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A haemochromatosis-causing HFE mutation is associated with SARS-CoV-2 susceptibility in the Czech population

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ABSTRACT

Background: Coronavirus disease (COVID-19), which is caused by the SARS-CoV-2 virus, has become a global pandemic. While susceptibility to COVID-19 is subject to several external factors, including hypertension, BMI, and the presence of diabetes, it is also genetically determined to a significant extent. Infectious agents require iron (Fe) for proper functioning. Carriers of mutations resulting in increased iron concentrations are understood to be at increased risk of COVID-19.

Methods: We examined *HFE* genotypes associated with hereditary haemochromatosis (rs1800562 and rs1799945 SNPs) in 617 COVID-19 patients (166 asymptomatic, 246 symptomatic and 205 hospitalised survivors) and 2 559 population-based controls.

Results: We found a higher frequency of the minor allele (Tyr282) of the rs1800562 polymorphism ($P < 0.002$) in patients compared to controls (8.5 % vs 5.5 %). Non-carriers of the minor allele were protected against SARS-Cov-2 infection (OR, 95 %CI; 0.59, 0.42–0.82). The frequency of minor allele carriers was almost identical across asymptomatic, symptomatic, and hospitalised survivors. The rs1799945 variant did not affect disease severity and its occurrence was almost identical in patients and controls (P between 0.58 and 0.84).

Conclusions: In conclusion, our results indicate that presence of the rs1800562 minor allele, which is associated with hereditary haemochromatosis (thus increased levels of plasma Fe), increases susceptibility to SARS-CoV-2.

1. Introduction

COVID-19 disease manifests as a result of infection with the SARS-CoV-2 virus [1]. According to the World Health Organization (covid19.who.int, accessed 16th November 2022), COVID-19 has resulted in almost 6 600 000 cumulative deaths around the world in the last three years. Although the infection is characterised by mild mortality, its extreme infectiousness has led to these high absolute mortality numbers.

Like other infections [2,3], select subpopulations are at increased risk of COVID-19. Disease occurrence and mortality are influenced by a wide spectrum of accompanying risk factors, of which non-Caucasian ethnicity, male sex, obesity, and diabetes mellitus (especially type 1) seem to be the most deleterious [4]. In addition, genetic predisposition has been widely discussed [5–8] as an important predictor of both susceptibility and severity.

Among the genetic variants understood to influence the course of COVID-19, those linked to HFE-related haemochromatosis have been cited on several occasions [6,9].

The hypothesis that HFE variability influences COVID-19 susceptibility and severity is plausible, given that iron is a crucial catalyst in several fundamental metabolic processes. An overload of iron is associated with poor prognoses and increased progression of disease in a number of viral infections [10,11]. A similar scenario has been proposed for COVID-19 [12] and, unsurprisingly, iron chelation has been discussed as a potential therapeutic goal in the treatment of the disease [13,14].

Abbreviations: COVID, coronavirus disease.

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Hereditary haemochromatosis (OMIM acc. No. 235200) is an autosomal recessive disease that affects how iron is metabolised in the body. Subjects affected with HFE are susceptible to increased iron resorption, leading to elevated plasma ferritin concentrations. Excess iron storage in tissues, predominantly the liver cells, can lead to serious complications including multiple organ dysfunction [15]. The nucleotide exchanges that cause haemochromatosis occur within the homeostatic iron regulator gene (HFE, OMIM acc. No. 613609). While the minor allele of the rs1800562 polymorphism (G845A, Cys282Tyr) is associated with increased risk of hereditary haemochromatosis, the minor allele of the rs1799945 polymorphism (C187G, Asp63His) is associated with a milder form. Importantly, the occurrence of *HFE* mutations significantly differs between ethnicities [16,17]. Although the rs1799945 polymorphism is present in different populations around the world, the more severe rs1800562 mutation predominates in European populations and is almost entirely absent from populations in other regions [18]. Even within Europe, significant inter-population differences exist. The highest frequency is in northwestern Europe with almost 10 % of variant/minor allele carriers, whereas frequency in the Balkan Peninsula is close to zero [18,19].

