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BRIEF REPORT

Interferon-Induced Transmembrane Protein 3 Genetic Variant rs12252-C Associated With Disease Severity in Coronavirus Disease 2019

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A major unanswered question in the current global coronavirus disease 2019 (COVID-19) outbreak is why severe disease develops in a small minority of infected individuals. In the current article, we report that homozygosity for the C allele of rs12252 in the interferon-induced transmembrane protein 3 (IFITM3) gene is associated with more severe disease in an age-dependent manner. This supports a role for IFITM3 in disease pathogenesis and the opportunity for early targeted intervention in at-risk individuals.

Keywords. COVID-19; severe pneumonia; rs12252; IFITM3.

Coronavirus disease 2019 (COVID-19) is a newly recognized illness caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which firstly emerged in December 2019 in the city of Wuhan in Hubei province, China. It has spread rapidly throughout Wuhan to other provinces in China and around the world. As of 29 February 2020, a cumulative total of 79 394 COVID-19 cases were reported in China, which led to 2838 deaths, according to the report of the World Health Organization–China Joint Mission on Coronavirus Disease 2019 (COVID-19) [1]. There is an urgent unmet need to understand why severe disease develops in some people. Heritable differences are known to modulate individual susceptibility to and severity of infectious disease [2, 3].

We hypothesized that a genetic variant of the interferon-induced transmembrane protein 3 (IFITM3) gene (*IFITM3*) was associated with the severity of COVID-19, specifically the single-nucleotide polymorphism rs12252. This genetic variant is common in Asian populations, and homozygosity for the C allele has been associated with influenza severity [4]. *IFITM3* encodes an immune effector protein critical to viral restriction and acts to restrict membrane fusion [5]. It is currently unknown whether *IFITM3* shows genetic association with the severity of COVID-19.

To investigate whether the *IFITM3* variant was associated with disease severity, we recruited a cohort of hospitalized patients with laboratory-confirmed COVID-19. These comprised a consecutive series of 80 patients admitted to Beijing Youan Hospital during January to and 2020. We phenotyped the patients into mild and severe disease groups, according to clinical definitions from the National Health Commission of China [1].

METHODS

Study Population

Patients were recruited from Beijing Youan Hospital, Capital Medical University, Beijing, between January 2020 and February 2020. All the candidates were hospitalized patients with laboratory-confirmed COVID-19. Their clinical data and whole blood samples were collected. The study was approved by Beijing Youan Hospital's institutional review board. Written informed consent was obtained from all patients.

Clinical Definitions

COVID-19 was diagnosed according to the diagnosis and treatment of COVID-19 recommended by the National Health Commission of China [6]. Laboratory confirmation was defined as a positive result at high-throughput sequencing or real-time reverse-transcription polymerase chain reaction assay of nasal and pharyngeal swab specimens [7]. Mild disease was defined as COVID-19 with the presence of fever, respiratory symptoms, and pneumonia at imaging. Severe disease was defined as COVID-19 with the additional presence of significant respiratory distress (respirations, >30/min), blood oxygen saturation <93%, ratio of arterial oxygen pressure to fraction of inspired oxygen <300 mm Hg, respiratory failure with mechanical ventilation, shock, or other organ failure requiring intensive care in the intensive care unit [6].

Sequencing and Genotyping of rs12252

Genomic DNA was extracted from peripheral blood mononuclear cells using the PureGene DNA Isolation Kit (Gentra System). The region encompassing the human *IFITM3* rs12252 sequence was amplified, sequenced, and analyzed as described elsewhere [4, 8].

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Statistical Analysis

Statistical analysis was performed using χ^2 and Fisher exact tests. Student *t* tests were used to compare values between mild and severe disease groups where data were normally distributed (evaluated with Kolmogorov-Smirnov test), and nonparametric *t* tests (Mann-Whitney tests) were used where data were not normally distributed. Statistical test differences were considered significant when *P* values were < .05. Analyses were performed using GraphPad Prism, version 7 (GraphPad Software). The age effect was determined using a linear model approach with patient age as dependent variable and disease severity as independent variable. The associations between categorical variables (age groups or genotypes) and disease severity were assessed using logistic regression. The analyses were performed using R software (version 3.6.0). Differences were considered statistically significant at $P < .05$.

