

Original Research

Association Between rs2200733 Polymorphism on Chromosome 4q25 and Atrial Fibrillation in a Greek Population

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Introduction: Atrial fibrillation (AF) is a common arrhythmia with evidence of genetic susceptibility. The rs2200733 single-nucleotide polymorphism (SNP) in a non-coding region on chromosome 4q25 has been associated with AF. The purpose of this case-control study was to examine the possible association of the rs2200733 polymorphism with AF in the Greek population.

Methods: A total of 295 individuals, 167 AF patients and 128 controls, were genotyped for the presence of the rs2200733 polymorphism using a polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLPs) method.

Results: The T/T genotype and the T allele were detected more frequently in patients with AF compared to controls (13.2% vs. 2.3%, $p=0.001$, and 29.6% vs. 17.9%, $p=0.001$), suggesting that the rs2200733 polymorphism increases susceptibility to AF in the Greek population. In a multivariate stepwise analysis that included many conventional precipitating factors for AF, T/T genotype and left atrium (LA) diameter were the only independent predictors of AF (OR 1.74, 95% CI: 1.40-2.98, $p=0.005$, and OR 2.88, 95% CI: 1.83-5.62, $p<0.001$, respectively). A trend of association was observed between the T/T genotype and lone AF ($p=0.08$).

Conclusions: Our results suggest that SNP rs2200733 confers a significant risk of AF in the Greek population, providing further support to the previously reported association between AF and rs2200733 polymorphism on chromosome 4q25.

Atrial fibrillation (AF) is the most common sustained cardiac arrhythmia, affecting 1-2% of the general population. Its prevalence increases with age, reaching approximately 5% at ages over 65 years and almost 10% at ages over 80 years.^{1,2} From the epidemiological point of view, AF is associated with an increased risk of stroke, heart failure, de-

mentia and death,¹ imposing a huge societal cost and also creating an enormous public health burden.² Antiarrhythmic therapy still remains the first-line option,³ although it is plagued by a high recurrence rate and a potential proarrhythmic effect, especially in individuals with underlying cardiac pathology. Surgery and catheter ablation have emerged as promising and

evolving techniques to treat the arrhythmia, yet their use is constrained by complexity, cost and a high recurrence rate.^{4,5}

Several epidemiological studies have demonstrated the familial aggregation of AF and also the increased vulnerability to this arrhythmia in the close relatives of individuals with AF, both suggesting a genetic contribution to the development of AF.⁶⁻⁹ However, familial AF accounts for only a small fraction of all cases of AF, whereas non-familial AF is the prevailing form of this arrhythmia.¹⁰ Until recently, the genetics of non-familial AF remained largely unexplored; the advent of genome-wide association studies (GWASs) has provided great insight into the molecular mechanisms implicated in this arrhythmia and has led to significant advances in our understanding of the genetic basis of this complex trait. In fact, GWASs for AF have led to the identification of novel variants that appear to confer increased susceptibility to sporadic AF.¹⁰ Among these, the common variant rs2200733 on chromosome 4q25 has been strongly and independently associated with an increased risk of AF in various ethnicities.¹¹⁻¹⁶ Notably, recent studies have also found that this variant is associated with recurrence of AF after catheter ablation,^{17,18} providing further evidence that this polymorphism is potentially of clinical relevance. The aim of this case-control study was to investigate the possible genetic association of the rs2200733 polymorphism with sporadic AF in the Greek population, as it has not been examined before in this ethnicity.

Methods

A total of 167 unrelated individuals diagnosed with AF and 128 control subjects were included in this study. All samples were of Greek origin and were collected from a number of different hospitals. The study conformed to the principles outlined in the Declaration of Helsinki and was approved by the Scientific Ethics Committee of the local hospitals. Written informed consent was obtained from all the participants.

