

Original Research Paper

Distribution of Alleles and Genotypes of ACE2 Gene Polymorphisms (rs2285666 and rs35803318) and Association with COVID-19 Among Populations in Kazakhstan

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Abstract: There is still no definitive confirmation regarding the association between ACE2 gene polymorphisms and COVID-19. This study was conducted to analyze the distribution of alleles and genotypes of the ACE2 gene rs2285666 and rs35803318 two polymorphisms and their possible association with COVID-19 among two ethnic groups, namely, the Kazakhs and Eastern Slavs, residing within the same geographic area in the city of Karaganda, Republic of Kazakhstan. This research was conducted for the first time in Kazakhstan. The genotyping was performed using the Amplification-Refractory Mutation System (ARMS) PCR. A substantial connection of rs2285666 with COVID-19 in the studied Kazakh and Eastern Slavs populations ($p > 0.05$) was not observed. It has been determined that certain alleles and genotypes serve as protective markers against COVID-19. It was determined that the G allele ($p = 0.0217$) of rs2285666 among Kazakh men reduced the likelihood of coronavirus infection. It was concluded that the C allele and CC genotype of rs35803318 have a statistically significant association with protection against COVID-19 among only Eastern Slavs. There were no differences in the alleles and genotypes of polymorphism between males and females. Thus, the analysis of the rs2285666 and rs35803318 polymorphisms has revealed that these two variants of the ACE2 gene do not exert an influence on the risk of contracting COVID-19.

Keywords: ACE2 Gene, SNP, COVID-19, SARS-CoV-2

Introduction

In December 2019, the World Health Organization (WHO) reported an unspecified pneumonia outbreak in Wuhan, Hubei province, China. In January 2020, WHO declared the COVID-19 epidemic caused by the SARS-CoV-2 virus to be a public health emergency of international concern (WHO, 2023).

Coronaviruses (CoVs) are (+) single-stranded RNA viruses that taxonomically belong to both the *Coronaviridae* family, as well as the *Coronavirinae* subfamily. As for the SARS-CoV-2, it is of the *Betacoronavirus* genus (Wu *et al.*, 2020a).

When in contact with human airways, the Spike proteins (S-proteins) of this virus can bind to surface receptors of sensitive cells, which mediate viral entry

into target cells for further replication. The SARS-CoV-2 surface S-protein attaches to a cell receptor and Angiotensin Converting Enzyme 2 (ACE2) on the human cell surface (Wrapp *et al.*, 2020; Wu *et al.*, 2020b; Zhou *et al.*, 2020). It has been established that the ACE2 receptors are massively found in the gastrointestinal tract (Harmer *et al.*, 2002), lung epithelium, and vascular endothelium (Hamming *et al.*, 2004). ACE2 is part of the complex Renin-Angiotensin System (RAS). One of the RAS' main biological functions is to control blood pressure levels. This system participates in the pathogenesis of various diseases, including cardiovascular diseases (Crowley and Coffman, 2012; Putnam *et al.*, 2012). Pinto *et al.* (2020) noted that ACE2 is highly expressed in the lungs of severe COVID-19 patients with comorbidities.

Angiotensin-converting enzyme 2 is encoded by the ACE2 gene. Li *et al.* (2020) reported that the ACE2 gene expression levels have no essential differences either in gender and age or between Asian and non-Asian races in various human tissues. rs2285666 and rs35803318 Single Nucleotide Polymorphisms (SNPs) relate to the ACE2 gene and are essential in the COVID-19 development. Mainly, rs2285666 is one of the most extensively studied ACE2 gene polymorphisms and is associated with a higher risk of developing hypertension, diabetes, and cardiovascular disease (Lu *et al.*, 2012; Malard *et al.*, 2013; Niu *et al.*, 2007; Zhong *et al.*, 2006; Pan *et al.*, 2018; Pinheiro *et al.*, 2019).

According to this study (Strafella *et al.*, 2020), homozygosity for rs2285666 polymorphism allele may increase the ACE2 expression in various brain tissues, which in turn affects the ACE2 functions in the brain. These results indicate that the ACE2 genetic variation may have a stronger effect on the COVID-19 symptoms, as well as the SARS-CoV-2 tissue tropism, rather than the predisposition to SARS-CoV-2.

In another study, the authors evaluated the influence of the ACE and ACE2 gene variants on the development of severe COVID-19 infection, defined as the need for hospitalization and/or intensive care (Martínez-Gómez *et al.*, 2022). The analysis revealed an association of the rs2285666 variant for the ACE2 gene with a higher risk of developing severe COVID-19, particularly among men.

Srivastava *et al.* (2020) revealed a relation of the rs2285666 polymorphism frequency with a low COVID-19 incidence, along with a low mortality rate among the Indian population.

Thus, the rs2285666 and rs35803318 genotypes and their alleles are important to comprehend the drivers of COVID-19 and may help to more precisely identify the groups of people at risk and severe disease. The studies on the association between the ACE2 gene variants and COVID-19 are ongoing and therefore, new data may help to more accurately understand the relationship between genotype and susceptibility to this disease. The objectives of this study are as follows: To analyze the genotypic and allelic frequencies of the rs2285666 and rs35803318 gene polymorphisms within two distinct ethnic groups, namely Kazakhs and Eastern Slavs, residing within the same geographical region; to determine whether there exists a statistically significant association between the identified genotypic and allelic frequencies of these polymorphisms and risk of contracting COVID-19.

