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HLA Alleles and *KIR* Genes in Romanian Patients with Chronic Hepatitis C

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ABSTRACT

Background & Aims: The role of natural killer (NK) cells in the defense against hepatitis C virus (HCV) infection involve both innate and adaptive immunity. NK cells express a large panel of inhibitory and activating receptors who bind human leukocyte antigen (HLA) class I receptors. Killer cell immunoglobulin-like receptors (KIRs) are the most polymorphic of these receptors being encoded by genes distributed differently in unrelated individuals. The aim of this study was to look at the immune response in chronic HCV patients by assessing *NK-KIR* genes and their corresponding *HLA* ligands.
Methods: We genotyped 127 chronically HCV-infected patients and 130 non-infected healthy individuals for both *KIR* genes and their *HLA* ligands. The *HLA-A, HLA-B, HLA-C* genotypes were analyzed using polymerase chain reaction high-resolution typing.
Results: *KIR2DL3, KIR2DL5, KIR2DS4* norm, *KIR3DL3, KIR2DP1, KIR3DP1* genes were significantly increased in the HCV group compared to healthy individual. Analysis of various *HLA* haplotypes revealed different HLA alleles associated with increased susceptibility to HCV infection. Thus, *HLA A*23:01* was more frequent in the patients' group than in the controls (p=0.030). At the same time *HLA B*44:02* and *C*04:02* were significantly elevated in HCV-positive patients (p=0.008 and respectively p= 0.007).
Conclusions: These results suggest that the expression of *KIR2DL3, KIR2DL5, KIR2DS4* norm, *KIR3DL3* genes and the association with *HLA* alleles such as *HLA A*23:01, B*44:02, C*04:02* may increase the patient susceptibility to chronic HCV infection.

Key words: hepatitis C virus infection – killer cell immunoglobulin-like receptors – human leukocyte antigen.

Abbreviations: HCV: hepatitis C virus; KIRs: killer cell immunoglobulin-like receptors; HCC: hepatocellular carcinoma; HLA: human leukocyte antigen; NK: natural killer cells; NKRs: NK receptors; PCR: polymerase chain reaction; SR: spontaneous resolution; SSP: sequence specific oligonucleotide.

INTRODUCTION

Currently worldwide around 71 million individuals are chronically infected with hepatitis C virus (HCV), some of them being under a significant risk of developing liver cirrhosis and hepatic tumors [1]. According to current estimates based on HCV serology, the prevalence of 4.5 - 4.9% of HCV infection in the general population in Romania represents one of the highest figures in Europe [2, 3].

Natural killer cells (NK) play a crucial role in the innate defense against tumors and viral infections also contributing to the immune response during chronic HCV infections [4-6]. NK cells activity is regulated by stimulatory and inhibitory NK receptors (NKRs) which consist mainly of inhibitory killer Ig–like receptors (KIRs) [7].

KIRs are encoded by multiple genes which may be present or absent in given individuals depending on their genotype. Thus, A haplotypes consist mostly of *KIR* genes for inhibitory receptors, whereas B haplotypes contain genes for both inhibitory and activating receptors [8].

The association of *KIR* frequencies with HCV infection was revealed in studies that linked several *KIR* genes such as *KIR2DL3* with spontaneous resolution (SR) of HCV infection [9-11]. Moreover, other studies also found that *KIR2DL2* and *KIR2DL3* were associated with the onset and progression of chronic HCV infection [12-14]. Most patients with HCV infection progress to chronic disease, which can lead to liver fibrosis and the subsequent occurrence of cirrhosis and hepatocellular carcinoma (HCC) [15, 16]. Saito et al. [17] found the frequencies of *KIR2DL2-HLA-C1* and *KIR2DS2-HLA-C1* are significantly higher in patients younger than 65 years with HCC.

KIRs recognize HLA class I molecules and it has been reported that *HLA* alleles are associated with the outcome of HCV infection. Previous studies have identified specific *HLA* class I alleles associated with HCV clearance, such as *HLA-A*03* and *HLA-A*09*, whereas *HLA-A*25* and *HLA-A*11* has been reported to be associated with viral persistence and with unfavorable treatment outcome in HCV infection [18]. *HLA-A*03*, *B*27, DRB1*01:01* and *DRB1*04:01* alleles were significantly associated with viral clearance [19].

