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BRIEF COMMUNICATION

Deletion of the NKG2C receptor encoding *KLRC2* gene and HLA-E variants are risk factors for severe COVID-19

Hannes Vietzen[1], Alexander Zoufaly[2], Marianna Traugott[2], Judith Aberle[1], Stephan W. Aberle[1] and Elisabeth Puchhammer-Stöckl[1] ✉

PURPOSE: Host genetic variants may contribute to severity of COVID-19. NKG2C$^+$ NK cells are potent antiviral effector cells, potentially limiting the extent of SARS-CoV-2 infections. NKG2C is an activating NK cell receptor encoded by the *KLRC2* gene, which binds to HLA-E on infected cells leading to NK cell activation. Heterozygous or homozygous *KLRC2* deletion (*KLRC2*^{del}) may naturally occur and is associated with a significantly lower or absent NKG2C expression level. In addition, HLA-E*0101/0103 genetic variants occur, caused by a single-nucleotide polymorphism. We therefore investigated whether the severity of COVID-19 is associated with these genetic variants.

METHODS: We investigated the distribution of *KLRC2* deletion and HLA-E*0101/0103 allelic variants in a study cohort of 361 patients with either mild ($N = 92$) or severe ($N = 269$) COVID-19.

RESULTS: Especially the *KLRC2*^{del}, and at a lower degree the HLA-E*0101, allele were significantly overrepresented in hospitalized patients ($p = 0.0006$ and $p = 0.01$), particularly in patients requiring intensive care ($p < 0.0001$ and $p = 0.01$), compared with patients with mild symptoms. Both genetic variants were independent risk factors for severe COVID-19.

CONCLUSION: Our data show that these genetic variants in the NKG2C/HLA-E axis have a significant impact on the development of severe SARS-CoV-2 infections, and may help to identify patients at high-risk for severe COVID-19.

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INTRODUCTION

Patients infected with SARS-CoV-2 may either show mild or even asymptomatic infection or they may develop severe and potentially lethal coronavirus disease 2019 (COVID-19). In the beginning of the SARS-CoV-2 pandemic, several risk factors for severe COVID-19 were reported, including older age, male gender, and comorbidities such as cardiovascular or respiratory system diseases[1]. However, these factors alone did not explain the differences in severity of COVID-19 observed between individuals. It was consequently hypothesized that host genetic factors may contribute to severity and overall clinical outcome of COVID-19[2]. In fact, a recent genome-wide association study (GWAS) shed light on the role of distinct single-nucleotide polymorphisms (SNP) for severe COVID-19[3].

Antiviral immune responses limit viral infections and influence the severity of viral diseases. Among these, natural killer (NK) cell responses are of major importance and significantly contribute to the early defense against pulmonary virus infections. NK cell activation depends on a panel of germline-encoded activating or inhibitory receptors. NKG2C$^+$ NK cells are characterized by the elevated expression of the activating CD94/NKG2C receptor and, in response to viral pulmonary infections, a subset of these highly potent NKG2C$^+$ NK cells migrates into the lung. A recently published study in COVID-19 patients demonstrated that NKG2C$^+$ NK cells were highly prevalent in patients with severe disease[4]. The interaction of CD94/NKG2C and its cellular ligand HLA-E further mediates cytotoxic NK cell responses and the release of pro-inflammatory effector molecules of NKG2C$^+$ cells against virus-infected cells[5]. Therefore variations of the *KLRC2* gene, which is encoding for the NKG2C receptor, may have substantial impact on the activation of NKG2C$^+$ NK cells and thereby on severity of COVID-19. It was shown that homozygous and heterozygous deletion of the *KLRC2* gene exist in about 4% and 32.4% of the white population[6]. Homozygous and heterozygous deletion of *KLRC2* is linked to decreased or absent expression of the NKG2C receptor and is associated with the severity of distinct virus infections[7]. However, it has not yet been investigated whether there is an association between *KLRC2* deletion and severity of COVID-19.

Also the cellular ligand HLA-E occurs in European populations in different variations, mostly as HLA-E*0101 or HLA-E*0103, which exhibit significant differences in cell surface expression levels[8]. In the present study we therefore investigated whether *KLRC2* deletion as well as specific HLA-E variants are significantly associated with the severity of COVID-19 in the individual host.