Materials and Methods

Study Design

The study included 2 ethnic groups at the age of ≥ 18 (96 Kazakhs and 80 Eastern Slavs) living in the city of Karaganda and the Karaganda region (Republic of Kazakhstan). All participants had not been vaccinated against COVID-19 in the last 12 months. Demographic characteristics of the study participants are provided in (Table 2).

The study was conducted in accordance with the recommendations of Helsinki ethical principles and approved by the local bioethics committee non-commercial joint-stock company "Karaganda Medical University" (protocol No. 2 dated 11 October 2022). Every participant provided written consent after being informed.

Venous blood samples were taken from voluntary participants from October 2022 and November 2022 and carried out in sterile evacuated tubes with K3-EDTA as an anticoagulant. Blood was centrifuged at 4000 rpm for 15 min at room temperature. The plasma was collected and placed into polypropylene tubes. The samples were tested to detect M and G class antibodies for SARS-CoV-2 by enzyme immunoassay (ELISA) using the SARS-CoV-2 IgG-ELISA-BEST and SARS-CoV-2-IgM-ELISA-BEST test systems (vector-best, Russia). Based on this method results, we divided each ethnic group's participants into 2 study groups according to the disease status: COVID-19 positive (p-COVID-19) with IgM >1 ng/mL, IgG >1 ng/mL, as well as COVID-19-negative (no-COVID-19) with IgG <1 ng/mL, IgM <1 ng/mL.

ACE2 Genotyping

Genomic DNA was extracted from venous blood samples using a "RIBO-prep" kit (AmpliSens, Russia). The extraction was conducted following the provided guidelines. To identify the Single Nucleotide Polymorphisms (SNPs) rs2285666 (A/C/T/G) and rs35803318 (C/T), we analyzed the isolated DNA by using the Amplification-Refractory Mutation System (ARMS) PCR. Genotyping was performed in a DTLite real-time PCR (DNA technology, Russia). The device was programmed with the following improving conditions: Initial denaturation cycle (94°C for 3 min), followed by 40 denaturation cycles (15s at 94°C), as well as annealing/extension rs2285666 (54°C) and rs35803318 (68°C) polymorphisms for 30s. PCR reactions were performed in a 25 μ L reaction mixture, which included 50 ng of DNA, 10 pmol of each primer (Lumiprobe, Russia), Taq-polymerase and appropriate buffer (GeneLab, Kazakhstan), dNTP (Thermo fisher scientific Baltics UAB, Lithuania) and SYBR Green (Sigma-Aldrich®, USA). The primer sequences are presented in (Table 1).

Table 1: List of primers for ARMS-PCR of rs2285666 and rs35803318

SNP	Primer sequence (5'-3')
rs2285666 C allele	FIP TTTCATAATCACTACTAAAAATTAGTAACC
rs2285666 A allele	RIP GAATGCTTATTACTTGAACCAGGCAT
rs2285666	FOP GTTAAAAGAGCATCTATGTGTTGAAAC
rs2285666	ROP AATACAATGAGCACCATCTACAGTACT
rs2285666 G allele	FIP TTTCATAATCACTACTAAAAATTAGTACCG
rs2285666 C allele	RIP GAATGCTTATTACTTGAACCAGGAAG
rs2285666	FOP TAAAAGAGCATCTATGTGTTGAAACA
rs2285666	ROP ATACAATGAGCACCATCTACAGTACTG
rs2285666 C allele	FIP CATAATCACTACTAAAAATTAGTATCC
rs2285666 T allele	RIP CAAGAATGCTTATTACTTGAACCAGGGAA
rs2285666	FOP GTTAAAAGAGCATCTATGTGTTGAAA
rs2285666	ROP AATTCTAAATACAATGAGCACCATCTA
rs35803318 T allele	FIP CAATGCCAACCACTATCACTCCCCTT
rs35803318 C allele	RIP CCATATGGCTGATTGTTTTGGAGTTTTG
rs35803318	FOP AAGTCTAGGAAAGGCCACTTACTTCTTCCG
rs35803318	ROP TTTCTGGGGATACAGCAACACTTGGAC

Table 2: Demographic characteristics of the study groups

Characteristics	Kazakhs			Eastern Slavs			
	p-COVID-19 N = 76	no-COVID-19 N = 20	p	p-COVID-19 N = 40	no-COVID-19 N = 40	p	
Gender (n, %)	Male	19 (25%)	8 (40%)	0.1843*	17 (42.5%)	23 (57.5%)	0.1797*
	Female	57 (75%)	12 (60%)		23 (57.5%)	17 (42.5%)	
	Min	18	19		18	32	
	Max	78	75	0.3312#	79	86	0.9714#
Age	Mean ± SD	42.83±14.18	45.75±14.48		50±15.58	51.05±14.86	
Median		43	46.5		51	49	

