Genetic variation of IFNL4 is associated with COVID-19.

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Abstract

COVID-19 currently represents a major public health problem. The causes that underlying susceptibility to infection in have not yet been determined. Interferons (IFNs) are intensively being investigated because of their antiviral properties. Among them, Interferon lambda 4 (IFNL4) has been reported to have antiviral activity against viral infections of the upper respiratory tract, Hepatitis virus C (HCV), and coronaviruses. The importance of this cytokine was shown by the fact that genetic variants of IFNL4 have been associated with viral clearance and response to IFNs-based therapies in HCV and other infections by RNA virus. In this study, we have investigated whether the rs12979860 polymorphism within the IFNL4 was also associated with COVID-19. Our findings show that the presence of the CC allele of rs12979860 was significantly lower in SARS-CoV-2 infected patients with regard to non-COVID-19 controls (38% vs 55%, p<0001). These results were not affected by sex, age, and severity of disease. These findings suggest that the CC allele may also confer protection against COVID-19. They may contribute to understanding the mechanisms of disease, the response to IFN-based treatments, and the racial differences observed in the disease.

Key Words: COVID-19, SARS-CoV-2, IFNL4, rs12979860

NOTE: This preprint reports new research that has not been certified by peer review and should not be used to guide clinical practice.
Introduction

COVID-19 is a disease caused by the SARS-CoV-2 coronavirus (1). Most of the infected patients have a favorable prognosis, although severe pulmonary and systemic inflammation can appear in a small proportion of patients that may cause multiple organ failure and death. The causes that determine susceptibility to infection have not yet been determined although severity seems to be associated with sex, age and certain comorbidities. To date, no effective treatments for COVID-19 have yet been established, and the efficacy of approved treatments remains limited (2).

Interferons (IFNs) are one of the most investigated compounds. Several studies have been conducted or are ongoing to investigate the efficacy of IFNs in the treatment of SARS-CoV-2 infection (3). IFNs play an important role in the early immune response to viral infections and have shown efficacy in controlling infections such as that mediated by hepatitis C virus (HCV) (4). Besides type I IFNs, type III IFNs also shown an important antiviral activity. Type I and type III IFNs induce the transcription of a similar set of IFN-stimulated genes with antiviral activities although through different receptors (5). The expression of Type III IFNs is mainly restricted to epithelial cells in contrast to the broad distribution of type I receptors. Among them, Interferon lambda 4 (IFNL4) has shown antiviral activity against viral infections of the upper respiratory tract, HCV, but also coronaviruses (5,6). Its importance is demonstrated by the fact that genetic variants of IFNL4 may condition the clearance of RNA viruses. In HCV infections, polymorphisms of the IFNL4 including rs12979860 were associated to response to IFN-based therapies and spontaneous viral clearance (5). Similar findings have been reported in viral infections of the upper respiratory tract (6). In this study, we investigated whether the rs12979860 single nucleotide polymorphism (SNP) within the IFNL4 was also associated to SARS-CoV-2 infection.
Patients and Methods

COVID-19 patients included in the study were attended in the Hospital San Pedro de Alcantara de Caceres, Spain. A diagnosis of COVID-19 was established on the basis of a suggestive clinical history and the detection of SARS-CoV-2 RNA in respiratory secretions. 177 patients were attended for COVID-19 among whom 45 had progressed to severe disease. Severe COVID-19 disease was defined as dyspnea, a respiratory rate of 30 or more breaths per minute, a blood oxygen saturation of 93% or less, a ratio of the partial pressure of arterial oxygen to the fraction of inspired oxygen (Pao2:Fio2) of less than 300 mm Hg, or infiltrates in more than 50% of the lung field, requiring support with at least high-flow nasal cannula oxygen therapy, noninvasive ventilation or mechanical ventilation. Samples to genotyping analysis were obtained after discharging from hospital; therefore, no death patients were included in the study. Control samples were obtained from volunteer donors before 2019 to avoid interference from possible undetected infected donors. Patients and controls gave their written informed consent and, when applicable, provided consent for banking and future analysis of biologic samples. The study was approved by the Hospital Ethical Committee for Clinical Research.

Genotyping analysis. Genomic DNA was isolated from whole blood using a Nucleospin blood kit (Macherey-Nagel, Duren, Germany). Genotyping to determining rs12979860 alleles was performed in a Stratagene 3005 MxP Quantitative PCR (Agilent Technologies, Inc., Santa Clara, CA) by using a Taqman genotyping mastermix (Thermofisher Scientific, Waltham, MA) and a commercial assay also from Thermofisher Scientific (Assay ID: C___7820464_10).

Statistical analysis. Frequency of the rs12979680 SNP among patients and controls were shows as were summarized as counts and percentages. Categorical variables were
compared using the chi-square test (two-sided) and Fisher's Exact Test (two-sided). A p value < 0.05 was considered to be statistically significant. Comparison analysis was to be performed by SPSS for Windows Release 17.0 (SPSS, Inc).

