CORRELATION OF ATRIAL FIBRILLATION WITH RENIN-ANGIOTENSIN-ALDOSTERONE SYSTEM GENE POLYMORPHISM

SHUXIN HOU, YINGMIN LU, DAMIN HUANG, XIAOHAN LUO, ZHAOXIA WANG, JINCHUN ZHANG, WEIPING XU
Department of Cardiology, XinHua (Chongming) Hospital Affiliated to School of Medicine, Shanghai Jiao Tong University, Shanghai 202150, China

ABSTRACT

Background: This study aimed to investigate renin-angiotensin-aldosterone system gene polymorphism in atrial fibrillation (AF) patients.

Materials and methods: 82 AF patients diagnosed in this survey were included for investigation, and 82 subjects without AF served as controls.

Results: There were 3 genotypes of AGT M235T in the subjects investigated: MM genotype (7.32% vs. 13.41%), MT (26.83% vs. 35.37%) and TT (65.85% vs. 51.22%). The TT genotype frequency and T allele frequency in AF group were significantly higher than in control group (P<0.05). Logistic regression analysis showed T allele increased the risk for AF by 1.57 times (P<0.05). There were 3 genotypes of ACE AluI/D in the subjects investigated: II genotype (17.07% vs. 46.34%), ID genotype (51.22% vs. 34.15%) and DD genotype (31.71% vs. 19.51%). The DD genotype frequency and D allele frequency in AF group were markedly higher than in control group (P<0.05). Logistic regression analysis showed D allele increased the risk for AF by 2.42 folds (OR=2.42, P<0.05). CYP112B2-344C/T showed TT, TC and CC genotypes, and their frequencies were 64.63%, 25.61% and 9.76%, respectively in AF group and 58.54%, 29.27% and 12.20%, respectively in control group. Allele T frequency was 77.44% and 73.17% in AF group and control group, respectively, and allele C frequency was 22.56% and 26.83% in AF group and control group, respectively. No significant differences were observed in the allele C and T frequencies between two groups (P>0.05). CYP112B2-344C/T gene polymorphism had no relationship with AF.

Conclusion: In AF adults of Chongming, AGT-M235T, angiotensin converting enzyme (ACE) AluI/D and CYP112B2-344C/T genes show polymorphisms, T allele of AGT-M235T gene and D allele of ACE AluI/D gene may increase the risk for AF, and CYP112B2-344C/T gene polymorphism has no relationship with AF.

Keywords: Atrial fibrillation; renin-angiotensin-aldosterone system; gene polymorphism.

DOI: 10.19193/0393-6384_2017_2_041

Received October 30, 2016; Accepted January 02, 2017

Introduction

Atrial fibrillation (AF) is the most serious atrial electrical activity disorder and one of the most common arrhythmias in clinical practice. The incidence of AF increases over age, and is 0.3%-0.4% in adults and 8.0%-11.0% in people aged 60-74 years1, 2. AF is a life-threatening arrhythmia, and may cause thromboembolism, increase the risk for stroke, promote the development of heart failure, and increase hospitalization, disability and mortality2, 3.

AF has a high incidence and usually threatens the human health, but the therapeutic efficacy is still poor in AF patients4, 5. Although catheter ablation and surgery have been used in the therapy AF,
their indications are limited, their cost is high and they are usually invasive, which significantly limit their wide application in clinical practice. Pharmacotherapy is still a major treatment for AF, but the recurrence rate is at a high level, up to about 60%. To date, the pathogenesis of AF is still poorly understood, and the ideal strategies for the therapy of AF have not been developed. Thus, to deeply investigate the pathogenesis and identify the targets are crucial for the effective therapy of AF.

In recent years, the incidence of familial AF increases, suggesting the genetic predisposition in AF. To date, ion channel related genes (such as KCNE1, KCNH2, KCND3, KCNN3, KCNJ5, SCN5A, SCN3B and HCN) and non-ion channel related genes (such as GJA5, GJA3 and PITX2) have been found to be closely related to the pathogenesis of AF. Gene therapy targeting these genes has been performed in animal models, achieving promising results (6-9).

