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Research article

HLA repertoire of 115 UAE nationals infected with SARS-CoV-2

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ABSTRACT

The class I and class II Human Leucocyte Antigens (HLA) are an integral part of the host adaptive immune system against viral infections. The characterization of HLA allele frequency in the population can play an important role in determining whether HLA antigens contribute to viral susceptibility. In this regard, global efforts are currently underway to study possible correlations between HLA alleles with the occurrence and severity of SARS-CoV-2 infection. Specifically, this study examined the possible association between specific HLA alleles and susceptibility to SARS-CoV-2 in a population from the United Arab Emirates (UAE). The frequencies of HLA class I (*HLA-A, -B,* and *-C*) and HLA class II alleles (*HLA-DRB1* and *-DQB1*); defined using Next Generation Sequencing (NGS); from 115 UAE nationals with mild, moderate, and severe SARS-CoV-2 infection are presented here. HLA alleles and supertypes were compared between hospitalized and non-hospitalized subjects. Statistical significance was observed between certain HLA alleles and supertypes and the severity of the infection. Specifically, alleles *HLA-B*51:01 and HLA-A*26:01* showed a negative association (suggestive of protection), whilst genotypes *HLA-A*03:01, HLA-DRB1*15:01,* and supertype B44 showed a positive association (suggestive of predisposition) to COVID-19 severity. The results support the potential use of HLA testing to differentiate between patients who require specific clinical management strategies.

1. Introduction

The Human Leucocyte Antigens (HLA) class I (HLA-A, HLA-B, and HLA-C) and class II (HLA-DRB1, HLA-DRA1, HLA-DQB1, HLA-DQA1, HLA-DPB1, and HLA-DPA1) genes of the Major Histocompatibility Complex (MHC) are important elements of the immune system. Proteins encoded by these genes play a role in initiating the host response against viral infections [1]. In this regard, studies have shown that specific HLA alleles and HLA haplotypes play a role in the severity as well as the progression of several autoimmune diseases [2] as well as viral diseases, including Human Immunodeficiency Virus (HIV) [3-7], hepatitis B [8,9], hepatitis C [10-12] and influenza [13-15].

Moreover, HLA class I and class II homozygosity or heterozygosity appear to play a role in viral disease susceptibility. In genetically isolated populations, homozygosity has been shown to lead to greater susceptibility to infection, whereas heterozygosity can increase resistance to infection [16,17]. The highly polymorphic

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nature of the HLA class I and HLA class II genes encode diverse protein variants that control the adaptive immune system. HLA class I molecules have two linked polypeptide chains, α and β–2 microglobulin. A peptide-binding groove exists between the α1 and α2 domains. The peptide-binding groove of the class I molecule accommodates peptides of 8 to 10 amino acids in length. When a virus infects a host cell, its proteins are degraded into small peptides. These viral peptides then bind to the binding groove of these HLA class I molecules and are exposed on the surface of the cell. The presence of those viral antigens is then recognized by leukocytes, and a cascade of immune response events is initiated to eliminate the virus [18]. HLA class II molecules have two polypeptides chains, an α and a β chain. The peptide-binding groove of HLA class II is made up of the α1 and β1 domains. It can accommodate up to 12 to 24 amino acids where the peptide binds in an extended conformation, with about a third of the peptide being approachable by the CD8 + T-cells [1].

Although limited, information on the association between HLA and COVID19 is gradually accumulating. Insights can also be gleaned from previous reports on the closely related SARS-CoV and MERS-CoV [18] strains of coronavirus. To date, HLA-B is the most polymorphic class I gene, followed by HLA-A and HLA-C [19]. Polymorphic sites situated in locations that encode the peptide-binding groove, which interacts with T-cell receptors, impacts the binding specificity of amino acids in the binding groove [1]. The frequencies of HLA variants differ between populations as a result of historical migrations leading to admixture and different selection pressures of past epidemics [1]. These factors, among others, have contributed to the selection of the HLA alleles with antigen peptide-binding properties for specific viral peptides. Previous research describing antigen presentation of SARS-CoV has revealed that several HLA variants contribute to the susceptibility of the infection. These HLA variants have included *HLA-B*46:01, HLA-B*07:03, HLA-DRB1*12:02* [20], and *HLA-C*08:01*, whereas *HLA-DRB1*03:01, HLA-C*15:02*, and *HLA-A*02:01* alleles were found to be protective against infection [18].

Genome sequencing of SARS-CoV-2 and SARS-CoV isolates has shown a high degree of homology at the nucleotide level between the two viruses, but there is a noticeable difference at the protein level [21]. Protein sequence alignment analysis revealed that there are two proteins; encoded by orf8 and orf10 in SARS-CoV-2; that have no homologous proteins in SARS-CoV, which may contribute to the differences in the HLA response to SARS-CoV and SARS-CoV-2 [21].

The highly infectious nature of SARS-CoV-2 and its rapid spread warrants the study of factors that contributed to the differences observed in the susceptibility and severity of SARS-CoV-2 among individuals. Severity risk factors include age, cardiovascular disease, and metabolic disorders comorbidity [22]. However, the role of genetic variations in individuals that account for the different clinical manifestations of the disease remains elusive [23]. Epidemiological studies have shown that while most cases of COVID-19 are mild in nature, the disease appears to be highly fatal in severe cases. A fatality rate of around 26% among hospitalized patients and 37% among those who have needed invasive mechanical ventilation has been reported in the United Kingdom [24]. Studies have shown that HLA class I genes or those that are in linkage disequilibrium with HLA class I genes might have an impact on the pathogenesis of the severe acute respiratory syndrome (SARS) in 2003 [20] and SARS-CoV-2 in 2019 [25-27]. Furthermore, our research has implicated *HLA-B*35* and *HLA-C*07*, as well as the 2-locus haplotype: *HLA -C*04-B*35* to SARS-CoV-2 infection in a mixed population that resides in the UAE.

The latest research points to genetic variants that contribute to immunity factors may also be playing a role in the manifestation of the observed variable clinical outcomes [28]. Knowledge of genetic biomarkers, including HLA, could assist in prioritizing public health intervention for vulnerable individuals in addition to providing individualized therapeutic targets [28]. Using Next Generation Sequencing (NGS) based HLA typing, this study investigated a possible correlation between the occurrence and severity of COVID-19 and certain HLA alleles and haplotypes in 115 COVID-19 patients from the UAE.