N-total number of the participants

*p values were calculated using the chi-square test

#p values were calculated using the mann-whitney U test significant difference at p<0.05

Statistical Analysis

Continuous data are presented as mean ± Standard Deviation (SD). Continuous variables for normal distribution were tested using the Shapiro-Wilk test. The categorical variables are described as frequencies and percentages. The comparison of continuous variables between the two groups was conducted using the Mann-Whitney U test. The Hardy-Weinberg Equilibrium (HWE) was calculated for each SNP in two groups separately using the Chi-Square test (χ^2 test) and the results were considered as deviating from the HWE at a significance level p<0.05. Fisher's exact test and the χ^2 test were used to compare genotype and allele frequencies. Associations between genotypes, alleles, and COVID-19 were assessed using Odds Ratios (OR) with a 95% confidence interval (95% CI). Tests of statistical significance were two-sided and considered significant when the p-value was <0.05. GraphPad Prism 8.4.3 software (graph-pad software, San Diego, CA, USA) was used to perform statistical analysis.

Results

The Characteristics of the Study Participants

As shown in Table 2, the average age among the Kazakh population in the p-COVID-19 group was higher compared to the no-COVID-19 ones (42.83±14.18 vs. 45.75±14.48 years; p = 0.3312). The number of females was significantly higher in comparison with males in the first group (75 vs. 25%) and (60 vs. 40%) in the second group respectively, but the difference between the two groups was not significant (p = 0.1843).

No statistically significant differences were found between the p-COVID-19 and no-COVID-19 groups concerning sex and age among Eastern Slavs. The percentages of males and females were 42.5 and 57.5% in the p-COVID-19 group, whereas 57.5 and 42.5% in the no-COVID-19 (p = 0.1797) respectively. The mean ± SD age was 50±15.58 years for the p-COVID-19 group and 51.05±14.86 years for the no-COVID-19 group (p=0.9714).

Association of ACE2 SNPs with COVID-19 Among Kazakh Population

The genotypes of rs2285666 in both groups deviated from Hardy-Weinberg equilibrium ($p = 0.0176$; $p = 0.0001$). In the no-COVID-19 group, rs35803318 did not deviate from the Hardy-Weinberg equilibrium ($p = 0.8007$), while the genotypes distribution of rs35803318 in the p-COVID-19 group was not consistent ($p = 0.0389$).

The results of genotype frequencies and allele distribution of each ACE2 gene's SNP and statistical analysis among the Kazakhs are illustrated in Table 3.

While analyzing, we did not find any statistically substantial distinctions in the frequency of genotypes and alleles of rs2285666 in both groups ($p > 0.05$). In our study, the rs2285666 polymorphism allele A was often found in two groups: P-COVID-19 (38.8%) and no-COVID-19 (42.5%).

Then, the groups were separated in accordance with their gender (i.e., male and female) to define if there were differences between the polymorphism and two groups of the same gender. When distributing the rs2285666 alleles among men and women of the Kazakh population, statistically distinguishable results were found in the C allele ($p = 0.0287$; $\chi^2 = 5.870$; OR = 6.300; 95% CI: 1.433-30.06), as well as the G allele ($p = 0.0217$; $\chi^2 = 5.870$; OR = 0.1176; 95% CI: 0.02097-0.5701) among men (Fig. 1).

The C allele frequency is significantly higher in the p-COVID-19 group (47%) compared to the no-COVID-19 group (12.5%). Thus, the C allele increases the likelihood of COVID-19 infection by 6.3 times. The G allele, on the

contrary, was more frequently found in the no-COVID-19 group (25%). It can be assumed that the G allele has protective properties.

Insignificant differences were found in genotype and allele frequencies of the ACE2 rs35803318 between the two groups. No gender associations have been found related to COVID-19.

Association of ACE2 SNPs with COVID-19 Among Eastern Slavs Population

The genotypes of rs35803318 variant in two groups were in HWE ($p = 0.4090$; $p = 0.9623$). However, rs2285666 failed the HWE in the p-COVID-19 group ($p = 0.0075$). This could be attributed to its connection with COVID-19. rs2285666 in the no-COVID-19 group did not deviate from the Hardy-Weinberg equilibrium ($p = 0.0848$).

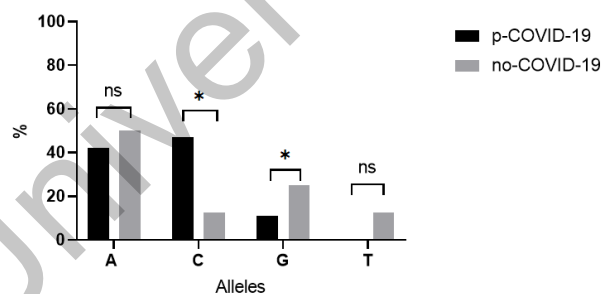


Fig. 1: rs2285666 allele frequency among Kazakh population men

Table 3: Distributions and association analysis of all analyzed genotypes and allele frequencies among the Kazakhs' COVID-19 positive and negative groups

