

The Role of Human Genetic Factors in the Natural Selection of Hepatitis C Virus' Dominant Genotype in Ethnically Close Populations of Buryats and Khalkha-Mongols

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The aim of the study was to assess the role of the innate immunity gene polymorphism in the population selection of hepatitis C virus (HCV) genotypes circulating in the ethnically close groups of Mongoloids: Buryats and Khalkha-Mongols.

Materials and Methods. Nucleotide polymorphisms of innate immunity genes were identified in 400 patients with chronic hepatitis C, including 200 people belonging to the ethnic group of Mongols living in Ulaanbaatar (Mongolia) and 200 to the ethnic group of Buryats living in Ulan-Ude (Republic of Buryatia). The control group (n=531) consisted of apparently healthy people comprising 220 Buryats and 311 Khalkha-Mongols. Genetic studies of twelve single-nucleotide polymorphisms of nine genes: *IFNL1* (rs30461); *IFNL3* (rs12979860 and rs8099917); *IFNL4* (rs368234815); *CD209* (rs4804803); *TLR3* (rs3775291 and rs13126816); *TLR7* (rs179008 and rs179009); *IFITM* (rs12252); *MyD88* (rs6853); *IFIH1* (rs1990760) have been performed in the mentioned selections of the sick and healthy individuals. When analyzing the genetic study results, frequencies of gene alleles and their combinations in the form of genotypes have been compared.

Results. The dominant prevalence of the HCV genotype 1 (98.0%) was found in the territory of Mongolia which appeared to be significantly higher ($p < 0.001$) than its prevalence in the territory of Buryatia (66.0%). Among the genetic factors which can influence the formation of the circulating genotype structures in Buryat and Mongolian population, single-nucleotide polymorphisms in three genes (*IFNL3*, *TLR3*, and *TLR7*), the frequency of which differed significantly in the examined cohorts, have been detected. In the ethnical Buryat group, the search for the candidate genes in patients with chronic hepatitis C genotype 1 and non-1 (2 or 3, 2/3) has established that T allele of rs179008 *TLR7* gene occurs 2 times more often in women with chronic hepatitis C infection caused by genotype 2/3 than by genotype 1 ($p = 0.04$).

Conclusion. A low prevalence of HCV genotypes 2 and 3 among the population in the territory of Mongolia is likely to be caused by a rare frequency of the mutant T allele of *TLR7* gene (rs179008) associated with the predisposition to HCV-2/3 infection, i.e. the situation that has been demonstrated in our work using the ethnical group of Buryats as an example.

Key words: viral hepatitis C; hepatitis C virus genotypes; gene polymorphisms; *IFNL1* (rs30461); *IFNL3* (rs12979860 and rs8099917); *IFNL4* (rs368234815); *CD209* (rs4804803); *TLR3* (rs3775291 and rs13126816); *TLR7* (rs179008 and rs179009); *IFITM* (rs12252); *MyD88* (rs6853); *IFIH1* (rs1990760).

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Introduction

The development of prophylactic measures and implementation of direct-acting antiviral agents into clinical practice have resulted in stabilization of the morbidity level of chronic viral hepatitis C (CHC). Currently, according to WHO data, the number of patients with CHC has essentially reduced from 160 to 71 million people [1, 2]. In 2016, the 69th World Health Assembly adopted the resolution in which the incidence of viral hepatitis in the world was declared to decrease significantly by 2030 up to its complete elimination. It should be taken into account that more than 50% of all infected people on the planet are concentrated in the countries of the Asian-Pacific region [1]. In Asia, the highest figures of hepatocellular carcinoma and the occurrence of liver cirrhosis formation in the outcome of viral hepatitis diseases are observed [1].

Hepatitis C virus (HCV) possesses a high variability and a wide genotypic diversity. Specific HCVs prevail in separate geographical regions. For example, genotype 1 (HCV-1) dominates in the countries of South and North America, Australia, and Europe (53–71%), genotype 3 (HCV-3) is the most prevalent in Asia (40%), while genotype 4 (HCV-4) in the Middle East and North Africa (about 71%) [3]. Despite a great deal of information connected with the spread of HCV genotypes in concrete countries, the mechanism of forming stable epidemic genotypes in restricted geographical areas is not completely clear.

