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ORIGINAL PAPER

MIF is a common genetic determinant of COVID-19 symptomatic infection and severity

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Summary

Background: Genetic predisposition to coronavirus disease 2019 (COVID-19) may contribute to its morbidity and mortality. Because cytokines play an important role in multiple phases of infection, we examined whether commonly occurring, functional polymorphisms in macrophage migration inhibitory factor (MIF) are associated with COVID-19 infection or disease severity.

Aim: To determine associations of common functional polymorphisms in *MIF* with symptomatic COVID-19 or its severity.

Methods: This retrospective case–control study utilized 1171 patients with COVID-19 from three tertiary medical centers in the USA, Hungary and Spain, together with a group of 637 pre-pandemic, healthy control subjects. Functional *MIF* promoter alleles (-794 $CATT_{5\text{-}8}$, *rs5844572*), serum MIF and soluble MIF receptor levels, and available clinical characteristics were measured and correlated with COVID-19 diagnosis and hospitalization. Experimental mice genetically engineered to express human high- or low-expression *MIF* alleles were studied for response to coronavirus infection.

Results: In patients with COVID-19, there was a lower frequency of the high-expression *MIF* $CATT_7$ allele when compared to healthy controls [11% vs. 19%, odds ratio (OR) 0.54 [0.41–0.72], $P < 0.0001$]. Among inpatients with COVID-19 ($n = 805$), there was a higher frequency of the *MIF* $CATT_7$ allele compared to outpatients ($n = 187$) (12% vs. 5%, OR 2.87 [1.42–5.78], $P = 0.002$). Inpatients presented with higher serum MIF levels when compared to outpatients or uninfected healthy controls (87 ng/ml vs. 35 ng/ml vs. 29 ng/ml, $P < 0.001$, respectively). Among inpatients, circulating MIF concentrations correlated with admission ferritin ($r = 0.19$, $P = 0.01$) and maximum CRP ($r = 0.16$, $P = 0.03$) levels. Mice with a human high-expression *MIF* allele showed more severe disease than those with a low-expression *MIF* allele.

Conclusions: In this multinational retrospective study of 1171 subjects with COVID-19, the commonly occurring -794 $CATT_7$ *MIF* allele is associated with reduced susceptibility to symptomatic SARS-CoV-2 infection but increased disease progression as assessed by hospitalization. These findings affirm the importance of the high-expression $CATT_7$ *MIF* allele, which occurs in 19% of the population, in different stages of COVID-19 infection.

Introduction

A prominent feature of coronavirus disease 2019 (COVID-19) is the significant variation in outcomes that individuals may experience after SARS-CoV-2 infection, which can range from asymptomatic presentation to severe illness requiring hospitalization and intensive care treatment.[1,2] Risk factors for severe disease include older age and underlying medical conditions, such as pre-existing immunosuppression and cardiopulmonary or metabolic disease.[1–3] Genetic variation in the host response to SARS-CoV-2 also may influence disease; however, few common genetic susceptibilities have been identified. The COVID Human Genetic Effort consortium reported defects in the type 1 interferon (IFN) response in eight loci governing type 1 IFN induction, amplification or response and estimated such defects to exist in 3–5% of cases of critical disease.[4,5] In the Genetics Of Mortality In Critical Care (GenOMICC) genome-wide association study, candidate single-nucleotide polymorphisms (SNPs) were identified and life-threatening disease was associated with high expression of tyrosine kinase 2 (*TYK2*) and low expression of an interferon receptor gene (*IFNAR2*).[6]

