IJTMGH
International Journal of Travel Medicine and Global Health

Int J Travel Med Glob Health. 2022 Sep;10(3):122-126
http://ijtmgh.com

doi 10.34172/ijtmgh.2022.22

Original Article

Open Access

Investigation of Polymorphisms of ACEII Gene in People With Coronavirus With Severe and Mild Symptoms or Asymptomatic

Fatemeh Arab[1], Mozhdeh Jafari Rad[2], Seyed-Alireza Esmaeili[2,3], Ali Mirhosseini[4], Mehran Moharreri[4], Ehsan Saburi[1,5*]

[1]Department of Medical Genetics and Molecular Medicine, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
[2]Immunology Research Center, Mashhad University of Medical Sciences, Mashhad, Iran
[3]Immunology Department, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
[4]Immunology Research Center, Inflammation and Inflammatory Diseases Division, Mashhad University of Medical Sciences, Mashhad, Iran
[5]Medical Genetic Research Center, Mashhad University of Medical Sciences, Mashhad, Iran

Corresponding Author: Ehsan Saburi, MD, Assistance Professor of Molecular Medicine, Medical Genetics and Molecular Medicine Department, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran. Tel: +98-9151048897, Email: Ehsansaburi@yahoo.com

Received May 6, 2022; **Accepted** July 25, 2022; **Online Published** August 11, 2022

Abstract

Introduction: Angiotensin-converting enzyme 2 (ACE2) is the central receptor of coronavirus disease 2019 (COVID-19) in host cells. Genetic polymorphisms in the ACE2 gene may promote cardiovascular disease and systemic inflammatory injury in a patient affected by COVID-19. Thus, the genetic background may account for the substantial inter-individual diversity in illness susceptibility or severity. Our study was conducted to find a significant relationship between ACE2 rs4646142 and rs2285666 polymorphisms and susceptibility to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).

Methods: In this study, we randomly selected 230 samples, including 76 patients with severe clinical symptoms and 154 patients with mild clinical symptoms (the positive case of COVID-19 was confirmed by real-time reverse transcriptase polymerase chain reaction [RT-PCR] assay). Then, we performed DNA extraction and investigated the polymorphisms of rs2285666 and rs4646142 by RFLP-PCR method with TaqI and Alu1 restriction enzymes.

Results: The study population included 107 men and 123 women, and the mean (±SD) age of the participants was 42.66±10.2. First, the levels of IgM and IgG were examined, and a significant association was observed in the level of IgM between the two groups of COVID-19 patients with mild and severe symptoms, as opposed to IgG. Meanwhile, no significant difference was observed between ACE2 rs4646142 and rs2285666 polymorphisms and the severity of COVID-19.

Conclusion: To better understand the genetic variations in people's susceptibility to COVID-19, this study was designed to evaluate the association between various ACE2 polymorphisms and the infection risk of SARS-CoV-2. However, no statistical difference was discovered.

Keywords: COVID-19, ACE2 Polymorphism, SNPs, rs2285666 Polymorphism, rs4646142 Polymorphism

Citation: Arab F, Jafari Rad M, Esmaeili SA, Mirhosseini A, Moharreri M, Saburi E. Investigation of polymorphisms of ACEII gene in people with coronavirus with severe and mild symptoms or asymptomatic. Int J Travel Med Glob Health. 2022;10(3):122-126. doi:10.34172/ijtmgh.2022.22.

