

Original Article

HLA-DRB1 and HLA-DQB1 genes and allergic broncopulmonary aspergillosis susceptibility and protection in patients with cystic fibrosis

Running Head: HLA and ABPA in cystic fibrosis

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ABSTRACT

Allergic bronchopulmonary aspergillosis (ABPA) is a hypersensitivity lung disease that affects patients with cystic fibrosis (CF) and asthmatic patients. Indeed, HLA-DRB1 alleles have previously been associated with ABPA-CF susceptibility although HLA-DQB1 allele associations were not clearly established. Our aim was to study HLA class II associations in our ABPA-CF patients and determine their role in susceptibility or protection. Patients with ABPA-CF, CF patients without ABPA, asthmatic patients without ABPA (AST) and healthy controls were included in this study. DNA was extracted by automatic extractor. HLA-DRB1 and -DQB1 genotyping was performed by luminex PCR-SSO method (OneLambda, CA). Allele specific PCR-SSP was also performed by high resolution analysis (OL, CA). Statistical analysis was performed by SPSS and Arlequin softwares. Both HLA-DRB1*15:01 and -DRB1*11:04 alleles showed an increased frequency in ABPA-CF patients with respect to AST, CF and controls, corroborating the data previously published. On the other hand, analysis of haplotypes revealed that almost all patients with ABPA-CF lacking DRB1*15:01 or DRB1*11:04 carry either DRB1*04, DRB1*11:01 or DRB1*07:01 alleles. In the HLA-DQB1 region, HLA-DQB1*06:02 allele was increased in these ABPA-CF patients with respect to AST and CF patients, and healthy controls, whereas HLA-DQB1*02:01 was decreased in ABPA-CF patients. These data confirm that a correlation exists between HLA-DRB1*15:01, -DRB1*11:04, DRB1*11:01, -DRB1*04 and -DRB1*07:01 alleles, and ABPA-CF susceptibility and suggest that HLA-DQB1*02:01 is an ABPA-CF resistance allele.

Key words: Allergic Bronchopulmonary aspergillosis; HLA; cystic fibrosis; asthmatic patients

List of Abbreviations: Human Leukocyte Antigen (HLA), Allergic Broncho-Pulmonary Aspergillosis (ABPA), Cystic fibrosis (CF), Asthmatic Patients without ABPA (AST).

Cystic fibrosis (CF) (MIM 219700) is the most common autosomal recessive disease in Caucasians (1). Chronic lung disease, pancreatic insufficiency and male infertility are the most characteristic clinical features. All of these phenotypic abnormalities are caused by mutations in the CF transmembrane conductance regulator (CFTR) gene (MIM 602421). A spectrum of CFTR mutations in CF patients from the Region of Murcia (Southeast of Spain) has previously been reported (2, 3).

On the other hand, allergic bronchopulmonary aspergillosis (ABPA) is a hypersensitivity lung disease that affects patients with CF and asthmatic patients and is caused by the colonization of the airways with the fungus *Aspergillus fumigatus* (4, 5). ABPA affects approximately 1-2% of AST and 7–9% of CF patients (6). The clinical features of ABPA include asthma, pulmonary infiltrates, bronchiectasis and pulmonary fibrosis. The immune and inflammatory responses against *Aspergillus fumigatus* antigens are characterized by an increase of total serum IgE, specific IgE and IgG antibodies, precipitating antibodies and eosinophilia (7). T cell reactivity in ABPA is characterized by the presence of CD4⁺ T cells producing IL-4 and IL-5 cytokines (8-10).

In this sense, associations between HLA class II antigens purified allergens and IgE responses have been reported (11, 12). Indeed, HLA-DRB1 alleles have previously been associated with ABPA susceptibility, although HLA-DQB1 allele associations were not clearly established (17, 18).

Our aim was to study HLA class II allele frequencies in our ABPA-CF patients and their comparison with those allele frequencies found in CF patients without ABPA, asthmatic patients without ABPA (AST) and healthy population to determine their role in susceptibility or protection.

