Correlations of SCN5A gene polymorphisms with onset of atrial fibrillation

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Abstract. – OBJECTIVE: The aim of this study was to analyze the correlations of the sodium channel, voltage-gated, type V, alpha subunit (SCN5A) gene polymorphisms with the onset of atrial fibrillation (AF), and its clinical significance.

PATIENTS AND METHODS: The quantitative Real Time-Polymerase Chain Reaction (QRT-PCR) amplification and the TaqMan analysis were utilized to analyze the composition of SCN5A genotypes and alleles in the peripheral blood mononuclear cells of 48 normal controls and 115 AF patients. Meanwhile, the differences in single nucleotide polymorphisms (SNPs), 1673 A>G, and 3666+69 G>C, between the AF group and the control group were analyzed using the χ^2-test. The high-risk factors influencing the AF attack were analyzed via logistic regression analysis, as well as univariate and multivariate analyses. In addition, the correlations of gene polymorphisms with high-risk factors (drinking and hypertension) for AF were verified by the χ^2-test.

RESULTS: There were statistically significant differences in the incidence rate of hypertension, the times of smoking and drinking, and the frequencies of palpitation and syncope between the AF group and the control group ($p<0.05$). The composition of genotypes and alleles of 1673 A>G and 3666+69 G>C in the AF group was significantly different from that of the control group ($p<0.05$). According to the results of the logistic regression analysis, as well as the univariate and multivariate analyses, drinking, and hypertension were associated with the occurrence of AF ($p<0.05$). Furthermore, statistically significant differences were observed in the composition of the gene polymorphisms 1673 A>G and 3666+69 G>C between patients with and without histories of drinking and hypertension ($p<0.05$).

CONCLUSIONS: There are significant differences in SCN5A gene polymorphisms 1673 A>G and 3666+69 G>C between AF patients and normal controls. Moreover, drinking and hypertension can influence the changes in the gene polymorphisms.

Key Words:
SCN5A, Gene polymorphism, Atrial fibrillation (AF), Risk factors, Drinking, Hypertension.

Introduction

Atrial fibrillation (AF) is one of the common forms of arrhythmia in clinical practices, with an estimated annual prevalence rate of ≥ 1% among adults worldwide. Previous studies have indicated that AF significantly increases the social and economic burden in the world. AF plays a predominant role in elevating the prevalence rate of persistent tachyarrhythmia in human beings[1]. Meanwhile, it has an apparent correlation with the occurrence of myocardial infarction and stroke. For example, some studies[2,3] have manifested that both the prevalence rate and severity of AF are raised to some extent in patients with myocardial infarction and stroke, seriously threatening patients' life. Some researchers have proved that AF is a multi-factorial disease regulated by genetic and non-genetic factors, including age, gender, hypertension, ischemic heart disease, heart failure, valvular heart disease, obesity, diabetes, hyperthyroidism, smoking, alcoholism, and pulmonary disease. These are considered as risk factors for the development of AF[4,5]. However, the pathogenesis of AF has not been thoroughly elucidated. With the rapid development of the sequencing techniques, a growing number of studies have discovered that great progress has been made in the research of the AF-related genes. Moreover, the role of genetics in AF becomes clearer[6]. Recently, massive rare variants of specific genes have been detected related to the occurrence of AF. Furthermore, it has also been revealed that the cardiac sodium channel serves as an important ion channel for rapid depolarization of cardiac action potential (AP). In this process, the sodium channel, voltage-gated, type V, and the α-subunit (SCN5A) gene polymorphisms induced by mutation are evidently correlated with arrhythmia.

SCN5A gene encodes an α-subunit of cardiac sodium channel, which is involved in the stable

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production and transmission of cardiac AP[7]. It is located on chromosome 3p21 and consists of 101,617 bases and 28 exons. SCN5A encodes 2,016 amino acids synthesizing the α-subunit of cardiac sodium channel. Remarkable increases in single nucleotide polymorphisms (SNPs) (1673 A>G and 3666+69 G>C) of the SCN5A gene, a molecular target of antiarrhythmic drugs, will increase the incidence rate of AF. Relevant studies[8-11] have illustrated that abnormally mutated 1673 A>G can alter channel gating kinetics and membrane transport capacity, can influence the activity of sodium channel, and trigger AF.

