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ACE I/D polymorphism in Czech first-wave SARS-CoV-2-positive survivors

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ABSTRACT

Background: Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) rapidly spread from China in 2019/2020 to all continents. Significant geographical and ethnic differences were described, and host genetic background seems to be important for the resistance to and mortality of COVID-19. Angiotensin-converting enzyme (ACE) insertion/deletion (I/D) polymorphism (rs4646994) is one of the candidates with the potential to affect infection symptoms and mortality.

Methods: In our study, we successfully genotyped 408 SARS-CoV-2-positive COVID-19 survivors (163 asymptomatic and 245 symptomatic) and compared them with a population-based DNA bank of 2,559 subjects.

Results: The frequency of *ACE* I/I homozygotes was significantly increased in COVID-19 patients compared with that in controls (26.2% vs. 21.2%; P = 0.02; OR [95% CI] = 1.55 [1.17–2.05]. Importantly, however, the difference was driven just by the symptomatic subjects (29.0% vs. 21.2% of the I/I homozygotes; P = 0.002; OR [95% CI] = 1.78 [1.22–2.60]). The genotype distribution of the *ACE* genotypes was almost identical in population controls and asymptomatic SARS-CoV-2-positive patients (P = 0.76).

Conclusions: We conclude that *ACE* I/D polymorphism could have the potential to predict the severity of COVID-19, with I/I homozygotes being at increased risk of symptomatic COVID-19.

1. Introduction

Infection by SARS-CoV-2 represents a new emerging healthy situation around the globe. The infection emerged in China probably at the end of 2019 and rapidly spread to all continents. The novel virus named SARS coronavirus 2 (SARS-CoV-2) [1] is associated with the pneumonia-associated disease known as COVID-19 (COronaVIrus Disease – 2019). In contrast to other coronavirus-associated diseases (SARS, MERS), COVID-19 is a far milder upper respiratory tract illness but has superior transmission capability [2,3].

Very quickly, it has been recognized that there are significant geographical and interethnic variations in COVID-19 prevalence [2,4,5] and COVID-19-associated mortality. The variability in the host immune system [6,7] caused by genetic background might partly explain the described prevalence differences.

Among the candidates with the potential to affect SARS-CoV-2 infection and COVID-19 symptoms and severity, the *ACE* gene is often mentioned and has been extensively examined in several studies (for example, see [8–10]).

ACE (angiotensin I-converting enzyme, alias kininase II; OMIM acc. No. 106180) is an important part of the renin–angiotensin–aldosterone system (RAAS). ACE2, another member of the RAAS family, is recognized as a gateway for SARS-CoV-2 particle entry into cells, serving as their receptor [11]. However, variants within ACE2 were not associated with the prevalence or severity of SARS during the 2003/2004 outbreak [12].

Within the Alu sequence of intron 16 of the *ACE* gene, there is a functional polymorphism, represented by an insertion/deletion (I/D) of 287 bp (rs4646994). The D allele is significantly associated with increased activity and concentration of the enzyme [13], but associations with blood pressure or with increased cardiovascular risk were found in some but not in all studies (summarized by [14–16]).

Insertion/deletion *ACE* polymorphisms exhibit some significant geographical and ethical variability, where the frequency of the I allele

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is increased in Asian and African populations in comparison to that in Caucasians.

As a detailed analysis of the COVID-19 course and I/D *ACE* polymorphism has not yet been performed, our study focused on analysis of the I/D polymorphism within the *ACE* gene in symptomatic and asymptomatic SARS-CoV-2-positive patients from the first COVID-19 wave in the Czech Republic.

2. Material and methods

2.1. Subjects

The *ACE* I/D polymorphism was genotyped in 410 COVID-19 subjects (Table 1) who tested positive for the presence of SARS-CoV-2 infection (and completely recovered at the time of sample collection – July 2020) during the first wave (app. March 2020 – June 2020) of the disease in the Czech Republic [17]. Of them, 164 were asymptomatic, and 246 were symptomatic (without hospitalization).

As controls, 2,559 adults were used (post-MONICA study [18,19]; random 1% population sample from 9 districts, stratified by age and sex). Their *ACE* genotypes were known from a previous study [20].

Written informed consent was provided by all subjects involved in the study. The study protocol was approved by the institutional ethics committee.

