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Association Between rs2200733 Polymorphism of PITX2 Gene and the Risk of Atrial Fibrillation

ABSTRACT

Background: As observed in recent genetic studies, PITX2 is one of the most popular genes with atrial fibrillation; single nucleotide polymorphism (rs2200733) at chromosome 4q25 (near PITX2) is found to be strongly associated with atrial fibrillation, but it has a difference among Chinese Han population. The basic aim of conducting this study is to find the correlation between PITX2 gene polymorphism and the risk of atrial fibrillation and to identify the possibility for early diagnosis of silent atrial fibrillation and high-risk atrial fibrillation.

Methods: The study included 98 cases of atrial fibrillation patients and 88 non-atrial fibrillation patients in Affiliated Hospital of Yangzhou University were enrolled in a case-control study. The single nucleotide polymorphism of rs2200733 at 4q25 near PITX2 was genotyped by polymerase chain reaction-restriction fragment length polymorphism analysis.

Results: A total of 98 patients with atrial fibrillation were genotyped, and the following frequencies were included in genotype percentages (44.9%, 50%, and 5.1%) while distribution of significant single nucleotide polymorphism rs2200733 consisted (29.55%, 53.41%, and 17.05%) which showed ($\chi^2=9.159$, $P=.01$). There was no significant difference in TC genotype frequency ($P=.642$), frequency of T allele ($\chi^2=7.447$, $P=.006$), and T allele was 1.806 times that of the control group (odds ratio = 1.806, 95% CI = 1.179-2.766, $P=.006$). According to logistic regression analysis, following results were concluded for TC genotype (odds ratio = 3.128, 95% CI = 1.053-9.287, $P=.04$), or TT genotype (odds ratio = 5.077, 95% CI = 1.653-15.595, $P=.005$) increased the risk of atrial fibrillation.

Conclusions: The genotype and allele frequency distribution of rs2200733 (T/C) near PITX2 is different in the atrial fibrillation group and the control group. The T allele is a risk factor for atrial fibrillation. Compared with the CC genotype, the TT genotype increased the risk of atrial fibrillation.

Keywords: Atrial fibrillation, PITX2, single nucleotide polymorphism, PCR-RFLP

ORIGINAL INVESTIGATION

INTRODUCTION

Atrial fibrillation (AF) is one of the most widely recognized arrhythmias in clinical practice. The epidemiology of AF shows that as of 2010, the total number of patients with AF worldwide reached 33.5 million.[1] Recently, the prevalence of AF in Europe and the United States is found to be 1–2%, which is different in other countries and regions. Because the prevalence of AF in Asia, North America, and Europe has been underestimated, the morbidity and incidence are higher than those in Asia.[2] Recently, a cluster sampling survey conducted by Zhou et al[3] (2004) in 14 natural populations showed that the overall prevalence of AF and the standardization rate was 0.61%, and there were differences in gender and age. Age grouping suggested that the prevalence of AF increased with age, and gender grouping suggested that the prevalence of AF in male patients was higher than that in females (0.9% vs. 0.7%; $P=.013$), which is close to the trend of relevant foreign data.[3]

Currently, various studies on the prevalence of AF are considered underestimated due to the missed detection of occult AF, as well as the low rate of hospital visits in patients with asymptomatic AF.[4] With the popularization of hypertension, coronary heart disease, diabetes, chronic kidney disease, valvular heart disease,

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heart failure, and other diseases in China, as well as the development of related hidden AF detection technology and the increase in the awareness of patients with arrhythmia, the future of AF. The prevalence of tremors will continue to increase. According to the 2016 European Society of Cardiology (ESC) guidelines for the management of AF, by 2030, the prevalence of AF in adults over the age of 20 will reach 3% populations with higher prevalence.[1] As the increased rate of AF is found, identifying high-risk groups for AF for early diagnosis is an important part of AF management. According to the genetic basis of AF, association is found with familial AF, heritability, and rapidly developing genome-wide association studies (GWAS) and risk loci is only the beginning of a long process of discovering the mechanisms by which these genetic variants increase AF risk.[5]

