The rs6817105 polymorphism on chromosome 4q25 is associated with the risk of atrial fibrillation in the Chinese Han population

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Abstract

Objective: Previous genome-wide association studies (GWASs) have identified rs6817105—a single nucleotide polymorphism (SNP) on chromosome 4q25—to be associated with the risk of atrial fibrillation (AF) in a European-descent population. We recently demonstrated this association in a large cohort of Japanese ancestry. Our present study was designed to determine this association in the Chinese Han population.

Methods: This case–control study included 597 AF cases and 996 AF-free controls, and rs6817105 SNPs were genotyped using the TaqMan allelic discrimination assay. Odds ratios (ORs) and 95% confidence intervals (95%CIs) were calculated in logistic regression models.

Results: The genotype distribution of rs6817105-CC was significantly more frequent in the AF patients than in the controls ($p=3.24\times10^{-32}$). In our study, logistic regression analysis showed a strong association between rs6817105 and the risk of AF (additive model: OR=2.22, 95%CI=1.89–2.61, $p=2.33\times10^{-22}$; dominant model: OR=2.96, 95%CI: 2.16–4.07, $p=2.03\times10^{-11}$; recessive model: OR=2.83, 95%CI=2.27–3.54, $p=4.00\times10^{-20}$). Stratification analyses showed a borderline statistical difference between subgroups of age for the association of rs6817105 with AF risk (p=0.049). However, further interactive analysis indicated no significant interaction between genotype of rs6817105 and age (p=0.178).

Conclusion: Our finding suggested that SNP rs6817105 may be associated with a high significant risk of AF in the Chinese Han population, although more replicative studies of larger sample size are needed to confirm this finding. *(Anatol J Cardiol 2016; 16: 662-6)*

Keywords: PITX2, rs6817105, Chinese Han population, polymorphism, atrial fibrillation

Introduction

Atrial fibrillation (AF) is the most common sustained cardiac rhythm disorder encountered in clinical practice. The prevalence of AF is approximately 0.4%–1% in the general population, and accounts for nearly one-third of hospitalizations for cardiac rhythm disturbance (1). AF is closely related to a 5-fold risk of stroke and a 3-fold incidence of congestive heart failure, contributing to higher morbidity and mortality (2, 3).

Although the electrocardiographic characteristics of AF have been described for over a century (4), the molecular basis of the arrhythmia remains unclear. Recently, multiple population-based studies have provided evidence of a genetic contribution to AF (5–7). Mutations in the cardiac ion channel genes were found to play a role in the development of AF (8–13), but these mutations account for only a small fraction of patients with AF (14, 15). Previous genome-wide association studies (GWAS) have indicated several significant candidate genes associated with this complex disorder, including genetic loci PITX2 on chromosome 4q25, ZFHX3 on chromosome 16q22, and KCNN3 on chromosome 1q21 (4). Among these loci, single nucleotide polymorphisms (SNPs) on chromosome 4q25 were first identified to be strongly associated with the risk in multiple cohorts and case–control studies (4). One SNP on chromosome 4q25, rs6817105 has been identified to be strongly associated with AF risk in individuals of European descent ($p=1.8\times10^{-74}$) (16, 17). Most recently, Lubitz et al. (18) also reported rs6817105 polymorphism along with three other SNPs in the 4q25 locus (rs1448818, rs4032974, and rs6838973) as susceptibility signals for AF in a large cohort of 64,683 individuals of European ancestry, including 3,302 individuals with prevalent AF and 3,869 individuals with incident AF and Japanese ancestry (11.309 individuals, 7.916 prevalent AF cases). We aimed to determine whether the SNP rs6817105 is also associated with AF in the Chinese Han population.

Methods

Study population

For this study, we recruited a total of 1,593 participants of Chinese Han origin, including 597 cases with all types of AF and

