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Genetic factors in development of cardiovascular diseases in women in menopause

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Objective. To determine the frequency of different allelic variants of the plasminogen activator inhibitor type 1 (PAI-1) and methylene tetrahydrofolate reductase (MTHFR) among postmenopausal women with coronary heart disease (CHD) and arterial hypertension (AH).

Materials and methods. There were examined 125 women of menopausal age with postmenopause duration from 3 to 18 years.The main group consisted of 32 women with CHD and 37 with hypertension.The control group consisted of 56 conditionally healthy women.The study of genetic polymorphism was performed using the method of allele-specific polymerase chain reaction, followed by hydrolysis of amplicons with an appropriate restriction endonuclease.

Results. This is a clear link between the risk of cardiovascular disease and an increase in the level of PAI-1 in the blood among postmenopausal women, due to the presence of the 4C allele of the PAI-1 gene and the genetic polymorphism of MTHFR in their genotype. Increasing of the concentration of PAI-1 is among the genetic causes of reduced fibrinolytic activity and tendency to thrombosis in women with menopausal syndrome, which is associated with the risk of myocardial infarction, diabetes mellitus type 1. The regulation of fibrinolysis is closely related to the concentration of homocysteine in the blood. One of the reasons for moderate hyperhomocysteinemia is the C677T mutation in the MTHFR gene. In women of the main group, 4G alleles in the PAI-1 gene and T alleles in the MTHFR gene are detected more often (38%) than in the control group (19%).In women with metabolic syndrome, the frequency of the "defective" 4G allele in the PAI-1 gene is high, with 29% of women having a homozygous genotype - 4G / 4G.

Conclusion. In addition to estrogen deficiency, genetic factors also play a role in the development of cardiovascular diseases in postmenopausal women.

Keywords:
postmenopause, gene polymorphism, plasminogen activator inhibitor, methylenetetrahydrofolate reductase, cardiovascular complications

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Cardiovascular diseases are the most frequent causes of death among women of menopausal age. In addition to the hormonal disorders associated with this period of life, at the present time it's beyond any doubt that there is a genetic predisposition to the development of cardiovascular diseases.

The study addresses the question of relationship between the risk of developing cardiovascular diseases and increasing the level of plasminogen activator inhibitor type 1 (PAI-1) in

the blood due to the presence in the 4G genotype of the allele of the PAI-1 gene and polymorphism of the 5,10-methylenetetrahydrofolate reductase gene (MTHFR) [20, 21].

The study of genetic factors that play a role in the development of cardiovascular diseases suggests that women of postmenopausal age constitute a high risk group for the listed diseases and this must be taken into account when choosing the optimal treatment method for menopausal disorders.

PAI-1 plays an important role in the regulation of fibrinolytic activity. Various diseases are associated with an increase in its blood level - hypertension of pregnant women, myocardial infarction, atherosclerotic vascular lesions [3, 4].

A positive correlation was also found between the carrier of the 4G allele and the risk of myocardial infarction in different age groups [7-9]. At the same time, it was noted that the concentration of growth factors, hormones in the blood, the presence of comorbidities and smoking can influence the results of studies. There is no single opinion regarding the role of 4G / 5G polymorphism in the development of hypertension [11, 12]. At the same time, in women with arterial hypertension, the 4G allele is detected more often than in healthy individuals [10].

MTHFR provides the conversion of folate to the biologically active form - 5-methyltetrahydrofolate, which is the most important donor of the methyl group in the body [17]. The nucleotide C→T substitution at the 677th position of the gene, accompanied by a noticeable alanine to valine at the 222nd position of the protein, leads to the synthesis of a thermolabile enzyme with reduced activity. A number of studies have shown that the presence of the T-allele in the genome, especially in the homozygous form, correlates with the risk of developing cardiovascular diseases, hypertension outside and during pregnancy [19]. At the same time, there are many publications in which there is no such connection between the C677T mutation and cardiovascular diseases [20].

The purpose of this study was to determine the frequency of different allelic variants of the PAI-1 and MTHFR genes in postmenopausal women with coronary heart diseaseand essential hypertension.

