ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Arterial Hypertension and Coronary Heart Diseases Development in Patients with Dyslipoproteinemia against Polymorphisms of GP IIIa Genes and Prothrombin Gene.

E. N. Kasapova*[1], G. N. Miandina[1], E. V. Tarasenko[1], S. P. Syatkin[2], I. P. Smirnova[2], A. S. Skorik[2], S. M. Chibisov[3], T. Yu. Zotova[3], M. L. Blagonravov[4], and E. V. Neborak[5].

[1]Department of Biology and General Genetics of PFUR. 117198, Moscow, Miklukho-Maklaya st., 8. Medical faculty
[2]T.T. Berezov Department of Biochemistry of PFUR. 117198, Moscow, Miklukho-Maklaya st., 8. Medical faculty
[3]Department of Pathological Physiology of PFUR. 117198, Moscow, Miklukho-Maklaya st., 8.Medical faculty
[4]Department of Ecology of PFUR. 117198, Moscow, Miklukho-Maklaya st., 8. Medical faculty
[5]Department of Biochemistry, Medical Faculty, Peoples' Friendship University of Russia.

ABSTRACT

The cause of a genetic predisposition to a variety of diseases, including coronary atherosclerosis and coronary heart disease is the presence of defective integrin receptors on the cell surfaces, as well as thrombophilia associated also with prothrombin gene mutations. This paper deals with the study of frequency of alleles of one of the integrins, GPIIIa glycoprotein (β- type III subunit), and prothrombin gene in patients with dyslipidemia. We have established that the presence of allele PLA2 of GPIIIa gene and allele G20210A of prothrombin gene in the genotype of a patient is a genetic risk factor for intensive development of coronary heart disease and hypertension in patients with dyslipidemia, which is associated with a higher incidence of complications and the need for carrying out more comprehensive antihypertensive therapy to stabilize blood pressure.

Keywords: GPIIIa gene, prothrombine gene, integrin, dyslipoproteinemia, coronary heart disease, arterial hypertension.

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Cardiovascular diseases are still the leading factor of mortality (about 1.2 mln. people annually) and disablement (31.2 per 10,000 people) in Russia [1]. One of the most important risk factors for coronary heart disease, hypertension and other cardiovascular complications is the lipid metabolism disorders. Assessment of the lipid metabolism disorders and probability of atherosclerosis development is commonly performed by determining in patients their level of total cholesterol and blood serum triglycerides, the concentrations of very low, low, and high density serum lipoproteins (VLDL, LDL, HDL), as well as their ratio called "atherogenic index". Low-density lipoproteins (LDL) and very low-density lipoproteins (VLDL) are major sources of lipid infiltration of the arterial walls, leading to the subsequent proliferation of connective tissue and the formation of fibrous plaques that both narrow the lumen and impair the physiological functions of the affected arteries, which further causes circulatory disorders. We have found that the protein component of one of the LDL classes is a cholesterol receptor responsible for its transfer from the plasma into the cells, whereas high-density lipoproteins (HDL), otherwise, perform acceptance of cholesterol from cell membranes and transfer it to the places of catabolism, inhibiting thereby lipid infiltration of the artery walls. To assess the probability of atherosclerosis, the so-called atherogenic index (or coefficient) (IA) is commonly used, equal to the ratio:

$$IA= (LDL + VLDL) : HDL$$

It is supposed that the process of atherogenesis starts at the IA value above 3.0-3.1 [2, 3].

Target levels of total cholesterol and LDL differ slightly according to the American and European guidelines: In the US, they are determined as 5.2 mmol/L and 2.6 mmol/L, and in Europe - as 5.0 mmol/L and 3.0 mmol/L, respectively [2].

Currently, the analyses, the cardiac patients undergo, are usually limited to total cholesterol and triglycerides tests, which makes it impossible to assess completely the disorders of lipid metabolism. For the diagnosis of dyslipidemia it is important to focus on the values of low-density lipoprotein, high-density lipoprotein, and atherogenic index.

