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ORIGINAL ARTICLE / KLİNİK ÇALIŞMA

Interrelation between the rs2200733 polymorphism of the *ATFB5* gene and atrial fibrillation in Uzbek patients

Özbek hastalarda *ATFB5* geninin rs2200733 polimorfizmi ile atriyal fibrilasyon arasindaki ilişki

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ABSTRACT

Objective: Atrial fibrillation (AF) is one of the most common cardiac arrhythmias and a major predictor of morbidity and mortality. AF is a polygenic and polyetiological disease. In various ethnic groups, the strongest and most independent relationship with the development of AF was found with the 4q25 locus, where the *ATFB5* gene is located. An analysis of the literature data showed that the carriage of the TT genotype of the rs2200733 *ATFB5* gene polymorphism is the most unfavorable genotype for the development of AF. The purpose of the study was to identify the prevalence of genotypes and alleles of the rs2200733 polymorphism of the *ATFB5* gene in Uzbek patients with AF.

Methods: The study included 69 Uzbek patients with paroxysmal (n=20) and persistent AF (n=49). The control group (n=30) was composed of Uzbek patients without AF. Genotyping for the carriage of allelic variants of the rs2200733 polymorphism of the *ATFB5* gene was performed using the Polymerase Chain Reaction-Restriction Length Polymorphism (PCR-RFLP) method. The distribution of the C and T alleles and the CC, CT, and TT genotypes of the rs2200733 polymorphism of the *ATFB5* gene in patients with AF and controls were compared.

Results: After genotyping 69 patients with AF, the following distribution of the *ATFB5* gene polymorphism rs2200733 was revealed: the CC genotype was detected in 35 (50.72%) patients, the CT genotype in 25 (36.23%) patients, and the TT genotype in 9 (13.05%) patients ($p<0.001$, $\chi^2=22.435$). Moreover, the C allele was detected in 95 (68.8%) patients, and the T allele was detected in 43 (31.2%) patients ($p<0.001$, $\chi^2=37.696$). The distribution of genotypes in the control group was as follows: the CC genotype was detected in 17 individuals (56.7%), the CT genotype was detected in 12 individuals (40%), and the TT genotype was detected in 1 individual (3.3%) ($p<0.001$, $\chi^2=20.100$). Moreover, the C allele was detected in 46 (76.7%) patients, and the T allele

ÖZET

Amaç: Atriyal fibrilasyon (AF), en yaygın kardiyak aritmilerden biridir ve morbidite ve mortalitenin önemli bir prediktörüdür. AF, poligenik ve çok nedenli bir hastalıktır. Çeşitli etnik gruplarda, AF gelişimi ile en güçlü ve en bağımsız ilişki, *ATFB5* geninin bulunduğu 4q25 lokusu ile bulunmuştur. Literatür verilerinin analizinde, rs2200733 *ATFB5* gen polimorfizminin TT genotipinin taşınmasının, AF gelişimi için en uygunsuz genotip olduğu gösterildi. Çalışmanın amacı, Özbek AF hastalarında *ATFB5* geninin rs2200733 polimorfizminin genotip ve alellerinin prevalansını belirlemekti.

Yöntemler: Çalışmaya paroksismal (n=20) ve persistan AF'si (n=49) olan 69 Özbek hasta dahil edildi. Kontrol grubu (n=30) AF'si olmayan Özbek hastalardan oluşturuldu. *ATFB5* geninin rs2200733 polimorfizminin alelik varyantlarının taşınması için genotipleme, Polimeraz Zincir Reaksiyonu-Restriksiyon Parçacık Uzunluk Polimorfizmi (PCR-RFLP) yöntemi kullanılarak gerçekleştirildi. AF hastalarında ve kontrollerde ATFB5 geninin rs2200733 polimorfizminin CC, CT ve TT genotipleri ve C ve T allellerinin dağılımı karşılaştırıldı.

Bulgular: AF'li 69 hastanın genotiplendirilmesinden sonra, *ATFB5* gen rs2200733 polimorfizminin aşağıdaki dağılımı ortaya çıktı: CC genotipi 35 hastada (%50.72), CT genotipi 25 hastada (%36.23) ve TT genotipi 9 (%13.05) hastada bulundu, $p<0.001$, $\chi^2=22.435$. Ayrıca 95 (%68.8) hastada C-alleli, 43 (%31.2) hastada T-alleli tespit edildi ($p<0.001$, $\chi^2=37.696$). Kontrol grubundaki genotiplerin dağılımı şu şekildedir: CC genotipi 17 kişide (%56.7), CT genotipi 12 (%40), TT genotipi ise 1 (%3.3) kişide bulundu ($p<0.001$, $\chi^2=20.100$). Ayrıca 46 (%76.7) hastada C alleli, 14 (%23.3) hastada T alleli saptandı ($p<0.001$, $\chi^2=32.033$). *ATFB5* geninin TT genotipinin AF'li hastalarda kontrollere kıyasla anlamlı ölçüde daha yaygın olduğu bulundu (%13.1'e karşı %3.3, $p=0.0001$).

