THROMBOSIS IN DIFFERENT LOCATIONS: CANDIDATE GENES AND THE FUNCTIONAL STATE OF THE HAEMOSTATIC SYSTEM

Tsvetovskaja G.A., Lifshits G.I., Chikova E.D., Belevantseva A.V., Voronina E.N., Novikova Y.V., Danilkina S.T.
Institute of chemical biology and fundamental medicine. Center of new medical technologies in Akademgorodok
Novosibirsk, Russia

Thrombotic diseases, such as ischemic disturbances of cerebral and coronary circulation and thrombotic diseases of the venous system, have spread widely in recent years. Many see the reasons of this pathology in disturbed hemostasis, hemorheology, as well as in the interaction of environmental factors and genetic predisposition [3, 4, 5, 8, 9].

Prevention, diagnostics and treatment of thrombotic conditions are the topical questions facing the modern medicine. At the same time, it has been proved, that the revealed markers of thrombinemia do not let identify the reason of predisposition to intravascular coagulation. Consequently, this method is not sufficient for selecting a pathogenetic therapy. In this regard, clinical practice focuses nowadays on tracking the causes of predisposition to recurrent thrombosis – finding «risk genes», responsible for the predisposition to multifactorial diseases, including the thrombotic ones [1, 6].

Aims and objectives

The aim of this study was to analyze the candidate genes in patients of the West Siberian region with a verified diagnose of thrombosis in different locations, and try to prove, that genetic tests give more diagnostic opportunities to cardiologists, phlebologists and other doctors in their everyday practice. We have examined the venous blood, estimated the functional state of the haemostatic system and thrombophilia predisposition genes.

Materials and methods

We observed 64 patients from the West Siberian region (Novosibirsk and the Novosibirsk region) with different forms of thrombophilia, aged between 6 and 71 years old. Our test persons were divided into two groups: 22 patients with arterial thrombosis – ischemic stroke and cardiosclerosis after a heart attack, and 42 patients with deep venous thrombosis (DVT). We studied 19 candidate genes (25 allelic variants), whose products are involved in coagulation cascade, fibrinolysis system, sustaining of vascular tone and methionine metabolism (folat cycle genes).

Results

Analysis of changes in haemostatic values – platelet, coagulation and fibrinolysis - in patients with ischemic stroke and deep venous thrombosis showed, that AT III activity, content of protein C, fibrinogen and D-dimer had no statistically considerable differences from the normal values. At the same time, in 73% of all patients from the both groups, a higher level of soluble fibrin complex (SFC) could be seen, which points to hemostasis activation, i.e. intensification of thrombin and fibrin formation. In both groups of patients, **a** higher functional platelet activity was observed, but the frequency of induced platelet activity among the patients with ischemic stroke was higher, than in the patients with DVT. It should be mentioned, that the higher platelet functional activity in patients with the deep venous thrombosis was induced by collagen, in patients with the arterial thromboses – by ADP and adrenalin.

The main candidate genes, which take part in thrombophilia development in the examined patients are listed in the following table.

Frequency rate of genetic polymorphisms in patients with thrombosis in different locations

Gene	Gene name, (polymorphic substitution)	Frequency (%)	Homozygous variant (%)
PAI1	Plasminogen activator inhibitor (675 5G -> 4G)	80,6	38,4
α2 PLI	Plasminogen inhibitor	50,0	53,8
PLAT	Tissue plasminogen activator (7351 C -> T)	34,4	20,0
MTHFD	Methylenetetrahydrofolate Dehydrogenase 1 (1958 A- >G)	76,6	47,8
MTHFR	Methylenetetrahydrofolate Reductase (C677T)	58,0	5,5
MTHFR	Methylenetetrahydrofolate Reductase (A1298C)	58,2	33,3
MTRR	Methionine synthase reductase (66A > G)	70,0	40,9
MTR	Methionine synthase (2756 A- >G)	10,3	0
NSO3	Endothelial NO–synthase (VNTR)	18,5	60,0
NSO3	Endothelial NO-synthase C->T (Glu298Asp)	42,8	16,6
Gp Ia	Integrin-alpha-2, glycoprotein 1a of platelets (807C- > T)	50,0	0
GpIIIa	Platelet glycoprotein IIIA, integrin beta 3, (1565 T- C) (Leu33Pro)	45,2	21,4
Gp Iba	Platelet glycoprotein Iba (VNTR)	0	
FGB	Fibrinogen-beta-peptide (455 G - >A)	37,5	8,3
FV	Leiden factor (1691 G->A (R506Q)	14,0	0
FII	II coagulation factor (20210 G - >A)	6,0	

