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ASSOCIATIVE RELATIONSHIP OF THE PLASMINOGEN ACTIVATOR INHIBITOR-1 GENE POLYMORPHISM WITH RISK FACTORS OF ISCHEMIC HEART DISEASE

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ABSTRACT

Research objective. To determine the relationship between allelic variants of the polymorphic marker of PAI-1 gene with the risk factors of ischemic heart disease (IHD) in Uzbek nationality.

Material and methods. 126 respondents were examined: 61 patients with stable angina (SA) aged 45 to 60 years (males) who were hospitalized in the cardiology department of the 1st TMA clinic in Tashkent. In the comparison group were 65 unrelated males of Uzbek nationality without clinical signs of coronary artery disease. The examined noted: arterial hypertension (AH) in 53 people, hypercholesterolemia (HCH) in 38 people, obesity in 23 people, smoking in 22 people.

The diagnosis –SA was verificated in compliance with classification of IHD accepted at the IV congress of cardiologists (2000) became criteria of including of patients. Functional class of SA was established on the basis of classification of stable angina of the Canadian society of cardiologists (1976) and exciting test veloergometry.

Criteria of an exception of a research- patients with unstable angina, myocardial infarction (MI), an acute and chronic heart, renal, liver failure, patients with an arrhythmia, acute disorders of a cerebral circulation, the diabetes mellitus, malignant neoplasms.

Results. The analysis of genotyping of the studied persons of the Uzbek nationality showed that, in patients with IHD mutagen allele 4G of gene PAI-1 in homozygous (4G/4G) and heterozygous (4G/5G) state what makes 21,3% and 47,5%, respectively, meets more than in group of healthy faces at which given distributions of genotypes made 9,2% and 38,5%. Points these data probability of influence of existence of 4G allele of PAI-1 gene, especially in a heterozygous state, to development of IHD.

5G/5G the group of healthy faces in number of 34 people in 52.3% of cases, than at 19 (31,1%) IHD patients caused a stir in the greatest occurrence of a favorable genotype. Thus, these differences have the high statistical importance and wear, nonrandom character ($p<0.05$).

Conclusion. The genotyping of the coagulation factor PAI-1 in the examined patients revealed the presence of unfavorable genotypes 4G/4G and 4G/5G was established more often in patients with coronary artery disease in 21.3% and 47.5% of cases, respectively, compared with the group of healthy individuals who This distribution of genotypes was 9.2% and 38.5%. Whereas, the prevalence rate of the 5G/5G genotype was prevalent in 52.3% of the control group over the spread of 31.1% of patients in the IHD.

Key words: Ischemic heart disease, genetic polymorphism, plasminogen activator inhibitor, 4G/5G, risk factors.

INTRODUCTION

Genetic variations of the serine proteinase inhibitor family E member 1 (*SERPINE1*) gene, which encodes plasminogen activator inhibitor 1, correlate with serum levels of its product and are associated with thrombophilia and coronary atherosclerosis. Various *SERPINE1* ;gene polymorphisms have been identified. However, only the functional 5G/4G polymorphism has been assessed in the context of aneurysmal subarachnoid hemorrhage (aSAH). We assessed associations of 6 *SERPINE1* polymorphisms with the clinical sequelae of aSAH. SERPINE1 gene polymorphisms were associated with delayed cerebral ischemia and functional outcome after aSAH. These associations may arise from alterations of plasminogen activator inhibitor 1 levels [1].

The risk of thrombotic events can also be affected by variations in Plasminogen Activator Inhibitor type 1 activity (PAI-1) [2]. This molecule belongs to the serpin family inhibitors and is one of the key inhibitors of plasmin generation in the plasma and tissues. It acts by inhibiting the activity of the fibrinolytic system, via inhibition of tPA (tissue plasminogen activator) and uPA (urokinase plasminogen activator). Localized on the chromosome 7, PAI-1 gene covers 16 Kb of longer and contains9 exons. It encodes for a protein of 50 KDa, composed by 379 amino acids [3]. A single insertion/deletion of a “G” at position − 675 in the promoter region of the gene gives rise to 4G and 5G alleles, which differ by their regulation of PAI-1 activity[4]. Numerous studies have found an association between SNPs in PAI-1 gene and the development of many diseases (HTA, stroke…); concerning (MI), results remain contradictory [5].

Plasminogen activator inhibitor-1 (PAI-1) is a protein of interest for both arterial remodeling and thrombotic risk as it regulates cell migration and vascular thrombosis. Elevated PAI-1 antigen levels have been identified as a potential biomarker for coronary artery disease and metabolic syndrome while being modulated by a number of atherosclerotic risk factors. Although linked by some studies as a marker of disease severity and prognosis, it remains to be understood whether it is also a mediator and/or therapeutic target of vascular disease. In this review, we discuss the current understanding of PAI-1 in vascular disease and its potential role in in-stent restenosis and stent thrombosis [6]

PAI-1 gene polymorphisms could change its generation level. Moreover it has been linked to worsening of some thrombotic diseases; as coronary heart disease, atherosclerosis and stroke [7]. Investigated the effects of overweight/obesity and lifestyle (smoking and alcohol intake) on plasma PAI-1 levels in 203 healthy men (age 44.5 ± 8.1) who visited our department for health check. These results suggest that overweight/obesity and unfavorable lifestyle such as smoking and heavy alcohol consumption may increase plasma PAI-1 levels and might be linked to the risk of ischemic heart disease [8].

High plasma *PAI-1* levels have been shown to be associated with atherosclerosis, restenosis, and in the pathogenesis of disorders associated with thrombotic events such as myocardial infarction and deep venous thrombosis [9,10,11].

It was reported that *PAI-1* gene polymorphism such as *4G*/*5G* and *4G*/*4G* genotypes has been associated with the higher gene expression and higher *PAI-1* levels in the circulation resulting in an increased risk for thrombotic events such as MI and stroke [12,13,14,15,16]

It has been proven that there are synergistic and cumulative effects of the 5G allele of PAI-1 polymorphism and the C allele of IL-6 polymorphism in smoking when determining the risk associated with coronary heart disease [17]. In a study by Hoekstra et al., 2002, the relationship of 4G / 5G polymorphism with intima media carotid artery parameters was studied in young smokers. [18].

The effect of RAS polymorphisms on t-PA and PAI-1 levels has been studied previously, but no data are available about the joint effects of *RAS* and bradykinin gene polymorphisms on markers of the fibrinolytic system [19]. The *I/D* polymorphism of the *ACE* gene has been associated with PAI-1 levels in apparently healthy persons https://www.sciencedirect.com/science/article/pii/S0888754306003223 - bib13, in patients with

hypertension [20], and in patients attending a metabolic ward [21] https://www.sciencedirect.com/science/article/pii/S0888754306003223 - bib14.