One of the highest incidence rates of viral hepatitis C in Asia is noted in Mongolia, HCV-1b (98.8%) being the most prevalent of all genotypes [4, 5]. The phenomenon of HCV-1 absolute predominance is not observed in the neighboring Buryatia, China or in other Asian countries [3]. Such a high concentration of one genotype in the population of Mongolia is rather difficult to explain by a specific epidemiology of viral hepatitis C in this country or by the “founder effect”. At the same time, in Russia, including the ethnically close Buryatia, the level of HCV-1 does not exceed 57.1% [6], and in China 56.8% [3].

A certain tolerance of the population to HCV-2/3 infection, when HCV-1 obtains selective advantage, is likely to be one of the explanations of this phenomenon. The given predisposition is supposed to be of the genetic nature and can be detected by the comparative study of the genetic polymorphism in the ethnically close population of Mongols and Buryats. The study of polymorphism of the innate immunity genes encoding the synthesis of membrane and intracellular adapter molecules, effectors of the immune response to HCV, may become important for the explanation of the causes of the natural selection of the dominant HCV genotype in the territory of Mongolia [7–9].

The aim of the study was to define the role of the polymorphism of innate immunity genes in the population selection of hepatitis C virus genotypes circulating in

the ethnically close groups of Mongoloids: Buryats and Khalkha-Mongols.

Materials and Methods

The investigations presented here have been carried out from 2013 to 2017 in the territory of Russia (Republic of Buryatia) and Mongolia. The material was collected in the medico-prophylactic settings of Buryatia and Mongolia: Republican Clinical Infectious Hospital (Ulan-Ude, Republic of Buryatia, Russia) and National Center for Communicable Diseases (Ulaanbaatar, Mongolia).

Single-nucleotide polymorphisms (SNP) in innate immunity genes were identified in 400 patients with CHC, including 200 people belonging to the ethnic group of Mongoloids living in Ulaanbaatar (Mongolia) and 200 to the ethnic group of Buryats living in Ulan-Ude (Republic of Buryatia, Russia). Among the representatives of the Mongoloid race, the ethnic group of Khalkha-Mongols is the most numerous making more than 85% of the total population [10].

Patients in both territories were selected using the method of continuous random sampling from those who addressed the Center of Hepatology of the Republican Clinical Infectious Hospital in Ulan-Ude (Republic of Buryatia, Russia) and National Center for Communicable Diseases in Ulaanbaatar (Mongolia). The diagnosis of CHC was established on the basis of history-taking, clinical examination, identification of hepatic transaminase activity, detection of anti-HCV IgG and HCV RNA. The control group (n=531) comprised apparently healthy people: 220 Buryats and 311 Khalkha-Mongols.

The genetic study was carried out in compliance with the Declaration of Helsinki (2013) and approved by the Ethics Committee of Irkutsk State Medical University. Informed consent for its conduction was obtained from all examined people.

Virus genotyping was performed using PCR method and AmpliSense-HCV-genotype kit (Central Research Institute of Epidemiology of Rospotrebnadzor of the Russian Federation).

To assess the probable effect of the genetic markers on the selection of a specific virus genotype dominant in the population, twelve SNPs of the nine genes were genetically studied in the given samples of the sick and healthy people: *IFNL1* (rs30461); *IFNL3* (rs12979860 and rs8099917); *IFNL4* (rs368234815); *CD209* (rs4804803); *TLR3* (rs3775291 and rs13126816); *TLR7* (rs179008 and rs179009); *IFITM* (rs12252); *MyD88* (rs6853); *IFIH1* (rs1990760). When choosing SNPs, the authors considered polymorphisms in the genes that encode the synthesis of the receptors or adapter molecules which are of great importance as antiviral factors of the innate immunity [11]. A preliminary analysis of the literature [7–9, 11, 12] showed the availability of signaling proteins whose genes have not been actually studied in viral hepatitis C but which play

an important role in providing inherited stability in other viral infections.

Molecular-genetic investigations were conducted using Rotor-Gene Q cycler (Qiagen, Germany) for real-time PCR. Definite gene sites were amplified with the help of appropriate primers. The genotypes of *IFNL3* gene were analyzed using AmpliSence reagent kit (Central Research Institute of Epidemiology of Rospotrebnadzor of the Russian Federation) to identify SNPs of rs8099917 and rs12979860 by a real-time PCR technique. To determine other genotypes, primers

and probes of original design synthesized in company Syntol and company Eurogen (Russia) were employed (Table 1). To make amplification more effective, a “hot start” technique with TaqF polymerase (Interlabservice, Russia) was used. The PCR included sample denaturation at 95°C for 15 min, and the following 45 cycles: 95°C for 15 s, 60°C for 30 s.

