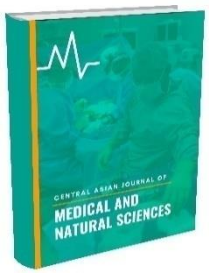
CENTRAL ASIAN JOURNAL OF MEDICAL AND NATURAL SCIENCES



Volume: 02 Issue: 06 | Nov-Dec 2021 ISSN: 2660-4159

http://cajmns.centralasianstudies.org

Frequency of Genotypes of the T-786C Polymorphic Marker of the ENOS3 Gene in Patients Depending on the Duration of the Disease

1. **O. O. Zhabbarov**
2. **Maksudova M. H.**
3. **Saydaliev R. S.**
4. **Tursunova L. D.**
5. **N. K. Khuzhaniyazova**

Received 29th Oct 2021,
Accepted 29th Nov 2021,
Online 25th Dec 2021

[1] Head of Department of Faculty and Hospital Therapy No. 2 of Tashkent Medical Academy, M.D., Associate Professor

[2] Associate Professor of Department of Faculty and Hospital Therapy #2 of Tashkent Medical Academy, Candidate of Medical Sciences.

[3] Senior lecturer of the Faculty and Hospital Therapy Department No.2 of Tashkent Medical Academy, Candidate of Medical Sciences.

[4] Assistant of Department of Faculty and Hospital Therapy #2 of Tashkent Medical Academy, Candidate of Medical Sciences

[5] Head of General Methodological Subjects Department, Abu Ali ibn Sina Public Health Technical School, Chilanzar

Abstract: This article presents the results of a study on the frequency of alleles and genotypes of the T-786C polymorphism of the ENOS3 gene in patients with diabetes under 10 years without DN (n=33), who constituted the main group compared with the control group of healthy individuals. Genotyping was performed by polymerase chain reaction. The study showed that association of C allele and CC genotype of ENOS3 gene plays a role in the development of diabetic nephropathy in patients with type 2 diabetes depending on the duration of the disease in the study group of the Uzbek nation.

Keywords: diabetic nephropathy, diabetes mellitus, nitric oxide synthase, endothelin-1, gene, polymorphism, allele, genotype

Introduction. Diabetes mellitus (DM) is a disease that accompanies a person for quite a long period of time. Since the emergence of this pathology and to this day it is of interest to many researchers because of its insufficient study. Despite the available successes in the treatment of DM, now, unfortunately, there is an increase in the incidence of both type 1 and type 2 DM. By 2040, the number of people aged 20-79 years with diabetes is projected to increase to 642 million [1].

The greatest danger of DM is certainly related to the complications that develop due to its damaging effects on blood vessels.

Diabetic nephropathy (DN) occupies an important place in this series, developing in approximately 20.1% of patients with type 1 diabetes and 6.3% of patients with type 2 diabetes [2]. In patients with type 2 diabetes, diabetic nephropathy ranks third among the causes of death after cardiovascular diseases and cancer pathologies [2].

To date, the leading cause of end-stage chronic kidney disease in both Europe and the USA and Japan is nephropathy associated with type 2 diabetes [3,4].

Diabetic nephropathy is represented by a complex of lesions of the arterioles, arteries, glomeruli and tubules of the kidneys [5, 6, 7, 8, 9]. DN is characterized by renal tissue damage in diabetes mellitus, which leads to the development of diffuse or nodular glomerulosclerosis, which, in turn, leads to the development of chronic renal failure (CRF).