Clinical studies have reported relationships between circulating levels of inflammatory cytokines, including IL-6, IL-2R, IL-10, IP-10/CXCL10 and MCP-1/CCL2, with progression to severe disease.[7,8] Cytokine blockade also improves outcomes in hospitalized patients, supporting the role of excessive inflammation in the development of severe COVID-19.[9,10] Macrophage migration inhibitory factor (MIF) is a pleiotropic cytokine with roles in both the initiation and the inflammatory progression of autoimmune and infectious diseases.[11–15] Increased levels of plasma MIF have been reported in COVID-19.[16] Notably, MIF is encoded in a functionally polymorphic locus that comprises a 4-nucleotide promoter microsatellite (-794 $CATT_{5\text{-}8}$, *rs5844572*), with higher CATT number associated with increased baseline and stimulus-activated *MIF* transcription (Figure 1).[17] The high-expression -794 $CATT_7$ allele occurs in approximately 20% of the population and has been associated with increased inflammatory end-organ damage in autoimmune diseases.[11,13,18] In the context of infection, where MIF contributes to pathogen clearance,[19] high-expression *MIF* alleles are associated with improved outcomes in community-acquired pneumonia and with reduced frequency of gram-negative, meningococcal and *Mycobacterial* sepsis.[14,15,20–22] In circumstances where inflammatory sequelae may dominate the clinical manifestations of infection, such as in severe malaria or in pneumococcal or West Nile virus meningitis, high-genotypic *MIF* expressers show worse outcomes.[15,23,24]

We analyzed the prevalence of *MIF* promoter variants in a retrospective case–control study of 1171 subjects with COVID-19 from three tertiary medical centers in the USA and Europe and show that the high-expression -794 $CATT_7$ allele is associated with reduced susceptibility to symptomatic SARS-CoV-2 infection but increased disease progression as assessed by hospitalization.

Materials and methods

Patients

Our study consisted of 1171 patients recruited from the Yale New Haven Health System, CT, USA ($n = 295$), the University of Pécs, Pécs, Hungary ($n = 294$) and the Universidad de Valladolid, Valladolid, Spain ($n = 582$). Recruited patients presented for evaluation with COVID-19-related symptoms during the first wave of the pandemic in 2020 and were confirmed to have SARS-CoV-2 infection based on a positive reverse transcription-quantitative polymerase chain reaction test. Hospitalization status was determined by chart review, and the outpatient designation limited to subjects not subsequently hospitalized. In the US cohort, 187 patients were admitted to Yale New Haven Health System and 108 patients were followed as outpatients. In the Hungarian cohort, 274 patients were admitted to the

Figure 1. Diagram of the human MIF gene showing its three exons, and the -173 G/C SNP (rs755622) and -794 $CATT_{5-8}$ (rs5844572) polymorphisms.

hospital and 20 patients were followed as outpatients. In the Spanish cohort, 521 patients were admitted, and 61 patients were outpatients. Frozen sera, demographic information, clinical history and laboratory data were collected and analyzed. Healthy control subjects ($n = 637$) were from a pre-pandemic database of *MIF* allele frequencies from US medical centers ($n = 519$)[25] and the Hungary and Spain study sites ($n = 118$). Controls were matched for age (61 ± 22 years) and sex (45% male). In the US, hospitalization and clinical data including 30 comorbid conditions were assessed using electronic medical records (Epic Systems Corporation, Verona, WI, USA) and coding from the International Classification of Diseases 10 (ICD-10) mapped to the Elixhauser comorbidity index.[26] Chronic respiratory diseases including chronic obstructive pulmonary disease (COPD) and cardiovascular diseases were analyzed in the Hungary patients. This study was approved by the Institutional Review Boards of all institutions (Yale: HIC#20000276790; Spain: CEIm de Valladolid Este, PI 20-1716; Hungary: 20800-6/2020/EÜIG).

Genotype analysis

Genomic DNA was isolated from serum using the easy-DNA kit (Invitrogen, Carlsbad, CA, USA) and two *MIF* promoter polymorphisms: the −794 $CATT_{5-8}$ microsatellite (*rs5844572*) and a -173 G/C SNP (*rs755622*), analyzed following methodologies described previously.[18] (It should be noted that neither the -794 $CATT_{5-8}$ variant nor the -173 SNP is represented in common GWAS platforms.) For ethnicity determination, the Illumina Infinium Global Screening Array-24 BeadChip (Illumina, CA, USA) was used and genotyping was done by Illumina GenomeStudio 2.0. Data were mapped using TRACE from the LASER package and a reference dataset of worldwide human relationships inferred from genome-wide patterns of variation.[27]

Serum MIF and soluble MIF receptor levels

Serum MIF and soluble MIF receptor (sCD74) levels were measured by enzyme-linked immunosorbent assay (ELISA) kits from R&D Systems (Minneapolis, MN, USA) and Invitrogen (Carlsbad, CA, USA), respectively. Healthy control sera were obtained from an existing Yale New Haven Health System biorepository matched for age and gender. All samples were obtained upon initial evaluation and analyzed in duplicate.