The primary materials were statistically processed by means of standard parametric and nonparametric tests in Statistica 6.1 program (StatSoft Inc., USA). SNPStatus program (<http://bioinfo.iconcologia.net/SNPStatus>) was

Table 1

Variable sites of innate immunity genes and primers for their identification in PCR

Gene	SNP	Localization/ substitution character	5'->3'primers and probes for real-time PCR
<i>IFNL1</i>	rs30461	Chromosome 19, exon/nonsynonymous, Asn188Asp	F GAAGGAGTAGGGCTCAGCCG R ACGCGAGACCTCAAATATGTGG 5'-(FAM)-GACGTTCTCAGACAC(A-LNA)GGT(C-LNA)C-(RTQ1) 5'-(R6G)-TGACGTTCTCAGACACA(G-LNA)GT(T-LNA)C-(BHQ2)
<i>IFNL4</i>	rs368234815	Chromosome 19, missense-mutation/—	F CCTGCTGCAGAAGCAGAGAT R GCTCCAGCGAGCGGTAGTG 5'-(R6G)-AT-CGC-A(G-LNA)A-(A-LNA)(G-LNA)G-CC-(BHQ1) 5'-(FAM)AT-CGC-A(G-LNA)(C-LNA)(G-LNA)-GC-CC-(BHQ1)
<i>CD209</i>	rs4804803	Chromosome 19, promotor zone/—	F ACTGTGTACACCCCTCCACTAG R AGGAAAGCCAGGAGGTCACA 5'(R6G)-CTGC-(C-LNA)(C-LNA)(A-LNA)-CCCTTGC-(BHQ1) 5'(FAM)-CTGC(C-LNA)(T-LNA)(ALNA)-CCCTTGC-(BHQ1)
<i>TLR3</i>	rs3775291	Chromosome 4, exon/nonsynonymous, Leu412Phe	F CCAAGAGAAAGCATCACTCTCTATTTTTG R GTTTGCGAAGTTTGACAAATGAAACATT 5'(R6G)-CCCTTACA(C-LNA)ATA(T-LNA)T(C-LNA)AACC-(BHQ2) 5'(FAM)-CCCTTACA(C-LNA)ATA(C-LNA)T(C-LNA)AACC-(RTQ1)
	rs13126816	Chromosome 4, intron/—	F GCAACGAAAAGGCAATCTAGAAGA R AAGTCTTGAAGTTTCAGTGAGCGA 5'(R6G)-AACCTCC(C-LNA)AT(T-LNA)TT(G-LNA)CTC(BHQ2) 5'(FAM)-ACCTCC(C-LNA)AT(C-LNA)TT(G-LNA)CTC(RTQ1)
<i>TLR7</i>	rs179008	Chromosome X, exon/nonsynonymous, Gln11Leu	F GGTGTTTCCAATGTGGACACTG R ACATCCAGAGTGACATCACAGG 5'(FAM)-TTATGTTAAAAAGGATAAGAATT(A-LNA)G(T-LNA)C-(RTQ1) 5'(R6G)-TTATGTTAAAAAGGATAAGAATT(T-LNA)G(T-LNA)C-(BHQ2)
	rs179009	Chromosome X, intron 2/—	F TTTGCTAAAGAGCTAAGATGCTAA R TTCAGCTGTCTAAACAGCATCC 5'(FAM)-GTAAGTACAAATACA(G-LNA)TC(T-LNA)TGG-(RTQ1) 5'(R6G)-GTAAGTACAAATACA(T-LNA)C(G-LNA)TGG-(BHQ2)
<i>IFITM</i>	rs12252	Chromosome 11, missense-mutation/—	F GGACACCATGAATCACAACACTGTC R TGAGCATTCCCTGGGGCCATA 5'-(FAM)TTCTTCTCTCTCTGTCAAC(A-LNA)G(C-LNA)G(RTQ1) 5'-(R6G)CTTCTTCTCTCTGTCAA(C-LNA)AG(T-LNA)G(BHQ2)
<i>MyD88</i>	rs6853	Chromosome 3, 3'UTR/—	F ATTTAGCTGCCATGCACCTGTC R GCGTACAAAACATGTAGAAGATGC 5'-(FAM)-GGGCATTTTAAAGCCAT(C-LNA)TC(A-LNA)AG-(RTQ1) 5'-(R6G)TGGGCATTTTAAAGCCA(T-LNA)CTC(G-LNA)A-(BHQ2)
<i>IFIH1</i>	rs1990760	Chromosome 2, exon/nonsynonymous, Ala946Thr	F CATTGTCAGATGATTTCCACATTTA R GATTCTTCCCTTGTACTTATAG 5'-(FAM)-TTTACATTGTAAGAGAAA(C-LNA)AAA(A-LNA)CAC-(RTQ1) 5'-(R6G)CTTACATTGTAAGAGAAA(C-LNA)AAA(G-LNA)CA-(BHQ2)

