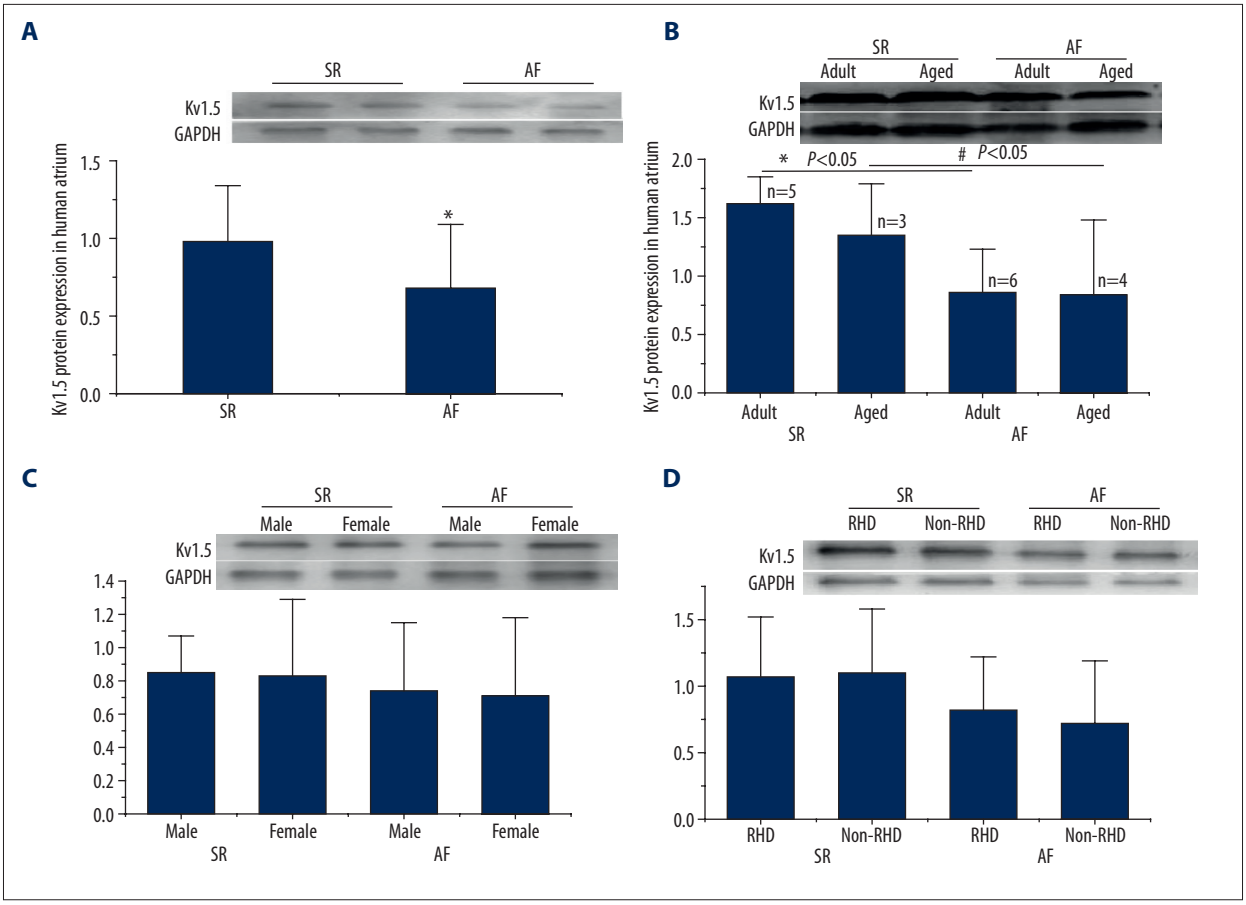

Figure 1. The protein expression of K_v1.5. (**A**) Ahows that the protein expression of K_v1.5 in SR and AF groups. (**B**) Shows that the relationship between the protein expression of K_v1.5 and aging. Subjects were picked from 4 groups: adult patients (n=5, mean age 53.2±2.6) and aged patients (n=3, age 65.0±3.0) in SR and adult patients (n=6, mean age 55.8±1.5) and aged patients (n=4, mean age 69.0±1.0) with AF. (**B**) Shows that K_v1.5 expression is significantly decreased in AF-aged group than SR-aged group, but there was no significant difference between AF-adult group and AF-aged group. (**C, D**) Show that the relationship of the protein expression of K_v1.5 with sex and RHD, respectively. Patients were divided into male and female group or non-RHD and RHD group in normal SR and AF, respectively. (**C, D**) Shows that there was no significant difference between non-RHD and RHD group or male and female groups.

could lead to atrial or ventricular hypertrophy, higher LVESD, LVEDD, or AAO, and lower mPG.