SNP	Genotype/ allele	p-COVID-19	no-COVID-19	OR (95%, CI)	χ^2 , df	p-value
		N = 76 n (%)	N = 20 n (%)			
rs2285666	AA	22 (29)	6 (30)	0.9506 (0.3140-2.876)	0.008492,1	>0.9999
	CC	19 (25)	2 (10)	3.000 (0.6863-13.93)	2.085,1	0.2254
	TT	2 (2.6)	2 (10)	0.2432 (0.03695-1.665)	2.153,1	0.1905
	GG	7 (9.2)	3 (15)	0.5749 (0.1313-2.227)	0.5687,1	0.4298
	CT	5 (6.6)	NA	3.154 (0.1673-59.44)	1.388,1	0.5802
	TG	4 (5.3)	NA	2.545 (0.1315-49.24)	1.098,1	0.5768
	AT	2 (2.6)	1 (5)	0.5135 (0.05785-7.803)	0.2934,1	0.5080
	AC	7 (9.2)	3 (15)	0.5749 (0.1313-2.227)	0.5687,1	0.4298
	AG	6 (8)	1 (5)	1.629 (0.2321-19.59)	0.1963,1	>0.9999
	CG	2 (2.6)	2 (10)	0.2432 (0.03695-1.665)	2.153,1	0.1905
	Allele A	59 (38.8)	17 (42.5)	0.8583 (0.4237- 1.699)	0.1797,1	0.7180
Allele C	52 (34.2)	9 (22.5)	1.791 (0.7793-4.184)	2.003,1	0.1844	
Allele G	26 (17.1)	9 (22.5)	0.7108 (0.3005-1.776)	0.6183,1	0.4903	
Allele T	15 (9.9)	5 (12.5)	0.7664 (0.2715-2.028)	0.2659,1	0.5729	
rs35803318	CC	14 (18.4)	6 (30)	0.5269 (0.1810-1.704)	1.287,1	0.3520
	TT	12 (15.8)	3 (15)	1.063(0.2669- 3.845)	0.05053,1	>0.9999
	CT	50 (65.8)	11 (55)	1.573 (0.5952-4.101)	0.7956,1	0.4371
	Allele C	78 (51.3)	23 (57.5)	0.7791 (0.3964-1.566)	0.4857,1	0.5939
	Allele T	74 (48.7)	17 (42.5)	1.284 (0.6355-2.592)	0.4857,1	0.5939

OR-odds ratio, CI-Confidence Interval; $p < 0.05$ values are considered significant

NA-not available

p-values were calculated using Fisher's exact test

Table 4: Distributions and association analysis of all analyzed genotypes and allele frequencies in the p-COVID-19 and no-COVID-19 groups among eastern Slavs

SNP	Genotype/ allele	p-COVID-19 N = 40 (n/%)	no-COVID-19 N = 40 (n/%)	OR (95%, CI)	χ^2	p-value
rs2285666	AA	13 (32.5)	19 (47.5)	0.5322 (0.2175-1.365)	1.875, 1	0.2537
	CC	17 (42.5)	12 (30)	1.725 (0.7089-4.378)	1.352, 1	0.3524
	TT	NA	NA	NA	NA	NA
	GG	NA	NA	NA	NA	NA
	CT	NA	NA	NA	NA	NA
	TG	NA	NA	NA	NA	NA
	AT	NA	NA	NA	NA	NA
	AC	10 (25)	9 (22.5)	1.148 (0.4291-3.188)	0.06903, 1	>0.9999
	AG	NA	NA	NA	NA	NA
	CG	NA	NA	NA	NA	NA
	Allele A	36 (45)	47 (58.75)	0.5745 (0.3071-1.075)	3.029, 1	0.1133
	Allele C	44 (55)	33 (41.25)	1.741 (0.9306-3.256)	3.029, 1	0.1133
	Allele G	NA	NA	NA	NA	NA
Allele T	NA	NA	NA	NA	NA	
rs35803318	CC	13 (32.5)	24 (60)	0.3210 (0.1268-0.8190)	6.084, 1	0.0243*
	TT	4 (10)	2 (5)	2.111 (0.4637-11.52)	0.7207, 1	0.6752
	CT	23 (57.5)	14 (35)	2.513 (1.000-6.118)	4.073, 1	0.0722
	Allele C	49 (61.25)	62 (77.5)	0.4589 (0.2386-0.8991)	4.972, 1	0.0390*
	Allele T	31 (38.75)	18 (22.5)	0.7732 (0.3582-1.700)	0.4312, 1	0.5577

p-values were calculated using Fisher's exact test

In the Eastern Slavs' group, we detected only A and C alleles of the ACE2 gene rs2285666 (Table 4).

The allele A predominated in the no-COVID-19 group (58.75%), whereas the allele C was more frequently found in the p-COVID-19 ones (55%). However, we did not reveal any association of rs2285666 with COVID-19. No significant differences in gender in the two groups were detected as well ($p > 0.05$).

