Inflammammasome Genetic Variants Are Associated with Protection to Clinical Severity of COVID-19 among Patients from Rio de Janeiro, Brazil

Nathalia Beatriz Ramos de Sá,1 Milena Neira-Goulart,1 Marcelo Ribeiro-Alves,2 Hugo Perazzo,2 Kim Mattos Geraldo,2 Maria Pia Diniz Ribeiro,2 Sandra Wagner Cardoso,2 Beatriz Grinszttein,2 Valdílêa G. Veloso,2 Artur Capão,3 Marilda Mendonça Siqueira,3 Ohanna Cavalcanti de Lima Bezerra,2 Cristiana Couto Garcia,3 Larissa Rodrigues Gomes,4 Andressa da Silva Cazote,1 Dalzila Vicaltina de Almeida,1 Carmem Beatriz Wagner Giacoia-Gripp,1 Fernanda Heloise Côrtes,1 and Mariza Gonçalves Morgado1

1Laboratory of AIDS & Molecular Immunology, Oswaldo Cruz Institute, FIOCRUZ, Rio de Janeiro, Brazil
2Laboratory of Clinical Research on STD/AIDS, National Institute of Infectology Evandro Chagas, FIOCRUZ, Rio de Janeiro, Brazil
3Laboratory of Respiratory Virus and Measles, Oswaldo Cruz Institute, FIOCRUZ, Rio de Janeiro, Brazil
4Center of Technological Development in Health (CDTS)/National Institute of Science and Technological for Innovation on Neglected Population Diseases (INCT-IDPN), FIOCRUZ, Rio de Janeiro, Brazil

Correspondence should be addressed to Nathalia Beatriz Ramos de Sá; nathalia.beatriz2008@gmail.com

Received 4 May 2022; Revised 18 August 2022; Accepted 24 August 2022; Published 5 September 2022

Academic Editor: Horacio Bach

Copyright © 2022 Nathalia Beatriz Ramos de Sá et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

COVID-19 has a broad spectrum of clinical manifestations, from asymptomatic or mild/moderate symptoms to severe symptoms and death. The mechanisms underlying its clinical evolution are still unclear. Upon SARS-CoV-2 infection, host factors, such as the inflammasome system, are activated by the presence of the virus inside host cells. The search for COVID-19 risk factors is of relevance for clinical management. In this study, we investigated the impact of inflammasome single-nucleotide polymorphisms (SNPs) in SARS-CoV-2-infected individuals with distinct severity profiles at clinical presentation. Patients were divided into two groups according to disease severity at clinical presentation based on the WHO Clinical Progression Scale. Group 1 included patients with mild/moderate disease (WHO < 6; n = 76), and group 2 included patients with severe/critical COVID-19 (WHO ≥ 6; n = 357). Inpatients with moderate to severe/critical profiles were recruited and followed-up at Hospital Center for COVID-19 Pandemic – National Institute of Infectology (INI)/FIOCRUZ, RJ, Brazil, from June 2020 to March 2021. Patients with mild disease were recruited at Oswaldo Cruz Institute (IOC)/FIOCRUZ, RJ, Brazil, in August 2020. Genotyping of 11 inflammasome SNPs was determined by real-time PCR. Protection and risk estimation were performed using unconditional logistic regression models. Significant differences in NLRP3 rs1539019 and CARD8 rs2043211 were observed between the two groups. Protection against disease severity was associated with the A/A genotype (OR_adj = 0.36; P = 0.032), allele A (OR_adj = 0.93; P = 0.010), or carrier-A (OR_adj = 0.45; P = 0.027) in the NLRP3 rs1539019 polymorphism; A/T genotype (OR_adj = 0.5; P = 0.045), allele T (OR_adj = 0.93; P = 0.018), or carrier-T (OR_adj = 0.48; P = 0.029) in the CARD8 rs2043211 polymorphism; and the A-C-G-C-C (OR_adj = 0.11; P = 0.018), A-C-G-G-G (OR_adj = 0.23; P = 0.003), C-C-G-C-C (OR_adj = 0.37; P = 0.021), and C-T-G-A-C (OR_adj = 0.04; P = 0.0473) in NLRP3 genetic haplotype variants. No significant associations were observed for the other polymorphisms. To the best of our knowledge, this is the first study demonstrating an association between CARD8 and NLRP3 inflammasome genetic variants and protection against COVID-19 severity, contributing to the discussion of the impact of inflammasomes on COVID-19 outcomes.
1. Introduction

At the end of 2019, a new disease emerged, described initially as an outbreak of viral pneumonia in individuals living in Wuhan, China [1]. Researchers identified a new coronavirus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), as the pathogen causing the outbreak [2]. The World Health Organization (WHO) named the associated coronavirus disease COVID-19 [3], which was raised to the pandemic category in March 2020 due to its fast dispersion around the world [4]. Globally, the mortality and incidence of SARS-CoV-2 have increased rapidly. Currently, the Americas are the continents most affected by the COVID-19 pandemic, and the United States of America (USA) and Brazil are the leaders in the numbers of cases to date [5]. According to official data from the WHO, more than 478 million individuals are already infected by SARS-CoV-2, and more than 6 million subjects have died due to COVID-19 worldwide [5, 6]. In Brazil, the first case was reported on February 26, 2020, and the first community transmission was identified on March 13, 2020. The country has accumulated more than 29 million reported cases, with more than 658,000 deaths, as of March 2022 [6]. SARS-CoV-2 vaccination started in January 2021 but was initially restricted to health care workers and elderly people. By April 2022, a total of 163 million individuals had been fully vaccinated, and more than 80 million subjects had received a booster vaccine dose, leading to a decrease in the incidence of severe disease and mortality [5].

The clinical presentation of COVID-19 can range from asymptomatic or mild/moderate flu-like symptoms to critical symptoms, such as severe acute respiratory syndrome (SARS), thromboembolism, sepsis, multiple organ failure, and death [6]. Although COVID-19 mortality rates vary among countries, older age and the presence of comorbidities have been strongly associated with more severe disease and death. The relationship between host genetics and the mechanisms underlying SARS-CoV-2 infection with the worst clinical evolution remains unclear [7–10].

In fact, during SARS-CoV-2 infection, host factors are activated by the presence of the virus inside host cells. Pattern recognition receptors (PRRs) recognize conserved virus fragments, known as pathogen-associated molecular patterns (PAMPs), and trigger the activation of several cellular components [11, 12]. Among the large family of PRRs are NOD-like receptors (NLRs), retinoic acid-inducible gene-I (RIG-I)-like receptors (RLRs), and Toll-like receptors (TLRs) [13, 14]. Some studies have already shown that the RLR family is an important PRR in the detection of coronaviruses [15, 16]. In addition, NLR receptors stand out due to their wide recognition of intrinsic or extrinsic stimuli, operating principally as cytoplasmic sensors [17]. These receivers lead to activation of the NF-κB signaling pathway, which culminates in the transcription of several molecules, such as gasdermin-D (GSDM-D), pro-IL-1β, and pro-IL-18, among others [18, 19]. These released molecules cause a wave of local inflammation involving increased secretion of proinflammatory cytokines and chemokines (e.g., IL-6, IFN-γ, CCL2, and CXCL10) [20, 21]. These and other cytokines have already been observed to be increased in SARS-CoV-2 infection, especially in more severe cases [20, 22].

The primary function of NLRs is to form a multiprotein complex known as the inflammasome. Inflammasomes are cytosolic multiprotein oligomers of the innate immune system that interact with several adapter proteins and are responsible for the activation of inflammatory responses, leading to activation of caspase-1 and inducing the release of the proinflammatory cytokines IL-1β and IL-18 [23]. Different PRRs (e.g., NLRP1 and NLRP3) can activate inflammasome assembly in response to specific stimuli, leading to inflammation and triggering the innate immune response. Inflammasome activation is strictly regulated by endogenous host proteins (e.g., CARD8 and HSP90) and by a variety of transcriptional and posttranscriptional mechanisms [24]. NLR inflammasomes comprise at least three components: a protein sensor (e.g., NLRP1 and NLRP3), an inflammatory caspase (e.g., caspase-1 and caspase-11), and an adapter molecule containing a CARD domain (e.g., ASC) [25]. In addition, twenty-two members of the NLR family have been described in humans and can be divided into four categories based on their functions: inflammasome formation, signal transduction, transcription activation, and autophagy [26].

The association between dysregulated inflammasome activity and the occurrence of certain human inflammatory diseases highlights the importance of this pathway in innate immune responses. As a regulatory mechanism, activation of inflammasome sensors (NLRP3 and AIM2) induces autophagy that subsequently impacts negatively the inflammasome function by inhibiting the formation and production of cytokines, such as IL1β, and degrading the inflammasome complex. Thus, autophagy accompanies inflammasome activation to limit inflammation by eliminating active inflammasomes [27]. As discussed in Sargazi et al., a potential correlation between SARS-CoV-2 and other coronavirus pathogens and autophagy has been suggested, indicating the relevance of targeting the autophagy pathway in the development of therapies for COVID-19 [28]. It has been shown that the SARS-CoV-2 proteins E, ORF3a, and ORF8b activate the NLRP3 inflammasome [17]. Mutations in inflammasome genes may lead to inflammatory disorders, such as chronic inflammation, autoimmunity, and viral infections [29–31]. For example, SNPs in the NLRP3 gene were found to be associated with a group of inflammatory disorders of genetic origin with exaggerated secretion of IL-1β [32].

Studies have already noted a relationship between inflammasome activation and COVID-19 [13, 33–36]. Inflammasome activation is one of the main theories to explain the cytokine storm that can occur during COVID-19, causing severe disease [13, 36]. NLRP3 activation in COVID-19 has already been described in tissues of COVID-19 patients. Additionally, higher levels of the inflammasome products IL-18 and Casp1p20 in COVID-19 patients were associated with severe disease [36]. Recently, it has been demonstrated that lung-resident macrophages infected with SARS-CoV-2 activate inflammasomes and release IL-1 and IL-18, leading to pyroptosis, which might contribute to lung inflammation [37].
However, data exploring the role of inflammasomes in SARS-CoV-2 infection remain scarce. Genetic factors contributing to the outcome of SARS-CoV-2 infection remain unclear; however, variants in specific sites of the ACE2 and TMPRSS2 genes, as well as the ABO locus, have already been considered genetic risk factors for COVID-19 outcomes [38–42]. Currently, new candidate genes have been described in the literature as influencing susceptibility to COVID-19. In this respect, Nia et al. showed that TNFα/ TNFβ polymorphisms might substantially affect COVID-19 susceptibility [43]. In a case-control study, Rokni et al. reported that carrying the A allele in TNFA-rs361525, the C allele in IL1RN-rs419598, and the A allele in IL6R-rs2228145 was related to susceptibility to developing COVID-19 [44]. These findings indicate that SNPs in several other candidate genes involved in the inflammatory response might also impact susceptibility to COVID-19. A recent study showed that two NLRP3 variants play an important role in severe and critical COVID-19 [45]. The search for risk and/or protection factors in severe COVID-19 is relevant for clinical management and deserves more investigation. Thus, in the present study, we investigated the impact of 11 single-nucleotide polymorphisms (SNPs) in NLRP3 rs10754558 (3′ UTR), rs4612666 (intronic region), rs1539019 (intronic region), rs3806268 (exonic region), and rs35829419 (exonic region); CARD8 rs2043211 (exonic region) and rs6509365 (intronic region); AIM2 rs2276405 (intronic region); CASP-1 rs572687 (intronic region); IFI16 rs1101996 (intronic region); and IL-1β rs1143634 (exonic region) inflammasome genes in SARS-CoV-2-infected individuals at distinct severity stages at clinical presentation in a public reference center for COVID-19 in Rio de Janeiro, Brazil.

