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Study of the Role of Polymorphism of ACE, GP1BA, PDE4D Genes and Clinical Features in the Development of Cerebrovascular Diseases

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Summary: The frequency of cerebrovascular diseases remains high in developed countries. Currently, studies are devoted to the study of factors predisposing to the development of cerebrovascular diseases, they include the study of both modified and not modified, including genetic ones. On the basis of the in Stroke center of N. I. Pirogov City Clinical Hospital №1, Moscow, two groups of patients were examined - 91 patients with chronic cerebral ischemia and 69 patients with ischemic stroke. Associations of polymorphisms of ACE, GP1BA, PDE4D genes with the course of cerebrovascular diseases were identified.

Keywords: Stroke, Chronic Cerebral Ischemia, Risk Factors, PDE4D, ACE, GP1BA

Introduction

Currently, special attention is paid to the study of risk factors that predispose to the development of various socially significant diseases, they include the study of both modified and not modified, including genetic ones.

According to WHO, chronic and acute forms of cerebrovascular diseases occupy leading positions in the overall morbidity structure throughout the world, especially in developed countries, and result in a high percentage of mortality and disability, thereby causing a high proportion of expenses for treatment and rehabilitation of this group of patients [1, 2].

In addition to vascular diseases of the brain, diseases leading to their development also occupy leading places, such as arterial hypertension, vascular atherosclerosis, and various forms of cardiac arrhythmias [3, 4, 5]. The progression of these diseases leads to damage to the vascular endothelium, which is the main regulatory component of the balance of blood coagulation and anticoagulation systems, which is the main pathogenetic element of pathological thrombosis and as a consequence of the development of thrombosis of both large and small vessels, leading to the development of not only acute strokes but also progression of chronic cerebral ischemia.

Considering the number and variety of factors and diseases leading to the development of cerebrovascular diseases in the world, the concept of a multidisciplinary approach to research is applied, these include areas in the clinical neurology, pharmacology, molecular biology, genetics, biochemistry and other disciplines.

A large amount of data has been accumulated on the influence of genetic factors in the development of cerebrovascular pathology [6] (Figure 1). The relationship of genetic polymorphism with the risk of ischemic stroke in general and its subtypes according to the TOAST [7] classification - large-artery atherosclerosis stroke, against the background of marked cerebral atherosclerosis and cardioembolic, caused by various types of cardiac rhythm disturbances was shown.

There is evidence from scientific studies on the association of the polymorphism of the PDE4D gene with the risk of development and prognosis of the disease of chronic cerebral ischemia and ischemic stroke, for different populations and age groups. The PDE4D gene encodes the enzyme phosphodiesterase,

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which is involved in the regulation of the cellular level of cAMP in many cell types, including vascular smooth muscle cells, endothelial cells, and macrophages. The enzyme activity affects the processes of inflammation, the response to vascular damage, the stability of the atherosclerotic plaque, the permeability of the blood-brain barrier.

The GPIb / V / IX complex is responsible for the adhesion of platelets to the damaged vessel walls at the first stage of hemostasis, and, thus, an increase in its expression can enhance the processes of thrombus formation. The allele C of the GP1BA gene (rs2243093, -5T / C) is characterized by a significant increase in the expression of the GPIb / V / IX complex on the platelet surface.Thus, the purpose of our work was to study the relationship of polymorphic markers in the genes ACE, GP1BA, PDE4D with the clinical features of cerebrovascular diseases - ischemic stroke and chronic cerebral ischemia.

Material and methods:
The study was conducted in Stroke center of N. I. Pirogov City Clinical Hospital №1, Moscow. 91 patients were included in the group of patients with chronic cerebral ischemia, with an average age of 63 ± 7.3 years, and women - 60% who had not previously suffered strokes. Among which were subgroups of patients according to the nature of changes in blood pressure: patients with a tendency to reduced blood pressure (less than 110 mm Hg systolic pressure) without drug therapy - 11 patients, patients with normal blood pressure (115 - 125 mm Hg). systolic blood pressure - 15 patients and 65 patients with a clinical diagnosis of arterial hypertension according to the current classification. Then, among the patients with arterial hypertension, two groups were distinguished according to their clinical features of arterial hypertension: with rapid progressive course, frequently hypertensive crises with high blood pressure (18 patients) and a slow flow persistently elevated blood pressure numbers that is difficult to correct medication (38 patients).

The group of patients who had ischemic stroke consisted of 69 patients, the average age was 63.5 ± 10 years, women - 52%, men 48%. From these patients, subgroups of patients with the main and most common causes leading to ischemic stroke — vascular atherosclerosis and heart rhythm disturbances — that completely met the criteria for TOAST, the classification used worldwide for verification of the pathogenetic subtype of ischemic stroke, were distinguished: 1) patients with large-artery atherosclerosis subtype of stroke (28 patients) and cardioembolic (against the background of the permanent form of atrial fibrillation - 26 patients) stroke, 15 patients had an unspecified pathogenetic subtype of ischemic stroke, in which, after further examination did not reveal the sources of embolism - atherosclerosis and heart rhythm disturbances. In the subgroup of patients with large-artery atherosclerosis subtype of stroke, the average score for admission on the NIHSS scale ranged from 5 to 15 points, in the subgroup with the cardioembolic subtype - from 10 points and above. There were also subgroups of patients with different degrees of stenosis of the brachiocephalic arteries: with occlusion and hemodynamically significant stenosis (more than 75%) (17 patients) and with hemodynamically insignificant stenosis (less than 75%) (42 patients). Further, a comparative analysis of these groups of patients (Figure 2). The patient's neurological status, the degree of cognitive impairment on the MMSE Mental Status Scale, and the stress state on the MacLean scale were assessed.

