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Association of several innate immune response gene polymorphisms with COVID-19 in Turkish population

Türk popülasyonunda çeşitli doğuştan bağışıklık tepkisi gen polimorfizmlerinin COVID-19 ile ilişkisi

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ABSTRACT

Aim: The coronavirus disease 2019 (COVID-19) was caused by severe acute respiratory syndrome 2 virus (SARS-CoV-2), has spread globally. Gender and age have been established as risk factors for severe COVID-19. However, these factors do not fully explain the effects on disease risk. According to researchers, single nucleotide polymorphisms (SNPs) on multiple genes could affect the severity of COVID-19. The progression of viral diseases depends on the characteristics of the patient's innate immunity. The effectiveness of the innate immune system depends on the patient's genetic factors, including SNPs in the *TLR*, *CCR5*, and *RIG-I* genes.

In this study, we researched the association of allele and genotype frequency in SNPs of COVID-19 patients with age and gender.

Materials and Methods: In our study, 200 patients with moderate COVID-19 were included. Single nucleotide polymorphisms (SNP) of *TLR3* (rs3775291, rs3775290, rs5743305), *TLR7* (rs179008), *TLR8* (rs3764880), *RIG-I* (rs12006123), and *CCR5* (rs1799987) were studied. SNPs were determined by restriction fragment length polymerase chain reaction (RFLP-PCR) methods.

Results: In the COVID-19 patients, we examined the patients were evaluated in terms of allele and genotype frequencies and the association between some parameters like age, and gender. In our results, *TLR3* rs5743305 AA genotype frequency (p=0.03) and *TLR7* rs179008 AA genotype frequency (p=0.03) were found to be significant in terms of age and gender.

Conclusions: These SNP data is assessed against disease risk to plan personalized pharmacological therapy for COVID-19 patients. The findings from this study will be useful for genome-wide association studies (GWAS).

Keywords: COVID-19, SNP, RFLP, innate immunity.

ÖZ

Amaç: *Coronavirüs hastalığı 2019 (COVID-19), şiddetli akut solunum sendromu 2 virüsünün (SARS-CoV-2) neden olduğu ve küresel olarak yayıldığı bir hastalıktır.*

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Cinsiyet ve yaş, ciddi COVİD-19 için risk faktörleri olarak belirlenmiştir. Ancak bu faktörler hastalık riski üzerindeki etkileri tam olarak açıklamamaktadır. Araştırmacılara göre, birden fazla gendeki tek nükleotid polimorfizmleri (SNP'ler), COVID-19'un şiddetini etkileyebilir. Viral hastalıkların ilerlemesi hastanın doğuştan gelen bağışıklığının özelliklerine bağlıdır. Doğuştan gelen bağışıklık sisteminin etkinliği, TLR, CCR5 ve RIG-I genlerindeki SNP'ler dahil olmak üzere hastanın genetik faktörlerine bağlıdır. Bu çalışmada, COVID-19 hastalarının SNP'lerindeki alel ve genotip sıklığının yaş ve cinsiyet ile ilişkisini araştırdık.

Gereç ve Yöntem: *Çalışmamıza orta şiddette COVİD-19 hastası 200 hasta dahil edildi. TLR3 (rs3775291, rs3775290, rs5743305), TLR7 (rs179008), TLR8 (rs3764880), RIG-I (rs12006123) ve CCR5'in (rs1799987) tek nükleotid polimorfizmleri (SNP) incelenmiştir. SNP'ler, kısıtlama fragmanı uzunluğu polimeraz zincir reaksiyonu (RFLP-PCR) yöntemleriyle belirlendi.*

Bulgular: *COVID-19 hastalarında allel ve genotip frekansları ile yaş, cinsiyet gibi bazı parametreler arasındaki ilişki açısından değerlendirilen hastaları inceledik. Sonuçlarımızda TLR3 rs5743305 AA genotip frekansı (p=0,03) ve TLR7 rs179008 AA genotip frekansı (p=0,03) yaş ve cinsiyet açısından anlamlı bulundu.*

Sonuç: *Bu SNP verileri, COVID-19 hastaları için kişiselleştirilmiş farmakolojik tedaviyi planlamak amacıyla hastalık riskine karşı değerlendirilebilir. Bu çalışmadan elde edilecek bulgular genom çapında ilişkilendirme çalışmaları (GWAS) için faydalı olacaktır.*

