

# The relationship between the clinical course of SARS-CoV-2 infections and *ACE2* and *TMPRSS2* expression and polymorphisms

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## Conflict of interest

None declared

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## Abstract

**Background.** The viral spike (S) protein and host *ACE2* and *TMPRSS2* genetic variations may act as a barrier to viral infections or determine susceptibility to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infections.

**Objectives.** We investigated the relationship between the expression patterns and polymorphisms of the *ACE2* and *TMPRSS2* receptor genes associated with coronavirus disease 2019 (COVID-19) and the clinical course of SARS-CoV-2 infections.

**Materials and methods.** We examined 147 COVID-19 patients (41 asymptomatic, 53 symptomatic and 53 cases treated in the intensive care unit (ICU)) and 33 healthy controls. The *ACE2* and *TMPRSS2* expression was determined using the One-Run RT-qPCR kit. Genotypic distributions of single nucleotide polymorphisms (SNPs) of *ACE2* and *TMPRSS2* were obtained using reverse transcription quantitative polymerase chain reaction (RT-qPCR).

**Results.** The expressions of *ACE2* and *TMPRSS2* were different between SARS-CoV-2-positive and -negative groups. The *ACE2* rs714205GG genotype and G-allele showed significant differences in the asymptomatic SARS-CoV-2-positive group. A significant correlation was found between the expression of *TMPRSS2* rs8134378GA, rs2070788GA, rs7364083GA, and rs9974589AC genotypes and SARS-CoV-2 positivity. The rs1978124 C-allele and rs8134378 A-allele expressions were significant in the symptomatic SARS-CoV-2-positive group. The *TMPRSS2* rs2070788GA expression was different in all patient groups compared to the control group. There was a difference between SARS-CoV-2-positive and -negative groups regarding the CTTA haplotype formed by *ACE2* variants. The AGCAG and AGAAG haplotypes formed by the *TMPRSS2* variants were more common in the asymptomatic patient group than in other patient groups.

**Conclusions.** Identifying the relationship between host genetic variants and COVID-19 susceptibility will contribute to further studies, enabling new vaccines and potential therapeutic approaches to be discovered.

**Key words:** single nucleotide polymorphisms, expressions, COVID-19, *ACE2* gene, *TMPRSS2* gene

## Background

The new type of severe acute respiratory syndrome (SARS) caused by coronavirus (CoV)-2 (2019-nCoV/SARS-CoV-2) led to a life-threatening coronavirus disease 2019 (COVID-19) pandemic all over the world, resulting in multiple organ failure, immune reactions and septic shock.<sup>1,2</sup> The effect of variations on susceptibility to SARS-CoV-2 infections and the severity of symptoms in certain populations have recently been one of the most emphasized areas, and it is thought that these variations may be an important factor in determining susceptibility to infections and severity of the disease.<sup>3</sup> The entry of SARS-CoV-2 into target cells takes place through the binding of the S1 unit of the viral spike (S) protein to the angiotensin-converting enzyme 2 (ACE2) surface receptors of the target cell, then cleaving the S1-S2 unit of the S protein through the transmembrane protease serine 2 (TMPRSS2) receptor, and facilitating the entry of the virus into the cell through membrane fusion of the unit containing S2.<sup>3–5</sup> Virus receptor binding is an important first step in viral infection.<sup>5</sup> Therefore, it is thought that variations may affect the expression patterns in host *ACE2* and *TMPRSS2* receptor genes, and the viral S protein may act as a barrier for viral infection and may determine the susceptibility to COVID-19 infections, affecting the course of the disease.<sup>4,6–9</sup>

The *ACE2* gene localized on chromosome Xp22 is expressed in tissues such as the colon and lung, but is more dominant in the heart, kidney and testicles. In addition to the predominance of respiratory system symptoms during infection, the development of complications, mostly in the heart and lungs, is explained by the abundant expression of the gene on type 2 pneumocytes, especially in the lungs.<sup>7,9–11</sup> Clinical studies have shown that *ACE1/ACE2* polymorphisms are associated with a risk for cardiovascular and pulmonary diseases.<sup>8,12</sup> Therefore, the co-existence of hereditary predispositions or common gene polymorphisms affecting the expression of *ACE1/ACE2* genes may cause increased capillary permeability in alveolar cells, coagulation, fibrosis, apoptosis, acceleration of lung damage, and pulmonary failure. Thus, although it is not always a rule, SARS-CoV-2 infections can be experienced much more severely in patients with existing chronic diseases.<sup>13</sup>

The *ACE2* is a polymorphic gene in the human genome with approx. 140 single nucleotide polymorphism (SNP) loci, some of which are associated with COVID-19.<sup>5</sup> In previous studies, special attention was drawn to rs2285666 (G8790A), which is in the 3<sup>rd</sup> intron of the *ACE2* and affects the expression of the gene with alternative splicing. It has been suggested that rs1978124 at intron 1 and rs714205 SNPs at intron 16 of the gene show a strong linkage disequilibrium with rs2285666. It has been stated that the rs73635825 variant causes significant differences in intermolecular interactions between the receptor and

S protein.<sup>5,9,11</sup> Additionally, polymorphisms in the *TM-PRSS2* gene localized at 21q22.3 may have greater importance in society in terms of the spread of influenza A and coronavirus infections. In this context, it has been stated that some SNPs in the *TMPRSS2* gene have functional significance by affecting the expression of the gene in genome-wide association studies.<sup>14,15</sup> Single nucleotide polymorphisms affecting proinflammatory and anti-inflammatory cytokine levels in cytokine genes have been indicated in the development of the “cytokine storm” in severe COVID-19 infection.<sup>16,17</sup>

## Objectives

The genetic differences observed in *ACE2* and *TMPRSS2* receptors, which play a role in the attachment of the virus to host cells, are important for the susceptibility of individuals to infection, and some SNPs in the *ACE2* may affect the susceptibility to SARS-CoV-2 infections by creating a predisposition for hypertension and other cardiovascular diseases.<sup>18</sup> Therefore, in our study, we aimed to determine the expression levels of *ACE2* and *TMPRSS2* in Turkish patients with SARS-CoV-2 infection, as well as the relationship between some common SNPs in these genes and the clinical course of the COVID-19 infection.

## Materials and methods

### Subjects

Individuals presenting to our hospital between December 2020 and May 2021 due to infection or contact with individuals infected with SARS-CoV-2, and who were tested for COVID-19 using the real-time polymerase chain reaction (PCR) method from a nasal-throat swab at the Gazi University (Ankara, Turkey) were included in this study.

Our case-control study followed the principles of the Declaration of Helsinki and was approved by the Gazi University Faculty of Medicine Clinical Research Ethics Committee (approval No. 2020-611).

The power analysis was conducted using G\*Power v. 3.1.9.7 software (<https://g-power.apponic.com/>) to determine the minimum sample size required to test the study hypothesis. Results indicated that the required total sample size (power = 0.80,  $\alpha$  = 0.05, effect size = 0.25) was 180. The eta squared ( $\eta^2$ ) was used to determine the effect size.