Renin-angiotensin-aldosterone system (RAAS) is an important humoral regulation system. Renin is an acid protease synthesized and secreted by renal juxtaglomerular cells. Renin enters the circulation via renal vein. The reduction in renal blood flow or reduction in plasma Na+ of any cause may lead to the increase in renin secretion. Renin acts on angiotensinogen in the plasma or tissues to produce angiotensin I (Ang I), which is then degraded into angiotensin II (Ang II) by angiotensin converting enzyme (ACE). RAAS mainly functions via Ang II which may act on systemic arterioles and venules to elevate blood pressure and increase the blood returning to the heart; increase the release of transmitters from the sympathetic nerve endings; increase the central sympathetic activity; promote the synthesis and release of aldosterone. RAAS play important roles in the regulation of blood pressure, cardiovascular homeostasis and fluid and electrolyte balance.

In addition, RAAS is also involved in multiple pathophysiological processes in a lot of cardiovascular diseases including hypertension, heart failure and arrhythmia. Over-activation of RAAS may cause atrial remodeling, resulting in AF (10-12). Angiotensinogen is the unique substrate of renin and the source of Ang II. ACE catalyzes the production of Ang II. Aldosterone synthase is a key enzyme involved in the synthesis of aldosterone. There is evidence showing that RASS inhibition may reduce the left atrial diameter in AF patients (13-15).

However, few studies have been conducted to investigate the relationship between AF and RAAS gene polymorphism, and no study has reported it in Shanghai.

In the present study, on the basis of epidemiological survey on AF in Chongming (a town in Shanghai), the AGT M235T, ACE AluI/D and CYP112B2-344C/T gene polymorphisms were detected in AF patients and controls, and the relationship between these gene polymorphisms and AF was further evaluated. Our findings may enrich the studies on the gene polymorphisms related to AF and provide evidence for the gene therapy of AF.

Material and methods

Population of the study
A total of 18 communities were randomly selected from 18 villages and towns in Chongming between May 2011 and April 2013, and cross sectional survey was employed to randomly recruited residents aged ≥20 years. After informed consent was obtained, epidemiological survey was conducted on AF. In this survey, a total of 122 patients were diagnosed with AF, but 40 patients were excluded due to the presence of cardiomyopathy, pulmonary heart disease, valvular disease, hyperthyroidism, fever, cancer and other systemic diseases. The remaining 82 patients were included as AF group, and there were 47 males and 35 females. Furthermore, 82 matched subjects without AF were also recruited in this survey as controls, and there were 47 males and 35 females. There was no kinship between AF patients and controls. Subjects included in this study had no history of consanguineous marriage or mixed marriage. Subjects in two groups matched in age, gender, race, smoking status, C-reaction protein (CRP), uric acid, region, and concomitant diseases (such as diabetes, hypertension, and coronary heart disease).

Epidemiological survey

Cross sectional survey was employed.

Blood collection
Fasting venous blood (5 ml) was collected from each subject in the morning. Serum was separated for the detection of blood glucose, lipids, liver function and CRP; plasma, red blood cells and white blood cells were separated by centrifugation.
at 3000 rpm for 10 min, and stored at -80°C for further DNA extraction.

**DNA extraction, and measurement of DNA concentration and purity**

Peripheral venous white blood cells were processed for the extraction of DNA with a kit (QIAGEN), and the extracted DNA was stored at -20°C. UV spectrophotometry was performed to detect the DNA concentration and purity. Identification of extracted DNA was conducted by 2% agarose gel electrophoresis.

**Design and synthesis of primers**

On the basis of previously reported sequences and the sequences in the GenBank, Primer 5 software was employed to design the primers for ANG, ACE and ALD (aldosterone) (16-20). Primers were synthesized in Shanghai Sangong Biotech Co., Ltd (Table 1).

<table>
<thead>
<tr>
<th>Genes</th>
<th>Primers</th>
</tr>
</thead>
<tbody>
<tr>
<td>AGT M235T</td>
<td>Forward 5'-CGTTTGTG-CAGGGCCTGGCCTCTC-3'</td>
</tr>
<tr>
<td></td>
<td>Reverse 5'-AGGGTGCTGGCTACACTG-GAACC-3'</td>
</tr>
<tr>
<td>ACE Alu/D</td>
<td>Forward 5'-CTGGAGACCACCTCC-CATCCTTTTCTC-3'</td>
</tr>
<tr>
<td></td>
<td>Reverse 5'-GATGTGGCCATCA-CATTGCTGACAT-3'</td>
</tr>
<tr>
<td>CYP112B2 -344C/T</td>
<td>Forward 5'-CAGAGGAGACCCCATGT-GAC-3'</td>
</tr>
<tr>
<td></td>
<td>Reverse 5'-CCTCCACCCCTGTTGACCC-3'</td>
</tr>
</tbody>
</table>

*Table 1*: Primers used for PCR of target genes.

