

The effect of ACE2 receptor, IFN- γ , and TNF- α polymorphisms on the severity and prognosis of the disease in SARS-CoV-2 infection

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Abstract

To investigate the effect of genetic variations in the angiotensin converting enzyme (ACE), interferon (IFNG) and tumor necrosis factor (TNF- α) genes on the severity of coronavirus disease (COVID-19). Between September and December 2021, 33 patients with COVID-19 were included in this prospective study. The patients were classified and compared according to disease severity: mild&moderate (n=26) vs severe&critical (n=7). These groups were evaluated to assess possible relationships with ACE, TNF- α and IFNG gene variations using univariate and multivariable analyses. The median age of the mild&moderate group was 45.5 (22–73), and that of the severe&critical group was 58 (49–80) years (p=0.014). Seventeen (65.4%) of the mild&moderate patients and 3 (42.9%) of severe&critical patients were female (p=0.393). According to results of univariate analysis, the percentage of patients with the c.418-70C>G variant of the ACE gene was significantly higher in the mild&moderate group (p=0.027). The ACE gene polymorphisms, c.2312C>T, c.3490G>A, c.3801C>T, and c.731A>G, were each only seen in separate patients with critical disease. The following variants were observed more frequently in the mild&moderate group: c.582C>T, c.3836G>A, c.511 + 66A>G, c.1488-58T>C, c.3281 + 25C>T, c.1710-90G>C, c.2193A> G, c.3387T>C for ACE; c.115-3delT for IFNG; and c.27C>T for TNF. It can be expected that patients carrying the ACE gene c.418-70C>G variant may present with a mild clinical manifestation of COVID-19. Several genetic polymorphisms may be associated with pathophysiology, as they appear to help predict COVID-19 severity and enable early identification of the patients requiring aggressive treatment.

Keywords

Coronavirus disease 2019, disease severity, gene variations, angiotensin converting enzyme, interferon, tumor necrosis factor

Introduction

Coronavirus disease 2019 (COVID-19), caused by the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), emerged in Wuhan, China, in December 2019 and became a worldwide pandemic.¹ Globally, as of 14 September 2022, there have been 607,083,820 confirmed cases of COVID-19, including 6,496,721 deaths, reported to the World Health Organization.²

COVID-19 is associated with a wide spectrum of symptoms, from common clinical manifestations such as fever, cough, and fatigue to the development of pneumonia and multi-organ dysfunction, which appear to be the leading causes of death.³ Various factors such as age,^{4,5} sex,^{6,7} comorbidities,^{8,9} immunity,^{10,11} and

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WHAT IS ALREADY KNOWN ON THIS TOPIC

- Coronavirus disease 2019 (COVID-19) is a pandemic viral disease, which is associated with a massive inflammatory response and a cytokine storm, leading to severe disease progression and organ damage.
- Several inflammatory markers, such as neutrophil and lymphocyte count, ferritin, D-dimer and C-reactive protein have been assessed to evaluate the effect of immune function on the progression of the disease.
- Some genetic polymorphisms on genes, such as Angiotensin converting enzyme (ACE), Interferon-gamma (IFN- γ), and tumor necrosis factor-alpha (TNF- α), have been shown a possible effect on the anti-viral response, inflammatory process, and disease progression.

WHAT THIS STUDY ADDS

- The studies investigating the correlation between genetic variations and the disease progression were on certain genes and their sequences, so eventually, there were inconsistencies among the results of these studies on the effect of the disease progression.
- There have been a few Turkish studies on these gene polymorphisms that have assessed the correlation between disease outcome and genetic variants.
- This study increases the evidence regarding the correlation between gene variants and disease severity, by adding novel variants through the sequencing of three primary genes associated with COVID-19, namely ACE, IFNG and TNF- α .
- In addition, genetic variations are closely linked with race and ethnicity, so sequencing of a group of patients from the genetically diverse Turkish population, definitely add novel variants into the pool of COVID-related gene variants.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE, OR POLICY