Two studies [6,9] focusing on links between polymorphisms and COVID-19 have found a putative association between the *HFE* rs1800562 variant and COVID-19 prevalence/mortality based on univariate (but not on multivariate) analysis.

Our study focuses on the direct analysis of two common polymorphisms at the *HFE* loci – G845A (rs1800562) and C187G (rs1799945) – in subjects with confirmed SARS-CoV-2 infection and in a population sample.

2. Materials and methods

HFE was genotyped in 617 subjects positively tested (PCR-based) for the presence of SARS-CoV-2 infection during the first wave (approx. March 2020 – September 2020) of the disease in the Czech Republic. Of these, 166 were asymptomatic, 246 were symptomatic (mild form, without hospitalisation) [20,21], and 205 were hospitalized non-fatal cases.

For the purpose of comparison, data from 2 559 adult subjects from the post-MONICA study [22,23] were extracted. We used the *HFE* genotypes of these individuals reported in a previous study [24]. Information on COVID-19 positivity/negativity was not provided.

Patients and population subjects were similar in age (48 ± 11 vs 46 ± 16 years), gender distribution (53 % vs 55 %), diabetes prevalence (8.2 % vs 9.9 %), and prevalence of hypertension (22.4 % vs 24.8 %). There were more obese subjects (28.7 % vs 32.7 %; $P < 0.05$) in the patient group.

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

DNA were isolated from whole EDTA blood using a slightly modified "salting-out" method [25]. The *HFE* polymorphisms rs1800562 and rs1799945 were genotyped using PCR-RFLP as previously described [26,27]. PCR was performed using the MJ Research DYAD Disciple PCR device and chemicals produced by Fermentas International, Inc. (Burlington, Ontario, Canada). Microplate array diagonal gel electrophoresis (MADGE) was performed using 0.5x TBE buffer in 10 % PAGE to separate the PCR fragments.

Statistical analysis was performed using https://www.hutchon.net/confidor.htm and https://www.socscistatistics.com (both accessed 17 October 2022). In cases where a particular subgroup of homozygotes contained<5 subjects, these were pooled with heterozygotes and analysed together. A value of $P < 0.05$ was considered significant.

3. Results

The genotyping call rates varied between 90.2 % and 99.8 %. Distribution of genotypes was in Hardy-Weinberg equilibrium for both controls and patients, including patient subgroups (P values between 0.25 and 0.96 for rs1799945 SNP and between 0.37 and 0.80 for rs1800562 SNP). The frequencies of individual genotypes were similar to other European Caucasian populations [17–19].

Carriers of the Tyr282 allele were more frequent ($P < 0.002$) among COVID-19 patients (Table 1). Subjects with at least one *HFE*-associated allele were at increased risk (OR, 95 %CI; 1.69, 1.21 – 2.35) of SARS-CoV-2 infection, and association was equally expressed in all subgroups of patients. We observed no gradient in genotype frequencies from asymptomatic through mildly symptomatic to hospitalised subjects, which suggests that this variant influences susceptibility to infection, but not severity of disease.

In contrast, genotypes of the His63Asp (rs1799945) variant (associated with the milder form of HFE) were almost identical in subjects tested positively for the SARS-CoV-2 virus and in the general population (all P values between 0.58 and 0.74). Similarly, we found no indication that this variant has the potential to influence COVID-19 severity (for genotype distributions based on disease course, see Table 1).

Analysis of *HFE* haplotypes (Table 2) did not improve COVID-19 prediction. This was largely due to the generally low prevalence of carriers of at least one minor allele and, especially, of compound heterozygotes.

4. Discussion

Inspired by several *in silico* studies, our work is the first to investigate *HFE* polymorphisms in real COVID-19 patients. Our results suggest that *HFE* variability may have an effect on susceptibility to the SARS-CoV-2 infection, but not on disease severity. Subjects with at least one minor allele from the rs1800562 *HFE* polymorphism displayed a higher probability of being infected with SARS-CoV-2.