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was detected in 14 (23.3%) patients (p<0.001, χ^2=32.033). The TT genotype of the *ATFB5* gene was found to be significantly more prevalent in patients with AF than in controls (13.1% vs 3.3%, p=0.0001).

Conclusion: The TT genotype of the rs2200733 polymorphism of the *ATFB5* gene was found to be significantly more prevalent in Uzbek patients with AF than in controls.

Sonuç: *ATFB5* geninin rs2200733 polimorfizminin TT genotipinin, kontrol bireylerine kıyasla AF'li Özbek hastalarda önemli ölçüde daha yaygın olduğu bulundu.

Atrial fibrillation (AF) is one of the most common cardiac arrhythmias and a major predictor of morbidity and mortality. According to a recent study, in 2020, more than 33 million people suffer from AF.[1]

AF is a polygenic and polyetiological disease. It can occur as the primary disease or secondary to many diseases. Moreover, the risk of inheriting AF increases in individuals who have at least 1 parent with a history of AF.[2]

The study of the role of polymorphisms of candidate genes for AF is especially important from the point of view of prognosis, in particular, to identify not only triggering factors responsible for the occurrence of acute forms of AF but also markers leading to its chronicity.

In various ethnic groups, the strongest and most independent relationship with the development of AF was found with the 4q25 locus, where the *ATFB5* and *PITX2* genes are located.[3-8] Moreover, the mechanism of influence of the 4q25 locus on the risk of developing AF is currently unknown. The main accepted hypothesis indicates the direct proximity of the *ATFB5* gene to the *PITX2* gene, which is involved in the asymmetric development of the heart during embryogenesis and is the main source of supraventricular arrhythmias. An analysis of literature data showed that the carriage of the TT genotype of the rs2200733 polymorphism of the *ATFB5* gene is the most unfavorable for the development of AF.[3,4,9]

In this regard, the purpose of this study was to identify the prevalence of genotypes and alleles of the rs2200733 polymorphism of the *ATFB5* gene in Uzbek patients with AF.

METHODS

The study included 69 Uzbek patients with paroxysmal AF (n=20) and persistent AF (n=49). Ethical approval for this study was obtained from the Ethics Committee of Republican Specialized Scientific and Practical Medical Center of Cardiology, written informed consent was obtained from all subjects before the study. At the time of inclusion in the study, in all patients with recurrent AF, the sinus rhythm was restored. The mean age of the patients was 60.1±11.6 years. The control group (n=30) consisted of Uzbek individuals aged 30-77 years. The presence of AF was excluded on the basis of anamnesis, the absence of symptoms of AF during physical examination, and a normal electrocardiogram at the time of inclusion in the study.

Abbreviations:

AF	*Atrial fibrillation*
AV	*Atrioventricular*
BMI	*Body mass index*
FC	*Functional class*
HR	*Heart rate*
PCR-RFLP	*Polymerase Chain Reaction-Restriction Length Polymorphism*
SD	*Standard deviation*
WPW	*Wolff-Parkinson-White syndrome*

A genetic profile with the inclusion of a family history was completed for each patient. Using registration cards, patients of Uzbek nationality with atrial fibrillation were selected.

The exclusion criteria were as follows: unstable angina, grade III-IV of stable angina, Functional Class III-IV (FC III-IV) of chronic heart failure, a history of heart surgery, rheumatic valve defects, an implanted pacemaker, prolonged QT interval >480 ms, sick sinus syndrome, a history of persistent ventricular arrhythmia, Wolff-Parkinson-White (WPW) syndrome, Brugada syndrome, arterial hypotension (systolic BP <95 mmHg), bradycardia (heart rate [HR] <60 bpm), left atrium size >45 mm, severe liver and kidney dysfunction, severe pulmonary failure, grade II-III AV block, left atrium thrombus, thyroid dysfunction, and decompensated diabetes mellitus.

