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Original Article

Implication of myddosome complex genetic variants in outcome severity of COVID-19 patients

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KEYWORDS
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Abstract *Background/purpose(s):* During a viral infection, the immune response is mediated by the toll-like receptors and myeloid differentiation Factor 88 (*MyD88*) that play an important role sensing infections such as SARS-CoV-2 which has claimed the lives of more than 6.8 million people around the world.
Methods: We carried out a cross-sectional with a population of 618 SARS-CoV-2-positive unvaccinated subjects and further classified based on severity: 22% were mild, 34% were severe, 26% were critical, and 18% were deceased. *Toll Like Receptor 7* (*TLR7*) single-nucleotide polymorphisms (rs3853839, rs179008, rs179009, and rs2302267) and *MyD88* (rs7744) were genotyped using TaqMan OpenArray. The association of polymorphisms with disease outcomes was performed by logistic regression analysis adjusted by covariates.
Results: A significant association of rs3853839 and rs7744 of the *TLR7* and *MyD88* genes, respectively, was found with COVID-19 severity. The G/G genotype of the rs3853839 *TLR7* was associated with the critical outcome showing an Odd Ratio = 1.98 (95% IC = 1.04−3.77). The results highlighted an association of the G allele of *MyD88* gene with severe, critical and deceased outcomes. Furthermore, in the dominant model (AG + GG *vs*. AA), we observed an Odd Ratio = 1.70 (95% CI = 1.02−2.86) with severe, Odd Ratio = 1.82 (95% CI = 1.04−3.21) with critical, and Odd Ratio = 2.44 (95% CI = 1.21−4.9) with deceased outcomes.
Conclusion: To our knowledge this work represents an innovative report that highlights the significant association of *TLR7* and *MyD88* gene polymorphisms with COVID-19 outcomes and the possible implication of the *MyD88* variant with D-dimer and IFN-α concentrations.

Introduction

During activation of the innate and adaptive immune responses, the participation of toll-like receptors (TLRs) is crucial in recognizing pathogen-associated molecular patterns (PAMPs).[1] TLRs 3, 7, and 8 are endolysosomal receptors that recognize double-stranded RNA and single-stranded RNA (TLR 7 and 8).[2] However, recognition of PAMPs by the TLR 7 and 8 is not sufficient to trigger an antiviral response since myeloid differentiation Factor 88 (MyD88) is required as an adaptor molecule to form the Myddosome complex, which initiates the signalling that leads to production of inflammatory cytokines such as TNF-α, IL-6, and Interleukin-1 (IL-1) family as well as type I IFNs like IFN-α.[3,4] Activation of MyD88 mediated by TLR7 and TLR8 is responsible for sensing infections by single-stranded RNA viruses such as HIV, influenza viruses, HCV, Sendai virus, and CBVs, among others.[5]

Considering that the SARS-CoV-2 virus has infected more than 761 million individuals and caused the death of 6.8 million who have developed COVID-19, the scientific community has devoted vast resources to examining the immunopathogenesis of the virus and therapeutic targets. *TLR7* is in the X-chromosome, and expressed on monocyte-macrophages and dendritic cells. Genetic variants of *TLR7* are associated with COVID-19 progression and patient outcomes, suggesting a role for *TLR7* in its pathogenesis.[6] Fallerini et al. reported *TLR7* loss-of-function variants that contribute to disease susceptibility in young males.[7] In addition, in four young males without a history of chronic disease, loss-of-function *TLR7* was found and associated with impaired type I and II IFN responses.[8] In COVID-19 patients, *TLR7* deficiency was reported in at least 1% of men under 60 years of age by Asano et al.[9]

A single nucleotide polymorphism (SNP) of the *MyD88* gene in the 3′ untranslated region (3′UTR) has been reported to be associated with diverse pathologies, such as Buerger's disease,[10] higher death risk at 90 days of septic shock,[11] and cardiovascular artery disease.[12] Some reports suggest the participation of *MyD88* in COVID-19.[13,14] Nevertheless, the efficacy of the antiviral response depends on both the molecular diversity of the pathogen and functional versatility and genetic variability of the myddosome.[15] In this context, we investigated the association of *TLR7* and *MyD88* gene variants with COVID-19 outcomes.

Methods

Setting and participants

We carried out a cross-sectional study. From June 2020–March 2021, unvaccinated patients during the first wave of SARS-CoV-2 infection, were recruited from the following hospitals of the Mexican Governmental Health System: Instituto Nacional de Rehabilitación "Luis Guillermo Ibarra Ibarra", Instituto Nacional de Cardiología "Ignacio Chávez", Hospital Central Militar, Instituto Nacional de Ciencias Médicas y Nutrición "Salvador Zubirán", Hospital General "Dr. Manuel Gea González", Hospital General ISSSTE "Tláhuac", and Hospital Central Norte Pemex.

Inclusion criteria were not familiar related, independent of gender, age $\geq$18 years, unvaccinated, and nonpregnant women with clinical manifestations of COVID-19 and positive qRT–PCR test. The exclusion criteria were incomplete clinical history. These individuals were classified according to previously described[16] according to Gandhi criteria as: mild, those ambulatory subjects with symptoms such as fever, headache, fatigue, odynophagia, cough, rhinorrhea, diarrhea, anosmia or dysgeusia, with or without dyspnea or pneumonia, not requiring hospitalization; severe, those hospitalized individuals with any of the following symptoms: tachypnea (FR $>$ 30 bpm), dyspnea for small efforts; and critical, those patients requiring invasive mechanical ventilation who could course with shock and multiorgan failure[17] The bioethics and research committees of the participating institutions approved this study. Written informed consent was obtained from each participant.

Blood samples

Blood samples were collected for DNA extraction and serum was obtained by centrifugation. Serum samples were stored immediately at -80 °C until further use.