Therefore, to reduce the risk of complications of coronary artery disease, hypertensive heart disease, and mortality from cardiovascular disease it is essential to carry out correction of different dyslipidemic states. One of the most effective lipid-lowering agents are statins. Statins cause the most accentuated reduction in total cholesterol and LDL, but, along with efficiency and ease of use, there is a high cost of the statin drugs and a number of side effects inhibiting the use of statins in general medical practice. Long-term use of statins caused in some patients complains of pain in the muscles sometimes accompanied by their atrophy, the loss of memory, and impaired immune functions [3].

Due to these circumstances, the assessment of the choice of drugs requires additional criteria to be used for selecting among patients with dyslipidemia a group of patients who primarily require more intensive monitoring and therapy, including the statin one.

The achievements of modern molecular genetics allow us to study the molecular and genetic mechanisms of complex traits, including multifactorial diseases, which are atherosclerosis, hypertension, coronary heart disease, and many others. One of possible approaches to the study of multifactorial diseases is based on the idea of the disease mechanism and is associated with the identification of candidate genes having potentially the greatest contribution to the pathogenesis.

Integrins, as surface receptors of cells, ensure interaction of cells with the extracellular matrix and with each other, as well as participate in the processes of blood coagulation, cells transfer into the area of inflammation, implantation and fetal development, metastasis of tumor cells, and apoptosis [4, 5]. Integrins consist of α-subunits (16 classes known) and β-subunits (8 classes known). Entering into noncovalent interactions, the α- andβ-subunits form heterodimers, thus ensuring the existence of integrin complexes with different specificity. A glycoprotein IIIa β-subunit (GPIIIa) is represented by two allelic forms: PLA1 and PLA2. Its average frequency in the population of allele PLA2 of GPIIIa gene for the European population is about 14%. PLA2 allele is characterized by the replaced thymine nucleotide with cytosine one at position 196 in the GPIIIa mRNA, which entails the incorporation of proline instead of leucine at position 33 of the protein molecule [6,

7]. We have found that the PLA2 allele is a cause of a genetic predisposition to a number of diseases, among which are the venous and arterial thrombosis, coronary cardiosclerosis, hereditary Glanzmann thrombasthenia, as well as some complications of pregnancy, and oncologic diseases [4, 8-10].

Prothrombin (coagulation factor II) is a major component of blood clotting. The enzymatic degradation of prothrombin leads to the formation of thrombin. This reaction is the first stage of the formation of blood clots. G20210A prothrombin gene mutation is characterized by replacement of nucleotide guanine (G) with nucleotide adenine (A) at position 20210. Increased expression of the mutant gene leads to increased levels of prothrombin 1.5-2 times higher than normal. The mutation is inherited by autosomal dominant type; hence, the thrombophilia occurs even in heterozygous carriers of the mutant gene. Thromboembolic diseases caused by disturbances in blood clotting lead also to cardiovascular diseases.

Objective of this paper was:

- to study the polymorphism of GP-IIIa gene in patients with dyslipidemia, and the possibility of using genetic predisposition, determined by PLA2 allele, as a criterion for determining the primary groups at risk of development of cardiovascular complications (coronary heart disease, hypertension) in these patients.
- to analyze an insertion-deletion polymorphism in the prothrombin gene in patients with dyslipidemia, ischemic heart disease, and hypertensive disease at their different stages.

Patient population

We have examined patients with dyslipidemia (the group included patients with a total cholesterol level above 5.0 mmol/L and/or atherogenic index above 3) having at admission the coronary heart disease and/or hypertension. The patients were discharged to outpatient treatment in a satisfactory condition in the absence of anginose attacks and with stable hemodynamics. When choosing the basic antihypertensive therapy, the target blood pressure was $\leq$ 140/90 mm Hg. For this purpose, we applied a monotherapy with antihypertensive drugs of the main groups, or, if necessary, a combined therapy with several groups of drugs.

MATERIALS AND RESEARCH METHODS

Obtaining the DNA-matrix

As a DNA-matrix, a genomic DNA was used obtained from peripheral blood cells by using the “Cytolysin" kit (Russia) according to the procedure attached thereto, or a dry drop of blood on the dry paper (3MM Whatman paper, England).

Polymerase chain reaction

Polymerase chain reaction (PCR) was performed by using a thermal cycler "Trepsonal" (Biometra, Germany) and "DNA Technology" (Russia).

