POSSIBLE EFFECT OF *OAS1* AND *TMPRSS6* BUT NOT *DPP4* AND *ZNF335* POLYMORPHISMS ON COVID-19 SEVERITY IN THE CZECH POPULATION

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SUMMARY

Objectives: The acute respiratory syndrome, known as COVID-19, is characterised by high morbidity and increased mortality. Genetic factors may partially explain the differences in susceptibility to and severity of COVID-19.

Methods: We have analysed common functional polymorphisms within the *OAS1* (rs4767027), *TMPRSS6* (rs855791), *DPP4* (rs3788979), and *ZNF335* (rs3848719) genes in SARS-CoV-2 positive subjects (n = 521, different disease severity) and in population controls (n = 2,559 subjects, COVID-19 status unknown).

Results: Neither *DPP4* nor *ZNF335* were associated with disease susceptibility or severity in the Czech population in any of the models used for calculation. T allele carriers of the *OAS1* polymorphism seem to be protective against symptomatic COVID-19 (p = 0.002 calculated for trend; asymptomatic, symptomatic, hospitalised). Similarly, within the *TMPRSS6*, minor TT homozygotes associated with lower plasma Fe concentrations were underrepresented in the overall patient group (p = 0.044; OR = 0.77, 95% CI: 0.59–0.99), and the difference was mainly driven by the severe COVID-19 subjects. In general, risky homozygotes of these two polymorphisms were less frequent than expected in the group of hospitalised COVID-19 survivors.

Conclusions: Common variants within *OAS1* (rs4767027) and *TMPRSS6* (rs855791) play some role in COVID-19 pathology in the Czech Caucasian population. Whether the depletion of minor allele carriers of these two variants is associated with increased COVID-19 mortality, needs to be analysed in an external confirmatory study.

Key words: COVID-19, DPP4, OAS1, polymorphism, SARS-CoV-2, severity, TMPRSS6, ZNF335

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INTRODUCTION

Severe acute respiratory syndrome RNA coronavirus 2 (SARS-CoV-2) infection occurred at the end of 2019 and spread rapidly from Wuhan, China, around the world. COVID-19 (COrona VIrus Disease) has so far (September 2023) affected over 760,000,000 people and is associated with almost 7,000,000 deaths*, with the Czech population being among the most affected worldwide (1, 2).

It has been quickly recognised that the susceptibility to this infection and the severity of the COVID-19 disease is increased in diabetics (especially of type 1), obese individuals and hypertonics (3, 4).

Similar to other infectious diseases (5, 6), it is widely discussed that COVID-19 susceptibility and severity are influenced not only by host immune system variability (7), but also, and importantly, by genetic factors (8–10). Number of common genetic variants (for example within the *ACE*, *APOE*, *CCR5*, *IFFITM3*, *TNFα* or common blood groups A, B or 0) have been associated with COVID-19 in studies with different designs.

Iron metabolism is closely related to immunity and host defence against infections in general, not excluding COVID-19 disease (11). Variability in the main genetic determinant of Fe plasma concentrations, the haemochromatosis associated *HFE* gene, has been connected with COVID-19 both in *in silico* (12) as well as in *in vivo* (13) studies.

*https://covid19.who.int

TMPRSS6 (coding for transmembrane protease, serine 6, OMIM acc. No. 609862) is the second most potent gene regulating iron homeostasis in the human body (14). The rs855791 (T > C) polymorphism is highly associated with iron levels (15) and the CC genotype, due to its association with increased Fe levels is therefore a plausible candidate to predispose the COVID-19 subjects to the more severe disease course.

Zinc finger-containing proteins (both belonging to SARS-CoV-2 or to the host) play an important role in antiviral defence as well as in regulation of the viral life cycle (16). ZNF proteins in general could promote the antiviral activity of immune cells and effectively suppress SARS-CoV-2 infection (17). The rs3848719 variant within one of them, namely *ZNF335* (zinc finger protein 335; OMIM acc. No. 610827), is one of the most powerful genetic determinants of the acute respiratory distress syndrome which (similarly to severe COVID-19) is characterized by pulmonary inflammation and hypoxaemia (18) and has been selected for a more detailed analysis.