HLA-B and *HLA-C* alleles have also been related to the outcome of HCV infection alone or in association with various *KIR* alleles. Thus *HLA-B*44, HLA-C*12*, and *KIR3DS1* were identified as predictive factors for SR of HCV [20]. In addition, *KIR2DS5* alleles were associated with SR [21]. Xiong et. al. [22] reported that *HLA B*15:01* and *B*13:02* are significantly higher in the HCV-infected group while *B*15:02* and *B*07:05* are significantly higher in the uninfected group. In a Chinese population, *HLA-A*02:01* and *DRB1*11:01* were associated with HCV spontaneous clearance [23].

Worldwide the genetic mechanisms responsible for the onset and the further development of chronic hepatitis C as well as their geographical variations are under intense research. At the same time, no studies have focused on the connection between the variability of *KIR/HLA* genes and HCV in Romanian patients.

In this context, the specific aim of the present study was to investigate the prevalence of *KIR* cluster and *HLA* ligands in Romanian patients with chronic hepatitis C and to explore possible associations between *KIR* genes and their respective *HLA* ligands.

METHODS

For this cross-sectional study we selected 127 patients (73 women and 54 men) that were diagnosed with HCV infection between 2017 and 2019, in the Department of Gastroenterology and Hepatology, Fundeni Clinical Institute, Romania. All patients were Romanian Caucasians and had a chronic HCV infection, diagnosed according to generally accepted criteria. Thus, serologic assays detecting specific antibodies to HCV, core antigen and assays that can detect and quantify HCV viral load, such as HCV RNA were routinely employed. General patients' characteristics including age, gender, age at infection onset and duration of disease are included in Table I.

Table I. Patients' characteristics

Characteristic	Patients n=127	Controls n =130
Age, years mean ± SD	58.2±11.9	54.6±11.6
Gender, male n (%)	54 (42.5%)	60 (46.2%)
Age at infection onset, years mean ± SD	51.1±11.6	-
Disease duration, years med (q1; q3)	5.0 (3.0; 10.0)	-

SD: standard deviation; q1, q3: 1st and 3rd quartiles

The control group consisted of 130 individuals with negative HCV serology Table I; there were no statistically significant differences in both gender or age between the cases and the controls individuals included in the present study.

Genomic DNA was isolated from 200µL of peripheral EDTA-anticoagulated blood using QIAamp® DNA Blood Mini Kit (Qiagen, Hilden, Germany), according to the manufacturer's instructions.

KIR and *HLA* were genotyped by PCR with sequence specific oligonucleotide (SSP) method probe protocols using commercial test kits described below.

NK cell inhibitors (*KIR2DL1, KIR2DL2, KIR2DL3, KIR2DL4, KIR2DL5, KIR3DL1, KIR3DL2* and *KIR3DL3*), NK cell activators (*KIR2DS1, KIR2DS2, KIR2DS3, KIR2DS4, KIR2DS5* and *KIR3DS1*) and 2 pseudogenes (*KIR2DP1* and *3DP1*) were examined using the PCR-SSP *KIR* genotyping kit (*KIR* Ready Gene, Inno-Train Diagnostik GmbH, Germany). Briefly the protocols were implemented as follows: the cycling program involved the initial denaturation at 96°C for 2 min followed by 10 cycles of denaturation at 96°C for 15 seconds, annealing at 65°C for 60 seconds; and by 20 cycles of denaturation at 96°C for 15 seconds, annealing at 61°C for 50 seconds, followed by elongation at 72°C for 30 seconds. Electrophoresis using 2.0% agarose gel was performed after PCR for interpreting positive bands in each well, based on the standard protocol available in the kit. The genotype of each individual was typed specifically.

HLA-A, HLA-B and *HLA-C* high-resolution genes were genotyped using AllSet+™ Gold SSP, OneLambda, USA and Taq DNA Polymerase (BioLabs, New England). After the analyzing of *HLA* alleles in the *HLA* database (https://www.ebi.ac.uk/ipd/imgt/hla/) we classified them into 3 different groups: C1, C2, and Bw4.

The C1 group of alleles observed in patients were *C*01, C*03, C*07, C*08, C*12, C*14* and *C*16*. The C2 group of alleles were C*02, C*04, C*05, C*06, C*15 and C*17. The group of alleles that belongs to the Bw4 group were B*13, B*15:17, B*27, B*32:01, B*37:01, B*38, B*44, B*49, B*51, B*52, B*53, B*57 and B*58. Only alleles observed in the patients were described.