used for statistical analysis of the candidate genetic studies. Each SNP was statistically analyzed according to different research models: codominant, dominant, recessive, overdominant, and log-additive. And the model with the least value of the Akaike information criterion for each gene polymorphism was assumed as the most probable [13]. Frequency of gene alleles and their combination in the form of genotypes was compared while analyzing the results of the genetic studies. Distribution of the genotypes across each locus was tested for the compliance with Hardy–Weinberg

law. OR (odds ratio) was calculated at 95% confidence interval. Wolf–Holden method was used to calculate OR at a zero value [14]. Significance was defined as $p \leq 0.05$.

Results

A comparative analysis of the clinical and laboratory indices in Mongols and Buryats with CHC is given in Table 2.

Clinical symptomatology did not show anything special in the course of CHC.

Table 2

Clinical and laboratory characteristics of patients with chronic hepatitis C in the ethnic groups of Buryats and Mongols (M±m)

Parameters	Buryats, n=200	Khalkha-Mongols, n=200	p
Age (years)	41.8±0.9	43.5±0.8	>0.05
Gender, male (%)	40.6±3.8	39.7±3.6	>0.05
Body mass (kg)	70.2±1.5	71.0±1.1	>0.05
Height (cm)	163.8±1.2	161.6±0.9	>0.05
Body mass index	26.2±0.9	27.2±0.8	>0.05
Supposed disease duration (years)	3.4±0.4	4.9±0.3	>0.05
Latent course (% (abs. number))	40.0±3.5 (80)	40.0±3.5 (80)	>0.05
Astenovegetative syndrome (% (abs. number))	54.0±3.5 (108)	58.0±3.5 (116)	>0.05
Heaviness and pains in the right hypochondrium (% (abs. number))	25.0±3.1 (50)	27.0±3.1 (54)	>0.05
Sclera icteritiousness (% (abs. number))	5.0±1.5 (10)	4.5±1.5 (9)	>0.05
Hepatomegaly (% (abs. number))	25.0±3.1 (50)	22.0±2.9 (44)	>0.05
Splenomegaly (% (abs. number))	9.5±2.1 (19)	9.5±2.1 (19)	>0.05
ALT (Units/L)	78.7±4.4	88.2±4.3	>0.05
AST (Units/L)	59.0±3.0	67.3±3.4	>0.05
Bilirubin (µmol/L)	15.0±0.6	14.0±0.4	>0.05
Neutrophils (×10 ⁹ /L)	2.9±0.2	3.0±0.2	>0.05
Erythrocytes (×10 ¹² /L)	4.83±0.5	4.93±0.1	>0.05
Leukocytes (×10 ⁹ /L)	6.1±0.2	6.0±0.1	>0.05
Thrombocytes (×10 ⁹ /L)	225.1±5.6	218.6±5.3	>0.05
Hemoglobin (g/L)	144.9±1.5	149.0±1.2	<0.05
Total cholesterol (mmol/L)	4.7±0.1	5.1±0.2	>0.05
Triglycerides (mmol/L)	1.2±0.1	1.4±0.2	>0.05
Glucose (mmol/L)	5.47±0.1	5.34±0.1	>0.05

Table 3

Proportion of hepatitis C virus genotypes having caused chronic viral hepatitis C in the ethnic group of Buryats and Mongols (M±m)

Region	Genotype 1		Genotype 2		Genotype 3	
	abs. number	%	abs. number	%	abs. number	%
Republic of Buryatia, n=200	132	66.0±3.3	10	5.0±1.5	58	29.0±3.2
Mongolia, n=200	196	98.0±1.0	2	1.0±0.7	2	1.0±0.7
p	<0.001		<0.05		<0.001	

Note: p — statistical significance of value differences in comparing the groups of Buryats and Khalkha-Mongols.