DPP4 (dipeptidyl peptidase IV alias CD26 or ADCP 2; OMIM acc. No. 102720), which is highly expressed in the human respiratory tract has been suggested to be a co-receptor important for the entry of the SARS-CoV-2 particles into cells. *DPP4* may also be involved in the development of the cytokine storm that leads to the fatal COVID-19 associated pneumonia (19, 20). Associations between lower levels of *DDP4* as well as between rs3788979 and the presence of COVID-19 have been described (21). In concert, the authors also reported lower level of plasmatic *DPP4* in TT homozygotes (21).

Finally, variants at the *OAS1* (2-prime,5-prime-oligoadenylate synthetase 1, OMIM acc. No. 164350) locus, which was transferred from *Homo neanderthalensis* to *Homo sapiens* (22, 23), appear to be of exceptional importance, as suggested by initial COVID-19-associated GWAS studies. *OAS1* plays a very important role in general protection against virus infection. *OAS1* acts as an activator of RNases, degrading mainly viral RNAs. As a result, protein synthesis is inhibited, and viral replication is impaired. The rs4767027 polymorphism is a member of a block of polymorphisms that affect *OAS1* activity.

Our case-control study investigated the above-mentioned variants and the risk of SARS-CoV-2 infection and COVID-19 severity in the Czech population.

MATERIALS AND METHODS

As cases, we have included a total of 521 SARS-CoV-2 positively tested adult subjects (aged 18–73 years) with different COVID-19 severity – 164 asymptomatic, 246 symptomatic and 111 hospitalised (13, 24–26). The frequencies of their genotypes have been compared with the results obtained at large general population sample of 2,559 subjects (aged 26–65 years at the time of the examination) (27, 28). Information on SARS-CoV-2 positivity/COVID-19 status was not available for this group. All subjects self-identified as "Caucasian".

DNA has been isolated from uncoagulated EDTA blood. Four SNPs (single nucleotide polymorphisms) of interest have been genotyped using the PCR-RFLP methods. Detailed oligonucleotide sequences, restriction enzymes used, and fragments characteristic of each allele are summarised in Table 1.

The OR (95% CI) was calculated and the distributions of individual genotypes and alleles between groups were compared in three models (2 × 3 or 2 × 2; MM vs. +m; MM vs. Mm vs. mm; +M vs. mm where "M" represents the major allele and "m" the minor allele) using the chi-squared test. For *DPP4* polymorphism, as there were less than 5 subjects in some subgroups +M vs. mm model has been omitted. Cochran-Armitage test has been used for calculations of trend between the SARS-CoV-2 subjects with different COVID-19 severity. Freely available statistical software** (fully compatible with SPSS statistical program; accessed 03/2023) has been used for evaluation. P-values less than 0.05 have been considered significant.

RESULTS

The compared groups (controls vs. patients) were similar in the frequencies of common COVID-19 risk factors, such as the prevalence of diabetes (8.2% vs. 9.9%) and hypertension (22.4% vs. 24.8%). Patients have been slightly more likely to be obese (32.7% vs. 28.7%; $p < 0.05$) compared to the general population (14). Also number of males was comparable in both groups (47% in patients and 45% in controls).

The detailed distribution of the examined genotypes within all analysed groups and subgroups is summarised in Table 2.

Table 1. *Genotyping details for analysis of SNPs within OAS1, TMPRSS2, DPP4 and ZNF335 genes*

Gene/SNP	Oligonucleotide sequences	PCR product	Enzyme	Size of restriction fragments (bp)	Allele
OAS1 rs4767027	5´ ttt cac cag cta tct tgg cac cct tag ccc 5´ aaa agg caa cac aag tgc att ttc cac cc	151 bp	MspI	151 121 + 30	T C
TMPRSS2 rs855791	5´ aac agg ggc tcc agg ctc ctg aga tct ca 5´ tgg tga tgt ggg cag cat cct ttc tcc c	249 bp	HaeIII	196 + 53 131 + 65 + 53	T C
DPP4 rs3788979	5´ ttt gta aag ata ccc tgt att gca agc aa 5´ ata gag tct tca tgt cca gga gag tga gcc c	339 bp	BsuRI	339 225 + 114	A G
ZNF335 rs3848719	5´ atc atg gcc tcc agg ctc tgc 5´ aat gtc cag ttc cac ctt ggc cc	171 bp	AluI	102 + 56 + 12 114 + 56	C T