Categorical values are expressed as numbers and percentage and quantitative ones as mean ±standard deviation (SD) or median (interval), med (q1; q3) according to their distribution. The alleles were numbered twice in homozygote patients. The differences between the *KIR* genotypes, *KIR-HLA* ligands, or *HLA-A, HLA-B,* and *HLA-C* alleles in HCV patients versus control group were assessed by Chi-square test. A p-value less than 0.05 was considered significant. The SPSS V25 software was used.

All participants provided their written informed consent to participate in this study, according to the ethical principles expressed in the Helsinki Declaration, and the study was approved by the Commission of Bioethics at the Fundeni Clinical Institute, Romania.

RESULTS

The present study analyzed the KIR genotypes in patients with chronic HCV (n=127) and in the control group (n=130). All 14 *KIR* genes and the two pseudogenes were assessed in both study groups (Table II). The frequency of *KIR2DL3,* *KIR2DL5, KIR2DS4* norm, *KIR3DL3* was significantly higher in patients with HCV than in respective controls. At the same time the two pseudogenes *KIR2DP1, KIR3DP1* were found to be statistically elevated in patients (p=0.001, respectively p≤0.001) versus respective controls (Table II).

Table II. Frequencies of KIR genotypes in patients and control groups.

KIR genes	Patients		Controls		p
	n	%	n	%	
2DL1	119	93.7	126	96.9	0.221
2DL2	83	65.4	70	53.8	0.060
2DL3	107	84.3	92	70.8	0.010
2DL4 norm	88	69.3	89	68.5	0.886
2DL4 deleted	91	71.7	90	69.2	0.670
2DL5	33	26.0	19	14.6	0.023
2DL5 null	35	27.6	47	36.2	0.139
2DS1	54	42.5	49	37.7	0.430
2DS2	76	59.8	66	50.8	0.144
2DS3	45	35.4	49	37.7	0.707
2DS4 norm	36	28.3	107	82.3	<0.001
2DS4 (del-22bp)	99	78.0	102	78.5	0.921
2DS5	41	32.3	39	30.0	0.693
3DL1	113	89.0	114	87.7	0.749
3DL2	124	97.6	130	100.0	0.078
3DL3	123	96.9	130	100.0	0.041
3DS1	40	31.5	49	37.7	0.297
2DP1	93	73.2	116	89.2	0.001
3DP1	29	22.8	130	100.0	<0.001
3DP1 norm	80	63.0	74	56.9	0.321

The Chi-square test was used to estimate the *KIR* genotypes between the HCV patients and the control group; n-number of cases with relevant genotypes; significance was set at a p value < 0.05.

Next, we divided patients in two age categories as proposed by Saito et al [17], respectively above and under 65 years. The frequencies of only two of the *KIR* genes searched were significantly different between the two age groups: *KIR2DL4del* was more frequent in younger [64 (81.0%) vs. 27 (56.3%), p=0.003), while *2DL5null* in older than 65 years patients (16 (20.3%) vs. 19 (39.3%), p=0.018].

The *HLA* class I (*HLA-A, HLA-B, HLA-C*) genotypes were analyzed using PCR-sequence-based high-resolution typing, to reveal the possible associations with HCV infection. Reliable genotyping was obtained for all patients and control groups. In total, 28 *HLA-A*, 48 *HLA-B*, and 29 *HLA-C* alleles were identified. At the *HLA-A* locus, 13 alleles showed frequencies of >1% accounting for 70% of the total *HLA-A* alleles identified.

Allele frequencies at the 4-digit level were analysed and at the HLA-A and HLA-B loci it was found that they are distributed differently between the two groups. *HLA-A*23:01* was significantly detected more often in the HCV-infected group than in the control group (p=0.03, Table III). Regarding *HLA-B* locus, *B*44:02* was statistically significant (p=0.008, Table IV).

The relation of *HLA-C* alleles with chronic HCV infected patients was also assessed. The study found that the presence of

Table III. Distribution of *HLA-A* alleles in chronic HCV-patients and the control group. Comparison of all *HLA-A* alleles at the 4-digit levels between chronic HCV patients and the control group.

