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SCIENTIFIC REVIEWS AND LECTURES

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GENETIC POLYMORPHISMS OF THE HEMOSTASIS SYSTEM

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ABSTRACT
Despite advances in treatment of chronic viral hepatitis, search of predictors of poor outcome is still needed. One of them is the hemostasis system. The decoding of the human genome has made it possible to determine genetic markers that lead to blood coagulation disorders. It is widely known that more than 10 single nucleotide polymorphisms (SNP) are responsible for some form of coagulation disorders. The genome-wide

association study (GWAS) has only come to the fore recently, and expansion of the roster of hemostasis genes is now possible.

Thrombophilia has a special role in the inherited disorders of hemostasis. The view of the role of thrombophilia changed since *FV Leiden*, *FII* and Antiphospholipid syndrome (APS) mutations were discovered. It was found that genetic anomalies of hemostasis cause thrombosis in 80-90 per cent of cases.

FGB, FII, FV, FVII, FXIII, ITGA2, ITGB3, MTHFR, PAI-1 and other genes of hemostasis are presented in the article. The article describes the role of these genes by developing thrombosis and thrombophilia, and considers protective SNPs.

A shared vision of the desired conditions of hemostasis genes affect chronic liver disease needs to be developed. Pathophysiological and biochemical mechanisms of allelic variants in thrombophilia genes of chronic liver disease are not exactly conventional. A better understanding of the role of hemostasis genes' allelic variants of processes of intrahepatic epithelium is essential for prognosis of the impact in the liver disease, treatment and clinical background.

Keywords: hemostasis system, coagulopathy, coagulation disorders, genetic factors, predisposition.

Relevance. According to WHO, 325 million people in the world have viral hepatitis, hepatitis B virus (HBV) and hepatitis C virus (HCV) account for 80% of hepatocellular carcinoma cases. Viral hepatitis related mortality is 1.34 million deaths per year, which is comparable to HIV/AIDS, tuberculosis and malaria [23]. In the Russian Federation the total number of people living with HBV and HBsAg carriers are estimated to be 5 million, the overall number of HCV infections at least 2 million people. According to Register "Chronic viral hepatitis in the Republic of Sakha (Yakutia)" 14 805 people with chronic viral hepatitis were registered since October 2017. A total of 6320 people had been registered as having HBV, 6619 – HCV, 1048 – HDV (hepatitis D virus), 646 – mixed infection, 382 patients with cirrhosis and 26 patients with primary liver cancer [2].

Since 1991, the program of WHO for immunization of the population from HBV is implemented in Russia. Antiviral therapy of viral hepatitis develops, but the incidence remains at the previous level. Despite advances in treatment of chronic viral hepatitis, search of predictors of poor outcome is still needed with a view to improving personalized therapy. One of them is hemostasis system. Hemostasis disorders at chronic liver disease lead to coagulation imbalance, at the same time it affects both primary and secondary hemostasis. Disruption of the hemostasis system in chronic liver diseases leads to a coagulation imbalance, and this affects both vascular platelet and coagulation hemostasis.It can lead either to bleedings, or to thromboses.

A genome-wide association study (GWAS) has only come to the fore recently. GWAS is an observational study of a genome-wide set of genetic variants in different individuals to see if any variant is associated with a trait. GWASs typically focus on associations between single-nucleotide polymorphisms (SNPs) and traits like major human diseases, but can equally be applied to any other genetic variants and any other organisms. Each person gives a sample of DNA, from which millions of genetic variants are read using SNP arrays. If one type of the variant (one allele) is more frequent in people with the disease, the variant is said to be associated with the disease. The associated SNPs are then considered to mark a region of the human genome that may influence the risk of disease [27].