**https://www.socscistatistics.com/tests/chisquare2/default2.aspx

Table 2. *Distribution of examined SNPs between SARS-CoV-2 positive subjects and Czech population*

	Population n (%)	COVID-19 total n (%)	COVID-19 asympto-matic n (%)	COVID-19 sympto-matic n (%)	COVID-19 hospitalised n (%)	p-value*	p-value	OR (95% CI)	p-value#
TMPRSS2/rs855791									
GG	838 (33.6)	157 (30.4)	52 (31.7)	75 (30.6)	30 (28.0)	0.156[1]	0.816[#]	1.00	
GT	1,168 (46.9)	278 (53.9)	81 (49.4)	132 (53.9)	65 (60.7)	0.011[2]	0.092[§]	1.27 (1.02–1.57)	0.028
TT	486 (19.5)	81 (15.7)	31 (18.9)	38 (15.5)	12 (11.2)	0.044[3]	0.012[±]	0.90 (0.67–1.19)	0.430
ZNF335/rs3848719									
GG	862 (32.3)	175 (33.9)	66 (40.2)	73 (29.8)	36 (33.6)	0.847[1]	0.305[#]	1.00	
GA	1,232 (49.1)	259 (50.2)	74 (45.1)	127 (51.8)	58 (54.2)	0.888[2]	0.341[§]	1.04 (0.84–1.28)	0.745
AA	415 (16.5)	82 (15.9)	24 (14.6)	45 (18.4)	13 (12.1)	0.491[3]	0.413[±]	0.97 (0.73–1.30)	0.853
DPP4/rs3788979									
CC	1,783 (71.9)	377 (73.2)	115 (71.0)	177 (72.0)	85 (79.4)	0.188[1]	0.797[#]	1.00	
CT	640 (25.8)	130 (25.2)	43 (26.5)	65 (26.4)	22 (20.6)	0.564[2]	0.993[§]	0.96 (0.77–1.20)	0.719
TT	56 (2.3)	8 (1.6)	4 (2.5)	4 (1.6)	0 (0.0)	0.314[3]	0.088[±]	0.68 (0.32–1.43)	0.302
OAS1/rs4767027									
CC	1,110 (44.8)	234 (44.9)	62 (37.8)	109 (44.3)	63 (56.8)	0.978[1]	0.101[#]	1.00	
CT	1,071 (43.3)	227 (44.5)	85 (51.8)	106 (43.1)	36 (32.4)	0.972[2]	0.945[§]	1.01 (0.82–1.23)	0.958
TT	294 (11.9)	60 (11.5)	17 (10.4)	31 (12.6)	12 (10.8)	0.816[3]	0.042[±]	0.97 (0.71–1.32)	0.838

p* – controls vs. all SARS-CoV-2 positive ([1]MM vs. +m; [2]MM vs. Mm vs. mm; [3]+M vs mm); p – [#]controls vs. COVID-19 asymptomatic; [§]controls vs. COVID-19 symptomatic; [±]controls vs. COVID-19 hospitalised, comparisons in MM vs. Mm vs. mm model; for *DPP4* in MM vs. +m model
p# 2 x 2 – chi-square test, identical comparison as for OR (95% CI) calculation
M – major allele; m – minor allele

The genotyping call rate varied between 96.7% and 98.8% in the population and between 98.7% and 99.8% in the patients group.

In general, we have found no significant associations between the investigated SNPs within the *DPP4* and *ZNF335* polymorphisms and susceptibility to the SARS-CoV-2 infection or COVID-19 severity (Table 2). For these two genes, no significant differences have been observed between the studied groups and no trends in COVID-19 subgroups have been suggested, regardless of the type of comparison.

In contrast, the variant at *TMPRSS6* seems to influence the course of COVID-19 in the Czech population. Minor TT homozygotes, associated with lower plasma Fe concentrations were slightly underrepresented in the entire group of patients (p = 0.044, OR = 0.77, 95% CI: 0.59–0.99 for TT vs. +G comparison). Interestingly, this significance is mainly driven by the group of hospitalised COVID-19 subjects, where only 11.2% (in comparison to 19.5% in population) of TT homozygotes have been detected (p = 0.032, OR = 0.52, 95% CI: 0.28–0.96 for TT vs. +G comparison).

