Hum Genet (2009) 126:843–849
DOI 10.1007/s00439-009-0737-3

ORIGINAL INVESTIGATION

Assessment of association of rs2200733 on chromosome 4q25 with atrial fibrillation and ischemic stroke in a Chinese Han population

Lisong Shi · Cong Li · Chuchu Wang · Yunlong Xia · Gang Wu · Fan Wang · Chengqi Xu · Pengyun Wang · Xiuchun Li · Dan Wang · Xin Xiong · Ying Bai · Mugen Liu · Jingyu Liu · Xiang Ren · Lianjun Gao · Binbin Wang · Qiutang Zeng · Bo Yang · Xu Ma · Yanzong Yang · Xin Tu · Qing Kenneth Wang

Received: 9 July 2009 / Accepted: 16 August 2009 / Published online: 26 August 2009

Abstract Atrial fibrillation (AF) is the most common arrhythmia in the clinical setting and an independent risk factor for stroke. Approximately 10 million Chinese people are affected by AF, but the genetic basis is largely unknown. A recent genome-wide association study in Iceland identified association between SNP rs2200733 on 4q25 and AF; however, many independent replication studies are essential to unequivocally validate this association. To assess the association between rs2200733 and AF as well as that between rs2200733 and ischemic stroke in a mainland Chinese Han population, we carried out case–control association studies with 383 AF patients versus 851 non-AF controls and 811 ischemic stroke patients versus 688 non-stroke controls. Highly significant association was detected between rs2200733 and AF in a Chinese Han population (allelic $P = 3.7 \times 10^{-11}$ with OR = 1.81; genotypic $P = 4.1 \times 10^{-12}$ with a dominant model). When the AF cases were divided into lone AF (32.6%) and other types of AF (67.4%), significantly stronger association was found with lone AF (OR = 2.40, $P = 1.3 \times 10^{-9}$ compared to OR = 1.59, $P = 6.2 \times 10^{-7}$ for other types of AF; $P = 0.02$ for two ORs). No significant association was found between rs2200733 and ischemic stroke. Our results suggest that SNP rs2200733 confers a highly significant risk of AF, but not ischemic stroke, in a more representative Chinese Han population in the mainland China.

Introduction

Atrial fibrillation (AF) is the most common cardiac arrhythmia in the clinical setting and is characterized with fast and irregular abnormal atrial electrophysiological activities. The prevalence of AF increases substantially with age, ranging from about 1% in young adults to nearly

L. Shi, C. Li, C. Wang, Y. Xia, and G. Wu contributed equally to this work.

Electronic supplementary material The online version of this article (doi:10.1007/s00439-009-0737-3) contains supplementary material, which is available to authorized users.

L. Shi · C. Li · C. Wang · F. Wang · C. Xu · P. Wang · X. Li · D. Wang · X. Xiong · Y. Bai · M. Liu · J. Liu · X. Ren · X. Tu (✉) · Q. K. Wang (✉)
Key Laboratory of Molecular Biophysics of Ministry of Education, College of Life Science and Technology, Center for Human Genome Research, Huazhong University of Science and Technology, Wuhan, China
e-mail: xtu@mail.hust.edu.cn

Q. K. Wang
e-mail: qkwang@mail.hust.edu.cn

Q. K. Wang
Center for Cardiovascular Genetics, Cleveland Clinic, Cleveland, OH, USA

Y. Xia · L. Gao · Y. Yang
First Affiliated Hospital of Dalian Medical University, Dalian, China

G. Wu · B. Yang
Renmin Hospital of Wuhan University, Wuhan, China

B. Wang · X. Ma
Institutions Affiliated to the National Population and Family Planning Commission, Beijing, China

Q. Zeng
Union Hospital of Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China

10% in those >80-year-old (Kannel et al. 1998). In the mainland China, approximately 10 million people are affected with AF (Hu and Sun 2008; Zhou and Hu 2008). AF is an independent risk factor for ischemic stroke (Wolf et al. 1991). In the mainland China, the prevalence of stroke in AF patients was much higher than that in non-AF population (Hu and Sun 2008; Zhou and Hu 2008). Common AF is associated with multiple risk factors, including age, valvular heart disease, hypertension, and left ventricular dysfunction (Go et al. 2001). Lone AF is referred to as AF which is not associated with other cardiovascular diseases. Lone AF accounts for nearly 30% of all AF cases (Brand et al. 1985; Lévy et al. 1999). Some AF patients have a positive family history, and several epidemiological studies suggest that genetic factors may play an important role in the development of AF, especially in patients with lone AF (Wang 2008).

