CORRELATION OF ATRIAL FIBRILLATION WITH SCN5A AND KCNE1 GENE POLYMORPHISM IN CHONGMING ADULTS OF SHANGHAI

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ABSTRACT

Aims: *To investigate the correlation of atrial fibrillation (AF) with SCN5A-A1673G and KCNE1-A112G gene polymorphism in Chongming adults of Shanghai.*

Materials and methods*: Cluster random sampling was performed in 18 communities of 18 villages, and inhabitants aged >20 years were screened. A total of 122 patients with AF who were recruited from this epidemiological study served as case group, and 122 subjects without AF from the same study served as controls. These subjects were recruited for case-control study at a ratio of 1:1. Polymerase chain reaction - restriction endonuclease fragment length polymorphism (PCR-RFLP) was performed to detect the SCN5A -A1673G and KCNEl-A112G (S38G) gene polymorphism for genotyping. Samples were randomly selected for sequencing to evaluate the reliability. SPSS version 17.0 was used for statistical analysis. The genotype frequency and Hardy-Weinberg equilibrium were evaluated. Chi square test was performed to compare the genotypes and allele frequency. A value of P<0.05 was considered statistically significant. Multivariate logistic regression analysis was employed to assess the correlation of AF and gene polymorphism.*

Results*: There were 3 genotypes of SCN5A-A1673G in the subjects investigated: AA genotype (38.33% vs. 58.33%), AG (45% vs. 30.83%) and GG (16.67% vs. 10.83%). In case group, G allele frequency was significantly higher than that in control group (39.17% vs. 26.25%), and GG genotype significantly influenced the AF (P<0.05). Logistic regression analysis showed G allele was associated with AF (R=1.46, 95%CI: 1.38-1.54). There were 3 genotypes of KCNEl-A112G in the subjects investigated: AA genotype (13.33% vs. 11.67%), AG genotype (25% vs. 38.33%) and GG genotype (61.67% vs. 50%). The frequency of GG genotype in case group was markedly higher than that in control group. The distribution of genotypes was different between 2 groups without statistical significance (P>0.05).*

Conclusion*: In Han Chinese of Chongming in Shanghai, AF is associated with SCN5A-A1673G gene polymorphism, but not with KCNE1-A112G (S38G) gene polymorphism.*

Key words*: Atrial fibrillation, gene polymorphism, polymerase chain reaction.*

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Introduction

Atrial fibrillation (AF) is the most common atrial arrhythmia in clinical practice and the most severe atrial electrophysiological disorder. AF not only influences the quality of life, but has high disability and mortality, significantly threatening human health[1, 2].

To elucidate the pathogenesis of AF is undoubtedly important for the prevention and treatment of AF. However, although AF has been a hot topic in the field of cardiovascular diseases, the pathogenesis of AF is still unclear. The identification of genes related to familial AF has promoted the investigation on the etiology of AF. Nevertheless, familial AF may not represent the whole AF family. AF in some patients is accompanied by organic heart diseases, such as Valvular heart disease, coronary heart disease, cardiomyopathy, hypertensive heart disease and chronic heart failure. Of note, not all the patients with organic heart diseases may develop AF. These findings suggest the genetic predisposition of AF.

In recent years, some studies have reported the association of genetic factors with AF[3, 4]. Increasing investigators focus on the molecular genetic mechanisms of AF. During the AF, there are changes in the electrophysiological properties of

the atrium, which is also known as electrophysiological remodeling (or ion remodeling). This is characterized by the shortened duration and reduced amplitude of action potentials of the atrium, shortened effective refractory period and reduced response of the atrium to changes in the heart rate. Following AF, the expression of genes related to ion channels (such as potassium channel, sodium channel and L-type calcium channel) changes significantly[3, 4]. Sodium channel is also known as voltage-dependent sodium channel (VDSC) and composed of α subunit and β subunit. The SCN5A gene encoding α subunit is highly expressed in the human heart and the α subunit is a major protein consisting the sodium channel. SCN5A gene is mapped to 3q21-q23[5], has 28 exons, encodes 2016 amino acids and has the molecular weight of 227 kD. Polymorphisms have been found at the intron/exon splicing region and the (CA)n repeats of intron 16. SCN5A is highly expressed in the human heart. There is evidence showing that not only SCN5 gene mutation may cause severe arrhythmia, but some common single nucleotide polymorphism (SNP) of SCN5A gene can also cause changes in the phenotypes of heart diseases related to heart sodium channel or increase the genetic susceptibility to arrhythmia[6].