In 2007, Gudbjartsson et al[6] found for the first time 2 single nucleotide polymorphisms (SNPs) on chromosome 4q25 in the Icelandic population through GWAS that were strongly associated with AF: rs2200733 and rs10033464. Two repeated trials were validated and a strong association was found between them in Icelandic, Swedish, European, and American population.[6] In Hong Kong, China, the association of rs10033464 was not significant. There are differences found in association with AF in repeated trials of the Chinese Han population. rs2200733 is located in the noncoding region, and there is no functional gene expression in the region of linkage disequilibrium. Its nearest PITX2 gene may be a potential candidate gene for AF.[7]

Genome-wide association studies uses millions of SNPs in the genome as molecular genetic markers to carry out control analysis or correlation analysis at the genome-wide level and to discover gene variants that affect complex traits through comparison.[8] Increased risk of AF in nonfamilial AF patients and the general population, which are located in coding sequences, mediate coded proteins, and some in noncoding regions and affect the expression of adjacent genes, was found. It affects the development of the cardiovascular system and normal cardiac electrical activity, ultimately contributing to the development of AF. In 2014, Tada et al[9] proposed the AF genetic risk score (AF-GRS) using 12 common SNPs associated with AF that had been identified by GWAS, which was used in 27 471 European ancestry patients. Population-involved prospective studies found that AF-GRS containing 12 SNPs was associated with paroxysmal AF, with a higher risk of AF in the highest genetic risk quintile than in the lowest quintile about twice as much. It can be seen that the study of related AF gene polymorphisms can predict the risk of AF, thereby providing a theoretical basis for genetic diagnosis.

In this study, a case–control study method was used to analyze the rs2200733 polymorphism of the PITX2 gene in the AF group and the control group by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) and gene sequencing. Whether the rs2200733 locus most strongly associated with AF is still relevant in the Han population in Yangzhou, China, provides a theoretical basis for the genetic diagnosis of the risk of AF. The purpose of this study is to integrate the knowledge of genetic risk factors into clinical practice and use genetic technology to diagnose AF, especially occult AF for early intervention, reduce the incidence of AF complications such as stroke and other embolic events, improve prognosis, and save medical costs in Chinese Han population.

HIGHLIGHTS

- Complete loss of PITX2 function results in a variety of cardiac malformations, including left atrial appendages, heterogenous atrioventricular and ventricular septal defects, and outflow tract defects including the anterior aorta.
- The PITX2 gene polymorphism rs2200733 (T/C) was closely associated with the risk of AF in the Han population in Yangzhou.
- PITX2 loss-of-function mutations predispose patients to AF by inducing a switch in the atrial and pulmonary myocardium to a sinus node-like phenotype.

METHODS

Study Population

Patients with Atrial Fibrillation

This study selected 98 patients with AF who were hospitalized in the Affiliated Hospital of Yangzhou University from May 2017 to June 2018.

Inclusion criteria: First, there is evidence of paroxysmal, persistent, or permanent AF (including ECG, 24-hour Holter monitoring, etc.). Second, the patients with non-rheumatic valvular heart disease were confirmed by echocardiography. Third, patients born and lived in Yangzhou for a long time who are of Han nationality and have no blood relationship with each other. Fourth, those with complete clinical data.

Exclusion criteria: Heart color Doppler ultrasound showed the existence of rheumatic heart disease, valvular heart disease, or a history of artificial or biological valve replacement. There were 30 patients with paroxysmal AF and 68 patients with non-paroxysmal AF.

Non-Atrial Fibrillation Control

This study selected 88 non-AF patients hospitalized in the Affiliated Hospital of Yangzhou University from May 2017 to June 2018 as the control group.

Inclusion criteria: Patients who have not been confirmed as having AF by electrocardiogram or 24-hour ambulatory electrocardiogram since birth, the patients with non-rheumatic valvular heart disease confirmed by echocardiography, and patients born and lived in Yangzhou for a long time who are of Han nationality and have no blood relationship with each other, and those with complete clinical data were included in the study.

Exclusion criteria: Same AF group.

Genotyping and Single Nucleotide polymorphism

The rs2200733 polymorphism locus information was found in the gene bank. According to the relevant literature data and

Table 1. The Primers Used for Polymerase Chain Reaction

		Primer Sequence
First round primers	Upstream primers	5′-TGAGATGTAGCAATGTAAACAGCTA-3′
	Downstream primers	5′-CCACTGCCCTAAGAGGTCCA-3′
Second round of primers	Upstream primers	5′-TGTAAACAGCTACTTTTTATATGATC-3′
	Downstream primers	5′-GGTAAGGAGCCTAGAGGACAGA-3′

This table shows the relevant literature data and single-nucleotide polymorphism site data, a 413-bp fragment containing the rs2200733 polymorphism site was amplified by nested polymerase chain reaction.