Recently, major advances have been made in identifying the genetic factors underlying AF. To date, several genetic loci have been mapped for monogenetic Mendelian AF by different research groups, including our group (Wang 2008; Lai et al. 2003; Tsai et al. 2008). Several AF genes have been identified; some genes encode ion channels (e.g. *SCN5A*, *KCNQ1*, *KCNE2*, *KCNJ2*, *KCNA5*, and *KCNH2*) (Wang 2008; Chen et al. 2003; Sébillon et al. 2003; Yang et al. 2004; Hong et al. 2005; Olson et al. 2005, 2006; Xia et al. 2005). Most interestingly two non-ion channel genes have been recently identified for AF, including *NUP155* reported by our group (Zhang et al. 2008) and *NPPA* (Hodgson-Zingman et al. 2008).

Although identification of genes for familial AF has provided enormous insights into the molecular pathogenic mechanisms of AF, they account for only a small proportion of AF cases. The most common form remains sporadic AF. The common AF is a complex disease which is caused by multiple genes, environmental factors, and gene–environment interactions. One large-scale case–control study using a genome-wide association strategy (GWAS) has been reported for AF in 2007. One single nucleotide polymorphisms (SNP) on chromosome 4q25, rs2200733, showed strong association with AF, and the finding was replicated in two other populations of European descent and a Chinese population from Hong Kong (Gudbjartsson et al. 2007). Follow-up studies replicated the 4q25 association with AF in additional five independent European populations (Anselmi et al. 2008; Kääb et al. 2009). However, the association in the Hong Kong Chinese population in the original GWAS report has not been replicated yet. Thus, it is unknown whether the 4q25 AF association remains significant in the most typical Chinese population from the mainland China because the Hong Kong population is derived mostly from a couple of provinces from Southern China. In this study, we tested the association between SNP rs2200733 and AF in a more representative Chinese Han population (GeneID). We further assessed the association of rs2200733 with lone AF in contrast to other types of AF.

It is interesting that SNP rs2200773 was found to be significantly associated with ischemic stroke (IS) in a separate GWAS from Iceland, and the finding was replicated in one German cohort and one UK cohort (Gretarsdottir et al. 2008). In this study, we also assessed the association between SNP rs2200733 and ischemic stroke in a Chinese population (GeneID).

Materials and methods

Ascertainment of patients

The Chinese GeneID population is a large cohort from the mainland China that aims to identify susceptibility genes for various cardiovascular diseases in the Chinese Han population. The study subjects have been enrolled from multiple hospitals in Wuhan, Dalian, and Beijing. The studies were approved by appropriate local institutional review boards on human subject research, and conformed to the guidelines set forth by the Declaration of Helsinki. Written informed consent was obtained from the participants. The participants completed a health questionnaire, and had fasted blood samples drawn. Genomic DNA was isolated from blood samples using standard protocols with the Wizard® Genomic DNA Purification Kit (Promega Corporation, Madison, WI, USA).

Diagnosis criteria

The AF cohort in GeneID consists of patients undergoing AF ablation as well as AF patients seen at the outpatient clinics over a period of last 5 years. Diagnosis of AF was based on standard diagnostic criteria (Fuster et al. 2006), and performed by a panel of cardiologists on the basis of electrocardiograms (ECG) and/or Holter ECG. Patients with other types of cardiac arrhythmias were excluded. Patients with cardiomyopathies and valvulopathies were also excluded based on data from echocardiography (Echo). AF patients were classified as "Paroxysmal AF", "Persistent AF", and "Permanent AF" according to ACC/AHA/ESC AF guidelines (Fuster et al. 2006). We limited the recruitment of AF patients to those affected men and women aged $\leq$75 years but with prior or current evidence of AF. An AF patient was diagnosed as "lone AF" if he/she had no hypertension, coronary artery disease (CAD), congenital heart disease, congestive heart failure, or diabetes. An individual was considered as an "AF control" if no AF, other types of arrhythmias, ischemic stroke,

valvulopathies, and cardiomyopathies were detected by ECG, Echo, or magnetic resonance imaging (MRI)/computed tomography (CT).

