

The significance of genetic mutations in the pathogenesis of the development of nephropathy in chronic kidney diseases of different etiologies

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Abstract. In modern medicine, a number of scientific studies are being conducted on the importance of genetic predisposition in the development of chronic kidney disease (CKD). This article presents the results of a study conducted to determine the level of APOL1 gene polymorphism in the pathogenesis of nephropathies of various etiologies in the Uzbek population. The analysis showed that the APOL1 gene polymorphism G1 and G2 mutations were found among the patients included in the study.

1 Introduction

In recent years, there is information that genetic predisposition, which is important in the pathogenesis of primary diseases leading to the development of SBK, is also important in the formation of nephropathies [1].

In 2010, Giulio Genovese and his colleagues discovered that there are two independent mutations in the APOL1 gene, G1 and G2. The results of the conducted research showed that the combination of these two forms in nephropathy, which develops in arterial hypertension, leads to the development of the terminal stage of SBK [2,10]. Podocyte dysfunction underlies the mechanism of APOL1 mutation-induced renal damage. It is also theorized that mutant forms of APOL1 dissolve kidney cell membranes to form clefts and impair podocyte function, similar to trypanosome lysis [7].

In high blood pressure, nephropathy is manifested by albuminuria, and from a morphological point of view, glomerulosclerosis and interstitial fibrosis are observed. Several mechanisms are involved in the formation of these processes. In particular, as a result of renal hemodynamic disorders, hyperfiltration and subsequent decrease in CFT, followed by the development of proteinuria. A number of authors (Fan et.al, 2014) observed this condition in hypertensive disease in experimental studies and substantiated that this pathological change is caused by Add3 gene mutation. In Add3 mutation, the myogenic response in renal vessels is disturbed, and as a result, the autoregulation of intrarenal hemodynamics is not realized [4].

APOL 1 is the only encoded signal peptide belonging to the apolipoprotein family and secretes splice isoforms into serum. Until the APOL 1 gene was studied in relation to kidney

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pathology, it was known as a trypanolytic factor present in the blood serum of humans and some species of primates and protecting them from trypanosomes. A large-scale GWAS study has shown that Africans with APOL1 gene mutations have a strong protection against the African trypanosomiasis that causes sleeping sickness, but they have a higher risk of developing nephropathies (GWAS, 2013).

Apolipoprotein L1 (APOL1) is a secondary component of cholesterol in high-density lipoprotein, which is mainly synthesized in the liver. APOL1 has also been found in vascular endothelium, heart, lung, podocytes, proximal tubule and artery endothelium of the kidney.[9]. This protein is involved in the formation of cholesterol esters in plasma. It ensures the leakage of cholesterol from cells, that is, APOL1 is important in the metabolism and transport of lipids in the human body, and plays an important role in the transport of cholesterol from peripheral cells to the liver. Several isoforms of the APOL1 gene have been studied to perform these functions.

APOL1 is a representative of the apolipoprotein family (there are 5 other representatives), a member of the bcl2 gene, which causes autophagic cell death. Studies have shown that increased levels of APOL1 cause cellular autophagy [8,11]. At the beginning of the 21st century, the human phenotype-specific G1 and G2 forms of the APOL1 gene were identified. The G1 isoform is the appearance of a pair of non-identical single nucleotide polymorphisms (SNPs), and the pair strand is nearly unstable, unbalanced. G2 is an internal deletion of amino acid residues N388 and Y389.

The importance of gene mutation patterns in kidney pathology has been deeply studied since the discovery of the existence of a population resistant to *Trypanosoma brucei rhodesiense*, a widespread causative agent among the black African population, and it has been shown that individuals with a G1, G2 phenotype genome do not suffer from African sleeping sickness. However, nondiabetic nephropathies, particularly focal segmental glomerulosclerosis, end-stage renal disease after arterial hypertension, and human immunodeficiency virus-associated nephropathies are more common in this population.

When a population at risk for APOL1-related nephropathy was studied among Africans, the disease was more common in Western populations. Among the Yoruba and Nigerian (West African) populations, the risk of meeting G1 and G2 alleles was 40% and 8%, respectively [5].