SNP site data, a 413-bp fragment containing the rs2200733 polymorphism site was amplified by nested PCR (Table 1) as shown in Figure 1. DNA was isolated from the blood genomic DNA extraction kit (0.1–1 mL) (DP318) of Tiangen Biochemical Technology (Beijing) Co., Ltd. was used to extract DNA from blood samples according to the instructions, and the concentration and purity were detected with a spectrophotometer. The sample was taken out of the −80°C refrigerator and dissolved at room temperature. of About 200 μL of blood sample was added into a 1.5 mL EP tube, 1 L of erythrocyte lysate was added, inverted and mixed well, and put in a centrifuge. The sample was centrifuged at 10 000 rpm for 1 minute and checked if the nuclei are precipitated. The remaining blood samples of the enrolled patients after the 5 items of coagulation function were routinely checked in the hospital, shaken, mixed, and aliquoted into 1.5 mL EP tubes, and stored in a −80°C refrigerator to avoid repeated freezing and thawing of the samples. The digestion reaction of the PCR products was noted and the restriction endonuclease should be placed on ice immediately after being taken out of the −20°C refrigerator and added in the last step, and the pipette tip must be replaced for each addition.

Statistical Analysis

Statistical analysis was performed using Statistical Package for Social Sciences software version 23 (Chicago, Ill, USA). The data of clinical categorical variables were expressed as frequency (n), and the χ^2 test was used for comparison between groups. Continuous variable data were tested for normal distribution. Normal distribution data were expressed as mean ± standard deviation (x ± s), and independent samples *t*-test was used for comparison between groups. The χ^2 test was used to verify whether the SNP loci were in Hardy–Weinberg equilibrium. Genotype and allele frequencies were compared between patients and controls using the χ^2 test. Logistic regression analysis was used to calculate the odds ratio (OR), 95% CI of genotype, and OR and 95% CI after adjusting for age, sex, history of coronary heart disease, and smoking history. The value of .05 means the difference is statistically significant.

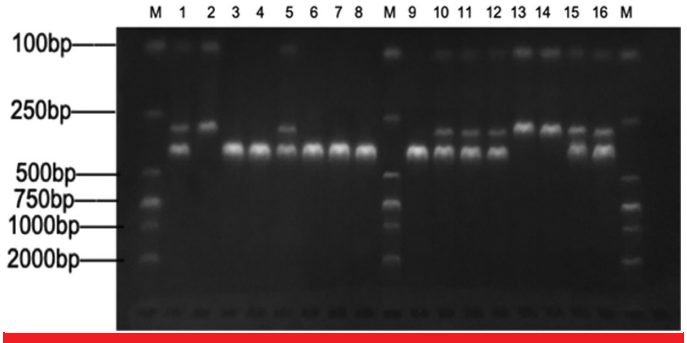

Figure 1. Gel electrophoresis of the restriction polymerase chain reaction product. Amplified DNA fragments of the PITX2 gene patients with atrial fibrillation digested with restriction enzyme MboI and produced bands. M: Marker; 1, 5, 10, 11, 12, 15, 16: TC genotype; 2, 13, 14: CC genotype; 3, 4, 6, 7, 8, 9:TT genotype.

RESULTS

As shown in Table 2, the basic clinical data of 98 patients with AF and 88 control patients were compared. The results showed that there was no statistical difference between the AF group and the control group in terms of gender, body mass index, diabetes, and dyslipidemia ($P > .05$), but there were differences in age, smoking history, hypertension history, coronary heart disease history, and stroke/TIA history difference ($P < .05$), and the AF group was higher than the control group.

