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Gene 865 (2023) 147325

Contents lists available at ScienceDirect

Gene

journal homepage: www.elsevier.com/locate/gene

Research paper

Inflammasome genes polymorphisms are associated with progression to mechanical ventilation and death in a cohort of hospitalized COVID-19 patients in a reference hospital in Rio de Janeiro, Brazil

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ARTICLE INFO

Edited by Negar Azarpira

Keywords:
COVID-19
Inflammasome single nucleotide polymorphisms (SNPs)
Risk factors

ABSTRACT

COVID-19 has a broad spectrum of clinical manifestations. We assessed the impact of single nucleotide polymorphisms (SNPs) of inflammasome genes as risk factors for progression to COVID-19 critical outcomes, such as mechanical ventilation support (MVS) or death. The study included 451 hospitalized individuals followed up at the INI/FIOCRUZ, Rio de Janeiro, Brazil, from 06/2020 to 03/2021. SNPs genotyping was determined by Real-Time PCR. We analyzed risk factors for progression to MVS (n = 174[38.6 %]) or death (n = 175[38.8 %]) as a result of COVID-19 by Cox proportional hazard models. Slower progression to MVS was associated with allele G (aHR = 0.66;P = 0.005) or the genotype G/G (aHR = 0.391;P = 0.006) in the NLRP3 rs10754558 or the allele G (aHR = 0.309;P = 0.004) in the IL1β rs1143634, while C allele in the NLRP3 rs4612666 (aHR = 2.342;P = 0.006) or in the rs10754558 (aHR = 2.957;P = 0.005) were associated with faster progression to death. Slower progression to death was associated to allele G (aHR = 0.563;P = 0.006) or the genotype A/G (aHR = 0.537;P = 0.005) in the CARD8 rs6509365; the genotype A/C in the IFI16 rs1101996 (aHR = 0.569;P = 0.011); the genotype T/T (aHR = 0.394;P = 0.004) or allele T (aHR = 0.68;P = 0.006) in the NLRP3 rs4612666, and the genotype G/G (aHR = 0.326;P = 0.005) or allele G (aHR = 0,68;P = 0.014) in the NLRP3 rs10754558. Our results suggest that inflammasome genetic variations might influence the critical clinical course of COVID-19.

Abbreviations: ACE2, Angiotensin-converting enzyme 2; AIM2, Absent in melanoma 2; ARDS, acute respiratory distress syndrome; CARD8, Caspase recruitment domain-containing protein 8; CASP-1, Interleukin-1 converting enzyme; CFTR, Cystic fibrosis transmembrane conductance regulator; COPD, Chronic obstructive pulmonary disease; COVID-19, coronavirus disease-2019; DNA, Deoxyribonucleic acid; GSDM-D, Gasdermin-D; HR, Hazard ratios; IFI16, Gamma-interferon-inducible protein 16; IFNγ, Interferon gamma; IL-18, Interleukin-18; IL-1α, Interleukin-1 alpha; IL-1β, Interleukin 1-beta; IL-23, Interleukin-23; IL-4, Interleukin-4; IL-6, Interleukin-6; IP-10, Interferon gamma-induced protein 10; MCP1, Monocyte chemoattractant protein 1; MVS, Mechanical ventilation support; NF-kB, Nuclear factor kappa B; NLRP3, Pyrin domain-containing 3; PRR, pattern recognition receptors; SAPS-III, Simplified Acute Physiology Score III; SARS-CoV-2, Severe acute respiratory syndrome coronavirus 2; SNPs, Single nucleotide polymorphisms; SOFA, Sequential Organ Failure Assessment; Th17, T helper 17; TLR3, Toll-like receptor 3; TLR7, Toll-like receptor 7; TLRs, Toll-like receptors; TMPRSS2, transmembrane serine protease 2; WHO, World Health Organization.

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https://doi.org/10.1016/j.gene.2023.147325
Received 17 November 2022; Received in revised form 23 January 2023; Accepted 27 February 2023
Available online 2 March 2023

1. Introduction

An outbreak of viral pneumonia caused by a new coronavirus, named SARS-CoV-2, was firstly described in Wuhan (China) in December 2019 (Zhu et al., 2020; Zhou et al., 2020). In March 2020, the World Health Organization (WHO) classified this outbreak as the coronavirus disease-2019 (COVID-19) pandemic**.** As June of 2022, more than 530 million individuals have been infected, and more than 6.3 million deaths occurred due to COVID-19 worldwide (WHO, 2022). Globally, mortality rates and incidence of SARS-CoV-2 have been increasing over time, especially in Europe and the region of the Americas. The United States of America (USA), India, and Brazil have been the top leaders in numbers of cumulative cases of COVID-19 (WHO, 2022). As a strategy of harm reduction of COVID-19, anti-SARS-CoV-2 vaccines became available by the end of 2020 in few countries. However, difficulty in implementing a policy for rapid population vaccination, especially in large low-to-middle income countries leads to the emergence of new virus variants (Karim and Karim, 2021; WHO, 2021).

The first SARS-CoV-2 infection case in Brazil was described on February 26, 2020, and the presence of community transmissions was reported as of March 13, 2020. Since the first case, Brazil has become an epicenter of COVID-19. Up to June 2022, Brazilian health authorities has reported more than 31 million reported cases, including more than 667,000 deaths (Ministério da Saúde, 2022; Delatorre et al., 2020). Mortality rates due to COVID-19 extremely varied among countries (Abou Ghayda, 2022). Older age and presence of comorbidities were the main factors associated with higher mortality in people with SARS-CoV-2 infection (Djaharuddin et al., 2021). In a prospective study conducted by our group, Perazzo *et al.* (2022) reported a high in-hospital high mortality rate (27 %) from June 2020 to March 2021, mainly driven by older age, need of significant ventilation support and high severity scores at hospital admission (Perazzo et al., 2022).

COVID-19 has a wide spectrum of clinical manifestations, ranging from asymptomatic or with mild symptoms to more severe forms that lead to death. Most of the patients with COVID-19 have mild or moderate diseases. However, 5–10 % might have severe and life-threatening disease courses (Gavriatopoulou et al., 2021). Severe disease can be characterized by dysregulated cytokine release (cytokine storm), pneumonia, and acute lung injury that rapidly progresses to acute respiratory distress syndrome (ARDS) and need of mechanical ventilation. Individuals with severe COVID-19 might also have thromboembolism, sepsis, multiple organ failure, and conditions that lead to death (NIH, 2022). Such a wide clinical spectrum suggests the influence of the host genetic background on disease susceptibility/resistance to SARS-CoV-2 infection and/or clinical evolution in distinct populations (Velavan et al., 2021; Ellinghaus et al., 2020; Fricke-Galindo and Falfán-Valencia, 2021). Previous analyses described that NLRP3, TLR7, CFTR, ACE2, TMPRSS2, and TLR3 polymorphisms might be associated with susceptibility to SARS-CoV-2 infection and/or COVID-19 disease severity (Devaux et al., 2020; Zguro et al., 2022).