Classically, there are three stages of diabetic nephropathy: microalbuminuria (MAU); proteinuria with preserved renal function; and chronic renal failure (CRF) [5,10,11]. However, the initial structural and functional changes start to develop before urinary albumin excretion increases (12). Modern advances in molecular medicine and experimental nephrology are leading to a gradual increase in knowledge about the more detailed mechanisms of AFU and PU development. And the main role of podocytes, the main components of the glomerular slit diaphragm, in these processes has been proved [12, 13]. There are works that demonstrate the relationship between the growth of AU and functional abnormalities in podocytes [12,14- 15]. It has been shown that these changes develop long before the detection of MAU and can be detected even in the short course of DM [16- 17]. Thus, podocytes are of interest for the development of methods to inhibit the development of DM [12]. Podocyte itself has a rather complex structural structure, which provides an extensive set of its functions and adaptive reactions under physiological conditions, but, in turn, makes this cell very sensitive to various damaging factors. As a result of exposure to pathogenic agents (metabolic, toxic, hemodynamic, genetic) podocytes undergo

Structural and functional changes (this phenomenon is referred to as "podocytopathy"). [18,19,20,21,22].

Diabetic nephropathy develops under the influence of a huge number of causes. But of the variety of mechanisms of DN development the most studied and provenare: *metabolic* (hyperglycemia, hyperlipidemia) and *hemodynamic* (intracollicular hypertension, arterial hypertension (AH)).

Hyperglycemia is **undoubtedly** one of the most important metabolic factors initiating renal damage. There are several pathways that ultimately lead to cell death.

Hyperlipidemia plays a major role in the development of DN, which is characterized by an increase in total cholesterol, low-density lipoproteins (LDL) and very-low-density lipoproteins (VLDL), a decrease in high-density lipoproteins (HDL) and also leads to renal disease. For a long time this factor was not taken into account, only after researches J.F. Moorhead and J.Diamond, hyperlipidemia began to be considered as a rather serious nephrotoxic factor [23-24]. Lipids filtered into the primary urine may cause damage to renal tubule cells [21, 25].

Today, the genetic theory of DN draws more attention, which suggests that predisposition plays an important role in both the development and progression of renal pathology [9]. That is, the activity of many damaging mediators, as well as enzymes involved in metabolism, is under genetic control. Thus, the realization of the damaging effect of a particular factor will depend on the nature of interaction between the genetically determined activity of this factor and the genetically determined susceptibility to its effects.

For example, genes involved in the development of DN have been identified: genes whose products are involved in the development of AH (angiotensinogen gene (AGT), renin (REN) gene, angiotensin-converting enzyme I (ACE) gene, endothelial nitric oxide synthase gene (eNOS3) [5].

Currently, the pathogenesis of micro- and macrovascular complications of DM is dominated by endothelial dysfunction, accompanied by deficiency of vasodilators - nitric oxide (NO), and activation of local vasoconstrictor secretion, such as endothelin-1 (E- 1). Therefore, the endothelial nitric oxide synthase gene (eNOS3) are of interest as candidate genes for diabetic nephropathy and CKD in type 2 diabetes.

The endothelium is known to regulate vascular tone through the release of vasodilator and vasoconstrictor factors and modulates the contractile activity of smooth muscle cells.

Endothelial dilatation factors include nitric oxide (NO). NO is the main vasodilator preventing tonic vasoconstriction of neuronal, endocrine or local origin. Under physiological conditions, NO is continuously involved in the adaptation of the vascular system to increased metabolic demands, physical exertion. In diseases, an excess of NO is responsible for an increase in peripheral vasodilation, while NO deficiency can lead to severe diseases, including arterial hypertension, coronary heart disease and atherosclerosis (also of the glomerular vasculature of the kidneys) [26,27].

NO prevents platelet adhesion and aggregation, monocyte adhesion, affects the vascular structure, which protects the vascular wall and prevents vascular remodeling in various pathological conditions. Nitric oxide is formed under the action of the enzyme NO synthase (NOS). NO synthase exists in the form of three main isoforms, which are named after the type of cells in which they were first discovered: neuronal NO synthase (nNOS or NOS I), endothelial NO synthase (eNOS or NOS III) and macrophage NO synthase or inducible NO synthase (iNOS or NOSII). Neuronal and endothelial NO synthases are enzymes with stable activity, whereas the activity of macrophage or inducible NO synthase is more regulated by cytokines. Endothelial NO synthase is stably expressed in endothelial cells. [28]

NO synthase inhibition leads to all the organic consequences of severe and prolonged arterial hypertension, including atherosclerosis and vascular organ damage. [29]