Humanized MIF mouse studies

The human and mouse MIF proteins show 90% amino acid identity and are interchangeable in human or mouse cell-based assays.[28,29] Two C57BL/6J mouse strains expressing the human high- or low-expression *MIF* alleles (e.g. MIF^{CATT7} and MIF^{CATT5} mice) were created using vector-based recombinant replacement of murine *Mif* by Taconic Biosciences (Rensselaer, NY, USA) (Supplementary Figure 1). Validation of human but not murine *MIF* mRNA expression was verified by qPCR, and -794 CATT-length dependent stimulated MIF production was confirmed *in vivo*. Test mice were infected intranasally with 1×10^7 p.f.u. of the murine MHV-A59 coronavirus strain and mortality followed.[30]

Statistical analysis

Descriptive data were presented as means ± standard deviations (SDs). Continuous variables were analyzed using the Student *t*-test or one-way ANOVA with the Dunnett's test for multiple comparisons as appropriate. Categorical variables were analyzed using the Chi-square test. A set of variables including age, sex, ethnicity and comorbidities were compared between patients with different *MIF* genotypes using the Chi-square test. Data were analyzed with SPSS version 28.0 (IBM, Armonk, NY, USA) and Prism 9 (GraphPad Software, Inc, San Diego, CA, USA). *P* values of 0.05 or less were considered statistically significant.

Results

Patient characteristics

The demographic characteristics of each of the studied cohorts are shown in Table 1. There was no difference between the mean age and sex distribution of healthy controls and patients. When subjects were grouped into inpatients and outpatients, the inpatients from the USA and Spain were significantly older than outpatients (US cohort: 64 ± 15 vs. 39 ± 12, $P < 0.001$; Spain cohort: 67 ± 15 vs. 53 ± 15 $P < 0.001$). In the Spain cohort, male sex also was significantly more frequent in inpatients than in outpatients (54% vs. 34%, $P = 0.008$).

MIF promoter polymorphisms and SARS-CoV-2 infection

We first determined potential associations between *MIF* promoter polymorphisms and risk for symptomatic COVID-19 using as reference information the frequency of *MIF* promoter alleles in a population of 637 healthy control subjects from a pre-pandemic reference database of *MIF* allele frequencies at US medical centers ($n = 519$)[25] and the Hungary and Spain study sites ($n = 118$). In accord with prior genetic and functional studies,[15,17,18] we grouped the low-expression -794 $CATT_{5,6}$ alleles together and analyzed their frequencies against the high-expression -794 $CATT_{7,8}$ alleles. (The high-expression -794 $CATT_8$ allele occurs rarely and was identified in only a single studied individual.) The frequencies in the studied and reference populations of the -794 $CATT_{5-8}$ alleles and a nearby -173 G/C SNP are shown in Supplementary Table 1. The frequency of high-expression -794 $CATT_{7,8}$ containing *MIF* genotypes was significantly lower in all COVID-19 patients when compared to the healthy controls (11% vs. 19%, OR 0.54 [0.41–0.72], $P < 0.0001$), as well as in the subgroups of COVID-19 inpatients (12%, OR 0.62 [0.47–0.84], $P = 0.002$) and COVID-19

outpatients (5%, OR 0.22 [0.12–0.45], $P < 0.0001$) (Table 2A). The frequency of the -173*C SNP was not significantly different in patients with COVID-19 compared to the healthy control group (Table 2B). The population frequency of the -794 $CATT_{5\text{-}8}$ alleles may be influenced by population stratification. As the US patient cohort comprised 21% African Americans subjects, we re-analyzed allele frequencies by ethnicity with a reference population[18] but observed no effect on the statistical associations between the frequency of the -794 $CATT_7$ or -173C* alleles and COVID-19 diagnosis. In the US patients, there was no difference in the frequency of -794 $CATT_{5\text{-}8}$ repeats or -173 SNP between Caucasian and African American subjects.

