Received: 4 January 2023 | Revised: 22 August 2023 | Accepted: 26 September 2023

DOI: 10.1111/tan.15251

ORIGINAL ARTICLE

HLA Immune Response Genetics WILEY

*HLA-DPB1*13:01* associates with enhanced, and *KIR2DS4*001* with diminished protection from developing severe COVID-19

Ticiana D. J. Farias[1,2] | **Silvia Brugiapaglia**[3] | **Susanna Croci**[4,5] | **Paola Magistroni**[6] | **Claudia Curcio**[3] | **Kristina Zguro**[5] | **Chiara Fallerini**[4,5] | **Francesca Fava**[4,5,7] | **Francesco Pettini**[5,8] | **Katherine M. Kichula**[1,2] | **Nicholas R. Pollock**[1,2] | **Neus Font-Porterias**[1,2] | **William H. Palmer**[1,2] | **Wesley M. Marin**[9] | **Margherita Baldassarri**[4,5] | **Mirella Bruttini**[4,5,7] | **Jill A. Hollenbach**[9] | **Audrey E. Hendricks**[1,10] | **Ilaria Meloni**[4,5] | **Francesco Novelli**[3,11] | **GEN-COVID Multicenter Study Group** | **Alessandra Renieri**[4,5,7,8] | **Simone Furini**[5] | **Paul J. Norman**[1,2] | **Antonio Amoroso**[6,12]

[1]Department of Biomedical Informatics, University of Colorado School of Medicine, Aurora, Colorado, USA

[2]Department of Immunology and Microbiology, University of Colorado School of Medicine, Aurora, Colorado, USA

[3]Department of Molecular Biotechnology and Health Sciences, University of Turin, Turin, Italy

[4]Medical Genetics, University of Siena, Siena, Italy

[5]Med Biotech Hub and Competence Center, Department of Medical Biotechnologies, University of Siena, Siena, Italy

[6]Immunogenetics and Transplant Biology, Azienda Ospedaliera Universitaria, Città della Salute e della Scienza di Torino, Turin, Italy

[7]Genetica Medica, Azienda Ospedaliero-Universitaria Senese, Siena, Italy

[8]Department of Biotechnology, Chemistry and Pharmacy, University of Siena, Siena, Italy

[9]Department of Neurology, UCSF Weill Institute for Neurosciences, University of California, San Francisco, San Francisco, California, USA

[10]Department of Mathematical and Statistical Sciences, and Department of Biomedical Informatics, University of Colorado School of Medicine, Aurora, Colorado, USA

[11]Molecular Biotechnology Center, University of Turin, Turin, Italy

[12]Department of Medical Sciences, University of Turin, Turin, Italy

Correspondence
Paul J. Norman, Department of Biomedical Informatics, University of Colorado School of Medicine, Aurora, CO 80045, USA.
Email: paul.norman@ucdenver.edu

Extreme polymorphism of HLA and killer-cell immunoglobulin-like receptors (KIR) differentiates immune responses across individuals. Additional to T cell receptor interactions, subsets of HLA class I act as ligands for inhibitory and activating KIR, allowing natural killer (NK) cells to detect and kill infected cells. We investigated the impact of HLA and KIR polymorphism on the

Ticiana D. J. Farias and Silvia Brugiapaglia are first co-authors.

Simone Furini, Paul J. Norman, and Antonio Amoroso are senior co-authors.

Francesco Novelli, Department of Molecular Biotechnology and Health Sciences, University of Turin, Turin 10126, Italy.
Email: franco.novelli@unito.it

Alessandra Renieri, Medical Genetics, University of Siena, Siena 53100, Italy.
Email: alessandra.renieri@unisi.it

Funding information
Conselho Nacional de Desenvolvimento Científico e Tecnológico, Grant/Award Number: 201608/2019-7; fastgrants; Italian Ministry of Education-University and Research-MIUR, Grant/Award Number: D15D18000410001; National Institutes of Health, Grant/Award Number: R01 AI158410; AIRC IG, Grant/Award Number: 19931; The Associazione Italiana Ricerca sul Cancro, Grant/Award Number: 27020; European Union - Next Generation EU - NRRP M6C2 - Investment 2.1 Enhancement and strengthening of biomedical research in the NHS, Grant/Award Number: PNRR-POC-2022-12375658; Fondazione Ricerca Molinette Onlus (CD38 and Associazione Ursula e Giorgio Cytron); Grant for Internalization, Grant/Award Number: CURC_GFI_22_01_FtoCC

[Correction added on 26 October 2023 after first online publication: *HLA-DPB1*013:01* was corrected to *HLA-DPB1*13:01* throughout the article.]

severity of COVID-19. High resolution HLA class I and II and KIR genotypes were determined from 403 non-hospitalized and 1575 hospitalized SARS-CoV-2 infected patients from Italy collected in 2020. We observed that possession of the activating *KIR2DS4*001* allotype is associated with severe disease, requiring hospitalization (OR = 1.48, 95% CI 1.20–1.85, $p_c = 0.017$), and this effect is greater in individuals homozygous for *KIR2DS4*001* (OR = 3.74, 95% CI 1.75–9.29, $p_c = 0.003$). We also observed the HLA class II allotype, *HLA-DPB1*13:01* protects SARS-CoV-2 infected patients from severe disease (OR = 0.49, 95% CI 0.33–0.74, $p_c = 0.019$). These association analyses were replicated using logistic regression with sex and age as covariates. Autoantibodies against IFN-α associated with COVID-19 severity were detected in 26% of 156 hospitalized patients tested. *HLA-C*08:02* was more frequent in patients with IFN-α autoantibodies than those without, and *KIR3DL1*01502* was only present in patients lacking IFN-α antibodies. These findings suggest that KIR and HLA polymorphism is integral in determining the clinical outcome following SARS-CoV-2 infection, by influencing the course both of innate and adaptive immunity.

KEYWORDS
COVID-19, HLA class II, *HLA-DPB1*, IFN-α antibodies, KIR2DS4

1 | INTRODUCTION

The symptoms of SARS-CoV-2 infection vary from none (asymptomatic) to multiple forms of coronavirus disease (COVID-19). Consequences include mild through severe disease, which can lead to respiratory failure and death.[1–3] Diversity of COVID-19 outcome is caused by differential immune responses to SARS-CoV-2 infection, driven in part by polymorphism of immune-response genes.[4,5] Class I and II HLA guide immune responses by presenting peptide fragments to $CD8^+$ T and $CD4^+$ T cells, enabling infected cells to be killed and antibodies to be produced. Additionally, some HLA allotypes can interact specifically with killer cell immunoglobulin-like receptors (KIR) expressed by natural killer (NK) cells and some T cells.[6] HLA and KIR are the most polymorphic genes in the human genome. Their extreme variation affects resistance to infectious disease and susceptibility to autoimmunity, among other conditions.[7–9] Combinatorial diversity of HLA and KIR has established effects on NK cell responses to respiratory infections, as demonstrated by H1N1 influenza and 2003 SARS-CoV.[10,11] In this context, HLA and KIR polymorphism likely impacts immune responses to SARS-CoV-2 and subsequent disease outcome.[12–14]

NK cells are lymphocytes that can recognize virus-infected cells and thereby influence the course of both innate and adaptive immunity.[15–17] NK cell functions are modulated by the balance of inhibitory and activating signals transduced from multiple cell surface receptors, of which inhibitory KIR are the most polymorphic and have dominant effect.[6,18] NK cells are educated during their development through ligation of inhibitory KIR, allowing them to respond to any HLA class I loss that may occur upon viral infection.[19] In addition to controlling acute viral infections, NK cells can also contribute to tissue pathogenesis. In severe COVID-19 patients, the NK cell gene expression profile is enriched toward pro-inflammatory cytokine production.[20–22] In addition, peripheral NK cells are reduced in number during COVID-19 and impaired in patients having severe disease.[22,23] Pro-inflammatory cytokines, including interferon-α (IFN-α), can also modulate NK cell function during SARS-CoV-2 infection.[24]

Genetically driven abnormal levels of IFN-α,[25] as well the presence of autoantibodies against IFN-α have been associated with COVID-19 severity.[26–29]

KIR and HLA are encoded by genes on separate chromosomes, and their combinatorial diversity thus depends on random segregation.[18] The KIR genomic region is characterized by gene presence/absence, copy number variation (CNV), and allele and haplotypic variability, any of which can impact NK cell functions.[9] KIR bind HLA ligands via their extracellular immunoglobulin-like domains (KIR2D have two, KIR3D have three) and those having long (L) cytoplasmatic tails transduce inhibitory signals. Transduction of activating signals by KIR having short (S) cytoplasmic tails is aided by accessory proteins.[30] Interaction of KIR with their specific HLA ligands modulates NK cell functions, for example, KIR3DL2 is an inhibitory receptor that binds *HLA-A*03* and *A*11*, and KIR2DS4 is an activating receptor that binds *HLA-A*11* and a subset of HLA-C allotypes.[9,18] Importantly, the interactions modulate a subset of NK cells having significant cytolytic activity and cytokine secretion.[15,19]

Focused analyses have identified that the distinct HLA variants associating with COVID-19 severity differ across populations.[4] Although the first genome-wide association studies (GWAS) of COVID-19 did not identify HLA variability in disease susceptibility,[31–33] a recent study in the UK showed, through imputation of HLA genotypes, that *HLA-DRB1*04:01* protects against developing severe disease.[34] On the other hand, KIR have remained largely intractable to GWAS studies. Nevertheless, presence and absence polymorphism has been reported associated with COVID-19 severity, and differential KIR expression levels by NK cells have been associated with specific clinical outcomes.[35–37] Due to limitations of HLA genotyping methods or KIR presence/absence analysis, limited sample size, different population ancestries and clinical outcomes evaluated, the role of combined HLA and KIR polymorphism in determining COVID-19 severity remains unclear. Population-based studies in Italy[38–42] and one association study with disease severity in Sardinia[35] have reported that HLA diversity could impact on disease severity and susceptibility. The aim of this study is to analyze HLA and KIR diversity in hospitalized and non-hospitalized COVID-19 from a large Italian cohort and its potential impact on NK cell function following SARS-CoV-2 infection. Additionally, we evaluated the impact of presence of IFN-α antibodies in hospitalized patients.