Molecular genetic research was performed on the basis of the Engelhardt Institute of Molecular Biology, Russian Academy of Sciences. DNA was isolated from blood by the standard method of phenol-chloroform extraction. Genotyping was performed by a two-step nest polymerase chain reaction (PCR) followed by allele-specific hybridization on a biochip, as described previously. The biochip is designed to identify 21 polymorphisms in the following genes: *ACE* (rs1799752), *FGB* (rs1800790), *F5* (rs6025), *F7* (rs6046), *F12* (rs1801020), *GP1BA* (rs2243093), *GPIIIa* (rs5918), *SERPINE1* (rs1799768), *MTHFR* (rs1801133), *CYP11B2* (rs1799998), *PON1* (rs662), *PON2* (rs1801282), *NOS2* (rs2297518), *NOS3* (rs1799983), *PDE4D* (rs966221, rs2910829), *HIF1a* (rs11549465, rs11549467), *LTA* (rs909253), *ALOX5AP* (rs4769874).

For statistical data processing, GraphPad InStat, version 3.05 (GraphPad SoftwareInc., USA) was used. A two-sided Fisher exact test was performed and the OR values (odds ratio, odds ratio) were calculated with a 95% confidence interval (95% CI). The critical level of statistical significance was assumed to be 0.05. The study protocol was approved by the ethical committee of Pirogov Russian National Research Medical University (RNRMU).

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Results and discussion:

When comparing allele frequencies, it was found that the T / T genotype of the PDE4D gene (rs966221, SNP83 C> T) is more common in patients with chronic cerebral ischemia and a progressive course of arterial hypertension (8 out of 17) compared with chronic cerebral ischemia and slow arterial hypertension the difference is statistically significant (OR = 6.22; CI = 1.86–20.79; p = 0.0036).

The identified T / T genotype association of the PDE4D gene (rs966221, SNP83 C> T) with the risk of developing a progressive course of chronic cerebral ischemia may be due to a change in the PDE4D enzyme activity. To clarify the role of SNP83C> T polymorphism of the PDE4D gene as a risk factor for the progressive flow of hypertension in patients with chronic cerebral ischemia requires further research.

Patients with the DD genotype of the ACE gene were more common in the group with cardioembolic stroke (12 patients out of 26) compared with patients with large-artery atherosclerosis subtype of stroke (3 patients out of 28), the differences were statistically significant (OR = 7.14, 95% CI = 1.72 -29.69, p = 0.0057). The frequency of allele D was also higher in the group of patients with cardioembolic stroke (61.5%) compared with patients with large-artery atherosclerosis subtype of stroke (37.5%) (OR = 2.67, 95% CI = 1.23-5.8, p = 0.02), the differences are statistically significant.

The D allele of the ACE gene, which encodes the angiotensin-converting enzyme (ACE), is characterized by increased expression compared to allele I. A number of studies and meta-analyzes have shown an association between the I> D polymorphism of the ACE gene and the risk of hemorrhagic and ischemic stroke development, predicted outcome of ischemic stroke. To date, the question of the role of the I / D polymorphism of the ACE gene in the development of ischemic stroke remains controversial. Our findings suggest that allele D may act as a genetic marker of the risk of developing a cardioembolic subtype of ischemic stroke.

When comparing groups of patients with different degrees of stenosis of the brachiocephalic arteries, the allele C of the GP1BA gene (rs2243093, -5T / C) was found to be more common in patients with occlusion of brachiocephalic arteries and severe hemodynamically significant stenosis (24%) compared with patients with hemodynamically insignificant stenosis (8%) (OR = 3.39, 95% CI = 1.12-10.25, p = 0.03). In the studied sample, there were no patients with the C allele in the homozygous state, but even the presence of this allele in the heterozygous state was associated with occlusion of the brachiocephalic arteries and severe hemodynamically significant stenosis: the TC genotype was detected in 8 of 17 patients with stenosis and in 7 of 42 patients with insignificant stenosis or without it (OR = 4.44, 95% CI = 1.27-15.54, p = 0.02).

According to various sources, data on the effect of GPIBA gene polymorphism on the risk of developing ischemic stroke are contradictory. In our study, an increased risk of thrombosis in the presence of the C1BA gene allele C was a significant risk factor for the development of stroke in combination with impaired hemodynamics in the main vessels (critical stenosis), including severe stenosis or occlusion of the brachiocephalic arteries.

