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Association of Polymorphic Markers of Leu28Pro Gene APOE and T-786C Gene ENOS3 in Diabetic Nephropathy in Uzbek Nation

Jabborov Ozimbay Otakhonovich

Tashkent Medical Academy, Tashkent, Uzbekistan

Abstract The aim of the present study was to determine the association of association of the Leu28Pro polymorphic markers of the APOE gene, T-786C of the NOS3 gene with the development of diabetic nephropathy, 129 patients with type 2 diabetes were examined. Patients in the main group: 65 patients with a disease duration of 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). Genotyping was carried out by polymerase chain reaction. The study showed the association of the Pro allele and the Leu/Pro genotype of the APOE gene and the C allele and the CC genotype of the NOS3 gene with a risk of developing diabetic nephropathy in patients with type 2 diabetes mellitus in the studied Uzbek nation.

Keywords Diabetic nephropathy, Gene, Polymorphism, Apolipoprotein, Nitric oxide synthase

1. Introduction

Diabetic nephropathy (DN) is a microvascular complication of diabetes mellitus, the development of which significantly worsens the course and further prognosis of the disease. Despite the similar incidence of DM in type 1 diabetes and type 2 diabetes, mortality from terminal renal failure in patients with type 2 diabetes is much higher - 45% compared with 5-10% of cases with type 1 diabetes [4,5,6,8,9].

The effectiveness of the treatment of this complication largely depends on the timeliness of its detection, which gives particular relevance and importance to the problem of studying the risk factors of DN and dictates the need to develop informative methods for its prediction.

According to modern concepts, DN develops under the influence of a whole complex of metabolic, hemodynamic, and also genetic factors, the interaction of which leads to the clinical manifestation of pathology.

Identification of markers associated with DN will allow the formation of risk groups for the development of DN even at the preclinical stage, expand the possibilities of early diagnosis and prevention of this complication.

* Corresponding author:
dok_azim66@mail.ru (Jabborov Ozimbay Otakhonovich)
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Of undoubted practical interest is also the study of factors of progression of DN with the aim of developing effective preventive measures and reducing the incidence of chronic renal failure (CRF). The cost of extracorporeal treatments for the terminal stages of nephropathy is extremely high [8,11,13]. Thus, the problem of renal complications of diabetes mellitus acquires, in addition to medical, important socio-economic importance.

The risk of nephropathy development is definitely determined by genetic factors. Only approximately 40-50% of patients with both type 1 diabetes and type 2 diabetes subsequently develop DN. Genetic factors may directly influence the development of DM and/or act in conjunction with genes affecting cardiovascular diseases. The search for genetic markers of susceptibility or, conversely, disease resistance is one of the most important tasks of medical science. [5,8,9]

This is determined by the fact that the establishment of such markers allows clinicians to form risk groups for diseases and, in some pathologies, to establish an individual prognosis or diagnosis (including before the clinical manifestation of disease). Evaluation of the role of a genetic marker in diabetes depends on racial and ethnic variations in the frequency of alleles and genotypes in the populations studied [11,15]. In recent years, the literature has been widely discussing the genetic risk of diabetes development and its complications depending on the genes of insulin resistance, genes that determine the reduced level of insulin, genes that affect lipid metabolism, polymorphism of the gene angiotensin-I-converting enzyme (ACE), and the gene

of endothelial NO-synthase (NOS) in patients with both types of diabetes mellitus [1,3,4].

Therefore, the individual concentrations and properties of the polypeptoprotein E and ENOS3 genes that induce endothelial dysfunction in lipid metabolism in individual patients are of interest for research as a candidate gene that determines the tendency for vascular complications in type 2 diabetes.

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

The polymorphism of the eNOS gene and APOE gene in case of type 2 diabetes and its macro- and microvascular complications in the Uzbek nationality has not been studied before.

Target. Evaluation of the contribution of the Leu28Pro polymorphic markers of the APOE gene and T-786C of the eNOS3 gene to the risk of developing diabetic nephropathy.

2. Material and Methods

In the Republican Scientific and Practical Center of nephrology on the basis of TMA Clinic III the main group of 129 patients of Diagnostic Diabetes-2 type were examined and the control group consisted of 110 healthy persons of the Uzbek nation, included on the principle of "case-control". Patients in the main group were distributed as follows: 65 patients with duration of the disease 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, without diabetic nephropathy (31 patients) and diabetic nephropathy (33 patients). The results of general blood and urine tests, lipid spectrum, glycemic profile, glycosylated hemoglobin, microalbuminuria, glomerular filtration rate (GFR) by CKD-EPI formula, endotheline-1 plasma level, EchoKG, SMAD and Doppler examination of kidney vessels were studied.

