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Molecular genetic characteristics of hemostasis in hemorrhagic fever with renal syndrome

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Abstract

Aim. To assess the predictive value of single-nucleotide polymorphisms of hemostasis and folate cycle genes in hemorrhagic fever with renal syndrome (HFRS).

Methods. 43 patients undergoing HFRS were examined based on the Republican clinical infectious diseases hospital in Izhevsk. Toxic shock syndrome (TSS) in the decompensated phase, pulmonary edema in the alveolar phase, and acute kidney injury (AKI) at stage F [RIFLE criteria (risk, injury, failure, loss, end-stage renal disease)] were registered as complications. Molecular analysis of patients' genomic DNA was performed after its isolation from peripheral blood cells. Genotyping was performed by using multiplex real-time PCR with conformationally restricted probes. Statistical analysis was performed by the licensed program SPSS 22.0; the significance level of difference between groups was determined using the nonparametric Mann–Whitney test (for quantitative variables) and the Fisher's exact test (for qualitative variables).

Results. The C/C genotype of the ITGB3:1565T/C gene (p=0.0278), and the C/C genotype of the MTHFR1298 A/C gene (p=0.0407) was less common in severe cases, while the G allele of FGB:–455G/A gene (p=0.046) and the T allele of the ITGB3:1565T/C gene (p=0.0166) was more frequent. More frequent detection of the 5G/4G genotype of the PAI-1:675 5G/4G gene was found in the case of TSS (p=0.0433). Genotype C/C of the ITGB3:1565T/C gene (p=0.0145) and a combination of pathological genotypes A/C and C/C of the MTHFR1298A/C gene (p=0.0004) are less common in the development of AKI at stage F.

Conclusion. The molecular genetic analysis makes it possible to identify patients with genotypes predisposing to a severe and complicated course of hemorrhagic fever with renal syndrome.

Keywords: gene polymorphism, hemorrhagic fever with renal syndrome.

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Background. Hemorrhagic fever with renal syndrome (HFRS) is a zoonotic disease widespread in the Udmurt Republic. It is caused by an RNA-containing[1] virus from the *Hantaviridae* family. Despite the significant genetic diversity of representatives of this family, the *Puumala* serotype is found predominantly on the territory of the republic [1,2].

The clinical presentation and course of HFRS is characterized by a well-known polymorphism. Typical mild and moderate cyclical forms of the disease are found. Also, severe cases of the disease are found complicated by infectious toxic shock, pulmonary edema, disseminated intravascular coagulation syndrome (DIC), acute renal failure, and lethal outcomes [2].

One of the variants for explaining the diversity of the clinical presentation of the disease may be the individuality of the human body reaction to hantavirus infection because of genetic characteristics. Thus, the associations of polymorphisms in the genes of proteins of the immune system (*MHC*, *TNF*, *IL1*) [3–7], endothelium (VE-cadherin, NOS) [8,9], hemostasis (*SERPINE1*, *ITGA2B*) [10,11], detoxification system (*CYP1A1*, *GSTP1*) [12], and their relationship with the severity of HFRS have been recently studied.

There is evidence that the haplotypes B*08-DRB1*03 [4,13] and B*46-DRB1*09, B*51-DRB1*09 in the *HLA* gene are associated with a more severe form of HFRS [14,15], and alleles

[1] RNA — ribonucleic acid.

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B*27 and DRB1*15 are associated with mild disease [16].

There are indications that allele A and genotype AA of polymorphism–308G>A (rs1800629) in *TNF* gene [6,7,17], genotype TT 1550T>C of *CDH5* gene (rs1049970) [8], allele G in polymorphism–844A>G (rs2227631) of the *SERPINE1* gene [10], the HPA3 B allele [18], the TT genotype of the gene *eNOS:894G/T* [9], the 1A2C and AG genotypes of the rs1048943 polymorphic loci of the *CYPIA1* gene and rs1695 of the GSTP gene affect the severity of HFRS [12].

Changes are known to occur in the level of expression of genes *GATA3* [19,20], T-BET, CD3, IFNβ, NFkB, STAT1, and MxA [20] in cell cultures after hantavirus infection.

