Association of *KCNE1* Genetic Polymorphisms with Atrial Fibrillation in a Chinese Han Population

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Objective: The purpose of this study was to investigate the association of the polymorphisms of the *KCNE1* gene with atrial fibrillation (AF) in a Chinese Han population. *Methods:* Three hundred seven AF patients and 330 age- and sex-matched controls were genotyped using the polymerase chain reaction–restriction fragment length polymorphism method for two single-nucleotide polymorphisms (rs1805127 and rs1892593) of the human *KCNE1* gene. *Results:* The frequencies of the AA, AG, and GG genotypes of rs1805127 were 11.7%, 50.0%, and 43.3%, respectively, in the AF group, whereas the ones in the control group had frequencies of 19.4%, 44.9%, and 35.8%, respectively. There were significant differences in frequencies of these three genotypes ($\chi^2 = 7.820$, $p = 0.016$) and G allele (65.8% vs. 58.2%; $\chi^2 = 8.266$, $p = 0.005$). The frequencies of AA, AG, and GG of rs1892593 were 38.4%, 47.9%, and 13.7% in the AF group, whereas the ones in the control group had frequencies of 37.8%, 48.5%, and 14.0%, respectively. There was no difference in distributions of frequencies of these three genotypes and allele ($\chi^2 = 0.051$, $p = 0.978$; $\chi^2 = 1.024$, $p = 0.837$, respectively) between AF patients and control subjects. We also found that rs1805127 was associated with left atrial diameter and left ventricular end diastolic diameter in AF patients ($\chi^2 = 24.883$, $p < 0.001$; $\chi^2 = 34.901$, $p < 0.001$, respectively). Logistic regression analysis showed that rs1805127 was an independent risk factor of AF in a Chinese Han population (odds ratio [OR] = 1.66, 95% confidence interval [CI]: 1.02–2.68 for AG; OR = 2.03, 95% CI: 1.24–3.31 for GG). *Conclusion:* The genetic polymorphism of *KCNE1* was associated with increased risk of AF in a Chinese Han population.

Introduction

ATRIAL FIBRILLATION (AF) is the most common sustained arrhythmia, and it results in significant morbidity and mortality. However, the pathogenesis of AF remains unclear up to date. Recently, more and more pieces of evidence indicated that AF is a multifactorial disease resulting from the interaction between environmental factors and genetics. Several studies demonstrated that the mutations in genes coding ion channels may be associated with parts of the familial AF (Otway *et al.*, 2007; Ellinor *et al.*, 2008). However, most studies indicated that risk factors for typical AF include advanced age, coronary artery disease (CAD), hypertension, and valvular heart disease (Lai *et al.*, 2002; Tsai *et al.*, 2004; Lasse *et al.*, 2008; Wang *et al.*, 2009).

KCNE1, a gene coding for humans, is a slowly activating component of the delayed rectifier potassium channel current (IKs), which plays an important role in atrial repolarization (Chen *et al.*, 2003), and was reported to be associated with AF in Europe recently (Olesen *et al.*, 2012). However, no replicated study was reported in other ethnic groups up to date. In the present study, we selected two tagging single-nucleotide polymorphisms (SNPs) (rs1805127 and rs1892593) to observe the relationship between KCNE1 and AF.

Participants and Methods

Participants

Participants diagnosed with AF were recruited at the First Teaching Hospital of the Xinjiang Medical University and the People's Hospital of Xinjiang Uygur Autonomous Region from 2008 to 2010. We enrolled 307 AF patients and 330 age- and sex-matched control participants in the present study. The diagnosis of AF was established according to ECG or ambulatory electrocardiogram and disease history. Informed consent was obtained from each individual according to a protocol approved by the Ethics Committee of the First Affiliated Hospital of the Xinjiang Medical University.