Selection of primers for amplification of the DNA target sequence was performed on the «Primer 3» program (http://www-genome.wi.mit.edu/cgi-bin/primer3/cgi). Design of primers was performed by using the nucleotide sequence of the GP3a gene (acc. M32672), described in the Genbank database.

GpIIIL – 1375 (5') gct cca atg tac ggg gta aa
GpIIIR - 1759 (5') ctc ctc aga cct cca cct tg

The concentration and the annealing temperature of synthesized primers was determined by the absorption spectrum of the samples at 260 nm using the computer program “Oligonucleotides concentration”, which considers the structure of an oligonucleotide. Internet address http://www.humgen.siobc.ras.ru.

Incubation samples contained (25 µl) primers per 1 µl of 10 µM (1.5 p.u./ml), 2.5 µl of restriction buffer 10x ("Bion", Russia), MgCl at a concentration of up to 3.5 mM, 2.5 µl of dNTP mixture 10x, 20 ng of DNA-matrix, and 0.5 un. Taq-polymerase.

The PCR program consisted of 35 cycles with following conditions of each: 94°C - 40 sec, 60°C - 30 sec, 72°C - 90 sec. After amplification, the samples underwent an overnight precipitation with 3 volumes of ethanol and were collected by centrifugation for 10 min. at 10 thous. g. The length of the amplification product of the GPIIIa polymorphic site was 384 bp.

To reveal mutations of G20210A prothrombin gene (coagulation factor II) we used allele-specific primers AS-P-WT (5'-CCATAGCACTGGGAGCATTGAGGATC-3') and AS-P-MT (5'-GTTCCGCACGCCTGAATAGCACTGGGAGCATTGAGGGTT-3'), as well as the common primer P-F (5'-GTTCCGCCTGAAGAAGTGGATACAGAA-3'). As a result of reaction with AS-P-WT and P-F primers, we obtained a product of 174 bp in length, and the reaction with AS-P-MT and P-F primers - 187 bp. The reaction mixture included 20 pmol of common reverse primer, 10 pmol of AS-P-WT primer, and 8 pmol of AS-P-MT primer. Amplification was performed under the following cycling conditions: 95°C - 2 min, 30 cycles (94°C - 30 sec, 60°C - 30 sec, 72°C - 30 sec), 72°C - 5 min.

Treating DNA with Mspl restriction endonuclease

The sample content was dissolved in water to a final volume of 100 µl of restriction mixture, and added thereto 5 un. of Mspl restriction endonuclease ("Promega", USA). After incubating the mixture for 1.5 hours at 37°C, 9 µl of 5M NaCl and 300 µl of ethanol were added. The mixture was incubated for 12 hours at -20°C, centrifuged for 10 min. at 10 thous. g., and released from supernatant. The precipitate was dissolved in 15 µl of buffer for polyacrylamide gel application.

Polyacrylamide gel electrophoresis

The PL1 and PLA2 alleles were identified by analyzing the DNA restriction fragment lengths. After processing the full-length amplification product of GPIIIa polymorphic site with *MSPI* endonuclease (length of 384 bp) the PLA1 allele restriction products have length of 295 bp, 83 bp, and 6 bp, the DNA restriction fragments of PLA2 allele have length of 175 bp, 120 bp, 83 bp, and 6 bp.

Restriction products were separated by electrophoresis in 10% polyacrylamide gel (PAAG) at 20mA and 250V for 1 hour; the gel was stained with silver nitrate according to standard procedures. We used a pUCI8 plasmid as a marker, treated with the Mspl restriction endonuclease (DNA fragment size ranges 501 to 34 bp).

During the prothrombin gene mutation analysis, the amplification products were separated by electrophoresis in 1.5% agarose gel. As the matrixes, we used DNA samples homozygous for the normal allele of the prothrombin gene.

Statistical analysis of results

The obtained data were processed with the use of STATISTICA 6.0 software package, StatSoft, USA. The significance of differences was assessed by nonparametric criteria “Obs/Exp”; the differences were considered significant at $p < 0.05$.

RESULTS AND DISCUSSIONS

We have examined 93 patients with dyslipidemia (the group included patients with a total cholesterol level above 5.0 mmol/L and/or atherogenic index above 3) aged 40 to 71 years (average age - 53.05 years).

