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ANALYSIS OF THE ROLE OF THE C807T POLYMORPHISM OF THE ITGA2 GENE WITH THE DEVELOPMENT OF VASCULAR THROMBOSES OF DIFFERENT LOCALIZATIONS.

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Abstract. The analysis of the genetic factor of the risk of developing vascular thrombosis of various localizations was carried out in 107 patients by studying the frequency of distribution of alleles and genotypes of the C807T polymorphism in the integrin alpha-2 (ITGA2) gene. In both groups, the actual distribution of C807T polymorphism genotypes corresponded to those expected at the Hardy-Weinberg equilibrium ($p<0.05$). A significant relationship was established between the risk of developing vascular thrombosis of various localization and the distribution of predisposing/protective variants of the C807T polymorphism genotypes in the ITGA2 gene. These data allow us to propose testing of this locus to predict the risk of thrombogenic complications in vascular thrombosis of various localizations (such as DVT, IS and MI).

Keywords: deep vein thrombosis of the lower extremities, ischemic stroke, myocardial infarction, genetic polymorphism C807T ITGA2.

The urgency of the problem. Despite the modern scientific progress in biology and medicine, thromboembolic diseases are still a global medical and social problem and are one of the main causes of mortality and disability of the population [6, 9]. Studies carried out in recent years have shown that every tenth inhabitant of the Earth experiences during his entire life such severe consequences of vascular thrombosis as ischemic stroke (IS), myocardial infarction (MI), deep vein thrombosis of the lower extremities (DVTL) [22]. The sudden onset and a very high rate of development of the thrombotic process are, perhaps, the main reasons that do not allow in most cases to effectively prevent the severe consequences of such a pathological process. In this regard, one of the most effective and promising methods of combating thromboembolic diseases (TEZ) is the timely implementation of preventive measures in persons with an increased risk of their development [1, 4, 11]. The studies carried out in recent years have significantly expanded our understanding of the molecular mechanisms of the formation of thrombogenic processes. This largely depends on the

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success in the study of hereditary thrombophilia as a genetic predisposition, which is the main cause of the endogenous risk of developing such complications [5, 7]. To date, there is a concept about the polygenic nature of hereditary thrombophilia, which shows the presence in most cases of ECD not one, but several genetic variants, which independently or jointly provoke the risk of developing such diseases [2, 12, 18]. All of the above substantiates the need for a fundamentally new approach to the study of the causes of genetic predisposition to thrombosis.

One of the most important tasks of studying the genetic nature of thromboembolic complications is to identify key regulator genes, in particular genes of the platelet hemostasis link, the products of which are the main products in the pathogenesis of vascular thrombosis of various localizations [10].

The integrin alpha-2 gene (ITGA2), also known as GPIa, is associated with altered platelet collagen binding receptor properties. This marker affects the adhesion of platelets to collagen and other substrates, and is also involved in the reorganization of the extracellular matrix. Genetic variants of ITGA2 can lead to changes in the kinetics of platelet adhesion. Having identified the genotype for this marker, it is possible to assess the genetic predisposition to myocardial infarction, stroke, vascular thromboembolism and take appropriate preventive measures [14].

Based on the above, we set ourselves the goal of assessing the frequency of carriage of the C807T genetic polymorphism of the platelet hemostasis gene ITGA2 in patients with vascular thrombosis of various localizations and its role in the development of thrombogenic complications.

Material and research methods. To solve the set tasks, we carried out a genetic study in 107 patients who were in the therapeutic, neurological and surgical departments of the clinic of the Andijan State Medical Institute and the Andijan branch of the Republican Scientific Center for Emergency Medical Aid, which made up the main group. In this study, all patients were divided into 3 subgroups, selected in accordance with the currently accepted recommendations: DVT of lower limb n = 35; AI n = 35; IM n = 37.

The control group consisted of 103 conditionally "healthy" persons without a TEZ at the present time and in anamnesis.

The diagnosis of DVT was carried out in accordance with the currently accepted recommendations and was established on the basis of the clinical picture and hemostasis, the results of Doppler ultrasound (USDG) of the vessels of the lower extremities. The diagnosis of IS was made on the basis of clinical and laboratory (hemostasological) data, the results of an MRI study. The diagnosis of myocardial infarction was made on the basis of the clinical picture, clinical and laboratory data, and ECG and ECHO-KG data.