Analyzing gene Gp-Ia integrin-alpha-2, polymorphic variant T Gp-Ia was revealed in 50% of all cases. As for polymorphic variant T of this gene, we observed, that platelets adhere to vessel walls faster, which can increase the risk of thrombophilia. Our data let regard variant T as a marker for high risk of thrombus formation, especially, in combination with gene defects – markers of endothelial dysfunction and folat cycle enzyme genes. Comparative analysis of distribution of alleles and genotypes of this polymorphic marker in patients with arterial and venous thromboses revealed no reliable differences.

Platelet glycoprotein GPIIIa (integrin β_3) encodes amino acid sequence of platelet receptor subunits for fibrinogen and Willebrand factor. A leicine-to-proline substitution, determined by the substitution of T by C in exon 2 of gene GPIIIa at position 1565, is accompanied by a higher platelet predisposition to aggregation, which increases the risk of cardio-vascular diseases. Polymorphic substitution 1565 T- > C was found in 45,2% of the examined patients of the both groups. None of the examined patients had polymorphic variant Gp1ba (VNTR) – gene that encodes amino acid sequence 1beta – subunits of specialized platelet receptors, which organize interaction between platelets and the wall of a damaged vessel or damaged surface of a atherosclerotic plaque.

Polymorphic substitution 675 5G -> 4G of the plasminogen activator inhibitor gene (PAI-1) points at the predisposition to endothelial dysfunction. The gene encodes protein – plasminogen activator inhibitor, which is one of the main components of the blood anticoagulation system. Polymorphic variant 4G, which is accompanied with increased gene expression and causes higher PAI-1 level in blood, was found in 80,6 % of the patients, both with arterial and venous thromboses. It is well known, that in endothelial dysfunction, fibrinolytic activity is mainly inhibited by stronger endothelial synthesis and PAI-1 secretion [2]. 45% of patients had a combination of PAI-1 gene polymorphism with gene α 2 PLI –plasminogen inhibitor, 34,8% had a polymorphic variant of tissue plasminogen activator (PLAT), polymorphic substitution 7351 C->T, which is a sign of falling expression of tissue plasminogen activator, leading to ineffective fibrinolysis.

We also estimated the predisposition for endothelial dysfunction analyzing mutation of genes, which regulate the vessel wall condition - NOS(e) endothelial NO-synthase, VNTR-polymorphism and polymorphic alternative C- T (Glu298Asp), as well as endothelin and hANP. Polymorphic variant of endothelial NO-synthase gene - NOS(e), VNTR-polymorphism and polymorphic substitution C -> T (Glu298 Asp) were registered in 42,8 and 18,5% of cases correspondingly, which could be a reason for reduced NO synthesis, and, as a result, growing vasoconstriction, lower vasodilation and higher predisposition to thrombus formation [9,10].

We studied the system of folat cycle genes, as they play an important pathogenetic role in thrombophilia development. Six folat cycle candidate genes (7 allelic variants) have been examined. Polymorphisms of genes MTHFD, MTRR and MTHFR were revealed frequently. The majority of homozygous variants were found in genes MTHFD and MTRR. It should be mentioned, that in most cases, we found combinations of several folat cycle gene polymorphisms. Three-genetic-polymorphism combination was observed in y 45,2% of patients, homozygous variants of one or two polymorphisms were revealed in 81,2% of patients. Combination of four polymorphisms was found at 22,6% of the examined patients, and two polymorphisms – at 19,3%. Obviously, the polymorphic variant combination is a serious risk factor of functional disturbances in folat cycle enzymes, which leads to excessive homocysteine accumulation in blood and raises its thrombogenicity dramatically (1, 6).

Our research revealed a relatively low percent of mutations in factor Leiden and prothrombin gene (II coagulation factor (20210 G - >A) - 14% and 8% correspondingly.

