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Original Article

Association between the *SCN5A* gene H558R polymorphism and atrial fibrillation in the Uygur and Han populations of Xinjiang

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Abstract: Atrial fibrillation (AF) is the most common and damaging arrhythmia in clinical practice. In recent years, an association between SCN5A gene polymorphisms and atrial fibrillation has received a significant amount of attention. The present study aimed to investigate the correlation between the H558R polymorphism in *SCN5A* gene and atrial fibrillation (AF) occurrence in Uygur and Han populations of Xinjiang, China. The study is a case-control study using traditional methods. We selected 100 Uygur and 100 Han patient cases in the Xinjiang region who had non-valvular atrial fibrillation as well as 100 Uygur and 100 Han cases of non-AF patients as a control group. DNA was prepared from peripheral blood samples, and PCR and DNA sequencing were applied to determine genotype and allele frequencies of SCN5A gene H558R polymorphism. In both Uygur and Han populations, genotype and allele frequencies were significantly different between cases and controls group (both $P < 0.05$). Moreover, genotype and allele frequencies differed significantly between Uygur AF patients and Han AF patients ($P < 0.05$). Logistic regression analysis showed that the AG+GG genotype was correlated with the occurrence of AF (odds ratio =2.19, 95% confidence interval =1.36-3.53, P=0.001). These findings suggest the existence of a correlation between the H558R polymorphism of *SCN5A* gene and AF occurrence in the Uygur and Han populations of Xinjiang, and indicate that the AG+GG genotype might be an independent risk factor for predisposition to AF.

Keywords: Atrial fibrillation, SCN5A, single nucleotide polymorphism, population

Introduction

Atrial fibrillation (AF) is the most common arrhythmia observed in clinical practice. It can lead to the formation of thrombus in peripheral arteries, increase the risk of heart failure and stroke, increase the length of hospital stay and patient mortality, and greatly affect a patient's quality of life. The occurrence rate of AF increases with age. In the United States, the AF occurrence rate in adults is 0.9% [1]; whereas in China it is about 0.7% [2]. The underlying mechanism behind AF is unknown. Some researchers suggest that its development is associated with genetic polymorphisms, and progress has been made into identifying correlations between single nucleotide polymorphisms (SNPs) and AF [3-6].

SCN5A encodes the α-subunit of the voltage-gated sodium channel in the heart, which plays a critical role in the generation and transmission of cardiac electrical impulses. Numerous studies in China and worldwide have indicated that the H558R SNP in *SCN5A* gene is closely related to the occurrence and progression of AF [7-10]. However, the distribution differences of this SNP between AF and non-AF patients of Uygur and Han populations have not yet been reported. The present study therefore investigated the relationship between the H558R polymorphism in *SCN5A* gene and AF occurrence among Uygur and Han populations.

Materials and methods

Subjects

The following patients, who were admitted to the Department of Cardiac Medicine, First Affiliated Hospital of Xinjiang Medical School during the period from February 2013 to August

2014, were included in the study: 100 Han AF patients, 100 Han non-AF patients, 100 Uygur AF patients and 100 Uygur non-AF patients. The criteria to include patients with atrial fibrillation were as follows: patients 18-75 years of age and diagnosed with atrial fibrillation for over 6 months by 12-lead electrocardiogram (ECG) or 24-hour Holter monitor. Atrial fibrillation's representation on ECG could include the following: the disappearance of P waves and replacement by small irregular baseline fluctuations with irregular shape and amplitude known as f-waves; a frequency of approximately 350 to 600 beats/min; or an extremely irregular ventricular heart rate. The normal ventricular heart rate of atrial fibrillation patients who do not receive drug treatment and have normal AV conduction is usually between 100 and 160 beats/minute. The QRS complexes are usually normal [11]. The control groups consisted of patients who were: (1) admitted to the hospital during the same period as the AF cases; (2) did not have a history of AF. Patients with infectious or non-infectious inflammatory disease, acute coronary syndrome, severe liver and kidney dysfunction, cancer, immune system diseases, tissue damage within a month and vascular events, surgery and stroke within six months were all excluded. The study was approved by the Institutional Review Board of the First Affiliated Hospital of Xinjiang Medical School. Informed consent was obtained from all patients.

DNA extraction

Venous blood samples (2 mL) were collected in test tubes containing ethylenediaminetetraacetic acid as an anticoagulant from all patients the morning after admission and overnight fasting. These blood samples were immediately frozen at -20°C. The whole blood genome extraction kit (Tiangen Biochemical Technology Co. Ltd., Beijing, China) was used for genomic DNA extraction.

