Biochemical Genetics
https://doi.org/10.1007/s10528-024-10722-8

ORIGINAL ARTICLE

The Association Between Genetic Variants in *ACE1*and *ACE2* Genes with Susceptibility to COVID-19 Infection

Hamid Abbaszadeh[1] · Hamid Kabiri-Rad[2] · Fariba Mohammadi[2] · Soheila Zangoie[3] · Mahdieh Rajabi-Moghaddam[4] · Shokouh Ghafari[5] · Masood Ziaee[6] · Davod Javanmard[6] · Ebrahim Miri-Moghaddam[7]

Received: 19 September 2023 / Accepted: 28 January 2024

Abstract

Angiotensin-converting enzyme 2 (ACE2) receptors facilitate the entry of the causative virus severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) into target cells. Some ACE gene variants have been suggested to be involved in COVID-19 pathogenesis. So, the aim was to assess the association between ACE1 rs4646994 and ACE2 rs2285666 genes polymorphisms and the susceptibility and severity of COVID-19. This case-control study was conducted on 197 patients with COVID-19 and 197 healthy controls. ACE-1 insertion/deletion (I/D) (rs4646994) and ACE2 rs2285666 genes polymorphisms were determined by the amplification refractory mutation system- polymerase chain reaction (ARMS-PCR) technique. The DD genotype of ACE1 I/D polymorphism was associated with increased susceptibility to COVID-19 infection ($p=0.012$), whereas the ID genotype of this polymorphism was associated with decreased susceptibility ($p=0.003$) (significance level=0.017). There was no significant association in allele and genotype distribution of ACE2 rs2285666 polymorphism between cases and controls. The ACE1 I/D polymorphism may be considered as a risk factor for COVID-19 susceptibility.

Keywords COVID-19 · SARS-CoV-2 · Angiotensin-converting enzyme 2 · Polymorphism · Iran

Introduction

Coronavirus disease 2019 (COVID-19), a respiratory disease emerged in December 2019, is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which has become a major public health problem in recent years throughout the world (Zhu et al. 2020). So far, more than 660 million people have been infected with

Extended author information available on the last page of the article

Published online: 13 February 2024

COVID-19 and more than 6.7 million have died due to COVID-19 and these figures are increasing day by day (Delanghe et al. 2020). COVID-19 can be asymptomatic or presents with mild symptoms to severe pneumonia, which can progress to respiratory failure or death due to adult respiratory distress syndrome (ARDS) (Wang et al. 2020a, 2020b; Hu et al. 2020). Hence more recent attention has focused on the identification of mechanisms that are involved in COVID-19 susceptibility and development (Harrison et al. 2020; Mi et al. 2020). One of the issues that emerged from these researches was that the prognosis of COVID-19 could varies widely depending upon various risk factors, such as male sex, older age, ethnicity, and comorbidities (e.g. hypertension (HTN), diabetes, obesity and etc.) (Fang et al. 2020; Gao et al. 2021; Sanyaolu et al. 2020). Furthermore, researchers hypothesized that the existence of common genes polymorphisms or inherited predispositions might place people at higher risk of COVID-19 from the beginning (Öztürk 2020). Some notable examples of this issue would be polymorphisms in the genes encoding the renin-angiotensin system (RAS) members that affect mutual expression levels of Angiotensin-converting enzyme 1 (ACE1) and 2 leading to increased capillary permeability, thrombosis, alveolar cells apoptosis, and accelerating lung damage in SARS-CoV-2 infection (Kenyon 2020; Pati et al. 2020; Yamamoto et al. 2021a, 2021b).

SARS-CoV-2 adsorption to susceptible target cells has been shown to be predominantly mediated by the interaction of ACE2 enzyme with the virus spike (S) protein (Verdecchia et al. 2020). ACE2 is an integral part of RAS expressing in various cells especially lung alveolar epithelial cells, which counteracts the effects of ACE1 (Hamming et al. 2007; Devaux et al. 2020). The ACE2 enzyme converts Angiotensin II (Ang II) to Ang 1–7 (Hamming et al. 2007). Ang II stimulates vasoconstriction, proliferation, inflammation, and fibrosis (Hamming et al. 2007; Wang et al. 2020a, 2020b). Data from several sources have identified the dysregulation of RAS members associated with COVID-19 pathogenesis and development (Yamamoto et al. 2021a, 2021b). It has been proposed that binding of SARS-CoV-2 to ACE2 may cause the down-regulation of ACE2 expression; this increases Ang II level that induces pro-inflammatory signals and triggers cytokine storm involved in ARDS and organ damage (Gemmati et al. 2020; Liu et al. 2020; Bakhshandeh et al. 2021).

In line with this, evidence suggests that the ACE2 rs2285666 polymorphism (transition G8790A) is among the most important ones that might affect COVID-19 (Devaux et al. 2020; Gemmati et al. 2020). This is exemplified in the work that shows its associations with comorbidities affecting COVID-19, such as HTN, type 2 diabetes mellitus (DM), and coronary heart disease, even though some issues remain almost inconclusive and ambiguous (Chaoxin et al. 2013; Pinheiro et al. 2019; Yang et al. 2015). The ACE2 rs2285666 polymorphism has been documented to affect the ACE2 gene expression through mRNA alternate splicing in the light of its location at the beginning of the intron 3 (Yang et al. 2015; Li 2012). ACE2 polymorphism might cause changes in the secondary structure of RNA and this change may result in disruption of ACE2 translation or protein stability, which in turn could result in altered binding of SARS-CoV-2 to the ACE2 receptor. The secondary structure of mRNA caused by ACE2 polymorphisms might make proteins more susceptible to proteases. Decreased ACE2 protein levels and the loss of protective effects of ACE2 pathway could contribute to the severe effects of SARS-CoV-2 infection. Unlike autosomal