SNPs selection and genotyping

Genomic DNA was isolated from peripheral blood white cells using a commercial kit column-based method (QIAmp 250 DNA Blood Mini Kit, Qiagen, Hilden, Germany). Genomic DNA samples at 10 ng/uL were deposited into genotyping OpenArray plates previously loaded with the genotyping primers and probes using the AccuFill System (Thermo Fisher Scientific). Real-time PCR amplification was carried out following the manufacturer's protocol using OpenArray technology in a QuantStudio 12 K flex System (Thermo Fisher Scientific). The results were analyzed using TaqMan Genotyper v1.6 software.

Statistical methods

The normality of the variable distribution was evaluated. For continuous variables, the Kruskal–Wallis test was used to compare nonparametric distributions among the studied groups, and the results were described using the median and interquartile range (IQR). The chi-squared test was performed for categorical variables. For all tests, a value of $p < 0.05$ was considered statistically significant. Hardy-Weinberg Equilibrium (HWE) was assessed for all polymorphisms in the mild group. For the *TLR7* polymorphisms the HWE was estimated in women of the mild group. Linkage disequilibrium (LD) among *TLR7* gene variants was assessed using HaploView software V4.2. A logistic regression analysis was used to evaluate the association between genetic variants and outcomes of COVID-19, adjusted by age, stratified by $<$ 60 years and $\geq$60 years old, sex, hypertension, type 2 diabetes, and obesity. The final models

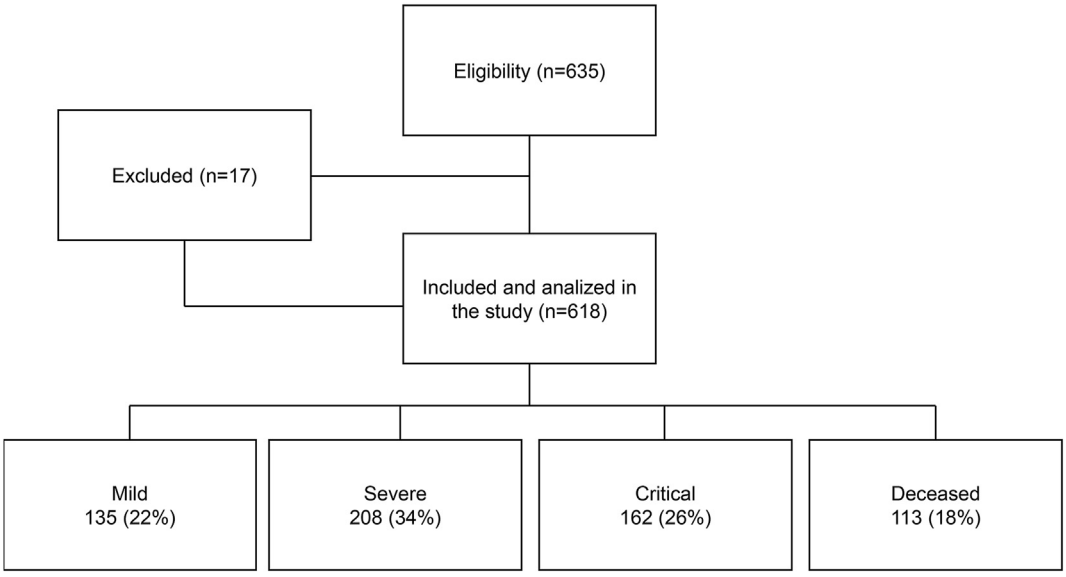

Figure 1. Flow-chart of participants in the study.

were evaluated using the Hosmer—Lemeshow goodness-of-fit test. The correction for multiple comparisons was 0.05/5(SNPs) = 0.001, which was considered statistically significant. The correlation between SNPs and clinical features was assessed by comparing their distribution among alleles and genotypes by the Kruskal—Wallis test and stratified by disease outcome. The analysis was performed using the STATA v.13 statistical package (StataCorp Texas, USA).

Results

Patients

In this study, 618 COVID-19 patients were enrolled and classified based on disease severity. Of these, 22% were mild, 34% were severe, 26% were critical, and 18% were deceased (Fig. 1).

The median age of patients was 52 years; however, in the mild group, the median age was 41 years, while the median age of the deceased group was 63 years. In this context, we found that 63% of the study population were males. Additionally, we showed the comorbidities and clinical symptoms in Table 1.

Clinical laboratory parameters are shown in Fig. 2. We observed an increasing trend of ferritin and Lactate Dehydrogenase (LDH) as the COVID-19 outcome severity increased. The median ferritin level in the mild group was 138.5 (ng/mL) (Interquartile Range (IQR) = 27.2—312.6) and 694.3 (ng/mL) in the deceased group (IQR = 398.05—1286.3). The median LDH was 152.5 (IQR = 124.5—199) in the mild group and 407 (U/L) (IQR = 322—488.4) in the deceased group. We observed

Table 1 Clinical and anthropometric characteristics of the study population.

	Total n = 618	Mild n = 135	Severe n = 208	Critical n = 162	Deceased n = 113	P value
Age[a]	52 (43—63)	41 (31—49)	53 (43—64)	52 (46—63)	63 (54—70)	<0.001
Gender						
Male	392 (63%)	67 (49%)	135 (65%)	116 (72%)	74 (65%)	0.001
Obesity	195 (31%)	20 (15%)	70 (34%)	67 (41%)	38 (34%)	<0.001
Type 2 Diabetes	191 (31%)	13 (10%)	75 (37%)	54 (33%)	49 (43%)	<0.001
Hypertension, n (%)	189 (31%)	14 (10%)	65 (32%)	61 (37%)	49 (43%)	<0.001
Heart rate, median (IQR), bpm^{+}	93 (81—105)	89 (78—100)	93 (80—105)	96 (87.5—110)	92 (81—104)	0.13
Oxygen saturation % (IQR)	87 (79—93)	94 (92—96)	87 (80—92)	83 (72—88)	81 (70—89)	<0.001
Fever, n (%)	274 (45%)	46 (34%)	101 (49%)	85 (52%)	42 (37%)	0.003
Cough, n (%)	429 (70%)	78 (58%)	145 (71%)	129 (80%)	77 (69%)	0.001
Dyspnoea, n (%)	379 (62%)	29 (21%)	150 (74%)	125 (77%)	75 (67%)	<0.001
Headache, n (%)	341 (56%)	80 (59%)	105 (52%)	109 (68%)	47 (42%)	<0.001
Odynophagia, n (%)	252 (41%)	59 (44%)	80 (39%)	75 (46%)	38 (34%)	0.19
Myalgia, n (%)	336 (55%)	68 (50%)	111 (55%)	105 (65%)	52 (46%)	0.013
Vomiting, n (%)	45 (7%)	8 (6%)	10 (5%)	18 (11%)	9 (8%)	0.13