Results

In order to analyze the role of rs12979680 in SARS-CoV-2 infection, we genotyped 177 patients diagnosed with COVID-19 among whom 45 had progressed to severe disease. Table 1 summarizes the population analyzed. In this study, we only considered sex and age as risk factors associated with SARS-CoV-2 infection.

| Characteristics | NON-COVID-19 (445) | Total COVID-19 (177) | Non-Severe COVID-19 (132) | Severe COVID-19 (45) |
|-----------------|-------------------|---------------------|--------------------------|---------------------|
| Gender:         |                   |                     |                          |                     |
| Male            | 217 (49%)         | 83 (47%)            | 62 (47%)                 | 21 (47%)            |
| Female          | 228 (51%)         | 94 (53%)            | 72 (53%)                 | 24 (53%)            |
| Age (years):    |                   |                     |                          |                     |
| < 65            | 59 ± 29 (57%)     | 68 ± 16 (42%)       | 67 ± 17 (47%)            | 72 ± 14 (73%)       |
| ≥ 65            | 192 (43%)         | 103 (58%)           | 70 (53%)                 | 33 (73%)            |

Table 1. Patients characteristics.

The distribution of the rs12979680 SNP between patients and controls is shown in table 2. The CC genotype was significantly decreased in COVID-19 patients with regard to controls (38% vs 55%, p=0.0001), suggesting a protective effect of this allele. The expression of CC was also decreased when analyzed by sex although it seemed to be most significative in females (33% vs 54%, p=0.001) than in males (43% vs 57%, p=0.039). The lower expression of CC in patients was also not affected by the age (<65 years: 40% vs 56%, p=0.016; ≥65 years: 36% vs 55%, p=0.004). Finally, the percentage
of COVID-19 patients expressing CC was not different between mild and severe disease (36% vs 42%, p=0.48).

| rs12979860 | NON-COVID-19 n=445 | COVID-19 n=177 | p value |
|------------|-------------------|----------------|---------|
| CC         | 247 (55%)         | 67 (38%)       | <0.001  |
| CT         | 161 (36%)         | 92(52%)        | <0.01   |
| TT         | 37 (8.3%)         | 18 (10%)       | 0.82    |

| Rs12979680 CC: | | | |
| Gender: | | | |
| Male | 124 (57%) | 36 (43%) | <0.05 |
| Female | 123 (54%) | 31 (33%) | <0.001 |
| Age: | | | |
| < 65 years | 143 (56%) | 29 (40%) | <0.05 |
| ≥ 65 years | 106 (55%) | 38 (36%) | <0.01 |
| Disease Severity: | | | |
| Non-Severe COVID-19 | 48 (36%) | | |
| Severe COVID-19 | 19 (42%) | 0.48 |

Table 2. Comparation of rs12979680 between COVID-19 patients and non-COVID-19 controls.

Discussion

In this study, we have found that the rs12979680 SNP within the IFNL4 is associated with COVID-19. Our finding suggests that the expression of the CC allele seems to confer protection against this disease. This observation correlated with the protection conferred by this allele during HCV and respiratory viral infections (4-6).
IFNL4 has been found to exert antiviral activity against HCV but also against several coronaviruses (5). However, it is paradoxical that the production of IFNL4 seems to be a disadvantage during HCV infection. Thus, the TT allele of the rs368234815 polymorphism disrupt the expression of IFNL4 (7). This inactivation of the IFNL4 gene has been correlated with the spontaneous clearance to HCV and with response to type I IFNs-based treatments. The rs12979680 polymorphism that we have analyzed is in strong linkage disequilibrium with rs368234815, and both polymorphisms have shown similar performance as predictors of viral clearance and to response to treatments with IFNs (8). Our findings suggest that similar to HCV and other viral infections, IFNL4 may play an important role in COVID-19. This may help to understand the disproportionate prevalence of COVID-19 in the black population with a lower presence of the protective CC allele than the white population (6, 9). The fact that there were no significant differences in CC expression between mild and severe disease suggests a role for this cytokine in the early stages, but not in disease progression.

As antiviral agents, IFNs are being investigated as treatments for COVID-19. However, their efficacy is somehow contradictory (3). One explanation proposed that the timing of IFN administration is important since early treatments with IFN seemed to decrease but late appeared to increase mortality. Our findings added a new complementary explanation. In HCV infection, it was demonstrated that the rs12979680 was associated with response to IFNs-based treatments. It may also be possible that this polymorphism may also affect responses to IFN-based treatments in COVID-19 patients. Thus, patients expressing the CC phenotype may be more responsive to these treatments. Furthermore, the fact that the expression of CC is lower in COVID-19 patients may mask the results obtained with IFNs-based treatments.
We believe that the data shown in this study is of great interest since it reports a role of IFNL4 in COVID-19, being rs12979680 polymorphism a potential marker of disease. However, further studies are needed to strengthen the presented observations. Thus, it would be necessary to analyze IFNL4 polymorphisms in asymptomatic people to help to decipher its role in infection. It would be also interesting to investigate the association of IFNL4 polymorphisms with reported COVID-19 comorbidities. Answering these questions may be of great interest to design treatments for SARS-CoV-2 infection but also for planning vaccination campaigns because of the shortage of vaccines.

Conclusions

We have found that the rs12979680 SNP within the IFNL4 is associated with COVID-19. We believe these findings may be useful in understanding the mechanisms of disease, but also in designing treatments and planning vaccination campaigns.

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