2 | METHODS

2.1 | COVID-19 cohort

We studied 1978 SARS-Cov-2 infected subjects of differing disease severity belonging to the Italian GEN-COVID Multicenter study (https://sites.google.com/dbm.unisi.it/gen-covid). This is a network of more than 40 Italian Hospitals which, between March 2020 and February 2021, collected plasma, serum, and blood samples as well as clinical data for COVID-19 research. Patients infected by SARS-CoV-2 were diagnosed using RT-PCR from nasopharyngeal swab.[43] Peripheral blood samples were collected in BD Vacutainer® PPT™ Plasma Preparation Tube K2E (EDTA). Plasma was isolated immediately using centrifugation and stored at −80°C in aliquots for subsequent analyses. DNA was extracted from EDTA peripheral blood samples using MagCore HF16 kit (Diatech Lab-Line, Jesi, Ancona, Italy) according to the manufacturer's instructions. Written informed consent was obtained from all patients. The GEN-COVID study was performed in accordance with the relevant international, EU, national, and institutional guidelines and approved in advance by the University Hospital (Azienda Ospedaliero-Universitaria Senese) Ethical Review Board, Siena, Italy (Prot n. 16,917, dated March 16, 2020). For association analysis, the cohort was divided into 403 patients having pauci-symptoms who were not hospitalized, and 1575 patients who were hospitalized due to severe COVID-19. For the latter, a subcategory of critical patients (required CPAP/BiPAP and high-flow oxygen therapy, or intubation, or the outcome was death) was analyzed where indicated. The demographic data are shown in Table 1.

2.2 | Sequencing HLA and KIR genes

HLA-class I and II and KIR genes were targeted for DNA sequencing using a well-established biotinylated DNA probe-based capture method,[44] with modifications as follows. Genomic DNA (500 ng from each sample) was fragmented enzymatically using the NEBNext® Ultra II FS module (E7810, New England Biolabs, Boston, MA) followed by NEBNext® Ultra II Ligation module (E7959, New England Biolabs, Boston, MA) with IDT xGen Stubby Adapters (Integrated DNA Technologies, Coralville, IA). Next, we performed post ligation cleanup and 800 bp DNA fragments were isolated by dual size selection with Agencourt AMPure XP beads (Beckman Coulter Life Sciences, Indianapolis, IN). Individual samples were labeled uniquely using 5 μL of 10 μM custom dual-index primers (Integrated DNA Technologies, Coralville, IA). Post amplification cleanup was based on the Kapa Hyper Prep protocol (Kapa Biosystems, Wilmington, MA). Equivalent quantities of each DNA library were pooled for enrichment. The KIR and HLA enrichment step[44] was modified as previously described.[45] Paired ends of 250 bp each were sequenced using a NovaSeq™ 6000 instrument and SP Reagent Kit (Illumina Inc, San Diego, CA). The read depth was greater than 150X

TABLE 1 Clinical and demographic frequencies of cohort and association with COVID-19 severity (logistic regression).

	Non-hospitalized $N = 403$ N (%)	Hospitalized $N = 1575$ N (%)	OR	CI (95%)	p
Clinical categories					
1. Non-hospitalized	403 (100%)	-	-		
2. Severe					
(a)	-	900 (57.1%)			
(b)	-	675 (42.9%)			
Sex					
Female	242 (60.0%)	564 (35.8%)	Ref		
Male	161 (40.0%)	1011 (64.2%)	2.67	2.14–3.35	<0.001
	$N = 403$ F (%)	$N = 1573$ F (%)			
Age categories					
<60 years	335 (83.1%)	611 (38.8%)	Ref		
≥60 years	68 (16.9%)	962 (61.2%)	7.89	5.99–10.52	<0.001
Age 18–29	35 (8.7%)	28 (1.8%)	-		
Age 30–39	61 (15.1%)	77 (4.9%)			
Age 40–49	125 (31.0%)	148 (9.4%)			
Age 50–59	114 (28.3%)	358 (22.8%)			
Age 60–69	43 (10.9%)	372 (23.6%)			
Age 70–79	20 (5.0%)	331 (21.0%)			
Age 80–89	4 (1.0%)	202 (12.8%)			
Age ≥ 90	0 (0.0%)	57 (3.6%)			

Note: The clinical categories were as follows: (1) non-hospitalized: asymptomatic or pauci-symptomatic, (2) Severe COVID-19 hospitalized patients (a) who did not require oxygen therapy, or oxygen support only and (b) patients who required CPAP/BiPAP and high-flow oxygen therapy, or intubation, or the outcome was death.

Abbreviations: CI, confidence interval; *N*, number of patients; OR, odds ratio; Ref, reference group for association study.

for all HLA and KIR genes. HLA alleles were determined from the sequence data using the consensus calls obtained from three algorithms: NGSengine® 2.10.0 (GenDX, Utrecht, the Netherlands), HLA Explore™ (Omixon Biocomputing Ltd. Budapest, Hungary) and *HLA*LA*.[46] In >99% of cases the three algorithms agreed at two fields of resolution. KIR copy number variation (CNVs) and alleles were obtained using the PING (Pushing Immunogenetics to the Next Generation) pipeline,[47] which operates in R.[48] We obtained 2 fields of resolution for all KIR and HLA alleles.

2.3 | Association of HLA and KIR polymorphism with COVID-19 hospitalization

We first inferred the genetic ancestry of individual HLA-A-B genotypes using a model-based clustering algorithm, STRUCTURE v2.3.4[49] as described previously.[50] We used HLA-A∼B data from the 1000 Genomes[51,52] and the present cohort, with a burn-in length of 10,000 and 20,000 MCMC resamples, and the number of assumed populations (K) set to 3. HLA-C was not included in the analysis, since STRUCTURE expects independent genetic markers, and HLA-B and -C are in strong LD and could bias the inference. Two-sample non-paired Wilcoxon tests were applied to compare the distribution of ancestry proportions between the hospitalized and non-hospitalized groups. We performed HLA and KIR association by comparing allele frequencies of hospitalized with non-hospitalized patients, using the BIGDAWG package for R.[53] For association studies, Fisher's exact test, odds ratio (OR), 95% confidence interval (95% CI) and p-values were calculated for any HLA and KIR alleles and haplotypes having frequency >2% across the cohort. We analyzed HLA class I genes: HLA-A, -B, -C, -E, -F, -G, and HLA class II genes: HLA-DRB1, -DQA1, -DQB1, -DPA1, -DPB1 for 403 non-hospitalized and 1575

hospitalized patients. For KIR, we analyzed KIR2DL1-5, KIR2DS1-5, KIR3DL1S1, KIR3DL2 for 402 non-hospitalized and 1569 hospitalized patients. KIR2DS4 alleles were combined as those expressing a functional receptor (KIR2DS4-full) or not (KIR2DS4-null).[54,55] HLA and KIR haplotypes were estimated using "haplostats," included in the BIGDAWG package.[53] The Bonferroni method was used to adjust for multiple tests, where $p < 0.05$ after correction (p_c) was considered significant. The p-values were corrected for 96 alleles for HLA and 57 alleles for KIR.

Any HLA or KIR genes having one or more alleles shown as significant on association analysis were then tested for sensitivity to confounders of sex and age >60 years old using logistic regression analysis using the "glm" function in R.[48] As this is a sensitivity test, we did not correct for multiple testing.[56] For any alleles identified through association analysis, we assessed if there was any effect due to ethnicity or recruitment location by including these factors as covariates using logistic regression.

2.4 | Interactions of KIR with HLA class I

Where indicated, we analyzed specific KIR allotypes in combination with their respective HLA-ligands. *HLA-A*03* and *-A*11* are ligands for KIR3DL2.[57] Any HLA-A or B allotypes carrying the Bw4 motif are ligands for KIR3DL1, with exceptions being *A*25* and *B*13*.[58] C1 + HLA-C and some C2 + HLA-C are ligands for KIR2DL2/3, and C2 + HLA-C are ligands for KIR2DS1 and KIR2DL1.[59,60] HLA ligands for KIR2DS2 are *A*11*, *C*01:02*, *C*16*.[61] HLA ligands for KIR2DS4 are *C*01:02*, *C*02:02*, *C*04:01*, *C*05:01*, *C*14:02*, *C*16:01*, and *A*11:01*.[54] The ligand for KIR2DS3 is unknown, and the only KIR2DS5 allotype present in the cohort (*2DS5*002*) does not bind to HLA class I.[62] In analyzing the lineages of KIR3DL1, allotypes of the 015-lineage or 005 lineage were combined as described.[63] *KIR3DL1*004* and others sharing residue-86 L were considered null alleles due to lack of cell-surface expression.[64]