- Discovering new variants in ACE, IFNG, and TNF- α genes will be helpful to categorize patients according to their severity for appropriate treatment and develop novel potent treatments for personalized therapies on severe patients.
- Testing these novel variants in routine clinical practice will save us money, by helping us for early diagnosis, starting aggressive treatment earlier and shortening the duration in intensive care.
- Novel variants in ACE, IFNG, and TNF- α genes have encouraged us and possibly other researchers in this field to test variants in other COVID-related genes.

genetic variations^{6,7} have been associated with COVID-19 severity. Several inflammatory biomarkers have been explored to assess immune function.^{12,13} Similar to various other critical infectious diseases, COVID-19 causes a massive inflammatory response and a subsequent cytokine storm that results in severe disease progression and organ damage.¹⁴

Many genetic polymorphisms have been linked to the severity of COVID-19 and some evident relationships have been described.⁶ Angiotensin converting enzyme type 2 (ACE2) receptors are found in pulmonary alveolar cells (type II), small intestine, colon, gallbladder, testes, brain stem, esophagus, heart, and blood vessels in the basal layer of the epidermis.¹⁵ The pathogenesis of COVID-19 begins with the binding of the virus to ACE2 receptors (Figure 1).¹⁶ The immune response triggered by the entry of SARS-CoV-2 into pulmonary tissue via ACE2 receptors determines the severity of the destruction and damage to alveolar cells and the reduction of pulmonary surfactant, all of which play critical roles in the clinical course of the disease.^{16,17} This role of ACE2 receptors has made them one of the most studied proteins for gene polymorphism research in COVID-19.^{18–20} Interferons (IFNs) are specific cytokines with basic antiviral functions that are secreted in response to various inflammatory stimuli and classified into three different subgroups.²¹ IFN- γ activates monocytes and macrophages, participates in antiviral responses that produce free radicals and is associated with proinflammatory cytokines such as tumor necrosis factor-alpha (TNF- α).²² IFN- γ and TNF- α contribute to the antiviral response and the inflammatory process.^{21,22} Similar to ACE, it has also been reported that some IFNG and TNF gene variations may also influence the severity of COVID-19.^{23,24} Identification of disease-related genetic variations in the ACE, TNF- α , and IFNG genes is critical for predicting patients whose clinical condition will be severe, and therefore, may contribute to elucidation of clinical outcomes and development of new therapeutic approaches. However, in studies investigating relationships between genetic variations and the clinical course of COVID-19, only certain genes and certain sequences have been investigated^{18–20} and there are inconsistencies between the results of these studies.^{25–29} Moreover, there are a few Turkish studies on previously published gene polymorphisms that have assessed relationships between COVID-19 and genetic factors.^{26,30,31} So hopefully, this study will increase evidence regarding the relationships between gene variants and disease severity, by adding novel variants through the sequencing of three primary genes associated with COVID-19, namely ACE, IFNG, and TNF- α . Genetic variations are closely linked with race and ethnicity, so sequencing of a patient group