[a] Kruskal—Wallis test. Chi square test.
IQR= Interquartile Range.

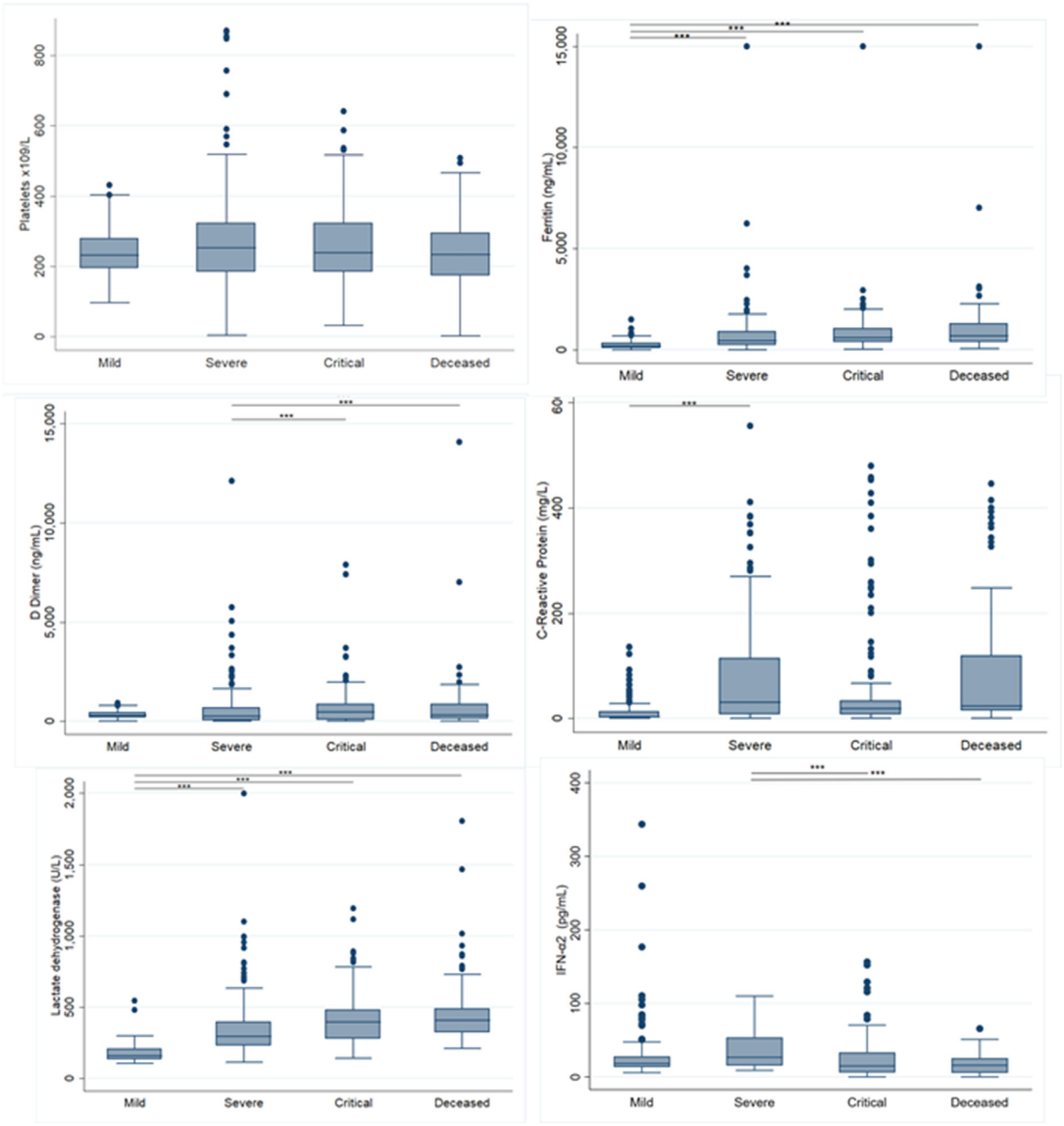

Figure 2. Laboratory values of the population study. (a) Platelets $\times 10^9$/L. (b) Serum ferritin concentrations (ng/ml). (c) D-dimer (ng/mL). (d) C-reactive protein (CRP) (mg/L) (e) Lactate dehydrogenase (LDH) (U/L).

a decreased level in IFN-α in mild outcome with 18.14(IQR = 13.5–27.9) versus deceased group 15.9(IQR = 5.9–26.7).

Allelic, genotypes and linkage disequilibrium

Table 2 shows the allelic and genotypic distribution of five SNPs on the *TLR7* and *MyD88* genes. We found statistically significant differences in the frequencies of SNPs in the *TLR7* gene, all SNPs were in HWE. We observed a strong LD between rs179008 and rs179009 variants, showing D′0.98 (Fig. 3).

