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Polymorphism of CYP2C9 Gene in Patients with Stable Angina Pectoris and its Significance in Pathogenesis of the Disease

Olesya V. Romaschenko*, Eduard A. Snegin, Nina I. Zhernakova, Petr K. Alferov, Lyudmila R. Zakirova, Vadim V. Rumbesht, Natalia D. Grischenko, Olga A. Osipova

Belgorod State University,85, Pobedy St., Belgorod, 308015, Russia
Corresponding author: Olesya V. Romaschenko
E-mail: Romashenko@bsu.edu.ru

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ABSTRACT

Introduction: This study was carried out in the direction of personalized medicine, based on identifying the genetic characteristics of patients, which can affect the pathogenesis of the disease and the effectiveness of pharmacotherapy.
Research tasks: To study of the polymorphism of the cytochrome CYP2C9*2 C430T gene in patients with stable angina pectoris and to evaluate the significance of this factor in pathogenesis of the disease.
Material and Methods: A study of 90 patients with stable angina pectoris was performed. The polymerase chain reaction was used to determine the gene polymorphisms of cytochrome CYP2C9*2 C430T. The condition of DNA was studied by the method of DNA comet assay.
Results and Discussion: The presence of gene polymorphisms by the alleles CYP2C9*2 C430T was found. They revealed a more severe clinical condition of patients with pathological genes by the alleles CYP2C9*2 C430T (CT genotype) in comparison with patients with normal genotype for this allele (CC): a higher functional class of angina pectoris, overweight, blood hypercoagulability, an increased level of personal anxiety and greater DNA damage.
Conclusion: polymorphism of the cytochrome CYP2C9*2 C430T gene is essential in the pathogenesis of angina pectoris: in patients with the pathological genotype of cytochrome CYP2C9*2 C430T gene there were revealed more severe clinical condition.
Keywords: polymorphism of the gene CYP2C9*2 C430T, stable angina pectoris, pathogenesis, personalized pharmacotherapy.
Correspondence:
Olesya V. Romaschenko
Belgorod State University, 85, Pobedy st., Belgorod, 308015
Russia
E-mail: Romashenko@bsu.edu.ru
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INTRODUCTION

Diseases of the cardiovascular system remain leaders in prevalence and mortality, despite all the efforts of doctors to follow patient management protocols and prescribe medications according to accepted standards [1,2]. Perhaps the effectiveness of pharmacotherapy may increase if the principles of personalized medicine are introduced in the treatment of patients with cardiovascular pathology [3,4]. Personalized pharmacotherapy based on identifying the genetic characteristics of patients is considered one of the top priorities in the development of modern medicine [5]. Today, the main base of drugs prescribing personalization is considered the knowledge about the genetic polymorphism of drugs biotransformation system [6]. One of such clinically significant enzymes of biotransformation is cytochrome CYP2C9, which is a protein with a molecular weight of 55 kDalton, consisting of 490 amino acid residues. The gene of this enzyme is located in the locus 10q24.1.24.3 of the 10th chromosome [7]. Cytochrome CYP2C9 is synthesized in liver cells and participates in biotransformation of 10% of therapeutically important drugs such as anticoagulant warfarin, anticonvulsant phenytoin, antidiabetic drug tolbutamide, nonsteroidal anti-inflammatory drugs (ibuprofen, diclofenac, etc.), losartan, etc. [8,9,10]. The pathological gene of cytochrome CYP2C9*2, causes the synthesis of the enzyme with altered catalytic activity (only 12% of the activity of normal CYP2C9) [6]. Therefore, carriers of the variant CUR2C9*2 are considered as "slow" metabolizers in both homozygous and heterozygous states: they have a reduced metabolism of drugs, which accumulate in high concentrations in the body, which can lead to undesirable drug reactions, including intoxication [11]. Along with the function of biotransformation of xenobiotics, cytochrome CYP2C9 also has the function of synthesizing from the polyunsaturated fatty acids of cell membranes of eicosanoids - local action hormones, highly active mediators, which take part in various processes, in particular, in hemostasis and vasodilation [12]. The ability of vessels to vasodilation is largely determined by the functional state of the endothelium. Endothelium of the vessels is the target organ for pharmacological correction of cardiovascular pathology both in the clinic and in the experiment [13,14,15,16,17,18,19,20,21,22,23,24]. In this regard, the study of polymorphism of genes that potentially affect the state of the vascular endothelium may be of scientific and practical interest.

The purpose of this study was to study of the polymorphism of the cytochrome CYP2C9*2 C430T gene in patients with stable angina pectoris and to evaluate the significance of this factor in pathogenesis of the disease.