Target PCR amplification

A total of 2 μl DNA template was used in a 20 μL PCR reaction containing the following: 10.0 μL master mix, 0.5 μL forward primer (5'-GCCAGTGGCACAAAAGACAGGCT-3'), 0.5 μL reverse primer (5'-GGAACTGCTGATCAGTTTGGGAGA-3'), and 7 μl ddH_2O. The reactions were gently vortexed to mix. PCR conditions were: initial denaturation at 95°C for 3 min, followed by 34 cycles of 95°C for 30 s, 60.6°C for 30 s, and 72°C for 1 min, with a final extension at 72°C for 5 min. The reactions were cooled to 4°C for at least 1 min. Amplified products (10 μl) were loaded onto 2% agarose electrophoresis gels to determine the sizes of the amplicons and whether the reactions were specific.

DNA sequencing

Amplified PCR products were sequenced by Shanghai Biotechnology Co. Ltd (Shanghai, China) to identify mutation sites.

Statistical analysis

The APSS16.0 software package was used for statistical analysis. Between-group comparisons of the categorical variables and the Hardy-Weinberg equilibrium (HWE) test were performed using the χ^2 test. Continuous variables were expressed as means ± standard deviation, and the between-group comparison was performed using the student's *t*-test. The non-conditional logistic regression model was used to analyze risk factors and confounding factors of AF. All tests were two-tailed tests and $P < 0.05$ was considered statistically significant.

Results

General comparison between cases and controls

Patient age and low density lipoprotein (LDL) levels were significantly different between cases and controls in both the Uygur and Han populations (both $P < 0.05$). No significant differences were observed regarding gender, smoking history, drinking history, systolic blood pressure (SBP), diastolic blood pressure (DBP), fasting blood sugar (FBG), fasting triglyceride (TG), and fasting high density lipoprotein (HDL) between the AF and control groups among either Uygur or Han individuals ($P > 0.05$) (**Table 1**).

Fitness test using the Hardy-Weinburg equilibrium

The χ^2 analysis showed that genotype and allele frequency distributions of *SCN5A* H558R were in HWE, suggesting that the sample in the present study represented a certain population structure.

Comparison of genotype and allele frequencies of the SCN5A H558R between cases and controls of Uygur and Han populations

PCR amplified a 500-bp product of the *SCN5A* H558R (**Figure 1**). Electrophoresis showed that

Table 1. Overall comparison between AF and non-AF groups of the Uygur and Han populations

Variables	Han Population			Uygur Population		
	Non-AF group	AF group	*P* value	Non-AF group	AF group	*P* value
Age/years old	53.97±4.97	63.49±10.5	< 0.001*	52.98±9.03	56.22±7.81	0.007*
Male/female	56/44	53/47	0.670	66/34	64/36	0.767
History of smoking	26 (26)	23 (23)	0.622	31 (31)	29 (29)	0.758
History of drinking	28 (28)	25 (25)	0.631	23 (23)	26 (26)	0.622
SBP/mmHg	119.91±15.78	123.2±16.08	0.146	124.86±15.87	123.86±18.18	0.679
DBP/mmHg	74.95±10.15	75.21±11.78	0.867	79.26±11.06	77.7±10.82	0.314
FBG/mmol/L	5.13±1	5.08±1.1	0.712	4.73±1.16	4.93±1.1	0.218
TG/mmol/L	1.52±0.61	1.59±0.54	0.399	1.79±0.74	1.59±0.76	0.066
HDL/mmol/L	1.24±0.35	1.17±0.29	0.121	1.37±0.56	1.26±0.48	0.135
LDL/mmol/L	2.22±1.28	2.6±0.64	0.008*	2.34±0.8	2.65±0.86	0.009*

Note: *indicated significant difference compared to control. Data presented as mean ± SD or Number of cases (%).

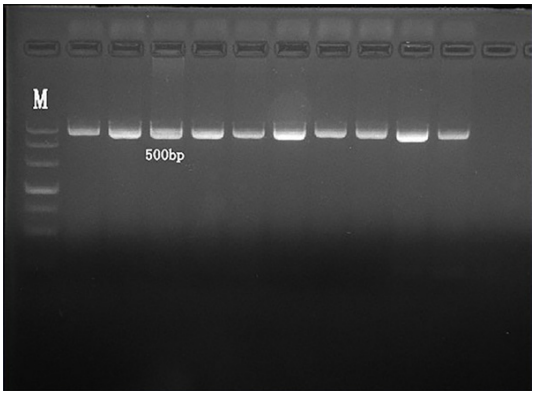

Figure 1. PCR products of *SCN5A* H558R amplification.

the product was distinct without non-specific bands, indicating that PCR products met experimental requirements. Three genotypes exist of the *SCN5A* H558R locus: AA, AG, and GG (**Figure 2**). In both the Uygur and Han populations, the genotype and allele frequencies were significantly different between case and control groups (P < 0.05). Additionally, the genotype and allele frequency was significantly different between Uygur and Han AF patients (P < 0.05). The AG genotype and G allele in the AF group had a significantly higher frequency than in the control group (**Tables 2**, **3**).