The frequencies of different genotypes at the rs2200733 locus in the AF group and the control group are shown in Table 3. Both the AF group and the control group were in line with the Hardy–Weinberg equilibrium, with *P* values of .176 and .727, respectively, indicating that the population was genetically balanced. The frequencies of TT, TC, and CC genotypes at rs2200733 (Figure 2) were 44.9%, 50%, and 5.1% in patients with AF, and 29.55%, 53.41%, and, 17.05% in controls, respectively. According to Pearson's chi-square statistical

Table 2. Baseline Clinical Characteristics of the AF Group and the Healthy Group

Parameters	AF Group (n = 98)	Control Group (n = 88)	*P*
Gender (M/F)	58/40	41/47	.086
Age(years)	73.54 ± 9.04	63.24 ± 10.18	<.001
BMI, kg/m^2	23.20 ± 3.34	23.70 ± 3.38	.316
Paroxysmal AF	30/68		
History of smoking	23/75	6/82	.002
Hypertension	73/25	44/44	.001
Diabetes mellitus	25/73	19/69	.530
Coronary heart disease	61/37	16/72	<.001
Stroke/TIA	45/53	11/77	<.001
Dyslipidemia	18/80	11/77	.271

The basic clinical data of 98 patients with AF and 88 control patients were compared. AF, atrial fibrillation; BMI, body mass index; TIA, transient ischemic attack.

Table 3. Genotype Frequency and Allele Frequency Distribution of Two Groups

Group	Number of Cases	Genotype Frequency, n (%)			Chi-Square	Allele Frequency, n (%)		HWE-*P*
		TT	TC	CC		T	C	
AF group	98	44 (44.90)	49 (50.00)	5 (5.10)		137 (69.90)	59 (30.10)	.176
Control group	88	26 (29.55)	47 (53.41)	15 (17.05)		99 (56.25)	77 (43.75)	.727
χ^2		4.656	0.216	6.892	9.159	7.447		
P		.031	.642	.009	.01	.006		
OR		1.943	0.872	0.262		1.806		
95% CI		1.059-3.564	0.490-1.552	0.091-0.753		1.179-2.766		

This table shows the frequencies of different genotypes at the rs2200733 locus in the AF group and the control group.
AF, atrial fibrillation; OR, odds ratio.

analysis, the genotype of the rs2200733 locus between the AF group and the control group was significantly different ($\chi^2=9.159$, $P=.01$). The frequency of TT genotype in the AF group was higher than that in the control group (2=4.656, $P=.031$), and the frequency of CC genotype was lower than that in the control group ($\chi^2=6.892$, $P=.009$), and the difference was statistically significant. There was no significant difference in TC genotype frequency between the 2 groups ($P=.642$). The frequency of T allele in patients with AF was significantly higher than that in normal controls ($\chi^2=7.447$, $P=.006$). The T allele was found to be a risk factor for AF, with a 1.806-fold higher risk of AF than the control group (OR=1.806, 95% CI=1.179-2.766, $P=.006$).

According to logistic regression analysis (Table 4) with the CC genotype as the reference, the population with the TC genotype (OR=3.128, 95% CI=1.053-9.287, $P=.04$) or the TT genotype (OR=5.077, 95 %CI=1.653-15.595, $P=.005$) increased the risk of AF, and the risk of TT genotype was greater. After adjusting for age, gender, history of coronary heart disease, hypertension, and smoking history, people with TT genotype still had an increased risk of AF (AOR*=4.557, 95% CI=1.129–18.396, $P^*=.033$), but the incidence of AF in people with TC genotype was not significantly different from CC genotype ($P=.259$). By establishing a genetic model, compared with genotype TT, genotype TC and CC can reduce the risk of AF (OR=0.515, 95% CI=0.281-0.944, $P=.032$). There was still

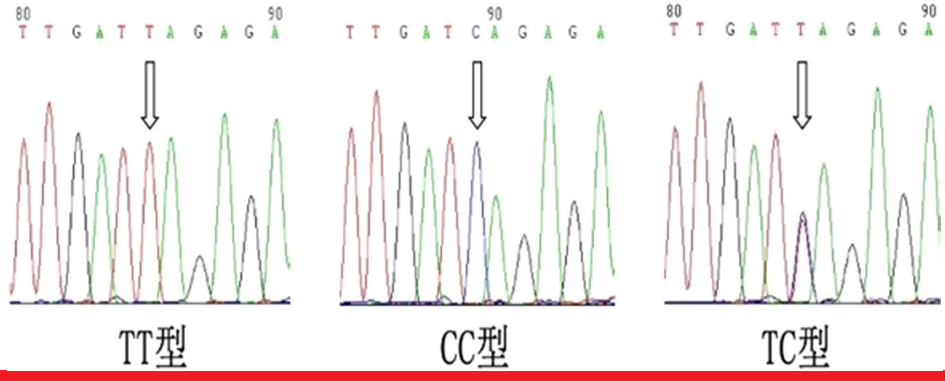

Figure 2. rs2200733 site gene sequencing diagram. The frequencies of TT, TC, and CC genotypes at rs2200733 (Figure 2) were 44.9%, 50%, and 5.1% in patients with AF, and 29.55%, 53.41%, and, 17.05% in controls, respectively.