2. Materials and methods

2.1. Enrolment and sample collection

Blood samples of 115 unrelated patients with COVID-19 were collected during their COVID-19 case review at the Sheikh Khalifa Medical Centre (SKMC), Abu Dhabi, UAE. All 115 patients were nationals of the UAE. Information about the study was provided to potential participants. Those who agreed to be part of the study provided their written consent on forms that were approved by the Abu Dhabi Health COVID-19 Research Ethics Committee (DOH/DQD/2020/538). Reciprocal approval was also granted by the Dubai Scientific Research Ethics Committee (DSREC-04/2020_09) and SEHA Research Ethics Committee (SEHA-IRB-005). Samples from minors (age < 18 years) were collected after obtaining signed assent forms from their parents. Blood was collected by an experienced phlebotomy nurse. A total of 2 ml of blood was collected from the cubital vein in an ethylenediaminetetraacetic acid (EDTA) tube. Samples were stored in a sealed biohazard bag and transported at 4 °C to the testing laboratories at Khalifa University, using a cool transport container.

2.2. Demographic data collection

Demographic data, including age, gender, and medical history (Table 1), were obtained with the aid of questionnaires. Clinical assessments of the participants provided by the healthcare providers included the determination of the level of severity (mild, moderate, and severe) and diagnosis of pneumonia which was confirmed using a chest x-ray. The group of participants that presented with mild or no disease symptoms did not require hospitalization (n = 82). The patients from the moderate group exhibited symptoms such as fever, cough, and pneumonia, which required hospitalization (n = 12). The severe group presented with critical clinical features such as high temperature, cough, pneumonia, shortness of breath, and required intensive care (n = 21).

2.3. DNA extraction and HLA sequencing

DNA was extracted from the EDTA tubes using the MagPurix system (Zinexts, Taiwan) as per the recommendations by the manufacturer. The quantity of the genomic DNA was determined by the dsDNA broad range fluorescence-based quantitation method (Denovix, Wilmington, USA). HLA genotyping was performed using the Holotype HLA 96/11 library kit (Omixon, Budapest, Hungary)

Table 1
Demographic data of the COVID-19 Abu Dhabi cohort, United Arab Emirates.

	Mild (n = 82)	Moderate and severe (n = 33)
Age mean in years	34 ± 14	58 ± 15
Gender		
Female – count (percentage)	27 (32.9%)	9 (27.2%)
Male– count (percentage)	55 (67%)	24 (72.7%)
Clinical outcome		
Pneumonia – count (percentage)	18 (22.2 %)	33 (100 %)
Death - count	0 (0%)	10 (30.3 %)

according to the manufacturer's protocol. The first step involved amplification of HLA class I and HLA class II loci by long-range Polymerase Chain Reaction (PCR). The PCR products of each individual were subsequently pooled for cleanup. The third step was the library preparation phase, which involved fragmentation, end repair, and ligation with indexed adaptors. Libraries were then combined into a single pooled library, and size selection was carried out using AMPure XP beads (Beckman Coulter, Massachusetts, USA). Quantification of the single pooled library was performed on the Viia 7 real-time PCR instrument (Applied Biosystems, Foster City, USA) using KAPA library quantification ROX low kit (Kappa Biosystems, Wilmington, USA). The final pooled library was then loaded onto the Illumina Miseq system (Illumina, San Diego, USA). FASTQ sequencing files were uploaded into the HLA Twin Software v4.2.0 (Omixon, Budapest, Hungary) for analysis. The software runs two independent computational algorithms to provide high confidence allele calling.

2.4. Statistical analysis

The samples were genotyped at a resolution of up to 4-fields using NGS. However, due to the sample size, the results in the second field resolution (subtype) were used for analysis and reported to minimize the number of tests, as analysis with high resolution would have generated too many unique 3-field allele assignments. Further, restricting the analysis to 2-field resolution allowed comparisons with published results.

The cohort was divided into two groups, hospitalized (patients with moderate to severe conditions) and non-hospitalized (patients who were asymptotic or had very mild symptoms).

Rare and very rare alleles were checked using the rare allele detector server (score < 4) available at (http://www.allelefrequencies.net), which detects rare and very rare alleles based on the established common and well-documented alleles in world populations [29].

Expected heterozygosity (He) and inbreeding coefficient (F_{is}) for each locus were determined using GenePop (stand-alone version 4.7.0 with the default settings) [31]. Hardy Weinberg Equilibrium test (HWE) was obtained using Genepop and Pypop [30] (the Guo and Thompson Hardy Weinberg test) [32]. Hardy-Weinberg equilibrium (HWE) was further tested using Genepop when H1 = excess heterozygosity and when H1 = defect heterozygosity using default settings (Markov chain parameters: dememorization = 10,000, batches = 20, iterations per batch = 5000).

Based on the proposed approach for analyzing immunogenetic data in case-control studies by Hollenbach, et al. [33], the Bridging ImmunoGenomic Data-Analysis Workflow Gaps (BIGDAWG) R package was used for case-control association analyses of individual HLA loci and amino acid level analysis [34].

HLA allelic association analyses was estimated using chi-square testing. Calculations of odds ratio (95% confidence interval), and P-values were performed using the R epicalc package implemented in BIGDAWG. The BIGDAWG software was designed for the analysis of highly polymorphic HLA data. The software combines rare alleles for multi-locus analyses with expected counts less than five (or degree of freedom do not allow for a test) into a common class (binning) for each locus and performs a goodness-of-fit test.

The corrected P values (P_c) for multiple comparisons were calculated using the Bonferroni method. The significance threshold (P = 0.05) was divided by the number of tested alleles after binning by BIGDAWG for the allelic association analysis (5 for HLA-A, 3 for HLA-C, 4 for HLA-B, 5 for HLA-DRB1, and 8 for HLA-DQB1), and the number of supertypes groups for the supertype association analysis (7 for *HLA-A*, 3 for *HLA-C* and 8 for *HLA-B* including the unclas-

sified group). All associations reported herein are based on the corrected P-value.

2.5. In silico prediction of HLA class I binding affinity to viral peptides

In order to perform in silico peptide binding prediction for the complete SARS-CoV-2 proteome (9,744 amino acids), we obtained FASTA-formatted protein sequence data from the National Center of Biotechnology Information (NCBI) database (https://www.ncbi.nlm.nih.gov/genome/browse#!/overview/Sars-cov-2). Specifically, the following 11 protein viral products were retrieved: ORF1ab (YP_009724389.1), surface glycoprotein (S) (YP_009724390.1), ORF3a (YP_009724391.1), envelope (E) (YP_009724392.1), membrane glycoprotein (M) (YP_009724393.1), ORF6 (YP_009724394.1), ORF7a (YP_009724395.1), ORF7b (YP_009725318.1), ORF8 (YP_009724396.1), nucleocapsid (N) (YP_009724397.2), and ORF10 (YP_009725255.1). Those sequences were k-meirzed to 8-, 9-, 10-, 11- and 12-mers and uploaded into the NetMHCpan v4.1 server [35].