Later, in other countries, the importance of APOL1 gene mutation patterns in the origin of nephropathies began to be studied. G. Genovese et al. (2010) analyzed the incidence of APOL1 gene mutations in 205 African-Americans diagnosed with focal segmental glomerulosclerosis confirmed by kidney biopsy and without a family history of the disease, and the cohort results were very low and large-scale studies were conducted. necessity is emphasized. When studying the incidence of APOL1 G1/G2-related nephropathies among white Africans (European-Americans), mutations were detected in 0.28% of patients with type 2 diabetes, compared to 0.33% in controls.[8].

Studies investigating the prevalence of APOL1 G1/G2 mutation-related kidney disease in Spanish and Asian populations have shown that Latin Americans In 0.3%, 0.4% of Asians have alleles that indicate the risk of developing end-stage nephropathy[5, 12].

One of the genes found to be important in nephropathies associated with arterial hypertension, studied in a number of scientific studies, is APOLLO1 is a gene. In epidemiologic studies of trypanosomiasis (sleeping sickness) among African Americans, rates of hypertensive and diabetic nephropathy were found to be higher than in Europeans.[6]. In numerous studies conducted APOLLO1 is a lysing factor in human serum, the presence of which has been shown to protect humans from trypanosome infection. This gene mutation simultaneously causes two pathologies in African Americans: sleeping sickness and hypertension, and diabetic nephropathy[9,11,13].

The purpose of the study is to study the incidence of APOL1 gene polymorphism in the prevention of chronic kidney diseases of various etiologies in the Uzbek population.

2 Research methods

APOL 1 gene is considered a new gene whose diagnostic and prognostic significance is being studied today, and the primers necessary for studying its mutation and polymorphism forms are not commercially available. Taking this into account, these gene primers were synthesized in the laboratory of the Institute of Chemistry of Plant Substances of the Academy of Sciences of the Republic of Uzbekistan in order to conduct molecular genetic research.

Prepared primers belong to the fields of molecular biology, medicine and pharmaceuticals, methods of laboratory diagnosis of nephropathy using PCR, and can be used in laboratory and research practice to identify mutations in the APOL 1 gene (apolipoprotein L1) during molecular genetic studies in the clinic.

Since there was no set of reagents for real-time PCR detection of APOL 1 gene mutation, it was synthesized in the following order. Synthesis of primers (oligonucleotides) was carried out on an ASM-2000 DNA synthesizer and using the phosphoramidite solid phase method. After synthesis, oligonucleotides were eluted from the solid phase with concentrated 30% aqueous ammonia at 60 °C for 2 h. The oligonucleotide solution was evaporated to remove ammonia and precipitated in 70% ethanol in the presence of 0.3 M sodium acetate and 50 mM magnesium chloride. Oligonucleotides were centrifuged at 14,000 rpm for 20 min. The precipitate was washed twice with 70% ethanol and dried at room temperature for 30 min.

Oligonucleotides were purified by polyacrylamide gel electrophoresis. For this, an 18% polyacrylamide gel containing 1:16 acrylamide/bisacrylamide + 7M urea and 1x tris-borate was prepared. A buffer solution containing 10 M urea, 10% glycerol, 1 mM EDTA, 10 mM tris buffer (Rn 7.5) was added to the primer solution. The prepared primer solution was heated at 95°C and poured into the wells for 1 minute. Gel electrophoresis was performed at 30 V/cm² for 4 hours. After that, the gel pieces were cut and eluted. Elimination was carried out at 8 °C for 12 h. The liquid part containing clean oligonucleotides was taken and precipitated with 70% ethanol for 20 minutes in a centrifuge at 14,000 revolutions per minute. The resulting oligonucleotides were diluted to a concentration of 20 µM in TE buffer pH 8.0.