According to the literature, the relationship between PAI-1 and tPA levels with stroke was investigated (function of the PAI-1 4G / 5G and -844G / A genotypes), as well as the relationship between these PAI-1 gene variants and stroke risk in a control study of 135 patients with ischemic stroke. Regression analysis demonstrated that 4G homozygosity (OR = 0.176), hypertension (OR = 6.288), and body mass index (OR = 1.325) were independent predictors of stroke. The protective effect of 4G allele against stroke suggests involvement of PAI-1 4G/5G polymorphism in stroke through a mechanism not related to fibrinolysis, possibly involving altered plaque stabilization, and/or through antagonism of tPA effects [22].

The fact that, essential hypertension predisposes to the procoagulant state characterized by hyperfibrinogenemia and hypofibrinolysis. Perindopril reduced fibrinogen levels in ACE II homozygotes due to its more potent inhibitory action on the renin-angiotensin system in such patients. It improved fibrinolysis by increasing t-PA levels regardless of ACE and PAI-1 genotype [23].

In one study, the effect of gene interaction on the MI risk gene was analyzed, combinations of G894T eNOS-4G / 5G PAI, G894T eNOS-T1131C APOA5 and 4G / 5G PAI-T1131C APOA5 were studied, and the dependence or absence of their only effect on the risk of heart attack was evaluated myocardium. Results suggest that the eNOS and APOA5 genes might be dependently associated with MI' risk of occurrence, as the *P* values of the associated genotype-combinations were lower than those obtained by the analysis of each gene separately. The associations of PAI gene with eNOS and APOA5 were statistically very significant; however, the *P* values of the associated genotypic-combinations were identical or superior to those obtained by the simple analysis of each one of them, indicating that their actions on MI risk are likely to be independent [24].

High PAI-1 levels after acute myocardial infarction (AMI) are associated with poor outcomes. The concentrations of insulin-like molecules, pro-inflammatory cytokines and insertion polymorphism (5G) / deletion (4G) in the promoter of the PAI-1 gene affect the level of circulating PAI-1. No evidence was found that subjects with 4G / 4G polymorphism had higher PAI-1 levels upon admission or 6 months after AMI. In these patients, PAI-1 levels are associated with concentrations of proinsulin-like molecules and pro-inflammatory cytokines [25].

To investigate whether the 4G/5G polymorphism of plasminogen activator inhibitor type 1 (PAI-1) and the −7351 C/T polymorphism of tissue-type plasminogen activator (t-PA) are associated with ischemic stroke, was conducted a case-control study of 190 hospital cases of first-ever ischemic stroke and 185 community-based controls. Findings do not indicate any association between the PAI-1 or t-PA polymorphisms and risk of stroke. Adding these results to previous studies in a meta-analysis indicated a strong association between this polymorphism and ischemic stroke (P = .0002), with no publication bias but with extreme heterogeneity. There was evidence of stroke association with the PAI-1 4G/5G locus [25].

From the analysis of published data, various relationships of the PAI-1 gene with the main symptoms of cardiovascular diseases and their risk factors are traced. For example, the 4G/5G genotypes and plasma PAI-1 levels were determined in 565 Chinese, 211 with and 354 without hypertension to study the genotype effect and the mode of gene–environment interaction. The present study showed that the 4G/4G genotype was associated with elevated plasma PAI-1 activity in Chinese patients with and without hypertension. The contribution of the PAI-1 genotype seemed larger in women. In hypertensive carrying the 4G/4G genotype, higher TG was correlated with higher PAI-1, suggesting a possible contribution of gene–environmental interaction to their high risk for atherothrombotic disease [26].

Also discovered clock and Bmal1 genes polymorphisms were independent risk factors of elevated plasm PAI-1 in hypertensive patients (the innovation fund project of Fujian Province Health Department) [27]

Prolonged euglobulin clot lysis time (ECLT) and increased level of plasminogen activator inhibitor-1 (PAI-1) were reported to be risk factors of arterial ischemic stroke (AIS) by some studies; however, these findings were not supported by other studies. The objective of this study was to determine the association of ECLT, PAI-1 level, and polymorphisms of 4G and 5G of PAI-1 gene to the development of ischemic stroke (IS) in Thai children. However, these results have not been confirmed by other studies. For example, in children from 1 to 18 years old in Thailand, the relationship of ECLT, the level of PAI-1 and the 4G and 5G polymorphisms of the PAI-1 gene with the development of ischemic stroke (II) was investigated. The PAI-1 level and 4G/5G polymorphism may not be a risk factor of AIS in this population. But it was found that 4G / 5G polymorphism was the most common PAI-1 genotype in this study [28].

In a study conducted by Tunisians to detect a link between PAI-1 and myocardial infarction (MI) gene polymorphism, MI was accelerated by acquired and hereditary risk factors, including G/A and 4G/5G polymorphisms in the plasminogen activator inhibitor inhibitor gene promoter -1 (PAI-1). This study indicates that the risk of MI was notably high in 4G carriers and a carriers with elevated plasma PAI-1, and were associated with reduced t-PA levels [29].

Currently, it is possible to predict the development of coronary heart disease (CHD) as a result of revealing a genetic predisposition to dyslipidemia with the development of vascular atherosclerosis, to a disturbance of the blood coagulation system and fibrinolysis, to endothelial dysfunction and remodeling of the vascular wall, hypertrophy and remodeling of the left ventricle myocardium. To the category of actively studied genetic factors, certain allelic variants of which may be associated with an increased risk of exacerbation of IHD, include polymorphic markers of genes encoding substances - regulators of vascular tone, hemostasis and lipid metabolism, in particular, endothelin-1 gene, endothelial nitric oxide synthetase 3 type, ACE inhibitor, fibrinogen, platelet glycoprotein receptors, lipoprotein lipase and others, since the level of these biologically active substances is an independent developmental RF cardio-vascular complications [30].

In some cases, a patient under 40-50 years old is expected to have a high risk of developing (MI), and the likelihood of developing this form of IHD with certain allelic variants of the genes increases sharply. It should be noted that a relatively small number of prospective studies allows us to establish the real prognostic value of candidate genes in comparison with the prognostic value of other factors of cardiovascular risk.

An elevated level of plasminogen activator inhibitor type I (PAI-1) is associated with a more severe course of IHD. The mechanism of this increase is still not clear. One possible hypothesis to explain this condition is its genetic predisposition. It is known that the level of mRNA transcription of the PAI-1 gene correlates with a specific allelic variant of this gene. This is what allows us to consider the PAI-1 gene as one of the possible candidate genes that determine the hereditary predisposition to IHD [31]. Most often, a mononucleotide polymorphic deletion / insertion marker located in the promoter region at position - 675 (4G (-675) 5G) is used in studies. Carriers of the 4G allele, both in the hetero- and homozygous state, have a higher level of plasma PAI-1 and a greater risk of developing acute coronary syndromes [32].

