BRIEF COMMUNICATION

HLA — Immune Response Genetics

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Association of *HLA-DRB1*09:01* with severe COVID-19

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HLA-A, -C, -B, and -DRB1 genotypes were analyzed in 178 Japanese COVID-19 patients to investigate the association of HLA with severe COVID-19. Analysis of 32 common HLA alleles at four loci revealed a significant association between *HLA-DRB1*09:01* and severe COVID-19 (odds ratio [OR], 3.62; 95% CI, 1.57–8.35; $p = 0.00251$ [permutation p value = 0.0418]) when age, sex, and other common HLA alleles at the DRB1 locus were adjusted. The *DRB1*09:01* allele was more significantly associated with risk for severe COVID-19 compared to preexisting medical conditions such as hypertension, diabetes, and cardiovascular diseases. These results indicate a potential role for HLA in predisposition to severe COVID-19.

KEYWORDS
association, coronavirus disease 2019, HLA, Japan, risk factor, severe acute respiratory syndrome coronavirus 2

Coronavirus disease 2019 (COVID-19) is caused by a newly discovered coronavirus, severe acute respiratory syndrome coronavirus (SARS-CoV)-2, and currently poses a major global public health problem. The clinical spectrum of SARS-CoV-2 infection ranges from asymptomatic to death.[1] Although it has been shown that major risk factors for severe symptoms and mortality in COVID-19 include advanced age and male sex[2,3] data on host genetic risk factors are limited. HLA alleles are associated with disease outcome in many infectious diseases, such as human immunodeficiency virus (HIV)[4] and malaria.[5] A genome-wide association study (GWAS) in Europe and United Kingdom showed no significant association of classical HLA alleles as well as single nucleotide polymorphisms (SNPs) in the HLA region with either COVID-19 or disease severity.[6,7] However, a recent study in Italy on the association of HLA with severe COVID-19 noted an association between HLA and severe COVID-19.[8] These contradictory findings warrant further study on the role of HLA in COVID-19 disease. Furthermore, the HLA region is the most variable in the human genome, and the influence of these alleles on the natural history of COVID-19 may differ among populations. The aim of this study was to investigate the association of HLA-A, -C, -B, and -DRB1 alleles with severe COVID-19 in Japanese.

We recruited 178 unrelated Japanese individuals with COVID-19, verified by a confirmed SARS-CoV-2 RNA polymerase-chain-reaction (PCR) nasopharyngeal swab test[9] at four hospitals in Japan: 147 patients from Yokohama Municipal Citizen's Hospital (Kanagawa), and 31 patients from three hospitals in Tokyo: Research Hospital, Institute of Medical Science, University of Tokyo ($N = 14$), National Center for Global Health and Medicine ($N = 9$), and JR Tokyo General Hospital ($N = 8$). Disease severity was categorized by chest computed tomography or X-ray and clinical care (Table 1). Mild disease was defined as asymptomatic or symptomatic without pneumonia. Moderate and severe disease was defined as the presence of pneumonia without or with the need for supplemental oxygen, respectively. Critical disease was defined as the need for ICU admission and/or mechanical ventilation. Patients with mild or moderate disease are classified as "non-severe," while those with severe or critical disease are grouped as "severe" for the study. Information on preexisting medical conditions—hypertension, diabetes, and cardiovascular diseases, was available for 174 patients. The study protocol was approved by the ethics committees of the National Institute of Infectious Diseases, University of Tokyo, and each hospital. Written informed consent was obtained from study participants and all samples were anonymized.

Genomic DNA was isolated from peripheral blood samples. HLA class I (HLA-A, -C, and -B) and class II (HLA-DRB1) four-digit allele typing was performed using the Luminex 200 system (Luminex, Austin, TX) and WAKFlow HLA Typing kit (Wakunaga, Hiroshima, Japan), which is specifically designed for HLA genotyping of Japanese individuals.

Age was expressed as medians and interquartile ranges (IQR). Categorical variables were summarized as counts and percentages. The associations between HLA alleles and clinical characteristics with severe COVID-19 were examined using logistic regression analysis (see Supporting Information, Appendix S1). The odds ratio (OR) and the 95% confidence interval (95% CI) were calculated from the beta coefficient and the SE estimated in the logistic regression analysis.