Correlation of clinical biomarkers with polymorphisms of the *TLR7* and *MyD88* genes

In a subsequent analysis with complete clinical data, we explored the distribution of the genotypes of the *TLR7* and *MyD88* genes. In this sense, we observed significant differences in ferritin (ng/mL), C reactive protein (mg/L) and LDH levels among the genotypes of the rs3853839, rs179008, and rs179009 variants of the *TLR7* gene; nevertheless, only ferritin and LDH showed significant differences for the rs2302267 variant (Table 3). Regarding rs7744 of the *MyD88* gene, significant differences were observed only in D-dimer

Table 2 Allelic and genotype *TLR7* and *MyD88* gene frequencies in the study population.

	Frequencies (%)					P[a]	HWE[b]
	Total (n = 618)	Mild (n = 135)	Severe (n = 208)	Critical (N = 162)	Deceased (n = 113)		
TLR7							
rs179008							
A	965 (78%)	199 (74%)	328 (79%)	254 (78%)	184 (81%)	0.20	
T	271 (22%)	71 (26%)	88 (21%)	70 (22%)	42 (19%)		
AA	451 (73%)	89 (66%)	156 (75%)	117 (72%)	89 (78%)	0.10	**0.07**[c]
AT	63 (10%)	21 (15%)	16 (8%)	20 (12%)	6 (5%)		
TT	104 (17%)	25 (18%)	36 (17%)	25 (15%)	18 (16%)		
rs179009							
A	743 (60%)	148 (55%)	253 (61%)	205 (63%)	137 (61%)	0.20	
G	493 (40%)	122 (45%)	163 (39%)	119 (37%)	89 (39%)		
AA	319 (52%)	56 (41%)	109 (52%)	92 (57%)	62 (55%)	**0.02**	**0.6**[c]
AG	105 (17%)	36 (27%)	35 (17%)	21 (13%)	13 (11%)		
GG	194 (31%)	43 (32%)	64 (31%)	49 (30%)	38 (34%)		
rs3853839							
C	793 (65%)	158 (59%)	265 (64%)	230 (71%)	140 (63%)	**0.01**	
G	443 (35%)	112 (41%)	147 (36%)	92 (29%)	84 (37%)		
CC	344 (56%)	62 (46%)	118 (57%)	105 (65%)	59 (53%)	**0.02**	**1**[c]
CG	105 (17%)	34 (25%)	29 (14%)	20 (12%)	22 (20%)		
GG	169 (27%)	39 (29%)	61 (29%)	37 (22%)	32 (28%)		
rs2302267							
T	1085 (88%)	241 (89%)	364 (87%)	281 (87%)	199 (88%)	0.87	
G	151 (12%)	29 (11%)	52 (13%)	43 (13%)	27 (12%)		
TT	521 (84%)	115 (85%)	174 (84%)	136 (85%)	96 (85%)	0.89	**0.17**[c]
TG	43 (7%)	11 (8%)	16 (8%)	9 (6%)	7 (6%)		
GG	54 (9%)	9 (7%)	18 (9%)	17 (10%)	10 (9%)		
MyD88							
rs7744							
A	954 (77%)	224 (83%)	315 (76%)	245 (76%)	170 (75%)	0.08	
G	282 (23%)	46 (17%)	101 (24%)	79 (24%)	56 (25%)		
A/A	370 (60%)	92 (68%)	120 (58%)	93 (57%)	65 (57%)		0.64
A/G	214 (35%)	40 (30%)	75 (36%)	59 (36%)	40 (35%)	0.34	
G/G	34 (5%)	3 (2%)	13 (6%)	10 (6%)	8 (7%)		

[a] Chi square test.
[b] HWE (Hardy Weinberg Equilibrium).
[c] HWE in women. Text in bold denotes statistical significance.

(ng/mL) (P = 0.03), with increasing levels among genotypes: for AA, a median of 281.3 (IQR 74.5—665.5); for AG, a median of 291 (ng/mL) (IQR = 69.5—683.5); and for GG, a median of 547.75 (ng/mL) (IQR = 260—845).

We performed the effect of *TLR7* alleles separately in males and females, we observed for the MAF allele of rs3853839 significant differences between C Reactive Protein (P = 0.009) observing a decreased level in the Minor Allele Frequency (MAF) with a median of 19.9 mg/L (IQR 6.01—79.7) and LDH (P = 0.01) with increasing levels in the MAF, median of 349 U/L (IQR 252—470) in men group. In this sense, we found significant differences in D-dimer (ng/mL) between women group (P = 0.01).

Logistic regression analysis

In the logistic regression analysis adjusted by age, sex, hypertension, type 2 diabetes, and obesity, we found a statistically significant association of rs7744 (1244 A > G) of the *MyD88* gene with outcome severity. Additionally, we observed an increase in the magnitude of the association according to COVID-19 outcome progression. It was shown a statistically significant association of the A/G genotype with an OR = 8.83 (95% CI = 1.82—42.23; P = 0.007) with fatal COVID-19 outcome. For the dominant model (AG + GG vs. AA), we observed an OR = 2.44 (95% CI = 1.21—4.9; P = 0.01) with deceased outcome. For the recessive model