The human ACE-2 (angiotensin-converting enzyme 2) molecule that is associated with TMPRSS2 (transmembrane serine protease 2) from the host cell interacts with the protein S (Spike) of the viral envelope, facilitating the entry of SARS-CoV-2 in target cells (Hoffmann et al., 2020). Host factors are activated by the presence of viruses in the cells. Among them cytosolic pattern recognition receptors (PRR) recognize virus fragments and trigger the activation of cellular components (Shi et al., 2015; Zhang et al., 2020), which may lead to activation of the NF-kB pathway, culminating in the transcription of several inflammasome molecules, such as the Pyrin domain-containing 3 (NLRP3), gasdermin-D (GSDM-D), pro-IL-1β, and pro-IL-18, among others (Tay et al., 2020; Tang et al., 2020). These released molecules cause a wave of local inflammation involving increased secretion of proinflammatory cytokines and chemokines (e.g., IL-6, IFNγ, MCP1, and IP-10) at inflammatory sites (Chen et al., 2020; Huang et al., 2020), culminating with the cytokine storm associated with the worsening with the disease and death (Hu et al., 2021). The activation of TLRs (Toll-like receptors) induces proinflammatory cytokines, such as IL-1α, IL-1β, IL-4, and IL-6, as well as interferons. The TLR-7 and TLR-3, endosomal pattern recognition receptors for viral RNA, have already been involved in the control of innate immunity during lung SARS-CoV-2 infection (Bortolotti et al., 2021).

The role of inflammasome activation was already demonstrated in the COVID-19 (Rodrigues, 2020). Therefore, inflammasome genetics can impact in outcomes of people with COVID-19. Mutations in the inflammasome genes may lead to inflammatory disorders, linked to constitutively high levels of IL-1β contributing to chronic inflammation (Keyel, 2014), including viral infections, such as HIV, tuberculosis, and hepatitis C (Pontillo et al., 2012; Toro et al., 2021; De Sá et al., 2022). Single nucleotide polymorphisms (SNPs) in the NLRP3 gene have already been associated with a group of inflammatory disorders of genetic origin with the exaggerated secretion of IL-1β and acting in synergy with IL-6 and IL-23, to induce the differentiation of Th17 cells (Lasigliè, 2011). Recent studies show that NLPR3 rs10157379, NLPR3 rs10754558, NLRP3 rs1539019, and CARD8 rs2043211 variants play an important role in severe and critical COVID-19 (Lasigliè, 2011; Maes et al., 2022). However, the role of SNPs in the progression to severe COVID-19 remains unclear. Thus, in the present study, we investigated the association of 11 SNPs of the NLRP3, Caspase recruitment domain-containing protein 8 (CARD8), Absent in melanoma 2 (AIM2), Interleukin-1 converting enzyme (CASP-1), Gamma-interferon-inducible protein 16 (IFI16), and Interleukin 1-beta (IL-1β) inflammasome genes with risk of progression to severe outcomes in subjects hospitalized with SARS-CoV-2 infection in a public reference hospital for COVID-19 in Rio de Janeiro, Brazil.

2. Materials and methods

This is a time-to-event study nested in the RECOVER-SUS study [NCT04807699], which is a prospective multicenter study coordinated by the Evandro Chagas National Institute of Infectious Diseases of the Oswaldo Cruz Foundation (INI/FIOCRUZ). The RECOVER-SUS study has been including individuals who were hospitalized with moderate, severe and critical COVID-19 in seven centers in Brazil. The RECOVER-SUS clinical cohort study and details of patient eligibility, enrollment, inclusion/exclusion criteria, and study design have been previously described (Perazzo et al., 2022). For the present study, we analyzed a subset of the RECOVER-SUS cohort of participants hospitalized at INI/FIOCRUZ from June 2020 to March 2021. Overall, data from 451 individuals hospitalized with COVID-19 at the INI-FIOCRUZ who agreed to provide biological samples for research analyses. Among them, 174 evolved to the use of mechanical ventilatory support, and 175 evolved to death. The individuals who were discharged without using MVS during hospital stay were analyzed as control groups in the descriptive analyses. However, for the time-to-event analyses all individuals were evaluated from symptoms onset to either any outcome (e.g., MVS, death) or hospital discharge.

The study protocol was approved by the Ethics Committee of the National Institute of Infectology Evandro Chagas (INI)/Oswaldo Cruz Foundation (FIOCRUZ), Rio de Janeiro, Brazil, under approval number CAAE: 32449420.4.1001.5262. All participants or their guardians signed an informed consent before enrolling in the study.

2.1. Clinical profiles at presentation

Clinical presentation was defined according to the WHO COVID-19 severity classification (Marshall et al., 2020), as moderate, severe, and critical COVID-19 within the first 24 hs of hospitalization. Moderate cases (WHO 4–5 classification) included in-hospital individuals with no oxygen therapy or oxygen by mask or nasal prong. Individuals who were hospitalized requiring oxygen support by NIV (noninvasive ventilation) or high flow, intubation, and mechanical ventilation, pO2/FiO2 $\geq$ 150

or SpO2/FiO2 ≥ 200, and mechanical ventilation pO2/FiO2 < 150 (SpO2/FiO2 < 200), or vasopressors were classified as severe (WHO 6–8 classification). Were classified as critical (WHO 9–10 classification) individuals requiring mechanical ventilation pO2/FiO2 < 150 and vasopressors, dialysis, and death, within the first 24 hs of hospitalization.

2.2. Genomic DNA extraction and quantification

The genomic DNA was extracted from the patient's whole blood cells, using QIAamp DNA Blood Mini Kit (Qiagen, Germany), according to the manufacturer's guidelines, and the degree of DNA purity and concentration in each sample was measured by spectrophotometry. Samples were stored at −20 °C.

2.3. Genotyping of SNPs in the inflammasome genes

We selected 11 SNPs in six inflammasome genes: CARD8 (rs2043211, rs6509365), AIM2 (rs2276405), IFI16 (rs1101996), CASP-1 (rs572687), IL-1β (rs1143634), and NLRP3 (rs10754558, rs1539019, rs4612666, rs3806268, and rs35829419). SNPs were genotyped through Taq Man assays using commercial kits (Applied Biosystems/AB and Vida Technologies, São Paulo, Brazil) at the ABI7500 Real-Time platform (Applied Biosystems/AB and Life Technologies). Allelic discrimination was carried out by employing the Thermo Fisher Connect Software.

2.4. Statistical analyses

For descriptive analyses, Mann-Whitney U tests were used to compare continuous numerical variables, and Fisher's exact tests were used for categorical demographic and baseline clinical variables.

Genotype and allele frequencies of each variant were determined by direct counting, and Hardy-Weinberg Estimation (HWE) deviations were evaluated by chi-square tests. Pairwise LD patterns were determined for each gene using r^2 statistics (cut from $r^2 \geq 0.8$). The haplotype frequencies, or even the allelic phase determination, were estimated by the expectation–maximization algorithm (EM algorithm). The estimation uncertainty was included in the statistical models applied for association analysis in the form of weights. Haplotype analyses similarly used the most common haplotype in our sample as a reference.

The incidence of mechanical ventilation use and death were analyzed based on the hospital medical record and on the "person-years" (pY) at risk based on time follow-up from the date of the first symptoms of COVID-19 to the date of the outcomes of death or use of mechanical ventilatory support. The incidences and their 95 % confidence intervals (95 % CI) were estimated according to asymptotic standard errors calculated from a Gamma distribution. The observation start date for time-to-event analyzes was defined as the onset of symptoms of COVID-19. Individuals who remained hospitalized without the need for mechanical ventilatory support or death were censored at the time of hospital discharge. The results of the time-to-event analyzes were presented in the form of hazard ratios (HR) with their 95 % CI, and the risks of progression for the events described above were estimated using Cox's proportional hazard models. In time-to-event analysis, the effects of the genetic traits of interest were corrected for phenotypic traits with at least one suggestion of association (p-value ≤ 0.1) with the outcome of interest, and marginal effects presented in the form of adjusted-HR (aHR). Multiple comparisons were corrected by the false discovery ratio (FDR) estimate. The risk proportionality assumption was tested using correlation analysis and chi-square tests based on the Schoenfeld scaled residuals and transformed survival times.