3. Results

The Abu Dhabi COVID-19 cohort in this study consisted of 115 UAE nationals infected with SARS-CoV-2. The age of the patients ranged from 16 to 80 years. Around seventy-one percent of the cohort were asymptomatic, while 28.6% had a moderate or severe infection. In total, 51 patients developed pneumonia: 18 out of 82 mild cases and 33 out of 33 moderate and severe cases. A summary of the demographics of the cohort can be found in Table 1. The correlation between age and COVID-19 severity was examined to assess the effect of age as a confounding factor in the study (see Fig. 1).

Table 2 lists the HLA class I (*HLA-A, -B, -C*) and class II (*HLA-DRB1, -DQB1*) allele counts of the non-hospitalized (n = 82) compared to the hospitalized (n = 33) group.

In total, we identified 40 *HLA-A* alleles, 32 *HLA-C* alleles, 38 *HLA-B* alleles, 41 *HLA-DRB1* alleles, and 17 *HLA-DQB1* alleles. However, due to low counts of some alleles, 35 alleles of *HLA-A*, 29 of *HLA-C*, 34 of *HLA-B*, 36 of *HLA-DRB1*, and nine of *HLA-DQB1* were combined (binned) into 5 common groups (*HLA-A* binned, *HLA-C* binned, *HLA-B* binned, *HLA-DRB1* binned, and *HLA-DQB1* binned)
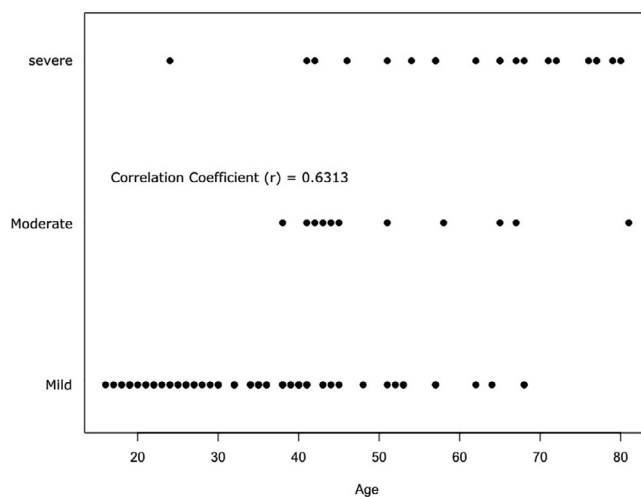

Fig. 1. A moderately positive correlation between Covid-19 severity and age in 115 UAE national patients with SARS-Cov-2 infection. Each point in the graph represents one individual. Four patients, 2 with mild, 1 with moderate, and 1 with severe symptoms, were excluded from the analysis due to the lack of necessary data (Age or date of birth).

Table 2
HLA Class I and class II allele count and statistical association in non-hospitalized and hospitalized SARS-CoV2 infected patients.

HLA Allele	Non-hospitalized (n = 82)	Hospitalized (n = 33)	OR	P-value	95% CI	Effect	SB (<50 nM)	WB (<500 nM)
HLA-A*02:01	8	11	2.28	0.0929	(0.77–7.01)		243	728
HLA-A*03:01	**2**	**10**	**8.75**	**0.0015**	**(1.73–84.34)**	**Risk**	249	729
HLA-A*24:02	9	2	0.31	0.1200	(0.03–1.57)		328	964
HLA-A*26:01	**13**	**0**	**0.00**	**0.0019**	**(0.00–0.44)**	**Protective**	347	986
HLA-C*04:01	9	8	1.92	0.2035	(0.60–6.01)		248	752
HLA-C*06:02	20	8	0.76	0.5551	(0.27–2.00)		239	726
HLA-B*08:01	3	7	3.31	0.0787	(0.71–20.62)		220	722
HLA-B*50:01	9	6	0.85	0.7707	(0.23–2.88)		193	621
HLA-B*51:01	**31**	**0**	**0.00**	**<0.0001**	**(0.00–0.10)**	**Protective**	**205**	**652**
HLA-DRB1*03:01	16	7	0.86	0.7502	(0.28–2.39)			
HLA-DRB1*07:01	16	7	0.86	0.7502	(0.28–2.39)			
HLA-DRB1*11:01	11	3	0.52	0.3229	(0.09–2.09)			
HLA-DRB1*15:01	**2**	**8**	**9.22**	**0.0012**	**(1.72–91.09)**	**Risk**		
HLA-DQB1*02:01	20	3	0.31	0.0611	(0.06–1.15)			
HLA-DQB1*02:02	13	5	0.91	0.8657	(0.24–2.95)			
HLA-DQB1*03:01	16	7	1.05	0.9140	(0.34–2.97)			
HLA-DQB1*03:02	12	2	0.37	0.1911	(0.04–1.78)			
HLA-DQB1*04:02	8	6	1.91	0.2502	(0.51–6.72)			
HLA-DQB1*05:01	6	7	3.11	0.0442	(0.83–11.87)			
HLA-DQB1*05:02	14	5	0.84	0.7463	(0.22–2.67)			

Alleles that showed statistical significance (corrected P-value < 0.05) are denoted in bold. OR: odds ratio, CI: confidence interval.

by BIGDAWG prior to computing the $\chi 2$ statistic. This resulted in a total of 5 alleles for *HLA-A*, 3 for *HLA-C*, 4 for *HLA-B*, 5 for *HLA-DRB1*, and 8 for *HLA-DQB1*.

Statistical analysis incorporating the binned groups resulted in some significant allele associations between the hospitalized and non-hospitalized groups (Table 2). From class I, *HLA-A*03:01* (P = 0.0015) was observed more frequently in the hospitalized groups while *HLA-A*26:01* (P = 0.0019) and *HLA-B*51:01* (P < 0.0001) genotypes were common among non-hospitalized patients and absent in the hospitalized group, indicating a possible protective effect. From class II, *HLA-DRB1*15:01* (P = 0.0021) was significantly higher in the non-hospitalized group.

Analysis for strong binding (SB) and weak binding (WB) of SARS-CoV-2 peptide binding affinity for each HLA class I allele are presented in Table 2. Strong binders (SB) are defined as having a percentile binding affinity rank (%rank) $\leq$ 0.5, while weak binders (WB) are defined as having a %Rank $\leq$ 2%. The total number of different peptides recognized as SB or WB for each HLA allele was then calculated to identify the HLA allele with the greater number of possible strong and weak viral peptide recognition. However, when the predicted peptide binding results were compared to the genotypic data of this study cohort, no direct correlation to severity was found (Table 2).