Table 1. Patient characteristics in the three studied cohorts

	Healthy controls,	**All patients,** $n = 1171$		
Total	$n = 637$	**US,** $n = 295$	**Hungary,** $n = 294$	**Spain,** $n = 582$
Age (mean ± SD)	61 ± 22	60 ± 18	64 ± 16	65 ± 16
Male (%)	45	51	57	52
Caucasian (%)	100	72	100	100
African-American (%)	0	21	0	0
Asian (%)	0	6	0	0
		Inpatients, $n = 982$		
Total		**US,** $n = 187$	**Hungary,** $n = 274$	**Spain,** $n = 521$
Age (mean ± SD)		64 ± 15	64 ± 16	67 ± 15
Male (%)		50	57	54
		Outpatients, $n = 189$		
Total		**US,** $n = 108$	**Hungary,** $n = 20$	**Spain,** $n = 61$
Age (mean ± SD)		39 ± 12	60 ± 16	53 ± 15
Male (%)		52	50	34

MIF promoter polymorphisms and COVID-19 hospitalization

We next examined the association between *MIF* alleles and hospitalization status as an indicator of COVID-19 severity. The frequencies of the high-expression -794 $CATT_{7,8}$ alleles were higher in inpatients when compared to the outpatients (12% vs. 5%; OR 2.87 [1.42–5.78], $P = 0.002$) (Table 2C). The frequency of the -173*C SNP was not significantly different between inpatients and outpatients with COVID-19 (Table 2D). The frequencies of these alleles in inpatients vs. outpatients in each study site are in Supplementary Table 2. Adjusting for the potential confounders of age and sex in the three study sites did not affect the allelic associations. The frequencies of comorbidities, specifically the 30 conditions of the Elixhauser comorbidity classification

Table 2. The -794 $CATT_{5\text{-}8}$ alleles are grouped into low-expresser ($CATT_{5,6}$) and high-expresser ($CATT_{7,8}$) variants

A Total	**Healthy controls,** $n = 617$	**All COVID-19 patients,** $n = 992$	**Inpatients,** $n = 805$	**Outpatients,** $n = 187$
−794 $CATT_{5,6}$	501 (81%)	881 (89%)	703 (88%)	178 (95%)
−794 $CATT_{7,8}$	116 (19%)	111 (11%)	102 (12%)	9 (5%)
Odds ratio		0.54	0.62	0.22
95% CI		0.41–0.72	0.47–0.84	0.12–0.45
P value		<0.0001	0.002	<0.0001

B Total	Healthy controls, $n = 579$	All COVID-19 patients, $n = 778$	Inpatients, $n = 664$	Outpatients, $n = 114$
-173 G	548 (95%)	774 (95%)	634 (95%)	110 (96%)
-173 C	31 (5%)	34 (5%)	30 (5%)	4 (4%)
Odds ratio		0.77	0.83	0.71
95% CI		0.48–1.28	0.51–1.42	0.25–1.94
P value		0.36	0.51	0.64

C Total		**Inpatients,** $n = 805$		**Outpatients,** $n = 187$
-794 $CATT_{5,6}$		703 (88%)		178 (95%)
v794 $CATT_{7,8}$		102 (12%)		9 (5%)
Odds ratio			2.87	
95% CI			1.42, 5.78	
P value			0.002	

D Total		Inpatients, n=664		Outpatients, n=114
-173 G		634 (96%)		110 (96%)
-173 C		30 (4%)		4 (4%)
Odds ratio			0.77	
95% CI			0.26–2.22	
P value			0.63	

Frequencies of the *MIF* -794 $CATT_{5\text{-}8}$ (A) and -173 G/C alleles (B) in all COVID-19 patients, COVID-19 inpatients, and COVID-19 outpatients were compared with frequencies in the healthy controls. Frequencies of *MIF* low- (-794 $CATT_{5,6}$) and high- (-794 $CATT_{7,8}$) expresser alleles (C) and the -173 G/C SNP (D) were compared between COVID-19 inpatients and outpatients.

schema[26] in the US patients, and chronic respiratory and cardiovascular diseases in the Hungary patients, did not differ significantly between patients with the *MIF* -794 $CATT_{5,6}$ and -794 $CATT_{7,8}$ alleles (Supplementary Tables 3 and 4).