The participant flow diagram for the study is shown in Fig. 1. The individuals included in the study were grouped as follows:

Group 1: Asymptomatic patients who were found to be positive for SARS-CoV-2 (n = 41);

Group 2: Symptomatic patients who were found to be positive for SARS-CoV-2 and did not require treatment in the intensive care unit (ICU) (n = 53);

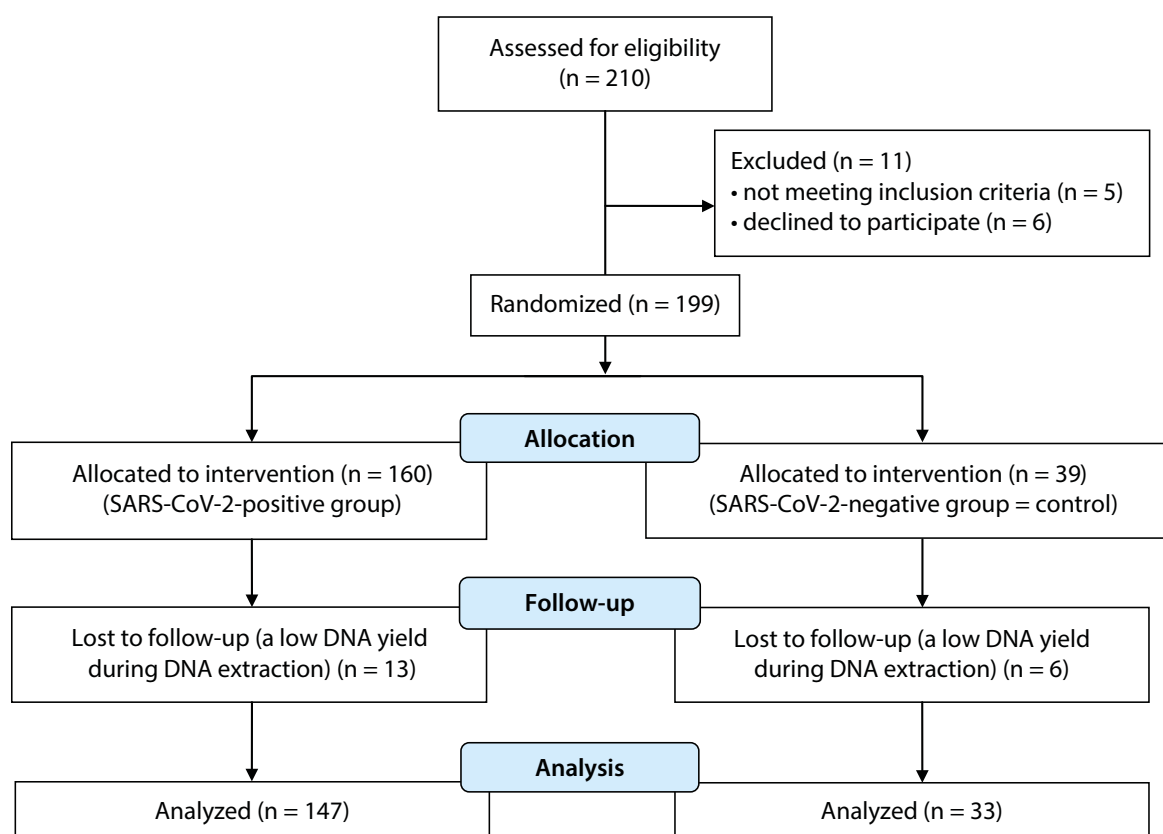


Fig. 1. CONSORT 2010 flow diagram

SARS-CoV-2 – severe acute respiratory syndrome coronavirus 2.

Group 3: Symptomatic patients who were found to be positive for SARS-CoV-2 and treated in the ICU (n = 53);

Group 4: Control group – individuals who had a history of contact with individuals determined to be SARS-CoV-2-positive, who were found to be SARS-CoV-2 negative (n = 33).

Peripheral venous blood samples of each patient who agreed to participate in the study had been stored in 4-milliliter ethylenediaminetetraacetic acid (EDTA) tubes at  $-80^{\circ}\text{C}$  until the beginning of the study.

## RNA extraction and reverse transcription quantitative PCR (RT-qPCR)

Total RNA was extracted from the peripheral venous blood samples using the NucleoSpin® RNA Blood kit (Macherey-Nagel GmbH & Co. KG, Düren, Germany), following the manufacturer's protocols. The concentration and quality of total RNA were assessed spectrophotometrically at 260 nm absorbance (NanoDrop 1000 Spectrophotometer; Thermo Fisher Scientific, Waltham, USA). We used the NCBI Primer-BLAST designing tool for primer design (<https://www.ncbi.nlm.nih.gov/tools/primer-blast/>). In primer design, care was taken to ensure that almost all primers had a similar melting temperature, and primers with prominent hairpins, homodimers or heterodimers were excluded.<sup>19</sup> The *ACE2* and *TMPRSS2* expressions were determined using the One-Run RT-qPCR kit (catalog No. 18R-01-100; SNP Biotechnology, Ankara, Turkey),

together with the specific primers for *ACE2* and *TMPRSS2* from the total RNA using the CFX96 Thermocycler (Bio-Rad, Hercules, USA). The sequences of oligonucleotides used for the RNA isolations of *ACE2* and *TMPRSS2* genes are given in Table 1. The expressions of *ACE2* and *TMPRSS2* were determined after RT-qPCR consisting of 50 cycles of 8 min at  $42^{\circ}\text{C}$ , 1 s at  $96^{\circ}\text{C}$  and 25 s at  $60^{\circ}\text{C}$  were normalized to the  $\beta$ -actin gene as a control. Each real-time PCR reaction was performed in duplicate. The gene expressions were analyzed using the Gene Study software (CFX96; Bio-Rad).

## Genomic DNA extraction and determination of SNPs

After obtaining genomic DNA from the 100  $\mu\text{L}$  of peripheral venous blood of the SARS-CoV-2-positive patient groups and control group using the DNA isolation kit (SNP Biotechnology), the genotype and allele distributions of rs714205, rs73635825, rs2285666, and rs1978124 in *ACE2*, and rs8134378, rs2070788, rs7364083, rs13052975, and rs9974589 in *TMPRSS2* were investigated using real-time PCR (CFX96; Bio-Rad), and haplotype analyzes were performed. The RT-qPCR mixture used per sample was prepared with 1.25  $\mu\text{L}$  of primer/probe, 12.5  $\mu\text{L}$  of TaqMan 2x PCR Mix, 9.375  $\mu\text{L}$  of RNase-free water, and 1.875  $\mu\text{L}$  of template DNA with a total reaction volume of 25  $\mu\text{L}$ , following the manufacturer's recommendations. While PCR amplification was performed,

**Table 1.** Sequences of oligonucleotides used in the multiplex polymerase chain reaction (PCR) assay for RNA isolation of the *ACE2* and *TMPRSS2* genes

Oligonucleotide name	Sequence
<i>ACTB</i> forward	5'-CCCAGCACAAATGAAGATCAAGATC-3'
<i>ACTB</i> reverse	5'-GGGTGTAACGCAACTAAGTCATAGTC-3'
<i>ACTB</i> molecular beacon	5'-FAM-AGATCATTGCTCCTCCTGAGCGCAAG-3'
<i>ACE2</i> forward	5'-GATCAGAGATCGGAAGAAGAAAAATAAGC-3'
<i>ACE2</i> reverse	5'-CTAAAAGGAGGTCTGAACATCATCAGTG-3'
<i>ACE2</i> molecular beacon	5'-FAM-AGAAAATCCTTATGCCTCCATCGATATTAGC-3'
<i>TMPRSS2</i> forward	5'-GAATGTGATGGTATTACGGACTG-3'
<i>TMPRSS2</i> reverse	5'-CTTGTAACACGACGTCAAGGACGAAG-3'
<i>TMPRSS2</i> molecular beacon	5'-TCGACAAATGAGGGCAGACGGCTAATC-3'

*ACTB* – human B-actin gene; *ACE2* – angiotensin-converting enzyme 2 gene; FAM – fluorescein; *TMPRSS2* – transmembrane protease serine 2 gene.

the genotypes were determined according to the high-resolution melting curve analysis by the glow of the fluorescent dye used (EvaGreen; Metabion, Martinsried, Germany). The genotyping was made according to the melting temperature ( $T_m$ ) of double-stranded DNA, which was denatured during PCR by increasing the temperature and the presence of DNA binding dye. Homozygous and heterozygous mutations cause the  $T_m$  to shift compared to a wild-type sample.<sup>19</sup>

## Statistical analyses

The statistical analysis of the data obtained at the end of the study was performed using the IBM SPSS v. 20 software (IBM Corp., Armonk, USA). Parametric variables were expressed as mean and standard deviation ( $M \pm SD$ ). The  $\eta^2$  was used to determine the effect size. To determine the differences between *ACE2* and *TMPRSS2* expression levels in the SARS-CoV-2-positive and -negative groups, we performed t-tests with Bonferroni correction (Supplementary Table 1). We also compared *ACE2* and *TMPRSS2* expression levels between the subgroups (asymptomatic patients, symptomatic patients, ICU-treated patients, and controls). As a result of the groups not being normally distributed, the Kruskal–Wallis test was used to compare the expression levels between the subgroups (Supplementary Tables 2 and 3). There were statistically significant differences between the subgroups. The homogeneity of variance was examined using Levene's test. Variances were not assumed equal; thus, a post hoc Dunn's test was used

to perform pairwise comparisons (Supplementary Table 4). The Hardy–Weinberg balance for the distributions of genotypes was calculated using the  $\chi^2$  test. A p-value <0.05 was considered statistically significant. The odds ratio (OR) and corresponding 95% confidence interval (95% CI) values were analyzed using multiple logistic regression tests in order to estimate the association of genotypes, allele frequencies and other variables with the occurrence and severity of COVID-19. Moreover, the correlation between COVID-19 and risk factors such as comorbidities, age, gender, *ACE2*, and *TMPRSS2* expressions was analyzed with a multinomial logistic regression model (Supplementary Table 5).