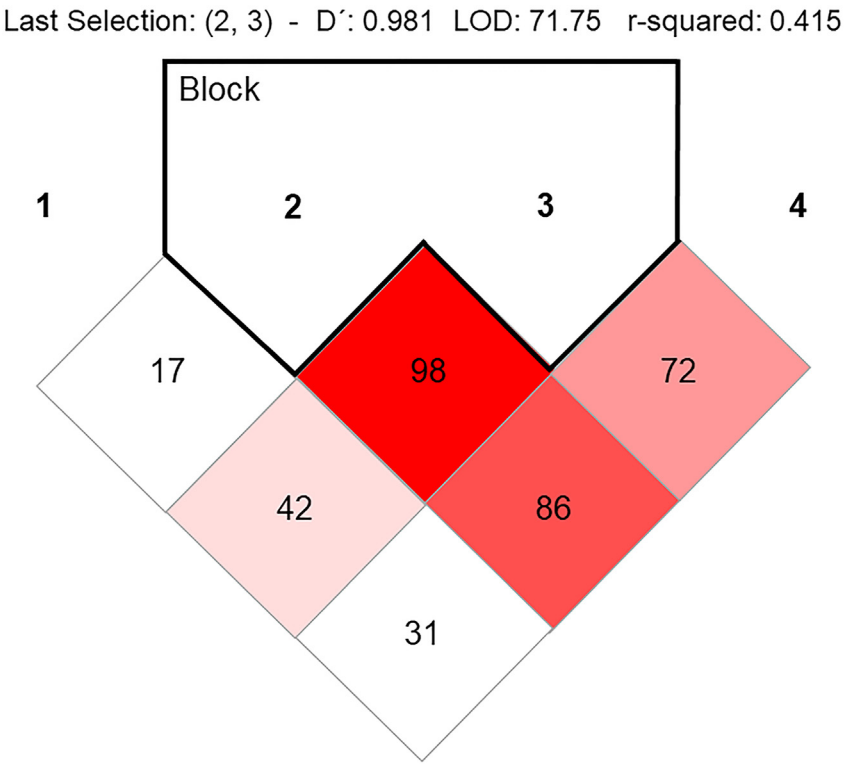

Figure 3. Linkage disequilibrium of TLR7 variants.

we found and OR = 6.75 (95% CI = 1.45–31.33; P = 0.01) with deceased outcome (Table 4).

Interestingly, for rs3853839 of the *TLR7* gene for the GG genotype under the codominant model we found an OR = 1.98 (95% CI = 1.04–3.77) with critical outcome, while for the recessive model (GG), we observed an OR = 1.91 (95% CI = 1.08–3.39) with critical outcome. In the log additive model, the OR was 1.42 (95% CI = 1.03–1.96) (Table 4). However, with the correction for multiple comparisons the allele G was associated with critical outcome (OR = 1.83; 95% IC = 1.21–2.75; P = 0.004). In a special analysis restricted only to young men (<60 years) without hypertension, type 2 diabetes, and obesity, the results showed an OR = 4.3 (95% CI = 1.11–16.52; P = 0.002) with critical outcome.

For the X-linked inheritance of the *TLR7* variants we performed a logistic regression for alleles stratified by gender and we found for G allele in the rs3853839 an OR = 2.49 (95% CI = 1.43–4.32; P = 0.001) with critical outcome in men (Table 5).

Discussion

Since COVID-19 pandemic emerged, multiple studies of susceptibility have been published showing different epidemiologic risks, such as non-communicable diseases.[18,19] In the present study, we found obesity, type 2 diabetes, and hypertension to be the most common comorbidities with a 31% frequency. In Mexico, the prevalence of obesity has been reported to be 36%, for type 2 diabetes 15.7%, and 30.2% for hypertension.[20] According to age and sex, Martínez-Martínez et al. reported a median age of 43.6 ± 17.07 years and an increased incidence of severe COVID-19 in men.[21] De la Cruz-Cano et al. reported a mean age of 59.62 years with a frequency of 60.52% for males.[22] In our study, the median age was 52 years (IQR, 43–63), and men accounted for a higher proportion of COVID-19 cases (63%).[18]

The main symptoms in our study population were cough (70%), dyspnoea (62%), and headache (56%); however, in other reports, the three main symptoms were headache (50%), arthralgia or myalgia (38%), and sore throat (36%).[18]

The susceptibility to COVID-19 has been described in multiple studies.[18,19] However, the genetic and molecular mechanisms are unclear. Previous studies on host genetics[23–25] have reported some *loci* that could affect the loss-of-function of immune molecules implicated in the response to infectious diseases. Reports have suggested that host genome variations play a role in COVID-19 outcomes.[26,27] In this sense, *TLR7* and *MyD88* gene variants could influence the susceptibility to infectious diseases.[28]

In our study, gene frequencies are similar to those reported in other populations. We found statistically significant differences in frequencies of *TLR7* gene SNPs, located on the X chromosome. The allele frequencies of rs3853839, located in the 3′UTR, were similar to the reported in HapMap for Mexican population[29]. This work represents the first report proposing the rs3853839 to increase the risk of developing severe outcomes in COVID-19.

Recently, rare variants of the *TLR7* gene were associated with COVID-19.[7,9,30,31] In this sense, Fallerini et al. (2021) identified these variants associated with COVID-19, especially in young males (<60 years old) hospitalized with supplemental oxygen (CPAP/BiPAP and intubated). This suggests that variants in the *TLR7* gene are responsible for

Table 3 Genotypes of *TLR7* and *MyD88* variant genes with clinical characteristics.