The structure of the resulting primers is as follows:

APOL1F – AGACGAGCCAGAGCCAATCTTC

APOL1R – CACCATTGCACTCCAACCTTGGC

The following composition was used to prepare a kit for PCR analysis (SINTOL, Russia): 1st PCR mixture, 2nd PCR mixture of APOL1 G1/G2, , SynTaq polymerase. 1-PCR mix 25 mM (NH₄)₂SO₄, 75 mM tris pH 8.8, 7.5 mM MgCl₂, 0.05% polysorbate 20, 0.01% sodium azide and the 2nd PCR mixture of APOL1 G1/G2 was composed of 50% glycerol, 2 µM APOL1F, 2 µM APOL1R, 2 µM dNTP, 0.05% polysorbate 20.

PCR analysis was performed in real-time on an Applied Biosystem, USA amplifier. The results of the analysis showed the presence or absence of APOL1 gene mutation by restriction analysis.

Results and their analysis. Apolipoprotein L1 is a gene family located on chromosome 22, which appeared late in the evolution of mammals (≈30-35 million years ago), and the functional gene is present in only a few species in the primate lineage. APOL 1 circulates in the blood as part of the high-density lipoprotein (HDL) complex. It is also found in large amounts in lung, pancreas, liver, kidney and placental tissue.

Extensive studies have concluded that chronic kidney disease associated with mutations in the APOL1 gene is associated with ethnicity, and is 3-4 times more common in African Americans than in whites. It is noteworthy that the G1 of the APOL1 gene [c.(1072A>G;

1200T>G] and G2 (c.1212_1217del6) mutations were found to be closely related to the degree of development of terminal stages of nondiabetic nephropathy and hypertensive nephropathy.

3 Results and discussion

This aspect of the studied gene mutations means that in the development of nephropathies of various etiologies, the study of these alleles as a risk factor may provide an opportunity to reduce the probability of developing SBK and complications with the terminal stage. In our research, we aimed to study the level and significance of APOL1 gene mutation patterns in hypertension, type 2 diabetes and chronic glomerulonephritis in the Uzbek population.

Of the 180 patients included in the study, 144 were included in the molecular genetic tests. In 36 patients, genetic testing was not performed because the pretreatment DNA samples were partially of poor quality.

170 healthy people were included in the control group and genetic tests were performed. According to the results of the research, APOL1 genes rs73885319, rs60910145 polymorphism and rs71785313 allele. The meeting rate was as follows. In the main group (n=144), rs73885319 was 1.4% in 2 patients, rs60910145 polymorphism 0.7% in 1 patient and rs71785313 allele. It was found in 1 0.7% of patients (Table 1).

Table 1. APOL1 gene Frequency of alleles and genotypes of rs73885319, rs60910145 and rs71785313 (in the main and control groups).

Groups		Genotype distribution number					
		rs73885319		rs60910145		rs71785313	
		Absolute	%	absolute	%	Absolutely	%
1	Main group n=144	2	1.4	1	0.7	1	0.7
2	Control group n=170	0		0		0	

The mutation was not detected in the study of the composition of blood taken from healthy people.

APOLLO1 it was determined that there are two independent mutations of the gene, G1 and G2. Depending on the occurrence of G1 and G2 mutational forms in the form of alleles and genotypes, a 3-level risk factor is defined.

G0/ G0 – no risk

G1/G0; G0/ G2 – low risk

G1/ G1- medium risk

G1/G2; G2/ G2 – high risk

We also analyzed the level of occurrence of G1 and G2 allelic (mutated) forms of the APOL1 gene in the later stages of the study (Table 2)

Table 2. The number of occurrences of APOL1 gene G1 and G2 allelic (mutated) forms in the studied groups and the control group (%).

Groups		Number of alleles				Genotypes meeting rate					
		G1		G2		G1+G1		G1+G2		G2+G2	
		n	%	n	%	n	%	n	%	N	%
1	Main group n=144	3	2.1	1	0.7	1	0.7	1	0.7	0	0.0
A	Hypertension disease n = 55	2	3.6	1	1.8			1	1.8		

Continuation of Table 2.