## Results

The demographic data of study groups are shown in Table 2. The multinomial logistic regression results of independent variables affecting COVID-19 severity are presented in Supplementary Table 6.

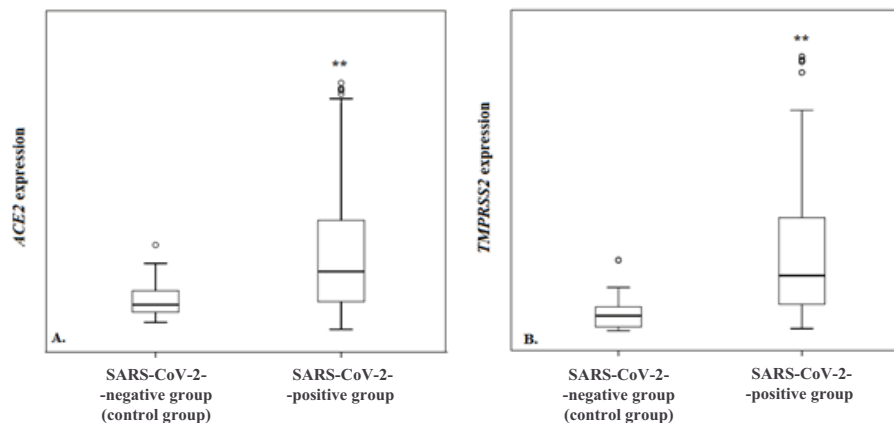
### *ACE2* and *TMPRSS2* expression

The *ACE2* expression was determined as  $1.34 \pm 0.14$  ( $M \pm SD$ ) in the control group and  $21.58 \pm 4.12$  in the SARS-CoV-2-positive group, with a statistical difference between the groups ( $p = 0.001$ ). The *TMPRSS2* expression was determined as  $1.20 \pm 0.15$  and  $132 \pm 41.61$  in the SARS-CoV-2-positive and -negative groups, respectively, and

**Table 2.** Demographic data of the study groups

Demographic and comorbidity data		Controls (n = 33)	Asymptomatic patients (n = 41)	Symptomatic patients (n = 53)	ICU-treated patients (n = 53)
Gender, n (%)	female	16 (48.5)	20 (48.8)	28 (52.8)	26 (49)
	male	17 (51.5)	21 (51.2)	25 (47.2)	27 (51)
Age ( $M \pm SD$ )		41.72 $\pm$ 8.16	42.65 $\pm$ 10.91	42.52 $\pm$ 9.66	67.15 $\pm$ 15.35
Comorbid disease*, n (%)		–	3 (7.3)	7 (13.2)	45 (84.9)

\* comorbid diseases: hypertension, cardiovascular diseases, diabetes mellitus, chronic lung diseases, kidney diseases, liver diseases, and malignancies; ICU – intensive care unit;  $M \pm SD$  – mean  $\pm$  standard deviation.

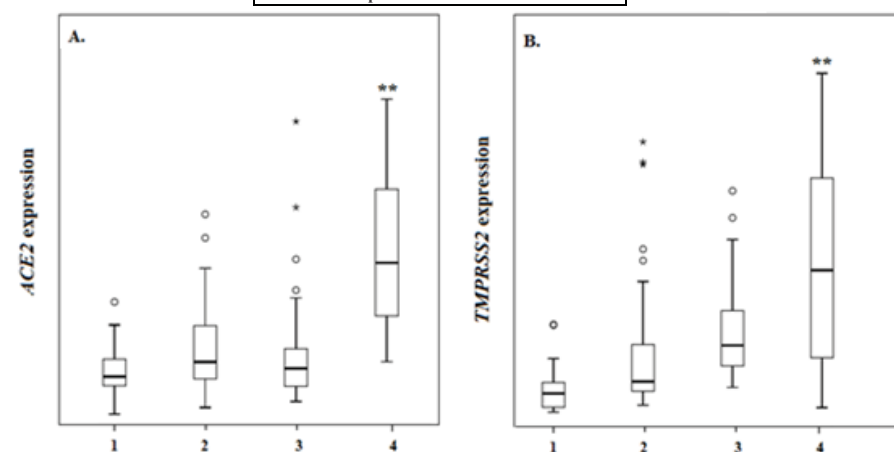


**Fig. 2.** *ACE2* (A) and *TMPRSS2* (B) expression levels in the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)-positive patient group and the control group (\*\* $p < 0.05$ ); t-test ( $t = 4.899$  for the *ACE2* and  $3.165$  for the *TMPRSS2*)

<i>ACE2</i>	Control group	SARS
Maximum	6.26	260.52
Q3	1.69	11.53
Median	0.94	3.05
Q1	0.62	1.07
Minimum	0.31	0.12

<i>TMPRSS2</i>	Control group	SARS
Maximum	7.64	3460.97
Q3	1.19	25.73
Median	0.67	4.24
Q1	0.19	1.18
Minimum	0.07	0.13

1. Control group patients with COVID-19
2. Asymptomatic patients with COVID-19
3. Symptomatic patients with COVID-19
4. COVID-19 patients treated in the ICU



**Fig. 3.** *ACE2* (A) and *TMPRSS2* (B) expression levels in study groups (\*\* $p < 0.05$ ); Kruskal–Wallis test (degrees of freedom (df) = 3 for the *ACE2* and *TMPRSS2*)

ICU – intensive care unit; COVID-19 – coronavirus disease 2019.

<i>ACE2</i>	Group 1	Group 2	Group 3	Group 4
Maximum	6.26	118.59	175.20	260.52
Q3	1.69	5.47	3.06	60.63
Median	0.94	1.39	1.26	11.54
Q1	0.62	0.90	0.77	4.15
Minimum	0.31	0.12	0.25	1.52

<i>TMPRSS2</i>	Group 1	Group 2	Group 3	Group 4
Maximum	7.64	727.90	207.35	3460.97
Q3	1.19	11.40	12.67	141.17
Median	0.67	1.22	4.34	5.65
Q1	0.19	0.77	2.44	0.68
Minimum	0.07	0.13	0.93	0.19

a significant difference was found between the 2 groups ( $p = 0.002$ ; Fig. 2).

The *ACE2* and *TMPRSS2* expressions were higher in the ICU-treated patient group compared to the control group ( $p = 0.001$ ). Although *ACE2* and *TMPRSS2* expressions were higher in the asymptomatic and symptomatic patient groups compared to the control group, a significant difference was only observed between the symptomatic patient group and the control group ( $p = 0.013$  and  $p = 0.041$ , respectively). The *ACE2* and *TMPRSS2* expressions were also higher in the ICU-treated patient group compared to the other patient groups ( $p = 0.001$  and  $p = 0.001$  for the *ACE2* gene, respectively;  $p = 0.020$  and  $p = 0.002$  for

the *TMPRSS2* gene, respectively). There was no difference between the asymptomatic and symptomatic patient groups in terms of *ACE2* and *TMPRSS2* expression ( $p = 0.456$  and  $p = 0.953$ , respectively; Fig. 3).