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996 AF-free controls by selecting hospitalized patients from the Department of Cardiology, the First Affiliated Hospital of Nanjing Medical University. Written informed consent was obtained from each individual. To confirm the credibility of this case–control study, we selected cases with the diagnostic criteria based on the medical history, clinical manifestation, and routine 12-lead electrocardiogram (ECG) or 24-h Holter ECG, whereas AF-free controls had no current symptoms and family history of AF (1). We also collected demographic and clinical information, including sex, age, and history of hypertension, diabetes, and coronary artery disease (CAD), from medical records from June 2010 to August 2013. All participants in this study were ethnic Han Chinese by checking their identification cards. For two groups, patients with advanced age (>90 years old), hyperthyroidism, cardiac valvulopathy, and severe cardiac dysfunction (NYHA class IV) were excluded. The results of 12-lead ECG, echocardiography, blood biochemical examination, thyroid function, blood pressure, and past hospital records were collected to assess the presence or absence of hypertension, CAD, hyperthyroidism, and diabetes mellitus in all participants. According to clinical characteristics, the patterns of AF were classified into paroxysmal AF (episodes that generally last ≤7 days), persistent AF (episodes that usually last >7 days), and permanent AF (long-standing AF, which cardioversion has failed or has not been attempted) (1). The term "lone AF" is generally apply to younger individuals (<60 years) without clinical or echocardiographic evidence of cardiopulmonary disease, including hypertension (1). We divided the patients with AF in this study according to the above classification. The study was approved by the Ethical Committee review board of Nanjing Medical University.

SNP genotyping

Genomic DNA was extracted from peripheral blood samples by proteinase K digestion, followed by phenol chloroform extraction (19). Genotyping was performed by the TaqMan allelic discrimination assay on a 7900HT real-time polymerase chain reaction system (7900HT Fast Real-Time PCR; Applied Biosystems, Foster City, CA, USA). The amplification results were determined by using SDS 2.3 Allelic Discrimination Software (Applied Biosystems). The success genotype calls (call rate) for this polymorphism was above 95%.

Statistical analysis

Differences in clinical characteristic between cases and controls were compared using Student's t-test for continuous variables and χ^2-test for categorical variables. The deviation of genotype distribution of polymorphisms from Hardy–Weinberg equilibrium and the different allele frequencies were also tested by χ^2-test in 2 groups. Logistic regression was utilized to assess the strength of relationship between rs6817105 and the risk of AF in the additive, dominant, and recessive models. Summary odds ratio (OR) and 95% confidence interval (95%CI) was used to pool the association between rs6817105 and AF risk among different ethnicities. The heterogeneity of associations between subgroups was evaluated using the χ^2-based Q-test. All statistical analyses were conducted with STATA 12.0 software (Stata Corporation, College Station, TX, USA). All significant tests were 2-tailed, and p<0.05 was set as statistically significant.

Table 1. Clinical characteristics of the study population

	AF (n=597)	Control (n=996)	*P*
Sex, male/female	397/200	674/322	0.630
Age, years, mean±SD	58.4±11.5	59.0±10.2	0.278
Paroxysmal AF, n (%)	383 (64.2)	NA	–
Persistent AF, n (%)	196 (32.8)	NA	–
Permanent AF, n (%)	18 (3.0)	NA	–
Lone AF, n (%)	71 (11.9)	NA	–
HTN, n (%)	260 (43.6)	267 (26.8)	6.18×10^{-12}
CAD, n (%)	48 (8.0)	51 (5.1)	0.019
DM, n (%)	53 (8.9)	28 (2.8)	9.55×10^{-8}

AF - atrial fibrillation; CAD - coronary artery disease; DM - diabetes mellitus; HTN - hypertension; NA- not available
Using student's t-test for continuous variables and χ^2 -test for categorical variables

Table 2. Distribution of genotype for polymorphism of rs6817105

Genotype[a]	AF	Control	P^b_{hwe}	*P*
CC	287 (48.1%)	263 (26.4%)	0.533	3.24×10^{-32}
CT	228 (38.2%)	488 (49.0%)		
TT	56 (9.4%)	245 (24.6%)		

[a]CC - homozygous for the risk allele.; CT - heterozygous; TT - homozygous for the referent allele
Genotyping calling rate: 98.4%
[b]*P* values for Hardy–Weinberg equilibrium tests in control group
P<0.05 was considered statistically significant
Using χ^2 -test for categorical variables

Results

Characteristics of the study groups

The demographic characteristics of 2 groups can be seen in Table 1. Among cases, patients with paroxysmal AF accounted for 64.2%, those with persistent AF accounted for 32.8%, and those with permanent AF accounted for 3.0%. Only a small part (11.9%) of all cases had lone AF. The average age was 58.4±11.5 years for cases and 59.0±10.2 years for controls, but there was no significant difference between them (p=0.278). The sex was matched well (p=0.630). Compared with AF-free controls, AF cases had a higher prevalence of risk factors such as hypertension, CAD, and diabetes (p<0.05).