T-786C ENO3 gene and Leu28Pro polymorphism tests of APOE gene were performed by Applied Biosystems 2720 (USA), using Litech (Russia) test systems according to the manufacturer's instructions.

The STATISTICA 6 program was used for statistical processing of the material. The data are presented in the form of mean values with standard deviation (M±SD). Normal distribution was checked by Kolmogorov-Smirnov Criterion. The relative risk of disease in carriers of a certain alley and genotype was calculated as an indicator of the odds ratio (OR - oddsratio). The OR value was calculated using the online calculator of the Medical Statistics program (http://medstatistic.ru/calculators.html).

Genotype distribution was checked for deviations from the Hardy-Weinberg equilibrium. The correlation coefficient r was calculated using the Spearman method. Differences at $p<0.05$ were considered statistically significant.

3. Results and Discussion

The frequency of alleles and genotypes of polymorphism T-786S of the ENOS3 gene in all patients (the main group) and the control sample is shown in Fig. 1.

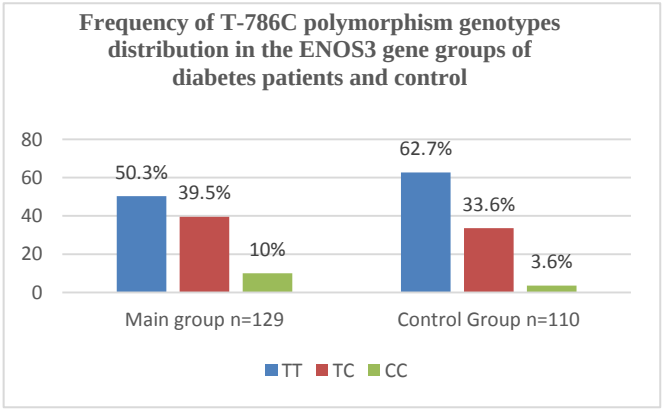

Figure 1

The prevalence of allele T in the studied basic and control groups 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, carriers of the C allele are 1.6 times more likely to develop the disease than carriers of the T allele ($\chi2 = 5.5$; $P = 0.02$; OR = 1.6; 95% CI 1.0844-2.524). Allel T ($\chi2 = 5.5$; $P = 0.02$; OR = 0.6; 95% CI 0.3962-0.9222) indicates that it has a protective effect on disease progression.

Table 1. The frequency of distribution of alleles and genotypes of polymorphismT-786C of the NOS3 gene in the main and control groups of 2-type diabetes patients

Alleys and genotypes	χ^2	P	OR	95% DI
T	5,5085	0,0189	0,6045	0,3962-0,9222
C	5,5085	0,0189	1,6544	1,0844-2,524
T/T	3,6702	0,0554	0,6035	0,3594-1,0132
T/C	0,888	0,346	1,29	0,7592-2,1919
C/C	3,7283	0,0535	2,9698	0,9392-9,3906

According to the results of the main and control groups, the frequency of TT, TS and CC genotypes distribution was 50.3%, 39.5%, 10% and 62.7%, 33.6% and 3.6%, respectively. According to the statistical calculation, CC genotype carriers are 2.9 times more likely to develop the disease than TT genotype carriers, and the difference between them has a reliable statistical significance ($\chi2 = 3.7$; $P = 0.05$; OR = 2.9; 95% CI 0.9392-9.3906). The TT genotype was significantly lower in the main group than in the control group by 50.3%, 62.7%, and showed a protective function against disease progression ($\chi2 = 3.7$; $P = 0.05$; OR = 0.6; 95% CI 0.3594-1.0132). The TC genotype was also significantly lower in the main group than in the control group, at 39.5% and 33.6% respectively, and did not play a significant role in the development of the pathology ($\chi2=0.9$; $P=0.3$; OR=1.29; 95% CI=0.7592-2.1919) (Table 1.). In our

study, we demonstrated an association between the carriage of the C-allelele (CC genotype) of the ENOS3 gene and diabetic nephropathy in patients with type 2 diabetes. The obtained results are consistent with the data of domestic and foreign authors, who showed that the carriage of C-allelel is an independent risk factor for DM in patients with type 2 diabetes in different ethnic groups [6]. According to the meta-analysis of 2014, in which the results of 32 studies published before 2013 were analyzed, the association of three eNOS3 limorphisms with DN development was revealed: 4b/a, T-786C and G984T. Polymorphisms 4b/a and T-786C showed a reliable association for all genetic models (OR=1,12-1,77 and 1,11-1,50, respectively). These data and the results of our study allow us to conclude that the eNOS3 gene plays an important role in the development of DN [13,14] in patients with type 2 diabetes mellitus in the studied Uzbek nation.

Similarly, the rate of occurrence of the Leu28Pro polymorphism alleles and genotypes in the main and control groups of the APOE gene in all patients surveyed is shown in Figure 2.

