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[1]A.M. Kalimagambetov[*], [2]A.Yu. Abenova,
[2]N.K. Degemerzanova, [3]T.S. Kiseleva, [3]A.K. Yerdenova

[1]School of Biology and Biotechnology, al-Farabi Kazakh National University, Almaty, Kazakhstan
[2]Perinatal Prevention Center, Astana, Kazakhstan
[3]Molecular genetics laboratory LLP «Tree Gene», Almaty, Kazakhstan
[*]E-mail: Aitkali.Kalimagambetov@kaznu.kz

The study of blood clotting gene polymorphism in thrombophilia

Abstract. The study of polymorphic variants of 11 genes of blood coagulation in women with suspected thrombophilic states revealed that the frequency of the homozygous form for the mutant allele of studied genes varies in the range of 0-50%. The highest frequency of homozygous genotype revealed in mutant allele 4G gene antagonist of tissue plasminogen activator SERPINE1 (PAI-1) – 50.0%, the frequency of the homozygous genotype for the mutant G allele of gene methionine synthase reductase MTRR was 31.82%, the frequency of the homozygous genotype for the mutant T allele of the gene of methylenetetrahydrofolate reductase MTHFR was 13,64%. The frequency of the 4G, G, and T mutant alleles according the given genes were 0.71, 0.56, 0.37, respectively. Heterozygous form for the mutant alleles was observed in all genes with the frequency from 2.27% to 43.18%. Homozygous form for mutation in F2, F5, F7 genes was not identified. The association of two or more homozygous genotypes for the mutant alleles of blood clotting in thrombophilia was established in 12 (27.27%) of women.
Key words: gene mutation, polymorphism, thrombophilia, PCR.

Introduction

Currently, a major global health and social problem is thrombophilia which is a pathological condition characterized by an increase in blood clotting and a tendency to thrombosis and thromboembolism, that is one of the major causes of death and disability in the population. Thrombophilia is divided conditionally to acquired (antiphospholipid syndrome, etc.) and hereditary. For the manifestation of hereditary forms of thrombophilia as diseases with hereditary predisposition, it is necessary to exposure environmental factors such as trauma, surgery, cancer, pregnancy, etc. [1].

By experts' estimate, every tenth person in the world experiences during lifetime serious consequences of thrombotic processes such as an acute myocardial infarction, an ischemic stroke, deep vein thrombosis, pulmonary embolism, atherosclerosis of the lower limbs, and other clinical manifestations of venous and arterial thrombosis [2].

In this regard, special attention of scientists are deserved by hereditary forms of the disease or abnormality of coagulation inhibitors or coagulation proteins, causing the state of pretrombosis and predisposition to thrombosis, because they can be found in young adults and often occur without clinical manifestations [3].

The development of modern biomedicine is characterized by wide implementation of molecular genetics approaches in study of the mechanisms of human diseases, the identification of disease's biomarkers. This approach allows developing methods for prevention and early pre-clinical treatment and reducing the risk of disease progress [4].

The aim of this work was the study of the frequency of polymorphic variants of blood coagulation genes in women with suspected thrombophilic states.

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Materials and methods

Testing of predisposition's genes to the development of thrombotic conditions is based on a comprehensive study of venous blood. 44 women aged 15 to 60 years were examined from January to July, 2014 in the genetic laboratory LLP «Tree gene», Almaty. Study of the features of gene polymorphism thrombophilia by ethnic lines was not carried out due to the fact that information on nationality was ambiguous.

Genetic testing was carried out in the directions of gynecologists, therapists, and self-appeal of women-trombophilia genes carriers.

Main reason for the survey was identification of genetic predisposition to hyperhomocysteinemia, thromboembolism, thrombosis, myocardial infarction, coronary heart disease, ischemic stroke, thromboembolic complications during pregnancy, atherosclerosis, hypertension, the applicability of oral contraceptives, and hormone replacement therapy.

Table 1 – The frequency of alleles, genotypes of polymorphic loci of studied thrombophilia genes