Table 4. SNP rs2200733 Logistic Regression Analysis of Gene Polymorphisms and Susceptibility to Atrial Fibrillation

rs2200733	Allele	Atrial Fibrillation Group	Control Group	OR Value (95% CI)	*P*	AOR* Value (95% CI)	*P**
Genotype	CC	5	15	1			
	TC	49	47	3.128 (1.053-9.287)	0.040	2.207 (0.558-8.723)	.259
	TT	44	26	5.077 (1.653-15.595)	0.005	4.557 (1.129-18.396)	.033
Stealth model	TT	44	26	1			
	TC+CC	54	62	0.515 (0.281-0.944)	0.032	0.425 (0.201-0.900)	.025
Dominant model	CC	5	15	1			
	TT+TC	93	73	3.822 (1.327-11.004)	0.013	3.072 (0.819-11.527)	.096
Additive model	TC	49	47	1			
	TT+CC	49	41	1.146 (0.644-2.040)	0.642	1.546 (0.749-3.194)	.239

The AOR* value and *P** value were adjusted for age, sex, history of hypertension, history of coronary heart disease, and history of smoking, respectively, after adjusting the OR value and *P*-value.

statistical significance after the factors of heart disease and smoking history (AOR* = 0.425, 95% CI = 0.201-0.900, *P** = .025).

DISCUSSION

Atrial fibrillation is a complex multifactorial disease caused by a combination of genetic and environmental factors.[10] Evidence has shown that a genetic predisposition for AF, especially in patients without underlying heart disease, is known as isolated AF. However, most patients with AF have a combination of common and rare genetic variants that predispose to clinically acquired cardiac or systemic disease, which is referred to as "classic" AF. The pathogenesis of AF is very complex, and various hypotheses have been put forward around the electrophysiological mechanism and pathophysiological mechanism, but none of them can fully explain the occurrence of AF. Advances in genetics in recent decades have provided us with an incompletely understood genetic mechanism for the existence of AF and confirmed the heterogeneity of AF.[11] This is found to be because of this heterogeneity, individual differences in disease mechanisms, and our inability to predict individual responses to therapy that current treatments for AF have limited success. The genetic research of AF provides new ideas and directions for the pathogenesis and treatment of AF and is more in line with the development trend of precision medicine focusing on individual differences of patients.

Early scientists discovered some single-gene mutations related to AF in familial AF through linkage and candidate gene research methods, and clearly located several causative genes related to single-gene AF, but this only explained AF.[12] A small subset of patients had tremors. In recent years, GWAS has developed rapidly and has become a standard procedure for finding the causative genes of complex diseases such as AF by typing hundreds of individuals with 500 000 to 1 million mono-nuclei in each individual. Nucleotide polymorphism markers were used to identify susceptibility loci in the genome that increase the risk of disease.[13]

rs2200733 is the first polymorphism locus associated with AF identified by GWAS. In addition to being thought to be associated with AF, Body et al[14] found that rs2200733 was also independently associated with postoperative AF after coronary artery bypass surgery in two independent cohorts. Husser et al[15] and Shoemaker et al[16] have successively demonstrated that rs2200733 can independently predict the recurrence of AF after catheter ablation in patients with isolated AF and typical AF, can be used as a clinical tool for selecting patients with AF ablation, and was validated in the Chinese Han population.[17] In addition, the researchers also found that rs2200733 was associated with ischemic stroke (OR = 1.26, $P = 2.18 \times 10^{-10}$) and was significantly associated with cardioembolic stroke (OR = 1.54, $P = 8.05 \times 10^{-9}$).[18] Again, the meta-analysis by Sun et al[19] confirmed this view. However, in a study of the Han population in southern China, rs2200733 was not found to be associated with ischemic stroke, but it was shown to be associated with systolic blood pressure in patients with ischemic stroke.[20] It is generally believed that the association of rs2200733 with ischemic stroke is due to its association with AF, although substantial evidence of AF is lacking in this subset of patients.