In continuing research in this direction, we studied the prevalence of a type I plasminogen activator inhibitor (PAI-1) among respondents of Uzbek nationality.

MATERIALS AND RESEARCH METHODS

126 respondents were examined: 61 patients with coronary heart disease (CHD) aged 45 to 60 years (males) who were hospitalized in the cardiology department of the 1st TMA clinic in Tashkent. In the comparison group were 65 unrelated males of Uzbek nationality without clinical signs of coronary artery disease. The examined noted: AH in 53 people, GHS in 38 people, obesity in 23 people, smoking in 22 people.

The average age in both groups was: 56.8 ± 6.40 years (42 to 66 years). Respondents included in the study suffered from ischemic heart disease of 11.4 ± 2.47 years. Analysis of DNA samples by the PAI-I gene (4G / 5G) was carried out by multiplex and standard

polymerase chain reaction and on thermal cyclers CG-1-96 “CorbettResearch” (Australia) and 2720 “AppliedBiosystems” (USA), using kits of LLC “GenoTehnologiya” ”and“ Litekh ”(Moscow), according to the manufacturers instructions. The SNP-express system is a set of reagents for detecting mutations (polymorphisms) in the human genome.

Two amplification reactions are conducted in parallel with a sample of isolated DNA, with two pairs of allele-specific primers. The results of the analysis allow us to give three types of conclusions: homozygote for allele 1; heterozygous; homozygote for the allele 2.

Mathematical Analysis of the Methods

The deviation of the distribution of genotypes from the canonical distribution of Hardy-Weinberg was estimated using the computer program “GenePop”. Predictive efficiency (AUC-classifier) was determined by the standard formula: AUC = (Se + Sp) / 2; where Se and Sp are the sensitivity and specificity of the polymorphic marker, respectively. Statistical processing of the results was carried out using the OpenEpi statistical software package (ver.9.3).

Statistical processing of data

The obtained data during the study were subjected to statistical processing on a Pentium-IV personal computer using the Microsoft Office Excel 2010 software package, including the use of the built-in statistical processing functions, as well as the STATISTICA 6.0 program. The statistical significance of the obtained measurements when comparing the average values was determined by the Student criterion (t) with the calculation of the probability of error (P) when checking the normality of the distribution (by the excess criterion) and the equality of the general variances (Fisher's F-test). For statistically significant changes, the confidence level was $p < 0.05$.

All patients were in hospital for in-patients and they were received pathogenetic and symptomatic treatment of coronary artery disease.

RESULTS

Now existence of the genetic predisposition to development of clottages caused by mutations and polymorphic options of the genes coding synthesis of participating proteins of haemostatic reactions is proved. Several tens of genetic options associated with disturbances in system of a hemostasis and risk of a clottage are taped. A polymorphism of an inhibitor of the activator of a plasminogen of the I type (PAI-I), a mutation of a factor of II – a prothrombin, factor V Leiden, and also MTHFR gene polymorphisms belong to number of the most important of them contributing to development of venous clottages.

In this regard, we for the first time studied the frequency of occurrence of different options of genotypes of a gene of PAI-1, clarification of its role in association with the main RF of IHD. Selection criteria for determination of additional prognostic criterion were patients with SA of Uzbek nationality.

The following data on the frequency of occurrence of alleles and genotypes of G5/G4 polymorphism of a gene of PAI in group of patients with SA and probands specified in tables 1-1.1 were obtained.

Table 1

Frequency of distribution of alleles of G5/G4 polymorphism of PAI gene in group of patients with IHD and healthy individuals

№	Groups	N	Frequency alleles			
			5G		4G	
			N	%	N	%
1	Main Group (n=61)	61	67	54,9	55	45,1
2	Control Group (n=65)	65	93	71,5	37	28,5

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Frequency of alleles of PAI-1 gene is investigated also in the control group consisting of 65 people (130 chromosomes). Frequency of 4G allele at healthy faces made 37(28,5) %. It is revealed 6 (9,2%) homozygous carrier and 25 (38,5%) heterozygous carriers of the given allele. Frequency of 5G allele at probands of this group made 93(71,5%). In a homozygous state it was revealed at 34 (52,3%) the person.

Population distribution of alleles of PAI-1 gene is investigated at 61 patients with IHD (122 chromosomes). Frequency of 4G allele in this group made 55(45,1%). 13 homozygous carriers and 29 heterozygous carriers of this allele are revealed. Frequency 5G allele at patients of the main group made 67(54,9%). This allele in a homozygous state was revealed at 34 people.

Table 1.1

Frequency of distribution of genotypes of G5/G4 polymorphism of PAI gene in group of patients with IHD and healthy individuals

Polymorphism	Patients with IHD (n=61)	Healthy individuals with risk factors of IHD (n=65)	OR	P
Genotypes				
4G/4G	13 (21,3%)	6 (9,2%)	2,66	
4G/5G	29 (47,5%)	25 (38,5%)	1,45	p<0.05
5G/5G	19(31,1%).	34 (52,3%)	0,41	

The analysis of genotyping of the studied persons of the Uzbek nationality showed that, at IHD patients mutagen of 4G allele of gene PAI-1 in a homozygous and heterozygous state what makes 21,3% and 47,5%, respectively, meets more than in group of healthy faces at which given distributions of genotypes made 9,2% and 38,5%. Points these data probability of influence of existence of 4G allele of PAI-1 gene, especially in a heterozygous state, to development of IHD, in particular of CVD.

5G/5G the group of healthy faces in number of 34 people in 52.3% of cases, than at 19 (31,1%) IHD patients caused a stir in the greatest occurrence of a favorable genotype. Thus, these differences have the high statistical importance and wear, nonrandom character (p<0.05).

Table 2.

Distribution of frequencies of genotypes under Hardy-Weinberg's law. The expected and observed frequencies of distribution of genotypes on DHW in the main and control groups:

№	Groups	Frequencies of distribution of genotypes			Total	χ2	P
		5G/5G	G5/G4	4G/4G			
1	Main Group (n=61)	19	29	13	n=61	7,00	0.03
	Expected frequency (n=61)	25.66	26.14	9.2			
2	Control Group (n=65)	34	25	6	n=65		
	Expected frequency (n=65)	27.34	27.86	9.8			
	Total	53	54	19	n=126		

Notes: χ2 =AMOUNT (observed - expected) 2/ expected = ((19-25,66)2/25,66)+((29-26,14)2/26,14)+((13-9,2)2/9,2)+((34-27,34)2/27,34)+((25-27,86)2/27,86)+((6-9,8)2/9,8)=7,00
Degree of freedom (df) = (number of columns-1)*(Number of lines-1)=(3-1)*(2-1)=2
Our indicator is in area p<0.05, calculated with the help of Microsoft Excel p=0.03

For this polymorphism at patients with SA and conditionally healthy donors, observed distribution of genotypes of this polymorphism correspond to theoretical and has rather high h_{obs} and h_{exp} level (the observed and expected heterozygosis) at Hardy-Weinberg's equilibrium ($p<0.05$) on the basis of the Chi-square indicator ($\chi 2=7,00$) it is established statistically significant differences on distribution of genotypes 5G/5G, G5/G4, 4G/4G between patients of the main group and probands of control group ($p<0.05$).