2. Materials and Methods

2.1. Study Design and Population. This is a case-control genetic study nested in the RECOVER-SUS study (NCT04807699), which is a prospective multicenter study that includes participants with SARS-CoV-2 infection who were hospitalized due to COVID-19 at “Instituto Nacional de Infectologia Evandro Chagas” of the “Fundação Oswaldo Cruz” (INI/FIOCRUZ). Patients 18 years or older with confirmed SARS-CoV-2 infection presenting moderate, severe, or critical COVID-19 profiles based on the WHO severity classification at clinical presentation were enrolled in the RECOVER-SUS cohort from June 2020 to March 2021. Details regarding patient eligibility, enrollment, inclusion/exclusion criteria, and the study design of the RECOVER-SUS clinical cohort study have been previously described [46]. For the present study, we analyzed a subset of the RECOVER-SUS cohort, including 451 patients who agreed to participate in the substudy, gave biological samples for the genetic analyses, and were recruited from June to October 2020 and from February to March 2021.

Additionally, a group with mild COVID-19 composed of 43 individuals 18 years or older with SARS-CoV-2 infection confirmed by RT-PCR with asymptomatic or mild disease severity (outpatients with COVID-19) were recruited in August 2020 by the Laboratory of Respiratory Virus and Measles – IOC/FIOCRUZ, Rio de Janeiro, Brazil, after disease resolution for blood collection.

For this study, both outpatients and hospitalized individuals without suspected, probable, or RT-PCR-confirmed SARS-CoV-2 infection, according to the WHO COVID-19 case definition, or those who did not sign the consent form were excluded.

This study was approved by the Ethics Committee of National Institute of Infectology Evandro Chagas (INI)/ Oswaldo Cruz Foundation (FIOCRUZ), Rio de Janeiro, Brazil, under the approval number CAAE 32449420.4.1001.5262 and the Oswaldo Cruz Institute (IOC)/Oswaldo Cruz Foundation (FIOCRUZ), Rio de Janeiro, Brazil, under the approval number CAAE 68118417.6.0000.5248. All participants or their legal representatives signed an informed consent form prior to enrollment in the study. All methods were performed in accordance with the relevant guidelines and regulations.

Demographic and clinical data and blood samples were collected at the study entry visit (baseline). Skin color was self-declared following the classification system employed by the Brazilian Institute of Geography and Statistics (IBGE) [47]. IBGE is an entity linked to the Brazilian Federal Government and is responsible for collecting Brazilian statistical, geographic, cartographic, geodetic, and environmental information.

2.2. Clinical Profiles at Presentation. Clinical presentation was defined as mild, moderate, severe, or critical COVID-19 according to the WHO severity classification [48]. The mild group (WHO < 4) included asymptomatic outpatients or those with mild symptoms, such as cough, chest pain, coryza, dyspnea, odynophagia, anosmia, ageusia, digestive symptoms, headache, and/or myalgia. The moderate group (WHO 4-5 classification) included hospitalized symptomatic patients with no need for oxygen therapy or oxygen by mask or nasal prong. The severe group (WHO 6-8 classification) included hospitalized patients requiring oxygen via NIV (noninvasive ventilation) or high flow, intubation, and mechanical ventilation P_{O2}/Fi_{O2} ≥ 150 or Sp_{O2}/Fi_{O2} ≥ 200, mechanical ventilation P_{O2}/Fi_{O2} < 150 (Sp_{O2}/Fi_{O2} < 200) or vasopressors. The critical group (WHO 9-10 classification) included patients requiring mechanical ventilation P_{O2}/Fi_{O2} < 150 and vasopressors, dialysis, or extracorporeal membrane oxygenation (ECMO) and patients who died.

2.3. Genomic DNA Extraction. DNA was extracted from whole blood using a QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Nordrhein-Westfalen, Germany) following the manufacturer’s instructions. The DNA concentration was determined using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Waltham, Massachusetts, USA). The filtrates containing the isolated DNA were stored at -20°C until the genomics analyses.

2.4. Single-Nucleotide Polymorphism Selection and Genotyping. We selected 11 SNPs in six inflammasome genes [49–51], considering the relevance of each gene in...
the inflammasome pathway: CARD8 (rs2043211 and rs6509365), AIM2 (rs2276405), IFI16 (rs1101996), CASP-1 (rs572687), IL-1β (rs1143634), and NLRP3 (rs10754558, rs1539019, rs4612666, rs3806268, and rs35829419). SNP genotyping was performed using commercially available TaqMan assays (Applied Biosystems/AB and Life Technologies) according to the manufacturer’s instructions (Applied Biosystems/AB and Life Technologies). Briefly, for qPCR, a final volume of 10 µL was used, and 4.50 µL of the genomic DNA, which was adjusted to 0.2 ng/µL, was placed in each well of a 96-well fast plate with 5 µL of the reaction buffer 2X TaqMan Master Mix and 0.5 µL of the 20X Assay Working Stock of each gene target. Genotyping was conducted with a reaction of one cycle of 95°C for 10 min for polymerase activation, one cycle of 95°C for 15 seconds for denaturation, and 60°C for 1 min for annealing/extension in an ABI7500 Real-Time PCR platform. Allelic discrimination was carried out using Thermo Fisher Connect Software. The SNP characteristics are listed in Supplementary Table 1.

2.5. Statistical Analyses. For statistical analyses, we divided the COVID-19 patients into two groups according to WHO scores < 6 or ≥ 6. Group 1 included patients with mild and moderate COVID-19 (n = 76; WHO < 6), and group 2 included patients with severe and critical COVID-19 (n = 357; WHO ≥ 6).

The Mann–Whitney U tests were used to compare baseline demographic and clinical, continuous numerical variables, and Fisher’s exact tests were used for categorical variables. In the SNP analyses, the frequencies of genotypes were determined by direct count, and χ² tests assessed deviations from HWE. Pairwise LD patterns were determined for each gene using r² statistics (cutoff of r² ≥ 0.8). The homozygous genotypes of the allele with the major frequency in our sample were compared with the other genotypes including the minor allele frequency allele (carriers) to better observe the differences caused by the variation. The protection/risk estimate is presented as adjusted odds ratios (aORs) with a 95% CI for each SNP and estimated through unconditional logistic regression models. We included any clinical phenotypic marker associated with COVID-19 as a confounder in modeling all other genetic analyses to eliminate any possible bias. Haplotype frequencies were estimated by maximum likelihood, and phase uncertainty was included in statistical models applied for association analyses. The most frequent haplotypes of the NLRP3 and CARD8 genes were considered references for the haplotype analyses. Multiple comparisons were corrected by estimations of false-discovery rates (FDRs). All statistical analyses were performed using R version 4.1.1 (R Core Team, 2021).

We post hoc estimated the statistical power of the study considering (a) the observed minor allele frequencies (MAFs) observed in our sample’s controls (i.e., those with WHO < 6) for the studied gene variants (SNPs); (b) the prevalence of severe COVID-19 prevaccination cases of 36.17% previously reported for the Brazilian population in the same period [52]; and (c) the ratio between controls and cases of 1:4.7 (or 76/357). We conducted simulations assuming that gene variants were under a genetic additive model with a 95% association with the disease genotype, given by the D’ linkage-disequilibrium measure. Simulations were conducted with R and packages “genetics” and “GeneticsDesign.”

3. Results

3.1. Sociodemographic and Clinical Characteristics. A total of 494 individuals with COVID-19 were included in this study. Of those, 61 patients with missing data needed for classification according to the WHO severity classification were excluded from the analysis. Thus, 433 individuals were included in this study and divided into two major groups according to the WHO severity classification, and their sociodemographic and clinical characteristics are depicted in Table 1. The mean age was 58 years (IQR = 21.79), with a mean of 50 years (IQR = 24.86) among the mild and moderate patients (WHO < 6 group) and 58 years (IQR = 22.64) among the severe and critical patients (WHO ≥ 6 group). Overall, 256 individuals (51.8%) were male, with 33 (43.4%) in the mild and moderate group and 189 (52.9%) in the severe and critical group. Regarding schooling, 145 (29.4%) of the individuals had a high school degree, with 15 (19.7%) in the mild and moderate group and 109 (30.5%) in the severe and critical group. The most common symptoms (>80%) in both groups were chest pain, diarrhea, abdominal pain, and nausea. In addition, the severe and critical groups presented a high frequency of (>80%) coryza, odynophagia, anosmia, loss of taste, headache, and myalgia as the most frequent symptoms. Most individuals included in this study (n = 323; 74.6%) were classified as having WHO severity scores between 6 and 8. The schooling, skin color, the comorbidity coronary artery disease, and some symptoms (fever, odynophagia, anosmia, loss of taste, and diarrhea) differed significantly between the groups. After correction by age, gender, skin color, schooling, diabetes mellitus, coronary artery disease, and obesity or previous bariatic disease comorbidities, wherever applicable, only oxygen supplementation or use of ventilatory support and a saturation level below 95% were significantly different between groups (Table 1).

3.2. Alleles, Genotypes, and Haplotype of Inflammasome Genes

3.2.1. Association of Alleles and Genotypes between the Groups. The genotypes, alleles, and carrier frequencies of all the studied SNPs associated with disease severity protection or risk are shown in Table 2. Genotype frequencies associated with the 11 SNPs analyzed were in Hardy-Weinberg equilibrium in both groups (Supplementary Table 1).