Serum MIF, sCD74 and inflammatory markers

We measured circulating MIF and soluble MIF receptor (sCD74) levels in sera obtained upon initial evaluation of outpatients or at hospital admission for inpatients in the US cohort. As expected from prior studies of critically ill patients with infection,[31] hospitalized patients presented with significantly higher MIF levels when compared to the outpatients (87 ± 56 ng/ml vs. 35 ± 27 ng/ml, $P < 0.0001$) or to the healthy controls (29 ± 13 ng/ml, $P < 0.0001$) (Figure 2A). Serum sCD74 concentrations can be elevated in severe illness and may reduce MIF bioactivity in circulation;[32] however, sCD74 levels did not differ between hospitalized and non-hospitalized COVID-19 patients (Figure 2C). Circulating MIF also has been reported to correlate with the high-expression -794 $CATT_{7,8}$ allele in conditions of sepsis or autoimmunity,[13,18,21] however we did not find a correlation between MIF or sCD74 levels and alleles in hospitalized and non-hospitalized COVID-19 patients (Figure 2B and D). We examined correlations between circulating MIF concentrations and inflammatory markers that were measured in the hospitalized US cohort. Ferritin levels measured during first 24 h of hospitalization ($r = 0.19$, $P = 0.01$) and mean of the ferritin ($r = 0.16$, $P = 0.03$) or IL-10 ($r = -2$, $P = 0.04$) level during the entire hospitalization showed correlation with circulating MIF (Figure 2E and F, Supplementary Figure 2). The maximum CRP level ($r = 0.16$, $P = 0.03$) measured during the hospitalization showed significant correlation with circulating MIF (Figure 2G). Statistically significant correlations were not observed between MIF and IL-6 or sIL2R levels, or between MIF and the presence of major COVID-19 co-morbidities such as older age or pre-existing immunosuppression, cardiopulmonary or metabolic disease (Supplementary Figure 2 and data not shown).

Influence of MIF alleles in experimental coronavirus infection

We sought to model the impact of the *MIF* promoter microsatellite in mice with experimentally established coronavirus infection by studying two mouse strains created by the recombinant replacement of mouse *Mif* with the high- (-794 $CATT_7$) and low- (-794 $CATT_5$) expression *MIF* alleles (Supplementary Figure 1). We infected mice intranasally with the murine MHV-A59 coronavirus strain[30] and observed that mice expressing the -794 $CATT_7$ allele suffered greater lethality than those with the -794 $CATT_5$ allele and showed increased levels of circulating MIF (Figure 3).

