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The Role of HLA-DRB1 Alleles in Pulmonary Cystic Fibrosis

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ABSTRACT

Cystic fibrosis (CF) is the most common lethal autosomal recessive disease in white Caucasians. It affects many organs including the lung, pancreas, and liver. Whilst CF is a monogenic disease, several studies revealed a complex relationship between genotype and clinical phenotype of diseases. We examined the expression of human leukocyte antigen (HLA) class II alleles among Iranian CF patients with disease-related microbial infection.

This study was conducted on 50 hospitalized CF patients (27 males, 23 females aged 15.5±6.5 years), and 50 healthy age- and gender-matched control subjects. 5ml whole blood was harvested and after isolation of genomic DNA, HLA-DRB1 subtypes were determined by single specific primer polymerase chain reaction methods.

HLA-DRB1*10 was less frequent and HLA-DRB1*04 and HLA-DRB1*11 was the most frequent allele in CF patients, but none reached significance. HLA-DRB1*04 allele was frequently seen among16 CF patients with high serum IgE levels (430.25±219.7 IU/mL) and 27 CF patients that were positive for Pseudomonas aeruginosa colonization. A total of 31 CF patients had candida Albicans colonization in whom HLA-DRB1*11 was mostly seen. A total of 3 CF patients had allergic bronchopulmonary aspergillosis and two were diabetic.

The DR4 and DR11 serotypes that recognize the HLA-DRB1*04 and HLA-DRB1*11 gene products respectively are not significantly enriched in the Iranian CF population. Further research should be conducted on DR4 and DR11 in CF patients to understand their possible role in infection and IgE expression.

Keywords: Cystic fibrosis; HLA-DRB1 chains; Pseudomonas aeruginosa; Staphylococcus aureus

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INTRODUCTION

Cystic fibrosis (CF) is an autosomal impairment caused by mutations in the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene located on chromosome 7q31.27.[1] This gene encodes a transmembrane chloride ion channel and mutations in this gene results in disturbed NaCl flux and fluid transportation into and out of the cells in various organs of the body including the lung, pancreas, gastrointestinal tract and reproductive organs. As a result, the secretions from these tissues are thick and sticky and sweat gland secretions contain high levels of salt.[1,2] The prevalence of CF is 1 in 2-3000 births and approximately 2000 gene mutations have been reported with the F508del mutation being the most common defect which is seen in 70% of CF cases.[3,4] This disorder has been observed in all races but is mainly reported in northern Europeans.[5] Atopy, as defined by a positive skin test, increased total serum IgE, and allergic symptoms are often observed in CF patients.[6,7]

The thick and sticky secretions in the respiratory tract of CF patients provide ideal conditions for chronic infections which cause more than 90% of all CF deaths.[8] The most common bacterial species found in CF patients are *Staphylococcus aureus (S. aureus)*, *Pseudomonas aeruginosa (P. aeruginosa)*, and *Burkholderia Cepacia*.[9] *P. aeruginosa* is the major bacterial agent causing CF lung infections and has been associated with disease progression. In addition, *Candida albicans (C. albicans)* is a common opportunist fungal infection that colonizes the respiratory tract of CF patients.[10] A previous study found a strong association between IgE and C. albicans and allergic bronchopulmonary aspergillosis (ABPA) which suggests that IgE may be an immunologic marker for the progression of ABPA in CF patients.[11]

Human Leukocyte Antigen (HLA) complex, which is located on chromosome 6, p21, plays an important role in regulating the immune system.[12] HLA is associated with a large number of diseases[13-19] including allergic diseases such as psoriasis and asthma where a link with HLA-DR4 and HLA-DR7 has been described.[20,21] The presence of HLA-DR7 increases the risk of autoimmune reactions in some patients. HLA-DR4 is the most frequently associated with autoimmune pancreatitis.[22,23]

HLA-DR is the only class II HLA reported to be up-regulated in CF patients with nasal polyps and associated with CF-related diseases such as coeliac disease.[24-26] Moreover, the HLA-DR4 and DR7 alleles were significantly higher in CF patients in Europe.[27] In the current study, we hypothesized that HLA class II alleles will be associated with susceptibility to CF, or CF infections, in Iranian CF patients. We aimed, therefore, to examine the association of HLA-DRB1 alleles with infectious agents such as *P. aeruginosa*, *S. aureus*, and *C. albicans* in correlation with serum levels of total IgE.