The only statistically significant difference of such index as the level of hemoglobin in the blood reflects the physiological reaction of the body to the climatic and geographical conditions of Mongols habitation. The city of Ulaanbaatar is situated on the intermontane plateau at a height of 1300–1350 m above sea level which is 600–800 m higher than Ulan-Ude [10].

The variety of HCV genotypes was studied based on the analysis of 200 PCR-positive blood samples from CHC patients living in Buryatia and 200 in Mongolia (Table 3). HCV-1 (98.0%) was established to prevail in Mongols with CHC which is significantly higher ($p < 0.001$) than in Buryats (66.0%). The proportion of

other genotypes in each of the compared groups was essentially lower. Thus, HCV-2 and HCV-3 made 2.0% in Mongols and 34.0% in Buryats ($p < 0.001$). In addition to the mentioned variants, single patients with the combination of two or more genotypes were revealed as well as those with anomalous HCV genotypes which were excluded from the statistical analysis in order to keep the sample representative.

At the first stage of our work, the prevalence of the variable sites of the examined genes in the populations of healthy people in the ethnic groups of Buryats and Mongols was compared (Table 4).

In these people, significant differences were

Table 4
Prevalence of single-nucleotide polymorphisms of innate immunity genes among healthy people in the ethnic groups of Buryats and Mongols

Gene, SNP	Genotypes/alleles	Mongols, n=311 (abs. number/%)	Buryats, n=220 (abs. number/%)	OR (95% CI)	p
IFNL1, rs30461	TT	273/87.8	180/81.8	1.0	—
	CT	33/10.6	36/16.4	1.65 (1.00–2.75)	0.051
	CC	5/1.6	4/1.8	1.21 (0.32–4.58)	0.78
	Dominant model	273	180	1.0	—
		38	40	1.60 (0.99–2.59)	0.056
	Recessive model	306	216	1.0	—
	5	4	1.13 (0.30–4.27)	0.85	
IFNL3, rs12979860	CC	255/82.0	171/77.7	1.0	—
	CT	48/15.4	38/17.3	1.18 (0.74–1.88)	0.49
	TT	8/2.6	11/5.0	2.05 (0.81–5.20)	0.12
	Dominant model	255	171	1.0	—
		56	49	1.30 (0.85–2.00)	0.22
	Recessive model	303	209	1.0	—
	8	11	1.99 (0.79–5.04)	0.14	
IFNL3, rs8099917	TT	259/83.3	166/75.5	1.0	—
	TG	48/15.4	46/20.9	1.50 (0.95–2.34)	0.078
	GG	4/1.3	8/3.6	3.12 (0.93–10.53)	0.054
	Dominant model	259	166	1.0	—
		52	54	1.62 (1.06–2.49)	0.026
	Recessive model	307	212	1.0	—
	4	8	2.90 (0.86–9.74)	0.072	
IFNL4, rs368234815	TT/TT	255/82.0	170/77.3	1.0	—
	TT/ΔG	48/15.4	40/18.2	1.25 (0.79–1.98)	0.34
	ΔG/ΔG	8/2.6	10/4.5	1.88 (0.73–4.85)	0.19
	Dominant model	255	170	1.0	—
		56	50	1.34 (0.87–2.05)	0.18
	Recessive model	303	210	1.0	—
	8	10	1.80 (0.70–4.65)	0.22	
CD209, rs4804803	AA	243/78.2	167/75.9	1.0	—
	AG	62/19.9	46/20.9	1.08 (0.70–1.66)	0.73
	GG	6/1.9	7/3.2	1.70 (0.56–5.14)	0.34
	Dominant model	243	167	1.0	—
		68	53	1.13 (0.75–1.71)	0.55
	Recessive model	305	213	1.0	—
	6	7	1.67 (0.55–5.04)	0.36	

