An analysis of the relationship between ACE2 genetic polymorphisms and the severity of COVID-19 disease in adult hospitalized patients - prospective observational study

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An analysis of the relationship between ACE2 genetic polymorphisms and the severity of COVID-19 disease in adult hospitalized patients - prospective observational study

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ABSTRACT

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) first emerged in Hubei province of China in December 2019 and spread rapidly to other parts of the world, causing the coronavirus disease 2019 (COVID-19). The angiotensin-converting enzyme-2 (ACE2) is recognized as the host receptor used by the SARS-CoV-2 virus to enter its target cells. Recent studies suggest that ACE2 gene polymorphisms might be candidates for genetic susceptibility of SARS-CoV-2 infection. The aim of the study was to evaluate the influence of ACE2 polymorphisms on COVID-19 disease risk and severity. In our study we confirmed that there is a statistically significant increased risk of a more severe disease course of SARS-CoV-2 infection associated with the need for hospitalization in the intensive care unit for patients with specific polymorphisms of the ACE2 gene. The most significant correlation was found for variant ACE2 rs2285666 (AA allele, OR=2.12, p=0.0189) and ACE2 rs2074192 (TT allele, OR=2.05,p=0.0016), and also for ACE2 rs4646174 (GG allele, OR=1.93, p=0.0016), ACE2 rs4646156 (TT allele OR=1.71, p=0.008) and ACE2 rs2158083 (TT allele OR=1.84, p=0.0025). In conclusion, our findings identify that certain ACE2 polymorphisms impact the severity of COVID-19 disease independently of other well-known risk factors.

Introduction

Severe acute respiratory syndrome virus 2 (SARS-CoV-2) has caused a global pandemic of respiratory illness, called Coronavirus disease 2019 (COVID-19). The disease first emerged in December 2019 in Wuhan Province of China, and in a few months, the virus spread rapidly to other parts of the world[1]. Based on the data from a COVID-19 Dashboard created by the Center for Systems Science and Engineering (CSSE) at Johns Hopkins University, at the time of writing this manuscript, there were over 244 million confirmed cases of SARS-CoV-2 infections and nearly 5 million deaths around the world.
SARS-CoV-2 belongs to a family of Coronaviruses, relatively large, enveloped viruses with non-segmental, positive-stranded RNA molecules. Most coronaviruses, such as 229E, NL63, HKU1, and OC43, typically cause mild respiratory infection[2,3,4]. However, past decades have brought us three highly lethal members of coronaviruses, including SARS-CoV-1, MERS-CoV, and novel SARS-CoV-2 that causes Severe Acute Respiratory Syndrome (SARS), Middle East Respiratory Syndrome (MERS), and Coronavirus disease 19 (COVID-19), respectively. In contrast, the SARS-CoV-2 is much more contagious than SARS-CoV-1 or MERS-CoV[5,6]. However, SARS-CoV-1 and MERS-CoV have higher mortality rates than novel SARSCoV-2[7]. Coronaviruses derived their name from the Latin word “crown”, which refers to the crown-like appearance of the virus seen under the electron microscope[8]. SARS-CoV-2 has four structural proteins: membrane (M), envelope (E), spike (S), and nucleocapsid (N) proteins. Like SARS-CoV-1, the surface spike protein of SARS-CoV-2 is essential for viral attachment and entry into the host cell via the angiotensin converting enzyme 2 (ACE2) receptor[9,10]. ACE2 receptor is highly expressed in capillary-rich organs, including lungs, which may explain the predominance of respiratory symptoms of the COVID-19[11,12,13]. ACE2 receptor is