The C allele and CC genotype rs35803318 frequency are considerably higher in the no-COVID-19 group. We found significant differences in both the C allele rs35803318 ($p = 0.0390$; $\chi^2 = 4.972$; OR = 0.4589; 95% CI: 0.2386-0.8991) and the CC genotype ($p = 0.0243$; $\chi^2 = 6.084$; OR = 0.3210; 95% CI: 0.1268-0.8190). With these results, we can assume the protective property of the C allele, as well as in the homozygous form. There was no statistical difference in the allelic and genotypic distribution among men and women ($p > 0.05$).

Discussion

The present study described the relationship between two potential effects of SNPs of the ACE2 gene and COVID-19 risk among Kazakh and Eastern Slavs in the Karaganda region. It should be noted that this study was conducted on a relatively small sample and only in two ethnic groups living in a certain region, which may limit the general applicability of the results obtained.

We have suggested that some persons may have a genetic susceptibility to SARS-CoV-2 infection. In addition, the ACE2 gene variants, involved in virus entry into cells, are of particular research interest.

According to Möhlendick *et al.* (2021), the presence of the GG genotype and the G allele in the ACE2 gene rs2285666 SNP was associated with either a confirmed two-fold increased likelihood of SARS-CoV-2 virus infection and a three-fold increase in getting severe disease or death from COVID-19, compared to the AA genotype carriers. However, their study found no relationship between gender, age, and risk of SARS-CoV-2 infection.

The study in the Polish population found that the AA genotype of rs2285666 may increase the risk of getting severe COVID-19 infection, while the GA genotype may serve as a "protective" variant (Sienko *et al.*, 2022). The presence of the A allele rs2285666 of the ACE2 gene is due to a higher risk of developing COVID-19 among patients (Sabater Molina *et al.*, 2022).

The study's authors revealed a positive relationship between the T rs2285666 allele and severe disease. In addition, they emphasized a stronger significance among men compared to a general study group (Martínez-Gómez *et al.*, 2022). Another study demonstrates that the presence of the T allele of the ACE2 gene rs2285666 is connected with a higher risk of developing severe forms of COVID-19 (Abdelsattar *et al.*, 2022). Khalilzadeh *et al.* (2022) found an association of rs2285666 with mortality and a considerably higher spread of the CC ACE2 rs2285666 genotype among patients who died, compared to the TT genotype. Alimoradi *et al.* (2022) found an association of rs2285666 with susceptibility to COVID-19 but did not define any association with disease severity.

According to the present study results, there was no association of rs2285666 polymorphism with COVID-19.

The findings are consistent with other studies which have found no association of rs2285666 and rs35803318 polymorphisms with susceptibility or severity of COVID-19 (Karakaş Çelik *et al.*, 2021; Torre-Fuentes *et al.*, 2021). In the examined Russian cohort, the researchers found no effect of rs2285666 and rs35803318 on susceptibility to COVID-19 or its severity (Shikov *et al.*, 2020). In this study, we revealed the statistical significance of the rs35803318 C allele ($p = 0.0390$; $\chi^2 = 4.972$; OR = 0.4589; 95% CI: 0.2386-0.8991) and CC genotype ($p = 0.0243$; $\chi^2 = 6.084$; OR = 0.3210; 95% CI: 0.1268-0.8190) among Eastern Slavs. However, we identified the C allele and CC genotype as protective markers. Our results are similar to those of Benetti *et al.* (2020). They found a higher polymorphism frequency among the control group participants rather than among the infected ones. Currently, there are few studies on the association of the rs35803318 polymorphism with COVID-19. Torre-Fuentes *et al.* (2021) reported that the rs35803318 variant was not definitely associated with the SARS-CoV-2 virus among the Spanish population.

In this study, the ACE2 rs2285666 alleles were more commonly detected in the p-COVID-19 group but did not differ statistically between the two groups. The gender analysis showed no statistically significant distinction was observed between the two groups of women. However, it showed the difference between the C allele ($p = 0.0287$; $\chi^2 = 5.870$; OR = 6.300; 95% CI: 1.433-30.06) and the G allele ($p = 0.0217$; $\chi^2 = 5.870$; OR = 0.1176; 95% CI: 0.02097-0.5701) among men in the Kazakh population. There were no significant statistical differences in the rs2285666 genotypes distribution in these two ethnic groups, as well as no association of polymorphism with the disease severity (Najafi and Mahdavi, 2023). In the Italian cohort, the authors did not find any significant association of the ACE2 variant with COVID-19 severity (Novelli *et al.*, 2020).

The authors from Turkey, Iraq, and Slovenia found no association between the rs2285666 polymorphism and the severity of COVID-19 (Duman *et al.*, 2022; Mahmood *et al.*, 2022; Jevnikar *et al.*, 2022). However, Mahmood *et al.* (2022) reported that the A allele was more commonly detected in the women's case group compared to the control group. According to our study, the G allele reduces the likelihood of infection among men of the Kazakh population.

In general terms, the ACE2 gene plays a significant role in determining an individual's resistance or susceptibility to COVID-19. Genetic variations within this gene could potentially play a central role in determining how vulnerable someone is to the virus and the severity of the illness they may experience. However, it's important to note that there isn't conclusive and comprehensive evidence yet to confirm the impact of ACE2 gene polymorphisms on susceptibility and resistance to SARS-CoV-2, their connection to the

severity of COVID-19 in relation to factors like concurrent diseases, gender, age, and different population groups.