rs2200733 is located in the noncoding region, and there is no functional gene expression in the region of linkage disequilibrium. Its nearest PITX2 gene may be a potential candidate gene for AF. During embryonic development in vertebrates, PITX2 is the final step in the left-side signaling pathway that establishes left-right asymmetric development of the body. Complete loss of PITX2 function results in a variety of cardiac malformations, including left atrial appendages, heterogenous atrioventricular and ventricular septal defects, and outflow tract defects including the anterior aorta. In humans and mice, PITX2 encodes 3 distinct protein isoforms through alternative splicing: PITX2a, PITX2b, and PITX2c. Only PITX2c is asymmetrically expressed in the developing heart and embryo, and specific deletion of this isoform can lead to previously described congenital malformations.[21]

Multiple studies have demonstrated that PITX2 plays an important role in the pathogenesis of AF.[22] Mommersteeg et al[23] and other studies found that PITX2c knockout mice could not form pulmonary myocardial sleeves. Myocardial sleeve is the myocardial tissue wrapped around the root of the pulmonary vein. It is the key anatomical site for ectopic excitation to cause focal AF, and it is also the starting position of clinical radiofrequency ablation. In addition, Kirchhof et al[21] also found that decreased PITX2c expression in mice shortened the duration of atrial action potentials and induced AF. In adult mice, PITX2-specific deletion enhances the expression of ion channels associated with AF, including increased expression of the potassium channel gene KCNQ1, which alters atrial repolarization, shortens the effective refractory period, and promotes action potentials triggering and conduction. In addition to altering the expression of atrial ion channels, PITX2 downregulates sinus node-specific gene expression, such as Shox2, HCN4, and Cav3.1, and upregulates gene programs characteristic of working myocardial phenotypes, such as Nkx2.5, Cx40, Cx43, ANP, and Kir2.1.[24] Thus, it can be speculated that PITX2 loss-of-function mutations predispose patients to AF by inducing a switch in the atrial and pulmonary myocardium to a sinus node-like phenotype to form an electrophysiological substrate favorable for AF.

PITX2 directs the asymmetric morphogenesis of the heart, participates in the formation of pulmonary veins, inhibits the development of the sinus node, and is involved in the regulation of genes that may lead to atrial arrhythmias, and is currently the strongest candidate causative gene for AF. These studies provide valuable insights into the underlying mechanisms of AF and loci and provide a framework for investigating other AF risk loci.

Currently, risk screening for AF is mostly based on clinical risk factors for AF. In 2009, Schnabel et al[25] invented the Framingham AF risk score based on the risk factors of AF and ECG PR interval as indicators to predict the 10-year

incidence of AF and has been validated in the Caucasian population. In 2011, Alonso et al[26] also proposed a new AF risk score based on risk factors for AF, which was validated in a prospective study of both Black and White populations. Later, to increase generality, the researchers formed the CHARGE-AF Consortium and conducted research in 3 community-based studies, the Framingham Heart Study (FHS), the Cardiovascular Health Study (CHS), and the Community A new AF risk score, the CHARGE-AF risk score, was obtained in the Atherosclerosis Risk in Communities (ARIC) study, which was validated in European and American populations of various ethnicities.[26] With the advancement of genomics, genetic screening of high-risk groups for AF has become possible. In 2014, Tada et al[9] proposed the AF-GRS using 12 common SNPs associated with AF that had been identified by GWAS. In a prospective study, AF-GRS containing 12 SNPs was found to be associated with paroxysmal AF, and the risk of AF in the highest genetic risk quintile was approximately twice as high as in the lowest quintile.[9]

It can be seen that the study of AF gene polymorphism can predict the risk of AF, thereby providing a theoretical basis for genetic diagnosis. However, the current related gene research is mostly based on the European and American Caucasian population, and the risk genes of AF based on the Chinese Han population still need to be further verified.