Genotyping of the single-nucleotide polymorphism rs2200733 of the *ATFB5* gene

Genotyping of samples for the carriage of allelic variants of the rs2200733 polymorphism of the *ATFB5* gene was performed by the Polymerase Chain Reac-

tion-Restriction Length Polymorphism (PCR-RFLP) method. DNA was isolated from whole blood using the Diatom DNA Prep 200 kit (Isogene, Russia) according to the standard protocol of the manufacturer. DNA samples were typed using the enzymatic amplification of DNA. For PCR amplification, the GenPak PCR Core kit (Isogene) was used. The application protocol was as follows: dNTP mix 0.5 mM-5 µL, 10x buffer-5 µL, H_2O-30 µL, 1.25 µL each of 10 µM forward and reverse primers, Taq DNA Polymerase 1 unit/µL-1 µL, and DNA 50 ng-2 µL. The following primer sequence was used: F-5′TATTCACAGGCTTCCCTC-TA-3′ and R-5′AATGCTGTGGGAACATAAAC-3. For PCR, the GeneAmp® 9700 amplifier (Applied Biosystems, USA) was used. Alleles and genotypes of polymorphic markers were determined by splitting amplicons with the Kzo9I restriction enzyme at 37°C for 16 hours. The final products were fractionated by electrophoresis in 3% agarose gel (UltraPure Agarose, Invitrogen, USA), followed by staining in ethidium bromide solution (0.01%) and visualization under ultraviolet light. The *ATFB5* gene is located at the 4q25 locus. As a result of amplification, the fragment consisting of 178 bp corresponded to the mutant TT genotype. Fragments of 109 and 69 bp corresponded to the CC genotype. The presence of three fragments of 178, 109, and 69 bp was evaluated as a heterozygous state of CT (Figure 1).

Statistical analysis

Statistical analysis was performed using the SPSS v22.0 (IBM Corp.; Armonk, NY, USA). The sample mean and standard deviation (SD) were determined. A comparative analysis of the frequencies of genotypes and alleles of the rs2200733 polymorphism of the *ATFB5* gene was performed using the χ^2 test. For all types of analysis, $p<0.05$ was considered statistically significant. The results are presented as mean±SD.

RESULTS

As shown in Table 1, patients with AF did not differ from the control group in sex, age, height, weight, or body mass index (BMI).

Analysis of the distribution of genotypes and alleles of the rs2200733 polymorphism of the *ATFB5* gene in patients with AF (group 1: n=69) and controls (group 2: n=30) showed that the frequency of occurrence of C and T alleles and the prevalence of CC, CT, and TT genotypes were similar (Table 2). The TT genotype of the *ATFB5* gene was found to be significantly more prevalent in patients with AF than that in controls (13.1% vs 3.3%, p=0.0001). It should be noted that the carriers of the TT genotype were 4 times more common among patients in group 1 than among patients in group 2.

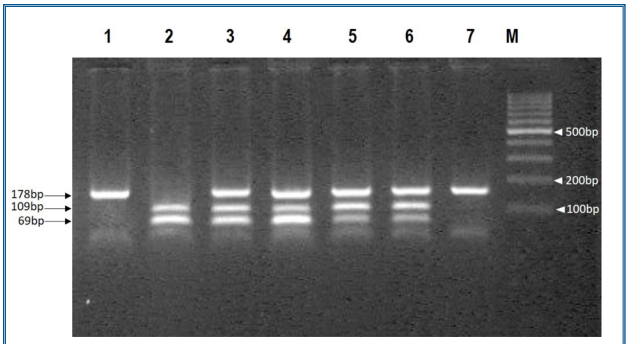

Figure 1. Gel electrophoresis of the restriction of polymerase chain reaction product. Amplified DNA fragments of the ATFB5 gene from patients with atrial fibrillation digested with restriction enzyme Kzo9I and produced the following bands: line 1 - TT genotype (homozygous for the mutant allele), line 2 - CC genotype (homozygous for the wild type allele), 3-6 CT - genotype (heterozygous), 7 - uncut PCR product, M - Molecular Weight Marker (100 bp ladder).

Table 1. Demographic and clinical characteristics of patients with AF and controls

Parameters	Group 1, patients with AF, mean±SD n=69	Group 2, control group, mean±SD n=30	*p*
Age, years	60.1±11.8	60.3±11.3	0.908
Weight, kg	87.5±15.1	82.5±13.6	0.126
Height, cm	171.2±9.1	170.2±8.1	0.605
BMI, kg/m^2	30.0±5.5	28.6±5.3	0.242
Men, n (%)	43 (62.3)	18 (60)	0.995
Arterial hypertension, n (%)	48 (69.6)	20 (66.7)	0.960
Coronary heart disease, n (%)	57 (82.6)	22 (73.3)	0.433
Diabetes mellitus, n (%)	6 (8.7)	3 (10)	0.933
Paroxysmal AF, n (%)	20 (28.9)		
Persistent AF, n (%)	49 (71.1)		
Isolated AF, n (%)	4 (5.8)		

AF: atrial fibrillation.