The expression of K_v1.5 protein of atrium

As shown in Figure 1A, the levels of K_v1.5 were significantly decreased to 0.68±0.41 in the AF group, and the levels in the normal control group were 0.98±0.36. In order to observe the relationship of K_v1.5 with ages, sex, heart disease in AF, subjects were picked from 4 groups: 51~60 years old patients (n=5, mean age 53.2±2.6 years) and over 60 years old patients (n=3, age 65.0±3.0 years) in SR and 51~60 years old patients (n=6, mean age 55.8±1.5 years) and over 60 years old patients (n=4, mean age 69.0±1.0 years) with AF. Meanwhile, patients were divided into non-RHD and RHD groups or male and female groups in normal SR and AF, respectively. Figure 1B shows that K_v1.5 expression is significantly decreased in AF-aged group than SR-aged group, but there was no significant difference between non-RHD and RHD group or male and female groups (Figure 1B–1D). Finally, linear regression was used to verify the correlation between the protein amount in AF and age, sex and RHD. Table 2 showed that the mount of K_v1.5 protein in patients with AF had no correlation with age, sex and RHD.

The expression of K_v1.5 mRNA of atrium

To investigate whether levels of *KCNA5* gene was altered in comparison to those in normal control, total RNA was extracted from the harvested atrial tissues, and RT-PCR was used to detect the gene expression. The results showed that *KCNA5* expression was no significant difference in the AF group when compared with the SR group (Figure 2A). We analyzed the relationship of *KCNA5* expression with ages, sex and RHD in AF. The results showed that there were not significant difference

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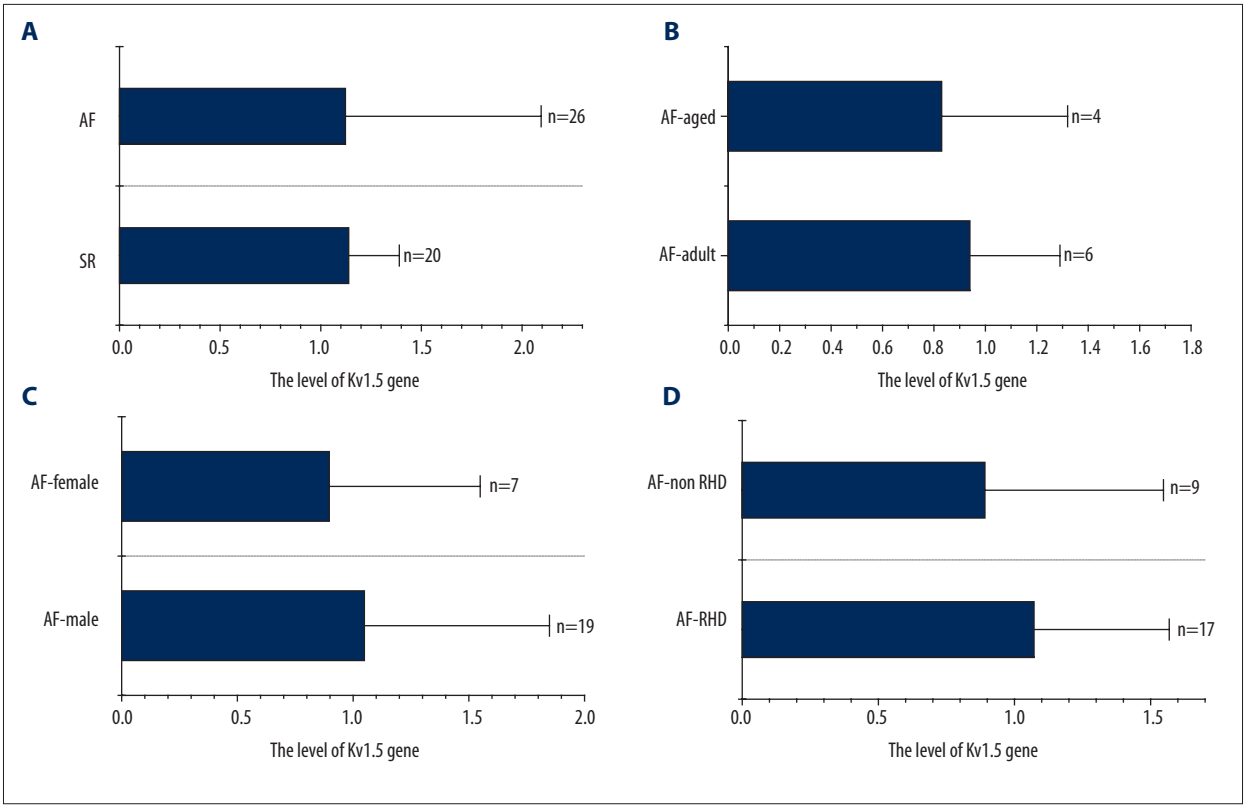

Figure 2. The mRNA expression of K_v1.5. (**A**) Shows that *KCNA5* expression was no significant difference in the AF group when compared with the SR group. (**B–D**) Shows that there were no significant differences in adult and aged group, non-RHD and RHD group, in men and women group of AF.

