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Examining the Correlation Between Patients with Atrial Fibrillation and eNOS G894T Polymorphism

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Objective: Atrial fibrillation (AF) is the most common sustained arrhythmia. AF is a serious health problem since it causes discomfort, heart failure, and strokes in patients. This study aims to identify the possible role of eNOS G894T polymorphisms in AF incidence.

Materials and Methods: Seventy-three individuals were included in the study, with a mean age of 63.47±9.10, who received an electrocardiogram (ECG) at the Elazığ Training and Research Hospital, Cardiology Department. Ninety-two individuals with a mean age of 60.71±9.14 who were confirmed to have no heart condition were included as the control group. Genomic DNA from both the control and AF groups was isolated from whole blood samples. eNOS G894T polymorphism was detected using real-time PCR analysis. This study was conducted between September and December 2018. Ethical approval was obtained from the Ethics Committee of University, dated 04/09/2018 and numbered 279476.

Results: A statistically significant difference in hypertension was found between the AF patient and control groups (p<0.023); however, no significant differences were observed for diabetes, smoking, and body mass index. The analysis of the G894T eNOS polymorphism revealed that the TT genotype was significantly more frequent in the patient group (59.27%) compared to the control group (3.27%) (p<0.0001), while the GG genotype was predominantly observed in the controls (63.04%). Additionally, the T allele frequency was markedly higher in patients than in controls (77.93% vs. 20.11%; OR: 14.02, 95% CI: 9.6-20.45; p<0.0001).

Conclusion: These findings indicate a strong association between the T allele and TT genotype of the G894T eNOS polymorphism and the disease.

Key Words: *Nitric oxide, eNOS G894T, atrial fibrillation*

Atriyal Fibrilasyon Olan Hastaların eNOS G894T Polimorfizmi ile İlişkisinin Araştırılması

Amaç: Atriyal fibrilasyon (AF), en yaygın görülen kalıcı aritmidir. AF, hastalarda rahatsızlık, kalp yetmezliği ve felçlere neden olması nedeniyle ciddi bir sağlık sorunudur. Bu çalışma, eNOS G894T polimorfizminin AF görülme sıklığı üzerindeki olası rolünü belirlemeyi amaçlamaktadır.

Gereç ve Yöntem: Bu çalışmaya Elazığ Eğitim ve Araştırma Hastanesi Kardiyoloji Kliniği'nde elektrokardiyogram (EKG) çekilen, yaş ortalaması 63,47±9,10 olan 73 birey dahil edilmiştir. Kalp rahatsızlığı olmadığı belirlenen, yaş ortalaması 60,71±9,14 olan 92 birey ise kontrol grubunu oluşturmuştur. Hem hasta hem de kontrol grubuna ait tam kan örneklerinden genomik DNA izole edilmiştir. eNOS G894T polimorfizmi gerçek zamanlı PCR analizi ile tespit edilmiştir. Çalışma Eylül – Aralık 2018 tarihleri arasında gerçekleştirilmiştir. Etik onay, 04.09.2018 tarihli ve 279476 numaralı Üniversite Etik Kurulu'ndan alınmıştır.

Bulgular: Hipertansiyon açısından AF hasta grubu ile kontrol grubu arasında istatistiksel olarak anlamlı bir fark bulunmuştur (p<0.023); ancak diyabet, sigara kullanımı ve vücut kitle indeksi açısından anlamlı bir fark gözlenmemiştir (p>0.004). G894T eNOS polimorfizmi analizinde, hasta grubunda TT genotipi anlamlı şekilde daha sık görülmüştür (%59.27) ve kontrol grubunda bu oran %3.27'dir (p<0.0001). GG genotipi ise büyük oranda kontrol grubunda tespit edilmiştir (%63.04). Ayrıca, T allel frekansı hasta grubunda kontrol grubuna göre oldukça yüksek bulunmuştur (%77.93 vs. %20.11; OR: 14.02, %95 GA: 9.6–20.45; p<0.0001).

Sonuç: Bu bulgular, G894T eNOS polimorfizminin T alleli ve TT genotipi ile hastalık arasında güçlü bir ilişki olduğunu göstermektedir.