a crucial element of the renin-angiotensin system (RAS) responsible for regulating blood pressure. Its catalytic domain has over 40% identity to its homolog angiotensin-converting enzyme (ACE)[14][15]. ACE is located in the lungs and converts inactive angiotensin I (AngI) to active angiotensin II (AngII)[16]. Angiotensin II binds to the AT1 receptor, causes vasoconstriction, and promotes inflammation or might enhance thrombosis via the AT4 receptor[17][18]. In contrast, the ACE2 enzyme catalyzes the hydrolysis of angiotensin II to inactive angiotensin (1-7) (Ang (1-7)), which is a vasodilator agent with antihypertensive and anti-proliferation properties[19][20]. Thus, the ACE2 is an important RAS negative factor and balances the ATII/Ang(1-7) ratio. The SARS-CoV-2 virus interacts with the ACE2 receptor and causes lower ACE2 availability and decreased breakdown of Ang II. Down-regulation of the ACE2 receptor might lead to severe acute respiratory failure with increased risk of cardiac injury and thrombosis.[21][22]

ACE2 gene is located on chromosome Xp22 and contains 20 introns and 18 exons. Several studies confirmed that ACE2 polymorphisms are associated with a risk of primary hypertension and other cardiovascular diseases[23][24]. The ACE2 variants associated with hypertension include rs2285666, rs879922, rs4646188, rs2106809, rs4240157, rs4830542, rs2158083, rs879922, rs1514283, rs2074192, rs4646155, rs4646176, rs4646174 and rs233575. While, left ventricular hypertrophy ACE2 polymorphisms include rs2106809, rs2074192, rs4646156, rs879922, rs4240157 and rs233575[25]. Cardiovascular diseases are well-known common co-morbidities of SARSCoV-2 infection that increase the risk of hospitalization and death.[26][27]

Based on the recent literature, it seems obvious that the ACE2 gene polymorphisms might be crucial candidates in understanding the genetic susceptibility for SARS-CoV-2 infection. The aim of presented study was to evaluate the influence of ACE2 gene polymorphisms (rs2074192, rs2158083, rs2285666, rs4646156, rs4646174) on COVID-19 disease risk and severity.

Results

The study involved 188 individuals that were positive for the SARS-CoV-2 virus between September 2020 and May 2021. Patients were classified according to the severity of the infection: Group I (101 patients) with no or mild symptoms, such as fever, cough, muscle aches, loss of pain/taste, who were treated at home or in isolation treatment places; Group II (87 patients) with a severe course of coronavirus disease that required hospitalization in the intensive care unit and mechanical ventilation. The general demographic and clinical characteristics of groups I and II are present in Table 1. In Group II the percentage of men were higher in (72.41%, $p < 0.001$), as was the BMI ($p=0.001$). The percentage of BMI categories in each group is present in Table 2. Additionally, there was a strong statistical significance between type 2 diabetes mellitus and severe SARS-CoV-2 infection outcome (OR=3.072, p=0.0428). The odds risk (OR) of being in Group II was 7.509 greater for patients with ischemic heart disease, but there was no statistical significance ($p>0.05$ and $p<0.1$). Interestingly, hypothyroidism was associated with low significance to a milder course of COVID19 disease (OR=0.208, p=0.006).

Association of genetic variants of ACE2 gene with the risk of severe COVID-19 disease

In our study, we confirmed that there is a statistically significant increased risk of a more severe disease outcome of SARS-CoV-2 infection associated with hospitalization in the intensive care unit for specific polymorphisms of the ACE2 gene (Table 3). The most significant correlation was found for variant ACE2 rs2285666 (AA allele, OR=2.12, p=0.019) and ACE2 rs2074192 (TT allele, OR=2.05, p=0.002), and for ACE2 rs4646174 (GG allele, OR=1.93, p=0.002), ACE2 rs4646156 (TT allele OR=1.71, p=0.008) and ACE2 rs2158083 (TT allele OR=1.84, p=0.003) (Fig1).

Association between ACE2 gene polymorphisms to general clinical characteristics and COVID-19 disease severity outcome

We analyzed the correlation between ACE2 polymorphisms and different demographic and clinical characteristics in study subjects. We found statistically significant correlation between two ACE2 polymorphisms: rs4646156 (TT>AT, p=0.033025) and rs2158083 (TT>TC, p=0.029705), and higher BMI value. There was no significant correlation between ACE2 polymorphisms and age or diabetes mellitus type 1 or type 2, hypertension, ischemic heart disease and other comorbidities.

