Features of Genotyping of Complications of The Metabolic Syndrome at The Preclinical Stage

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Received: 07 May 2019; Accepted: 25 May 2019


ABSTRACT

Use of the principles personalized medicine for preventing such widespread conditions as metabol-ic syndrome (MS) representing a complex of pathogenetically interrelated metabolic disorders - abdominal obesity, hypertension, insulin resistance, dislipidemia, actual, as according a number of large epidemiological studies it with products versus about 20 to 45% of fatal complications.

MS, allocation of a separate pathology is of great clinical value, claim about Since, on the one hand, this condition is reversible, that is, with appropriate therapy, it is possible to reduce the se-verity of its main manifestations, on the other hand, the presence of MS acts as the main cause of high cardiometabolic risk, combining the risk cardiovascular diseases and the risk of developing diabetes mellitus (DM) type 2, which are the main causes of increased mortality in industrialized countries. With the development of MS, there is a 5-fold increase in the risk of type 2 diabetes and a 2-fold increase in the risk of developing cardiovascular diseases over the next 5-10 years. In ad-dition, in patients with MS, the risk of stroke increases by 2-4 times, by 3-4 times myocardial in-farction, the risk of death from these diseases increases by 2 times compared with patients without MS, regardless of the history of cardiovascular events.

Based on the monitoring of individuals after genetic testing and a critical analysis of the results of a prospective genetic analysis, information about the possibilities of pre-symptomatic (prognostic) genetic testing becomes more and more practical. The obtained genotyping data allowed to evaluate the effectiveness of preclinical diagnosis and determine the tactics of treating patients.

Materials and Methods: Release of DNA for the subsequent genotyping is made of whole blood by sets of «S-Sorb» reagents of the «Sintol» company with use of a sorbent of dioxide of silicon for 135 patients of State-funded health institution "Central City Clinical Hospital" Veliky Novgo-rod, from them 101 patients – with stroke, 34 patients – with a hypertension, obesity, diabetes 2 types.

For studying of genetic predisposition to development of complications of a metabolic syndrome the complex of genetic polymorphisms is allocated. Genotyping on the following genes was carried out:

- Blood pressure regulation system - Thr174Met (rs4762) polymorphism of the angiotensinogen gene (AGT);
- Hemostasis system - polymorphism G-455A (rs1800790) of the fibrinogen gene (FGB);
- The system of regulation of the inflammatory process - polymorphism C174G (rs1800795) of the gene for interleukin-6 (IL6).

Single nucleotide genetic polymorphisms of the studied genes were revealed using the “real-time” polymerase chain reaction using «Sintol» reagent kits with specific oligonucleotide primers and TaqMan probes.
Keywords
Complications of a metabolic syndrome, Genotyping.

Introduction
One of the important results of deciphering the human genome is the fast development of a qualitatively new section of medical science - molecular medicine, within which problems of diagnostics, treatment and prevention of diseases are solved at the level of genes and their expression products - DNA, RNA, proteins.

Inherited polymorphic gene changes play a crucial role in determining the unique biochemical profile of each person, in assessing his hereditary predisposition to various multifactorial diseases. Genetic polymorphism is a mutation that is not directly related to hereditary diseases, but can significantly affect a person’s susceptibility to this or that multifactorial pathology. This is explained by the fact that genetic polymorphism is far from always neutral and can lead to the appearance of protein products with altered functional activity.

The emergence of most multifactorial diseases is determined by genetic polymorphism not in one, but in many genes at once, which are realized only with the presence of corresponding unfavorable environmental factors. Testing allelic markers and possible phenotypic adjustment of their functions can significantly reduce the number of people who actually develop a multifactorial disease (atherosclerosis, type 2 diabetes, myocardial infarction, hypertension, etc.). The pre-symptomatic identification of individuals at high risk for multifactorial pathology and its primary prevention are the main tasks of predictive (predictive) medicine.

A characteristic feature of molecular medicine as medicine, based on data on the molecular structure of the human genome, is its individual character. It is aimed at correcting the pathological process in a very specific person, taking into account the unique features of his genome. Its other major feature is a pronounced preventive focus. Full information about the genome can be obtained long before the onset of the disease. Appropriate preventive measures can completely eliminate or largely prevent the development of a serious illness.