Anahtar Kelimeler: *Nitrik oksit, eNOS G894T, atriyal fibrilasyon*

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Introduction

Arrhythmia is an irregular heart rhythm, meaning that is an abnormal heart rhythm. It usually occurs in those people not possessing any previous abnormalities in their hearts. The heartbeat becomes fast and the person feels it easily. It is quite noticeable during excitement or any physical activity; furthermore, heart rhythms might be irregular but slow. There is a need that arrhythmias should be examined genetically. People who

have arrhytmias in their families are also more likely to experience arrhytmias. Defects occuring in genes is a factor in the emergence of the ailment. Moreover, the use of drug,smoke, stress and caffeine intake might cause arhythmia (1). Atrial fibrillation (AF) is the most frequent clinical arrhythmia whose prevalence varies between society and countries (0.1-4% and 2.8-14%). The incidence of AF is 1% in youngsters, of 10% in the ages over 80 and is increasing with age (2, 3). AF can lead to serious complications, stroke, and congestive heart failure (4, 5). Atrial fibrillation (AF) results from as a result of heart rhythm abnormality, atrial electrical or structural reconstruction (6). It is found that AF is often associated with other cardiovascular diseases such as hypertension, ischemic heart disease, valvular heart disease, and heart failure. Even though AF is originally thought to be a non-genetic disorder, the scientific evidence points out that it possesses an inherited link in many patients (6). Lineage-based studies have illustrated that an single gene mutations and single nucleotide polymorphisms contribute to AF development importantly. Either variations or polymorphisms in ion channels, calcium retention proteins, fibrosis, conduction and inflammatory genes may, for instance, create distinct individuals sensitive to electrical or structural remodeling and ultimately fibrillation in the atrium (7, 8). Alterations occuring in endothelium-derived Nitric Oxide (eNO) production have been associated with a variety of diseases (9, 10). In humans, the presence of distinct polymorphisms in the eNOS gene can be genetically determined. NO, synthesized by endothelial cells, contributes to the vasodilation process and blood pressure regulation (11). The eNOS gene comprising of 25 introns and 26 exons is mapped in the 7q35-36 chromosome and encodes 135 kDa (kilodaltons) containing 1203 amino acids (12, 13). The eNOS gene is highly polymorphic, and its G894T (Glu298Asp) variant in exon 7 having been suggested to be responsible for reduced NO synthesis and EH development is the most seen polymorphism (14). Mentioned variant changes the primary structure of the protein and possesses the potency to directly change one or more functional properties of the enzyme. According to two different studies, it has been showed that Asp-containing eNOS protein at position 298 exposed to selective proteolytic cleavage in endothelial cells and vascular tissues (15, 16). If this finding is right, cleaved fragments are expected to be lack of NO synthase activity, but two other reports suggest that this observation may be a work (17, 18). The levels of plasma NO are primarly regulated thanks to the activity of eNOS. In ENOS gene, certain polymorphisms, a great majority of those are related to important proportion of variability in plasma NO levels can be seen (19). The peresence of AT / C point mutation at position 786 in the promoter region suppresses the transcription of eNOS gene, showing a> 50% decrease in promoter activity (20, 21). This functional variant can change the amount of NO produced by endothelial or eNOS activity and affect either subcellular targeting or interaction with other regulatory proteins. Likewise, a series of consecutive iterative polymorphism repeats in intron 4 have been associated with alterations in plasma NO concentration. Lately, genetic variations have been linked to AF in studies (22, 23). The eNOS enzyme plays a crucial role in the cross-regulation of oxidative stress, which is partially in relation to AF pathogenesis (24). In line with this information, the aim of our current study was to elucidate the relationship between AF and eNOS G894T.

Materials and Methods

Research and Publication Ethics: All procedures on human studies are in accordance to ethical standards of University Ethics Committee (Approval date: 04/09/2018; approval #: 279476).