Introduction

Coronaviruses are pathogens affecting both humans and animals. At the end of 2019, a new type of coronavirus was identified that caused numerous cases of pneumonia in Wuhan, a city in China.[1] This virus spread quickly and caused an epidemic in China. As of May 17, 2022, more than 519105112 confirmed cases of Corona and 6266324 confirmed deaths have been reported.[2] The World Health Organization (WHO) named this disease COVID-19, which is short for coronavirus disease 2019.[3] Several risk factors have been proposed concerning the host and COVID-19 disease, including the patient's age, diabetes, cardiovascular diseases, malignancy, lung diseases, and immune system weakness.[4,5] The mechanism of SARS-Cov-2 disease depends on entry through angiotensin-converting enzyme 2 (ACE2). In this context, several studies have been performed on this enzyme and its effects on the severity of COVID-19.[6] It was proposed that higher COVID-19 infection susceptibility is relevant to the expression of the target ACE2 receptor in the virus-exposed epithelium.[7]

The ACE2 enzyme, as a surface receptor, plays a role in many physiological functions.[8] The highest expression of ACE2 is

found in kidney, small intestine, heart, thyroid, adipose tissue, lung, etc.[9] ACE2 is located as an ectoenzyme on the surface of endothelium and other cells. Although apparently, the main substrate of this enzyme is angiotensin II, it is known that this enzyme may have other physiological substrates. As a result, it can perform various functions in different places.[10] Regulation of ACE2 expression and function is important for the the treatment of many diseases, including hypertension, cardiovascular disease, diabetes, lung injuries, and fibrotic diseases.[11] Research on ACE2 polymorphisms and their relationship with COVID-19 shows that polymorphisms of this gene can be effective in cardiovascular and pulmonary diseases by changing the level of ACE2 expression.[12] The risk of hospitalization and death in those exposed to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is substantially correlated with hypertension and cardiovascular illness, which are prominent comorbidities in COVID-19.[13]

Determining the elements that contribute to COVID-19's development or severe COVID-19 in some people will help to ascertain the disease's pathophysiology and implement the most effective new treatment strategies because the genetic or environmental factors that increase susceptibility to SARS-CoV-2 remain unclear. To this end, the current study aimed to investigate the existence of common ACE2 gene polymorphisms, i.e., rs4646142 and rs2285666, to find an effective relationship between the severity of COVID-19 and these polymorphisms.

Methods

In this study, a group of 230 subjects was selected in a case-control study, including 76 patients with severe clinical symptoms of coronavirus admitted to Ghaem Hospital and 154 patients with a definite diagnosis of COVID-19 with mild symptoms. Inclusion criteria included being over the age of 20, being hospitalized at Ghaem Hospital in Mashhad, and having a definite diagnosis of COVID-19 based on a positive RT-PCR (reverse transcriptase polymerase chain reaction) result for nasal and pharyngeal swab samples. Exclusion criteria included the death of the patient before taking the sample; lack of patient satisfaction at any stage of the study; underlying diseases such as diabetes, high blood pressure, pulmonary and renal diseases, malignancies, and immune system defects; age less than 20 years; and over 60 years old.

DNA Extraction and Genotyping

In tubes containing EDTA, 5 mL of blood was collected from each patient to extract genomic DNA (Pars Tous, Mashhad, Iran) from the whole blood. A 2 % (w/v) agarose gel electrophoresis and the absorbance at 260/280 nm were used to assess the DNA yield and purity. Genotyping of ACE2 rs4646142 and rs2285666 polymorphisms was performed by the PCR and restriction fragment length polymorphism (RFLP).

PCR and RFLP

PCR was carried out with particular primers in a final volume of 12 µL for each polymorphism, containing PCR master mix, 2x: 6 µL, forward primer, 10 µM: 0.25 µL, and reverse primer, 10 µM: 0.25 µL, DNA template, 2 µL, and nuclease-free water, 3.5 µL (Table 1). Taq1 restriction enzyme was utilized to identify rs4646142 polymorphism. Following digestion, the G allele is identified by the 311 and 108 bp fragments, while the C allele is identified by the 419 bp fragment (GG: 311+ 108 bp CC: 419 bp GC: 419+311+ 108 bp). And Alu1 restriction enzyme was used to identify rs2285666 polymorphism. After incubation for 16 h at 37°C, fragments of 281 and 185 bp identify the T allele, and a band of 466 bp identifies the C allele (CC: 466 bp TT: 281+185bp CT: 466+281+ 185bp).

