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Assessing the Relationship between Ace and Nos3 Gene Polymorphism and Chronic Cerebrovascular Insufficiency

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Abstract: Association between ACEI/D, NOS3 4a/4b, and NOS3 C/T 786 gene polymorphisms and chronic cerebrovascular insufficiency (CCVI) was investigated in this study. The aim was to establish the relationship between these gene polymorphisms and CCVI and to explore the gene-gene interaction associated with this pathology. A total of 110 individuals were comprehensively examined, including 68 patients with CCVI as the main group and 42 healthy individuals as the control group. Gene polymorphisms of ACEI/D, NOS3 4a/4b, and NOS3 C/T 786 were determined using the polymerase chain reaction (PCR) method. The study found a significant dominance of the D allele and D/D genotype of the ACE gene and the 4b allele and 4b/4b genotype in CCVI patients, especially in stages III and IV. The highest occurrence of stages III and IV CCVI was observed in carriers of the I/I and D/D genotypes in combination with 4b/4b.

Keywords: *chronic cerebrovascular insufficiency, gene polymorphisms in angiotensin-converting enzyme, nitric oxide synthase, vascular disease, genetic variation, cerebrovascular disease, genetic susceptibility, neurovascular disorders, cerebrovascular pathology.*

1. Introduction:

Cerebrovascular diseases are one of the medical and social problems in most economically developed countries around the world. Due to their prevalence and serious complications, such as stroke leading to severe disability of patients and deterioration of their quality of life, chronic cerebrovascular insufficiency (CCVI) is the most common manifestation of cerebrovascular pathology, which is often encountered by every neurologist in their practice. The development of cerebrovascular insufficiency (CVI) is closely related to the involvement of the extra- or intracranial portion of the internal carotid arteries (ICA) and vertebral arteries (angiopathies), which can manifest as acute or chronic cerebral blood flow disorders. It should be emphasized that chronic and prolonged changes in the brain caused by the gradual deterioration of cerebral blood flow and changes in its metabolic needs lead to the development of acute cerebral blood flow disorders.

One of the important and promising areas in the study of genetic predisposition to various diseases, including cardiovascular diseases, are the so-called candidate genes. Vasodilation and vasoconstriction lead to smooth muscle contraction, thus affecting blood pressure. Among them, genes encoding the elements of the renin-angiotensin-aldosterone system (RAAS) are considered to be the main candidate genes, such as the angiotensin-

converting enzyme (ACE) polymorphism I/D and endothelial nitric oxide synthase (eNOS) polymorphisms 4b/4a and C/T 786. Angiotensin-1 is formed in the bloodstream and quickly converted to octapeptide angiotensin-2, which depends on the level of circulating ACE in the blood on the surface of the vascular endothelium, and inactivates bradykinin, a vasodilator protein. In carriers of the I/I and I/D genotypes, the level of the enzyme ACE is normal, whereas in carriers of the D/D genotype, the level of the enzyme is often elevated, even in healthy carriers. In a study of 428 healthy individuals, it was shown that people with the bb genotype have a NO level approximately twice as high as those with the other genotypes.

One of the key mechanisms triggering the pathogenesis of ischemic cerebrovascular disease is the disturbance of the functional properties of the endothelium, which leads to changes in the tone of the vascular wall and further progression of the pathological process. Timely diagnosis plays an important role in identifying this pathological condition and preventing the development of cerebral circulation disorders. Neurovisualization methods are essential for diagnosing vascular symptoms, and the most reliable method is computerized X-ray or, preferably, magnetic resonance imaging with angiography of the brain, as well as Doppler or duplex scanning of the brachiocephalic arteries. There is increasing speculation about the possible involvement of genetic mechanisms in the combination of etiological factors in sporadic small vessel disease in CADASIL. Any disturbances in cerebral blood flow should be a reason for detailed examination of the main vessels of the brain. Early recognition and timely treatment will reduce the incidence of cerebrovascular catastrophes.

