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Significance of *IL-1Ra* and *IL-6* gene variants in Turkish patients with Crimean–Congo hemorrhagic feverUmut Safiye Say Coskun¹✉, Ayse Feyda Nursal², Ferdi Gunes³, Aydın Rustemoglu⁴, Abdullah Yayli⁵, Nevin Karakus⁴, Huseyin Sener Barut⁶, Serbulent Yigit⁴¹Tokat Gaziosmanpasa University, Medicine Faculty, Department of Medical Microbiology, Tokat, Turkey²Hitit University, Medicine Faculty, Department of Medical Genetics, Corum, Turkey³Obstetrics and Pediatrics Hospital, Clinic of Infectious Disease, Aydın, Turkey⁴Tokat Gaziosmanpasa University, Medicine Faculty, Department of Medical Biology, Tokat, Turkey⁵Tokat Gaziosmanpasa University, Medicine Faculty, Microbiology Laboratory, Tokat, Turkey⁶Tokat Gaziosmanpasa University, Medicine Faculty, Department of Infectious Disease, Tokat, Turkey

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ABSTRACT

Objective: To investigate the association between *IL-1Ra* variable number of tandem repeat (rs2234663), *IL-6* -597GA (rs1800797), *IL-6* -572GC (rs1800796) and the risk of Crimean-Congo hemorrhagic fever (CCHF) in the Turkish patients. **Methods:** This study included 50 patients infected with CCHF and 50 healthy controls. These variants were genotyped using polymerase chain reaction and/or restriction fragment length polymorphism method. **Results:** The distribution of the *IL-6* -572GC genotypes and alleles varied significantly between the patients and the controls. The subjects carrying *IL-6* -572GC GG genotype and G allele had increased risk of developing CCHF compared to the control group ($P=0.006$, $P=0.014$, respectively). *IL-6* -572GC GC genotype was higher in the controls than the patients ($P=0.006$). For the triple genotype combinations, the 1/2-GC-GG genotype combination was detected more frequently in the control group than CCHF patients ($P=0.016$). *IL-6* (-572/-597) GG-GG genotype was significantly higher in the patient group ($P=0.015$), while the GC-GG genotype was significantly lower in the patient group ($P=0.005$). Additionally, the G-G haplotype was significantly higher in the patient group ($P=0.042$), whereas C-G was found to be significantly lower in the patients than the control group ($P=0.037$). **Conclusions:** The results of this study suggest the *IL-6* -572GC variant might be genetic markers of sensitivity to CCHF in the Turkish population and may facilitate greater protection against the disease.

1. Introduction

Crimean-Congo hemorrhagic fever (CCHF) is a serious infectious disease with a mortality rate of 3%-50% worldwide. This infection is caused by RNA virus from the *Nairovirus* species of the

Bunyaviridae family[1]. This virus is endemic in especially Middle Asia, and Southeastern Europe. CCHF infection is manifested by microvascular deterioration and afflicted homeostasis. Although

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✉First and corresponding author: Assist. Prof. Dr. Umut Safiye Say Coskun, Tokat Gaziosmanpasa University Faculty of Medicine, Department of Medical Microbiology, Tokat, Turkey.

Tel: +90 505 5410856

Fax: +90 356 252 16 25

E-mail: umut.saycoskun@gop.edu.tr

the pathophysiology of CCHF is not understood properly. The clinic and severity of the disease emerge from endothelial damage and impaired immune response[2].

Interleukin-1 (IL-1) is important for inducing inflammatory and immunological process[3]. IL-1Ra is an antagonist of the pro-inflammatory cytokines and inhibits the activity of IL-1. Therefore IL-1Ra lowers the detrimental actions of IL-1 throughout the inflammatory process. It has been reported that a variable number of tandem repeats (VNTR) of 86 bp variant (rs2234663) are located in intron 2 of the *IL-1Ra* gene. This variant results in the presence of five alleles and each of them corresponds to a distinct number of repeats. The number of repeats has functional importance since repeats consist of binding sites for transcription factors[4].