The clinical characteristics of 178 patients with COVID-19 are presented in Table 1. The median age of the 178 patients was 57.5 years (IQR 44.0–70.0), ranging from 20 to 94 years, and 106 of 178 patients (60%) were male. All cases, with the exception of one patient, were symptomatic, and 170 of 178 (96%) were hospitalized. Seventy-three and 105 patients were defined as severe and nonsevere COVID-19, respectively. It should be noted that the actual proportion of nonsevere cases to severe cases in Japanese patients with COVID-19 is larger than that in Table 1, since hospitalized patients were mainly recruited in this study. Advanced age and male sex were risk factors for severe COVID-19 (Table S1). Twenty of 95 (21%) individuals less than 60 years of age versus 53/83 (64%) of individuals greater than 60 years of age presented with severe COVID-19. Diabetes was significantly associated with severe COVID-19 when age and sex were adjusted (OR, 3.87; 95% CI, 1.53–9.79; $p = 0.00434$), while hypertension and cardiovascular disease were not (Table S1).

A total of 103 HLA alleles were detected at four HLA loci, HLA-A, -C, -B, and -DRB1, in our study cohort. The allele frequencies of the *DRB1*09:01* allele in severe and nonsevere cases are presented in Table S2. We selected 32 common HLA alleles (frequency > 0.05) for the association analysis of which *HLA-DRB1*09:01* was significantly associated with risk of severe COVID-19 (OR, 3.62; 95% CI, 1.57–8.35; $p = 0.00251$ [permutation p value = 0.0418]) when age, sex, and other common HLA alleles at the DRB1 locus were adjusted. (Table 2 and Figure S1).

To examine the effect of preexisting medical conditions on the association between *DRB1*09:01* and COVID-19 severity, hypertension, diabetes, and cardiovascular disease were included as co-variables in the logistic regression model for potential confounding. The *DRB1*09:01* allele was still significantly associated with

TABLE 1 Clinical characteristics of the study patients, stratified by the disease severity

Characteristic	All COVID-19 patients (N = 178)	Patients with severe disease (N = 73)	Patients with nonsevere disease (N = 105)
Age (years)			
Median (interquartile ranges)	57.5 (44.0–70.0)	68.0 (59.0–77.0)	49.0 (35.0–60.0)
Distribution no. (%)			
20–29 years	17 (9.6)	0 (0.0)	17 (16.2)
30–39 years	18 (10.1)	1 (1.4)	17 (16.2)
40–49 years	30 (16.9)	9 (12.3)	21 (20.0)
50–59 years	30 (16.9)	10 (13.7)	20 (19.0)
60–69 years	33 (18.5)	20 (27.4)	13 (12.4)
70–79 years	32 (18.0)	19 (26.0)	13 (12.4)
80–89 years	15 (8.4)	11 (15.1)	4 (3.8)
90–99 years	3 (1.7)	3 (4.1)	0 (0.0)
Male sex no. (%)	106 (59.6)	47 (64.4)	59 (56.2)
Severity no. (%)			
Mild: Symptomatic without pneumonia	30 (16.9)	-	30 (28.6)
Moderate: Symptomatic with pneumonia, no oxygen therapy	75 (42.1)	-	75 (71.4)
Severe: Symptomatic with pneumonia, conventional oxygen therapy	51 (28.7)	51 (69.9)	-
Critical: Admission to intensive care unit (ICU) or use of mechanical ventilation	22 (12.4)	22 (30.1)	-
Pre-existing medical conditions—no. (%)[a]			
Hypertension	48 (27.6)	30 (42.9)	18 (17.3)
Diabetes	33 (19.0)	24 (34.3)	9 (8.7)
Cardio vascular diseases	10 (5.7)	9 (12.9)	1 (1.0)
DRB1 genotype—no. (%)[b]			
09:01/09:01	5 (2.8)	5 (6.8)	0 (0.0)
09:01/X	41 (23.0)	19 (26.0)	22 (21.0)
X/X	132 (74.2)	49 (67.1)	83 (79.0)

[a]Information on pre-existing medical conditions was acquired from 174 patients (70 severe cases and 104 nonsevere cases) with COVID-19.
[b]X stands for any other DRB1 allele.

severe COVID-19 (OR, 2.66; 95% CI, 1.22–5.80; $p = 0.0139$; Model 6, Table S1). In this model, diabetes again showed a significant association with severe COVID-19.

Since a high degree of linkage disequilibrium (LD) is observed in the HLA region, the *DRB1*09:01* allele may be a marker for causal SNPs in nearby genes. To examine this, LD coefficients (r^2) between *DRB1*09:01* and 22,881 SNPs surrounding the HLA-DRB1 locus were calculated (Figure S2). Eight SNPs were found to be in strong LD ($r^2 > 0.95$) with *DRB1*09:01*. Since these SNPs are located close to the HLA-DRB1 locus, the significant association of *DRB1*09:01* with severe COVID-19 is unlikely to be caused by SNPs on other genes. Genotyping of the DRB1 locus, unlike SNPs, while direct, is costly and time consuming. Since rs375979285, rs75314265, and rs79572840 showed perfect LD ($r^2 = 1$) with *DRB1*09:01*, each of these can be used as a proxy for *DRB1*09:01*. In all populations studied in the 1000 Genomes Project (1KG) phase 3,[10] two SNPs, rs117108573 and rs117501019, exhibit relatively high degree of LD with *DRB1*09:01* ($r^2 = 0.94$). The geographic distributions of rs117108573-T and rs117501019-T alleles (Figure S3) are expected to reflect that of *DRB1*09:01* presented in Table S3. In populations where the frequency of rs117108573-T or rs117501019-T is high, such as East Asian populations, it may be considered as a marker for risk of severe COVID-19.