Conclusion. To sum up, among the population of Novosibirsk and the Novosibirsk region, platelet glycoprotein and endothelial NO-synthase genes, as well as genes that encode endothelial proteins of fibrinolysis and folat cycle system, are the mostly frequent revealed thrombophilia-related polymorphic sites. The obtained data prove, that one of the key elements of thrombosis pathogenesis in different locations is the inhibition of fibrinolytic blood activity. Mutations in tissue plasminogen activator gene (PLAT) combined with mutations of the most important fibrinolysis inhibitors *PAI 1 и* α 2 PLI, create serious conditions for a lower plasmin level in blood and cleaning the clogged blood vessels. Higher risk of thrombus formation and pulmonary embolism have PAI1-allele carriers, especially in combination with additional genetic defects – mutations in folat cycle genes (MTHFD, MTPP, MTHFR), platelet glycoprotein genes (GP III a, Gp-Ia) and genes, responsible for the vessel wall regulation.

Endothelial dysfunction, which determines the ineffective fibrinolysis, combined with the above mentioned risk genes of thrombophilia, can turn the predisposition into the pathology. Despite the fact, that arterial and venous thromboses develop differently, we found some syntrope genes involved in thrombophilia development, i.e. polymorphic genes responsible for both arterial and venous thromboses. We do not rule out the possibility, that the polymorphic gene frequency rate in patients with different forms of thrombophilia could change, if a larger number of people would be examined. As for the population of the West Siberian region – patients with pulmonary embolism, post-thrombotic disease, recurrent thrombosis, patients who need cava filter, testing of the above mentioned genes is getting more and more

important, as it lets determine a pathogenetically reasonable therapy and reduce the number of thrombosis recurrences. Besides, diagnostics of predisposition for thrombophilia lets considerably reduce the number of the post-operative thromboembolic venous complications, which are challenging for modern medicine and life threatening for patients after surgeries.

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THE CONJUNCTIVIS CYTOMORPHOLOGY UNDER THE SECONDARY «DRY EYE» CONDITIONS, HAVING ACCOMPANIED BY THE CHRONIC OCULAR ISCHEMIC SYNDROME

Yanchenko S.V.
Kuban State Medical University,
The Eye Diseases Department
Krasnodar, Russia

The Aim

To study the conjunctivis cytomorphology at the secondary «dry eye» (SDE) patients under the chronic ocular ischemic syndrome (COIS) conditions.

Materials & Methods

The 64 moderately severe SDE patients (by Brzhevsky V.V., 2003), having had the COIS symptoms, and the 25 healthy volunteers at the age of 66,4±3,3 years have been examined. They have defined in the conjunctivis imprints, having received by means of the original instrument use for the measured cellular material sampling (e.g. Yanchenko S.V. and co –authors 2008), and having stained by the May–Grunvald method: the epithelial cells average number with the alteration symptoms (EA) in the form of the karyopyknosis and the karyorrhexis, and the goblet cells (GC) average number in the one field of view (calculating on 100 random selected cytological objects). The tissular entropy factor (TEF) has been measured by the computer morphometry method by Avtandilov G.A. (1990), having permitted, objectively, to judge on the tissue structural irregularity level under the SDE conditions (e.g. Yanchenko S.V. and co – authors 2008). The 25 persons without the ophthalmopathology symptoms have been entered into the 1-st group, the 64 SDE patients with the COIS symptoms presence have been entered into the 2-nd group. All the results have been processed by the variation statistics methods.

Results

The singular epithelial cells presence with the alteration symptoms (e.g. 2,1±0,04) have been registered in the conjunctivis imprints of the 1-st group. The GC quantity has been made up (5±0,05). The tissular entropy factor (TEF) has been equal to 1,3±0,002 standard units. On the contrary, the EA average number at the 2 – nd group patients has been increased for 24% (e.g. $p<0,001$), the GC quantity has been lowered for 20% (e.g. $p<0,001$), the TEF has been made up 2,1±0,009 (that is for 62% higher of the similar value under the standard conditions; e.g. $p<0,001$).

Conclusion

It has been registered the cells number increase with the alteration symptoms and the GC average number lowering at the SDE patients under the COIS conditions, that is quite typical and for the other SDE clinical versions. These results are quite natural, as in spite of the factors variety, having promoted to the