2.5 | Detection of interferon-α specific antibodies in COVID-19 patients

Plasma from 156 hospitalized patients was subject to IFN-α specific autoantibody detection. The clinical and demographic data and results are presented in Tables S1 and S2. Differences between subjects with or without IFN-α antibodies were compared using Pearson's χ^2 using R, with $p < 0.05$ as the threshold for significance. The titer of antibodies against one or more of the 12 IFN-α subunits was measured using ELISA. Human recombinant proteins of IFN-α (Vinci-Biochem, Firenze, Italy) were coated onto flat-bottomed plates and incubated overnight at 4°C, with the following concentrations: IFNA1 5 μg/mL, IFNA2 5 μg/mL, IFNA4 10 μg/mL, IFNA5 8 μg/mL, IFNA6 20 μg/mL, IFNA7 10 μg/mL, IFNA8 8 μg/mL, IFNA10 5 μg/mL, IFNA14 8 μg/mL, IFNA16 20 μg/mL, IFNA17 20 μg/mL, IFNA21 10 μg/mL, all in 0.1 mol/L Na_2CO_3. Plasma was diluted at 1:500 in PBS containing 1% bovine serum albumin (BSA) and 0.05% Tween-20. Anti-human HRP (GE Healthcare, Milan, Italy) diluted 1:3000 was then added followed by tetramethylbenzidine (Tebu Bio, Magenta, Italy) incubation. Rabbit polyclonal antibody against human IFN-α (Vinci-Biochem) was included as appropriate positive control. The Delta values of human plasma were calculated by subtracting the optical density of coated wells from the uncoated wells to account for background signal. We compared HLA and KIR allele frequencies between patients with and without IFN-α specific antibodies, analyzing alleles that were >2% in frequency ($N = 150$).

2.6 | Estimation of HLA binding affinity for SARS-CoV-2 Spike derived peptides

We performed in silico prediction of the affinity for HLA class II allotypes of peptide fragments derived from SARS-CoV-2 Spike protein. We used netMHCIIpan v4.0, setting $a < 0.5\%$ rank for strong binders.[65] The Spike protein of SARS-CoV-2 alpha (B.1.1.7) virus strain was used (UniProtKB P0DTC2).[66] Sliding windows for peptide fragments were set at 15 amino acids in length.[65] Viable HLA class II allotypes[67,68] were subdivided into two groups: "broad" or "limited" presenters, with the threshold being the median number of distinct peptides predicted to bind across all HLA-DP, DQ or DR allotypes. Multivariate transformation and visualization was performed using the function "prcomp" and package "factoextra" (https://cloud.r-project.org/package=factoextra/); the two first principal components (PC) were visualized.[48]

3 | RESULTS

3.1 | *HLA-DPB1*13:01* is associated with protection from severe COVID-19

To investigate the impact of HLA polymorphism on SARS-CoV-2 infection course, we analyzed a large cohort

TABLE 2 HLA alleles and haplotypes showing frequency differences, comparing non-hospitalized with severe COVID-19 patients, in an Italian cohort.

Alleles	Non-hospitalized $2N = 806$ Obs. (%)	Severe $2N = 3150$ Obs. (%)	OR	CI (95%)	p	p_c
*HLA-B*08:01*	52 (6.5%)	142 (4.5%)	0.68	0.49–0.97	0.023	1.000
*HLA-C*07:01*	139 (17.2%)	433 (13.7%)	0.76	0.62–0.95	0.012	1.000
*HLA-C*07:02*	48 (6.0%)	253 (8.0%)	1.38	1.00–1.94	0.047	1.000
*HLA-DPB1*13:01*	41 (5.1%)	83 (2.6%)	**0.49**	**0.33–0.74**	**0.0002**	**0.019**
*HLA-DQB1*03:03*	36 (4.5%)	90 (2.9%)	0.63	0.42–0.96	0.020	1.000
HLA class II haplotypes						
*DPA1*01:03∼DPB1*13:01*	3 (0.4%)	5 (0.2%)	0.43	0.08–2.75	0.229	1.00
*DPA1*02:01∼DPB1*13:01*	39 (4.8%)	75 (2.4%)	**0.48**	**0.32–0.73**	**0.0002**	**0.002**
*DPA1*02:02∼DPB1*13:01*	0 (0%)	3 (0.1%)	-			

Note: - = not calculated, p_c = Bonferroni correction for 96 HLA alleles, 10 DPA1∼DPB1 or 10 DQA1∼DQB1 haplotypes analyzed. In bold, $p_c < 0.05$.
Abbreviations: CI, confidence interval; OR, odds ratio.

from Italy, collected in early 2020 and comprised of 403 individuals with mild disease (non-hospitalized) and 1575 patients hospitalized with severe COVID-19. We analyzed 11 HLA genes, comprising highly polymorphic HLA class I (HLA-A, -B, -C), less polymorphic HLA class I (−E, -F, and -G) and HLA class II (HLA-DPA1, -DPB1, -DQA1, -DQB1, -DRB1). In this cohort, we observed 96 HLA alleles having greater than 2% frequency (Table S3). HLA-B is the most variable HLA class I gene, having 16 alleles, followed by HLA-C (13 alleles) and HLA-A (12 alleles). HLA-G has five alleles in the cohort and HLA-E and HLA-F each have two alleles. For HLA class II, HLA-DQB1 is the most variable gene with 12 alleles, followed by HLA-DRB1 (11 alleles), HLA-DQA1 and HLA-DPB1 (10 alleles), and HLA-DPA1 (3 alleles). No differences in genetic ancestry proportions were observed between the severe COVID-19 and non-hospitalized patients (Figure S1).

We compared the HLA allele frequencies of hospitalized with non-hospitalized COVID-19 patients. Regarding the less polymorphic HLA class I genes (HLA-E, -F, and -G), no significant frequency differences were found in relation to disease severity (Table S3). In analyzing highly polymorphic HLA class I, *HLA-B*08:01* and *-C*07:01* were more frequent in the non-hospitalized patients, whereas *-C*07:02* was more frequent in hospitalized patients (Table S3). These differences were not significant after p-value correction ($p_c > 0.05$, Table 2). Of note, the frequencies of the HLA class I ligands for KIR did not differ between hospitalized and non-hospitalized patients (Table S3). Regarding HLA class II, *HLA-DQB1*03:03* and *HLA-DPB1*13:01* were more frequent in non-hospitalized than hospitalized patients (Table S3). However, only *DPB1*13:01* remained statistically significant after Bonferroni correction (OR = 0.49, 95% CI 0.33–0.73, $p = 0.0002$, $p_c = 0.019$, Table 2). The results were stable when including sex, age, and ethnicity as covariates (OR = 0.50, 95% CI 0.32–0.80, $p = 0.003$, Table S4). In addition, *HLA-DPB1*13:01* remained associated when adjusted by the hospital of recruitment (OR = 0.35, 95% CI 0.14–0.92, $p = 0.032$, Table S4). Finally, we evaluated if *HLA-DPB1*13:01* could protect from the most severe outcomes, including patients that need mechanical breathing assistance (CPAP/BiPAP), intubation, or when the outcome was death. Indeed, *HLA-DPB1*13:01* was more frequent in non-hospitalized patients than in these critical cases (OR = 0.46, 95% CI 0.26–0.83, $p = 0.010$, Table S5).

HLA class II molecules are expressed as heterodimers, with specific alleles encoding the respective α and β subunits occurring in genomic proximity and having high linkage disequilibrium (LD). *HLA-DPB1*13:01* can be observed in high LD with one of three -DPA1 alleles (*-DPA1*02:01*, *-DPA1*02:02*, or *-DPA1*01:03*).[69] The *HLA-DPB1*13:01∼HLA-DPA1*02:01* combination was more frequent in non-hospitalized than hospitalized patients (OR = 0.48, 95% CI 0.32–0.73, $p = 0.0002$, $p_c = 0.002$, Table 2). Concurrence of *HLA-DPB1*13:01* with *HLA-DPA1*01:03* was also more frequent in non-hospitalized than hospitalized patients, although in low frequency (0.4% vs. 0.2%). The *HLA-DPB1*13:01∼DPA1*02:02* haplotype was not analyzed as it was present in only three patients (Table 2). As neither of the DPA1 alleles associated with disease state, these findings suggest *DPB1*13:01* is driving the observed protection from severe COVID-19.