The ischemic stroke cohort in GeneID consists of patients enrolled from the Stroke Specialty Clinic at three hospitals in Beijing. Diagnosis of ischemic stroke was based on the World Health Organization (WHO) criteria (Goldstein et al. 1989) by a panel of clinical neurologists. All patients had MRI or CT performed. ECG data were missing for the majority of ischemic stroke patients; thus, it was impossible to classify the patients with ischemic stroke into other sub-categories. Controls for ischemic stroke were defined as individuals without a history of stroke, and had normal MRI/CT imaging at the time of evaluation.

Genotyping of SNP rs2200733

SNP rs2200733 was genotyped using a Rotor-Gene™ 6000 High Resolution Melt system (Corbett Life Science, Concorde, NSW, Australia). Genotyping was performed in a total of 25 μL polymerase chain reaction (PCR) volume containing 1 μL of LC Green dye, 5 pmol of each primer, 25 ng of genomic DNA, 2.5 μL of 10 × PCR buffer with 1.5 mmol/L $MgCl_2$, 5 mmol deoxynucleotide triphosphates, and 1 U of *Taq* polymerase. Two positive controls for each genotype (T/T, T/C, and C/C) were included in each run. A total of 24 cases and controls were randomly selected for verification of genotyping results using direct DNA sequence analysis. DNA sequence analysis was performed with forward and/or reverse primers using the BigDye® Terminator v3.1 Cycle Sequencing Kits on an ABI PRISM® 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA).

Statistical analysis

SNP rs2200733 genotypes were tested for Hardy–Weinberg equilibrium among controls using PLINK v1.05. Allelic and genotypic association of rs2200733 with AF was assessed using Pearson's 2 × 2 and 2 × 3 contingency table χ^2 test (SPSS, version 13.0). Odds ratios (ORs) and 95% confidence intervals (CIs) were estimated using the χ^2 test (SPSS, version 13.0). When the case–control samples were stratified, Breslow–Day tests were performed to analyze the homogeneity between ORs from each subgroup (SPSS, version 13.0). Multivariate analysis was performed by incorporating age, sex, hypertension (HT), and CAD as covariates by using multivariate logistic regression (SPSS, version 13.0). Empirical *P* values were determined using the PLINK v1.05 program with 100,000 Monte–Carlo simulations.

Power analysis

Power analysis was carried out using nQuery Advisor version 6.0.

Results

Power analysis

We studied 383 AF patients and 851 non-AF controls from the GeneID population consisting of the typical, mainland Chinese Han individuals covering 10 provinces from Central and Northern China. The average age at diagnosis was 58 ± 14 years for cases and 59 ± 11 years for controls (Table 1). Among the AF cases, 62.9% was classified as paroxysmal AF, 33.2% as persistent AF, and only 3.9% as permanent AF; 32.6% of patients had lone AF; 13.5% of AF patients had ischemic stroke and 6.0% had positive family history (Table 1). The male/female ratio and the rate of CAD and hypertension are shown in Table 1. For power analysis, we utilized the known population parameters of an OR of 1.42 and T allele frequency of 0.528 in the Hong Kong cohort from the previously published report (Gudbjartsson et al. 2007). Our study with 383 AF cases and 851 controls can provide statistical power of 97.9% at a Type I error rate of 0.05, which suggests that our sample size provides sufficient power to identify the association between SNP rs2200733 and AF.

Our ischemic stroke study involved 811 patients with ischemic stroke and 688 non-stroke controls from the GeneID population. The average age at diagnosis was 61 ± 11 years for cases, and 61 ± 9 years for controls (Table 1). When using the setting of an OR of 1.4 and C allele frequency of 0.472 as reported previously (Gudbjartsson et al. 2007; Gretarsdottir et al. 2008), our study cohort can provide a statistical power of 99.6% at 0.05 Type I error, which strongly suggests that our GeneID cohort has sufficient power to identify the association between rs2200733 and ischemic stroke.

Allelic association of rs2200733 and AF in a Chinese Han population

There was no deviation from Hardy–Weinberg equilibrium for SNP rs2200733 in the control groups ($P > 0.05$; supplementary Table 1). We found highly significant association between SNP rs2200733 and AF in the Chinese GeneID population (Table 2). The T allele confers risk of AF. The *P* value for allelic association reached 3.7×10^{-11} with an OR of 1.81 and 95% CI from 1.51 to 2.15 (Table 2). The homozygous OR for rs2200733 was 2.83 (Table 2).

