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

Yonghong Zhang,1,2,a Ling Qin,3,a Yan Zhao,1,4 Ping Zhang,2 Bin Xu,1 Kang Li,1 Lianchun Liang,1 Chi Zhang,1 Yanchao Dai,1 Yingmei Feng,1 Jiaping Sun,1 Zhongjie Hu,1 Huiqing Xiang,1 Julian C. Knight,2,4 Tao Dong,1,4 and Ronghua Jin1

1Beijing Youan Hospital, Capital Medical University, Beijing, People’s Republic of China, 2Chinese Academy of Medical Science Oxford Institute, University of Oxford, Oxford, United Kingdom, 3Wellcome Centre for Human Genetics, Nuffield Department of Medicine, University of Oxford, Oxford, United Kingdom, and 4MRC Human Immunology Unit, MRC Weatherall Institute of Molecular Medicine, University of Oxford, Oxford, United Kingdom

INTERFERON-INDUCED TRANSMEMBRANE PROTEIN 3 (IFITM3) is a cellular protein predominantly expressed in the lung and represents an immune effector protein critical to viral restriction [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].
Statistical Analysis

Statistical analysis was performed using χ² 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; β = 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                  | Total (N = 80) | Mild Disease (n = 56) | Severe Disease (n = 24) | Died (n = 3) | PValue 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) | <.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 (8750)              | 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) | | | | | |
| Respiration/min                | 20 (20–21)    | 20 (20–20)           | 21 (20–24.75)          | 23 (20–25)  | <.001   |
| SaO₂ (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   |
| PaO₂/FiO₂ (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; PaO₂/FiO₂, ratio of arterial oxygen partial pressure to fraction of inspired oxygen; SaO₂, arterial oxygen saturation.

aData represent no. (%) of patients unless otherwise specified.

bP-values comparing severe and mild disease groups were calculated by means of χ² 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.
homzygous 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

Author contributions. Study conception and design: Y. Zhang, T. D., and R. J. Performance of experiments and data analysis: Y. Zhang, L. Q., Y. Zhao, P. Z., and J. C. K. Clinical sampling and data collection: B. X., K. L., L. L., C. Z., Y. D., Y. F., J. S., Z. H., H. X., and R. J. Writing, review, and/or revision of the manuscript: Y. Zhang, J. C. K., and T. D.

Financial support. This work was supported by the Beijing Natural Science Foundation (grants 7191004 and 7202069); the Beijing Municipal Science & Technology Commission (grant Z171100001017078); the Beijing Municipal Administration of Hospitals (grants DFL20181701 and ZYLX201711); the Beijing Key Laboratory (grant BZ0373); the National Key Research and Development Project (grant 2020YFC0841700); the Chinese Academy of Medical Sciences Innovation Fund for Medical Sciences (grant 2018-I2M-2-002); the Medical Research Council (MRC), United Kingdom (grant MR/L018942/1 to T. D. and the MRC Human Immunology Unit Core); the Nuffield Department of Medicine, Oxford University (T. D.); the Wellcome Trust (investigator award (204969/Z/16/Z to J. C. K.); and the NIHR Oxford Biomedical Research Centre with Wellcome Trust (grants 090532/Z/09/Z and 203141/Z/16/Z to J. C. K. and core facilities of the Wellcome Centre for Human Genetics).

Potential conflicts of interest. All authors: No reported conflicts. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

1. Report of the WHO-China Joint Mission of Coronavirus Disease 2019 (COVID-19). https://www.who.int/docs/default-source/coronaviruse/who-china-joint-mission-on-covid-19-final-report.pdf. Accessed 16–24 February 2020.
2. Chapman SJ, Hill AV. Human genetic susceptibility to infectious disease. Nat Rev Genet 2012; 13:175–88.
3. Zhao X, Sehgal M, Hou Z, et al. Identification of residues controlling restriction versus enhancing activities of IFITM proteins on entry of human coronaviruses. J Virol 2018; 92:e01535–17.
4. Zhang YH, Zhao Y, Li N, et al. Interferon-induced transmembrane protein-3 genetic variant rs12252-C is associated with severe influenza in Chinese individuals. Nat Commun 2013; 4:1418.
5. Brass AL, Huang IC, Benita Y, et al. The IFITM proteins mediate cellular resistance to influenza A H1N1 virus, West Nile virus, and dengue virus. Cell 2009; 139:1243–54.
6. The National Health Commission of China. The diagnosis and treatment of COVID-19. http://www.nhc.gov.cn/yzygj/s7653p/202002/d4b895337e19445f8d728fcafe1e3e13a.shtml. Accessed 8 February 2020.
7. Guan WJ, Ni ZY, Hu Y, et al; China Medical Treatment Expert Group for Covid-19. Clinical characteristics of coronavirus disease 2019 in China. N Engl J Med 2020; 382:1708–20.
8. Zhang Y, Makvandi-Nejad S, Qin L, et al. Interferon-induced transmembrane protein-3 rs12252-C is associated with rapid progression of acute HIV-1 infection in Chinese MSM cohort. AIDS 2015; 29:889–94.
9. Makvandi-Nejad S, Laurenson-Schafer H, Wang L, et al. Lack of truncated IFITM3 transcripts in cells homozygous for the rs12252-C variant that is associated with severe influenza infection. J Infect Dis 2018; 217:257–62.