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eNOS3 - As One Of The Genetic Aspects Of The Development Of Chronic Kidney Disease

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Annotation. Chronic kidney disease (CKD) affects approximately 10% of the global adult population. Various genetic and environmental risk factors contribute to kidney disease, complicating the identification of underlying pathophysiologic mechanisms. CKD is based on the development of nodular or diffuse glomerulosclerosis, with its terminal stage characterized by chronic renal failure (CRF). This article discusses the clinical and genetic aspects of CKD and their relationships in the early stages of the disease, as well as the cumulative effect of metabolic, hemodynamic, and genetic factors on disease development.
Keywords: chronic kidney disease, endothelin-1, gene, polymorphism, alleles, microalbuminuria genotype, glomerular filtration rate, intrarenal hemodynamics.

Introduction. The medical and social significance of chronic kidney disease (CKD) is underscored by the widespread prevalence of kidney pathology in the population, the serious risk of cardiovascular complications, the high mortality rate, and the need for expensive replacement therapy methods. [23,28,33].

Currently, nephrologists and therapists are placing great emphasis on improving the early diagnosis of chronic kidney disease. This interest is driven by the need for timely pharmacological and non-pharmacological interventions to address the main manifestations of renal pathology and to slow the progression of the disease to terminal renal failure, which requires dialysis therapy and organ transplantation.[1,11,17]. To unify approaches to the diagnosis, treatment, and prevention of kidney diseases, the term "chronic kidney disease" has been established since 2002. The criteria for CKD include structural or functional damage to the kidneys, with or without a decrease in glomerular filtration rate (GFR) for three months or more, or a GFR of less than 60 ml/min/1.73 m², also lasting more than three months[6,19,22,]. CKD occupies a special place among chronic non-communicable diseases, with its prevalence comparable to socially significant diseases such as essential hypertension and diabetes mellitus. CKD is diagnosed in more than 50 million people worldwide[16,18,20].

Nephrosclerosis serves as the morphological basis for the progression of chronic kidney diseases. Its development is characterized by the loss of functional kidney tissue due to hyperactivation of apoptosis processes and the death of functionally significant cells, such as podocytes and tubular epithelial cells. This is accompanied by the proliferation of functionally inactive connective tissue due to excessive cell proliferation and hyperproduction of extracellular matrix (ECM) [3, 12, 26].

Currently, significant attention is given to disorders of vascular endothelial function in the progression of chronic kidney disease, both locally in the kidneys and systemically. Numerous experimental and clinical studies have demonstrated that activated vascular endothelium is the structural and functional unit that integrates inflammation processes with intravascular coagulation, fibrinolysis, and rheological disorders in the kidneys5,24]. Furthermore, endothelial dysfunction is recognized as one of the leading factors in the pathogenesis of kidney diseases, whether of immune or non-immune origin. Several studies have explored the role of endothelial dysfunction in the development of pathological processes in patients with diabetic nephropathy, hypertension, and various forms of glomerulonephritis[2,4,15].

An analysis of the scientific literature suggests a commonality of pathological processes that induce endothelial damage, including ischemia, oxidative stress, hemodynamic disorders, and neurotransmitter effects. These factors contribute to the disruption of endothelial integrity, vasoconstriction, the development of mediators of proliferation, adhesion and inflammation, increased thrombosis, and ultimately actively

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stimulate sclerotic processes in renal tissue. Thus, endothelial dysfunction can be considered an independent risk factor for the progression of CKD[7,14,21].

Therefore, the gene for endothelial nitric oxide synthase (eNOS3) is of interest as a candidate gene for CKD. It is important to study and identify the relationship between eNOS gene polymorphism and the development and progression of CKD, as well as to determine the genetic determinacy of their risk factors in the Uzbek population. The polymorphism of the eNOS gene in CKD among the Uzbek nationality has not been studied before.

The aim. To assess the role of the polymorphic marker of the eNOS3 gene in the risk of developing chronic kidney disease (CKD) and to study the functional state of the kidneys in patients with glomerular and interstitial diseases. Additionally, the study aims to determine the correlation between these factors at an early stage of CKD development in individuals of Uzbek nationality.

Material and methods. The study included 85 patients with glomerular and interstitial diseases who were hospitalized in the Department of Nephrology of the National Medical Center of the Republic of Uzbekistan in the period from March 2023 to October 2024. The control group consisted of 32 healthy individuals of the Uzbek nation, included according to the "case-control" principle.

The patients in the main group were distributed as follows: the first group of 42 patients without signs of CKD, the second group of 43 patients with signs of CKD. The T-786C polymorphism of the eNOS3 gene was analyzed using a programmable thermal cycler manufactured by Applied Biosystems (Model 2720, USA), with test systems from Litech (Russia), according to the manufacturer's instructions.

The following indicators were assessed: general blood and urine test results, microalbuminuria, glomerular filtration rate (GFR) calculated using the CKD-EPI formula, endothelin-1 levels in blood plasma, and parameters of intrarenal hemodynamics, including Dopplerographic parameters: Pulsatility Index (PI), Resistance Index (RI), and maximum velocity (Vmax).

Statistical analysis was performed using the STATISTICA 6 software. The data are presented as means with standard deviation (M ± SD). The normality of the distribution was evaluated using the Kolmogorov-Smirnov test. The relative risk of disease in carriers of specific alleles and genotypes was calculated as an odds ratio (OR). The OR value was computed using the online calculator from the Medical Statistics program (http://medstatistic.ru/calculators.html).