genes (e.g., ACE1), X-linked ACE2 gene cannot present heterozygous condition and its advantages in males in case of polymorphisms and mutations (Gemmati et al. 2020). This is a good illustration of the male carrier of G-allele that shows a lower enzymatic activity of ACE2 rather than A-allele counterpart (Pinheiro et al. 2019; Wu et al. 2017). Interestingly it has been demonstrated that the patients with DD genotype characterized by a higher ACE1 enzyme activity in conjunction with GG-females or G-males that have reduced ACE2 activity, are more susceptible to HTN mainly in relation to widely known cardiovascular risk factors, for instance dyslipidemia, old age, and diabetes (Pinheiro et al. 2019). In line with this, the mentioned study has provided a deeper insight into this issue that an excessively activated RAS induced by inherited predispositions influencing the ACE1/ACE2 equilibrium and in association with various risk factors might lead to the disturbance of normal tissue homeostasis (Gemmati et al. 2020; Pinheiro et al. 2019). Taken together, this condition can lead to organs dysfunction (e.g., lungs) in the case of SARS-CoVs that induce ACE1/ACE2 imbalance through the binding of ACE2 receptor (Gemmati et al. 2020; Pinheiro et al. 2019).

In addition, the ACE1 rs4646994 polymorphism can also play an important role in addressing the issue of inherited predispositions (Itoyama et al. 2004; Marshall et al. 2002; Rigat et al. 1990). The ACE1 enzyme is a homologue of ACE2 that increases blood pressure by converting the Ang I to Ang II and consequently resulting in the control of body fluid volume (Coates 2003). The ACE1 rs4646994 polymorphism is an insertion/deletion (I/D) genetic variation defined as the presence or absence of a 287-base pair Alu sequence in intron 16 (Gemmati et al. 2020). In this research, one of the causal factors leading to the study of rs4646994 was that its association with alterations in serum ACE1 levels and activity, which is a crucial factor in COVID-19 pathogenesis (Rigat et al. 1990; Mizuiri et al. 2001). In this context, previous research has established that 47% of serum ACE1 phenotypic variation is strongly dependent upon the I/D polymorphism which this can be seen in the case of DD genotype showing the highest serum/tissue ACE1 levels, ID genotype having intermediate levels, and the II variant expressing the lowest ones (Rigat et al. 1990; Mizuiri et al. 2001). The DD genotype of this polymorphism has been also associated with comorbid conditions related to COVID-19, such as HTN, diabetes, and lung disorders (e.g., asthma) (Yamamoto et al. 2021a, 2021b; Gard 2010). The above-mentioned polymorphism is also considered as a risk factor for ARDS and SARS (Itoyama et al. 2004; Marshall et al. 2002; Jerng et al. 2006). By way of illustration, Itoyama et al. showed that the D allele frequency is significantly higher in hypoxic SARS group in comparison with the non-hypoxic ones (Itoyama et al. 2004). However, this differs from the findings presented by Chan et al. that showed no significant differences in I/D polymorphism between SARS patients and control group (Chan et al. 2005). Other authors also supported the higher frequency of ACE1 DD genotype in ARDS patients compared to the controls and determined its association with mortality in them (Marshall et al. 2002). Analysis of genotype frequencies in another article also succeeded to show the better chance of survival in ARDS patients with II genotype (Jerng et al. 2006).

Considering various effects of genetic polymorphisms on the susceptibility of different populations and ethnicities to COVID-19, this study aimed to evaluate how

certain genetic variants in the ACE1 rs4646994 and ACE2 rs2285666 genes affect COVID-19 in the east region of Iran.

Material and Method

Study Population

This case-control research was conducted on 197 hospitalized patients newly diagnosed with COVID-19 referred to Vali-Asr Hospital (Birjand, Iran) between June 2021 to September 2021 (mean age: 59.18 years, range 2–94). The confirmed COVID-19 patients were cases with positive results of quantitative PCR tests for Alpha and Delta variants of SARS-CoV-2 (Huang et al. 2020).

Patients with COVID-19 were categorized into two groups based on the severity of the disease. The disease severity of COVID-19 was defined according to the patients' symptoms, laboratory tests and imaging findings. Hospitalized patients with clinical symptoms or present pneumonia on chest computed tomography (CT) scan were considered as moderate patients ($n=154$) while severe to critical cases ($n=43$) were defined as having the following criteria: severe respiratory distress, respiratory rate ≥ 30 (breaths/minute), O2 saturation (SpO2) $\leq 90\%$ at rest, Horovitz-quotient (P/F ratio) ≤ 300 mmHg and lung involvement > 50 (Wei 2020). We also studied 197 age-matched volunteer individuals as the control group (mean age 70.01 years, range 50–81) without any history of COVID-19 confirmed by RT-PCR test for SARS-CoV-2.

Clinical characteristics of individuals were collected through questionnaires which included demographic information, such as age, body mass index (BMI), smoking habits, and the presence of comorbidities diabetes mellitus, coronary artery disease, HTN, and chronic obstructive pulmonary disease (COPD)). Other clinical data were collected from the participants' medical records. Some individuals were excluded from the study based on the presence of any infectious and immune-related disorder. written informed consent was obtained from all participants in this study. The Ethics Committee of Birjand University of Medical Sciences, Birjand, Iran approved the study (Ethical code: IR.BUMS.REC.1399.060).