In the study groups, *ACE2* and *TMPRSS2* expressions did not differ according to gender ( $p > 0.05$ ). There was no significant difference in terms of clinical severity of the disease according to gender among the patient groups ( $p = 0.956$  and  $p = 0.458$  respectively).

The mean age was higher in the SARS-CoV-2-positive patient group who were treated in the ICU compared to the other patient and control groups ( $p = 0.001$ ). Also, there was a significant difference between the clinical



course of infection and age in the SARS-CoV-2-positive patient groups ( $p = 0.001$ ). To determine the relationship between patient age and *ACE2* and *TMPRSS2* expressions, the patients were divided into 3 different age groups: 20–40 years, 40–60 years and over 60 years of age. The *ACE2* expression in the over 60 years of age patient group was higher than in the 2 other age groups ( $p = 0.004$  and  $p = 0.039$ , respectively). The *TMPRSS2* expression was higher in patients over 60 years of age compared to patients aged 20–40 years ( $p = 0.049$ ), but not different from patients aged 40–60 years ( $p = 0.415$ ).

The presence of comorbid diseases was more common in those treated in the ICU than in the other patient groups ( $p = 0.001$ ). It was determined that *ACE2* and *TMPRSS2* expression levels increased in the presence of comorbid diseases in the SARS-CoV-2-positive patient group ( $p = 0.001$  and  $p = 0.02$ , respectively). There was no difference between *ACE2* and *TMPRSS2* expressions and the presence of comorbid diseases in the asymptomatic and symptomatic patient groups ( $p = 0.795$  and  $p = 0.311$  for the *ACE2* gene, respectively;  $p = 0.469$  and  $p = 0.302$  for the *TMPRSS2* gene, respectively). Higher *ACE2* and *TMPRSS2* expression levels were detected in the presence of comorbid diseases in the ICU-treated patient group ( $p = 0.019$  and  $p = 0.018$ , respectively).

### *ACE2* and *TMPRSS2* SNPs

The sum of the genotypes obtained for each of the *ACE2* and *TMPRSS2* SNPs in our study groups was equal to 1, and the genotype and allele distributions were in the Hardy–Weinberg equilibrium. The genotype and allele distributions of *ACE2* SNPs were similar in the SARS-CoV-2-positive and -negative groups ( $p > 0.05$ ;  $p$ -values are given in Table 3). When the genotype and allele distributions of *TMPRSS2* polymorphisms were examined, the expressions of rs2070788GA, rs7364083GA and rs9974589AC genotypes were higher in the SARS-CoV-2-positive group ( $p$ -values = 0.001, 0.036 and 0.024, respectively) compared to the control group (Table 3).

Although the rs714205GG genotype was more common in asymptomatic, symptomatic and ICU-treated patients than in the control group, a statistical difference was observed only in the asymptomatic patient group ( $p = 0.049$ ). Similarly, the expression of rs714205 G-allele was found to be higher in the asymptomatic patient group ( $p = 0.032$ ). In the symptomatic patient group, the expressions of rs1978124 C-allele, rs8134378GA genotype and A-allele were statistically different compared to the other patient and control groups ( $p = 0.032$ , 0.014 and 0.006, respectively). The expression of rs2070788GA genotype was different in all groups compared to the control group ( $p = 0.039$ , 0.001 and 0.001, respectively). The expressions of rs7364083GA and rs9974589AC genotypes were statistically different in the symptomatic patient group compared to the other patient and control groups ( $p = 0.003$  and 0.005, respectively; Table 4).

No significant relationship was found between SNPs investigated in our study and *ACE2* and *TMPRSS2* expression levels ( $p > 0.05$ ).

The multinomial logistic regression results of independent variables affecting genotypic distribution are shown in Supplementary Table 7.

### *ACE2* and *TMPRSS2* haplotype frequencies

Based on the CCTA haplotype formed by the wild-type alleles of *ACE2* variants, 9 and 8 haplotypes with frequencies above 5% were detected in the patient and control groups, respectively. The CCTA haplotype was the highest in the SARS-CoV-2-positive patient group, and the CCTA and CCCA haplotypes were similar in the control group. While the CTTA haplotype showed a statistical difference between the SARS-CoV-2-positive patient group and the control group ( $p = 0.02$ ), there was no difference between the SARS-CoV-2-positive patient groups in terms of *ACE2* haplotype frequencies ( $p > 0.05$ ;  $p$ -values are given in Table 5). Thirty haplotypes were identified in the SARS-CoV-2-positive patient groups with *TMPRSS2* variants, 16 haplotypes were identified in the control group, and GGAGG consisting of wild-type alleles was taken as the reference haplotype. There was no statistical difference between the SARS-CoV-2-positive patient and control groups in terms of haplotype distributions ( $p > 0.05$ ;  $p$ -values are given in Table 6).

In the SARS-CoV-2-positive asymptomatic patient group, AGCAG and AGAAG haplotypes had a higher frequency than those in the other patient groups (symptomatic and ICU-treated,  $p = 0.03$  and  $p = 0.01$ , respectively).

## Discussion

Variations in the nucleotide sequences of the 2 host genes, *ACE2* and *TMPRSS2*, indispensable in the introduction of coronavirus into host cells, may alter the expression and functionality of these proteins.<sup>20</sup> Although recent studies have attempted to associate these variants with susceptibility to SARS-CoV-2 infections,<sup>5,21,22</sup> there is not yet sufficient evidence that rare variants in *ACE2* can modulate susceptibility to SARS-CoV-2 infections. However, *TMPRSS2*, which plays a role in the proteolytic cleavage of the SARS-CoV-2 S proteins and thus facilitates the entry of the virus into the host cell, contains many variants of different frequencies among human populations.<sup>20</sup> Therefore, the relationship between the risk and susceptibility of SARS-CoV-2 infections and different polymorphisms of *ACE2* and *TMPRSS2* and expression levels was investigated in COVID-19 patients and a control group. According to our results, *ACE2* and *TMPRSS2* expressions were significantly increased in the SARS-CoV-2-positive patient group compared to the control group, and the expressions of the genes were higher in the ICU-treated

**Table 3.** Genotype and allele distribution of *ACE2* and *TMPRSS2* polymorphisms in severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)-positive and -negative groups

dbSNP ID			SARS-CoV-2-negative* (n = 33)	SARS-CoV-2-positive (n = 147)	OR	95% CI	df	p-value
rs714205	genotype	CC	24	98	1	reference	–	–
		CG	6	18	0.735	0.263–2.050	1	0.556
		GG	3	31	2.531	0.713–8.978	1	0.151
	allele	C	54	214	1	reference	–	–
		G	12	80	1.714	0.872–3.372	1	0.118
rs73635825	genotype	AA	32	146	1	reference	–	–
		AG	1	1	0.219	0.013–3.597	1	0.288
		GG	0	0	–	–	–	–
	allele	A	65	293	1	reference	–	–
		G	1	1	0.221	0.014–3.581	1	0.288
rs2285666	genotype	CC	22	98	1	reference	–	–
		CT	5	17	0.763	0.254–2.291	1	0.630
		TT	6	32	1.197	0.446–3.212	1	0.721
	allele	C	49	213	1	reference	–	–
		T	17	81	1.096	0.597–2.014	1	0.767
rs1978124	genotype	TT	13	74	1	reference	–	–
		CT	8	28	0.615	0.230–1.642	1	0.332
		CC	12	45	0.659	0.277–1.569	1	0.346
	allele	T	34	176	1	reference	–	–
		C	32	118	0.712	0.417–1.218	1	0.215
rs8134378	genotype	GG	29	107	1	reference	–	–
		GA	4	37	2.507	0.826–7.609	1	0.105
		AA	0	3	–	–	–	–
	allele	G	62	251	1	reference	–	–
		A	4	43	2.655	0.919–7.677	1	0.071
rs2070788	genotype	GG	11	32	1	reference	–	–
		GA	4	72	7.535	2.39–23.734	1	<b>0.001</b>
		AA	18	43	1.218	0.506–2.932	1	0.660
	allele	G	26	136	1	reference	–	–
		A	40	158	1.324	0.768–2.282	1	0.312
rs7364083	genotype	GG	6	22	1	reference	–	–
		GA	12	83	2.470	0.106–5.750	1	<b>0.036</b>
		AA	15	42	1.310	0.446–3.849	1	0.624
	allele	G	24	127	1	reference	–	–
		A	42	167	1.320	0.760–2.294	1	0.324
rs13052975	genotype	GG	23	98	1	reference	–	–
		GA	8	45	1.320	0.548–3.178	1	0.535
		AA	2	4	0.469	0.081–2.720	1	0.399
	allele	G	54	241	1	reference	–	–
		A	12	53	0.990	0.495–1.978	1	0.976
rs9974589	genotype	AA	6	28	1	reference	–	–
		AC	12	81	2.664	1.137–6.242	1	<b>0.024</b>
		CC	15	38	1.842	0.635–5.345	–	0.261
	allele	A	24	137	1	reference	–	–
		C	42	157	1.527	0.880–2.650	1	0.132