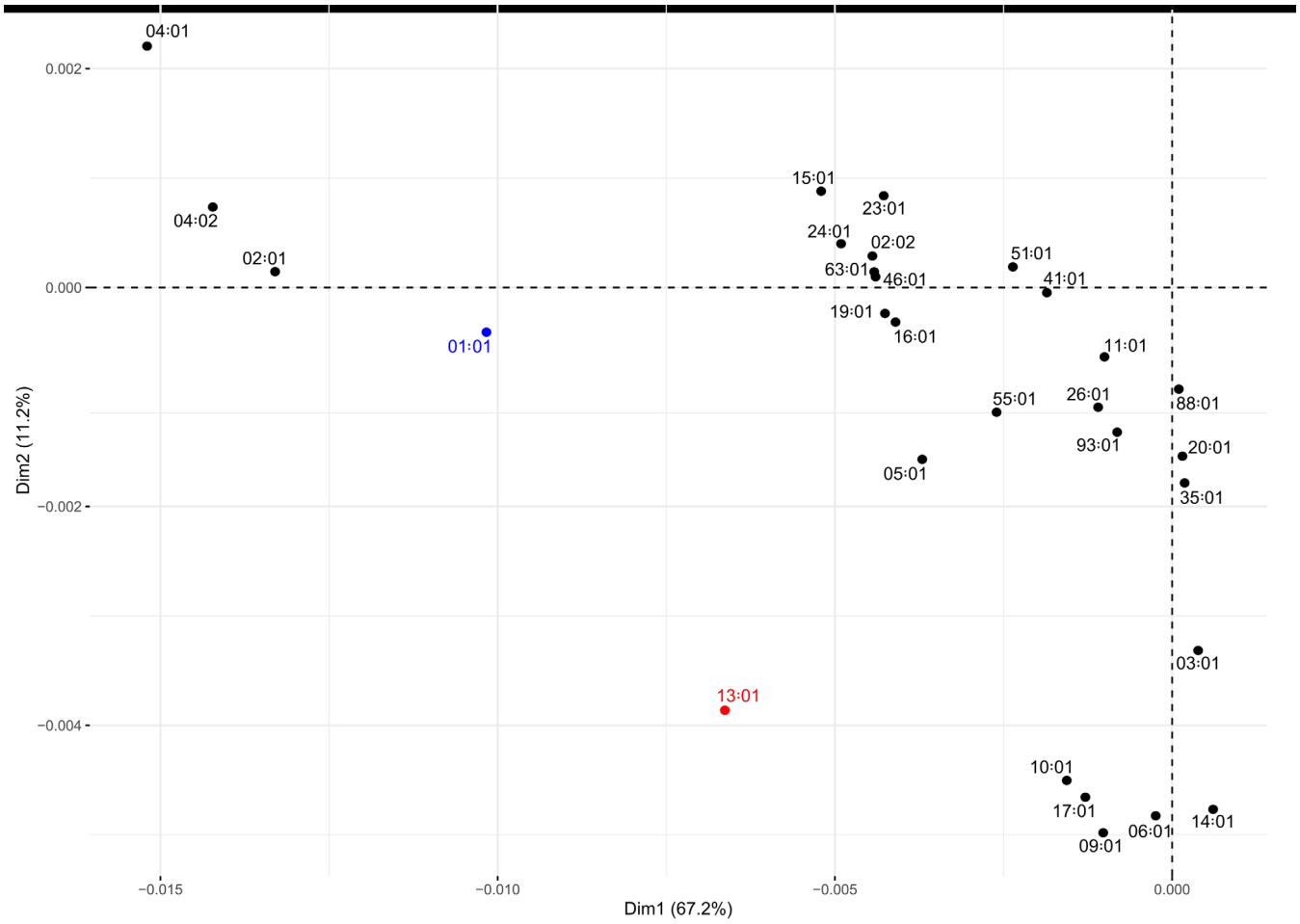

FIGURE 1 HLA-DPβ1*13:01 is predicted to bind a distinct repertoire of SARS-CoV-2 Spike protein derived peptide fragments. Shown is a principal component analysis (PCA) plot of the peptide fragments predicted to bind the HLA-DPβ1 allotypes indicated. DPβ1*13:01 is shown in red and DPβ1*01:01 in blue.

3.2 | HLA-DPβ1*13:01 has distinct peptide and NK cell receptor binding properties

Antibodies against SARS-CoV-2 proteins help control infection and disease severity.[26,29] As the differential peptide binding characteristics of HLA class II allotypes guide production of antibody specificity, we estimated the binding affinities of peptides derived from the Spike protein for each potential HLA-class II allotype (Table S6). HLA allotypes were divided according to the number of distinct Spike peptides they could present (broad, limited, or none). By this measure, the HLA-DPα1*02:01∼DPβ1*13:01 heterodimer was classified as a broad binder (Table S6). The other two HLA-DPβ1*13:01 heterodimers observed in our cohort (DPα1*01:03 or DPα1*02:02) were also predicted to be broad Spike peptide binders (Table S6). Suggesting differential peptide binding contributes to differential disease risk, the Spike-derived peptide binding repertoire of DPβ1*13 clusters distinctly from that of other DP molecules (Figure 1). This finding suggests HLA-DPβ1*13:01-Spike peptide complexes encourage production of a distinct antibody specificity profile. Some HLA-DP allotypes can also bind NKp44, which is an NK cell activating receptor.[70] Among these molecules HLA-DPα1*02:01∼DPβ1*13:01 and DPα1*02:01∼DPβ1*01:01 are the strongest NKp44 binders.[70] As we saw no association with disease for *HLA-DPB1*01:01* (Table S3), and interaction with Nkp44 is peptide-specific,[70] this distinction could also be explained by the differential peptide repertoires of DPβ1*13:01 and DPβ1*01:01. Although it is important to note that the in silico analysis assessed peptide binding without regard to their immunogenic properties, these findings imply that DPβ1*13 can influence both antibody production and NK cell driven killing in response to SARS-CoV-2 infection. An alternative proposed mechanism is that DPβ1*13:01 may be able to resist downregulation by SARS-CoV-2 immunomodulatory ORF7a.[71]

TABLE 3 KIR alleles and haplotypes associated with COVID-19 severity, comparing non-hospitalized to hospitalized patients.

	Non-hospitalized $2N = 804$ F (%)	Hospitalized $2N = 3138$ F (%)	OR	CI (95%)	p	p_c
(a) Alleles						
*KIR2DL4*00102*	109 (13.6%)	558 (17.8%)	1.38	1.10–1.74	0.004	0.228
*KIR2DL4*00802*	142 (17.7%)	466 (14.9%)	0.81	0.66–1.01	0.049	1.000
*KIR2DS3*00103*	94 (11.7%)	474 (15.1%)	1.34	1.06–1.72	0.014	0.798
*KIR2DS3*00201*	18 (2.2%)	124 (4.0%)	1.80	1.08–3.15	0.020	1.000
*KIR2DS4*00101*	120 (14.9%)	648 (20.7%)	**1.48**	**1.20–1.85**	**0.0003**	**0.017**
*KIR2DS4*00601*	148 (18.4%)	472 (15.0%)	0.78	0.64–0.97	0.019	1.000
*KIR3DL2*00201*	99 (12.3%)	526 (16.8%)	1.43	1.14–1.82	0.002	0.120
*KIR3DL1*00201*	80 (10.0%)	393 (12.5%)	1.30	1.00–1.69	0.045	1.000
*KIR3DL1*00401*	125 (15.5%)	384 (12.2%)	0.76	0.61–0.95	0.013	0.741
(b) Genotype						
*2DS4*null/*null*	289 (71.9%)	997 (63.5%)	Ref.			
*2DS4*full/*null*	106 (26.4%)	477 (31.6%)	1.34	1.03–1.76	0.034	0.067
*2DS4*full/*full*	7 (1.7%)	76 (4.8%)	**3.74**	**1.75–9.29**	**0.0017**	**0.003**
(c) Haplotypes						
*2DS4*00101∼3DL2*00201*	91 (12.2%)	523 (16.3%)	**1.44**	**1.14–1.84**	**0.0019**	**0.019**
*2DL4*00102∼3DL1*00201∼2DS4*00101∼3DL2*00201*	72 (9.0%)	348 (11.1%)	1.27	0.97–1.68	0.080	1.000
*2DL4*00102∼3DL1*01502∼2DS4*00101∼3DL2*00201*	23 (3.0%)	140 (4.5%)	1.52	0.97–2.47	0.061	1.000
*2DL4*00102∼3DL1*02901∼2DS4*00101∼3DL2*00201*	0 (0.0%)	14 (0.4%)	-			

Note: p_c = Bonferroni correction for 57 alleles; 2 KIR2DS4 genotypes, 9 KIR2DS4∼KIR3DL2 haplotypes, or 10 2DL4∼3DL1∼2DS4∼3DL2 haplotypes analyzed. In bold $p_c < 0.05$.

Abbreviations: CI, confidence interval; F, allele or haplotype frequency; OR, odds ratio; Ref, Genotype of reference.

3.3 | *KIR2DS4*001* increases the risk of developing severe COVID-19

We analyzed 12 KIR genes KIR2DL1, KIR2DL2/3, KIR2DL4, KIR2DL5, KIR2DS1, KIR2DS2, KIR2DS3/5, KIR2DS4, KIR3DL1/S1 and KIR3DL2 to high resolution through sequencing. In our cohort, 57 alleles were observed having >2% allele frequency (Table S3). KIR3DL2 was the most variable gene observed, with 11 alleles, followed by KIR3DL1/S1 (8 alleles), KIR2DL4 (7 alleles), KIR2DS4 and KIR2DL1 (6 alleles), KIR2DL2/3, KIR2DL5A/B and KIR2DS3/5 (5 alleles), and KIR2DS1 and KIR2DS2 (2 alleles). Of the alleles identified, *KIR2DL4*00102*, *KIR2DS3*00103*, *KIR2DS3*00201*, *KIR2DS4*00101*, *KIR3DL2*00201*, and *KIR3DL1*00201* were associated with increased risk of hospitalization, and *KIR2DL4*00802*, *KIR2DS4*00601*, *KIR3DL1*00401* with decreased risk (Table 3). However only *KIR2DS4*00101* remained associated with COVID-19 severity after correction for multiple testing (OR = 1.48, 95% CI 1.20–1.85, $p_c = 0.017$, Table 3). We therefore analyzed KIR2DS4 using logistic regression with age >60 and sex as covariates, which showed that carriers of *KIR2DS4*00101* were significantly more frequent in hospitalized than non-hospitalized individuals (OR = 1.48, 95% CI = 1.14–1.93, $p = 0.003$, Table 4). The results were stable when including sex, age, and ethnicity as covariates (OR = 1.4, 95% CI 1.07–1.83, $p = 0.014$, Table S4). In addition, *KIR2DS4*001* remained nominally significant when adjusted by the hospital of recruitment (OR = 1.62, 95% CI 1.00–2.66, $p = 0.052$, Table S4). Again, we evaluated if *KIR2DS4*001* could associate with protection from the most severe outcomes, including patients that need mechanical breathing assistance (CPAP/BiPAP), intubation, or when the outcome was death. Indeed, *KIR2DS4*001* was more frequent in non-hospitalized patients than these critical cases (OR = 1.51, 95% CI 1.10–2.08, $p = 0.010$, Table S5).