In this study, both NLRP3 rs1539019 and CARD8 rs2043211 polymorphisms were associated with protection against disease severity in SARS-CoV-2-infected individuals (Table 2). For NLRP3 rs1539019, the association was with carrying the A/A genotype (ORadj = 0.36 [95% CI, 0.14–0.92], P = 0.033), allele A (ORadj = 0.93 [95% CI, 0.88–0.98], P =
Table 1: Sociodemographic and clinical features associated with presentation of either mild and moderate COVID-19 symptoms or severe and critical COVID-19 symptoms (n = 433).

| Features                        | WHO scale | Mild and moderate group (N = 76) | Severe and critical group (N = 357) | aOR^a (95% CI) | \( P \) value^b | Adjusted \( P \) value^b |
|---------------------------------|-----------|---------------------------------|------------------------------------|----------------|----------------|--------------------------|
| Gender; n (%)                   |           |                                 |                                    |                |                |                          |
| Female                          |           | 43 (56.58%)                     | 168 (47.06%)                       |                |                | Reference                |
| Male                            |           | 33 (43.42%)                     | 189 (52.94%)                       | 1.58 (0.82-3.02) | 0.169          | 1                        |
| Skin color; n (%)               |           |                                 |                                    |                |                |                          |
| White                           |           | 36 (47.37%)                     | 64 (17.93%)                        |                |                | Reference                |
| Brown                           |           | 32 (42.11%)                     | 229 (64.15%)                       | 2.76 (1.32-5.77) | 0.014          | 0.562                    |
| Other                           |           | 8 (10.53%)                      | 64 (17.93%)                        | 2.78 (0.95-8.16) | 0.063          | 1                        |
| Age; n (%)                      |           |                                 |                                    |                |                |                          |
| 40-60                           |           | 25 (36.76%)                     | 135 (39.59%)                       | 0.49 (0.13-1.91) | 0.270          | 1                        |
| 18-40                           |           | 22 (32.35%)                     | 39 (11.44%)                        |                |                | Reference                |
| 60-80                           |           | 18 (26.47%)                     | 139 (40.76%)                       | 1 (0.25-4.06)  | 1              | 1                        |
| 80-90.6                         |           | 3 (4.41%)                       | 28 (8.21%)                         | 2.22 (0.11-43.98) | 1              | 1                        |
| Schooling; n (%)                |           |                                 |                                    |                |                |                          |
| High school                     |           | 15 (21.43%)                     | 109 (37.85%)                       | 2.77 (1.19-6.46) | 0.036          | 1                        |
| Low education                   |           | 17 (24.29%)                     | 134 (46.53%)                       | 2.14 (0.92-4.99) | 0.078          | 1                        |
| Diabetes mellitus; n (%)        |           |                                 |                                    |                |                |                          |
| No                              |           | 66 (86.84%)                     | 235 (65.83%)                       |                |                | Reference                |
| Yes                             |           | 10 (13.16%)                     | 122 (34.17%)                       | 2.01 (0.8-5.05) | 0.135          | 1                        |
| Systemic arterial hypertension; n (%) |       |                                 |                                    |                |                | Reference                |
| No                              |           | 59 (77.63%)                     | 177 (49.58%)                       |                |                | Reference                |
| Yes                             |           | 17 (22.37%)                     | 180 (50.42%)                       | 1.57 (0.7-3.51) | 0.270          | 1                        |
| Coronary artery disease; n (%)  |           |                                 |                                    |                |                | Reference                |
| No                              |           | 67 (88.16%)                     | 347 (97.2%)                        |                |                | Reference                |
| Yes                             |           | 9 (11.84%)                      | 10 (2.8%)                          | 0.21 (0.05-0.89) | 0.033          | 1                        |
| Obesity or previous bariatric surgery; n (%) |     |                                 |                                    |                |                | Reference                |
| No                              |           | 69 (90.79%)                     | 291 (81.51%)                       |                |                | Reference                |
| Yes                             |           | 7 (9.21%)                       | 66 (18.49%)                        | 1.14 (0.45-2.87) | 0.786          | 1                        |
| O2 supplementation or ventilatory support; n (%) |     |                                 |                                    |                |                | Reference                |
| No                              |           | 45 (59.21%)                     | 47 (13.17%)                        |                |                | Reference                |
| Yes                             |           | 31 (40.79%)                     | 310 (86.83%)                       | 5.24 (2.34-11.76) | <0.001         | 0.002                    |
| Saturation below 95%; n (%)     |           |                                 |                                    |                |                | Reference                |
| No                              |           | 9 (11.84%)                      | 191 (53.5%)                        |                |                | Reference                |
| Yes                             |           | 42 (55.26%)                     | 149 (41.74%)                       | 8.06 (3.12-20.82) | <0.001         | 0.001                    |
| Fever; n (%)                    |           |                                 |                                    |                |                | Reference                |
| No                              |           | 34 (44.74%)                     | 208 (58.26%)                       |                |                | Reference                |
| Yes                             |           | 45 (59.21%)                     | 225 (63.03%)                       | 2.05 (1.05-4.01) | 0.035          | 1                        |
| Cough; n (%)                    |           |                                 |                                    |                |                | Reference                |
| No                              |           | 31 (40.79%)                     | 132 (36.97%)                       |                |                | Reference                |
| Yes                             |           | 64 (84.21%)                     | 325 (91.04%)                       | 0.97 (0.49-1.9) | 0.925          | 1                        |
| Chest pain; n (%)               |           |                                 |                                    |                |                | Reference                |
| Yes                             |           | 12 (15.79%)                     | 32 (8.96%)                         | 0.55 (0.22-1.36) | 0.195          | 1                        |
| Coryza; n (%)                   |           |                                 |                                    |                |                | Reference                |
| No                              |           | 59 (77.63%)                     | 327 (91.6%)                        |                |                | Reference                |
0.010), or carrier-A (ORadj = 0.45 [95% CI, 0.22-0.91], P = 0.027). For CARD8 rs2043211, the association was with carrying the A/T genotype (ORadj = 0.5 [95% CI, 0.25-0.99], P = 0.046), allele T (ORadj = 0.93 [95% CI, 0.88-0.99], P = 0.018), or carrier-T (ORadj = 0.48 [95% CI, 0.25-0.93], P = 0.029).

The frequency of the T allele in the NLRP3 rs4612666 polymorphism was slightly different between the group of patients with mild and moderate disease (29.61%) and those with severe to critical disease (38.52%) (ORadj = 1.05 [95% CI, 1.00-1.11], P = 0.062). Additionally, the T/T genotype showed a frequency of 5.26% in the mild and moderate group and 16.81% in the severe and critical group, constituting a genetic marker with a trend for the risk of disease severity in SARS-CoV-2-infected individuals (ORadj = 3.41 [95% CI, 0.93-12.59], P = 0.065).

The SNPs in CARD8 (rs6509365), IFI16 (rs1101996), CASP-1 (rs572687), IL-1β (rs1143634), AIM2 (rs2276405), and NLRP3 (rs3806268, rs35829419, and rs10754558) did not reveal any significant associations with disease severity risk and/or protection in SARS-CoV-2-infected individuals.

### Table 1: Continued.

| Features | WHO scale Mild and moderate group | Severe and critical group | aOR (95% CI) | P value | Adjusted P value |
|----------|----------------------------------|----------------------------|-------------|---------|-----------------|
| Dyspnea; n (%) | Yes | 17 (22.37%) | 0.8 (0.31-2.03) | 0.635 | 1 |
| | No | 40 (52.63%) | Reference |
| | Yes | 36 (47.37%) | 1.67 (0.84-3.34) | 0.143 | 1 |
| | No | 59 (77.63%) | Reference |
| Odynophagia; n (%) | Yes | 17 (22.37%) | 0.35 (0.13-0.98) | 0.046 | 1 |
| | No | 46 (60.53%) | Reference |
| Anosmia; n (%) | Yes | 30 (39.47%) | 0.34 (0.16-0.74) | 0.006 | 0.266 |
| | No | 50 (65.79%) | Reference |
| Loss of taste; n (%) | Yes | 26 (34.21%) | 0.42 (0.19-0.94) | 0.034 | 1 |
| | No | 61 (80.26%) | Reference |
| Diarrhea; n (%) | Yes | 15 (19.74%) | 0.3 (0.12-0.73) | 0.008 | 0.343 |
| | No | 68 (89.47%) | Reference |
| Abdominal pain; n (%) | Yes | 8 (10.53%) | 0.39 (0.11-1.32) | 0.129 | 1 |
| | No | 65 (85.53%) | Reference |
| Nausea; n (%) | Yes | 11 (14.47%) | 0.47 (0.15-1.46) | 0.193 | 1 |
| | No | 48 (63.16%) | Reference |
| Headache; n (%) | Yes | 28 (36.84%) | 0.5 (0.24-1.04) | 0.063 | 1 |
| | No | 44 (57.89%) | Reference |
| Myalgia; n (%) | Yes | 32 (42.11%) | 0.69 (0.34-1.37) | 0.288 | 1 |
| Outcomes; n (%) | No hospt | 43 (56.58%) | Reference |
| | Hospt | 33 (43.42%) | 357 (100%) | NC |

a Odds ratios were adjusted by skin color, schooling, gender, age, and associated comorbidities, such as diabetes mellitus, coronary artery disease, and obesity or previous bariatric disease. P values were calculated using the unconditional logistic regression model. Associations were considered significant at *P* < 0.05.

n: number of individuals in each group; aOR: adjusted odds ratio; 95% CI: 95% confidence interval; NC: not calculated; Hospt: hospitalized; No hospt: not hospitalized.

