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Short communication

Variant-genetic and transcript-expression analysis showed a role for the chemokine-receptor *CCR5* in COVID-19 severity

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ABSTRACT

The chemokine receptor CCR5 has been implicated in COVID-19. CCR5 and its ligands are overexpressed in patients. The pharmacological targeting of CCR5 would improve the COVID-19 severity. We sought to investigate the role of the CCR5-Δ32 variant (rs333) in COVID-19. The *CCR5*-Δ32 was genotyped in 801 patients (353 in the intensive care unit, ICU) and 660 healthy controls, and the deletion was significantly less frequent in hospitalysed COVID-19 than in healthy controls ($p = 0.01$, OR = 0.66, 95%CI = 0.49–0.88). Of note, we did not find homozygotes among the patients, compared to 1% of the controls. The *CCR5* transcript was measured in leukocytes from 85 patients and 40 controls. We found a significantly higher expression of the *CCR5* transcript among the patients, with significant difference when comparing the non-deletion carriers (controls = 35; patients = 81; $p = 0.01$). ICU-patients showed non-significantly higher expression than no-ICU cases. Our study points to *CCR5* as a genetic marker for COVID-19. The pharmacological targeting of CCR5 should be a promising treatment for COVID-19.

1. Introduction

The C-C chemokine-receptor-5 (CCR5) is expressed by several cell types, including T cells, macrophages and dendritic cells. CCR5 might play a role in the inflammatory response to coronaviruses infection. Human dendritic cells infected by SARS-CoV-1 showed increased CCR5 expression [1]. The levels of ccr5 mRNA were increased in mice infected with the SARS-Cov [2]. Mice deficient in ccr5 infected with SARS-CoV-1 exhibited defects in directing inflammatory to the airway [3]. Mice lacking the ccr5 infected with a neurotropic coronavirus showed

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reduced macrophage infiltration and demyelination [4]. CCR5 has been implicated in COVID-19. A single-cell RNA sequencing on nasopharyngeal and bronchial samples from patients with moderate or critical disease and healthy controls pointed to CCR5 pathways as suppressors of immune hyperactivation in critical COVID-19 [5]. Leronlimab (a CCR5 blocking antibody) reduced the SARS-Cov-2 viral load and plasma IL-6 and restored the CD4/CD8 ratio in severe COVID-19 [6–8]. The CCR2b/CCR5 antagonist cenicriviroc showed anti SARS-Cov-2 activity [9].

Approximately 1% of Europeans are homozygotes for a *CCR5* deletion (Δ32) and do not express the receptor. CCR5 is the receptor for human immunodeficiency virus, and Δ32 homozygotes are resistant to CCR5-tropic HIV infection.

Individuals who are Δ32-carriers would also have a reduced inflammatory response that might explain its association with several immune-mediated diseases. The first COVID-19 Genome Wide Association study (GWAs) reported a strong association with chromosome 3 markers in the chemokine-receptor gene cluster [10]. *CCR5* maps to this region and could thus partly explain the observed association with polymorphisms in this region. The genotyping of patients from the first pandemic wave (March-April 2020) confirmed the association with the chromosome 3 markers and showed a reduced frequency of the Δ32 variant among the COVID-patients (results published as a no peer-reviewed preprint) [11].

We sought to investigate the differences in *CCR5* transcript levels in leukocytes from COVID-19 patients and healthy individuals, and whether the *CCR5*-Δ32 was associated with the risk of developing COVID-19 or was a genetic modifier of disease severity in a large cohort of patients and controls from the two first pandemic waves.

2. Methods

2.1. Patients and controls

We genotyped 801 COVID-19 hospitalised patients (353 severe, requiring admission in the intensive care unit, ICU) and 650 age-matched population controls. These controls were randomly chosen from the general population during the years 2010–2015 and were CCR5-genotyped to determine the allele and genotype frequencies in our elderly population. In this work they were studied with the only purpose of defining the *CCR5* variant frequencies in our elderly population, and data about their current COVID-19 status was not available [12].

A second group of controls was composed by 210 elderly subjects followed in the period March-December 2021 with no disease (n = 120; all negative in blood antibody test) or mild symptoms that did not require hospitalization (n = 90; confirmed by PCR). They were recruited to investigate a putative role of the variants in the resistance to SARS-CoV-2 infection.