Conclusion

In this pilot study analyzes the links of the ACE2 gene SNPs with the risk of COVID-19 within the context of two ethnic groups. According to our study results, there were no significant associations of the analyzed rs2285666 variant with COVID-19 between both groups ($p > 0.05$). The significance of the C allele rs35803318 and CC genotype was found among Eastern Slavs but hereby identified them as protective markers. There were no significant differences in the polymorphism alleles and genotypes among men and women. However, it was defined that the G allele ($p = 0.0217$) among men in the Kazakh population reduce the odds of infection. The C allele ($p = 0.0287$; $\chi^2 = 5.870$; OR = 6.300; 95% CI: 1.433-30.06) increases the likelihood of COVID-19 infection. Thus, the analysis of ACE2 gene polymorphisms has demonstrated that these SNPs are not associated with the risk of developing COVID-19. However, the obtained results indicate the potential for conducting further research on the association between the rs2285666 and rs35803318 polymorphisms and COVID-19 with a larger sample population in Kazakhstan. This study contributes to the scientific understanding of the role of genetic factors in susceptibility to COVID-19 among these ethnic groups, which may hold significance for future research and public health measures. The ARMS PCR method is not without limitations. It may be more susceptible to human error compared to automated genotyping methods. In addition, we understand that our study includes restricted sample sizes, as well as the no-COVID-19 thoroughly selected group participants. Before our conclusions become common, they are required to be confirmed by independent studies.

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Author's Contributions

Anar Bisseneva: Performed the experiments, data analysis, and interpretation, and drafted the manuscript. Final revision of the manuscript.

Konstantin Li: Conceived and designed this study. Performed the experiments. Supervision. Provided critical revision of the manuscript.

Gayane Pogossyan: Conceived and designed this study, supervision. Provided critical revision of the manuscript.

Valeriya Protas: Performed the experiments. Data interpretation, critical and final revision of the manuscript.

Ethics

This article is original and contains unpublished material. The corresponding author confirms that all of the other authors have read and approved the manuscript and that no ethical issue is involved.

References

- Abdelsattar, S., Kasemy, Z. A., Ewida, S. F., Abo-Elhoud, R. A. A., Zytoon, A. A., Abdelaal, G. A., ... & Kamel, H. F. M. (2022). ACE2 and TMPRSS2 SNPs as Determinants of Susceptibility to and Severity of, a COVID-19 Infection. *British Journal of Biomedical Science*, 79, 10238.
<https://doi.org/10.3389/bjbs.2021.10238>
- Alimoradi, N., Sharqi, M., Firouzabadi, D., Sadeghi, M. M., Moezzi, M. I., & Firouzabadi, N. (2022). SNPs of ACE1 (rs4343) and ACE2 (rs2285666) genes are linked to SARS-CoV-2 infection but not with the severity of the disease. *Virology Journal*, 19(1), 48.
<https://doi.org/10.1186/s12985-022-01782-6>
- Benetti, E., Tita, R., Spiga, O., Ciolfi, A., Birolo, G., Bruselles, A., ... & Pinto, A. M. (2020). ACE2 gene variants may underlie interindividual variability and susceptibility to COVID-19 in the Italian population. *European Journal of Human Genetics*, 28(11), 1602-1614.
<https://doi.org/10.1038/s41431-020-0691-z>
- Crowley, S. D., & Coffman, T. M. (2012). Recent advances involving the renin-angiotensin system. *Experimental Cell Research*, 318(9), 1049-1056.
<https://doi.org/10.1016/j.yexcr.2012.02.023>
- Duman, N., Tuncel, G., Bisgin, A., Bozdogan, S. T., Sag, S. O., Gul, S., Kiraz, A., Balta, B., Erdogan, M., Uyanik, B., Canbek, S., Ata, P., Geckinli, B. B., Arslan Ates, E., Alavanda, C., Yesim Ozdemir, S., Sezer, O., Ozgon, G. O., Gurkan, H., ... Temel, S. G. (2022). Analysis of ACE2 and TMPRSS2 coding variants as a risk factor for SARS-CoV-2 from 946 whole-exome sequencing data in the Turkish population. *Journal of Medical Virology*, 94(11), 5225-5243.
<https://doi.org/10.1002/jmv.27976>
- Hamming, I., Timens, W., Bulthuis, M. L. C., Lely, A. T., Navis, G. V., & van Goor, H. (2004). Tissue distribution of ACE2 protein, the functional receptor for SARS coronavirus. A first step in understanding SARS pathogenesis. *The Journal of Pathology: A Journal of the Pathological Society of Great Britain and Ireland*, 203(2), 631-637.
<https://doi.org/10.1002/path.1570>
- Harmer, D., Gilbert, M., Borman, R., & Clark, K. L. (2002). Quantitative mRNA expression profiling of ACE 2, a novel homologue of angiotensin converting enzyme. *FEBS Letters*, 532(1-2), 107-110.
[https://doi.org/10.1016/S0014-5793\(02\)03640-2](https://doi.org/10.1016/S0014-5793(02)03640-2)
- Jevnikar, K., Lapajne, L., Petrovič, D., Meglič, A., Logar, M., Vidovič Valentinčič, N., ... & Mekjavić, P. J. (2022). The role of ACE, ACE2 and AGTR2 polymorphisms in COVID-19 severity and the presence of COVID-19-related retinopathy. *Genes*, 13(7), 1111.
<https://doi.org/10.3390/genes13071111>
- 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. *Journal of Medical Virology*, 93(10), 5947-5952.
<https://doi.org/10.1002/jmv.27160>
- Khalilzadeh, F., Sakhaee, F., Sotodehnejadnematalahi, F., Zamani, M. S., Ahmadi, I., Anvari, E., & Fateh, A. (2022). Angiotensin-converting enzyme 2 rs2285666 polymorphism and clinical parameters as the determinants of COVID-19 severity in Iranian population. *International Journal of Immunogenetics*, 49(5), 325-332.
<https://doi.org/10.1111/iji.12598>
- Li, M. Y., Li, L., Zhang, Y., & Wang, X. S. (2020). Expression of the SARS-CoV-2 cell receptor gene ACE2 in a wide variety of human tissues. *Infectious Diseases of Poverty*, 9(02), 23-29.
<https://doi.org/10.1186/s40249-020-00662-x>
- Lu, N., Yang, Y., Wang, Y., Liu, Y., Fu, G., Chen, D., ... & Zheng, Y. (2012). ACE2 gene polymorphism and essential hypertension: An updated meta-analysis involving 11,051 subjects. *Molecular Biology Reports*, 39, 6581-6589.
<https://doi.org/10.1007/s11033-012-1487-1>
- Mahmood, Z. S., Fadhil, H. Y., Hussein, T. A. A., & Ad'hiah, A. H. (2022). Severity of coronavirus disease 19: Profile of inflammatory markers and ACE (rs4646994) and ACE2 (rs2285666) gene polymorphisms in Iraqi patients. *Meta Gene*, 31, 101014.
<https://doi.org/10.1016/j.mgene.2022.101014>