Logistic regression analysis of the SCN5A H558R among Uygur and Han populations

The unconditional logistic regression model was applied to AF risk factors including age, sex, history of smoking, history of drinking, blood pressure, fasting blood sugar, triglyceride, total cholesterol, HDL, and LDL. In both the Han and Uygur populations, the AG+GG genotype was correlated with AF occurrence (OR=2.72, 95% CI=1.25-5.96, P=0.012) and (OR=2.37, 95% CI=1.22-4.63, P=0.011), respectively. The AG+GG genotype was also correlated with AF occurrence when both Uygur and Han populations were considered together (OR=2.19, 95% CI=1.36-3.53, P= 0.001) (**Tables 4**, **5**).

Discussion

SCN5A encodes the α-subunit of the voltage-gated sodium channel. It is located on chromosome 3p21 and has 28 exons. The α-subunit consists of 2,016 amino acids with a molecular weight of 227 kDa. It has four homologous structural domains (DI-DIV) joined by an inter-domain cytoplasmic linker [12, 13]. Each domain contains six transmembrane segments (S1-S6), of which S4 serves as the voltage sensor being rich in positively-charged residues. Membrane depolarization causes the transmembrane movement of S4 segment, which activates the sodium channel [14, 15].

SCN5A mutations can lead to sodium channel functional defects or blockage of protein expression or transportation, resulting in a weakened influx of total sodium ions, disequilibrium of the transmembrane ionic current during the phase 1 action potential, the disappearance of the plateau during the phase 2 action potential in epicardial cells, a shortened action potential, and eventual cardiac arrhythmia [16]. To date, many cardiac diseases have been

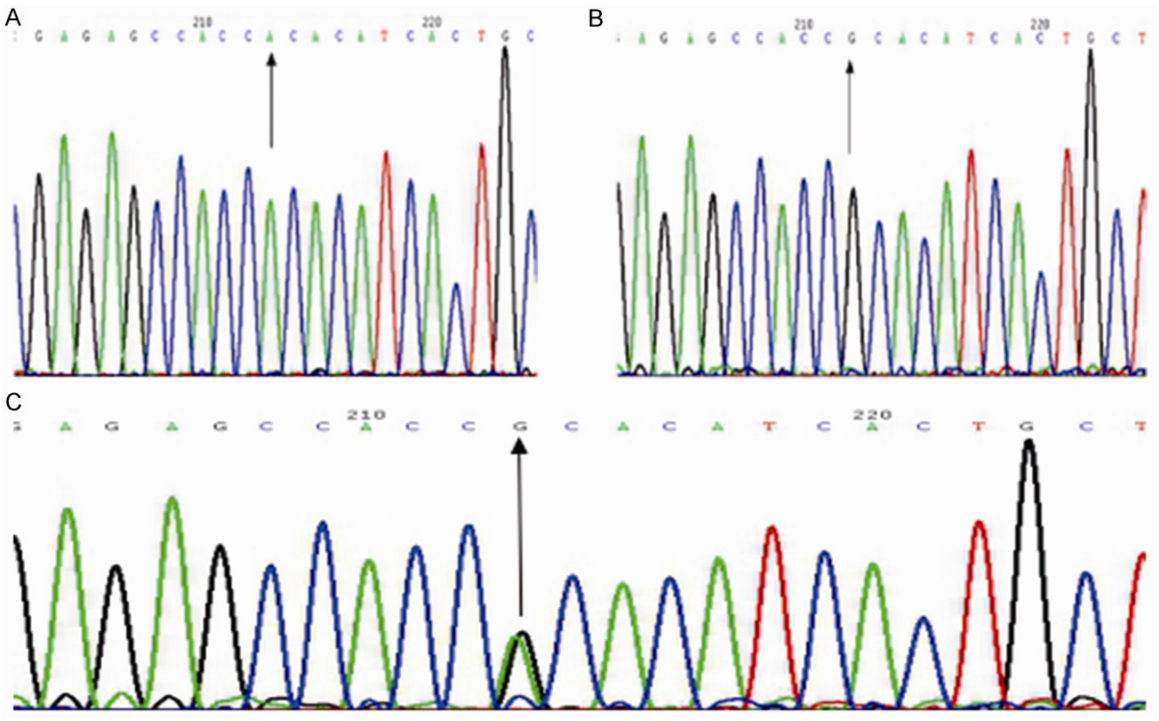

Figure 2. Sequencing results of the *SCN5A* H558R SNP. A: Homozygous AA wild-type; B: Mutated homozygous GG genotype; C: Heterozygous AG genotype.