All the participants were Caucasian from the region of Asturias (Northern Spain, total population 1 million). The study was approved by the Ethics Committee of Principado de Asturias (Oviedo, Spain), and all the patients or their representatives gave their consent.

2.2. CCR5 genotyping and transcript analysis

The *CCR5*-Δ32 (rs333) was genotyped in patients and controls following a previously reported procedure (**supplementary figure**) [12]. The *CCR5* transcript-level (cDNA) was determined within leukocytes of 85 patients (45 ICU, 40 no-ICU) and 40 of the 120 SARS-CoV-2 negative controls. For the patients the blood for RNA isolation was obtained at the time of hospital admission. Each cDNA was amplified by triplicate with *CCR5* and *ACTB* Taqman expression assays (Thermo Fisher) and the mean cycle threshold (Ct) was calculated (**suppl. files**). The ratio of the Ct *CCR5/ACTB* indicated the *CCR5* expression, with lower values corresponding to increased transcript levels.

Table 1
Main values in the COVID-19 patients and controls. Multiple logistic regression p-values, Odds ratios (OR) and 95% confidence intervals (CI) were calculated including age, sex, hypertension and the corresponding genotypes. Severe cases were those in need of critical care support, including high-flow oxygen, positive-pressure ventilation (either invasive or non-invasive) or vasoactive drugs. The anthropometric values and comorbidities were obtained from the participants medical records.

	COVID N = 801	**CONTROLS N = 650**	**ICU N = 353**	**NO-ICU N = 448**	**NO or mild disease N = 210**
Male	480 (60%)	356 (54%)	268 (76%)	212 (47%)	88 (42%)
Mean age, SD years	66 ± 15	68 ± 7	66 ± 11	62 ± 16	61 ± 11
Age IQ range years	53–75	65–76	50–75	60–75	55–78
Hypertension	382 (48%)	224 (34%)	212 (60%)	170 (38%)	68 (32%)
Exitus	88 (11%)	–	75 (21%)	13 (3%)	–
***CCR5* Δ32bp rs333**					
Δ / Δ	0	5 (1%)	0	0	2 (2%)
Δ / WT	96 (12%)	106 (16%)	35 (10%)	61 (14%)	32 (15%)
WT / WT	705 (88%)	539 (82%)	318 (91%)	387 (86%)	176 (83%)
Δ32 allele frequency	0.06	0.09	0.05	0.10	0.09
p, OR (95%CI) *	**p = 0.01, OR = 0.66 (0.49–0.88)**		**p = 0.11, OR = 0.70 (0.45–1.08)**		

* Logistic-regression, Δ32 – carriers vs. WT/WT genotypes.

2.3. Statistical analysis

The statistical analysis was performed with the R-project free software (www.r-project.org). Logistic regression (linear generalized model, LGM) was used to compare mean values and frequencies between the groups.

3. Results and discussion

There was a significantly lower frequency of *CCR5*-Δ32 among the COVID-19 patients compared to population controls: **p = 0.01, OR = 0.66 (0.48–0.91)** (Table 1). The post-hoc power of the study was 76.5%. The difference was mainly due to the reduced frequency of *CCR5*-Δ32 carriers in the severe-ICU group, significantly lower than in the non-severe patients (10% vs. 14%). Of note, we did not find deletion-homozygotes among the patients compared to 1% in the controls. We did not find significant differences between hypertensives and normotensives, and mean age did not differ between the genotypes (**suppl. table**). A total of 88 patients were exitus, 75 (21%) and 13 (3%) of the ICU and no-ICU patients, respectively. The *CCR5*-Δ32 was associated with a non-significantly reduced death frequency in the ICU (17 vs 22%; p = 0.53) and no-ICU (2% vs 3%; p = 0.21) (suppl. table).

We studied a total of 120 individuals with no evidence for SARS-CoV-2 during the two 2021 pandemic waves, and 90 cases with PCR-confirmed infection and mild symptoms. The main finding in this group was a variant-frequency similar to the found in the general controls (0.09). There was one *CCR5*-Δ32 homozygote in each group, with carrier frequencies of 16% (19/120) and 16% (14/90). The frequency in the SARS-CoV-2 mild cases suggested that the absence of *CCR5* does not fully protect against infection, but could reduce the risk of severe symptoms. Compared to the total controls (n = 860) patients showed a lower frequency of *CCR5*-Δ32-carreirs (OR = 0.67, 95%CI = 0.51–0.89).