In this study, the genetic tests were performed for the peripheral blood samples of AF patients and normal controls. Meanwhile, SNPs 1673 A>G and 3666+69 G>C were analyzed as well. The aim of this study was to explore the correlations of the SCN5A gene polymorphisms with AF onset, thus providing specific targets for the laboratory studies and clinical treatments of AF patients in China.

Patients and Methods

Patients

A total of 115 AF patients aged (48.34 ± 5.02) years old and 48 normal controls aged (45.93 ± 4.85) years old were enrolled in this research. The epidemiological information of the enrolled participants was recorded, including: age, gender, body mass index (BMI), dyspnea, incidence rate of hypertension, times of smoking and drinking, as well as frequencies of palpitation and syncope. Informed consent was obtained from each subject before the study. The exclusion criteria were as follows: participants with congenital heart disease, trauma, or cardiovascular diseases induced by surgery. This study was approved by the Ethics Committee of Tianjin Union Medical Center.

Main reagents

The main reagents were TRIzol lysis buffer (Sigma-Aldrich, Saint Louis, MO, USA), peripheral blood mononuclear cell separation medium (Hao Yang, Tianjin, China), TaKaRa reverse transcription kit (TaKaRa, Otsu, Shiga, Japan), dNTP reagent (Promega, Madison, WI, USA), deoxyribonucleic acid (DNA) polymerase, and amplification primers of GAPDH, as well as 1673 A>G and 3666+69 G>C of SCN5A gene (synthesized by SBS).

Extraction of Total Ribonucleic Acid (RNA) and Acquisition of Complementary DNA (cDNA) Via Reverse Transcription

Venous whole blood (4 mL) was collected into an ethylenediaminetetraacetic acid (EDTA) anticoagulant tube. Subsequently, the intermediate monocytes were obtained using peripheral blood mononuclear cell separation medium. Next, the cells were washed twice, added with 1 mL TRIzol (Invitrogen, Carlsbad, CA, USA) and lysed on ice for 30 min. After that, the cells were added with chloroform, followed by centrifugation to obtain total RNA. The concentration of the extracted RNA was detected using a spectrophotometer. The reverse transcription system (20 μL) was prepared according to the instructions of the kits. In accordance with detected RNA concentration, the sample concentration of 1000 ng/mL was determined by adding the corresponding volume. The reagents in the TaKaRa kit (TaKaRa, Otsu, Shiga, Japan) were added according to the requirements, and the volume was added to 20 μL using diethyl pyrocarbonate (DEPC)-treated water (Beyotime, Shanghai, China). The reverse transcription conditions were as follows: 37°C for 15 min, 85°C for 5 min, cooling down to 4°C and then the reaction was terminated. The reverse transcription product cDNA was stored at -80°C for use.

Quantitative Real Time-Polymerase Chain Reaction (QRT-PCR) Amplification

The amplification system (25 μL) was prepared according to the kit instructions, including: 1 μL cDNA template, 1 μL forward primer, 1 μL reverse primer, 12 μL 2x Taq Master Mix and 10 μL DEPC-treated water. The system was mixed by vortex, and the liquid on the tube wall was centrifuged and precipitated. QRT-PCR amplification conditions were as follows: preheating at 94°C for 2 min, followed by 94°C for 40 s, 55°C for 30 s and 72°C for 30 s, for a total of 40 cycles. After that, the gel electrophoresis was conducted under a voltage of 120 V for 30 min. Finally, the imaging analysis was performed using a gel imager (Bio-Rad, Hercules, CA, USA). Primers used were shown in Table I.

Statistical Analysis

The Statistical Product and Service Solutions (SPSS) 22.0 (IBM Corp., Armonk, NY, USA) was used for all statistical analyses. The enumeration data in the epidemiological characteristics of patients were subjected to the χ^2-test, while the measurement data were subjected to inde-

Table I. Primer sequences used in PCR.