Elucidation of the gene network of each multifactorial disease, identification of central genes and modifiers in it, analysis of the association of their alleles with the disease, development of a complex of preventive measures for a particular patient on this basis constitute the conceptual and methodological basis of predictive medicine. As a result of the examination, information can be obtained about a certain degree of risk of developing these diseases, and the physician, taking into account the results of molecular genetic analysis, can work out the tactics of pathogenetically justified proactive therapy, that is, make the necessary correction to correct the inborn metabolic defect.

Candidate genes that determine susceptibility to the development of metabolic syndrome complications
Cardiovascular diseases (CVD) are one of the most important medical and social problems world-wide and occupy one of the main places in the structure of total mortality [1]. Among them, stroke and coronary heart disease (IHD) most often lead to the death of the patient. In addition, in recent years, the prevalence of these diseases has increased, especially among able-bodied population, which often leads to the occurrence of persistent impairment of life and disability of patients. Therefore, along with the treatment of diseases, preventerology (disease prevention) is becoming increasingly important, including using modern molecular genetic research methods.

According to the literature, among the candidate genes that can determine the propensity to develop complications of the metabolic syndrome can be divided into several groups: genes of the blood pressure regulation system, genes of the hemostasis system, and genes of the inflammatory process regulation system.

Blood pressure regulation system
Protein products of the genes of the renin-angiotensin system are important regulators of blood pressure and homeostatic function of the kidneys, ensuring the maintenance of vital processes in the body. The development of hypertension is predicted taking into account the possible increase in the tone of resistive vessels due to increased formation of angiotensin II, due to a genetically determined increase in the synthesis of angiotensinogen.

Angiotensinogen - The AGT gene encodes an angiotensinogen protein - serum globulin of the alpha globulin fraction, produced mainly by the liver cells, from which angiotensin I is produced by renin. The gene is on chromosome 1 (1q42.2) [3]. The AGT gene encodes the synthesis of angiotensinogen, which is a whey protein of the a-globulin fraction, with a molecular weight of about 65 kDa. AGT is synthesized mainly by the liver, adipocytes of adipose tissue, its synthesis is con-trolled by estrogens, glucocorticoids, and thyroid hormones. The proteolytic enzyme renin contributes to the transformation of AGT into inactive angiotensin-I. Under the action of angiotensin-converting enzyme and a number of alternative pathways involving chymases, cathepsin G, tonin and other serine proteases, angiotensin-I is converted into the biologically active substance angio-tensin-II, which realizes its effect through angiotensin receptors [4,5]. The most significant from a clinical point of view, the variants of the AGT gene are due to point nucleotide polymorphisms, leading to amino acid substitutions in the 174 and 235 codons of the gene -T174M (rs4762) and M235T (rs699), respectively. They determine an increased level of angiotensinogen expression, which is regarded as a risk factor for the development of arterial hypertension. The incidence of unfavorable variants 174M and 235T in Caucasians is 10–15% and 15–20%, respectively.

It has now been established that angiotensins are biopeptides with a broad physiological spectrum of action. They have vasopressor effects, regulate the level of blood pressure and renal filtration, water-salt metabolism, participate in the regulation of stress...
reactions. Angiotensins are able to affect the smooth muscle cells of the vascular wall, causing vasoconstrictor reactions; on vascular endothelium, changing the production of NO and endothelin; on the myocardium, increasing its contractions, etc.

**Hemostasis system**
Fibrinogen (factor I) is one of the main factors of the coagulation system, which is involved in the process of hemostasis. In addition to its role in the coagulation reaction, fibrinogen is involved in the pathogenesis of atherosclerosis, promoting adhesion of platelets and leukocytes to the endothelial surface and modulating plasmin binding to its receptor. Data from epidemiological studies and meta-analyses show that elevated plasma levels of fibrinogen are associated with an increased risk of coronary artery disease and myocardial infarction (myocardial infarction). An increase in plasma fibrinogen per 1 g/l is associated with a more than twofold increase in the risk of coronary artery disease, stroke and vascular mortality [6]. High plasma concentration of fibrinogen is considered an independent predictor of the risk of myocardial infarction [7].

