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BRIEF COMMUNICATION

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HLA allele frequencies and susceptibility to COVID-19 in a group of 99 Italian patients

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With the aim to individuate alleles that may reflect a higher susceptibility to the disease, in the present study we analyzed the HLA allele frequency distribution in a group of 99 Italian patients affected by a severe or extremely severe form of COVID-19. After the application of Bonferroni's correction for multiple tests, a significant association was found for *HLA-DRB1*15:01*, *-DQB1*06:02* and *-B*27:07*, after comparing the results to a reference group of 1017 Italian individuals, previously typed in our laboratory. The increased frequencies observed may contribute to identify potential markers of susceptibility to the disease, although controversial results on the role of single HLA alleles in COVID-19 patients have been recently reported.

KEYWORDS

COVID-19, HLA, Disease susceptibility

COVID-19 is a severe acute respiratory syndrome caused by SARS-CoV-2 that affected in the last few months more than 12 million individuals around the world (https://covid19.who.int/). Although many studies have been addressed to elucidate the genetics and genomics characteristics of the virus, different aspects of the disease are still unknown, such as its impact on patients' susceptibility, the progression and the clinical outcome of the disease.[1-3]

Ongoing researches are trying to elucidate the mechanisms potentially responsible for inducing the immune response against SARS-Cov-2, including the role of HLA alleles in the affected individuals.[4] The HLA complex constitutes a specific group of molecules expressed on the cell surface, crucial for the recognition of nonself molecules by the acquired immune system. Their essential function is to bind and display antigens derived from pathogens on the cell surface and present them to the appropriate T lymphocytes, triggering an immune response.[5,6]

Different scientific contributions were recently published in the literature addressing the potential relevance of HLA polymorphism and susceptibility to pandemic viruses, with particular interest in the SARS-Cov-2 infection, focusing the attention on differences of HLA allele frequency and distribution of HLA peptide binders in populations.[4,7-10] Up to date conflicting results were published in the Literature. A recent study by Ellinghaus et al, investigating a large cohort of COVID-19 patients from Italy and Spain, using a genome-wide association study approach, did not find any significant evidence of HLA involvement. On the contrary Kaghuri et al in the version of the manuscript posted on 7 May 2020, in medRxiv, identified seven HLA susceptibility alleles.

Although a low sample size might confer to a statistic analysis insufficient power, particularly for systems with multiple alleles such as HLA, we analyzed the HLA alleles frequency distribution in a group of 99 COVID-19 Italian patients, with the aim to individuate alleles that may reflect a higher susceptibility to the disease.

The median age of the 99 analyzed patients was 63 years (range: 2-92 years), including 10 children with a severe form of the disease, but none of them with Kawasaki-like syndrome .[11,12] The majority of the enrolled patients were males (61 males, 38 females). Patients were subdivided into two groups according to the state of the disease: (a) severe, characterized by a respiratory impairment, requiring noninvasive ventilation; (b) extremely severe, defined as respiratory failure, requiring invasive ventilation and intensive care unit admission.

Next-generation sequencing molecular HLA typing was performed on patients DNA (Symphonia, Qiagen) using the AllType kit (One Lambda, Canoga Park, California). These kits use a single multiplexed polymerase chain reaction to amplify the full HLA-A/B/C/DQA1/DPA1 gene sequences and from exon 2 to the 3′UTR of the HLADRB1/3/4/5/DQB1/DPB1 genes. Libraries were run on the Ion S5 platform using Thermo Fisher Scientific reagents. Reads were analyzed using the HLA TypeStream Visual Software (One Lambda), ver. 1.3, updated to the IPD-IMGT/HLA last database release, 3.40.0.[13]

In order to contextualize the potential susceptibility of the infection to the specific population, results were compared to a reference group of 1017 Italian individuals, previously typed in our laboratory, representative of HLA allele frequency distribution in Italy.[14-16] Differences between the allele frequencies of the two groups were analyzed using a 2×2 contingency table calculating the chi-square statistic and Yates correction. The strength of association between HLA alleles was estimated by 95% confidence intervals; *P* values were corrected for multiple comparisons according to the Bonferroni's method (pc).