IL-6 is a significant regulator of acute phase response. IL-6 is synthesized by activated macrophages, monocytes, and lymphocytes. It has been reported that IL-6 expression could be genetically controlled by polymorphisms found at positions rs1800795, rs1800796 and rs1800797 (aka IL-6 -174GC, -572GC, -597GA)[5].

Owing to different cytokines which have an important function in the control of immune response and defense against viruses, we aimed at exploring the correlation between *IL-1Ra* VNTR (rs2234663), *IL-6* -597GA (rs1800797), and *IL-6* -572GC (rs1800796) variants and CCHF susceptibility in a Turkish cohort.

2. Materials and methods

2.1. Ethical approval

The current study was approved by the Ethics Committee of Tokat Gaziosmanpasa University (number 16/KA EK/031).

2.2. Study population

Fifty CCHF patients [21 female, 29 male; mean age: (37.94±12.54) years] with RNA positivity were recruited from Infectious Diseases and Clinical Microbiology Department at Tokat Gaziosmanpasa University in Tokat, Turkey. Detailed clinical history and laboratory examinations were evaluated for each patient. In addition, alanine transaminase (ALT), aspartate transaminase (AST), platelet count (PLT) of patients were also evaluated. Fifty age-matched healthy individuals were recruited as controls [28 female, 22 male; mean age of (36.68±12.32) years]. Control group were selected from those with no history of acute or chronic disease and the absence of any other viruses, disorders. Subjects living in the Central Black Sea Region, older than 18 years were included in the present study. There was no significant difference in terms of age and gender among patients and the control.

2.3. Diagnosis of CCHF infection

All the serum specimens were stored at -20 °C until testing. RNA

was collected with the Bosphore CCHF virus Quantification Kit (Anatolia Geneworks, Turkey). Lyophilized proteinase K and carrier RNA were liquefied with buffers and RNase free water. The carrier RNA was mixed with RNase free water (1 000 µL). Patient serum (400 µL) was added to carrier RNA (1 mg/mL, 10 µL) and proteinase K (10 mg/mL, 20 µL). RNA isolation was performed on the Magnesia 16 isolation device (Anatolia Geneworks, Turkey) in accordance with the manufacturer's recommendation. All collected RNAs were stored at +4 °C if used within a short period of time or at -20 °C.

2.4. Genotyping

Genomic DNA was isolated from venous blood using a DNA extraction kit following the manufacturer's standard protocol (Sigma-Aldrich, St.Louis, MI, USA). *IL-1Ra* VNTR variant was detected according to Tarlow *et al*[4]. Genotyping for *IL-1Ra* VNTR variant was performed using polymerase chain reaction (PCR). PCR was performed in a 25 µL final volume (25 pM of each primer, 0.1 mM of dNTP, 0.5 µg of genomic DNA, 1.5 mM of MgCl₂, 2.5 µL of PCR buffer and 1.5 unit of Taq DNA polymerase) PCR steps were implemented as follows: initial denaturation at 94 °C for 4 min; 30 cycles of denaturation at 94 °C for 45 s, annealing at 51 °C for 30 s, and extension at 72 °C for 45 s; and final extension at 72 °C for 5 min. Two oligonucleotide primers F: 5'-CTC AGC AAC ACT CCT AT-3' and R: 5'-TTC CAC CAC ATG GAA C-3' based on flanking region of the *IL-1Ra* were used. The PCR products were analyzed by 3% agarose gel electrophoresis. Five different alleles of *IL-1Ra* were defined as follows: allele 1, four repeats (410 bp); allele 2, two repeats (240 bp); allele 3, five repeats (500 bp); allele 4, three repeats (325 bp); allele 5, six repeats (595 bp).

