Role of ACE2 genetic polymorphisms in susceptibility to SARS-CoV-2 among highly exposed but non infected healthcare workers

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Abstract
We aim to evaluate the role of single-nucleotide polymorphisms of the angiotensin-converting enzyme 2 in susceptibility to SARS-CoV-2 infection. We included 28 uninfected but highly exposed healthcare workers and 39 hospitalized patients with COVID-19. Thirty-five SNPs were rationally selected. Two variants were associated with increased risk of being susceptible to SARS-CoV-2: the minor A allele in the rs2106806 variant (OR 3.75 [95% CI 1.23–11.43]) and the minor T allele in the rs6629110 variant (OR 3.39 [95% CI 1.09–10.56]). Evaluating the role of genetic variants in susceptibility to SARS-CoV-2 infection could help identify more vulnerable individuals and suggest potential drug targets for COVID-19 patients.

Background

Considering the great expansion of the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), the presence of highly exposed people who have not yet acquired the infection is striking. Previous studies suggest that genetic factors along with other risk factors can determine an individual's susceptibility to respiratory tract infections; therefore they may contribute to the very high transmissibility of SARS-CoV-2, as well as to the susceptibility of each individual to infection [1]. However, these factors are largely unknown. The development of new preventive strategies for COVID-19 would be greatly facilitated by the identification of host genetic pathways and DNA polymorphisms that modulate the risk of infection [2].

SARS-CoV-2 binds to the angiotensin-converting enzyme 2 (ACE2) receptor for entry into the host cells [3]. It has been hypothesized that the ACE2 gene constitutes a genetic risk factor for SARS-CoV-2 infection [4]. In this regard, variations in the expression of ACE2 may play a significant role in determining an individual's susceptibility to COVID-19 [1]. Moreover, several single-nucleotide polymorphisms (SNPs) of the ACE2 gene previously reported to be associated with other disorders, such as hypertension and other cardiovascular diseases, are thought to influence susceptibility to SARS-CoV-2 infection [5,6]. This phenomenon has been observed in other infections, one example being the genetic variants of the dipeptidyl peptidase-4 receptor in the case of MERS-CoV [7].

Our objective is to evaluate the role of ACE2 SNPs in the susceptibility to SARS-CoV-2 infection in a sample of highly exposed healthcare workers and patients with COVID19.

Methods

Study design and data sources

We classified participants as being susceptible or non-susceptible to SARS-CoV-2 infection. Susceptible individuals were hospitalized patients with confirmed SARS-CoV-2 infection by PCR in nasopharyngeal swabs, sputum, or lower respiratory tract secretions,

within the first 7 days from the onset of symptoms. Non-susceptible participants were healthy healthcare workers who had been on duty for at least three months in COVID19 wards or intensive care units and had had at least three high-risk exposures to SARS-CoV-2 [8], without having experienced symptoms suggestive of SARS-CoV-2 infection, persistently negative PCR SARS-CoV-2 testing, and absence of SARS-CoV-2 IgM and IgG in plasma by indirect chemiluminescence immunoassay (Vircell, Granada, Spain). The most frequent type of exposures were largely unprotected exposure to aerosol-generating procedures or patient secretions, and close contact without face masks with other confirmed cases of COVID-19. We excluded subjects unable to provide informed consent or witnessed oral consent with the written consent by a representative. The study was carried out at the Ramón y Cajal University Hospital in Madrid (Spain) and was approved by the local Research Ethics Committee (ceic.hrc@salud.madrid.org, approval number 095/20).

Endpoint

The primary endpoint was the association between the ACE2 SNPs analyzed and the susceptibility to SARS-CoV-2 infection.

Genotyping of SNPs in ACE-2 gene

SNPs in ACE-2 were identified based on existing literature and human genome sequence databases. A search using the dbSNP database of NCBI yielded more than 17,000 SNPs for ACE2 gene. In order to reduce this vast number of SNPs, we performed a literature search in Pubmed database in May 2020, including the terms "ACE2, SNP" or "ACE2, polymorphism" and achieved 206 and 107 results, respectively. Then articles including information related to "COVID-19", "SARS-CoV-2", "mortality" and "cardiovascular disease", "diabetes mellitus", "obesity", and "hypertension" were further assessed. Thirty-five SNPs were identified and rationally selected [9] (Supplemental Table 1). Total DNA was extracted from peripheral blood using QIAamp DNA Mini Kit (Qiagen), quantified using a NanoDrop-1000 (Thermo-Fisher, Waltham, MA), and diluted to 20 ng/μL concentration. Genotyping of the selected SNP of

ACE-2 was performed using Sequenom's MassARRAY platform (San Diego, CA, USA) and the iPLEX® Gold assay design system at the Spanish National Genotyping Center (CeGen; http://www.cegen.org/). Samples Na1086, Na11994 and Na11995 from Coriell Institute (www.coriell.org) were used as positive controls. The primers used in this study are summarized in Supplemental Table 2.