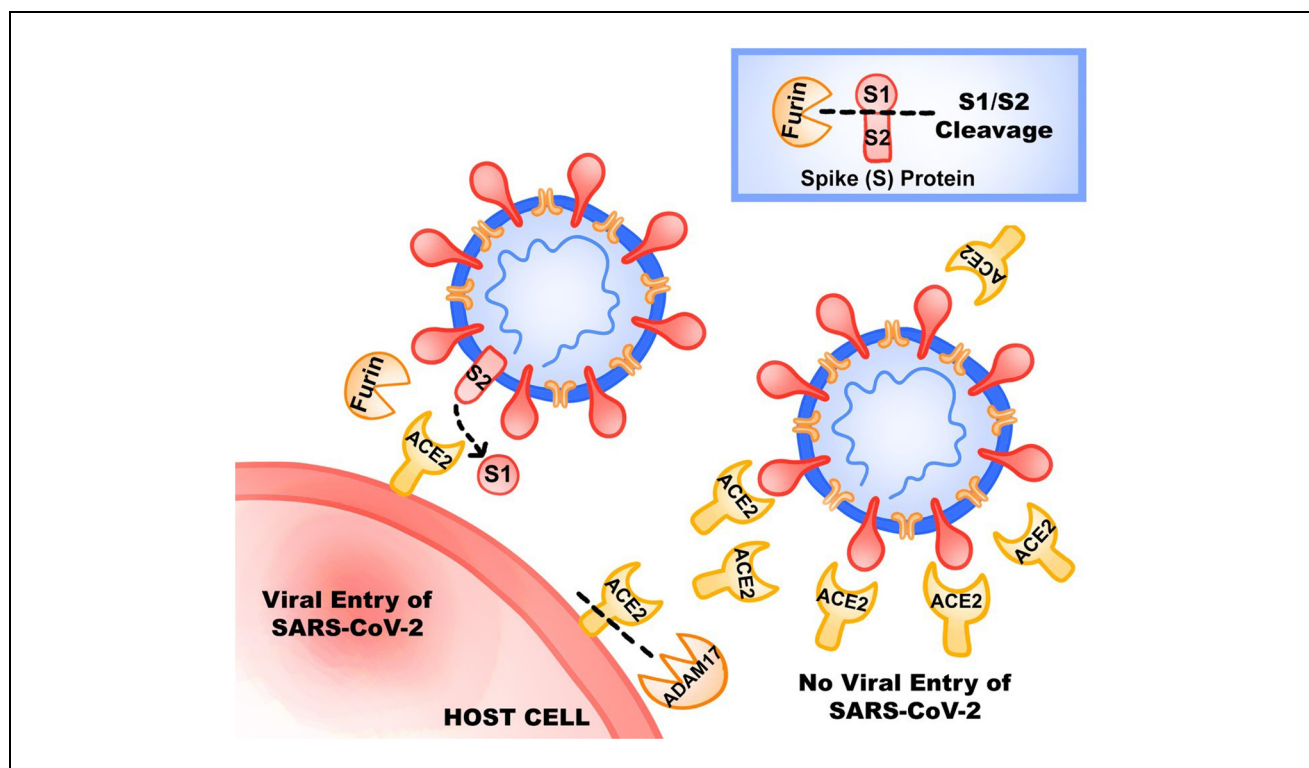


Figure 1. Mechanism of entry of SARS-CoV-2 virus into host cell by binding to ACE2 receptor.

from the genetically diverse Turkish population will definitely add novel variants into the pool of COVID-related gene variants.

As such, we aimed to investigate the angiotensin converting enzyme (ACE), interferon (IFNG), and tumor necrosis factor (TNF- α) gene variations in patients with COVID-19, and to assess their relationships with disease severity.

Patients and methods

Ethics

This prospective study was conducted after obtaining approval from the Ethics Committee of the Medical Faculty of Ufuk University (2020708/04), Ankara, Turkey. Written informed consent was obtained from all patients included in the study. The study was carried out according to the principles of the Declaration of Helsinki and its later amendments.

Participants and grouping

A total of 33 patients with a diagnosis of COVID-19 confirmed by polymerase chain reaction between September and December 2021 at the Chest Diseases Department of Ufuk University Dr. Rıdvan Ege

Hospital were included in the study. Patients younger than 18 years or older than 80 years, those vaccinated against SARS-CoV-2, pregnant or breastfeeding patients, immunosuppressive patients, and patients with malignancy were excluded from the study. We recruited patients who were not vaccinated and became ill when COVID-19 first began to spread (before the first variants appeared).

First, the patients were divided into four groups. Mild disease (group 1, $n = 17$) was defined as presence of mild clinical symptoms with or without mild signs of pneumonia on imaging. Moderate disease (group 2, $n = 9$) was defined as presence of fever, moderate respiratory symptoms with radiological signs of pneumonia. Severe disease (group 3, $n = 3$) was defined as the presence of severe clinical symptoms, shortness of breath ($RR \geq 30$ breaths/min), $SpO_2 \leq 93\%$ in ambient air, $PaO_2/FiO_2 \leq 300$ mmHg with more than 50% significant lesion progression on imaging findings (within 24–48 h). Finally, critical disease (group 4, $n = 4$) was defined as the presence of respiratory failure and/or septic shock and/or multi-organ failure requiring mechanical ventilation.³² However, due to the small number of patients in some groups for statistical comparison, two main disease severity groups were formed and compared: mild&moderate vs severe&critical.³²

Table 1. Comparison of groups with respect to analyzed parameters and genetic variations.