Anahtar Sözcükler: *COVID-19, SNP, RFLP, doğuştan bağışıklık.*

INTRODUCTION

Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) has progressed in different manifestations in humans, caused Coronavirus Disease 2019 (COVID-19) in December 2019. The disease has varied from asymptomatic infection to serious infection and even death. These symptoms are caused by various reasons. Viral parameters and host factors such as people's health status, age, gender, smoking, immunological status, diabetes, hypertension, cardiovascular disease, chronic respiratory disease, cancer, and genetic factors affected clinical symptoms and infection outcomes (1).

The induction of T cell responses and the reprogramming of innate immune cells play a pivotal role in shaping the immune system's response to COVID-19. These influences can have various effects, including suppression, promotion, or alteration of the clinical characteristics of the disease. The innate immune system, as the first line of defense, triggers potent antiviral responses upon detecting invading viruses, ultimately leading to the activation of adaptive immunity. This antiviral response is initiated when pathogen-associated molecular patterns (PAMPs) present in viral proteins and nucleic acids activate host-pathogen recognition receptors (PRRs). SARS-CoV-2, as an enveloped virus with a single-stranded, positive-sense RNA genome (+ssRNA), possesses one of the largest RNA virus genomes (26-32 kb). Within host cells, multiple signaling networks have evolved to detect and respond to viral infections. This paper also attempts to elucidate the role of this virus in activating PRRs, with Toll-like receptors (TLRs) being one class of PRRs involved in recognizing the virus, along with retinoic acid-inducible gene I (RIG-I).The activation of these receptors triggers signaling cascades that lead to the expression of various cytokines and chemokines, which are critical in shaping the immune response to COVID-19 (3).

TLRs serve as a crucial link between the innate and adaptive immune systems. Among them, endosomal TLRs are considered potential entry points for SARS-CoV-2 (4, 5). TLR3, TLR7, and TLR8 are transmembrane proteins found on the surface of endosomes. TLR3 specifically detects retroviral double-stranded (ds)RNA, triggering the production of inflammatory cytokines and type I interferons (IFNs) with antiviral properties. On the other hand, TLR7 and TLR8 are responsible for recognizing single-stranded RNA (ssRNA) (6,7). In addition to TLRs, the innate immune system also employs other pattern recognition receptors (PRRs), such as RIG-I-like receptors. RIG-I, for instance, recognizes dsRNA and, when activated, induces the expression of interferon genes upon sensing viral RNA in the cytosol (8). Moreover, the gene C-C chemokine receptor 5 (CCR5) has been associated with the severity of COVID-19. CCR5 plays an indispensable role in both innate and adaptive immune responses (9).

Numerous single nucleotide polymorphisms (SNPs) located within pattern recognition receptor (PRR) genes have been linked to different levels of susceptibility to infectious, inflammatory, and allergic diseases. For many PRRs, the exact molecular mechanisms through which these SNPs impact receptor functions remain unclear. Genome-wide association studies (GWAS) have piqued the interest of researchers, offering a promising avenue for unraveling the connections between SNPs and disease risk in the context of COVID-19 infection (10).

We aimed to evaluate the relationship between *TLR3* (rs5743305, rs3775290, rs3775291), *TLR7* (rs179008), *TLR8* (rs3764880), *RIG-I* (rs12006123) *CCR5* (rs1799987) polymorphisms and the age and sex of the patients during COVID-19 infection. We believe that our results will contribute to GWAS studies.

MATERIALS and METHODS

Study Population

This prospective study involved patients who sought treatment at Tepecik Training and Research Hospital (TEAH) for COVID-19 infection between March 1, 2021, and February 1, 2022. The study received ethical approval from the Ethics Committee of Izmir Health Sciences University TEAH on 08/02/2021 (Approval No: 2021/02-03), and informed consent was obtained from all participants. The study included 200 hospitalized patients who had been confirmed as COVID-19 positive through quantitative real-time polymerase chain reaction (qRT-PCR) testing conducted in the microbiology laboratory at TEAH. It's worth noting that the patients in the study group had not received vaccination at the time of blood sample collection. All patients in our study group exhibited symptoms ranging from moderate to severe infection. Since it is uncertain whether individuals can contract COVID-19 more than once, we determined allele frequencies by referencing the NCBI SNP database, which compiles data from previous studies, and compared them with our results.