SNPs of hemostasis system genes

Genes	Localization	SNPs	Allels	Effects
FGB	*4q31.3*	*C148T*	*T*	high level of fibrinogen in blood, increase in probability of blood clots formation
			C	normal level of fibrinogen in blood
FII	*11p11.2*	*G20210A*	*A*	high level of a prothrombin in blood, increase in probability of blood clots formation
			G	normal level of prothrombin in blood
FV	*1q24.2*	*G1691A*	*A*	steady active form of FV to action of enzymes that leads to hypercoagulation
			G	normal coagulation
FVII	*13q34*	*G10976A*	*A*	decrease in probability of thromboses
			G	normal coagulation
FXIII	*6p25.1*	*G103T*	*T*	decrease in probability of thromboses
			G	normal coagulation
ITGA2	*5q11.2*	*C807T*	*T*	increase in speed of adhesion of platelets that results in the increased risk of thrombophilia.
			C	normal coagulation
ITGB3	*17q21.32*	*T1565C*	*C*	increase in speed of platelet adhesion, low efficiency of acetilsalicylic acid
			T	normal coagulation
MTHFR	*1p36.22*	*C677T*	*T*	folate cycle disorders, cardiovascular diseases
			C	normal level of folat
PAI-1	*7q22.1*	*5G(-675)4G*	*4G*	decrease fibrinolytic activities, increased probability of blood clots formstion
			5G	normal fibrinolytic activities

Due to interpretation of human genome structure it became possible to define the genetic markers causing hemostasis system disorders. Markers which are revealed by molecular genetic analysis represent variants of genes that cause changes of gene activity or modification of protein product. In certain conditions it can lead to hemostasis system disorders [27]. Thrombophilia has the special role in the inherited disorders of hemostasis. Barkagan Z. S. and Momot A. P. (2001) define thrombophilia as disorders of hemostasis and hemorheology, which are characterized by the increased tendency to thrombose occurance with ischemia in blood vessels [9]. The overall picture of the role of thrombophilia in the pathogenesis of thrombosis changed after *FV Leiden*, *FII* and Antiphospholipid syndrome (APS) mutations were discovered.

Today, more than 10 SNPs of hemostasis system are widely known (Table 1).

Genetic polymorphisms of fibrinogen. Fibrinogen is made and secreted into the blood primarily by liver hepatocyte cells. Endothelium cells are also reported to make what appears to be small amounts of fibrinogen but this fibrinogen has not been fully characterized; blood platelets and their precursors, bone marrow megakaryocytes, while once thought to make fibrinogen, are now known to take up and store but not make the glycoprotein. The final secreted, hepatocyte-derived glycoprotein is composed of two trimers with each trimer composed of three different polypeptide chains, the fibrinogen alpha chain (also termed as the Aα or α chain) encoded by the FGA gene, the fibrinogen beta

chain (also termed as the Bβ or β chain) encoded by the FGB gene, and the fibrinogen gamma chain (also termed as the γ chain) encoded by the FGG gene. All three genes are located on the long or «p» arm of human chromosome 4.

T. Cronjé et al (2017) studied 6000 representatives of the people of Tswana living in South Africa. They found that *FGB (854A)* and *FGG (rs1049636)* were significantly connected with the general fibrinogen, and *FGA (rs2070011)* related to high concentrations of fibrinogen γ among indigenous people of South Africa. FGB (-148T) was associated with a large diameter of fiber, and *FGA* was associated with high concentrations of fibrinogen. In this research, *FGA (rs2070011)* and *FGG (rs1049636)* were less significant in terms of their influence on the maximum absorption [15].

A.P. Reiner et al (2006) studied 5115 Euro-Americans and showed what rs1049636 in 9 *FGG* (C-allele) introne and rs1800791 (minor A-allele) in primotor *FGB* are connected with increase in the general level of fibrinogen [4]. SNP rs1800791 increase the levels of fibrinogen by linking nuclear proteins with the *FGB* promotors (Van't Hooft et al, 1999) [33]. Lovely et al (2011) studied 5124 people of European origin, and found SNPs rs7681423 and rs1049636 located in the field of fibrinogen splaysing in 9th intron of *FGG* gene. It is the second most important SNP which is connected with fibrinogen levels. In addition, there was a relation between high levels of fibrinogen and rs2070011 in the field of FGA promotor [3]. However, as a result of studies of 3891 Europeans, Mannila M.N. et al (2006) obtained contradictory results: decrease in levels of fibrinogen was related with increase in number of alleles [13].