Isolation of DNA from peripheral blood was carried out using a commercial kit of reagents "AmpliPrime RIBO-prep" (LLC "Interlabservice", Russia) according to the manufacturer's instructions. Testing of the rs1126643 polymorphism of the ITGA2 gene was carried out by allele-specific PCR in Real-Time format on a Rotor-Gene Q device (Quagen, Germany), using a commercial test kit from Syntol LLC (Russia), in accordance with the manufacturer's instructions.

Statistical processing of the results was performed using the standard software package OpenEpi V.9.2

The results obtained and their discussion. The results of calculating the deviations of the observed and expected frequencies of distribution of alleles and genotypes of the C807T polymorphism in the ITGA2 gene in the main and control groups showed that in the studied groups the actual distribution of the C807T polymorphism genotypes corresponded to those expected at Hardy-Weinberg equilibrium (RHB) ($p < 0.05$).

The data obtained show the mutual totality of the studied groups for the C807T polymorphism in the ITGA2 gene. In the studied groups of patients with vascular thrombosis of various localizations and in the control sample, there was no heterogeneity between the actually observed and theoretically expected values of the genotypes of the polymorphic variant C807T in the ITGA2 gene.

The associative analysis of the C807T locus in the ITGA2 gene in the studied groups of patients with DVT, IS, MI, and control was carried out using the case-

control design. The obtained results of isolation of this locus are presented in Table 1 and indicate the presence of a significant contribution of the unfavorable T allele and the associated T807T genotype to the development of vascular thrombosis of various localizations. In the general group of patients and controls, the proportion of C and T alleles was 55.1% and 44.9% versus 70.9% and 29.1%, respectively. Statistical processing revealed a significant decrease in the frequency of the wild C allele and a significant increase in the unfavorable T allele in patients with vascular thrombosis of various localizations as compared to conventionally healthy donors. The calculated odds ratio showed that the chance of detecting a functional unfavorable allele T among respondents in the main group increased significantly - 2 times compared with representatives of the control sample ($\chi 2 = 11.1$; $p = 0.001$; OR = 2.0; 95% CI: 1.32-2.96). This OR value was considered as a sign of an increased risk factor for thrombosis.

Table 1. Frequency of distribution of alleles and genotypes of C807T polymorphism in the ITGA2 gene in patient and control groups.

№	Group	Allele frequency Genotype distribution frequency				Allele frequency Genotype distribution frequency					
		C		T		C/C		C/T		T/T	
		N	%	n	%	N	%	n	%	n	%
1	The main group n = 107 of them:	118	55.1	96	44.9	34	31.8	50	46.7	23	21.5
1.1	DVTn=35	38	54.3	32	45.7	10	28.6	18	51.4	7	20.0
1.2	IS n=35	39	55.7	31	44.3	12	34.3	15	42.9	8	22.9
1.3	MI n=37	41	55.4	33	44.6	12	32.4	17	45.9	8	21.6
2	Control group n = 103	146	70.9	60	29.1	51	49.5	44	42.7	8	7.8

Table 2. Associative relationship of the C807T polymorphism of the ITGA2 gene with the development of TES.

Study groups Alleles and genotypes Statistical difference in relation to the control group	Study groups Alleles and genotypes Statistical difference in relation to the control group	Study groups Alleles and genotypes Statistical difference in relation to the control group			
		OR	95% CI:	χ^2	p-value
Main group (n = 107)	C	0.5	0.34 – 0.76	11.1	0.0008*
	T	2.0	1.32 – 2.96		
	C/C	0.5	0.27 – 0.83	6.8	0.009*
	C/T	1.7	0.94 – 3.09	3.1	0.08
	T/T	6.1	2.39 – 15.63	15.8	0.0001*
Deep vein thrombosis of the lower extremities (n = 35)	C	0.5	0.28 – 0.85	6.5	0.01*
	T	2.0	1.17 – 3.58		
	C/C	0.4	0.18 – 0.93	4.6	0.03*
	C/T	2.1	0.87 – 4.99	2.8	0.09
	T/T	4.5	1.32 – 15.12	6.3	0.01*
Ischemic stroke (n = 35)	C	0.5	0.30 – 0.90	5.4	0.02*
	T	1.9	1.11 – 3.38		
	C/C	0.5	0.24 – 1.18	2.4	0.12
	C/T	1.4	0.61 – 3.42	0.7	0.4
	T/T	4.2	1.33 – 13.62	6.5	0.01*
Myocardial infarction (n = 37)	C	0.5	0.30 – 0.88	5.9	0.015
	T	2.0	1.13 – 3.39		
	C/C	0.5	0.22 – 1.08	3.2	0.07
	C/T	1.6	0.71 – 3.81	1.3	0.25
	T/T	3.3	1.13- 9.49	5.2	0.02*