Snp	Site	Primers	Length
1673 A>G	Exon 12	F: 5'-CCCTCAATGCTCTGAGAAGTTT-3' R: 5'-TTTGGACTTGGCACTGGTGATCA-3'	237
3666 + 69 G>C	Intron 20	F: 5'-CGCCACCCCCATCATCGTAG-3' R: 5'-TGACCTGACTTTCCAGCTGGAGA-3'	367

pendent-samples *t*-test. Hardy-Weinberg genetic equilibrium test was adopted for participants. The χ^2-test was applied to analyze the composition of the genotypes and alleles. The multiple logistic regression analysis was used for the statistical analysis of the high-risk factors for AF onset. The univariate and multivariate regressions were utilized to analyze the correlations of clinical features of AF patients with gene polymorphisms. The χ^2-test was conducted to analyze the status of drinking and hypertension, as well as two gene polymorphisms of SCN5A. $p \leq 0.05$ was considered statistically significant.

Results

Epidemiological Characteristics of Recruited Subjects

The epidemiological characteristics of the recruited subjects were shown in Table II. No significant differences were observed in age, gender, BMI, occurrence of dyspnea, and electrocardiogram characteristics of sinus rhythm between the AF group and the control group ($p>0.05$). Patients in the AF group exhibited significantly higher incidence rate of hypertension, more time of smoking and drinking, as well as greater frequencies of palpitation and syncope, than the control group ($p<0.05$).

Hardy-Weinberg Genetic Equilibrium Test for Recruited Subjects

The Hardy-Weinberg genetic equilibrium test (χ^2-test) was adopted to analyze the genotype composition of 1673 A>G and 3666+69 G>C in the AF group and the control group. No statistically differences were observed between the AF group and the control group, suggesting that both 1673 A>G and 3666+69 G>C met the genetic equilibrium law (Table III).

Composition of Genotypes and Alleles of 1673 A>G/3666+69 G>C

To clarify the composition of the genotypes of the two polymorphisms in the AF group and the control group, qRT-PCR and gel electrophoresis were performed for peripheral blood mononuclear cells of recruited subjects. Meanwhile, the composition of genotypes was subjected to the χ^2-test.

Table II. Clinical features of study population.

	AF group (115)	Control group (48)	F/χ^2	*p*
Age	48.34±5.02	45.93±4.85	2.73	0.24
Gender (male/female)	68±47	23±25	1.73	0.19
BMI (kg/m^2)	26.64±3.65	24.07±2.94	3.65	0.068
Hypertension (+/-)	51/64	13/35	4.35	0.040
Smoking (+/-)	78/37	26/22	5.70	0.017
Drinking (+/-)	46/69	11/37	4.35	0.037
Dyspnea (+/-)	17/98	2/46	3.71	0.054
Palpitation (+/-)	53/62	12/36	6.28	0.012
Syncope (+/-)	36/79	4/44	9.65	0.002
Electrocardiogram characteristics of sinus rhythm			23.21	<0.001
Ventricular rate (min^{-1})	73.52±22.17	71.17±19.43	2.93	0.21
PR interval (ms)	172.64±32.42	175.53±33.28	1.67	0.35
QRS duration (ms)	109.55±55.63	102.74±17.89	3.31	0.17
QT interval (ms)	426.88±29.63	419.63±36.18	3.08	0.19

Table III. Genetic equilibrium test.

Gene	Genotype	AF group			Control group		
		n=117	χ^2	*p*	n=50	χ^2	*p*
1673 A>G	AA	49	0.05	0.98	15	1.83	0.40
	AG	51			17		
	GG	15			16		
3666+69 G>C	GG	16	0.26	0.88	19	0.19	0.91
	GC	49			21		
	CC	50			8		

The results indicated that statistically significant differences were observed in the genotypes and alleles of 1673 A>G and 3666+69 G>C between the AF group and the control group ($p<0.05$). All these findings implied that the occurrence of AF might be correlated with gene changes to some extent (Table IV).

Logistic Regression Analysis of Risk Factors for AF

Based on collected epidemiological characteristics, age, gender, BMI, hypertension, smoking, drinking, dyspnea, palpitation, syncope, and the electrocardiogram characteristics of sinus rhythm were assigned (Table V). Subsequently, the above epidemiological characteristics of the recruited subjects were analyzed via multivariate logistic regression analysis. The results demonstrated that the statistically significant differences were observed in hypertension, smoking, drinking, dyspnea, palpitation, and syncope between the AF group and the control group ($p<0.05$), all of which were risk factors for AF (Table VI).