3.2.2. Association of Haplotypes between the Groups. With respect to the NLRP3 genetic haplotype variants (rs1539019 - rs4612666 - rs3806268 - rs35829419 - rs10754558), carrying the A-C-G-C-G (ORadj = 0.11 [95% CI, 0.02-0.69], P = 0.018), A-C-G-C-G (ORadj = 0.23 [95% CI, 0.09-0.62], P = 0.004), C-C-G-C-C (ORadj = 0.37 [95% CI, 0.16-0.86], P = 0.022), and/or C-T-G-A-C (ORadj = 0.04 [95% CI, 0.00-0.48], P = 0.047) haplotypes were associated with protection against disease severity in SARS-CoV-2-infected
| Genes SNP (rs) | Genes | Alleles and genotypes | Mild and moderate group | Severe and critical group | aOR<sup>a</sup> (95% CI) | P value<sup>b</sup> |
|---------------|-------|-----------------------|-------------------------|---------------------------|--------------------------|-----------------|
|              | CARD8 | rs2043211 A/A          | 35 (46.05)              | 202 (56.58)               | Reference                |                 |
|              |       | A/T                   | 32 (42.11)              | 130 (36.41)               | 0.5 (0.25-0.99)          | 0.046           |
|              |       | T/T                   | 9 (11.84)               | 25 (7)                    | 0.42 (0.13-1.4)          | 0.157           |
|              |       | A                     | 102 (67.11)             | 534 (74.79)               | Reference                |                 |
|              |       | T                     | 50 (32.89)              | 180 (25.21)               | 0.93 (0.88-0.99)         | 0.018           |
|              |       | Noncarrier-A          | 9 (11.84)               | 25 (7)                    | Reference                |                 |
|              |       | Carrier-A             | 67 (88.16)              | 332 (93)                  | 1.76 (0.56-5.54)         | 0.337           |
|              |       | Noncarrier-T          | 35 (46.05)              | 202 (56.58)               | Reference                |                 |
|              |       | Carrier-T             | 41 (53.95)              | 155 (43.42)               | 0.48 (0.25-0.93)         | 0.029           |
|              |       | A                     | 35 (46.05)              | 180 (50.42)               | Reference                |                 |
|              |       | A/G                   | 31 (40.79)              | 142 (39.78)               | 0.7 (0.35-1.4)           | 0.314           |
|              |       | G/G                   | 10 (13.16)              | 35 (9.8)                  | 0.53 (0.18-1.57)         | 0.253           |
|              |       | A                     | 101 (66.45)             | 502 (70.31)               | Reference                |                 |
|              |       | G                     | 51 (33.55)              | 212 (29.69)               | 0.96 (0.9-1.01)          | 0.135           |
|              | CARD8 | rs6509365 A/A          | 35 (46.05)              | 180 (50.42)               | Reference                |                 |
|              |       | A/G                   | 31 (40.79)              | 142 (39.78)               | 0.7 (0.35-1.4)           | 0.314           |
|              |       | G/G                   | 10 (13.16)              | 35 (9.8)                  | 0.53 (0.18-1.57)         | 0.253           |
|              |       | A                     | 101 (66.45)             | 502 (70.31)               | Reference                |                 |
|              |       | G                     | 51 (33.55)              | 212 (29.69)               | 0.96 (0.9-1.01)          | 0.135           |
|              |       | Noncarrier-A          | 10 (13.16)              | 35 (9.8)                  | Reference                |                 |
|              |       | Carrier-A             | 66 (86.84)              | 322 (90.2)                | 1.6 (0.57-4.47)          | 0.372           |
|              |       | Noncarrier-G          | 35 (46.05)              | 180 (50.42)               | Reference                |                 |
|              |       | Carrier-G             | 41 (53.95)              | 177 (49.58)               | 0.66 (0.35-1.27)         | 0.217           |
|              |       | C/C                   | 73 (96.05)              | 345 (96.64)               | Reference                |                 |
|              |       | C/T                   | 3 (3.95)                | 12 (3.36)                 | 0.95 (0.19-4.74)         | 0.951           |
|              |       | C                     | 149 (98.03)             | 702 (98.32)               | Reference                |                 |
|              |       | T                     | 3 (1.97)                | 12 (1.68)                 | 1.02 (0.83-1.24)         | 0.878           |
|              | AIM2  | rs2276405 Noncarrier-C | 76 (100)                | 76 (100)                  | Reference                |                 |
|              |       | Carrier-C             | 357 (100)               | 357 (100)                 | 0.3055                   | 0.306           |
|              |       | Noncarrier-T          | 73 (96.05)              | 345 (96.64)               | Reference                |                 |
|              |       | Carrier-T             | 3 (3.95)                | 12 (3.36)                 | 0.95 (0.19-4.74)         | 0.951           |
|              |       | C/C                   | 38 (50)                 | 166 (46.5)                | Reference                |                 |
|              |       | A/A                   | 9 (11.84)               | 42 (11.76)                | 0.62 (0.22-1.78)         | 0.376           |
|              |       | C/A                   | 29 (38.16)              | 149 (41.74)               | 0.72 (0.35-1.46)         | 0.360           |
|              |       | C                     | 105 (69.08)             | 481 (67.37)               | Reference                |                 |
|              |       | A                     | 47 (30.92)              | 233 (32.63)               | 0.98 (0.92-1.04)         | 0.445           |
|              |       | Noncarrier-C          | 9 (11.84)               | 42 (11.76)                | Reference                |                 |
|              |       | Carrier-C             | 67 (88.16)              | 315 (88.24)               | 1.37 (0.51-3.68)         | 0.533           |
|              |       | Noncarrier-A          | 38 (50)                 | 166 (46.5)                | Reference                |                 |
|              |       | Carrier-A             | 38 (50)                 | 191 (53.5)                | 0.7 (0.36-1.36)          | 0.287           |
|              |       | G/G                   | 52 (68.42)              | 243 (68.07)               | Reference                |                 |
|              |       | A/A                   | 5 (6.58)                | 14 (3.92)                 | 1.44 (0.26-7.86)         | 0.675           |
|              |       | G/A                   | 19 (25)                 | 100 (28.01)               | 1.57 (0.75-3.28)         | 0.228           |
|              |       | G                     | 123 (80.92)             | 586 (82.07)               | Reference                |                 |
|              |       | A                     | 29 (19.08)              | 128 (17.93)               | 1.03 (0.97-1.11)         | 0.347           |
|              |       | Noncarrier-G          | 5 (6.58)                | 14 (3.92)                 | Reference                |                 |
|              |       | Carrier-G             | 71 (93.42)              | 343 (96.08)               | 0.8 (0.15-4.28)          | 0.796           |
|              |       | Noncarrier-A          | 52 (68.42)              | 243 (68.07)               | Reference                |                 |
|              |       | Carrier-A             | 24 (31.58)              | 114 (31.93)               | 1.56 (0.77-3.15)         | 0.219           |
|              |       | G/G                   | 47 (61.84)              | 228 (63.87)               | Reference                |                 |
|              |       | A/A                   | 2 (2.63)                | 9 (2.52)                  | 0.53 (0.08-3.39)         | 0.503           |
|              |       | G/A                   | 27 (35.53)              | 119 (33.33)               | 1.17 (0.58-2.39)         | 0.660           |

<sup>a</sup> OR: Odds Ratio

<sup>b</sup> P value: Probability value
Table 2: Continued.

| Genes SNP (rs) | Alleles and genotypes | Mild and moderate group $N=76$ | Severe and critical group $N=357$ | aOR$^a$ (95% CI) | $P$ value$^b$ |
|----------------|------------------------|-------------------------------|-----------------------------------|------------------|--------------|
| NLRP3 rs1539019 |                        |                               |                                   |                  |              |
| G             | 121 (79.61)            | 575 (80.53)                   | Reference                         |                  |              |
| A             | 31 (20.39)             | 137 (19.19)                   | 1 (0.93-1.07)                     |                  |              |
| 0.925         |                        |                               |                                   |                  |              |
| Noncarrier-G  | 2 (2.63)               | 10 (2.8)                      | Reference                         |                  |              |
| Carrier-G     | 74 (97.37)             | 347 (97.2)                    | 1.98 (0.31-12.49)                 | 0.467            |              |
| Noncarrier-A  | 47 (61.84)             | 229 (64.15)                   | Reference                         |                  |              |
| Carrier-A     | 29 (38.16)             | 128 (35.85)                   | 1.1 (0.55-2.18)                   | 0.79             |              |
| C/C           | 24 (31.58)             | 146 (40.9)                    | Reference                         |                  |              |
| C/A           | 17 (22.37)             | 48 (13.45)                    | 0.36 (0.14-0.92)                  | 0.033            |              |
| C             | 35 (46.05)             | 163 (46.66)                   | 0.48 (0.23-1.03)                  | 0.061            |              |
| C/A           | 83 (54.61)             | 455 (63.73)                   | Reference                         |                  |              |
| NLRP3 rs4612666 |                      |                               |                                   |                  |              |
| C             | 69 (45.39)             | 259 (36.27)                   | 0.93 (0.88-0.98)                  | 0.010            |              |
| A             | 17 (22.37)             | 48 (13.45)                    | Reference                         |                  |              |
| Carrier-C     | 59 (77.63)             | 309 (63.73)                   | 1.79 (0.81-3.97)                  | 0.152            |              |
| Noncarrier-A  | 24 (31.58)             | 146 (40.9)                    | Reference                         |                  |              |
| Carrier-A     | 52 (68.42)             | 211 (36.2)                    | 0.45 (0.22-0.91)                  | 0.027            |              |
| C/C           | 35 (46.05)             | 142 (39.78)                   | Reference                         |                  |              |
| C/T           | 37 (48.68)             | 155 (43.42)                   | 1.42 (0.72-2.82)                  | 0.316            |              |
| T/T           | 4 (5.26)               | 60 (16.81)                    | 3.41 (0.93-12.59)                 | 0.065            |              |
| C             | 107 (70.39)            | 439 (61.48)                   | Reference                         |                  |              |
| T             | 45 (29.61)             | 275 (38.52)                   | 1.05 (1-1.11)                     | 0.062            |              |
| NLRP3 rs3806268 |                     |                               |                                   |                  |              |
| Carrier-T     | 41 (53.95)             | 215 (60.22)                   | 1.66 (0.86-3.21)                  | 0.131            |              |
| G/G           | 31 (40.79)             | 135 (37.82)                   | Reference                         |                  |              |
| A/A           | 10 (13.16)             | 50 (14.01)                    | 1.34 (0.46-3.94)                  | 0.593            |              |
| G/A           | 35 (46.05)             | 172 (48.18)                   | 1.4 (0.7-2.79)                    | 0.342            |              |
| G             | 97 (63.82)             | 442 (61.9)                    | Reference                         |                  |              |
| A             | 55 (36.18)             | 272 (38.1)                    | 1.02 (0.97-1.08)                  | 0.476            |              |
| Noncarrier-G  | 10 (13.16)             | 50 (14.01)                    | Reference                         |                  |              |
| Carrier-G     | 66 (86.84)             | 307 (85.99)                   | 0.9 (0.3-2.46)                    | 0.835            |              |
| Noncarrier-A  | 31 (40.79)             | 135 (37.82)                   | Reference                         |                  |              |
| Carrier-A     | 45 (59.21)             | 222 (62.18)                   | 1.39 (0.72-2.68)                  | 0.332            |              |
| C/C           | 73 (96.05)             | 340 (95.24)                   | Reference                         |                  |              |
| A/A           | 0 (0)                  | 1 (0.28)                      | Reference                         |                  |              |
| C/A           | 3 (3.95)               | 16 (4.48)                     | 0.73 (0.18-3.02)                  | 0.665            |              |
| C             | 149 (98.03)            | 696 (97.48)                   | Reference                         |                  |              |
| NLRP3 rs35829419 |                  |                               |                                   |                  |              |
| A             | 3 (1.97)               | 18 (2.52)                     | 0.98 (0.84-1.15)                  | 0.827            |              |
| Noncarrier-C  | 0 (0)                  | 1 (0.28)                      | Reference                         |                  |              |
| Carrier-C     | 76 (100)               | 356 (99.72)                   | NC                               |                  |              |
| Noncarrier-A  | 73 (96.05)             | 340 (95.24)                   | Reference                         |                  |              |
| Carrier-A     | 3 (3.95)               | 17 (4.76)                     | 0.75 (0.18-3.08)                  | 0.689            |              |
| C/C           | 26 (34.21)             | 149 (41.74)                   | Reference                         |                  |              |
| NLRP3 rs10754558 |                |                               |                                   |                  |              |
| C/G           | 43 (56.58)             | 164 (45.94)                   | 0.7 (0.35-1.4)                    | 0.313            |              |
| G/G           | 7 (9.21)               | 44 (12.32)                    | 1.14 (0.38-3.4)                   | 0.819            |              |
| C             | 95 (62.5)              | 462 (64.71)                   | Reference                         |                  |              |
individuals (Table 3). No haplotype of the CARD8 genetic variants was associated with risk and/or protection against disease severity in COVID-19 (Table 3). These analyses were performed considering the most frequent haplotype of the NLRP3 (C-T-G-C-G haplotype) and CARD8 (AA) genes as references.

Considering the observed minor allele frequencies (MAFs) of 0.02 (rs2276405/T and rs35829419/A), 0.19 (rs572687/A), 0.20 (rs11134364/A), 0.30 (rs4612666/T), 0.31 (rs1101996/C), 0.33 (rs2043211/T), 0.34 (rs6509365/G), 0.36 (rs3806268/A), 0.38 (rs10754558/G), and 0.45 (rs1539019/A) observed in our sample's controls (i.e., those with WHO < 6) for the studied gene variants (SNPs) and the prevalence of severe COVID-19 precuation cases of 36.17% previously reported for the Brazilian population in the same period [52], we estimated statistical powers for the study under a genetic additive model framework of 80% for MAFs between 0.19 and 0.34 to accept aORs ≥ 2.6 (or ≤ 0.38). For MAFs, between 0.36 and 0.45 was sufficient to accept aORs greater or equal to 2.8 (or ≤ 0.36) with equal estimated power. Only for the variant with an extremely low MAF, of 0.02, the sample size was insufficient. Indeed, we estimated 80% statistical power for this MAF only for aORs > 4 (<0.25).