Objective: The objective of this study is to investigate the association between molecular-genetic results of ACEI/D, NOS3 4a/4b, and NOS3 C/T 786 gene polymorphisms in patients with chronic cerebrovascular insufficiency. The study aims to utilize the obtained results to gain a better understanding of the genetic factors involved in the development of this disease. The identification of genetic risk factors associated with chronic cerebrovascular insufficiency can facilitate early diagnosis and personalized treatment approaches for patients. Therefore, the present study aims to contribute to the understanding of the complex molecular mechanisms underlying cerebrovascular diseases, ultimately leading to the development of more effective diagnostic and therapeutic strategies [1].

2. Research Methods.

We conducted a comprehensive examination of 110 individuals, including 68 patients with chronic cerebrovascular insufficiency in the main group. The control group consisted of 42 healthy individuals. Among the 68 patients, 41 (60%) were male and 27 (40%) were female. The age of patients ranged from 26 to 85 years old, with an average of 62.9±0.8 years (61.7±1.2 years for males and 62.4±1.9 years for females). In the control group, there were 28 (67%) male patients with an average age of 59.8±1.2 years, and 14 (33%) female patients with an average age of 56±0.89 years.

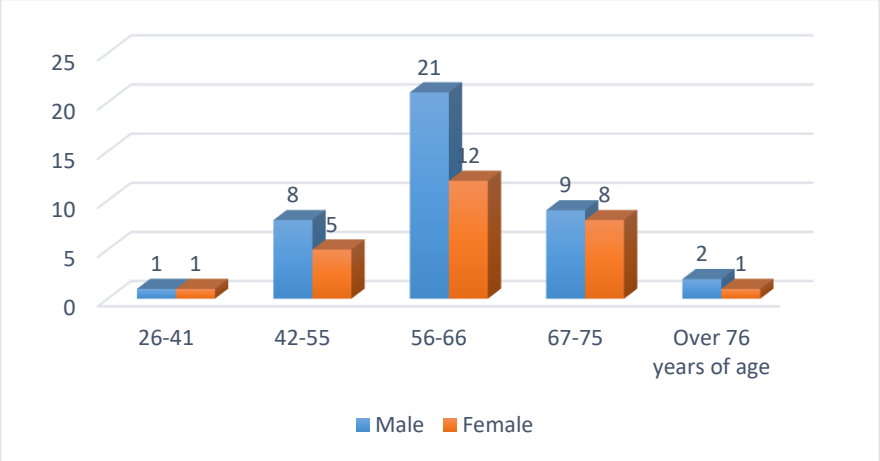

Fig.1. Distribution of patients by sex and age

Analysis based on gender revealed a higher prevalence of males in both groups. Among individuals aged 56-66 years, CCVI was significantly more common, and the number of male patients exceeded that of female patients.

The patients were examined in the Department of Angioneurology at the 2nd Clinic of the Tashkent Medical Academy and in the Department of Vascular Neurology and Vascular Surgery at the 1st Republican Clinical Hospital (fig. 1).

Clinical and paraclinical examinations were conducted for all patients. The degree of cerebrovascular insufficiency was determined based on the classification by A.V. Pokrovsky (1979). Paraclinical methods included MRI of the brain using angiography and duplex scanning of the brachiocephalic vessels. Molecular genetic studies were also conducted using polymerase chain reaction (PCR) at the Department of Molecular Medicine and Cell Technology of the National Institute of Genetics and Public Health of the Ministry of Health of Uzbekistan. Standard PCR with detection of amplification products on programmable thermocyclers from "Applied Biosystems" (USA) and "Corbett Research" (Quagen, Germany) was performed using DNA samples [3; 4; 6]. The Pearson chi-square test was used to determine differences in qualitative features. The Hardy-Weinberg law was applied to calculate allele and genotype frequencies. In both the main and control groups, the D allele (58% and 52%) was observed more frequently than the I allele (42% and 48%). The frequency of the NOS3 gene variant 4b/4a showed that the 4b allele was more common in both groups (86% and 98%), while the 4a allele was less frequent (14% and 2%). In the C/T 786 variant of the NOS3 gene, the T allele was more common in both groups (80% and 88%), while the C mutation allele was less frequent (20% and 12%).