This study included 73 patients who underwent ECG (electrocardiography) and diagnosed with atrial fibrillation clinically applied to the Internal Medicine clinic of Fırat University hospital and 92 healthy control groups, similar to patients group in terms of age and gender, without any arrhythmia problems determined by EKG. In the study, the patient was diagnosed by the light of the criteria constituted by the Turkish Cardiology Association. Primers were designed for mutation of interest (G894T), which were used to screen predefined polymorphism in relevant gene region via real-time polymerase chain reaction (RT-PCR). In all subjects, blood samples were drawn in EDTA tubes (3 cc). DNA was isolated by using genomic DNA kits (PureLink) in Molecular Genetic Laboratory. DNA samples were stored at -20°C until analyzed. In samples obtained, SNP screen was performed by using appropriate primer sets and probes (AccuPower Dual Star qPCR PreMix K-6100) via amplification of target areas. Concentration and purity level of DNA isolated were determined by DS-11+ NanoDrop spectrophometer. The suitability of DNA concentration for master mix used for QPCR amplification was also tested. In this study, Bioneer ExiCycler Q-PCR and real-time PCR devices were used. The devices were controlled with appropriate equipments throughout study. Wild-type sequences and gene mutation group were used to identify gene alleles which encode same characteristics but result in different features due to variation in codes.

Statistical Analysis: Statistical analyses were performed using SPSS version 22.0 (Chicago, USA). The Kolmogorov-Smirnov and Shapiro-Wilk tests were used to assess the normality of continuous variables. Student's t-test was used to analyze continuous variables with a normal distribution. The Chi-square test was used to analyze categorical data. Numerical data are expressed as mean ± standard deviation, and categorical data are expressed as percentages. A *p*-value of <0.05 was considered statistically significant.

Table 1. Distribution of risk factors for the control and atrial fibrillation groups and descriptive information

		Control	Patient	*p* value (<0.05)
Age		63.47±9.10	60.71±9.14	
		n (%)	n (%)	
Sex	Female	31 (42.5)	40 (43.5)	
	Male	42 (57.5)	52 (56.5)	
Hypertension	Yes	46 (63.0)	70 (76.1)	**0.023**
	No	27 (37.0)	22 (23.9)	
Smoking	Yes	17 (23.3)	29 (31.5)	**0.720**
	No	56 (76.7)	63 (68.5)	
Diabets Mellitus	Yes	25 (34.2)	37 (40.2)	**0.652**
	No	48 (65.8)	55 (59.8)	
BMI, kg/m^2		25.3 ± 3.2	25.5±4.5	**0.68**

n: Patient number, *p*: Degree of significance between groups, BMI: Body Mass Index

Table 2. Allelic and genotypic distribution of G894T eNOS polymorphism between cases and controls

		Patient 73 n (%)	Control 92 n (%)	(95% CI)	*p* values (<0.05)
Genotypes	GG	3 (3.49%)	68 (63.04%)	1	<0.0001
	GT	27 (37.24%)	31 (33.69%)	20.2 [7.7-52.4]	<0.0001 *
	TT	43 (59.27%)	3 (3.27%)	332.5 [98.2–1125.4]	<0.0001 *
Allele frequency	G	32 (22.07%)	147 (79.89)	1	<0.0001 *
	T	113 (77.93%)	37 (20.11)	14.02 [9.6–20.45]	<0.0001 *

Results

The study included 73 Atrial Fibrillation patients, 56.5% of which was male and 43.5% of which was female. The average age of the cases was calculated as 60.71±9.14. The control group comprised of 57.5% male and 42.5% female. The average age was determined as 63.47±9.10. The data of the cases and controls are illustrated in Table1 demographically. Genotype and allele distributions of eNOS G894T gene polymorphisms belonging to the patient and control groups are exhibited in Table 2. It has been found that G894T polymorphism (p<0.0001) of the eNOS gene was statistically significant as compared to the patient group controls. Moreover, when allele frequencies of G894T polymorphism of the patient and control groups were compared, an important distinctness was determined in both alleles (proportion test, p=0.0001). When hypertension, gender, habits and biochemical (diabetes, body mass index) parameters were taken into consideration to evaluate whether they create a significant difference, only first one (hypertension) made it. There was no difference observed in other distributions (p> 0.05), which is stated in Table 2.

