Examining The Frequency Distribution Of Genotypes T-786c Polymorphic Marker Of The Enos3 Gene And I/D Of The Ace Gene At Diabetic Nephropathy In Patients With Type 2 Diabetes

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Abstract

Summary: This article presents the results of a study of 129 patients with type 2 diabetes (main group)and 110 healthy subjects (control group) to determine whether the polymorphic markers T-786Cof the ENOS3 gene and AluIns/DelI>D of the ACE gene are associated with the development of diabetic nephropathy (DN). Patients in the main group: 65 patients with disease duration up to 10 years without diabetic nephropathy (33 patients, group 1) and with diabetic nephropathy (32 patients), 64 patients with diabetes lasting more than 10-20 years without diabetic nephropathy (31patients, group 3) and diabetic nephropathy (33 patients). Genotyping was carried out by polymerase chain reaction. The study showed that the association of C allele and CC genotype of ENOS3 gene and D allele and I/D genotype of ACE gene play a significant role in the development of diabetic nephropathy in patients with type 2 diabetes in the studied Uzbek nation.

Key words: diabetic nephropathy, diabetes mellitus, gene, polymorphism, allele, genotype.

Introduction. Diabetic nephropathy (DN) is a microvascular complication of diabetes mellitus (DM), the development of which significantly worsens the course and further prognosisof the disease [18,15,19]. In DN, small blood vessels in the filtering apparatus of the kidneys areaffected, further leading to increased amounts of protein excreted in the urine (proteinuria)(6,5,14). DN develops in 13-15% of individuals in the general population and is much morecommon, up to 40-50%, in at-risk groups, which include patients with T2DM (2). TheInternational Diabetes Federation predicts that the number of people with diabetes in the world will increase to 587 million by 2035, 95% of whom will have T2DM [6,7,16].

In recent years, the risk of nephropathy has definitely been determined by genetic factors. Only about 40-50% of patients with both type 1 diabetes and type 2 diabetes subsequently developDN [8,11,13,20].

Genetic factors may directly influence the development of DN and/or act together with genes affecting cardiovascular disease. Search
genetic markers of susceptibility or, conversely, resistance to disease is one of the most pressing challenges in medical science. [5,9,12]

This is because the establishment of such markers makes it possible for clinicians to form risk groups for disease development and, for some pathologies, to establish an individual prognosis or diagnosis (including before the clinical manifestation of the disease). Evaluation of the role of a particular genetic marker in DM depends on racial and ethnic variations in allele and genotype frequencies in the populations studied [3,10,17]. In recent years, the genetic risk of diabetes and its complications depending on genes of insulin resistance, genes that determine reduced insulin levels, polymorphism of angiotensin-I converting enzyme (ACE) gene, gene of endothelial NO synthase (NOS) in patients with both types of diabetes are widely discussed in the literature [1,2,4,5,9].

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

eNOS and ACE gene polymorphisms in type 2 diabetes and its macrovascular and microvascular complications have not previously been studied in the Uzbek population.

Objective. To assess the contribution of eNOS3 and ACE gene polymorphic marker in the risk of diabetic nephropathy in type 2 diabetes in persons of Uzbek ethnicity.

Material and methods

A core group of 129 patients with type 2 DM and a control group of 110 healthy individuals of the Uzbek nation included according to the case-control principle were examined in the Republican Scientific and Practical Centre of Nephrology at the TMA clinic III. The 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) and 64 patients with diabetes lasting more than 10-20 years, with no diabetic nephropathy (31 patients) and diabetic nephropathy (33 patients). The parameters studied were the results of general blood and urine tests, lipid spectrum, glycaemic profile, glycosylated haemoglobin, microalbuminuria, glomerular filtration rate (GFR) using the CKD-EPI formula, plasma endothelin-1 level, EchoCG, CMAD and Doppler study of the renal vessels.

The T-786C polymorphism of the ENOS3 gene and the AluIns/DelI>D polymorphism of the ACE gene were tested on an AppliedBiosystems programmable thermal cycler
2720 (USA), using Litech test kits (Russia), according to the manufacturer's instructions.

STATISTICA 6 software 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 the Kolmogorov-Smirnov criterion. The 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).