Out of 68 patients, 45 (66%) underwent reconstructive surgeries for pathological tortuosity of extracranial arteries. In the remaining cases, conservative treatment was administered, including antioxidants, metabolics, blood rheology improving drugs, and cerebroprotective agents. Among the 68 patients, 12 (18%) had varying degrees of stenosis in the internal carotid arteries, while the other 56 (82%) had pathological deformations in the internal carotid arteries. Of these, 43 (77%) cases of pathological tortuosity of the carotid arteries were associated with stenosis, with such cases occurring in 32 (58%) men and 24 (42%) women.

Genes	N	Frequency of genotype spreads						
		Main group		Control group				
		N	%	N	%	P	χ^2	OR
Gen ACE I/D	I/I	23	34	12	29	0,7	0,08	1,2
	I/D	11	16	15	37	0,02	4,8	0,4
	D/D	34	50	14	34	0,1	2,0	1.5
Gen NOS34a/4b	4b/4b	49	72	40	95	0,006	7,5	0,7
	4b/4a	19	28	2	5	0,006	7,5	5,8
	4a/4a	-	-	-	-	-	-	-
Gen NOS3 C/T 786	T/T	44	65	31	76	0,3	0,9	0,8
	T/C	21	31	10	24	0,6	0,3	1,2
	C/C	3	4	-	-			

Table 1. Frequency of ACE (I/D), NOS3 4a/4b and C/T 786 genotypes in patients with CHF

Table 1 shows the distribution of three ACE gene genotypes: I/I, I/D, and D/D, with varying frequency in both the study and control groups. The D/D genotype is the most commonly observed in the study group (50%), followed by I/I (34%) and I/D (16%). In contrast, the control group is more likely to have the I/D genotype (37%), followed by D/D (34%) and I/I (29%). Comparison of the study and control groups revealed that the homozygous D/D mutation genotype is more common in patients than in healthy individuals (P=0.1, χ2=2.0, OR=1.5), and carriers of this genotype are 1.5 times more likely to develop CAD than those who do not have this genotype. Similarly, the non-mutational homozygous I/I genotype increases the risk of CAD by 1.2 times

compared to the control group. The heterozygous I/D variant is more frequently observed in healthy individuals (16% vs. 37%).

Research has identified two genotypes for the NOS3 gene: 4b/4b and 4a/4b, with no homozygous 4a/4a mutations detected in either group. The distribution of genotypes in both the study and control groups showed that the non-mutational 4b/4b variant prevails (72% and 95%) over the heterozygous 4a/4b variant (28% and 5%) (P=0.006; $\chi 2$=7.5; OR=5.8). Carriers of this genotype are 5.8 times more likely to develop CAD than those who do not have this genotype.

In the investigation of the NOS3 C/T786 gene, three genotypes were identified in the study group and two genotypes in the healthy control group since no mutation of this gene was found in the control group. The distribution of genotypes in both groups showed that the non-mutational T/T variant prevails (65% and 76%) over the heterozygous T/C variant (31% and 24%) (P=0.6, $\chi 2$=0.3, OR=1.2). Carriers of this genotype are 1.2 times more likely to develop CAD.

The homozygous C/C mutation variant was found only in the study group (4%).