DNA Isolation

Genomic DNA was extracted from peripheral blood (200 µl) using standard protocols with a DNA Blood isolation Kit (GeneAll, South Korea) and following the manufacturer's instructions. The purity of DNA samples was measured using NanoDrop Spectrophotometer (Thermo Scientific, USA). The purity of the DNA samples was between 1.80 and 1.90 and their concentrations were above 30 ng/µL.

Detection of *TLR3, TLR7, TLR8, CCR5*, and *RIG-I* Genotypes by PCR-RFLP

The *TLR3* (rs5743305, rs3775290, rs3775291), *TLR7* (rs179008), *TLR8* (rs3764880), *CCR5* (rs1799987) and *RIG-I* (rs12006123) genetic variants were characterized by Polymerase Chain Reaction (PCR) followed by RFLP detection. The sequences of primers and the restriction enzymes used are presented in the supplementary (Table-1). All primers were synthesized by Oligomer Biotechnology (Oligomer Biotechnology, Turkey). The amplification protocol is presented in the supplementary (Table-2). The amplified samples were electrophoresed on 1.5% agarose gel stained with 0.01% of ethidium bromide and they were visualized under an ultraviolet transilluminator. PCR products were incubated with restriction enzymes overnight at 37°C. Restriction products were separated using 3% agarose gel electrophoresis and the fragments were visualized and stained with ethidium bromide (Figure-1).

Statistical Analyses

Statistical analyses of the data were performed using the Statistical Package for the Social Sciences (SPSS) version 25.0. The Mann-Whitney U test for continuous variables and Pearson's 2 test and Fisher's exact test for categorical variables were used to compare the distributions of demographical features between the groups. Statistical significance of the differences in genotypes and alleles of polymorphism of genes were calculated using a chi-squared test (χ^2). All p values <0.05 were regarded as statistically significant.

Table-1. Primers and restriction enzyme used in the RFLP genotyping of each polymorphism.

Gene	SNP (rs number)	Primers (5' to 3')	Restriction enzyme
TLR3	rs5743305	F: CCCAATGGATAGATGTGAGGGACAGTCA R: CTCTTTGTGGGCTCCAGCTTCAGCGAG	Hinf
	rs3775290	F:TCACTTGCTCATTCTCCCTTA R:GGACCAAGGCAAAGGAGTTC	TaqI
	rs3775291	F:TCACTTGCTCATTCTCCCTTA R:GGACCAAGGCAAAGGAGTTC	HpyF3I
TLR7	rs179008	F:CTTCTACCCTCTCGAAAGC R: TAGGAAACCATCTAGCCCC	XapI (ApoI)
TLR8	rs3764880	F: GTGTGTGTCTGATTTGGGTTG R: TTTCTAGGCTCACACCATTTG	Hin1 II (NlaIII)
RIG-I	rs12006123	F: TTGCTGATGCTTCAAAGAGCTTAGTCCG R: AAGTTTCTCTGGCTCCCCGCCTGC	Cfr42I (SacII)
CCR5	rs1799987	F: TTGTTTCCGTTTACAGAGAACAATAA R: GCGAAAAGAATCAGAGAACAGTT	SduI (Bsp1286I)

SNP, single nucleotide polymorphism; RFLP, restriction fragment length polymorphism; F, forward primer; R, reverse primer; bp, base pairs; underlined base indicates a deliberate mismatch to incorporate a restriction enzyme site.

Table-2. The run protocols of the genes.

a) *TLR3* (rs5743305) amplification protocol.