The extreme polymorphism of HLA alleles results in a vast diversity of peptide-binding HLA specificities and low population coverage for any given peptide-binding HLA specificity [36]. Several researchers have found that aggregating HLA alleles into supertypes is useful in studies of disease association [36,37], and in designing specific cytotoxic T-lymphocyte (CTL) vaccine candidates for SARS [38]. This increases the power of the studies by grouping large numbers of rare alleles according to their functional relevance [37]. In this regard, the differences between the hospitalized and the non-hospitalized group based on HLA class I supertypes were estimated.

To test for HLA supertype association, the revised and updated classification of HLA class I supertypes for locus A and B described by Sidney, et al. [36], which covers most of the HLA class I polymorphism, was used. Only those subtypes [36] that were identified based on the stringency of selection (i.e. reference panel) were considered in the analysis. Unlike HLA class I, class II supertypes have been less extensively studied due to the complexity of their struc-

ture. In this regard, this paper only discusses class I supertype associations.

After correcting for multiple comparison, the results of this study showed that supertype B44 (including *HLA-B*18:01, -B*40:02, -B*41:01, -B*44:02, -B*44:03, B*45:01, -B*50:01*) is significantly associated to SARS-CoV-2 severity (see Fig. 2).

In the current study, an overall higher than expected level of homozygosity for *HLA-A, HLA-B, HLA-C, HLA-DRB1,* and *HLA-DQB1* was observed in the 115 infected patients after applying the Hardy-Weinberg test using PyPop (Table 3). Further, we compared the heterozygosity level between hospitalized and non-hospitalized patients and found that *HLA-A* and *HLA-B* loci showed a statistically significant association with severity (Fig. 3). Others have highlighted associations between homozygosity of HLA class I alleles with age at death in COVID-19 patients [39].

The inbreeding coefficient (F_{is}) for the cohort ranged from 0.19 to 0.35 for the Weir, and Cockerham estimates and from 0.12 to 0.37 with Robertson and Hill estimates, where an estimate up to 0.1 demonstrates a low degree of inbreeding and a zero represents individuals with arbitrarily remote common ancestors (Table 4).

Locus level association analysis results showed that *HLA-A* (P < 0.001) and *HLA-B* (P < 0.001) are significantly associated with COVID-19 severity (Table 5).

From the amino acid position association analysis, multiple positions from locus *HLA-A* and *HLA-B* were found in a significant association with the disease (P < 0.001) while *HLA-C* had one position, namely position.121, that was marginally significant. From class II locus, two positions from *HLA-DRB1* [position.13 (P = 0.007), position.72 (P = 0.0012)] and one position from *HLA-DQB1* [position.57 (P = 0.028)] were associated with the disease. Table 6 summarizes all amino acid associations.

4. Discussion

Since the outbreak of SARS-CoV-2 in 2019, HLA variability analysis has been the focus of many groups to identify possible genetic variations that contribute to the severity and progression of the disease [25-27]. However, most of these findings do not align, making it difficult to draw any conclusions about the role of HLA in COVID-19 infection. In this regard, it is important to consider the fact that different allele frequencies observed in association with COVID-19 might vary between racial backgrounds [25-28]. Thus,
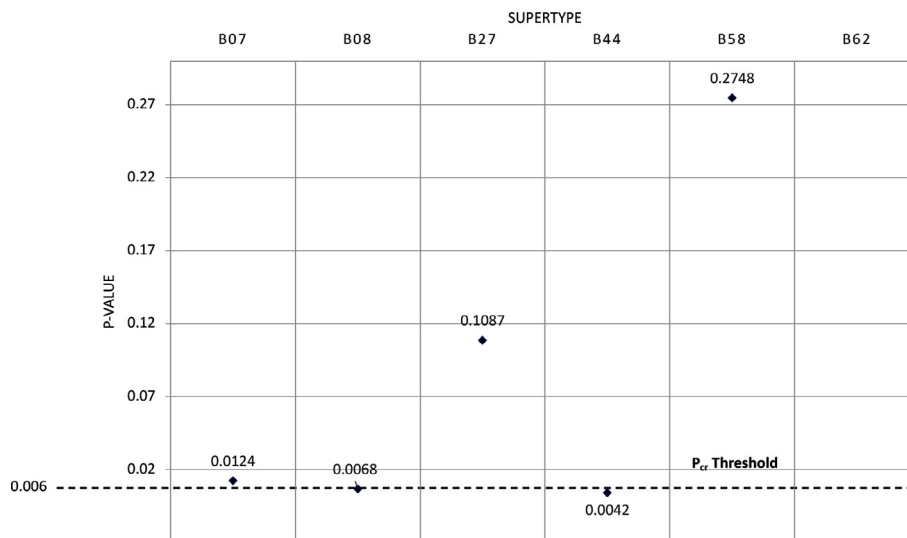
Fig. 2. The association of HLA-B supertypes on the severity of SARS-CoV-2 infection in the current cohort. The dots represent uncorrected P-values and the dotted line represents the P-corrected treshhold (Pcr = 0.006). Only B44 supertypes are associated with SARS-CoV-2 infection.

Table 3
Hardy-Weinberg test for heterozygote deficiency and excess for each HLA locus in COVID-19 group using Genepop.

| | | | F_{is} estimation | |
Locus	P-value	S.E.	W&C	R&H
HLA-A	0.0000	0.0000	0.1935	0.1159
HLA-B	0.0000	0.0000	0.2450	0.1621
HLA-C	0.0000	0.0000	0.2234	0.2690
HLA-DRB1	0.0000	0.0000	0.3505	0.3725
HLA-DQB1	0.0001	0.0001	0.3024	0.2826

Hardy Weinberg test when H1 = heterozygote deficit

| | | | F_{is} estimation | |
Locus	P-value	S.E.	W&C	R&H
HLA-A	0.9997	0.0003	0.1935	0.1159
HLA-B	1.0000	0.0000	0.2450	0.1621
HLA-C	1.0000	0.0000	0.2234	0.2690
HLA-DRB1	1.0000	0.0000	0.3505	0.3725
HLA-DQB1	1.0000	0.0000	0.3024	0.2826

Hardy Weinberg test when H1 = heterozygote excess

| | | | F_{is} estimation | |
Locus	P-value	S.E.	W&C	R&H
HLA-A	0.0017	0.0017	0.1935	0.1159
HLA-B	0.0000	0.0000	0.2450	0.1621
HLA-C	0.0000	0.0000	0.2234	0.2690
HLA-DRB1	0.0000	0.0000	0.3505	0.3725
HLA-DQB1	0.0029	0.0005	0.3024	0.2826

F_{is} estimation: inbreeding coefficient. S.E.: standard error, R&H: Robertson & Hill value, W&C: Weir & Cockerham value.

bioinformatics models such as peptide binding prediction could potentially be used to obtain more conclusive findings [27,40].