Unlike previous studies showing connection between levels of fibrinogen and other SNP in intron 9 of *FGG*, rs2066865 and rs13800791 in the field of *FGB* promotor (Mannila et al, 2007), R.C. Kotzé et al (2015) did not find connection between rs1049636 *FGG*, rs2070011 *FGA* and properties of the clot, which shows that the observed differences are result of genotypes differences of fibrinogen levels. Other SNPs analyzed in this research (rs1049636 and rs2070011) also did not have association with properties of the clot. Observed increase in the maximum absorption can be result of increase in concentration of fibrinogen for rs1800787, though it cannot be proved because of small amount of samples of alleles homozygous minor carriers [19].

Genetic polymorphisms *G20210A* (rs1799963) of *FII.* The F2 gene encodes the prothrombin protein (also called coagulation factor II). Prothrombin occurring in blood plasma is an essential component of the blood-clotting mechanism. Prothrombin is transformed into thrombin by a clotting factor known as factor X or prothrombinase; thrombin then acts to transform fibrinogen, also present in plasma, into fibrin, which, in combination with platelets from the blood, forms a clot.

SNP *G20210A* is caused by replacement of the nucleotide of guanine (G) by adenine (A) in *FII* gene position 20210; it leads to the raised gene expression in A-allele. The surplus production of prothrombin is risk factor of myocardial infarction, thromboses, and pulmonary embolism which often lead to death. Unfavorable allele of polymorphism (A) has autosomal dominant inheritance pattern. It means that the increased risk of thrombophilia might be even in case of heterozygotic form of polymorphism. S.R. Poort et al (1996) studied 418 persons of European origin with thrombosis aggravated family anamnesis. They found that allele A carriers have high risk of death during the postoperative period, as well as during cancer therapy and other diseases [28]. Symptoms of thrombophilia are shown at heterozygotic carriage, especially in case of combination with Leyden mutation. If the patient with chronic viral hepatitis has mutation of *FII (G20210A)* gene, the high speed of fibrosis can be explained both as formation of microblood clots in tissue of liver, and as effects of thrombin which is both the mitogen and the activator of star-shaped cells of liver.

In research of E. E. Starostina et al *FII* (*G20210A*) was found more often in group with "fast" fibrosis than in group with "slow" fibrosis [1].

A. Kallel et al (2016) studied 1290 people, and found that GG genotype is associated with high frequency myocardial infarctions among men [6].

In metaanalyse B. Jin et al (2011) did not find assosition between polymorphism of *FII (G20210A)* and the ischemic heart disease (IHD) among the Asian population, but they found out that polymorphism of *FII (G20210A)* increases risk of IHD among the European population [35]. In metaanalyse M. Dziadosz and L. V. Baxi found that SNP FII (G20210A) occurs among Asian population (in China, South Korea and Japan), however among aboriginals of the Middle East it is less than among Ashkenazi Jews (the frequency of this polymorphism is 2.5-12.25% and correlates with frequency of thromboses [11].

Results of this kind were obtained by S. K. Pandey et al (2012). They studied prevalence of *FII (G20210A)* among aboriginals of India with sickle-cell anaemia. However, this disease was associated with polymorphism of *G1691A* (rs6025) of *FV* gene – Leiden mutation [29].

In metaanalyse C. Li et al (2017) analysed 34 researches with participation of 14,611 patients with myocardial infarction (MI) and 84,358 healthy people. Statistically significant correlations between *FII (G20210A)* and MI was found during nucleotide replacement A>G. SNP of *FII (G20210A)* increases MI risk with the age. The metaanalyse showed that SNP of *FII (G20210A)* can represent risk factor for MI [31].

