ORIGINAL RESEARCH

DOI 10.25789/YMJ.2022.78.01

УДК 616.9:616.61–002.151 (07)

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POLYMORPHISM RS1127327 OF THE MICRORNA-146A TARGET GENE CCDC6 ASSOCIATED WITH A REDUCED RISK OF SEVERE HEMORRHAGIC FEVER WITH RENAL SYNDROME IN PATIENTS FROM THE VOLGA-URAL REGION OF RUSSIA

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The association analysis of polymorphic variants common for miRNA-146a, miRNA-218, miRNA-410, miRNA-503 target genes was performed in patients with hemorrhagic fever infected with PUUV. It has been revealed that the genotype rs1127327*AA of the CCDC6 gene is associated with a reduced risk of severe disease form. Further studies of the network of genes that are targets of various miRNAs are needed to elucidate the molecular mechanisms that can influence the onset and development of HFRS.

Keywords: miRNA, target genes, hemorrhagic fever with renal syndrome.

Introduction. Orthohantaviruses are RNA viruses that belong to the most widespread zoonotic viruses transmitted by rodents (10). These viruses are the etiological agents of two clinical forms of human disease: hemorrhagic fever with renal syndrome (HFRS) in Eurasia and hantavirus cardiopulmonary syndrome in America. Between 150,000 and 200,000 cases of orthochantavirus caused disease are reported worldwide each year, with most cases of hemorrhagic fever occurring in Asia, mostly in China (7). In the Russian Federation, HFRS occupies the leading place among all natural focal human diseases. In the Russian Far East, the annual incidence of HFRS is caused by two orthochantaviruses: Hantaan (genetic variants of Far East and Amur) and Seoul (6). Puumal virus (PUUV) is the main hantavirus causing hemorrhagic fever with renal syndrome in Europe. The number of patients diagnosed with HFRS in Europe is increasing and amounts to more than 3,000 cases per year (10). The Volga Federal District accounts for almost 90% of all cases of HFRS registered in the Russian Federation. The Republic of Bashkortostan, the largest source of HFRS in the Volga Federal District, has particularly high rates (6). Despite numerous attempts to study the disease, the pathogenesis of viral hemorrhagic fevers remains largely unknown. The course and outcome of the disease are thought to depend on the viral load, the genetic profile of patients, and the immune response (7). Active research is underway to address gaps in knowledge about the pathogenesis of HFRS. There are still no test systems for predicting the course of the disease with high accuracy, sensitivity and specificity. Promising markers in this respect could be microRNAs, which are endogenously expressed RNA molecules 18-22 nucleotides long that inhibit gene expression at the post-transcriptional level by binding to the 3′-translated mRNA target region and play an essential role in various biological processes, including cell cycle, apoptosis, cell proliferation and differentiation. Although the role of microRNAs in various viral infections has been extensively studied in recent years, there are only a few publications examining the role of circulating RNAs and microRNAs in Hantaan virus infection. A number of microRNAs (miR-146a, miR-410, miR-218, miR-503) have been identified that may be involved in the pathogenesis of HFRS (4,8,9,11). It is known that alteration of microRNA interaction with binding site resulting from single nucleotide substitution can modify expression of target genes involved in the initiation and development of various diseases (1), therefore, scientists put emphasis on identification of target genes for each microRNA.

The aim of the study is to analyze polymorphic variants in target genes, which are common for microRNA-146a, microRNA-218, microRNA-410, microRNA-503, which play a role in pathogenesis of HFRS, to find markers of HFRS risk.

Material and methods. Eighty-six individuals with moderate and eighty-eight patients with severe forms of HFRS and 115 healthy individuals were included in the study. All patients were hospitalized in infectious diseases hospitals in Ufa from 2018 to 2021 and gave voluntary informed consent to participate in the study. The diagnosis of HFRS was based on clinical findings (fever>38°C, acute kidney damage, thrombocytopenia) and was confirmed serologically. Patient blood samples were collected during hospitalization (mainly during the oliguric period).