3.3. Inflammase Gene Polymorphisms and COVID-19-Associated Comorbidities. As the individuals included in the study had several comorbidities (Table 1), e.g., diabetes mellitus (DM), systemic arterial hypertension (SAH), coronary artery disease (CAD), and obesity or previous bariatric surgery (Ob), they were taken into consideration in all analyses between the groups (mild and moderate group vs. severe and critical group), as shown in the footnotes of all tables. Furthermore, to make sure that the results found were not influenced by the associated comorbidities, we performed an analysis with the general population of our study and the comorbidities present in the cohort.

The association of the major comorbidities identified in the COVID-19 individuals included in the present study with the inflammasome SNPs analyzed here is presented in Supplementary Tables 2S-4S. Briefly, carrier-A in the CARD8 rs2043211 polymorphisms (OR_{adj} = 0.17 [95% CI, 0.04-0.85], P = 0.031) was associated with protection against CAD (Table 2S). Similarly, protection against DM was associated with carrier-C (OR_{adj} = 0.47 [95% CI, 0.24-0.91], P = 0.024) in the NLRP3 rs1539019 polymorphism (Table 3S). Protection against obesity was associated with carrying the G/A genotype (OR_{adj} = 0.42 [95% CI, 0.23-0.78], P = 0.006) or carrier-A (OR_{adj} = 0.48 [95% CI, 0.27-0.85], P = 0.012) in the NLRP3 rs3806268 polymorphisms, whereas a slightly increased risk for obesity was observed for those carrying the A allele in the NLRP3 rs35829419 polymorphism (OR_{adj} = 1.21 [95% CI, 1.02-1.44], P = 0.029) (Table 4S). Concerning the analysis of the inflammasome haplotypes (Table 5S), carriers of the NLRP3 C-T-G-C-G haplotype had an increased risk for CAD (OR_{adj} = 1.82 [95% CI, 2.43-5.75], P = 0.002). No other association between comorbidities and inflammasome haplotypes was observed (data not shown). No significant associations between hypertension and the SNPs included in this study were observed (data not shown).

It is important to point out that none of the inflammasome polymorphisms found to be associated with CAD, DM, or obesity comorbidities showed any significant association when comparing the COVID-19 mild/moderate group with the severe/critical group (Tables 2 and 3).

4. Discussion

Innate immune receptors are essential in the sensing of infectious organisms, continuously monitoring the extracellular milieu as well as intracellular compartments. The inflammatory process in cells is often mediated by inflammasomes, which are cytosolic multiprotein oligomers of the innate immune system [53]. Inflammasomes tend to aggregate in response to various endogenous or exogenous stimuli and orchestrate the development of local and/or systemic inflammation [53, 54]. The mechanism of inflammasome activation in COVID-19 is still poorly explored. However, Rodrigues et al. showed that the NLRP3 inflammasome is activated in hospitalized patients infected with SARS-CoV-2 [36]. This suggests a role of the NLRP3 inflammasome in the pathophysiology of the disease, as a marker.

### Table 2: Continued.

| Genes SNP (rs) | Alleles and genotypes | Mild and moderate group | Severe and critical group | aOR* (95% CI) | P valueb |
|---------------|-----------------------|------------------------|--------------------------|--------------|---------|
| G            |                       | 57 (37.5)              | 252 (35.29)              | 0.99 (0.94-1.04) | 0.711   |
| Noncarrier-C |                       | 7 (9.21)               | 44 (12.32)               | Reference    |         |
| Carrier-C    |                       | 69 (90.79)             | 313 (87.68)              | 0.71 (0.26-1.96) | 0.510   |
| Noncarrier-G |                       | 26 (34.21)             | 149 (41.74)              | Reference    |         |
| Carrier-G    |                       | 50 (65.79)             | 208 (58.26)              | 0.77 (0.39-1.5) | 0.435   |

*Odds ratios were adjusted by skin color, schooling, gender, age, and associated comorbidities, such as diabetes mellitus, coronary artery disease, and obesity or previous bariatric disease. **P values were calculated using the unconditional logistic regression model. Associations were considered significant at a value of **P < 0.05. 1The rs1143634 polymorphism in the IL-1β gene determination was not possible for one individual in the hospitalized group. n: number of individuals in each group; aOR: adjusted odds ratio; 95% CI: 95% confidence interval; NC: not calculated; A, T, G, and C: each allele count, irrespective of the genotype; Carrier-A: total of genotypes with the A allele; Carrier-T: total of genotypes with the T allele; Carrier-C: total of genotypes with the C allele; Carrier-G: total of genotypes with the G allele; Noncarrier-A: total of genotypes without the A allele; Noncarrier-T: total of genotypes without the T allele; Noncarrier-C: total of genotypes without the C allele; Noncarrier-G: total of genotypes without the G allele.
of disease severity and a potential therapeutic target for COVID-19. Toldo et al. identified the presence of inflammasomes in the lungs of patients with fatal COVID-19 [55]. On the other hand, several studies of the genes involved in assembling inflammasome complexes have attempted to explain their role in the heterogeneity of disease. For the same infection, some individuals are more susceptible to developing the disease, while others remain asymptomatic [25]. The present study demonstrated that genetically specific profiles (alleles, genotypes, and haplotypes) of NLRP3 rs1539019 and CARD8 rs2043211 polymorphisms were associated with protection against disease severity in SARS-CoV-2-infected individuals. Our data suggest that these SNPs might modulate inflammasome activation, contributing to protection against disease severity. Indeed, in a recent study [45], two other NLRP3 SNPs, NLRP3 rs10157379 and rs10754558 polymorphisms, were associated with an important role in severe acute respiratory syndrome (SARS) and severe and critical COVID-19 [45]. There was no significant association between NLRP3 rs10754558 and decreased

| Genes SNP (rs) | NLRP3 rs1539019 rs4612666 rs3806268 rs35829419 rs10754558 | CARD8 rs2043211 rs6509365 |
|----------------|--------------------------------------------------|--------------------------|
| **CTGCC**      | 26 (17.11)                                       | 99 (65.13)               |
| **ACACC**      | 10 (6.58)                                        | 3 (1.97)                 |
| **ACACG**      | 21 (13.82)                                       | 2 (1.32)                 |
| **ACGCC**      | 4 (2.63)                                         | 2 (1.32)                 |
| **ACGCG**      | 20 (13.16)                                       | 0 (0)                    |
| **ATGAG**      | 2 (1.32)                                         | 0 (0)                    |
| **ATGCC**      | 12 (7.89)                                        | 3 (1.97)                 |
| **ATGGC**      | 10 (6.58)                                        | 0 (0)                    |
| **CCACC**      | 17 (11.18)                                       | 17 (11.18)               |
| **CCACG**      | 5 (3.29)                                         | 5 (3.29)                 |
| **CCGAC**      | 2 (1.32)                                         | 0 (0)                    |
| **CCGCC**      | 24 (15.79)                                       | 24 (15.79)               |
| **CCGCG**      | 6 (3.95)                                         | 6 (3.95)                 |
| **CTACC**      | 1 (0.66)                                         | 1 (0.66)                 |
| **CTACG**      | 1 (0.66)                                         | 1 (0.66)                 |
| **CTGAC**      | 1 (0.66)                                         | 1 (0.66)                 |
| **CTGAG**      | 0 (0)                                            | 0 (0)                    |
| **CTGCG**      | 2 (1.32)                                         | 2 (1.32)                 |
| **AA**         | 99 (65.13)                                       | 99 (65.13)               |
| **AG**         | 3 (1.97)                                         | 0 (0)                    |
| **TA**         | 2 (1.32)                                         | 2 (1.32)                 |
| **TG**         | 48 (31.58)                                       | 48 (31.58)               |

*Odds ratios were adjusted by skin color, schooling, gender, age, and associated comorbidities, such as diabetes mellitus, coronary artery disease, and obesity or previous bariatric disease. *P* values were calculated using the unconditional logistic regression model. Associations were considered significant at a value of *P* < 0.05. aOR: adjusted odds ratio; 95% CI: 95% confidence interval; NC: not calculated; n: number of individuals in each group.

| Genes SNP (rs) | NLRP3 rs1539019 rs4612666 rs3806268 rs35829419 rs10754558 | CARD8 rs2043211 rs6509365 |
|----------------|--------------------------------------------------|--------------------------|
| **CTGCC**      | 26 (17.11)                                       | 99 (65.13)               |
| **ACACC**      | 10 (6.58)                                        | 3 (1.97)                 |
| **ACACG**      | 21 (13.82)                                       | 2 (1.32)                 |
| **ACGCC**      | 4 (2.63)                                         | 2 (1.32)                 |
| **ACGCG**      | 20 (13.16)                                       | 0 (0)                    |
| **ATGAG**      | 2 (1.32)                                         | 0 (0)                    |
| **ATGCC**      | 12 (7.89)                                        | 3 (1.97)                 |
| **ATGGC**      | 10 (6.58)                                        | 0 (0)                    |
| **CCACC**      | 17 (11.18)                                       | 17 (11.18)               |
| **CCACG**      | 5 (3.29)                                         | 5 (3.29)                 |
| **CCGAC**      | 2 (1.32)                                         | 0 (0)                    |
| **CCGCC**      | 24 (15.79)                                       | 24 (15.79)               |
| **CCGCG**      | 6 (3.95)                                         | 6 (3.95)                 |
| **CTACC**      | 1 (0.66)                                         | 1 (0.66)                 |
| **CTACG**      | 1 (0.66)                                         | 1 (0.66)                 |
| **CTGAC**      | 1 (0.66)                                         | 1 (0.66)                 |
| **CTGAG**      | 0 (0)                                            | 0 (0)                    |
| **CTGCG**      | 2 (1.32)                                         | 2 (1.32)                 |
| **AA**         | 99 (65.13)                                       | 99 (65.13)               |
| **AG**         | 3 (1.97)                                         | 0 (0)                    |
| **TA**         | 2 (1.32)                                         | 2 (1.32)                 |
| **TG**         | 48 (31.58)                                       | 48 (31.58)               |

AOR: adjusted odds ratio; 95% CI: 95% confidence interval; NC: not calculated; n: number of individuals in each group.

| Genes SNP (rs) | NLRP3 rs1539019 rs4612666 rs3806268 rs35829419 rs10754558 | CARD8 rs2043211 rs6509365 |
|----------------|--------------------------------------------------|--------------------------|
| **CTGCC**      | 26 (17.11)                                       | 99 (65.13)               |
| **ACACC**      | 10 (6.58)                                        | 3 (1.97)                 |
| **ACACG**      | 21 (13.82)                                       | 2 (1.32)                 |
| **ACGCC**      | 4 (2.63)                                         | 2 (1.32)                 |
| **ACGCG**      | 20 (13.16)                                       | 0 (0)                    |
| **ATGAG**      | 2 (1.32)                                         | 0 (0)                    |
| **ATGCC**      | 12 (7.89)                                        | 3 (1.97)                 |
| **ATGGC**      | 10 (6.58)                                        | 0 (0)                    |
| **CCACC**      | 17 (11.18)                                       | 17 (11.18)               |
| **CCACG**      | 5 (3.29)                                         | 5 (3.29)                 |
| **CCGAC**      | 2 (1.32)                                         | 0 (0)                    |
| **CCGCC**      | 24 (15.79)                                       | 24 (15.79)               |
| **CCGCG**      | 6 (3.95)                                         | 6 (3.95)                 |
| **CTACC**      | 1 (0.66)                                         | 1 (0.66)                 |
| **CTACG**      | 1 (0.66)                                         | 1 (0.66)                 |
| **CTGAC**      | 1 (0.66)                                         | 1 (0.66)                 |
| **CTGAG**      | 0 (0)                                            | 0 (0)                    |
| **CTGCG**      | 2 (1.32)                                         | 2 (1.32)                 |
| **AA**         | 99 (65.13)                                       | 99 (65.13)               |
| **AG**         | 3 (1.97)                                         | 0 (0)                    |
| **TA**         | 2 (1.32)                                         | 2 (1.32)                 |
| **TG**         | 48 (31.58)                                       | 48 (31.58)               |
COVID-19 severity risk or protection in our cohort. Although both studies included Brazilian individuals, our study was focused on people from the Southeast and North regions, whereas the study of Maes et al. included COVID-19 patients from one city in South Brazil, with a predominance of Caucasian ethnicity in their study group, while self-declared brown individuals predominated in our cohort. We do not know if this difference in ethnicity predominance between the two studies contributed to the differences in our results.