Statistical power for the Cox proportional hazard analyses was estimated for the different events (i.e., clinical outcomes) of interest in our sample assuming the minor allele frequencies among controls (individuals who had the best outcome) of the observed genetic variants (SNPs) and significance level of 0.05 (Chow et al., 2008).

Statistical analyzes were performed using the R software (version 4.1.1) and its "genetics" and "survival" packages.

3. Results

3.1. Sociodemographic and clinical characteristics

Clinical and sociodemographic characteristics of participants at hospital admission comparing discharge and death outcomes according to the use or not of MVS are described in Table 1.

Data from 451 participants were analyzed, where 277 (61.4 %) were discharged and 174 (38.6 %) died during the hospital stay. Overall, most of the participants were male (53.2 %). The overall median age was 60 years (IQR = 21.84), with a median of 55 years (IQR = 20.83) in the group of individuals who entered MVS and were discharged and 67 years (IQR = 17.05) in the group of individuals who entered MVS and died. The frequencies of the individuals according to gender, skin color, and schooling were not distinct among the groups. Among the comorbidities, the frequencies of chronic obstructive pulmonary disease (COPD), HIV infection, hepatic cirrhosis, and transplant were equally distributed between the discharge and death outcomes, according to the use or not of MVS. Overall, 374 individuals (82.9 %) required some use of oxygen support at clinical presentation, with significant differences among groups (p-value < 0.001). Several common symptoms at clinical presentation (greater than50 %) were observed for the COVID-19 individuals (Table 1), but only dyspnea (312 individuals; 69.2 %), fever (243 individuals; 53.9 %), and headache (70 individuals; 15.5 %) showed statistically significant differences among groups.

Accordingly, to the mortality predictor scores performed at clinical presentation, the group of individuals who entered MVS and died also had the highest number of patients with the highest SOFA and SAPS-III scores. In SAPS-III scores, this same group was the one with the highest number of individuals with scores greater than 46 with 106 patients (77.9 %), which predicts a higher mortality rate. We used three cuts to represent disease severity ranges in this mortality predictor scores. For the Glasgow Coma Scale, the cutoff was made at a score of 0–9, indicating severe trauma, 9–13 moderate, and 13–15 minor (McDougal, 2009). For the SOFA, we used for cutoff a score of 0–7 indicating a mortality rate of 37 %, a score of 7–10 a mortality rate of 60 %, and 10–24 greater than 90 % (Ferreira et al., 2001). In the SAPS-III score the cutoff was made a score of 30–46, indicating mortality of < 3 % and 46–101 with a mortality rate of greater than 70 % (Sakr et al., 2008).

To identify clinic confounders in the genetic association study, we performed a phenotypic analysis of the clinical data in the outcome of mechanical ventilation (Table S1) and death (Table S2) using Cox proportional hazard models. Based on that, we found that the faster progression to use MVS was associated with age ranges of 60–80 years (aHR 3.213; P = 0.001) and 80–90 years (aHR 2.921; P = 0.011); having the comorbidity COPD (aHR = 2.619; P < 0.001), and cough (aHR = 1.509; P = 0.009). Meanwhile dyspnea was associated with a slower progression to use MVS. The mortality predictor score, Glasgow scale cat 13–15 (aHR = 0.279; P = 0.001) was associated with a slower progression to use MVS. The SOFA cat 7–10 (aHR = 4.085; P < 0.001) and 10–24 (aHR = 10.481; P = 0.001) were associated with a risk for a faster progression to use MVS, as well the SAPS-III cat 46–101 (aHR = 3.134; P < 0.001). Similarly, faster progression to death was associated with age ranges of 60–80 years (aHR = 3.569; P = 0.001) and 80–90 years (aHR 4.029; P = 0.001), active cancer (aHR = 5.033; P = 0.024) and transplant (aHR = 15.726; P = 0.007). The mortality predictor score, SOFA cat 7–10 (aHR = 2.073; P < 0.001) and 10–24 (aHR = 2.249; P = 0.013) were associated with a risk for a faster progression to death, as well the SAPS-III cat 46–101 (aHR = 2.673; P < 0.001).

For the genetic analyses, we selected confounder variables, given they were at least suggestively associated with progression to the outcomes, such as age, diabetes mellitus, COPD, active cancer, current smoking, transplant, Glasgow Scale, SOFA, and SAPS-III, for progression

Table 1
Sociodemographic and clinical features for all individuals included in the study categorized according to mechanical ventilation support (MVS) use. (N = 451).