According to the anamnestic data collected, none of the patients had received antiviral treatment before blood collection. All patients with HFRS were infected with PUUV. DNA from patients with HFRS was isolated by phenol-chloroform extraction. MicroRNA targets were determined using the mirdsnp database (http://mirdsnp.ccr.buffalo.edu/search.php#). In the first step we analyzed all possible targets for each microRNA separately - microRNA-146a, microRNA-218, microRNA-410 and microRNA-503 and found that polymorphic variants of the CCDC6 gene were "overlapping" targets for all of the above microRNAs. The polymorphic loci common to all microRNA targets selected for the study were genotyped by Taq-man allelic discrimination on a CFX96 Touch™ Real-Time PCR Detection System instrument. The results allelic discrimination were analyzed using the CFX96 Touch™ Real-Time PCR Detection System software. Pairwise comparison of genotype and allele frequencies in the patient and healthy person groups was performed using the χ2 criterion (P) for 2x2 contingency tables with Yates correction/

Results and discussion. We analyzed rs1127327, rs3802695, rs10821594, rs11540401, rs16914105 polymorphisms in the CCDC6 gene. The distribution of genotype frequencies for all polymorphic loci corresponded to Hardy-Weinberg equilibrium (p > 0.05). The clinical characteristics of the patients included in the study are shown in Table 1.

A comparison of allele and genotype frequencies between a sample of severe HFRS patients and a control group of healthy individuals revealed that the AA genotype of the polymorphic locus rs1127327 of the *CCDC6* gene was associated with a reduced risk of severe disease (OR=0.25; CI95%=0.07-0.9; p=0. 03), whereas the CC genotype demonstrated only a slight decrease in the frequency of occurrence in the patient group compared to controls and was not a risk marker for the development of HFRS (p>0.05, Table 2). Analysis of the distribution of polymorphic variants rs3802695, rs10821594, rs11540401, rs16914105 in the *CCDC6* gene in patients with HFRS and control individuals showed no statistically significant results (p>0.05). The *CCDC6* gene, which is the target of all microRNAs studied, including microRNA-146a, is located on the long arm of chromosome 10 (10q21) and contains 9 exons that encode a 3 KB transcript showing an open reading frame of 475 amino acids. The *CCDC6* gene promoter, located 259 bp upstream of the ATG site, controls its expression in various human tissues.

CCDC6 protein is a ubiquitously expressed proapoptotic protein. *CCDC6* is phosphorylated at T434 by ATM kinase, which stabilizes the protein in the nucleus in response to DNA damage. Loss of the *CCDC6* region recognized by ATM kinase or complete deficiency of the protein determines an increase in cell survival, allows DNA synthesis and promotes the transition to mitosis after exposure to genotoxic stress (2). Currently, the effect of the rs1127327 polymorphic variant on miR-146a expression has not been described, but it is known that, for example, the G/C polymorphism (rs2910164) in the pre-miR-146a sequence reduces pre and mature miR-146a in the presence of the C allele by 1.9 and 1.8 times, respectively, compared to the G allele. The C allele was also shown to prevent nuclear factor binding to pre-miR-146a, and decreased miR-146a resulted in less effective inhibition of target genes involved in the Toll-like receptor complex and cytokine signaling pathway (*TRAF6, IRAK1*) and *CCDC6* (3).

It is well known that miR-146a is involved in innate immunity and inflammatory responses in viral infection.

