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Sh.G. Ismayilova, Sh.S. Ibrahimova, S.R. Muradova, E.I. Haziyev, L.Kh. Safaraliyeva, A.N. Humbatova, A.P. Mammadova
Azerbaijan Medical University, Baku, Azerbaijan

ASSOCIATION OF ENDOTHELIAL NITRIC OXIDE SYNTHASE GENE POLYMORPHISMS WITH END-STAGE RENAL DISEASE

e-mail: mic_amu@mail.ru

This study aimed to assess the association of the endothelial nitric oxide synthase 786T>C (rs2070744) and 894G>T (rs1799983) polymorphisms with end-stage renal disease risk in an Azerbaijani cohort. The study included 120 end-stage renal disease patients and 100 healthy controls. The results revealed that the T allele of the endothelial nitric oxide synthase 786T>C polymorphism significantly increased the risk of end-stage renal disease (p=0.002). Moreover, the T allele in both the endothelial nitric oxide synthase 786T>C and 894G>T polymorphisms was associated with a higher risk of macroalbuminuria (OR=5.93; p=0.013) and microalbuminuria (OR=2.56; p=0.024). These findings suggest that the T alleles in the endothelial nitric oxide synthase polymorphisms play a crucial role in chronic kidney disease progression, and genetic screening for these variants may help identify patients at greater risk for end-stage renal disease in Azerbaijani population.

Key words: chronic kidney diseases, eNOS gene polymorphisms, T-786C, G894T, end-stage renal disease.

Ш.Г. Ісмаїлова, Ш.С. Ібрагімова, С.Р. Мурадова, Е.І. Газієв, Л.Х. Сафаралієва, А.Н. Гумбатова, А.П. Мамедова

АСОЦІАЦІЯ ПОЛІМОРФІЗМІВ ГЕНА ЕНДОТЕЛІАЛЬНОЇ СИНТАЗИ ОКСИДУ АЗОТУ З ТЕРМІНАЛЬНОЮ СТАДІЄЮ НИРКОВОЇ ХВОРОБИ

Метою даного дослідження була оцінка асоціації поліморфізмів ендотеліальної синтази оксиду азоту 786T>C (rs2070744) та 894G>T (rs1799983) з ризиком термінальної стадії ниркової недостатності в азербайджанській когорті. До дослідження було включено 120 пацієнтів із термінальною стадією ниркової недостатності та 100 здорових осіб контрольної групи. Результати показали, що алель Т поліморфізму ендотеліальної синтази оксиду азоту 786T>C значно збільшує ризик термінальної стадії ниркової недостатності (p=0,002). Більш того, алель Т у поліморфізмах ендотеліальної синтази оксиду азоту 786T>C і 894G>T був пов'язаний з більш високим ризиком макроальбумінурії (OR=5,93; p=0,013) та мікроальбумінурії (OR=2,56; p=0,024). Ці результати показують, що алелі Т у поліморфізмах ендотеліальної синтази оксиду азоту відіграють вирішальну роль у прогресуванні хронічної хвороби нирок, і генетичний скринінг на ці варіанти може допомогти виявити пацієнтів із підвищеним ризиком термінальної стадії ниркової недостатності у популяції Азербайджану.

Ключові слова: хронічні захворювання нирок, поліморфізм гена eNOS, T-786C, G894T, термінальна стадія ниркової недостатності.

Chronic kidney disease (CKD) affects approximately 13.4 % of the global population [1]. As kidney function declines, CKD can progress to end-stage renal disease (ESRD). Patients with ESRD require renal replacement therapy, leading to significant medical expenses and a reduced quality of life [10]. Identifying risk factors for kidney disease is therefore crucial. Recognized risk factors for CKD include genetic predisposition, diabetes, hypertension, and a family history of the condition [9]. Additionally, research has shown that various gene polymorphisms influence the risk of developing CKD [2]. Patients with ESRD necessitate renal replacement therapy, which incurs substantial medical costs and results in a marked decline in quality of life. Consequently, the timely implementation of interventions to halt or slow the progression of CKD is critical for improving patient outcomes and minimizing the burden of the disease.