In this regard, we consider it urgent to conduct a molecular genetic study of single-nucleotide polymorphism (SNP) of genes of the blood coagulation system and the metabolism of folates in HFRS patients to establish their influence on the characteristics of the clinical presentation of the disease.

SNP is a one-nucleotide difference in the deoxyribonucleic acid (DNA) sequence in the human genome and result from point mutations. It is believed that SNP of genes do not lead independently to diseases but only affect the susceptibility to diseases and impart characteristics in their course.

The **aim** of the study was to evaluate the prognostic value of SNP of genes of the hemostasis system and folate cycle in HFRS.

Materials and methods. The study was based on the results of a retrospective study conducted in the spring and summer period of 2019. Patients with HFRS were hospitalized at the Republican Clinical Infectious Diseases Hospital in Izhevsk [21]. All patients lived on the territory of a natural focus, and in all cases, the diagnosis of HFRS was confirmed serologically by using enzyme-linked immunosorbent assay (ELISA) [2].

Inclusion criteria were admission to inpatient treatment within the first 48 hours from the onset of the disease, absence of chronic somatic pathology, clinical and epidemiological signs of HFRS upon admission, age between 25 and 66 years, smoking or alcohol abuse.

Subsequent exclusion criteria consisted of negative ELISA (serological ruling out of HFRS) and mild course of the disease.

All patients received the same type of pathogenetic therapy; the treatment was supplemented according to clinical guidelines in the event of complications. A scoring system was used to assess the severity of HFRS, which takes into account hemodynamic and uropoietic disorders, the emergence of hemorrhagic syndrome, the presence of cerebral or lung edema, serum creatinine levels, and kidney rupture [22]. Infectious toxic shock (ITS) in the decompensated stage, pulmonary edema in the alveolar stage, acute kidney injury (AKI) class F according to the RIFLE[2] classification were registered as complications [2,23].

The study was approved by the local ethical committee of the Izhevsk State Medical Academy (Protocol No. 654 dated 04/23/2019). Written informed consent was taken from all patients to participate in the study.

Patients were divided into two groups during the pilot study: group 1 and group 2 (Tables 1,2):

– Group 1 included 13 patients with a history of moderate (uncomplicated) clinical form of the disease.

– Group 2 included 30 patients with severe and complicated course of HFRS.

Both the groups were comparable in age (39 [34.0; 45.0] years in group 1, and 42.5 [37.0; 52.0] years in group 2) ($p = 0.255$).

Molecular genetic analysis of the genomic DNA of patients was performed when taking control blood tests upon discharge from the hospital from peripheral blood cells using a set of reagents RealBest Extraction 100. A set of reagents RealBest-Genetics Hemostasis (12) was used to determine gene polymorphisms. In total, we analyzed SNP of 12 genes that are significant for the functioning of the blood coagulation system, fibrinolysis, vascular wall, and platelets (Table 3).

Genotyping was performed using real-time multiplex polymerase chain reaction with conformationally blocked probes. A CFX96 recording amplifier (Bio-Rad, USA) was used. Information on the frequencies of SNP alleles for Caucasians was obtained from the results of studies conducted as part of international projects.

Clinical and laboratory data were saved in the Microsoft Office Excel database. The licensed SPSS 22.0 software was used for statistical analysis. Nonparametric Mann–Whitney test (for quantitative variables) and Fisher's exact test (for qualitative variables) were used to determine the significant differences between the groups [21].

Results. Clinical and laboratory characteristics of patients enrolled in the study and the incidence of complications of HFRS in the groups compared are presented in Tables 1 and 2. The data presented indicated that there were significantly more pronounced manifestations of renal dam-

[2] The abbreviation RIFLE is formed by the initial letters of each of the successively distinguished stages of acute kidney injury, namely risk, injury, failure, loss of function, and end-stage renal disease.