Thus, as a result of the study, loci associated with the development of cardioembolic and large-artery atherosclerosis subtypes of stroke according to TOAST criteria were identified. Polymorphic markers were identified in the genes GP1BA and ACE, which are associated with the clinical features of arterial hypertension and with the pathogenetic subtypes of ischemic stroke, in the presence of unmodified risk factors.

The identified clinical and genetic markers of the risk of the development and nature of the cerebrovascular process are of interest for further study, as they are of particular importance for predicting the course of the disease.

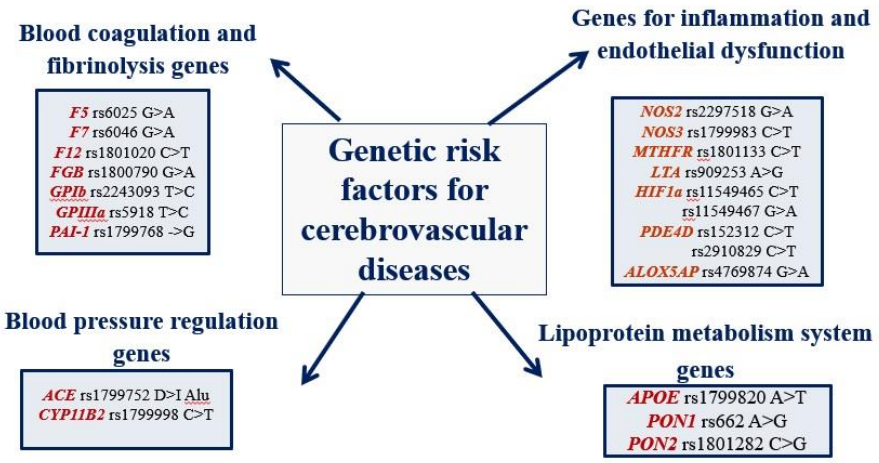

Figure 1. Genetic risk factors for cerebrovascular diseases

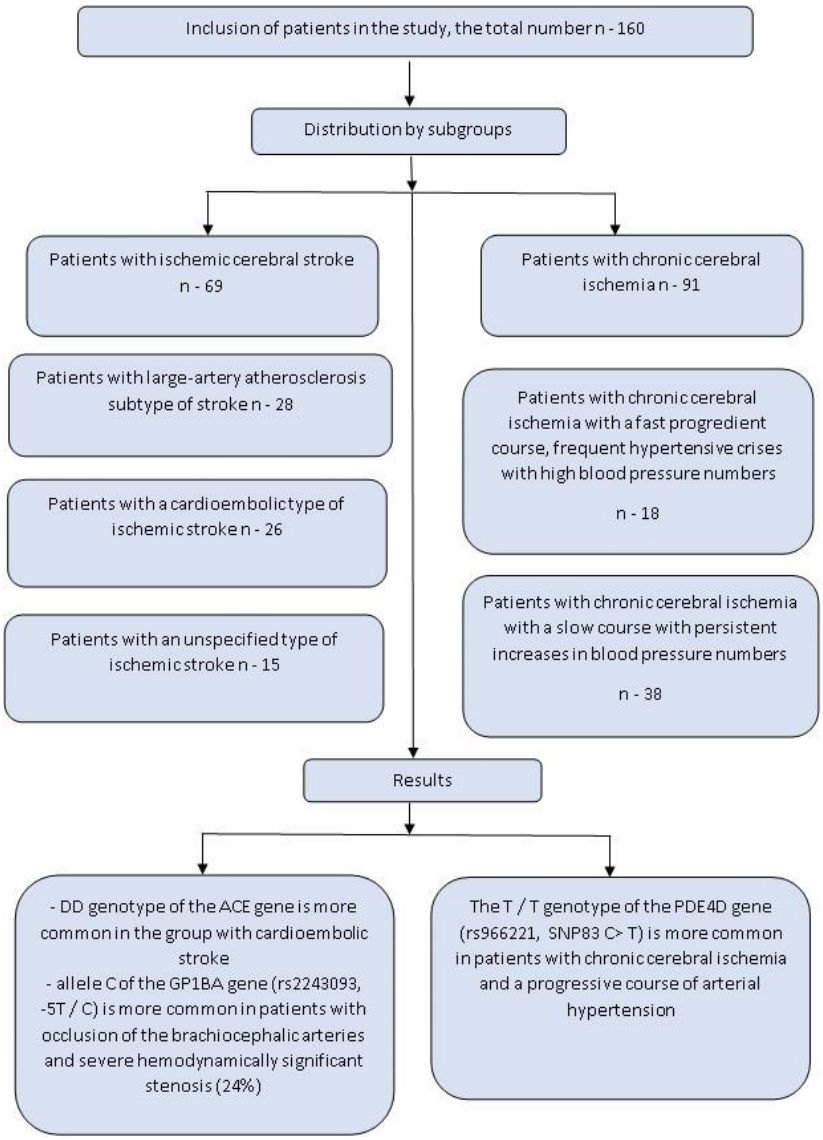

Figure 2. Study Design

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