Statistical analysis

A quality control procedure was performed on each of the SNPs: genotype call rate and minor allele frequency were calculated and a visual inspection of the clusterplots was carried out. For each SNP, a logistic regression analysis was performed to assess the genetic association with susceptibility. SNPs were coded under a dominant genetic model, in which both heterozygous and rare allele homozygous were combined and compared without correction for multiple comparisons with the genotype homozygous for the most frequent allele. Gender was included in the regression model as a covariate. Analysis was performed using the SNPassoc library for R software and Stata v16.0 (StataCorp LP, College Station, TX).

Results

Study population

We included 67 participants, 39 susceptible and 28 non-susceptible individuals. The sample of susceptible subjects was representative of a medium-aged population (median age 58 [IQR 44–84] years) with a slightly higher representation of women (56%). Of them, 59% had a mild infection and 41% had COVID19 who required hospitalization. Non-susceptible healthcare workers had a median age of 43 (IQR 36–51) years and 78% were women.

SNPs in ACE2 and SARS-CoV-2 susceptibility

Of the total 35 SNPs analyzed (**Supplemental Table 1**), two showed an association with SARS-CoV-2 susceptibility. The minor A allele in the rs2106806 variant (AA or GA vs. GG genotype) was associated with an increased risk of being susceptible to SARS-CoV-2 infection (OR 3.75 [95% CI 1.23–11.43], $p = 0.016$). In the same way, the minor T allele in the rs6629110

Table 1. SNPs from ACE2 gene showing an association with SARS-CoV-2 susceptibility.

SNP	Genotype	Susceptible, n (%)		OR (95% CI)	p-value
		No	Yes		
rs2106806	GG	17 (63)	15 (38.5)	Ref.	Ref.
	GA + AA	10 (37)	24 (61.5)	3.75 (1.23–11.43)	0.0156
rs6629110	CC	15 (55.6)	14 (35.9)	Ref.	Ref.
	CT + TT	12 (44.4)	25 (64.1)	3.39 (1.09–10.56)	0.0289

ACE2, angiotensin-converting enzyme 2; CI, confidence interval; OR, odds ratio; SNP, single-nucleotide polymorphism.

variant (TT or TC vs. CC genotype) was associated with an increased infection susceptibility (OR 3.39 [95% CI 1.09–10.56], *p* = 0.029) (Table 1).

Discussion

In this observational work, we aimed to identify SNPs in the ACE2 gene that could confer increased susceptibility to SARS-CoV-2 infection. Two variants were associated with a 3- to a 4-fold greater risk of COVID19: rs6629110 and rs2106806.

Because ACE2 is the target molecule of SARS-CoV-2 for cell entry, higher levels of ACE2 expression are expected to lead to higher levels of SARS-CoV-2 viremia. The Genotype-Tissue Expression (GTEx) database revealed that SNP rs6629110 was associated with greater expression of ACE2 [5], although researchers were not able to confirm the link between variants in the ACE2 gene and COVID-19 [10]. The SNP rs2106806 was among those included in a recent study aimed at describing a transcription regulatory network within the ACE2 locus in the context of SARS-CoV-2 infection, but no correlation was found [11]. Here, we show that the minor A allele within rs2106806 increases the risk of critical COVID-19.

The allele frequency of the ACE2 SNP rs6629110 is much higher in East Asian than in European populations [5,10] but its importance during SARS-CoV-2 infection remains unknown. This variant has been associated with high expression of ACE2 in tissues such as the tibial artery [10] but, to our knowledge, an association with increased expression of ACE2 in lung tissue has not been described so far and there is no association between the variant and expression of ACE2 in nasal epithelium. Hence, this is the first report linking the rs6629110 variant with COVID19 susceptibility. However, it requires external validation in other studies.

Because the mechanisms protecting low-susceptible individuals to acquire SARS-CoV-2 remain poorly understood, the main strength of our study is a novelty. We exploited a striking phenomenon, i.e. subjects without evidence of having been infected with SARS-CoV-2 despite repeated high-risk exposures to explore gene variants associated with disease susceptibility. Given the multifactorial nature of high-risk exposures, the extent of exposure to SARS-CoV-2 cannot be measured today in clinical studies, which is a limitation of our study. Because of the definition we used to classify non-susceptible individuals, we think, however, that it is very likely that this group had multiple high-risk exposures. The main limitation is the rather low number of individuals assessed, which precluded us to use a genome-wide association approach. In contrast to GWA studies, which generate thousands of untargeted variables that demand a meticulous correction for false discovery rate, we rationally selected the SNPs to be analyzed. Issues such as exposure imply that studies of genetic susceptibility to infectious diseases will have modest effect sizes and, therefore, that statistical power will be at a premium [12]. Then the greater inherent power of a rational selection of candidate SNPs may make it preferable over GWA. Indeed, because we rationally selected the SNPs in the ACE2 to be assessed based on the background literature, we did not use Bonferroni corrections to adjust for multiple comparisons, as has been recommended for targeted analyses [13]. Admittedly, the fact that the associations would not remain statistically significant after adjustment by false discovery rate demands caution when interpreting the results. The study should be considered as hypothesis-generating and the findings must be validated in larger studies.

In summary, we describe two candidate variants in the ACE2 gene that were predictive of a greater risk of SARS-CoV-2 infection. Evaluating the role of genetic variants in susceptibility to SARS-CoV-2 infection could help identify more vulnerable individuals and suggest potential drug targets for COVID-19 patients.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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