Gene, SNP	Genotypes/alleles	Mongols, n=311 (abs. number/%)	Buryats, n=220 (abs. number/%)	OR (95% CI)	p
TLR3, rs3775291	GG	134/43.1	128/58.2	1.0	—
	AG	152/48.9	83/37.7	0.57 (0.40–0.82)	0.0023
	AA	25/8.0	9/4.1	0.38 (0.17–0.84)	0.014
	Dominant model	134 177	128 92	1.0 0.54 (0.38–0.77)	— 0.00061
	Recessive model	286 25	211 9	1.0 0.49 (0.22–1.07)	— 0.067
	TLR3, rs13126816	GG	224/72.0	136/61.8	1.0
AG		80/25.7	75/34.1	1.54 (1.06–2.26)	0.025
AA		7/2.3	9/4.1	2.12 (0.77–5.82)	0.14
Dominant model		224 87	136 84	1.0 1.59 (1.10–2.30)	— 0.013
Recessive model		304 7	211 9	1.0 1.85 (0.68–5.05)	— 0.22
TLR7*, rs179008		AA	182/98.4	119/90.1	1.0
	AT	2/1.1	10/7.6	7.65 (1.65–35.52)	0.0025
	TT	1/0.5	3/2.3	4.59 (0.47–44.63)	0.15
	Dominant model	182 3	119 13	1.0 6.63 (1.85–23.75)	— 0.00097
	Recessive model	184 1	129 3	1.0 4.28 (0.44–41.60)	— 0.17
	TLR7*, rs179009	CC	155/83.8	105/79.6	1.0
CT		26/14.1	25/18.9	1.42 (0.78–2.59)	0.25
TT		4/2.1	2/1.5	0.74 (0.13–4.10)	0.73
Dominant model		155 30	105 27	1.0 1.33 (0.75–2.36)	— 0.33
Recessive model		181 4	130 2	1.0 0.70 (0.13–3.86)	— 0.68
IFITM3, rs12252		CC	68/21.9	56/25.5	1.0
	CT	177/56.9	109/49.5	0.75 (0.49–1.15)	0.18
	TT	66/21.2	55/25.0	1.01 (0.61–1.67)	0.96
	Dominant model	68 243	56 164	1.0 0.82 (0.55–1.23)	— 0.34
	Recessive model	245 66	165 55	1.0 1.24 (0.82–1.86)	— 0.31
	MyD88, rs6853	AA	295/94.9	205/93.2	1.0
AG		16/5.1	15/6.8	1.35 (0.65–2.79)	0.42
GG		0/0	0/0	—	—
Dominant model		295 16	205 15	1.0 1.35 (0.65–2.79)	— 0.42
Recessive model		311 0	220 0	1.0 —	— —
IFIH1, rs1990760		CC	201/64.6	128/58.2	1.0
	CT	90/28.9	75/34.1	1.31 (0.90–1.91)	0.16
	TT	20/6.5	17/7.7	1.33 (0.67–2.64)	0.41
	Dominant model	201 110	128 92	1.0 1.31 (0.92–1.87)	— 0.13
	Recessive model	291 20	203 17	1.0 1.22 (0.62–2.38)	— 0.56

* For the genes mapped on X chromosome genotype distribution was performed only for females.

Table 5

Prevalence of single-nucleotide polymorphisms of *TLR7* (rs179008) gene among patients with chronic hepatitis C of genotype 1 and non-1 compared to patients with chronic hepatitis C (non-1 genotype) in the ethnic group of Buryat females (abs. number/%)

Genotype of <i>TLR7</i> * (rs179008) gene	Chronic hepatitis C, genotype 1, n=79	Chronic hepatitis C, non-1 genotype, n=41	OR (95% CI)	p
AA	69/87.3	30/73.2	1.0	—
AT	7/8.9	8/19.5	2.63 (0.87–7.91)	0.078
TT	3/3.8	3/7.3	2.30 (0.44–12.06)	0.31
Dominant model	69	30	1.0	—
	10	11	2.53 (0.97–6.59)	0.053
Recessive model	76	38	1.0	—
	3	3	2.00 (0.39–10.38)	0.40
A allele	145/91.8	68/82.9	1.0	—
T allele	13/8.2	14/17.1	2.30 (1.02–5.15)	0.0397

Note: p — statistical significance of value differences in comparing patients with chronic hepatitis C genotype 1 and CHC non-1 genotype; * statistical significance of value differences for the genes mapped on X chromosome, genotype distribution was performed for females only.

established to be in the frequency of one SNP gene encoding interferon- λ 3 synthesis and three SNP genes encoding toll-like endosomal pattern recognition receptors (TLR). Thus, in healthy Mongols, GG genotype rs3775291 of *TLR3* gene occurred more rarely (OR=0.54; CI=0.38–0.77; p=0.0006). But significantly more frequently were TT genotype rs8099917 of *IFNL3* (OR=1.62; CI=1.06–2.49; p=0.026), GG genotype rs13126816 of *TLR3* (OR=1.59; CI=1.10–2.30; p=0.013), and AA genotype rs179008 of *TLR7* (OR=6.63; CI=1.85–23.75; p=0.00097). The latter genotype also differed by the fact that 98.4% of the representatives of Mongol ethnic group were carriers of the dominant AA homozygous variants and only in three examined people minor T allele was detected in the genotype composition.