RESULTS

Demographic and phenotypic data are shown in Table 1. Mild disease (n = 56) was defined as the presence of fever, respiratory symptoms, and pneumonia seen with imaging. In patients with severe disease (n = 24), significant tachypnea, hypoxia, respiratory failure, or other organ failure also developed. The patient cohort was broadly representative of published clinical reports in the outbreak to date in terms of case mix and severity [7]. We found that blood oxygen saturation was significantly lower in patients with severe disease (77% vs 96.7% in patients with mild disease; $P = .001$), and the ratio of arterial oxygen partial pressure to the fraction of inspired oxygen was significantly lower (223.5 ± 45.8 vs 466.7 ± 135.6 mm Hg, respectively; $P < .001$). Seven patients in the severe disease group received intensive care in the intensive care unit; 6 were mechanically ventilated, and 3 of the 6 died.

We found evidence of a clear relationship between age and disease severity. The median age was 49.5 years (interquartile range, 37.75–63.5; Table 2), and comparing patients with mild or severe disease, the median age increased from 43.5 to 67.5 years ($P < .001$; $\beta = 20.8$; Supplementary Figure 1). We observed that 14 (58.3%) of the 24 patients with severe disease (including all patients who died) were >63.5 years old (third quantile), compared with only 6 (10.7%) of the 56 patients with mild disease ($P < .001$; odds ratio [OR], 11.7; Table 2). The age-related difference in disease severity was also profound when we restricted the analyses to the median age (≥49.5 or <49.5 years) of the cohort ($P < .001$; OR, 13.6).

To determine whether the homozygous C-allele carriers associate with the severity of COVID-19, we genotyped the cohort by sequencing a 300–base pair locus spanning rs12252. Among all hospitalized patients, we found that 35% were

Table 1. Patient Demographics by Clinical Phenotype

Characteristic	Patients Hospitalized With COVID-10, No. (%)[a]				*P* Value[b]
	Total (N = 80)	Mild Disease (n = 56)	Severe Disease (n = 24)	Died (n = 3)	
Age, median (IQR), y	49.5 (37.75–63.5)	43.5 (34–56.5)	67.5 (57.75–74.25)	86 (80.5–88.5)	<.001
Male sex	33 (41.25)	24 (42.86)	9 (37.5)	1 (33.3)	.66
Preexisting conditions					
Diabetes	9 (11.25)	5 (8.93)	4 (16.67)	1 (33.33)	.44
Hypertension	21 (26.25)	9 (16.07)	12 (50)	2 (66.67)	.002
Cancer	4 (5)	2 (3.57)	2 (8.33)	0 (0)	.58
Chronic liver disease	2 (2.5)	2 (3.57)	0 (0)	0 (0)	>.99
Presenting symptoms					
Fever	62 (77.50)	41 (73.21)	21 (87.50)	2 (66.67)	.16
Cough	51 (63.75)	33 (58.93)	18 (75.00)	2 (66.67)	.17
Expectoration	26 (32.50)	15 (26.79)	11 (45.83)	2 (66.67)	.10
Vomiting	1 (1.25)	0 (0)	1 (4.17)	0 (0)	.30
Diarrhea	1 (1.25)	1 (1.79)	0 (0)	0 (0)	>.99
Physiological variables, median (IQR)					
Respirations/min	20 (20–21)	20 (20-20)	21 (20–24.75)	23 (20–25)	<.001
Sao_2 (n = 56)	94.95 (88.125–97.625)	97.2 (95.5–98.1)	88.0 (79.6–90.9)	79.6 (77.6–80.3)	<.001
Pao_2/Fio_2 (n = 45)	386.5 (261.85–472.0)	449.5 (379.1–494.3)	211.35 (192–260)	193.8 (187–200)	<.001
ICU admission	7 (8.75)	0 (0)	7 (29.17)	3 (100)	<.001
Mechanical ventilation	6 (7.5)	0 (0)	6 (25.00)	3 (100)	<.001
Death within 28 d	3 (3.75)	0 (0)	3 (12.5)	3 (100)	.02

