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SHORT COMMUNICATION

ABCA3 and LZTFL1 Polymorphisms and Risk of COVID-19 in the Czech Population

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Summary

SARS-CoV-2 infection, which causes the respiratory disease COVID-19, has spread rapidly from Wuhan, China, since 2019, causing nearly 7 million deaths worldwide in three years. In addition to clinical risk factors such as diabetes, hypertension, and obesity, genetic variability is an important predictor of disease severity and susceptibility. We analyzed common polymorphisms within the *LZTFL1* (rs11385942) and *ABCA3* (rs13332514) genes in 519 SARS-CoV-2-positive subjects (164 asymptomatic, 246 symptomatic, and 109 hospitalized COVID-19 survivors) and a population-based control group (N = 2,592; COVID-19 status unknown). Rare *ABCA3* AA homozygotes (but not A allele carriers) may be at a significantly increased risk of SARS-CoV-2 infection [P = 0.003; OR (95 % CI); 3.66 (1.47-9.15)]. We also observed a borderline significant difference in the genotype distribution of the *LZTFL1* rs11385942 polymorphism (P = 0.04) between the population sample and SARS-CoV-2-positive subjects. In agreement with previous studies, a nonsignificantly higher frequency of minor allele carriers was detected among hospitalized COVID-19 subjects. We conclude that a common polymorphism in the *ABCA3* gene may be a significant predictor of susceptibility to SARS-CoV-2 infection.

Keywords

COVID-19 • *LZTFL1* • *ABCA3* • Polymorphism • Susceptibility

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SARS-CoV-2 infection of unclear origin [1], which causes coronavirus disease 2019 (COVID-19), spread rapidly from Wuhan, China at the end of 2019. The infection has a relatively low mortality rate but is highly infectious. To date, it has been implicated in 765 000 000 cases and almost 7 million deaths worldwide (https://covid19.who.int; accessed on May, 2023). The population of the Czech Republic is among the most affected (https://covid19.who.int) [2]. The heterogeneity of the host immune system [3, 4] is responsible for the highly variable course of the disease. In a significant proportion of cases the infection is asymptomatic, while symptomatic individuals usually suffer from fever, cough, and, in some cases, gastrointestinal irritation. But under certain, albeit poorly understood, circumstances, COVID-19 infection can lead to severe pneumonia and subsequent death of the infected subjects.

Clinical risk factors such as obesity, diabetes, and hypertension [5,6] are among the factors that worsen the susceptibility and prognosis of the disease. However, genetic background also plays an important role in

disease susceptibility and severity [7,8]. For example, variants in genes encoding angiotensin-converting enzyme-I, apolipoprotein E, the AB0 blood group system, chemokine receptor 5 or oligoadenylate synthase 1 have been reported to predict COVID-19 susceptibility or severity [7,8].

Given the long list of genes that could potentially influence disease progression, we decided to investigate the role of two preselected genes associated with lung function, *ABCA3* and *LZTFL1*.

ABCA3 (ATP-binding cassette transporter subfamily A member 3; OMIM 601615) is a candidate gene that has yet to be extensively studied in relation to COVID-19. COVID-19 is a respiratory infection that, in severe cases, can lead to lung inflammation. Therefore, *ABCA3* would seem a logical and plausible contributor to COVID-19 susceptibility and/or severity. As a lung-specific phospholipid transporter critical for intracellular surfactant synthesis, storage, and homeostasis [9], *ABCA3* plays a vital role in proper lung function. For example, *ABCA3* mutations can be lethal in newborns [10], while several common *ABCA3* variants (including rs13332514) are associated with neonatal respiratory distress [11].

According to genome-wide association study (GWAS), a cluster of variants at the 3p21.31 locus (including rs11385942, a single A insertion/deletion polymorphism), known to influence the expression of leucine zipper transcription factor-like 1 (*LZTFL1*; OMIM 606568), is associated with an increased risk of hospitalization in COVID-19 patients [12]. This finding was subsequently confirmed in several ethnically different populations [13,14]. *LZTFL1* encodes a protein that is highly expressed in the lung. Expression of this protein, which is involved in regulating the function of airway cilia, has been shown to correlate with bronchial epithelial cell differentiation [15].

To investigate the potential effects of the above variants on the development of COVID-19 in the Czech population, two groups of adult subjects were genotyped.

First, 519 subjects with a positively tested (PCR-based) result for the presence of the SARS-CoV-2 virus infection were included. Of these patients, 164 subjects were asymptomatic, 246 were symptomatic (mild form without hospitalization) [16-18], and 109 were hospitalized non-fatal cases.