Analysis on Correlations of Gene Polymorphisms 1673 A>G and 3666+69 G>C With Risk Factors in AF Patients

The above results manifested significant correlations of gene polymorphisms 1673 A>G and 3666+69 G>C with the onset of AF. To further investigate whether high-risk factors for AF had certain associations with gene polymorphisms, the multivariate and univariate analyses were adopted for the correlations of the two gene polymorphisms with epidemiological characteristics of patients. As shown in Table VII, drinking, palpitation, and syncope were markedly correlated with gene polymorphism 1673 A>G. As shown in Table VIII, hypertension, palpitation, and syncope exhibited evident correlations with gene polymorphism 3666+69 G>C. Since syncope and palpitation were characteristic clinical features after AF attack, the multiple logistic regression analysis results suggested that drinking and hypertension were high-risk factors for AF.

Analysis on Correlations of Gene Polymorphisms With Drinking and Hypertension

The χ^2-test was utilized for the correlation analysis on the composition of gene polymorphism 1673 A>G and drinking status among all recruited subjects. The results revealed that the gene polymorphism 1673 A>G had prominent differences between drinking and non-drinking people ($p<0.05$) (Table IX). The same method was then applied for the correlation analysis on the composition of gene polymorphism 3666+69 G>C and hypertension status. Of note, similar prominent differences were observed ($p<0.05$) (Table X). The multiple logistic regression analysis, as well as univariate and multivariate regression analyses, implied that drinking and hypertension were risk factors for AF. Furthermore, they could affect the genetic composition of 1673 A>G and 3666+69 G>C.

Discussion

SCN5A is a gene closely associated with the occurrence of heart diseases. It encodes α-subunit of the voltage-gated sodium channel, and it is also responsible for the uplink phase of rapid depolarization of cardiac AP[12]. Multiple studies have demonstrated that mutations of the SCN5A gene can result in loss-of-function or dysfunction of the sodium channel. Meanwhile, it can significantly increase the incidence rates of ventricular fibrillation, dilated cardiomyopathy, cardiac conduction disease, and congenital sick sinus syndrome[13,14].

Table IV. Composition of genotypes and alleles of 1673A>G and 3666+69 G>C.

Gene	Genotype	AF group		Control group				AF group			Control group			
		n	%	n	%	χ^2	*p*	Allele	n	%	n	%	χ^2	*p*
1673 A>G	AA	49	42.61	15	31.25	9.09	0.011	A	149		44		12.40	<0.001
	AG	51	44.35	17	35.42			G	81		56			
	GG	15	13.04	16	33.33									
3666+69 G>C	GG	16	13.91	19	39.58	17.25	<0.001	G	81		59		19.03	<0.001
	GC	49	42.61	21	43.75			A	149		37			
	CC	50	43.47	8	16.67									

Table V. Variable assignments for logistic regression analysis.

Variable	Assignment
Age	<46=1, ≥46=0
Gender (male/female)	Male=1, female=0
BMI (kg/m^2)	<25=1, ≥25=0
Hypertension (+/-)	Yes=1, No=0
Smoking (+/-)	Yes=1, No=0
Drinking (+/-)	Yes=1, No=0
Dyspnea (+/-)	Yes=1, No=0
Palpitation (+/-)	Yes=1, No=0
Syncope (+/-)	Yes=1, No=0
Ventricular rate (min^{-1})	<72=1, ≥72=0
PR interval (ms)	<174=1, ≥174=0
QRS duration (ms)	<105=1, ≥105=0
QT interval (ms)	<422=1, ≥422=0

Table VI. Logistic regression analysis of risk factors.

Independent variable	β value	S*	Odds ratio (OR)	Confidence 95% interval (95% CI)	p
Hypertension	3.31	0.76	9.13	1.97-15.23	0.0017
Smoking	1.92	0.43	4.61	1.32-6.56	0.042
Drinking	2.68	0.53	4.42	1.54-7.21	0.035
Palpitation	3.72	0.81	5.31	2.17-7.86	0.0012
Syncope	2.97	0.68	4.97	1.79-6.54	0.013

Table VII. Univariate and multivariate regression analyses on gene polymorphism 1673 A>G.