\* individuals who had a history of contact with individuals determined to be SARS-CoV-2-positive, who were found to be SARS-CoV-2-negative using polymerase chain reaction (PCR), and who were not infected with SARS-CoV-2 (control group). OR – odds ratio; 95% CI – 95% confidence interval; df – degrees of freedom; dbSNP – Single Nucleotide Polymorphism Database. Values in bold indicate statistical significance.

**Table 4.** Genotype and allele distributions of *ACE2* and *TMPRSS2* polymorphisms in the study groups including severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)-positive patient groups consisting of asymptomatic, symptomatic and ICU-treated individuals, and a SARS-CoV-2-negative group

dbSNP ID	SARS-CoV-2-negative* (n = 33)	SARS-CoV-2-positive asymptomatic patients				SARS-CoV-2-positive symptomatic patients				SARS-CoV-2-positive symptomatic and ICU-treated patients			
		n = 41	OR	95% CI	p-value	n = 53	OR	95% CI	p-value	n = 53	OR	95% CI	p-value
rs714205	CC	25	1	reference	–	40	1	reference	–	33	1	reference	–
	CG	4	0.650	1.160–2.553	0.527	6	0.600	0.174–2.073	0.419	8	0.970	0.297–3.162	0.959
	GG	12	3.840	0.963–15.319	<b>0.049</b>	7	1.400	0.330–5.933	0.648	12	0.209	0.739–11.449	0.127
	C	54	1	reference	–	86	1	reference	–	74	1	reference	–
	G	28	2.333	1.076–5.061	<b>0.032</b>	20	1.098	0.496–2.428	0.818	32	1.946	0.919–4.122	0.082
rs73635825	AA	40	1	reference	–	53	1	reference	–	53	1	reference	–
	AG	1	0.800	0.048–13.295	0.876	0	–	–	–	0	–	–	–
	GG	0	–	–	–	0	–	–	–	0	–	–	–
	A	81	1	reference	–	106	1	reference	–	106	1	reference	–
	G	1	0.793	0.049–12.917	0.870	0	–	–	–	0	–	–	–
rs2285666	CC	26	1	reference	–	40	1	reference	–	32	1	reference	–
	CT	3	0.508	0.109–2.368	0.388	6	0.660	0.181–2.412	0.530	8	1.100	0.318–3.810	0.880
	TT	12	1.692	0.545–5.252	0.363	7	0.642	0.192–2.148	0.472	13	1.490	0.491–4.516	0.481
	C	55	1	reference	–	86	1	reference	–	72	1	reference	–
	T	27	1.415	0.690–2.903	0.344	20	0.670	0.321–1.399	0.287	34	1.361	0.685–2.703	0.378
rs1978124	TT	19	1	reference	–	30	1	reference	–	25	1	reference	–
	CT	7	0.599	0.174–2.060	0.416	12	0.650	0.215–1.965	0.445	9	0.585	0.183–1.875	0.367
	CC	15	1.169	0.303–2.411	0.767	11	0.397	0.140–1.130	0.083	19	0.823	0.307–2.206	0.699
	T	45	1	reference	–	72	1	reference	–	59	1	reference	–
	C	37	1.145	0.598–2.192	0.684	34	0.502	0.267–0.944	<b>0.032</b>	47	0.946	0.457–1.568	0.596
rs8134378	GG	35	1	reference	–	32	1	reference	–	41	1	reference	–
	GA	6	1.243	0.320–4.830	0.754	19	4.444	1.350–14.624	<b>0.014</b>	12	2.122	0.622–7.241	0.230
	AA	0	–	–	–	3	–	–	–	0	–	–	–
	G	76	1	reference	–	83	1	reference	–	94	1	reference	–
	A	6	1.224	0.331–4.530	0.762	25	4.784	1.583–14.459	<b>0.006</b>	12	1.979	0.610–6.415	0.255



**Table 4.** Genotype and allele distributions of *ACE2* and *TMPRSS2* polymorphisms in the study groups including severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)-positive patient groups consisting of asymptomatic, symptomatic and ICU-treated individuals, and SARS-CoV-2-negative group – cont.

dbSNP ID	SARS-CoV-2-negative* (n = 33)	SARS-CoV-2-positive asymptomatic patients				SARS-CoV-2-positive symptomatic patients				SARS-CoV-2-positive symptomatic and ICU-treated patients			
		n = 41	OR	95% CI	p-value	n = 53	OR	95% CI	p-value	n = 53	OR	95% CI	p-value
rs2070788	GG	11	1	reference	–	9	1	reference	–	12	1	reference	–
	genotype				<b>0.039</b>	32	12.000	3.369–42.748	<b>0.001</b>	26	7.800	2.221–27.389	<b>0.001</b>
	AA	18	1.125	0.385–3.291	0.830	12	1.227	0.391–3.854	0.726	15	1.309	0.450–3.806	0.621
	allele				–	50	1	reference	–	50	1	reference	–
rs7364083	A	46	1.204	0.623–2.327	0.581	56	1.374	0.736–2.563	0.319	56	1.374	0.736–2.563	0.319
	GG	6	1	reference	–	5	1	reference	–	11	1	reference	–
	genotype				0.151	38	4.750	1.695–13.309	<b>0.003</b>	23	1.513	0.572–4.001	0.404
	AA	15	1.154	0.298–4.467	0.836	10	1.250	0.299–5.230	0.760	19	1.447	0.435–4.821	0.547
rs13052975	G	34	1	reference	–	48	1	reference	–	45	1	reference	–
	A	48	1.240	0.636–2.415	0.528	58	1.448	0.760–2.294	0.250	61	1.262	0.670–2.379	0.471
	GG	24	1	reference	–	37	1	reference	–	37	1	reference	–
	genotype				0.330	16	1.243	0.459–3.364	0.668	15	1.166	0.427–3.180	0.765
rs9974589	AA	2	1.437	0.220–9.405	0.705	0	–	–	–	1	0.311	0.027–3.624	0.351
	G	62	1	reference	–	90	1	reference	–	89	1	reference	–
	A	20	1.452	0.650–3.241	0.363	16	0.940	0.517–1.708	0.84	17	0.860	0.381–1.937	0.715
	allele				–	8	1	reference	–	12	1	reference	–
rs9974589	AC	20	1.923	0.686–5.394	0.214	35	4.375	1.555–12.310	<b>0.005</b>	26	2.167	0.805–5.831	0.126
	CC	13	1.538	0.422–5.606	0.514	10	2.000	0.531–7.539	0.306	15	2.000	0.594–6.730	0.263
	A	36	1	reference	–	51	1	reference	–	50	1	reference	–
	allele				0.581	55	1.623	0.864–3.042	0.132	56	1.224	0.832–2.934	0.165

\* individuals who had a history of contact with individuals determined to be SARS-CoV-2-positive, who were found to be SARS-CoV-2-negative by polymerase chain reaction (PCR), and who were not infected with SARS-CoV-2 (control group); ICU – intensive care unit; OR – odds ratio; 95% CI – 95% confidence interval; dbSNP – Single Nucleotide Polymorphism Database. Values in bold indicate statistical significance.