Discussion

The aim of this prospective observational study was to report data regarding the possible association between ACE2 gene polymorphisms and disease severity in patients with SARS-CoV-2 infection. The results of our study demonstrate a statistically significant correlation between the ACE2 receptor gene rs2074192, rs2158083, rs2285666, rs4646156, rs4646174 polymorphisms, and the severity of COVID-19 in adult patients. To our best knowledge, we are the first authors that investigated these five ACE2 polymorphisms together in a wet lab fashion. The data found in other studies vary between these ACE2 gene variants. In our study, we found a strong correlation between ACE2 rs2074192 TT-genotype and poor outcome of patients with severe form of COVID-19. Our findings are consistent with a pilot study of Cafiero et al., who identified a higher frequency of T-allele of ACE2 rs2074192 in symptomatic vs. asymptomatic Italian patients[28]. Also, a recent study that included 1644

French-Canadian and British patients showed that the T allele was associated with the severity of COVID-19 disease in obese smoking males[29]. Moreover, the rs2074192 T-allele is well known for its relation to cardiovascular risk and hypertension, common risk factors for COVID-19 disease[30].

In a study by Möhlendick et al. the authors postulated that ACE2 rs2285666 GG-genotype is associated with severe COVID-19 outcome, whereas AA-genotype could have a "protective" role[31]. In contrast, we found opposite conclusions in our Polish population as rs2285666 AA-genotype increases the risk of being in the severe group of patients, and GA-genotype could be a "protective" variant. Also, our results contradict two different studies performed recently. The first study in which Gomez et al. compared 204 controls with 204 COVID-19 patients (137 non-severe and 67 severe) and reported no association between ACE2 rs2285666 polymorphism with COVID-19 severity in the Spanish population[32]. However, they found that ACE1 DD genotype, which was not investigated in our study, together with hypertension, hypercholesterolemia, and hypertensive male gender, were associated with poor outcomes of COVID-19 disease[32]. The second study in which Çelik et al. compared 155 COVID-19 patients via severity of the disease and found no relation of ACE2 rs2106809 and rs2285666 variants to the outcome of COVID-19 disease[33]. In contrast to the results of both above-mentioned studies, our patient's groups were more homogenous due to strict exclusion criteria, e.g., older age, severe obesity, and comorbidities, which was intentively used to optimize our findings. Our study revealed the correlation of rs4646156 and rs2158083 with higher BMI values. The ACE2 rs4646156 variant is known to be associated with diabetes mellitus type 2 and high total cholesterol (TC) levels, which are well known to be more frequent in obese patients[3435]. The severity of SARS-CoV-2 infection is well known to be associated with age, male sex, and some pre-existing diseases, including diabetes mellitus, hypertension, and other cardiovascular diseases. However, we found no significant correlation between ACE2 polymorphisms and age or diabetes mellitus type 1 or type 2, hypertension, ischemic heart disease, and other comorbidities. Sze et al. performed a meta-analysis, which included 18,728,893 patients from 50 studies, and found that individuals from Asian and Black ethnicities had a higher risk of SARS-CoV-2 infection than White individuals[36]. Also, both the rate of ICU admission and death rate were higher in the Asian population. Although our study involved a Polish population of the study subjects, further studies comparing different ethnicities would be required.

In addition, some of the limitations of our study should be mentioned. Firstly, our sample size is limited, and further research in a larger group of patients is essential for validating our results. Secondly, the study subjects were limited to ethnicity and race of the Polish population. A future study in a more diverse patient population might explain some differences during COVID-19 in different ethnic, racial or geographic backgrounds. However, despite its limitations this study sheds an important light onto the association between the ACE2 gene polymorphisms and the severity of COVID-19.

