

## ASSOCIATION BETWEEN CHROMOSOME 4Q25 POLYMORPHISM RS2200733 AND THE INCIDENCE OF ATRIAL FIBRILLATION IN BULGARIAN PATIENTS

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### **Summary**

Atrial fibrillation (AF) is the commonest type of arrhythmia seen in everyday clinical practice, which leads to a significant increase in both morbidity and mortality. Its incidence increases with age and tends to turn into an epidemic. The cause of AF in 10-20% of cases remains unknown. Several mutations and polymorphism that might be responsible for the development of AF have been found, including single nucleotide polymorphisms (SNPs) – rs2200733 and rs10033464 in the long arm of the fourth chromosome. These polymorphisms are selected on the basis of genome-wide association study in Iceland from 2007, the results from which were later confirmed in 4 other large populations. The rs2200733 is a common noncoding polymorphism, described in National Center for Biotechnology Information (NCBI) database dbSNP like NC\_000004.12:g.110789013C>T, with a frequency of the less common allele between 0.1 and 0.24. In order to investigate the association between the rs2200733 polymorphism in chromosome 4q25 and the development of AF, we studied the frequency of this polymorphism in patients with heart diseases from the Pleven region, and thus evaluate the relationship between the individual genotype and the clinical condition of the patients. We carried out a case-control study on 80 patients: 40 with AF and 40 without AF- from the Pleven region. None of these had structural heart disease. The study was conducted between November 2015 and November 2017. With deoxyribonucleic acid (DNA) analysis, we determined rs2200733 polymorphism, using a TaqMan-based polymerase chain reaction (PCR). The Cochran-Armitage trend test, the Chi-Squared Pearson correlation, Fisher test we used confirmed the statistically significant association between the rs2200733 polymorphism in chromosome 4q25 and the development of AF. In the population examined, the genotypic frequencies were as follows: CC – 45 (56.2%), CT – 19 (23.8%), TT – 16 (20%), with value of Chi-Square ( $\chi^2$ ) 24.496, df=2,  $p<0.001$ . Screening for SNPs could be a useful marker for the detection of patients predisposed to AF.

**Keywords:** atrial fibrillation, chromosome, single nucleotide polymorphisms

### **Introduction**

Atrial fibrillation (AF), the most common arrhythmia seen in everyday clinical practice, is caused by chaotic electrical impulses in the atria [1]. Vulpian was the first to describe the irregular atrial electric activity in

1874, calling it ‘fremissement fibrillaire’. In 1906, William Einthoven published the first electrocardiogram of AF [2, 3]. In 2010, the worldwide prevalence of AF among men was 20.9 million, 12.6 million – among women, with its incidence being higher in the developed countries. The number of people suffering from this arrhythmia in the European Union is estimated at 14-17 million. Its incidence increases with age, and among those over 80, it is 8-15% [4]. AF increases both morbidity and mortality, with a fivefold increase of the risk of ischemic stroke and threefold for heart failure. Risk factors for the development of AF are age, arterial hypertension, heart failure, ischemic heart disease, valvular diseases, cardiomyopathies, congenital heart diseases, thyroid gland pathology, chronic obstructive pulmonary disease (COPD), diabetes, sleep apnea, renal failure [4]. In 10-20% of cases, the cause remains unknown. Evidence exists for the role of genes in the pathogenesis of AF. In 1997, Brugada reported the chromosome locus found first (10q22-q24) in a family with AF [5, 6]. Other mutations and polymorphisms have also been found to be connected with the development of AF, such as the single nucleotide polymorphisms (SNPs) in the long arm of the fourth chromosome (chromosome 4q) [7]. They are thought to be the most common genetic variation. SNP is the substitution of a single base in the deoxyribonucleic acid (DNA) molecule. Two SNPs (rs2200733 and rs10033464B) have been identified in chromosome 4q. For the disease to occur, one SNP is not enough, but it alone or with other SNPs might increase the risk for the development of AF [6]. SNPs in genes coding cardiac ion channels, connexin 40, RAAS might lead to AF. The gene closest to an SNP is PITX2, which plays a vital role in the foetal development of the heart and the differentiation of the left atrium [8].

To investigate the link between rs2200733 polymorphism in chromosome 4q25 and the development of AF, we studied the frequency of this polymorphism in patients from the Pleven region with or without AF, so that we could determine the relationship between the individual genotype and the clinical condition of the patient.