TABLE 2 Association of HLA-A, -C, -B, and -DRB1 alleles with severe COVID-19 in symptomatic Japanese patients

Locus	Allele	Severe (2N = 146) no. (%)[a]	Nonsevere (2N = 210) no. (%)[a]	OR (95% CI)[b]	p[c]
A	A*02:01	14 (0.10)	26 (0.12)	0.885 (0.299–2.62)	0.826
	A*02:06	12 (0.08)	14 (0.07)	1.16 (0.345–3.91)	0.809
	A*11:01	15 (0.10)	17 (0.08)	1.76 (0.579–5.33)	0.320
	A*24:02	55 (0.38)	70 (0.33)	1.75 (0.716–4.27)	0.220
	A*26:01	12 (0.08)	16 (0.08)	1.66 (0.502–5.45)	0.407
	A*31:01	11 (0.08)	13 (0.06)	1.49 (0.451–4.95)	0.511
	A*33:03	13 (0.09)	27 (0.13)	0.945 (0.306–2.92)	0.921
	Others	14 (0.10)	27 (0.13)	ND	ND
C	C*01:02	18 (0.12)	34 (0.16)	0.980 (0.364–2.63)	0.967
	C*03:03	19 (0.13)	29 (0.14)	0.884 (0.309–2.53)	0.818
	C*03:04	19 (0.13)	21 (0.10)	2.05 (0.753–5.58)	0.160
	C*04:01	9 (0.06)	11 (0.05)	1.57 (0.416–5.96)	0.505
	C*07:02	20 (0.14)	27 (0.13)	1.09 (0.392–3.04)	0.866
	C*08:01	8 (0.05)	11 (0.05)	1.45 (0.395–5.34)	0.574
	C*12:02	18 (0.12)	25 (0.12)	1.59 (0.585–4.31)	0.364
	C*14:02	10 (0.07)	8 (0.04)	3.13 (0.848–11.6)	0.0868
	C*14:03	11 (0.08)	21 (0.10)	1.25 (0.386–4.04)	0.712
	Others	14 (0.10)	23 (0.11)	ND	ND
B	B*07:02	12 (0.08)	13 (0.06)	1.65 (0.542–5.03)	0.377
	B*15:01	13 (0.09)	15 (0.07)	1.23 (0.434–3.46)	0.701
	B*35:01	13 (0.09)	24 (0.11)	0.510 (0.204–1.27)	0.149
	B*40:01	9 (0.06)	16 (0.08)	1.38 (0.443–4.29)	0.579
	B*40:02	12 (0.08)	7 (0.03)	2.96 (0.927–9.45)	0.0669
	B*44:03	11 (0.08)	20 (0.10)	0.954 (0.344–2.65)	0.927
	B*51:01	14 (0.10)	11 (0.05)	2.31 (0.760–7.01)	0.140
	B*52:01	17 (0.12)	25 (0.12)	1.48 (0.618–3.57)	0.377
	B*54:01	5 (0.03)	16 (0.08)	0.364 (0.106–1.26)	0.110
	Others	40 (0.27)	63 (0.30)	ND	ND
DRB1	DRB1*01:01	10 (0.07)	13 (0.06)	1.64 (0.531–5.08)	0.388
	DRB1*04:05	15 (0.10)	26 (0.12)	1.93 (0.783–4.75)	0.153
	DRB1*08:03	7 (0.05)	14 (0.07)	0.792 (0.253–2.49)	0.690
	DRB1*09:01	29 (0.20)	22 (0.10)	3.62 (1.57–8.35)	0.00251[d]
	DRB1*13:02	12 (0.08)	19 (0.09)	1.56 (0.548–4.45)	0.404
	DRB1*15:01	17 (0.12)	16 (0.08)	1.55 (0.595–4.01)	0.371
	DRB1*15:02	16 (0.11)	25 (0.12)	1.68 (0.673–4.20)	0.266
	Others	40 (0.27)	75 (0.36)	ND	ND

Abbreviation: ND, not determined.

[a]The count and the frequency of each HLA allele are shown.