Table 1 Clinical characteristics of the GeneID study population

Characteristic	Case	Control
Atrial fibrillation		
Total number of samples	383	851
Sex (male/female)	237/146	561/290
Age[a] (Mean ± SD)	58 ± 14	59 ± 11
Category		
Paroxysmal (%)	241 (62.9)	N/A
Persistent (%)	127 (33.2)	N/A
Permanent (%)	15 (3.9)	N/A
Lone AF (%)	125 (32.6)	N/A
Hypertension[b] (%)	148 (38.6)	482 (56.6)
CAD[c] (%)	68 (17.8)	96 (11.3)
Stroke history[d] (%)	52 (13.5)	N/A
Family history[e] (%)	23 (6.0)	N/A
Ischemic stroke		
Total number of samples	811	688
Sex (male/female)	476/335	435/253
Age[a] (Mean ± SD)	61 ± 11	61 ± 9

SD standard deviation; *AF* atrial fibrillation; *CAD* coronary artery disease

[a] Age for the case group refers to as the age at diagnosis of AF or ischemic stroke; age for the control group refers to as the age at which the study subject was enrolled into the study

[b] Hypertension was diagnosed by the criteria of blood pressure higher than 140/90 mmHg

[c] CAD is diagnosed by coronary angiography

[d] Stroke was diagnosed by neuroimaging, including CT or MRI

[e] Family history was defined as the finding that at least one first-degree relative of the proband was diagnosed as being affected with the disease

The association between SNP rs2200733 and AF was more significant in the lone AF group (OR = 2.40, $P = 1.3 \times 10^{-9}$) than in the other AF group (OR = 1.59, $P = 6.2 \times 10^{-7}$).

When the cases and controls were divided into a male group and a female group, the association remained significant in both groups with P values of 8.5×10^{-8} (OR = 1.83) and 8.6×10^{-5} (OR = 1.77), respectively (Table 2; Fig. 1). Then, the cohort was divided into different age groups, and the P values for association were 7.7×10^{-9} (OR = 2.07) for individuals with the age of <60 years and 3.6×10^{-4} (OR = 1.57) for older individuals (≥60 years). Association for AF without CAD was significant ($P = 5.9 \times 10^{-11}$, OR = 3.0), whereas that for AF with CAD became non-significant ($P = 0.155$). Association for AF without hypertension was significant ($P = 3.3 \times 10^{-8}$, OR = 1.96), whereas that for AF with hypertension was less significant ($P = 4.5 \times 10^{-4}$, OR = 1.61) (Table 2).

Genotypic association of rs2200733 and AF in a Chinese population

We found statistically significant genotypic association between rs2200733 and AF. The association was more significant with the assumption of a dominant or additive model ($P = 4.1 \times 10^{-12}$ and 9.3×10^{-11}, respectively) than a recessive model ($P = 4.2 \times 10^{-4}$) (Table 3).

To adjust for potential confounding factors, we performed a multivariate logistic regression analysis. Both allelic and genotypic association between SNP rs2200733 and AF remained highly significant after adjusting for possible risk factors, including age, gender, CAD, and hypertension (Tables 2, 3). Empirical P values were estimated by performing 100,000 Monte–Carlo simulations and found to be significant (Tables 2, 3).

Lack of association between rs2200733 and ischemic stroke in a Chinese Han population

We carried out a large case–control association study with 811 ischemic stroke patients and 688 controls to assess a previously reported association (Gretarsdottir et al. 2008) between SNP rs2200733 and ischemic stroke. No significant P values were obtained for either allelic or genotypic association between rs2200733 and ischemic stroke ($P = 0.42$ and 0.45, respectively; Tables 2, 3). The association remained non-significant in either male or female cohorts.

We also analyzed the genotyping data by dividing the cases into early onset (<60 years, 39.8%) and late onset (≥60 years, 60.2%) ischemic stroke patients. The association between rs2200773 and late onset ischemic stroke was not significant ($P > 0.05$; Tables 2, 3). A significant P value was obtained with the early onset group only for both allelic ($P = 0.01$, risk allele C OR = 1.32, 95% CI = 1.07–1.64) and genotypic frequencies ($P = 0.005$ with a recessive model) (Tables 2, 3). The association remained significant after adjusting for covariates of sex and age and after 100,000 Monte–Carlo simulations (Tables 2, 3). It is notable that the risk allele for early onset ischemic stroke is C in the GeneID population (Table 2), instead of the T allele for risk of ischemic stroke in the European populations.

Discussion

A 2007 GWAS report identified significant association between SNP rs2200733 on chromosome 4q25 and AF in an Icelandic population, and further studies replicated the association in a Sweden cohort, a US cohort, and a Chinese population from Hong Kong (Gudbjartsson et al. 2007).