TLR7				
rs3853839	CC	CG	GG	P[a]
Oxygen saturation %	88 (80–93)	89 (82–95)	86.5 (78–93)	0.09
Platelets $\times 10^9$/L	230 (167–295)	260 (204–321)	240 (186–308)	0.18
Ferritin (ng/mL)	**493.7(259.8–904.3)**	**230.6(96.6–489.3)**	**511.5(257–987.9)**	**<0.001**
D Dimer (ng/mL)	278.5 (61.4–690)	348.5 (120–677)	319 (90.3–754)	0.76
C-Reactive Protein (mg/L)	**20.05(6.8–90)**	**13.03(3.1–31.73)**	**19.67(6.11–61.73)**	**0.04**
DHL (U/L)	**279.5(202–436.5)**	**251.9(159.2–354)**	**327.1(228–453)**	**<0.001**
IFNα (pg/mL)	17.9 (9.7–34.3)	15.5 (9.6–23.6)	18.2 (11.4–40.9)	0.21
rs179008	AA	AG	GG	
Oxygen saturation %	87 (78–93)	90 (83–94)	89 (80–94)	0.15
Platelets $\times 10^9$/L	238.5 (185–310)	250 (193–289)	231 (164–300)	0.41
Ferritin (ng/mL)	**463(219–888.3)**	**159.9(42.1–503.2)**	**529.1(259.8–1000)**	**<0.001**
D Dimer (ng/mL)	313 (74.7–672.4)	263 (77–616)	294.35 (84–828)	0.62
C-Reactive Protein (mg/L)	**18.98(5.30–66.96)**	**10.2(3.53–24.81)**	**20.83(5.5–123.49)**	**0.01**
DHL (U/L)	**309.5(214–448)**	**233.95(147–323)**	**313.9(221.5–442)**	**0.001**
IFNα (pg/mL)	18.12 (10.8–34.7)	19.8 (9.8–31.8)	16.05 (9.8–26.1)	0.66
rs179009	AA	AG	GG	
Oxygen saturation %	87 (77–92)	90 (82–94)	88 (79–93)	0.09
Platelets $\times 10^9$/L	237 (184–305)	252 (198–320)	226 (174–300)	0.13
Ferritin (ng/mL)	**486.5(237–894.1)**	**170.35(46–433.9)**	**580.3(259.8–1021.6)**	**<0.001**
D Dimer (ng/mL)	297 (73–627)	286 (77–666)	322.7 (87–793.43)	0.46
C-Reactive Protein (mg/L)	**18.03(5.39–62.32)**	**11.05(2.6–28.42)**	**22.35(7.1–90)**	**0.006**
DHL (U/L)	**308(217–443.7)**	**219.5(154–323)**	**334.2(238–456.5)**	**<0.001**
IFNα (pg/mL)	17.6 (10.8–34.3)	20.4 (12.3–31.8)	17.6 (10.4–33.8)	0.6
rs2302267	TT	TG	GG	
Oxygen saturation %	87 (79–93)	90.5 (83–95)	85 (71–93)	0.05
Platelets $\times 10^9$/L	235 (180–307)	250 (204–336)	230 (185–295)	0.30
Ferritin (ng/mL)	**457.3(205.1–885.9)**	**212.25(61–390.6)**	**584.4(387.7–1042.1)**	**<0.001**
D Dimer (ng/mL)	325.5 (83.15–751)	260 (77–564)	162.05 (44.1–465)	0.07
C-Reactive Protein (mg/L)	18.34 (5.3–62.9)	9.7 (5–71.55)	20.24 (5.72–163.72)	0.32
DHL (U/L)	**306.30(211–438)**	**209.15(158.5–269.7)**	**360.75(250–480)**	**<0.001**
IFNα (pg/mL)	18.2 (10.7–33.8)	14.3 (9.9–19.7)	17.6 (11.9–50.4)	0.31
MyD88				
rs7744	AA	AG	GG	
Oxygen saturation %	88 (80–93)	87 (77–93)	83 (71–92)	0.09
Platelets $\times 10^9$/L	237 (178–303)	233 (184–304.5)	271 (204–331)	0.39
Ferritin (ng/mL)	487 (184.6–888.3)	438.3 (225–865.4)	359.3 (208.4–558.9)	0.81
D Dimer (ng/mL)	**281.3(74.5–665.5)**	**291(69.5–683.5)**	**547.75(260–845)**	**0.03**
C-Reactive Protein (mg/L)	17.12 (4.65–82.9)	17.65 (5.42–51.74)	24.5 (18.31–38.51)	0.30
DHL (U/L)	304 (202–438.5)	293.6 (212.1–436.5)	324 (276–389)	0.62
IFNα (pg/mL)	18.16 (11.8–37.2)	17.6 (9.7–27.4)	17.02 (6.14–22.6)	0.16

[a] Kruskal–Wallis test. Chi square test.
IQR = Interquartile Range.

severely affecting young males with COVID-19.[7] Moreover, van de Veerdonk FL and Netea MG (2021) suggested that screening *TLR7* variants in patients and their relatives could be a potential therapeutic strategy during interferon gamma treatment.[32]

A recent work showed that *TLR7* is responsible for IFN-α production in response to SARS-CoV-2,[33] that could explain why some variants of the *TLR7* gene are implicated in immune activation during COVID-19. The type I IFN response is associated with severe disease that has been demonstrated in patients with inborn errors of type I IFN.[34] A deficiency in the signaling pathway would result in abrogated innate and adaptive immune responses, like we can observe, the IFN-α decreased in the deceased group suggesting that the study of these SNPs could be associated with a deficiency of signaling pathway in the antiviral immune responses of COVID-19.

On the other hand, the *MyD88* gene is located on chromosome 3p22 locus. Variants in this gene are associated with diverse pathologies, such as ulcerative colitis and Buerger's disease.[10,35] The rs7744 variant is located at the 3′UTR of the *MyD88* gene and might play a key role in the severity of COVID-19. In the present work, we found a significant association of rs7744 with the severity of COVID-19, increasing the odds ratio of severity in the codominant and log additive models. MyD88 is implicated

Table 4 Association of *TLR7* and *MyD88* polymorphisms with COVID-19 outcomes.