Thus, *FII (G20210A)* is not enough for formation of thrombophilia among Asian population and concedes on value to *FV (G1691A).*

***FVII* gene** encodes coagulation factor III which is a cell surface glycoprotein. This factor enables cells to initiate blood coagulation cascades, and it functions as a high-affinity receptor for the coagulation factor VII. This protein is the only thing in the way of coagulation which has no congenital disease.

***FV (G1691A)* (rs6025).** The *FV* gene provides instructions for making a protein called coagulation factor V. Coagulation factors are a group of related proteins that make up the coagulation system, a series of chemical reactions that form blood clots. The factor V protein is made primarily by cells in the liver. The protein circulates in the bloodstream in an inactive form until the coagulation system is activated by an injury that damages blood vessels. When coagulation factor V is activated, it interacts with coagulation factor X. The active forms of these two coagulation factors form a complex that converts an important coagulation protein called prothrombin to its active form, thrombin. Thrombin then converts a protein called fibrinogen into fibrin, which is the material that forms the clot.

The functional importance of genetic marker of *FV (G1691A)* was well described earlier. Inflammation in liver tissue at chronic hepatitises is associated with activation of coagulation system which is more expressed at patients with Leiden mutation and results in hyperactivity of thrombin and fibrin deposits. Thrombin is mitogen for liver star-shaped cells therefore start of coagulation cascade can stimulate star-shaped cells and fibrosis.

The metaanalyse of X. Shang et al (2013) revealed that Leiden mutation plays an important role in formation of an osteonecrosis of a femur, but not among Asian population [25].

In their research E. E. Starostina et al found that patients with fast rate of liver fibrosis more often have heterozygotic genotype *GA* of *FV* gene in comparison

with patients with slow rate of liver fibrosis [1].

In their research P. Angchaisuksiri et al (2000) found that the prevalence of SNP *FII (G20210A)* and *FV (G1691A)* is lower among Asians than among Caucasians. The low prevalence of these two mutations can explain the low frequency of thrombosis of deep veins in the Thai population [30].

In research of P.M. Ridker (USA, 1997) it was found that *FV (G1691A)* meets less often among the Asians living in the territory of the USA than among Caucasian Americans [14].

In research of A.A. Dashti et al (2011) it was found that Leiden mutation is present among the Iranian or Iraqi origin Kuwait citizens, and this mutation is not found among indigenous Arab Kuwait citizens [10].

De Stefano V. et al (1998) received similar results. They found that Leiden mutation is absent among Africans, Asians and races with Asian origin, such as Indians, Eskimos and Polynesians [12].

***FVII (G10976A*).** The *FVII* gene provides instructions for making a protein called coagulation factor VII. Coagulation factors are a group of related proteins that are involved in the coagulation system, which is a series of chemical reactions that form blood clots. After an injury, clots seal off blood vessels to stop bleeding and trigger blood vessel repair. Coagulation factor VII is made primarily by cells in the liver. The protein circulates in the bloodstream in an inactive form until the coagulation system is turned on (activated) by an injury that damages blood vessels. Activated coagulation factor VII helps to turn on other coagulation factors in turn. This step-wise process ultimately promotes the conversion of an important coagulation protein called fibrinogen into fibrin, which is the material that forms blood clots.

N.A. Zakai et al (2011) studied 815 cases of a stroke. SNP rs6046, rs3093261 (*FVII*); rs4918851, rs3781387 (*HABP2*); rs3138055 (*NFKB1A*); rs4648004 (*NFKB1*) related to ischemic stroke (p <0,01). SNP rs6046 and rs3093261 related to levels of VIIc factor. Ratios between SNP and ischemic stroke did not depend on levels of VIIc factor. The variation of genes related to VII factor, and levels of VIIc factor related to risk of ischemic stroke in elderly cohort, that indicates a potential causal role of VII factor in the etiology of ischemic stroke [7].