Table 2 Analysis of allelic association of rs2200733 T allele with AF and ischemic stroke in the GeneID population

Phenotype (case/control)	Frequency of T allele (case/control)	OR (95% CI)	T allele homozygous OR	*P* value	*P*-cor[a]	*P*-adj[b]	*P*-emp[c]
Atrial fibrillation							
Entire cohort (383/851)	0.646/0.503	1.81 (1.21–3.20)	2.83	3.7×10^{-11}	/	1.3×10^{-10}	$<10^{-6}$
Male (237/561)	0.654/0.508	1.83 (1.47–2.29)	2.89	8.5×10^{-8}	1.0×10^{-6}	1.0×10^{-7}	$<10^{-6}$
Female (146/290)	0.634/0.493	1.77 (1.33–2.37)	2.77	8.6×10^{-5}	0.001	3.0×10^{-4}	2.3×10^{-4}
Age[d] <60 (195/426)	0.664/0.488	2.07 (1.62–2.66)	3.61	7.7×10^{-9}	9.2×10^{-8}	3.3×10^{-8}	$<10^{-6}$
Age[d] ≥60 (188/425)	0.628/0.518	1.57 (1.23–2.01)	2.20	3.6×10^{-4}	0.004	4.0×10^{-5}	1.4×10^{-5}
Lone AF (125/851)	0.708/0.503	2.40 (1.80–3.20)	4.88	1.3×10^{-9}	1.6×10^{-8}	1.2×10^{-8}	$<10^{-6}$
Other AF (258/851)	0.616/0.503	1.59 (1.30–1.94)	2.50	6.2×10^{-7}	7.4×10^{-6}	2.4×10^{-5}	1.5×10^{-5}
AF with stroke (52/851)	0.721/0.503	2.56 (1.65–3.97)	12.21[e]	1.5×10^{-5}	1.8×10^{-4}	1.1×10^{-4}	8.2×10^{-5}
AF w/o stroke (331/851)	0.634/0.503	1.72 (1.43–2.06)	2.50	8.4×10^{-9}	1.0×10^{-7}	1.3×10^{-8}	$<10^{-6}$
AF with HT (148/482)	0.622/0.505	1.61 (1.23–2.10)	2.29	4.5×10^{-4}	0.005	8.5×10^{-4}	8.2×10^{-4}
AF w/o HT (235/369)	0.662/0.500	1.96 (1.54–2.49)	3.30	3.3×10^{-8}	4.0×10^{-7}	6.7×10^{-8}	$<10^{-6}$
AF with CAD (68/96)	0.610/0.531	1.38 (0.88–2.16)	1.92	0.16	0.88	0.49	0.24
AF w/o CAD (315/755)	0.654/0.499	1.90 (1.56–2.30)	3.00	5.9×10^{-11}	7.1×10^{-10}	2.4×10^{-10}	$<10^{-6}$
Ischemic stroke							
Entire cohort (811/688)	0.496/0.511	1.06 (0.92–1.22)	0.89	0.43	/	0.43	0.45
Male (476/455)	0.479/0.514	1.15 (0.96–1.14)	0.90	0.14	0.45	0.14	0.20
Female (335/233)	0.521/0.506	0.94 (0.75–1.19)	1.13	0.61	0.98	0.53	0.75
Age[d] <60 (323/361)	0.454/0.524	1.32 (1.07–1.64)	0.57	0.010	0.04	0.012	0.012
Age[d] ≥60 (488/327)	0.525/0.497	0.90 (0.73–1.09)	1.24	0.27	0.72	0.32	0.31

AF atrial fibrillation; *CAD* coronary artery disease; *HT* hypertension

[a] Corrected *P* values were obtained using Bonferroni's correction

[b] Adjusted *P* values were obtained using multivariate logistic regression analysis

[c] Empirical *P* values were obtained by performing 100,000 Monte–Carlo simulations

[d] Age for the case group refers to as the age at diagnosis of disease; age for the control group refers to as the age at which the subject was enrolled into the study

[e] May be false value due to small sample size

Later, the association in Caucasian populations was further replicated in German, Italian, Netherland, and US Framingham/Vanderbilt cohorts (Anselmi et al. 2008; Kääb et al. 2009). On the other hand, the association in the Hong Kong population has not been replicated yet. In the present study, we have carried out a large-scale case–control association study with 383 Chinese AF patients and 851 matched controls for SNP rs2200733. The results showed highly significant association between SNP rs2200733 and AF in the GeneID Chinese Han population. Both allelic and genotypic associations were highly significant even after adjustment for covariates (Tables 2, 3). The Chinese population in Hong Kong was derived mainly from Guangdong and Guangxi Provinces in Southern China. This study has expanded the association between rs2200733 and AF to the more representative mainland Chinese Han population with nearly 10 million AF patients (Hu and Sun 2008; Zhou and Hu 2008).