Table 2. Genotype and allele frequencies of *SCN5A* H558R in Uygur and Han populations

Genotype frequency	Uygur population		x^2 vale	*P* value	Han population		x^2 value	*P* value
	Case group	Control group			Case group	Control group		
Genotype frequency								
AA	57	74		0.023*	43	61	7.376	0.025
AG	41	23			49	36		
GG	2	3			8	3		
Allele frequency								
A	155	171	4.245	0.039	135	158	6.749	0.009
G	45	29			65	42		

*Likelihood ratio test.

Table 3. Genotype and allele frequencies of *SCN5A* H558R in the Uygur AF and Han AF

Group	Genotype frequency			Allele frequency	
	AA	AG	GG	A	G
Han AF	43	49	8	135	65
Uygur AF	57	41	2	155	45
x^2 value	6.271			5.016	
P value	0.043			0.025	

associated with mutations in *SCN5A*, including long QT syndrome [17], Brugada syndrome [18, 19], sick sinus syndrome and atrial fibrillation [20, 21]

Chen et al. [9] previously found that the *SCN5A* H558R SNP was correlated with isolated AF and illustrated a possible mechanism by which AF was aggravated by H558R. They used functional expression and whole cell patch clamp technology to show that H558R decreased the sodium currents, which caused a decrease in atrioventricular conduction. This in turn shortened the wavelength of the atrial reentry, and favored both single-circuit and multiple-circuit reentry, leading to AF.

Sequencing results from our study revealed the existence of three *SCN5A* H558R genotypes, and the significant difference in the distribution

Table 4. Logistic regression analysis of *SCN5A* H558R in Uygur and Han populations

Genotype	AF group (n, %)	Non-AF group (n, %)	OR value (95% CI)	*P* value
Han population				
AA	43 (43.0)	61 (61.0)	1	
AG	49 (49.0)	36 (36.0)	2.77 (1.23-6.25)	0.014
GG	8 (8.0)	3 (3.0)	1.40 (0.56-3.48)	0.473
AG+GG	57 (57.0)	39 (39.0)	2.72 (1.25-5.96)	0.012
Uygur population				
AA	57 (57.0)	74 (74.0)	1	
AG	41 (41.0)	23 (23.0)	2.65 (1.33-5.29)	0.006
GG	2 (2.0)	3 (3.0)	0.82 (0.28-2.38)	0.716
AG+GG	43 (43.0)	26 (26.0)	2.37 (1.22-4.63)	0.011

Table 5. Logistic regression analysis of *SCN5A* H558R considering Uygur and Han populations together

Genotype	AF group (n, %)	Non-AF group (n, %)	OR value (95% CI)	*P* value
AA	100 (50.0)	135 (67.5)	1	
AG	90 (45.0)	59 (29.5)	2.20 (1.35-3.58)	0.001
GG	10 (5.0)	6 (3.0)	1.36 (0.73-2.54)	0.328
AG+GG	100 (50.0)	65 (32.5)	2.19 (1.36-3.53)	0.001

of these between cases and controls in both the Uygur and Han populations ($P < 0.05$). Logistic regression analysis found that the AG+GG genotype was correlated with AF occurrence in both the Han and Uygur populations, as well as when both populations were collectively analyzed. Thus, the H558R polymorphism of SCN5A gene appears to be correlated with AF occurrence, with the G allele likely causing predisposition to AF. Our results are consistent with a previous case-control study by Chen et al. [9] in which H558R was correlated with AF, and the G allele shown to be an independent risk factor. Our study also revealed significant frequency differences in genotypes and alleles of H558R between Uygur and Han AF patients in Xinjiang ($P < 0.05$). This could be explained by differences in genetics, lifestyles, and locations of the two populations.

The present study showed that age differences were significant between the AF group and control group in both Uygur and Han populations; in other words, that the AF group had a significantly higher mean age than the control group in both populations. This indicates that age is a risk factor for AF, which is consistent with the results found previously in studies from China and worldwide. This may reflect the modified cardiac structure and electrical activities that occur with increasing age, as well as the fact that weight often increases with age.

A limitation of the present study was its small number of regions and sample size. It therefore should be repeated in a larger area with an increased population to understand the underlying AF mechanisms at the genomics level. Nevertheless, we confirmed that the H558R polymorphism of SCN5A gene is significantly correlated with AF occurrence in both the Uygur and Han populations of Xinjiang. Such results not only provide molecular genetics data for further study of this correlation, but also the theoretical rationale for conducting additional genetic studies.

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Disclosure of conflict of interest

None.

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