Features			Discharge (N = 277)		Death (N = 174)		[a]*P*-value
		Overall N = 451	**Without MVS (N = 239)**	**With MVS (N = 38)**	**Without MVS (N = 37)**	**With MVS (N = 137)**	
Gender; n (%)	Female	211 (46.8 %)	116 (48.5 %)	23 (60.5 %)	18 (48.6 %)	54 (39.4 %)	0.102
	Male	240 (53.2 %)	240 (53.2 %)	123 (51.5 %)	15 (39.5 %)	19 (51.4 %)	
Skin Color; n (%)	White	75 (16.6 %)	75 (16.6 %)	37 (15.5 %)	7 (18.4 %)	5 (13.5 %)	0.155
	Brown	274 (60.8 %)	274 (60.8 %)	144 (60.3 %)	25 (65.8 %)	18 (48.6 %)	
	Other	35 (7.8 %)	26 (10.8 %)	3 (7.9 %)	2 (5.4 %)	4 (2.9 %)	
Age; n (%)		60.25 (IQR = 21.84)	55.19 (IQR = 18.83)	55.45 (IQR = 20.83)	69.76 (IQR = 18.88)	67.48 (IQR = 17.05)	< **0.001**
	(18–40)	45 (10.9 %)	33 (15.1 %)	5 (13.9 %)	3 (10.7 %)	4 (3.1 %)	
	(40–60)	159 (38.6 %)	104 (47.5 %)	18 (50 %)	3 (10.7 %)	34 (26.4 %)	
	(60–80)	176 (42.7 %)	72 (32.9 %)	12 (33.3 %)	15 (53.6 %)	77 (59.7 %)	
	(80–90)	32 (7.8 %)	10 (4.6 %)	1 (2.8 %)	7 (25 %)	14 (10.9 %)	
Schooling; n (%)	University education	51 (11.3 %)	51 (11.3 %)	34 (14.2 %)	6 (15.8 %)	2 (5.4 %)	0.127
	High school	140 (31 %)	75 (31.4 %)	14 (36.8 %)	10 (27 %)	41 (29.9 %)	
	Low Education	205 (45.4 %)	108 (45.2 %)	14 (36.8 %)	16 (43.2 %)	63 (48.9 %)	
	Illiterate	27 (6 %)	13 (5.4 %)	0 (0 %)	4 (10.8 %)	10 (7.3 %)	
Diabetes Mellitus; n (%)	No	313 (69.4 %)	177 (74.1 %)	27 (71.1 %)	25 (67.6 %)	84 (61.3 %)	0.08
	Yes	138 (30.6 %)	62 (25.9 %)	11 (28.9 %)	12 (32.4 %)	53 (38.7 %)	
Coronary Artery Disease; n (%)	No	440 (97.6 %)	234 (97.9 %)	37 (97.4 %)	34 (91.9 %)	135 (98.5 %)	0.128
	Yes	11 (2.4 %)	5 (2.1 %)	1 (2.6 %)	3 (8.1 %)	2 (1.5 %)	
Systemic arterialhypertension; n (%)	No	231 (51.2 %)	135 (56.5 %)	19 (50 %)	18 (48.6 %)	59 (43.1 %)	0.093
	Yes	220 (48.8 %)	104 (43.5 %)	19 (50 %)	19 (51.4 %)	78 (56.9 %)	
COPD; n (%)	No	422 (93.6 %)	232 (97.1 %)	34 (89.5 %)	35 (94.6 %)	121 (88.3 %)	**0.007**
	Yes	29 (6.4 %)	7 (2.9 %)	4 (10.5 %)	2 (5.4 %)	16 (11.7 %)	
HIV; n (%)	Negative	406 (90 %)	210 (87.9 %)	38 (100 %)	26 (70.3 %)	132 (96.4 %)	< **0.001**
	Positive	24 (5.3 %)	16 (6.7 %)	0 (0 %)	3 (8.1 %)	5 (3.6 %)	
Hepatical Cirrhosis; n (%)	No	449 (99.6 %)	239 (100 %)	38 (100 %)	35 (94.6 %)	137 (100 %)	< **0.001**
	Yes	2 (0.4 %)	0 (0 %)	0 (0 %)	2 (5.4 %)	0 (0 %)	
Transplant; n (%)	No	450 (99.8 %)	239 (100 %)	38 (100 %)	36 (97.3 %)	137 (100 %)	**0.011**
	Yes	1 (0.2 %)	0 (0 %)	0 (0 %)	1 (2.7 %)	0 (0 %)	
Oxygen supplementation or ventilatory support	Yes	374 (82.9 %)	177 (74.1 %)	38 (100 %)	22 (59.5 %)	137 (100 %)	< **0.001**
	No	77 (17.1 %)	62 (25.9 %)	0 (0 %)	15 (40.5 %)	0 (0 %)	
Fever; n (%)	Yes	243 (53.9 %)	141 (59 %)	23 (60.5 %)	14 (37.8 %)	65 (47.4 %)	**0.025**
	No	208 (46.1 %)	98 (41 %)	15 (39.5 %)	23 (62.2 %)	72 (52.6 %)	
Cough; n (%)	Yes	275 (61 %)	156 (65.3 %)	24 (63.2 %)	20 (54.1 %)	75 (54.7 %)	0.178
	No	176 (39 %)	83 (34.7 %)	14 (36.8 %)	17 (45.9 %)	62 (45.3 %)	
Chest Pain; n (%)	No	411 (91.1 %)	217 (90.8 %)	36 (94.7 %)	36 (97.3 %)	122 (89.1 %)	0.374
	Yes	40 (8.9 %)	22 (9.2 %)	2 (5.3 %)	1 (2.7 %)	15 (10.9 %)	
Coryza; n (%)	No	415 (92 %)	215 (90 %)	35 (92.1 %)	35 (94.6 %)	130 (94.9 %)	0.354
	Yes	36 (8 %)	24 (10 %)	3 (7.9 %)	2 (5.4 %)	7 (5.1 %)	
Dyspneia; n (%)	Yes	312 (69.2 %)	158 (66.1 %)	33 (86.8 %)	19 (51.4 %)	102 (74.5 %)	**0.003**
	No	139 (30.8 %)	81 (33.9 %)	5 (13.2 %)	18 (48.6 %)	35 (25.5 %)	
Odynophagy; n (%)	No	435 (96.5 %)	226 (94.6 %)	37 (97.4 %)	37 (100 %)	135 (98.5 %)	0.127
	Yes	16 (3.5 %)	13 (5.4 %)	1 (2.6 %)	0 (0 %)	2 (1.5 %)	
Anosmia; n (%)	No	403 (89.4 %)	209 (87.4 %)	34 (89.5 %)	37 (100 %)	123 (89.8 %)	0.148
	Yes	48 (10.6 %)	30 (12.6 %)	4 (10.5 %)	0 (0 %)	14 (10.2 %)	
Loss Of Taste; n (%)	No	411 (91.1 %)	211 (88.3 %)	34 (89.5 %)	37 (100 %)	129 (94.2 %)	**0.053**
	Yes	40 (8.9 %)	28 (11.7 %)	4 (10.5 %)	0 (0 %)	8 (5.8 %)	
Diarrhea; n (%)	No	411 (91.1 %)	210 (87.9 %)	36 (94.7 %)	36 (97.3 %)	129 (94.2 %)	0.07
	Yes	40 (8.9 %)	29 (12.1 %)	2 (5.3 %)	1 (2.7 %)	8 (5.8 %)	
Abdominal Pain; n (%)	No	438 (97.1 %)	232 (97.1 %)	37 (97.4 %)	37 (100 %)	132 (96.4 %)	0.706
	Yes	13 (2.9 %)	7 (2.9 %)	1 (2.6 %)	0 (0 %)	5 (3.6 %)	
Nausea; n (%)	No	427 (94.7 %)	225 (94.1 %)	36 (94.7 %)	36 (97.3 %)	130 (94.9 %)	0.884
	Yes	24 (5.3 %)	14 (5.9 %)	2 (5.3 %)	1 (2.7 %)	7 (5.1 %)	
Headache; n (%)	Yes	70 (15.5 %)	45 (18.8 %)	7 (18.4 %)	1 (2.7 %)	17 (12.4 %)	**0.048**
	No	381 (84.5 %)	194 (81.2 %)	31 (81.6 %)	36 (97.3 %)	120 (87.6 %)	
Myalgia; n (%)	No	354 (78.5 %)	180 (75.3 %)	27 (71.1 %)	31 (83.8 %)	116 (84.7 %)	0.094
	Yes	97 (21.5 %)	59 (24.7 %)	11 (28.9 %)	6 (16.2 %)	21 (15.3 %)	
Some thrombosis	Yes	1 (0.2 %)	1 (0.2 %)	0 (0 %)	0 (0 %)	0 (0 %)	NC
	No	450 (99.8 %)	239 (100 %)	38 (100 %)	37 (100 %)	136 (99.3 %)	
Glasgow Scale		15 (IQR = 0)	15 (IQR = 0)	15 (IQR = 0)	15 (IQR = 0)	15 (IQR = 1)	< **0.001**
Glasgow scale cat	(0,9]	11 (2.9 %)	2 (0.9 %)	1 (3.2 %)	1 (3.2 %)	7 (6.9 %)	< **0.001**
	(9,13]	23 (6.1 %)	2 (0.9 %)	2 (6.5 %)	2 (6.5 %)	17 (16.7 %)	

(*continued on next page*)

Table 1 (*continued*)

Features			Discharge (N = 277)		Death (N = 174)		[a]*P*-value
		Overall N = 451	**Without MVS (N = 239)**	**With MVS (N = 38)**	**Without MVS (N = 37)**	**With MVS (N = 137)**	
	(13,15]	345 (91 %)	211 (98.1 %)	28 (90.3 %)	28 (90.3 %)	78 (76.5 %)	
SOFA		3 (IQR = 4)	2 (IQR = 1)	3 (IQR = 4)	4 (IQR = 4)	6 (IQR = 5)	**< 0.001**
SOFA cat	(0,7]	379 (85 %)	229 (97 %)	30 (81.1 %)	29 (78.4 %)	91 (66.9 %)	**< 0.001**
	(7,10]	53 (11.9 %)	6 (2.5 %)	6 (16.2 %)	8 (21.6 %)	33 (24.3 %)	
	(10,24.1]	14 (3.1 %)	1 (0.4 %)	1 (2.7 %)	0 (0 %)	12 (8.8 %)	
SAPS-III		46 (IQR = 14.75)	43 (IQR = 9)	43 (IQR = 13)	51 (IQR = 14)	57 (IQR = 21)	**< 0.001**
SAPS-III cat	(30,46]	230 (51.6 %)	164 (69.5 %)	22 (59.5 %)	14 (37.8 %)	30 (22.1 %)	**< 0.001**
	(46,101]	216 (48.4 %)	72 (30.5 %)	15 (40.5 %)	23 (62.2 %)	106 (77.9 %)	