Covariates

We collected information on each subject's medical history and lifestyle characteristics using standardized questionnaires. All participants underwent a standardized physical

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TABLE 1. CHARACTERISTICS OF THE PARTICIPANT

Variables	AF (n = 303)	Control (n = 328)	t or χ^2	p Value
Gender (M/F)	165/142	177/153	0.001	0.978
Age (year)	63.3 ± 11.3	63.5 ± 5.7	0.294	0.769
BMI (kg/m^2)	24.2 ± 3.8	24.3 ± 3.7	0.362	0.718
LVEF	59.3 ± 7.0	60.1 ± 6.9	1.451	0.147
LAD (mm)	44.0 ± 7.6	40.6 ± 7.9	−5.462	<0.001
LVEDD (mm)	49.8 ± 5.6	49.0 ± 5.7	−1.814	0.070
Smoking (n, %)	70 (22.8)	74 (22.04)	0.013	0.909
Drinking (n, %)	44 (14.3)	45 (13.6)	0.064	0.800
Hypertension (n, %)	181 (59.0)	190 (57.6)	0.125	0.724
CAD (n, %)	85 (27.7)	90 (27.3)	0.014	0.907
Diabetes (n, %)	30 (9.70)	30 (8.80)	0.001	0.975

AF, atrial fibrillation; BMI, body–mass index; LVEF, left ventricular ejection fraction; LAD, left atrial diameter; LVEDD, left ventricular end diastolic diameter; CAD, coronary artery disease.

examination performed by experienced research staff. Height was measured to the nearest 0.1 cm, and weight was measured with a standard scale in the upright position to the nearest 0.1 kg. Hypertension was defined as a systolic blood pressure of ≥140 mmHg and/or a diastolic blood pressure of ≥90 mmHg, on at least two separate occasions, or antihypertensive treatment. Diabetes mellitus was defined as the presence of an active treatment with insulin or an oral antidiabetic agent; for patients on dietary treatment, documentation of an abnormal fasting blood glucose or glucose tolerance test based on the World Health Organization criteria was required for establishing this diagnosis (Xie et al., 2011b). Smoking status classifications were defined as current smokers and never-smokers. Alcohol drinking was defined as current drinking and never drinking.

Echocardiography evaluation using a 7.5 MHz linear-type B-mode probe (Siemens) was undertaken by a specialist to evaluate heart morphology, including left atrial diameter (LAD) and left ventricular end diastolic diameter (LVEDD) and the cardiac function (left ventricular ejection fraction [LVEF]) on a day close to the day of blood biochemistry analysis (within 2 days).

Biochemical analysis

Serum concentrations of triglycerides, total cholesterol, plasma glucose, high-density lipoprotein cholesterol, and low-density lipoprotein cholesterol were measured using standard methods in the Central Laboratory of First Affiliated Hospital of the Xinjiang Medical University as described previously (Xie et al., 2009, 2010a, 2010b, 2011a).

Genotyping

We screened the data for the Tag SNPs on the International HapMap Project Website: http://hapmap.org/. Using Haploview 4.2 software and the HapMap phrase II database, we obtained two tagging SNPs (rs1805127 and rs1892593) for Chinese Han using minor allele frequency ≥0.1 and linkage disequilibrium patterns with $r^2 \geq 0.8$ as a cutoff. These two SNPs are located in one haplotype block and represent a 48-kb region of the KCNE1 gene.

Genomic DNA was extracted from the peripheral blood leukocytes using a DNA extraction kit (Beijing Bioteke Co. Ltd). Genotyping was confirmed by polymerase chain reaction–restriction fragment length polymorphism analysis. The primers of these two SNPs were designed by the use of primer premier 5.0. For rs1805127, the sense primer was 5′-AGACAGTTCAGCAGGG-3′, while the antisense primer was 5′-ATGGTTTTCAACGACA-3′. For rs1892593, the sense primer was 5′-TGGGCTCTATTTTCAG-3′, while the antisense primer was 5′-CCATTGGTCATTTTCC-3′. The annealing temperature was 50°C for rs1805127, and it was 48°C for rs1892593. Restriction endonucleases MSPAI and NciI were used for the genotyping of rs1805127 and rs1892593, respectively.