Initiation denaturation	94 ^{0}C	1	5 min
Denaturation Annealing	94 ^{0}C		45 sec
Extension	64 ^{0}C	30	45 sec
	72 ^{0}C		45 sec
Final Extension	72 ^{0}C	1	5 min

b) *TLR3* (rs3775291, rs3775290) *CCR5* (rs1799987), *TLR7* (rs179008), *TLR8* (rs3764880) amplification protocol

Initiation denaturation	94 ^{0}C	1	5 min
Denaturation Annealing	94 ^{0}C		45 sec
Extension	61 ^{0}C	30	45 sec
	72 ^{0}C		45 sec
Final Extension	72 ^{0}C	1	5 min

c) *RIG I* (rs12006123) amplification protocol

Initiation denaturation	94 ^{0}C	1	5 min
Denaturation Annealing	94 ^{0}C		45 sec
Extension	65 ^{0}C	35	45 sec
	72 ^{0}C		45 sec
Final Extension	72 ^{0}C	1	5 min

RESULTS

The study population included 200 cases with mild infection COVID-19 symptoms, of which 99 were male and 101 were female. The study population was divided into three age groups: young (18–30), medium (31–49), and elderly (50–69). Three participant groups consisted of 24.5% of the young (n=49), 54% of the middle-aged (n=108), and 21.5% of older people (n=43). *TLR3* (rs5743305, rs3775290, rs3775291), *TLR7* (rs179008), *TLR8* (rs3764880), *CCR5* (rs1799987) and *RIG-I* (rs12006123) genotype and allele frequencies and their effects on COVID-19 patients were investigated (Table-3). These analyzes were evaluated according to the age and gender of the patients (Table-4).

In the context of age and gender, the investigated SNPs TLR3 (rs5743305) and TLR7 (rs179008) exhibited statistically significant AA genotypes among COVID-19 patients. Notably, the older male COVID-19 patient group displayed a higher prevalence of these genotypes compared to other groups (Table-4) (Figure-1).

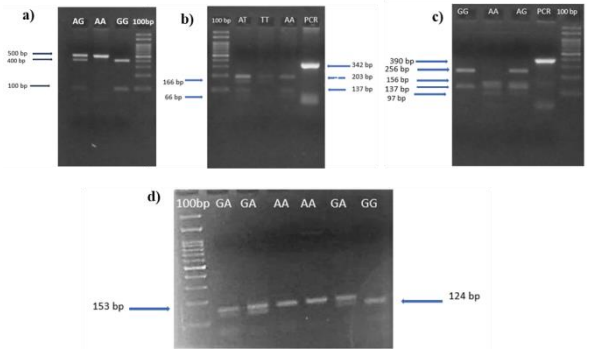

Figure-1. Gel electrophoresis, showing PCR-RFLP results after digestion with the restriction enzyme.

a)CCR5 rs1799987 (Line1:AG, Line2:AA, Line3:GG, Line4:Marker 100bp), b)TLR7 rs179008 (Line1:Marker 100bp, Line2:AT, Line3:TT, Line4: AA, Line5: PCR product), c)TLR 8 rs3764880 (Line1:GG, Line2:AA, Line3:AG, Line4: PCR product, Line5: Marker100bp), d) RIG rs12006123 (Line1:Marker 100bp, Line2:GA, Line3:GA, Line4: AA, Line5: AA, Line6: GA, Line7:GG)

Table-3. Genotype and allele frequencies of *TLR3, TLR7, TLR8, CCR5* and *RIG-I* SNPs in COVID-19 patients.

Gene/SNP	Genotype / Allele	Genotype / Allele Frequencies N (%)	Control Genotype / Allele Frequencies* (%)
TLR3 **rs5743305**	TT	69 (34.5)	-
	TA	92 (46)	-
	AA	39 (19.5)	-
	A	170 (42.5)	36.206
	T	230 (57.5)	63.794
TLR3 **rs3775290**	CC	114 (57)	-
	CT	68 (34)	-
	TT	18 (9)	-
	C	296 (74)	70.8094
	T	104 (26)	74.913**
TLR3 **rs3775291**	CC	115 (57.5)	-
	CT	64 (32)	-
	TT	21 (10.5)	-
	C	251 (62.75)	71.6526
	T	149 (37.25)	28.3474
TLR7 **rs179008**	AA	154 (77)	-
	AT	19 (9.5)	-
	TT	27(13.5)	-
	A	327 (81.75)	80.022
	T	73 (18.25)	19.978
TLR8 **rs3764880**	AA	91 (45.5)	-
	AG	60 (30)	-
	GG	49 (24.5)	-
	A	245 (61.25)	70.6278
	G	155 (38.75)	293722
CCR5 **rs1799987**	AA	44 (22)	-
	AG	94 (47)	-
	GG	62 (31)	-
	A	182 (45.5)	54.227
	G	218 (54.5)	45.773
RIG-I **rs12006123**	GG	38 (19)	-
	GA	150 (75)	-
	AA	12 (6)	-
	A	174 (43.5)	20.0594
	G	226 (56.5)	79.9406

*These data were obtained from the National Center for Biotechnology Information.