The NLRP3 rs1539019 polymorphism is an intronic variation whose function is still not entirely understood. However, several studies have reported that intronic polymorphisms may be associated with susceptibility/resistance to several diseases, such as rheumatoid arthritis [56], type II diabetes [57], and coronary artery disease [58]. One explanation for this is that many transcription factors bind to intronic sites that may play a role in regulating gene expression. In a study by Chung et al., the C allele of the NLRP3 rs1539019 polymorphism was found to be associated with the risk of renal cell carcinoma [59]. Additionally, Estefanous et al. reported that rs1539019 is associated with susceptibility to hepatitis C and a lower response to IFN treatment, depending on the allele and/or genotype. Moreover, Dehghan et al. reported a statistically significant association between the NLRP3 rs1539019 polymorphism and the risk of cardiovascular disease [60, 61]. To the best of our knowledge, our study is the first to demonstrate an association of the genotype A/A, allele A, or carrier-A in the NLRP3 rs1539019 variant with protection against disease severity in SARS-CoV-2-infected individuals. What is still unclear is the exact molecular mechanisms by which the NLRP3 rs1539019 polymorphism plays a protective effect in the outcome of COVID-19. It is possible that many transcription factors bind to intronic sites that may play a role in regulating gene expression [62]; therefore, we suggest that this polymorphism may involve an area containing a positive regulatory sequence. However, this hypothesis needs to be confirmed in further functional investigations.

The NLRP3 inflammasome, also known as NALP3 and cryopyrin, is currently the most studied inflammasome and is considered the main study model of these cytoplasmic complexes. The NLRP3 gene is located on the long arm of chromosome 1q44 and reacts to a diverse set of endogenous or exogenous stimuli [63]. NLRP3 has been linked to the pathogenesis of several diseases, including [1] metabolic disorders, such as type 2 diabetes [64], obesity [65], and autoimmune and inflammatory diseases [66–68]; neurological diseases [69]; and [2] diseases caused by viral pathogens, such as HIV [50], influenza A [70], and SARS-CoV [71]. SNPs in the NLRP3 gene have already been associated with a group of inflammatory disorders of genetic origin [32, 72]. Other inflammasomes and molecules related to the activation cascade (e.g., CARD8, AIM2, IFI16, CASP-1, and IL-1β) have also been found to be associated with a variety of infections and metabolic diseases. They may affect the function of the NLRP3 inflammasome [73, 74]. From a functional perspective, the results of another study showed that SARS-CoV-2 upregulates the expression of genes involved in inflammatory processes, such as NLRP3, while downregulating the genes in the autophagic pathway [28].

Caspase recruitment domain–containing protein (CARD) 8 mediates inflammasome activation in response to various pathogen-associated signals [24]. CARD8 plays an important role in apoptosis regulation, inhibition of the activation of NF-κB and caspase-1, and cytokine regulation [75]. CARD8 polymorphisms have been associated with several diseases, such as HIV-1 [76], ischemic stroke [77], and type 2 diabetes mellitus [78].

The CARD8 rs2043211 polymorphism has already been associated with risk and/or protection against several diseases, such as cardiovascular disease [79], atherosclerotic coronary artery disease [80], and inflammatory diseases, such as inflammatory bowel disease [81]. The rs2043211 variant of CARD8 is an A > T transversion on the template strand that introduces a premature stop codon, which results in the expression of a severely truncated CARD8 protein; therefore, this variant is unable to suppress NF-κB activity, which leads to high constitutive levels of pro-IL-1β [82]. In a recent study by our group, we verified an association between the CARD8 variant rs2043211 and protection against immune reconstitution inflammatory syndrome (IRIS) associated with HIV-TB coinfection (de Sá et al., 2022 unpublished data). Although COVID-19 has an important inflammatory profile [83] and constitutive increases in pro-IL-1β contribute to the cytokine storm that worsens the clinical status of patients [84], in our study, we found that the allele T, carrier-T, or genotype A/T in CARD8 rs2043211 polymorphisms is associated with protection against disease severity in individuals infected with SARS-CoV-2. One explanation for this is the interaction between CARD8 and NLRP3 [85]. Roberts et al. reported that a combination of CARD8 rs2043211 and NLRP3 rs35829419 has a protective effect against Crohn’s disease, which is an inflammatory disease, by preventing the NLRP3 inflammasome from excessively producing interleukin-1β [85]. In our study, both CARD8 rs2043211 polymorphisms and NLRP3 rs1539019 polymorphisms had a protective effect against disease severity; thus, we hypothesized that this protective effect could be explained by their interaction, although the mechanism underlying this positive association with protection against disease severity in SARS-CoV-2 is not fully elucidated.

To the best of our knowledge, this is one of the first studies demonstrating an association between CARD8 genetic variants and protection against disease severity in SARS-CoV-2-infected individuals. Studies linking CARD8, NLRP3, and SARS-CoV-2 infection are still scarce due to the recent emergence of this pathogen. One recent study showed that the inflammasome is robustly activated in SARS-CoV-2-infected hospitalized individuals [36]. In addition, several studies have indicated that the inflammasome may be involved in the pathogenesis of the disease [13, 33–36].

In the present study, we classified patients at presentation according to the WHO severity classification and identified CARD8 and NLRP3 polymorphisms associated with protection against COVID-19 severity. No polymorphisms
associated with a higher risk of disease severity were observed in our analyses.

Although selected inflammasome polymorphisms were associated with protection/susceptibility to some comorbidities observed in our study group (coronary artery disease, diabetes mellitus, and obesity), none of them showed a significant association when comparing the mild/moderate COVID-19 group with the severe/critical COVID-19 group. In our study, we found that the CARD8 rs2043211 polymorphism was associated with protection against coronary artery disease. Several studies have tried to demonstrate the role of this SNP in CAD, but no consistent association has been described thus far [80, 86, 87]. We also found that carrier-C in the NLRP3 rs1539019 polymorphism and the G/A genotype of the NLRP3 rs3806268 polymorphism were associated with protection against diabetes mellitus and obesity, respectively; on the other hand, carrying the A allele in the NLRP3 rs35829419 polymorphism was associated with a risk of obesity. To the best of our knowledge, this is the first time that these polymorphisms have been associated with these comorbidities.

Some limitations of the current study should be noted, mainly concerning the limited sample size of the mild/moderate group. Moreover, although the Brazilian population has an extensive mixture of ethnic/racial origins, the frequency of these and other SNPs is not consistent throughout the different populations in the world, justifying large further international studies or meta-analyses using already published data from different countries to assess the associations of genetic background with COVID-19 clinical profiles and outcomes. Future studies combining inflammasome genetic polymorphisms and functional analysis will be of foremost relevance to better understand the role of this cytoplasmic protein complex and its downstream effector inflammatory factors in the outcomes of SARS-CoV-2 infection.

Concerning the impact of the COVID-19 vaccination in the individuals analyzed in the present group, it is important to note that the participants included in 2020 were not vaccinated. For those recruited in 2021 (until March), we have no information on vaccination status [46]; however, the inpatients recruited in this period had WHO scores of 8-9, which eliminated any bias potentially caused by vaccination in the association between SNPs and disease protection observed in our study. Moreover, in Brazil, administration of the COVID-19 vaccine began in 2021 (end of January) exclusively for elderly people (>80 years) and health care workers, and only 2.0% of the Brazilian population was fully vaccinated on the date of censure for this analysis (March 31, 2021) [88].

5. Conclusion

The present study is the first to report an association between the NLRP3 rs1539019 polymorphism and CARD8 rs2043211 polymorphisms and protection against disease severity in SARS-CoV-2-infected individuals. We conclude that inflammasome genetic variants influence the COVID-19 clinical outcomes among the patients included in our study. Our work highlights the importance of genetic variations in inflammasome genes in the clinical evolution of COVID-19.

Data Availability

The databases used and/or analyzed during the current study would be available from the corresponding author on reasonable request after anonymization.

Disclosure

The funding agencies played no role in the design of the study, data collection, analysis, or interpretation, nor in writing the manuscript.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors’ Contributions

NBRDS, MNG, ASC, OCLB, CCG, ASC, and LRG organized the samples and databank and performed the experiments. NBRDS, MNG, MRA, and MGM analyzed and interpreted data. MRA performed the statistical analyses. NBRDS, MNG, MRA, and MGM wrote the manuscript. HP, KMG, MPDR, SWC, CCG, BG, and VGV contributed to the acquisition of data for the patients. HP, KMG, MPDR, SWC, BG, VGV, AC, MMS, OCLB, CCG, LRG, ASC, DVA, CBGG, and FHC revised the manuscript. NBRDS and MGM designed the experiments. MGM and FHC conceived, supervised, and provided infrastructure for the entire study. All authors read and agreed with the contents and submission of this manuscript. Nathalia Beatriz Ramos de Sá and Milena Neira-Goulart contributed equally to this work.

Acknowledgments

The authors are thankful to all patients who agreed to participate in this study and their families, the frontline health care workers at INI/FIOCRUZ Hospital, and the RECOVER study team in Rio de Janeiro. We are also in debt to Sylvia Lopes Maia Teixeira for helpful discussion. The study was supported by the Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ) (Grant numbers SEI-260003/00268/2020 and SEI-260003/013002/2021) and INOVA FIOCRUZ/Fundação Oswaldo Cruz (Grant numbers 48401996705881 and 48402179226880). NBRS and CCG are recipients of INOVA FIOCRUZ/Fundação Oswaldo Cruz, HP is recipient of FAPERJ (E-26/201.351/2021), MGM is recipient of CNPQ (314064/2018-4) and FAPERJ (E-26/201.177/2021), and BG is recipient of CNPQ (305789/2019-8) and FAPERJ (E-26/202.915/2018).

Supplementary Materials

Table S1: characteristics of inflammasome SNPs included in the study. Table S2: unconditional logistic multiple
regression model of risk and protection genetic factors for coronary artery disease in SARS-CoV-2-infected individuals in our cohort (n = 433). Table S3: unconditional logistic multiple regression model of risk and protection genetic factors for diabetes mellitus in SARS-CoV-2-infected individuals in our cohort (n = 433). Table S4: unconditional logistic multiple regression model of risk and protection genetic factors for obesity or previous bariatric disease in SARS-CoV-2-infected individuals in our cohort (n = 433). Table S5: association analyses among NLRP3 and CARD8 inflammasome haplotype frequencies and risk/protection factors for coronary artery disease in SARS-CoV-2-infected individuals. (Supplementary Materials)

References

[1] H. Lu, C. W. Stratton, and Y. W. Tang, "Outbreak of pneumonia of unknown etiology in Wuhan, China: the mystery and the miracle," Journal of Medical Virology, vol. 92, no. 4, pp. 401-402, 2020.