MATERIALS AND METHODS

We included a total of 361 Austrian COVID-19 patients (median age: 69 years, 45% female), who were confirmed positive for SARS-CoV-2 by polymerase chain reaction (PCR) from respiratory swabs between 17 February and 17 April 2020 at the Center of Virology, Medical University of Vienna. A total of 92/361 (25.5%) patients showed only minor symptoms and stayed in home quarantine ("nonhospitalized"), 190/361 (52.6%) patients were hospitalized with severe COVID-19 symptoms but never required intensive care ("hospitalized non-ICU"), and 79/361 (21.9%) patients were severely affected and needed intensive care as defined earlier[9] ("hospitalized ICU"). In addition, we included 260 Austrian SARS-CoV-2 negative control individuals (median age: 63 years, 50% female), from whom respiratory swabs were obtained from SARS-CoV-2 surveillance.

[1]Center for Virology, Medical University of Vienna, Vienna, Austria. [2]Department of Medicine IV, Kaiser Franz Josef Hospital, Vienna, Austria. ✉email: elisabeth.puchhammer@meduniwien.ac.at

SARS-CoV-2 detection

Viral RNA was as isolated from respiratory swabs of COVID-19 patients using NucliSens EasyMag extractor (BioMérieux, Marcy-l'Étoile, France). SARS-CoV RNA was eluted in 50 µl nuclease-free H_2O, followed by the quantification by a recently published qPCR[10].

Genotyping

Genomic DNA was isolated from respiratory swabs of COVID-19 patients and controls using NucliSens EasyMag extractor (BioMérieux). DNA was eluted in 50 µl of nuclease-free H_2O. HLA-E*0101/0103 genotypes were determined by a Taqman assay and *KLRC2*$^{wt/del}$ variants were determined by touchdown PCR as described previously[6,7]. As internal controls, genomic DNA obtained from the HeLa, HEK-293T, and K562 (all ATCC, Manassas, VA, USA) were used, as recommended previously[6]. Randomly chosen amplicons from all *KLRC2* and HLA-E variants were routinely selected, sequenced on a 3130 genetic analyzer (Applied Biosystems, Foster City, CA, USA), and analyzed with Geneious Prime 2019 (Biomatters, Auckland, New Zealand).

Statistical analysis

The distribution of the patient's gender, comorbidities, and genetic variants was compared by χ^2 test. Patient age was assessed by analysis of variance (ANOVA) and Dunn post test. Correlation of the genetic variants and comorbidities was assessed using χ^2 test. For multivariable analysis, a general main effects log-linear model with genetic variables, gender, and age groups (<60, 60–70, 70–80, >80 years) was used to identify combined genetic variables associated with the risk for severe SARS-CoV-2 infections, who were hospitalized or hospitalized in an ICU. *P* values <0.05 were considered significant. Statistical analyses were performed using IBM SPSS Statistics 24. The study was approved by the local ethics committee (EK number 1389/2019).

RESULTS

In our study cohort, hospitalized COVID-19 patients with and without requiring ICU treatment were significantly older, were more likely to be males, and had significantly more comorbidities such as obesity, hypertension, chronic obstructive pulmonary disease (COPD), and coronary artery (CAD) disease compared with nonhospitalized COVID-19 patients (Table 1). This confirms previous data that male gender, higher age, and comorbidities are risk factors for severe COVID-19[1].

From SARS-CoV-2 infected patients and controls, *KLRC2*$^{wt/del}$ variants were determined and 65% of overall control persons encoded for the *KLRC2*$^{wt/wt}$, 33% for the *KLRC2* $^{wt/del}$, and 2% for the *KLRC2*$^{del/del}$ variant. Hospitalized COVID-19 patients and patients requiring intensive care showed a significantly lower frequency of the *KLRC2*$^{wt/wt}$ variant compared with nonhospitalized COVID-19 patients and controls (Fig. 1a). Presence of the NKG2C^{del} allele was significantly associated with severe COVID-19 requiring hospitalization and with hospitalization in ICU ($p = 0.0006$ [odds ratio; OR = 2.6] and $p < 0.0001$ [OR = 7.1], respectively, χ^2 test). Male and female COVID-19 patients carrying the *KLRC2*^{del} allele were equally at risk for hospitalization compared with the nonhospitalized patients (Table S1). We further tested the distribution of the *KLRC2*^{del} variants between the age groups, as defined in "Materials and Methods," and found a significantly higher frequency of the *KLRC2*^{del} allele in hospitalized and ICU COVID-19 patients throughout the age groups (Table S2).