**Table 5.** *ACE2* rs714205 (C/G), rs2285666 (C/T), rs1978124 (T/C), and rs73635825 (A/G) haplotype frequencies in the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)-positive patient and control groups

Haplotype	SARS-CoV-2-negative (n = 60), n (%)	SARS-CoV-2-positive (n = 225), n (%)	OR	95% CI	df	p-value
CCTA	16 (26.6)	81 (36)	1	reference	–	–
CCCA	16 (26.6)	46 (20.4)	0.568	0.260–1.241	1	0.15
CTTA	<b>7 (11.6)</b>	<b>10 (4.4)</b>	<b>0.282</b>	<b>0.093–0.852</b>	1	<b>0.02</b>
GTTA	6 (10)	27 (12)	0.889	0.316–2.501	1	0.82
GTCA	5 (8.3)	31 (13.7)	1.225	0.413–3.629	1	0.71
GCTA	4 (6.6)	11 (4.8)	0.543	0.154–1.922	1	0.34
GCCA	3 (5)	9 (4)	0.593	0.144–2.433	1	0.46
CTCA	3 (5)	10 (4.4)	0.658	0.163–2.663	1	0.55
CCTG	0 (0)	1 (0.4)	–	–	–	–

OR – odds ratio; 95% CI – 95% confidence interval; df – degrees of freedom. Haplotypes are given according to the localization of microsatellite markers on the X chromosome. Values in bold indicate statistical significance.

**Table 6.** *TMPRSS2* rs13052975 (G/A), rs2070788 (G/A), rs9974589 (A/C), rs7364083 (G/A), and rs8134378 (G/A) haplotype frequencies between severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)-positive patient and control groups

Haplotype	SARS-CoV-2-negative (n = 101), n (%)	SARS-CoV-2-positive (n = 818), n (%)	OR	95% CI	df	p-value
GGAGG	14 (13.9)	95 (11.6)	1	reference	–	–
GACAG	21 (20.8)	106 (13.0)	0.744	0.358–1.545	1	0.42
GGAAG	7 (6.9)	81 (9.2)	1.705	0.657–4.429	1	0.27
GAAGG	7 (6.9)	68 (8.3)	1.432	0.549–3.736	1	0.46
GAAAG	7 (6.9)	74 (9.0)	1.558	0.598–4.056	1	0.36
GACGG	6 (5.9)	71 (8.7)	1.495	0.573–3.896	1	0.41
GGCGG	8 (7.9)	69 (8.4)	1.271	0.505–3.197	1	0.61
GGCAG	7 (6.9)	71 (8.7)	1.495	0.573–3.896	1	0.41
AACAG	6 (5.9)	46 (5.7)	1.130	0.408–3.130	1	0.81
AGAGG	5 (5.0)	24 (3.0)	0.707	0.232–2.157	1	0.54
AGCAG	2 (2.0)	12 (1.5)	0.884	0.179–4.374	1	0.88
AGCGG	3 (3.0)	16 (1.9)	0.786	0.203–3.046	1	0.72
AGAAG	3 (3.0)	13 (1.6)	0.639	0.161–2.526	1	0.52
GGAGA	3 (3.0)	34 (4.2)	1.670	0.452–6.172	1	0.44
GACAA	1 (1.0)	30 (3.7)	4.421	0.558–35.031	1	0.15
AACAA	1 (1.0)	8 (1.0)	1.179	0.137–10.154	1	0.88

OR – odds ratio; 95% CI – 95% confidence interval; df – degrees of freedom. Haplotypes are given according to the localization of microsatellite markers on the chromosome 21.

group compared to the asymptomatic and symptomatic COVID-19 patient groups (Fig. 3). The data obtained from patients with a more severe clinical course of COVID-19 support the claim that *ACE2* and *TMPRSS2* genes may be directly related to the severity of COVID-19. Especially since the *ACE2* receptor is the target molecule for the entry of SARS-CoV-2 into cells, and the *TMPRSS2* is the main protease facilitating the entry of SARS-CoV-2 into host cells, the increased expression of both genes indicates that these patients have more severe SARS-CoV-2 viremia. In other words, it can be said that there is a cause-effect relationship. This important finding suggests that in the future, inhibition strategies targeting *ACE2* or *TMPRSS2*

at the gene or receptor level may be developed and used as an antivirus and/or therapeutic approach to reduce the entry of SARS-CoV-2 into host cells and minimize the mortality rate.

The localization of the *ACE2* gene on the X chromosome leads to the fact that females are potentially heterozygous for the expression of this gene and males are hemizygous.<sup>23</sup> Therefore, it is natural that there are differences in *ACE2* expression between males and females in theory, yet in practice and in our study, no difference was observed between the genders in terms of *ACE2* expression. Although, it is argued that the reactions of females to SARS-CoV-2 viremia may be different due

to the localization of inflammation-related genes, including innate and adaptive immune response-related genes on the X chromosome.<sup>24</sup> The gender difference between females and males and the fact that males were hemizygous in terms of *ACE2* did not have any effect on the more severe course of COVID-19 in our study groups. Similarly, Alimoradi et al. showed that gender was not significantly associated with the severity and incidence of COVID-19.<sup>5</sup> The mean age and the presence of comorbid diseases in COVID-19 patients in the ICU-treated group differed compared to the other groups in our study. The *ACE2* and *TMPRSS2* expression levels were higher in the SARS-CoV-2-positive patients over 60 years old. The *ACE2* and *TMPRSS2* expression levels were different in ICU-treated patients with comorbid diseases compared to those without comorbid diseases.

Of the *ACE2* polymorphisms, only the expression of rs714205GG genotype and G-allele showed a significant difference in the SARS-CoV-2-positive asymptomatic group, suggesting that this variant may be associated with a lighter clinical course. In the SARS-CoV-2-positive symptomatic patient group, the expression of rs1978124 C-allele was statistically different from other groups. According to this result, it can be concluded that the rs1978124 C-allele is effective in the symptomatic course of infection. However, taking into account the patient's immunity and comorbid diseases, such interpretation is appropriate. In addition, other possibilities should be considered, such as gene–RNA interactions and epigenetic factors, where there may be other *ACE2* polymorphisms or interactions of different genes that may affect *ACE2* receptor function. Möhlendick et al. reported that carriers of the *ACE2* rs2285666GG genotype or G-allele have a two-fold increased risk for SARS-CoV-2 infections compared to the AA genotype.<sup>9</sup> This conclusion was also supported by Alimoradi et al.<sup>5</sup> In our study, the rs2285666 G- and A-alleles were not found in the patient and control groups, and there was no difference between the groups in terms of the determined C- and T-alleles. However, this result does not reflect the whole population, and allele frequencies may vary between populations. Therefore, the susceptibility of different ethnic groups to SARS-CoV-2 may vary in relation to different genotypes.

According to our results, the expression of *TMPRSS2* rs2070788GA, rs7364083GA and rs9974589AC genotypes showed significant differences in SARS-CoV-2-positive patients. Especially regarding the rs2070788GA genotype, there was a significant difference in all SARS-CoV-2-positive patient groups. Therefore, we believe that the presence of rs2070788GA is associated with SARS-CoV-2 sensitivity rather than the clinical course of COVID-19. The minor allele frequencies (MAFs) of rs7364083 and rs9974589 differed in populations according to the genome aggregation database (gnomAD) (<https://gnomad.broadinstitute.org/>). In our study groups, the frequency of variant alleles rs7364083 and rs9974589 was found to be higher, which

is similar to the literature.<sup>25</sup> Moreover, the rs7364083GA and rs9974589AC genotypes were higher in the SARS-CoV-2-positive groups, and a statistical difference was observed only in the symptomatic patient group. Thus, the rs7364083GA and rs9974589AC genotypes may be associated with SARS-CoV-2 susceptibility and may correlate with the clinical course of COVID-19 infections. The rs8134378 A-allele, which differs significantly in SARS-CoV-2-positive symptomatic patients, may also be associated with infection sensitivity.

