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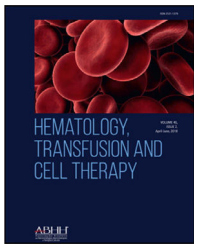

Original article

Association between *FY*02N.01* and the severity of COVID-19: initial observations

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ABSTRACT

Introduction: The pro-inflammatory immune response underlies severe cases of COVID-19. Antigens of the Duffy blood group systems are receptors for pro-inflammation chemokines. The *ACKR1* c.-67T>C gene variation silences the expression of Duffy antigens on erythrocytes and individuals presenting this variant in homozygosity have impaired inflammatory response control. Our aim was to evaluate the association between the *ACKR1* c.-67T>C and the severity of COVID-19.

Methods: This was a retrospective single-center case-control study, enrolling 164 participants who were divided into four groups: 1) Death: COVID-19 patients who died during hospitalization; 2) Hospital Discharge: COVID-19 patients who were discharged for home after hospitalizations; 3) Convalescent Plasma Donors: COVID-19 patients who were not hospitalized, and; 4) Controls: patients with diagnosis other than COVID-19. Patients were genotyped for the *ACKR1* c.-67T>C (*FY*02 N.01* allele) and the frequency of individuals presenting the altered allele was compared between the groups.

Results: The groups significantly differed in terms of the percentage of patients presenting at least one *FY*02N.01* allele: 36.8% (Death group), 37% (Hospital Discharge group), 16.1% (Convalescent Plasma group) and 16.2% (Control group) ($p = 0.027$). The self-declared race ($p < 0.001$) and the occurrence of in hospital death ($p = 0.058$) were independently associated with the presence of the *FY*02N.01* allele. Hypertension ($p < 0.001$), age ($p < 0.001$) and the presence of at least one *FY*02N.01* allele ($p = 0.009$) were independently associated with the need for hospitalization.

Conclusion: There is a suggestive association between the presence of the *FY*02N.01* and the severity of COVID-19. This may be a mechanism underlying the worse prognosis for Afro-descendants infected with SARS-CoV-2.

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Introduction

The SARS-CoV2 epidemic began in December 2019 in China and quickly spread around the world, leading the scientific community to mobilize towards understanding the mechanisms of infection and transmission of the SARS-CoV-2 virus, as well as unveiling the exacerbated immune response that underlies severe cases of the disease.

The imune response against COVID-19 is not completely understood. It is known that genetic factors and clinical comorbities are directly associated with the severity of symptoms and disease progression. T cell response has been shown to play an essential role in the protective response against the SARS-CoV-2 virus. The CD4 T lymphocytes stimulate B lymphocytes to produce neutralizing antibodies and to recruit CD8 T cytotoxic lymphocytes that will eliminate the infected cells.[1] When the immune response is ineffective, the innate immune cells continue to release cytokines, chemokines and acute phase proteins, inducing a hyperinflammatory response with massive cellular homing to the infeccted tissues. This exacerbated response may induce the acute respiratory distress syndrome and the collapse of other multiple organs. An ineffective initial immune response and the maintenance of a pro-inflammatory response are responsible for the disease severity, which can culminate in the patient's death.[2]

Racial ancestry is apparently associated with the severity of the COVID-19 infection. In Louisiana, a statistical survey found that African American patients accounted for 59% of COVID-19 deaths, while the total population of African descent in the state corresponded to 33%. Although socioeconomical factors underlie the heterogeneity of COVID-19 severity presented by patients of Caucasian or African racial background, it is known that individuals of African ancestry have a stronger inflammatory response, in comparison to those of European ancestry.[3,4] Furthermore, previous studies have demonstrated that individuals of African ancestry have a higher frequency of variants related to pro-inflammatory cytokines, justifying an increase in the release of these cytokines, mainly the TNF-α. [3]

The Duffy blood group system consists of five antigens disposed on a multipass membrane glycoprotein called the Atypical Chemokine Receptor 1 (ACKR1), also known as the Duffy Antigen Receptor for Chemokines or DARC.[5-7]. The Duffy antigens are present on the red blood cell (RBC) surface, as well as on the post-capillary venules of most tissues.[8] The Duffy blood group system plays an important role in the immune response, as Duffy antigens are high-affinity receptors for the CC and CXC class of pro-inflammatory chemokines, especially the IL-8, which is an inflammatory chemokine involved in neutrophil activation and trafficking.[5,9-11] In such a manner, Duffy antigens (DARC) act as a blood chemokine sink.