Genotype distribution

The distributions of the SNP rs6817105 C/T genotypes among AF subjects and controls are shown in Table 2. The genotyping calling rate was 98.4% for rs6817105. We used Hardy–Weinberg equilibrium (HWE) to analyze the genotype distribution, which was in agreement with the HWE in the control group (p=0.533).

Table 3. Association between SNP rs6817105 and AF in the different ethnicities

Ethnicity backgrounds	AF Risk/ Referent Allele	AF Risk Allele Frequency	OR (95%CI)	*P*	$OR_{adjusted}$ (95%CI)	$P_{adjusted}$	$P_{heterogeneity}$
European (18)	C/T	0.13	1.64 (1.55,1.73)	1.8×10^{-74}	1.60 (1.52,1.68)	1.2×10^{-80}	<0.001
Japanese (18)	C/T	0.47	– –	–	1.90 (1.74,2.08)	5.4×10^{-46}	–
Chinese	C/T	0.70	–	–	**2.22 (1.89,2.61)**	**2.33×10^{-22}**	–
Dominant model	CC /CT+TT	NA	–	–	2.96 (2.16,4.07)	2.03×10^{-11}	–
Recessive model	CC +CT/TT	NA	–	–	2.83 (2.27,3.54)	4.00×10^{-20}	–
Pooled OR	C/T	NA	–	–	**1.86 (1.53,2.27)**	<0.001	–

SNP - single nucleotide polymorphism; AF - atrial fibrillation; CI - confidence interval; OR - odds ratio
OR adjusted = OR adjusted for all loci in the study of Lubitz SA et al. or adjusted for age, gender, hypertension, diabetes, and coronary artery disease in this study
P heterogeneity = The *P* value for heterogeneity in the different ethnicity backgrounds; *P*<0.05 was considered statistically significant
Using logistic regression in the additive, dominant as well as recessive models

Table 4. Stratification analysis on the association of rs6817105 with AF risk

Characteristics	OR	95% CI	*P* [a]	*P* [b]
Sex				0.881
Male	2.20	1.81–2.67	2.71×10^{-15}	
Female	2.26	1.68–3.03	7.14×10^{-8}	
Age				0.049
<59	1.87	1.48–2.36	1.19×10^{-7}	
≥59	2.61	2.06–3.30	1.05×10^{-15}	
HTN				0.104
No	2.00	1.63–2.43	1.63×10^{-11}	
Yes	2.66	2.01–3.52	7.93×10^{-12}	
CAD				0.313
No	2.17	1.83–2.57	2.13×10^{-19}	
Yes	3.36	1.46–7.70	4.24×10^{-3}	
DM				0.923
No	2.21	1.87–2.61	6.90×10^{-21}	
Yes	2.31	0.96–5.56	0.061	

CAD - coronary artery disease; CI - confidence interval; DM - diabetes mellitus; HTN - hypertension; OR - odds ratio
[b]Adjusted for sex, age, hypertension, diabetes, and coronary artery disease
[b]*P* for heterogeneity test using the χ^2-based Q test
P<0.05 was considered statistically significant
using the χ^2-based Q-test for heterogeneity analysis of two subgroups

Table 5. Interaction between rs6817105 genotype and age on AF risk

Age (years)	Genotype	AF	Control	OR	*P* [a]
<59	TT	30	103	1	
<59	TC/CC	242	341	2.40 (1.53,3.75)	1.24×10^{-4}
≥59	TT	26	142	0.55 (0.30,0.99)	4.65×10^{-2}
≥59	TC/CC	273	410	1.93 (1.24,3.014)	3.63×10^{-3}
P for multiplicative interaction					0.178

AF - atrial fibrillation; OR - odds ratio
aAdjusted for sex, age, hypertension, diabetes, and coronary artery disease
P<0.05 was considered statistically significant
Using logistic regression for interaction analysis

Association between SNP rs6817105 and AF Risk

Table 3 reveals that rs6817105 was strongly and significantly associated with an increased risk of AF in the additive model (OR=2.22, 95%CI=1.89–2.61, p<0.001), the recessive model (OR=2.83, 95%CI=2.27–3.54, p<0.001), as well as the dominant model (OR=2.96, 95%CI=2.16–4.07, p<0.001). The pooled relative risk of AF risk with rs6817105 is 1.86 (95%CI=1.53–2.27, p<0.001; I2=93.2%) in the random effects model (Table 3). The p value of heterogeneity between Chinese Han population and Japanese population is 0.122 (not shown). We further performed stratification analyses based on sex, age, hypertension, diabetes, and CAD. As shown in Table 4, the p values for association were 1.19×10^{-7} (OR=1.87) for individuals aged <59 years and 1.05×10^{-15} (OR=2.61) for older individuals (≥59 years). A borderline significant difference between subgroups of age was observed for the association of rs6817105 with AF risk (p for heterogeneity =0.049). However, no significant interaction was detected between genotype of rs6817105 and age (p for multiplicative interaction =0.178, Table 5).