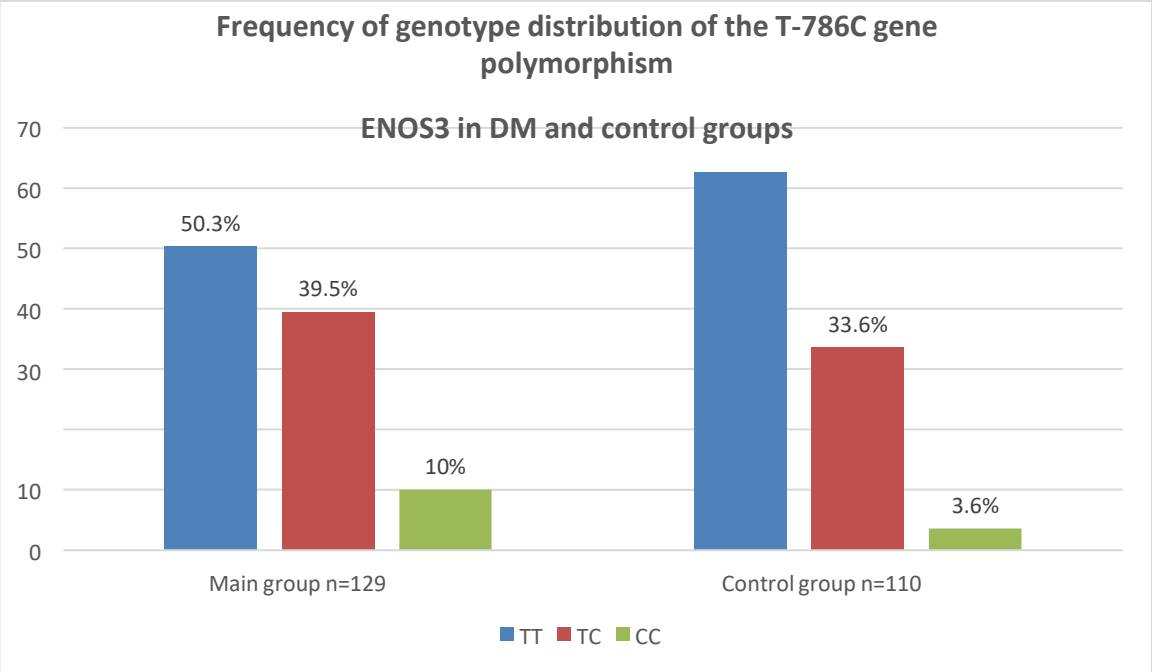
The distribution of genotypes was checked for deviation from Hardy-Weinberg equilibrium. Correlation coefficient r was calculated by Spearman's method. Differences were considered statistically significant at $p<0.05$.

All patients signed an informed consent before the examination.

Results and discussion

The allele and genotype frequencies of the T-786S polymorphism of the ENOS3 gene in allpatients (treatment group) and controls are shown in Figure 1.

Figure 1.



The prevalence of the T allele in the main and control groups studied was 70.1% and 79.5%, respectively. The prevalence of the unfavourable C allele was 29.8% and 20.4%, respectively. According to the statistical calculation, in

C allele carriers were 1.6 times more likely to develop the disease than T allele carriers (χ^2 = 5.5; P = 0.02;OR = 1.6; 95% CI 1.0844-2.524). The T allele (χ^2 = 5.5; P = 0.02; OR = 0.6; 95% CI 0.3962-0.9222) indicates that it has a protective effect on disease progression.

Table 1. Frequency of alleles and genotypes of the T-786C polymorphism of the eNOS3 gene in themain and control groups of type 2 DM patients.

Alleles and genotypes	Number of people surveyed alleles and genotypes				χ2	P	OR	95% CI
	main group N	%	Control group N	%				
T	181	70,1	175	79.5	5,5085	0,0189	0,6045	0,3962-0,9222
C	77	29,8	45	20.4	5,5085	0,0189	1,6544	1,0844-2,524
T/T	65	50,3	69	62,7	3,6702	0,0554	0,6035	0,3594-1,0132
T/C	51	39,5	37	33.6	0,888	0,346	1,29	0,7592-2,1919
C/C	13	10,0	4	3.6	3,7283	0,0535	2,9698	0,9392-9,3906

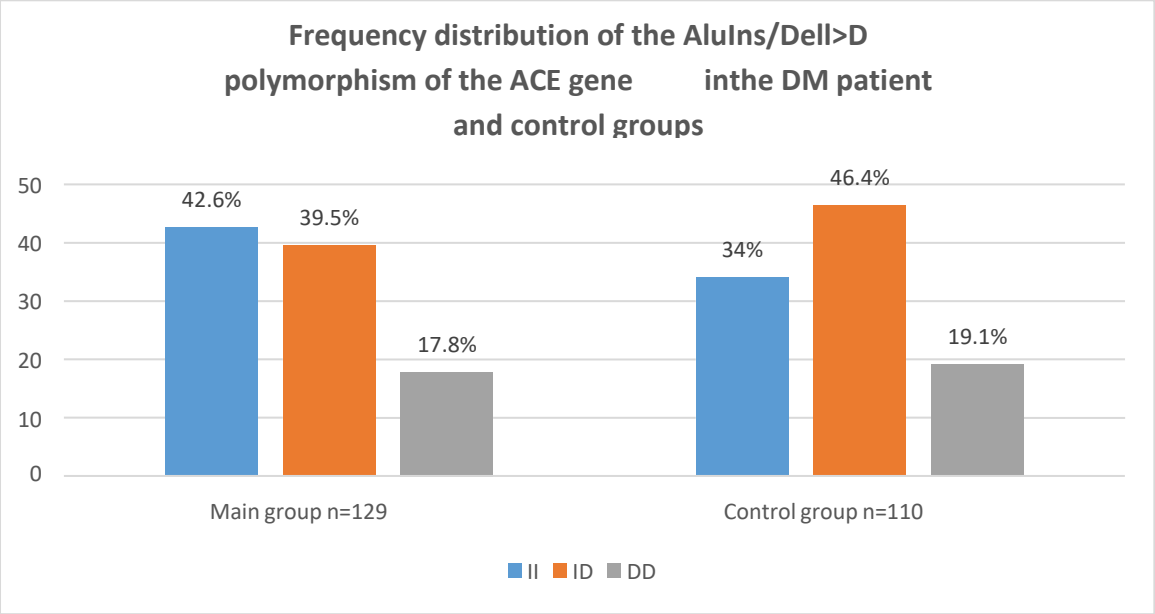
The frequency of TT, TC and CC genotypes was 50.3%, 39.5%, 10% and 62.7%, 33.6%, 3.6%, respectively, according to the results of the core and control groups. According to statistical calculation, carriers of the CC genotype were 2.9 times more likely to develop the disease than carriers of the TT genotype, and the difference between them was statistically significant (χ^2 = 3.7;P = 0.05; OR = 2.9; 95% CI 0.9392-9.3906).