In 2007, a USA study[6] showed the distribution of three genotypes of A1673G (H558R) of SCN5A (HH, HR and RR) was markedly different between patients and controls, and the presence of R allele could significantly increase the risk for isolated AF. However, few studies have been conducted to investigate the relationship between SCN5A gene polymorphism and AF in China because the polymorphism varies among races and regions. Thus, this study was undertaken to investigate the SCN5A-A1673G polymorphism in AF patients of Chongming, a city of Shanghai in China.

Potassium current involves the whole repolarization of action potential and depolarization in the diastolic phase (phases 1-4 of action potential) of the myocytes. Potassium current plays an important role in the normal electrophysiological activities of myocytes. A total of 5 genes encoding the β subunit of voltage-dependent potassium channel have been identified in humans. These 5 genes have similarities in the gene structure and the function of encoded proteins. These proteins are also known as KCNE family including KCNE1-KCNE5. The KCNE1 expression is the highest and KCNE1 gene is a major gene encoding the β subunit.

The β subunit encoded by KCNE1 gene may bind to the α subunit encoded by KCNQ1 gene to form the slowly activating delayed rectifier potassium channel which may product slowly activating delayed rectifier potassium channel current (Iks). KCNE1-A112G (S38G) gene is mapped to 21(21q22.1-22.2). Lai et al[7] for the first time found that the 112A→G polymorphism at an exon of KCNEl gene (S38G; alteration of serine to glycine) and reported that the G allele at site 112 was associated with AF. Zeng et al[8] investigated this polymorphism and found the action potential was comparable under normal conditions between cells with 2 different alleles and KCNEl-S38G has no significant influence on Iks. These findings were inconsistent with what reported by Lai et al. Chongming city is composed of Chongming Island, Hengsha Island and Changxing Island which are alluvial islands at Yangtze River estuary. In Chongming city, there are relatively isolated transportation and small population mobility, and no studies have been undertaken to investigate AF in adults in Chongming city.

This study aimed to investigate the distribution of SCN5A -A1673G and KCNEl-A112G in adults of Chongming city, explore the correlation of SCN5A -A1673G and KCNEl-A112G with AF in adults of this area, which may provide evidence for further elucidation of genetic mechanisms of AF and molecular and gene therapy of AF. In addition, our findings may be helpful for the prevention and treatment of AF.

Materials and methods

Subjects

From May 2011 to April 2013, 18 communities were randomly selected from 18 villages of Chongming city and cardiovascular epidemiological survey on AF and relevant factors was performed in residents aged ≥20 years. AF patients were recruited into study group. A total of 122 patients were diagnosed with AF, including 67 males and 55 females. In addition, 122 subjects without AF were also included as controls (67 males and 55 females). All the patients and controls were Han Chinese and not related, and had no family history of intermarriage. Subjects were matched at a ratio of 1:1 on the basis if age, gender, race, smoking status, uric acid, CRP, region and concomitant diseases (diabetes mellitus, hypertension, coronary heart disease, and valvular disease).

DNA extraction

Peripheral blood (5 ml) was collected from each subject in both groups, and whole genomic DNA was extracted with DNA extraction kit and rapid salting-out process. DNA was stored at -20°C.

Expansion and pKCNE1 S38Grimer design

According to the sequences of SCN5A-A1673G and KCNE1 S38G in GenBank, primers were designed independently, with Prime 5. Primers were as follows: SCN5A-A1673G: 5'-GGCGAGA-GAGAGAGCGACC-3' (forward), 5'-GTGACTG-GAGGGCGCTGTA-3' (reverse); KCNE1 A112G: 5'-GTGACGCCCTTTCTCACCA-3' (forward), 5'-CCCCTCACCCCTTACAACA-3' (reverse). The reaction mixture (20 μl) included 10 pmol/L primer, 3 μl of genomic DNA, 2 μl of 10×Buffer, 25mM MgCl2, 2.0 mM dNTPs, and 1 μl of TaqDNA polymerase. PCR was performed as follows: pre-denaturation at 95°C for 5 min, 40 cycles of denaturation at 94°C for 1 min, annealing at 56°C for 1 min and extension at 72°C for 1 min, and a final extension at 72°C for 8 min. Products were stored at 4°C[4-6].