Statistical analyses

Analyses were carried out using SPSS version 17.0 (SPSS). The Hardy–Weinberg equilibrium was assessed using Chi-square analyses. Measurement data are shown as means ± standard deviation, and the differences between AF patients and control subjects were assessed by independent-sample t-test. The Chi-square test was used to analyze the differences in enumeration data between AF patients and control subjects, as well as the differences in distributions of genotypes and alleles between AF patients and control subjects. Logistic regression analyses were used to assess the contribution of the major risk factors.

Results

Participant characteristics

Table 1 shows the clinical characteristics of AF patients (n = 307) and control (n = 330) subjects. The LAD was significantly different between the two groups (p < 0.01), whereas age, sex, body–mass index (BMI), LVEF, LVEDD, smoking, drinking, hypertension, diabetes, and coronary artery disease were not significantly different between these two groups (all p > 0.05) (Table 1).

The genotype distribution of each SNP did not show significant difference from the Hardy–Weinberg equilibrium values (Table 2). Table 3 shows the distribution of the genotypes

TABLE 2. HARDY–WEINBERG EQUILIBRIUM ANALYSIS OF TWO SINGLE-NUCLEOTIDE POLYMORPHISMS IN BOTH ATRIAL FIBRILLATION AND CONTROL SUBJECTS

Group	N	rs1805127				rs1892593			
		AA	AG	GG	p	AA	AG	GG	p
AF	Real	36	138	133	0.982	118	147	42	0.724
	Prospective	39.91	138.17	132.91		120.31	144.06	43.40	
Control	Real	64	148	118	0.155	124	160	46	0.623
	Prospective	57.66	160.56	111.78		126.03	155.81	48.15	

TABLE 3. GENOTYPES AND ALLELE DISTRIBUTION OF MI PATIENTS AND CONTROL SUBJECTS

		Genotype (n, %)				Allele frequency		
Groups	n	AA	AG	GG	p	A	G	p
rs1805127					0.016			0.005
AF	307	36 (11.73)	138 (44.95)	133 (43.32)		210 (34.20)	404 (65.80)	
Control	330	64 (19.39)	148 (44.85)	118 (35.75)		276 (41.82)	384 (58.18)	
rs1892593					0.975			0.837
AF	307	118 (38.44)	147 (47.88)	42 (13.68)		383 (62.38)	231 (37.62)	
Control	330	124 (37.58)	160 (48.49)	46 (13.94)		408 (61.82)	252 (38.18)	

MI, myocardial infarction.

and alleles of the two SNPs. For total subjects, the genotype and the allele distribution of rs1805127 differed significantly between the AF patients and control subjects ($\chi^2 = 7.820$, $p < 0.05$ and $\chi^2 = 8.266$, $p < 0.01$, respectively). However, the genotype and the allele distributions of rs1892593 were not different between the AF patients and control subjects. Logistic regression analysis showed that after adjusting the confounders, such as diabetes, hypertension, age, sex, smoking, and drinking, the rs1805127 remained significantly associated with AF (odds ratio [OR] = 1.66, 95% confidence interval [CI]: 1.02–2.68 for AG; OR = 2.03, 95% CI: 1.24–3.31 for GG).

Table 4 shows the characteristics of the participants according to rs1805127 genotypes in the AF group. The LAD and LVEDD were significantly higher in the GG genotype compared with that in the AA and AG genotypes ($\chi^2 = 24.883$, $p < 0.001$; $\chi^2 = 34.901$, $p < 0.001$, respectively). There were no differences in other characteristics such as age, sex, BMI, LVEF, smoking, drinking, hypertension, CAD, and diabetes.

Discussion

In the present study, we found that rs1805127 of the *KCNE1* gene was associated with AF in a Chinese Han population. The G allele carriers have an increased risk of AF compared with the AA genotype of rs1805127. To the best of our knowledge, this is the first study to explore the association of the *KCNE1* gene with AF in a Chinese population.