We then analyzed the patients' HLA-E*0101/0103 variants. Overall, 22.3% and 30.4% of the controls were homozygous for the HLA-E*0101/0101 and HLA-E*0103/0103, respectively, while 47.3% showed the heterozygous HLA-E*0101/0103 variant. Hospitalized

Table 1. Characteristics of the study cohort.

	Nonhospitalized $N = 92$	Hospitalized (non-ICU) $N = 190$	Hospitalized (ICU) $N = 79$	*p* value[a]
Female gender (%)	$N = 54$ (58%)	$N = 78$ (41%)	$N = 31$ (39%)	$P = 0.02$ Nonhospitalized vs. hospitalized: $p = 0.007$ Nonhospitalized vs. ICU: $p = 0.01$ Hospitalized vs. ICU: $p = 0.79$
Median age (years, min–max)	45 (18–93)	74.4 (18–99)	78.3 (20–97)	$P < 0.0001$ Nonhospitalized vs. hospitalized: $P < 0.0001$ Nonhospitalized vs. ICU: $P < 0.0001$ Hospitalized vs. ICU: $P < 0.0001$
Comorbidities	$N = 5$ (5.4%)	$N = 20$ (10.5%)	$N = 12$ (15.2%)	$P < 0.0001$
Obesity (%)	$N = 1$ (1.1%)	$N = 85$ (44.7%)	$N = 36$ (45.6%)	Nonhospitalized vs. hospitalized: $P < 0.0001$
Hypertension (%)	$N = 1$ (1.1%)	$N = 44$ (23.1%)	$N = 21$ (26.5%)	Nonhospitalized vs. ICU: $P < 0.0001$
COPD (%)	$N = 0$ (0%)	$N = 31$ (16.3%)	$N = 13$ (16.4%)	Hospitalized vs. ICU: $p = 0.61$
CAD (%)	$N = 85$ (91.3%)	$N = 15$ (7.9%)	$N = 12$ (15.2%)	
None (%)				

CAD coronary artery disease, *COPD* chronic obstructive pulmonary disease, *ICU* intensive care unit.
[a]Differences were assessed with the χ^2 (gender and comorbidities) and analysis of variance (ANOVA) and Dunn post test (age).

