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Study of Morphofunctional Parameters of Blood Cells in Ischemic Stroke Using a Genome-wide Research

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Biomarkers can play many useful roles in modern neurology. Early diagnosis and immediate therapy are important factors for reducing the degree of brain tissue damage in ischemic stroke, reduces the risk of death from stroke. In the current study, apolipoprotein CIII (ApoCIII), a biomarker of ischemic stroke, was found.

Keywords: Biomarkers; brain; fibrinolysis; DNA; ischemic stroke; genome-wide sequencing.

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1. INTRODUCTION

Cardiovascular diseases continue to be the main cause of disability and death in all countries of the world. According to WHO estimates, in 2012, CVD claimed the lives of 17.5 million people, which accounted for 31% of all deaths in the world. According to WHO data for 2021, more than half of the cases caused by sudden deaths in the world are caused by coronary heart disease and stroke. These diseases have not lost their leading positions over the past decades. According to Russian statistics, Russians die most often due to diseases related to the cardiovascular system. Ischemic pathology accounts for 28.4 percent of deaths [1]. In this regard, in recent years, scientists have been actively searching for biological markers that would allow identifying the atherosclerotic process at the early stages of its occurrence, and thereby screening patients with further stratification of risk groups [2].

As part of the search for universal diagnostic markers that would allow diagnosing and predicting the course of stroke with different clinical manifestations, it is necessary to update the study of genetic markers of oxidative stress as predictors of cardiovascular catastrophes [3]. Then develop technologies for routine diagnostics based on new data suitable for implementation in general medical practice [4].

In the foreign literature there are indications of associations of the development of ischemic stroke and its more severe course, including the carriage of polymorphic gene variants: glutathione peroxidase (GPXC599T) [5], hypoxia-induced factor (HIF1a C1772T), NADPH-H oxidase (p22phox C242T), manganese superoxide dismutase (MnSOD C47T) [6].

Substantiation of the prospects for the study of biomarkers of cardiovascular catastrophes of various genesis

The methods currently used in the diagnosis of cardiovascular brain catastrophes are not effective enough and, moreover, take too much time. There is a need to search for new types of diagnostics that will be much better than today's existing protocols and tools [7].

The discovery of sensitive and specific biomarkers of ischemic stroke of the brain will improve the results of the therapeutic strategy and will help to assess the progress or complications of the disease [8]. Relevant diagnosis of ischemic stroke within the first 4.5 hours after the appearance of the first symptoms makes it possible to start treatment with recombinant tissue plasminogen activators [9], which limits the magnitude of negative changes in the brain, increases the effectiveness of treatment [10].

The studied potential biomarkers are substances involved in the processes of coagulation and fibrinolysis, as well as molecules released from damaged vascular endothelial cells, nerves and cardiac tissue. The analyzed substances are characterized by oxidative stress, apoptosis, excitotoxicity and damage to the blood-brain barrier.

In addition to the CRP protein, Pentraxin-3 (PTX3) is a protein of the acute phase of inflammation. However, unlike CRP, this is not produced in the liver, but is generated locally at the crash site [11]. The synthesis of PTX-3 is under the stimulating influence of interleukin-1β (IL-1β), TNF-α and lipopolysaccharides, while interleukin-4 (IL-4), interleukin-13 (IL-13) and IFN-γ inhibit the production of PTX-3-process [12]. Elevated levels of pentraxin-3 are independently associated with increased mortality after ischemic stroke, so it could be used as a prognostic factor [13].

Another well-known acute phase protein is lipocalin associated with neutrophil gelatinase (NGAL). Its secretion increases with inflammation and damage to the endothelium. Studies show that NGAL may be useful for distinguishing ischemic stroke from hemorrhagic stroke [14].

Another representative who claims to become a biomarker in the diagnosis of cardiovascular catastrophe: osteoprotegrin - glycoprotein, which is a representative of the superfamily of tumor necrosis factor α receptors. In recent years, data on the possible role of osteoproterin in the development of cardiovascular diseases have begun to appear in the literature. Its role in increasing the level of osteoprotegerin in the development and progression of atherosclerosis and as a consequence of coronary heart disease, strokes and chronic heart failure has been proven [15].