The *IL-6* -597GA (rs1800797) and -572GC (rs1800796) variants were genotyped by PCR based-restriction fragment length polymorphism (RFLP) analysis. The primers used for *IL-6* -597GA and -572GC variants were (F): 5'-CAG CAG CCA ACC TCC TCT AA-3' and (R) 5'-AAA CCA GAC CCT TGC ACA AC-3'. The cycling conditions for *IL-6* -597GA and -572GC variants were 40 cycles of 30 s at 95 °C, 30 s at 62 °C and 1 min at 72 °C. As the *IL-6* -597GA, the G to A transition was identified with *Fok* I, the A allele yielded fragments of 136 and 88 bps. As the *IL-6* -572GC, the G to C transversion was identified by digestion with *Bsr*BI, producing for the G allele fragments of 150 and 74 bps.

2.5. Statistical analysis

All statistical analyses were evaluated by Statistical Program Version 15.0 (SPSS Inc., Chicago, Illinois, USA) and OpenEpi info software package program. Genotype distributions and allele frequencies were evaluated by *Chi*-square statistic and Fisher's exact tests. *P* < 0.05 value was considered significant.

3. Results

This study represented 50 CCHF patients and 50 healthy subjects as the control group. The clinical features of the cases were demonstrated in Table 1. The genotypic and allelic frequencies of this VNTR variant were shown in Table 2. *IL-1Ra* VNTR variants were recognized as five types of alleles: 1 (four repeats, 410 bp), 2 (two repeats, 240 bp), 3 (five repeats, 500 bp), 4 (three repeats, 325 bp) and 5 (six repeats, 595 bp). In the present study, because of the differences of 86-bp tandem repeat number, five types of alleles could be established in this study. *IL-1Ra* 1 and 2 alleles were the most common meanwhile, other alleles were rare. The genotype frequencies of the VNTR *IL-1Ra* variant in the patients were as follows: 28 individuals (56%) were 1/1 genotype, 16 (32%) were 1/2 genotype, 2 (4%) were 2/2 genotype, 3 (6%) were 1/3 genotype, 1 (2%) were 2/5 genotype. The genotype frequencies of the VNTR *IL-1Ra* variant in the controls were as follows: 27 individuals (54%) were 1/1 genotype, 16 (32%) were 1/2 genotype, 5 (10%) were 2/2 genotype, 1 (2%) was 2/4 genotype and 1 (2%) was 2/3 genotype. Genotypic and allelic frequencies of the *IL-1Ra* VNTR variant did not show significant differences between the study groups ($P > 0.05$).

Table 1

Clinical characteristics of patients [n (%)].

Characteristics	Patients (n=50)
Serum AST (IU/mL)	
>700	10 (20)
<700	40 (80)
Serum ALT (IU/mL)	
>900	5 (10)
<900	45 (90)
PLT ($\times 10^6/L$)	
<20 000	24 (48)
>20 000	26 (52)
Survival	
Exitus	8 (16)
Living	42 (84)
Status	
Severe	27 (54)
Mild	23 (46)

ALT: alanine transaminase; AST: aspartate transaminase; PLT: platelet count.

Table 2

Genotype and allele frequencies of *IL-1Ra* VNTR variant in groups [n (%)].

Indices	Patients (n=50)	Controls (n=50)
Genotypes		
1/1	28 (56)	27 (54)
1/2	16 (32)	16 (32)
1/3	3 (6)	0
2/2	2 (4)	5 (10)
2/4	0	1 (2)
2/3	0	1 (2)
2/5	1 (2)	0
Alleles		
1	75 (75)	70 (70)
2	21 (21)	28 (28)
3	3 (3)	1 (1)
4	0	1 (1)
5	1 (1)	0

The distributions of genotype and allele frequencies of the *IL-6* -597GA and -572GC in CCHF and the control group were shown in Table 3. Genotypic and allelic frequencies of the *IL-6* -597GA variant did not show significant differences between the study groups ($P > 0.05$). A significant difference was observed in the *IL-6* -572GC genotype and allele distributions between groups. CCHF infected patients had a significantly higher frequency of *IL-6* -572GC GG genotype ($P=0.006$; $OR: 3.69$, 95% $CI: 1.53-8.89$) and G allele ($P=0.014$; $OR: 2.60$, 95% $CI: 1.26-5.37$) than healthy controls. *IL-6* -572GC GC genotype was more frequent in control group compared to CCHF patient group ($P=0.006$; $OR: 0.24$, 95% $CI: 0.09-0.64$) (Table 3).