The diagnosis of AF was based on electrocardiograms (ECG) and/or Holter ECG data following standard diagnostic criteria.¹⁹ Patients presenting with AF assumed to be secondary to other cardiac or extracardiac diseases, such as cardiomyopathies, valvulopathies, pulmonary and thyroid pathologies, as well as patients with atrial flutter not related to AF, were excluded from the study. The control group included age-matched individuals without a history

of AF, but with a high prevalence of risk factors associated with this arrhythmia. Our controls had no signs or symptoms of AF on physical examination and showed sinus rhythm on the ECG. AF was classified as paroxysmal, persistent, or permanent, according to the ACC/AHA/ESC AF guidelines.¹⁹ Lone AF was defined as AF occurring in the absence of cardiac or systemic disease.¹⁹

All participants had a standard ECG recording and underwent transthoracic and/or transoesophageal echocardiography for measurement of left atrial (LA) diastolic diameter. Patients with disease symptoms at age <60 years were considered as having early-onset AF, while patients with disease symptoms at age ≥60 years were considered as having late-onset AF. The presence of hypertension, coronary artery disease (CAD), diabetes mellitus (DM), and smoking status was recorded in both our study groups, as well as the body mass index (BMI), calculated as weight in kilograms divided by height in meters squared.²⁰

Single-nucleotide polymorphism genotyping

Genomic DNA was extracted in all subjects from peripheral blood leukocytes, using the Wizard Genomic DNA Purification Kit (Promega). Rs2200733 genotypes were determined using the Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) method. The region containing the rs2200733 polymorphism was amplified with the following set of primers: right 5'-TATTCACAGGCTTCCCTCTA-3' and left 5'-AATGCTGTGGGAACATAAAC-3. The resulting PCR product (178 bp) was used for RFLP analysis. The PCR conditions used for the rs2200733 polymorphism were 94°C for 10 min, followed by 32 cycles of 94°C for 30 s, 65°C – 0.5°C/cycle for 30 s, 72°C for 45 s, and a final extension step of 72°C for 10 min. The restriction enzyme BfuCI (New England BioLabs) was used for the genotyping of the rs2200733 polymorphism. Mutated homozygotes T/T were represented by an undigested PCR 178 bp product. Mutated heterozygotes C/T were represented by the fragments 178+109+69 bp, and the wild-type homozygotes C/C were represented by the fragments 109+69 bp. All fragments were separated on 3% agarose gels and visualised with UV light after ethidium bromide staining.

Statistical analysis

Statistical analysis was conducted using the SPSS 14.0

software package (SPSS Inc., Chicago, Illinois). Continuous variables are summarized as mean \pm standard deviation (SD); categorical variables are presented as absolute numbers and percentages. Genotype and allele frequencies were compared between patients and controls, as well as their subgroups, using the chi square test. Multivariate stepwise logistic regression was performed, incorporating age, DM, hypertension, CAD, BMI, LA diameter, sex, and rs2200733 genotype as covariates. Odds ratio (OR) and 95% confidence interval (CI) were calculated and a p-value <0.05 was considered significant.

Results

The demographic and clinical characteristics of the study participants are summarized in Table 1. Patients with AF were less often male, had a lower incidence of CAD, and had a larger LA diameter compared to controls.

We applied a dual approach to assess the relation between the rs2200733 genotype and presence of AF: (i) a genotype-based approach and (ii) an AF-based approach, as detailed below.

Examining first all study subjects as one group, the proportion of the T/T genotype was lower compared to the C/C and C/T genotypes (8.5% vs. 59.3% vs. 32.2%, respectively). However, AF was more common in carriers of the T/T genotype compared to individuals with the C/T or C/C genotypes (88% vs. 53.6%, $p<0.001$) (Figure 1).

We also compared the genotype and allele distributions for the rs2200733 polymorphism in patients

with AF versus controls. The T/T genotype was more common in patients with AF compared to controls (13.2% vs. 2.3%, respectively, $p=0.001$) (Figure 2). Similarly to the results pertaining to genotypes, when we focused on the alleles, the presence of T allele was higher in patients with AF compared to controls (29.6% vs. 17.9%, $p=0.001$) (Figure 3).