Table 1. Clinical and laboratory characteristics of patients included in the study

Parameters assessed	Patients of group 1. HFRS of moderate severity (n = 13)	Patients of group 2. HFRS of severe (complicated) course (n = 30)	Significance of differences between groups
Age. years	5.0 [5.0; 6.0]	22.0 [21.0; 26.0]	<0.001
Men	39.0 [39.0; 39.0]	39.0 [39.0; 39.5]	0.457
Women	6.0 [5.0; 8.0]	7.0 [6.0; 8.0]	0.15
HFRS course severity. points	0	16.67	0.1174
Body temperature (max.). °C	53.85	76.67	0.1345
Duration of fever > 37°C. days	84.62	96.67	0.1543
Hemorrhagic syndrome (skin bruise. epistaxis). %	4.77 [4.42; 5.07]	4.75 [4.1; 5.36]	1.0
Lumbar pain. %	10.9 [8; 13.3]	15.1 [10.9; 19.1]	0.012
Oliguria. anuria. %	8.0 [5.0; 12.0]	7.0 [4.0; 14.0]	0.851
Blood erythrocytes. $\times 10^{12}$/L	17.0 [15.0; 21.0]	13.0 [8.0; 17.0]	0.056
Blood leukocytes. $\times 10^{9}$/L	64.0 [53.0; 83.0]	58.5 [38.7; 87.0]	0.751
Stab neutrophils. %	20.0 [17.0; 31.0]	11.5 [6.0; 18.0]	0.003
Lymphocytes. %	5.0 [5.0; 6.0]	22.0 [21.0; 26.0]	<0.001
Platelets. $\times 10^{9}$/L	39.0 [39.0; 39.0]	39.0 [39.0; 39.5]	0.457
Erythrocyte sedimentation rate. mm/h	6.0 [5.0; 8.0]	7.0 [6.0; 8.0]	0.15
Proteinuria > 0.030 g/L. %	100	100	—
Proteinuria. g/L	502.0 [396.0; 1016.0]	1920.0 [1205.0; 3307.0]	<0.001
Leukocyturia > 4 per field of view. %	7.69	60	0.0015
Erythrocyturia > 2 per field of view. %	30.77	76.67	0.0042
Renal epithelial cells > 2 per field of view. %	23.08	83.33	0.0001
Urea >8.5 mmol/L. %	46.15	96.67	0.0001
Urea. mmol/L	8.3 [6.5; 10.7]	32.95 [16.4; 48.6]	<0.001
Creatinine > 100 μmol/L. %	61.54	100	0.0003
Creatinine. μmol/L	132.0 [98.0; 143.0]	432.75 [211.4; 701.0]	<0.001
Antibodies to HFRS virus of IgM class (+). %	100	100	—

Note: HFRS — hemorrhagic fever with renal syndrome; Ig — immunoglobulin.

Table 2. Frequency of registration of hemorrhagic fever with renal syndrome complications in patients of the compared groups

Complications	Patients of group 1, HFRS of moderate severity (n = 13)	Patients of group 2, HFRS of severe (complicated) course (n = 30)	Significance of differences between groups
Lethality, %	0	3.33	0.5054
Infectious toxic shock, %	0	33.33	0.0175
Pulmonary edema, %	0	20	0.0822
RIFLE class F acute kidney injury, %	7.69	63.33	0.0008
Hemodialysis, %	0	10	0.2372

Table 3. Results of the study of single-nucleotide polymorphisms of genes of the hemostasis system and the folate cycle in patients with hemorrhagic fever with renal syndrome