2.2. DNA analysis

DNA was isolated from EDTA-treated blood [21]. The *ACE* I/D polymorphism was genotyped as described in detail by Rigat et al. [22]. PCR products of ~ 490 bp and ~ 200 bp characterise the I and D alleles, respectively. All D/D subjects were regenotyped with *ACE* I-specific oligonucleotides according to Ueda et al. [23] to avoid the misgenotyping of some I/D heterozygotes as D/D homozygotes. Based on confirmatory genotyping, 51 (3.8%) controls and 4 (0.9%) patients primarily genotyped as D/D were reclassified as I/D. PCR was performed on an MJ Research DYAD Disciple PCR device (MJ Research, MA, US), and all chemicals were produced by Fermentas International Inc. (Burlington, Ontario, Canada).

2.3. Statistical analysis

Statistical analysis has been performed using the www.socscistatistics.com tools (accessed 12/2020).

3. Results

The achieved call rate was 99.5% for COVID-19 patients and 98.2% for controls. In both the patient and control groups, the genotype frequencies were in agreement with Hardy-Weinberg equilibrium.

We observed significant differences in genotype frequencies of the *ACE* I/D polymorphisms between the patients and controls (for additional details, see Table 2).

The frequency of *ACE* I/I homozygotes was significantly increased in the entire group of COVID-19 patients compared with that in the controls (26.2% vs. 21.2%; P = 0.02; OR [95% CI] = 1.55 [1.17–2.05] for I/I vs. + D comparison).

Table 1
General characteristics of controls and COVID-19-positive subjects.

	Controls	Patients
N	2,579	410
Age (years)	48 ± 11	44 ± 15
% female	53.4	54.7
Diabetes (%)	8.2	7.8
Hypertension (%)	22.3	13.3

Importantly, however, the difference was driven by the symptomatic subjects only (29.0% vs. 21.2% of I/I homozygotes; P = 0.005; OR [95% CI] = 1.51 [1.13–2.02] for I/I vs + D comparison; P = 0.0025; OR [95% CI] = 1.78 [1.22–2.60] for D/D vs. I/I comparison).

The genotype distribution of the *ACE* genotypes was almost identical in population controls and asymptomatic SARS-CoV-2-positive patients (P = 0.79; OR [95% CI] 1.05 [0.72–1.54]; for additional details, see Table 2).

4. Discussion

In our study, we detected a significant effect of the *ACE* insertion/deletion polymorphism on the risk of symptomatic SARS-CoV-2 infection/COVID-19.

The I/D polymorphism within the *ACE* gene is among the heavily discussed inherited variants with the potential to influence the COVID-19 outcome [8,10,24,25], but especially as papers mostly have no direct patient data the conclusions are not uniform but rather contradictory. Some papers are suggesting the D allele and others suggesting the I allele as deleterious.

Mostly, the ecological studies used mortality data from different dates, which could lead to misreporting as the pandemia has different course due to time in different countries. Further, there are important regional differences in spread of pandemia within the countries and finally, for identical countries, different I/D ratios and different models (dominant or recessive) were used for calculation.

For example, our results are in agreement with the results described by Delanghe et al. [8,26]. They compared the numbers of fatalities, related both per million inhabitants and per number of total cases, within the first COVID-19 wave in data from dozens of European countries and recognized the I/I genotype as deleterious.

The functional link between *ACE* I/D polymorphism and infectivity and pathogenicity of the SARS-Cov-2 virus has been recently proved by Jacobs et al. [27]. In I/I homozygotes, increased ACE2 protein levels in lung epithelium has been detected – this can facilitate the virus entry into the host organism as ACE2 is used by the virus as gateway for cell entry.

Studies performed on Asian populations [24,28,29], reported opposite results and suggested a D/D genotype as a predictive marker of COVID-19 severity. These discrepancies could be caused by the fact, that there could be ethnicity specific allelic effect similar to the effect of response on ACE inhibitor treatment (see below, [30]. Different conclusions reached are indirectly confirmed by the fact that some nonwhite ethnicities with increased COVID-19 mortality exhibit an increased population frequency of the I allele.

However, the studies are very difficult to compare. The major difference between our and these studies is in the protocol performed. Furthermore, these studies have generally extrapolated the available data about the *ACE* I/D genotype frequencies and publicly available data about the prevalence of the disease. In contrast to the abovementioned studies, we have direct access to the patient DNA, COVID −19 status and we have the possibility to compare the ACE genotype frequencies directly with the large and population based sample. The abovementioned studies focused on mortality cases, which is in contrast with our study, which is focused on survivors and compares symptomatic and asymptomatic subjects. In contrast, we focused on concrete subjects, individuals who tested positive for suffering from SARS-CoV-2 infection, and linked the exact genotype with disease symptoms. Despite these differences, we have obtained similar results.