The FGB gene encoding the β-chain of fibrinogen can certainly be considered as one of the important candidate genes, since fibrinogen serves as a substrate for thrombin, and as a result of its proteolytic cleavage, a fibrin clot is formed. Fibrinogen plays an important role in the process of platelet aggregation, that is, it is one of the main factors causing the blood plasma viscosity. In the promoter region of the FGB gene in position -455, single nucleotide polymorphism G/A was found. The prevalence of such polymorphism is from 5 to 10% in the general population. The polymorphic marker G (-455) A of the FGB gene is associated with the level of fibrinogen in the blood plasma. It is known that the presence of the -455A allele leads to an increase in gene expression by 1.2–1.5 times, as a result, to an increased level of fibrinogen in the blood by 10–30% and an increased risk of developing coronary artery disease, acute coronary syndrome, peripheral artery disease and stroke in adults [8,9].

In vitro studies have shown that the FGB -455A allele, associated with an increased level of fibrinogen, disrupts the binding to the repressor protein, resulting in an increase in the FGB chain transcription [10].

**The system of regulation of the inflammatory process**
In a fundamental and clinical study, evidence was obtained that inflammatory mechanisms play a central role in the pathogenesis and progression of atherosclerosis, plaque separation, thrombosis, and stroke.

Elevated levels of IL-6 are associated with the development [13] and severity of coronary disease [14], with the development of processes in the atherosclerotic plaque and the subsequent acute coronary event [15]. There are opinions that, despite the fact that such processes are characterized by the activity of proteins of the acute phase, an increase in the concentration of IL-6 may be an additional marker of the development of the pathological process in the vascular bed [16].

A number of studies have identified significant imbalances between pro-inflammatory (TNFα, IL-6, IL-8) and anti-inflammatory cytokines (IL-4) in patients with ischemic heart disease (IHD) and dyslipidemia (elevated cholesterol (CH) and low-density lipoprotein (LDL)). A linear relationship was found between the serum IL-6 level and the level of VLDL [17]. IL-6 is believed to affect adipose tissue function and plasma lipid levels [18]. According to some data, up to 30% of serum IL-6 is secreted by adipose tissue, which cannot be ignored, since overweight is one of the risk factors for cardiovascular disease and the development of an acute coronary event - possibly due to the activation and intensification of the inflammatory process [19, 20].

The process of inflammation is accompanied by the production of a wide range of cytokines and other mediators that form a regulatory network in which individual elements have a synergistic or antagonistic effect, which affects the pathological process, variants of its course and outcome. In this context, interleukin-6 (IL-6) as a key mediator of the inflammatory response, produced mainly by activated macrophages, may play an important role in the cumulative processes of inflammation and atherogenesis. IL-6 has a diverse and very significant effect on many organs and systems of the body: blood, liver, immune and endocrine systems, as well as metabolism. IL-6 has been shown to affect the synthesis of proteins of the acute phase of inflammation by hepatocytes and contributes to an increase in the concentration of C-reactive protein (CRP), one of the markers for the development of cardiovascular disease and the development of acute myocardial infarction [11]. It should be noted that the peak concentration of CRP correlates with the maximum increase in the concentration of IL-6 [12].

The results of genotyping obtained at this stage of the study are presented in the figure.

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**Figure 1:** Distribution of the examined individuals depending on allelic variants of the studied genes.

The relationship between the increase in systolic pulse and mean blood pressure with the level of IL-6 was established. Individual differences in the plasma level of IL-6 were shown, and screening
studies revealed that the level of IL-6 was higher in those who subsequently suffered a myocardial infarction [21].

The C174G polymorphism in the promoter region of the IL-6 gene has been shown to affect gene transcription and, accordingly, expression of this cytokine, which ultimately controls the level of interleukin-6 circulating in the blood [22].

**Conclusion**

The analysis of the literature on the study of the pathogenetic mechanisms of the development of the metabolic syndrome and its complications and the previous national and foreign studies on the participation of genetic polymorphisms in the development of this pathology was carried out. Fatal complications of metabolic syndrome: blood pressure regulation system, hemostasis system and inflammatory process regulation system. The search for candidate genes for the development of metabolic syndrome complications will be continued with the expansion of the list of studied genetic markers.

Based on the analysis of the results obtained, informative markers have been allocated to assess the risk of the development of fatal complications of the metabolic syndrome in the population of able-bodied age in Veliky Novgorod.

An individual approach to the patient, based on an adequate interpretation of the results of genetic research and their comparison with clinical, laboratory and instrumental research data, will allow early diagnosis of genetically determined diseases and offer the most effective preventive and therapeutical measures to prevent the development of the pathological process.

**References**