- Malard, L., Kakinami, L., O'Loughlin, J., Roy-Gagnon, M. H., Labbe, A., Pilote, L., ... & Paradis, G. (2013). The association between the angiotensin-converting enzyme-2 gene and blood pressure in a cohort study of adolescents. *BMC Medical Genetics*, *14*(1), 1-7.
<https://doi.org/10.1186/1471-2350-14-117>
- Martínez-Gómez, L. E., Herrera-Lopez, B., Martínez-Armenta, C., Ortega-Pena, S., Camacho-Rea, M. D. C., Suarez-Ahedo, C., ... & Lopez-Reyes, A. (2022). ACE and ACE2 gene variants are associated with severe outcomes of COVID-19 in men. *Frontiers in Immunology*, *13*, 812940.
<https://doi.org/10.3389/fimmu.2022.812940>
- Möhlendick, B., Schönfelder, K., Breuckmann, K., Elsner, C., Babel, N., Balfanz, P., ... & Kribben, A. (2021). ACE2 polymorphism and susceptibility for SARS-CoV-2 infection and severity of COVID-19. *Pharmacogenetics and Genomics*.
<https://doi.org/10.1097/FPC.0000000000000436>
- Najafi, M., & Mahdavi, M. R. (2023). Association investigations between ACE1 and ACE2 polymorphisms and severity of COVID-19 disease. *Molecular Genetics and Genomics*, *298*(1), 27-36.
<https://doi.org/10.1007/s00438-022-01953-8>
- Niu, W., Qi, Y., Hou, S., Zhou, W., & Qiu, C. (2007). Correlation of angiotensin-converting enzyme 2 gene polymorphisms with stage 2 hypertension in Han Chinese. *Translational Research*, *150*(6), 374-380.
<https://doi.org/10.1016/j.trsl.2007.06.002>
- Novelli, A., Biancolella, M., Borgiani, P., Cocciadiferro, D., Colona, V. L., D'Apice, M. R., ... & Novelli, G. (2020). Analysis of ACE2 genetic variants in 131 Italian SARS-CoV-2-positive patients. *Human Genomics*, *14*, 1-6.
<https://doi.org/10.1186/s40246-020-00279-z>
- Pan, Y., Wang, T., Li, Y., Guan, T., Lai, Y., Shen, Y., ... & Liu, C. (2018). Association of ACE2 polymorphisms with susceptibility to essential hypertension and dyslipidemia in Xinjiang, China. *Lipids in Health and Disease*, *17*(1), 1-9.
<https://doi.org/10.1186/s12944-018-0890-6>
- Pinheiro, D. S., Santos, R. S., Jardim, P. C. V., Silva, E. G., Reis, A. A., Pedrino, G. R., & Ulhoa, C. J. (2019). The combination of ACE I/D and ACE2 G8790A polymorphisms reveals susceptibility to hypertension: A genetic association study in Brazilian patients. *PloS One*, *14*(8), e0221248.
<https://doi.org/10.1371/journal.pone.0221248>
- Pinto, B. G., Oliveira, A. E., Singh, Y., Jimenez, L., Gonçalves, A. N., Ogava, R. L., ... & Nakaya, H. I. (2020). ACE2 expression is increased in the lungs of patients with comorbidities associated with severe COVID-19. *The Journal of Infectious Diseases*, *222*(4), 556-563.
<https://doi.org/10.1093/infdis/jiaa332>
- Putnam, K., Shoemaker, R., Yiannikouris, F., & Cassis, L. A. (2012). The renin-angiotensin system: A target of and contributor to dyslipidemias, altered glucose homeostasis and hypertension of the metabolic syndrome. *American Journal of Physiology-Heart and Circulatory Physiology*, *302*(6), H1219-H1230.
<https://doi.org/10.1152/ajpheart.00796.2011>
- Sabater Molina, M., Nicolás Rocamora, E., Bendicho, A. I., Vázquez, E. G., Zorio, E., Rodriguez, F. D., ... & Gimeno Blanes, J. R. (2022). Polymorphisms in ACE, ACE2, AGTR1 genes and severity of COVID-19 disease. *PLoS One*, *17*(2), e0263140.
<https://doi.org/10.1371/journal.pone.0263140>
- Shikov, A. E., Barbitoff, Y. A., Glotov, A. S., Danilova, M. M., Tonyan, Z. N., Nasykhova, Y. A., ... & Glotov, O. S. (2020). Analysis of the spectrum of ACE2 variation suggests a possible influence of rare and common variants on susceptibility to COVID-19 and severity of outcome. *Frontiers in Genetics*, *11*, 551220.
<https://doi.org/10.3389/fgene.2020.551220>
- Sienko, J., Marczak, I., Kotowski, M., Bogacz, A., Tejchman, K., Sienko, M., & Kotfis, K. (2022). Association of ACE2 Gene Variants with the Severity of COVID-19 Disease-A Prospective Observational Study. *International Journal of Environmental Research and Public Health*, *19*(19), 12622.
<https://doi.org/10.3390/ijerph191912622>
- Srivastava, A., Bandopadhyay, A., Das, D., Pandey, R. K., Singh, V., Khanam, N., ... & Chaubey, G. (2020). Genetic association of ACE2 rs2285666 polymorphism with COVID-19 spatial distribution in India. *Frontiers in Genetics*, 1163.
<https://doi.org/10.3389/fgene.2020.564741>
- Strafella, C., Caputo, V., Termine, A., Barati, S., Gambardella, S., Borgiani, P., ... & Cascella, R. (2020). Analysis of ACE2 genetic variability among populations highlights a possible link with COVID-19-related neurological complications. *Genes*, *11*(7), 741.
<https://doi.org/10.3390/genes11070741>
- Torre-Fuentes, L., Matías-Guiu, J., Hernández-Lorenzo, L., Montero-Escribano, P., Pytel, V., Porta-Etessam, J., ... & Matías-Guiu, J. A. (2021). ACE2, TMPRSS2 and Furin variants and SARS-CoV-2 infection in Madrid, Spain. *Journal of Medical Virology*, *93*(2), 863-869.
<https://doi.org/10.1002/jmv.26319>
- WHO. (2023). Coronavirus disease (COVID-19) Weekly Epidemiological Updates and Monthly Operational Updates. Situation Reports.
<https://www.who.int/emergencies/diseases/novel-coronavirus-2019/situation-reports>