The majority of Afro-Americans (approximately two thirds) show a T-to-C exchange in the *ACKR1* gene, encoding the Duffy antigens.[12] This *ACKR1* c.-67T>C gene variation silences the expression of Duffy antigens on RBCs and, in homozygosity, predicts the Duffy-null phenotype Fy(a-b-). Duffy null individuals present higher sensibility to monocyte mobilization by the CCL2 and, consequently, a decrease in the inflammatory response control.[10,13-15]

Our aim was to evaluate the association between the *ACKR1* c.-67T>C single nucleotide variation and the severity of the COVID-19 infection.

Methods

Patient recruitment

Patients were recruited at the *Fundação Pró-Sangue* from March to June 2020 and divided into three groups: 1) Death Group: patients who were hospitalized due to COVID-19 who died during the hospital stay; 2) Hospital Discharge Group: patients that were hospitalized due to COVID-19 who were discharged for home; 3) Convalescent Plasma Group: patients who had confirmed diagnosis of COVID-19 with no need for hospitalization and who were selected for convalescent plasma (CP) donation, based on the positive results of the anti-SARS-CoV-2 ELISA assay (OrthoClinical Diagnosis), and; 4) Control Group: patients who did not present COVID-19 and who were randomly selected. The study was conducted according to the Helsinki principles.

DNA extraction

DNA was individually extracted from all selected samples, using a genomic kit (PureLink, Invitrogen), following the manufacturer's instructions. The purity and concentration of the purified material were evaluated by spectrophotometry (Model 1000 spectrophotometer, Nanodrop). DNA samples were diluted to a final concentration of 100ng/mL for the genotyping experiments.

Genotyping of ACKR1 c.-67T≥C

The samples were genotyped for the c.-67T>C polymorphism of the *FY*B* promoter region (*FY*02 N.01* allele) and classified as Mutated/Mutated (MM), Mutated/Wild Type (M/W) and Wild-type/Wild-tipe (W/W). The RFLP genotyping protocol was performed, using the Ban I enzyme, following the protocol described elsewhere.[16]

Statistical analysis

Categorical variables were compared between the groups (Death, Hospital Discharge, Convalescent Plasma and Control), using the Chi-Square test. Continuous variables were compared between the same groups, using the ANOVA test, with the Tukey method as *post-hoc*. Multivariable analysis was performed through logistic regression. All tests were held in the SPSS software (20th version). A *p*-value less than 0.05 was considered significant.

Results

Characteristics of the COVID-19 patients

A total of 164 patients were enrolled in the study. The included participants were divided into the four studied

Table 1 – Clinical and demographical characteristics of the studied cohort of patients.