A multivariable stepwise analysis was performed to determine the predictors of AF, including relevant clinical, echocardiographic, and genetic variables (age, DM, hypertension, CAD, BMI, LA diameter, sex, and rs2200733 genotype). LA diameter (OR 2.88, 95% CI: 1.83-5.62, $p<0.001$) and T/T genotype (adjusted OR 1.74, 95% CI: 1.40-2.98, $p=0.005$) were the only independent predictors of AF, with LA diameter emerging as the more powerful. Notably, LA diameter did not differ between AF patients with T/T genotype com-

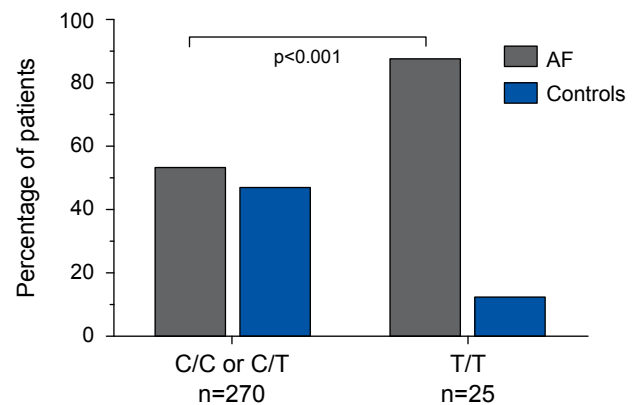


Figure 1. Prevalence of atrial fibrillation (AF) in individuals with the T/T genotype (right columns) compared to individuals with either C/C or C/T genotype (left columns).

Table 1. Demographic and clinical characteristics of patients with atrial fibrillation (AF) and controls.

Variable	AF patients (n=167)	Controls (n=128)	p
Age (years)	65.6 \pm 9.8	65.2 \pm 9.8	0.79
BMI (kg/m ²)	24.9 \pm 2	24.6 \pm 3	0.54
Male sex (%)	54.4	73.9	0.04
Hypertension (%)	62.8	50	0.08
Coronary artery disease (%)	23.9	45.4	0.001
Diabetes mellitus (%)	22.1	26.1	0.51
Current smoker (%)	21.4	20	0.26
LA diameter (mm)	45.0 \pm 3.9	42.6 \pm 2.7	<0.001
Age of AF onset (years)	60.8 \pm 10.1	–	–
AF category:			
Paroxysmal (%)	60.2	–	–
Persistent (%)	18.6	–	–
Permanent (%)	21.2	–	–
Lone AF (%)	23	–	–

Data are expressed as mean \pm SD or percentage. BMI – body mass index; LA – left atrium.