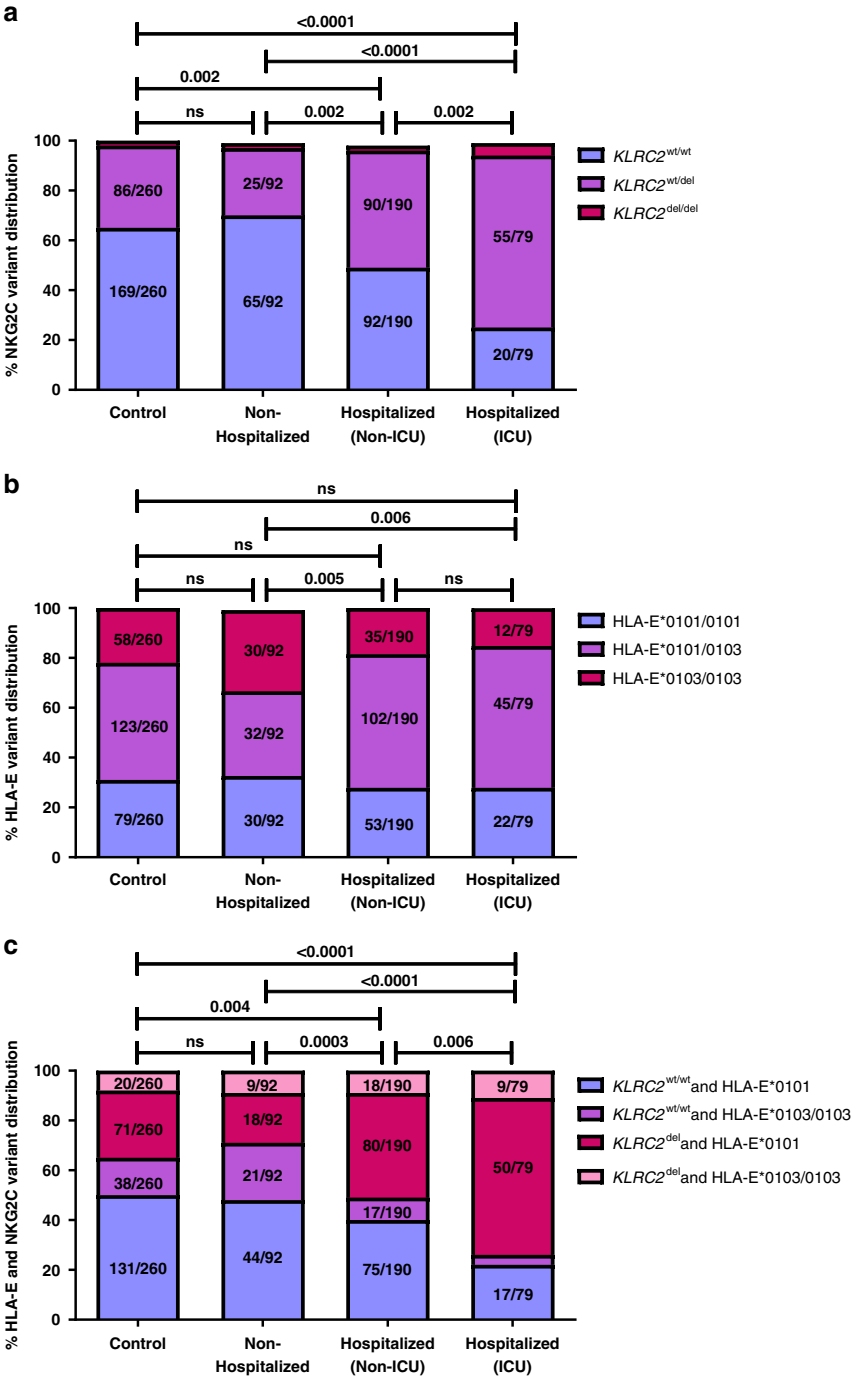

Fig. 1 Distribution of *KLRC2* and HLA-E variants in patients with different disease severities of COVID-19. Distribution of *KLRC2* (**a**,**c**) or HLA-E (**b**,**c**) variants, between control persons ($N = 260$), nonhospitalized ($N = 92$), hospitalized non-ICU ($N = 190$) as well as hospitalized ICU ($N = 79$) COVID-19 patients. Bars represent the relative frequency of (**a**) $KLRC2^{wt/wt}$, $KLRC2^{wt/del}$, and $KLRC2^{del/del}$; (**b**) HLA-E*0101/0101, HLA-E*0101/0103, and HLA-E*0103/0103; (**c**) combined $KLRC2^{wt/wt}$ or $KLRC2^{del}$ as well as the HLA-E*0103/0103 or HLA-E*0101 variants for each group. χ^2 test was used for statistical comparison between variants. del deletion, ICU intensive care unit, wt wild type.

and ICU patients carried more often the heterozygous HLA-E*0101/0103 variant, compared with patients with mild COVID-19 (Fig. 1b). The presence of the HLA-E*0101 allele was significantly associated with hospitalization and hospitalization in ICU ($p = 0.01$ [OR = 2.1] and $p = 0.01$ [OR = 2.7], respectively, χ^2 test). We then also tested the distribution of the HLA-E alleles between male and female COVID-19 patients and between the age groups. Male and female COVID-19 patients carrying the HLA-E*0101 allele, were equally at risk for hospitalization (Table S1). We further compared the distribution of the HLA-E*0101/0103 variants between the age groups and found a higher frequency of the HLA-E*0101 allele in hospitalized and ICU COVID-19 patients among all age groups (Table S2).

We then also evaluated whether $KLRC2^{wt/del}$ and HLA-E*0101/0103 variants are associated with relevant pre-existing comorbidities. No significant association between the comorbidities and the $KLRC2^{wt/del}$ and HLA-E*0101/0103 variants were found (all $p > 0.05$, χ^2 test).

We then analyzed the association between combined HLA-E and *KLRC2* variants and COVID-19 severity. The *KLRC2*^{del} and HLA-E*0101 allele were significantly overrepresented in hospitalized and ICU COVID-19 patients (Fig. 1c). We further performed a multivariable analysis including the presence of the individual *KLRC2* and HLA-E alleles, as well as age and gender of the different patient groups as independent variables. Age >60 years ($p = 0.001$ and $p < 0.001$), *KLRC2*^{del} ($p = 0.001$ and $p < 0.001$), and HLA-E*0101 alleles ($p = 0.006$ and $p = 0.006$) contributed significantly to the overall probability for hospitalization and intensive care treatment. Overall, we found no correlation between the genetic variants and the patients' age or gender in the individual study cohorts (all $p > 0.05$, χ^2 test). The *KLRC2*^{del} as well as the HLA-E*0101/0103 variants were thus found to be independent risk factors for COVID-19.