	Death(38)	Hospital discharge(27)	Covalescent plasmaDonor(62)	Controls(37)	*p*-value
Gender (male)	23 (60.5%)	13 (48.1%)	33 (53.2%)	17 (45.9%)	0.607
Age	57.37 ± 14.891	50.63 ± 17.6	36.18 ± 10.5	50 ± 18.38	< 0.001
FY c.-67T>C					0.079
W/W	24 (63.2%)	17 (63%)	52 (83.9%)	31 (83.8%)	
W/M	10 (26.3%)	8 (29.6%)	5 (8.05%)	4 (10.8%)	
M/M	4 (10.5%)	2 (7.4%)	5 (8.05%)	2 (5.4%)	
Grouped c.-67T>C genotype					
Only wild alleles	24 (63.2%)	17 (63%)	52 (83.9%)	31 (83.8%)	0.027
One or more mutated alleles	14 (36.8%)	10 (37%)	10 (16.1%)	6 (16.2%)	
Hypertension (yes/no)	11 (37.9%)/ 18 (62.1%)	11 (52.4%)/ 10 (47.6%)	3 (4.8%)/ 59 (95.2%)	-	<0.001
Diabetes (yes/no)	9 (31%)/ 20 (69%)	3 (14.3%)/ 18 (85.7%)	0 (0%)/ 62 (100%)	-	<0.001
Self-declared race					
Caucasian	28 (73.7%)	17 (65.4%)	47 (75.8%)	28 (75.7%)	0.509
African	10 (26.3%)	9 (34.6%)	12 (19.4%)	8 (21.6%)	
Other	0	0	3 (4.8%)	1 (2.7%)	

groups: 1) Death: n = 38; 2) Hospital Discharge: n = 27; 3) Convalescent Plasma: n = 62, and; 4) Controls: n = 37.

Table 1 summarizes the demographical and clinical characteristics of the included patients.

Patients who died during hospitalization were significantly older (57.37 ± 14.89 years old) and presented higher rates of diabetes (31%). The prevalence of systemic hypertension was higher in the Death and Hospital Discharge groups, in comparison to the Convalescent Plasma group ($p < 0.001$). The self-declared race did not significantly differ between the groups.

Duffy null genotype

The frequency of the W/M genotype was higher in the Death and Hospital Discharge groups (26.3% and 29.6%, respectively), in comparison to the Convalescent Plasma and Control groups (8.05% and 10.8%), without reaching statistical significance ($p = 0.079$). Groups significantly differed in terms of the percentage of patients presenting at least one *FY*02N.01* allele: 36.8% (Death group), 37% (Hospital Discharge group), 16.1% (Convalescent Plasma group) and 16.2% (Control group) ($p = 0.027$).

The multivariable analysis was performed considering the presence of the *FY*02N.01* allele as a dependent variable. The self-declared race ($p < 0.001$) and the occurrence of in-hospital death ($p = 0.058$) were independently associated with the presence of the *FY*02N.01* allele. Another multivariable analysis was made, considering hospitalization as a dependent variable. Hypertension ($p < 0.001$), age ($p < 0.001$) and the presence of at least one *FY*02N.01* allele ($p = 0.009$) were independently associated with this endpoint.

Discussion

This study evaluated the association between the c.-67T>C variation of the *ACKR1* gene (*FY*02N.01*), which silences the expression of the DARC receptor on the RBC membrane and the severity of the COVID-19 cases. It was demonstrated that: 1) the *FY*02N.01* allele was more frequent among patients with COVID-19 requiring hospitalization, in comparison to the group of patients who did not require a hospital stay; 2) the African self-declared race and death secondary to COVID-19 were independently associated with the presence of at least one *FY*02N.01* allele, and; 3) the *FY*02N.01* in homozygosity or heterozygosity, older age and the presence of hypertension were associated with the need for hospitalization of COVID-19 patients.

Up to now, it has been demonstrated that the immune response against the SARS-CoV-2 virus mainly involves the production of type I and type III interferon (IFN) and cytokines

by the innate immune cells which are present in the inflammatory sites. These cytokines are capable of inhibiting the viral replication and recruiting lymphocytes through the release of chemokines. These lymphocytes can trigger a specific immune response against the virus. However, SARS-CoV-2 is capable of reducing the IFN-I and IFN-III pahthways, preventing the antiviral response and inducing the production of multiple pro-inflammatory cytokines (IL-1B, IL-6, TNF and IL- 1RA), which activate a compensatory mechanism, predominantly dependent on monocyte/macrophage hyperactivation.[2,17]