Polymorphisms	Severe			Critical			Deceased		
	OR[a]	95% CI	P	OR[a]	95% CI	P	OR[a]	95% CI	P
TLR7									
rs179008									
A	Reference			Reference			Reference		
T	0.86	0.56–1.30	0.48	0.94	0.60–1.47	0.79	0.67	0.37–1.19	0.17
AA	Reference			Reference			Reference		
AT	0.54	0.21–1.37	0.19	1.40	0.54–3.61	0.48	0.91	0.25–3.24	0.89
TT[c]	0.93	0.48–1.81	0.84	0.85	0.41–1.77	0.67	0.61	0.24–1.52	0.28
AT + TT[d]	0.74	0.42–1.32	0.32	1.26	0.66–2.40	0.47	0.75	0.32–1.78	0.52
TT[r]	0.94	0.47–1.87	0.87	0.97	0.45–2.11	0.95	0.66	0.22–1.17	0.45
Log additive	0.89	0.64–1.24	0.51	0.85	0.54–1.34	0.50	0.82	0.49–1.37	0.46
rs179009									
A	Reference			Reference			Reference		
G	0.74	0.52–1.06	0.10	0.79	0.54–1.17	0.25	0.86	0.53–1.38	0.54
AA	Reference			Reference			Reference		
AG	0.65	0.29–1.46	0.30	0.38	0.15–0.99	0.05	0.35	0.11–1.14	0.08
GG[c]	0.70	0.39–1.26	0.24	0.81	0.44–1.4	0.50	0.91	0.43–1.93	0.82
AG + GG[d]	0.69	0.41–1.14	0.15	0.67	0.38–1.17	0.16	0.71	0.36–1.41	0.33
GG[r]	1.08	0.64–1.82	0.75	1.19	0.67–2.09	0.54	1.42	0.71–2.83	0.31
Log additive	0.82	0.62–1.09	0.19	0.87	0.64–1.17	0.37	0.91	0.63–1.32	0.63
rs3853839									
C	Reference			Reference			Reference		
G	1.09	0.75–1.56	0.64	**1.83**	**1.21–2.75**	**0.004**	1.36	0.83–2.23	0.21
CC	Reference			Reference			Reference		
CG	0.83	0.34–1.99	0.68	1.16	0.41–3.25	0.78	2.02	0.56–7.20	0.27
GG[c]	1.09	0.61–1.95	0.75	1.98	1.04–3.77	0.03	1.57	0.70–3.52	0.26
CG + GG[d]	0.86	0.50–1.48	0.59	1.85	0.98–3.49	0.05	1.17	0.56–2.43	0.67
GG[r]	1.49	0.91–2.44	0.10	1.91	1.08–3.39	0.03	1.28	0.63–2.58	0.49
Log additive	1.09	0.82–1.45	0.51	1.42	1.03–1.96	0.03	1.22	0.83–1.81	0.30
rs2302267									
T	Reference			Reference			Reference		
G	0.72	0.40–1.28	0.26	1.10	0.61–1.99	0.73	0.64	0.29–1.40	0.26
TT	Reference			Reference			Reference		
TG	1.34	0.48–3.72	0.56	1.24	0.38–4.02	0.72	1.23	0.42–3.55	0.70
GG[c]	0.52	0.19–1.41	0.20	1.14	0.43–2.98	0.88	1.01	0.40–2.51	0.98
TG + GG[d]	0.71	0.35–1.44	0.34	1.03	0.49–2.16	0.93	0.71	0.25–1.97	0.51
GG[r]	0.76	0.29–2.00	0.58	1.53	0.58–4.02	0.38	0.53	0.15–1.85	0.32
Log additive	0.82	0.53–1.28	0.40	1.11	0.70–1.74	0.64	0.77	0.42–1.40	0.39
MyD88									
Rs7744									
A	Reference			Reference			Reference		
G	1.58	1.01–2.45	0.04	1.76	1.10–2.82	0.02	2.45	**1.38–4.34**	**0.002**
A/A	Reference			Reference			Reference		
A/G	1.66	0.96–2.84	0.06	1.69	0.94–3.05	0.08	2.03	0.97–4.25	0.06
G/G[c]	2.34	0.58–9.40	0.23	3.51	0.86–14.24	0.07	8.83	**1.82–42.23**	**0.007**
A/G + G/G[d]	1.70	1.02–2.86	0.04	1.82	1.04–3.21	0.03	2.44	**1.21–4.9**	**0.01**
G/G[r]	1.96	0.49–7.77	0.33	2.91	0.73–11.64	0.13	6.75	**1.45–31.33**	**0.01**
Log additive	1.61	1.02–2.54	0.04	1.75	1.09–2.84	0.02	2.41	**1.35–4.32**	**0.003**

[a] Adjusted for age, sex, hypertension, type 2 diabetes, and obesity. d: dominant inheritance model, the reference group is formed by the major allele homozygote genotype; r: recessive inheritance model, the reference group is formed by the major allele homozygote and heterozygote genotypes. The text in bold denotes statistical significant.

in TLR/interleukin-1 receptor (IL-1R) family signalling in response to pathogens and injury.[36] In a review of *MyD88* as a therapeutic target for inflammatory lung diseases, the authors reported that mice deficient in this gene have inflammatory responses in models such as endotoxin-induced acute respiratory distress, allergic asthma, tobacco smoke inflammation,[37] bronchitis and lung fibrosis.[38]

Table 5 Association of *TLR7* polymorphisms with COVID-19 outcomes in men.

Polymorphisms	Severe			Critical			Deceased		
	OR[a]	95% CI	P	OR[a]	95% CI	P	OR[a]	95% CI	P
				TLR7					
rs179008									
A	Reference			Reference			Reference		
T	1.06	0.63—1.82	0.82	0.66	0.36—1.20	0.17	0.77	0.37—1.59	0.48
rs179009									
A	Reference			Reference			Reference		
G	0.78	0.49—1.22	0.28	0.59	0.35—98	0.04	0.99	0.54—1.82	0.99
rs3853839									
C	Reference			Reference			Reference		
G	1.22	0.76—1.98	0.4	**2.49**	**1.43—4.32**	**0.001**	1.42	0.74—2.7	0.28
rs2302267									
T	Reference			Reference			Reference		
G	0.79	0.37—1.68	0.55	1.72	0.80—3.68	0.15	0.88	0.33—2.34	0.82

[a] Adjusted for age, hypertension, type 2 diabetes, and obesity.