**Polymerase chain reaction-restriction fragment length polymorph (PCR-RLFP)**

20 pmol/ul primer solution was prepared.

PCR of AGT M235T gene: following reagents, sample and primers were added to a PCR tube: distilled water (19 ul), 2.0 mM dNTPs (2 ul), 10× buffer (2 ul), forward primer (1 ul), reverse primer (1 ul), TaqDNA polymerase (1 ul) and DNA template (2 ul). The total volume of the mixture for PCR was 30 ul. PCR was conducted under following conditions: pre-denaturation at 94°C for 3 min, 35 cycles of denaturation at 94°C for 30 s, annealing at 68°C for 1 min and extension at 72°C for 1 min, and a final extension at 72°C for 10 min. RLFp was not required because the 16th intron of ACE Alu/D gene has an Alu insertion (I) and deletion (D).

PCR of CYP112B2-344C/T gene: Following reagents, sample and primers were added to a PCR tube: distilled water (14 ul), 2.0 mM dNTPs (2 ul), 10× buffer (2 ul), 25mM MgCl2 (2 ul), forward primer (1 ul), reverse primer (1 ul), TaqDNA polymerase (1 ul) and DNA template (2 ul). The total volume of the mixture for PCR was 25 ul. PCR was conducted under following conditions: pre-denaturation at 92°C for 4 min, 38 cycles of denaturation at 92°C for 1 min, annealing at 68°C for 1 min and extension at 72°C for 1 min, and a final extension at 72°C for 10 min. The PCR products in 20 ul of mixture were subjected to RLFp: PCR products (8 ul), HaeIII restriction endonuclease, 10× buffer and sterilized deionized water. Digestion was performed at 45°C for 8 h.

**Gene polymorphism analysis**

PCR products and products after digestion were subjected to ethidium bromide containing agarose gel electrophoresis. Five samples were randomly selected for sequencing (ABI3730XL). The sequences obtained were compared with those in GenBank (http://www.ncbi.nlm.nih.gov/). Results showed the obtained sequences were same to those from GenBank.

**Statistical analysis**

Data were double-checked and input into Excel. Statistical analysis was performed with SPSS (Statistical Product and Service Solutions, compiled by Binrong Ma) version 17.0. Quantitative data are expressed as mean ± standard deviation (±S) and comparisons were done with Student t test between groups.
Comparisons of qualitative data and Hardy-Weinberg equilibrium test were performed with chi-square test. Logistic regression model was employed to screen the potential confounding factors including age, gender, smoking, drinking, region, blood biochemical parameters and concomitant cardiovascular diseases (hypertension, coronary heart disease and diabetes mellitus). Paired design was used in this study, and thus confounding factors related to AF were not identified. Logistic regression was employed to analyze the correlation of gene polymorphisms with AF. A value of P<0.05 was considered statistically significant.

Results

Baseline characteristics of AF patients and controls

From May 2011 to April 2013, a total of 18 communities were randomly selected from 18 villages and towns of Chongming city, and 14885 residents, aged ≥20 years, were recruited. There were 7277 males and 7608 females; 14802 Han Chinese and 83 Chinese of minority). Of these subjects, 122 were diagnosed with AF. Finally, 82 AF patients were included after exclusion of concomitant cardiomyopathy, pulmonary heart disease, valvular disease, hyperthyroidism, fever, cancer and other systemic diseases. There were 47 males and 35 females in AF patients.

In addition, 82 subjects without AF were also recruited from the survey as controls, and there were 47 males and 35 females. These subjects were recruited for case-control study at a ratio of 1:1.

Results showed there were no marked differences in the age, gender, uric acid, CRP, left atrial diameter, left ventricular ejection fraction, blood pressure, blood lipid, coronary heart disease, diabetes and hypertension between two groups (P>0.05). Subjects in both groups matched in above parameters and the influence of confounding factors was excluded (Table 2).