[a] *P*-value were calculated using the unconditional logistic regression model. Associations were considered significant with a value of $P < 0.05$. N: number of individuals in each group. MVS: Mechanical Ventilation Support. NC: not calculated. COPD: Chronic obstructive pulmonary disease. SOFA: Sequential Organ Failure Assessment. SAPS III: Simplified Acute Physiology Score III. Cat: Category of small categories. Glasgow scale cat (0,9): severe trauma; (9,13): moderate trauma; (13,15): mild trauma/normal. SOFA cat (0,7): corresponded to mortality rate of 37 %; (7,10): corresponded to mortality rate of 60 %; (10,24): corresponded to mortality rate greater than 90 %. SAPS3 cat (30,46): corresponded to mortality of < 3 %; (46,101): corresponded to mortality rate of greater than 70 %.

to death, and age, systemic arterial hypertension, COPD, active cancer, obesity or previous bariatric surgery, HIV, Glasgow Scale Cat, SOFA, and SAPS-III for progression to use of mechanical ventilation in phenotypic analyses. We believe that including these variables in the genetic analysis was sufficient to eliminate potential sample biases.

3.2. Alleles, genotypes, and haplotypes associated with time to the event of mechanical ventilatory support (MVS)

Genotype frequencies of the 11 SNPs analyzed in the present study are in Hardy-Weinberg equilibrium (Table S3). The genotypes, alleles, carrier frequencies and haplotypes of the studied SNPs among the individuals who were submitted to MVS (N = 175) which had statistically significant results are shown in Table 2. The remaining ones are shown in Table S4.

The time of progression in weeks from the beginning of the symptoms until the use of MVS is described in Table S5. In this study, we observed that carrying the G allele (aHR = 0.391; P = 0.006), or the G/G genotype (aHR = 0.66; P = 0.005) in the NLRP3 rs10754558 variant or carrying the G allele in the IL1β rs1143634 variant (aHR = 0.309; P = 0.004), were associated with a slower progression to the use of MVS. We also performed the haplotype analysis and, considering the genetic variants of NLRP3 (rs1539019 - rs4612666 - rs3806268 - rs35829419 - rs10754558), carrying the A-T-G-A-G haplotype (aHR = 10.241; P < 0.001) was associated with a risk for faster progression to MVS use while carrying the C-T-G-C-G haplotype (aHR = 0.206; P = 0.024) was associated with a slower time to progression to use MVS. These analyzes of haplotypes were performed considering the most frequent haplotype of the NLRP3 (C-T-G-C–C) gene as references. All haplotype analyses are shown in Table S6.

Assuming the observed rates of use of ventilatory support in our cohort of approximately 83 %, and the minor allele frequencies among controls, i.e., those who did not required ventilatory support, of 0.02 to 0.4, for the n-sample of 451 patients and significance level (alpha of

Table 2
Analysis of alleles, genotypes, and haplotypes of COVID-19 individuals from the beginning of symptoms to the progression to mechanical ventilation outcome that showed significant results, using Cox proportional hazard models or time-to-event analyses.

Genes	SNP	Alleles /Genotype/Haplotypes	[a]pY	Crude Incidence/pY (CI95 %)	Mechanical Ventilation aHR (CI95 %)	[b]*P*-value
NLRP3	rs10754558	C/C	7.88	10.28 (8.16–12.77)	Reference	
		C/G	9.4	7.98 (6.28–10.01)	0.748 (0.51–1.096)	0.136
		G/G	3.55	5.35 (3.22–8.36)	**0.391 (0.2–0.763)**	**0.006**
		C	25.16	9.42 (8.26–10.7)	Reference	
		G	16.5	6.85 (5.64–8.23)	**0.66 (0.443–0.983)**	**0.005**
		No carrier C	3.55	5.35 (3.22–8.36)	Reference	
		Carrier C	17.28	9.03 (7.67–10.56)	2.202 (1.16–4.182)	0.16
IL1β	rs1143634	G/G	14.6	7.4 (6.07–8.93)	Reference	
		A/A	0.33	17.96 (6.59–39.1)	3.213 (1.358–7.603)	0.088
		G/A	5.86	10.24 (7.81–13.18)	1.02 (0.683–1.523)	0.922
		No carrier G	0.37	18.94 (7.61–39.02)	Reference	
		Carrier G	20.46	8.21 (7.02–9.55)	**0.309 (0.14–0.683)**	**0.004**
		No carrier A	14.63	7.45 (6.12–8.99)	Reference	
		Carrier A	6.2	10.65 (8.24–13.55)	1.113 (0.76–1.63)	0.583
NLRP3	rs1539019	**ATGAG**	0.02	40.58 (1.03–226.12)	**10.241 (1.375–76.25)**	**< 0.001**
	rs4612666	**CTGCG**	2.04	1.96 (0.54–5.03)	**0.206 (0.049–0.856)**	**0.024**
	rs3806268					
	rs35829419					
	rs10754558					

[a] pY:person-years; [b]P-value were calculated using the Cox's proportional risk model. Associations were considered significant with a value of $P < 0.05$. N: number of individuals in each group; aHR: adjusted Hazard ratio; CI95 %: 95 % confidence interval; NC: not calculated. Adjusted Hazard ratio were adjusted by age, systemic arterial hypertension, COPD, active cancer, obesity or previous bariatric surgery, HIV, Glasgow Scale Cat, SOFA, and SAPS3. 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 T allele; Carrier-C = total of genotypes with the C allele; Carrier-G = total of genotypes with the G allele; No carrier-A = total of genotypes without the A allele; No carrier-T = total of genotypes without the T allele; No carrier-C = total of genotypes without the C allele; No carrier-G = total of genotypes without the G allele.

0.05) we obtained statistical powers (1 - beta) greater than 99 % for the Cox proportional hazard analyses for the events of use of ventilatory support (data not shown).

3.3. Alleles, genotypes, and haplotypes associated with time to the event of death

Genotypes, alleles, and carrier frequencies of all the studied SNPs comparing COVID-19 individuals who evolve to death (N = 174) are shown in Table 3. In addition, the median time of weeks to progression to death is described in Table S7.

We observed that carrying the C allele of the NLRP3 rs10754558 variant increases the progression to death (aHR = 2.957; P = 0.005). Carrying the C allele of the NLRP3 rs4612666 variant also increases the progression to death (aHR = 2.342; P = 0.006). Moreover, the following alleles and genotypes were associated with a slower progression to death, as follows: carry either the G/G genotype (aHR = 0.323; P = 0.005) or the G allele (aHR = 0.688; P = 0.014) of the NLRP3 rs10754558 variant; the T/T genotype (aHR = 0.394; P = 0.004) or the T allele (aHR = 0.68; P = 0.006) of NLRP3 rs4612666 variant; the A/G genotype (aHR = 0.537; P = 0.005) or the G allele of the CARD8 rs6509365 variant (aHR = 0.563; P = 0.006), and the A/C genotype of the IFI16 rs1101996 variant (aHR = 0.569; P = 0.033). SNPs in the CARD8 (rs2043211), CASP-1 (rs572687), AIM2 (rs2276405), and NLRP3 (rs3806268, rs35829419, and rs1539019) genes did not reveal any significant association with the studied outcomes. We also performed the haplotype analysis of inflammasome SNP associated with the time to death, as shown in Table 4.