The increase in plasmatic cytokine levels is apparently correlated with the prognosis of the disease, especially the high levels of IL-6. It was found that the increase in this cytokine leads to a worse prognosis of the disease, with bilateral pulmonary involvement and an increase in body temperature consistent with an acute inflammatory response. In a study conducted in China, it was found that the levels of this cytokine were 2.9 times higher in patients with the most severe COVID-19 illness and that, when these patients were treated with the IL-6 inhibitor Tocilizumab, there was an improvement in the clinical condition, without any adverse effects or death.[18,19]

Duffy antigens are chemokine receptors that bind with high affinity to inflammatory chemokines, including the CCL2 and CCL11, removing and absorbing the excess of chemokines present in the inflammatory focus.[15] The Duffy null phenotype, represented serologically by the Fy(a-b-), is observed in Afro-descendants and, in this situation, secondary to the c.-67T>C variation of the *ACKR1* gene (*FY*02N.01*) in homozygosity.[20]

The Duffy-null phenotype disturbs the balance that controls the plasma chemokine levels, leading to hypersensitivity to the chemokine signal. Studies in mice that received a transfusion of Duffy-negative erythrocytes showed these animals evolved with more intense neutrophilic infiltrate, increased levels of cytokines and increased vascular permeabilization in the lungs, when compared to mice that received convencional Duffy-positive erythrocytes. Moreover, it has been demonstrated Duffy null individuals are more sensitive to monocyte mobilization induced by CCL2.[13,21]

In the present study, we detected that 37% of the patients with severe COVID-19 presented at least one *FY*02N.01* allele and, consequently, a reduced expression of Duffy antigens on the erythrocyte membrane. Hospitalized COVID-19 patients also presented more frequently at least one *FY*02N.01* allele, in comparison to CP donors (37% versus 16.1%, Table 1). One possible explanation for that association is that the decrease in Duffy receptors on the RBC surface impacts the chemokine clearance and potentiates the inflammatory response, with greater impairment of COVID-19 target organs, resulting in a more severe phenotype.

The Duffy null phenotype is more prevalent among people of African ancestry, who are also prone to the development of severe complications of COVID-19.[4] It is known that individuals of African descent have a marked pro-inflammatory response, when compared to individuals of Caucasian descent. Several genomic studies have already described that African people have a higher frequency of variants related to pro-inflammatory cytokines and a lower frequency of variants related to anti-inflammatory cytokines.[15] The decrease in Duffy receptors in erythrocytes due to the *FY*02N.01* may be one contributing factor to the worse prognosis of people of African descent infected with COVID-19. In our results, self-declared race was not associated with the severity of the COVID-19 disease. However, self-declared race was independently associated with the presence of the *FY*02N.01* allele, reinforcing the association between this genetic variation and the African genome.

This study has some limitations, the most important of which is the sample size, which was not large and possibly justified the lack of statistical significance in the comparison of the *FY*02N.01* genotype frequency between the studied groups. Furthermore, data referring to the body mass index was not available for the included patients, nor was the socioeconomical status. Finally, the control group, comprising patients with a diagnosis other that COVID-19, was not matched to the COVID-19 groups, in terms of the severity of the illness. Moreover, the illness presented by these patients may correlate with the Duffy null genotype and this was not addressed by the present study.

Conclusion

In conclusion, there is an association between the presence of the *FY*02N.01* allele and the need for hospitalization of COVID-19 patients. This result sheds light on a possible role played by Duffy receptors on the removal of chemokines from the infected patients.

Conflicts of interest

The authors declare no conflicts of interest.

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REFERENCES

1. Li G, Fan Y, Lai Y, Han T, Li Z, Zhou P, et al. Coronavirus infections and immune responses. J Med Virol. 2020;92(4):424–32.
2. Maggi E, Canonica GW, Moretta L. COVID-19: unanswered questions on immune response and pathogenesis. J Allergy Clin Immunol. 2020;146(1):18–22.
3. Nedelec Y, Sanz J, Baharian G, Szpiech ZA, Pacis A, Dumaine A, et al. Genetic ancestry and natural selection drive population differences in immune responses to pathogens. Cell. 2016;167(3):657-69 e21.
4. Price-Haywood EG, Burton J, Fort D, Seoane L. hospitalization and mortality among black patients and white patients with Covid-19. N Engl J Med. 2020;382(26):2534–43.
5. Neote K, Mak JY, Kolakowski Jr. LF, Schall TJ. Functional and biochemical analysis of the cloned Duffy antigen: identity with the red blood cell chemokine receptor. Blood. 1994;84(1):44–52.