*KIR2DS4*00101* encodes a receptor that is expressed on the cell surface (KIR2DS4-full allotype) whereas the remaining alleles observed in the cohort, *KIR2DS4*003*, **004*, **006*, and **010*, share a 22 bp deletion resulting in lack of cell surface expression (KIR2DS4-null allotype).[54,55] To evaluate if the presence or absence of

TABLE 4 Logistic regression analysis of KIR gene candidates from screening study, using sex and age >60 years old as covariates.

Alleles	Non-hospitalized $N = 402$ f (%)	Hospitalized $N = 1569$ f (%)	OR	CI (95%)	p
*KIR2DS4*00101*	113 (28.1%)	572 (36.5%)	**1.48**	**1.14–1.93**	**0.003**
*KIR2DS4*006*	133 (33.1%)	427 (27.2%)	**0.74**	**0.57–0.96**	**0.021**
KIR allotypes					
*KIR3DL1*00201/*01502/*02901*	104 (25.9%)	520 (33.1%)	**1.44**	**1.10–1.89**	**0.008**
*KIR2DS4*full*	115 (28.6%)	574 (36.6%)	**1.45**	**1.12–1.89**	**0.005**
*KIR2DS4*null*	394 (98.0%)	1490 (95.0%)	**0.32**	**0.14–0.66**	**0.004**

Note: In bold $p < 0.05$. No p correction applied. The *KIR2DS4*null* genotype has 2 copies of alleles having a 22 bp deletion (*KIR2DS4*003*; **004*; **006*, **010*) or absence of the gene.

Abbreviations: CI, confidence interval; f, carrier frequency; OR, odds ratio.

functional KIR2DS4 molecules could impact disease severity, we divided KIR2DS4 alleles according to their expression ability. We observed an increase in susceptibility from none, through one, to two copies of a functional KIR2DS4, with two copies remaining significantly associated after multiple testing correction (OR = 3.74, 95% CI 1.75–9.29, $p_c = 0.003$, Table 3). This observation was repeated using logistic regression, where functional KIR2DS4 presence (risk) remained significant (OR = 1.45, 95% CI = 1.12–1.9, $p = 0.005$, Table 4).

Because the KIR genes are densely packed and the receptors co-expressed,[72] we sought to identify any effect of LD on the association analysis. By example, *KIR2DS4*00101* occurs in strong LD with *KIR3DL2*00201*,[73] which was nominally associated with increased hospitalization risk (Table 3). Extending the analysis to KIR3DL1, which is located adjacently 5′ from KIR2DS4,[72] we observed that KIR3DL1 alleles **00201*, **01502*, and **02901* when linked to *KIR2DS4*00101* were more frequent in hospitalized than non-hospitalized patients (Table 3). These three alleles belong to the 015-like lineage of KIR3DL1/S1,[63] but no significant association was observed when we combined all alleles described for this lineage (Table S7). Importantly, the three distinct haplotypes associated with increased risk for hospitalization are characteristic to populations having distinct genetic ancestry background, *3DL1*00201* in Europeans, *3DL1*01502* in Asians and *3DL1*02901* in Amerindians.[9] This observation implies the *2DS4*00101* allotype association with severe COVID-19 is independent of genetic ancestry. Thus, these results suggest the *KIR2DS4*00101* is the causal variant associated with increased risk of COVID-19 hospitalization, whereas the KIR3DL2 and KIR3DL1/S1 associations are driven by LD with this allele.

Ligation of activating KIR by HLA class I on the surface of infected cells can contribute to NK cell stimulation.[6,15] Although *KIR2DS4*001* remained significantly associated with severe COVID-19 in presence of either of its cognate ligands (OR = 1.41, 95% CI 1.04–1.91, $p = 0.028$, Table 5), we observed no change in the relative risk from determining this association without regard to ligands (OR = 1.48, Table 4). Indeed, we observed a similar odds ratio upon conditioning for the cognate ligands of KIR2DS4 (OR = 1.35, Table 5). Interestingly however, we observed that individuals possessing two or more activating KIR in combination with their respective HLA class I ligand were more frequent in hospitalized than non-hospitalized patients (OR = 1.94, 95% CI 1.36–2.80, $p = 3.1 \times 10^{-04}$, Table 5). This latter finding implicates activating KIR in COVID-19 pathogenesis. Together, the findings suggest the association of *KIR2DS4*001* with COVID-19 severity is independent of the known HLA class I ligands for KIR2DS4, or that the specificity of HLA class I ligation by KIR2DS4 is altered during infection, due to peptide repertoire changes.[74–76]

3.4 | Specific HLA and KIR variants may associate with IFN-α specific antibody production

Autoantibodies against IFN-α can negatively impact infection clearance and contribute to severity of COVID-19.[24,26] Among 156 severe COVID-19 patients analyzed in our cohort, 26% had autoantibodies recognizing one or more IFN-α subunits (Figure S2). No differences were found for any considered variables except for age, where antibody positive patients were younger (57.3 ± 15.0) than antibody negative patients (63.1 ± 14.2, $p = 0.025$; Table S1). Additionally, female patients produced autoantibodies that can recognize a greater diversity of IFN-α subunits when compared to male patients ($p = 0.041$; Table S2). Among these 156 patients, 150 had HLA and KIR genotype information.

In this cohort, we did not observe any HLA class II alleles to be associated with IFN-α specific antibody

TABLE 5 KIR + ligand combinations associating with severe COVID-19.

Receptor + ligand combination	Non-hospitalized $N = 402$ f (%)	Hospitalized $N = 1569$ f (%)	OR	CI (95%)	p
*KIR2DS4*001*					
*2DS4*00101* + ligand	73 (18.2%)	374 (23.8%)	**1.41**	**1.04–1.91**	**0.028**
*2DS4*00101* + no ligand	40 (10.0%)	198 (12.6%)	1.35	0.92–2.01	0.133
KIR2DS1–4 + HLA ligands					
Absent or 1 pair	357 (88.8%)	1278 (81.5%)	Ref		
2 to 4 pairs	**45 (11.2%)**	**291 (18.5%)**	**1.94**	**1.36–2.80**	**3.1×10^{-04}**

Note: By logistic regression association analysis, using sex and age > 60 years old as covariates. In bold p-values < 0.05. No p-value correction applied. HLA ligands for respective KIR are given in Methods.
Abbreviations: CI, confidence interval; f, carrier frequency; OR, odds ratio; Ref, Allotype of reference.

TABLE 6 HLA and KIR alleles and haplotypes with presence of anti-Interferon-α levels in hospitalized COVID-19 patients.

	IFN-α negative antibody $N = 114$ $2N = 228$ F (%)	IFN-α positive antibody $N = 36$ $2N = 72$ F (%)	OR	CI (95%)	p	p_c
Alleles						
*HLA-C*08:02*	4 (1.7%)	6 (8.3%)	5.09	1.16–25.1	0.007	0.44
*KIR3DL1*01502*	13 (5.7%)	0 (0.0%)	-			
Haplotype						
*HLA-B*14:02∼HLA-C*08:02*	2 (0.9%)	6 (8.3%)	10.97	1.87–116.48	0.003	0.05

Note: p_c = p-adjusted using Bonferroni correction for 88 HLA alleles and 17 HLA-B∼HLA-C haplotypes analyzed.
Abbreviations: CI, confidence interval; OR, odds ratio.

production (Table 6). Among HLA class I alleles, *HLA-C*08:02* was enriched in patients who generated IFN-α antibodies, compared to those without them (OR = 5.09, 95% CI 1.16–25.1, $p = 0.007$). Additionally, the haplotype *HLA-C*08:02* with *HLA-B*14:02* was more frequent in IFN-α antibody positive than negative patients (8.3% vs. 0.9%, $p = 0.003$, Table 6). Neither of these findings remained significant after correcting for multiple tests ($p_c > 0.05$). Of interest, *KIR3DL1*01502* was present in 5.7% patients lacking IFN-α antibody, but in none of the antibody positive patients (Table 6). The absence of this low frequency allele in the IFN-α antibody positive group could be due to sample size limitation ($N = 36$). These results therefore suggest that specific HLA and KIR alleles may affect production of IFN-α antibodies, but the findings should be replicated in a larger cohort with multiple tests correction applied.

4 | DISCUSSION

Presentation of pathogen-derived peptides by HLA, and their recognition by immune cell receptors, is critical for survival through driving both innate and adaptive immunity.[8,18,77] Although the exceptional polymorphism of HLA (and KIR) is driven by pathogen diversity, only rarely are specific alleles identified that protect from clinical infection by a given pathogen.[78] More commonly identified are those that, by comparison with other alleles, associate with differential disease severity following infection.[79,80] We therefore analyzed a cohort of individuals confirmed to be infected with SARS-CoV-2, to determine any impact of specific HLA or KIR alleles on the likelihood of developing severe COVID-19. We showed that *HLA-DPB1*13:01* is associated with less severe disease outcome (not being hospitalized), whereas *KIR2DS4*001* is associated with development of severe disease requiring hospitalization. These findings imply that specific variants of HLA and KIR have differential effect on the innate and adaptive responses to SARS-CoV-2 infection, as observed for other viral infections.[9]

As expressible forms of KIR2DS4, most commonly *KIR2DS4*001*, are found at relatively high frequency across populations worldwide,[9,54] the findings in the Italian cohort are likely to be replicated in other populations. Indeed, we identify haplotypes containing *KIR2DS4*001* that are rare in Europeans, but common in other

populations, that are contributing to the disease risk. Although *HLA-DPB1*13:01* allele frequency is low in the Italian cohort, this allele can reach up to 20% or 30% in East Asian and South American Amerindian populations, respectively.[81] Similar to our study, *HLA-DRB1*15* has a well-established association with Multiple Sclerosis, and the frequency of this allele varies to the same degree as *DPB1*13:01* across populations, but the association remains.[82] Thus, reporting associations with low worldwide allele frequency variants may stimulate replication studies in other cohorts, where these variants are more common and potentially have a higher impact.