Table 2. Relation of K_v1.5 remodeling and age, sex, RHD of patients with AF (*P* value, *n*=26).

Parameters	mRNA	Protein
Age	0.076	0.637
Sex	0.156	0.411
RHD	0.799	0.878

in adult and aged group, non-RHD and RHD group, in men and women group of AF (Figure 2B–2D, Table 2).

Discussion

In this study, we found that aging population and rheumatic heart disease increased the risk of incident AF, and men may be more prone to AF than women. The K_v1.5 protein expression was reduced in chronic AF but mRNA levels were not different between AF and SR patients. The changes of K_v1.5 protein and mRNA levels were mostly related to AF, not cardiac disease, age or gender, might be a risk factor to this ion channel remodeling.

Comparisons with previous observations of KP_v1.5 remodeling in AF

I_{Kur} is found to be much more abundant in atria than in ventricles [11]. Therefore, it has been the primary foci of many researchers regarding atrial electrical remodeling and sensitively potential target of atrial selective drugs for anti-AF. Van Wagoner et al. [12] reported that K_v1.5 proteins were decreased in AF, which was confirmed by Brundel et al. [4] and this study. However, we did not find differences in the mRNA levels of K_v1.5 between AF and SR patients. Similar results were reported by Grammer et al. [6] and Brundel et al. [13], but Lai et al. [7] and Oh et al. [14] reported that K_v1.5 mRNA was reduced in AF patients. There are 3 possible expiations for this discrepancy. Firstly, incidence of AF varies widely in different populations[15]. Our samples all came from Han Chinese, whose prevalence of AF is 0.7–1.1%[15]. The differences in AF risk across individuals as well as racial groups are likely to be related to their genetic factors [15]. Secondly, drugs administer to treat cardiovascular diseases might also influence the gene expression and its translation. The mount of mRNA is greatly different in different periods of AF. Many reports showed that remodeling of ion channels occurs during persistent AF but not during paroxysmal AF. Finally, hypertension and other factors

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might have the influence on K_v1.5 protein expression. Since the number is limited, we did not analyze it.

Difference of K_v1.5 expressing in different site of atrium

In this study, we collected the right atrial appendages as a proxy. Van Wagoner et al. [12] found that the expression of K_v1.5 protein was reduced in both the left and the right atrial appendages of AF patients, and the changes had no significant difference between 2 sites. Lai et al. [7] detected the mRNA levels of the right atrial free wall, right atrial appendage, left atrial free wall and left atrial appendage, respectively. He found that the mRNA levels of the genes (including K_v1.5) among the 4 atrial sampling sites were not significantly different in the same patient. Although the trigger and maintenance of AF were mainly in left atrium, right atrial appendage is more accessible for sampling than the left atrial (LA) appendage[4,16,17]. Because the majority of studies investigating human tissue are restricted to the RA only [18], collecting both right and left atrial appendages is difficult in clinics. Therefore, we hypothesized that the tissues from right atrial appendages have the same presentation as the tissues from left atrial appendages.

Relation of K_v1.5 Remodeling and diseases and other factors

We analyzed the influence of all clinical parameters. The results are summarized in Table 1. We showed that there is an obvious positive correlation between AF and some clinical parameters, including gender, age, rheumatic cardiac disease, LA diameter, RV diameter, LVESD and LVEDD. Notably, more and more women suffer with AF nowadays[19].

The further study showed that age, gender and heart disease, had no significant effects on the expression of K_v1.5 proteins and genes. As all we know, the incidence and prevalence of AF increases with age [20]. Alterations in structure and function of atrial ion channels associated with aging provide the substrate for AF[21]. However, Lai et al. [7] reported that AF was the only factor that influenced K_v1.5 mRNA amount; sex, age, right atrial pressure, pulmonary wedge pressure, and the rheumatic heart disease had no significant effects on the expression of the genes (K_v1.5), which is similar to ours.

Limitations of the study

The number of patients is small. Therefore, the present data cannot be extrapolated uncritically to all patients with AF. In addition, drugs might affect the ion channel protein and gene expression.