In conclusion, we demonstrated the rs2074192, rs2158083, rs2285666, rs4646156, and rs4646174 polymorphisms of the ACE2 gene are associated with the severity of COVID-19 disease. Also, the ACE2 rs4646156 and rs2158083 variants were correlated with higher BMI values.

Methods

In this prospective observational study, we enrolled adult patients of both sexes, who were positive for the SARS-CoV-2 virus, confirmed with PCR standardized testing, who were symptomatic and who were not vaccinated. To minimize the possible interferences, such as older age or severe obesity, on the results we optimize groups via exclusion criteria for study subjects. The exclusion criteria were as follows: (1) age over 68 years; (2) BMI > 35 kg/m2 except for relatively younger patients or patients without severe chronic illness (3) chronic kidney disease (CKD) treated with dialysis; (4) unknown and/or decompensated hypertension; (5) unknown and/or decompensated diabetes mellitus.

According to the manufacturer's protocol, genomic DNA was isolated from peripheral blood leukocytes using a commercially available PureLink™ Genomic DNA Mini Kit (Thermo Fisher Scientific, Waltham, MA, USA). Briefly, 200 μl of each blood sample was digested using proteinase K and RNase for 10 min at 55°C. Afterward, the lysis buffer and ethanol were added, and the samples were loaded onto a silica-based membrane. After washing and elution steps, the DNA concentration was measured using Epoch Microplate Spectrophotometer (BioTek, Winooski, VT, United States). Analysis of the ACE2 (rs2074192, rs2158083, rs2285666, rs4646156, rs4646174) polymorphisms was performed by real-time PCR method using LightCycler®96 system (Roche Diagnostics). Sets of LightSNiPs (TibMolbiol, Germany) to determine ACE2 polymorphisms contained appropriate concentrations of specific primers and probes for the amplified fragment and were prepared according to the manufacturer's instructions. The PCR program was initiated at 95°C for 10 min. Each PCR cycle comprised a denaturation step at 95°C for 10s, an annealing step at 60°C for 10 s, and an elongation step at 72°C for 15s (45 cycles). The final stage was the melting of products as a result of temperature rise to 95ºC. The reaction composition of a single sample was as follows: H2O - 6.7 ul, LightSNiP - 0.5 ul, LightCycler480 Genotyping Master - 1 ul , MgCl2 (25mM) - 0.8 ul, DNA (50 ng) - 1 ul. The analysis of the genotyping was based on the melting curve using LightCycler®96 Basic Software.

Statistical analysis was performed on raw data using Statistica 13 software package (TIBCO Software Inc., 3307 Hillview Avenue Palo Alto, CA 94304, USA). Polymorphisms in study groups were analyzed using frequency tables and Pearson's chi-squared, as well as nonparametric R Spearman, rang tests. Odds ratios and confidence intervals for polymorphisms and

concomitant diseases were calculated using the creator of logistic regression. They all were analyzed as one-factor risk evaluation and in conjunction as a multifactor set. Continuous variables were analyzed with the use of t-Student test and Kruskal-Wallis ANOVA rang test. Statistical tests were chosen according to the input data and the cause-effect analytic questions. Statistical significance was set at $p < 0.05$.

All methods were carried out in accordance with relevant guidelines and regulations. All experimental protocols were approved by the Ethical Committee of the Pomeranian Medical University in Szczecin (Approval number: KB-0012/88/2020; approval date, June 22.06.2020). The study was conducted in accordance with the Helsinki Declaration (1975, revised 2000). We obtained approval from the Ethical Committee of the Pomeranian Medical University in Szczecin to perform our study without a written informed consent from all study subjects due to the observational nature of the study (Approval number KB-0012/88/2020; approval date, 22.03.2021).