**The frequency of C>T substitution.

Table-4. Association of SNPs with susceptibility to COVID-19 and phenotype.

***TLR3* rs5743305**	**AA**	**F**	**M**	***p***	**TA**	**F**	**M**	***p***	**TT**	**F**	**M**	***p***
	Y	10.25%	10.25%		**Y**	9.78%	15.21%		**Y**	15.94%	8.69%	
	MA	38.46%	20.51%	**0.03**	**MA**	21.73%	28.26%	>0.05	**MA**	33.33%	24.63%	>0.05
	A	2.56%	17.49%		**A**	15.21%	9.78%		**A**	5.79%	11.59%	
***TLR3* rs3775290**	**CC**	**F**	**M**	***p***	**CT**	**F**	**M**	***p***	**TT**	**F**	**M**	***p***
	Y	12.28%	10.52%		**Y**	11.76%	17.64%		**Y**	15.78%	0	
	MA	31.57%	25.43%	>0.05	**MA**	26.47%	20.58%	0.52	**MA**	42.10%	15.79%	0.69
	A	4.38%	15.78%		**A**	11.76%	11.76%		**A**	10.52%	15.79%	
***TLR3* rs3775291**	**CC**	**F**	**M**	***p***	**CT**	**F**	**M**	***p***	**TT**	**F**	**M**	***p***
	Y	10.43%	13.04%		**Y**	17.18%	12.5%		**Y**	9.52%	4.76%	0.26
	MA	33.91%	22.60%	0.14	**MA**	26.56%	25%	0.17	**MA**	28.57%	19.04%	
	A	7.82%	12.17%		**A**	4.68%	14.06%		**A**	9.52%	28.57%	
***TLR7* rs179008**	**AA**	**F**	**M**	***p***	**AT**	**F**	**M**	***p***	**TT**	**F**	**M**	***p***
	Y	12.33%	12.98%		**Y**	15.78%	5.26%		**Y**	11.11%	11.11%	
	MA	29.87%	24.02%	**0.03**	**MA**	47.36%	10.52%	0.30	**MA**	25.92%	25.92%	0.25
	A	5.84%	14.93%		**A**	21.05%	0		**A**	3.70%	22.22%	
***TLR8* rs3764880**	**AA**	**F**	**M**	***p***	**AG**	**F**	**M**	***p***	**GG**	**F**	**M**	***p***
	Y	12.08%	16.48%		**Y**	13.33%	1.66%		**Y**	12.24%	16.32%	
	MA	23.07%	29.67%	0.14	**MA**	51.66%	10%	0.47	**MA**	20.40	26.53%	0.12
	A	3.29%	15.38%		**A**	16.66%	6.66%		**A**	2.04%	22.44%	
***CCR5* rs1799987**	**AA**	**F**	**M**	***p***	**AG**	**F**	**M**	***p***	**GG**	**F**	**M**	***p***
	Y	13.63%	11.36%		**Y**	15.95%	12.76%		**Y**	6.45%	11.29%	
	MA	31.81%	18.18%	0.6	**MA**	30.85%	23.40%	0.72	**MA**	30.64%	25.80%	0.25
	A	11.36%	13.63%	0	**A**	4.25%	12.76%		**A**	8.06%	17.74%	
***RIG -I* rs12006123**	**AA**	**F**	**M**	***p***	**GA**	**F**	**M**	***p***	**GG**	**F**	**M**	***p***
	Y	33.33%	0		**Y**	12%	14.66%		**Y**	7.89%	5.26%	
	MA	16.66%	41.66%	0.06	**MA**	30.66%	22.66%	0.11	**MA**	36.84%	18.42%	0.19
	A	8.33%	0		**A**	7.33%	12.66%		**A**	5.26%	26.31%	

F: Female, M: Male, Y:Young, MA:Middle-Aged, A:Adult

DISCUSSION

COVID-19 was first reported in Turkey on March 11, 2020, and it rapidly spread throughout the country. It has become apparent that distinct ethnic and genetic backgrounds can have an impact on the severity of the disease. These backgrounds also exhibit variations in single nucleotide polymorphisms (SNPs). Thus in the present study, we aimed to investigate the frequencies of several innate immune response gene polymorphisms and whether they were associated with the age and gender of COVID-19 severity in a Turkish population.