DNA Extraction and Genotyping

Genomic DNA was isolated from the leukocytes of blood specimens using a manual procedure (salting-out). The purity of extracted DNA was evaluated by Epoch microplate spectrophotometer (Epoch, BioTek, USA) and samples were kept at −20 °C until use. In this study, we used amplification refractory mutation system PCR (ARMS-PCR) assay to detect mentioned polymorphisms. The ARMS primers were designed by the Primer3 software. The primers sequences were as follow: ACE1 rs4646994, forward primer 5′-CTGGAGACCACTCCCATCCTTTCT -3′; reverse primer 5′-GATGTGGCCATCACATTCGTCAGAT-3′ and for ACE2 rs2285666, forward inner primer (T allele) 5′-TAATCACTACTAAAAATTAGTATCC −3′; reverse inner primer (C allele) 5′-GCTTATTACTTGAACCAGGGAA-3′; forward outer

primer 5′-AAGTAAATGTGATACAATTTACAAG-3′; reverse outer primer 5′-AA AGGATATCTTTATATTAGCATTC-3′. Amplification conditions comprised of an initial denaturing stage 95 °C (5 min) and followed by 35 cycles of denaturation at 95 °C (45 s), annealing at 50 and 63 °C (45 s) for rs2285666 and rs4646994, respectively, and extension at 72 °C (30 s); as well as a final extension stage at 72 °C (5 min). PCR products were electrophoresed on 1.5% agarose gel stained with safe stain dye and visualized by a gel documentation system (Fig. 1).

Statistical Analysis

Data were analyzed using SPSS software (version 22, SPSS Inc., Chicago, IL, USA). The normality of the article data was assessed by Kolmogorov–Smirnov statistic test. The continuous quantitative variables were described as mean ± standard deviation (SD). Accordingly, the student's T-test or Mann-Whitney U analysis was conducted to compare continuous quantitative variables depending on the data distribution between groups. Statistical analysis of differences between categorical variables was performed by Chi-square test (or Fisher's exact test). We measured the odds ratio (OR) at 95% confidence intervals (CI) by logistic regression analysis to describe the strength of association. Associations between genomic variants and COVID-19 susceptibility, severity, as well as mortality were assessed in different models, including dominant, recessive and co-dominant. In the light of the ACE2 gene location on the X-chromosome, female and male individuals were analyzed separately in the study. Hardy–Weinberg equilibrium (HWE) for the accordance of genotype frequencies distribution was analyzed using the chi-square test. For all tests, p-values less than 0.05 were considered statistically significant.

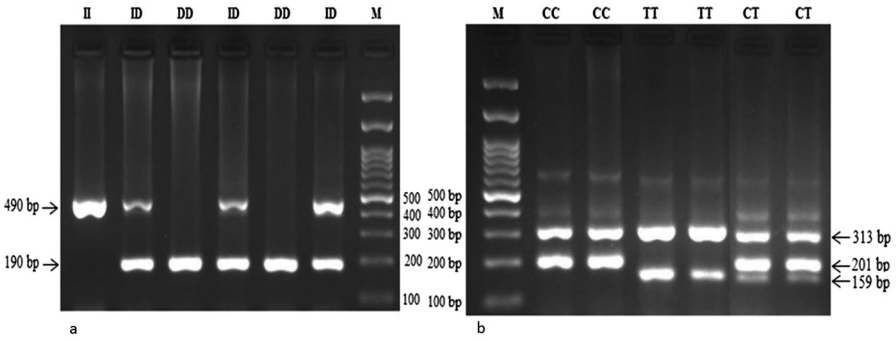

Fig. 1 **a** Representative agarose gel electrophoresis of ACE1 rs4646994 polymorphism, Lane M; 100-bp DNA ladder; II genotype was showed as a single band at 490 bp (lane marked with II), the ID genotype; two bands at 490 and 190 bp (lanes marked with ID) and the DD genotype; a single band at 190 bp (lanes marked with DD). **b** Agarose gel electrophoresis of ACE2 rs2285666 polymorphism; Lane M; 100-bp ladder; CC genotype was showed as two bands at 313 and 201 bp (lanes marked with CC), TT genotype; two bands at 313 and 159 bp (lanes marked with TT), and the CT genotype; three bands at 313, 201 and 159 bp (lanes with marked CT)

Results

The demographic data of COVID-19 cases were summarized in Table 1. The control group composed of 104 (52.8%) men and 93 (47.2%) women with a median age of 64 (IQR: 28) at the beginning of the investigation. As correlational analyses showed, COVID-19 appeared to be unaffected by age and sex. Furthermore, the overall mean BMI in controls was 26.43 ± 5.37 kg/m^2, which did not significantly vary from the patients.

In addition, we compared patients based on the disease severity. The results, as shown in Table 1, demonstrated that there is a statistically significant difference in median age between moderate (58 yrs.; IQR: 27) and severe to critical patients (72 yrs.; IQR: 35) ($p = 0.002$). Furthermore, the chi-square test showed that the percentage of COPD in severe to critical patients was remarkably higher than in moderate ones ($p = 0.002$). The other variables, such as sex, smoking habit, HTN, DM, and CAD were not found significant between groups (Table 1).