This study found that the distribution of TT and CC genotypes at the rs2200733 locus was significantly different between the AF group and the control group. Among them, the TT genotype AF group was higher than the control group, and the CC genotype AF group was lower than the control group. The frequency of T allele in patients with AF was significantly higher than that in the control group, and the T allele was a risk factor for AF (95% CI = 1.179-2.766, $P = .006$). According to logistic regression analysis, people with TC genotype (OR = 3.128, 95% CI = 1.053-9.287, $P = .04$) or TT genotype (OR = 5.077, 95% CI = 1.653-15.595, $P = .005$) and compared with the CC genotype, the risk of AF was increased, and the increased risk of the TT genotype was greater. After adjusting for age, gender, history of coronary heart disease, hypertension, and smoking history, people with TT genotype still had an increased risk of AF (AOR* = 4.557, 95% CI = 1.129-18.396, $P^* = .033$), but the incidence of AF in people with TC genotype was not significantly different from CC genotype ($P = .259$). By establishing a genetic model, compared with genotype TT, genotype TC and CC can reduce the risk of AF (OR = 0.515, 95% CI = 0.281-0.944, $P = .032$). Statistical significance was found after the factors of heart disease and smoking history (AOR* = 0.425, 95% CI = 0.201-0.900, $P^* = .025$).

CONCLUSIONS

The findings of this study concluded that the PITX2 gene polymorphism rs2200733 (T/C) was closely associated with the risk of AF in the Han population in Yangzhou. Compared with the CC genotype, the TT genotype increased the risk of AF. Therefore, the occurrence of AF can be predicted by detecting the genotype of high-risk groups of AF, which provides a theoretical basis for genetic diagnosis.

Ethics Committee Approval: This study was approved by the Ethics Committee of the Affiliated Hospital of Yangzhou University (approval year and no: 2017-YKL5-28-007).

Informed Consent: Written informed consent was obtained from all participants who participated in this study.

Peer-review: Externally peer-reviewed.

Author Contributions: Yin Shen Han and Yuan Xiaochen — Methodology; Yin Shen Han, Yuan Xiaochen, Abass Mahamoud Ahmed, Zhuchao, Wangjun — Validation, formal analysis, and investigation; Yuan Xiaochen, Abass Mahamoud Ahmed, and Yin Shen Han — Writing, review, and editing. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

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REFERENCES

1. Kirchhof P, Benussi S, Kotecha D, et al. 2016 ESC Guidelines for the management of atrial fibrillation developed in collaboration with EACTS. *Kardiol Pol*. 2016;74(12):1359-1469. [CrossRef]
2. Huang C, Zhang S, Huang D, Hua W. Atrial fibrillation: current understanding and treatment recommendations-2018. *Zhong Guo Xin Zang Qi Bo Xin Dian Sheng* Li Za Zhi. 2018;32:315-368.
3. Zhou ZQ, Hu DY, Chen J, Zhang RH, Li KB, Zhao XL. An epidemiological survey of atrial fibrillation in China. *Zhonghua Nei Ke Za Zhi*. 2004;43(7):491-494.
4. Boriani G, Valzania C, Biffi M, Diemberger I, Ziacchi M, Martignani C. Asymptomatic lone atrial fibrillation-how can we detect the arrhythmia? *Curr Pharm Des*. 2015;21(5):659-666. [CrossRef]
5. Nielsen JB, Fritsche LG, Zhou W, et al. Genome-wide study of atrial fibrillation identifies seven risk loci and highlights biological pathways and regulatory elements involved in cardiac development . *Am J Hum Genet*. 2018;102(1):103-115. [CrossRef]
6. Gudbjartsson DF, Arnar DO, Helgadottir A, et al. Variants conferring risk of atrial fibrillation on chromosome 4q25. *Nature*. 2007;448(7151):353-357. [CrossRef]
7. Zhao LQ, Zhang GB, Wen ZJ, et al. Common variants predict recurrence after nonfamilial atrial fibrillation ablation in Chinese Han population. *Int J Cardiol*. 2017;227:360-366. [CrossRef]
8. Hirschhorn JN, Daly MJ. Genome-wide association studies for common diseases and complex traits. *Nat Rev Genet*. 2005; 6(2):95-108. [CrossRef]
9. Tada H, Shiffman D, Smith JG, et al. Twelve–single nucleotide polymorphism genetic risk score identifies individuals at increased risk for future atrial fibrillation and stroke. *Stroke*. 2014;45(10):2856-2862. [CrossRef]
10. Tsai CT, Lai LP, Hwang JJ, Lin JL, Chiang FT. Molecular genetics of atrial fibrillation. *J Am Coll Cardiol*. 2008;52(4):241-250. [CrossRef]
11. Darbar D, Roden DM. Genetic mechanisms of atrial fibrillation: impact on response to treatment. *Nat Rev Cardiol*. 2013;10(6):317-329. [CrossRef]
12. Magnani JW, Rienstra M, Lin H, et al. Atrial fibrillation: current knowledge and future directions in epidemiology and genomics. *Circulation*. 2011;124(18):1982-1993. [CrossRef]
13. Abraham G, Inouye M. Genomic risk prediction of complex human disease and its clinical application. *Curr Opin Genet Dev*. 2015;33:10-16. [CrossRef]