In recent years, studies have been conducted that have shown that a high concentration of osteoprotegrin in blood plasma correlates with

the severity of atherosclerotic lesions of peripheral arteries, the severity of heart failure and carotid artery stenosis, unstable angina and acute myocardial infarction [16].

Elderly patients with chronic heart failure were examined as part of a large randomized placebo-controlled CORONA trial (2011). The obtained results demonstrated the relationship of osteoprotegrin levels with age, low body mass index, functional class of chronic heart failure, left ventricular ejection fraction, heart rate, glomerular filtration rate, levels of very low density lipoproteins, triglycerides, NT-proBNP, C-reactive protein [17]. The authors also showed that the level of osteoprotegrin is a risk factor for the progression of heart failure and an increase in the frequency of hospitalizations [18].

However, the findings should be interpreted with caution: despite the large sample size and the large number of outcomes studied, the study was conducted among patients older than 60 years with systolic heart failure and the results obtained cannot be applied in patients with chronic heart failure and preserved left ventricular ejection fraction [19]. In the same study, the effect of statins (rosuvastatin) on the concentration of osteoprotegrin was studied. Rosuvastatin significantly improved the lipid composition of the blood in patients, but the level of osteoprotegrin did not change statistically significantly [20].

At the same time, B. Nellemann and co-authors published data that low doses of statins reduce the level of the marker in patients with type 2 diabetes with microalbuminuria and hypercholesterolemia [17].

Research objectives:

- To identify patterns of changes in ischemic stroke at the genetic, molecular and cellular levels;
- To assess the risk of stroke in the population of different ethnic groups (ethnic Russians and Caucasian nationalities) in the North Caucasus Federal District and people of different ages;
- Statistically process patient data on the main indicators of blood clotting: plasma and cellular clotting factors;
- If possible, to identify new targets (markers) of blood clotting when exposed to drugs administered during stroke (thrombolytics, antiplatelet agents and anticoagulants) on the morphofunctional parameters of platelets;
- If possible, identify a new gene marker for the prevention and diagnosis of ischemic stroke.

2. MATERIALS AND METHODS

The immediate subject of the search for the identification of associative links are single-nucleotide (point) bases in genes (SNP) that determine the components of a particular molecular biological system. Substitution of one nucleotide in one or both alleles of a gene causes the mutation effect. There is a decrease in the functional activity of the encoded molecule, therefore, options for strengthening the function or its complete disappearance are much less possible.

Based on the known pathogenetic mechanisms of ischemic stroke development, the research is aimed at the main enzyme-metabolic systems: inflammatory cascade, hemorheological reactions, oxidative stress.

The main method of research is genome-wide sequencing. Massive parallel sequencing, otherwise referred to as next generation sequencing (NGS), refers to the methods that have appeared in the last decade. Previously, for sequencing, the method of termination of the growing chain developed by Frederick Sanger. Sequencing platforms using the method Sanger, reads the nucleotide sequence with high accuracy, but low productivity. NGS technologies allow simultaneously determine the nucleotide sequences of many different DNA strands during the implementation of processes synthesis or ligation of DNA that provides reading billion nucleotides per day.

To date, within the framework of the associative approach, more than 300 candidate genes for the development of ischemic heart disease, as well as over 32 loci identified in the course of GWAS studies. However, despite the huge amount of work on this topic, performed in leading laboratories around the world, data on the influence of certain genes on the development of ischemic heart disease in most cases are contradictory nature. The reason for this is ethnic differences; pleiotropic effect genes; the presence of subclinical phenotypes; unaccounted environmental factors, influencing the phenotypic manifestation of genetic characteristics individual, etc.