Table 1 Clinical and demographic features of COVID-19 patients between moderate and severe to critical cases

Parameter	Moderate cases ($n = 154$)	Severe to critical cases ($n = 43$)	*P* value
Age*(years), median [IQR]	58 (Yang et al. 2015)	72 (Marshall et al. 2002)	**0.002**
Sex, n (%)			
M/F	92 (59.7)/ 62 (40.3)	21 (48.8)/ 22 (51.2)	0.201
BMI (kg/ m^2), mean ± SD	26.59 ± 5.44	25.95 ± 5.27	0.492
BMI classification, n (%)			0.243
Underweight	11 (7.1)	6 (14)	
Normal	57 (37)	12 (27.9)	
Overweight	40 (26)	16 (37.2)	
Obese	40 (26)	7 (16.3)	
Extremely obese	6 (3.9)	2 (4.7)	
Shortness of breath, n (%)	70 (45.5)	19 (44.2)	0.883
Smoking habit, n (%)	8 (5.2)	0 (0)	0.127
Hypertension, n (%)	44 (28.6)	9 (20.9)	0.318
Diabetes, n (%)	14 (9.1)	3 (7)	0.662
CAD, n (%)	8 (5.2)	2 (4.7)	0.886
COPD*, n (%)	9 (5.8)	9 (20.9)	**0.002**
Laboratory tests			
PT*(sec), median [IQR]	12.5 [1.5]	13.5 [1.9]	**0.014**
PTT(sec), median [IQR]	37 (Sanyaolu et al. 2020)	38 (Pati et al. 2020)	0.223
CRP(mg/L), median [IQR]	39.5 (Mizuiri et al. 2001)	43.9 (Pinheiro et al. 2019)	0.309
ESR(mm/h), median [IQR]	29.7 (Pouladi and Abdolahi 2021)	32.6 (Tikellis and Thomas 2012)	0.494

The p values indicate the statistical significance between the moderate and severe to critical cases

Abbreviations: IQR, Interquartile Range; M, male; F, female; BMI, Body Mass Index; SD, Standard Deviation; CAD, Coronary Artery Disease; COPD, Chronic Obstructive Pulmonary Disease; PT, Prothrombin Time; PTT, Partial Thromboplastin Time; CRP, C Reactive Protein; ESR, Erythrocyte Sedimentation Rate

The p value significance showe in bold formate

The results showed that the median age of patients in intensive care unit is significantly higher than that of infectious disease ward (72 yrs. [IQR: 36] vs. 58.5 yrs.; [IQR: 28]; $p=0.006$). Moreover, ICU-admitted patients compared to other ones had a higher percentage of COPD (22% vs. 5.8%; $p=0.004$). Besides these results, it has been found that 9.64% (19 out of 197) of the COVID-19 study population died during follow-up. Mann-Whitney U analysis showed that the median age of the non-survivors was significantly higher than that of the survivors (73 yrs. [IQR: 29] vs. 60 yrs. [IQR: 29]; $p=0.002$). In addition, there was a significant difference in the mean BMI between the non-survivors (23.78 ± 5 kg/m^2) and survivors (26.73 ± 7.3 kg/m^2) ($p=0.023$). We also compared in these subgroups the laboratory and clinical parameters. The results showed an elevation in the time of coagulation test (prothrombin time (PT)) and a higher percentage of COPD in the non-survivor group ($p=0.015$ and $p=0.05$, respectively).

In the next part, the genotype distribution and the allele frequency of ACE2 rs2285666 and ACE1 rs4646994 polymorphisms in COVID-19 subjects and the control group were analyzed (Table 2). In COVID-19 patients, the DD, ID and II genotype frequencies of ACE1 gene polymorphism were 48.4%, 33.1% and 18.5% respectively whereas the genotypes in control group were distributed as follows: 35.5%, 48.4% and 16.1%. There was a statistically significant difference in ACE1 genotypes distribution between the patients and control individuals ($p=0.01$). No significant differences in ACE1 allelic frequencies between COVID-19 patients and controls were observed. As shown in Table 2, no statistically significant difference in genotype and allelic distribution of ACE2 rs2285666 was observed between cases

Table 2 Genotype and allele distribution of ACE1 and ACE2 genes in the control and case groups and according to the severity of the disease in the case group

SNP	Control N (%)	Covid-19 cases N (%)	*P value*	COVID-19 cases ($n=197$)		*P value*
				Moderate (n=154) %	severe to critical (n=43) %	
Rs4646994[1]						
DD	66 (35.5)	89 (48.4)	**0.01**	69 (48.3)	20 (48.8)	0.737
ID	90 (48.4)	61 (33.1)		46 (32.1)	15 (36.6)	
II	30 (16.1)	34 (18.5)		28 (19.6)	6 (14.6)	
I	150 (40.3)	129 (35.1)	0.139	102 (35.7)	27 (32.9)	0.647
D	222 (59.7)	239 (64.9)		184 (64.3)	55 (67.1)	
Rs2285666[2]						
CC	50 (54.3)	41 (50)	0.694	31 (50.8)	10 (47.6)	0.583
CT	36 (39.1)	37 (45.1)		26 (42.6)	11 (52.4)	
TT	6 (6.5)	4 (4.9)		4 (6.6)	0 (0)	
C	272 (70.8)	281 (73.6)	0.399	224 (74.2)	57 (67.8)	0.249
T	112 (29.2)	101 (26.4)		78 (25.8)	27 (32.2)	

The p values indicate the statistical significance between the moderate and severe to critical cases

1. There were 13 and 11 participants with missing data for rs4646994 polymorphism in COVID-19 patients and control group, respectively

2. There were 4 and 5 pm in COVID-19 patients and control group, respectively

The p value significance showe in bold formate

and controls ($p>0.05$). We observed that the ACE1 DD genotype increased the risk of COVID-19 infection in a dominant genetic model (OR = 1.703; 95% CI: 1.123–2.584, $p=0.012$, DD vs. II + ID; significance level: 0.017). In addition, it has been demonstrated that the ACE1 ID genotype decreases the risk of COVID-19 infection in a co-dominant model (OR = 0.529; 95% CI: 0.347–0.806, $p=0.003$, ID vs. DD + II; significance level: 0.017). As shown in Table 2, no statistically significant difference in genotype and allelic distribution of ACE2 rs2285666 was observed between cases and controls ($p>0.05$). Because of the ACE2 gene location on the X chromosome, HWE for ACE2 rs2285666 was only tested in women and indicated no significant deviation from expectations ($p=0.884$). The distribution of ACE1 genotypes in patients and control individuals was compatible with HWE ($p=0.195$).