Gene	Gene marker	Allele frequency		Genotype frequency, % (n)		
FGB -fibrinogen (the clotting factor I)	G(-455)A	G	A	G/G	G/A	A/A
		0.79	0.21	75.0 (33)	20.45 (9)	4.54 (2)
F2-prothrombin (the coagulation factor II)	G20210A	G	A	G/G	G/A	A/A
		0.99	0.01	97.73 (43)	2,27 (1)	-
F5 – the factor V clotting (Leiden mutation)	G1691A	G	A	G/G	G/A	A/A
		0.99	0.01	97.73 (43)	2,27 (1)	-
F7 – the blood clotting factor VII	G10976A	G	A	G/G	G/A	A/A
		0.87	0.13	75.0 (33)	25.0(11)	-
F13 A1- the blood clotting factor XIII	G103T	G	T	G/G	G/T	T/T
		0.85	0.15	90.91 (40)	6.82 (3)	2.27 (1)
SERPINE1 (PAI-1) – antagonist of tissue plasminogen activator	5G(-675)4G	5G	4G	5G/5G	5G /4G	4G/4G
		0.29	0.71	40.91 (18)	9.09 (4)	50.0 (22)
ITG A2 – alpha2 integrin (platelet receptor to collagen)	C807T	C	T	C/C	C/T	T/T
		0.70	0.30	52.27 (23)	38.64 (17)	9.09 (4)
ITG B3 – beta integrin (the platelet fibrinogen receptor)	T1565C	T	C	T/T	C/T	C/C
		0.79	0.21	81.82 (36)	13.64 (6)	4.54 (2)
MTHFR – methylenetetrahydrofolatereductase	C677T	C	T	C/C	C/T	T/T
		0.63	0.37	43.18 (19)	43.18 (19)	13.64 (6)
MTR – methionine synthase	A2756G	A	G	A/A	A/G	G/G
		0.74	0.26	59.09 (26)	34.09 (15)	6.82 (13)
MTRR – methionine synthase reductase	A66G	A	G	A/A	A/G	G/G
		0.44	0.56	31.82 (14)	36.36 (16)	31.82 (14)

11 genes of predisposition to thrombophilia, whose products are involved in the coagulation cascade, fibrinolysis system, maintaining vascular tone, the metabolism of methionine and folate: FGB – the clotting factor I, F2 – prothrombin (the coagulation factor II), F5 – the factor clotting V (Leiden mutation), F7 – the blood clotting factor VII, F13 A1- the blood clotting factor XIII, SERPINE1 (PAI-1) – antagonist of tissue plasminogen activator, ITG A2 -alfa2 integrin (platelet collagen receptor), ITG B3-beta integrin (platelet fibrinogen receptor), MTHFR – methylenetetrahydrofolatereductase, MTR – methionine synthase, MTRR – methionine synthase reductase were determined in every woman [5].

Genotyping was carried out by Real time PCR method in cycler Biorad CFX11 [6-8].

Results and their discussion

The results of the frequency of gene polymorphisms which are responsible for predisposition to thrombophilia are presented in Table 1.

Despite the small size of the study sample, a number of features of polymorphisms in studied thrombophilia genes were identified. As seen from Table 1, the frequency of homozygous genotype in the mutant allele forms ranged from 0% to 50%.

The highest frequency of homozygous genotype detected in the mutant allele of the gene antagonist of tissue plasminogen activator SERPINE1 (PAI-1) – 50.0%, the frequency of homozygous genotype in the mutant allele of methionine synthase reductase MTRR was 31.82%, the frequency of homozygous genotype in the mutant methylenetetrahydrofolatereductase in mutant allele of MTHFR gene was 13,64%.

The frequency of the studied genes' mutant alleles was 0.71, 0.56, 0.37, respectively. Carrier of the mutant allele, heterozygous form of the studied genes was observed in all genes with the frequency of occurrence from 2.27 to 43.18 percent. Homozygous form for F2, F5, F7 genes mutations was not identified in the studied group. None of the surveyed women had a homozygous form for the normal allele in all studied genes. It is noted the presence of at least one heterozygotes for mutant allele for alleged thrombophilic states. An association of two or more homozygous genotypes in the mutant allele (Table 2) was shown in 12 (27.27%) cases.

Detected high frequency of homozygous genotypes mutant in the alleles of genes MTRR, PAI1, MTHFR – 83.33%, 58.33%, 41.67%, respectively. Unconditionally, a combination of genetic polymorphisms of the mutant genes is an important factor that increases the risk of thrombophilia.

Table 2 – Association of homozyguous genotypes in the mutant alleles of genes of the blood clotting system

Individuals	Genes
1	FGB, ITG B3, MTRR
2	PAI1, MTRR, MTHFR
3	F13, MTRR, MTHFR
4	PAI1, MTHFR
5	FGB, ITG A2, PAI1, MTRR, MTR
6	PAI1, MTRR
7	ITG A2, PAI1, MTRR
8	ITG A2, ITG B3, MTR
9	MTRR, MTHFR
10	PAI1, MTRR
11	PAI1, MTRR
12	MTRR, MTHFR

Establishing of polymorphisms' combinations that increase the risk of thrombotic complications will greatly expand the understanding of the molecular mechanisms of the hereditary thrombophilia formation and its prevalence in the general population [9].

Conclusion

Thus, the results show the necessity of molecular genetics analysis to identify genetic predisposition to thrombosis. At the same time, the level diagnostics on a limited number of genes that are considered markers of hereditary thrombophilia, such as: MTHFR C677T, FV5 G1691A (Leiden), F2 G20210A, MTR A2756G and ITG A2 C807T, is not informative enough. Testing the maximum of possible number of genes of predisposition to thrombophilia should be carried out in order to reveal hidden forms of pathology in individuals and preventing thrombotic conditions and resulting complications.

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