Distribution of frequencies of genotypes of a polymorphic marker 4G (-675)5G of PAI-1 gene in groups of IHD patients depending on a functional class of SA are presented in table 3.

Table 3

Distribution of frequencies of genotypes of a polymorphic marker 4G(-75)5G of PAI-1 gene in subgroups of IHD patients.

Groups	GenePAI-1,detected genotypes, n (%)			Total	χ^2	P
	5G/5G	5G/4G	4G/4G			
Patients with SA FC II	6	14	4	n=24	1,85	0.39
Expected	7.48	11.41	5.11			
Patients with SA FC III	13	15	9	n=37		
Expected	11.52	17.59	7.89			
Total	19	29	13	n=61		

Notes: χ^2 =AMOUNT (observed - expected)^2/observed = 1,85
Degree of freedom (df)= (Number of columns-1)*(Number of lines-1)=(3-1)*(2-1)=2
On the $p<0.05$ significance level, with 2nd degree of freedom, the number in the table must be equal to 5,99. But we have 1,85.
Our indicatorpis in the areap>0.05, calculated in calculations with the help of Microsoft Excel p=0.39

Assessment of occurrence of various genotypes of a polymorphic marker 4G(-675)5G of PAI-1 gene, established that differences between distribution 5G/5G,5G/4G,4G/4G of genotypes depending on FC of SA weren't reliable as HI – a square on persen turned out $\chi 2=1,85$ that corresponds ($p>0.05$). On the basis of these results it is possible to assume that, existence of heterozygous and homozygous mutagen genotypes of a gene of PAI-1 doesn't influence disease severity in a particular on degree of FC of SA.

When genotyping of the studied groups in relation to the frequency of occurrence of a favorable homozygous genotype 5G/5G of PAI gene, in group of patients of CHD, the low frequency of this genotype in 31,1% cases, in comparison to statistically significant frequent occurrence of a genotype 5G/5G in 52,3% cases among healthy faces is taped. Occurrence at patients of SA of a heterozygous polymorphic genotype 5G/4G of PAI gene, made statistically significantly 47,5% of cases, in comparison with control group in 38,5% cases ($p<0.05$) that demonstrates probability of high prevalence of this genotype at persons of the Uzbek nationality.

The frequency distribution of genotypes corresponded to Hardy-Weinberg equilibrium. In this regard, was made a comparison of the main clinical parameters and risk factors for atherosclerosis in patients with different genotypes of the polymorphic marker 4G (-675) 5G of the PAI-1 gene in the group of patients with coronary artery disease.

Table 4

The vain clinical characteristics of patients with different genotypes of the polymorphic marker *4G (-675) 5G* of the *PAI-1* gene

Parameters	Genotype *4G/4G* (n=13)	Genotype *4G/5G* (n=29)	Genotype *5G/5G* (n=19)	P
Age	59,4±2,46	56,7±1,50	57,6±3,01	p> 0.05
Hereditary history, n(%)	8(61,5)	19(65,5)	11(57,8)	p> 0.05
AH, n(%)	11(84,6)	27(93,1)	15(78,9)	p> 0.05
BMI>25 кг/м^2, n(%)	12(92,3)	26(89,6)	14(73,6)	p<0.05*
HCh, n(%)	11(84,6)	18(62,1)	9 (47,4)	p<0.05*
ADS, n(%)	12 (92,3)	28 (96,5)	14 (73,6)	p> 0.05
Hypodynamia, n (%)	9 (69,2)	17 (58,6)	11 (57,9)	p<0.05*
Smoking, n(%)	8(61,5)	9(31,0)	5(26,3)	p<0.05*

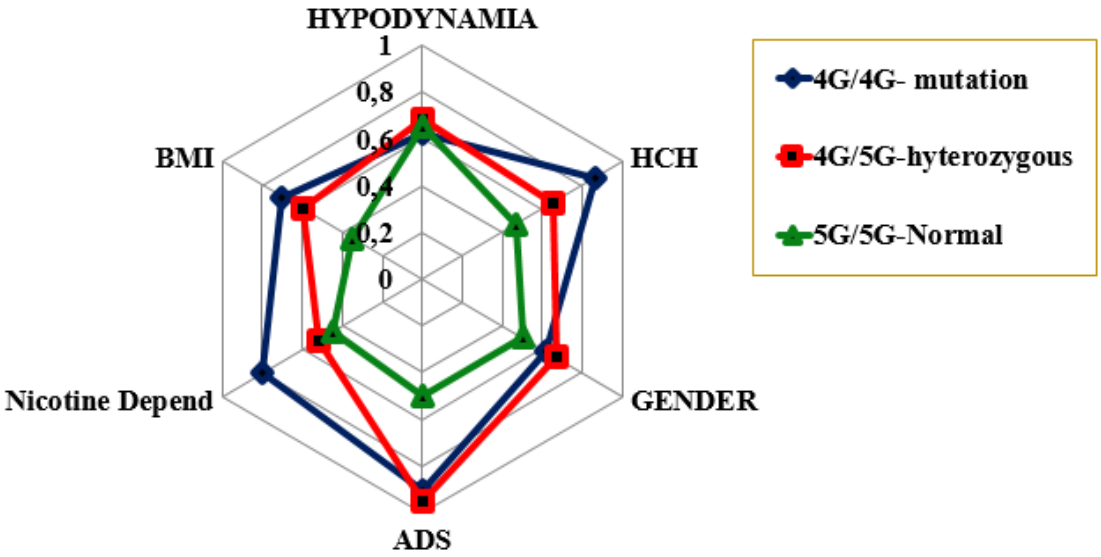
Note: * - P <0.05 significant difference between the indicators.

The analysis of interrelation of not modified and modified risk factors with 5G/5G, 4G/5G and 4G/4G genotypes of PAI-1 gene established that genotyping between groups considerably didn't differ depending on age whereas heriditary burdeness on IHD met at a heterozygous polymorphic genotype (61,5%) more often, and also a homozygous mutagen genotype (65,5%) of patients with IHD, at healthy faces the frequency of genetic predisposition between probands with various genotypes appeared almost equally. Among patients of the main group with genotypes 4G/4G an obesity of various degree is taped in 46,2% of cases, at a genotype of 4G/5G in 37,9% and at 5G/5G 26,3%, therefore is rather more often at patients with SAwith an obesity including the third degree of PAI-1 gene mutation was genotyped.