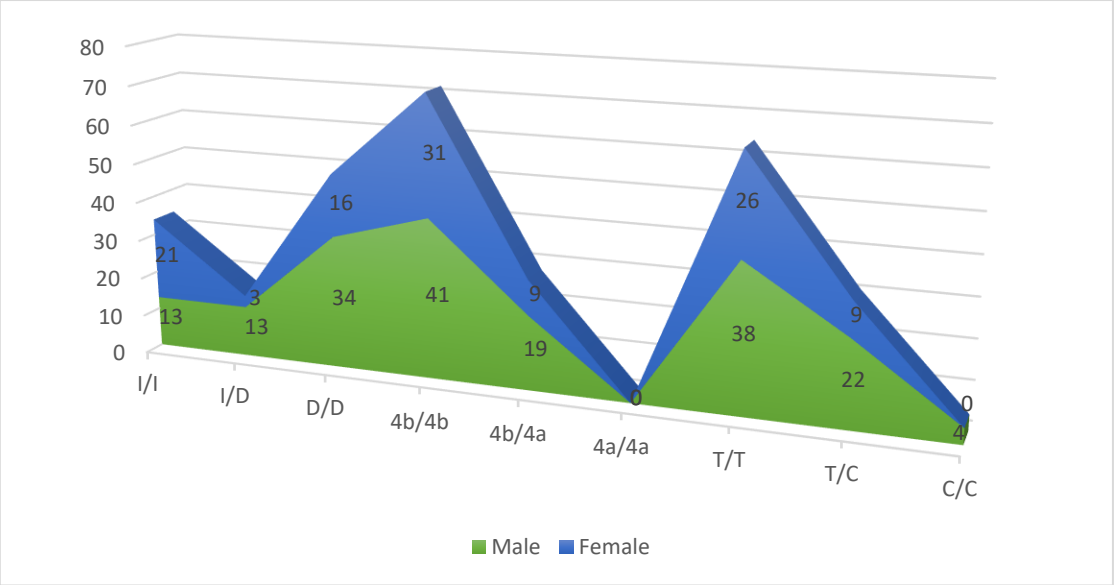

Figure 2. Incidence of combination of ACE (I/D), NOS3 4a/4b and NOS3 C/T 786 genotypes in patients with CHF as a function of gender

Figure 2 shows that, with the exception of the ACE gene genotype I/I, which is more prevalent in females, males predominate in all types of gene polymorphisms. The mutant variant 4a/4a was not detected. It is worth noting that the C/C genotype (mutant variant) of the NOS3 gene was found exclusively in male patients [14; 15; 16; 17].

All patients were divided according to the degree of cerebrovascular insufficiency (CVI) using the classification of A.V. Pokrovsky: there were no cases of asymptomatic CVI (stage I) in our patients. CVI stage II was detected in 15%, stage III in 46%, and stage IV in 39% of cases.

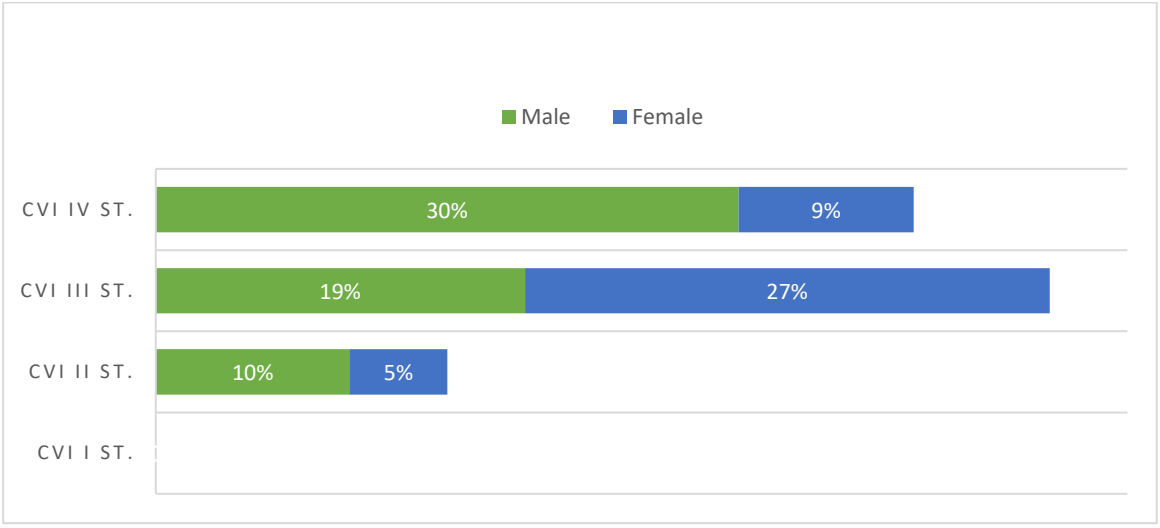

Fig.3. Frequency of CVI depending on gender.

The analysis of the frequency of occurrence of chronic cerebrovascular insufficiency by gender (Figure 3) revealed that stages II and IV of CCVI are more common in male patients (10% and 30%) compared to female patients (5% and 9%). However, the incidence of stage III CCVI is higher in female patients (27%) than in male patients (19%).