Material and methods

There were examined 125 women of menopausal age with a postmenopause duration from 3 to 18 years. The main group consisted of 32 women with coronary heart disease (IHD) or after myocardial infarction (MI) - group IA, and 37 women with arterial hypertension (AH) - IB group. Parents of 47 women of these groups also had diseases of the cardiovascular system (in 25 - myocardial infarction, in 43 - hypertension). The control group consisted of 56 conditionally healthy women who did not suffer from coronary heart disease and hypertension and matched for age. The study of genetic polymorphism was performed using the method of allele-specific polymerase chain reaction, followed by hydrolysis of the corresponding restriction amplicons. Identification of allelic variants was performed by the presence of a recognition site for the corresponding restriction endonuclease using agarose gel electrophoresis.

Results and discussion

Our studies have shown that in postmenopausal women, both with myocardial infarction in anamnesis and with hypertension, genotypes containing the 4G allele in the homo- or heterozygous form were encountered more often than in the control group (4G/4G or 4G/5G).

Table 1. Frequency of PAI-1 and MTHFR allele genotypes among women with cardiovascular diseases

Genotype and allele	Control group (n = 56)		Ischemic heart disease (n = 32)		Arterial hypertension (n = 37)	
	abc.	%	abc.	%	abc.	%
Genotype PAI-1 4G/4G	11	19,6	6	25,5	12	32,4
4G/5G	24	42,8	25	68,1**	23	62,2
5G/5G	21	37,5	1	6,4**	2	5,4**
Allele4G	46	41,0	37	57,8	47	63,5*
5G	66	59,0	27	42,2	27	36,5
Genotype MTGFR CC	35	62,5	13	40,6	14	37,8
CT	21	37,5	14	43,8	18	48,6
TT	0	0	5	15,6**	5	13,5**
Allele C	91	81,3	40	62,5	46	62,2
T	21	18,7	24	37,5**	28	37,8**

Note. * — p< 0,05; ** — p< 0,01.

Among these women the frequency of the 4C-allele was also higher than in the group of conditionally healthy women. The frequency of 4G / 4G homozygotes only is most pronounced among patients with hypertension (Table 1).

A significant and reliable dependence of both MI and AG on the MTHFR genetic polymorphism was also revealed. In both study groups, patients with a homozygous TT genotype were detected more often than in the control group. A significantly higher overall T-allele frequency ($p < 0.01$) was found in these groups. The presence of such a pronounced connection between the MTHFR polymorphism and PAI-1 with cardiovascular diseases is possibly due to the fact that only postmenopausal women were in the main group and more careful selection of women in the control group.

At the same time, it should be noted that while analyzing the clinical course of postmenopause in patients with most severe hypertension, we were able to note manifestations of the neurovegetative symptom complex and depressive symptoms.The metabolic disorders prevailed among women with IHD and MI.

We analyzed whether there is an association of metabolic syndrome with the polymorphism of the gene PAI-1. The obtained data coincided with the results of epidemiological studies, which revealed a link between the level of PAI-1 in blood plasma and metabolic disorders. In particular, a number of studies indicate a correlation between increased levels of PAI-1 and the presence of metabolic syndrome [1]. Sources of PAI-1 in insulin resistance can be adipose tissue. The frequency of the 4G allele of the PAI-1 gene among women with metabolic disorders was 73.7%. Of this number 16 (29%) women were homozygous carriers with a 4G allele, 27 were heterozygous (4G / 5G) and 12 were homozygous carriers of 5G / 5G (Table 2). It should also be noted that 3 of 16 patients (4G / 4G) had a myocardial infarction in anamnesis, 3 of them had IHD, and 6 women had hypertension.

Table 2. The frequency of polymorphism of the gene inhibitor of cell plasminogen activator type 1 among women with metabolic syndrome in postmenopausal

Genotype and allele	Quetelet index, kg / mg	Immunoreactive insulin, MCED / ml	Total cholesterol, mmol / l	Triglycerides, mmol / l	High density lipoproteins, mmol / l	Low density lipoproteins, mol / l
4G/4G (n = 16)	28,31±0,56	15,61±0,63	5,8 ±0,04	2,43±0,69	0,6±0,12	5,4±0,4
4G/5G (n = 27)	28,36±0,36	10,78±0,36	4,5±0,12	3,81±0,02	0,9±0,04	4,3±0,23
5G/5G (n = 12)	25,4±0,27	8,9±0,01	3,86±0,1	0,86±0,12	0,9±0,03	1,53±0,9

As can be seen from the table, disorders of carbohydrate metabolism were manifested by an increase of the level of immunoreactive insulin and the value of the Quetelet index. Blood lipid abnormalities were characterized by an increase of total cholesterol and dyslipidemia, in particular, an increase of triglycerides, low-density lipoproteins, and a decrease of high-density lipoproteins. More pronounced metabolic disorders occurred among female carriers of the 4G allele in the homozygous form: for example, immunoreactive insulin was 15.6 μED/ml, low density lipoproteins - 5.4 mmol/l, high density lipoproteins - 0.6 mmol/l.