B	Chronic glomerulonephritis n=41	1	2.4			1	2.4			
S	Type II diabetes n=48	-		-		-		-		-
2	Control group n=170	0		0		0		0		0

In general analysis by main group(n=144) G1 allele in 3 patients (2.1%), while the G2 allele it was found in 1 patient (0.7%). When genotypes are studied G1+G1 genotype in 1 case (0.7%) and G1+G2 genotype in 1 case (observed in 0.7%) ($r > 0.05$). G2+G2 genotype was not recorded.

When the obtained results were analyzed by the studied groups, the following changes were revealed. Among patients with AG I degree (n= 55), G1 allele was detected in 2 (3.6%), G2 in 1 (1.8%), G1+G2 genotype was detected in 1 patient (1.8%) ($r < 0.001$). Chronic glomerulonephritis is studied in the early stages 1 out of 41 patients (2.4%) had G1 allele and 1 patient (2.4%) had G1+G1 genotype. Among patients in the compensation period of type II diabetes APOL1 gene mutation patterns were not found.

The distribution of alleles and genotypes for all changes in patient groups and healthy control groups showed significant deviations according to the Hardy-Weinberg law. Indicators in the main group corresponded to the Hardy-Weinberg law, i.e. APOL1 genes 73885319 (c.1072A>G;) allele (mutation) form transferred A/A-0.98, A/G- 0.014, G/G-0, and the losing result A/A-0.98, A/G- 0.013, G/G-0 ($\chi^2 = 0.07$, $df =$, $p = 0.93$) (Table 3). APOL1 genes 60910145 (1200T>G) and the observed allele (mutation) form T/T - 0.993, T/G - 0.007 and G/G-0, total 0.99, pending T/T - 0.993, T/G - 0.007 and G/G-0 ($\chi^2 = 0.02$, $df =$, $p = 0.9$) (Table 4).

APOL1 gene G1 mutation form and G2 (c.1212_1217del6) of the allele (mutation) form when we analyzed the distribution, the results showed significant deviations according to the Hardy-Weinberg law

Table 3. APOL1 genes 73885319 (c.1072A>G;) theoretical (expected) and observed prevalence of the allele (mutation) form (according to Hardy-Weinberg).

Main group					
Alleles (polymorphisms)	Number of alleles encountered				
1072A	0.99				
1072G	0.01				
Haplotype form	Number of haplotypes		ch2	P	df
	Observed	Expected			
A/A	0.9861	0.9862	0.000	0.93	1
A/G	0.014	0.0138	0.000		
G/G	0.00	0.00	0.007		
Total	1.0	1.00	0.007		

Table 4. APOL1 genes 60910145 (1200T>G) theoretical (expected) and observed number of occurrences of the allelic (mutation) form (according to Hardy-Weinberg)

Main group					
Alleles (polymorphisms)	Number of alleles encountered				
1200T	0.99				
1200G	0.01				
Haplotype form	Number of haplotypes		ch2	P	Df
	Observed	Expected			
T/T	0.993	0.993	0.000	0.9	1
T/G	0.007	0.007	0.000		
G/G	0.00	0.00	0.002		

Everything	0.99	100.00	0.002		
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APOL1 gene G1 mutation c.1072A>G and 1200T>G allele haplotype meeting observed T/T - 0.993, T/G - 0.007 and G/G-0 , total 0.99, pending -/- 0.993, +/- - 0.007 and +/-0 ($\chi^2=0.02$, $df=1$, $p=0.9$). APOLLO1 gene G2 (c.1212_1217del6) the same indicators were observed in the analysis of allele (mutation) forms. According to the results of our research, APOL1 gene mutations were detected in 2.8% of cases. From forms of polymorphisms rs73885319 1.4%; rs60910145- 0.7%; The rs71785313 allele was detected in 0.7% of cases.

In this study, we tried to evaluate the number of occurrences of APOL1 gene G1 and G2 mutation forms in the studied Uzbek population and their role in the development of nephropathies of different etiologies.

4 Conclusion

In conclusion, it can be said that this gene is also present in the Uzbek population, and its G1+G1 and Identification of G1+G2 genotypes in our population, this gene mutation has diagnostic and prognostic significance in the development of the terminal stage of CKD.

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