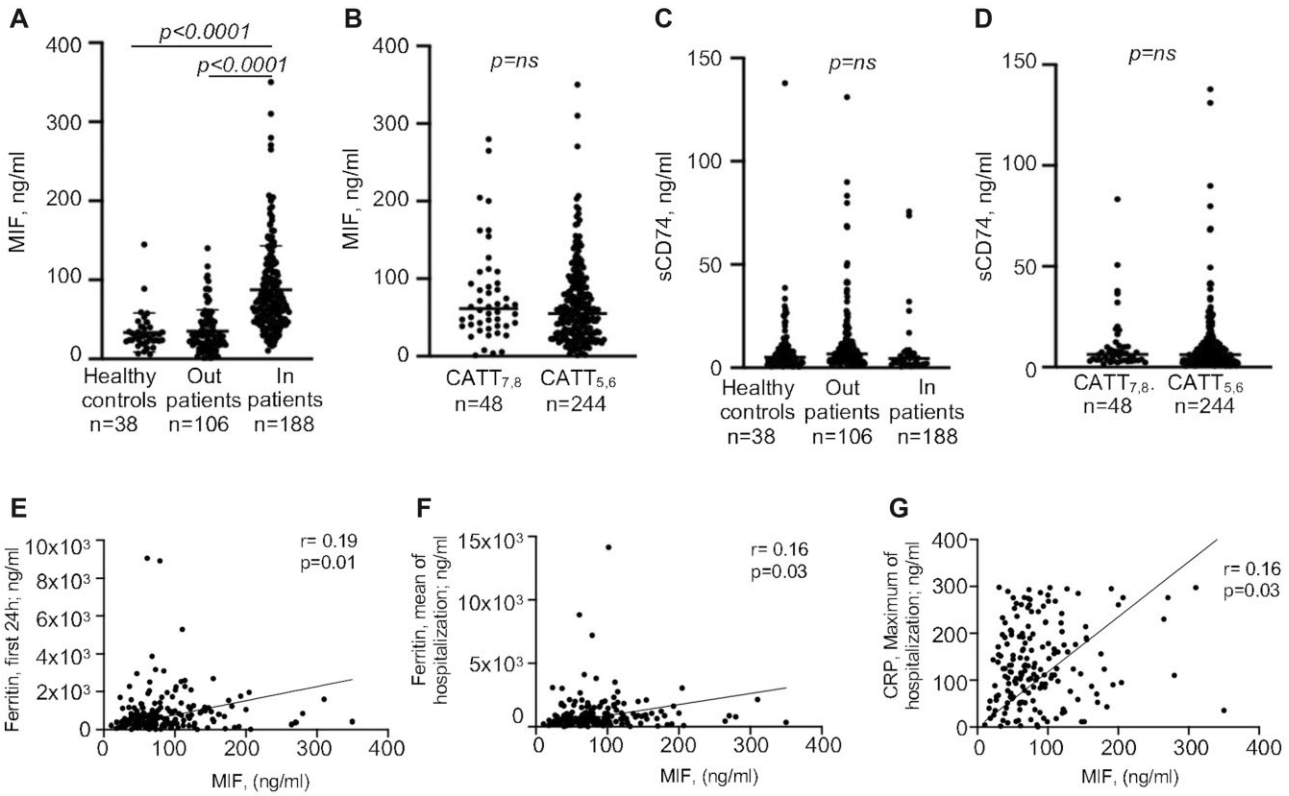

Figure 2. Circulating concentrations of MIF and sCD74 in outpatients and inpatients, and between all patients with the -794 $CATT_{7,8}$ alleles vs. the -794 $CATT_{5,6}$ alleles. Circulating concentrations of MIF (**A, B**) and sCD74 (**C, D**) in healthy controls, outpatients and inpatients, and between all patients (B, D) with the -794 $CATT_{7,8}$ alleles vs. the -794 $CATT_{5,6}$ alleles. (A) Healthy controls: mean ± SD, 29 ± 13 ng/ml; outpatients: 35 ± 27 ng/ml; and inpatients: 87 ± 56 ng/ml (mean ± SD). (B) MIF levels were similar between all patients with the $CATT_{7,8}$ allele vs. the $CATT_{5,6}$ alleles (72 ± 56 ng/ml vs. 67 ± 52 ng/ml, respectively). (C) Serum sCD74 levels were similar among healthy controls, outpatients and inpatients (10 ± 18 ng/ml vs. 9 ± 15 ng/ml vs. 12 ± 17 ng/ml, respectively), and (D) between all patients with the -794 $CATT_7$ allele vs. the -794 $CATT_{5,6}$ alleles (11 ± 17 ng/ml vs. 10 ± 16 ng/ml, respectively); ns: not significant. Correlation between serum ferritin levels with MIF concentrations in 163 COVID-19 patients measured in the first 24 h of hospital admission (**E**) and as a mean of hospitalization duration (**F**). Correlation between maximum serum CRP level during hospitalization with MIF concentration in the same COVID-19 patient population (**G**).