Variables	Patients (n = 33)		p Value
	Mild&Moderate (n = 26)	Severe&Critical (n = 7)	
Age, years	45.5 (22–73)	58 (49–80)	0.014
Sex, female	17 (65.4%)	3 (42.9%)	0.393
White blood cell count ($10^3/\mu\text{L}$)	8.09 (3.16–13.40)	10.44 (4.92–13.88)	0.123
Neutrophil count ($10^3/\mu\text{L}$)	5.36 (2.00–9.6)	6.47 (3.00–9.6)	0.252
Lymphocyte count ($10^3/\mu\text{L}$)	1.81 (0.90–4.28)	1.40 (0.70–2.71)	0.201
Neutrophil to lymphocyte ratio	2.69 (1.07–8.73)	5.71 (1.74–11.29)	0.086
Platelet count ($10^3/\mu\text{L}$)	229 (127–454)	281 (141–430)	0.291
Hemoglobin (g/dL)	13.9 (11.8–16.5)	14.9 (12.1–16.4)	0.201
Ferritin ($\mu\text{g/L}$)	193.5 (25–1289)	695 (103–3072)	0.012
D-dimer ($\mu\text{g/mL}$)	285 (140–1051)	1700 (950–2700)	<0.001
C-reactive protein (mg/L)	29.5 (5–360)	141 (102–235)	0.001
Aspartate aminotransferase (U/L)	23 (13–87)	39 (26–51)	0.046
Alanine aminotransferase (U/L)	29 (9–137)	48 (29–162)	0.117
Albumin (g/L)	36.6 (28.5–42.8)	34.95 (26–43.8)	0.739
Any comorbidity	11 (42.3%)	6 (85.7%)	0.085
Hypertension	5 (19.2%)	3 (42.9%)	0.320
Diabetes	5 (19.2%)	3 (42.9%)	0.320
Asthma	3 (11.5%)	1 (14.3%)	1.000
Obesity	3 (11.5%)	3 (42.9%)	0.093
Chronic renal failure	1 (3.8%)	0 (0%)	1.000
Atherosclerotic heart disease	1 (3.8%)	1 (14.3%)	0.384
Chronic obstructive pulmonary disease	1 (3.8%)	0 (0%)	1.000
Heart failure	2 (7.7%)	0 (0%)	1.000
Cerebrovascular disease	0 (0%)	0 (0%)	N/A
Hospitalization	11 (42.3%)	6 (85.7%)	0.085
Length of hospital stay, days	0 (0–21)	13 (0–27)	0.003
Genetic variations			
ACEc.582C>T	3 (11.5%)	1 (14.3%)	1.000
rs4298			
ACEc.2312C>T	0 (0.0%)	1 (14.3%)	0.212
rs780755664			
ACEc.3490G>A	0 (0.0%)	1 (14.3%)	0.212
rs145579007			
ACEc.3801C>T	0 (0.0%)	1 (14.3%)	0.212
rs144242912			
ACEc.3836G>A	1 (3.8%)	0 (0.0%)	1.000
rs4980			
ACEc.511 + 66A>G	21 (80.8%)	3 (42.9%)	0.068
rs4296			
ACEc.1488-58T>C	4 (15.4%)	1 (14.3%)	1.000
rs4311			
ACEc.418-70C>G	13 (50.0%)	0 (0.0%)	0.027
rs4295			
ACEc.3281 + 25C>T	2 (7.7%)	0 (0.0%)	1.000
rs4354			
ACEc.1710-90G>C	1 (3.8%)	0 (0.0%)	1.000
rs529300793			
ACE2193A>G	4 (15.4%)	0 (0.0%)	0.555
rs4331			
ACEc.3387T>C	10 (38.5%)	0 (0.0%)	0.073
rs4362			
ACEc.731A>G	0 (0.0%)	1 (14.3%)	0.212
rs3730025			
TNFc.27C>T	1 (3.8%)	0 (0.0%)	1.000
rs377627218			
IFNGrs2234686	15 (57.7%)	4 (57.1%)	1.000
c.115-3delT			

Categorical data summarized as n (%) of group, continuous data summarized with median (min–max) values.
p values in bold indicate statistical significance.