Discussion

We carried out a case–control study with 597 Chinese AF patients and 996 ethnically and geographically matched controls for SNP rs6817105. A strong significant association between rs6817105 and AF was detected in the Chinese Han population. In overall, both allelic and genotypic associations were strongly significant after adjustment for covariates (Table 3). Previous studies have identified rs6817105 as an AF susceptibility locus in European and Japanese populations (16–18). That suggests that 4q25 locus and rs6817105 specifically has an association with AF in different ethnicities. The present study has expanded the association between rs6817105 and AF to the Chinese Han population. It appears that SNP rs6817105 has a more robust effect on AF in the Chinese Han population (OR=2.22, 95%CI=1.89–2.61, p<0.001) than in the Caucasian population (OR=1.64, 95%CI=1.55–1.73, p<0.001) and the Japanese population (OR=1.60, 95%CI=1.52–1.68, p<0.001). The p value for heterogeneity is significant (p<0.001, I2=93.2%), indicating that the effect of SNP rs6817105 on AF was significantly stronger in the Chinese Han population than in Caucasian and Japanese populations. It is worth noting that the C allele of rs6817105 is much more frequent in our study population (the allelic frequency is 0.70) than in those of European descent (the allelic frequency is 0.13) and Japanese population (the allelic frequency is 0.47), which may account for the different estimated risks in different ethnic populations (18). In addition, the results of further stratification and interaction analysis based on some risk factors of AF (age, sex, diabetes, hypertension, and CAD) showed that SNP rs6817105 was not associated with these factors, which demonstrated rs6817105 was independently related to AF. For the last decade, nine non-coding SNPs associated with increased risk of AF have been investigated to be possible signals for the causative genes that lie in proximity to these SNPs (20). Previous GWASs were performed in Icelandic population to demonstrate 2 sequence variants (rs2200733 and rs10033464) on chromosome 4q25 associated with AF by Gudbjartsson et al. (16) in 2007. The similar results of associations between these SNPs and AF were replicated in four populations in a large study (21). Subsequently, Gudbjartsson et al. (16) and Shi et al. (22) reported a significant association between rs2200733 and AF in the populations of Chinese Han ancestry. To date, the 4q25 locus has been most comprehensively studied (4). In recent years, it was reported that the SNP rs6817105 is approximately 150kb upstream of the PITX2 gene on chromosome 4q25 (17). Although the association between rs6817105 on 4q25 and AF was demonstrated in the present and previous studies, the potential mechanism is still unknown. Several transgenic approaches have provided evidences that PITX2 dysfunction might contribute to AF (23). Therefore, the most possible actions of rs6817105 on the development of AF may occur through regulating the PITX2 gene (17). Pitx2 is a homeobox transcription factor, displaying a specific expression pattern during embryogenesis. Its pivotal role in left–right signaling has been unraveled by gain and loss of function experiments (24). Constitutive deletion of PITX2C and myocardium-specific deletion of PITX2 in mice resulted in a default program for sinus node formation in the left corresponding regions (24). Additionally, several investigations clarified that PITX2 also plays an essential role in the development of pulmonary vein myocardium (25). However, the exact action of SNP rs6817105 on PITX2 requires further study.

Study limitations

Our study has several limitations. First, it is the first time that an associated between SNP rs6817105 and AF was identified in the Chinese Han population. Although hundreds of patients were enrolled in our study, more larger-scale studies are needed to confirm our finding. Second, our study subjects, consecutively recruited from Jiangsu Province of eastern China, only partially represent the entire Chinese Han population. Third, the sample size of "lone AF" was limited. So, it was unavailable for investigating "lone AF" only. Moreover, functional genomic studies would be required to clarify the specific underlying mechanism.

Conclusion

In conclusion, our study showed a highly significant association between the SNP rs6817105 and AF in the mainland Chinese Han population, thus expanding on previous reports of the association in European and Japanese descent populations.

Conflict of interest: None declared.

Peer-review: Externally peer-reviewed.

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