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None

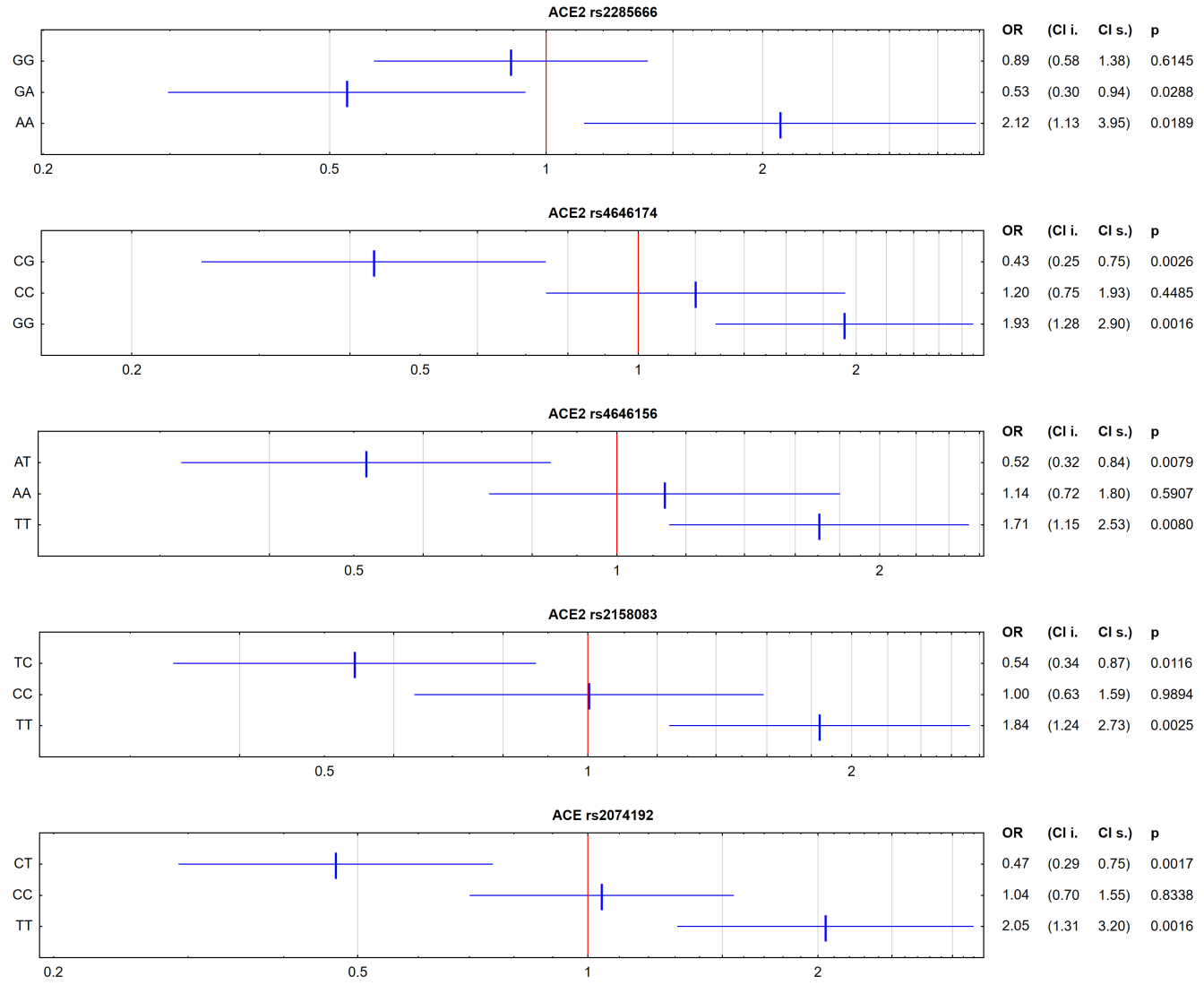
Author contributions statement

JS - designed the study, collected the data, analyzed the results, wrote the manuscript, provided funding and supervised project administration. IM - collected the data, analyzed the results, MK - designed the study, collected the data, analyzed the results, provided critical revision of the manuscript. AB analyzed and interpreted the patient data regarding genetic analysis, revised the manuscript critically. KT – performed statistical analysis, analyzed the results, provided graphics and critical revision of the manuscript. MS - analyzed the results and provided critical revision of the manuscript. KK – participated in the design of the study, collected the data, analyzed the results, made substantial contributions to the conception of the work and critically revised the manuscript. All the authors read and approved the final manuscript and agree to be accountable for all aspects of the work.