The endothelial nitric oxide synthase gene, eNOS3, is located on the long arm of chromosome 7 (7q36.1) and consists of 26 exons [30]. It has three studied polymorphism variants: G894T, 4b/a, and T-786C. In the experiment, the presence of alleleC position 786 of eNOS3 gene promoter was found to reduce its activity by 52%, while the resulting lack of eNOS3 is the cause of reduced synthesis and release of NO and endothelial dysfunction [31,32]. It has been shown that the presence of the C allele and CC genotype is an independent risk factor for CHD and MI in European and Japanese populations [33,34], as well as for the development of DN in patients with type 2 DM [35].

It is of interest to study and reveal the relationship between eNOS gene polymorphism as a predictor of the development and progression of DN in patients with type 2 DM and determine the genetic determinism of their risk factors in the Uzbek ethnic group.

eNOS gene polymorphism in type 2 DM and its macrovascular and microvascular complications has not been previously studied in the Uzbek ethnic group.

Objective. To assess the contribution of polymorphic marker T-786C of eNOS3 gene in the risk of diabetic nephropathy in patients depending on the duration of the disease in type 2 DM in persons of Uzbek ethnicity.

Material and methods

In the Republican Scientific and Practical Center of Nephrology on the base of TMA III clinic, 129 patients with type 2 DM and the control group consisted of 110 healthy persons of Uzbek nation were examined in the main group, included according to the "case-control" principle. Patients in the main group were distributed as follows: 65 patients with disease duration up to 10 years, without diabetic nephropathy (33 patients) and with diabetic nephropathy (32 patients), 64 patients with diabetes lasting more than 10-20 years, with no diabetic nephropathy (31 patients) and diabetic nephropathy (33 patients).

We studied the frequency of alleles and genotypes distribution of T-786C polymorphism of ENOS3 gene in patients with diabetes up to 10 years without DN (CPN) who constituted the main group in comparison with the control group of healthy individuals.

The following parameters were also studied: results of general blood and urine tests, lipid spectrum, glycemic profile, glycosylated hemoglobin, microalbuminuria, glomerular filtration rate (GFR) according to CKD-EPI formula, endothelin-1 level in blood plasma, EchoCG, CMAD and Doppler study of renal vessels.

T-786C polymorphism of the ENOS3 gene was tested on a programmable thermal cycler by AppliedBiosystems 2720 (USA) using Litech test systems (Russia), according to the manufacturer's instructions.

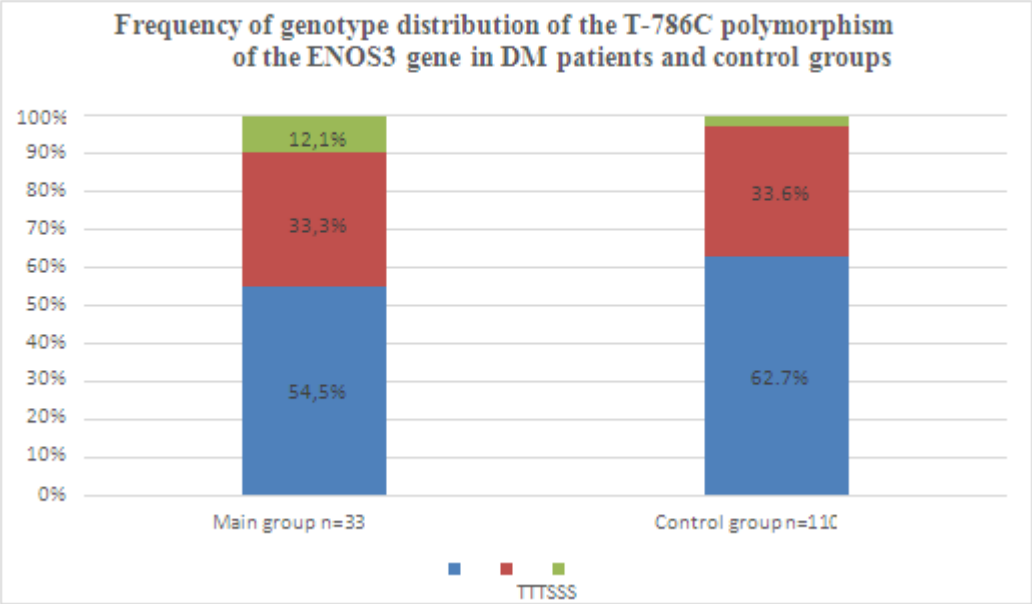
STATISTICA 6 program was used for statistical processing of the material. The data are presented as mean values with standard deviation (M±SD). Normality of distribution was checked by Kolmogorov-Smirnov criterion. Relative risk of disease among carriers of a particular allele and genotype was calculated as an odds ratio (OR). The OR value was calculated using an online medical statistics calculator (http:// medstatistic.ru/calculators.html).