In addition to the well-known protective effect against HIV-1, this

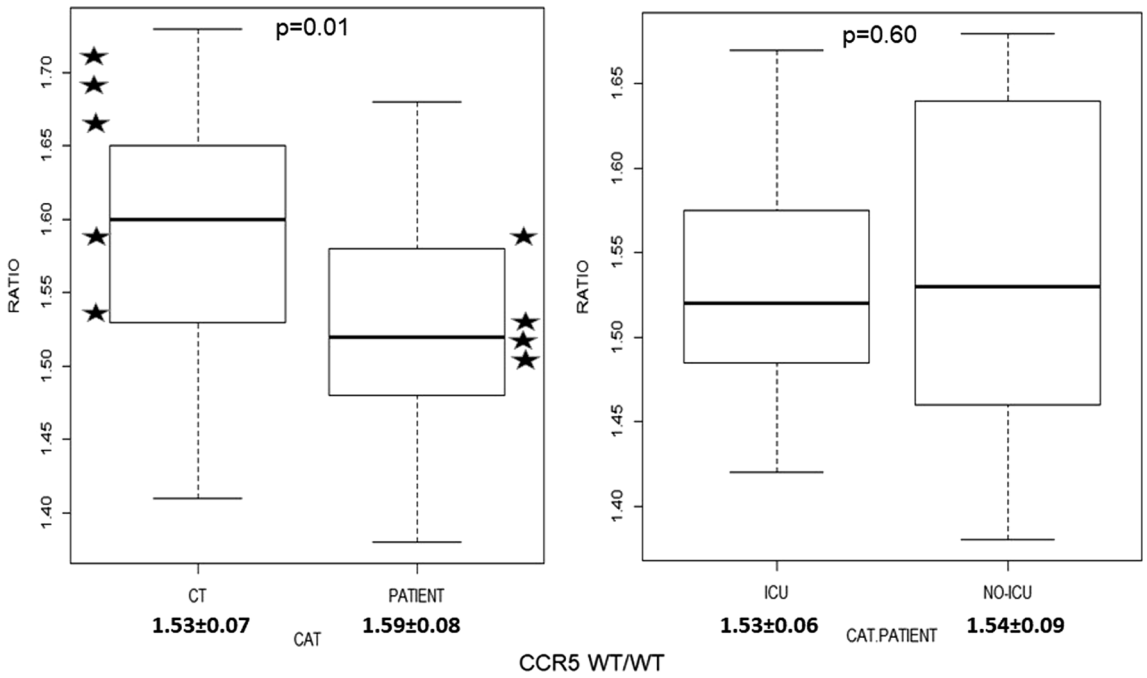

Fig. 1. Expression rate of *CCR5* cDNA in total leukocytes, relative to the normalizing gene (*ACTB*). Patients and matched controls. The box-plots corresponded to Wt/Wt homozygotes, and the stars indicate values for the *CCR*5 Δ32 heterozygotes (5 controls and 4 patients). Lower *CCR5/ACTB* cycle threshold (Ct) ratios indicate higher *CCR5*-transcript levels.

variant has been investigated in reference to the clinical outcomes of several viral infections, such as hepatitis B and influenza A [13,14]. Because CCR5 is not a recognised receptor for SARS-Cov-2 the most likely explanation for the protective effect of Δ32 in COVID-19 is an attenuated inflammatory response among the CCR5-deletion carriers.

We investigated the total leukocyte transcript in severe-cases and controls. We did not find significantly different mean rations ($p = 0.07$) between the two groups. When comparing only wt/wt individuals, controls ($n = 35$) showed significantly higher mean ratio ($p = 0.01$) than patients ($n = 81$) (Fig. 1). ICU patients showed lower mean ratios compared to non-ICU patients (1.59 ± 0.13 vs. 1.53 ± 0.11; $p = 0.01$), but the difference was non-significant for the wt/wt patients (Fig. 1). Thus, the increased expression of *CCR5* in circulating leukocytes was a marker for COVID-19 severity. The main limitation was that we did not measured the transcript expression in different leukocyte populations. Also, only 4 patients and 5 controls were Δ32 heterozygotes and we could thus not confirm a difference in *CCR5* levels between the genotypes, although deletion carriers had higher ratios (lower transcript expression).