Previous studies have suggested that the *ACE2* rs2285666 A-allele is associated with increased *ACE2* expression in healthy individuals as well as in patients with diabetes and cerebral stroke.<sup>9,26</sup> Gómez et al. declared that there was no difference in terms of *ACE2* rs2285666 variants in COVID-19 patients with mild and severe course of the disease, but this variant was associated with hypertension in the elderly population.<sup>12</sup> In patients with multiple sclerosis who have a SARS-CoV-2 infection, *TMPRSS2* rs61735792 and rs61735794 variants are reported to be associated with the severity of the infection.<sup>27</sup> In our study, 13.2% of the SARS-CoV-2-positive symptomatic patients and 84.9% of the ICU-treated patients had at least 1 comorbid disease such as hypertension, cardiovascular disease, diabetes mellitus, chronic lung diseases, kidney diseases, liver diseases, and malignancies. In the SARS-CoV-2-positive patient groups with comorbid diseases, *ACE2* and *TMPRSS2* expressions were higher. In the ICU-treated patient group, *ACE2* and *TMPRSS2* expression levels were higher in the presence of comorbid diseases. There was no relationship between the SNPs examined and *ACE2* and *TMPRSS2* expression levels, but it should not be ignored that there may be other genetic factors, such as other intragenic variations, regulatory genes and epigenetic factors that may affect *ACE2* and *TMPRSS2* expression levels.

Gemmati et al. suggest a strong linkage disequilibrium between *ACE2* rs1978124, rs714205 and rs2285666 variants.<sup>11</sup> According to our results, the CTTA haplotype frequency formed with *ACE2* variants in the SARS-CoV-2-positive patient group was lower than in the control group. Therefore, the CTTA haplotype may be more resistant to SARS-CoV-2 infections. In terms of the *TMPRSS2* haplotypes, although there was no difference between the SARS-CoV-2-positive patient groups and the control group, AGCAG and AGAAG haplotypes were identified more frequently in the asymptomatic SARS-CoV-2-positive patient group compared to the other patient groups. Therefore, these haplotypes may have a role in a milder course of COVID-19.

Martínez-Sanz et al. reported that *ACE2* rs2106806 and rs6629110 variants may be responsible for SARS-CoV-2 infection susceptibility in hospital staff not infected with SARS-CoV-2 and in hospitalized COVID-19 patients.<sup>18</sup> Similarly, Hou et al. stated that polymorphisms in *ACE2* and *TMPRSS2* genes may be associated with genetic susceptibility to COVID-19.<sup>4</sup> Irham et al. suggested that

there is an increase in *TMPRSS2* expression associated with rs464397, rs469390, rs2070788, and rs383510 variations in lung tissue, which is the major infection site for SARS-CoV-2, and this increase may affect infection severity as well as SARS-CoV-2 sensitivity.<sup>28</sup> According to our study, the high frequency of the rs2070788GA genotype in the SARS-CoV-2-positive group and the increased *TMPRSS2* expression detected in the ICU-treated group support the view that *TMPRSS2* variants affect the expression of the gene and increase the susceptibility to SARS-CoV-2 infections. Irham et al. showed that there is a higher *TMPRSS2* expression in lung tissues in the rs2070788GG genotype.<sup>28</sup> In our study, although there was no relationship between *ACE2* and *TMPRSS2* expressions and genotypes in the peripheral venous blood of the patient and control groups, it was not possible to evaluate *ACE2* and *TMPRSS2* expressions in target tissues, especially the lungs.

Abdelsattar et al. reported that *ACE2* rs2285666 and *TMPRSS2* rs12329760 variants may be associated with COVID-19 disease severity.<sup>29</sup> However, in our study, no finding reported the relationship between *ACE2* rs2285666 genotype and allele frequency with disease severity. Pandey et al. stated that SARS-CoV-2 host sensitivity in South Asian population is similar to the Western Eurasian population, and this sensitivity is associated with the *TMPRSS2* gene.<sup>30</sup> Thus, there is a significant relationship between rs2070788 G-allele and the COVID-19 mortality rate. In our study, the frequency of the rs2070788GA genotype was also found to be high in the SARS-CoV-2-positive patient groups, especially in the symptomatic and ICU-treated patients, but it was detected at a significantly different frequency from the control group. Therefore, we believe that this variant is associated with COVID-19 disease severity, but it is quite difficult to associate the *TMPRSS2* expression level with this variant alone, and it is more appropriate to conduct multicenter studies from different populations to confirm this finding.

Hussain et al. reported that *ACE2* variants such as rs73635825 and rs143936283 may create a positive prognosis for COVID-19 course in some individuals.<sup>6</sup> The *ACE2* rs73635825 variant, which is quite rare, was found in only 1 patient in our study group, and no difference was observed between the groups.

It is suggested that there is no relationship between *ACE2* expression and variants and severity of COVID-19 and gender in the Italian population. However, *TMPRSS2* expression and variants differed according to gender and may be effective in the prognosis of the disease.<sup>24</sup> According to our results, *ACE2* and *TMPRSS2* expressions, variants and the severity of infection did not differ between the genders in the SARS-CoV-2-positive patient and control groups. However, it would be more appropriate to support these results with similar studies in a larger study population.

Kim and Jeong reported that *ACE2* rs2074192 and *TMPRSS2* rs2298659 showed a higher correlation compared to other *ACE2* and *TMPRSS2* variants, while *IFITM3*

rs6598045 was associated with COVID-19-related mortality rates.<sup>31</sup> We found that *ACE2* rs714205 may be effective in the milder clinical manifestation of COVID-19, and even *ACE2* rs1978124 and *TMPRSS2* rs8134378, rs2070788, rs7364083, and rs9974589 may be effective in varying degrees of symptomatic courses of COVID-19. We also demonstrated a correlation between changes in *ACE2* and *TMPRSS2* expression levels and the clinical findings of COVID-19.

## Conclusions

In conclusion, our study demonstrated that genetic factors of the host may affect the sensitivity and clinical course of COVID-19. Since SARS-CoV-2 is a new virus on which studies have been conducted for the last 2 years, it is a long process to define the genetic factors affecting infection sensitivity. Conducting studies aimed at determining genetically-based prognostic factors that will enable the early detection of individuals at high risk who require urgent medical treatment for COVID-19 is even more important, especially during epidemic periods. Studies in different populations in which the number of patients, examined genes and polymorphisms are increased will provide more information about the genetic variations at the receptor level and host genetic characteristics that may be effective in the sensitivity and clinical course of COVID-19.

## Limitations

The main limitation of our study is that *ACE2* and *TMPRSS2* expression levels can only be studied in peripheral venous blood. We also observed a total of 9 SNPs in these 2 genes. Moreover, the possible role of host genetics on SARS-CoV-2 vaccine efficacy was not evaluated. Future research should address the correlation between host genetic factors and the response to the SARS-CoV-2 vaccine.

## Conclusions

The data of our study shed light on the establishment of genetic biomarkers in the predetermination of susceptible populations for COVID-19, the identification of new and effective drug targets for COVID-19 patients, and the development of new vaccines.

## Supplementary data

The supplementary materials are available at <https://doi.org/10.5281/zenodo.7826506>. The package contains the following files:

Supplementary Table 1. T-test (*ACE2* and *TMPRSS2* expression levels in the SARS-CoV-2-positive and negative groups).



Supplementary Table 2. Normal distribution test (*ACE2* and *TMPRSS2* expression levels between the subgroups (asymptomatic patient, symptomatic patient, ICU-treated, and control groups)).

Supplementary Table 3. Kruskal–Wallis test results.

Supplementary Table 4. Post hoc tests results.


Supplementary Table 5. Assumption checking results.


Supplementary Table 6. Multinomial logistic regression results of independent variables affecting COVID-19 disease severity.


Supplementary Table 7. Multinomial logistic regression results of independent variables affecting genotypic distribution.