This classification was expected to provide a chance to better assess potential relationships between gene variations and disease severity.

Data collection, laboratory, and genetic analyses

Demographic data, comorbidities, and hospitalization status and duration of the patients who met inclusion criteria were recorded. Routine orders after the confirmation of COVID-19 included complete blood count (neutrophil count ($10^3/\mu\text{L}$), lymphocyte count ($10^3/\mu\text{L}$), platelet count ($10^3/\mu\text{L}$), ferritin ($\mu\text{g/L}$), D-dimer ($\mu\text{g/mL}$), C-reactive protein (mg/L), aspartate aminotransferase (U/L), and alanine aminotransferase (U/L)). All quantifications were performed in our clinical chemistry department via use of routine devices and routine methods. Complete blood count, lymphocyte count, and platelet count were measured by Sysmex XE-5000 according to manufacturer's recommendations (Sysmex Corporation, Japan). Ferritin, D-dimer, C-reactive protein, aspartate aminotransferase and alanine aminotransferase were measured by Cobas E601 according to manufacturer's recommendations (Roche Diagnostics, Switzerland).

Genomic DNA was extracted from patients using a DNA isolation kit (QIAamp DNA Blood Mini Kits, Qiagen, Hilden, Germany). The exon/exon-intron junctions of ACE, TNF- α , IFNG genes were sequenced using next generation sequencing (NGS) performed on an Illumina Miseq Sequencer (Illumina Inc., San Diego, CA, USA), following the manufacturer's instructions. The generated library was sequenced on the Illumina Miseq NGS platform to achieve a minimum reading depth of $20\times$ for $>98\%$ of the targeted bases. The hg19 (GRCh37) sequence was used as reference for identifying genetic variants. Raw data was obtained in FASTQ and VCF formats, and analyses were performed with VCF data using the NextGENe (Version 2.4.2.3/SoftGenetics LLC State College/Pennsylvania/USA) and Geneticist Assistant (Version 1.8.1.0/SoftGenetics LLC-USA). All identified variants were evaluated with respect to their pathogenicity and causality, and were categorized into classes according to the American College of Medical Genetics and Genomics guidelines.³³

Statistical analysis

The SPSS version 25 software (IBM, Armonk, NY, USA) was used for all statistical analyses which were subject to a significance threshold of $p \leq 0.05$. Normality of distribution in continuous variables was assessed with the Shapiro–Wilk test. Since the great majority conformed to non-normal distribution, all

continuous variable comparisons were performed with the non-parametric Mann–Whitney U test. The Fisher's exact test was used to compare categorical variable frequencies between groups. Continuous data were summarized with median (min–max) values, categorical data were summarized with absolute and relative frequencies (n, %).

Results

There were 26 patients in the mild&moderate group and seven patients in the severe&critical group. The mean age of all patients was 50.79 ± 12.14 years, the median age of the mild&moderate group was 45.5 (22–73), and that of the severe&critical group was 58 (49–80) ($p = 0.014$). Seventeen (65.4%) of the mild&moderate patients and 3 (42.9%) of the severe&critical patients were female ($p = 0.393$). According to univariate analysis results, median ferritin ($p = 0.012$), D-dimer ($p < 0.001$), C-reactive protein ($p = 0.001$), aspartate aminotransferase ($p = 0.046$) and length of hospital stay ($p = 0.003$) were higher in the severe&critical group compared to the mild&moderate group. The percentage of patients with the c.418-70C>G variant of the ACE gene was significantly higher in the mild&moderate group ($p = 0.027$). There was no significant difference between the two groups in terms of patient distribution with respect to other genetic variations (Table 1).