The present study was subject to multiple methodological limitations, including sample size, uneven age distribution, and existing comorbidities in some of the patients. Due to the limited sample size, adjustment for age between hospitalized and the non-hospitalized group was not possible. Furthermore, haplotype frequency calculation resulted in many single count haplotypes due to the small sample size. Thus, risk haplotypes could not be discussed. The UAE population has been shown to be admixed [41]. However, controlling for population stratification was not done in this study due to the small sample size.

Nevertheless, HLA allelic associations must be analyzed with caution due to the number of alleles under investigation, as there is a high risk of obtaining a significant allele by chance. Therefore,

adjustment for multiple comparisons is necessary. Most HLA allelic associations lost significance after statistical correction for multiple testing in this study. Similarly, a large study on 835 patients from Italy and 775 patients from Spain revealed no significant allele associations with either COVID-19 disease occurrence or severity after statistical adjustment [42]. A study by Littera, et al. [43] on 182 Sardinian COVID-19 patients showed no substantial differences in HLA alleles between subjects and the control group [37]. Conversely, in this study, several interesting and clinically relevant observations can be made from the statistical comparison between the hospitalized and non-hospitalized groups.

Among the most frequent alleles (subtype) in this cohort is *HLA-B*35* (13%) (Including *HLA-B*35:01/02/03/05/08/36*) and *HLA-C*07* (Including *HLA-C*07:01/02/10/18/23/29/31*) (30%). Similarly, in a recent report to characterize the COVID-19 disease in the UAE,
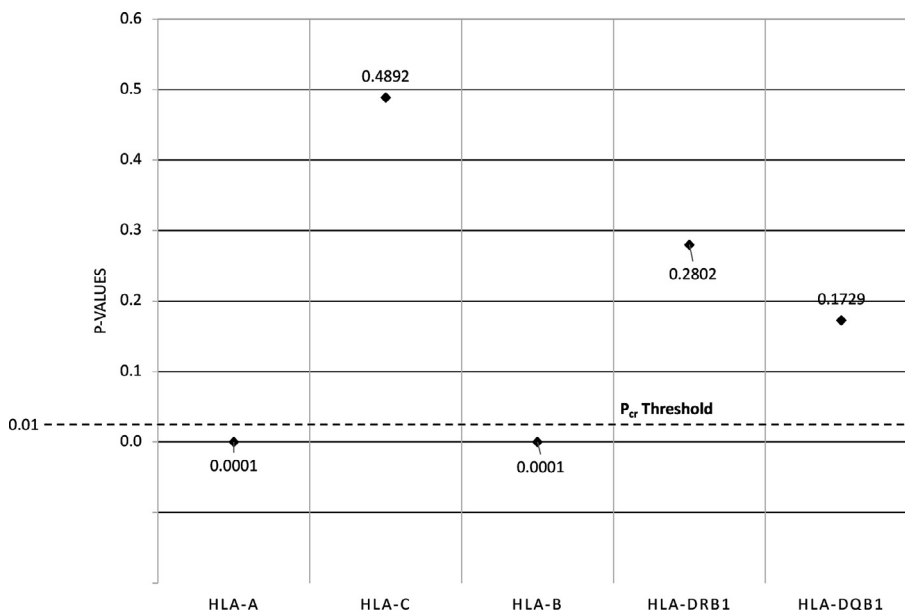
Fig. 3. The association of heterozygosity level in HLA loci to the severity of COVID-19. heterozygosity is defined as having the same 2-field allele in a specific locus and was tested using Pypop.

Table 4
Guo and Thompson Hardy-Weinberg test of 115 COVID-19 patients using PyPop.

Locus	Observed Heterozygotes	Expected Heterozygotes	Observed Homozygotes	Expected Homozygotes	P-value
HLA-A	49	101	59	6.65	0.0000*
HLA-C	83	103	29	8.62	0.0000*
HLA-B	44	91	53	6	0.0000*
HLA-DRB1	82	109	35	8.18	0.0000*
HLA-DQB1	69	90	31	10	0.0000*

Table 5
Summarized locus association testing results obtained using BIGDAWG software. Loci denoted in bold are significant after correcting for multiple comparisons.

Locus	$\chi 2$	P-value	Significance
HLA-A	**22.73**	**<0.001**	**S**
HLA-C	1.77	0.413	NS
HLA-B	**33.82**	**<0.001**	**S**
HLA-DRB1	11.00	0.027	NS
HLA-DQB1	9.96	0.191	NS

NS: Not significant, S: Significant

our group observed higher than expected frequencies of *HLA-B*35* and *HLA-C*07* in infected patients with ethnic backgrounds from South Asia. The same allele, *HLA-B*35*, has been identified in other viral respiratory diseases, including influenza A (H3N2) in Northeast India [15] and the development of isolated pulmonary hypertension in patients with scleroderma in Europeans [44].

Additionally, more than half of the *HLA-B*35* carriers (60%) in the current cohort carried this allele in the haplotype *HLA-B*35 ~ C*04* (6%). In a study by our group, the same haplotype was the most frequent in a multiethnic cohort and was observed in 11 out of the 15 nationalities (Tay et al., 2021 submitted).

To increase the power and confidence of HLA viral association studies, it is important to combine HLA typing with case-control studies and T cell binding assays in addition to systematic prediction of the peptide-binding groove of different alleles [27,40]. On this basis, genotypic data from this study were compared with theoretical viral peptide-binding predictions using in silico analysis methods.

Bioinformatic peptide-binding models of HLA class I have been successfully employed in multiple SARS-CoV-2 studies [26,39,45,46]. Unfortunately, HLA class II peptide binding prediction models still have problems, and that is why they have not been discussed in this study [27].

In theory, those who present alleles that are predicted to have a high affinity to bind to viral peptides would only develop mild symptoms of the disease, as their HLA molecules will be more efficient in presenting the peptides to the immune system.

Greater susceptibility to severe SARS-CoV-2 infection was observed in subjects who carried the class I allele *HLA-B*51:01* (P < 0.0001). Interestingly, *HLA-B*51:01* is the second common HLA-B allele in the general population of Abu Dhabi with a frequency as high as 13.5% after *HLA-B*50:01* [19]. However, no patient from the hospitalized group carried this allele, whilst it was frequent in the non-hospitalized cohort. Nevertheless, our SARS-CoV-2 peptide binding prediction showed that *HLA-B*51:01* binding capacity is limited to 205 for SB and 652 for WB.