DISCUSSION

Our data provide evidence that NKG2C-driven NK cell responses might be a main factor limiting virus infection and severity of COVID-19 and that the deletion of the activating NKG2C/CD94 receptor is associated with development of severe COVID-19.

Limited data are so far available concerning the NK cell response against SARS-CoV-2. Recently, it was shown that NK cell counts were significantly reduced in patients with severe compared with those with mild COVID-19[11]. In addition, NK cells from patients with severe COVID-19 showed increased expression of the inhibitory NKG2A$^+$ receptor[11], which is associated with a functional exhaustion of NK cells. As the deletion of the activating NKG2C receptor is linked to decreased or lacking expression of the NKG2C receptor[7], it is feasible that this deletion is further associated with severe COVID-19. The present data underline the important role of a potent antiviral NKG2C$^+$ NK cell response against SARS-CoV-2 and highlight that the NKG2C$^+$ mediated NK cell response may significantly contribute to prevent severe COVID-19.

Epithelial cells of the respiratory tract are the main targets of viral entry due to their high-level expression of the cellular ACE2 receptor[12]. Interestingly, a recently published study demonstrated the upregulation of HLA-E in lung epithelial cells in moderate and severe COVID-19 patients[4]. It is thus reasonable that the NKG2C-mediated NK cell responses represent an important mechanism of immune defense against SARS-CoV-2 infected cells in the respiratory tract. Remarkably, NKG2C is also expressed on a small subset of differentiated CD8 + T cells[13], which may further influence the defense against SARS-CoV-2.

In our study, we also found an increased frequency of the heterozygous HLA-E*0101/0103 variant and the HLA-E*0101 allele in patients with severe, compared with those with mild COVID-19. Recently published in vitro studies have demonstrated that cell surface expression levels are lower for HLA-E*0101/0101 than for HLA-E*0103/0103[8], which may result in a delayed or decreased NKG2C$^+$ NK cell response, and which would support the present data. A recent genome-wide association study (GWAS) identified distinct genetic risk factors for severe COVID-19[3]. The *KLRC2*$^{wt/del}$ variants were not included in these analyses. However, the SNP responsible for the HLA-E*0101/0103 variants, was included and not found among the high-level risk factors for severe COVID-19[3], which is in contrast to our findings. Notably, the association of the HLA-E*0101 allele and severe COVID-19 was especially evident when we analyzed the HLA-E variants together with *KLRC2*$^{wt/del}$, which may explain the discrepancies between the studies. Furthermore analyses in independent and larger study cohorts may reveal whether these discrepancies in the HLA-E variants are due to a potential masking by the cross-replicating associations on a genome-wide level.

The extent of comorbidities such as obesity, hypertension, COPD, or CAD was not different between patients with or without the need for ICU treatment, and thus not a confounding factor. In contrast, patients with mild COVID-19 in our study cohort rarely had comorbidities and thus further studies are required to analyze the impact of comorbidities in combination with the *KLRC2* and HLA-E variants on the development of severe infections.

In conclusion, the finding that the *KLRC2* deletion especially appears to be a significant individual risk factor for severe COVID-19 highlights the significance of the NK cell response against SARS-CoV-2. Further studies are needed to elucidate in detail the impact of the NKG2C$^+$ NK cell–mediated immune responses on the course of COVID-19. Revealing these NK cell–driven antiviral mechanisms could be of high importance for gaining further insight in the protective immune responses against SARS-CoV-2.

DATA AVAILABILITY

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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AUTHOR CONTRIBUTIONS

H.V. and E.P.-S. conceived and planned the experiments. H.V. carried out the experiments. H.V., A.Z., M.T., J.A., S.W.A., and E.P.-S. contributed to the interpretation of the results. E.P.-S. took the lead in writing the manuscript.

All authors provided critical feedback to the manuscript. All authors reviewed the final manuscript.

ETHICS DECLARATION

The study was approved by the ethics committee of the Medical University of Vienna (EK number 1389/2019) and the institutional review board of the Medical University of Vienna (EK number 1389/2019). According to the review board, no informed consent was required from the patients, as only leftover and stored samples from routine laboratory diagnosis were used in the retrospective study.

COMPETING INTERESTS

The authors have declared no competing interests.

ADDITIONAL INFORMATION

The online version of this article (https://doi.org/10.1038/s41436-020-01077-7) contains supplementary material, which is available to authorized users.

Correspondence and requests for materials should be addressed to E.P.-S.

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