The most observed variant in all groups was the ACE gene c.511 + 66A>G variant; however, this variant was not observed in any patient with critical disease. ACE gene variants, c.2312C>T, c.3490G>A, c.3801C>T, and c.731A>G, were observed in four separate patients with critical disease. It was noted that the c.582C>T, c.3836G>A, c.511 + 66A>G, c.1488-58T>C, c.418-70C>G, c.3281 + 25C>T, c.1710-90G>C, c.2193A>G, and c.3387T>C variants of the ACE gene were mostly present in patients with mild and/or moderate clinic. Whole gene sequencing of the TNF gene was performed, but only the c.27C>T variant was observed in a single patient with mild clinical findings. The entire IFNG gene was sequenced, but only the c.115-3delT variant was observed in our population. This variant was the second most frequently observed variant and was present in all groups, but most frequently in the mild&moderate group (15/19) (Table 2).

Discussion

Clarifying the genetic and clinicodemographic factors associated with COVID-19 susceptibility, clinical severity and inter-individual variability in response to

infection are crucial in the investigation of the pathophysiology of the disease, in the prediction of patients with higher likelihood for severe clinical course, and to potentially develop new treatment strategies or administer aggressive therapy.^{5,6} For this purpose, we investigated ACE, TNF- α , and IFNG whole gene sequencing in patients grouped according to COVID-19 severity. As a result, only ACE gene c.418-70C>G variant was found to be significantly associated with COVID-19 severity. This variant was significantly higher in the mild&moderate patients. Besides, The ACE gene polymorphisms, c.2312 C>T, c.3490G>A, c.3801C>T, and c.731A>G, were each only seen in separate patients with critical disease. Although the ACE 731 A>G variation is associated with critical outcome, the presence of IFN c.115-3delT variation eased the clinical condition of the patient.

In addition, the following variants were observed more frequently in the mild&moderate group: for ACE: c.582 C>T, c.3836G>A, c.511 + 66A>G, c.1488-58T>C, c.3281 + 25C>T, c.1710-90G>C, c.2193A>G, c.3387T>C; for IFNG: c.115-3delT; and for TNF: c.27C>T variant.

The SARS-CoV-2 virus enters the host cell through the binding of its spike protein to ACE-2. Therefore, variations in the ACE-2 sequence that affect its interaction with the SARS-CoV-2 virus may be associated with susceptibility.^{34,35} The coexistence of hereditary predispositions in ACE genes or common genetic polymorphisms affecting these genes may also cause increased capillary permeability, fibrosis, coagulation and apoptosis, and accelerated lung injury in alveolar cells, all of which can influence COVID-19 disease severity.^{36,37} In the present study, the ACE gene c.418-70C>G variant was significantly more common in patients with mild&moderate COVID-19 than those with severe&critical disease. ACE gene variants, c.2312C>T, c.3490G>A, c.3801C>T, and c.731A>G, were each seen in patients with critical disease; whereas, the c.582C>T, c.3836G>A, c.511 + 66A>G, c.1488-58T>C, c.3281 + 25C>T, c.1710-90G>C, c.2193A>G, and c.3387T>C variants were mostly seen in patients with mild&moderate disease. ACE gene variants were more common compared to TNF- α and IFNG gene variants. The association between SARS-CoV-2 infection and ACE gene variants has been shown in many previous studies.^{18,19,26,38–40} The literature shows that the frequency of ACE1 Del/Del genotype,⁴¹ the ACE2 rs2074192 T allele⁴² and the ACE2 rs2285666 G/G genotype²⁵ were associated with COVID-19 severity. Möhlendich et al. found that the G/G genotype and the G-allele for ACE2 rs2285666 were associated with an increased risk of SARS-CoV-2 infection (two-fold) and a risk of mortality (three-fold),

and that the G-allele was an independent factor for severe disease.²⁵ In another study, it was shown that the ACE1 (rs4343) and ACE2 (rs2285666) genes were associated with COVID-19 susceptibility, but not with the severity of the disease.⁴³ Conversely, the presence of the ACE-2 rs2285666 variant in the Indian population¹⁸ and the presence of variants rs2074192 and rs1978124 in the Spanish population¹⁹ showed a protective effect by reducing susceptibility and mortality. However, other studies have revealed that ACE I/D, DD, ACE-2 receptor rs2106809 and rs2285666 polymorphisms were not associated with COVID-19 severity.^{20,26,38,39} These differing results in studies may be due to genetic differences between populations and/or variations in the severity and number of patients examined. In the present study, we performed ACE whole gene sequencing. Although only the c.418-70C>G polymorphism was found to be significantly associated with less-severe COVID-19, we think that the relationships between other variations that were identified in this study and the severity of COVID-19 should be investigated in future studies involving different populations.