TLRs play a pivotal role in detecting viral particles and triggering the innate immune system (4). In previous studies, *TLR3* has been shown to play a protective function in infections caused by COVID-19 viruses such as SARS-CoV and MERS-CoV (1). The rs5743305 polymorphism is located on the promoter region of the *TLR3* gene and is thought to affect transcriptional activity. In our study, a significant difference was observed in AA genotype in gender-related age distribution. The rs5743305 polymorphism is placed in the promoter region of the *TLR3* gene and is thought to affect transcriptional activity (9). In a study conducted with enterovirus 71 in the Chinese population, no significant difference was found in terms of genotypes when the rs5743305 polymorphism was compared with 180 patients and 201 controls (11). In our study, a significant difference was observed in AA genotype in age distribution depending on gender.

The *TLR3* gene rs3775290 polymorphism is present in exon 4. substitution of C to T at this position in rs3775290 results in an amino acid

change from phenylalanine to leucine at position 459 of the protein, which changes the *TLR3* outer region and thus affects the ligand-receptor interaction. Alseoudy et al. showed that males with the TT genotype of the *TLR3* rs3775290 polymorphism may be more susceptible to COVID-19 pneumonia than females with the same genotype (12). However, according to our findings, there was no significant difference in the distribution of TT genotype according to gender. Considering the studies on different viral infections, Huang et al. both the T allele and TT genotype of *TLR3* rs3775290 were found to be statistically significant in chronic hepatitis (CHB), HBV-associated liver cirrhosis (LC), and hepatocellular carcinoma (HCC) (13). Mosaad et al. showed that *TLR3* rs3775290 heterozygous CT genotype may be a risk factor for chronic HCV infection (14). The CC genotype of *TLR3* (rs3775290) was significantly associated with dengue susceptibility among East Indian patients (15). In our study, the most common genotype frequency in COVID-19 patients was CC. It is the second most common CT genotype.

The amino acid change from leucine to phenylalanine at position 412 of the protein results from the substitution of G to A at the rs3775291 site. Barkhash et al. demonstrated a link between the presence of the G allele in *TLR3* SNP rs3775291 and susceptibility to tick-borne encephalitis virus (TBEV) in the Russian population (16). Allele T at the TLR3 rs3775291 locus has been linked to an increased risk of HBV infection, according to research (17,18). In our study, the C (62.75%) allele was found to be higher than the T (37.25%) genotype. Furthermore, a molecular insertion study of the rs3775291 variant compared with the wild-type version showed poor recognition of SARS-CoV-2 dsRNA, implying that immune protection may be compromised (19). TLR3 (rs5743305), which was significant as a result of our study, plays a role in escaping the immune system through the TNF receptor-related factor (TRAF3) gene when the COVID-19-related pathway is examined in the KEGG pathway. TRAF3 stimulates the non-canonical IKK-related kinases TBK1 and IKKε, leading to IRF3 dimerization, nuclear translocation, DNA binding, and activation of IRF-dependent antiviral genes (20). In conclusion, we hypothesized that differences in minor allele frequency rs3775291 between ethnic groups may play a role in SARS-COV-2 susceptibility related to gender and age, but no significant difference was found.