Connection of mutagen genotypes with smoking appeared to be one of the most significant as among patients with heterozygous genotype smokers in 61,5% of cases, in comparison with patients with a polymorphic genotype and a homozygous genotype of wild allele 5G made 31% and 26,3% of smokers, respectively prevailed. Existence and degree of expression of the accompanying ADS at patients of the main group, prevailed at persons with mutagen 4G/4G in 92,4% and a polymorphic 4G/5G genotype in 96,5% of cases, and at patients with a genotype 5G/5G ADS in rather smaller degree is revealed at 73,6% of cases. HCH was also significant RF interconnected with mutagen genotypes as most high cholesterin was registered at patients with SA with PAI-1 gene mutation (84,6%), in comparison with patients having a polymorphic gene of PAI-1 (62,1%) and a favorable gene of PAI-1 (47,4%). At patients with SA with all options of genotypes significant differences on existence of a hypodynamia and degree of physical activity it isn't established.

For assessment of the interconnected influence of mutagen genotypes of a gene of PAI-1 in associations with IHD RF such as smoking, the accompanying ADS, obesities, HCH and a hypodynamia, and also with genetic predisposition the correlation analysis represented in fig. 1 was carried out.

Fig. 1 Correlation analysis of RF and PAI-1 gene's genotypes



The analysis of correlation communication of a mutagen homozygous genotype showed that this mutation of a gene of PAI-1 has strong direct correlation with ADS (r= +0,9), smoking (r= +0,8), HCH (r= +0,86) and an obesity (r= +0,7), and also average correlation communication with a hypodynamia (r= +0,62) and genetic predisposition (r= +0,62). The polymorphic heterozygous genotype of a gene of PAI-1 had strong direct correlation link only with ADS (r= +0,95) whereas had an average correlation with a hypodynamia (r= +0,68), genetic predisposition (r= +0,67), HCH (r= +0,65), an obesity (r= +0,6) and smoking (r= +0,52). Less moderate the favorable homozygous genotype 5G/5G with all FR had correlation communication: hypodynamia (r= +0,65), ADS (r= +0,5), genetic predisposition (r= +0,5), HCH (r= +0,47), smoking (r= +0,45) and obesity (r= +0,45).

The research of system parameters of a hemostasis depending on genotyping of a gene of PAI-1 was conducted only at patients of the main group. Comparison of key parameters of a coagulogram at patients with different genotypes of PAI-1 is presented in table 5.

Table 5

Key parameters of hemostasis system at patients with various genotypes of a polymorphic marker 4g (-675) 5g of PAI-1 gene

Indicator	**Genotype *4G/4G* (n=13)**	**Genotype*4G/5G* (n=29)**	**Genotype *G/5G* (n=19)**	**P**
Haemotocrit	48.6±10.79	48.3±6.46	48.2±7.20	p> 0.05
Trombin time	24.3±1.84	23.1±3.90	21.9±11.46	p>0.05
Fibrinogen	278.4±42.84	248.8±73.94	273.5±77.84	p>0.05
PI	129.4±2,16	122.2±2.24	108.9±2.15	p<0.05*
PT	8.5±1.52	9.0±1.74	10.1±5.79	p<0.05*
aPTT	35.4±20.44	34.1±9.44	34.5±16.74	p>0.05
INR	0.92±0.11	0.96±0.13	1.11±0.10	p>0.05

Note:* - P<0,05 reliable difference of indicators.

Based on the evaluation indicators of coagulogram in patients with SA, revealing statistically significant deviation indicators of the external coagulation cascade: the shortening of prothrombin time (PT) and the increase of PI, patients with genotype 4G / 4G PT and PI was 8.5 ± 1.52 and 129.4±2.16, in genotype 4G / 5G was 9.0 ± 1.74 and 122.2±2.24, respectively, that indicates the likelihood of patient adherence to a hypercoagulable state.

While in terms of Trombin time, aPTT, fibrinogen, INR has not revealed significant differences between the study groups depending on genotyping.

Thus, the presence of a heterozygous genotype 5G> 4G polymorphic marker PAI-1 gene, especially the 4G / 4G mutation in was associated with adherence to hypercoagulability in patients with SA, which in turn increases the risk of thrombosis. The results of this study provide the basis for the improvement of methods of diagnosis and prognosis of early manifestation of coronary heart disease and the development of thromboembolic complications.

AUTHORS' CONTRIBUTION

Nurillaeva N.M. and Khasanova N.A conceived of the presented data. Nurillaeva N.M., Khasanova N.A, Zokirova M.B. and Nuritdinova N.B. contributed to the interpretation of the results. Khasanova N.A and Nurillaeva N.M. wrote the paper with input from all authors. Nurillaeva N.M. and Tseluyko V.I. supervised the findings of this work. All authors discussed the results and commented on the manuscript.

DISCUSSION

In this study we define the frequency of the prevalence of RF such as smoking, anxiety depressive syndrome, obesity, hypercholesterinemia and hypodynamia, and the presence of stress in 63 patients with IHD, in particular SA and 65 healthy individuals in Uzbek nationality, based genotyping gene SERPIN1.

When CAD combined with ADS and psychological distress components, the summation of their effect is expected, which may be accompanied by worsening of the clinical course of CAD according to the morphological characteristic of platelets, in the direction of enhancing the activity of the thrombocyte link of the hemostasis cascade [33].

The results of this study showed that the presence and severity of RF such as ADS, obesity and hypodynamia authentically prevalent in patients with SA ($p<0.05$), inparticular in FC III for compairing with probands in control group.

Differences in the frequency of occurrence smokers between the main and control groups were not statistically significant, while the degree of nicotine dependence in patients SA was significantly higher compared to the level of the NA smokers in healthy subjects ($p <0.005$). The presence of stress was almost the majority of the test, but the main group was dominated by the average level of stress, and in the control group most probands revealed lower levels of stress ($p <0.05$).

Studies have shown that the hereditary risk factors contribute significantly to the development of coronary heart disease. Thus, during the decoding of the human genome, researchers are paying attention to the molecular genetics of thrombosis and atherosclerosis to improve the understanding of the pathophysiology of arterial thrombosis. Rangeidentifiedcertaingenesthatcontributetotheriskofdevelopingthedisease.Currently, we proved the existence of a genetic predisposition to thrombosis, caused by mutations and polymorphic variants of genes coding for the synthesis of proteins participating hemostatic reactions. It revealed several dozens of genetic variants associated with disturbances in the hemostatic system and the risk of thrombosis. Among the most important of these predisposing to venous thrombosis are polymorphism plasminogen activator inhibitor type I (PAI-I), which is widely studied makes it possible for the risk of coronary vascular pathologies of venous thrombosis and other cardiovascular disorders.