The association between *CCR5*-Δ32 and COVID-19 seems plausible considering the reported association between chromosome 3p21.31 variants and COVID-19 [10]. If the protective effect of this variant is confirmed by others the *CCR5* variants might serve as valuable markers to identify individuals predisposed to severe COVID-19. A limitation of our study was that we did not provide a functional link between the *CCR5* deletion and COVID-19 outcomes, and we cannot conclude whether this variant was associated with an overall resistance to SARS-Cov-2 infection or a reduced inflammatory response, or both. Although the CCR5 has not a recognised role in SARS-CoV-2 binding to the host cell, a preliminary study showed that the CCR5 inhibitor Maraviroc was able to decrease the extent of viral-cell fusion and the viral load [15]. However, this effect was in contrast with other work that found that Maraviroc did not show any anti-SARS-CoV-2 activity [9].

To determine whether CCR5 has a direct role in SARS-CoV-2 infection studies with mice expressing the human viral receptor (ACE-2) and lacking ccr5 should be of upmost relevance [16].

It has been reported that ccr5-deficient mice showed an impaired memory CD4 + T-cell response, and *CCR5*-Δ32 homozygotes developed a significantly reduced T-cell response after activation with anti-CD3 and anti-CD28 [17]. This study suggested that the absence of CCR5 might result in a less efficient antibody response, and could thus result in lower efficacy of natural and vaccine-induced immunization. The lack of CCR5 might thus exhibit a dual effect, protecting from severe COVID-19 immediately after the infection (innate immunity) and reducing the long-term antiviral adaptive response.

To our knowledge only two recent studies reported frequencies of *CCR5*-Δ32 in COVID-19. In a cross-sectional study among stem cell donors registered in Germany the *CCR5* genotype was available for 110,544 donors who were tested at least once for SARS-CoV-2. The distribution of *CCR*5-Δ*32* genotypes (*CCR5-Δ32* carriers vs. homozygous wild-type) did not differ significantly between individuals with or without SARS-CoV-2 infections, nor between individuals with or without symptomatic infections, severe respiratory disease, or respiratory hospitalizations. The authors concluded that the *CCR5-Δ32* mutation do not determine the risk of SARS-CoV-2 infections nor the disease course [18]. There are several differences between this and our study that could explain the different conclusions. Among others, the study design (cross-sectional vs case-control). Also, in the German study no data about the age-distribution among the mutation-carriers was provided, while our study involved hospitalised patients who were mainly of advanced age. Another study genotyped 416 Czech first-wave SARS-CoV-2-positive infection survivors (164 asymptomatic and 252 symptomatic) and compared them with a population cohort ($n = 2404$). The authors found a significantly higher *CCR*5-Δ32 frequency in asymptomatic SARS-CoV-2-positive compared to COVID-19-symptomatic patients, and concluded the CCR5 deletion might predict the severity of SARS-CoV-2 infection [19].

Finally, some authors have suggested that the frequency of the *CCR5* deletion might correlate with the SARS-CoV-2 infection and death. Thus, countries with higher deletion frequency could show the highest infection and COVID-19 mortality rates [20,21]. Our work and others did not support this conclusion [19]. Moreover, Spain has one of the lowest *CCR5*-Δ32 frequencies while having one of the highest worldwide infection and mortality rates (https://www.worldometers.info/coro

navirus) (suppl. figure). We studied the best characterised *CCR5* functional variant, but other polymorphisms have been related with gene expression and the HIV-infection outcomes [22]. In view of our results, these and other *CCR5*-linked polymorphisms are putative markers for adverse course in COVID-19.

4. Conclusion

The pharmacological blockade of *CCR5* has been associated with an improvement of the symptoms in a group of severe COVID-19 patients. Our study showed a significant increased expression of *CCR5*-transcript among severe COVID-19 patients compared to healthy controls. The common *CCR5* deletion was significantly protective against severe COVID-19, particularly among deletion-homozygotes. The genetic analysis of *CCR5*-variants, such as Δ32, might help to identify patients with a higher susceptibility to severe COVID-19.

5. Contributorship

All the authors contributed to this work by recruiting the patients and performing the genetic and statistical analysis. E.C. wrote the ms. and takes full responsibility for the accuracy of the data. All the authors approved the submission of this ms.

6. Data accessibility

To facilitate the revision of our results by other researchers, an excel file with the data is provided as supplementary file.

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.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.intimp.2021.107825.

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