As presented in Table 2, the statistical comparison drawn among patients clarified no significant differences in genotype and allele frequencies of both mentioned polymorphisms between the two subgroups classified according to the disease severity (moderate COVID-19 patients vs. severe to critical ones). Moreover, we investigated the variants among survivors and non-survivors and demonstrated that the genotype and allelic distribution of both polymorphisms show no statistically significant differences (Table 3).

Discussion

In the present the study, we analyzed the association of ACE1 rs4646994 and ACE2 rs2285666 with COVID-19 susceptibility and severity. We observed that the frequency of DD genotype of ACE1 rs4646994 was higher in COVID-19 patients compared to control individuals. No statistically significant difference in genotype and allelic distribution of ACE2 rs2285666 polymorphism was observed between

Table 3 Genotype and allele distribution of ACE1 and ACE2 patients according to the disease mortality

SNP Genotype/allele	COVID-19 cases ($n=197$)		*P value*
	non-survivors (n = 19) %	survivors (n = 178) %	
Rs4646994[1]			
DD	11 (57.9)	78 (47.3)	0.09
ID	8 (42.1)	53 (32.1)	
II	0 (0)	34 (20.6)	
I	8 (21.1)	121 (36.7)	**0.056**
D	30 (78.9)	209 (63.3)	
Rs2285666[2]			
CC	3 (33.3)	38 (52.1)	0.470
CT	6 (66.7)	31 (42.5)	
TT	0 (0)	4 (5.5)	
C	24 (66.7)	257 (73.4)	0.385
T	12 (33.3)	93 (26.6)	

The p values indicate the statistical significance between the non-survivors and survivors

1. There were 13 participants with missing data for rs4646994 polymorphism in COVID-19 patient

2. There were 4 participants with missing data for rs2285666 polymorphism in COVID-19 patient

The p value significance showe in bold formate

COVID-19 patients and the control group. We did not observe any association between these polymorphisms and the risk of severe disease.

There is evidence that RAS plays pivotal roles in COVID-19 pathogenesis. A well-known example of this is the involvement of ACE2 enzyme as the cellular receptor of SARS-CoV2 that its expression regulating the individuals' susceptibility to the infection. In addition, the literature on COVID-19 has highlighted the ACE1/ACE2 activity imbalance, which could play a role in COVID-19 severity. Previous studies have also explored the relationships between the functional ACE1/ACE2 genes polymorphisms and the risk of comorbidities, such as cardiovascular and pulmonary diseases that their findings shed new lights on the RAS contributions to the susceptibility and outcomes of COVID-19. The results of present study suggest that the DD variant of ACE1 rs4646994 polymorphism might be regarded as a risk factor for COVID-19 infection; therefore, in principle, in areas with a high prevalence of DD genotype, the administration of ACE1 inhibitors would modulate the impact of effective phenotype (DD genotype; the highest ACE1 activity). This work contributes to existing knowledge of the DD genotype involvement in HTN and increased ACE1 activity by providing the mentioned result. Noticeably, it concludes that an overly activated ACE1 enzyme induced by inherited predispositions, such as ACE1 rs4646994, in combination with various risk factors (e.g. advanced age) might lead to an organ dysfunction through ACE2 receptor suppression by SARS-CoV-2 and the destroyed ACE1/ACE2 equilibrium. On the contrary, variants of ACE2 rs2285666 polymorphism do not seem to be considered a risk factor for COVID-19 infection and have no impact on COVID-19 disease susceptibility in this study. Since the frequency of ID genotype was higher in control individuals in the present study, it seems that this genotype has a protective role against COVID-19 infection. It is interesting to note that the protective role of the ID genotype against COVID-19 emerged in the study might be on account of the decreased expression levels of ACE1 enzyme in comparison with the DD genotype in individuals. These findings can at least justify a part of the different susceptibility of people to COVID-19 infection.

In a study by Kouhpayeh et al. in the southeast of Iran (Kouhpayeh et al. 2021), the II genotype of ACE1 rs4646994 polymorphism and the I allele decreased the risk of COVID-19 infection; in contrast, in our study the ID genotype decreased the risk of COVID-19 infection and no significant difference in allelic distribution was found. The reason for this inconsistency can be related to the ethnic differences between the studied populations in two regions of Iran or the used techniques of investigating polymorphisms in two studies (RFLP versus ARMS-PCR). In both studies, there was not any association between this polymorphism and the severity of the disease. In both studies, a significantly higher age was observed in patients with severe disease than a non-severe disease.

The observed higher frequency of DD genotype of ACE1 rs4646994 polymorphism in the COVID-19 patients in our study was similar to that reported in a variety of other countries, including Brazil, Saudi Arabia, Finland, Russia, Spain, Italy and Turkey (Mir et al. 2021). In contrast, the II genotype of this polymorphism is the dominant genotype in East Asian populations (Korean, Chinese, Taiwanese and Japanese populations) (Saab et al. 2007). This suggests that the genotype distribution of ACE1 rs4646994 polymorphism varies between different populations and geo-

graphic regions; this variation can explain at least part of the observed differences in COVID-19 disease prevalence in different countries.