Carry the haplotypes A-C-A-C–C (aHR = 2.344; P = 0.002), A-C-G-C–C (aHR = 2.174; P = 0.042), C–C-A-C–C (aHR = 2.026; P = 0.002), C-T-A-C–C (aHR = 2.068; P = 0.012) or A-T-G-A-G (aHR = 34.611; P < 0.001) of the NLRP3 genetic variants (rs1539019 - rs4612666 - rs3806268 - rs35829419 - rs10754558) was associated with a higher risk for faster progression to death. These analyzes of haplotypes were performed considering the most frequent haplotype of the NLRP3 (C-T-G-C–C) gene as references.

It is worth noting that carrying the NLRP3 haplotype A-T-G-A-G was associated with an expressive faster progression of participants to MVS and death. No haplotypes of the CARD8 gene were associated with progression to the analyzed outcomes.

Assuming the observed rates of death in our cohort of approximately 61 %, and the minor allele frequencies among controls, i.e., those who were discharged after COVID-19, of 0.02 to 0.4, for the n-sample of 451 patients and significance level (alpha of 0.05) we obtained statistical powers (1 - beta) greater than 97 % for the Cox proportional hazard analyses (data not shown).

4. Discussion

COVID-19 severity and death have been mainly associated with older ages, male sex, and the presence of comorbidities, such as obesity, smoking, type-2 diabetes mellitus, and others (Ferreira et al., 2001; Sakr et al., 2008). Host factors are key to determining disease severity and progression in a disease as heterogeneous as COVID-19 (Wiersinga et al., 2020). In the present study, we showed that carrying the allele C in selected NLRP3 inflammasome SNPs (rs10754558 or rs4612666 variants) was associated with faster progression to death among in-hospitalized COVID-19 individuals. Moreover, it was also highlighted in our study that carrying the NLRP3 haplotype A-T-G-A-G was associated with an expressive risk for a faster progression to disease severity, as measured in the use of MVS and death. The pivotal role of NLRP3 inflammasome activation in the SARS-CoV-2 infection and COVID-19 has already been described (Wu et al., 2020; Wiersinga et al., 2020; López-Reyes et al., 2020). Rodrigues et al (Rodrigues, 2020) showed that the NLRP3 inflammasomes are active in individuals with COVID-19 and that the magnitude of this activation was associated with COVID-19 outcome. Moreover, it is described that NLRP3 inflammasome formation and a consequent hyperreactive immune response is considered the main cause of dysregulated immune response against SARS-COV-2 infection, contributing to the cytokine storm and severity and worse clinical outcome of COVID-19 (Rodrigues, 2020). Thus, potential drugs for blocking NLRP3-mediated inflammation are under development and/or clinical testing (Amin et al., 2022).

We acknowledge that several studies on inflammasome activation and COVID-19 outcomes have been published. However, as of our knowledge, only two studies associated inflammasome genetic polymorphisms and COVID-19 clinical profiles showing a risk effect of the NLRP3 rs10754558 C/G genotype, increasing the risk towards severe COVID-19 and mortality (Maes et al., 2022). Likewise in a recent study published by our group we show that NLRP3 rs1539019 A/A genotype, allele A or carrier A and CARD8 rs2043211 A/T genotype, allele T or carrier T were associated with protection against disease severity (de Sá et al., 2022). Supporting our results, where NLRP3 rs10754558 carrying the C allele was associated with a faster progression to death, Maes et al (Maes et al., 2022) also found a risk effect of the NLRP3 rs10754558 C/G genotype in severe COVID (Maes et al., 2022). However, in our study, the NLRP3 rs10754558 G/G genotype or G allele was associated with a protective effect, increasing the time until both, the use of MVS and death. We hypothesized that the difference in ethnicity predominant between the two studies is contributing to the differences in the results. In our study the individuals are predominantly self-declared brown/biracial individuals, with people from Southeast and North regions, and although both studies include Brazilian individuals, their study is focused on people from the South of Brazil, with the predominance of whites. Besides that, Toro *et al.* (2021) observed that individuals with the C/C genotype were prevalent among the individuals with Hepatitis C Virus and had a 58 % chance higher of developing hepatitis due to the virus (Toro et al., 2021). Studies of viral pathogens corroborate these findings, where the G allele and the G/G genotype of the NLRP3 rs10754558 variant were associated with a protective role against HIV-1 infection susceptibility for example, which corroborates our findings (Pontillo et al., 2012; Amin et al., 2022).

Other inflammasomes genetic variants included in our study, such as NLRP3 rs4612666, CARD8 rs6509365, IFI16 rs1101996, and the IL1β gene rs1143634 variants, were associated with protection or risk for a faster or slower progression to the severe outcomes of use of mechanical ventilation support or death in SARS-CoV-2 infected individuals. Our data suggest that these SNPs might modulate inflammasome activation, contributing to a worse or better prognosis in disease progression to severe outcomes.

Our study found that the carrier C allele of the NLRP3 rs4612666 variant was associated with faster progression to death. Indeed, Ehtesham *et al.* (2021) found that the C allele in this variant of the gene NLRP3 is associated with clinical characteristics and severe disease activity of systemic lupus erythematosus, an inflammatory autoimmune disease (Ehtesham et al., 2021). Also, Hitomi et al. (Hitomi, 2009) found the rs4612666 variant was significantly associated with susceptibility to food-induced anaphylaxis and found that the C allele showed higher transcriptional enhancer activity and mRNA stability. Therefore, we hypothesize, that one of the possible reasons for the C allele of the NLRP3 rs4612666 variant to be associated with faster progression to death, is that this variant could increase NLRP3 mRNA stability and enhance NLRP3 activity, which subsequently led to a series of inflammatory reactions, contributing to a faster progression to this outcome. However, further studies need to be carried out to confirm this hypothesis (Ehtesham et al., 2021; Hitomi, 2009). We also found in this same variant of NLRP3 that T/T genotype or allele T is associated with protection in time to progression to death. However, in the literature, most studies show a negative association of the T/T genotype and allele T of NLRP3 rs4612666 in several diseases (Perri et al., 2021; Zhao et al., 2017; Cheng et al., 2018). We assume that the association with protection in our study is attributed to the geographical/ethnic variation

Table 3
Analysis of alleles and genotypes of COVID-19 individuals from the beginning of symptoms to the progression to death outcome using Cox proportional hazard models or time-to-event analyses.