Genetic factors are among the risk factors for the development of CKD in patients with T2D [3]. It has been suggested that there is a genetic predisposition to the formation of diabetic kidney disease, and the role of endothelial nitric oxide synthase (eNOS) gene polymorphisms is believed to be crucial in the development of this condition [11]. Studies on potential genetic associations related to pathologies genetically linked to oxidative stress have shown that genetic variations in vascular oxidative enzymes can influence the redox balance, leading to significant inter-individual differences in vascular oxidative stress. In particular, studies on genetic variants related to endothelial nitric oxide synthase (eNOS), such as 786T>C (rs2070744) and 894G>T (rs1799983), in diabetic patients across different populations have yielded contradictory results [2, 3]. Polymorphisms in the eNOS gene can influence the production of nitric oxide (NO), which plays a crucial role in regulating vascular tone, blood circulation, and kidney function. Impaired NO production may contribute to the development of vascular and kidney diseases, including chronic kidney disease [8]. The T-786C and 894G>T polymorphisms are among the most studied variations in the eNOS gene, and their effects on eNOS activity may be associated with the progression of CKD.These

polymorphisms have been linked to nephropathy in various studies. However, the relationship between ESRD risk and the polymorphisms in eNOS remains debatable. While some research indicates that different eNOS genotypes are associated with ESRD, other studies propose that there may be no significant link to ESRD risk [5, 8, 9].

The purpose of the study was to evaluate the frequency of these polymorphisms in the Azerbaijani population and their potential role in the development of end-stage renal disease among patients with diabetic kidney disease.

Material and methods. The study included 120 patients, comprising 64 women (52.38 %) with a mean age of 56.7±4.21 years and 56 men (47.62 %) with a mean age of 54.8±5.81 years who were enrolled from the outpatient and inpatient departments of the Educational Therapeutic Clinic at Azerbaijan Medical University between January 2024 and September 2024. Diagnosis of end-stage renal disease was confirmed by at least two nephrologists based on medical history and review of clinical records. All participants had a GFR<15 mL/min. Serum creatinine levels were measured in the Nephrology Department using the Jaffe reaction method.

The control group comprised 100 healthy individuals (50 men and 50 women; mean age±SD: 56.5±8.23), all of whom attended the health center for routine visits.

Blood samples were obtained from all participants using sterile vacuum tubes containing ethylenediaminetetraacetic acid (EDTA) for genomic DNA extraction. Genomic DNA was isolated from the leukocyte fraction with EDTA and from whole blood samples using the phenol-chloroform method.

For the analysis of the G894T polymorphism in exon 7 of the endothelial nitric oxide synthase (eNOS) gene, two primers were used: forward primer 5'-AAG GCA GGA GAC AGT GGA TGG A-3' and reverse primer 5'-CCC AGT CAA TCC CTT TGG TGC TCA-3'. For the T786C polymorphism of the eNOS gene (rs2070744), the primers used were forward 5'-CACCTGCATTCTGGGAACTGTA-3' and reverse 5'-GCCGCAGTAGCAGAGAGAC-3'. To digest the PCR products, 5 units of the restriction endonuclease MboI were added and incubated overnight at 37°C. The digested PCR products were analyzed by electrophoresis on a 3.0% agarose gel. The three genotypes (GG, GT, and TT) of the eNOS G894T polymorphism were identified based on fragment sizes of 250 bp, 250 bp/165 bp/90 bp, and 165 bp/90 bp, respectively. For the T786C polymorphism, the fragment sizes for genotypes TT, TC, and CC were 140 bp, 90 bp, and 50 bp, respectively.