Matsunga et al.[35] proposed that this variant could be implicated with high levels of MyD88 as binding site of miRNA is closely located to rs7744 resulting in a non-regulated cleavage of mRNA and impair protein synthesis. However, to understand the function of rs7744, further studies are necessary. Given the impact of the association seen between rs7744 *MyD88* gene variant and fatal outcomes of COVID-19, as well as the locus of this polymorphism, we wanted to predict its functional impact on miRNA transcriptional regulation of *MyD88* gene. We explored the PolymiRTS database to search for miRNA that recognize the 3′UTR sequence where the variant falls.[39] In this sense, two miRNA (miR-6866-5p and miR-877-5p) can bind to the MyD88 mRNA of rs7744 variant ancestral allele; while with the minor allele these miRNAs lose their target. Then, the minor allele generates a target sequence for two miRNA (miR-520g-5p and miR-6837-3p). This points out the functional impact over *MyD88* gene expression of the rs7744 variant, and further functional studies are needed to corroborate which miRNA is involved in the high risk of developing fatal outcomes for COVID-19.

MyD88 plays a critical role in protecting hosts against different pathogens, such as viruses, any dysfunction of this protein adaptor might result in abrogated innate and adaptive immune responses. In the context of immunity against severe acute respiratory syndrome coronaviruses,

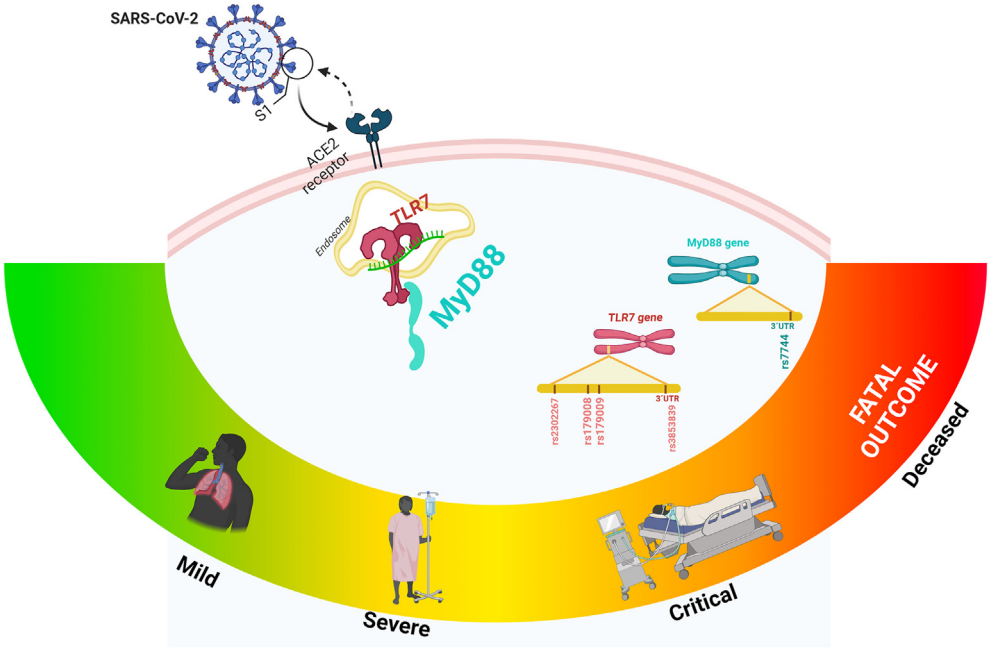

Figure 4. The MyD88 rs7744 variant and TLR7 rs3853839 are involved in COVID-19 progression.

Sheahan et al. reported that *MyD88* knockout mice showed enhanced pulmonary pathology and a higher mortality rate after infection with the novel human SARS-CoV from 2003,[40] highlighting the pivotal role of this adaptor protein in providing immune protection against respiratory viruses. In a different study, Seo et al. reported that mice deficient in *MyD88* showed significant susceptibility to primary influenza infection compared to their wild type counterparts. In the same study, the authors suggested that the absence of *MyD88* could be correlated with a decreased production of pro-inflammatory cytokines, particularly Th1 cytokines, which could result in impaired T-cell mediated antiviral responses.[41]

Despite the key role of *MyD88* in protecting hosts against infections, it has been reported that this adaptor protein can induce excessive inflammation and accelerate diseases,[42,43] a phenomenon that is very relevant in the context of SARS-CoV-2. It has been reported that a large number of patients have cytokine release syndrome that triggers pathology progression. Therefore, it is relevant to take these findings further and understand whether mutations in the *MyD88* gene could be correlated with disease progression caused by a lack of pro-inflammatory cytokines or excessive systemic inflammation.

The present study has some limitations, such as the inability to access all laboratory information from the study population. As well as, it is important to look for data on the cytokine and chemokine status of the patients included in this study. Another limitation is that we do not know patients smoking status, and the association could be modified. Another limitation that we hope to address soon is evaluating the role of TRIF in the observed correlations in this study, given that TRIF is an important adaptor protein that plays a significant role triggering immune responses against single-stranded RNA viruses mediated by TLR7/8.

In conclusion, our results suggest that the *MyD88* rs7744 variant and *TLR7* rs3853839 are involved in COVID-19 progression. The identification of susceptibility variants to COVID-19 may lead to develop a personalized treatment (Fig. 4).

Author contributions

ALR, LEMG, GAMN and CP were the main contributors in the design, acquisition, management and interpretation of the data and writing the article. ALR acquisition of the financial support for the project and leadership responsibility for the research. PVC, JMRP, ALR, GAMN performed the formulation of overarching research. TTL, RPVV, GVA, FJMR, MMMM, EBG, JFMV, JG, RCZ and JMRP conducting a research and investigation evidence and process. JPRH, RPS, JMF, MLM, DMZA, GJVZ, AMC, LRT, RFC, PMMM and LELJ liaised with patients and provided access to samples, laboratory and clinical information. BHL, SOP and YSK performance the DNA extraction. OSML, LEMG, AHB performed the genotyping. CMA, DML, MCCR verification the replication/reproducibility of the results/experiments. JJM, MVF, CSA, MVA and LEMG maintain research data. LEMG performed the statistical analysis in STATA. GEJG creation of images. LEMG and DML drafted the manuscript. CP, GAMN and ALR reviewed and edited the manuscript. All authors reviewed and approved the final manuscript.

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Conflict of interest statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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