The found differences in the frequency of these four candidate genes in healthy people of both ethnical groups provided the basis for their further investigations as potential genetic factors for predisposition to HCV-1 infection and tolerance to HCV-2/3. The comparative analysis of SNPs in the mentioned genes in the Buryats and Mongols ethnic groups with HCV-1-induced CHC has established some regular patterns. TT genotype rs8099917 of *IFNL3* (65.9 and 77.7% in Buryats (p=0.005), 73.5 and 83.3% in Mongols (p=0.002), respectively) and GG genotype rs3775291 of *TLR3* (47.0 and 61.8% in Buryats (p=0.007), 56.1 and 72.0% in Mongols (p=0.0002)) occurred in both groups significantly less rarely than in healthy people.

Comparison of the genotype SNP variety of genes *IFNL3* (rs8099917), *TLR3* (rs3775291 and rs13126816), *TLR7* (rs179008) in CHC patients infected with HCV-1 and HCV-2/3 was performed only in the cohort of the sick Buryats as only single CHC patients with non-1

genotype of HCV are revealed in Mongolia. The analysis conducted did not permit us to established association between virus genotypes and SNP genotypes of the examined genes. However, the allele analysis found significantly higher frequency of T allele among Buryat females infected with non-1 genotype of HCV (Table 5).

Discussion

The investigation showed that the structure of HCV genotypes was essentially different in Mongolia and in neighboring Buryatia. Dominant prevalence of HCV-1 (98.0%) was detected in the territory of Mongolia which is significantly higher (p<0.001) than its prevalence in the territory of Buryatia (66.0%). As the groups were fully comparable in their clinical, laboratory, and age-sex indices, it is natural to assume that the predominance of virus genotype 1 among Mongols may be caused by polymorphism of innate immunity genes which create selective advantages for the circulation of the given genotype or prevent HCV-2/3 circulation due to genetic tolerance to the infection. The first works on this subject performed in ethnical groups of Caucasians were the investigations of Wietzke-Broun with co-authors (2006) and Askar with co-authors (2009) [15, 16]. In one of them, the SNP of the interferon regulatory factor *IRF-1* gene, which provides genetic resistance to HCV-3a, was described [15]. In another work, a TT homozygous genotype rs3775291 of the *TLR3* gene whose carriers are not ill with HCV-1 was detected [16]. The search for a mechanism of this phenomenon has led the authors to the conclusion that active reactions of innate immunity underlie the resistance of some people to HCV-1a. In the people of the Caucasian race having TT genotype

rs377529, a strong expression of the *TLR3* gene in the hepatic cells was found which provides a subsequent activation of the interferon regulatory factor-3 (IRF-3) and nuclear factor kB (NF-kB) leading to the intensive production of interferon- β and proinflammatory cytokines [16].

SNPs of the three genes (*IFNL3*, *TLR3*, and *TLR7*), the frequency of which significantly differed in the examined cohorts, were detected in the present work among the genetic factors which can influence formation of the structures of the circulating HCV genotypes in the population of Buryats and Mongols.

Genotypes of *IFNL3* variable sites are referred to one of the first polymorphisms described in viral hepatitis C having association with a spontaneous clearance of the virus and a response to the antiviral treatment with interferon and ribavirin [17]. In the present investigation, it has been ascertained that TT genotype of rs8099917 in *IFNL3* gene favorable for the spontaneous HCV clearance is significantly more common in the population of healthy Mongols than in Buryats.

Other SNPs, the distribution of which significantly differed among healthy Mongols and Buryats, were localized in genes *TLR3* (rs3775291, rs13126816) and *TLR7* (rs179008). TLRs are referred to pattern recognition receptors and actively participate in triggering and regulation of cytokine inflammation. *TLR3* and *TLR7* receptors are localized transmembraneously on the endosome whereto the virus gets after internalization. These receptors recognize single- and double-stranded RNA, which is formed in the process of replication and particle assembly of practically all RNA-containing viruses. Polymorphism of *TLR* genes can influence both virus recognition (processing) and activation of the signaling pathway causing inflammation (signaling). The comparison of the polymorphisms of both genes showed significant differences in their frequency in the examined ethnical groups. Thus, examination of practically healthy population of Mongols and Buryats revealed three candidate genes the SNPs of which may influence the structure of HCV genotypes circulating in the population.