IFN- γ plays an important role in the antiviral response and the inflammatory process. This shows that IFNs and their receptors are heavily involved in COVID-19 disease, particularly in polymorphisms of genes that affect IFN- λ (type III) expression.⁴⁴ We sequenced the whole IFNG gene, but only the c.115-3delT variant was observed as a common finding (79%) in patients with mild or moderate clinical manifestations. However, there was no significant relationship between the occurrence of this genotype and the clinical severity of COVID-19. In a study conducted by Chong and colleagues in 2006, the A allele of the IFN- γ + 874 polymorphism was associated with SARS susceptibility in the population. However, in the same study, no significant correlation was observed with the TNF- α -308 variant.⁴⁵ The effect of the IFN- γ + 874A/T polymorphism in SARS depends on its role in regulating IFN- γ production. It is possible that high IFN- γ production may impair antiviral response to SARS-CoV, making these individuals more susceptible to viral infection.⁴⁵ IFN λ 3 rs12979860 CC and rs368234815 TT variants were associated with higher efficiency in clearing RNA viruses.⁴⁶ Similar results were noted with the rs8099917, rs12980275 variants of IFN λ 3.²⁸ However, in a study on the rs12979860 variant, the CC genotype was associated with a markedly increased susceptibility to SARS-CoV-2 infection.²³ Arguably, the TC genotype was associated with higher mortality and a more severe disease course.²³ However, there seems to be an inconsistency in the effects of IFN λ 4 polymorphisms on the course of COVID-19. In addition, polymorphism at the rs368234815 TT/ Δ G locus resulted in decreased IFN λ 4

Table 2. The distribution of all positive gene variants according to disease severity.

Gene	Variant/SNP	Mild n = 50 (56.8%)	Moderate n = 25 (28.4%)	Severe n = 7 (8.0%)	Critical n = 6 (6.8%)	Total n = 88 (100%)
ACE	c.582C>T p.Asn194Asn NM_000789.4 rs4298, HET SYNONYMUS	2	1	1		4
ACE	c.2312C>T p.Thr771Met rs780755664 HET	—	—	—	1	1
ACE	c.3490G>A- p.Gly1164Arg NM_000789.4 rs145579007 HET	—	—	—	1	1
ACE	c.3801C>T p.Ile1267Ile NM_000789.4 dbSNPrs144242912	—	—	—	—	—
ACE	c.3836G>A p.Arg1279Gln NM_000789.4 dbSNPrs4980	—	1	—	—	1
ACE	c.511 + 66A>G chr17-61556527 A>G NM_000789.4 dbSNPrs4296	12	9	3	—	24
ACE	c.1488-58T>C chr17-61560763 T>C NM_000789.4 dbSNPrs4311	3	1	1	—	5
ACE	c.418-70C>G NM_000789.4 dbSNPrs4295	8	5	—	—	13
ACE	c.3281 + 25C>T NM_000789.4 dbSNPrs4354	1	1	—	—	2
ACE	c.1710-90G>C Exon 11 rs529300793 Homozygote	1	—	—	—	1
ACE	c.2193A>G p.Ala731Ala NM_000789.4 dbSNPrs4331	2	2	—	—	4
ACE	c.3387T>C p.Phe1129Phe NM_000789.4 dbSNPrs4362	8	2	—	—	10
ACE	c.731A>G p.Tyr244Cys NM_000789.4 dbSNPrs3730025	—	—	—	1	1
TNF	c.27C>T p.Asp9Asp NM_000594.3.8 dbSNPrs377627218	1	—	—	—	1
IFNG	c.115-3delT chr12-68552041 TA>T NM_000619.3 dbSNPrs2234686 rs550844533	12	3	2	2	19