MATERIALS AND METHODS

Patient's Assembly

Our study was conducted on 50 CF patients (27 male, 23 female, 15.5±6.5 years) who were referred to the Masih Daneshvari Hospital in Tehran-Iran from March 2017 to April 2018. The diagnosis of CF was confirmed using a combination of physical examination, clinical history (based on the ERS guidelines 2017), and lab tests as described previously.[28] Patients with diagnostic criteria other than CF were excluded from this study. 50 age- (9.96±7.19) and gender-matched healthy individuals were also examined as a control group. Healthy individuals with a family background of CF were excluded. The study was approved by the Ethical committee of Masih Daneshvari Hospital IR.SBMU.NRITLD.1395.234 and complied with the national legislation and Declaration of Helsinki guidelines. Written informed consent was obtained from study participants and all necessary patient/participant consent and the appropriate institutional forms have been archived.

Demographic data including age, gender, age at diagnosis, diabetes, gallstones, lung function tests (FVC and FEV1 obtained within the previous 6 months), and PaCO2 and PaO2 for these patients are given in Table 1.

DNA Extraction

Peripheral whole blood (5 mL) was collected in an EDTA tube. DNA extraction was performed using a salting-out method and the extracted samples were diluted with Tris 1M and H_2O. The DNA concentration was determined using a Nanodrop 2000 (Thermo

Fisher, USA), and the samples were stored at -70°C until analysis.

Microbiological Studies

Sputum samples were analyzed for *P. aeruginosa*, *S. aureus*, and *C. Albicans* colonization by culture on Mannitol Salt Agar (*S. aureus*), on Muller-Hinton Agar (*P. aeruginosa*), or in SC medium (*C. Albicans*) in the Hospital microbiology center.

Serum IgE Levels

Serum IgE levels were measured by ELISA (PishtazTeb Zaman, Tehran, Iran).

HLA Typing

HLA-DRB1 subtypes were determined by single specific primer polymerase chain reaction (SSP-PCR methods) (Texas BioGene, USA) as described previously.[29] The PCR product was loaded onto a 1% agarose gel and the expression of the various alleles was visualized by Gel DOC (Bio-RAD, USA) according to the manufacturer's instructions. Identification of each allele from the electrophoretic bands was performed by Morgan™ SSPal HLA typing analysis software Ver 2.5. In SSP-PCR, only primers that are fully complementary to the target sequence are amplified, and the presence of an amplified DNA fragment represents the presence of the specific allele in the genomic DNA.

Statistical Analysis

Statistical analyses for frequencies of each allele were performed using the chi-square test and the confidence interval of the relative risk was done using the Koopman asymptotic score Method in GraphPad Prism (version 8.02) for HLA-DRB1 typing. Results were considered significant when $p \leq 0.05$.

Table 1. Demographic data of cystic fibrosis (CF) patients

	Cystic fibrosis patient	Healthy control subjects
Demographic data		
Number of subjects	50	50
Age (mean±SD) years	15.5±6.5	9.96 ± 7.19
Age at diagnosis, yr	8±7.3	—
Sex		
male	27	27
female	23	23
Respiratory data		
FVC, % pred	49.3±22.5	—
FEV1, % pred	47.2±24.9	—
PaO2, mm Hg	47.1±23.8	—
PaCO2, mm Hg	43.6±14.2	—
Comorbidities		
IgE, IU/mL	210.59±246	—
Diabetes, n	2	—
Gallstone, n	2	—
Microbiological data		
P. aeruginosa colonization, n	27	—
Staphylococcus aureus, colonization, n	12	—
Candida colonization, n	31	—

FEV1: forced expiratory volume in 1 second, FVC: Forced vital capacity,
Normal serum levels of IgE for adults are < 150 UI/mL.

RESULTS

Analysis of the HLA-DR *B1-16 Alleles

The presence of all HLA-DRB1-16 alleles was analyzed and representative results are shown (Figure 1).

HLA-DRB1 Allele Frequency

The distribution of all alleles in CF patients and healthy controls are shown in Table 2. HLA-DRB1*10 is less frequent and HLA-DRB1*11 and HLA-DRB1*04 are potentially more frequent in CF patients. Also, HLA-DRB1*09 and HLA-DRB1*12 are less frequent and HLA-DRB1*11 is more frequent in healthy subjects. However, none of these differences reached statistical significance ($p \geq 0.05$). Furthermore, a non-significant effect of gender or age on HLA distribution was seen.