Importantly, a large cohort study performed in England detected that ACE inhibitor treatment was associated with a significantly (with a HR of approximately 0.7) reduced risk of COVID-19 [30], even after multiple adjustments. Furthermore, it cannot be excluded that the *ACE* I/D polymorphism could play different roles in different stages of infection and disease as well as in different ethnicities, as ACE inhibitors seem to be protective against COVID-19 in white UK subjects but in contrast

Table 2
Distribution of the *ACE* rs4646994 I/D polymorphism within the control and COVID-19-positive subject groups.

ACE	Controls		COVID-19 asymptomatic		COVID-19 symptomatic		OR Controls vs. asymptomatic	P	P*	OR Controls vs. symptomatic	P	P*
	N	%	N	%	N	%						
I/I	547	21.2	36	22.1	71	29.0	1.15 (0.72–1.83)	0.55	0.46*	1.78 (1.22–2.60)	0.002	0.03*
I/D	1331	51.6	87	53.4	123	50.2	1.14 (0.79–1.68)	0.49	0.76#	1.27 (0.91–1.78)	0.16	0.008#
D/D	701	27.2	40	24.5	51	20.8	1.00		0.98§	1.00		0.005§
I/I	547	21.2	36	22.1	71	29.0	1.05 (0.72–1.54)	0.79		1.51 (1.13–2.02)	0.005	
+D	2032	78.8	127	77.9	174	71.0	1.00			1.00		
+I	1878	72.8	123	75.5	194	79.2	1.14 (0.80–1.66)	0.46		1.42 (1.03–1.96)	0.03	
D/D	701	27.2	40	24.5	51	20.8	1.00			1.00		

P* for D/D vs. + I*; D/D vs. I/D vs. I/I#, +D vs. I/I§ comparisons.

even deleterious in nonwhite UK subjects. It would be of interest to study whether there is some interaction between *ACE* genotypes and ACE inhibitors in susceptibility to symptomatic COVID-19.

Among other factors, male sex, obesity, diabetes and nonwhite ethnicity are reported to be associated with an increased risk of COVID-19 [4]. Within the control group, we did not detect any association between the *ACE* I/D polymorphism and blood pressure, body mass index or the presence of diabetes mellitus, suggesting a context-independent association with disease.

The possibility of predicting symptomatic COVID-19 using a simple, quick and inexpensive test based on individual subject DNA polymorphisms could be very important in general testing to distinguish between subjects with high and low risk of disease and, importantly, for the estimation of the severity of the disease. It is certain that the analysis of one polymorphism will not be sufficient, and the examination of a wide list of polymorphisms will be necessary. Creation of the genetic risk score reflecting the host ability to affect the individual's sensitivity to COVID-19 could be important.

The COVID situation has evoked a wave of interest focused on potential host genetic predisposition to the disease. The list of suggested polymorphisms [6] with potential to affect SARS-CoV-2 susceptibility is long (including, for example, genes for vitamin D receptor, MBL and CLEC4M), but so far, undoubtedly confirmed and widely replicated genetic determinants of SARS-CoV-2 infection and COVID-19 severity remain unknown.

Genetic susceptibility to COVID-19 was also examined using the genome-wide association approach [31]. This study detected a few useful signals within the AB0 blood-group system, suggesting a benefit in blood group 0 and increased risk associated with blood group A. The second strongest signal was localized at the 3p21.31 locus, where promising candidates, such as genes for two chemokine receptors, CCR9 and CXCR6, or the gene for SLC6A20, which interacts with ACE2, are localized.

Nonetheless, the differences in the prevalence of disease suggest possible nonuniversality of the involved genes/variants, as suggested by our results, where the *ACE* I/I genotype is associated with the symptomatic course of the disease.

5. Conclusion

The results of our study suggest that *ACE* I/I homozygotes may be at an increased risk of symptomatic COVID-19, at least in East European Caucasians.

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CRediT authorship contribution statement

Jaroslav A. Hubacek: Conceptualization, Formal analysis, Funding acquisition, Project administration, Writing - original draft. **Ladislav Dusek:** Conceptualization, Funding acquisition, Project administration, Supervision, Writing - review & editing. **Ondrej Majek:** Formal analysis, Investigation, Supervision, Writing - review & editing. **Vaclav Adamek:** . **Tereza Cervinkova:** Data curation, Investigation, Writing - review & editing. **Dana Dlouha:** Writing - review & editing, Investigation, Methodology, Writing - review & editing. **Vera Adamkova:** Conceptualization, Data curation, Funding acquisition, Project administration, Supervision, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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