HLA-A	HCV patients 254 alleles		Controls 260 alleles		p
	n	%	n	%	
*01:01	29	11.4	33	12.7	0.657
*02:01	59	23.2	71	27.3	0.287
*02:05	3	1.2	0	0	0.079
*02:06	1	0.4	0	0	0.311
*02:09	1	0.4	0	0	0.311
*02:11	2	0.8	2	0.8	0.981
*02:17	1	0.4	1	0.4	0.987
*03:01	32	12.6	30	11.5	0.712
*03:02	1	0.4	0	0	0.311
*11:01	26	10.2	24	9.2	0.701
*23:01	9	3.5	2	0.8	0.030
*24:02	34	13.4	30	11.5	0.526
*24:03	0	0	2	0.8	0.161
*24:07	0	0	2	0.8	0.161
*25:01	1	0.4	3	1.2	0.327
*26:01	12	4.7	16	6.2	0.475
*29:01	3	1.2	0	0	0.079
*29:02	2	0.8	0	0	0.152
*30:01	6	2.4	4	1.5	0.499
*30:02	1	0.4	0	0	0.311
*31:01	4	1.6	4	1.5	0.973
*32:01	12	4.7	15	5.8	0.596
*33:01	2	0.8	3	1.2	0.672
*33:03	1	0.4	6	2.3	0.061
*66:01	2	0.8	1	0.4	0.549
*68:01	9	3.5	9	3.5	0.960
*68:02	0	0	2	0.8	0.161
*74:01	1	0.4	0	0	0.311

The Chi-square test was used to estimate the differences between HCV patients and the control groups; n: number of cases with relevant genotypes; significance was set at a p value < 0.05.

*HLA-C*04:02* was more frequent in chronic HCV patients than in the control group (p=0.007). At the same time, *HLA-C*03:04* was found to be significantly more prevalent in the control group than in the HCV-infected group (p=0.034, Table V).

When HCV patients were stratified accordingly to age (65 years) groups, the frequencies of *HLA A*23:01* were significantly higher in younger patients (p=0.017), while *HLA B*49:01* was in older than 65 years HCV patients (p=0.027). No significant difference was found for other *HLA-A, -B*, or *-C* alleles or for any of the *HLA* ligands tested in this research.

The frequencies of *KIR* and their *HLA-C* ligands were analyzed for their statistical associations with chronic hepatitis C. KIR-ligands are HLA molecules that can be grouped into three major categories based on their amino acid sequence: HLA-C1, HLA-C2 and HLA-B molecules. HLA-C group 1 (HLA-C1) having asparagine at position 80 is recognized by inhibitory KIR2DL1 and HLA-C group

Table IV. Distribution of *HLA-B* alleles in chronic HCV-patients and the control group. Comparison of all *HLA-B* alleles at the 4-digit levels between chronic HCV patients and the control group.

HLA-B	HCV patients 254 alleles		Controls 260 alleles		p
	n	%	n	%	
*07:02	14	5.5	14	5.4	0.949
*07:05	2	0.8	0	0	0.152
*08:01	12	4.7	17	6.5	0.373
*13:01	1	0.4	0	0	0.311
*13:02	8	3.1	13	5.0	0.289
*14:02	5	2.0	8	3.1	0.424
*15:01	3	1.2	9	3.5	0.087
*15:03	1	0.4	0	0	0.311
*15:08	1	0.4	1	0.4	0.987
*15:17	0	0	1	0.4	0.322
*18:01	29	11.4	20	7.7	0.151
*18:03	0	0	1	0.4	0.322
*18:04	1	0.4	0	0	0.311
*27:02	2	0.8	8	3.1	0.060
*27:03	1	0.4	0	0	0.311
*27:05	6	2.4	8	3.1	0.619
*35:01	15	5.9	20	7.7	0.421
*35:02	4	1.6	5	1.9	0.763
*35:03	11	4.3	12	4.6	0.876
*35:05	1	0.4	0	0	0.311
*35:08	3	1.2	1	0.4	0.304
*37:01	3	1.2	3	1.2	0.977
*38:01	9	3.5	12	4.6	0.539
*39:01	5	2.0	6	2.3	0.790
*39:06	0	0	3	1.2	0.086
*40:01	1	0.4	3	1.2	0.327
*40:02	5	2.0	8	3.1	0.424
*40:06	5	2.0	3	1.2	0.456
*41:01	5	2.0	3	1.2	0.456
*41:02	2	0.8	1	0.4	0.549
*41:03	2	0.8	0	0	0.152
*44:02	26	10.2	11	4.2	0.008
*44:03	6	2.4	4	1.5	0.499
*44:05	3	1.2	1	0.4	0.304
*44:06	1	0.4	0	0	0.311
*47:01	2	0.8	4	1.5	0.428
*48:01	3	1.2	0	0	0.079
*49:01	8	3.1	3	1.2	0.118
*50:01	1	0.4	2	0.8	0.576
*51:01	25	9.8	24	9.2	0.813
*51:07	1	0.4	0	0	0.311
*51:08	0	0	1	0.4	0.322
*52:01	10	3.9	10	3.8	0.958
*55:01	4	1.6	4	1.5	0.973
*56:01	3	1.2	6	2.3	0.330
*57:01	1	0.4	1	0.4	0.987
*58:01	3	1.2	7	2.7	0.215
*73:01	0	0	2	0.8	0.161