As previously reported, individuals with the DD genotype have significantly higher blood ACE1 levels compared to the I/D and I/I genotypes (Rigat et al. 1990; Gard 2010). In COVID-19 patients, high levels of ACE1 enzyme in the blood of individuals with the DD genotype might lead to the production of Ang II. In addition, the down-regulation of ACE2 caused by the binding of the SARS-CoV-2 spike protein increases Ang II and its consequent harmful effects (Soy et al. 2020; Imai et al. 2005). Studies performed on Asians showed that the patients with the low DD genotype and a higher II genotype frequency have a statistically significant lower mortality (Pati et al. 2020; Yamamoto et al. 2020; Verma et al. 2021). Our findings support the work of mentioned research but in this area linking D allele with mortality, even though ACE1 tends to be distinctively distributed between the survivors and non-survivors despite it is not statistically significant (p value = 0.056). It is not far from the expectation that with the increase of the studied population, the results will become significant. In the studies by Gómez et al. and Karakas et al., in agreement with our findings, they reported that the ACE1 rs4646994 polymorphism was not associated with the severity of COVID-19 infection (Martínez-Gómez et al. 2022; Karakaş Çelik et al. 2021).

In contrast to our findings, in a study by Srivastava et al. on Indian population, it has been reported there is a significant correlation between the alternate allele (T-plus strand or A-minus strand) of ACE2 rs2285666 polymorphism and a lower COVID-19 infection as well as case-fatality rate. This inconsistency can be attributed to ethnic differences or the impact of other risk factors, variables and environmental conditions on the disease susceptibility.

Comparison of the findings with those of other studies confirms that older age and comorbidities, such as COPD correlate with increased risk of COVID-19 severity and mortality (Verdecchia et al. 2020; Lippi and Henry 2020).

Conclusion

In conclusion, this study strengthens the idea that there is a link between genetic factors and COVID-19. Taken together, ACE1 gene ID polymorphism should be investigated as a predictive biomarker. It has also proven beneficial to COVID-19 pandemic management. To achieve a clear understanding of this field, further research on various ethnicities is recommended.

Acknowledgements This work was supported by the Birjand University of Medical Sciences, Birjand, Iran (Grant number: 5385). The authors would like to sincerely thank all participants who willingly participated in this research.

Author Contributions All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by H.A., H.K.R., F.M., S.Z., M.R.M., S.G., D.J., and E.M.M. The first draft of the manuscript was written by E.M.M. and H.A. and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Funding This work was supported by the Birjand University of Medical Sciences, Birjand, Iran (Grant number: 5385).

Data Availability The manuscript has no associated data.

Declarations

Ethical Approval The Ethics Committee of Birjand University of Medical Sciences, Birjand, Iran approved the study (Ethical code: IR.BUMS.REC.1399.060).

Consent to Participate A written informed consent was obtained from all participants in this study.

Consent for Publication Not applicable.

Competing Interests The authors declare no competing interests.

References

Bakhshandeh B, Sorboni SG, Javanmard A-R, Mottaghi SS, Mehrabi M-R, Sorouri F, Abbasi A, Jahanafrooz Z (2021) Variants in ACE2; potential influences on virus infection and COVID-19 severity. Infect Genet Evol 90:104773

Chan KA, Tang NL, Hui DS, Chung GT, Wu AK, Chim SS, Chiu RW, Lee N, Choi K, Sung YM (2005) Absence of association between angiotensin converting enzyme polymorphism and development of adult respiratory distress syndrome in patients with severe acute respiratory syndrome: a case control study. BMC Infect Dis 5:1–6

Chaoxin J, Daili S, Yanxin H, Ruwei G, Chenlong W, Yaobin T (2013) The influence of angiotensin-converting enzyme 2 gene polymorphisms on type 2 diabetes mellitus and coronary heart disease. European Review for Medical & Pharmacological Sciences, p 17

Coates D (2003) The angiotensin converting enzyme (ACE). Int J Biochem Cell Biol 35:769–773

Delanghe JR, De Buyzere ML, Speeckaert MM (2020) ACE1 polymorphisms are more important confounders in the spread and outcome of COVID-19 in comparison with ABO polymorphism. Eur J Prev Cardiol 27:1331–1332

Devaux CA, Rolain J-M, Raoult D (2020) ACE2 receptor polymorphism: susceptibility to SARS-CoV-2, hypertension, multi-organ failure, and COVID-19 disease outcome. J Microbiol Immunol Infect 53:425–435

Fang X, Li S, Yu H, Wang P, Zhang Y, Chen Z, Li Y, Cheng L, Li W, Jia H (2020) Epidemiological, comorbidity factors with severity and prognosis of COVID-19: a systematic review and meta-analysis. Aging 12:12493

Gao Yd, Ding M, Dong X, Zhang Jj, Kursat Azkur A, Azkur D, Gan H, Sun Yl, Fu W, Li W (2021) Risk factors for severe and critically ill COVID-19 patients: a review. Allergy 76:428–455

Gard PR (2010) Implications of the angiotensin converting enzyme gene insertion/deletion polymorphism in health and disease: a snapshot review. Int J Mol Epidemiol Genet 1:145

Gemmati D, Bramanti B, Serino ML, Secchiero P, Zauli G, Tisato V (2020) 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 21:3474

Gómez J, Albaiceta GM, García-Clemente M, López-Larrea C, Amado-Rodríguez L, Hermida T, Enriquez AI, Herrero P, Melón S (2020) M.E. Alvarez-Argüelles, Angiotensin-converting enzyme (ACE1, ACE2) gene variants are associated with COVID19 severity depending on the hypertension status, MedRxiv, 2020.2006. 2011.20128033

Hamming I, Cooper ME, Haagmans BL, Hooper NM, Korstanje R, Osterhaus AD, Timens W, Turner A, Navis G, van Goor H (2007) The emerging role of ACE2 in physiology and disease. J Pathology: J Pathological Soc Great Br Irel 212:1–11