We studied in the group the allelic distribution of GPIIIa gene, and the incidence of coronary heart and hypertension diseases. Severity of hypertensive disease was estimated on the maximum values of systolic blood pressure (degree 1: 140-160 mm Hg; degree 2: 160-180 mm Hg; degree 3: above 180 mm Hg), as well as in terms of antihypertensive therapy applied to each individual patient.

It should be noted that many drugs can cause the appearance of or exacerbate the existing hyperlipidemic disorders. The above relates primarily to certain drugs used for treatment of hypertension and angina. The most important hyperlipidemic properties are common to thiazide diuretics and β-blockers, while preparations of angiotensin-converting enzyme inhibitors, calcium antagonists, and indapamide have no effect on serum lipid concentration. However, not at all β-adrenergic blockers have negative effects. A range of

ISSN: 0975-8585

multicenter studies have shown that the selective β-adrenergic blocker bisoprolol no effect on total cholesterol and triglycerides, and high-density lipoprotein level increased significantly by 9% ($p<0.05$) [11]. Given the above, the antihypertensive therapy in patients with dyslipidemia was selected with the use of ACE inhibitors (Diroton), calcium antagonists (Verapamil), indapamide and selective β-adrenergic blocker bisoprolol (Concor). The coronary heart disease therapy involved the use of nitrate drugs (Kardiket) for indications.
The study of the allelic distribution of GPIIIa gene in the above group of patients detected no homozygous for the PLA2 allele. Homozygous for the PLA1 allele and PLA1/PLA2 heterozygous genotypes had ratio of 63% and 37%, respectively (Table 1).

Table 1. Main criteria of the patient population due to their genotype

Criteria	PLA1/A1	PLA1/A2
1. **The number of patients,** (persons) (% of total number studied)	58 (63%)	34 (37%)
2. **Average age,** (years)	53.20	54.69
3. **Cholesterol level,** (mmol/L)	6.43	6.38
4. **Atherogenic index**	3.48	3.30
5. **CHD incidence** (% of number of patients in group)	28 (48.2%)	20 (58.8%)
6. **CHD complication frequency** (AMI, AF) (% of total CHD patients in group)	10 (37%)	14 (70%)
7. **Arterial hypertension** (group incidence)	48 (83%)	34 (100%)

Acronyms: AMI - acute myocardial infarction in the anamnesis.
AF - atrial fibrillation, persistent or paroxysmal.

Among homozygous for the PLA1 allele the average level of total cholesterol was 6.43 mmol/L, the average atherogenic index was 3.48. Coronary heart disease in this group was observed in 28 patients (48.2%), including one complicated by myocardial infarction and/or by rhythm disturbance as in atrial fibrillation - in 10 patients (37%).

Among heterozygous for the PLA1/A2 allele the average level of total cholesterol was 6.43 mmol/L, the average atherogenic index was 3.30. Coronary heart disease in this group was observed in 20 patients (58.8%), including one complicated by myocardial infarction and/or by rhythm disturbance as in atrial fibrillation - in 14 patients (70%).

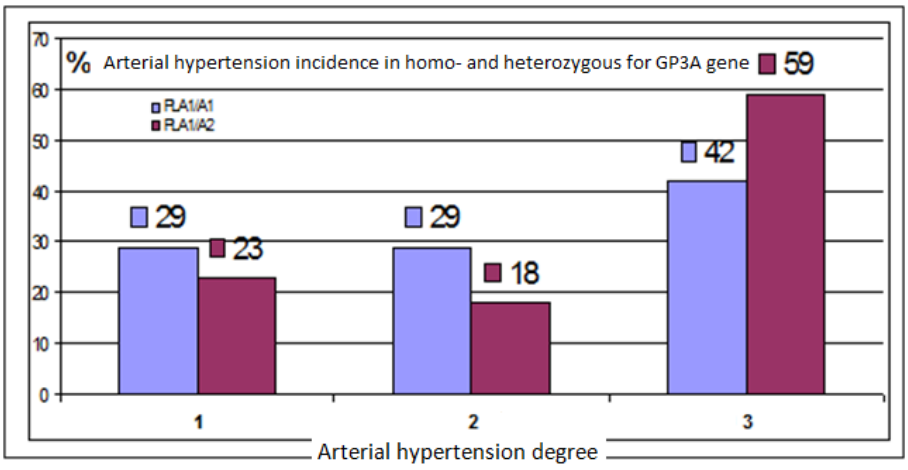

Figure 1. Severity of hypertension in homo- or heterozygous for the GPIIIa gene.

degree 1 arterial hypertension: Blood pressure= 140-160/90 mm Hg.
degree 2 arterial hypertension: Blood pressure= 160-180/90 mm Hg.
degree 3 arterial hypertension: Blood pressure> 180/90 mm Hg.