Minor alleles of SNP rs2146751, rs10665, rs1755685, rs6039 in *FVII* site reduce FVII level, and minor alleles rs964617 and rs762636 increase FVII level. SNP rs6046 leads to amino-acid replacement of Arg353Glu which reduces functional activity of protein of VII factor. Minor allele – *402A*, (rs510317) resulting from SNP *402GA* in FVII gene pro-motor increases activity of transcription. Rs510317 is associated with increased level of VIIc factor in plasma and increased risk of thromboses among Caucasian populations.

In metaanalyse X. Mo et al (2011) thirty-nine researches of SNP *FVII (R353Q)* (rs6046), *FVII (HVR4)* and *FVII (-323Ins10)* (rs36208070) were registered. The research of SNP *FVII (R353Q)* included 9151 cases of Coronary artery disease (CAD) and 14,099 people of control group, the research of SNP *FVII (HVR4)* included 2863 cases of CAD and 2727 people in control group, the research of SNP *FVII (-323Ins10)* included 2,862 cases and 4240 people in control group. Statistically significant association was found between *FVII (R353Q)* and CAD in Asian populations. Association for SNP *FVII (HVR4)* was not revealed [5].

Mutation of *F8, F9, F10, F11, F12* genes. Mutations in the *F8* gene cause hemophilia A, the most common form of bleeding disorder. More than 1,300 alterations of this gene have been identified. Some of these mutations change single DNA building blocks (base pairs) in the gene, while others delete or insert multiple base pairs. The most common mutation in people with severe hemophilia A is the rearrangement of genetic material called inversion. This inversion involves a large segment of the *F8* gene.

Mutations in the *F9* gene cause a type of hemophilia called hemophilia B. More than 900 alterations of this gene have been identified. The most common mutations change single DNA building blocks (base pairs) in the gene. Several rare mutations in the F9 gene cause an increased sensitivity (hypersensitivity) to a drug called warfarin.

Mutations in genes of X (*F10*), XI (*F11*), XII (*F12*) factors cause bleeding. However, it is not met very often. At least two mutations in the F12 gene are associated with hereditary angioedema type III.

SNP *G103T* (*F13A1*). The F13A1 gene provides instructions for making one part, the A subunit, of a protein called factor XIII. Factor XIII in the bloodstream is made of two A subunits (produced from the F13A1 gene) and two B subunits (produced from the F13B gene). When a new blood clot forms, the A and B subunits separate from one another, and the A subunit is cut (cleaved) to produce the active form of factor XIII (factor XIIIa). The active protein links together molecules of fibrin, the material that forms the clot, which strengthens the clot and keeps other molecules from breaking it down. Studies suggest that factor XIII has additional functions, although these are less understood than its role in blood clotting. Specifically, factor XIII is likely involved in other aspects of wound healing, immune system function, maintaining pregnancy, bone formation, and the growth of new blood vessels (angiogenesis).

At least 140 mutations in the *F13A1* gene have been found to cause inherited factor XIII deficiency, a rare bleeding disorder. Without treatment, affected individuals have a greatly increased risk of abnormal bleeding episodes, including life-threatening bleeding inside the skull (intracranial hemorrhage). *F13A1* gene mutations severely reduce the amount or activity of the A subunit of factor XIII. In most people with these mutations, the level of functional factor XIII in the bloodstream is less than 5 percent of normal. This loss of factor XIII activity weakens new blood clots and prevents them from stopping blood loss effectively.

The metaanalyse of Li J. et al (2015) included five researches: 382 cases and 352 controls. The prevalence of homozygous genotype of *Val/Val* of wild type was 64.9% (248 of 382) in group of patients and 75.9% (267 of 352) in control group. After the comprehensive analysis results showed that *F13A1 (Val34Leu)* is the link with thromboses, and women who had *Val/Val* genotype for *F13A1 (Val34Leu)* were not prone to abortions [18].