Peripheral blood DNA

Peripheral blood (5 ml) was collected from each subject in both groups, and whole genomic DNA was extracted with DNA extraction kit. UV spectrophotometry was performed to evaluate the DNA purity and concentration by measuring the optical density at 260 nm and 280 nm. The DNA purity and concentration were determined according to the OD260 and the ratio of OD260/OD280. Results showed the DNA concentration was 20-50 mg/ml, and the OD260/OD280 was 1.8-2.0, suggesting that the extracted DNA met the requirements in following experiments. DNA samples were selected for subsequent 2% agarose gel electrophoresis. The DNA bands were clear, and no other bands were observed (Fig. 1).

Allele frequency and genotype distribution

The AGT M235T gene was 163 bp in length after PCR, and 2 fragments sized 163 bp and 140 bp, respectively, were found after digestion with Tht III and agarose gel electrophoresis. The gene with fragment sized 163 bp alone was wide type (MM genotype), the gene with 2 fragments sized 140 bp and 163 bp, respectively, was heterozygous (MT genotype); the gene with 1 fragment sized 140 bp was mutant (TT genotype). There were MM genotype, MT genotype and TT genotype. The theoretical number of different genotypes, M allele and T allele, and Hardy-Weinberg equilibrium test was

Table 2: Clinical characteristics of subjects in both groups.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>AF patients</th>
<th>Controls</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (M/F)</td>
<td>47/35</td>
<td>47/35</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>66.1±15.2</td>
<td>59.4±10.4</td>
<td>0.05</td>
</tr>
<tr>
<td>SBP</td>
<td>120±15.6</td>
<td>117±7.2</td>
<td>0.07</td>
</tr>
<tr>
<td>DBP</td>
<td>72±13.6</td>
<td>73.5±5.9</td>
<td>0.06</td>
</tr>
<tr>
<td>LAD</td>
<td>42±6.5</td>
<td>27±3.7</td>
<td>0.21</td>
</tr>
<tr>
<td>LVEF</td>
<td>63.5±9.3</td>
<td>67.0±5.3</td>
<td>0.17</td>
</tr>
<tr>
<td>TC</td>
<td>4.35±0.72</td>
<td>4.60±0.67</td>
<td>1.06</td>
</tr>
<tr>
<td>TG</td>
<td>1.24±0.95</td>
<td>1.12±0.45</td>
<td>1.17</td>
</tr>
<tr>
<td>CRP</td>
<td>4.76±5.90</td>
<td>3.78±4.25</td>
<td>1.62</td>
</tr>
<tr>
<td>UA</td>
<td>141±12.86</td>
<td>128±22.73</td>
<td>0.06</td>
</tr>
<tr>
<td>Total</td>
<td>82</td>
<td>82</td>
<td></td>
</tr>
</tbody>
</table>

Notes: SBP: systolic blood pressure; DBP: diastolic blood pressure, LAD: left atrial diameter, LVEF: left ventricular ejection fraction; TC: total cholesterol; TG: Triglycerides; CRP: C-reactive protein; UA: uric acid.
performed. Results showed $P > 0.05$, suggesting that the genotype distribution in both AF group and control group met the genetic equilibrium (Table 3). That is, the samples selected reflect the characteristics of general population.

There were significant differences in the genotype and allele distributions between AF group and control group. The frequency of MM genotype was 7.32% and 13.41% in AF group and control group, respectively, that of MT genotype was 26.83% and 35.67% in AF group and control group, respectively, and that of TT genotype was 65.85% and 51.22% in AF group and control group, respectively. The M allele frequency was 20.73% and 31.10% in AF group and control group, respectively and the T allele frequency was 79.27% and 68.90% in AF group and control group, respectively. The TT genotype frequency and T allele frequency in AF group were significantly higher than in control group ($P<0.05$) (Table 4). Logistic regression analysis showed T allele increased the risk for AF by 1.57 folds (OR=1.57, $P<0.05$).

The PCR products of ACE AluI/D gene had two fragments sized 490 bp and 190 bp, respectively. Gene with 490 bp fragment alone belongs to II genotype, gene with 190 bp fragment alone belongs to DD genotype, and gene with both 490 bp fragment and 190 bp fragment belongs to ID genotype. There were II genotype, ID genotype and DD genotype. Hardy-Weinberg equilibrium testing showed the genotype distribution in both AF group and control group met the genetic equilibrium ($P>0.05$) (Table 5).