Thrombophilia gene polymorphism		Patient group with moderate severity (n = 13)	Patient group with severe course (n = 30)	Significance of differences between groups
F5:1691 G/A	G/A. %	7.69	10	0.8109
	A/A. %	0	0	—
	A. %	3.85	5	0.8155
F2:20210 G/A	G/A. %	0	3.33	0.5054
	A/A. %	0	0	—
	A. %	0	1.67	0.5079
F7:10976 G/A	G/A. %	30.77	30	0.9598
	A/A. %	7.69	0	0.1243
	A. %	23.08	15	0.3647
F13:c.103 G/A	G/T. %	38.46	26.67	0.4393
	T/T. %	7.69	6.67	0.9035
	T. %	26.92	20	0.4773
FGB:–455 G/A	G/A. %	46.15	30	0.3074
	A/A. %	15.38	3.33	0.1543
	A. %	38.46	18.33	0.046
PAI-1:–675 5G/4G	5G/4G. %	46.15	46.67	0.9753
	4G/4G. %	53.85	53.33	0.9753
	4G. %	76.92	76.67	0.9794
MTHFR:1298 A/C	A/C. %	30.77	36.67	0.7094
	C/C. %	23.08	3.33	0.0407
	C. %	38.46	21.67	0.1061
MTHFR:677 C/T	C/T. %	46.15	46.67	0.9753
	T/T. %	7.69	3.33	0.533
	T. %	30.77	26.67	0.6969
MTR:2756 A/G	A/G. %	38.46	30	0.5866
	G/G. %	7.69	6.67	0.9035
	G. %	26.92	21.67	0.5962
MTRR:66 A/G	A/G. %	53.85	43.33	0.5256
	G/G. %	15.38	36.67	0.1629
	G. %	42.31	58.33	0.1712
ITGB3:1565 T/C	T/C. %	30.77	20	0.4427
	C/C. %	15.38	0	0.0278
	C. %	30.77	10	0.0166
ITGA2:807 C/T	C/T. %	38.46	36.67	0.911
	T/T. %	7.69	26.67	0.1601
	T. %	26.92	45	0.1153

Note: Plasmic hemostasis component, coagulation system: F5 — Leiden factor; F2 — prothrombin; F7 — proconvertine; F13 — coagulation factor 13; FGB — fibrinogen. Fibrinolysis: PAI-1 — a type 1 plasminogen activator inhibitor. Vascular hemostasis component, folate cycle: MTHFR — methylenetetrahydrofolate reductase; MTR — methionine synthase; MTRR — methionine synthase reductase. Platelet component: ITGB3 — platelet receptor of fibrinogen encoding the β_3 integrin protein; ITGA2 — a platelet receptor to collagen encoding the α_2 integrin protein.

age (proteinuria, pathological urinary sediment) and impaired renal nitrogen excretion (the degree of increase in creatinine and urea levels) in the severe course of the disease. More than half of patients with severe HFRS had RIFLE class F AKI, and every third patient had a clinical presentation of ITS.

Table 3 presents the frequencies of SNP of the genes of the hemostasis system and folate cycle in HFRS patients. Comparison of the distribution frequencies of homozygous mutations in the groups indicates their probable relationship with the severity of HFRS.

Thus, in the patients of group 2, the C/C genotype of the gene *ITGB3:1565T/C* (p =>^# 0.0278) and the C/C genotype of the gene *MTHFR1298A/C* (p = 0.0407) were less common. Also, in severe HFRS (in the patients of group 2) more frequent detection of the G allele of the gene *FGB:−455G/A* (p = 0.046) and the T allele of the gene *ITGB3:1565T/C* (p = 0.0166) was noted.

A more frequent detection of the 5G/4G genotype of the gene *PAI-1:675* 5G/4G was found in the case of ITS in the group 2 patients (p = 0.0433).

The study revealed that in the case of class F AKI (RIFLE criteria), the C/C genotype of the gene *ITGB3:1565T/C* (p = 0.0145) and the combination of the A/C and C/C genotypes of the gene *MTHFR1298A/C* (p = 0.0004) were found almost two times less often. Also, with AKI, more frequent detection of pathological genotypes A/G and G/G of the *MTRR66A/G* gene was recorded (p = 0.0011). It was found that in the group of patients with HFRS complicated with AKI, the C allele of the gene *ITGB3:1565T/C* (p = 0.0204) was less common, the C allele of the *MTHFR1298 A/C* gene was less often detected (p = 0.0012), and the G allele of the gene *MTRR66A/G* was more often detected (p = 0.0065).

The influence of some genes on certain clinical and laboratory parameters of HFRS has been established.

Thus, C/T and T/T mutations in the gene *ITGA2:807C/T* cause a longer fever [with normal C/C polymorphism, its course is 6.0 [4.0; 7.0] days, 8.0 [6.5; 10] days with a heterozygous C/T mutation (p = 0.009), and 8.0 [6.0; 8.0] days with homozygous T/T mutation (p = 0.045)]. The C/C genotype in the gene *MTHFR:677C/T* causes a low platelet count on admission [with normal C/C polymorphism, it is 62.0 [39.0; 79.0] × 10^9/L, 56.0 [40.5; 88.5] × 10^9/L with heterozygous C/T mutation (p = 0.548), and 111.5 [92.0; 131.0] × 10^9/L with homozygous T/T mutation (p = 0.049)].