Our data highlight the important role of hepatic iron concentration in the progression of infection. They are also consistent with previous findings on, for example, HIV infection (reviewed by Drakesmith and Prentice [28]), where iron overload is associated with poor prognoses.

Iron, an essential element in processes such as nucleic acid synthesis and ATP generation, has the potential to affect virus viability. *HFE* variants that cause haemochromatosis, a condition also known as "iron overload", are therefore likely to be implicated in influencing COVID-19 susceptibility. Our results confirmed an increase in the prevalence of haemochromatosis in SARS-CoV-2-positive subjects.

It has been observed [16,17] that the prevalence of *HFE* minor alleles is highest in Caucasians (especially populations in northern Europe), much lower in Asians, and completely absent in black Africans. However, black Africans and Caribbeans are most at risk from COVID-19-associated mortality and morbidity [29,30]. Intriguingly, a recent study detected a significant admixture of the *HFE* Tyr282 allele in African Americans, reflecting the significant effect of both paternal and maternal European ancestry on this ethnic group [31]. Considering the contradictions in these findings, further detailed analysis of the role of *HFE* in influencing susceptibility to SARS-CoV-2 infection in non-Caucasian ethnicities is warranted. Of course, other genes as well as lifestyle and socio-economic inequalities [29,32] play also an important role in increasing the prevalence of COVID-19 in non-Caucasian ethnicities.

The Cys282Tyr mutation first emerged no earlier than 6 000 years ago as a consequence of the Neolithic agricultural revolution (another clinically relevant HFE mutation, His63Asp, is thought to predate Cys282Tyr by thousands of years) [18]. With its ability to increase iron absorption from food by almost threefold, the Cys282Tyr mutation constituted an adaptive advantage. The rapid spread of the mutation during the Neolithic age has been explained [18,33] as an adaptive response of farmers migrating from the warm climates of the Middle East to the cooler environments of Europe and/or due to a dietary shift from meat-based foods high in iron to cereal-based foods low in iron. Like

Table 1
Distribution of HFE polymorphisms in SARS-CoV-2-positive subjects and controls.

HFE	Population		COVID-19 total		COVID-19 asymptomatic		COVID-19 symptomatic		COVID-19 hospitalised		P	OR
rs1799945	N	%	N	%	N	%	N	%	N	%		
CC	1875	73.4	453	74.4	123	74.5	186	75.7	144	72.7	0.62*	1.05 (0.86–1.29)
CG	629	24.6	145	23.8	39	23.6	54	22.0	52	26.3	0.74#	1.06 (0.74–1.52)
GG	51	2.0	11	1.8	3	1.8	6	2.4	2	1.0	0.58§	1.12 (0.83–1.52)
											0.84±	0.97 (0.70–1.34)
rs1800562												
GG	2180	94.5	559	91.0	151	91.5	223	90.7	185	91.1	0.002*	0.59 (0.42–0.82)
GA	124	5.4	54	8.8	14	8.5	22	8.9	18	8.9	0.11#	0.62 (0.35–1.12)
AA	3	0.1	1	0.2	0	0	1	0.4	0	0	0.02§	0.56 (0.35–0.90)
											0.05±	0.60 (0.36–1.00)

2x2 chi-square test for M/M vs + m subjects; * controls vs all SARS-CoV-2 positive; # controls vs COVID-19 asymptomatic; § controls vs COVID-19 symptomatic; ± controls vs COVID-19 hospitalised.
M – major allele; m – minor allele.

Table 2
Distribution of *HFE* haplotypes (rs1800562 and rs1799945) in SARS-CoV-2-positive subjects and controls.

Population		rs1799945					
		CC		CG		GG	
		N	%	N	%	N	%
rs1800562	GG	1563	67.9	562	24.4	49	2.1
	GA	109	4.7	14	0.6	0	0.0
	AA	4	0.2	0	0.0	0	0.0
COVID-19		rs1799945					
		CC		CG		GG	
		N	%	N	%	N	%
rs1800562	GG	401	66.9	136	22.7	15	2.5
	GA	38	6.3	8	1.3	0	0.0
	AA	1	0.2	0	0.0	0	0.0

mutations that cause familial hypercholesterolaemia [34,35], this adaptive advantage later declined as a consequence of cultural and environmental improvements.