- Wrapp, D., Wang, N., Corbett, K. S., Goldsmith, J. A., Hsieh, C. L., Abiona, O., ... & McLellan, J. S. (2020). Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation. *Science*, 367(6483), 1260-1263. <https://doi.org/10.1101/2020.02.11.944462>
- Wu, F., Zhao, S., Yu, B., Chen, Y. M., Wang, W., Song, Z. G., ... & Zhang, Y. Z. (2020a). A new coronavirus associated with human respiratory disease in China. *Nature*, 579(7798), 265-269. <https://doi.org/10.1038/s41586-020-2008-3>
- Wu, A., Peng, Y., Huang, B., Ding, X., Wang, X., Niu, P., ... & Jiang, T. (2020b). Genome composition and divergence of the novel coronavirus (2019-nCoV) originating in China. *Cell Host and Microbe*, 27(3), 325-328. <https://doi.org/10.1016/j.chom.2020.02.001>
- Zhong, J., Yan, Z., Liu, D., Ni, Y., Zhao, Z., Zhu, S., ... & Zhu, Z. (2006). Association of angiotensin-converting enzyme 2 gene A/G polymorphism and elevated blood pressure in Chinese patients with metabolic syndrome. *Journal of Laboratory and Clinical Medicine*, 147(2), 91-95. <https://doi.org/10.1016/j.lab.2005.10.001>
- Zhou, P., Yang, X. L., Wang, X. G., Hu, B., Zhang, L., Zhang, W., ... & Shi, Z. L. (2020). A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature*, 579(7798), 270-273. <https://doi.org/10.1038/s41586-020-2012-7>