2.1 Cell Samples

1 ml of peripheral blood (about 200 µl is used for the first analysis) in a test tube for coagulological studies (vacutainer with sodium citrate), hematological studies (with EDTA and K3), biochemical studies (EDTA K3) and genetic studies (EDTA K3) [21]. DNA isolation is carried out using commercially available kits (DNeasy Blood and Tissue Kit, Qiagen) in accordance with the manufacturer's recommendations. The study material is genotype II of the ACE polymorphic gene (I/D), which is an independent predictor of a favorable 12-month outcome of the STEMI. The carriage of the Ser allele of the polymorphic gene ADRB1 Ser49Gly is associated with an increase in the frequency of adverse cardiovascular events within 12 months after STEMI. Polymorphisms of the genes SLCO1B1 (val174ala), CYP2C19*2, CYP2C19*3, ADRB1 (Arg389Gly), LIPS (C514T) do not affect the 12-month prognosis after STEMI.

200 samples of biological material (blood, saliva) were used in the work.

The following scenarios are set in the work:

1) Selection of calculation protocols capable of describing experimental data for the objects under consideration and predicting the properties of new protein systems with the required accuracy; 2) Analysis of polymorphisms in ACE and AGT genes; 3) In silico modeling, using neural networks and supercomputer calculations, the development of myocardial infarction, based on the data obtained after DNA sequencing.

Methods of determination: 1) Genome-wide sequencing); 2) PCR and restriction analysis; 3) Targeted gene sequencing.

Target markers: apolipoprotein CIII (ApoCIII) is normally present in blood plasma at a concentration of 0.1 g/l, and is mainly found in VLDL and in HDL and LDL particles. This is due to atherogenesis.

3. RESULTS AND DISCUSSION

In our study, apolipoprotein CIII (Apo CIII), a biomarker of ischemic stroke, was found. The clinical significance of all candidate markers should be thoroughly tested in future controlled clinical trials with analysis focused on focused clinical questions. biomarkers of cerebral circulation, such as NR2-peptide and NR2-antibodies, can become key components of a successful treatment strategy and monitoring of disease outcomes.

More than 95% of plasma ApoAII is associated with HDL, which is ~ 20% of the total mass of HDL. Concentration ApoAII in human blood plasma at normal levels lipids is ~ 30 mg / dL and is largely determined by genetic factors. Studies of the physiological effects of ApoAII have proven their participation in the reverse transport of cholesterol, as well as their antioxidant, anti-inflammatory and other properties, due to which HDL is believed to have antiatherogenic properties. Our study found no significant differences in the distribution of genotypes and alleles of lipid metabolism genes (APO CIII, APO E) in hypertensive patients who underwent stroke, and in a group of healthy children and adolescents, which may indicate the absence of the relationship between the polymorphism of lipid metabolism genes and the development of stroke in patients with hypertension. Required further study of their influence on the development of cardiovascular pathology. An extremely important role in maintaining normal hemostasis is played by the vascular endothelium. One of the factors leading to its damage and, accordingly, to increased thrombus formation, is an increase in the concentration of homocysteine.

In an expanded study, an attempt was made to identify new targets (markers) of blood clotting in ischemic stroke, morphofunctional parameters of platelets, which may be useful in the treatment of AI and prevention of venous thromboembolism.

Target markers were found: apolipoprotein CIII (Apo CIII) is normally present in blood plasma at a concentration of 0.1 g/l, and is mainly found in VLDL and in HDL and LDL particles. This is due to atherogenesis.

Based on the known pathogenetic mechanisms of ischemic stroke development, the research is aimed at the main enzyme-metabolic systems: inflammatory cascade, hemorheological reactions, oxidative stress, molecular mechanisms of signaling interactions [22].

4. CONCLUSION

Biomarkers can play many useful roles in modern neurology. Early diagnosis and immediate therapy are important factors for reducing the degree of brain tissue damage in

ischemic stroke, reduces the risk of death from stroke. In addition to the clinical benefits, the financial benefits cannot be ignored, because when creating new diagnostic systems to determine the cardiovascular threat, doctors will be able to find such patients much faster, provide appropriate treatment and prevent economic gaps in the economy and job losses in case of illness or death.

CONSENT

It is not applicable.

ETHICAL APPROVAL

As per international standard or university standard written ethical approval has been collected and preserved by the author(s).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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