[2] N. Zhu, D. Zhang, W. Wang et al., "A novel coronavirus from patients with pneumonia in China, 2019," The New England Journal of Medicine, vol. 382, no. 8, pp. 727–733, 2020.

[3] World Health Organization (WHO), Laboratory testing of human suspected cases of novel coronavirus (nCoV) infection, 2020.

[4] World Health Organization (WHO), Situation Report-51 SITUATION IN NUMBERS total and new cases in last 24 hours, 2020.

[5] WHO WHO, "Weekly epidemiological update on COVID-19 [Internet]," 2022 [cited 2022 Mar 25]. Available from: https://www.who.int/publications/m/item/weekly-epidemiological-update-on-covid-19—22-march-2022.

[6] Worldometer, "COVID-19 coronavirus pandemic," [Internet]. 2022. Available from: https://www.worldometers.info/coronavirus/.

[7] H. Ritchie, E. Mathieu, L. Rodés-Guirao et al., "Coronavirus pandemic (COVID-19). Our World Data [Internet]," 2020 Mar 5 [cited 2021 Sep 12]; Available from: https://ourworldindata.org/coronavirus.

[8] D. Meintrup, S. Borgmann, K. Seidl et al., "Clinical medicine specific risk factors for fatal outcome in critically ill COVID-19 patients: results from a European multicenter study," J Clin Med, vol. 10, no. 17, 2021.

[9] M. M. Minashkin, N. Y. Grigortsevich, A. S. Kamaeva et al., "The role of genetic factors in the development of acute respiratory viral infection COVID-19: predicting severe course and outcomes," Biomedica, vol. 10, p. 549, 2022.

[10] M. Sabater Molina, E. Nicolás Rocamora, A. I. Bendichio et al., "Polyomorphisms in ACE, ACE2, AGTR1 genes and severity of COVID-19 disease," PLoS One, vol. 17, no. 2, p. e0263140, 2022.

[11] J. Shi, Y. Zhao, K. Wang et al., "Cleavage of GSDMD by inflammatory caspases determines pyroptotic cell death," Nature, vol. 526, no. 7575, pp. 660–665, 2015.

[12] H. Zhang, L. Zeng, M. Xie et al., "TMEM173 drives lethal coagulation in sepsis," Cell Host & Microbe, vol. 27, no. 4, pp. 556–570.e6, 2020.

[13] D. F. van den Berg and A. A. te Velde, "Severe COVID-19: NLRP3 inflammasome dysregulated," Frontiers in Immunology, vol. 11, 2020.

[14] M. Manik and R. K. Singh, "Role of toll-like receptors in modulation of cytokine storm signaling in SARS-CoV-2-induced COVID-19," Journal of Medical Virology, vol. 94, no. 3, pp. 869–877, 2022.

[15] A. Park and A. Iwasaki, "Type I and Type III Interferons - Induction, Signaling, Evasion, and Application to Combat COVID-19," Cell Host & Microbe, vol. 27, no. 6, pp. 870–878, 2020.

[16] E. de Wit, N. van Doremalen, D. Falzarano, and V. J. Munster, "SARS and MERS: recent insights into emerging coronaviruses," Nature Reviews. Microbiology, vol. 14, no. 8, pp. 523–534, 2016.

[17] M. B. Calado, C. E. da Silva Santana, and S. Crovella, "Do inflammasome impact COVID-19 severity?," Virus Disease, vol. 32, no. 3, pp. 410–420, 2021.

[18] D. Tang, P. Comish, and R. Kang, "The hallmarks of COVID-19 disease," PLoS Pathogens, vol. 16, no. 5, article e1008536, 2020.

[19] M. Z. Tay, C. M. Poh, L. Rénia, P. A. MacAry, and L. F. P. Ng, "The trinity of COVID-19: immunity, inflammation and intervention," Nature Reviews Immunology, Nature Research, vol. 20, no. 6, pp. 363–374, 2020.

[20] G. Chen, D. Wu, W. Guo et al., "Clinical and immunologic features in severe and moderate coronavirus disease," The Journal of Clinical Investigation, vol. 82, article 137244, 2019.

[21] C. Huang, Y. Wang, X. Li et al., "Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China," The Lancet, vol. 395, no. 10223, pp. 497–506, 2020.

[22] M. Aziz, R. Fatima, and R. Assaly, "Elevated interleukin-6 and severe COVID-19: a meta-analysis," Journal of Medical Virology, vol. 92, no. 11, pp. 2283–2285, 2020.

[23] V. A. K. Rathinam and K. A. Fitzgerald, "Inflammasome complexes: emerging mechanisms and effector functions," Cell, vol. 165, no. 4, pp. 792–800, 2016.

[24] S. M. Man and T.-D. Kanneganti, "Regulation of inflammasome activation," Immunological Reviews, vol. 265, no. 1, pp. 6–21, 2015.

[25] M. B. Figueira, D. S. de Lima, A. L. Boechat et al., "Single-nucleotide variants in the AIM2 – absent in melanoma 2 gene (rs1103577) associated with protection for tuberculosis," Frontiers in Immunology, vol. 12, p. 604975, 2021.

[26] Y. K. Kim, J. S. Shin, and M. H. Nahm, "NOD-like receptors in infection, immunity, and diseases," Yonsei Medical Journal, vol. 57, no. 1, p. 5, 2016.

[27] C. S. Shi, K. Shenderov, N. N. Huang et al., "Activation of autophagy by inflammatory signals limits IL-1β production by targeting ubiquitinated inflammasomes for destruction," Nature Immunology, vol. 13, no. 3, pp. 255–263, 2012.

[28] S. Sargazi, R. Sheervalilou, M. Rokni, M. Shirvaliloo, O. Shahraki, and N. Rezaei, "The role of autophagy in controlling SARS-CoV-2 infection: an overview on virophagy-mediated molecular drug targets," Cell Biology International, vol. 45, no. 8, pp. 1599–1612, 2021.

[29] P. A. Keyel, "How is inflammation initiated? Individual influences of IL-1, IL-18 and HMGB1," Cytokine, vol. 69, no. 1, pp. 136–145, 2014.

[30] K. Van Reeth, "Cytokines in the pathogenesis of influenza," in Veterinary Microbiology, pp. 109–116, Elsevier, 2000.

[31] S. M. Yamada and A. Pontillo, "The genetics behind inflammasome regulation," Mol Immunol [Internet], vol. 145, pp. 27–42, 2022.
[32] D. Lasigliè, E. Traggiai, S. Federici et al., “Role of IL-1 beta in the development of human TH17 cells: lesson from NLRP3 mutated patients,” PLoS One, vol. 6, no. 5, p. e20014, 2011.

[33] H. Hoel, L. Heggelund, D. H. Reikvam et al., “Elevated markers of gut leakage and inflammasome activation in COVID-19 patients with cardiac involvement,” Journal of Internal Medicine, vol. 289, no. 4, pp. 523–531, 2021.

[34] S. Amor, L. Fernández Blanco, and D. Baker, “Innate immunity during SARS-CoV-2: evasion strategies and activation trigger hypoxia and vascular damage,” Clinical and Experimental Immunology, vol. 202, no. 2, pp. 193–209, 2020.

[35] A. Elhabyan, S. Elayacoub, E. Sanad, A. Abukhadra, A. Elhabyan, and V. Dinu, “The role of host genetics in susceptibility to severe viral infections in humans and insights into host genetics of severe COVID-19: a systematic review,” Virus Research, vol. 289, article 198163, 2020.

[36] T. S. Rodrigues, K. S. G. de Sá, A. Y. Ishimoto et al., “Inflammasomes are activated in response to SARS-cov-2 infection and are associated with COVID-19 severity in patients,” The Journal of Experimental Medicine, vol. 218, no. 3, 2020.

[37] E. Seifik, R. Qu, C. Junqueira et al., “Inflammasome activation in infected macrophages drives COVID-19 pathology,” Nature, vol. 606, no. 7914, pp. 585–593, 2022.

[38] L. Wulandari, B. Hamidah, C. Pakpahan et al., “Initial study on TMPRSS2 p.Val160Met genetic variant in COVID-19 patients,” Human Genomics, vol. 15, no. 1, p. 29, 2021.

[39] Y. Hou, J. Zhao, W. Martin et al., “New insights into genetic susceptibility of COVID-19: an ACE2 and TMPRSS2 polymorphism analysis,” vol. 18, no. 1, 2020.

[40] T. P. Velavan, S. R. Pallerla, J. Rüter et al., “Host genetic factors determining COVID-19 susceptibility and severity,” eBioMedicine, vol. 72, article 103629, 2021.

[41] N. Alimoradi, M. Sharqi, D. Firouzabadi, M. M. Moezzi, and N. Firouzabadi, “SNPs of ACE1 (rs4343) and ACE2 (rs2285666) genes are linked to SARS-CoV-2 infection but not with the severity of disease,” Virology Journal, vol. 19, no. 1, pp. 1–9, 2022.

[42] M. Rokni, M. Heidari Nia, M. Sarhadi et al., “Association of TMPRSS2 gene polymorphisms with COVID-19 severity and mortality: a case-control study with computational analyses,” Applied Biochemistry and Biotechnology, vol. 194, no. 8, pp. 3507–3526, 2022.

[43] M. Heidari Nia, M. Rokni, S. Mirinejad et al., “Association of polymorphisms in tumor necrosis factors with SARS-CoV-2 infection and mortality rate: a case-control study and in silico analyses,” Journal of Medical Virology, vol. 94, no. 4, pp. 1502–1512, 2022.

[44] M. Rokni, M. Sarhadi, M. Heidari Nia et al., “Single nucleotide polymorphisms located in TNFA, IL1RN, IL6R, and IL6 genes are associated with COVID-19 risk and severity in an Iranian population,” Cell Biology International, vol. 46, no. 7, article 1109, 1127 pages, 2022.

[45] M. Maes, W. L. Tedesco Junior, M. A. Lozovoy et al., “In COVID-19, _NLRP3_ inflammasome genetic variants are associated with critical disease and these effects are partly mediated by the sickness symptom complex: a nomothetic network approach,” Molecular Psychiatry, vol. 27, no. 4, pp. 1945–1955, 2022.

[46] H. Perazzo, S. W. Cardoso, M. P. Ribeiro et al., “Correction to ‘In-hospital mortality and severe outcomes after hospital discharge due to COVID-19: A prospective multicenter study from Brazil’ [Lancet Reg Health Am. 2022 Jul; 11:100244] DOI: 10.1016/j.lana.2022.100244,” Lancet Regional Health-Americas, vol. 11, p. 100300, 2022.

[47] Instituto Brasileiro de Geografia e Estatística, Características etnico - raciais da população: classificação e identidades. Estudos e Análises: informação demográfica e socioeconômica, pp. 83–99, 2013.

[48] J. C. Marshall, S. Murthy, J. Diaz et al., “A minimal common outcome measure set for COVID-19 clinical research,” The Lancet Infectious Diseases, vol. 20, no. 8, pp. e192–e197, 2020.