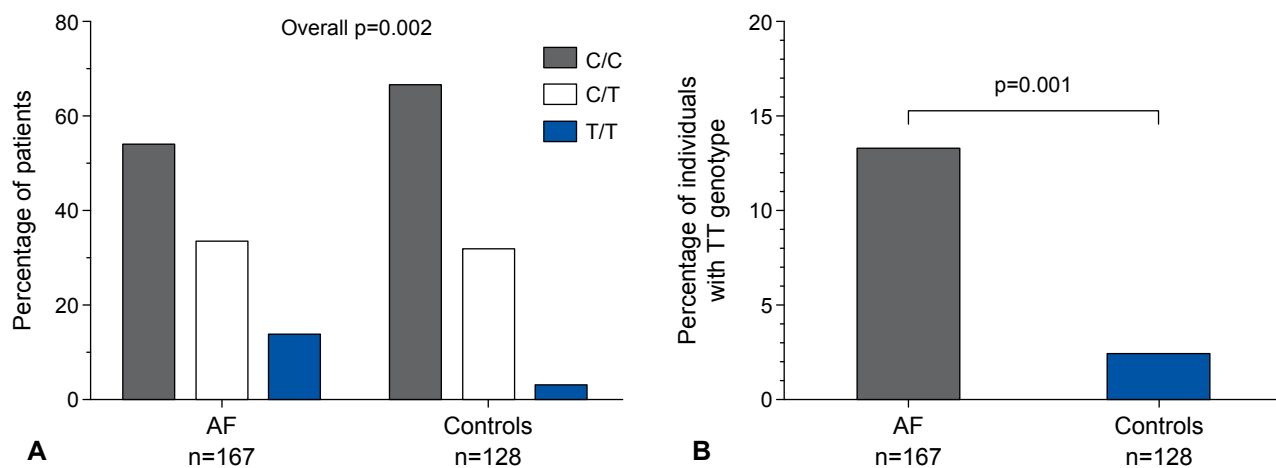


Figure 2. A: Proportion of patients with atrial fibrillation (AF) (left) and controls (right) with each of the three rs2200733 genotypes. B: Patients with AF more commonly carried the T/T genotype compared to controls without AF.

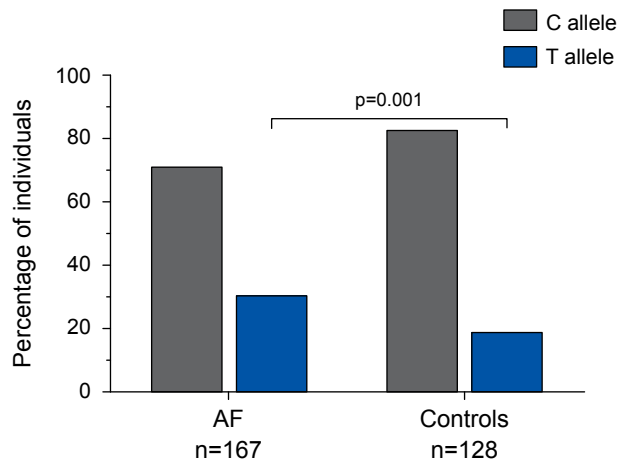


Figure 3. Patients with atrial fibrillation (AF) were more commonly carriers of the T allele compared to controls.

pared to those with C/T or C/C genotype (44.5 ± 3.9 vs. 45 ± 4.0 mm, respectively, $p=0.68$).

A search for possible clinical correlates of the different rs2200733 genotypes in patients with AF revealed that T/T genotype tended to be more frequent in patients with lone than in those with secondary AF ($p=0.08$). However, no significant differences were found in the prevalence of the rs2200733 genotypes in relation to age, sex, hypertension, CAD, DM, BMI, or age of AF onset (data not shown).

Discussion

In the present study, evidence is provided for the association between AF and the rs2200733 polymor-

phism on chromosome 4q25 in a Greek population with AF.

In the last decade, several GWASs have identified rs2200733, an SNP at 4q25, to be the most common chromosomal variant present in patients with AF.¹⁰ Nevertheless, a large percentage of the heritability of AF remains unexplained. This is probably attributable, not only to an unknown number of yet unidentified common variants, but also to the fact that common variants, such as rs2200733 SNP, often have small to modest effects. It is also likely that the heritability of AF is the result of a multifactorial combination, including common and rare variants, and also gene–gene and gene–environment interactions.^{21,22} In addition, genetic polymorphisms may reveal different distributions among diverse ethnic groups, suggesting that ethnic variation may play an important role in the genetic predisposition to human diseases such as AF.¹⁰ Therefore, it is important for such associations to be tested in various populations of diverse origin, who also exhibit various precipitating risk factors. For instance, differences have been reported among different populations in the frequency of the polymorphic rs2200733 allele in a Polish AF group¹⁴ and two German groups with AF.^{12,18} Similarly, a landmark analysis of common variants on chromosome 4q25 associated with an increased risk of AF demonstrated a higher frequency and a stronger association of the studied variants with AF in a Chinese population compared to two populations of European origin.¹¹ Because genetic polymorphisms may reveal different distributions and have different clinical implications among diverse ethnic groups,¹⁰ several