No differences were found in the incidence of mutations of the genes under study in patients during the study, depending on the development of pulmonary edema, which is a characteristic complication of HFRS.

Discussion. The pathogenesis of HFRS is based on virus-induced immune-mediated damage to endothelial cells. Damage to the endothelium is accompanied by an increase in capillary permeability, edema of the interstitial tissue, plasma transudation, the development of hemoconcentration, and thrombogenesis. The endothelial damage and its disintegration result in the following: dynamic impairment of microcirculation in individual organs, the development of AKI, the occurrence of respiratory distress syndrome, ITS, and the emergence of thrombocytopenia and DIC syndrome characteristic of HFRS.

It is expected that in the pathogenesis of HFRS proteins of the hemostatic system will be of key importance, and the imbalance of the system will aggravate the vascular and hemorrhagic manifestations of the disease.

The effect of SNP mutations of some genes of the blood coagulation system and the folate cycle on the course and frequency of complications in HFRS were evaluated in this study.

In the course of the work, the association of the G allele of the gene *FGB:–455G/A*, the T allele of the gene *ITGB3:1565T/C* with a severe course of the disease was shown for the first time; the 5G/4G genotype of gene *PAI-1:675 5G/4G* with the development of ITS; the T allele of the gene *ITGB3:1565T/C*, the A allele and genotype A/A of the *MTHFR1298A/C* gene, the pathological genotypes A/G and G/G, and the G allele of the gene *MTRR:66A/G* with the development of AKI were shown.

The–455 G/A polymorphism of the fibrinogen (FGB) gene leads to an increase in fibrinogen production and causes the development of thrombophilia. This can be a factor for the development of coronary heart disease, peripheral arterial disease, acute coronary syndrome, and stroke [24,25].

The gene *ITGB3* (glycoprotein-platelet receptor for fibrinogen) is known to encode the synthesis of the β_3-chain of the GP2b/3a integrin complex involved in intercellular interactions. Allele C causes increased platelet adhesion and increases the risk of acute coronary syndrome [26]; it affects miscarriage [27] and decreases the effectiveness of acetylsalicylic acid (aspirin) [28].

The pathological polymorphism of the *PAI-1* gene (plasminogen activator of plasma) causes a higher concentration of the plasminogen activator inhibitor and reduces the activity of the anticoagulant system. The 4G/4G genotype is associated with an increased risk of thrombogenesis [29]; *PAI-1* is detected in high concentrations in the tissues of deceased patients with sepsis [30].

Mutation of the *MTHFR* gene affects the development of thrombosis, in particular, in the renal veins. Pathological polymorphism causes hyperhomocysteinemia in renal pathology [31], and a change in the *MTRR* gene affects the lesion of the coronary arteries [32].

No other studies showed the effect of mutations in the *ITGA2* gene (platelet receptor gene for collagen) on the severity of the course of HFRS [11].

The extremely high frequency of the 675 5G/4G heterozygous mutation in the *PAI-1* gene in both groups of patients is noteworthy. This phenomenon cannot be explained within the framework of the study performed, but theoretically, this mutation may be associated with a greater predisposition to the occurrence of HFRS.

CONCLUSIONS

1. Predictors of severe HFRS include the presence of the G allele of the gene *FGB:–455G/A* and the T allele of the gene *ITGB3:1565T/C*, as well as the absence of the C/C genotype of the gene *ITGB3:1565T/C* and the C/C genotype of the gene *MTHFR1298A/C*.

2. Genotype 5G/4G of gene *PAI:675 5G/4G* predisposes the development of ITS.

3. Severe AKI develops more often in patients with HFRS with A/G and G/G genotypes, as well as the G allele of the *MTRR66A/G* gene, and much less often in patients with the C/C genotype of the gene *ITGB3:1565T/C* and A/C and C/C genotypes of the gene *MTHFR1298A/C*.

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