Statistical Analysis

Statistical analysis of the data was carried out using SPSS version 16 software with Student *t* test, and Mann-Whitney U statistical tests. Using the chi-square test, the Hardy-Weinberg equilibrium was evaluated. *P* values ≤0.05 were considered statistically significant.

Results

This study consisted of 107 men and 123 women, and the mean (±SD) age of the participants was 42.66±10.2. The oldest patient was 66 years old, and the youngest was 20 years old. Also, the mean (±SD) body mass index (BMI) of patients was 28.24±4.63. As we expected, there was no significant difference in age, sex, and BMI between the two groups of patients, one with severe symptoms and the other with mild symptoms. In addition, all patients were checked for their IgM and IgG levels. However, as shown in Table 2, there was a significant difference in IgM levels between these two groups in contrast with IgG levels.

The distribution of all genotypes for rs2285666 polymorphism of ACE2 gene is represented in Table 3. Allele and genotypes frequencies of rs2285666 polymorphism in the two groups of severe and mild COVID-19 are displayed in Table 3. There was no statistically significant association between the two groups based on genotypes and allele frequency for rs2285666 polymorphism ($P=0.58$).

Because the ACE2 gene is located on chromosome X, Hardy-Weinberg principle cannot be applied to this gene at this sample size. On the other hand, in inheritance models, rs2285666 polymorphism was not statistically correlated with COVID-19 in inheritance models (Table 4).

As shown in Table 5, there was also no significant difference in genotypes and allele frequencies for rs4646142 polymorphism ($P=0.37$). The distribution of each genotype for the rs4646142 polymorphism is shown in Table 5. In addition, the haplotype analysis between these two groups showed no significant relevance between haplotypes and severity of COVID-19 in patients (Table 6).

Discussion

COVID-19 caused a great pandemic, resulting in millions of deaths. Apart from advances in infectious disease control, treatment, risk factors, and pathology, there is still no exclusive treatment for COVID-19.[14] Numerous studies point to ACE2 as the main COVID-19 receptor in host cells.[15] Some specific genetic changes in the ACE2 sequence may affect the ability of the virus to enter the cell more effectively by modifying its

expression level or increasing its binding affinity for SARS-CoV-2.[16] The site of ACE2 rs2285666 polymorphism is in the intronic-consensus splicing nucleotides, and could thus affect the processing of ACE2 total RNA to mRNA and, ultimately, is the volume of produced protein.[17] Thus, numerous SNPs in the ACE2 gene have been examined for a possible correlation with susceptibility to COVID-19.[18-20] Despite the studies conducted on different types of ACE2 and its effect on susceptibility to COVID-19 in diverse populations, knowledge in this field is still limited. Our study was conducted to find a significant relationship between ACE2 rs4646142 and rs2285666 polymorphisms and susceptibility to SARS-CoV-2. But unfortunately, no correlation was found.

The present study investigated the rs2285666 polymorphism of the ACE2 gene in patients with mild and severe COVID-19. rs2285666 polymorphism showed no significant correlation with the susceptibility to COVID-19 ($P=0.58$). Several investigations have demonstrated a significant link between ACE2 polymorphism and COVID-19 susceptibility.[19,21] Gómez et al revealed that ACE1-DD genotype ($P=0.049$) could be linked to severe COVID-19. However, the ACE2 sequencing did not identify coding sequence variations that would account for an elevated risk of COVID-19 development. They claimed that hypertension affected the severity of COVID-19.[22] Another investigation explained the potential of the rs2285666 polymorphism to raise the risk of genetic susceptibility to COVID-19.[23] Möhlendick et al revealed that the G allele of ACE2 rs2285666 was strongly linked to a nearly two-fold more substantial risk of contracting SARS-CoV-2 infection and a three-fold increased risk of developing a severe illness or death by COVID-19.[24] These findings were verified by Sabater Molina et al. Their research revealed that different genetic variations of ACE2 were linked to the fatal clinical outcomes and mortality groups of COVID-19 patients.[25]