Gene Association			**CCVI 2**	**CCVI 3**	**CCVI 4**	**Total**
ACE I/D	**NOS3 4a/4b**	**NOS3 C/T786**				
Genotype I/I	4b/4b	T/T	2(3%)	9(13%)	4(6%)	15(22%)
	4b/4b	T/C	-	2(3%)	1(1,4%)	3(4,4%)
	4a/4b	T/T	-	-	1(1,4%)	1(1,4%)
	4a/4b	T/C	1(1,4%)	2(3%)	-	3(4.4%)
	4b/4b	C/C	-	-	1(1,4%)	1(1,4%)
Total			3(4,4%)	13(19%)	7(10,2)	23(34%)
Genotype I/D	4b/4b	T/T	-	2(3%)	1(1,4%)	3(4,4%)
	4a/4b	T/T	-	1(1,4%)	-	1(1,4%)
	4b/4b	T/C	-	1(1,4%)	2(3%)	3(4,4%)
	4a/4b	T/C	1(1,4%)	-	2(3%)	3(4,4%)
	4b/4b	C/C	-	1(1,4%)	-	1(1,4%)
Total			1(1,4%)	5(7%)	5(7%)	11(16%)
Genotype D/D	4b/4b	T/C	2(3%)	1(1,4%)	-	3(4,4%)
	4a/4b	T/T	1(1,4%)	2(3%)	2(3%)	5(7,4%)
	4b/4b	T/T	2(3%)	7(10%)	10(15%)	19(28%)
	4a/4b	T/C	1(1,4%)	2(3%)	3(4%)	6(8,4%)
	4b/4b	C/C	-	1(1,4%)		1(1,4%)
Total			6(9%)	13(19%)	15(22%)	34(50%)
Summary			10 (15%)	31 (46%)	27 (39%)	68 (100%)

Table 2. Manifestation of gene-gene interactions depending on the stage of CHMN

The study of gene-gene interactions of the investigated polymorphisms in relation to the degree of CCVI revealed (Table 2) that the mutant homozygous polymorphisms D/D and I/I of the ACE gene in combination with the 4b/4b and T/T polymorphisms of the NOS3 gene (13% and 6% of cases, 10% and 15%) were more frequently observed in patients with CCVI III and IV stages. The association of the I/D genotype of the ACE

gene with all genotypes (4b/4a) and (C/T786) of the NOS3 gene showed the lowest incidence rate of CCVI [10; 11; 12; 13].

3. Discussion:

Our data revealed that the D allele and DD genotype of the ACE gene were more frequent than the I allele and II and ID genotypes in the main group of patients. These results coincide with those of other authors [2,10], but there are contradictory data [7], where the frequency of the ID genotype is higher than that of the II and DD genotypes. Our results showed that the DD genotype was more common in men, while the II genotype was more common in women, compared to the results of authors [8], where no sex differences were detected for this polymorphism. In English and Chinese populations, the 4a allele of the eNOS 4a/4b gene polymorphism is considered an important risk factor for stroke development. The association between the –786T/T genotype and the risk of developing stroke is determined in African Americans [5; 9]]. These data are similar to our findings, where carriers of the 4b/4a and T/T genotypes of the NOS3 gene were more likely to have HSMN IV stage (strokes).

4. Conclusions of Genetic Polymorphisms Associated with Chronic Cerebrovascular Disease: Insights from Allele and Genotype Frequencies Analysis

1. The distribution of allele and genotype frequencies of the 4b/4a polymorphism of the NOS3 gene and I/D polymorphism of the ACE gene in patients and healthy individuals showed that the D allele and D/D genotype of the ACE gene and the 4b allele and 4b/4b genotype of the NOS3 gene are more common in patients with chronic cerebrovascular disease (CCVD) than in the control group.
2. The D/D genotype of the ACE gene and 4b/4b genotype of the NOS3 gene are more likely to cause III and IV stages of CCVD.
3. Carriers of the I/I and D/D genotypes in combination with the 4b/4b genotype have the highest incidence of III and IV stages of CCVD.
4. The combination of heterozygous genotypes (I/D and 4b/4a) has a low incidence of CCVD.
5. Chronic cerebrovascular insufficiency of II and IV stages was more commonly observed in male patients, while III stage was more commonly observed in female patients.

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