That is, the samples selected reflect the characteristics of general population. There were significant differences in the three genotypes. The frequency of II genotype was 17.07% and 46.34% in AF group and control group, respectively, that of ID genotype was 51.22% and 34.15% in AF group and control group, respectively, and that of DD genotype was 31.71% and 19.51% in AF group and control group, respectively. The I allele frequency was 42.68% and 63.41% in AF group and control group, respectively and the T allele frequency was 57.32% and 36.59% in AF group and control group, respectively. The DD genotype frequency and D allele frequency in AF group were significantly higher than in control group ($P<0.05$) (Table 6). Logistic regression analysis showed D allele increased the risk for AF by 2.42 folds (OR=2.42, $P<0.05$).

The PCR products of CYP112B2 -344C/T gene were 537 bp in length, and there were TT, TC and CC genotypes after digestion with HaeIII and electrophoresis. Gene with 273 bp fragment alone was TT genotype, gene with two 202 bp fragments was CC genotype, and gene with 273 bp fragment and 202 bp fragment was CT genotype. Hardy-Weinberg equilibrium testing showed the genotype distribution in both AF group and control group met the genetic equilibrium ($P>0.05$) (Table 7).

That is, the samples selected reflect the characteristics of general population. There were significant differences in the three genotypes.

---

### Table 3: Hardy-Weinberg equilibrium test of AGT M235T genotype frequency.

<table>
<thead>
<tr>
<th>Group</th>
<th>Genotype n/f (%)</th>
<th>χ²</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MM</td>
<td>MT</td>
<td>TT</td>
</tr>
<tr>
<td>AF</td>
<td>Actual</td>
<td>6/7,32</td>
<td>22/26,83</td>
</tr>
<tr>
<td></td>
<td>Theoretical</td>
<td>3.52/4.30</td>
<td>26.89/32.87</td>
</tr>
<tr>
<td>Control</td>
<td>Actual</td>
<td>11/13.41</td>
<td>29/35.37</td>
</tr>
<tr>
<td></td>
<td>Theoretical</td>
<td>7.93/9.67</td>
<td>35.14/42.85</td>
</tr>
</tbody>
</table>

### Table 4: Distribution of AGT M235T genotype and allele.

<table>
<thead>
<tr>
<th>Group</th>
<th>Genotype n/f (%)</th>
<th>χ²</th>
<th>$P$</th>
<th>Allele n/f (%)</th>
<th>χ²</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MM</td>
<td>MT</td>
<td>TT</td>
<td>M</td>
<td>T</td>
<td></td>
</tr>
<tr>
<td>AF</td>
<td>6/7,32</td>
<td>22/26,83</td>
<td>54/65,85</td>
<td>34/20,73</td>
<td>130/79,27</td>
<td>8.22</td>
</tr>
</tbody>
</table>

### Table 5: Hardy-Weinberg test of ACE AluI/D genotype frequency.

<table>
<thead>
<tr>
<th>Group</th>
<th>Genotype n/f (%)</th>
<th>χ²</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>II</td>
<td>ID</td>
<td>DD</td>
</tr>
<tr>
<td>AF</td>
<td>Actual</td>
<td>14/17.07</td>
<td>42/51.22</td>
</tr>
<tr>
<td>Control</td>
<td>Actual</td>
<td>38/46.34</td>
<td>28/34.15</td>
</tr>
<tr>
<td></td>
<td>Theoretical</td>
<td>32.98/40.21</td>
<td>38.05/46.40</td>
</tr>
</tbody>
</table>
The frequency of TT genotype was 64.63% and 58.54% in AF group and control group, respectively, that of TC genotype was 25.61% and 29.27% in AF group and control group, respectively, and that of TT genotype was 9.76% and 12.20% in AF group and control group, respectively. The T allele frequency was 77.44% and 73.17% in AF group and control group, respectively and the T allele frequency was 22.56% and 26.83% in AF group and control group, respectively. There were no marked differences in the distributions of different genotypes, T allele and C allele between two groups (P>0.05, Table 8). These genotypes and alleles had no relationship with AF.

**Discussion**

AF has a high prevalence and a high incidence of complications, which significantly threaten the human health. In past about 100 years, studies have confirmed that atrial remodeling is a key step in the occurrence and development of AF\(^3,21\). RAAS play important regulatory roles in the atrial remodeling and pathogenesis of AF. AGT gene is mapped to 1q42-43 and 12 kb in length, and it composed of 5 exons and 4 introns. AGT M235T polymorphism occurs in the 2nd exbon causing the transformation of thymine (T) into cytosine (C) at 704 nucleotide and the transformation of methionine (Met) into threonine (Thr) at codon 235.