The following statistical methods were used for data analysis: for quantitative variables, the mean (M) and standard deviation (σ) were calculated, and the results are presented as M±σ. To assess differences between groups, the Student's t-test for independent samples was applied to parametric data. For categorical data, such as genotype distribution, the chi-square (χ^2) test was used. This test helps to determine if there is a statistically significant difference in the frequency of different genotypes and alleles between groups.

Statistical significance was considered for p-values <0.05, indicating significant differences between groups. To evaluate the association between eNOS gene polymorphisms and the ESRD the odds ratio (OR) was calculated. This measure evaluates the specific event (e.g., macroalbuminuria) occurring in a group with a particular genotype compared to a control group. Additionally, a confidence interval (CI) was calculated, which indicates the range of values within which the true odds ratio is likely to fall with a certain level of confidence.

Results of the study and their discussion. According to results obtained, both systolic and diastolic blood pressure levels were significantly elevated in patients with end-stage renal disease compared to those in the control group (p<0.05). The key demographic characteristics of all participants included in the study are summarized in Table 1.

Furthermore, serum levels of low-density lipoprotein cholesterol (LDL-C) and total cholesterol were markedly higher in patients with ESRD than in healthy control group participants (p=0.013 and p=0.025, respectively). Creatinine levels were also significantly elevated in ESRD patients compared to control group (p=0.014), while the glomerular filtration rate (GFR) was notably lower in this subgroup (p=0.001).

The study of genotype and allele frequencies of the eNOS 786T>C polymorphisms in patients with ESRD revealed that the frequency of the CC genotype in the ESRD group was 42.5 %, while in the control group it was only 22.0 % (p=0.034). According to the results, the frequency of the C allele in the ESRD group was 80.8 %, which differed significantly compared to the healthy group (p=0.002). However, the frequency of the C allele did not differ statistically between women and men (p=0.062). These results suggest that the CC genotype significantly increases the risk of ESRD.

The presence of the C allele was associated with a higher risk of developing ESRD compared to the control group (OR=1.85; 95 % CI=1.171–2.924), suggesting a statistically significant association between the C allele and an increased risk of ESRD. These findings suggest that the eNOS 786T>C polymorphism may play a role in the development of CKD, highlighting the need for further research on the impact of the eNOS genotype on kidney function. Thus, the presence of the eNOS CC genotype and the C allele may serve as significant biomarkers for identifying patients at risk of developing CKD.

Table 1

Demographic characteristics of study groups

Parameters		ESRD (n=120) M±σ	Control group (n=100) M±σ	p
Age		55.3±4.21	56.5±8.23	0.161
Sex	Female	64 (52.38 %)	50 (50.0 %)	0.053
	Male	256 (47.62 %)	50 (47.5 %)	0.055
BMI (kg/m²)		27.3±4.43	22.5±4.19	0.061
SBP (mmHg)		140.3±8.57	110.8±10.43	0.028
DBP (mmHg)		99.3±6.42	77.4±7.33	0.021
Creatinine (mg/dl)		9.6±2.28	0.67±0.38	0.014
Total cholesterol(mg/dl)		262.0±43.32	157.4±28.73	0.025
Triglyceride (mg/dl)		167.1±31.43	144.6±43.3	0.013
LDL-cholesterol (mg/dl)		114.8±18.3	67.2±28.8	0.073
HDL-cholesterol (mg/dl)		40.3±4.61	79.2±4.78	0.013
UACR, (mg/g)		437.3±32.82	27.98±2.31	0.001
GFR–ml/min/1,73m^2		8.5±5.39	112.3±10.36	0.001

Note: p<0.05 indicates statistically significant differences between the groups.

The frequencies of the genotypes and alleles of the eNOS 894G>T polymorphism are as following: GG was 24.2 % in ESRD group and 40 % in control group (p=0.05), TT was 45 % in ESRD group and 13 % in control group (p=0.001). The frequencies of G allele were 55 % and 63.8 % in ESRD and control groups, respectively (p=0.014). The T allele also demonstrated statistically significant differences between groups: 75.8 % and 36.2 %, respectively (p=0.002).