Mutation of *VWF gene*. The VWF gene provides instructions for making a blood clotting protein called von Willebrand factor. This protein contains regions that attach (bind) to specific cells and proteins during the formation of a blood clot. After an injury, clots protect the body by sealing off damaged blood vessels and preventing further blood loss. Cytogenetic Location: 12p13.31, which is the short (p) arm of chromosome 12 at position 13.31. Molecular Location: base pairs 5,948,874 to 6,124,675 on chromosome 12. More than 300 mutations in the VWF gene have been found to cause von Willebrand disease. It is deficient or defective in von Willebrand disease and is involved in a large number of other diseases, including thrombotic thrombocytopenic purpura, Heyde's syndrome, and possibly hemolytic-uremic syndrome.

Von Willebrand Factor's primary function is binding to other proteins, in particular factor VIII, and it is important in platelet adhesion to wound sites. It is not an enzyme and, thus, has no catalytic activity. VWF binds to a number of cells and molecules. Factor VIII is bound to

VWF while inactive in circulation; factor VIII degrades rapidly when not bound to VWF. Factor VIII is released from VWF by the action of thrombin. In the absence of VWF, factor VIII has a half-life of 1-2 hours; when carried by intact VWF, factor VIII has a half-life of 8-12 hours. VWF binds to collagen, e.g., when it is exposed in endothelial cells due to damage occurring to the blood vessel. Endothelium also releases VWF which forms additional links between the platelets' glycoprotein Ib/IX/V and the collagen fibrils. VWF binds to platelet gpIb when it forms a complex with gpIX and gpV; this binding occurs under all circumstances but is most efficient under high shear stress (i.e., rapid blood flow in narrow blood vessels, see below). VWF binds to other platelet receptors when they are activated, e.g., by thrombin (i.e., when coagulation has been stimulated).

W. Tang et al performed a genetic association study of *FVIIIc* and VWF that assessed 50,000 SNPs in 18,556 European Americans (EAs) and 5,047 African Americans (AAs) from five population-based cohorts. Previously unreported associations for *FVIIIc* were identified in both AAs and EAs with KNG1 (most significantly associated SNP rs710446, Ile581Thr, Ile581Thr, EAs and AAs). Significant associations for *FVIIIc* were also observed with rs12557310 in EAs, and with rs2236568 in AAs [16].

J. Song et al (2016) studied the connection of atherosclerosis and *ST3GAL4* as well as their connection with Willebrand's factor and VIII factor among 12117 subjects. 14 SNP of *ST3GAL4* rs2186717, rs7928391 and rs11220465 related to levels of Willebrand factor and with activity of VIII factor after adjustment on age, body mass index, hypertension, diabetes, smoking and blood group [8].

In many foreign researches it is established that homozygotes on minor allele of pro-motor SNP of VWF (3268GA) rs7966230 have higher Willebrand factor levels than homozygotes on widespread allele in population of healthy people. Minor allele of this SNP is associated with arterial thrombosis and with the increased risk of CAD among people with progressing atherosclerosis. Minor allele VWF (1793G) have carriers 2.6-fold, and carriers of WF (793GG) genotype – 3.5-fold increase in risk of CAD.

P.M. Ridker (1997) identified a number of SNPs participating in regulation of multimeasure *FV* size. When genotyping young patients with the first episode of an acute CAD or an ischemic stroke it was established that minor alleles of SNP rs4764478 (A/T), rs216293 (R/a) and rs1063857 (T2385C) were associated with substantial increase of the FV levels and risk of arterial thrombosis and cardiovascular diseases irrespective to other classical factors. SNP rs1063856 (*G2365A, Thr789Ala*) in the domain which participates in multimerization and linking with VIII factor is associated with FV level and risk of arterial thromboses. This association is revealed among healthy people and patients with CAD. SNP rs1063856 is associated with CAD risk among young patients with type 1 diabetes among Caucasian population. Euro-Americans have significant association between FV level and FVIII (Gly2705Arg) (rs7962217), because of disorders of FV multimeasure. This evidence points to the causal link between VWF and arterial thrombosis. Statistically significant correlations between *VWF* and CAD were found among high risk population [14].