It is worth noting that the frequency of the risk allele T of SNP rs2200733 in the Caucasian populations deviates greatly from that in the Chinese population, <30 to >50%, respectively (Hapmap data, http://www.hapmap.org). These results suggest that SNP rs2200733 is a more common genetic risk factor in the Chinese population than in the Caucasian populations.

We compared the frequency of rs2200733 T allele in the previously published Hong Kong Chinese AF cases (60.5% of 333 cases) (Gudbjartsson et al. 2007) and that in our GeneID cases (65.6% of 383 cases), and found a significant difference ($P = 0.003$). No significant difference was found between two control groups ($P = 0.09$). It appears that SNP rs2200733 has a stronger effect on AF in our GeneID population (OR = 1.81, 95% CI = 1.51–2.15) than on Hong Kong population (OR = 1.42, 95% CI = 1.16–1.73). The *P* value for homogeneity of ORs was statistically significant ($P = 0.024$) (Fig. 1), suggesting that the effect of SNP rs2200733 on AF was significantly greater in the mainland Chinese population than in Hong Kong population. Further studies with large sample sizes are needed to determine the varying effects of SNP

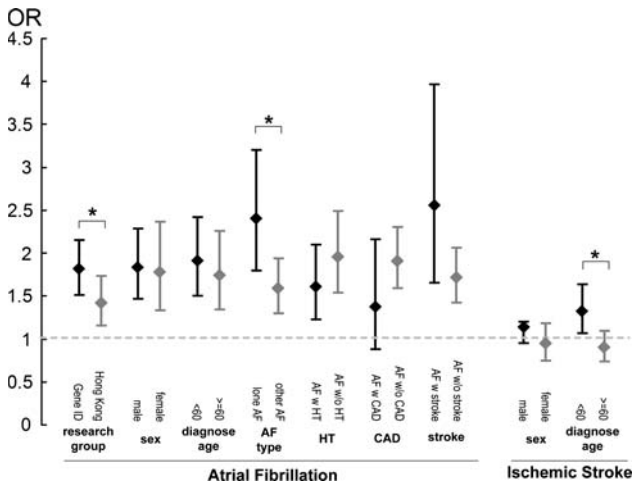

Fig. 1 Comparison of odds ratios (ORs). *Vertical axis* OR; *horizontal axis* different groups of patients with AF or ischemic stroke subdivided based on various criteria. Each *filled rhombus* with a *line segment* refers to the OR and 95% CI for each subgroup. The *asterisk* denotes the statistical significant difference between ORs of subgroups by homogeneity test ($P < 0.05$)

Table 3 Analysis of genotypic association of rs2200733 with atrial fibrillation and ischemic stroke under different models of inheritance in the GeneID population

Model	Observed P	Corrected P^a	Adjusted P^b	Empirical P^c
Atrial fibrillation				
Dominant	4.1×10^{-12}	8.2×10^{-11}	1.7×10^{-11}	$<1.0 \times 10^{-6}$
Recessive	4.2×10^{-4}	8.4×10^{-4}	0.0016	5.3×10^{-4}
Additive	9.3×10^{-11}	/	1.0×10^{-9}	$<1.0 \times 10^{-6}$
Ischemic stroke (entire cohort)				
Dominant	0.24	0.42	0.23	1.00
Recessive	0.99	0.99	0.99	0.26
Additive	0.45	/	0.46	0.57
Ischemic stroke (age[d] <60)				
Dominant	0.18	0.55	0.19	0.30
Recessive	0.005	0.020	0.006	0.006
Additive	0.019	0.038	0.013	0.019
Ischemic stroke (age[d] ≥60)				
Dominant	0.463	0.92	0.54	0.306
Recessive	0.267	0.71	0.32	0.261
Additive	0.504	0.75	0.33	0.583

[a] Corrected P values were obtained using Bonferroni's correction

[b] Adjusted P values were obtained using multivariate logistic regression analysis

[c] Empirical P values were obtained by performing 100,000 Monte–Carlo simulations

[d] Age for the case group refers to as the age at diagnosis of disease; age for the control group refers to the age at which the subject was enrolled into the study

rs2200733 in different ethnic background and whether the difference in this study may be partly due to the small sample size of the Han Chinese in the Hong Kong study by Gudbjartsson et al. (2007) and the GeneID study.