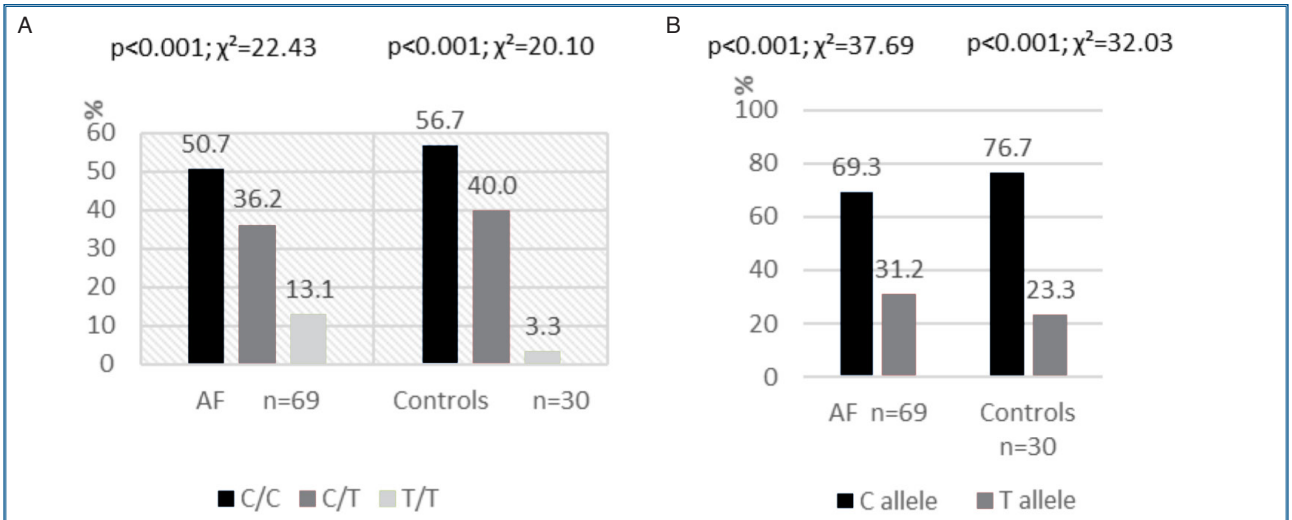

Figure 2. Distribution of **(A)** genotypes and **(B)** alleles of the rs2200733 polymorphism of the *ATFB5* gene among Uzbek patients with atrial fibrillation and controls.

Table 2. Prevalence of genotypes and alleles of the rs2200733 polymorphism of the *ATFB5* gene among patients with atrial fibrillation and controls

Genotypes and alleles	Group 1, patients with AF n=69	Group 2, control group n=30	*p*
CC genotype, n (%)	35 (50.7)	17 (56.7)	0.745
CT genotype, n (%)	25 (36.2)	12 (40)	0.896
TT genotype, n (%)	9 (13.1)	1 (3.3)	0.0001
C allele, n (%)	95 (68.8)	46 (76.7)	0.344
T allele, n (%)	43 (31.2)	14 (23.3)	0.344

AF: atrial fibrillation.

On the basis of the genotyping of 69 patients in group 1, the following distribution of the rs2200733 polymorphism of the *ATFB5* gene was obtained: the CC genotype was detected in 35 (50.72%) patients, the CT genotype was detected in 25 (36.23%) patients, and the TT genotype was detected in 9 (13.05%) patients ($p<0.001$, $\chi^2=22.435$) (Figure 2). Moreover, the C allele was detected in 95 (68.8%) patients, and the T allele was detected in 43 (31.2%) patients ($p<0.001$, $\chi^2=37.696$). Thus, a significant prevalence of the C allele of the rs2200733 polymorphism of the *ATFB5* gene was detected in Uzbek patients with AF. The obtained results indicated the predominance of the CC genotype and C allele of the rs2200733 polymorphism of the *ATFB5* gene in Uzbek patients with AF.

A similar analysis was carried out in controls (group 2), where a similar pattern of the distribution of genotypes and alleles of the rs2200733 polymorphism of the *ATFB5* gene was obtained. The distribution of genotypes was as follows: the CC genotype was detected in 17 (56.7%) individuals, the CT genotype was detected in 12 (40%) individuals, and the TT genotype was detected in 1 (3.3%) individuals ($p<0.001$, $\chi^2=20.1$) (Figure 2). Moreover, the C allele was detected in 46 (76.7%) patients, and the T allele was detected in 14 (23.3%) patients ($p<0.001$, $\chi^2=32.033$). The results obtained for controls also demonstrated significant prevalence of the C allele and the CC genotype of the rs2200733 polymorphism of the *ATFB5* gene.