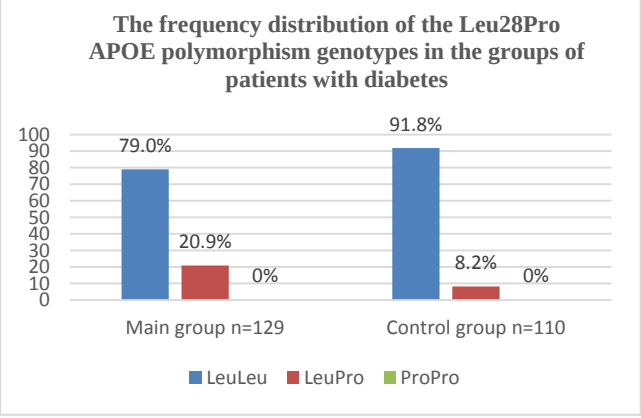

Figure 2

In our study, we studied the distribution of genotypes and alleles of the polymorphic marker Leu28Pro of the APOE gene in primary and control patients. The prevalence of the Leu allele in the main and control groups was 89.5% and 95.95%, respectively. The incidence rate of the functional adverse Pro allele was 10.4% and 4.1%, respectively. The statistical report shows that the Pro allele carriers are 2.7 times more likely to develop the disease than the Leu allele carriers, and the difference between them is a reliable statistical value (χ^2 =6.9; P =0,008; OR=2,7; 95% CI 1,2597-5,9608). The Leu allele indicates that it has a protective effect against the progression of the disease. (χ^2 =6,9; P=0,008; OR=0,4; 95% CI 0,1678-0,7938). According to the results of the main and control groups, the distribution of Leu/Leu, Leu/Pro genotypes was 79.0%, 20.9% and 91.8%, 8.2%, but the Pro/Pro genotype in our analysis of the mutational genotype. According to statistical calculated, the probability of disease in carriers of the Leu/Pro genotype is 2.9 times higher than in carriers of the Leu/Leu genotype, and the difference between them is statistically significant. (χ^2 =7,5; P=0,006; OR=2,9; 95% CI 1,3308-6,6311).

Table 2. The frequency of distribution of alleles and genotypes of Leu28Pro polymorphism of the APOE gene in the main and control groups

Allelys and genotypes	χ^2	P	OR
Leu	6,9278	0,0085	0,3649
Pro	6,9278	0,0085	2,7403
Leu/Leu	7,5421	0,006	0,3366
Leu/Pro	7,5421	0,006	2,9706
Pro/Pro			

The Leu/Leu genotype was significantly lower in the main group than in the control group, by 79.0%, 91.8% and showed a protective function against disease progression (χ^2 =7,5; P=0,006; OR=0,3; 95% CI 0,1508-0,7515). (Tab. 1.) In our study, the association between carriage of the Pro allele (Leu/Pro genotype) of the APOE gene and diabetic nephropathy in patients with type 2 diabetes has been demonstrated. The results obtained are consistent with the data of domestic and foreign authors who showed that Carriage of the Pro allele is an independent risk factor for DM in patients with type 2 diabetes in various ethnic groups [1,2,6].

An analysis of data from foreign studies also indicates a connection between the APOE gene polymorphic marker e2/e3/e4 and the development of DM in both DM 2 and DM 1, which may indicate that lipid metabolism disorders can play a significant role in the pathogenesis of MD. In earlier the study revealed the association of the polymorphic marker e2/e3/e4 of the APOE gene with the development of MD in type 2 diabetes, that the carriage of the e3 allele and the e3/e3 genotype of the e2/e3/e4 marker of the apolipoprotein E gene (APOE) is a factor in the increased risk of MD in DM 1 [1]. Japanese authors have described the association of this marker with the progression of kidney damage in DM 2 from MAU to proteinuria, where the e2 allele is considered as an independent risk factor progression of DN [3,4,7,12].

These data and the results of our study allow us to conclude that the polymorphic marker of the APOE gene Leu28Pro and the polymorph marker of the ENOS gene T786C play an important role in the study of DN in patients with type 2 diabetes in the Uzbek population. However, it should be noted that genes involved in vascular tone regulation in microvascular products, such as ACE and ENOS3, play a crucial role in the pathogenesis of the early stages of DN.

4. Conclusions

Thus, the study revealed that the involvement of endothelial encoding factor genes (NOS3) and lipid metabolism genes is a reliable combination of the risk of diabetic nephropathy in patients with type 2 diabetes, whose expression products play an important role in the pathogenesis of kidney injury in diabetes. The results of this

study indicate that further study of the molecular basis of diabetes development and progression will lead to the development of new promising directions in the prevention of this pathology.

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