Restriction digestion and genotyping

Products from PCR for SCN5A-A1673G were digested with McrI restriction endonuclease. The reaction mixture (20 μl) included 2 μl of 10× Buffer, 1 μl of McrI restriction endonuclease, 12 μl of products, and 5 μl of distilled water, and digestion was done at 50 °C for 12-16 h. Then, products after digestion was subjected to agarose gel electrophoresis at 120 V for 40 min, and bands were observed and analyzed with gel analysis system. Representative photographs were captured and analyzed. Identification of SCN5A-A1673G genotypes: AA genotype, AG genotype and GG genotype[4-7].

Products from PCR for KCNE1- A112G (S38G) were digested in MspAl 1 restriction endonuclease. The reaction mixture (20 μl) included 2 μl of 10× Buffer, 1 μl of MspAl 1 restriction endonuclease, 10 μl of products, and 7 μl of distilled water, and digestion was done at 50 °C for 16 h. Then, products after digestion was subjected to 2.5% agarose gel electrophoresis at 125 V for 40 min, and bands were observed and analyzed with gel analysis system. Representative photographs were captured and analyzed. Identification of KCNE1 -A112G genotypes: AA genotype, AG genotype and GG genotype.

After genotyping, 10% of samples were randomly selected and genotyping was performed again. The consistence rate was 100%. At the same time, products from PCR were subjected to ABI3730XL and then compared with those from database (http: //www. ncbi. him. Nih. gov/).

Statistical analysis

Quantitative data were expressed as mean ± standard deviation and comparisons were done with student t test. Qualitative data were compared with chi square test. SPSS version 17.0 was employed for statistical analysis. Hardy-Weinberg equilibrium was tested. Logistic regression model was used to screen potential confounding factors such as gender, age, region, blood biochemistry, concomitant cardiovascular diseases (hypertension, coronary heart disease, diabetes and valvular disease), smoking and drinking. Paired design was employed in the present study, and thus confounding factors were not found to be associated with AF. Logistic regression analysis was used to evaluate the correlation of gene polymorphism with AF. A value of $P<0.05$ was considered statistically significant.

Results

General information of subjects in two groups

A total of 18 communities were randomly selected from 18 villages by cluster sampling, and epidemiological surgery was done in a total of 14885 subjects aged ≥20 years (males: 7277, females: 7608; Han: 14802, Minority: 83). A total of 122 patients were diagnosed with AF (males: 67, females: 55) and then recruited into study group. At the same time, subjects without AF were included as controls (males: 67, females: 55).

Patients were matched with controls at a ratio of 1:1, and comparisons were done with t test. There were no marked differences in the age, uric acid, CRP, gender, left atrial size, left ventricular ejection fraction, blood pressure, blood lipid, coronary heart disease, diabetes mellitus, hypertension and valvular disease ($P>0.05$) between patients and controls. This suggests that the study design avoids the influence of confounding factors (Table 1).

Extraction of DNA from blood

Genomic DNA was extracted from white blood cells and then subjected to 3% agarose gel electrophoresis for 60 min at 100 V. Results showed the band of genomic DNA was clear and no other bands were observed.

Group	Gender (M/F)	Age	SBP	DBP	LA	LVEF	TC	TG	CRP	UA	n
Patients	67/55	66.2±15.4	120±15.4	74±14.6	42±6.8	63.8±9.2	4.36±0.71	1.25±0.96	4.75±5.96	140±17.96	122
Controls	67/55	59.5±10.2	118±7	73.2±5.4	27.2±3.6	67.2±5.4	4.62±0.68	1.11±0.46	3.82±4.23	126±23.93	122
P	0.1	0.05	0.06	0.09	0.2	0.7	1.09	2.07	1.81	0.07	

Table 1: Clinical information of subjects in 2 groups.