Table1

Clinical manifestations of the oliguric period of HFRS depending on the severity of the disease

Symptoms	Disease form				Total	
	Moderate		Severe			
	N	%	N	%	N	%
Oligoanuria	84	97.7	88	100	74	84
Weakness	86	100	88	100	88	100
Lower back pain	84	97.6	88	100	86	97.7
Abdominal pain	57	66.3	80	90.9	67	75.7
Nausea, vomiting	52	60.4	87	98.9	67	76.1
Petechial rash	36	41.9	73	83	52	59.3
Hemorrhagic enanthema	39	45.3	71	80.7	53	59.1
Bleeding at injection sites	17	19.7	67	76.1	39	44.3
Hemorrhages in the sclerae	6	6.9	15	17	9	10.2
Nosebleed	4	4.7	21	23.9	11	12.5
Microhematuria	86	100	88	100	88	100
Macrohematuria	7	8.1	22	25	12	13.6
Proteinuria	86	100	88	100	88	100

Table2

Distribution of allele and genotype frequencies of polymorphic locus rs1127327 of the CCDC6 gene in the group of HFRS patients and controls

Genotype, allele	Patients		Control		χ2	P-value	OR	95% CI
	n	pi	n	pi				
AA	3	3.4	14	12	4.99	0.025	3.9	1.09-14.1
AC	43	48.9	44	38	2.29	0.13	0.65	0.37-1.1
CC	42	47.7	57	50	0.06	0.79	1.07	0.6-1.8
A	49	27.8	72	31.3	0.57	0.45	0.84	0.55-1.3
C	127	72.2	158	68.7	0.57	0.45	1.18	0.76-1.8

Note. OR is the odds ratio, 95% CI is the lower and upper bounds of the 95% confidence interval for OR, p-value is the significance level of the criterion.

However, little is known about the effect of miR-146a on PUUV virus-induced infection and the molecular mechanisms by which this effect occurs. Previously, researchers demonstrated that live Hantaan virus can induce the expression of miR-146a, NFkBp65 and downstream proinflammatory cytokines. According to the literature, the miR-146a promoter has two binding sites to NF-kB, so it is suggested that NF-kB-dependent expression of miR-146a may be a regulator of innate immune responses (8). In a study by Qing-Zhou Chen et al. scientists concluded that NF-kB-dependent induction of miR-146a is also found in Hantavirus infection because HTNV NP/GP proteins promoted miR-146a and NF-kB promoter activity (8).

It is also known that miR-146a suppresses IFN-b expression. Interferons can provide paramount protection against viral infections in vertebrates, and activated NF-kBp65 can directly determine early IFNb production after viral infection. miR-146a has been shown to regulate type I IFN through negative feedback via NF-kB. High doses of Hantaan virus were also known to suppress interferon secretion, and virus ANDV- and virus SNV-encoded proteins had the potential ability to inhibit IFN-b induction and signal transduction (13). Tula hantavirus (TULV), ANDV, and New York hantavirus-1 (NY-1V) Gn proteins, but not PHV, suppress IFN-b production by inhibiting phosphorylation of IRF3 via TBK1 kinase early in viral infection (5), which also promotes HTNV replication.

The effect of Pumala virus on IFN secretion has not been studied, but it can be assumed that the described mechanism is universal, as many viruses can promote viral replication and proliferation by inhibiting IFN secretion in host cells (8).

Human miR-146 occurs in two different forms: miR-146a, localized on chromosome 5q33, and miR-146b, located on chromosome 10q24. Because the mature forms differ by only 2 nucleotides, many of the predicted target genes are common to both microRNAs, but each also has specific targets unique to the particular microRNA. The two related miR-146s are regulated differently, with miR-146a (but not miR-146b) being strongly induced by lipopolysaccharide. It has been suggested that miR-146 plays a role in Toll-like receptor and cytokine signaling and thus in the immune response, and there is evidence that miR-146a is regulated by NF-kappa B (12).

Conclusion. This study shows that the AA genotype of the polymorphic locus rs1127327 of the CCDC6 gene, which is a common target of non-xc microRNAs, is associated with a decreased risk of HFRS in patients from Bashkortostan (OR=0.25; CI95%=0.07-0.9; p=0.03). Nevertheless, further studies of the network of genes that are targets of different microRNAs are needed to elucidate the molecular mechanisms that may influence the occurrence and development of HFRS.

Funding. The study was carried out with the financial support of the Russian Foundation for Basic Research and the State Foundation for Basic Research in the framework of scientific project № 21-515-53017

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