Table 3

Genotype and allele frequencies of *IL-6* -597GA and *IL-6* -572GC variants in groups [n (%)].

Indices	Patients (n=50)	Controls (n=50)	OR, 95% CI	P
<i>IL-6</i> -597GA Genotypes				
GG	41 (82)	39 (78)		>0.05
GA	7 (14)	9 (18)		>0.05
AA	2 (4)	2 (4)		>0.05
Alleles				
G	89 (89)	87 (87)		>0.05
A	11 (11)	13 (13)		>0.05
<i>IL-6</i> -572GC Genotypes				
GG	40 (80)	26 (52)	3.69, 1.53-8.89	0.006
GC	7 (14)	20 (40)	0.24, 0.09-0.64	0.006
CC	3 (6)	4 (8)		
Alleles				
G	87 (87)	72 (72)	2.60, 1.26-5.37	0.014
C	13 (13)	28 (28)		

Clinical and laboratory data of patients were also analyzed according to *IL-1Ra*, *IL-6* -572GC, and *IL-6* -597GA genotype distribution and shown in Table 4. Triple, binary genotype and haplotype combinations for the *IL-1Ra*, *IL-6* -572GC, and *IL-6* -597GA loci were demonstrated. The 1/2-GC-GG genotype combination was demonstrated more frequently in the control group than CCHF patients (18% vs. 2%, $P=0.016$; $OR: 0.09$, 95% $CI: 0.01-0.75$) (Table 5). There was a significant difference between patients and the control group with the frequencies of binary genotypic and haplotypic characteristics, especially for the *IL-6* (-572/-597) GG-GG and GC-GG loci. The GG-GG genotype was significantly higher in the patients (68% vs. 42%, $P=0.015$; $OR: 2.93$, 95% $CI: 1.31-6.59$). The GC-GG genotype was significantly lower in the patient group compared to the control (8% vs. 32%, $P=0.005$; $OR: 0.18$, 95% $CI: 0.06-0.60$). Additionally, the G-G haplotype was detected significantly higher in the patient group ($P=0.042$; $OR: 1.99$; 95% $CI: 1.07-3.71$). The other haplotype, C-G, was significantly lower in patients than in the control group ($P=0.037$; $OR: 0.41$, 95% $CI: 0.19-0.90$) (Table 6).

Table 4

Clinical and laboratory data for *IL-1Ra*, *IL-6 -572GC*, and *IL-6 -597GA* genotypes (n).

Indices	AST		ALT		PLT		Status		
	>700	<700	>900	<900	<20 000	>20 000	Severe	Mild	
<i>IL-1Ra</i> VNTR Genotypes									
1/1	8	20	4	24	13	15	15	13	
1/2	1	15	1	15	8	8	9	7	
1/3	0	3	0	3	1	2	1	2	
2/2	0	2	0	2	1	1	1	1	
2/5	1	0	0	1	1	0	1	0	
Alleles									
2	2	19	1	20	11	10	12	9	
1	17	58	9	66	35	40	40	35	
3	0	3	0	3	1	2	1	2	
5	1	0	0	1	1	0	1	0	
<i>IL-6 -572GC</i> Genotypes									
G/G	9	31	4	36	18	22	21	19	
G/C	1	6	1	6	4	3	4	3	
C/C	0	3	0	3	2	1	2	1	
Alleles									
G	19	68	9	78	40	47	46	41	
C	1	12	1	12	8	5	8	5	
<i>IL-6 -597GA</i> Genotypes									
G/G	8	33	4	37	20	21	22	19	
G/A	2	5	1	6	3	4	4	3	
A/A	0	2	0	2	1	1	1	1	
Alleles									
G	18	71	9	80	43	46	48	41	
A	2	9	1	10	5	6	6	5	

ALT: alanine transaminase; AST: aspartate transaminase; PLT: platelet count.