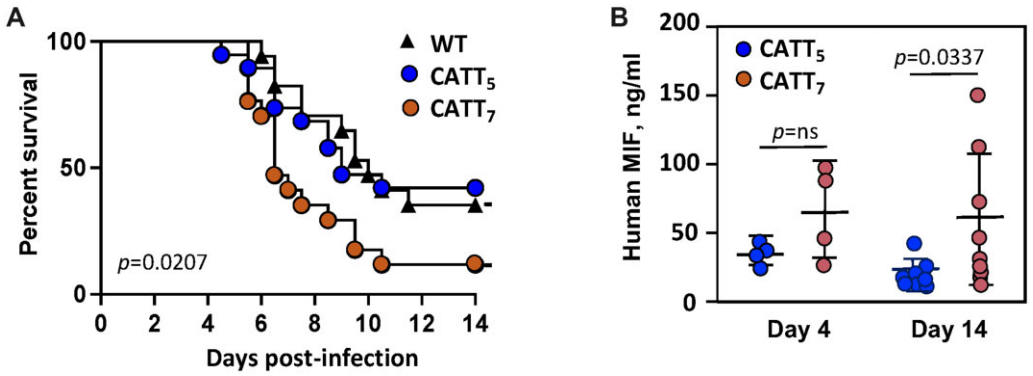

Figure 3. Impact of the *MIF* promoter microsatellite in mice with experimentally established coronavirus infection. (**A**) Kaplan–Meyer survival plot showing enhanced lethality to coronavirus infection in mice encoding the human *MIF* -794 $CATT_7$ allele ($CATT_7$) when compared to the -794 $CATT_5$ *MIF* allele ($CATT_5$). Infection was established in all test mice by intranasal administration of 1×10^7 p.f.u. of the MHV-A59 coronavirus strain. *P* values by log-rank test statistic for MIF^{CATT7} mice versus MIF^{CATT5} or wild-type mice (WT: with an endogenous murine *Mif* gene); $n = 17–19$ mice per each tested group. (**B**) Circulating human MIF levels measured in individual mice post-infection. Day 4: $CATT_5 = 35.1 \pm 6.5$ ng/ml, $CATT_7 = 64.6 \pm 33.9$ ng/ml ($n = 4$ mice/group). Day 14: $CATT_5 = 19.6 \pm 10.8$ ng/ml, $CATT_7 = 55.1 \pm 48.6$ ng/ml ($n = 8–9$ mice/group). Mean ± SD with *P* values by Mann–Whitney; ns, not significant.

Discussion

A major challenge for the care of patients with COVID-19 is variability in the progression and manifestations of the disease, and in predicting those who may be at greatest risk for severe disease and require hospital care. There also is limited understanding for why upwards of 50% of individuals experience asymptomatic infection.[33] Multiple factors, including pre-existing immunosuppression, advanced age, diabetes, obesity and cardiopulmonary disease may increase susceptibility to infection and to the morbidity and mortality of COVID-19.[1–3] Nevertheless, these conditions do not account for the full risk and the unpredictability of acquiring infection or in developing severe disease.

The immune system and its cytokines have an essential role in host defense by orchestrating barrier defenses to pathogen invasion, recruiting a protective inflammatory response and influencing the differentiation of adaptive immunity. MIF is constitutively expressed by a variety of cell types, including respiratory epithelium, and circulating and tissue macrophages. It is rapidly released upon innate sensing and acts to upregulate multiple pathogen response pathways. MIF additionally inhibits the activation-induced apoptosis of immune cells to sustain strong inflammatory responses.[14,21,34]

Functional *MIF* promoter variants occur commonly, with the high-expression -794 $CATT_7$ allele present in ~20% of healthy control populations.[18,25] In the current study, high-genotypic *MIF*-expressing individuals appear to have a reduced rate of symptomatic COVID-19 but suffer more severe disease, as assessed by hospital admission. These finding are in accord with studies of *MIF* genetics in other infectious disease scenarios. In one example, genetic *MIF* deficiency was observed experimentally to be associated with reduced ability to clear nasal carriage of *Streptococcus pneumoniae*,[12] but high-expression alleles were associated with unfavorable outcome in a study of invasive pneumococcal disease, where an excessive inflammatory response is clinically injurious.[15] MIF expressed within respiratory epithelium and resident immune cells thus may play a critical role in limiting SARS-CoV-2 dissemination into the lung. Once the pulmonary infection becomes established however, MIF's role in orchestrating inflammatory responses may be deleterious and contribute to more severe disease manifestations. Similarly, high-expression *MIF* alleles appear to protect older adults from developing gram-negative bacteremia, potentially by up-regulating the innate sensor TLR-4[35] but correlate with the overall morbidity and mortality of gram-negative sepsis.[20,36] As many of the severe manifestations of COVID-19 result from tissue-damaging inflammation, the present findings suggest that the high-expression -794 $CATT_7$ *MIF* allele exerts a similar dual influence on disease, with a protective role on the initial acquisition of the virus but a detrimental effect once infection becomes established by promoting excessive inflammation.