ISSN: 0975-8585

Hypertensive heart disease was in 48 people (83%) in the heterozygotes PLA1/A1, including: degree 1 hypertension - 14 people (29%), degree 2 hypertension - 14 people (29%), and degree 3 hypertension - 20chel (42%). In the group of heterozygotes PLA1/A2 the hypertension was observed in 34 persons (100%), including: degree 1 hypertension - 8 people (23.6%), degree 2 hypertension - 6 people (17.6%), and degree 3 hypertension - 20 people (58.8%). Results are shown in Figure 1.

When choosing the antihypertensive therapy, the target blood pressure was ≤ 140/90 mm Hg. For this purpose, we applied a monotherapy with antihypertensive drugs of the main groups, or, if necessary, a combined therapy. In addition to the number of combinable group of drugs we have considered the applied daily dosages of antihypertensive drugs (in % of the maximum daily therapeutic dose). It should be noted that we analyzed the doses of the drugs applied at the time of discharge from the hospital for further outpatient treatment, i.e., considered the volume of long-term basic outpatient treatment necessary to maintain stable hemodynamics at BP of ≤140 / 90 mm Hg. Some of patients - with symptomatic paroxysmal hypertension - did not need long-term continuous antihypertensive treatment after their discharge from hospital. Results are shown in Tables 2 and 3.

Table 2. Volume of basic antihypertensive therapy (constant administration) as a percentage of the maximum therapeutic daily dose in groups of patients with dyslipidemia of different genotypes

Group of drugs	**Patients PLA1/A1** (% of the maximum therapeutic daily drug dose)	**Patients PLA1/A2** (% of the maximum therapeutic daily drug dose)
ACE-inhibitors (100%= Diroton 10 mg/day)	31.56%	72.44%
Diuretics (100%= Arifon 2.5 mg/day)	46.05%	84.70%
β-*blockers* (100%= Concor 10 mg/day)	14.34%	30.14%
Calcium channel blockers (100%= Verapamil 10 mg x2/day)	15.14%	7.14%

Table 3. Number of antihypertensive drugs of main groups used in combination during the selection of arterial hypertension basic therapy,% of the total number of patients in the group of homo- and heterozygotes for GPIIIa

Number of drug groups (acc. Table 2) used in the combined basic AH treatment	**Patients PLA1/A1** (% of total patients in group)	**Patients PLA1/A2** (% of total patients in group)
0 (symptomatic arterial hypertension, requiring no continuous use of antihypertensive drugs)	11	0
One group	27.60	29.61
Two groups	43.14	20.32
Three groups	11.85	37.14
Four groups	0	16.04

In the same group of patients with dyslipidemia, described above, we carried out the study of polymorphism in the G20210A prothrombin gene. We examined 61 people, among which 3 (5%) patients were homozygous G20210A/A for defective allele, 4 (6.5%) were heterozygous G20210AG, and 54 people (88.5%) were homozygous G20210G/G for the normal allele. We have established that the presence of the mutant allele in the genotype of G20210A prothrombin gene complicates the coronary heart disease, causes an increase in the frequency of CHD complications such as arrhythmias and acute myocardial infarction. Results are shown in Table 4.