Although some studies have shown a link between the peptide binding affinity and the severity of the disease [26,39,46,47], this study did not reveal a clear link between the degree of affinity between individual HLA molecules and the evolution of the disease in this study. Similarly, other reports did not confirm a link between in silico peptide binding prediction and genotypic data of patients [45].

NetMHCpan 4.1 server [35] is a validated algorithm for T-cell epitope prediction and has been recently updated. A combination of binding affinity thresholds that offers the best precision and specificity was used [35,48]. However, it is important to acknowledge the limitation of this computational method. The peptide

Table 6
Summarized significant amino acid position association analysis results obtained using BIGDAWG.

Locus	Position	χ^2	P-value	Locus	Position	χ^2	P-value
A	Position.9	12.67	0.005	B	Position.12	11.22	0.001
A	Position.65	5.54	0.019	**B**	**Position.24**	**35.94**	**<0.001**
A	Position.70	4.52	0.034	B	Position.37	11.38	0.001
A	Position.74	6.58	0.010	B	Position.46	11.38	0.001
A	**Position.76**	**18.79**	**<0.001**	B	Position.67	9.26	0.002
A	**Position.77**	**18.42**	**<0.001**	B	Position.70	10.88	0.001
A	**Position.79**	**14.68**	**<0.001**	B	Position.71	10.88	0.001
A	**Position.80**	**14.68**	**<0.001**	**B**	**Position.72**	**31.33**	**<0.001**
A	**Position.81**	**14.68**	**<0.001**	B	Position.86	11.18	0.001
A	**Position.82**	**14.68**	**<0.001**	**B**	**Position.93**	**38.77**	**<0.001**
A	**Position.83**	**14.68**	**<0.001**	**B**	**Position.94**	**38.77**	**<0.001**
A	**Position.97**	**23.42**	**<0.001**	**B**	**Position.108**	**18.62**	**<0.001**
A	Position.105	7.93	0.005	B	Position.126	6.77	0.009
A	Position.107	6.58	0.010	**B**	**Position.144**	**15.64**	**<0.001**
A	Position.109	9.09	0.003	B	Position.165	4.77	0.029
A	**Position.114**	**32.77**	**<0.001**	**B**	**Position.169**	**27.32**	**<0.001**
A	Position.143	6.48	0.011	B	Position.176	15.04	0.001
A	**Position.145**	**30.79**	**<0.001**	**B**	**Position.184**	**21.57**	**<0.001**
A	Position.146	6.48	0.011	B	Position.190	9.90	0.002
A	**Position.152**	**14.03**	**<0.001**	B	Position.193	9.90	0.002
A	**Position.176**	**17.83**	**<0.001**	DRB1	Position.13	14.00	0.007
C	Position.121	6.21	0.045	DRB1	Position.72	10.88	0.012
B	Position.9	8.82	0.012	DQB1	Position.57	9.13	0.028
B	Position.11	6.26	0.012				

Loci denoted in bold are significant associations after correcting for multiple comparisons.

binding recognition is complex and is affected by many factors, including the relative expression of individual viral proteins [48]. Furthermore, there is currently limited data on experimentally determined SARS-CoV-2 immunogenic T-cell epitope and peptide HLA restriction [48], and our analysis relied on the available information in published literature.

Iturrieta-Zuazo, et al. [27] suggested that the degree of HLA homozygosity could play a role in the severity of the SARS-CoV-2 infection as their results showed a higher percentage of HLA homozygosity in Spanish patients with severe infection in loci A and C when compared to moderate and mild patients [27]. This is based on the evidence that individuals with heterozygosity at certain HLA loci may have better resistance to infectious diseases when compared to the corresponding homozygotes [49]. A study of 6,311 HIV-1 infected individuals reported better resistance of *HLA-B* heterozygosity against HIV-1 [17]. The study suggested that heterozygosity in HLA alleles increases the probability of carrying the most protective HLA alleles [5]. Similarly, a statistically significant association between COVID-19 severity and homozygosity at *HLA-A* and *HLA-B* (Fig. 3) was observed in this study.

In conclusion, the present study investigated the association of HLA allele and haplotype variations in Emirati patients with respect to COVID-19 severity. Alleles *HLA-B*51:01, HLA-A*26:01* were significantly higher in the non-hospitalized patients, suggesting a protective association, whilst genotypes *HLA-A*03:01, HLA-DRB1*15:01,* and supertype B44 showed a significant correlation with disease severity. Overall, we could not establish a clear link between in silico peptide binding prediction of HLA class I and clinical outcome. Suffice to say, many other genetic and epigenetic factors independent of the HLA could play a role in the immunopathogenesis of SARS-CoV-2. Despite some shortcomings, this study contributes to the global effort to understand the immunological pathway of SARS-CoV-2 severity and occurrence. Further investigation with a larger cohort and diverse populations is necessary to clarify the role of HLA in SARS-CoV-2 infection. This will further assist in developing specific vaccination and personalizing treatments.

5. Availability of data

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

6. The UAE COVID-19 Collaborative Partnership

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Author contribution

H.A.; a graduate student at Khalifa University; was primarily responsible for managing this project and developed the objectives with her advisors, G.K.T. and H.S.S. H.A. collated the data and performed the analyses outlined in this study. H.A. and G.K.T. analyzed, and constructed the figures, with H.A. preparing the first draft of the manuscript. H.A. and S.E carried out the laboratory assays used in the study at the Center for Biotechnology. M.U. and N.A were responsible for the recruitment of the patients who contributed data to the study by collecting demographic data. H. F. J., A. F., E. A., S. E., A.T. S., M. M. A. H., B. H. M., M. U., N. A. provided critical review during manuscript preparation. All authors on the primary list contributed to the data interpretation or critically reviewed the manuscript and approved the final manuscript for submission. All investigators of the UAE COVID-19 Collaborative Partnership provided comments on the study in some form or were involved in developing overall research questions for a larger pro-

ject that was conceived to study the role of the virus and host in COVID-19 in the United Arab Emirates.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

[1] P. Cruz-Tapias, J. Castiblanco, J. Anaya, Major histocompatibility complex: Antigen processing and presentation, in: J. Anaya, Y. Shoenfeld, A. Rojas-Villarraga (Eds.), Autoimmunity: From Bench to Bedside, El Rosario University Press, Colombia, 2013.

[2] H. Al Naqbi, A. Mawart, J. Alshamsi, H. Al Safar, G.K. Tay, Major histocompatibility complex (MHC) associations with diseases in ethnic groups of the Arabian Peninsula, Immunogenetics 73 (2) (2021) 131–152.

[3] P.R. Goulder, B. Walker, HIV and HLA class I: an evolving relationship, Immunity 37 (3) (2012) 426–440.