Footnotes: SBP: systolic blood pressure; DBP: diastolic blood pressure; LA: Left atrial diameter; LVEF: Left ventricular ejection fraction; TC: Total cholesterol; TG: Triglyceride; CRP: C reaction protein; UA: uric acid.

Allele frequency and distribution of genotypes After agarose gel electrophoresis, results showed 3 genotypes of SCN5A-A1673G were identified: AA, AG and GG, the frequency of which was 38.33% vs. 58.33%, 45% vs. 30.83% and 16.67% vs. 10.83%, respectively. The allele A frequency was the highest (0.83%vs.73.75%), and the allele G frequency was 39.17% vs. 26.25%. The allele and genotype frequency met the Hardy-Weinberg equilibrium in patients and controls. In patients, G allele frequency was higher than that in controls, and GG genotype had significant influence on AF (P<0.05). This shows this genotype and allele frequency were marked different between patients and controls (P<0.05) (Table 2).

A1673G	Alleles /frequency %			χ2	P	Alleles / frequency %		χ2	P
	AA	AG	GG			A	G		
Patients	46/38.33	54/45	20/16.67	8.02	0.02	146/60.83	94/39.17	8.52	0.01
Controls	70/58.33	37/30.83	13/10.83			177/73.75	63/26.25		

Table 2: Alleles and genotype distribution of SCN5A-A1673G in two groups.

A112G	Alleles /frequency %			χ2	P	Alleles /frequency %		χ2	P
	AA	AG	GG			A	G		
Patients	16/13.33	30/25	74/61.67	2.01	0.55	62/25.83	178/74.17	0.01	0.08
Controls	14/11.67	46/38.33	60/50			74/30.83	166/69.17		

Table 3: Alleles and genotype distribution of KCNE1- A112G in two groups.

Logistic regression analysis revealed that allele G was closely related to AF (OR=1.46, 95%CI: 1.38-1.54).

After agarose gel electrophoresis, results showed 3 genotypes of KCNE1-A112G (Figure 1): AA, AG, and GG, and the genotype frequency was 13.33% vs. 11.67%, 25% vs. 38.33% and 61.67% vs. 50%, respectively. Allele G frequency was the highest (74.17% vs. 69.17%) and allele A frequency was 25.83% vs. 30.83%. This allele and genotype frequency met Hardy-Weinberg equilibrium. No significant difference was observed in this genotype and allele frequency between patients and controls (P<0.05) (Table 3). Logistic regression analysis revealed that KCNEl-A112G allele had no influence on AF (P>0.05).

Discussion

The voltage gated sodium channel in the heart may produce inward sodium current (INa) which forms the rapidly rising phase of action potential of the myocytes and plays an important role in the contraction-excitation coupling of myocytes. SCN5A gene encodes the β subunit of voltage gated sodium channel in the heart and may induce the rapid increase in action potential of myocytes and promote the rapid transmission of pulse among myocytes. Studies have shown that SCN5A gene mutation may cause several arrhythmias including Brugada syndrome, long OT syndrome, progressive Cardiac conduction disease and AF[9, 10]. Chen et al found that A1673G gene polymorphism was able to increase the risk for AF. Cellular functional expression and patch clamp were used in their study, and results showed A1673G could reduce the sodium current density to decrease the atrioventricular conduction velocity, which in turn shortened the atrial reentrant wavelength and promoted the single path

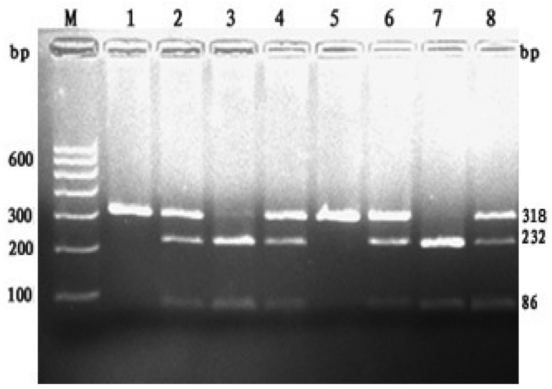

Fig. 1: Genotypes of KCNE1 A112G gene after electrophoresis. AA genotype: 1, 5; AG genotype: 2, 4, 6, 8; GG genotype: 3, 7.