The distribution of genotypes was tested for deviations from Hardy-Weinberg equilibrium. The correlation coefficient (r) was calculated using Spearman's method, with differences considered statistically significant at $p < 0.05$.

Results and their discussion. During the study, the functional state of the kidneys in the examined groups was assessed based on the results of albuminuria (AU), urea, creatinine, endothelin-1, GFR, and renal vascular Dopplerography. Comparative analysis of groups 1 and 2 revealed that AU was significantly higher in group 2 compared to group 1 (33.17±2.45 vs. 101.46±18.14; $p<0.05$).

Furthermore, an increase in the amount of AU in urine showed a significant positive correlation ($p<0.05$) with blood creatinine ($r=0.40$) and the resistance index (RI) of renal vessels, while GFR ($r=-0.42$) and endothelin-1 ($r=-0.43$) exhibited significant negative correlations ($p < 0.05$).

Table 1

Laboratory results between groups

Laboratory indicators	Group 1	Group 2
AU	33,16±2,45	101,44±18,14*
Endothel-1	6,52±0,17	4,60±0,26*
Creatinine	70,86±2,36	142,67±20,25*
eGFR	90,14±13,17	54,9±12,55*
RI	0,63±0.01	0,74±0.01*

Note: * - confidence ($p<0.05$).

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Understanding the pathogenetic mechanisms underlying the formation of nephrosclerosis in chronic kidney disease (CKD) requires analyzing the correlations between various factors. The correlation between blood endothelin-1 and other progression factors—such as proteinuria, creatinine, and urea levels—varies depending on the stage of CKD. In the initial stages, the duration and severity of renal blood flow disorders are crucial, as indicated by a direct correlation with the duration of CKD and the resistance index level[9,13].

In the subsequent stages of the disease, damage to the glomerular filter becomes prominent, manifested by increased permeability and deteriorating kidney excretory function. This is confirmed by a direct correlation with the levels of proteinuria, creatinine, and blood urea.

Table 2 presents the frequency of alleles and genotypes of the T-786C polymorphism of the eNOS3 gene in all patients (main group) and the control sample. The prevalence of the T allele in the studied and control groups was 70.2% and 79.6%, respectively, while the incidence of the unfavorable C allele was 29.8% and 20.4%. Statistical analysis revealed that carriers of the C allele have a 1.6-fold statistically significant higher probability of developing the disease compared to carriers of the T allele (χ^2=5.5; p=0.02; OR=1.6; 95% CI: 1.0843–2.523). Conversely, the T allele (χ^2=5.5; p=0.02; OR=0.6; 95% CI: 0.3962–0.9221) appears to have a protective effect against disease progression.

Table 2

The frequency of distribution of alleles and genotypes of polymorphism at–786C of the eNOS3 gene in the main and control groups

Alleles and genotypes	The number of alleles and genotypes examined				χ^2	P	OR	95% CI
	the main group		control					
	N	%	N	%				
T	119	70,2	51	79,6	5,5075	0,0289	0,6045	0,3961-0,9221
C	51	29,7	13	20,4	4,3152	0,0174	1,6544	1,0843-2,523
T/T	43	50,6	20	62,6	3,7103	0,0537	0,6035	0,3593-1,0131
T/C	33	38,8	11	33,7	0,794	0,352	1,29	0,7591-2,1918
C/C	9	10,5	1	3,7	3,672	0,0543	2,9698	0,9391-9,3905

According to the results from the main and control groups, the frequency distribution of the TT, TC and CC genotypes was 50,6%, 38,8% and 10,5%, and 62,6%, 33,7%, and 3,7%, respectively. Statistical analysis indicated that carriers of the CC genotype are 2.9 times more likely to develop the disease compared to carriers of the TT genotype, with this difference being statistically significant (χ^2 = 3.7; p = 0.05; OR = 2.9; 95% CI: 0.9391–9.3905).

In the main and control groups, the TT genotype was distributed as follows: 50.3% and 62.7%, and showed a protective function against disease progression (χ^2 =3.7; p=0.05; OR=0.6; 95% CI: 0.3593–1.0131). The TC genotype was also significantly lower in the main group compared to the control group, with frequencies of 39.4% and 33.7%, respectively, but it did not show a significant effect on the development of the pathology (χ^2=0.7; p=0.3; OR=1.29; 95% CI: 0.7591–2.1918) (Table 2).

Our study demonstrated an association between the carrier of the C allele (CC genotype) of the NOS3 gene and chronic kidney disease in patients with glomerular and interstitial diseases.

These findings, along with the results of our study, allow us to conclude that the NOS3 gene plays a significant role in the development of chronic kidney disease [13] in the Uzbek population studied.

Increased albuminuria, angiotensin-converting enzyme (ACE) levels, and endothelin-1 (ET-1) indicate the presence of generalized endothelial dysfunction (ED) and can be utilized for the early detection of renal pathology and the prevention of cardiovascular complications.

Conclusion. The study revealed a significant association between the risk of chronic kidney disease in patients with glomerular and interstitial diseases and genes encoding endothelial factors (NOS3), whose expression products play a role in the pathogenesis of kidney damage. Changes in the functional state of the kidneys occur even at the microalbuminuria stage of CKD. The appearance of microalbuminuria or a decrease in estimated

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glomerular filtration rate (eGFR) indicates a well-established pathological process in the kidneys, limiting the potential for reversal.

In this context, the search for polymorphisms of genes that predispose individuals to the development of CKD before the onset of disease complications, as well as the exploration of new laboratory and instrumental methods, represents a promising direction for the prevention of this pathology.

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October 2024

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