We evaluated the HLA and KIR associations in a large cohort of patients from a single ancestry, whereas the majority of previous studies performed were (i) restricted to HLA-A, -B, -C, -DRB1 and -DQB1,[78] (ii) in small sample size cohorts,[40] (iii) with distinct disease categories (i.e., patients versus population controls),[34] and/or (iv) in other ancestries.[83] Moreover, no previous study analyzed KIR at high resolution. These factors render it difficult to compare across studies[4] and, for these reasons, this is the first time that *DPB1*13:01* and *KIR2DS4*001* are being suggested to have roles in development of severe COVID-19. In other studies of Europeans, *HLA-DRB1*04:01* associated with protection from severe disease[34] and from developing any symptoms.[78] Although HLA-DPB1 variability was not evaluated in either study that identified *DRB1*04:01*, the lead SNP in the GWAS (rs9271609)[34] occurs in weak LD with *DPB1*13:01* in a European ancestry population ($D' = 0.032$, $r^2 < 0.001$).[84] Thus, we tentatively suggest these other cohorts may replicate our findings.

Our finding that increased severity risk is associated with KIR2DS4 is consistent with the observed preferential expansion of NK cells expressing KIR2DS4 in severe COVID-19, which was observed in multiple studies, also irrespective of ligand presence.[36,37,85] Together these results are compatible with a previous study suggesting individuals lacking specific inhibitory KIR/ligand combinations, whilst possessing activating KIR, were more prone to severe disease.[83] Together with the correlation we observed of increasing activating receptor quantity with disease severity, these observations suggest the unusual expansion of KIR2DS4$^+$ NK cells during severe disease could result in NK cells becoming impaired during SARS-CoV-2 infection,[86] or that excess activation could lead to collateral damage.

Cytokine expression profiles have revealed distinct signatures of NK cells in COVID-19 severity, in which NK cell function, cytokine release, and pro-inflammatory pathways differ between moderate and severe cases.[21,22,24] Among them, IFN-α pathway expression levels were enriched in NK cells of severe when compared to moderate COVID-19 patients. In addition, soluble IFN-α suppresses NK cell production of IFN-γ, affecting the immune response, even after decreasing IFN-α antibody titers in these patients.[24] Antibodies neutralizing IFN-α, thereby preventing its activity toward SARS-CoV-2, were previously observed in 10.2% of COVID-19 patients with life-threatening disease.[26] In our cohort, we observed that 26% of hospitalized COVID-19 patients had IFN-α specific antibodies in the plasma, albeit detected equally across severity subgroups. In addition, we observed the frequency of a haplotype containing *HLA-C*08:02* and *B*14:01* was elevated in patients having IFN-α autoantibodies. Although their role in autoantibody production is not clear, specific HLA class I allotypes could participate in presenting autoantigens to CD8+ T cells, indirectly activating B-cells, as observed in psoriasis.[87] Due to the small sample size of the IFN-α autoantibody group, this result requires investigation in a larger study.

As a limitation of our study, we stratified our samples according to hospitalization status, dividing the patients with mild (or pauci-) symptoms that could be treated at home from those patients who needed hospital assistance. It is important to emphasize that the hospitalized group involves a variety of symptoms, as well presence of comorbidities, such as autoimmunity, cardiovascular diseases, diabetes type 2, and obesity. Due to lack of comorbidity data available for all the patients, we did not subdivide the cohort into smaller groups. It would be important to analyze a larger sample stratified by comorbidities to evaluate the impact of HLA and KIR polymorphism on severity. Moreover, we performed p-value correction using the Bonferroni method, considering all HLA and KIR alleles with frequency higher than 2%. In the HLA and KIR regions that both have high linkage disequilibrium this correction may be conservative, contributing to false-negative results (type II error) that might not fully resolve the complexity of HLA and KIR polymorphism in COVID-19 severity.

In summary, we show specific HLA and KIR variants have capacity to influence COVID-19 severity. In a large cohort from Italy, we observed that presence of *HLA-DPβ1*13:01*, which can interact with both CD4+ T cell and NK cell receptors, associates with protection from, whereas KIR2DS4, which stimulates NK cells, increases the risk of developing severe COVID-19. Our study highlights the complexity of HLA and KIR polymorphism in shaping the course of SARS-CoV-2 infection, through possible impact both on the innate and the adaptive immune response.

AUTHOR CONTRIBUTIONS

Conceptualization: Ticiana D. J. Farias, Silvia Brugiapaglia, Francesco Novelli, Alessandra Renieri, Simone Furini, Antonio Amoroso, Paul J. Norman; Data Curation:

Ticiana D. J. Farias, Silvia Brugiapaglia, Susanna Croci, Katherine M. Kichula, Nicholas R. Pollock, Kristina Zguro, Mirella Bruttini, Francesco Novelli, Alessandra Renieri, Simone Furini, Paul J. Norman, Antonio Amoroso; Formal Analysis: Ticiana D. J. Farias, Silvia Brugiapaglia, Paola Magistroni, Claudia Curcio, Kristina Zguro, Chiara Fallerini, Francesco Pettini, Katherine M. Kichula, Nicholas R. Pollock, Neus Font-Porterias, Francesco Novelli, Alessandra Renieri, Simone Furini, Antonio Amoroso, Paul J. Norman; Investigation: Ticiana D. J. Farias, Silvia Brugiapaglia, Susanna Croci, GEN-COVID, Jill A. Hollenbach, Audrey E. Hendricks, Francesco Novelli, Alessandra Renieri, Simone Furini, Antonio Amoroso, Paul J. Norman; Methodology: Ticiana D. J. Farias, Silvia Brugiapaglia, Paola Magistroni, Claudia Curcio, Katherine M. Kichula, Nicholas R. Pollock, William H. Palmer, Wesley M. Marin, Jill A. Hollenbach, Audrey E. Hendricks, Francesco Novelli, Alessandra Renieri, Simone Furini, Antonio Amoroso, Paul J. Norman; Resources: Ticiana D. J. Farias, Silvia Brugiapaglia, Francesca Fava, Margherita Baldassarri, GEN-COVID, Francesco Novelli, Alessandra Renieri, Simone Furini, Paul J. Norman, Antonio Amoroso; Supervision: Ilaria Meloni, Francesco Novelli, Alessandra Renieri, Simone Furini, Paul J. Norman, Antonio Amoroso; Writing – Original Draft Preparation: Ticiana D. J. Farias, Paul J. Norman. All authors reviewed, edited, and approved this manuscript.

ACKNOWLEDGMENTS

This work was supported by a grant from fastgrants.org, and by NIH R01 AI158410 (to PJN). TDJF received an International Postdoctoral fellowship from Conselho Nacional de Desenvolvimento Científico e Tecnológico (PDE – process number 201608/2019-7). FN was supported by AIRC IG grant (n. 19931). SB received a Fellowship from Fondazione Ricerca Molinette, Torino, Italy. AA was supported by the Italian Ministry of Education-University and Research-MIUR, Progetto strategico di Eccellenza Dipartimentale #D15D18000410001 (to the Department of Medical Sciences, University of Turin).

We also thank the MIUR project "Dipartimenti di Eccellenza 2018–2020" of the Department of Medical Biotechnologies University of Siena, Italy, and "Bando Ricerca COVID-19 Toscana" project to Azienda Ospedaliero-Universitaria Senese. We thank Intesa San Paolo for the 2020 charity fund dedicated to the project N B/2020/0119 "Identificazione delle basi genetiche determinanti la variabilità clinica della risposta a COVID-19 nella popolazione italiana." The Italian Ministry of University and Research for funding within the "Bando FISR 2020" in COVID-19 for the project "Editing dell'RNA contro il Sars-CoV-2": hackerare il virus per identificare bersagli molecolari e attenuare l'infezione – HACKTHECOV) and the Istituto Buddista Italiano Soka Gakkai for funding the project "PAT-COVID: Host genetics and pathogenetic mechanisms of COVID-19" (ID n. 2020-2016_RIC_3). We thank EU project H2020-SC1-FA-DTS-2018-2020, entitled "International consortium for integrative genomics prediction (INTERVENE)" – Grant Agreement No. 101016775.

We are grateful to Wietse Mulder of GenDX and Atilla Bérces of Omixon for the use of their respective software packages. We thank all patients and hospital staff for their participation in this study. This study is part of the GEN-COVID Multicenter Study, https://sites.google.com/dbm.unisi.it/gen-covid, the Italian multicenter study aimed at identifying the COVID-19 host genetic bases. Specimens were provided by the COVID-19 Biobank of Siena, which is part of the Genetic Biobank of Siena, member of BBMRI-IT, of Telethon Network of Genetic Biobanks (project no. GTB18001), of EuroBioBank, and of RD-Connect. We thank the CINECA consortium for providing computational resources and the Network for Italian Genomes (NIG; http://www.nig.cineca.it) for its support. We thank private donors for the support provided to AR (Department of Medical Biotechnologies, University of Siena) for the COVID-19 host genetics research project (D.L n.18 of March 17, 2020). We also thank the COVID-19 Host Genetics Initiative (https://www.covid19hg.org/).