In the studied main group of patients with vascular thrombosis of various localizations and control of the frequency of C807C, C807T, T807T genotypes were: 31.8%, 46.7% and 21.5% versus 49.5%, 42.7% and 7.8%, respectively. A significant decrease in the number of ancestral homozygote C807C in the main group compared with the control group was revealed, which indicates the association of this variant with the absence of risk and the possible protective effect of this genotype in relation to the formation of DVTHL, IS and MI ($\chi 2 = 6.9$; $p = 0.01$; OR = 0.5 ; 95% CI: 0.27-0.83) (Table 2).

There was a tendency to an increase in the proportion of carriers of the unfavorable C807T genotype among patients compared with the control group - 46.7% versus 42.7%, respectively ($\chi2 = 3.1$; $p = 0.08$; OR = 1.7; 95% CI: 0.94-3.09). The detection of such a genotype poses a small risk of thrombosis (Table 2).

The frequency of the unfavorable genotype T807T among patients with vascular thrombosis of various localizations was significantly higher than in the control group - 6.1 times (21.5% versus 7.8%, respectively, with $\chi2 = 15.8$; $p = 0.0001$; OR = 6.1; 95% CI: 2.39-15.6), which indicates the presence of an increased risk of thrombogenic complications with the carriage of this genotypic variant.

Dividing the main group of patients into subgroups increases the OR values and allows a more accurate assessment of the level of association. Therefore, the next stage of our work was a comparative analysis of the polymorphism of the genotypes of the C807T polymorphism in the ITGA2 gene in the subgroups of patients with DVT, IS and MI.

As can be seen from Table 1, in patients with DVT of lower limb, in the studied group of patients and controls, the proportion of C and T alleles was 54.3% and 45.7% versus 70.9% and 29.1%, respectively. Statistical processing of the results revealed a significant decrease in the frequency of the favorable C allele and a significant increase in the unfavorable T allele in patients with DVT of lower limb compared to conventionally healthy donors. The calculated odds ratio showed that the chance of detecting a functional unfavorable allele T in respondents with DVT of lower limb significantly increased 2 times compared with representatives of the control group ($\chi2 = 6.5$; $p = 0.01$; OR = 2.0; 95% CI: 1.17-3.58). This value indicates an increased risk factor for thrombosis. The frequencies of C807C, C807T, T807T genotypes in the studied groups of patients with DVT of lower limb and control were: 28.6%, 51.4% and 20.0% versus 49.5%, 42.7% and 7.8%, respectively. As can be seen, the frequency of the ancestral genotype C807C among patients with DVT of lower limb was significantly lower than in the control group ($\chi2 = 4.6$; $p = 0.03$; OR = 0.4; 95% CI: 0.18-0.93), which indicates a protective effect of this genotype against formation of TGVNK. Compared with the control, in the DVTL subgroup, a

tendency to an increase in the content of the unfavorable C807T genotype was revealed ($\chi 2$ = 2.8; p = 0.09; OR = 2.1; 95% CI: 0.87-4.99). A significant high frequency of the unfavorable homozygous T807T genotype was revealed among patients with DVT of lower limb ($\chi 2$ = 6.3; p = 0.01; OR = 4.5; 95% CI: 1.32-15.12), which indicates a high risk of thrombotic complications with the carriage of this genotype in the ITGA2 gene (Table 2).