## Materials and Methods

A survey on 80 patients from the region of Pleven was conducted; all admitted to the University multiprofile hospital for active treatment (UMHAT) “Dr. Georgi Stranski” in Pleven between November 2015 and November 2017. The patients were divided into two groups: 40 cases and 40 controls. All the patients were aged between 18 and 65 and had no structural heart disease. Those in the first group had AF with or without arterial hypertension, and those in the second without AF, were admitted to the hospital because of poor control of arterial hypertension. The exclusion criteria were severe left ventricular hypertrophy, ischemic heart disease, cardiomyopathies, valvular diseases, obesity, thyroid gland disorders, virus infection, chronic pulmonary diseases, diabetes, and alcohol and drug abuse. Informed consent from the patients was obtained, as well as permission from the local ethics committee. Blood samples for DNA analysis were collected, and TaqMan-based polymerase chain reaction (PCR) was used to determine the presence of another other s 2200733 polymorphism. Two methods of DNA analysis were used: primary DNA extraction from fresh blood samples, and isolation with streptavidin colons of frozen blood samples. Genotyping of the rs2200733 polymorphism in chromosome 4q25 was achieved with TaqMan-method (Life Technologies), using the platform PicoReal 96-Real-time PCR and consequent analysis of the allelic profiles, performed with specialized software (Thermoscientific). The following statistical methods were used: Cochran-Armitage trend test and Pearson’s Chi-Square test. As the results for the dominant/recessive models were different due to biological or technical causes, the results were adjusted to the Hardy-Weinberg equilibrium to minimise the bias.

## Results

In the group of 80 patients from the region of Pleven we investigated, three genotypes were observed: homozygous for allele C-normal type (45 patients), heterozygous for allele CT (19 patients), and homozygous for allele T-mutant type (16 patients). From the target group, 13

patients (32%) were homozygous for allele C, 11 (28%) were heterozygous for allele CT, and 16 (40%) were homozygous for allele TT. From the control group, 33 (82%) were homozygous for allele C, 7 (18%) were heterozygous for allele CT, and none of them was homozygous for the mutant allele T.

The allele frequencies were analysed (Table 1, Figure 1).

Chi-Square=33.272 with 1 degree of freedom,  $p < 0.001$  demonstrated a strong connection between SNPs and AF.

For the same table, the Fisher exact test showed statistically significant association.

We checked data for Hardy-Weinberg equilibrium (HWE), using the calculator available from

<http://www.oege.org/software/hwe-mr-calc.shtml> [9].

For our patients, the genotypic frequencies were as follows: CC 45, CT 19, TT 16, which showed that in our group of cases and controls the HWE was not observed, and the results

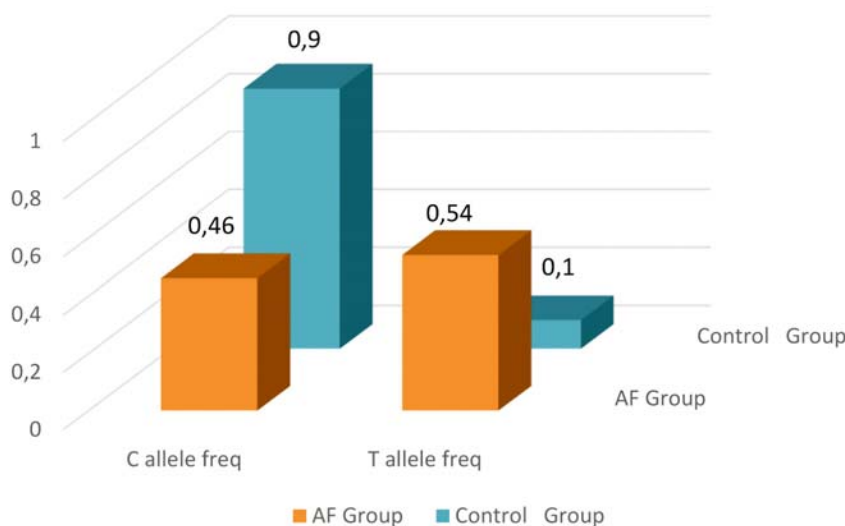
**Table 1.** Table of the allelic frequency differences and Chi-Square with Yates' correction

	C allele freq	T allele freq
<b>Group AF</b>	0.46 (n=37)	0.54 (n=43)
<b>Group Controls</b>	0.9 (n=72)	0.1 (n=8)

obtained were  $\chi^2=16.43$ ,  $p < 0.001$ .

Three cases present the absence of each of the three genotype groups. For example, line 1 showed the potential absence of the common homozygote group when the Hardy-Weinberg was used. In each case, the number in red is the result of an adjustment to the expected number under Hardy-Weinberg equilibrium when taking under consideration the observed number for the other two groups (Table 2).

That is why we used the Cochran-Armitage trend test (Sasieni, 1997) [10], and the results from this test showed that statistically there was a significant link between the groups and genotypes. Chi-Square=24.496,  $df=2$ ,  $p < 0.001$  (Table 3, Figure 2).