In our study, the exchange at aminoacid 63 was not associated with increased susceptibility to SARS-CoV-2 infection or COVID-19 severity (unlike the mutation located at *HFE* amino acid 282). In comparison with the major *HFE*-associated exchange at 282, this iron-associated disturbance in metabolism is mild. It should also be noted that there is another rare exchange within the *HFE* gene (Ser65/Cys, A193 → T, rs1800730). However, the prevalence of the minor Cys65 allele [27,36] is too low (1–2 % among the general population) to be of interest when screening COVID-19 risk at a population level. Moreover, its effect on *HFE* haematochromatosis development is similar to the effect of the H63D mutation.

Increasing the cellular iron pool by downregulating *HFE* expression (and subsequently hepcidine) is understood to promote the general persistence of viruses. It has also been advanced that excess iron decreases the viability of HIV-infected cells, and elevates the activity of reverse transcriptase, which is important for virus replication [37].

Interestingly, thus far only one case study [38] has proposed an association between *HFE* Tyr282 homozygosity and COVID-19, which corresponds with our findings.

It is clear that host genetic variability is important in COVID-19 pathology. However, the list of host genetic variants potentially associated with COVID-19 susceptibility and severity is not long. Of these variants, one of the most extensively examined (despite inconclusive results) is an insertion-deletion polymorphism within the angiotensin-converting enzyme [21,39–41], which bears similarity with ACEII, the major gateway for the cellular entry of SARS-CoV-2.

AB0 blood groups are also associated with COVID-19 (reviewed by Pendu et al., [42]) - subjects with blood group A are at highest risk.

Interestingly, recent studies using GWAS data have identified variants within two loci transferred to the human genome from Neanderthals. The first loci, at OAS1 [43], activates RNAses, which in turn degrade viral RNAs and impair viral replication. The second, LZTFL1 [44], is expressed in respiratory endothelium cells, which are among the main cellular targets of SARS-CoV-2 infection.

Our study has several week points, whose shall be addressed in subsequent investigations. We were not able to carry out a more detailed statistical analysis (adjusting for confounding factors) due to incomplete demographic data - in about 20 % of cases, it was not possible to obtain the required characteristics in sufficient details. Also, the wide age range among patients positively tested for SARS-CoV-2 may have had a bearing on our findings, since age is a major determinant of COVID-19 severity. Biochemical parameters, associated with haemochromatosis, as plasma levels of Fe or ferritin, were not available in the patients, as this analysis is not routinely performed at critical care units, where hospitalised subjects have been treated.

The simultaneous analysis of *HFE* mutation presence and plasma levels of ferritin/iron could be of extreme interest in this case, as it is known, that the disease penetrance is not absolute and not all mutation carriers have pathologically increased plasma iron levels.

Finally, the fact that there is no gradient in minor allele frequency according the disease severity, the possibility (rather unlikely) of propensity to be undergo testing cannot be fully excluded.

Identifying individuals at increased risk of, or genetically protected against, SARS-Cov-2 infection is an important factor in mitigating disease prevalence and severity. To that end, genetic analysis represents a quick and low-cost solution for determining susceptible persons.

In conclusion, we contend that the rs1800562 *HFE* mutation, associated with elevated iron concentration in plasma, increases the risk of SARS-CoV-2 infection, but not COVID-19 severity. Further independent studies are needed in order to confirm the global validity of this association.

CRediT authorship contribution statement

J.A. Hubacek: Conceptualization, Formal analysis, Funding acquisition, Project administration, Writing – original draft. **T. Philipp:** Investigation, Methodology, Writing – review & editing. **V. Adamkova:** Conceptualization, Data curation, Funding acquisition, Project administration, Supervision, Writing – review & editing. **O. Majek:** Formal analysis, Investigation, Supervision, Writing – review & editing. **L. Dusek:** Conceptualization, 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.

Data availability

Data will be made available on request.

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