The slow delayed rectifier current (IKs) is important for cardiac repolarization. One of the current key functions is to prevent excessive action potential prolongation during adren-

ergic stimulation. The single-transmembrane segment β-subunit KCNE1 modulates the function of the six-transmembrane segment, pore-forming α-subunit Kv7.1 (Barhanin *et al.*, 1996; Sanguinetti *et al.*, 1996). Within the heart, KCNE1 is the major interacting β-subunit associating with Kv7.1. The interaction between these proteins determines IKs properties and modulates current characteristics (eliminating ionic current inactivation, increasing unitary conductance, and slowing activation) (Yang and Sigworth, 1998; Kurokawa *et al.*, 2001). Most of the interactions underlying this modulation have been localized to the transmembrane domain and the C-terminus of KCNE1 (Tapper and George, 2000; Melman *et al.*, 2002; Kang *et al.*, 2008). Therefore, the *KCNE1* gene was considered as a candidate gene of cardiac arrhythmia.

The *KCNE1* gene was located on 21q22.1–22.2 (Gaborit *et al.*, 2005). Several studies indicated that the genetic polymorphism of *KCNE1* was associated with many cardiac arrhythmia diseases, such as familial long-QT syndrome, Jervell syndrome (Splawaki *et al.*, 2000; Teng *et al.*, 2004; Nishio *et al.*, 2009; Fatini *et al.*, 2010), drug-secondary long-QT syndrome (Paulussen *et al.*, 2004), and nonfamilial arrhythmias (Lai *et al.*, 2002; Lou *et al.*, 2007). rs1805127 (S38G) is located in exon 3 of the *KCNE1* gene and changes serine to glycine. Ehrlich *et al.* (2005) demonstrated that the rs1805127 G-allele carriers have a higher prevalence of tachyarrhythmia resulting from decreased IKs current, prolonged action potential duration, and mild prolonged relative refractory period. Lai *et al.* (2002) reported that the *KCNE1* gene G allele was very common in AF patients compared with that of control subjects. Prystupa *et al.* (2006) found that the *KCNE1* gene polymorphism was associated with lone AF. Our previous study indicated that in the Uygur population, there was a higher frequency of G allele of rs1805127 in AF patients compared with that in control subjects (Yao *et al.*, 2011). However, several studies (Zeng *et al.*, 2005; Xu *et al.*, 2008) suggested that the *KCNE1* gene G38S polymorphism was not found to be associated with AF. In the present study, we also found that the G38S polymorphism was associated with AF in a Chinese Han population; after adjustment of confounders, the difference remains significant. In addition, we also found that the LAD and LVEED were increased in GG genotype carriers compared with AG or AA genotype carriers in AF patients. Although left atrial enlargement is an important risk factor for the occurrence of AF (Henry *et al.*, 1976; Vaziri *et al.*, 1994), the association of rs1805127 with left atrial enlargement remains unclear.

In conclusion, rs1805127 was found to be associated with the prevalence of AF in a Chinese Han population.

TABLE 4. CHARACTERISTICS OF PARTICIPANTS ACCORDING TO RS1805127 GENOTYPES IN THE ATRIAL FIBRILLATION GROUP

Variables	AA	AG	GG	p Value
Sex (M/F)	56/44	153/133	133/118	0.874
Age (years)	63.8 ± 7.47	62.6 ± 8.82	63.9 ± 9.36	0.067
BMI (kg/m²)	24.9 ± 3.13	24.0 ± 3.62	24.2 ± 4.13	0.096
LVEF (%)	60.7 ± 7.43	59.2 ± 7.21	60.0 ± 6.33	0.135
LAD (mm)	38.6 ± 7.82	41.4 ± 6.94	44.6 ± 8.26	< 0.001
LVEDD (mm)	46.4 ± 4.99	49.7 ± 5.31	51.3 ± 5.29	< 0.001
Smoking (n, %)	22 (22.0)	77 (26.9)	57 (22.7)	0.431
Drinking (n, %)	8 (8.0)	44 (15.4)	37 (14.7)	0.168
Hypertension (n, %)	60 (60.0)	168 (58.7)	143 (57.0)	0.851
CAD (n, %)	25 (25.0)	76 (26.6)	74 (29.5)	0.628
Diabetes (n, %)	14 (14.0)	21 (7.30)	26 (10.40)	0.130

Author Disclosure Statement

No competing financial interests exist.

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