[49] A. Pontillo, M. S. Carvalho, A. J. Kamada et al., “Susceptibility to Mycobacterium tuberculosis infection in HIV-positive patients is associated with CARD8 genetic variant,” JAIDS Journal of Acquired Immune Deficiency Syndromes, vol. 63, no. 2, pp. 147–151, 2013.

[50] A. Pontillo, L. A. Brandão, R. L. Guimarães, L. Segat, E. Athanasakis, and S. Crovella, “A 3’UTR SNP in NLRP3 gene is associated with susceptibility to HIV-1 infection,” Journal of Acquired Immune Deficiency Syndromes, vol. 54, no. 3, pp. 236–240, 2010.

[51] A. Pontillo, T. M. Oshiro, M. Girardelli, A. J. Kamada, S. Crovella, and A. J. S. Duarte, “Polymorphisms in Inflammasome genes and susceptibility to HIV-1 infection,” BASIC Trans Sci., vol. 59, no. 2, pp. 121–125, 2012.

[52] F. A. Zeiser, B. Donida, C. A. da Costa et al., “First and second COVID-19 waves in Brazil: a cross-sectional study of patients’ characteristics related to hospitalization and in-hospital mortality,” Lancet Regional Health-Americas, vol. 6, p. 100107, 2022.

[53] P. Broz and V. M. Dixit, “Inflammasomes: mechanism of assembly, regulation and signalling,” Nature Reviews Immunology, Nature Publishing Group, vol. 16, no. 7, pp. 407–420, 2016.

[54] J. Kaivol, T. A. Nyman, and S. Matikainen, “Inflammasomes and SARS-CoV-2 infection,” Viruses, vol. 13, no. 12, p. 2513, 2021.

[55] S. Toldo, R. Bussani, V. Nuzzi et al., “Inflammasome formation in the lungs of patients with fatal COVID-19,” Inflammation Research, vol. 70, no. 1, pp. 7–10, 2021.

[56] B. Pakzad, F. Yousefisadr, H. Karimzadeh, M. Mousavi, E. Noormohamadi, and R. Salehi, “Single nucleotide polymorphism rs5029937 in TNFAIP3 gene is correlated with risk of rheumatoid arthritis,” Medical Journal of the Islamic Republic of Iran, vol. 35, 2021.

[57] D. M. Lehman, D. J. Fu, A. B. Freeman et al., “A single nucleotide polymorphism in MGEA5 encoding O-GlcNAc-selective N-Acetyl-β-D-glucosaminidase is associated with type 2 diabetes in Mexican Americans,” Diabetes, vol. 54, no. 4, pp. 1214–1221, 2005.

[58] S. AbdulAzeez, A. N. Al-Nafie, A. Al-Shehri et al., “Intronic polymorphisms in the CDKN2B-AS1 gene are strongly associated with the risk of myocardial infarction and coronary artery disease in the Saudi population,” International Journal of Molecular Sciences, vol. 17, no. 3, p. 395, 2016.

[59] C.-J. Chung, B.-Y. Bao, Y.-C. Lin et al., “Polymorphism of nucleotide binding domain-like receptor protein 3 (NLRP3) increases susceptibility of total urinary arsenic to renal cell carcinoma,” Scientific Reports, vol. 10, no. 1, 2020.

[60] S. Z. K. Estfanous, S. A. Ali, S. M. Seif, S. H. A. Soror, and D. H. A. Abdelaziz, “Inflammasomes genes’ polymorphisms in Egyptian chronic hepatitis C patients: influence on vulnerability to
infection and response to treatment,” *Mediators of Inflammation*, vol. 2019, Article ID 3273645, 12 pages, 2019.

[61] A. Dehghan, Q. Yang, A. Peters et al., “Association of novel genetic loci with circulating fibrinogen levels: a genome-wide association study in six population-based cohorts: Dehghan genome-wide association study on fibrinogen,” *Circ Cardiovasc Genet*, vol. 2, no. 2, pp. 125–133, 2009.

[62] G. Euskirchen, T. E. Royce, P. Bertone et al., “CREB binds to multiple loci on human chromosome 22,” *Molecular and Cellular Biology*, vol. 24, no. 9, pp. 3804–3814, 2004.

[63] F. Martinon, A. Mayor, and J. Tschopp, “The inflammasomes: guardians of the body,” *Annual Review of Immunology*, vol. 27, no. 1, pp. 229–265, 2009.

[64] H. Yaribeygi, M. T. Mohammadi, R. Rezaee, and A. Sahebkar, “Fenofibrate improves renal function by amelioration of NOX-4, IL-18, and p53 expression in an experimental model of diabetic nephropathy,” *Journal of Cellular Biochemistry*, vol. 119, no. 9, pp. 7458–7469, 2018.

[65] H. Y. Kim, H. J. Lee, Y. J. Chang et al., “Interleukin-17-producing innate lymphoid cells and the NLRP3 inflammasome facilitate obesity-associated airway hyperreactivity,” *Nature Medicine*, vol. 20, no. 1, pp. 54–61, 2014.

[66] M. S. J. Mangan, E. J. Olhava, W. R. Roush, H. M. Seidel, G. D. Glick, and E. Latz, “Erratum: Targeting the NLRP3 inflammasome in inflammatory diseases,” *Nature reviews. Drug discovery. NLM (Medline)*, vol. 17, no. 9, p. 688, 2018.

[67] J. B. de Alencar, J. M. Zacarias, P. Y. Tsuneto et al., “Influence of inflammasomes NLRP3, and IL1B and IL2 gene polymorphisms in periodontitis susceptibility,” *PLoS One*, vol. 15, no. 1, 2020.

[68] J. Manuel Sánchez-Maldonado, M. Martinez-Bueno, H. Canhão et al., “NFkB2 polymorphisms associate with the risk of developing rheumatoid arthritis and response to TNF inhibitors: results from the REPAIR consortium,” *Scientific Reports*, vol. 10, no. 1, pp. 1–13, 2020.

[69] K. M. von Herrmann, L. A. Salas, E. M. Martinez et al., “NLRP3_ expression in mesencephalic neurons and characterization of a rare NLRP3_ polymorphism associated with decreased risk of Parkinson’s disease,” *NPJ Parkinson’s Disease*, vol. 4, no. 1, pp. 1–9, 2018.

[70] I. C. Allen, M. A. Scull, C. B. Moore et al., “The NLRP3 inflammasome mediates in vivo innate immunity to influenza A virus through recognition of viral RNA,” *Immunity*, vol. 30, no. 4, pp. 556–565, 2009.

[71] X. Wu, Y. Li, C. B. Song et al., “Increased expression of ST2 in early HIV infected patients attenuated the IL–33 induced T cell responses,” *Frontiers in Immunology*, vol. 9, p. 2850, 2018.

[72] Z. Heidari, S. Salimi, M. Rokni et al., “Association of IL-1 β, NLRP3, and COX-2 gene polymorphisms with autoimmune thyroid disease risk and clinical features in the Iranian population,” *BioMed Research International*, vol. 2021, Article ID 7729238, 10 pages, 2021.

[73] V. N. C. Leal, E. C. Reis, and A. Pontillo, “Inflammasome in HIV infection: lights and shadows,” *Molecular Immunology*, vol. 118, pp. 9–18, 2020.

[74] H. Canhão et al., “CARD8 rs2043211 with NALP3 rs35829419 in Crohn’s disease,” *Genes and Immunity*, vol. 11, no. 4, pp. 351–356, 2010.

[75] S. Ito, Y. Haru, and T. Kubota, “CARD8 is a negative regulator for NLRP3 inflammasome, but mutant NLRP3 in cryopyrin-associated periodic syndromes escapes the restriction,” *Arthritis Research & Therapy*, vol. 16, no. 1, p. R52, 2014.

[76] M. Razmara, S. M. Srinivasula, L. Wang et al., “CARD-8 Protein, a New CARD Family Member That Regulates Caspase-1 Activation and Apoptosis,” *Journal of Biological Chemistry*, vol. 277, no. 16, pp. 13952–13958, 2002.

[77] J. Lv, X. Jiang, J. Zhang, X. Peng, and H. Lin, “Combined polymorphisms in genes encoding the inflammasome components NLRP3 and CARD8 confer risk of ischemic stroke in men,” *Journal of Stroke and Cerebrovascular Diseases*, vol. 29, no. 8, article 104874, 2020.

[78] F. Tsetos, A. Roumeliotis, X. Tsekmekidou et al., “Genetic variation in CARD8, a gene coding for an NLRP3 inflammasome-associated protein, alters the genetic risk for diabetic nephropathy in the context of type 2 diabetes mellitus,” *Diabetes & Vascular Disease Research*, vol. 17, no. 6, p. 147916412097089, 2020.

[79] H. Huang, Q. Bi, H. Wei, B. Luo, and Y. He, “Association between capspace recruitment domain-containing protein 8 rs2043211 polymorphism and cardiovascular disease susceptibility: a systematic review and meta-analysis,” *Anatolian Journal of Cardiology*, vol. 20, no. 2, pp. 70–76, 2018.

[80] G. V. Paramel, L. Folkersen, R. J. Straatbrudge et al., “CARD8 gene encoding a protein of innate immunity is expressed in human atherosclerosis and associated with markers of inflammation,” *Clinical Science*, vol. 125, pp. 401–407, 2013.

[81] J. Liu, Y. Y. Liu, J. Liu et al., “Association between CARD8 rs2043211 polymorphism and inflammatory bowel disease: a meta-analysis,” *Immunological Investigations*, vol. 44, no. 3, pp. 253–264, 2015.

[82] D. C. Ko, K. P. Shukla, C. Fong et al., “A genome-wide in vitro bacterial-infection screen reveals human variation in the host response associated with inflammatory disease,” *American Journal of Human Genetics*, vol. 85, no. 2, pp. 214–227, 2009.

[83] A. U. Anka, M. I. Tahir, S. D. Abubakar et al., “Coronavirus disease 2019 (COVID-19): an overview of the immunopathology, serological diagnosis and management,” *Scandinavian Journal of Immunology*, vol. 93, no. 4, p. e12998, 2021.

[84] B. Admou, “COVID-19 et marqueurs immunologiques pertinents,” *The Pan African Medical Journal*, vol. 39, p. 40, 2021.

[85] R. L. Roberts, R. K. G. Topless, A. J. Phipps-Green, R. B. Gearry, M. L. Barclay, and T. R. Merriman, “Evidence of interaction of CARD8 rs2043211 with NALP3 rs35829419 in Crohn’s disease,” *Genes and Immunity*, vol. 11, no. 4, pp. 351–356, 2010.

[86] D. Zhou, X. Wang, T. Chen et al., “The NLRP3 rs10754558 polymorphism is associated with the occurrence and prognosis of coronary artery disease in the Chinese Han population,” *BioMed Research International*, vol. 2016, Article ID 3185397, 9 pages, 2016.

[87] M. García-Bermúdez, R. López-Mejías, C. González-Juanatey et al., “CARD8 rs2043211 with NALP3 rs35829419 in Spanish rheumatoid arthritis patients,” *DNA and Cell Biology*, vol. 32, no. 1, pp. 28–33, 2013.

[88] Coronavirus (COVID-19), “Vaccinations - Our World in Data,” 2022 Jul 28. Available from: [https://ourworldindata.org/covid-vaccinations?country=BRA](https://ourworldindata.org/covid-vaccinations?country=BRA).