In addition, distinct genotypes of the *OAS1* polymorphism also appear to be beneficial in the case of COVID-19 severity. In general, there were no differences between the population and all SARV-CoV-2 positive subjects in our study. However, when subjects with different disease course have been examined in detail, carriers of at least one *OAS1* T allele (TT homozygotes + CT heterozygotes) were less frequent among hospitalised COVID-19 survivors (p = 0.014, OR = 0.62, 95% CI: 0.42–0.91; comparison with the population). Finally, there was a significant decreasing trend (p = 0.002) in the incidence of T allele carriers among asymptomatic subjects over symptomatic to hospitalised patients.

It is important to emphasise that we did not detect an increased frequency of the genotypes putatively associated with severe COVID-19 in the hospitalised subjects for either of the above-mentioned polymorphisms.

DISCUSSION

The COVID-19 pandemic that we have witnessed has shown that our modern society desperately needs the support of the public health sector as a field that has in its charge very important decisions that shape the life of the whole society. The widest possible knowledge of this new disease (including the genetic determination of disease susceptibility and severity) and its possible impact on our health is essential for the right decisions to be taken by the appropriate authorities as well as clinicians.

In our study, two out of four examined common genetic polymorphisms were associated with COVID-19 susceptibility or severity.

Several studies have associated *OAS1* loci to not only with COVID-19 (22, 23), but also with several other viral infections (29). In general, minor alleles within the *OAS1* loci are suggested to be associated with COVID-19 severity (30). In our study, minor allele carriers are most common between asymptomatic SARS-CoV-2. Taken together with our results, this suggests that carriers of *OAS1* minor alleles may be in fact at increased risk of COVID-19 mortality, not just hospitalisation, but a study focused on deceased COVID-19 subjects has not been performed so far. Unfortunately, we cannot confirm this theory as we also lack a group of COVID-19 deceased subjects.

Rather surprisingly, no study focusing on the potential association between *TMPRSS6* variability and COVID-19 has been described to date, so the unique finding of our study needs to be further confirmed. The association found is indirectly supported by the fact, that iron metabolism undoubtedly plays in the pathogenesis of COVID-19 an important role (11).

In contrast, *DPP4* and *ZNF335* variants were not associated with COVID-19 susceptibility or severity.

We were the first to try to find an association between *ZNF335* variability and COVID-19. Our findings do not support the idea, that this zinc finger containing protein, potentially influencing viral cycle (16), will play an highly important role in this pathology.

Finally, we have not confirmed the results of the Mexican study (21) suggesting that the *DPP4* rs3788979 TT genotype is associated with the severity of COVID-19. The fact that the number of subjects we have examined overcome five times the number of patients and ten times the number of controls included in this study (21) suggests that the original study was probably prone to the false positive result. Another possibility is that ethnicity may interact significantly with this variant and COVID-19, and that the ethnic differences between our and Mexican study are in the background of the observed discrepancies.

We wish also to point to several study limitations. Firstly, as the population samples have been collected due to the pre-COVID-19 era, we do not have data about the SARS-CoV-2 positivity testing for controls. However, vast majority of COVID-19 studies suffer on the identical limitation, and also our previous studies focused on the COVID-19 (13, 24–26) have successfully implemented the identical group of controls. Secondly, our subgroup of examined patients have been collected retrospectively (for ethical reasons), thus only COVID-19 survivors are included. We cannot exclude, that the depletion of minor allele carriers of *OAS1* and *TMPRSS2* variants between hospitalised subjects is a consequence of the increased COVID-19 mortality. In subsequent studies, a group of COVID-19 non-survivors needs to be analysed.

CONCLUSION

We conclude that it is likely, that common variants within *OAS1* and *TMPRSS6*, but not within *DPP4* and *TMPRSS2* play an important role in COVID-19 pathology in the Czech Caucasian population. To confirm the utility of our observations, it is necessary to perform further external validation studies.

Conflicts of Interest

None declared

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Adherence to Ethical Standards

The institutional ethic committee has approved the protocol of the study (docket No. 9877/21, approved 2021/04/14). All participating subjects signed an informed consent for genetic testing. Data were handled strictly anonymously.

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