Conclusions

We found that AF is associated with down-regulation of K_v1.5 proteins and age-related alterations in atrial tissues were attributed to the increased expression of K_v1.5. However, the specific factors initiating K_v1.5 channel remodeling is unclear. It may be associated with abnormal energy metabolism, losing control of ion current, or others in AF, which still need to further study.

References:

1. Heeringa J, van der Kuip DA, Hofman A et al: Prevalence, incidence and lifetime risk of atrial fibrillation: The rotterdam study. Eur Heart J, 2006; 27: 949–53
2. Nattel S, Burstein B, Dobrev D: Atrial remodeling and atrial fibrillation: Mechanisms and implications. Circ Arrhythm Electrophysiol, 2008; 1(1): 62–73
3. Allessie M, Ausma J, Schotten U: Electrical, contractile and structural remodeling during atrial fibrillation. Cardiovasc Res, 2002; 54: 230–46
4. 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: 684–90
5. Bosch RF, Zeng X, Grammer JB et al: Ionic mechanisms of electrical remodeling in human atrial fibrillation. Cardiovasc Res, 1999; 44: 121–31
6. Grammer JB, Bosch RF, Kuhlkamp V, Seipel L: Molecular remodeling of kv4.3 potassium channels in human atrial fibrillation. J Cardiovasc Electrophysiol, 2000; 11: 626–33
7. Lai LP, Su MJ, Lin JL et al: Changes in the mrna levels of delayed rectifier potassium channels in human atrial fibrillation. Cardiology, 1999; 92: 248–55
8. Van Wagoner DR, Pond AL, McCarthy PM et al: Outward k+ current densities and kv1.5 expression are reduced in chronic human atrial fibrillation. Circ Res, 1997; 80: 772–81
9. January CT, Wann LS, Alpert JS et al: 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: 2071–104
10. Schiller NB, Shah PM, Crawford M et al: Recommendations for quantitation of the left ventricle by two-dimensional echocardiography. American society of echocardiography committee on standards, subcommittee on quantitation of two-dimensional echocardiograms. J Am Soc Echocardiogr, 1989; 2(5): 358–67
11. Tamkun MM, Knoth KM, Walbridge JA et al: Molecular cloning and characterization of two voltage-gated k+ channel cdnas from human ventricle. FASEB J, 1991; 5: 331–37
12. Van Wagoner DR, Pond AL, McCarthy PM et al: Outward k+ current densities and kv1.5 expression are reduced in chronic human atrial fibrillation. Circ Res, 1997; 80: 772–81
13. Brundel BJ, Van Gelder IC, Henning RH et al: Alterations in potassium channel gene expression in atria of patients with persistent and paroxysmal atrial fibrillation: Differential regulation of protein and mrna levels for k+ channels. J Am Coll Cardiol, 2001; 37(3): 926–32
14. Billman GE: Vernakalant, a mixed sodium and potassium ion channel antagonist that blocks k(v)1.5 channels, for the potential treatment of atrial fibrillation. Curr Opin Investig Drugs, 2010; 11: 1048–58
15. Lau CP, Tse HF, Siu CW, Gbadebo D: Atrial electrical and structural remodeling: Implications for racial differences in atrial fibrillation. J Cardiovasc Electrophysiol, 2012; 23(Suppl.1): S36–40
16. Gao M, Wang J, Wang Z et al: An altered expression of genes involved in the regulation of ion channels in atrial myocytes is correlated with the risk of atrial fibrillation in patients with heart failure. Exp Ther Med, 2013; 5: 1239–43

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17. Voigt N, Heijman J, Wang Q et al: Cellular and molecular mechanisms of atrial arrhythmogenesis in patients with paroxysmal atrial fibrillation. Circulation, 2014; 129: 145–56
18. Slagsvold KH, Johnsen AB, Rognmo O et al: Comparison of left versus right atrial myocardium in patients with sinus rhythm or atrial fibrillation – an assessment of mitochondrial function and microrna expression. Physiol Rep, 2014; 2(8): pii: e12124
19. Trovato GM, Pace P, Cangemi E et al: Gender, lifestyles, illness perception and stress in stable atrial fibrillation. Clin Ther, 2012; 163: 281–86
20. Xu GJ, Gan TY, Tang BP et al: Age-related changes in cellular electrophysiology and calcium handling for atrial fibrillation. J Cell Mol Med, 2013; 17: 1109–18
21. Dun W, Boyden PA: Aged atria: Electrical remodeling conducive to atrial fibrillation. J Interv Card Electrophysiol, 2009; 25: 9–18

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