ISSN: 0975-8585

Table 4. Main criteria of patients with dyslipidemia against the G20210A prothrombin gene polymorphism

Criteria	Homozygous G20210A/A for defective allele	Heterozygous G20210AG	Homozygous G20210G/G for the normal allele
1. **The number of patients,** (persons) (% of total number studied)	**3 (5%)**	**4 (6.5%)**	**54 (88.5%)**
2. **Cholesterol level,** (mmol/L)	**5.10**	**5.29**	**6.05**
3. **Atherogenic index**	**3.9**	**3.48**	**3.23**
4. **CHD incidence** (% of number of patients in group)	**1 (33%)**	**3 (75%)**	**31 (57%)**
5. CHD complication frequency (% of total CHD patients in subgroup)	**1 (100%)**	**1 (30%)**	**14 (45%)**
6. **Hypertension incidence** (% of number of patients in group)	**3 (100%)**	**4 (100%)**	**50 (92.5%)**
7. The average hypertension degree (degree 1 = 140-160 mm Hg, degree 2 = 160-180 mm Hg, degree 3 > 180 mm Hg)	**2.33**	**2.0**	**2.06**

CONCLUSION

In the result of this study, we have established that the presence of PLA2 allele of GPIIIa gene and G20210A allele of prothrombin gene in patients with dyslipidemia is a genetic risk factor for intensive development of coronary heart disease and hypertension as such. These alleles determine the complicated and unstable progress of these diseases. Patients with dyslipidemia have PLA2 alleles of GPIIIa gene and G20210A prothrombin gene associated with greater frequency of complications; these patients require multicomponent therapy and high doses of drugs of different antihypertensive groups, and longer treatment for their blood pressure stabilization. Thus, the GPIIIa PLA2 carriers are a genetically determined group of patients requiring more active, long-sustained and multicomponent antihypertensive therapy.

Determination of genotype of the GPIIIa gene and prothrombin gene in patients with dyslipidemia can be used as an additional criterion in predicting the severity of cardiovascular diseases, in assessing the risk of possible complications, in deciding on the amount of the basic anti-hypertensive and cardiotropic therapy, as well as the appropriateness of lipid-lowering drugs for patients with dyslipidemia.

REFERENCES

1. Gurvich I.N. health status of the population of Russia: 1989 - 2003 // Russian Journal "AIDS, Cancer and Social Health". - 2005. - Vol.9. - p. 72-83.
2. Thompson, G.R. Handbook of Hyperlipidaemia. G.B..: Current Science 1994. 258p.
3. Libov I.A., Gultikova O.S. Some clinical aspects of the use of statins for the treatment of patients with coronary heart disease and hyperlipidemia.// Moscow MedicalJournal. - Dec. 2001. - p. 14-15.
4. Radzinskii V.E., Itkes A.V. et al. Prediction of gestosis and fetal growth retardation of fetal and maternal genotypes // Obstetrics and Gynecology. - 2003. - No.10. - p. 23 - 26.
5. Zotova T.Iu, Miandina G.I., Frolov V.A., Komarov A.G., Zotov A.K. Effect of ITGB3 gene polymorphism on the incidence of hypertension in patients with acute coronary syndrome // Clinical Medicine. - 2013. - Vol. 91, No.8 - p. 22-24.
6. Lansa F., Kieffer N., Phillips D.R. et al. Characterization of the human platelet glycoprotein IIIa gene. Comparison with fibropectin receptor beta-subunit gene.//J Biol Chem. - 1994. – V. 61. - P.812-814.
7. Newman P.J., Derbes R.S., Aster R.H. The human platelet alloantigens, PLA1 and PLA2, are associated wiht a leucine33/proline33 amino acid polymorfism in membrane glycoprotein IIIa, and are distinguishable by DNA typing.//J Clin Invest. - 1989. – V. 83. - P. 1778-1781.
8. Weiss D., Tayback M. et al. Polymorfism of platelet glycoprotein receptor as an inherited risk factor for coronari trombosis.// N. Engl. J. – 1996, V.334. – P. 1990-996.
9. Ridker P.M., Hennekens C.H. et al. PLA1/PLA2 polymorfism of platelet glycoprotein IIIa and risks of myocardial infarction, stroke and venous trombosis.// N. Engl. J. – 1997. –V.349. – P.385-388.

10. Beer J.H., Pederiva S. Genetics of platelet receptor singlle-nucleotide polymorfism: clinical implications in trombosis.// J. Neural Transm. – 2000. – V.107. – P. 266-272.
11. Tereshchenko S.N., Akimova O.S. Using the β-blockers in patients with relative contraindications to their use.// Heart. - 2002. - Vol.1. - p. 180.