As shown in Table 1, after applying Bonferroni's correction, a significant association was found for three

TABLE 1 Comparison of HLA allele frequencies between a group of 99 COVID-19 patients and a group of 1017 Italian individuals

Healthy Italian individuals (2034 haplotypes)			COVID-19 Italian patients (198 haplotypes)				
Allele	**N**	***F%***	**Allele**	**N**	***F%***	***P***	**pc**
*B*27:07*	2	0.10	*B*27:07*	4	2.02	0.00001	0.004
*B*58:01*	41	2.02	*B*58:01*	10	5.05	0.01317	ns
*C*06:02*	228	11.21	*C*06:02*	9	4.55	0.005356	ns
*DRB1*07:01*	291	14.31	*DRB1*07:01*	17	8.59	0.0339	ns
*DRB1*15:01*	94	4.62	*DRB1*15:01*	20	10.10	0.0015	0.048
*DQB1*06:02*	74	3.64	*DQB1*06:02*	15	7.58	0.0001	0.0016

Abbreviations: F%, allele frequency, in percent; N, number of alleles; ns, not significant; *P*, value of chi-square statistic analysis; pc, statistic value after Bonferroni's correction.

TABLE 2 HLA allele frequencies in 99 COVID-19 Italian patients

Locus A	N	*F%*	Locus B	N	*F%*	Locus C	N	*F%*	Locus DRB1	N	*F%*	Locus DQB1	N	*F%*
*A*01:01*	24	12.12	*B*07:02*	12	6.06	*C*01:02*	5	2.53	*DRB1*01:01*	7	3.54	*DQB1*02:01*	13	6.57
*A*01:02*	1	0.51	*B*07:05*	1	0.51	*C*02:02*	7	3.54	*DRB1*01:02*	10	5.05	*DQB1*02:02*	13	6.57
*A*02:01*	44	22.22	*B*07:06*	1	0.51	*C*03:02*	4	2.02	*DRB1*03:01*	13	6.57	*DQB1*03:01*	60	30.30
*A*02:05*	6	3.03	*B*08:01*	8	4.04	*C*03:03*	7	3.54	*DRB1*04:01*	2	1.01	*DQB1*03:02*	12	6.06
*A*03:01*	23	11.62	*B*13:02*	1	0.51	*C*04:01*	39	19.70	*DRB1*04:02*	2	1.01	*DQB1*03:03*	5	2.53
*A*03:02*	2	1.01	*B*14:01*	1	0.51	*C*04:09:N*	1	0.51	*DRB1*04:03*	5	2.53	*DQB1*03:04*	1	0.51
*A*11:01*	16	8.08	*B*14:02*	10	5.05	*C*05:01*	5	2.53	*DRB1*04:04*	1	0.51	*DQB1*03:05*	1	0.51
*A*23:01*	9	4.55	*B*15:01*	4	2.02	*C*06:02*	9	4.55	*DRB1*04:05*	5	2.53	*DQB1*04:02*	8	4.04
*A*24:02*	28	14.14	*B *15:17*	3	1.52	*C*07:01*	32	16.16	*DRB1*04:07*	2	1.01	*DQB1*05:01*	19	9.60
*A*25:01*	1	0.51	*B*18:01*	19	9.60	*C*07:02*	15	7.58	*DRB1*04:11*	1	0.51	*DQB1*05:02*	14	7.07
*A*26:01*	6	3.03	*B*18.03*	1	0.51	*C*07:04*	3	1.52	*DRB1*07:01*	17	8.59	*DQB1*05:03*	11	5.56
*A*29:01*	1	0.51	*B*27:02*	1	0.51	*C*07:06*	1	0.51	*DRB1*08:01*	5	2.53	*DQB1*06:01*	3	1.52
*A*29:02*	1	0.51	*B*27:05*	1	0.51	*C*07:18*	4	2.02	*DRB1*08:02*	1	0.51	*DQB1*06:02*	15	7.58
*A*30:01*	3	1.52	*B*27:07*	4	2.02	*C*07:119*	1	0.51	*DRB1*08:04*	1	0.51	*DQB1*06:03*	11	5.56
*A*30:02*	6	3.03	*B*35:01*	14	7.07	*C*07:783*	1	0.51	*DRB1*09:01*	1	0.51	*DQB1*06:04*	10	5.05
*A*30:04*	2	1.01	*B*35:02*	9	4.55	*C*08:02*	10	5.05	*DRB1*10:01*	1	0.51	*DQB1*06:09*	2	1.01
*A*31:01*	4	2.02	*B*35:03*	10	5.05	*C12:02*	2	1.01	*DRB1*11:01*	19	9.60		198	100,00
*A*32:01*	8	4.04	*B*35:08*	3	1.52	*C*12:03*	22	11.11	*DRB1*11:03*	1	0.51			
*A*33:01*	5	2.53	*B*35:12*	1	0,51	*C*14:02*	9	4.55	*DRB1*11:04*	28	14.14			
*A*33:03*	2	1,01	*B*35:43*	1	0.51	*C*15:02*	11	5.56	*DRB1*11:15*	1	0.51			
*A*68:01*	6	3.03	*B*37:01*	1	0.51	*C*15:05*	2	1.01	*DRB1*12:01*	3	1.52			
	198	100.00	*B*38:01*	5	2.53	*C*16:01*	2	1.01	*DRB1*13:01*	12	6.06			
			*B*38:02*	1	0.51	*C*16:02*	3	1.52	*DRB1*13:02*	13	6.57			
			*B*39:01*	4	2.02	*C*16:04*	1	0.51	*DRB1*13:03*	4	2.02			
			*B*39:06*	3	1.52	*C*17:01*	2	1.01	*DRB1*14:01*	2	1.01			
			*B*40:02*	1	0.51		198	100,00	*DRB1*14:06*	1	0.51			
			*B*41:01*	4	2.02				*DRB1*14:54*	8	4.04			
			*B*44:02*	4	2.02				*DRB1*15:01*	20	10.10			
			*B*44:03*	4	2.02				*DRB1*15:02*	2	1.01			
			*B*45:01*	1	0.51				*DRB1*15:04*	1	0.51			
			*B*49:01*	10	5.05				*DRB1*16:01*	8	4.04			