SNP *5G (-675)4G* (*PAI-1).* Plasminogen activator inhibitor-1 (PAI-1) also known as endothelial plasminogen activator inhibitor or serpin E1 is a protein that is encoded by the SERPINE1 gene. Heterozygotic variant 5G (-675)4G prevails among population. Therefore, this SNP has no independent diagnostic value. Its effect is possible to estimate in combination with other factors contributing to development of pathology, for example, in combination with FGB (-467A). The allelic variant (-675)4G is followed by higher activity of gene, than *(-675)5G*, that causes higher concentration of *PAI-1* and reduction of fibrinolytic system activity. Homozygous *4G(-675)4G* is associated with increase in risk of thrombosis, pre-eclampsia, placenta disorders and spontaneous abortion.

In *SERPIN1* gene coding *PAI-1* SNP are identified: insertion/deletion of guanosine in position *4G(-675)5G (rs1799889), G(-844)A (rs2227631), c43GA (rs6092) and p.I17V (rs6090)* which change concentration of PAI-1 in plasma.

In the research of R. Natesirinilkul et al (2014) the level of PAI-1 and polymorphism 4G/5G among the Thai children did not show statistically significant interrelation with ischemic stroke. However, the mutation was found in 69-80% of the examined [22]. In the research of Li X. et al (2015) three variants (rs8093048, rs9946657, rs9320032) of PAI-2 gene were found in 407 patients with CAD and 518 people of the control groups of Chinese provinces of Han, and there was statistically significant with CAD [34].

K.N. Kim et al (2012) studied the link between SNP of PAI-1 of and hypertension among the Korean women and found correlation [32].

In the research of E. E. Starostina et al SNP of PAI-1 (5G (-675)4G) was more often detected among patients with "fast" fibrosis HCV-infection than among patients with "slow" fibrosis (55.62% against 47.16%) [1].

The interrelation between the speed of progressing of liver fibrosis among patients with HCV and polymorphic markers of other genes (MTHFR (C677T), FVII (G10976A), FXIII (C103T), GA2 (C807T), GB3 (T1565C)) is not revealed. Besides, Starostina et al found combinations of FII(GA)-FV(GG) and FII (GG)-FV(GA) meet more often in groups with "fast" fibrosis than in groups with "slow" fibrosis.

SNP T1565C (ITGB3). The ITGB3 gene provides instructions for making the beta3 subunit of a receptor protein called integrin alphaIIb/beta3 (αIIbβ3), which is found on the surface of small cell fragments called platelets. Platelets circulate in blood and are an essential component of blood clots. The beta3 subunit attaches (binds) to the alphaIIb subunit, which is produced from the ITGA2B gene, to form integrin αIIbβ3. It is estimated that 80,000 to 100,000 copies of integrin αIIbβ3 are present on the surface of each platelet.

Polymorphisms of the genes coding the proteins which are not entering the classical scheme of hemostasis. The results of GWAS which are carried out by Klarin D. at al (2017) demonstrate that the genes which are not link with coagulation can promote risk of venous thromboembolism (VTE). In metaanalyse researchers of INVENT Consortium gave the first instruction on the fact that genes out of coagulation cascade of TSPAN15 and SLC44A2 promote risk of VTE. In this research it was revealed that rs4602861 in ZFPM2 also promote risk of VTE. Multitype-2 protein is the known factor of transcription critical for hematopoiesis and development of heart. The locus is related with the circulating level of the growth factor of an endothelium of vessels (Vascular endothelial growth factor, VEGF), and recent data demonstrate that VEGF can be crucial for permission of venous blood clot. In total ZFPM2 can affect risk of VTE by means of modulation of the circulating VEGF and violation of balance of thrombosis in a venous system [17].