Table 5

Haplotype frequencies in patients and controls [n (%)].

Haplotypes (3 sites)	Patients (n=50)	Controls (n=50)
(<i>IL-1Ra</i> VNTR)- (<i>IL-6 -572GC</i>)- (<i>IL-6 -597GA</i>)		
1/1-GG-GG	18 (36)	12 (24)
1/1-GG-GA	2 (4)	1 (2)
1/1-GG-AA	1 (2)	1 (2)
1/1-GC-GG	3 (6)	5 (10)
1/1-GC-GA	2 (4)	3 (6)
1/1-GC-AA	1 (2)	1 (2)
1/1-CC-GG	1 (2)	2 (4)
1/1-CC-GA	0	2 (4)
1/2-GG-GG	11 (22)	5 (10)
1/2-GC-GG	1 (2)	9 (18)
1/2-CC-GG	1 (2)	0
1/2-GG-GA	3 (6)	2 (4)
1/3-GG-GG	2 (4)	0
1/3-CC-GG	1 (2)	0
2/2-GG-GG	2 (4)	2 (4)
2/2-GC-GG	0	2 (4)
2/2-GG-GA	0	1 (2)
2/4-GG-GG	0	1 (2)
2/3-GG-GG	0	1 (2)
2/5-GG-GG	1 (2)	0

Table 6

Comparative analysis of *IL-6 -572GC* and *-597GA* haplotypes.

Indices	Patients	Controls	P	OR, 95% CI
Combined genotypes [n (%)]				
GG-GG	34 (68)	21 (42)	0.015	2.93, 1.31-6.59
GG-GA	5 (10)	4 (8)		
GG-AA	1 (2)	1 (2)		
GC-GG	4 (8)	16 (32)	0.005	0.18, 0.06-0.60
GC-GA	2 (4)	3 (6)		
GC-AA	1 (2)	1 (2)		
CC-GG	3 (6)	2 (4)		
CC-GA	0	2 (4)		
Combined haplotypes (%)				
G-G	0.783 3	0.638 7	0.042	1.99, 1.07-3.71
G-A	0.086 0	0.081 3		
C-G	0.106 7	0.231 3	0.037	0.41, 0.19-0.90
C-A	0.023 3	0.048 7		

4. Discussion

The pathogenesis of CCHF is not well established. Within minutes after the invasion of the host by the pathogenic agent, the innate immune system is activated and the host defense remains active during the infection. Endothelial damage may take place either directly by the invasion of viruses or indirectly owing to chemokines and cytokines emerged from the activated mononuclear cells[1].

Pro-inflammatory cytokines induce adaptive immunity and facilitate T-cell regulation and tolerance formation. *IL-1Ra* is involved in terminating the acute pro-inflammatory event and prevents chronic

inflammation from injuring healthy cells. IL-1Ra levels are increased in inflammatory conditions and correlate with their activity and therapies[6]. The significance of IL-1Ra as an anti-inflammatory mediator is also supported by data on patients with mutations in this protein, in whom loss of IL-1Ra leads to very serious systemic inflammation[7]. The relationship between IL-1Ra and various viral infections was evaluated in the literature. Some studies reported that the release of high amounts of the IL-1Ra is correlated with the fatal outcome of Ebola virus infection in humans[8,9] or with high viral titers and mortality in mice infected with mouse-adapted Ebola virus[10]. Ebola virus belongs to the same family as CCHF virus. Also, it was detected that blood IL-1Ra levels were higher in Dengue virus-infected patients as compared to controls[11]. Lai *et al.* indicated IL-1Ra was associated with the development of liver inflammation, which was reduced in patients with hepatitis B related liver failure and invertedly associated with disease severity[12].