In contrast, Karakaş Çelik et al. found that the distribution of genotype and allele frequencies of the ACE2 receptor gene rs2285666 SNP was not statistically significant in COVID-19 patients.[26] Similarly, in the population studied by Pouladi et al, although they showed that different polymorphisms could have different susceptibilities to COVID-19, no statistical difference was discovered between the rs2285666 polymorphism and the risk of infection with SARS-CoV-2.[27] Differences in population size or ethnic features may help explain some of the discrepancies in the results.

Table 1. Primer Sequence for ACE2 Gene Polymorphisms (5'→3')

Polymorphisms	Primer Sequence
rs4646142	F: CGAAGACAGTAGTAGAAGGTTAGG R:TGGAGATTACCTGAGTTTTCC
rs2285666	F: CATGTGGTCAAAAGGATATCT R: AAAGTAAGGTTGGCAGACAT

Table 2. Comparison of Study Characteristics Based on Frequency

Variable	Severity of COVID-19		*P* Value
	Severe (n=76)	Mild (n=154)	
Gender			0.5[a]
Male	33	74	
Female	43	80	
Age (Mean ± SD)	41.58±10.30	43.19±9.86	0.96[b]
Serum immunoglobulin levels (Mean ± SD)			
IgG	1.35±2.49	1.44±2.71	0.33[b]
IgM	0.73±0.76	0.25±0.37	≤0.01[b]
BMI (Mean ± SD)	28.48±4.36	28.12±4.77	0.15[b]

[a] Pearson chi-square; [b] *t* test.

Table 3. Allele and Genotypes Frequencies of rs2285666

Group	A	G	A/A	G/A	G/G	*P* Value
Mild symptom	76	232	8	60	86	-
Severe symptom	28	124	2	24	50	-
All samples	104	356	10	84	136	0.58

Table 4. Inheritance Models of ACE Gene Polymorphism

Model	Genotype	Severity of COVID-19	Mild COVID-19	*P* Value	OR (95% CI)
Codominant	G/G	50(65.8%)	86(55.8%)	0.29	1
	G/A	24(31.6%)	60(39%)		0.69 (0.38-1.24)
	A/A	2(2.6%)	8(5.2%)		0.43(0.09-2.10
Dominant	G/G	50(65.8%)	86(55.8%)	0.15	1
	G/A-A/A	26(34.2%)	68(44.2%)		0.66 (0.37-1.16)
Recessive	G/G-G/A	74(97.4%)	146(94.8%)	0.35	1
	AA	2(2.6%)	8(5.2%)		0.49(0.10-2.38)
Overdominant	G/G-A/A	52(68.4%)	94(61%)	0.27	1
	G/A	24(31.6%)	60(39%)		0.72(0.40-1.29)
Log-additive	-	-	-	0.12	0.68(0.41-1.11)

Abbreviation: OR, odds ratio.

Study Limitation

One of the limitations of this study was the small sample size of patients. Therefore, further studies in this area in different populations seem necessary because understanding ACE2 gene polymorphisms and their effects on gene expression can be an explanation for some people with severe COVID-19 without underlying disease compared to similar people with mild or no symptoms.

Conclusion

Our study did not support the correlation between two ACE2 polymorphisms (rs4646142 and rs2285666) and severity of

Table 5. Allele and Genotypes Frequencies of rs4646142

Group	C	G	C/C	G/C	G/G	*P* Value
Mild symptom	28	280	0	28	126	-
Severe symptom	8	144	0	8	68	-
All samples	36	424	0	36	194	0.37

Table 6. Haplotype Analyses of ACE2 Gene Polymorphisms

Haplotype		OR	Frequency	*P* Value
Rs4646142	Rs2285666			
G	G	1	0.7195	-
G	A	0.77 (0.42-1.33)	0.2022	0.35
C	G	0.81 (0.28-2.28)	0.0544	0.68
C	A	0.03 (0.0-25125452.29)	0.0239	0.73

Abbreviation: OR, odds ratio.