In meta-analyze which was carried out by K.C. Desch (2015), the following data were provided: the research found associations with alleles in genes of *F12, KNG1* among 1477 individuals (a gene of a kininogen 1) and HRG (histidine rich glycoprotein), all proteins were earlier described as a part of coagulative cascade. The subsequent larger research (9240 individuals) found additional signals in ABO, *FV*, and C6orf10 (an open frame of reading of chromosome 6), everything,

except C6orf10, had the known functions in the cascade of coagulation [24].

In GWAS-research Huang et al found associations for the activator of a fabric plasminogen (tPA) and inhibitor of the activator of plasminogen 1 (PAI-1) (more than 20000 individuals). The t-PA levels were statistically significantly connected with alleles of genes of two complex proteins of SNARE STX2 and STXBP5. These two loci were also connected with the FV levels [21].

In the research of M. Sabater-Lleal et al (2013) SNP rs4129267, rs6734238 and rs1154988 located in loci of IL6R, IL1F10/IL1RN and PCCB were considerably connected with fibrinogen level in blood and risk of development of cardiovascular diseases [26]. Q. Ma et al (2014) found significant associations with alleles in PLG locus (plasminogen) and LPA (apolipoprotein) and SIGLEC14 (sialic acid binding Ig like lectin 14) [20]. Also, in GWAS researches connection with disorders in hemostasis system and genes of PROCR was found (protein receptor C), EDEM2 (ER degradation-enhancing alpha-mannosidase-like protein 2), GCKR (regulatory protein of a glucokinase) and BAZ1B (Bromodomain Adjacent To Zinc Finger Domain 1B) [17].

Conclusion. All in all, common understanding of the influence of hemostasis genes polymorphism on chronic liver diseases is not yet been established. Pathophysiological and biochemical mechanisms of allelic variants impact of thrombophilia genes on progress and development of complications of this group of diseases, such as portal hypertensia, hepatocellular carcinoma, cryoglobulinemia, et al. are not well understood. While examining these issues the existence of other factors influencing rates of liver fibrosis (race, sex, age, virus genotype, presence of associated diseases, addictions, et al.) should be taken into consideration. Accordingly, a major concern has studying the influence of *FII G20210A* and *FV G1691A* which are not often found among population. A better understanding of the role of hemostasis genes' allelic variants in processes of intrahepatic epithelium is essential for prognosis of the impact the liver disease, as well as treatment and clinical background.

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GENETIC HETEROGENEITY OF PH-NEGATIVE CHRONIC MYELOPROLIFERATIVE DISEASES

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ABSTRACT

Chronic myeloproliferative diseases are clonal diseases of the hematopoietic system, characterized by uncontrolled proliferation of myeloid line cells. Classic Ph-negative chronic myeloproliferative diseases include polycythemia vera, essential thrombocythemia and primary myelofibrosis. Main complications that occur in patients with chronic MPD include thrombosis and transformation to secondary acute myeloid leukemia. A key factor of pathogenesis of this group of diseases is presented by activation of the JAK-STAT signaling pathway due to *JAK2* and *MPL* gene mutations, as well as mutation of *CALR* gene. These mutations play an important role in diagnosis and defining of disease prognosis and scoring possible complications. *JAK2*V617F mutation was demonstrated to be the most important risk factor for thrombosis, but did not have any affect in overall survival. *CALR*-positive patients with essential thrombocythemia and primary myelofibrosis have better prognosis than those with *JAK2* mutation. The worst prognosis has «triple-negative» patients with primary myelofibrosis.

Keywords: chronic myeloproliferative diseases, *JAK2, MPL, CALR.*