Additional information

Competing interests The authors declare that they have no competing interest.

Figure 1. Box plots of ACE2 polymorphisms



Legend: OR, odds ratio; Cl confidence interval;

Table 1. General demographic and clinical characteristics

Characteristics	Group I	Group II	OR (95%)	P-value
Age (mean± SD)	54.17 ± 3.86	53.07 ± 10.07	NA	0.340
Female, n (%)	69 (68.31)	24 (27.59)	0.42 [0.307-0.576]	< 0.001
Male, n (%)	32 (31.68)	63 (72.41)	2.379 [1.736-3.260]	< 0.001
BMI (mean± SD)	26.10 ± 4.44	28.23 ± 3.97	NA	< 0.001
Hypertension, n (%)	33 (17.65)	36 (19.25)	1.22 [0.90-1.64]	0.195
Diabetes mellitus type 2, n (%)	4 (2.13)	12 (6.38)	3.072 [1.037-9.102]	0.043
Hypothyroidism,, n (%)	16 (15.8)	4 (4.56)	0.208 [0.068-0.638]	0.006

Legend: OR - odds ratio; Cl - confidence interval; NA - not applicable

Table 2. BMI categories in Group I and Group II

BMI	Group	Number of Patients	Cumulative number	Percentage (%)	Cumulative %	100% - %
BMI < 20	I	6	6	5.940	5.941	100.000
BMI ≥20 to <25	I	39	45	38.614	44.554	94.059
BMI ≥25 to <30	I	42	87	41.584	86.139	55.445
BMI ≥30	I	14	101	13.861	100.000	13.861
Undefined	I	0	101	0	100.000	0.000
BMI < 20	II	1	1	1.149	1.149	100.000
BMI ≥20 to <25	II	17	18	19.540	20.690	98.851
BMI ≥25 to <30	II	41	59	47.126	67.816	79.310
BMI ≥30	II	28	87	32.184	100.000	32.184
Undefined	II	0	87	0	100.000	0.000

$$BMI = weight(inkg)/height^2(inm^2)$$

Table 3. ACE2 polymorphism genotypes

ACE2 polymorphism	Group I [n (%)]	Group II [n (%)]	OR (95%)	P-value
rs2285666				
AA	8 (4.26)	16 (8.51)	2.12 [1.13-3.95]	0.019
GG	71 (37.70)	60(31.91)	0.89[0.58-1.38]	0.029
GA	22 (11.70)	11 (5.85)	0.53 [0.30-0.94]	0.614
rs4646174				
GG	44 (23.40)	57 (30.32)	1.93 [1.28-2.90]	0.002
CG	31 (16.49)	9(4.79)	0.43[0.25-0.75]	0.03
CC	26 (13.83)	21 (11.17)	1.20 [0.75-1.93]	0.449
rs4646156				
TT	42 (22.34)	53 (28.19)	1.71 [1.15-2.53]	0.008
AT	34 (18.09)	13 (6.91)	0.52 [0.32-0.84]	0.008
AA	25 (13.30)	21 (11.17)	1.14 [0.72-1.80]	0.591
rs2158083				
TT	39 (20.74)	53 (28.19)	1.84 [1.24-2.73]	0.003
TC	35 (18.62)	14 (7.45)	0.54 [0.34-0.87]	0.012
CC	27 (14.36)	20 (10.64)	1.00 [0.63-1.59]	0.989
rs2074192				
TT	20 (10.64)	34 (18.09)	2.05 [1.31-3.20]	0.002
CT	36 (19.15)	14 (7.45)	0.47 [0.29-0.75]	0.002
CC	45 (23.94)	39 (20.74)	1.04 [0.70-1.55]	0.834

Legend: OR - odds ratio; Cl - confidence interval;