Harrison AG, Lin T, Wang P (2020) Mechanisms of SARS-CoV-2 transmission and pathogenesis. Trends Immunol 41:1100–1115

Hoffmann M, Kleine-Weber H, Schroeder S, Krüger N, Herrler T, Erichsen S, Schiergens TS, Herrler G, Wu N-H, Nitsche A (2020) SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor, cell, 181 271–280. e278

Hu Y, Sun J, Dai Z, Deng H, Li X, Huang Q, Wu Y, Sun L, Xu Y (2020) Prevalence and severity of corona virus disease 2019 (COVID-19): a systematic review and meta-analysis. J Clin Virol 127:104371

Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, Zhang L, Fan G, Xu J, Gu X (2020) Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China, the lancet. 395:497–506

Imai Y, Kuba K, Rao S, Huan Y, Guo F, Guan B, Yang P, Sarao R, Wada T (2005) Leong-Poi, Angiotensin-converting enzyme 2 protects from severe acute lung failure. Nature 436:112–116

Itoyama S, Keicho N, Quy T, Phi NC, Long HT, Van Ban V, Ohashi J, Hijikata M, Matsushita I, Kawana A (2004) ACE1 polymorphism and progression of SARS, biochemical and biophysical research communications. 323:1124–1129

Jerng J-S, Yu C-J, Wang H-C, Chen K-Y, Cheng S-L, Yang P-C (2006) Polymorphism of the angiotensin-converting enzyme gene affects the outcome of acute respiratory distress syndrome. Crit Care Med 34:1001–1006

Karakaş Çelik S, Çakmak Genç G, Pişkin N, Açikgöz B, Altinsoy B, Kurucu İşsiz B, Dursun A (2021) Polymorphisms of ACE (I/D) and ACE2 receptor gene (Rs2106809, Rs2285666) are not related to the clinical course of COVID-19: a case study. J Med Virol 93:5947–5952

Kenyon C (2020) ACE-1 I/D polymorphism associated with COVID-19 incidence and mortality. An ecological study

Kouhpayeh HR, Tabasi F, Dehvari M, Naderi M, Bahari G, Khalili T, Clark C, Ghavami S, Taheri M (2021) Association between angiotensinogen (AGT), angiotensin-converting enzyme (ACE) and angiotensin-II receptor 1 (AGTR1) polymorphisms and COVID-19 infection in the southeast of Iran: a preliminary case-control study. Translational Med Commun 6:1–10

Kuba K, Imai Y, Rao S, Gao H, Guo F, Guan B, Huan Y, Yang P, Zhang Y, Deng W (2005) A crucial role of angiotensin converting enzyme 2 (ACE2) in SARS coronavirus–induced lung injury. Nat Med 11:875–879

Li Y-y (2012) Lack of association of ACE2 G8790A gene mutation with essential hypertension in the Chinese population: a meta-analysis involving 5260 subjects. Front Physiol 3:364

Lippi G, Henry BM (2020) Chronic obstructive pulmonary disease is associated with severe coronavirus disease 2019 (COVID-19), respiratory medicine. 167:105941

Liu Y, Yang Y, Zhang C, Huang F, Wang F, Yuan J, Wang Z, Li J, Li J, Feng C (2020) Clinical and biochemical indexes from 2019-nCoV infected patients linked to viral loads and lung injury. Sci China Life Sci 63:364–374

Marshall RP, Webb S, Bellingan GJ, Montgomery HE, Chaudhari B, McAnulty RJ, Humphries SE, Hill MR, Laurent GJ (2002) Angiotensin converting enzyme insertion/deletion polymorphism is associated with susceptibility and outcome in acute respiratory distress syndrome. Am J Respir Crit Care Med 166:646–650

Martínez-Gómez LE, Herrera-López B, Martinez-Armenta C, Ortega-Peña S, Camacho-Rea MdC, Suarez-Ahedo C, Vázquez-Cárdenas P, Vargas-Alarcón G, Rojas-Velasco, J.M.J.F.i.i. (2022). Fragoso, ACE and ACE2 gene variants are Associated with severe outcomes of COVID-19 in men. 450.

Mi J, Zhong W, Huang C, Zhang W, Tan L, Ding L (2020) Gender, age and comorbidities as the main prognostic factors in patients with COVID-19 pneumonia. Am J Translational Res 12:6537

Mir MM, Mir R, Alghamdi MAA, Alsayed BA, Wani JI, Alharthi MH, Al-Shahrani AM (2021) Strong association of angiotensin converting enzyme-2 gene insertion/deletion polymorphism with susceptibility to SARS-CoV-2, hypertension, coronary artery disease and COVID-19 disease mortality. J Personalized Med 11:1098

Mizuiri S, Hemmi H, Kumanomidou H, Iwamoto M, Miyagi M, Sakai K, Aikawa A, Ohara T, Yamada K, Shimatake H (2001) Angiotensin-converting enzyme (ACE) I/D genotype and renal ACE gene expression. Kidney Int 60:1124–1130

Öztürk R (2020) COVID-19: pathogenesis, genetic polymorphism, clinical features and laboratory findings. Turk J Med Sci 50:638–657

Pati A, Mahto H, Padhi S, Panda AK (2020) ACE deletion allele is associated with susceptibility to SARS-CoV-2 infection and mortality rate: an epidemiological study in the Asian population. Clin Chim Acta 510:455–458

Pinheiro DS, Santos RS, Jardim PCV, Silva EG, Reis AA, Pedrino GR, Ulhoa CJ (2019) The combination of ACE I/D and ACE2 G8790A polymorphisms revels susceptibility to hypertension: a genetic association study in Brazilian patients. PLoS ONE 14:e0221248