Studies have shown that T allele carriers have increased serum AGT, which is further degraded into Ang II by ACE, leading to the increase in Ang II. Ang II binds to Ang II receptor (AT1) on fibroblasts to regulate the proliferation of fibroblasts, induce the mRNA expression of type I and III collagens, increase the synthesis and secretion of type I and III collagens via the extracellular signal-regulated kinase of mitogen-activated protein kinase, resulting in atrial fibrosis. In addition, the increased Ang II binds to AT1 to activate AMPK system, leading to the elevation of gap junction protein synthesis; increased Ang II binds to AT1 to increase intracellular calcium via G-protein coupled recep-
The increase in ACE may promote the degradation of AGT into Ang II, leading to the elevation of Ang II and finally the atrial fibrosis. ACE gene is mapped to 17q23 and 21 kb in length. It contains 26 exons and 25 introns. Studies have shown that ACE gene has insertion/deletion (I/D) polymorphism at 16 intron, which is related to the serum ACE activity, Ang II, myocardial hypertrophy and arrhythmia. DD genotype increases the ACE activity and Ang II, and then promotes myocardial hypertrophy and arrhythmia\(^{(25-27)}\). In 2011, Topal et al found ACE-AluD significantly increased the incidence of AF\(^{(28)}\). In our study, the frequencies of DD genotype and D allele in AF group increased markedly when compared with control group \((P<0.05)\), and D allele increased the risk for AF by 2.42 folds \((OR=2.42, P<0.05)\), which were consistent with previous findings.

Aldosterone may regulate the ions movement and the expression of collagens, involving the myocardial remodeling. Aldosterone inhibitor is able to delay or reverse myocardial remodeling and prevent AF\(^{(29)}\). Aldosterone synthase is a key enzyme in the synthesis of aldosterone. CYP11B2 gene is mapped to 8q22 and 7 kb in length. It contains 9 exons and 8 introns. To date, CYP11B2-344C/T polymorphism has been found in the promoter region \((\text{exchange between cytosine and thymine at -344})\).

In recent years, some studies focused on the relationship between CYP11B2-344C/T polymorphism and hypertension, and results reveal that CYP11B2-344C/T polymorphism is closely related to the hypertension and myocardial hypertrophy \((30, 31)\). However, few studies have been conducted on the relationship between CYP11B2-344C/T polymorphism and AF. A recent study of Lu et al found CYP11B2-344C/T polymorphism was not associated with AF\(^{(32)}\). In the present study, results showed no significant differences in the frequencies of CYP11B2-344C/T genotypes between AF group and control group \((P>0.05)\), suggesting that the CYP11B2-344C/T polymorphism is not related to AF.

Taken together, in the present study conducted in 82 AF patients and 82 matched controls, results show AGT M235T, ACE, Alu I/D and CYP11B2-344C/T polymorphisms in AF adults of Chongming, Shanghai, and the AGT-235T and ACE-AluD polymorphism may increase the risk for AF, but CYP11B2-344C/T polymorphism is not associated with AF. On the basis of epidemiological survey, the subjects in our study are representative, but the sample size is small. In addition, the gene polymorphism usually involves several sites, and AF is usually as a result of interaction between polygenic inheritance and environment. Thus, the relationship between gene polymorphism and AF is still poorly understood, and more studies with large sample size are required to confirm our findings.

**Acknowledgements**

This study was supported by the Yingmin Lu Foundation of the Committee of Shanghai Municipal Health and Family Planning (No. 20124241).

**Compliance with ethics guidelines**

Shuxin Hou, Yingmin Lu, Damin Huang, Xiaohan Luo, Zhaoxia Wang, Jinchun Zhang, Weiping Xu declare that they have no conflict of interest. All procedures followed were in accordance with the ethical standards of Institutional Review Board of XinHua (Chongming) Hospital Affiliated to School of Medicine, Shanghai Jiao Tong University and with the Helsinki Declaration of 1975, as revised in 2000. Informed consent was obtained from all patients for being included in the study.

**References**

6) Yao J, Ma YT, Xie X, Liu F, Chen BD. Association of KCNE1 genetic polymorphisms with atrial fibrillation.

Corresponding author
YINGMIN LU AND WEIPING XU
Department of Cardiology, XinHua (Chongming) Hospital
Affiliated to School of Medicine, Shanghai Jiao Tong University,
No. 25, Nannmengang Road
Shanghai 202150
(China)