Circulating MIF levels correlate with APACHE II severity scores in bacterial sepsis[37] and together with IL-6, IL-8 and extracellular nicotinamide phosphoribosyl transferase (eNAMPT) predict mortality in ARDS.[38] We observed a significant difference in serum MIF concentrations between the outpatient and the inpatient groups. Correlations between circulating MIF levels and *MIF* genotype have been reported in examples of autoimmunity or chronic infection,[13,18,21] however we did not observe this in our study. This finding may be due to the inadequacy of plasma in reflecting *MIF* expression at sites of tissue inflammation, temporal variation in cytokine expression and clinical heterogeneity at the time of blood sampling. Plasma MIF levels did correlate with circulating ferritin and CRP, which is a useful integrator of sustained inflammatory signaling in many clinical settings including COVID-19.[2]

The present findings must be viewed in the context of the limitations of the study. We used a retrospective case–control study design to investigate functional *MIF* alleles in COVID-19 and relied on a database of *MIF* allele frequencies collected previously in healthy controls. The controls were matched for gender, age and ethnicity, and pre-dated the emergence of SARS-CoV-2, which obviates the concern of inadvertently including subjects with undiagnosed infection. The healthy controls nevertheless may not be representative of the populations from which cases were recruited. We studied a large number of cases from three tertiary academic centers and used hospital admission as a surrogate for severe disease, which is simple to assess but assumes comparable admission criteria. Spurious associations may occur in gene association studies, however selection of the candidate *MIF* gene and the designation of low- and high-expression alleles was supported by multiple prior studies.[11,13,15,17,18,20–25] By the nature of a gene association study, we cannot ascribe causality to a particular *MIF* allele. However, the observed direction of the

gene effect is consistent with prior genetic and functional findings[11–13,15–17,20–25] and agree with an evaluation of the -794 $CATT_7$ *MIF* allele in a model of experimentally established coronavirus infection. Additional validation studies are warranted, including an investigation of the specific impact of *MIF* promoter variants in the context of established vulnerabilities to COVID-19 such as immunosuppression, older age, and cardiopulmonary and metabolic diseases. Our studied patients were predominantly Caucasian, and closer investigation of other ethnic groups may inform specific associations between *MIF* alleles and COVID-19 in these populations.

Conclusions

Finally, the present findings suggest the possibility of using *MIF* allele determination for risk stratification, especially in the difficult circumstances of pandemic conditions, as well as the potential application of MIF-directed therapeutic approaches in genetically at-risk individuals.[39,40]

Supplementary material

Supplementary material is available at *QJMED* online.

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Authors contributions

J.J.S., W.F., J.P.-Y. and M.P. analyzed the biospecimens. L.L. and A.W. provided technical expertise and supervision. K.I.-W., M.P. and H.Q. performed the mouse studies. J.J.S., J.G., S.U., J.K. and J.L. performed the statistical analysis. J.J.S., W.S., O.K. and A.G. extracted the clinical data. H.Z., I.K. and A.I.K. supervised the statistical analysis. A.B.G., M.S., A.V.W. and A.O. provided oversight and suggestions. S.C.D. and M.E.A. created the mouse model. S.C.D., A.B.G., D.B.O., P.H., A.G., A.I.K., I.K. and R.B. provided conceptual input and supervision. J.J.S. and R.B. drafted the manuscript, which was reviewed by all authors.

Data availability

All data generated or analyzed during this study are included in this published article (and its Supplementary materials) and will be available upon request to R.B.

Conflict of Interest: RB and LL are inventors on patent describing the therapeutic use of MIF antagonists.

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