14. Body SC, Collard CD, Shernan SK, et al. Variation in the 4q25 chromosomal locus predicts atrial fibrillation after coronary artery bypass graft surgery. *Circ Cardiovasc Genet*. 2009;2(5): 499-506. [CrossRef]
15. Husser D, Adams V, Piorkowski C, Hindricks G, Bollmann A. Chromosome 4q25 variants and atrial fibrillation recurrence after catheter ablation. *J Am Coll Cardiol*. 2010;55(8):747-753. [CrossRef]
16. Benjamin Shoemaker MB, Muhammad R, Parvez B, et al. Common atrial fibrillation risk alleles at 4q25 predict recurrence after catheter-based atrial fibrillation ablation. *Heart Rhythm*. 2013;10(3):394-400. [CrossRef]
17. Chen F, Yang Y, Zhang R, et al. Polymorphism rs2200733 at chromosome 4q25 is associated with atrial fibrillation recurrence after radiofrequency catheter ablation in the Chinese Han population. . *Am J Transl Res*. 2016;8(2):688-697.
18. Gretarsdottir S, Thorleifsson G, Manolescu A, et al. Risk variants for atrial fibrillation on chromosome 4q25 associate with ischemic stroke. *Ann Neurol*. 2008;64(4):402-409. [CrossRef]
19. Sun L, Zhang Z, Xu J, Xu G, Liu X. Chromosome 4q25 variants rs2200733, rs10033464, and rs1906591 contribute to ischemic stroke risk. *Mol Neurobiol*. 2016;53(6):3882-3890. [CrossRef]
20. Su L, Shen T, Xie J, et al. Association of GWAS-Supported Variants rs2200733 and rs6843082 on chromosome 4q25 with ischemic stroke in the Southern Chinese Han population. *J Mol Neurosci*. 2015;56(3):585-592. [CrossRef]
21. Kirchhof P, Kahr PC, Kaese S, et al. PITX2c is expressed in the adult left atrium, and reducing Pitx2c expression promotes atrial fibrillation inducibility and complex changes in gene expression. *Circ Cardiovasc Genet*. 2011;4(2):123-133. [CrossRef]
22. Martin RI, Babaei MS, Choy MK, et al. Genetic variants associated with risk of atrial fibrillation regulate expression of PITX2, CAV1, MYOZ1, C9orf3 and FANCC. *J Mol Cell Cardiol*. 2015;85:207-214. [CrossRef]
23. Mommersteeg MT, Brown NA, Prall OW, et al. Pitx2c and Nkx2-5 are required for the formation and identity of the pulmonary myocardium. *Circ Res*. 2007;101(9):902-909. [CrossRef]
24. Qiu XB, Xu YJ, Li RG, et al. PITX2C loss-of-function mutations responsible for idiopathic atrial fibrillation. *Clinics (Sao Paulo)*. 2014;69(1):15-22. [CrossRef]
25. Schnabel RB, Sullivan LM, Levy D, et al. Development of a risk score for atrial fibrillation (Framingham Heart Study): a community-based cohort study. *Lancet*. 2009;373(9665):739-745. [CrossRef]
26. Alonso A, Krijthe BP, Aspelund T, et al. Simple risk model predicts incidence of atrial fibrillation in a racially and geographically diverse population: the CHARGE-AF consortium. *J Am Heart Assoc*. 2013;2(2):e000102. [CrossRef]