In patients with IS in the studied groups of patients and controls, the proportion of C and T alleles was 55.7% and 44.3% versus 70.9% and 29.1%, respectively (Table 1). The calculated odds ratio showed that the chance of detecting a functional unfavorable allele T in respondents with IS significantly increased (1.9 times more) compared with representatives of the control group ($\chi 2$ = 5.4; p = 0.02; OR = 1.9; 95% CI: 1.11- 3.38). The frequencies of C807C, C807T, T807T genotypes rs1126643 of the ITGA2 gene in the studied subgroup of patients with IS and controls were 34.3%, 42.9% and 22.9% versus 49.5%, 42.7% and 7.8%, respectively. As can be seen, the frequency of the ancestral genotype C807C among patients with IS was also slightly lower than in the control group ($\chi 2$ = 2.4; p = 0.12; OR = 0.5; 95% CI: 0.24-1.18). There was a significant increase (4.2 times) in the amount of unfavorable homozygous T807T genotype in patients with IS compared with the control group - 22.9% versus 7.8%, respectively ($\chi 2$ = 6.5; p = 0.01; OR = 4.2; 95% CI: 1.33-13.62) ...

The same picture was observed in the subgroup of patients with MI. The calculated relative risk of developing myocardial infarction was observed when an unfavorable C807T genotype was detected and sharply increased in the presence of an unfavorable T allele and a mutant T807T genotype.

When a functional unfavorable allele T was detected, the risk of developing this pathology significantly increased (2.0 times more) in patients with MI compared with representatives of the control group ($\chi 2$ = 5.9; p = 0.015; OR = 2.0; 95% CI: 1.13 - 3.39) ...

The distribution frequencies of C807C, C807T, T807T genotypes in the studied subgroup of patients with MI and controls were: 32.4%, 45.9% and 21.6% versus 49.5%, 42.7% and 7.8%, respectively.

An insignificant increase in the unfavorable C / T genotype was found among patients in the subgroup with MI; in the presence of an unfavorable C / T genotype, the relative risk of developing this pathology increased 1.6 times as compared with the control group ($\chi 2 = 1.3$; $p = 0.025$; OR = 1.6; 95% CI: 0.71 - 3.81). ...

The calculated relative chance of detecting a functional "weak T807T genotype among patients with MI compared to conventionally healthy individuals was OR = 3.3 at 95% CI: 1.13-9.498 ($\chi 2 = 5.2$; $p = 0.02$).

Thus, in patients with DVT, IS and MI, compared with the control group, a significant increase in the incidence of the unfavorable homozygous T807T genotype of the rs1126643 polymorphism in the ITGA2 gene with a significant risk of developing TES was revealed, which can be considered a factor of increased risk of thromboembolic complications leading to the development of data. pathologies.

Our results are consistent with the data of the authors [13, 19, 20], where a significant association of the C807T polymorphism in the ITGA2 gene with the risk of development, formation and clinical variants of various thromboembolic complications leading to vascular complications of various localizations was found. At the same time, our results do not completely coincide with the opinion of the authors [8, 23], where the association between this polymorphism and impairment of the hemostatic system, the formation of thrombosis in cardiovascular pathology is critically examined. Thus, Nikolopoulos GK et all. 2007 [17] in their studies did not find a correlation between the ITGA2 C807T polymorphism and the incidence of ischemic stroke. Recent studies have reported opposite results regarding the association of polymorphisms in two integrin genes, ITGA2 and ITGB3, with ischemic stroke [15].

The reason for this uncertainty and ambiguity of research results may be a population peculiarity, insufficient substantiation of the effect of the ITGA2 gene on the change in the rate of platelet adhesion. Perhaps, the studied polymorphism is not a

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"main" one, but a modifier that affects a region of the DNA sequence of the ITGA2 gene.

At the same time, some researchers believe that genotyping for individual polymorphisms is not informative for the clinical assessment of the individual risk of developing thrombotic disease and its complications [16, 21]. Therefore, it is advisable to carry out genotyping of patients for other markers of hemostasis disorder. They consider the characterization of not only individual candidate genes, but also the complex interactions between them, as a more pathogenetically grounded approach to the study of such diseases.

The discrepancy between the data of various studies is also explained by the fact that the genetic risk of thromboembolic diseases is not based on the effect of one gene, but on interactions between pathophysiological pathways controlled by several genes and other external risk factors [3]. Therefore, we consider it necessary to continue the work on the study of complex molecular mechanisms leading to the development of ECD with a complex etiology.

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