Further investigations were focused on the analysis of the mentioned four polymorphisms in the three candidate genes *IFNL3*, *TLR3*, and *TLR7* in the population of Mongols and Buryats ill with CHC caused by various virus genotypes. A more frequent occurrence of the favorable TT genotype rs8099917 of *IFNL3* gene among healthy people in comparison with patients with CHC caused by HCV-1 among both Mongols and Buryats confirms a well-known fact about genetic resistance of carriers of the given genotype to HCV infection [17]. The obtained data showed that application of rs8099917 SNP of *IFNL3* gene as a prognostic factor for Caucasians and Negroids is acceptable for the ethnic groups of Mongoloids living in the territory of Buryatia and Mongolia.

In addition to the SNP of *IFNL3* gene, one more

variable site rs13126816 of *TLR3* gene, the GG genotype of which marks the resistance to HCV-1-induced viral hepatitis C. This SNP was also universal and concerned both ethnical groups. Carriers of GG genotype had a lower risk of getting sick compared to other individuals: 2 times among Mongols and 1.8 times among Buryats. The previous study of this SNP in Caucasians [18] showed that macrophages of the people carrying G allele are capable of quick and intense production of interferon- β . According to the study performed in the USA in the ethnic groups of Caucasians, Afro-Americans and Latin Americans [18], rs13126816 G allele of *TLR3* gene was associated with a high frequency of HCV-1 spontaneous clearance. The same SNP was associated with the resistance of individuals to herpesvirus [19]. Thus, two SNPs (rs8099917 *IFNL3* and rs13126816 *TLR3*), the wild genotypes of which determine the resistance of their carriers to HCV-1 infection, were detected in the examined groups.

The search for gene genotypes involved in the reactions of innate immunity in patients with CHC caused by HCV genotypes 1 and 2/3 did not find their significant differences in the groups. But at the same time, the analysis by separate alleles has established that rs179008 T allele of *TLR7* gene occurred two times more in women with CHC caused by genotype 2/3 than by genotype 1 ($p=0.04$). The rs179008 SNP of *TLR7* gene is localized in exon 3 of the X chromosome. This SNP is nonsynonymous by its character and is characterized by the substitution of glutamine (Gln) for leucine (Leu) in the encoded protein. As a result, the signaling peptide of *TLR7* receptor displays functional degeneracy [20]. In response to synthetic inducers in the dendrite cells and hepatocytes of the minor T allele carriers, low expression of *IFNL1* mRNA, *IL-10R β* , and *IL-28R α* is observed [21]. Besides, T allele is associated with a high viral load, poor response to antiviral therapy, and predisposition to a rapid progression of HIV infection in women [21, 22]. Mononuclears of healthy people, carriers of T allele, weakly produce interferon- α *in vitro* in response to the interferon inducers [22]. Higher frequency of the mutant *TLR7* gene in women with CHC caused by virus genotype 1 is obviously the consequence of a weak interferon and cytokine response to the infection.

HCV-2/3 is known to display a higher sensitivity to interferon- α in contrast to HCV-1 and HCV-4. Genetic disorder of interferon- α synthesis in response to HCV-2/3 infection is rather an important factor of providing a long-term persistence of the given virus genotypes. A rare presence of TT genotype rs179008 of *TLR7* gene (0.5%) among the Mongolian population determines population resistance to HCV-2/3 infection and stipulates absolute domination of HCV-1. The causes of the absence of the minor mutant rs179008 T allele of *TLR7* gene require additional investigations and may lie in the specific genotype structure not only of HCV but other viruses in which reactions of innate immunity play a significant role. A copy number of *TLR7* gene, the frequency of which

among the Mongolian population remains unexplored, may be of some importance.

Conclusion

The investigations performed enabled us to detect a candidate *TLR7* gene, the variable sites of which (rs179008) occur with various frequency in Buryat women with virus genotype 1 and non-1 (2/3). T allele frequency is more than twice greater in patients with non-1 genotype (17.1 and 8.2%, respectively). A low prevalence of HCV genotypes 2 and 3 among the population in Mongolia territory is likely to be caused by a rare frequency of the mutant T allele (rs179008) of *TLR7* gene associated with the predisposition to HCV-2/3 infection demonstrated in our work using the ethnical group of Buryats as an example.

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