Research Highlights

What Is Already Known?

Expression of the ACE2 receptor gene may influence susceptibility to COVID-19. There is no obvious evidence that polymorphisms of the ACE2 gene are directly related to the severity of COVID-19. Interindividual differences in COVID-19 severity might be related to epigenetic mechanisms of ACE2 receptor gene expression.

What Does This Study Add?

This study was performed to find a significant relationship between ACE2 polymorphisms and individual predisposition to COVID-19 severity. Our data suggest that the ACE2 rs4646142 and rs2285666 polymorphisms are not associated with the risk of developing severe COVID-19.

COVID-19. The survey of polymorphisms explains how the genetic background of people affects their vulnerability to diseases. Due to the inconsistency in the results of various studies, more research in polymorphisms is necessary. It is therefore suggested that this study be conducted in a larger population with different polymorphisms.

Authors' Contributions

FA, SAE and ES were involved in search strategy and drafting. FA, MJR, AM and MM were contributed in sample collection and laboratory operation. SAE and ES supervised the project, revised and edited the manuscript. All authors read and approved the final manuscript.

Conflict of Interest Disclosures

The authors declare that they have no competing interests.

Ethical Approval

An informed consent form was obtained from all patients before sampling. If the patient did not agree at any stage of the research, the patient sample was excluded from the study process. Current study was approved by Mashhad University of Medical Sciences ethics committee with code of IR.MUMS.REC.1399.259.

Funding/Support

The authors would like to appreciate Research Deputy of Mashhad University of Medical Sciences for supporting this study (IR.MUMS.REC.1399.259).

Acknowledgements

The authors thank Dr. Amir Reza Momeni Rustaee and Dr. Houshang Rafatpanah for their great help.