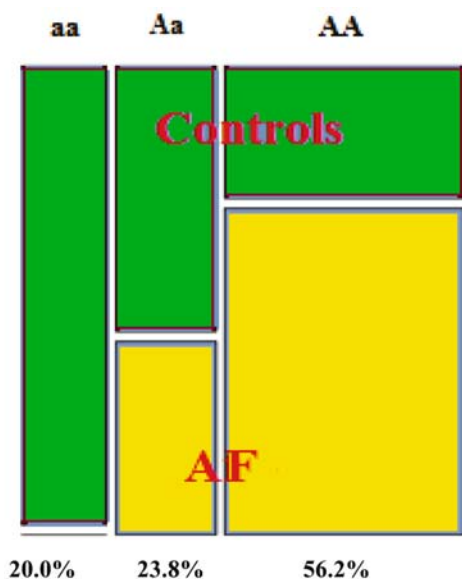
**Figure 1.** Figure of allelic frequency differences and Chi-Square with Yates' correction

**Table 2.** Solutions for perfect HWE, under a model of ascertainment (+/-) of one group

Genotype	Common Hz	Heterozygotes	Rare Hz	C allele freq	T allele freq
Common Hz	<b>5.64</b>	19	16	0.37	0.63
Heterozygotes	45	<b>53.67</b>	16	0.63	0.37
Rare Hz	45	19	<b>2.01</b>	0.83	0.17

**Table 3.** Cochran-Armitage Test for Trend, showing the connection between the examined group and the genotypes

	TT	CT	CC	Sum
AF	16 (20.0%)	11(13.8%)	13 (16.2%)	40 (50.0%)
Control	0 (0.0%)	8 (10.0%)	32 (40.0%)	40 (50.0%)
Sum	16 (20.0%)	19 (23.8%)	45 (56.2%)	80 (100.0%)



**Figure 2.** Cochran-Armitage Test for Trend, showing the connection between the examined group and the genotypes

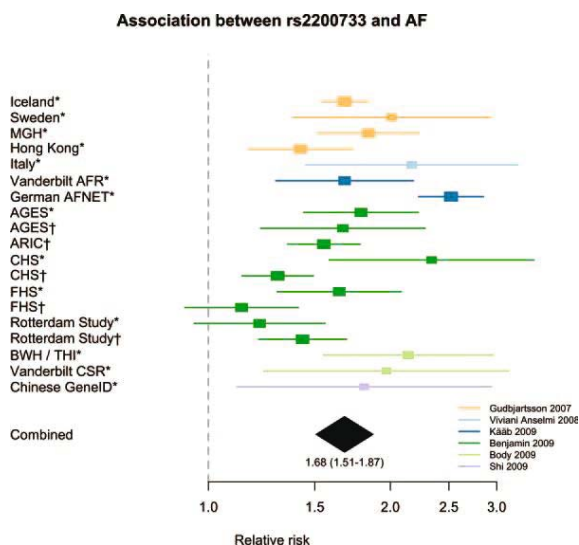
## Discussion

The pathogenesis of AF is not yet fully understood. It is known that certain genetic factors play a role in the mechanism of its genesis. Several loci and genes were found, polymorphisms in which could lead to AF. One such example is the SNPs in 4q. SNPs in genes that encode ion channels in the heart, connexin 40, and RAAS might be responsible for the development of AF. A survey among patients with AF who underwent radiofrequency ablation procedures in the period 2008–2012 in three centres – Wanderbilt, Leipzig, Massachusetts – proved that the rs2200733 polymorphism in 4q increases the risk for AF 1.4 times [11].

In a group of patients from Hong Kong in 2007, a strong connection was identified between the presence of 4q25 SNP and the development of AF. The same results were reported from a smaller-scale trial in Italy [12].

The results from multiple surveys have confirmed the correlation between the rs2200733

polymorphism and the risk for AF (Figure 3) [12].



Each copy of the allele of this polymorphism increases the risk 1.68 times. The magnitude of this effect is comparable to other risk factors like age, arterial hypertension, diabetes [12]. In the experimental group, it was proved that the presence of a particular polymorphism is related to the development of AF. Our survey confirmed current data, i.e. that there exists a connection between SNPs in 4q and the occurrence of AF. The exact effects of these SNPs on its development need to be further investigated. PITX2 is the closest gene to an SNP, and it has a crucial role in the embryogenesis of the heart. Studies worldwide have shown that there is a connection between the C/T polymorphism in 4q and the incidence of AF. Although the guidelines of the European Society of Cardiology on AF do not recommend routine genetic testing in screening for AF, in the future, it might have a significant contribution to diagnosis, treatment and prophylaxis of AF.

## Conclusions

Prevention plays an essential role in reducing the complications of AF. Why some patients

with arterial hypertension develop AF while others do not is a question with no answer yet. New genetic surveys have shed light on the pathogenesis of idiopathic AF. Confirmation of the influence of these genetic factors would lead to an earlier diagnosis, better prevention and treatment for AF.

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