For abbreviations see Table III

Table V. Distribution of *HLA-C* alleles in chronic HCV-patients and the control group. Comparison of all *HLA-C* alleles at the 4-digit levels between chronic HCV patients and the control group.

HLA-C	HCV patients 254 alleles		Controls 260 alleles		p
	n	%	n	%	
*01:01	0	0	1	0.4	0.322
*01:02	16	6.3	15	5.8	0.801
*02:01	1	0.4	0	0	0.311
*02:02	21	8.3	21	8.1	0.937
*03:02	2	0.8	5	1.9	0.267
*03:03	5	2.0	6	2.3	0.79
*03:04	3	1.2	11	4.2	0.034
*04:01	36	14.2	36	13.8	0.915
*04:02	7	2.8	0	0	0.007
*05:01	9	3.5	8	3.1	0.768
*05:02	1	0.4	0	0	0.311
*06:02	19	7.5	25	9.6	0.387
*07:01	39	15.4	37	14.2	0.720
*07:02	9	3.5	18	6.9	0.086
*07:04	4	1.6	3	1.2	0.681
*08:01	3	1.2	0	0	0.079
*08:02	3	1.2	8	3.1	0.138
*08:03	2	0.8	0	0	0.152
*12:02	10	3.9	13	5.0	0.560
*12:03	28	11.0	29	11.2	0.963
*14:02	4	1.6	4	1.5	0.973
*15:02	18	7.1	13	5.0	0.320
*15:05	2	0.8	2	0.8	0.981
*15:11	0	0	1	0.4	0.322
*16:01	3	1.2	1	0.4	0.304
*16:02	2	0.8	1	0.4	0.549
*16:04	1	0.4	0	0	0.311
*17:01	2	0.8	0	0	0.152
*17:03	4	1.6	2	0.8	0.395

For abbreviations see Table III

2 (HLA-C2); lysine at position 80 is recognized by specific KIR2DL2 and KIR2DL3 [24, 25]. *HLA-B* alleles can be classified as either *Bw4* or *Bw6*. Bw4 is defined by the presence of leucine at position 82 and arginine at position 83 [26] whereas, Bw6 is defined by the presence of asparagine, leucine, arginine and glycine at positions 80-83, respectively [27]. The total number of HLA ligands is shown in Table VI and revealed no significant differences between the HCV patients and the control group.

DISCUSSION

The present study examined the expression of *HLA* alleles and *KIR* genes in Romanian patients with chronic hepatitis C. Reported results found that the association of *KIR2DL3, KIR2DL5, KIR2DS4* norm, *KIR3DL3* genes in chronic HCV patients is statistically significant. At the same time patients and

Table VI. Distribution of gene and genotype frequencies of *KIR-HLA* ligands in controls when compared to HCV patients. The following HLA-ligands: *C1, C2, C1C1, C1C2, C2C2, Bw4, Bw6* and their associations with HCV infection, were analyzed in HCV patients and the control group.

HLA ligands	HCV patients n=127		Controls n=130		p
	n	%	n	%	
C1	97	76.4	103	79.2	0.582
C2	88	69.3	83	63.8	0.355
C1C1	39	30.7	47	36.2	0.355
C1C2	58	45.7	56	43.1	0.676
C2C2	29	22.8	27	20.8	0.688
Bw4	82	64.6	80	61.5	0.615
Bw6	45	35.4	50	38.5	0.615

For abbreviations see Table III

control groups were genotyped for *HLA-A, HLA-B, HLA-C*. Among the studied HCV patients, the following *HLA* alleles *A*23:01, B*44:02* and *C*04:02* were associated with HCV infection.