The distribution of genotypes was tested for deviation from Hardy-Weinberg equilibrium. Correlation coefficient r was calculated by Spearman's method. Differences were considered statistically significant at $p<0.05$. All patients signed an informed consent prior to the examination.

Results and discussion

The frequency of alleles and genotypes of the T-786S polymorphism of the ENOS3 gene in the patients of the main group and the control sample are shown in Figure 1.

Fig.1



The prevalence of the T allele in the studied main and control groups was 71.2% and 79.5%, respectively. The prevalence of the unfavorable C allele was 28.7% and 20.4%, respectively. According to statistical calculation, carriers of the C allele were 1.5 times more likely to develop the disease than carriers of the T allele (χ^2= 2.02; P = 0.15;OR= 1.5; 95% CI 0.8411-2.9385). The T allele (χ^2=

2.02; P = 0.15; OR = 0.6; 95% CI 0.3403-1.189) indicates that it has a protective effect on disease progression.

According to the results of the main and control groups, the frequency of TT, TC and CC genotypes was 54.5%, 33.3%, 12.1% and 62.7%, 33.6%, 3.6% respectively. According to statistical calculation, carriers of the CC genotype were

3.65 times more likely to develop the disease than carriers of the TT genotype, and the difference between them had a significant statistical significance (χ^2=3.5; P=0.06; OR=3.6; 95% CI 0.8312-1.5513). The TT genotype was significantly lower in the main group than in the control group, 54.5%, 62.7%, and showed protective function against disease progression ($^2\chi$=0.7; P=0.4; OR=0.7; 95% CI 0.3247-1.5659). The TC genotype was also significantly lower in the main group than in the control group, 33.3% and 33.6%, respectively, and did not play a significant role on disease progression ($^2\chi$=0.001; P=1.0; OR=1.0; 95% CI 0.4324-2.2506) (Table 1).

Table 1: Frequency of alleles and genotypes distribution of T-786C polymorphism of NOS3 gene in the main and control groups of patients with type 2 DM

Alleles and genotypes	Number of alleles and genotypes surveyed				χ2	P	RR	95% CI	OR	95% CI
	major group N%		Control group N%							
T	47	71,2	175	79.5	2,0297	0,1542	0,8952	0,3673-2,1815	0,6361	0,3403-1,189
C	19	28,7	45	20.4	2,0297	0,1542	1,4074	0,5775-3,4297	1,5721	0,8411-2,9385
T/T	18	54,5	69	62.7	0,7132	0,3984	0,8696	0,2698-	0,713	0,3247

								2,8033		- 1,5659
T/C	11	33,3	37	33.6	0,001	0,9702	0,991	0,2854-3,4405	0,9865	0,4324 - 2,2506
C/C	4	12,1	4	3.6	3,4602	0,0629	3,3333	0,7453-14,907	3,6552	0,8312 - 15,513

Our study demonstrated an association between C-allele (CC genotype) carriage of the ENOS3 gene and diabetic nephropathy in type 2 DM patients. The results revealed that C-allele carriage is an independent risk factor for DN in patients with type 2 DM. According to a 2014 meta-analysis, which analyzed the results of 32 studies published before 2013, the association of three eNOS3 gene polymorphisms with the development of DN: 4b/a, T-786C and G984T. The 4b/a and T-786C polymorphisms showed a significant association for all genetic patterns (OR=1.12-1.77 and 1.11-1.50, respectively). These data and the results of our study suggest that eNOS3 gene plays an important role in the development of DN [13] in patients with type 2 diabetes mellitus of the studied Uzbek nation depending on the duration of the disease.