or multipath reentrant circuit, resulting in AF. This electrophsiological effect provides a theoretical basis for the atrial ectopic beat induced AF. In addition, studies also indicated that the distribution of three genotypes of H558R (A1673G) gene (HH, HR and RR) was significantly different between AF patients and controls (50% vs. 63%, 40% vs. 33 and 10% vs. 4%, respectively; OR=1.6). Logistic regression analysis showed R allele increased the risk for isolated AF by 1.6 folds[6]. Thus, there is relationship between gene mutation and AF. In the present study, classic case control design was employed, aiming to elucidate the role of genetic mutation of SCN5A gene in AF of Han Chinese in Chongming city. A total of 122 AF patients and 122 controls were recruited into present study, and the number of subjects was identical between study group and control group which may reduce the influence of confounding factors and minimize the bias. Chi square test was used to compare the genotypes and alleles of SCN5A-A1673G between patients and controls. Results showed there were marked differences in the genotypes and alleles of SCN5A-A1673G between patients and controls ($P<0.05$). Logistic regression analysis revealed that allele G was closely associated with AF (OR=1.46). In the present study, we for the first time investigated the pathogenesis of AF, which fills the gaps in researches in the area of AF. Our findings may provide evidence for further investigations on the pathogenesis of AF, molecular diagnosis of AF, prediction model of AF and prevention of AF.

A total of 5 KCNE genes (KCNE1-5) have been identified in human genome. In vitro study has shown that the binding of KCNQl to KCNEl is closely related to the electrophysiology of Iks, which is ascribed to that Iks is composed of KCNQl encoded α subunit and KCNEl encoded β subunit. Lundquist et al[11] found that the expression of members of KCNE family shows following trend in the human myocardium: KCNEI>KCNE4>KCNE5～KCNE3>>KCNE2. In the atrium, KCNE1 and KCNE3-5 are highly expressed. At cellular level, α subunit and β subunit of KCNE1 exert distinct effects on Iks, and to increase the KCNE1 copies has no influence on Iks. Moreover, the influence of KCNE2 on Iks is also not significant. KCNE3 can increase the outward current of Iks to a certain extent. It has been confirmed that KCNE2-R27C, KCNE3-R53H and KCNQI-S140GS140G are related to the familial AF[8], and these mutations are found in KCNE and KCNQ1. KCNEl-A112G (S38G) is the first polymorphism site related to AF. In the present study, results showed, although more AF patients had KCNEl-112G, there were no marked differences in the allele frequency and genotype distribution. Logistic regression analysis showed KCNEl-112G allele had no influence on AF. Above findings indicate that KCNEl-112G allele is not an absolute risk factor of AF. In Han Chinese of Chongming city, more subjects have KCNEl-112G allele, but there was no significant difference in the KCNEl-A112G (S38G) distribution between AF patients and controls. Our findings were consistent with the report of Zeng et al[8], but inconsistent with those from the study of Lai et al[7]. The KCNEl-A112G might vary among races and regions and there was no marked difference in the KCNEl-A112G distribution between AF patients and non-AF subjects. KCNEl-A112G is not one of absolute risk factors of AF ($P>0.05$). In addition, findings from in vitro studies on cells may be different from those from humans, and KCNEl-A112G might have no influence in vivo. AF is caused by multiple factors, and we will further investigate the effect of other molecular genetic factors on AF and the interaction between AF and KCNEl-A112G, which may provide evidence for the molecular genetic mechanisms of AF.

In the present study, random cluster sampling was performed for epidemiological survey to reduce the sampling error. Patients and controls matched in confounding factors were recruited into present study for the detection of gene polymorphism, which may reduce the influence of confounding factors and decrease the bias, leading to acquire more reliable results. However, our study was different from a majority of studies on AF inpatients. Results showed SCN5A- A1673G gene polymorphism was related to AF, and G allele may

increase the risk for AF significantly. However, KCNEl-A112G gene polymorphism was not associated with AF. Our findings preliminarily elucidate the epidemiology of AF in Chongming city and enrich the understanding of genetic mechanisms of AF. Our findings fill the gaps in researches in the area of AF and provide scientific evidence for prevention and control of AF in Chongming city of China.

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