Variable Univariate analysis (n=117)	Classification	OR (95% CI)	p
Gene polymorphism 1673 A>G	GA	0.966 (0.436-1.86)	0.045
Age	<46=1, ≥46=0	0.048 (0.022-0.57)	0.44
Gender (male/female)	Male=1, female=0	0.64 (0.41-0.72)	0.23
BMI (kg/m^2)	<25=1, ≥25=0	0.065 (0.022-0.184)	0.33
Hypertension (+/-)	Yes=1, No=0	1.84 (0.90-4.26)	0.0011
Smoking (+/-)	Yes=1, No=0	1.29 (0.34-3.27)	0.0071
Drinking (+/-)	Yes=1, No=0	1.54 (0.43-4.61)	0.0021
Dyspnea (+/-)	Yes=1, No=0	0.54 (0.23-1.18)	0.067
Palpitation (+/-)	Yes=1, No=0	1.33 (0.43-3.22)	0.0021
Syncope (+/-)	Yes=1, No=0	1.42 (0.51-3.73)	0.0016
Gene polymorphism 1673 A>G	GA	1.27 (0.56-2.24)	0.032
Drinking	<24=1, ≥24=0	1.57 (1.34-3.73)	0.0019
Palpitation	<135/<85=1, ≥135/85=0	1.74 (0.53-4.36)	0.0014
Syncope	Yes=1, No=0	1.66 (0.33-3.84)	0.018
Smoking (+/-)	Yes=1, No=0	1.29 (0.34-3.27)	0.0071
Drinking (+/-)	Yes=1, No=0	1.54 (0.43-4.61)	0.0021
Dyspnea (+/-)	Yes=1, No=0	0.54 (0.23-1.18)	0.067
Palpitation (+/-)	Yes=1, No=0	1.33 (0.43-3.22)	0.0021
Syncope (+/-)	Yes=1, No=0	1.42 (0.51-3.73)	0.0016
Gene polymorphism 1673 A>G	GA	1.27 (0.56-2.24)	0.032
Drinking	<24=1, ≥24=0	1.57 (1.34-3.73)	0.0019
Palpitation	<135/<85=1, ≥135/85=0	1.74 (0.53-4.36)	0.0014
Syncope	Yes=1, No=0	1.66 (0.33-3.84)	0.018

Table VIII. Univariate and multivariate regression analyses on gene polymorphism 3666+69 G>C.

Variable Univariate analysis (n=117)	Classification	OR (95% CI)	*p*
Gene polymorphism 3666+69 G>C	GC	0.95 (0.56-1.94)	0.033
Age	<46=1, ≥46=0	0.056 (0.031-0.66)	0.375
Gender (male/female)	Male=1, female=0	0.67 (0.42-0.73)	0.17
BMI (kg/m2)	<25=1, ≥25=0	0.062 (0.012-0.18)	0.313
Hypertension (+/-)	Yes=1, No=0	2.14 (0.83-4.44)	0.001
Smoking (+/-)	Yes=1, No=0	1.48 (0.31-3.32)	0.024
Drinking (+/-)	Yes=1, No=0	1.57 (0.57-3.10)	0.017
Dyspnea (+/-)	Yes=1, No=0	0.48 (0.11-1.01)	0.23
Palpitation (+/-)	Yes=1, No=0	2.10 (0.38-4.76)	<0.001
Syncope (+/-)	Yes=1, No=0	1.87 (0.49-3.87)	0.01
Gene polymorphism 3666+69 G>C	GA	1.42 (0.68-2.39)	0.015
Hypertension	<24=1, ≥24=0	2.37 (1.23-3.51)	<0.001
Palpitation	<135/<85=1, ≥135/85=0	1.55 (0.48-4.16)	0.0013
Syncope	Yes=1, No=0	1.03 (0.23-3.42)	0.038

Table IX. Analysis on correlations of gene polymorphism 1673 A>G with drinking among people.

Gene	Genotype	Drinking		Non-drinking			
		n	%	n	%	χ^2	*p*
1673 A>G	AA	33	57.89	31	29.25		
	AG	16	28.07	52	49.06	12.81	0.002
	GG	8	14.04	23	21.70		

Table X. Analysis on correlations of gene polymorphism 1673 A>G with drinking among people.