TABLE 2 (Continued)

Locus A	N	F%	Locus B	N	F%	Locus C	N	F%	Locus DRB1	N	F%	Locus DQB1	N	F%
			*B*50:01*	2	1.01				*DRB1*16:02*	1	0.51			
			*B*51:01*	24	12.12					198	100,00			
			*B*51:07*	1	0.51									
			*B*51:08*	1	0.51									
			*B*52:01*	1	0.51									
			*B*53:01*	4	2.02									
			*B*55:01*	5	2.53									
			*B*56:01*	1	0.51									
			*B*57:01*	5	2.53									
			*B*57:03*	1	0.51									
			*B*58:01*	10	5.05									
			*B*73:01*		0.00									
				198	100,00									

Abbreviations: F%, allele frequency, in percent; N, number of alleles.

alleles: *HLA B*27:07* (0.10% vs 2:02%—P = .00001; pc = 0.004), *DRB1*15:01* (4.62% vs 10.10%—P = 0.0015; pc = 0.0480), *DQB1*06:02* (3.64% vs 7.58%—P = .0001; pc = 0.016). A positive association was also observed for *B*58:01* (2.02% vs 5.05%—P = 0.01317, pc = 10 012) that was lost after the application of Bonferroni's correction. Two different alleles were on the contrary negatively associated with SARS-CoV-2; *C*06:02* (11.21% vs 4.5%—P = .005356, pc = 0.1339) and *DRB1*07:01* (14.31% vs 8.59%—P = 0.0339, pc = 1.087), that, however, did not maintain the statistical significance after the application of the Bonferroni's correction. In Table 2, we also show the HLA alleles distribution of the 99 COVID-19 patients.

The increased frequencies that we observed for *DRB1*15:01* and *DQB1*06:02*, in strong linkage disequilibrium with each other, in the 99 severe affected COVID-19 Italian patients were not in line with the results obtained on a larger survey studied by Ellinghaus et al[10] that did not show any association between HLA and COVID-19, but confirmed those published by Kachuri et al[7] identifying these two alleles among seven HLA susceptibility alleles. Despite the small sample size, that might represent a risk for a false positive finding, we believe that our observations may be interesting to share for contributing to verify the potential relevance of specific HLA alleles interacting with SARS-CoV-2, prior to a specific mandatory multicenter study that is planned to be performed in the near future.

CONFLICT OF INTEREST

The authors have declared no conflicting interests.

AUTHORS CONTRIBUTION

A.N., G.N., F.L. and M.A. designed the study, analyzed the data and wrote the manuscript; P.R., F.L., A.C. and M.A. selected and cured patients; M.B., L.L., C.P., V.L.C. provided genetic laboratory data; R.C. and S.B. critically reviewed the manuscript. All authors read and approved the final version of the manuscript.

ETHICS STATEMENT

Biological samples used in this study were collected according to the ethical procedures of the EFACOVID2.0 research program promoted by the University of Rome Tor Vergata. This program ensures that the work is carried out with the highest regard for ethical issues and with respect to the rights, integrity and privacy of patients. All consent, material/information storage and distribution procedures

have been approved by the local Ethics Committees (CEI PTV protocol no. 50/20). SARS-CoV-2 positive patients who are offered participation in a research study sign an informed consent prepared ad hoc, which provides detailed information on the type of test, the implications of the genetic results and the possible psychosocial implications. As regards the participation of children in the research, consent and authorization are expected to be signed by the parents in accordance with the rules laid down by the Ethics Committee of the Bambino Gesù Hospital in Rome (http://www.ospedalebambinogesu.it/en/home).

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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