Lone AF is AF without detectable cardiovascular disease such as congestive heart failure, high blood pressure, CAD, etc. Approximately 30% of AF cases belong to lone AF. Genetic factors were assumed to play a more important role in the development of lone AF than of AF associated with other cardiovascular diseases (Ellinor et al. 2005; Arnar et al. 2006). We tested this hypothesis using the GeneID population. When we divided the total AF cases into "lone-AF" and "other AF" groups, the frequency of the risk T allele was higher in the lone AF group (70.8%) than in the other AF group (61.6%) ($P = 0.013$). The OR for the "lone AF" group was 2.40, whereas that for the "other AF" group was 1.59. A significant difference was identified between these two ORs ($P = 0.022$) (Fig. 1). These results suggest that the influence of genetic factors is stronger for lone AF than for other types of AF and that rs2200733 confers a stronger risk in the lone AF population than in other types of AF populations.

The OR in AF cases diagnosed under 60 years of age was slightly higher than AF cases over 60 years (2.07 vs. 1.57), but the difference was not significant ($P = 0.12$) (Table 2; Fig. 1). We noted that the proportion of lone AF cases in the two different age groups differed greatly: 48.2 versus 17%, respectively (P value was 1.8×10^{-10}). Hypertension is known to be a risk factor for AF. The association between rs2200733 and AF was significant in both AF with hypertension and AF without hypertension (Table 2). The results suggest that hypertension may not influence the contribution of rs2200733 to risk of AF. CAD is also a known risk factor for AF. The association between rs2200733 and AF was significant in AF group without CAD but not in the AF group with CAD (Table 2). The results suggest that SNP rs2200733 may not confer risk of AF in those patients also affected with CAD.

SNP rs2200733 was also identified in a 2008 GWAS for ischemic stroke in Iceland. The association was replicated in a German cohort and a UK cohort (Gretarsdottir et al. 2008). Our results suggest, however, that rs2200733 is not a risk factor for ischemic stroke in the Chinese population ($P = 0.43$). The association analysis was further carried out with the cases divided into two groups: one group with early onset ischemic stroke (<60 years of age) and the other group with late onset disease (≥60 years of age). A significant P value was obtained for early onset ischemic stroke but not with late onset disease. Despite the significant P value for the association between rs2200733 and early onset ischemic stroke, the risk allele in the GeneID early onset ischemic stroke cohort is the C allele, instead of the T allele in the European populations. The results

suggest that the association between rs2200733 and early onset ischemic stroke is likely a false positive finding, or that rs2200733 is not a causative so that two different alleles of this SNP in different ethical populations are in linkage disequilibrium with the risk allele of an unidentified causative SNP. Thus, future studies in both Caucasian and Chinese populations are needed to further assess the association of SNP rs2200733 with ischemic stroke.

In conclusion, we found highly significant association between SNP rs2200733 on chromosome 4q25 and AF in the mainland Chinese Han population. The results expanded the association between rs2200733 and AF from a Hong Kong population to a more representative Chinese Han population.

Acknowledgments We thank all patients and normal control individuals for their participation of this study. We thank Drs. Dingfeng Su and Baofeng Yang for their help. This work was supported by the China National Basic Research Program (973 program 2007CB512000 and 2009CB521901), China National 863 Scientific Program (2006AA02Z476) and National Natural Science Foundation of China (30670857; 30800457). This study was approved by the Institutional Review Board of College of Life Science and Technology, Huazhong University of Science and Technology.

References

Anselmi CV, Novelli V, Roncarati R et al (2008) Association of rs2200733 at 4q25 with atrial flutter/fibrillation diseases in an Italian population. Heart 94:1394–1396

Arnar DO, Thorvaldsson S, Manolio TA et al (2006) Familial aggregation of atrial fibrillation in Iceland. Eur Heart J 27:708–712

Brand FN, Abbott RD, Kannel WB et al (1985) Characteristics and prognosis of lone atrial fibrillation. JAMA 254:3449–3453

Chen YH, Xu SJ, Bendahhou S et al (2003) KCNQ1 gain-of-function mutation in familial atrial fibrillation. Science 299:251–254

Ellinor PT, Yoerger DM, Ruskin JN et al (2005) Familial aggregation in lone atrial fibrillation. Hum Genet 118:179–184