MATERIALS AND METHODS

A total of 90 patients with IHD: stable angina pectoris were examined: 63 men and 27 women aged from 37 to 81 years (mean age of patients was 59.26 ± 0.74 years). Clinical examination of patients in the initial status was carried out, when they entered the cardiology departments of the Belgorod Regional Clinical Hospital of St. Joasaph. Each participant was acquainted with the research program and signed informed consent. In the majority of patients angina pectoris was associated with hypertension - 80 (89.4%), rhythm disorders - 22 (24.4%), postinfarction cardiosclerosis - 44 (48.8%), chronic heart failure - 85 (94.4%), in some - with type II diabetes - 21 (23.1%). The diagnosis of stable angina pectoris was verified after clinical, instrumental, laboratory examination in accordance with

the recommendations of the European Society of Cardiology (ESC) (2013) [1].

The program of examination of patients included the implementation of general clinical methods of investigation, instrumental and laboratory, including electrocardiography, echodoplercardiography, coronary angiography, treadmill test, general and biochemical blood tests with determination of coagulogram, glucose, potassium, creatinine and other parameters according to the recommendations of the ESC (2013) [1].

Gene polymorphisms of CYP2C9*2 C430T was determined by polymerase chain reaction using presets reagents of firm "Liteh" (Russia) in the Center of genomic selection of Belgorod State University. DNA was isolated from blood leukocytes from patients.

The condition of DNA was studied by the method of DNA comet assay in the Center of genomic selection of the Belgorod State University. The number of damaged cells (%) and the maximum degree of DNA damage (1-4 units) were determined by the method of DNA comet assay [25].

In the summary tables only reliable data were entered for further analysis. The statistical processing of the material was carried out by the method of variational statistics. The difference between the two groups was assessed according to Student's t-test. The results were considered statistically significant at $p < 0.05$. The criterion $\chi 2$ was used to estimate the correspondence of the sample distribution to predetermined distributions (the Hardy-Weinberg law). To evaluate the results of the DNA comet assay method, CometAssay software was used. During the calculations, the programs "Microsoft Excel 2007" and "SPSS for Windows 11.0" were used.

RESULTS AND DISCUSSION

When analyzing the CYP2C9*2 C430T gene, 75 people (83%) were found to be homozygous for the normal allele (CC genotype), and 15 people (17%) were heterozygous (genotype CT), i.e. together with the normal allele are pathological one (Figure 1.). Homozygous people with the pathological allele (TT genotype) were not found in the sample. Thus, the frequency of occurrence of the pathological allele is 0.092, and the normal one is 0.908. There is a small excess of heterozygotes (coefficient of inbreeding F = -0.102), as well as a low value of the index of genetic variability of the Shannon index (I = 0.308). However, in this case the genotypic structure of the sample under study is generally consistent with the Hardy-Weinberg law ($\chi 2 = 0.953$, P = 0.329, Df = 1), which allows us to speak about the relative stability of the population for a given gene.

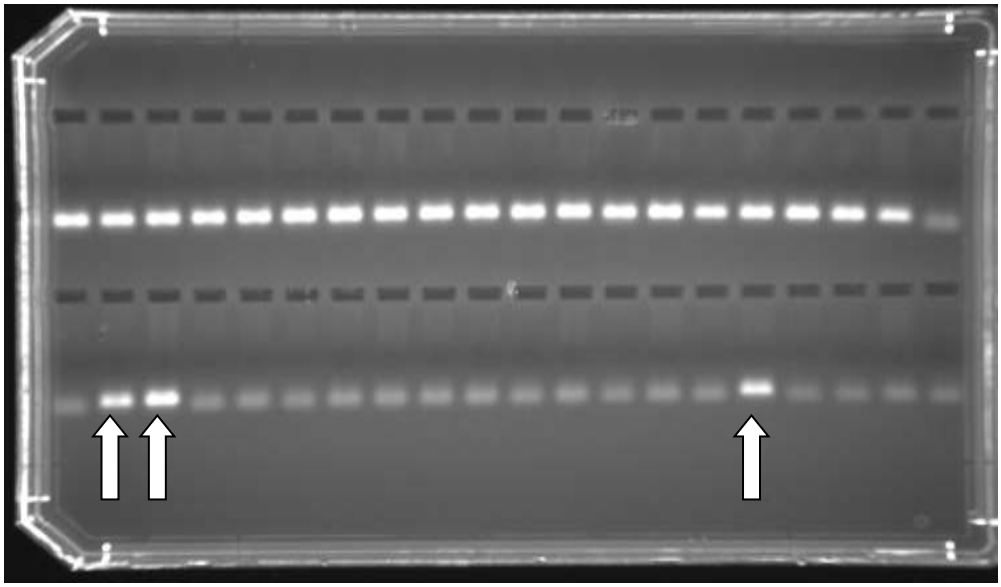

Figure 1: Electrophoregram of polymorphism of the gene CYP2C9 * 2 C430T in patients with ischemic heart disease.

Note: The arrows denote heterozygotes along the pathological allele of the CT. The unmarked positions correspond to homozygotes over the normal CC allele.