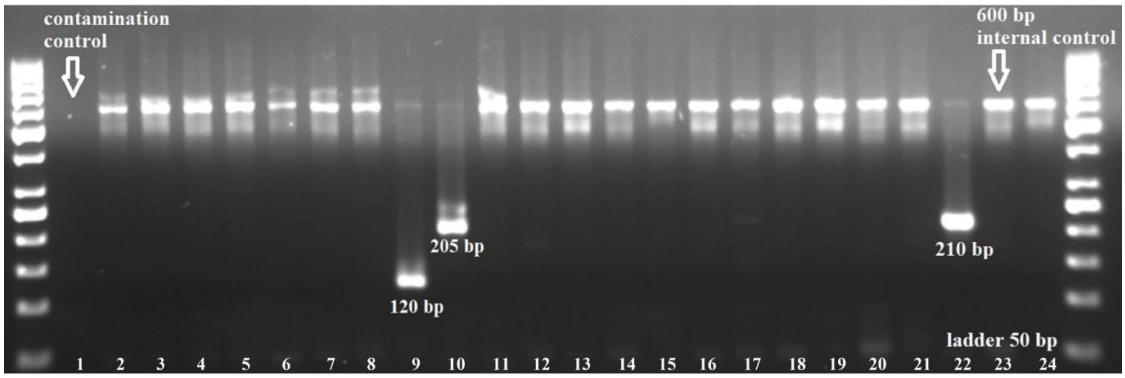

Figure 1. Representative gel electrophoresis of PCR products. Gel electrophoresis showing amplification profile of DNA samples of cystic fibrosis (CF) patients. The product size in bp refers to the amplification of a selective allele. The presence of different Positive amplification in each sample indicates a specific allele. In this figure amplification in wells number 9, 10, and 22 shows DRB1*11:01, DRB1*11:05 allele. Detection of allele-specific amplified bands in 1% agarose gel by single specific primer-polymerase chain reaction (SSP-PCR). The internal control represents a conserved region of the housekeeping gene (provided in the kit) and serves as an indication

Table 2. Frequency of HLA-DRB1 in control and cystic fibrosis (CF) patients

	CF patients		Healthy controls			
	n	**%**	**n**	**%**	***p***	**RR**
HLA-DRB1						
*DRB1*01*	2	4	5	10	0.23	0.55
*DRB1*03*	9	18	7	14	0.58	1.15
*DRB1*04*	15	30	9	18	0.16	1.35
*DRB1*07*	8	16	9	18	0.79	0.93
*DRB1*08*	5	10	3	6	0.46	0.46
*DRB1*09*	2	4	0	0	0.15	2.04
*DRB1*10*	1	2	3	6	0.30	0.48
*DRB1*11*	22	44	18	36	0.41	1.17
*DRB1*12*	2	4	0	0	0.15	0.42
*DRB1*13*	11	22	11	22	0.99	1
*DRB1*14*	8	16	6	12	0.56	1.17
*DRB1*15*	11	22	9	18	0.61	1.12
*DRB1*16*	4	8	7	14	0.33	0.70

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HLA-DRB1 and the Level of Total IgE

16 individuals had high serum levels of IgE (430.25±219.7 IU/mL) and these subjects most commonly expressed the HLA-DRB1*04 allele (Table 3). Three CF patients had ABPA, of which two were HLA-DRB1*04+, however, the small number of patients did not allow for further analysis.

HLA-DRB1 Frequency and *P. Aeruginosa* and *C. Albicans* Colonization

27/50 CF patients were colonized with *P. aeruginosa* within the lung and the HLA-DRB1*04 allele was the most frequent allele present in these subjects (Table 3). In contrast, 31/50 CF patients were colonized by *C. albicans* with the most frequent allele being HLA-DRB1*11 (Table 3).

HLA-DRB1 Frequency and *C. Albicans* with Level of Total IgE and *P. Aeruginosa* with Level of Total IgE

14/50 patients had high levels of serum IgE and were colonized by *C. Albicans*. The HLA-DRB1*04 and HLA-DRB1*11 alleles were the most frequently found in these patients (Table 3). In contrast, the HLA-DRB1*04 allele was the most frequent allele present in the 10 *P. Aeruginosa* positive CF patients with high serum levels of total IgE (Table 3). The frequency of HLA-DRB1alleles, other microbial colonization, and serum levels of IgE among CF patients are shown in Table 3.