[4] P.U. Cameron, S.A. Mallal, M.A.H. French, R.L. Dawkins, Major histocompatibility complex genes influence the outcome of HIV infection. Ancestral haplotypes with C4 null alleles explain diverse HLA associations, Hum. Immunol. 29 (4) (1990) 282–295.

[5] M. Carrington, G. Nelson, M. Martin, T. Kissner, D. Vlahov, J. Goedert, et al., HLA and HIV-1: Heterozygote Advantage and B*35-Cw*04 Disadvantage, Science 283 (1999) 1748.

[6] K. Diouf, A. Sarr, G. Eisen, S. Popper, S. Mboup, P. Kanki, Associations between MHC class I and susceptibility to HIV-2 disease progression, J. Human Virology 5 (2002) 1.

[7] F. Pereyra, X. Jia, P. McLaren, A. Telenti, P. de Bakker, B. Walker, et al., The major genetic determinants of HIV-1 control affect HLA class I peptide presentation, Science 330 (2010) 1551.

[8] L.i. Wang, Z.-Q. Zou, K. Wang, Clinical Relevance of HLA Gene Variants in HBV Infection, J. Immunology Res. 2016 (2016) 1–7.

[9] P. Araujo, G. Gonçalves, F. Latini, O. Ferreira, L.C. Porto, J.A. Barreto, M.J.C. Girao, R.S. Diaz, KIR and a specific HLA-C gene are associated with susceptibility and resistance to hepatitis B virus infection in a Brazilian population, Cell Molecular Immunology 11 (6) (2014) 609–612.

[10] M.A. Montes-Cano, J.L. Caro-Oleas, M. Romero-Gómez, M. Diago, R. Andrade, I. Carmona, J.A. Reina, A. Núñez-Roldán, M.F. González-Escribano, HLA-C and KIR genes in hepatitis C virus infection, Hum Immunology 66 (11) (2005) 1106–1109.

[11] S.K. Yoon, J.Y. Han, C.-W. Pyo, J.M. Yang, J.W. Jang, C.W. Kim, U.I. Chang, S.H. Bae, J.Y. Choi, K.W. Chung, H.S. Sun, H.B. Choi, T.-G. Kim, Association between human leukocytes antigen alleles and chronic hepatitis C virus infection in the Korean population, Liver International 25 (6) (2005) 1122–1127.

[12] I. Ivic, N. Bradaric, N. Puizina-Ivic, D. Ledina, B. Luksic, R. Martinic, HLA-Cw7 allele as predictor of favorable therapeutic response to interferon-alpha in patients with chronic hepatitis C, Croatian Medical Journal. 48 (2007) 807.

[13] D. Frleta, C. Yu, E. Klechevsky, A.-L. Flamar, G. Zurawski, J. Banchereau, et al., Influenza virus and poly(I:C) inhibit MHC class I-restricted presentation of cell-associated antigens derived from infected dead cells captured by human dendritic cells, J. Immunology (Baltimore, Md. 1950) 2009 (182) (1950) 2766.

[14] A.C.M. Boon, G. de Mutsert, Y.M.F. Graus, R.A.M. Fouchier, K. Sintnicolaas, A.D. M.E. Osterhaus, G.F. Rimmelzwaan, Sequence variation in a newly identified HLA-B35-restricted epitope in the influenza A virus nucleoprotein associated with escape from cytotoxic T lymphocytes, J. Virol. 76 (5) (2002) 2567–2572.

[15] M. Dutta, P. Dutta, S. Medhi, B. Borkakoty, D. Biswas, Polymorphism of HLA class I and class II alleles in influenza A(H1N1)pdm09 virus infected population of Assam, Northeast India, J. Med. Virol. 90 (5) (2018) 854–860.

[16] J.M. Blackwell, S.E. Jamieson, D. Burgner, HLA and infectious diseases, Clin. Microbiol. Rev. 22 (2) (2009) 370–385.

[17] Arora J, Pierini F, McLaren P, Carrington M, Fellay J, Lenz T: HLA Heterozygote Advantage against HIV-1 Is Driven by Quantitative and Qualitative Differences in HLA Allele-Specific Peptide Presentation. Molecular biology and evolution 2020;37:639.

[18] X. Li, M. Geng, Y. Peng, L. Meng, S. Lu, Molecular immune pathogenesis and diagnosis of COVID-19, J. Pharm. Anal. 10 (2) (2020) 102–108.

[19] González-Galarza F, Takeshita L, Santos E, Kempson F, Maia M, da Silva Aet al. : Allele frequency net 2015 update: new features for HLA epitopes, KIR and disease and HLA adverse drug reaction associations. Nucleic acids research 2015;43:D784.

[20] M. Lin, H.-K. Tseng, J.A. Trejaut, H.-L. Lee, J.-H. Loo, C.-C. Chu, P.-J. Chen, Y.-W. Su, K.H. Lim, Z.-U. Tsai, R.-Y. Lin, R.-S. Lin, C.-H. Huang, Association of HLA class I with severe acute respiratory syndrome coronavirus infection, BMC Med. Genet. 4 (1) (2003), https://doi.org/10.1186/1471-2350-4-9.

[21] J. Xu, S. Zhao, T. Teng, A.E. Abdalla, W. Zhu, L. Xie, Y. Wang, X. Guo, Systematic Comparison of Two Animal-to-Human Transmitted Human Coronaviruses: SARS-CoV-2 and SARS-CoV, Viruses 12 (2) (2020) 244, https://doi.org/10.3390/v12020244.

[22] J. Zheng, SARS-CoV-2: an Emerging Coronavirus that Causes a Global Threat, Int. J. Biological Sci. 16 (10) (2020) 1678–1685.

[23] R. Jordan, P. Adab, K. Cheng, COVID-19: risk factors for severe disease and death, BMJ 368 (2020) m1198.

[24] Docherty A, Harrison E, Green C, Hardwick H, Pius R, Norman Let al. : Features of 20 133 UK patients in hospital with covid-19 using the ISARIC WHO Clinical Characterisation Protocol: prospective observational cohort study. BMJ 2020;369:m1985.

[25] W. Wang, W. Zhang, J. Zhang, J. He, F. Zhu, Distribution of HLA allele frequencies in 82 Chinese individuals with coronavirus disease-2019 (COVID-19), HLA 96 (2020) 194.

[26] Nguyen A, David J, Maden S, Wood M, Weeder B, Nellore Aet al. : Human leukocyte antigen susceptibility map for SARS-CoV-2. MedRxiv 2020:2020.03.22.20040600.