Reference

1. Dhama K, Khan S, Tiwari R, et al. Coronavirus disease 2019-COVID-19. Clin Microbiol Rev. 2020;33(4):e00028-20. doi:10.1128/cmr.00028-20.
2. World Health Organization (WHO). WHO Coronavirus (COVID-19) Dashboard. https://covid19.who.int/.
3. Chen Z, Zheng W, Wu Q, et al. Global diversity of policy, coverage, and demand of COVID-19 vaccines: a descriptive study. BMC Med. 2022;20(1):130. doi:10.1186/s12916-022-02333-0.
4. Rashedi J, Mahdavi Poor B, Asgharzadeh V, et al. Risk factors for COVID-19. Infez Med. 2020;28(4):469-474.
5. Saburi E, Abazari MF, Hassannia H, et al. The use of mesenchymal stem cells in the process of treatment and tissue regeneration after recovery in patients with COVID-19. Gene. 2021;777:145471. doi:10.1016/j.gene.2021.145471.
6. Bosso M, Thanaraj TA, Abu-Farha M, Alanbaei M, Abubaker J, Al-Mulla F. The two faces of ACE2: the role of ACE2 receptor and its polymorphisms in hypertension and COVID-19. Mol Ther Methods Clin Dev. 2020;18:321-327. doi:10.1016/j.omtm.2020.06.017.
7. Wan Y, Shang J, Graham R, Baric RS, Li F. Receptor recognition by the novel coronavirus from Wuhan: an analysis based on decade-long structural studies of SARS coronavirus. J Virol. 2020;94(7):e00127-20. doi:10.1128/jvi.00127-20.
8. Ashraf UM, Abokor AA, Edwards JM, et al. SARS-CoV-2, ACE2 expression, and systemic organ invasion. Physiol Genomics. 2021;53(2):51-60. doi:10.1152/physiolgenomics.00087.2020.
9. Ni W, Yang X, Yang D, et al. Role of angiotensin-converting enzyme 2 (ACE2) in COVID-19. Crit Care. 2020;24(1):422. doi:10.1186/s13054-020-03120-0.
10. Lubbe L, Cozier GE, Oosthuizen D, Acharya KR, Sturrock ED. ACE2 and ACE: structure-based insights into mechanism, regulation and receptor recognition by SARS-CoV. Clin Sci (Lond). 2020;134(21):2851-2871. doi:10.1042/cs20200899.
11. Turner AJ. ACE2 cell biology, regulation, and physiological functions. In: Turner AJ, Unger T, Steckelings UM, dos Santos RA, eds. The Protective Arm of the Renin Angiotensin System (RAS). New York: Elsevier; 2015. p. 185-189. doi: 10.1016/b978-0-12-801364-9.00025-0.
12. Gintoni I, Adamopoulou M, Yapijakis C. The impact of ACE and ACE2 gene polymorphisms in pulmonary diseases including COVID-19. In Vivo. 2022;36(1):13-29. doi:10.21873/invivo.12672.
13. Zhou F, Yu T, Du R, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. Lancet. 2020;395(10229):1054-1062. doi:10.1016/s0140-6736(20)30566-3.
14. Cucinotta D, Vanelli M. WHO declares COVID-19 a pandemic. Acta Biomed. 2020;91(1):157-160. doi:10.23750/abm.v91i1.9397.
15. Wu J, Deng W, Li S, Yang X. Advances in research on ACE2 as a receptor for 2019-nCoV. Cell Mol Life Sci. 2021;78(2):531-544. doi:10.1007/s00018-020-03611-x.
16. Fan Z, Wu G, Yue M, et al. Hypertension and hypertensive left ventricular hypertrophy are associated with ACE2 genetic polymorphism. Life Sci. 2019;225:39-45. doi:10.1016/j.lfs.2019.03.059.
17. Sama IE, Ravera A, Santema BT, et al. Circulating plasma concentrations of angiotensin-converting enzyme 2 in men and women with heart failure and effects of renin-angiotensin-aldosterone inhibitors. Eur Heart J. 2020;41(19):1810-1817. doi:10.1093/eurheartj/ehaa373.
18. Wooster L, Nicholson CJ, Sigurslid HH, Lino Cardenas CL,

Malhotra R. Polymorphisms in the ACE2 locus associate with severity of COVID-19 infection. medRxiv [Preprint]. June 22, 2020. Available from: https://www.medrxiv.org/content/10.1101/2020.06.18.20135152v1.
19. Djukic T, Stevanovic G, Coric V, et al. GSTO1, GSTO2 and ACE2 polymorphisms modify susceptibility to developing COVID-19. J Pers Med. 2022;12(3):458. doi:10.3390/jpm12030458.
20. Li J, Wang Y, Liu Y, et al. Polymorphisms and mutations of ACE2 and TMPRSS2 genes are associated with COVID-19: a systematic review. Eur J Med Res. 2022;27(1):26. doi:10.1186/s40001-022-00647-6.
21. Wang J, Zou H, Corpe C. An observed association between angiotensin-converting enzyme 2 polymorphisms and COVID-19 severity in China. J Infect. 2022;84(1):e21-e22. doi:10.1016/j.jinf.2021.10.020.
22. 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.
23. 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.
24. 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.
25. Sabater Molina M, Nicolás Rocamora E, Bendicho AI, et al. Polymorphisms in ACE, ACE2, AGTR1 genes and severity of COVID-19 disease. PLoS One. 2022;17(2):e0263140. doi:10.1371/journal.pone.0263140.
26. Karakaş Çelik S, Çakmak Genç G, Pişkin N, et al. 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. 2021;93(10):5947-5952. doi:10.1002/jmv.27160.
27. Pouladi N, Abdolahi S. Investigating the ACE2 polymorphisms in COVID-19 susceptibility: an in silico analysis. Mol Genet Genomic Med. 2021;9(6):e1672. doi:10.1002/mgg3.1672.