The TT genotype was significantly lower in the main group than in the control group,50.3%, 62.7%,

and showed a protective function against disease progression (χ^2 = 3.7; P = 0.05;OR = 0.6; 95% CI 0.3594-1.0132). The TC genotype was also significantly lower in the maingroup than in the control group, 39.5% and 33.6% respectively, and did not play a significant roleon the development of pathology (χ^2 = 0.9; P = 0.3; OR = 1.29; 95% CI 0.7592- 2.1919) (Table 1).

The allele and genotype frequencies of the AluIns/DelI>D polymorphism of the ACE gene in all patients (main group) and the control sample are shown in figure 2.

Fig.2



The prevalence of allele I in the first and second groups studied was 56.0% and 79.5%, respectively. The prevalence of the unfavourable D allele was 43.9% and 20.9%, respectively. According to the statistical calculation, carriers of the D allele were 2.9 times more likely to develop the disease than carriers of the I allele (x^2 =7.6; P=0.006; OR=2.9; 95% CI 1.353-6.452). Allele I (x^2 =7.6; P=0.006; OR=2.9; 95% CI 1.353-6.452) indicates that it has a protective effect on disease progression.

Table 2Frequency distribution of alleles and genotypes of the AluIns/DelI>D gene polymorphism ACE in the first and third groups of type 2 DM patients

Alleles and genotypes	Number of people surveyed alleles and genotypes				χ2	P	OR	95% CI
	First group N %		Group three N %					
I	37	56,0	49	79,0	7,652	0,006	0,339	0,155-0,739
D	29	43,9	13	20,9	7,652	0,006	2,954	1,353-6,452
I/I	11	33,3	21	67,7	7,57	0,006	0,238	0,084-0,677
I/D	15	45,4	7	22,5	3,707	0,054	2,857	0,965-8,46
D/D	7	21,2	3	9,6	1,613	0,204	2,513	0,587-10,76

The I/I, I/D and D/D genotypes were 33.3%, 45.4%, 21.2% and 67.7%, 22.5% and 9.6%,respectively, according to the results of the first and third groups. According to By statistical calculation, ID genotype carriers were 2.8 times more likely to develop the disease than I/I genotype carriers and the difference between them had significant statistical significance (x^2 =0.02; P=0.9; OR=1.1; 95% CI 0.529-2.113). Genotype II was significantly lower in group I than group III by 33.3%, 67.7% and showed protective function against disease progression (x^2 =7.52; P=0.006; OR=0.2; 95% CI 0.084-0.677). The D/D genotype was also significantly lower in group 3 than in

group 1, 45.4% and 22.5% respectively and played a significant role in the development of pathology (x^2 =1.6; P=0.2; OR=2.5; 95% CI 0.587-10.76). (Table 2).

Our study demonstrated an association between C-allele (CC genotype) carriage of the ENOS3 gene and diabetic nephropathy in type 2 DM patients. An association was also found between carriage of the D allele (DD genotype) of the ACE gene and diabetic nephropathy in type 2 DM patients. These results are consistent with those of national and international authors who have shown that D-allele carriage is an independent risk factor for DN in patients with type 2 diabetes in different ethnic groups (6).

These data and the results of our study suggest that genotypes of the polymorphic marker t- 786c of the ENOS3 gene and AluIns/DelI>D of the ACE gene play an important role in the development of DN in type 2 diabetes patients in the studied Uzbek nation.

Conclusion

Thus, the study found a significant association of the risk of diabetic nephropathy in patients with type 2 diabetes with genes encoding components of RAS - angiotensin-converting enzyme (ACE) gene and genes encoding endothelial factors (eNOS3), whose expression products play a role inthe pathogenesis of kidney damage in diabetes.

The results of this study indicate the importance of further research into the molecularbasis of the development and progression of DN will lead to the development of promising new directions in the prevention of this pathology.

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