Conclusion

Consequently, postmenopausal women have a clear link between the risk of developing cardiovascular diseases and an increase in the level of PAI-1 in the blood, due to the presence of the 4I allele of the PAI-1 gene and the genetic polymorphism MTHFR in their genotype. The findings confirm that, in addition to estrogen deficiency, genetic factors also play a role in the development of cardiovascular diseases in postmenopausal women. The choice of treatment for menopausal disorders should be based on the principles of evidence-based medicine, taking into account the identification of anamnestic data on family predisposition to cardiovascular diseases.

In addition, it can be considered expedient to determine MTHFR and PAI-1 among premenopausal women with a history of a family history of predisposition to cardiovascular diseases, and to strictly follow the use of hormone replacement therapy.

To address the issue about the method of treatment of menopausal disorders, it is necessary to know the state of the hemostatic system. Our studies have shown that women with menopausal syndrome have significant impairments in the adhesive-coagulation properties of

platelets, which play a large role in the development of vascular pathology in the elderly.

In addition to hypercoagulation there is a decreasing of fibrinolytic properties of the blood associated with the development of atherosclerosis and thrombotic vascular diseases among postmenopausal women.

Among the genetic causes of a decrease in fibrinolytic activity and the tendency to thrombogenesis, there is an increase in the concentration of PAI-1, which is associated with the risk of myocardial infarction, type 1 diabetes. The process of regulation of fibrinolysis is closely related to the concentration of homocysteine in the blood — a product of demethylated methionine and transsulfurization. We were interested in the frequency of genetically determined disorders in the hemostasis system. One of the reasons for moderate hyperhomocysteinemia may be a mutation 677 (C→T) in the MTHFR gene.

While investigating of the frequency of polymorphic alleles of the PAI-1 and MTHFR genes in women with cardiovascular diseases and varicose veins, we saw that all of these women had a significantly higher frequency of 4G alleles in the gene PAI-1 and T-alleles in the gene MTHFR than in the control group. These studies have shown once again that disorders in the hemostasis system are closely related to disorders in the cardiovascular system, with thrombotic diseases and their development can often be genetically determined. It is necessary to take into account and conduct research on the identification of genetic factors among women in menopause, especially in the presence of aggravated family history. In addition, we were able to verify that there is an association of metabolic syndrome with the polymorphism of the PAI-1 gene.

Among women with metabolic syndrome, the frequency of the "defective" 4G allele was high, and 29% of women had a homozygous genotype - 4G / 4G. In addition, women of postmenopausal age have significant adhesive - aggregation activity of platelets and their aggregation ability. This is an indication of the risk of intravascular coagulation and thrombosis.

These data suggest that violations in homeostatic systems are not only internal, but also intersystem in nature. The increasing frequency of cardiovascular diseases with age is associated both with the hemostasis system and the state of lipid metabolism.

Studies of the lipid profile in postmenopausal women showed a certain regularity. Hypercholesterolemia, hypertriglyceridemia and a decrease of high-density lipoproteins are most pronounced among women of older age groups, especially after 60 years, as well as atherogenic index.

A particularly unfavorable fact is the combination of lower HDL and high levels of triglycerides, which significantly increases the risk of myocardial infarction. It is also important that these disorders of the lipid spectrum are all the more pronounced the heavier the severity of menopausal syndrome. The literature data show that lipids play a large role in the carbohydrate metabolism. Intersystem disorders of homeostasis are confirmed by our studies of carbohydrate metabolism in women with menopausal syndrome.

Consequently, an increase in the concentration of glucose and insulin resistance was increased in women with menopausal syndrome and also correlated with age, the duration of postmenopause and the severity of menopausal syndrome. As known insulin resistance leads to the development of atherogenic disorders in vascular endothelium with the development of hypertension and a decrease in vascular elasticity.

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