Fuster V, Rydén LE, Cannom DS et al (2006) ACC/AHA/ESC 2006 Guidelines for the Management of Patients with Atrial Fibrillation: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines and the European Society of Cardiology Committee for Practice Guidelines (Writing Committee to Revise the 2001 Guidelines for the Management of Patients With Atrial Fibrillation): developed in collaboration with the European Heart Rhythm Association and the Heart Rhythm Society. Circulation 114:e257–e354

Go AS, Hylek EM, Phillips KA et al (2001) Prevalence of diagnosed atrial fibrillation in adults: national implications for rhythm management and stroke prevention: the AnTicoagulation and Risk Factors in Atrial Fibrillation (ATRIA) Study. JAMA 285:2370–2375

Goldstein M, Barnett HJM, Orgogozo JM et al (1989) Stroke—1989. Recommendations on stroke prevention, diagnosis, and therapy. Report of the WHO Task Force on Stroke and other Cerebrovascular Disorders. Stroke 20:1407–1431

Gretarsdottir S, Thorleifsson G, Manolescu A et al (2008) Risk variants for atrial fibrillation on chromosome 4q25 associate with ischemic stroke. Ann Neurol 64:402–409

Gudbjartsson DF, Arnar DO, Helgadottir A et al (2007) Variants conferring risk of atrial fibrillation on chromosome 4q25. Nature 448:353–357

Hodgson-Zingman DM, Karst ML, Zingman LV et al (2008) Atrial natriuretic peptide frameshift mutation in familial atrial fibrillation. N Engl J Med 359:158–165

Hong K, Bjerregaard P, Gussak I et al (2005) Short QT syndrome and atrial fibrillation caused by mutation in KCNH2. J Cardiovasc Electrophysiol 16:394–396

Hu D, Sun Y (2008) Epidemiology, risk factors for stroke, and management of atrial fibrillation in China. J Am Coll Cardiol 52:865–868

Kääb S, Darbar D, van Noord C et al (2009) Large scale replication and meta-analysis of variants on chromosome 4q25 associated with atrial fibrillation. Eur Heart J 30:813–819

Kannel WB, Wolf PA, Benjamin EJ et al (1998) Prevalence, incidence, prognosis and predisposing conditions for atrial fibrillation: population-based estimates. Am J Cardiol 82:2N–9N

Lai LP, Lin JL, Huang SK (2003) Molecular genetic studies in atrial fibrillation. Cardiology 100:109–113

Lévy S, Maarek M, Coumel P et al (1999) Characterization of different subsets of atrial fibrillation in general practice in France: the ALFA study. The College of French Cardiologists. Circulation 99:3028–3035

Olson TM, Michels VV, Ballew JD (2005) Sodium channel mutations and susceptibility to heart failure and atrial fibrillation. JAMA 293:447–454

Olson TM, Alekseev AE, Liu XK et al (2006) Kv1.5 channelopathy due to KCNA5 loss-of-function mutation causes human atrial fibrillation. Hum Mol Genet 15:2185–2191

Sébillon P, Bouchier C, Bidot LD et al (2003) Expanding the phenotype of LMNA mutations in dilated cardiomyopathy and functional consequences of these mutations. J Med Genet 40:560–567

Tsai CT, Lai LP, Hwang JJ et al (2008) Molecular genetics of atrial fibrillation. J Am Coll Cardiol 52:241–250

Wang QK (2008) Atrial fibrillation: genetic consideration. In: Natale A, Jalife J (eds) Atrial fibrillation from bench to bedside. Humana Press, New Jersey, pp 133–144

Wolf PA, Abbott RD, Kannel WB (1991) Atrial fibrillation as an independent risk factor for stroke: the Framingham Study. Stroke 22:983–988

Xia M, Jin Q, Bendahhou S, He Y et al (2005) A Kir2.1 gain-of-function mutation causes human atrial fibrillation. Biochem Biophys Res Commun 332:1012–1019

Yang Y, Xia M, Jin Q et al (2004) Identification of a KCNE2 gain-of-function mutation in patients with familial atrial fibrillation. Am J Hum Genet 75:899–905

Zhang X, Chen S, Yoo S et al (2008) Mutation in nuclear pore component NUP155 leads to atrial fibrillation and early sudden cardiac death. Cell 135:1017–1027

Zhou Z, Hu D (2008) An epidemiological study on the prevalence of atrial fibrillation in the Chinese population of mainland China. J Epidemiol 18:209–216