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that supports the findings of this study are available in the supplementary material of this article.

ORCID

Ticiana D. J. Farias https://orcid.org/0000-0002-3606-2428
Francesca Fava https://orcid.org/0000-0002-4363-2353
Wesley M. Marin https://orcid.org/0000-0002-2334-0396
Paul J. Norman https://orcid.org/0000-0001-8370-7703

REFERENCES

1. Guan WJ, Ni ZY, Hu Y, et al. Clinical characteristics of coronavirus disease 2019 in China. *N Engl J Med*. 2020;382(18):1708-1720.
2. Rello J, Storti E, Belliato M, Serrano R. Clinical phenotypes of SARS-CoV-2: implications for clinicians and researchers. *Eur Respir J*. 2020;55(5):2001028.
3. Zhang X, Tan Y, Ling Y, et al. Viral and host factors related to the clinical outcome of COVID-19. *Nature*. 2020;583(7816):437-440.
4. Srivastava A, Hollenbach JA. The immunogenetics of COVID-19. *Immunogenetics*. 2023;75(3):309-320.

5. Francis JM, Leistritz-Edwards D, Dunn A, et al. Allelic variation in class I HLA determines CD8(+) T cell repertoire shape and cross-reactive memory responses to SARS-CoV-2. *Sci Immunol*. 2022;7(67):eabk3070.
6. Pende D, Falco M, Vitale M, et al. Killer Ig-like receptors (KIRs): their role in NK cell modulation and developments leading to their clinical exploitation. *Front Immunol*. 2019;10:1179.
7. Shiina T, Hosomichi K, Inoko H, Kulski JK. The HLA genomic loci map: expression, interaction, diversity and disease. *J Hum Genet*. 2009;54(1):15-39.
8. Dendrou CA, Petersen J, Rossjohn J, Fugger L. HLA variation and disease. *Nat Rev Immunol*. 2018;18(5):325-339.
9. Pollock NR, Harrison GF, Norman PJ. Immunogenomics of killer cell immunoglobulin-like receptor (KIR) and HLA class I: coevolution and consequences for human health. *J Allergy Clin Immunol Pract*. 2022;10(7):1763-1775.
10. Aranda-Romo S, Garcia-Sepulveda CA, Comas-García A, et al. Killer-cell immunoglobulin-like receptors (KIR) in severe A (H1N1) 2009 influenza infections. *Immunogenetics*. 2012;64(9): 653-662.
11. Wang C, Xia CQ. The involvement of natural killer cells in the pathogenesis of severe acute respiratory syndrome. *Am J Clin Pathol*. 2004;121(4):507-511.
12. Nguyen A, David JK, Maden SK, et al. Human leukocyte antigen susceptibility map for severe acute respiratory syndrome coronavirus 2. *J Virol*. 2020;94(13). doi:10.1128/jvi.00510-20
13. Parker R, Partridge T, Wormald C, et al. Mapping the SARS-CoV-2 spike glycoprotein-derived peptidome presented by HLA class II on dendritic cells. *Cell Rep*. 2021;35(8):109179.
14. Weingarten-Gabbay S, Klaeger S, Sarkizova S, et al. Profiling SARS-CoV-2 HLA-I peptidome reveals T cell epitopes from out-of-frame ORFs. *Cell*. 2021;184(15):3962-3980.e3917.
15. Vivier E, Raulet DH, Moretta A, et al. Innate or adaptive immunity? The example of natural killer cells. *Science*. 2011; 331(6013):44-49.
16. Jost S, Altfeld M. Control of human viral infections by natural killer cells. *Annu Rev Immunol*. 2013;31:163-194.
17. Ali A, Gyurova IE, Waggoner SN. Mutually assured destruction: the cold war between viruses and natural killer cells. *Curr Opin Virol*. 2019;34:130-139.
18. Parham P, Moffett A. Variable NK cell receptors and their MHC class I ligands in immunity, reproduction and human evolution. *Nat Rev Immunol*. 2013;13(2):133-144.
19. Anfossi N, Andre P, Guia S, et al. Human NK cell education by inhibitory receptors for MHC class I. *Immunity*. 2006;25(2): 331-342.
20. Zhang S, Cooper-Knock J, Weimer AK, et al. Multiomic analysis reveals cell-type-specific molecular determinants of COVID-19 severity. *Cell Syst*. 2022;13:598-614.e6.
21. Maucourant C, Filipovic I, Ponzetta A, et al. Natural killer cell immunotypes related to COVID-19 disease severity. *Sci Immunol*. 2020;5(50). doi:10.1126/sciimmunol.abd6832
22. Leem G, Cheon S, Lee H, et al. Abnormality in the NK-cell population is prolonged in severe COVID-19 patients. *J Allergy Clin Immunol*. 2021;148(4):996-1006.e1018.
23. Carsetti R, Zaffina S, Piano Mortari E, et al. Different innate and adaptive immune responses to SARS-CoV-2 infection of asymptomatic, mild, and severe cases. *Front Immunol*. 2020;11: 610300.
24. Kramer B, Knoll R, Bonaguro L, et al. Early IFN-alpha signatures and persistent dysfunction are distinguishing features of NK cells in severe COVID-19. *Immunity*. 2021;54(11):2650-2669.e2614.
25. Zhang Q, Bastard P, Liu Z, et al. Inborn errors of type I IFN immunity in patients with life-threatening COVID-19. *Science*. 2020;370(6515). doi:10.1126/science.abd4570
26. Bastard P, Rosen LB, Zhang Q, et al. Autoantibodies against type I IFNs in patients with life-threatening COVID-19. *Science*. 2020;370(6515). doi:10.1126/science.abd4585
27. Bastard P, Zhang Q, Zhang SY, Jouanguy E, Casanova JL. Type I interferons and SARS-CoV-2: from cells to organisms. *Curr Opin Immunol*. 2022;74:172-182.
28. Goncalves D, Mezidi M, Bastard P, et al. Antibodies against type I interferon: detection and association with severe clinical outcome in COVID-19 patients. *Clin Transl Immunol*. 2021; 10(8):e1327.
29. Bastard P, Gervais A, Le Voyer T, et al. Autoantibodies neutralizing type I IFNs are present in ~4% of uninfected individuals over 70 years old and account for ~20% of COVID-19 deaths. *Sci Immunol*. 2021;6(62). doi:10.1126/sciimmunol.abl4340
30. Campbell KS, Purdy AK. Structure/function of human killer cell immunoglobulin-like receptors: lessons from polymorphisms, evolution, crystal structures and mutations. *Immunology*. 2011;132(3):315-325.
31. Pairo-Castineira E, Clohisey S, Klaric L, et al. Genetic mechanisms of critical illness in COVID-19. *Nature*. 2021;591(7848): 92-98.
32. Severe Covid GG, Ellinghaus D, Degenhardt F, et al. Genome-wide association study of severe Covid-19 with respiratory failure. *N Engl J Med*. 2020;383(16):1522-1534.
33. Degenhardt F, Ellinghaus D, Juzenas S, et al. Detailed stratified GWAS analysis for severe COVID-19 in four European populations. *Hum Mol Genet*. 2022;31:3945-3966.
34. Kousathanas A, Pairo-Castineira E, Rawlik K, et al. Whole-genome sequencing reveals host factors underlying critical COVID-19. *Nature*. 2022;607(7917):97-103.
35. Littera R, Chessa L, Deidda S, et al. Natural killer-cell immunoglobulin-like receptors trigger differences in immune response to SARS-CoV-2 infection. *PloS One*. 2021;16(8):e0255608.
36. Bernal E, Gimeno L, Alcaraz MJ, et al. Activating killer-cell immunoglobulin-like receptors are associated with the severity of coronavirus disease 2019. *J Infect Dis*. 2021;224(2):229-240.
37. Casado JL, Moraga E, Vizcarra P, et al. Expansion of CD56 (dim)CD16(neg) NK cell subset and increased inhibitory KIRs in hospitalized COVID-19 patients. *Viruses*. 2021;14(1). doi:10.3390/v14010046
38. Correale P, Mutti L, Pentimalli F, et al. HLA-B*44 and C*01 prevalence correlates with Covid19 spreading across Italy. *Int J Mol Sci*. 2020;21(15). doi:10.3390/ijms21155205
39. Guerini FR, Bolognesi E, Lax A, et al. HLA allele frequencies and association with severity of COVID-19 infection in northern Italian patients. *Cells*. 2022;11(11). doi:10.3390/cells11111792
40. Novelli A, Andreani M, Biancolella M, et al. HLA allele frequencies and susceptibility to COVID-19 in a group of 99 Italian patients. *HLA*. 2020;96:610-614.
41. Pisanti S, Deelen J, Gallina AM, et al. Correlation of the two most frequent HLA haplotypes in the Italian population to the