Transcription activity of IL-1Ra is enhanced with the number of repeat because of increase in the binding sites for transcription factors[4]. The amount of IL-1Ra synthesis in human endothelial cells with the 2/2 genotype is three times less than in cells with the 1/1 genotype; an intermediate level of this protein is recorded in cells with the 1/2 genotype[13]. Zhang *et al.* reported IL-1Ra 1/2 genotype and IL-1Ra allele 2 were significantly lower in chronic hepatitis B than the control group[14]. Witkin indicated amounts of HIV-1 RNA in the blood were significantly lower in the patients who were IL-1Ra 2 homozygous compared to the other IL-1Ra genotypes[15].

In the present study, we analyzed the possible correlation of *IL-1Ra* VNTR, *IL-6* -572GC, and -597GA variants with CCHF in Turkish patients. There are not many studies on this issue in the literature. We observed there was not a significant difference in terms of *IL-1Ra* genotype and allele frequencies between the patients and the healthy control group ($P > 0.05$).

IL-6 has an important role in immunoregulation. Also, IL-6 deficiency can lead to dysfunctional innate and adaptive immunity as opposed to many infections[16]. In 2017, Meng *et al.* indicated that serum level of IL-6 was elevated in patients with HBV-infection[17]. Elevated IL-6 levels might indicate more active hepatic necroinflammation and be correlated with the manifestation and severity in chronic HBV infection. IL-6 also plays a role as a growth factor for Epstein-Barr Virus-infected B cells, leading to elevated Ig synthesis[18]. Another study conducted in 2017 indicated Epstein-Barr virus-induced gene 3 (EBI3) can support IL-6 functions by mediating trans-signaling[19]. Sghaier *et al.* found that carriage of IL-6 rs1800796 GG genotype, IL-6 rs1474358 C-allele, and IL-6 rs1800797 A-allele was more frequent in chronic hepatitis C virus infection than hepatocellular carcinoma[20]. Attar *et al.* demonstrated that there was a significant difference in the frequencies of *IL-6* -174GC GG genotype and G allele between HBV infected patients and the control group[21]. Also, a significant association between IL-6 variant was shown by Fabris *et al.* in HBV infection[22] and Cussigh *et al.* in HCV infection[23]. Wujcicka *et al.* showed the prevalence rate of *IL-6* -174GC GC genotype was significantly

decreased among the human cytomegalovirus infected, compared to the uninfected control subjects in 2017[24]. There was a significant relationship between the IL-6 rs1818879 GA heterozygous genotype and serious influenza A (H1N1) infection[25]. For *IL-6* -572GC, the controls with spontaneous clearance of HBV have significantly higher allele G and GG genotype frequency compared to the chronic HBV infection patients[26].

In this study, it was found that the patients carrying *IL-6* -572GC GG genotype and G allele had 3.69 and 2.60-fold increased risk developing CCHF compared to the control group, respectively. *IL-6* -572GC GC genotype was higher in control group than patients ($P=0.006$). This may be considered as heterozygous advantage. Besides, we evaluated the clinical and laboratory parameters of patients according to genotype distribution of these variants and triple, binary genotype and haplotype combinations for these loci. It was found that 1/2-GC-GG genotype combination was more often in control than in the CCHF patients ($P=0.016$). For *IL-6* gene variants, the GG-GG genotype was significantly higher in the patient group ($P=0.015$), although the GC-GG genotype was significantly lower in the patient group ($P=0.005$). Also, G-G haplotype was higher in the patient group ($P=0.042$), while C-G was lower in patients ($P=0.037$). It was thought GC-GG genotype and C-G haplotype facilitate greater protection against the disease.

To our knowledge, the present study is the first evaluation that investigated the relationship between *IL-1Ra* VNTR, *IL-6* -572GC, -597GA variants and CCHF in a Turkish cohort. This study showed the *IL-6* -572GC variant might be a strong genetic sensitivity marker for the development of CCHF in Turkish population. We believe that present study will serve as a premise for similar research. In future trials, both other cytokine and cytokine receptor gene variants should be investigated for the comprehension of the factors that have an important function in CCHF disease.

Conflict of interest statement

The authors declare no conflict of interests.

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