An analysis of the distribution of genotypes and alleles of the rs2200733 polymorphism of the *ATFB5* gene in group 1 (n=69) and group 2 (n=30) showed that the frequency of occurrence of the C and the T alleles and the prevalence of CC and CT genotypes were similar for both the groups (Table 2). In contrast, the TT genotype of the *ATFB5* gene was found to be significantly more prevalent in group 1 than that in group 2 (13.1% vs 3.3%, p=0.0001). It should be noted that the carriers of the TT genotype were roughly 4 times more common among patients in group 1 than among controls.

DISCUSSION

To date, many loci associated with the development of AF have been identified.[10] Recently, up to 9 ge-

netic polymorphisms have been identified that are associated with a risk of AF development.[11] However, over the past decade, several studies of genome-wide associations have identified the mononucleotide polymorphism rs2200733 in the *ATFB5* gene at the 4q25 locus as the most common chromosomal variant present in patients with AF.[12] In particular, Kääb et al.,[4] in their study conducted on four cohorts of European population, confirmed a reliable relationship between AF and intergenic variants of the single-nucleotide polymorphisms rs2200733 and rs10033464 on chromosome 4. Another group of scientists led by Gudbjartsson et al.[3] identified 2 genes at the 4q25 locus in individuals of European descent, which were significantly related to AF, including the *ATFB5* gene polymorphism rs2200733 that we studied. In a meta-analysis conducted by Ferrán et al.,[9] the association of TT genotype carriage of polymorphism rs2200733 of the *ATFB5* gene was also confirmed to be associated with a risk of developing AF. The same group of researchers revealed an association between the polymorphism rs2200733 of the *ATFB5* gene and atrial fibrillation and atrial dilatation in Spanish individuals. In a Greek population of patients with AF, the TT genotype of the rs2200733 polymorphism of the *ATFB5* gene was more common than the CT or CC genotypes (88% vs 53.6%, p<0.001).[13]

In our study, we only analyzed the distribution of genotypes and alleles of the rs2200733 polymorphism of the *ATFB5* gene in patients with AF and in controls, to further explore in future studies the possible association of this polymorphism with the risk of AF development in Uzbek population. The prevalence of genotypes and alleles of the rs2200733 polymorphism among all patients included in this study coincides with the results of previous studies conducted in different European countries.[9,13] Our data are consistent with the results of a meta-analysis[14] and indicate a possible relationship between the rare mutant variant (TT genotype) and a 4-fold increase in the risk of AF development.

This study is the first genetic project to study the association of the rs2200733 polymorphism of the *ATFB5* gene in patients with AF in the Uzbek population. The obtained results indicate a predominance of both the CC genotype and C allele of the rs2200733 polymorphism of the *ATFB5* gene, both among Uzbek patients with AF and controls. In contrast, the TT genotype of the *ATFB5* gene was found to be significantly more prevalent in Uzbek patients with AF than in controls. At the same time, it should be noted that in patients with AF, the T allele was more common than in controls (without AF), but the differences did not reach statistical significance. This was due to the small number of patients studied.

Thus, the prognostic value of the rs2200733 polymorphism of the *ATFB5* gene is undeniable because the TT genotype of the rs2200733 gene was more common among AF patients than among controls, which is also consistent with the results of previous studies conducted in other populations. These data allow considering the TT genotype and T allele of rs22007333 polymorphism of the *ATFB5* gene as a factor of the assumed risk of developing AF in Uzbek population.

The AF is a polyetiological and polygenic disease, and the predisposition to AF is associated with multiple factors, including environmental factors, as well as with an individual set of genetic polymorphisms of candidate genes. Furthermore, ethnicity can undoubtedly play a significant role, which underlies various variations of genetic polymorphisms of candidate genes.

Limitations

The limitations of our study include the small number of patients. Due to the small sample size, these results are presented as generating a hypothesis and should be confirmed by larger studies. In addition, asymptomatic episodes of AF in the control group cannot be completely excluded.

Conclusion

The TT genotype of the rs2200733 polymorphism was found to be significantly more prevalent in Uzbek patients with AF than that in controls.

Ethics Committee Approval: Ethics committee approval was received for this study from the Ethics Committee of Republican Specialized Scientific and Practical Medical Center of Cardiology (Approval Date: February 5, 2018; Approval Number ЭК-2018/04).

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Conflict-of-interest: None.

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Keywords: Atrial fibrillation; *ATFB5* gene; rs2200733 polymorphism

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