For comparison, 2,592 adult subjects (selected as the general population aged 28-65 years at the time of examination) from the post-MONICA study [19] were genotyped. Information on COVID-19 positivity and negativity was not available for these subjects. The study protocol complied with the 1964 Declaration of Helsinki and its subsequent amendments and was approved by the Ethics Committee of the Institute for Clinical and Experimental Medicine and Thomayer University Hospital. All subjects were self-reported Caucasians and provided their signed informed consent to take part in the DNA analysis.

Individual SNPs have been screened by the PCR-RFLP methodology, using the oligonucleotides 5′ CCA TGC AGA TGG CCC TTG GCC CCT TGG; 5′ TCC CTG GTG CTC GCC TTC CTG CTG TG***A*** T and the restriction enzyme HinfI (rs13332514; NC_000016.10:2317334:
G:A; restriction site present in the case of the G allele) and 5′ TTT TCT CAC CAG TCA TCT ACT GAC AGT GG; 5′ TCT AAG CAC AGT CAC AGC ACA TCA GA***T*** and restriction enzyme DpnI/MboI (rs11385942; NC_000003.12:45834967:AA:AAA, restriction site present in the case of the deletion allele). Mismatched nucleotides, creating appropriate restriction sites, are in bold italics. For full details of the PCR conditions and cycling temperatures, please contact the corresponding author.

Statistical analysis was performed using www.socscistatistics.com (accessed March/2023). In cases where a subgroup of homozygotes contained less than five subjects, these were pooled with heterozygotes and analyzed together. A P value of < 0.05 was considered significant.

As described previously [18], patients and controls were similar in age (48±11 and 46±18 years), sex distribution, and the prevalence of diabetes and hypertension. More obese subjects were detected among patients ($P < 0.05$).

Genotyping call rates varied between 95.3 % and 99.6 % and the allele frequencies of both polymorphisms were similar to those found in other Caucasian populations (according to the National Institutes of Health SNP database; www.ncbi.nlm.nih.gov/snp/) and in the general population. Genotype distribution was consistent with Hardy-Weinberg equilibrium.

We observed significant differences in the genotype frequencies of both genes between patients and the population.

Rare minor *ABCA3* homozygotes were more common in patients (1.5 %) than in population controls (0.4 %), suggesting that these subjects may be at increased risk of COVID-19 infection [$P = 0.003$; OR (95 % CI); 3.66 (1.47-9.15)]. The association was observed only in the case of the recessive comparison (AA homozygotes vs G allele carriers), but no differences

were observed in the prevalence of heterozygotes between groups (P = 0.22) (see Table 1 and Fig. 1. for further details and comparisons). This observation is novel and unique. No association between variants within the *ABCA3* gene and COVID-19 has yet been described. Surprisingly, *ABCA3* variants have thus far not been considered potential predictors of COVID-19 severity, despite their acknowledged roles in abnormal lung function. On the other hand, GWAS have not found any association between *ABCA3* loci and COVID-19. Therefore, it is possible that the low population frequency of the minor allele of rs13332514 makes it difficult to meet the minimum P value threshold of 10^{-8} required by GWASs, especially if the disease is only associated with the minor allele in a recessive model.

In the case of *LZTFL1* variability, carriers of at least one minor allele appear to be, in general, slightly protective (P = 0.04) against SARS-CoV-2 infection. However, when we examined subjects according to disease severity, carriers of at least one insertion allele were underrepresented (P = 0.04) among non-hospitalized SARS-CoV-2 subjects. This finding was valid both in comparison with hospitalized COVID-19 patients [OR (95 % CI); 0.56 (0.32-0.98)] and with the general population [P = 0.008; OR (95 % CI); 0.65 (0.48-0.90)]. These frequencies suggest that subjects with the insertion allele are unlikely to have an increased susceptibility to the disease. However, if they do become infected, a severe course of the disease can be expected (see Table 1 for more details). This overrepresentation of the minor allele in hospitalized COVID-19 patients has been reported previously [13, 14, 20] and is consistent with the different distributions in our subjects who tested positive for SARS-CoV-2.