During the pandemic, it is seen that males need more intensive treatment and die at a higher rate than females. Male gender is considered a risk factor. The fact that females have a higher immunological response may explain the difference in immunomodulator expression between genders. *TLR7* and *TLR8* are PRRs encoded by the X chromosome. *TLR7* has also been associated with SARS-CoV-1 and MERS-CoV infections as a PRR (21). Variations of *TLR7* may have done damage at the start of the pandemic, therefore we aimed to detect the efficacy of SNPs of TLR. In plasmacytoid dendritic cells, *TLR7* SNP rs179008 downregulates *TLR7* protein. TLR7 SNP rs179008 reduces TLR7 protein in plasmacytoid dendritic cells by regulating mRNA translation (22). When the COVID-19-related pathway is examined in the KEGG pathway, TLR7 participates in the cytokine storm through IL-1 receptor-associated kinase (IRAK) (20). In our study, a significant difference was found when the AA, AT, and TT genotypes were evaluated according to gender. It was observed that AA genotype differed significantly in age distribution depending on gender. Alseoudy et al. in their study of COVID-19, *TLR7* rs179008 of the patient group in the study populations had the AA genotype. They reported that people with the TT genotype compared to the control groups may be associated with the disease. When they compared the different genotypes of *TLR7* rs179008 by sex, they said that men with the AA genotype may be at higher risk of contracting COVID-19 infection than women with the same genotype (12). Although there is no significant difference similar to these, the number of male patients with AA genotype was higher in our study group than in females, and the AA genotype was significantly higher in males than in other genotypes (80.8%).

The TLR8 rs3764880 variant is known to affect isoform expression and elevate protein levels in human monocytes. In a study conducted by others, it was found that in female patients, individuals with the AG genotype and G allele for TLR8 were more common among those hospitalized in the intensive care unit compared to patients who were treated in the clinical service ($p < 0.05$) (23).In your study, a significant difference was also observed when evaluating the AA, AG, and GG genotypes based on

gender. Additionally, in the age distribution based on gender, the AG genotype was more prevalent in females than in males. Furthermore, previous research has suggested that the A allele of TLR8 rs3764880 is a risk allele for the development of chronic HCV infection in both sexes (24). These findings underscore the potential impact of TLR8 rs3764880 genetic variations on the clinical outcomes of COVID-19, particularly in relation to gender and age, in your study population.

Other polymorphisms affecting *CCR5* expression, such as rs1799987 G/A, should be considered in patients with COVID-19. The *CCR5* rs1799987 A genotype is associated with increased *CCR5* expression (25). Shieh et al. showed that individuals homozygous for the rs1799987 A/A genotype had significantly higher numbers of T cells expressing *CCR5* (26). The *CCR5* rs1799987 A allele is common worldwide, ranging from 32% to 66% of populations (27). Bagci et al. determined that the *CCR5* rs1799987 A allele frequency in the Turkish population was 54.5% (28). The results of our study of the *CCR5* rs1799987 polymorphism detected a higher frequency of AG genotypes in COVID-19 patients. In addition, the frequency of the *CCR5* rs1799987 A allele in COVID-19 patients was found to be 45.5% in our study. The G allele has less promoter activity than the A allele in vitro, with individuals with the AA genotype having more *CCR5* expression on the surface of leukocytes than individuals with other genotypes. If the rs1799987 A allele binds to higher *CCR5* expression, it may have an impact on the treatment of COVID-19 patients.

Research on the RIG-I rs12006123 gene polymorphism is relatively limited in the literature. In a study on the measles virus, Clifford et al. identified the CC genotype as the most common for the RIG-I gene rs12006123 SNP (29). While there is a COVID-19 study associated with this particular SNP, your study found the GA genotype to be the most common in your population. It's noteworthy that there was no significant difference observed in terms of genotypes with respect to gender and age distribution in your study. The diversity of genetic variants in different populations and the multifaceted nature of immune responses to viral infections highlight the importance of conducting genetic studies like yours to better understand how specific genetic factors may influence the susceptibility and clinical outcomes of COVID-19 in various groups.

CONCLUSION

Determining SNP genotype and allele frequencies and evaluating their relationship with the development of the infection is crucial for tailored patient management. A better understanding of the immune genetic factors underlying the phenotypic response to infection with SARS-CoV-2 is a promising strategy for future diagnostics. To our knowledge, this is the first published study to date to explore the association between *RIG-I* and *CCR5* genes and COVID-19 in the Turkish population. However, a thorough evaluation of our data involves taking the consideration of additional clinical factors. In addition, we can get an improved understanding of how prevalent they are in this disease by investigating how polymorphic variants affect COVID-19 individuals based on gender and age distribution. Assessing the hereditary risks in the local population is required for a personalized diagnosis and forecasts of the complex infection course.

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