differential regional incidence of Covid-19. *J Transl Med*. 2020; 18(1):352.
42. Amoroso A, Magistroni P, Vespasiano F, et al. HLA and AB0 polymorphisms may influence SARS-CoV-2 infection and COVID-19 severity. *Transplantation*. 2021;105(1):193-200.
43. Daga S, Fallerini C, Baldassarri M, et al. Employing a systematic approach to biobanking and analyzing clinical and genetic data for advancing COVID-19 research. *Eur J Hum Genet*. 2021;29(5):745-759.
44. Norman PJ, Hollenbach JA, Nemat-Gorgani N, et al. Defining KIR and HLA class I genotypes at highest resolution via high-throughput sequencing. *Am J Hum Genet*. 2016;99(2):375-391.
45. Tao S, Kichula KM, Harrison GF, et al. The combinatorial diversity of KIR and HLA class I allotypes in Peninsular Malaysia. *Immunology*. 2020;162:389-404.
46. Dilthey AT, Mentzer AJ, Carapito R, et al. HLA*LA-HLA typing from linearly projected graph alignments. *Bioinformatics*. 2019;35(21):4394-4396.
47. Marin WM, Dandekar R, Augusto DG, et al. High-throughput interpretation of killer-cell immunoglobulin-like receptor short-read sequencing data with PING. *PLoS Comput Biol*. 2021;17(8):e1008904.
48. R: a language and environment for statistical computing. version 2.9.0. 2009.
49. Falush D, Stephens M, Pritchard JK. Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. *Genetics*. 2003;164(4): 1567-1587.
50. Norman PJ, Hollenbach JA, Nemat-Gorgani N, et al. Co-evolution of human leukocyte antigen (HLA) class I ligands with killer-cell immunoglobulin-like receptors (KIR) in a genetically diverse population of sub-Saharan Africans. *PLoS Genet*. 2013;9(10):e1003938.
51. Abi-Rached L, Gouret P, Yeh JH, et al. Immune diversity sheds light on missing variation in worldwide genetic diversity panels. *PloS One*. 2018;13(10):e0206512.
52. Durbin RM, Altshuler D, Durbin RM, et al. A map of human genome variation from population-scale sequencing. *Nature*. 2010;467(7319):1061-1073.
53. Pappas DJ, Marin W, Hollenbach JA, Mack SJ. Bridging ImmunoGenomic Data Analysis Workflow Gaps (BIGDAWG): an integrated case-control analysis pipeline. *Hum Immunol*. 2016; 77(3):283-287.
54. Graef T, Moesta AK, Norman PJ, et al. KIR2DS4 is a product of gene conversion with KIR3DL2 that introduced specificity for HLA-A*11 while diminishing avidity for HLA-C. *J Exp Med*. 2009;206(11):2557-2572.
55. Maxwell LD, Wallace A, Middleton D, Curran MD. A common KIR2DS4 deletion variant in the human that predicts a soluble KIR molecule analogous to the KIR1D molecule observed in the rhesus monkey. *Tissue Antigens*. 2002;60(3): 254-258.
56. Rothman KJ. No adjustments are needed for multiple comparisons. *Epidemiology*. 1990;1(1):43-46.
57. Hansasuta P, Dong T, Thananchai H, et al. Recognition of HLA-A3 and HLA-A11 by KIR3DL2 is peptide-specific. *Eur J Immunol*. 2004;34(6):1673-1679.
58. Saunders PM, Pymm P, Pietra G, et al. Killer cell immunoglobulin-like receptor 3DL1 polymorphism defines distinct hierarchies of HLA class I recognition. *J Exp Med*. 2016;213(5):791-807.
59. Fan QR, Long EO, Wiley DC. Crystal structure of the human natural killer cell inhibitory receptor KIR2DL1-HLA-Cw4 complex. *Nat Immunol*. 2001;2(5):452-460.
60. Hilton HG, Guethlein LA, Goyos A, et al. Polymorphic HLA-C receptors balance the functional characteristics of KIR haplotypes. *J Immunol*. 2015;195(7):3160-3170.
61. Blunt MD, Khakoo SI. Activating killer cell immunoglobulin-like receptors: detection, function and therapeutic use. *Int J Immunogenet*. 2020;47(1):1-12.
62. Blokhuis JH, Hilton HG, Guethlein LA, et al. KIR2DS5 allotypes that recognize the C2 epitope of HLA-C are common among Africans and absent from Europeans. *Immun Inflamm Dis*. 2017;5:461-468.
63. Parham P, Norman PJ, Abi-Rached L, Guethlein LA. Variable NK cell receptors exemplified by human KIR3DL1/S1. *J Immunol*. 2011;187(1):11-19.
64. Pando MJ, Gardiner CM, Gleimer M, McQueen KL, Parham P. The protein made from a common allele of KIR3DL1 (3DL1*004) is poorly expressed at cell surfaces due to substitution at positions 86 in Ig domain 0 and 182 in Ig domain 1. *J Immunol*. 2003;171(12):6640-6649.
65. Reynisson B, Alvarez B, Paul S, Peters B, Nielsen M. NetMHCpan-4.1 and NetMHCIIpan-4.0: improved predictions of MHC antigen presentation by concurrent motif deconvolution and integration of MS MHC eluted ligand data. *Nucleic Acids Res*. 2020;48(W1):W449-W454.
66. UniProt C. UniProt: the universal protein knowledgebase in 2021. *Nucleic Acids Res*. 2021;49(D1):D480-D489.
67. Kwok WW, Kovats S, Thurtle P, Nepom GT. HLA-DQ allelic polymorphisms constrain patterns of class II heterodimer formation. *J Immunol*. 1993;150(6):2263-2272.
68. Tollefsen S, Hotta K, Chen X, et al. Structural and functional studies of trans-encoded HLA-DQ2.3 (DQA1*03: 01/DQB1*02:01) protein molecule. *J Biol Chem*. 2012; 287(17):13611-13619.
69. Hollenbach JA, Madbouly A, Gragert L, et al. A combined DPA1∼DPB1 amino acid epitope is the primary unit of selection on the HLA-DP heterodimer. *Immunogenetics*. 2012;64(8):559-569.
70. Niehrs A, Garcia-Beltran WF, Norman PJ, et al. A subset of HLA-DP molecules serve as ligands for the natural cytotoxicity receptor NKp44. *Nat Immunol*. 2019;20(9):1129-1137.
71. Zhou Z, Huang C, Zhou Z, et al. Structural insight reveals SARS-CoV-2 ORF7a as an immunomodulating factor for human CD14(+) monocytes. *iScience*. 2021;24(3):102187.
72. Wilson MJ, Torkar M, Haude A, et al. Plasticity in the organization and sequences of human KIR/ILT gene families. *Proc Natl Acad Sci U S A*. 2000;97(9):4778-4783.
73. Amorim LM, Augusto DG, Nemat-Gorgani N, et al. High-resolution characterization of KIR genes in a large North American cohort reveals novel details of structural and sequence diversity. *Front Immunol*. 2021;12:674778.
74. Sim MJW, Rajagopalan S, Altmann DM, Boyton RJ, Sun PD, Long EO. Human NK cell receptor KIR2DS4 detects a conserved bacterial epitope presented by HLA-C. *Proc Natl Acad Sci USA*. 2019;116(26):12964-12973.
75. Naiyer MM, Cassidy SA, Magri A, et al. KIR2DS2 recognizes conserved peptides derived from viral helicases in the context

of HLA-C. *Sci Immunol*. 2017;2(15). doi:10.1126/sciimmunol.aal5296
76. Das J, Khakoo SI. NK cells: tuned by peptide? *Immunol Rev*. 2015;267(1):214-227.
77. Touraine JL, Betuel H, Souillet G, Jeune M. Combined immunodeficiency disease associated with absence of cell-surface HLA-A and -B antigens. *J Pediatr*. 1978;93(1):47-51.
78. Augusto DG, Murdolo LD, Chatzileontiadou DSM, et al. A common allele of HLA is associated with asymptomatic SARS-CoV-2 infection. *Nature*. 2023;620:128-136.
79. Khakoo SI, Thio CL, Martin MP, et al. HLA and NK cell inhibitory receptor genes in resolving hepatitis C virus infection. *Science*. 2004;305(5685):872-874.
80. Martin MP, Qi Y, Gao X, et al. Innate partnership of HLA-B and KIR3DL1 subtypes against HIV-1. *Nat Genet*. 2007;39(6):733-740.
81. Gonzalez-Galarza FF, McCabe A, Santos E, et al. Allele frequency net database (AFND) 2020 update: gold-standard data classification, open access genotype data and new query tools. *Nucleic Acids Res*. 2020;48(D1):D783-d788.
82. Hollenbach JA, Oksenberg JR. The immunogenetics of multiple sclerosis: a comprehensive review. *J Autoimmun*. 2015;64:13-25.
83. Maruthamuthu S, Rajalingam K, Kaur N, et al. Individualized constellation of killer cell immunoglobulin-like receptors and cognate HLA class I ligands that controls natural killer cell antiviral immunity predisposes COVID-19. *Front Genet*. 2022;13:845474.
84. Machiela MJ, Chanock SJ. LDlink: a web-based application for exploring population-specific haplotype structure and linking correlated alleles of possible functional variants. *Bioinformatics*. 2015;31(21):3555-3557.
85. Rendeiro AF, Casano J, Vorkas CK, et al. Profiling of immune dysfunction in COVID-19 patients allows early prediction of disease progression. *Life Sci Alliance*. 2021;4(2):e202000955.
86. Wilk AJ, Rustagi A, Zhao NQ, et al. A single-cell atlas of the peripheral immune response to severe COVID-19. *Nat Med*. 2020;26:1070-1076.
87. Prinz JC. Human leukocyte antigen-class I alleles and the autoreactive T cell response in psoriasis pathogenesis. *Front Immunol*. 2018;9:954.

SUPPORTING INFORMATION

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

How to cite this article: Farias TDJ, Brugiapaglia S, Croci S, et al. *HLA-DPB1*13:01* associates with enhanced, and *KIR2DS4*001* with diminished protection from developing severe COVID-19. *HLA*. 2024;103(1):e15251. doi:10.1111/tan.15251