[27] I. Iturrieta-Zuazo, C.G. Rita, A. García-Soidán, A. de Malet Pintos-Fonseca, N. Alonso-Alarcón, R. Pariente-Rodríguez, A. Tejeda-Velarde, S. Serrano-Villar, J.L. Castañer-Alabau, I. Nieto-Gañán, Possible role of HLA class-I genotype in SARS-CoV-2 infection and progression: A pilot study in a cohort of Covid-19 Spanish patients, Clinical immunology 219 (2020) 108572, https://doi.org/10.1016/j.clim.2020.108572.

[28] E. Di Maria, A. Latini, P. Borgiani, G. Novelli, Genetic variants of the human host influencing the coronavirus-associated phenotypes (SARS, MERS and COVID-19): rapid systematic review and field synopsis, Human Genomics 14 (2020) 30.

[29] Hurley CK, Kempenich J, Wadsworth K, Sauter J, Hofmann JA, Schefzyk Det al. : Common, intermediate and well-documented HLA alleles in world populations: CIWD version 3.0.0. HLA 2020;95:516.

[30] A.K. Lancaster, R.M. Single, O.D. Solberg, M.P. Nelson, G. Thomson, PyPop update–a software pipeline for large-scale multilocus population genomics, Tissue Antigens 69 (Suppl 1) (2007) 192.

[31] F. Rousset, GENEPOP'007: A complete re-implementation of the GENEPOP software for Windows and Linux, Mol. Ecol. Resour. 8 (1) (2008) 103–106.

[32] S.W. Guo, E.A. Thompson, Performing the exact test of Hardy-Weinberg proportion for multiple alleles, Biometrics 48 (2) (1992) 361, https://doi.org/10.2307/2532296.

[33] J.A. Hollenbach, S.J. Mack, G. Thomson, P.A. Gourraud, Analytical methods for disease association studies with immunogenetic data, Methods Mol Biol 882 (2012) 245.

[34] D.J. Pappas, W. Marin, J.A. Hollenbach, S.J. Mack, Bridging ImmunoGenomic Data Analysis Workflow Gaps (BIGDAWG): An integrated case-control analysis pipeline, Hum. Immunol. 77 (3) (2016) 283–287.

[35] 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:W449.

[36] J. Sidney, B. Peters, N. Frahm, C. Brander, A. Sette, HLA class I supertypes: a revised and updated classification, BMC Immunol 9 (1) (2008) 1, https://doi.org/10.1186/1471-2172-9-1.

[37] K. MacDonald, K. Fowke, J. Kimani, V. Dunand, N.D. Nagelkerke, T.B. Ball, J. Oyugi, E. Njagi, L. Gaur, R. Brunham, J. Wade, M. Luscher, P. Krausa, S. Rowland-Jones, E. Ngugi, J. Bwayo, F. Plummer, Influence of HLA Supertypes on Susceptibility and Resistance to Human Immunodeficiency Virus Type 1 Infection, J. Infect. Dis. 181 (5) (2000) 1581–1589.

[38] C. Sylvester-Hvid, M. Nielsen, K. Lamberth, G. Roder, S. Justesen, C. Lundegaard, P. Worning, H. Thomadsen, O. Lund, S. Brunak, S. Buus, SARS CTL vaccine candidates; HLA supertype-, genome-wide scanning and biochemical validation, Tissue Antigens 63 (5) (2004) 395–400.

[39] M. Shkurnikov, S. Nersisyan, T. Jankevic, A. Galatenko, I. Gordeev, V. Vechorko, A. Tonevitsky, Association of HLA Class I Genotypes With Severity of Coronavirus Disease-19, Front Immunol 12 (2021) 641900.

[40] M.S. Sohail, S.F. Ahmed, A.A. Quadeer, M.R. McKay, In silico T cell epitope identification for SARS-CoV-2: Progress and perspectives, Adv. Drug Deliv. Rev. 171 (2021) 29–47.

[41] G.K. Tay, A. Henschel, G. Daw Elbait, H.S. Al Safar, Genetic Diversity and Low Stratification of the Population of the United Arab Emirates, Front. Genet. 11 (2020).

[42] D. Ellinghaus, F. Degenhardt, L. Bujanda, M. Buti, A. Albillos, P. Invernizzi, et al., Genomewide Association Study of Severe Covid-19 with Respiratory Failure, N Engl. J. Med. 383 (2020) 1522.

[43] R. Littera, M. Campagna, S. Deidda, G. Angioni, S. Cipri, M. Melis, D. Firinu, S. Santus, A. Lai, R. Porcella, S. Lai, S. Rassu, R. Scioscia, F. Meloni, D. Schirru, W. Cordeddu, M.A. Kowalik, M. Serra, P. Ragatzu, M.G. Carta, S. Del Giacco, A. Restivo, S. Deidda, S. Orrù, A. Palimodde, R. Perra, G. Orrù, M. Conti, C. Balestrieri, G. Serra, S. Onali, F. Marongiu, A. Perra, L. Chessa, Human Leukocyte Antigen Complex and Other Immunogenetic and Clinical Factors Influence Susceptibility or Protection to SARS-CoV-2 Infection and Severity of the Disease Course. The Sardinian Experience, Front. Immunology 11 (2020) 605688.

[44] S. Lenna, D. Townsend, F. Tan, B. Kapanadze, M. Markiewicz, M. Trojanowska, et al., HLA-B35 upregulates endothelin-1 and downregulates endothelial nitric oxide synthase via endoplasmic reticulum stress response in endothelial cells, J. Immunology (Baltimore, Md.: 1950) 2010 (184) (1950) 4654.

[45] R. Secolin, T.K. de Araujo, M.C. Gonsales, C.S. Rocha, M. Naslavsky, L.D. Marco, M.A.C. Bicalho, V.L. Vazquez, M. Zatz, W.A. Silva, I. Lopes-Cendes, Genetic variability in COVID-19-related genes in the Brazilian population, Hum. Genome Var. 8 (1) (2021), https://doi.org/10.1038/s41439-021-00146-w.

[46] C.A.M. La Porta, S. Zapperi, Estimating the Binding of Sars-CoV-2 Peptides to HLA Class I in Human Subpopulations Using Artificial Neural Networks, Cell Syst. 11 (4) (2020) 412–417.e2.

[47] Warren RL, Birol I: Retrospective in silico HLA predictions from COVID-19 patients reveal alleles associated with disease prognosis. medRxiv : the preprint server for health sciences 2020:2020.10.27.20220863.

[48] Copley HC, Gragert L, Leach AR, Kosmoliaptsis V: Influence of HLA class II polymorphism on predicted cellular immunity against SARS-CoV-2 at the population and individual level. bioRxiv 2020:2020.12.24.424326.

[49] M. Lipsitch, C. Bergstrom, R. Antia, Effect of human leukocyte antigen heterozygosity on infectious disease outcome: the need for allele-specific measures, BMC Med. Genet. 4 (2003) 2.