Table 3. Various microbial colonization and level of IgE among cystic fibrosis (CF) patients

	P. Aeruginosa	*C. Albicans*	Total IgE	*S. aureus*	High total IgE & *C. Albicans*	High total IgE & *P. Aeruginosa*
HLA-DRB1	(n)	(n)	(n)	(n)	(n)	(n)
*DRB1*01*	0	0	1	1	0	0
*DRB1*03*	4	7	3	1	3	2
*DRB1*04*	11	9	9	5	5	5
*DRB1*07*	3	5	2	3	2	0
*DRB1*08*	2	3	0	0	0	0
*DRB1*09*	0	1	0	0	0	0
*DRB1*10*	0	0	0	0	0	0
*DRB1*11*	8	11	6	9	5	1
*DRB1*12*	0	1	1	0	1	1
*DRB1*13*	7	8	4	1	4	4
*DRB1*14*	5	7	3	1	3	3
*DRB1*15*	8	9	2	5	2	1
*DRB1*16*	3	3	1	0	1	1

DISCUSSION

We report no significant enrichment of allele frequency in Iranian children with CF although there was a trend toward the HLA-DRB1*11 and HLA-DRB1*04 alleles being more common. In contrast, in subjects with high serum IgE levels and colonized with Pseudomonas or Candida, HLA-DRB1*11 and HLA-DRB1*04 were expressed as the most frequent alleles respectively. CF children with high levels of serum CRP expressed lower levels of HLA-DR and HLA-DQ.

Although early studies indicated an association between HLA-DR4 and CF,[27] our current data that shown there were no significant differences in the distribution of the alleles in 50 Iranian CF patients compared with the control group is in line with other, more recent, studies where no association between HLA class 1 and 2 with CF have been observed.[6,30-36] Future studies should examine whether there is any link to disease severity.

Our results show a higher frequency of HLA-DRB1*04 in patients with *P. aeruginosa* colonization.

Pseudomonas is the most important factor in causing infection in CF patients[37] and this may directly reflect CFTR dysfunction within the membrane decreasing the expression of defense proteins and enhancing susceptibility to this infection.[38] Previous studies indicate that HLA-DR7 is the most frequent allele in patients with CF colonized with pseudomonas[27] suggesting that the resistance and susceptibility to *P. aeruginosa* could be attributed to the HLA class 2 loci in CF patients. The mechanism(s) by which reduced expression of HLA-DR and HLA-DQ in CF patients[1] provides suitable conditions for *P. aeruginosa* colonization is uncertain.

The HLA-DRB1*04 allele was more abundant in the 16 CF patients that expressed high levels of serum IgE. These data are in contrast to the greater prevalence of DR7 as the most frequent allele in a French population of CF patients with high levels of IgE.[27] In our patients, the DR4 allele was observed to a greater extent among patients with both high IgE levels and *P. aeruginosa* colonization.

ABPA is often observed in patients with asthma and CF due to the colonization of *Aspergillus fumigates (A. fumigatus)* in the lower respiratory system.[39] The pathogenesis of ABPA includes reduced mucociliary clearance, impairment of fungicidal proteins, attenuated levels of complement in the respiratory tract, and suppression of phagocytosis.[40] In our study only 3 patients out of the 12 colonized with *aspergillus* had ABPA. Although HLA-typing among these patients showed that 2 out of 3 patients had an HLA-DRB1*04 allele the numbers are too small to draw valid conclusions. However, previous studies have suggested that a high frequency of DR2 and DR5 is related to the development of ABPA.[41] Moreover, 84% of patients with ABPA-CF have HLA-DRB1*15:01, DRB1*11:04, DRB1*11:01, DRB1*07:01, and DRB1*04 alleles in comparison with CF and asthma.[42] In contrast, HLA-DQB1*02:01 was found as an ABPA-resistant allele.[42]

Certain alleles of class II HLA may affect T helper cell (Th1, Th2) responses to *A. fumigatus*. The Th1 response and the subsequent production of antibodies may have a protective effect against *aspergillus* and activating a Th2 response leads to the release of cytokines and immunoglobulins which cause allergic inflammation.[42-44] Indeed, the T-cell response to *aspergillu*s allergens causes a Th2 response with the secretion of IL-4, IL-5, and IL-13 cytokines for IgE production.[41] Increased IL-5 release can eventually lead to reduced production of interferon (IFN)-γ[41-43] and so antigen-presentation by HLA-DRB1*04 may skew the immune response towards a Th2 response with an associated increase in total IgE levels.[45]

In conclusion, we found no significant association between HLA-DRB1*04 and HLA-DRB1*11 and CF although maybe there is an association with disease phenotypes including species of colonization and IgE levels.

Due to the small sample size and the Inaccessibility to the new HLA typing technique in this study, large and multicentral studies and also utilizing new techniques such as Sequence-based typing (SBT) are proposed to verification the impact of specific HLA class 2 alleles on susceptibility to CF.

CONFLICT OF INTEREST

The authors have no conflicts of interest.

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