It has been shown that level PAI-1 in plasma of blood associated with polymorphism in the promoter of the gene PAI-1, which is a single nucleotide deletion / insertion guanine (4G / 5G). People who are homozygous for the 4G allele have higher levels of PAI-1 in plasma, and homozygous for the 5G allele lower.

Currently, intensive work on the study of the role and frequency of occurrence mutagenic genotypes of genes coagulation balance in the development of arterial thrombosis and ischemic heart disease pathogenesis. The data in the literature, a comparative analysis of the frequency genotypes risk of MI with levels of the disease in some European countries.

Due to the relatively frequent of occurrence of mutagenic genotype 4G / 4G of the gene PAI-I, we first studied the frequency of occurrence of different variants of genotypes of the gene PAI-1, the identification of its role in the development of coronary heart disease in association with major RF.

Population distribution of alleles of the gene PAI-1 was studied in 61 patients with SA. 4G allele frequency in this group accounted for 45.1% of cases, while the 5G allele frequency was 54.9%. allele frequency of PAI-1 was studied as a control group consisting of 65 people. 4G allele frequency in healthy individuals accounted for 28.5% of cases, whereas the 5G incidence was 71.5%.

In a stratified analysis by ethnicity [34] noted in their meta-analysis that Asians and Caucasians subjects carrying the 4G allele had higher risk of cardiovascular diseases, when Festa et al. reported that the PAI 4G/5G genotype explained 0.63% of the variability of circulating PAI-1 levels in non-Hispanic whites, 0.99% in Hispanics, and 2.37% in blacks, and that there interaction analyses did not show any statistical differences in the relation between PAI-1 levels and the 4G/5G genotype by ethnicity [35].

Our study sample was not in HWG equilibrium for the distribution of 4G/5G PAI polymorphism among cases and controls ($P = 0.003$ and $P < 0.05$ respectively). For that reason, we cannot say that the distribution in our sample study describes the real one of this variant among Moroccan MI patients. Thus, we cannot conclude about its association with MI risk among Moroccan MI patients [36].

Analysis of genotyping investigated people of Uzbek population showed that, in patients with coronary artery disease mutagenic allele 4G of gene PAI-1 in the homozygous and heterozygous state, that is 21.3% and 47.5%, respectively, found in more than a group of healthy individuals who have a given distribution genotypes was 9.2% and 38.5% .These data indicates the likelihood of the presence of the allele 4G of gene PAI-1, especially in the heterozygous state for progression IHD, in particularly SA.

In addition, studying the relation of these polymorphisms to the level of active PAI-1 in Egyptian patients presenting to a single tertiary center in Cairo. On studying the effect of the presence of combined mutant genotypes or alleles in CAD patients, STEMI patients showed significantly higher frequencies for the presence of combined mutant alleles of PAI-1 4G/5G and PAI-1C/G polymorphisms (p 0.016) raising the possibility of multifactorial genetic alteration predisposition to acute coronary syndrome [37].

Based on the index chi-square ($\chi 2 = 7{,}00$) establishment of statistical significant differences in distribution genotypes 5G/5G, 4G/5G, 4G / 4G patients between the main group and the control group of probands ($p < 0.05$). When genotyping study groups with respect to the frequency of occurrence of favorable homozygous genotype 5G/5G gene PAI-1 in patients with SA identified low incidence of this genotype in 31.1% of cases, compared to the frequent of occurrence of a statistically significant genotype 5G/5G in 52.3% cases among healthy individuals.

Occurrence in patients with SA heterozygous polymorphic genotype 5G/4G of gene PAI-1 was statistically significant 47.5% of cases, compared with the control group, 38.5% in cases ($p < 0.05$) that gives evidence high probabilities of genotype prevalence in persons of Uzbekistan nationality. When evaluating the frequency of occurrence of mutations of gene PAI-1 fitted that

frequency mutagenic homozygous genotype 4G/4G in patients was significantly higher (21.3%) compared with the control group (9.2%).

Also important the fact that the data obtained in the study indicate the importance of early detection presence of polymorphic and mutagenic genotypes of gene PAI-1 in the Uzbek population.

In the study, we first studied the incidence of mutagenic genotypes of gene PAI-1 and clarify its role in association with major risk factors of IHD in the development of SA.

Given the multifactorial pathogenesis of coronary artery disease, that is the presence of many RF that lead to the development and progression of the disease, were analyzed for the presence of the polymorphism associations 4g (-675) 5g gene PAI-1 with clinical and anamnestic data, such as smoking, concomitant ADS, obesity, hypercholesterinemia, and physical inactivity in the studied groups.

Based on the findings on the relationship mutagenic genotypes of PAI-1 gene with RF such as ADS, smoking, hypercholesterinemia and obesity, as well as considering the relatively frequent of occurrence of homozygous 4G / 4G genotype of gene PAI-1 at the Uzbek people, it can be assumed about the interconnected influence on the development of IHD mutation of gene PAI-1 in combination with dominantly RF. Not enough importance is the fact that, in the course of the study detected predictive values polymorphic marker 4G (-675) 5G of gene PAI-1 in association with RF: ADS, smoking, obesity and hypercholesterinemia in the development of IHD, in particular the SA.

In order to assess the likely impact of genotypes of polymorphic marker4G (-675) 5G of gene PAI-1 on the flow and the risk of adverse complications of SA, such as blood clot organization analyzed the basic parameters of the coagulation balance in patients with SA.

Discovering new CAD risk factors at the level of genetic regulation of the coagulation and fibrinolysis cascades was subjected to intense research investigations. These research studies aimed at a better prediction and consequently prevention of this critical condition. Many polymorphisms have been implicated in the pathophysiology of CAD [38]. Among these are: polymorphisms of PAI-1 at different sites of the gene, which are important in determining PAI-1 activity thus controlling the balance between coagulation and fibrinolysis [15].

Study of the hemostatic system parameters depending on genotyping of gene PAI-1 was performed only in patients of the main group. The study detected presence of heterozygous genotype 5G>4G polymorphic marker of gene PAI-1, especially the 4G /4G mutation was associated with adherence to the hypercoagulability in patients with SA, which in turn increases the risk of thrombosis. The results of this study provide the basis for the improvement of methods of diagnosis and prognosis of early manifestation of IHD and the development of thromboembolic complications.

The results of this study showed that polymorphism genotype 1.5 times increased risk of developing coronary heart disease. Given the relatively frequent of occurrence of polymorphism 4G (-675) 5G gene PAI-1 in healthy individuals, as well as its association with RF such as smoking, obesity, hypercholesterinemia, and ADS, you can expect that in places without timely data, other modifications have on the probability distribution function of IHD in particular SA of probands in this group.