Abbreviations: COVID-19, coronavirus disease 2019; ICU, intensive care unit; IQR, interquartile range; Pao_2/Fio_2, ratio of arterial oxygen partial pressure to fraction of inspired oxygen; Sao_2, arterial oxygen saturation.

[a]Data represent no. (%) of patients unless otherwise specified.

[b]*P* values comparing severe and mild disease groups were calculated by means of χ^2 and Fisher exact tests. Student *t* tests were used where data were normally distributed (evaluated with Kolmogorov-Smirnov test), and nonparametric *t* tests (Mann-Whitney tests) where data were not normally distributed.

Table 2. Logistic Regression Analysis for Genotype Distributions (CC vs CT/TT) Between Mild and Severe COVID–19 Cases

Characteristics	All patients (n = 80)	Mild (n = 56)	Severe (n = 24)	Died (n = 3)	*P* value[a]	OR[a]	*P* value[b]	OR[b]
Age, median (IQR), y	49.5 (37.75–63.5)	43.5 (34–56.5)	67.5 (57.75–74.25)	86 (80.5–88.5)	4.04E–05	11.67		
≥63.5 (third quantile)	20 (25)	6 (10.7)	14 (58.3)	3 (100)				
<63.5	60 (75)	50 (89.3)	10 (41.7)	0 (0)				
Gender					.656	1.25		
Male	33 (41.25)	24 (42.86)	9 (37.5)	1 (33.3)				
Female	47 (58.75)	32 (57.14)	15 (62.5)	2 (66.7)				
Genotype, rs12252					.069	2.50	.0093	6.37
CC	28 (35)	16 (28.57)	12 (50)	2 (66.7)				
CT	37 (46.25)	30 (53.57)	7 (29.17)	1 (33.3)				
TT	15 (18.75)	10 (17.86)	5 (20.83)	0 (0)				

Abbreviations: IQR, interquartile range; OR, odds ratio.

[a]*P* values and odds ratios comparing severe and mild infection patients were calculated by logistic regression.

[b]*P* values and odds ratios comparing severe and mild infection patients were calculated by logistic regression, and adjusted by age groups.

homozygous for the CC allele (46.25% CT heterozygotes and 18.75% TT homozygotes). Adjusting for age on regression analysis, we found a significant difference between mild and severe disease groups, with an association between homozygosity for the C allele (CC vs CT/TT) and disease severity (P <.0001; OR, 6.37; Table 2). In addition, 2 of the 3 patients who died carried the CC genotype. However, the frequency of CC genotype (28.6%) observed in our mild patient groups is similar to that in general Beijing population (26.2%), according to the International Genome Sample Resource (IGSR) (https://www.internationalgenome.org/1000-genomes-browsers).

DISCUSSION

These findings require further validation but represent important early evidence in a carefully phenotyped cohort for a role of IFITM3 in the severity of COVID-19. Our findings support a hypothesis that host defects in control of intracellular viral replication may result in more prolonged and severe disease. The mechanism of action of this synonymous variant of IFITM3 is still unknown; a truncated version of IFITM3 suggested by earlier work on influenza is unlikely to lead to the differences in viral restriction previously seen [9]. Larger cohort studies are needed to confirm this genetic association with severity of COVID-19, but the data presented here support the need for such genetic analyses in different global populations, with the opportunity for early targeted intervention in persons at risk if robust genetic biomarkers can be established and potential therapeutic targets highlighted.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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