analyses have been performed focused on confirming previously demonstrated associations in specific ethnic groups^{11,13-16} The independent genetic association studies of rs2200733 with AF have increased the quantity of genetic data, which could be used in a future meta-analysis, increasing the statistical power of the final conclusions. The present study is the first to examine the genetic association of the rs2200733 polymorphism with AF in a Greek population. Both genotype distribution and allele frequencies for this variant were significantly associated with AF compared with controls, confirming the importance of this polymorphism as a risk factor for AF.

The mechanism by which the 4q25 locus confers AF risk is currently unknown. The most popular and widely accepted hypothesis points to the close proximity of this variant to the *PITX2* gene, which is involved in the left–right asymmetric development of the heart during embryogenesis,²³ as well as in the development of pulmonary vein myocardium, a major source of atrial arrhythmogenesis.²⁴ In mice, *PITX2* haplo insufficiency has been associated with atrial arrhythmias,²⁵ whereas the expression of the *PITX2c* isoform, which is predominantly expressed in the adult left atrium, has been found to be decreased in patients with sustained AF, suggesting a *PITX2* loss of function mechanism in AF.²⁶ Notably, in a recent study a novel *PITX2c* missense mutation was associated with lone AF via a loss-of-function mechanism.²⁷

In our Greek cohort, statistically significant differences were found in both genotype and allele frequencies regarding the rs2200733 polymorphism. Both the T/T genotype and the T allele were overrepresented in the AF compared to the control group—a result which is in line with reports of previous studies carried out in different European populations.²⁸ The predictive value of the rs2200733 polymorphism was also strong, as AF was more common in carriers of the T/T genotype compared to individuals with the C/T or C/C genotypes (88% vs. 53.6%, $p < 0.001$). After multivariable analysis, the T/T genotype remained an independent risk factor for AF, along with LA diameter. However, there was no statistically significant difference in LA diameter between AF patients with T/T genotype and those with C/T or C/C genotype, indicating a lack of interaction between these two parameters.

Although the influence of genetic factors is believed to be stronger in lone AF than in other types of AF,^{15,29,30} this was not detected in the present study. Nevertheless, there was still a positive trend for an as-

sociation of the T/T genotype with lone AF patients ($p = 0.08$), suggesting that a potential association of the rs2200733 variant with AF subphenotypes should be further examined in larger population studies so that more definite conclusions may be drawn.

Limitations

The main limitation of the present study is the small number of individuals who were enrolled, especially those with lone AF. Because of the small sample size, these results are presented as hypothesis-generating and need to be confirmed by larger studies. However, the size of the sample will add to meta-analyses performed in the long term, accentuating the role of the above mentioned polymorphism in AF. Undetected asymptomatic AF episodes in the control group cannot be excluded, although a detailed history was taken in order to eliminate this eventuality. The results of the present study are in complete agreement with previous ones,¹¹⁻¹⁶ reconfirming the putative importance of this polymorphism in AF. A linkage disequilibrium and haplotype analysis for the rs2200733 polymorphism and other identified SNPs in the *PITX2* gene would also be of particular interest.

Conclusions

We found a significant association between SNP rs2200733 on chromosome 4q25 and AF in a Greek population. The rs2200733 polymorphism was strongly and independently associated with an increased risk of AF, without being affected by known risk factors for AF. This is the first study carried out in the Greek ethnicity, adding to the growing pool of data examining this variant in different ethnic groups. Although the exact mechanism linking this variant to AF remains currently unknown, our results provide additional evidence about the importance of the rs2200733 polymorphism in AF, further emphasising the need for future studies examining the genetic and functional role of this polymorphism in AF.

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