A comparative analysis was performed between groups of patients with the normal and pathological gene CYP2C9*2 C430T. A number of significant differences were found (Tables 1,2.). In the carriers of the pathological cytochrome CYP2C9*2 C430T gene (CT genotype), the following features were observed: a higher functional class of angina, excess body weight, a tendency to hypercoagulation of blood (despite the fact that all patients took antiplatelet agents), a significant relationship with the psychological profile - increased the level of personal anxiety, the DNA in the original status is more damaged.

Table 1: Comparative analysis of groups of patients with stable angina pectoris having a normal and pathological gene CYP2C9*2 C430T

Index	Patients with normal genome (CC)	Patients with a pathological gene (CT)	p
Weight, kg	85.03±2.07	99.24±4.46	0.002
Quetelet index, kg / m2	29.90±0.65	33.14±1.37	0.020
Prothrombin index, %	96.29±1.22	100.00±0	0.003
international normalized attitude	1.07±0.03	1.00±0	0.036
DNA: the number of damaged cells, %	3.43±1.13	11.00±3.21	0.020

Comment: the reliability of the differences was assessed by the Student's t-test

Table 2: Comparative analysis of groups of patients with stable angina pectoris having a normal and pathological gene CYP2C9*2 C430T

Index	Patients with normal genome (CC)	Patients with a pathological gene (CT)	p
Functional class of angina pectoris	2.33/ 3.00 (2.00; 3.00)	2.79/ 3.00 (2.00; 3.00)	0.070
The degree of obesity	0.64/ 0.00 (0.00; 1.00)	1.24/ 1.00 (0.00; 2.00)	0.039
Personal anxiety, scores	48.28/ 47.00 (41.50; 54.50)	53.14/ 52.50 (47.75; 58.25)	0.061
DNA: the maximum degree of damage	1.60/ 2.00 (0.50; 2.00)	2.45/ 2.00 (2.00; 3.00)	0.023

Comment: The numerator is the arithmetic mean, the denominator is the median, 25% and 75% quartile. The reliability of the differences was evaluated according to the Mann-Whitney U criterion.

As mentioned above, cytochrome CYP2C9 has the function of biotransformation of xenobiotics and the function of synthesizing from the polyunsaturated fatty acids of cell membranes of eicosanoids - local action hormones, highly active mediators, which take part in various processes, in particular, in hemostasis and vasodilation [12]. The biochemistry of the reaction is as follows: under the action of phospholipase A2 from phospholipids of endothelial cell membranes, arachidonic acid is released, which in three ways is converted into various eicosanoids-prostaglandins under the action of cyclooxygenase, leukotrienes by lipoxygenase and epoxyicosotriene acids by cytochromes via the P450 monooxygenase pathway. These epoxyicosotriene acids (EETs) reduce blood clotting, reduce platelet aggregation, produce vasodilation, stimulate angiogenesis, have an anti-inflammatory effect, and also protect the heart from "damage" in ischemia-reperfusion. EETs produce these functional effects by activating receptor-mediated signaling pathways and ion channels [12].

The presence of the pathological CYP2C9*2 gene in humans causes the synthesis of the so-called "slow" enzyme of cytochrome 2C9, which in turn leads not only to a decrease in the biotransformation rate of drugs, as mentioned above [11], but also to a violation of eicosanoid synthesis, which can lead to increased blood clotting, increased platelet aggregation, vasoconstriction, pro-inflammatory effects (excessive formation of free radicals of oxygen). The phenomenon of association of the presence of the pathological gene CYP2C9*2 with a higher functional class of angina in patients with ischemic heart disease, the tendency of blood to hypercoagulability, greater DNA damage, can be explained by a significant decrease in the synthesis of eicosanoids-epoxyoxy-isotrenic acids and activation of free-radical oxidation, which can destroy DNA. Excess body weight contributes to stagnation of blood and its thickening, increased personal anxiety is a known risk factor for cardiovascular disease [26,27]. In other words, according to our study, the reduced activity of cytochrome CYP2C9 observed in carriers of the pathological gene CYP2C9*2 C430T is associated with a more severe course of angina pectoris.

CONCLUSION

Polymorphism of the cytochrome CYP2C9*2 C430T gene is essential in the pathogenesis of angina pectoris: in patients with the pathological genotype of cytochrome CYP2C9*2 C430T gene there were revealed more severe clinical condition: a higher functional class of angina pectoris, overweight, blood hypercoagulability, an increased level of personal anxiety and greater DNA damage. The obtained results can be used in planning personalized approaches to pharmacotherapy of patients with stable angina pectoris.

MAIN FINDINGS

1. In the study of cytochrome CYP2C9*2 C430T gene polymorphism the relative stability of the population for this gene was found: 75 patients (83%) were found to be homozygous for the normal allele (CC genotype), 15 patients (17%) were heterozygous (genotype CT) and no any homozygous patient with the pathological allele (TT genotype) was found.
2. In patients with stable angina pectoris having a pathological cytochrome CYP2C9 * 2 C430T gene (CT

genotype), in comparison with patients with normal genotype for this allele (CC), a higher functional class of angina pectoris, overweight, blood hypercoagulability, an increased level of personal anxiety and greater DNA damage were revealed.

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