Conclusion

Thus, the study revealed a significant association of the risk of diabetic nephropathy in patients with type 2 diabetes with genes encoding endothelial factors (NOS3), the expression products of which play a role in the pathogenesis of kidney damage in diabetes. The results of this study indicate the importance of further study of the molecular basis for the development and progression of DN, which will lead to the development of new promising directions in the prevention of this pathology.

List of references

1. Ogurtsova K, da Rocha Fernandes JD, Huang Y, et al. IDF Diabetes Atlas: Global estimates for the prevalence of diabetes for 2015 and 2040. *Diabetes Res Clin Pract.* 2017;128:40-50.
2. Dedov I.I., Shestakova M.V., Vikulova O.K. Epidemiology of diabetes mellitus in the Russian Federation: clinical and statistical analysis according to the federal register of diabetes mellitus. Diabetes mellitus. 2017; 20(1): 13- 41.
3. Remuzzi G, Schieppati A, Ruggenenti P. Clinical practice. Nephropathy in Patients with Type 2 Diabetes. *N Engl J Med*. 2002; 346(15): 1145-1151.
4. Shishko O.N., Mokhort T.V., Konstantinova E.E. et al. The role of vascular endothelial growth factor in the pathogenesis of diabetic nephropathy. Medical Journal. 2013. 1(43): 132-135.
5. Smirnov IE, Kucherenko AG, Smirnova GI Badalyan AR Diabetic nephropathy. The Russian Journal of Pediatrics. 2015; 18(4): 43-50.
6. Reutens A.T.. Epidemiology of diabetic kidney disease. *Med Clin North Am*. 2013; 97(1): 1-18.(In Australia.).
7. Dedov I.I., Kuraeva T.L., Peterkova V.A. Diabetes mellitus in children and adolescents. - Moscow: GEOTAR-Media; 2007.
8. Dedov I.I., Shestakova M.V. Diabetes mellitus: diagnosis, treatment, prevention. MOSCOW: MIA; 2011.
9. Baranov A.A., Namazova-Baranova L.S., Ilyin A.G. et al. Scientific research in pediatrics: directions, achievements, prospects. Russian Pediatric Journal. 2013; 5: 4-14.
10. Satirapoj B. Nephropathy in diabetes. *Adv Exp Med Biol.* 2012; (771): 107-122.