In our analysis of the association between the T allele and the risk of ESRD, the T allele compared to the G allele showed a higher risk (OR=2.00; 95 % CI=1.27–3.16).

A comparison between the TT genotype and the GG genotype showed a higher risk of developing macroalbuminuria in patients with the TT genotype (OR=5.93; 95 %CI=1.722–9.383; p=0.013). The risk of developing microalbuminuria in patients with the TT genotype is 2.56 times higher than in the control group (p=0.024) (Table 2).

Table 2

Odds ratios and confidence intervals for the genotypes and alleles of eNOS 894G>T

eNOS 894G>T	UACR=30–300mg/g OR (95 % CI)	UACR≥300mg/g OR (95 % CI)	Control group OR (95 % CI)
Genotypes			
GG	n=19	n=10	n=40
GT	0.446(0.261–2.117) p=0.581, n=16	0.571 (0.391–3.653) p=0.614, n=21	n=47
TT	2.561 (1.04–8.165) p=0.024, n=22	5.933 (1.722–9.383) p=0.013, n=32	n=13
Alleles			
G	n=35	n=31	n=87
T	1.574 (0.90–2.77) p=0.061, n=38	2.479 (1.29−4.73) p=0.012, n=53	n=60

Note: p-values < 0.05 indicate statistically significant differences between the control group. UACR–urine albumin-creatinine ratio.

The presence of the T allele is associated with an increased risk of elevated urine albumin-creatinine ratio (UACR) levels. The OR for the T allele in the UACR=30–300 mg/g group compared to the control group was 1.574, with a 95 % CI of 0.90–2.77 (p=0.061). Although this result does not reach statistical significance, it indicates a potential trend toward an increased risk, warranting further investigation

In the UACR≥300 mg/g group, the OR was 2.479, with a 95 % CI of 1.29–4.73 (p=0.012), indicating a statistically significant association between the T allele and macroalbuminuria.

CKD is a clinical condition caused by various pathophysiological mechanisms, such as glomerulonephritis, diabetic nephropathy, hypertensive nephrosclerosis, and autoimmune disorders. While the specific cause of glomerular damage is less critical in predicting progression to ESRD, genetic and environmental factors significantly influence the outcome [1, 4]. Notably, the rate of renal function decline in CKD patients varies widely among individuals due to a combination of factors. Although the mechanism explaining this potential connection is not fully understood, it is believed that variants of the eNOS gene cause defects in NO synthesis and may reduce NO levels, increasing susceptibility to glomerular diseases and impairing kidney function [6, 7]. This has been shown in studies confirming the role of the polymorphism –786)T>C in the progression of kidney diseases [2].

NO is essential for regulating the microcirculatory bed of the glomeruli, sodium processing by the kidneys, and renin secretion, all of which are vital for kidney function. Impaired eNOS secretion is recognized as a key factor in various cardiovascular and renal diseases, such as hypertension, ischemic heart disease, thromboembolic disorders, and atherosclerosis [8, 9].

This study highlights the critical importance of investigating eNOS polymorphisms and their potential interactions with other genetic variants that may influence renal outcomes. The findings provide a significant contribution to the understanding of the mechanisms underlying susceptibility to and progression of CKD. This, in turn, can facilitate the identification of novel pharmacological targets aimed at mitigating the risk of CKD development and progression.

Conclusion

The results of the study demonstrate a statistically significant association between the eNOS 894G>T and 786T>C polymorphisms and the risk of developing CKD. These findings offer valuable insights into the role of these polymorphisms in CKD pathogenesis. Furthermore, they underscore the need for future research to integrate these findings with biochemical assessments of oxidative stress biomarkers and DNA damage. Such studies should involve larger, more diverse populations across different ethnic groups to validate these associations and expand the generalizability of the findings. In conclusion, we found significant associations between eNOS polymorphisms and ESRD. Our findings suggest that eNOS polymorphisms may be relevant to the genetic component of CKD that leads to ESRD in Azerbaijani population.

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