We are aware of the limitations of our study, particularly the lack of a fourth group consisting of deceased COVID-19 patients. It would have been interesting to investigate whether the *ABCA3* polymorphism would be a potential predictor of mortality in these individuals. Additionally, the numbers of patients within each patient subgroup were relatively small, especially considering that the frequency of the minor *ABCA3* homozygotes in the general population was less than 1 %. Although we did not adjust for potential confounding factors, we consider it unlikely that this omission would have significantly altered our findings for two reasons. Firstly, our study groups had similar clinical characteristics; secondly, within the population group, we did not detect any association between the polymorphisms studied and major clinical COVID-19 risk factors such as diabetes, obesity (BMI), or hypertension. In addition, these factors have yet to be associated with the variability of *ABCA3* or *LZTFL1* in the literature. Finally, COVID-19 status was not known in controls; nevertheless, the cohort provide representative information about genotype frequencies for Czech population. Potential confounding from differential SARS-CoV-2 ascertainment rate cannot be excluded in patients with mild disease.

In summary, we present our findings for two genes encoding proteins important for proper lung function. Our study is the first to associate a common variant within the *ABCA3* gene with susceptibility to SARS-CoV-2 infection. Whether the association is valid in other populations needs to be confirmed in subsequent studies.

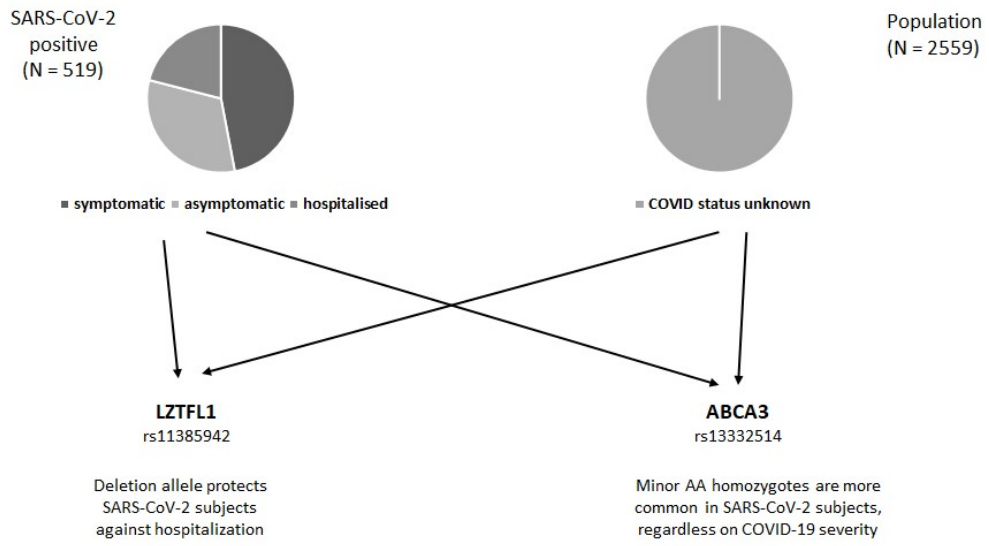

Fig. 1. Schematic overview of the study results.

Table 1. Distribution of *ABCA3* and *LZTFL1* genotypes among SARS-CoV-2 positive subjects and the general population.

LZTFL1	**Population**		**COVID-19 total**		**COVID-19 asymptomatic[#]**		**COVID-19 symptomatic[§]**		**COVID-19 hospitalised[±]**		**P***	**OR**	**P**
rs11385942	N	%	N	%	N	%	N	%	N	%			
AA/AA	2016	81.6	423	85.5	133	86.9	206	87.3	84	79.2			0.10[#]
AA/AAA	433	17.5	67	13.5	18	11.8	28	11.9	21	19.8	0.04	0.76 (0.58 – 0.99)	0.03[§]
AAA/AAA	21	0.9	5	1.0	2	1.3	2	0.8	1	0.9			0.54[±]
ABCA3													
rs13332514													
GG	2107	81.9	430	83.2	138	84.1	202	82.1	90	84.1			0.47[#]
GA	455	17.7	79	15.2	24	14.6	40	16.3	15	14.0	0.003	3.66 (1.47 – 9.15)	0.93[§]
AA	11	0.4	8	1.5	2	1.2	4	1.6	2	1.9			0.56[±]

P* 2x2 chi-square test for M/M vs +m subjects (*LZTFL1*) or +M vs m/m subjects (*ABCA3*); controls vs all SARS-CoV-2 positive, [#] controls vs COVID-19 asymptomatic; [§] controls vs COVID-19 symptomatic; [±] controls vs COVID-19 hospitalized, M – major allele; m – minor allele

Conflict of Interest

There is no conflict of interest.

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