Discussion

Even though studies illustrate that atrial fibrillation is mostly inherited, many aspects of atrial fibrillation have not been elucidated yet. The studies that investigate the variants and candidate genes associated with atrial fibrillation continue. AF has become a gradually important arrhythmia owing to bringing to increased mortality and morbidity (25). It leads to the heart to beat very quickly, very slowly or irregularly as a consequence of the dysfunction the electrical impulses taking a role in the heart beats. AF takes a role in mediation of atrial electrical and mechanical remodeling, and in this process, the role of oxidative stress becomes

more and more prominent (26). Nitric oxide traditionally possesses a defensive role in the vascular endothelium and has a protection role the organs against oxidative stress. However, it has been revealed that the balance system of the nitric oxide / oxidative stress is complex. When eNOS is debar from the crucial cofactor tetrahydrobiopterin or the substrate L-arginine, it is able to make large amounts production of reactive oxygen types such as peroxynitrite rather than NO, and it is leading to the separation of NOS and might bring about tissue damage that regulates pathological remodeling of the myocardium (27, 28). According to the recent studies (22, 23) genetic variations have been linked to AF. Endothelial nitric oxide synthase (eNOS) enzyme takes a significant role in the cross-regulation of oxidative stress related to AF pathogenesis partially (26). According to the study conducted by Gensini F. et al. on AF patients in 2003, it was point out that Enos did not have any involment in the pathogenesis of AF, even if it was obtained ACE / D allele related results (28). Caucasian patients with AF revealed that ACE insertion / deletion polymorphism genotype distribution and allele frequency were significantly different between patients and controls. Whlist the ACE DD genotype is significantly associated with AF risk, analysis of ENOS polymorphisms reported no significant difference in terms of genotype distribution and allele frequency between patients and controls. Philip et al. (29) found that eNOS shows a significant effect on phenylephrine. Even though identified mutations of atrial fibrillation provide great insight into the pathogenesis of atrial fibrillation, certain common single nucleotide polymorphisms (SNPs) might be of broad interest relatively. Genome-wide association studies (GWAS) revealed that three different genetic loci on chromosomes 1q21, 4q25 and 16q22 have been linked to atrial fibrillation. According to association studies, some common SNPs in genes which are responsible for encoding cardiac ion channel proteins, calcium processing protein, connexin 40 and taking a role in the renin-angiotensin system (30, 31), and inflammatory or anti-inflammatory pathways might give rise to have a predisposition to the development of atrial fibrillation. Olesen et al. (32) found that the GWAS associations of SNPs at three loci on chromosomes 4q25, 7p31, and 12p12 in a patient population with early-onset single atrial fibrillation. However, the population included in the study was relatively small to elucidate the relationship sufficiently. Larger scale analysis would be a potent study to assess the risk of atrial fibrillation in those who have SNPs. Wutzler et al. (33) examined variations in the human soluble epoxide hydrolase gene which is responsible for recurrence of atrial fibrillation after catheter ablation and ultimately identified the rs751141 polymorphism of EPHX2. The gene is associated with a significantly increased risk of recurrence of atrial fibrillation after catheter ablation. Karimi et al. (2022) reported a significant association between the eNOS G894T polymorphism and coronary slow flow phenomenon in a cohort of 267 patients in Iran (34). In a review conducted by Wang et al. in 2023, the role of genetic polymorphisms-especially following catheter ablation-in the recurrence of atrial fibrillation (AF) was emphasized. It was noted that various genetic variants, including the eNOS G894T polymorphism, may influence the risk of AF recurrence and contribute to the development of personalized treatment strategies (35, 36). These results might point to a potential and vital role of these common variants in classifying catheter ablation by genotype and might guide distinct treatments in the coming years.

Although the genetic background of atrial fibrillation has not yet been fully elucidated, our findings contribute to the understanding of the disease's genetic structure. In line with previous studies, our results showed a significant association between the eNOS G894T polymorphism and atrial fibrillation, with the TT genotype and T allele being more prevalent in the patient group ($p<0.0001$). The observed difference in hypertension prevalence between the patient and control groups ($p<0.023$) may also support the potential interplay between cardiovascular risk factors and genetic predisposition. We suggest that ethnic differences may explain the inconsistencies observed in some studies. In conclusion, eNOS G894T variants may serve as potential genetic risk factors for atrial fibrillation. However, further large-scale and multi-ethnic studies are needed to clarify this association and better understand the underlying mechanisms.

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