Pouladi N, Abdolahi S (2021) Investigating the ACE2 polymorphisms in COVID-19 susceptibility: an in silico analysis. Mol Genet Genom Med 9:e1672

Rigat B, Hubert C, Alhenc-Gelas F, Cambien F, Corvol P, Soubrier F (1990) An insertion/deletion polymorphism in the angiotensin I-converting enzyme gene accounting for half the variance of serum enzyme levels. J Clin Investig 86:1343–1346

Saab Y, Gard P, Overall A (2007) The geographic distribution of the ACE II genotype: a novel finding. Genet Res 89:259–267

Saengsiwaritt W, Jittikoon J, Chaikledkaew U, Udomsinprasert W (2022) Genetic polymorphisms of ACE1, ACE2, and TMPRSS2 associated with COVID-19 severity: a systematic review with meta-analysis. Rev Med Virol 32:e2323

Sanyaolu A, Okorie C, Marinkovic A, Patidar R, Younis K, Desai P, Hosein Z, Padda I, Mangat J, Altaf M (2020) Comorbidity and its impact on patients with COVID-19, SN comprehensive clinical medicine, 2 1069–1076

Soy M, Keser G, Atagündüz P, Tabak F, Atagündüz I, Kayhan S (2020) Cytokine storm in COVID-19: pathogenesis and overview of anti-inflammatory agents used in treatment. Clin Rheumatol 39:2085–2094

Tikellis C, Thomas M (2012) Angiotensin-converting enzyme 2 (ACE2) is a key modulator of the renin angiotensin system in health and disease, International journal of peptides, (2012)

Verdecchia P, Cavallini C, Spanevello A, Angeli F (2020) The pivotal link between ACE2 deficiency and SARS-CoV-2 infection. Eur J Intern Med 76:14–20

Verma S, Abbas M, Verma S, Khan FH, Raza ST, Siddiqi Z, Ahmad I, Mahdi F (2021) Impact of I/D polymorphism of angiotensin-converting enzyme 1 (ACE1) gene on the severity of COVID-19 patients. Infect Genet Evol 91:104801

Wang D, Hu B, Hu C, Zhu F, Liu X, Zhang J, Wang B, Xiang H, Cheng Z, Xiong Y (2020a) Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus–infected pneumonia in Wuhan. China Jama 323:1061–1069

Wang K, Gheblawi M, Oudit GY (2020b) Angiotensin converting enzyme 2: a double-edged sword. Circulation 142:426–428

Wei P-F (2020) Diagnosis and treatment protocol for novel coronavirus pneumonia (trial version 7). Chin Med J 133:1087–1095

Wu YH, Li JY, Wang C, Zhang LM, Qiao H (2017) The ACE 2 G8790A polymorphism: involvement in type 2 diabetes mellitus combined with cerebral stroke. J Clin Lab Anal 31:e22033

Yamamoto N, Ariumi Y, Nishida N, Yamamoto R, Bauer G, Gojobori T, Shimotohno K, Mizokami M (2020) SARS-CoV-2 infections and COVID-19 mortalities strongly correlate with ACE1 I/D genotype. Gene 758:144944

Yamamoto N, Nishida N, Yamamoto R, Gojobori T, Shimotohno K, Mizokami M, Ariumi Y (2021a) Angiotensin-Converting Enzyme (ACE) 1 Gene Polymorphism and Phenotypic Expression of COVID-19 Symptoms, Genes, 12

Yamamoto N, Yamamoto R, Ariumi Y, Mizokami M, Shimotohno K, Yoshikura H (2021b) Does genetic predisposition contribute to the exacerbation of COVID-19 symptoms in individuals with comorbidities and explain the huge mortality disparity between the East and the West? Int J Mol Sci 22:5000

Yang M, Zhao J, Xing L, Shi L (2015) The association between angiotensin-converting enzyme 2 polymorphisms and essential hypertension risk: a meta-analysis involving 14,122 patients. J Renin-Angiotensin-Aldosterone Syst 16:1240–1244

Zhang H, Penninger JM, Li Y, Zhong N, Slutsky AS (2020) Angiotensin-converting enzyme 2 (ACE2) as a SARS-CoV-2 receptor: molecular mechanisms and potential therapeutic target. Intensive Care Med 46:586–590

Zhu N, Zhang D, Wang W, Li X, Yang B, Song J, Zhao X, Huang B, Shi W, Lu R (2020) A novel coronavirus from patients with pneumonia in China, 2019. New England journal of medicine

Authors and Affiliations

Hamid Abbaszadeh[1] · Hamid Kabiri-Rad[2] · Fariba Mohammadi[2] · Soheila Zangoie[3] · Mahdieh Rajabi-Moghaddam[4] · Shokouh Ghafari[5] · Masood Ziaee[6] · Davod Javanmard[6] · Ebrahim Miri-Moghaddam[7]

✉ Ebrahim Miri-Moghaddam
miri4@bums.ac.ir

1 Department of Oral and Maxillofacial Pathology, Faculty of Dentistry, Birjand University of Medical Sciences, Birjand, Iran

2 Cellular and Molecular Research Center, Birjand University of Medical Sciences, Birjand, Iran

3 Department of Biochemistry, Institute of Biochemistry and Biophysics, University of Tehran, Tehran, Iran

4 Department of Pathology, Faculty of Medicine, Birjand University of Medical Sciences, Birjand, Iran

5 Cellular and Molecular Research Center, Faculty of Medicine, Shahrekord University of Medical Sciences, Shahrekord, Iran

6 Infectious Diseases Research Center, Birjand University of Medical Sciences, Birjand, Iran

7 Department of Molecular Medicine, Faculty of Medicine, Cardiovascular Diseases Research Center, Birjand University of Medical Sciences, Birjand, Iran, 9717853577