In the course of establishing the risk of developing IHD in particular in the presence of SA the homozygous 4G/ 4G genotype of gene PAI-1 is increased by more than 2.7 times, indicating the importance of the presence detection early mutation gene PAI-1 in population Uzbekistan. This indicates that the genotype contributes significantly to the development of the disease and is the most informative for determining genetic predisposition to the development of coronary artery disease and adverse complications.

Assessment of the results obtained in this study, can this essential life on the assumption that the early development of IHD in healthy people, especially at untimely eliminate the dominant risk factors, since the establishment of this correlation relationship mutagenic genotype of gene PAI-1 with smoking, obesity, ADS and HCH. This fact must be allow in the optimization

methods of early primary and secondary prevention of coronary heart disease in particular in fight with the RF.

CONCLUSION

The genotyping of the coagulation factor PAI-1 in the examined patients revealed the presence of unfavorable genotypes 4G/4G and 4G/5G was established more often in patients with coronary artery disease in 21.3% and 47.5% of cases, respectively, compared with the group of healthy individuals who This distribution of genotypes was 9.2% and 38.5%. Whereas, the prevalence rate of the 5G / 5G genotype was prevalent in 52.3% of the control group over the spread of 31.1% of patients in the IHD.

In patients with IHD of persons with 4G/5G and 4G/4G genotypes of the PAI-1 gene, there was a high incidence in patients with controlled PR: anxiety-depressive syndrome in 96,5% and 92,4%, obesity in 37,9% and 46,7%, smoking in 61,5% and 31,0%, HCS in 62,1% and 84,6% of cases, respectively

The presence of the heterozygous genotype 5G> 4G of the polymorphic marker of the PAI-1 gene and in particular of the 4G / 4G mutation is associated with a direct predisposition to hypercoagulation. In the presence of the 4G / 5G polymorphic genotype, the PAI-1 gene increases the risk of developing coronary artery disease by a factor of 1.5, 4G / 4G PAI1 gene increases the risk of CHD development and the number of adverse complications by more than 2.7 times.

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Conflict of interests

The authors declare that they received no support from any organization for the submitted work and that they had no financial relationships with any organization that might have an interest in the submitted work.

Literature

1. Hendrix P, Foreman PM, Harrigan MR, Fisher WS, Vyas NA, Lipsky RH, et al. Association of Plasminogen Activator Inhibitor 1 (SERPINE1) Polymorphisms and Aneurysmal Subarachnoid Hemorrhage // World Neurosurg. 2017 Sep;105:672–7.
2. Nakamura S, Nakamura I, Ma L, Vaughan DE, Fogo AB. Plasminogen activator inhibitor-1 expression is regulated by the angiotensin type 1 receptor in vivo // Kidney Int. 2000;58(1):251–9.
3. Hassani Idrissi H, Hmimech W, Diakite B, Korchi F, Baghdadi D, Habbal R, et al. Association of G894T eNOS, 4G/5G PAI and T1131C APOA5 polymorphisms with susceptibility to myocardial infarction in Morocco // Meta Gene. 2016;9:56–61.
4. Nordt TK, Lohrmann J, Bode C. Regulation of PAI-1 expression by genetic polymorphisms. Impact on atherogenesis // Thromb Res [Internet]. 2001 Sep 30 [cited 2019 Dec 14];103 Suppl 1:S1-5. Available from: http://www.ncbi.nlm.nih.gov/pubmed/11567663
5. Ding J, Nicklas BJ, Fallin MD, de Rekeneire N, Kritchevsky SB, Pahor M, et al. Plasminogen activator inhibitor type 1 gene polymorphisms and haplotypes are associated with plasma plasminogen activator inhibitor type 1 levels but not with myocardial infarction or stroke // Am Heart J [Internet]. 2006 Dec 1 [cited 2019 Dec 14];152(6):1109–15. Available from:

https://www.sciencedirect.com/science/article/abs/pii/S0002870306005278?via%3Dihub
6. Jung RG, Simard T, Labinaz A, Ramirez FD, Di Santo P, Motazedian P, et al. Role of plasminogen activator inhibitor-1 in coronary pathophysiology //Thromb Res. 2018 Apr;164:54–62.
7. Muller JE, Tofler GH, Stone PH. Circadian variation and triggers of onset of acute cardiovascular disease // Circulation. 1989;79(4):733–43.
8. Sasaki A, Kurisu A, Ohno M, Ikeda Y. Overweight/Obesity, Smoking, and Heavy Alcohol Consumption Are Important Determinants of Plasma PAI-1 Levels in Healthy Men // Am J Med Sci. 2001 Jul;322(1):19–23.
9. Figueras J, Monasterio J, Domingo E, Meneses B, Nieto E, Cortadellas J, et al. Prothrombotic profile in patients with vasospastic or non vasospastic angina and non significant coronary stenosis // Thromb J. 2011;9:1–7.
10. Deyoung MB, Tom C, Dichek DA. Formation in Balloon-Injured Rat Carotid Arteries. 2001;1:1972–7.
11. Katrancıoğlu N, Karahan O, Küçükkurtulgan H, Sanrı US, Kılıç AT. PAI-1 4G/4G gene polymorphism is associated with higher serum lipid level in Turkish population. Cumhur Tıp Derg [Internet]. 2011;33(3):307–11. Available from: http://dergipark.ulakbim.gov.tr/cumucmj/article/viewFile/938/1008000989
12. Assawamakin A, Sriratanaviriyakul N, Lalerd Y, Thongnoppakhun W, Praditsap O, Tongsima S, et al. Meta-analysis of the plasminogen activator inhibitor-1 (PAI-1) gene with insertion/deletion 4G/5G polymorphism and its susceptibility to ischemic stroke in Thai population // Asian Biomed. 2012;6(2):203–17.
13. Boekholdt SM, Bijsterveld NR, Moons AHM, Levi M, Büller HR, Peters RJG. Genetic variation in coagulation and fibrinolytic proteins and their relation with acute myocardial infarction: A systematic review // Circulation. 2001;104(25):3063–8.
14. Balta G, Altay C, Gurgey A. PAI-1 gene 4G/5G genotype: A risk factor for thrombosis in vessels of internal organs // Am J Hematol. 2002;71(2):89–93.
15. Eriksson P, Kallin B, Van't Hooft FM, Bavenholm P, Hamsten A. Allele-specific increase in basal transcription of the plasminogen- activator inhibitor 1 gene is associated with myocardial infarction // Proc Natl Acad Sci U S A. 1995;92(6):1851–5.
16. Spronk HMH, van der Voort D, ten Cate H. Blood coagulation and the risk of atherothrombosis: A complex relationship // Thromb J. 2004;2:1–10.
17. Sarecka B, Zak I, Krauze J. Synergistic effects of the polymorphisms in the PAI-1 and IL-6 genes with smoking in determining their associated risk with coronary artery disease // Clin Biochem. 2008 May;41(7–8):467–73.
18. Hoekstra T, Geleijnse JM, de Waart F, Nederhand R, Kluft C, Kok FJ, et al. The 4G/5G-polymorphism in the PAI-1 gene is not associated with markers of atherosclerosis in male smokers // Thromb Res. 2002 Aug;107(3–4):115–9.
19. Asselbergs FW, Williams SM, Hebert PR, Coffey CS, Hillege HL, Navis G, et al. Epistatic effects of polymorphisms in genes from the renin-angiotensin, bradykinin, and fibrinolytic systems on plasma t-PA and PAI-1 levels // Genomics. 2007 Mar;89(3):362–9.
20. Jeng JR, Harn HJ, Yueh KC, Jeng CY, Shieh SM. Plasminogen activator inhibitor-1 and angiotensin I converting enzyme gene polymorphism in patients with hypertension // Am J Hypertens. 1998;11(2):235–9.
21. Margaglione M, Grandone E, Vecchione G, Cappucci G, Giuliani N, Colaizzo D, et al. Plasminogen Activator Inhibitor-1 (PAI-1) Antigen Plasma Levels in Subjects Attending a Metabolic Ward: Relation to Polymorphisms of PAI-1 and Angiontensin Converting Enzyme (ACE) Genes // Arterioscler Thromb Vasc Biol [Internet]. 1997 Oct [cited 2019 Nov 19];17(10):2082–7. Available from: https://www.ahajournals.org/doi/10.1161/01.ATV.17.10.2082
22. Saidi S, Slamia LB, Mahjoub T, Ammou SB, Almawi WY. Association of PAI-1 4G/5G and -844G/A Gene Polymorphism and Changes in PAI-1/tPA Levels in Stroke: A Case-Control Study