In the past years a number of studies have focused on the influence of NK cells specific receptors on the outcome of HCV infection [9, 28, 29]. Thus, Khaboo et. al. [9] described a protective role for *KIR2DL3/KIR2DL3-HLA-C1C1* in both Caucasians and African Americans. This data was confirmed by Romero et. al. [28] and Knapp et. al. [10] in intravenous drug users. It is already demonstrated that both *KIR2DL3* and *KIR2DL2* bind *HLA-C1*. *KIR2DL3* binds *HLA-C1* with lower affinity than does *KIR2DL2* [30]. Inhibitory genes such as *KIR2DL2* and *KIR2DL3* segregate as alleles at a single locus and based on this, a weaker inhibitory receptor-ligand (*KIR2DL3-HLA-C1*) interaction would be protective. This protective effect has been shown only among individuals homozygous for *KIR2DL3*, but not in *KIR2DL2/KIR2DL3* heterozygotes or *KIR2DL2* homozygotes. It is also known, that *KIR2DL3* and *KIR2DS4* were found to be significantly more prevalent among HCV patients, when compared to controls [29]. This observation was also confirmed by our results: *KIR2DL3* was more prevalent among HCV patients than in controls. However, other studies, have shown opposite results: *KIR2DL3* gene was more prevalent among patients with persistent infection [31, 32]. Inhibitory *KIR2DL5* was reported with a higher frequency in chronically infected patients [33]. In the present study, *KIR2DL5* was significantly higher in patients with HCV than in the control group. Further studies are required for understanding HLA ligand, its function, and its association with HCV infection.

Previous studies have also identified the potential protective impact of common *HLA-A* alleles against persistent infection [23, 34, 35].

The association with *HLA-A*03* was observed in spontaneous clearance of HCV and the association with *HLA-A*25* predict an unfavorable treatment outcome in HCV infection [18]. Other reports showed that *HLA-A*01* and *HLA-A*02* conferred unfavorable outcomes over the course of infection. In the Egyptian population *HLA-A*09* was associated with protection, whereas *HLA-A*11* was with viral persistence against HCV [18, 19, 34]. In the current study only *HLA-A*23:01* was associated with HCV infection.

*HLA-B *57*-mediated antiviral immunity confers protection for HCV and HIV infections. This suggests a common shared mechanism of a successful immune response against persistent viruses [36-38]. Neumann-Haefelin et al. [39] note that the protection associated with the *HLA-B*27* allele has been linked to an epitope identified in genotype 1 infections but was not identified in genotype 3 infections in German patients. We also looked for the links between the *HLA-B*57* association with chronic HCV. This finding was present in both Caucasians and African-Americans, but in our selected Romanian patients, no statistically significant associations were found between *HLA B*57* and HCV.

The impact on the outcome of HCV infection was also observed regarding *HLA-C* alleles. Frias et al. [20] found that the combination of *HLA-B*44* and *HLA-C*12* alleles and the *KIR3DS1* genotype are associated with HCV chronicity in HIV patients. In our study, we found that the *HLA-B*44:02* and *HLA-C*04:02* were associated with HCV infection.

KIR and *HLA* alleles were associated with response to antiviral treatment. *HLA B*44, DRB*02, DRB*07* were associated with a higher response rate in peginterferon alpha associated with ribavirin treatment [40] and *KIR2DS3* has been reported to be associated with the failure to respond to HCV treatment [41].

Considering our current results, in a future study we will focus on the possible associations between certain *HLA* alleles and treatment response in patients with HCV infection.

Our study has several limitations consisting of the absence of *HLA-* class II genotyping and a relatively small number of patients. Due to the retrospective nature of the study, exposure risk and factors, were not analyzed in this study. A further study will expand on the present results and address the above limitations.

CONCLUSIONS

Our results support the potential role of *KIR* genes and *KIR-HLA* association in the control of HCV infection in the Romanian population. The association between *HLA* class I alleles and HCV indicate a potential role for the innate immune system in HCV pathogenesis. The biologic functions of activating or inhibitory KIRs could be influenced by other factors in different population groups suggesting that the ethnic genetic variations of HLA may greatly affect the host immune responses against HCV. The underlying mechanism of spontaneous HCV clearance is quite complex and requires further research.

Conflicts of interests: None to declare.

Authors' contribution: L.D.U collected and analyzed the data, drafted the manuscript. B.C. drafted the work. M.D. and I.C. conceived and designed the study. A.D. statistical performed the statistical analysis. All authors critically revised the manuscript, approved the final version to be published, and agree to be accountable for all aspects of the work.

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