[b]The odds ratio (OR) and the 95% confidence interval (CI) were calculated from the beta coefficient and the standard error estimated in the logistic regression analysis.

[c]p-value was obtained using logistic regression analysis adjusted for age and sex.

[d]p_{perm} = 0.0418 in DRB1*09:01.

The present study revealed a significant association of the HLA class II allele, *DRB1*09:01*, with risk of severe COVID-19 in a cohort of Japanese patients. Five (7%) of 73 patients with severe COVID-19 and none of 105 nonsevere cases were homozygous for *DRB1*09:01* (Table 1). The ORs for diabetes and *DRB1*09:01* were 3.21 and 2.66, respectively (Model 6, Table S1). Although the OR for one copy of the *DRB1*09:01* allele was smaller than that for diabetes, the OR for *DRB1*09:01* homozygote (two copies of *DRB1*09:01* allele) was estimated to be 7.07 (= exp (2 × log[2.66])), implying that homozygous *DRB1*09:01* could be a more important risk factor for severe COVID-19 than preexisting medical conditions.

This study showed an association of *DRB1*09:01* with COVID-19 severity but did not address the impact of *DRB1*09:01* on the susceptibility to SARS-CoV-2 infection. Indeed, the allele frequency of *DRB1*09:01* (14.2%) in study participants is equivalent to that reported for the general Japanese population (14.5%),[11] suggesting that this allele is not a risk-factor for SARS-CoV-2 infection.

Previous studies have reported associations of several HLA class II alleles on SARS-CoV disease.[12,13] However, the role of HLA in COVID-19 disease remains controversial. One recent study in Italy reported associations between *DRB1*15:01* and *DQB1*06:02* and COVID-19[8], however, other studies have found no associations between HLA and disease.[6] The present study suggests that SARS-CoV-2-mediated disease progression could also be affected by HLA class II polymorphisms. The major role of HLA class II molecules is to process exogenous peptides for presentation to CD4+ T cells, which play a crucial role in antiviral cellular and humoral immunity. It has been reported that robust SARS-CoV-2-specific CD4+ T-cell responses are induced following infection and correlate with the number of plasmablasts and SARS-CoV-2-specific IgG and IgA levels.[14,15] Thus, an association between *DRB1*09:01* and severe COVID-19 found in the present study may be attributed to skewed CD4+ T-cell responses in *DRB1*09:01*+ patients. Further studies comparing SARS-CoV-2–specific CD4+ T-cells in infected individuals with and without the *DRB1*09:01* allele are ongoing.

Our study is the first in Asia, to our knowledge, to describe an association between HLA and severe COVID-19. The allele frequencies of *DRB1*09:01* estimated from the SNP genotype data were high in 1KG-East Asian populations, but low in 1KG-European populations (Table S3). According to the Allele Frequency Net Database (http://www.allelefrequencies.net/), the *DRB1*09:01* allele is very rare in Spanish and Italian populations (0.3% and 0.6%, respectively), and the allele frequency is low (1.2 ~ 1.4%) in the United Kingdom. Therefore, previous GWASs in European populations[6,7] may not have detected association signals in the HLA class II region due to a lack of statistical power. However, the effect size of *DRB1*09:01* was modest, and the present study did not provide strong evidence against the null hypothesis (i.e., the permutation *p*-value was slightly below 0.05). Future studies with larger sample sizes are therefore needed to replicate the association of *DRB1*09:01* in Japanese or East Asians and to explore other HLA alleles associated with risk of severe COVID-19 in populations worldwide.

CONFLICT OF INTEREST
The authors declare no conflicts of interest associated with this manuscript.

AUTHOR CONTRIBUTIONS
A. Kawana-Tachikawa, J. Ohashi, T. Matano, T. Suzuki, Y. Takahashi designed the study. A. Anzurez, M. Koga, K. Nakayama-Hosoya, M. Nakamura-Hoshi, S. Seki, T. Matsumura, T. Takano, T. Onodera, Y. Adachi, S. Moriyama, and K. Terahara performed the experiments. A. Anzurez, J. Ohashi, I. Naka, M. Isshiki, and Y. Watanabe performed data analysis. N. Tachikawa, Y. Yoshimura, H. Sasaki, H. Horiuchi, N. Miyata, K. Miyazaki, M. Koga, K. Ikeuchi, H. Nagai, M. Saito, E. Adachi, H. Yotsuyanagi, S. Kutsuna, A. Kawashima, Y. Miyazato, N. Kinoshita, C. Kouno, and K. Tanaka recruited patients, and collected and analyzed clinical data. J. Ohashi and A. Kawana-Tachikawa wrote the manuscript. All authors have read and approved the manuscript.

DATA AVAILABILITY STATEMENT
The data of HLA genotype and COVID-19 severity for each subject are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.