Genes	SNP	Alleles /Genotype	[a]pY	Crude Incidence/pY (CI95 %)	Death aHR (CI95 %)	[b]*P*-value
NLRP3	rs10754558	C/C	12.56	5.97 (4.7–7.48)	Reference	
		C/G	13.72	5.68 (4.49–7.09)	0.924 (0.616–1.386)	0.703
		G/G	5.04	4.17 (2.58–6.37)	**0.323 (0.147–0.709)**	**0.005**
		C	38.85	5.87 (5.13–6.68)	Reference	
		G	23.81	5.04 (4.18–6.03)	**0.688 (0.453–1.044)**	**0.014**
		No carrier C	5.04	4.17 (2.58–6.37)	Reference	
		Carrier C	26.29	5.82 (4.93–6.82)	**2.957 (1.397–6.26)**	**0.005**
NLRP3	rs4612666	C/C	12.42	6.28 (4.96–7.84)	Reference	
		C/T	13.73	5.32 (4.17–6.68)	0.856 (0.557–1.317)	0.48
		T/T	5.17	4.45 (2.82–6.67)	**0.394 (0.207–0.749)**	**0.004**
		C	38.58	5.94 (5.19–6.76)	Reference	
		T	24.08	4.94 (4.09–5.91)	**0.68 (0.457–1.012)**	**0.006**
		No carrier C	5.17	4.45 (2.82–6.67)	Reference	
		Carrier C	26.15	5.77 (4.89–6.77)	**2.342 (1.279–4.288)**	**0.006**
NLRP3	rs1539019	C/C	12.95	5.48 (4.28–6.92)	Reference	
		A/A	4.46	5.16 (3.27–7.74)	0.643 (0.337–1.223)	0.178
		C/A	13.92	5.75 (4.56–7.15)	0.796 (0.523–1.209)	0.284
		C	39.82	5.58 (4.87–6.36)	Reference	
		A	22.83	5.52 (4.6–6.57)	0.817 (0.554–1.206)	0.231
		Non carrier C	4.46	5.16 (3.27–7.74)	Reference	
		Carrier C	26.87	5.62 (4.76–6.59)	1.381 (0.752–2.535)	0.298
NLRP3	rs3806268	G/G	11.56	5.1 (3.89–6.58)	Reference	
		A/A	4.8	5.63 (3.71–8.19)	1.23 (0.658–2.3)	0.517
		G/A	14.97	5.88 (4.72–7.24)	1.363 (0.894–2.078)	0.15
		G	38.09	5.41 (4.7–6.2)	Reference	
		A	24.57	5.78 (4.87–6.81)	1.155 (0.779–1.711)	0.314
		No carrier G	4.8	5.63 (3.71–8.19)	Reference	
		Carrier G	26.53	5.54 (4.68–6.51)	0.979 (0.555–1.728)	0.941
NLRP3	rs35829419	C/C	29.81	5.6 (4.79–6.52)	Reference	
		A/A	0.03	33.2 (0.84–185)	NC	
		C/A	1.49	4.03 (1.48–8.77)	0.955 (0.382–2.385)	0.922
		C	61.1	5.56 (4.99–6.19)	Reference	
		A	1.55	5.16 (2.23–10.17)	0.956 (0.27–3.393)	0.932
		No carrier C	0.03	33.2 (0.84–185)	Reference	
		Carrier C	31.3	5.53 (4.73–6.42)	NC	
CARD8	rs6509365	A/A	15.43	5.57 (4.46–6.88)	Reference	
		A/G	13.32	5.48 (4.3–6.89)	**0.537 (0.346–0.832)**	**0.005**
		G/G	2.58	5.82 (3.26–9.6)	0.707 (0.333–1.501)	0.367
		A	44.18	5.55 (4.87–6.28)	Reference	
		G	18.47	5.58 (4.55–6.76)	0.74 (0.486–1.127)	0.11
		No carrier G	15.43	5.57 (4.46–6.88)	Reference	
		Carrier G	15.89	5.54 (4.44–6.82)	**0.563 (0.373–0.85)**	**0.006**
CARD8	rs2043211	A/A	17.65	5.67 (4.61–6.89)	Reference	
		A/T	11.82	5.24 (4.02–6.72)	0.759 (0.49–1.177)	0.218
		T/T	1.86	6.46 (3.34–11.29)	0.886 (0.377–2.081)	0.781
		A	47.12	5.56 (4.91–6.28)	Reference	
		T	15.54	5.54 (4.43–6.84)	0.859 (0.541–1.364)	0.315
		No carrier A	1.86	6.46 (3.34–11.29)	Reference	
		Carrier A	29.47	5.5 (4.68–6.41)	1.01 (0.437–2.334)	0.982
AIM2	rs2276405	C/C	30.18	5.57 (4.76–6.47)	Reference	
		C/T	1.14	5.26 (1.93–11.44)	1.171 (0.424–3.235)	0.761
		C	61.51	5.56 (4.99–6.18)	Reference	
		T	1.14	5.26 (1.93–11.44)	1.167 (0.284–4.785)	0.607
		No carrier T	30.18	5.57 (4.76–6.47)	Reference	
		Carrier T	1.14	5.26 (1.93–11.44)	1.171 (0.424–3.235)	0.761
CASP1	rs572687	G/G	21.23	5.79 (4.82–6.91)	Reference	
		A/A	0.94	6.37 (2.34–13.87)	1.069 (0.367–3.112)	0.903
		G/A	9.16	4.92 (3.59–6.58)	0.865 (0.549–1.362)	0.531
		G	51.61	5.64 (5.01–6.32)	Reference	
		A	11.04	5.16 (3.91–6.69)	0.925 (0.547–1.564)	0.666
		No carrier G	0.94	6.37 (2.34–13.87)	Reference	
		Carrier G	30.38	5.53 (4.72–6.43)	0.908 (0.312–2.643)	0.859
IFI16	rs1101996	C/C	13.51	6 (4.76–7.45)	Reference	
		A/A	3.74	6.15 (3.9–9.22)	1.136 (0.65–1.986)	0.655
		A/C	14.08	4.97 (3.88–6.28)	**0.569 (0.369–0.88)**	**0.011**
		C	41.09	5.65 (4.94–6.42)	Reference	
		A	21.56	5.38 (4.45–6.45)	0.911 (0.616–1.347)	0.59
		No carrier C	3.74	6.15 (3.9–9.22)	Reference	
		Carrier C	27.58	5.47 (4.64–6.42)	0.684 (0.402–1.162)	0.16

(*continued on next page*)

Table 3 (*continued*)

Genes	SNP	Alleles /Genotype	[a]pY	Crude Incidence/pY (CI95 %)	Death aHR (CI95 %)	[b]*P*-value
IL1β	rs1143634	G/G	20.27	5.18 (4.24–6.27)	Reference	
		A/A	0.74	6.74 (2.19–15.73)	2.055 (0.818–5.166)	0.126
		G/A	10.23	6.16 (4.73–7.88)	1.073 (0.704–1.636)	0.743
		No carrier G	0.84	7.19 (2.64–15.64)	Reference	
		Carrier G	30.49	5.51 (4.71–6.41)	0.5 (0.217–1.151)	0.103
		No carrier A	20.36	5.21 (4.26–6.3)	Reference	
		Carrier A	10.97	6.2 (4.81–7.86)	1.135 (0.759–1.698)	0.538

[a] pY:person-years; [b]P-value were calculated using the Cox's proportional risk model. Associations were considered significant with a value of $P < 0.05$. N: number of individuals in each group; aHR: adjusted Hazard ratio; CI95 %: 95 % confidence interval; N: number of individuals in each group; aHR: were adjusted by age, diabetes mellitus, COPD, active cancer, current smoking, transplant, Glasgow Scale, SOFA, and SAPS3. 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 T allele; Carrier-C = total of genotypes with the C allele; Carrier-G = total of genotypes with the G allele; No carrier-A = total of genotypes without the A allele; No carrier-T = total of genotypes without the T allele; No carrier-C = total of genotypes without the C allele; No carrier-G = total of genotypes without the G allele.

Table 4
Analyses among NLRP3 and CARD8 inflammasome haplotypes frequencies in Cox proportional hazard models for time to progression of death due to COVID-19.