Gene	Genotype	Hypertension		Non-hypertension			
		n	%	n	%	χ^2	*p*
3666+69 G>C	GG	19	18.27	16	27.12		
	GC	44	41.51	26	44.07	2.59	0.27
	CC	41	38.68	17	28.81		

For instance, the dysfunction of the sodium channel can trigger dysfunction of excitatory electrical signals. The disorders of the SCN5A-encoded signaling pathway may cause a decreased function of the cytoskeletal proteins binding to their ligands as well. This may finally lead to myocardial dilation, remodeling, and dilated cardiomyopathy[12,15,16]. As a multi-factorial disease, AF is probably triggered when the threshold is reached under the action of genetic and environmental factors. In this study, the gene variations of 1673 A>G and 3666+69 G>C in SCN5A were analyzed. Meanwhile, we aimed to clarify the role of the genetic variation factors in AF. By exploring the associations of the allele and genotypes of gene polymorphisms 1673 A>G and 3666+69 G>C with AF, our results indicated that the allele and genotypes of the two gene polymorphisms in AF group were notably different from those in the control group. This manifested that the polymorphisms induced by SCN5A gene variation might be the major cause of AF. Previous studies have found that the SCN5A gene regulates the expression level of the α-subunit of sodium channel. Its polymorphisms in many exons have been found to be related to the occurrence of heart diseases[17]. Therefore, the correlations of 1673 A>G and 3666+69 G>C changes with the changes in myocardial sodium channel-related proteins can be analyzed at protein expression level in subsequent studies. All these findings might provide relevant targets for the treatment of AF and other heart diseases.

AF is a disease influenced by both environment and gene. It is also particularly important to analyze its epidemiological characteristics in the study of its pathogenesis. In this paper, the epidemiological analysis in the AF group and the control group manifested that some statistically significant differences were observed in hypertension, smoking, drinking, syncope, and palpitation ($p<0.05$). However, in view of the fact that syncope and palpitation are characteristic clinical features after AF attack, the high-risk factors for AF include hypertension, smoking, and drinking, rather than those two factors. According to the aforementioned analyses, the gene polymorphisms 1673 A>G and 3666+69 G>C of SCN5A were prominently correlated with the onset of AF. Considering that epidemiological characteristics are closely related to AF[18-20], such as hypertension, smoking, and drinking, a logistic regression analysis was adopted for the two gene polymorphisms and epidemiological characteristics. It was discovered that hypertension, smoking, drinking, syncope, and palpitation showed significant correlations with AF. According to the univariate and multivariate analyses on the correlations of gene polymorphism 1673 A>G with relevant factors, we found that drinking, palpitation, and syncope were significantly associated with 1673 A>G gene polymorphism. Numerous studies have demonstrated that syncope and palpitation are clinical features instead of risk factors. Therefore, the correlations between drinking and genotypes and alleles of 1673 A>G among all recruited subjects were analyzed. The results indicated that the gene composition of 1673 A>G had statistical differences between drinking and non-drinking people. The same analytical methods were utilized to analyze the correlations of the gene polymorphism 3666+69 G>C with risk factors. It was revealed that hypertension exerted a close correlation with gene polymorphism 3666+69 G>C. Moreover, the gene composition of 3666+69 G>C was statistically different between hypertension and non-hypertension people. Hence, in addition to genetic factors that are capable of affecting the differential expression of SCN5A gene loci, it was conjectured that drinking and hypertension could increase the incidence of gene polymorphisms and trigger AF and other heart diseases by controlling gene mutation and recombination at the SCN5A gene loci.

Conclusions

We showed that there are apparent SCN5A gene polymorphisms 1673 A>G and 3666+69 G>C in the peripheral blood cells of AF patients. This finding can be influenced by a variety of high-risk factors, including hypertension and drinking. Therefore, by analyzing the differences in the genotypes and allele, as well as high-risk factors, can help to find out the proteins, genetic, and environmental factors influencing SCN5A gene expression. Furthermore, the above findings can provide a new treatment direction for the pathogenesis and clinical research of AF.

Conflict of Interests

The Authors declared that they have no conflict of interests.

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