11. Shestakova MV, Chugunova LA, Shamkhalova MS, Dedov I.I. Diabetic nephropathy: advances in diagnosis, prevention, treatment. Diabetes mellitus. 2005; 3: 22-25.
12. Bobkova I.N., Shestakova M.V., Shchukina A.A. Diabetic nephropathy - focus on podocyte damage. Nephrology. 2015; 19(2): 33-43.
13. Shankland SJ. Podocyte′s response to injury: role in proteinuria and sclerosis.
14. *Kidney Int.* 2006; 69: 2131-2147.
15. Stitt-Cavanagh E, MacLeod L, Kennedy CRJ. The podocyte in diabetic kidney disease. *The Scientific World Journal.* 2009; 9: 1127-1139.
16. Wolf G, Chen S, Ziyadeh FN. From the periphery of glomerular capillary wall toward the center of disease: podocyte injury comes of age in diabetic nephropathy. *Diabetes.* 2005; 54(6): 1626-1634.
17. Ziyadeh FN, Wolf G. Pathogenesis of podocytopathy and proteiuria in diabetic glomerulopathy. *Current Diabetes Reviews.* 2008; 4: 39-45.
18. Steffes MW, Schmidt D, Mccrery R, Basgen JM. Glomerular cell number in normal subjects andin type 1 diabetic patients. *Kidney International.* 2001; 59: 2104- 2113.
19. Patari A, Forsblom C, Havana M, et al. Nephrinuria in diabetic nephropathy of type 1 diabetes. *Diabetes.* 2003; 52: 2969-2974.
20. Lewko B, Stepinski J. Hypergliycemia and mechanical stress: Targeting the renal podocyte. *J Cell Physiol.* 2009; 221(2): 288-295.
21. Diez-Sampedro A, Lenz O, Fornoni A. Podocytopathy in diabetes: a metabolic and endocrine disorder. *Am J Kidney Dis.* 2011; 58(4): 637-646.
22. Zakharyina O.A., Tarasov A.A., Babaeva A.R. Actual aspects of drug prophylaxis and treatment of diabetic angiopathy. Drug Bulletin. 2012; 5(45): 14- 22.
23. Shestakova M.V. Diabetic nephropathy: results of the XX century, prospects for the XXI century. Diabetes mellitus. 2000; 1: 15-18.
24. Moorhead JF. Lipids and the pathogenesis of kidney disease. *Am J Kidney Dis.*
25. 1991; 27(1): 65-70.
26. Diamond J. Focal and segmental glomerulosclerosis: analogies to atherosclerosis. *Kidney Int.* 1988; 33: 917-924.
27. Dedov I.I., Shestakova M.V. Algorithms of specialized medical care for patients with diabetes mellitus. Moscow: Endocrinology Research Center; 2007.
28. Zanatta C.M., Crispim D., Sortica D.A., et al. Endothelin-1 gene polymorphisms and diabetic kidney disease in patients with type 2 diabetes mellitus // Diabetology and Metabolic Syndrome. 2015. Vol. 19, №7. P. 103-110.
29. Zeravica R., Ilinicic B., Cabarkapa V., et al. Plasma Endothelin-1 Levels and albuminuria in Patients with Type 2 Diabetes Mellitus // Medical Review. 2016. Vol. LXIX, NO. 5-6. P. 140-145.
30. Zanatta C.M., Veronese F.V., Loreto M.S., et. al. Endothelin-1 and Endothelin A Receptor Immunoreactivity is Increased in Patients with Diabetic Nephropathy // Renal Failure. 2012. Vol.34, №3. P. 308-315.
31. Yoon Y., Song J., Hong S.H., et. al. Plasma nitric oxide concentrations and nitric oxide synthase gene polymorphisms in coronary artery disease // Clinical Chemistry. 2000. Vol. 46, №10. P. 1626-1630.

32. Uryasiev O.M., Shakhanov A.V. The role of polymorphism of nitric oxide synthases in the formation of comorbid pathology - bronchial asthma and hypertension // Kazan Medical Journal. 2017. T. 98, №2. C. 226-232.
33. Dellamea B.S., Pinto L.C., Leitao C.B., et. al. Endothelial nitric oxide synthase gene polymorphisms and risk of diabetic nephropathy: a systematic review and meta-analysis // Bio Med Central Medical Genetics. 2014. Vol. 15. P. 9-23.
34. Ezzidi I., Mtiraoui N., Mohamed M.B., et al. Association of endothelial nitric oxide synthase Glu298Asp, 4b/a, and -786T>C gene variants with diabetic nephropathy // Journal of Diabetes and its Complications. 2008. Vol. 22, №5. P. 331-338.
35. Colombo M.G., Paradossi U., Andreassi M.G., et al. Endothelial nitric oxide synthase gene polymorphisms and risk of coronary artery disease // Clinical Chemistry. 2003. Vol. 49, №3. P. 389-395.
36. Nakayama M., Yasue H., Yoshimura M., et al. T(- 786)C mutation in the 5'- flanking region of the endothelial nitric oxide synthase gene is associated with myocardial infarction, especially without coronary organic stenosis // American Journal of Cardiology. 2000. Vol. 86, №6. P. 628-634.
37. Shestakova M.V., Shakhmalova M.S. Diabetic nephropathy: clinic, diagnosis, treatment. Moscow 2009.