Genes	Haplotypes	[a]pY	Crude Incidence/pY (IC95 %)	Death aHR (CI95 %)	[b]*P*-value
NLRP3	CTGCC	16.61	5.3 (4.25–6.53)	Reference	
rs1539019	**ACACC**	2.98	7.71 (4.89–11.58)	**2.344 (1.282–4.285)**	**0.002**
rs4612666	ACACG	11.59	5.52 (4.25–7.05)	0.827 (0.516–1.325)	0.458
rs3806268	**ACGCC**	2.21	6.33 (3.46–10.62)	**2.174 (0.918–5.152)**	**0.042**
rs35829419	ACGCG	2.45	4.49 (2.24–8.04)	0.92 (0.351–2.413)	0.775
rs10754558	**ATGAG**	0.04	28.1 (0.71–156.54)	**34.611 (4.502–266.058)**	**<0.001**
	ATGCC	2.16	4.62 (2.22–8.5)	0.424 (0.186–0.966)	0.234
	ATGCG	0.85	3.55 (0.73–10.36)	0.394 (0.091–1.714)	0.122
	CCACC	7.06	5.95 (4.29–8.04)	**2.026 (1.215–3.378)**	**0.002**
	CCACG	2.37	4.22 (2.02–7.76)	1.036 (0.419–2.564)	0.926
	CCGAC	0.03	33.2 (0.84–185)	NC	
	CCGCC	6.77	6.79 (4.97–9.06)	**2.074 (1.289–3.336)**	**<0.001**
	CCGCG	2.81	6.41 (3.8–10.14)	0.895 (0.341–2.347)	0.802
	CTACC	0.37	8.06 (1.66–23.55)	**2.068 (0.616–6.942)**	**0.012**
	CTACG	0.05	NC	NC	
	CTGAC	0.09	11.41 (0.29–63.6)	NC	
	CTGAG	0.88	5.71 (1.85–13.32)	1.562 (0.537–4.543)	0.505
	CTGCG	2.22	3.6 (1.56–7.1)	0.851 (0.321–2.252)	0.708
CARD8	AA	44.11	5.55 (4.88–6.3)	Reference	
rs2043211	AG	3.01	5.66 (3.29–9.05)	0.521 (0.286–0.948)	0.225
rs6509365	TA	0.07	NC	NC	NC
	TG	15.46	5.56 (4.45–6.87)	0.827 (0.595–1.147)	0.213

[a] pY:person-years; [b]P-value were calculated using the Cox's proportional risk model. Associations were considered significant with a value of $P < 0.05$. aHazard ratio: adjusted Hazard ratio. CI95 %: 95 % confidence interval; aHR: were adjusted by age, diabetes mellitus, COPD, active cancer, current smoking, transplant, Glasgow Scale, SOFA, and SAPS3; NC: not calculated.

between our study and all others. Most existing studies with this NLRP3 variant are in Chinese populations. Further validation is needed to clarify this question.

The A/G genotype or carrier G of CARD8 rs6509365 was associated with slower progression to death in our study. Da Silva *et al.* (2019) also showed that A/G genotype and carrier allele G of this variant in the gene CARD8 were significantly more common in healthy volunteers than in sporadic melanoma malignancy patients, suggesting a protective effect of this variant on melanoma development (da Silva et al., 2016). Furthermore, it has already been shown that rs6509365 A > G could reduce CARD8 gene expression (Ko et al., 2009). So, we can hypothesize that genotype of rs6509365 could represent a protective factor in the progression to death, due to the dual role exerted by CARD8 in the NLRP3 inflammasome apoptosis suppression (Pathan et al., 2001).

IFI16 gene is a key DNA sensor that triggers downstream type I interferon (IFN-I) production and antiviral immunity (Li et al., 2019) IFI16 rs1101996 is an intron polymorphism, and after extensive research on associations of this polymorphism with other diseases, we found no data. Therefore, for the first time, we report the association of the A/C genotype of the IFI16 rs1101996 variant with a slow progression to death in individuals with COVID-19.

IL-1β is a potent proinflammatory cytokine crucial for host-defense responses to infection (Lopez-Castejon and Brough, 2011). Our study found that the carrier G allele of the IL1β rs1143634 polymorphism is associated with a slower progression to MVS. Song et al. (Song, 2021) found that the genotype G/A is associated with a decreased risk of gastric cancer and may be a protective factor (Song, 2021). Also, Kotsa *et al.* (2021) found that the genotype G/A predisposes men to lower total fat mass and body mass index (BMI) (Mikhailova and Ivanoshchuk, 2021). Although we did not observe any association in our study with progression to the outcomes with obesity, it is already widely known that obese patients can have worse outcomes with COVID-19 infection, including respiratory failure, needs for mechanical ventilation, and higher mortality (Mikhailova and Ivanoshchuk, 2021; Sanchis-Gomar et al., 2020; Zhou et al., 2019). Based on this we hypothesize that the fact the G/A genotype of the IL1β rs1143634 polymorphism is associated with non-obese individuals may be linked with our finding that carrying the G allele is associated with slower progression to MVS outcome in individuals with COVID-19.

5. Conclusion

The present study reports that genetic polymorphisms of inflammasomes are associated with the progression to the use of MVS or death. Thus, we show that NLRP3 rs10754558 and rs4612666 variants, the CARD8 rs6509365 variant, the IFI16 rs1101996 variant, and the IL1β

rs1143634 variant were associated with a slower/faster progression to the use of MVS or death outcomes. The haplotypes of the NLRP3 gene variants included in this study was also associated with the progression to MVS or death events.

6. Funding

The study was supported by Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro - FAPERJ (Grant number SEI-260003/002689/2020; SEI- 260003/013002/2021 and SEI-260003/019710/2022) and INOVA FIOCRUZ/Fundação Oswaldo Cruz (Grant number 48401996705881). Hugo Perazzo is recipient of FAPERJ (E-26/201.351/2021), Mariza Gonçalves Morgado is recipient of CNPQ (314064/2018-4) and FAPERJ (E-26/201.177/2021), and Beatriz Grinsztejn is recipient of CNPQ (305789/2019-8) and FAPERJ (E-26/202.915/2018). The funding agencies played no role in the design of the study, data collection, analysis, or interpretation, nor in writing the manuscript.

7. Institutional Review Board Statement

The study protocol was approved by the Ethics Committee of the National Institute of Infectology Evandro Chagas (INI)/Oswaldo Cruz Foundation (FIOCRUZ), Rio de Janeiro, Brazil, under approval number CAAE: 32449420.4.1001.5262. All procedures were performed in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national).

8. Informed Consent Statement

All patients in the study were aware of and agreed to participate in the research and signed an informed consent form.

9. Data Availability Statement

The data that support the findings of this study are available upon request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

CRediT authorship contribution statement

Milena Neira-Goulart: Conceptualization, Methodology, Validation, Investigation, Data curation, Writing – original draft. **Nathalia Beatriz Ramos de Sá:** Conceptualization, Methodology, Validation, Investigation, Data curation, Writing – original draft, Supervision, Project administration, Funding acquisition. **Marcelo Ribeiro-Alves:** Methodology, Software, Formal analysis, Data curation, Writing – review & editing. **Hugo Perazzo:** Investigation, Data curation, Writing – review & editing. **Kim Mattos Geraldo:** Investigation, Data curation, Writing – review & editing. **Maria Pia Diniz Ribeiro:** Investigation, Data curation, Writing – review & editing. **Sandra Wagner Cardoso:** Investigation, Data curation, Writing – review & editing. **Beatriz Grinsztejn:** Investigation, Data curation, Writing – review & editing, Funding acquisition. **Valdiléa G. Veloso:** Investigation, Data curation, Writing – review & editing, Funding acquisition. **Larissa Rodrigues Gomes:** Investigation, Methodology, Writing – review & editing, Funding acquisition. **Andressa da Silva Cazote:** Investigation, Methodology, Writing – review & editing, Funding acquisition. **Dalziza Victalina de Almeida:** Investigation, Methodology, Writing – review & editing, Funding acquisition. **Carmem Beatriz Wagner Giacoia-Gripp:** Investigation, Methodology, Writing – review & editing, Funding acquisition. **Fernanda Heloise Côrtes:** Investigation, Methodology, Writing – review & editing, Funding acquisition. **Mariza Gonçalves Morgado:** Conceptualization, Methodology, Validation, Data curation, Writing – original draft, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgments

The authors are thankful to all patients who agreed to participate in this study and their families, the frontline health care workers of the INI/Fiocruz Hospital, and the RECOVER study team in Rio de Janeiro.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.gene.2023.147325.

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