6. Chaudhuri A, Zbrzezna V, Polyakova J, Pogo AO, Hesselgesser J, Horuk R. Expression of the Duffy antigen in K562 cells. Evidence that it is the human erythrocyte chemokine receptor. J Biol Chem. 1994;269(11):7835–8.
7. Murphy PM. The molecular biology of leukocyte chemoattractant receptors. Annu Rev Immunol. 1994;12:593–633.
8. Sippert EA, de Oliveira e Silva C, Visentainer JE, Sell AM. Association of Duffy blood group gene polymorphisms with IL8 gene in chronic periodontitis. PLoS One. 2013;8(12):e83286.
9. Hadley TJ, Peiper SC. From malaria to chemokine receptor: the emerging physiologic role of the Duffy blood group antigen. Blood. 1997;89(9):3077–91.
10. Darbonne WC, Rice GC, Mohler MA, Apple T, Hebert CA, Valente AJ, et al. Red blood cells are a sink for interleukin 8, a leukocyte chemotaxin. J Clin Invest. 1991;88(4):1362–9.
11. Cartron JP, Bailly P, Le Van Kim C, Cherif-Zahar B, Matassi G, Bertrand O, et al. Insights into the structure and function of membrane polypeptides carrying blood group antigens. Vox Sang. 1998;74(Suppl 2):29–64.
12. Novitzky-Basso I, Rot A. Duffy antigen receptor for chemokines and its involvement in patterning and control of inflammatory chemokines. Front Immunol. 2012;3:266.
13. Mangalmurti NS, Xiong Z, Hulver M, Ranganathan M, Liu XH, Oriss T, et al. Loss of red cell chemokine scavenging promotes transfusion-related lung inflammation. Blood. 2009;113(5):1158–66.
14. Hansell CA, Hurson CE, Nibbs RJ. DARC and D6: silent partners in chemokine regulation? Immunol Cell Biol. 2011;89(2):197–206.
15. Yao S, Hong CC, Ruiz-Narvaez EA, Evans SS, Zhu Q, Schaefer BA, et al. Genetic ancestry and population differences in levels of inflammatory cytokines in women: role for evolutionary selection and environmental factors. PLoS Genet. 2018;14(6):e1007368.
16. Denomme GAR, Reid ME. Molecular Protocols in Trasfusion Medicine. Academic Press; 2000.
17. Blanco-Melo D, Nilsson-Payant BE, Liu WC, Uhl S, Hoagland D, Moller R, et al. Imbalanced host response to SARS-CoV-2 drives development of COVID-19. Cell. 2020;181(5):1036-45 e9.
18. Coomes EA, Haghbayan H. Interleukin-6 in Covid-19: a systematic review and meta-analysis. Rev Med Virol. 2020;30(6):1–9.
19. Liu T, Zhang J, Yang Y, Ma H, Li Z, Zhang J, et al. The role of interleukin-6 in monitoring severe case of coronavirus disease 2019. EMBO Mol Med. 2020;12(7):e12421.
20. Hoher G, Fiegenbaum M, Almeida S. Molecular basis of the Duffy blood group system. Blood Transfus. 2018;16(1):93–100.
21. Mayr FB, Spiel AO, Leitner JM, Firbas C, Schnee J, Hilbert J, et al. Influence of the Duffy antigen on pharmacokinetics and pharmacodynamics of recombinant monocyte chemoattractant protein (MCP-1, CCL-2) in vivo. Int J Immunopathol Pharmacol. 2009;22(3):615–25.