// J Stroke Cerebrovasc Dis. 2007 Jul;16(4):153–9.
23. Jastrzębska M, Widecka K, Naruszewicz M, Ciechanowicz A, Janczak-Bazan A, Foltyńska A, et al. Effects of perindopril treatment on hemostatic function in patients with essential hypertension in relation to angiotensin converting enzyme (ACE) and plasminogen activator inhibitor-1 (PAI-1) gene polymorphisms // Nutr Metab Cardiovasc Dis. 2004 Oct;14(5):259–69.
24. Hassani Idrissi H, Hmimech W, Diakite B, Korchi F, Habbal R, Nadifi S. Synergic predisposing effect of G894T (eNOS), 4G/5G (PAI) and T1131C (APOA5) polymorphisms to myocardial infarction // Gene Reports. 2018 Jun;11:165–9.
25. Panahloo A, Mohamed-Ali V, Gray RP, Humphries SE, Yudkin JS. Plasminogen activator inhibitor-1 (PAI-1) activity post myocardial infarction: the role of acute phase reactants, insulin-like molecules and promoter (4G/5G) polymorphism in the PAI-1 gene // Atherosclerosis. 2003 Jun;168(2):297–304.
26. Jeng J-R. Association of PAI-1 gene promoter 4g/5g polymorphism with plasma PAI-1 activity in Chinese patients with and without hypertension. //Am J Hypertens. 2003 Apr;16(4):290–6.
27. Huan W, Jiuying L, Hui C. GW25-e0783 Association between Plasma PAI-1 Levels and Clock Genes Polymorphisms in Primary Hypertensions // J Am Coll Cardiol. 2014 Oct;64(16):C175.
28. Natesirinilkul R, Sasanakul W, Chuansumrit A, Kadegasem P, Visudtibhan A, Wongwerawattanakoon P, et al. Global Fibrinolytic Activity, PAI-1 Level, and 4G/5G Polymorphism in Thai Children with Arterial Ischemic Stroke // J Stroke Cerebrovasc Dis. 2014 Nov;23(10):2566–72.
29. Abboud N, Ghazouani L, Saidi S, Belhaj Khlifa S, Addad F, Mahjoub M, et al. A016 Association of PAI-1 4G/5G and -844G/A gene polymorphisms and changes in PAI-1/TPA levels in myocardial infarction. A casecontrol study // Arch Cardiovasc Dis. 2009 Mar;102:S12.
30. Boeva O. konstitutsionalnie i geneticheskie faktori v prognozirovanii riska povtornix obostreniy ishemicheskoy bolezni serdsa // Avtoreferat.Stavropol, 2008.- 342 s
31. Zateyshchikov D.A., Selesneva N.D., Minouchkina L.O., O.Yu.Kudryashova, SereginYu.V., Nosikov V.V., Barinov V.G., Cimbalova T.E., Sidorenko B.A. Plasminogen inhibitor activator gene 4G(-675)5G polymorphism and hemostasis in patients with ischemic heart disease // Trombi,krovotochivost I bolezni sosudov 2002
32. Kumagai N, Takahashi N, Kinoshita M, Tsunematsu S, Tsuchimoto K, Saito H, et al. Polymorphisms of NS5B protein relates to early clearance of hepatitis C virus by interferon plus ribavirin: a pilot study // J Viral Hepat. 2004;11:225--35.
33. Abdumalikova F.B. Influence of psychological and personality characteristics of patients with coronary artery disease on the phenotype of platelets // european journal of pharmaceutical and medical research 2019;6(5):662–6.
34. Zhang H, Dong P, Yang X, Liu Z. Is Associated With Coronary Artery Disease Risk : a Meta-Analysis Commonly Studied Functional Variant in the // Int J Clin Exp Med 2014;7(10):3777–88.
35. Festa A, D'Agostino R, Rich SS, Jenny NS, Tracy RP, Haffner SM. Promoter (4G/5G) plasminogen activator inhibitor-1 genotype and plasminogen activator inhibitor-1 levels in blacks, hispanics, and non-hispanic whites: The Insulin Resistance Atherosclerosis Study // Circulation. 2003;107(19):2422–7.
36. Hassani Idrissi H, Hmimech W, Diakite B, Korchi F, Baghdadi D, Habbal R, et al. Association of G894T eNOS, 4G/5G PAI and T1131C APOA5 polymorphisms with susceptibility to myocardial infarction in Morocco // Meta Gene. 2016 Sep;9:56–61.
37. Al-Wakeel H, Sewelam N, Khaled M, Abdelbary A. Impact of PAI-1 4G/5G and C > G polymorphisms in acute ST elevation myocardial infarction and stable angina patients: A single center Egyptian study // Egypt J Med Hum Genet. 2018 Oct;19(4):325–31.
38. Martínez-Quintana E, Chirino R, Nieto-Lago V, Pérez-Jiménez P, López-Ríos L, Rodríguez-González F. Prognostic value of ACE I/D, AT1R A1166C, PAI-I 4G/5G and GPIIIa a1/a2 polymorphisms in myocardial infarction // Cardiol J. 2014;21(3):229–37.