Data were given as frequency (n).

expression and apparently increased the risk of acquiring SARS-CoV-2 and was suggested to cause higher viral load due to reduced viral clearance.²⁷ However, rs368234815 was discovered to have a protective function against COVID-19, as patients were reported to have higher survival rates.²⁸ The c.115-3delT variant may be associated with a mild clinical manifestation of COVID-19, but this needs to be supported by further studies in larger and/or different populations.

TNF- α is a proinflammatory cytokine produced mainly by macrophages and lymphocytes.⁴⁷ The main physiological effect of TNF- α is to recruit neutrophils and monocytes to promote the immune and inflammatory response.⁴⁸ TNF- α gene variants have been widely studied and previous findings show relationships between TNF- α gene variants and the development of autoimmune diseases, infectious diseases, and cancer.^{49,50} In the present study, TNF- α gene polymorphisms were studied in SARS-CoV-2 infection. Only the c.27C>T variant of the TNF gene was seen in one patient with mild clinical manifestations. Saleh et al. studied TNF- α polymorphism G-308, a promoter polymorphism, in 900 COVID-19 patients and found that the AA genotype of TNF- α was associated with greater severity and susceptibility to SARS-CoV-2 infection.²⁴ Galindo et al. also studied TNF (rs1800629 and rs361525), TNFRSF1A (rs767455 and rs1800693), TNFRSF1B (rs1061622 and rs3397) polymorphisms and they reported that TNF and TNFRSF1B gene polymorphisms affect the plasma levels of soluble TNFR1 and TNFR2, which are implicated in COVID-19 severity.²⁹ TNF- inhibitors may be an effective therapy for preventing disease progression in severe SARS-CoV-2 infection, and therefore, determining associations between TNF gene polymorphisms and disease severity will be helpful for the selection of candidates who may benefit from anti-TNF therapy.

The most important advantages of the study are: (i) All regions of the ACE, IFNG and TNF genes were sequenced, enabling detection of all possible variants of the relevant genes. (ii) Patients who were vaccinated were not included in the study, and therefore, the direct influence of genetic factors on disease severity could be examined. (iii) Since the number of studies conducted in genetics and COVID-19 relationship in Turkey is quite limited,^{26,30,31} we think that our findings can contribute to the demonstration of regional genetic differences on the subject.

However, some potential limitations should be considered when interpreting the results. First, the results cannot be generalized to the whole population, as it is a single-center study. Secondly, the small number of participants prevented detailed comparison of disease severity groups and may have adversely affected statistical analyses. The small number of participants is due to the fact

that sequencing all regions of these three genes poses both financial and time-related constraints. In addition, the small number of participants may have caused inconsistencies in the results of multivariable regression analysis.

In conclusion, the ACE gene c.418-70C>G variant was found to be significantly associated with mild&moderate COVID-19. Apart from this, the c.2312C>T, c.3490G>A, c.3801C>T, and c.731A>G polymorphisms of the ACE gene may be associated with critical disease; whereas the c.582C>T, c.3836G>A, c.511 + 66A>G, c.1488-58T>C, c.3281 + 25C>T, c.1710-90G>C, c.2193A> G, and c.3387T>C variants of the ACE gene may be associated with less-severe disease. Additionally, the IFNG c.115-3delT variant and the TNF c.27C>T variant may be associated with mild or moderate COVID-19 course. It can be expected that patients carrying the ACE gene c.418-70C>G variant may present a milder clinical manifestation of COVID-19. Together with prior data, it is clear that further comprehensive studies in larger and/or different groups are necessary to assess whether these gene polymorphisms can help explain the pathophysiological mechanisms of the disease.

Author contributions

Planning and design: ESG, BC, EEA

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Ethics committee approval

This prospective study was conducted after obtaining the approval of the Ethics Committee of the Medical Faculty of Ufuk University (2020708/04), Ankara, Turkey.

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