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## References

- World Health Organization (WHO). WHO Coronavirus (COVID-19) Dashboard. <https://covid19.who.int/>. Geneva, Switzerland: World Health Organization (WHO). Accessed June 30, 2023.
- Hoffmann M, Kleine-Weber H, Schroeder S, et al. SARS-CoV-2 cell entry depends on *ACE2* and *TMPRSS2* and is blocked by a clinically proven protease inhibitor. *Cell*. 2020;181(2):271–280.e8. doi:10.1016/j.cell.2020.02.052
- Suh S, Lee S, Gym H, et al. A systematic review on papers that study on single nucleotide polymorphism that affects coronavirus 2019 severity. *BMC Infect Dis*. 2022;22(1):47. doi:10.1186/s12879-022-07034-w
- Hou Y, Zhao J, Martin W, et al. New insights into genetic susceptibility of COVID-19: An *ACE2* and *TMPRSS2* polymorphism analysis. *BMC Med*. 2020;18(1):216. doi:10.1186/s12916-020-01673-z
- Alimoradi N, Sharqi M, Firouzabadi D, Sadeghi MM, Moezzi MI, Firouzabadi N. SNPs of *ACE1* (rs4343) and *ACE2* (rs2285666) genes are linked to SARS-CoV-2 infection but not with the severity of disease. *Virology*. 2022;19(1):48. doi:10.1186/s12985-022-01782-6
- Hussain M, Jabeen N, Raza F, et al. Structural variations in human *ACE2* may influence its binding with SARS-CoV-2 spike protein. *J Med Virol*. 2020;92(9):1580–1586. doi:10.1002/jmv.25832
- Devaux CA, Rolain JM, Raoult D. *ACE2* receptor polymorphism: Susceptibility to SARS-CoV-2, hypertension, multi-organ failure, and COVID-19 disease outcome. *J Microbiol Immunol Infect*. 2020;53(3):425–435. doi:10.1016/j.jmii.2020.04.015
- Imai Y, Kuba K, Ohto-Nakanishi T, Penninger JM. Angiotensin-converting enzyme 2 (*ACE2*) in disease pathogenesis. *Circ J*. 2010;74(3):405–410. doi:10.1253/circj.CJ-10-0045
- Möhlendick B, Schönfelder K, Breuckmann K, et al. *ACE2* polymorphism and susceptibility for SARS-CoV-2 infection and severity of COVID-19. *Pharmacogenet Genomics*. 2021;31(8):165–171. doi:10.1097/FPC.0000000000000436
- Lukassen S, Chua RL, Trefzer T, et al. SARS-CoV-2 receptor *ACE2* and *TMPRSS2* are primarily expressed in bronchial transient secretory cells. *EMBO J*. 2020;39(10):e105114. doi:10.15252/embj.20105114
- Gemmati D, Bramanti B, Serino ML, Secchiero P, Zauli G, Tisato V. COVID-19 and individual genetic susceptibility/receptivity: Role of *ACE1/ACE2* genes, immunity, inflammation and coagulation. Might the double X-chromosome in females be protective against SARS-CoV-2 compared to the single X-chromosome in males? *Int J Mol Sci*. 2020;21(10):3474. doi:10.3390/ijms21103474
- Gómez J, Albaiceta GM, García-Clemente M, et al. Angiotensin-converting enzymes (*ACE*, *ACE2*) gene variants and COVID-19 outcome. *Gene*. 2020;762:145102. doi:10.1016/j.gene.2020.145102
- Paniri A, Hosseini MM, Akhavan-Niaki H. First comprehensive computational analysis of functional consequences of *TMPRSS2* SNPs in susceptibility to SARS-CoV-2 among different populations. *J Biomol Struct Dyn*. 2021;39(10):3576–3593. doi:10.1080/07391102.2020.1767690
- Bhattacharyya C, Das C, Ghosh A, et al. Global spread of SARS-CoV-2 subtype with spike protein mutation D614G is shaped by human genomic variations that regulate expression of *TMPRSS2* and *MX1* genes [published as a preprint on May 5, 2020]. doi:10.1101/2020.05.04.075911
- Xia S, Yan L, Xu W, et al. A pan-coronavirus fusion inhibitor targeting the HR1 domain of human coronavirus spike. *Sci Adv*. 2019;5(4):eaav4580. doi:10.1126/sciadv.aav4580
- Badawi A. Hypercytokinemia and pathogen–host interaction in COVID-19. *J Inflamm Res*. 2020;13:255–261. doi:10.2147/JIR.S259096
- Velavan TP, Pallerla SR, Rüter J, et al. Host genetic factors determining COVID-19 susceptibility and severity. *EBioMedicine*. 2021;72:103629. doi:10.1016/j.ebiom.2021.103629
- Martínez-Sanz J, Jiménez D, Martínez-Campelo L, et al. Role of *ACE2* genetic polymorphisms in susceptibility to SARS-CoV-2 among highly exposed but not infected healthcare workers. *Emerg Microbes Infect*. 2021;10(1):493–496. doi:10.1080/22221751.2021.1902755
- Erali M, Wittwer CT. High resolution melting analysis for gene scanning. *Methods*. 2010;50(4):250–261. doi:10.1016/j.jymeth.2010.01.013
- Matsuyama S, Nao N, Shirato K, et al. Enhanced isolation of SARS-CoV-2 by *TMPRSS2*-expressing cells. *Proc Natl Acad Sci U S A*. 2020;117(13):7001–7003. doi:10.1073/pnas.2002589117
- Benetti E, Tita R, Spiga O, et al. *ACE2* gene variants may underlie interindividual variability and susceptibility to COVID-19 in the Italian population. *Eur J Hum Genet*. 2020;28(11):1602–1614. doi:10.1038/s41431-020-0691-z
- Suryamohan K, Diwanji D, Stawiski EW, et al. Human *ACE2* receptor polymorphisms and altered susceptibility to SARS-CoV-2. *Commun Biol*. 2021;4(1):475. doi:10.1038/s42003-021-02030-3
- Pinheiro DS, Santos RS, Jardim PCB, et al. The combination of *ACE1/D* and *ACE2 G8790A* polymorphisms reveals susceptibility to hypertension: A genetic association study in Brazilian patients. *PLoS One*. 2019;14(8):e0221248. doi:10.1371/journal.pone.0221248
- Klein SL, Flanagan KL. Sex differences in immune responses. *Nat Rev Immunol*. 2016;16(10):626–638. doi:10.1038/nri.2016.90
- Asselta R, Paraboschi EM, Mantovani A, Duga S. *ACE2* and *TMPRSS2* variants and expression as candidates to sex and country differences in COVID-19 severity in Italy. *Aging (Albany NY)*. 2020;12(11):10087–10098. doi:10.18632/aging.103415
- Wu YH, Li JY, Wang C, Zhang LM, Qiao H. The *ACE2 G8790A* polymorphism: Involvement in type 2 diabetes mellitus combined with cerebral stroke. *J Clin Lab Anal*. 2017;31(2):e22033. doi:10.1002/jcla.22033
- Torre-Fuentes L, Matías-Guio J, Hernández-Lorenzo L, et al. *ACE2*, *TMPRSS2*, and Furin variants and SARS-CoV-2 infection in Madrid, Spain. *J Med Virol*. 2021;93(2):863–869. doi:10.1002/jmv.26319
- Irham LM, Chou WH, Calkins MJ, Adikusuma W, Hsieh SL, Chang WC. Genetic variants that influence SARS-CoV-2 receptor *TMPRSS2* expression among population cohorts from multiple continents. *Biochem Biophys Res Commun*. 2020;529(2):263–269. doi:10.1016/j.bbrc.2020.05.179
- Abdelsattar S, Kasemy ZA, Ewida SF, et al. *ACE2* and *TMPRSS2* SNPs as determinants of susceptibility to, and severity of, a COVID-19 infection. *Br J Biomed Sci*. 2022;79:10238. doi:10.3389/bjbs.2021.10238
- Pandey RK, Srivastava A, Singh PP, Chaubey G. Genetic association of *TMPRSS2* rs2070788 polymorphism with COVID-19 case fatality rate among Indian populations. *Infect Genet Evol*. 2022;98:105206. doi:10.1016/j.meegid.2022.105206
- Kim YC, Jeong BH. Strong correlation between the case fatality rate of COVID-19 and the rs6598045 single nucleotide polymorphism (SNP) of the interferon-induced transmembrane protein 3 (*IFITM3*) gene at the population-level. *Genes (Basel)*. 2020;12(1):42. doi:10.3390/genes12010042