MATERIALS AND METHODS

Patients and controls

Patients with ABPA-CF ($n=38$), CF patients without ABPA ($n=46$) and AST ($n=306$) were included in this study and recruited at the University Hospital “Virgen de la Arrixaca” from the Murcia Region, in the Southeast of Spain. CF mutational analysis was performed by genetic service of our hospital, as previously reported (2, 3). AST patients were also diagnosed as previously reported (15, 16). Indeed, a total of 176 unrelated healthy Caucasoid blood donors (CS), living in the same area, were also used as control group. Patients with ABPA fulfilled the criteria for the diagnosis, as outlined by Patterson *et al* (17). In these patients ABPA was diagnosed by the presence of recurrent wheezing, the presence of chest radiographic infiltrates, peripheral blood eosinophilia, immediate *Aspergillus fumigatus* skin reactivity, positive precipitating antibodies against *Aspergillus fumigatus* antigens, elevated serum total IgE levels of greater than 1000 IU/ml, and IgE and IgG anti-*Aspergillus fumigatus* antibodies. All subjects gave their informed consent prior to their inclusion in this study to the collection and storage of blood, isolation of DNA, and determination of gene polymorphisms. Indeed, the study was approved by the local medical committee.

DRB1 and DQB1 genotyping

DNA was extracted by Maxwell16 extractor (Promega, WI) as previously published (18). HLA-DRB1 and -DQB1 genotyping was performed by luminex PCR-SSOP methodology (OneLambda, CA), according to the manufacturers recommended procedure, as previously published (19). In addition, allele specific PCR-SSP (OL, CA) was also performed by high resolution analysis, as previously published (20).

Statistical analysis

Statistical analysis of allele frequencies distribution between groups was performed by SSPS v15.0 (Chicago, IL) and Arlequin V2.0 (University of Geneva) softwares, as previously published (18, 20). Categorical data were analyzed using the Fisher's exact test and the likelihood ratio χ^2 test. P -value <0.05 was considered as significant. P values were corrected by Bonferroni correction (P_c), as previously published (18).

RESULTS

Allele frequencies in AST, CF and healthy control groups were very similar and no significant differences were found between these compared populations.

However, both HLA-DRB1*15:01 ($P_c=0.03$) and -DRB1*11:04 (borderline, $P_c=0.07$) alleles showed an increased frequency in ABPA-CF patients respect to controls, CF patients and AST patients corroborating the data previously published by Chauhan *et al* (12) (Table 1).

On the other hand, analysis of haplotypes revealed that almost all patients with ABPA-CF lacking DRB1*15:01 or DRB1*11:04 carry either DRB1*04, DRB1*11:01 or DRB1*07:01 alleles ($P_c=0.04$, ABPA-CF *vs* AST).

Thus, these data showed that 84% of ABPA-CF patients carried either DRB1*15:01, DRB1*11:04, DRB1*11:01, DRB1*07:01 and/or DRB1*04 alleles in a significantly higher frequency than those found in controls, CF patients and AST patients (Table 1). Indeed, the DRB1*03:01 allele frequency was decreased in ABPA-CF patients with respect to controls, CF patients and AST patients, although not significant.

The remaining HLA-DRB1 alleles showed no significant differences between the compared populations. No DRB1*15:03 allele, reported by Chauhan *et al*. (12), was found in our control and patient population.

HLA-DQB1*06:02 allele was also increased in ABPA-CF patients with respect to AST patients, CF patients and healthy controls, being the HLA-DQB1*06:02 allele frequency the highest found in ABPA-CF patients with respect to controls, CF patients and AST ($P_c=0.03$ ABPA-CF vs. AST, CS), whereas the HLA-DQB1*02:01 allele frequency was decreased in ABPA-CF patients ($P_c=0.04$ ABPA-CF vs. AST, CF, CS)(Table 1).

HLA-DRB1*15:01 presents strong linkage with HLA-DQB1*06:02. Therefore, the observed increasing in this HLA-DQB1 allele may simply reflect the high frequency of the DRB1*15:01 allele in patients with ABPA-CF.

DISCUSSION

This study, as a whole, provides new information that identify HLA-DQ2 serological subtypes appointed by Chauhan *et al.* (12), being the HLA-DQB1*02:02 subtype an eventual allele for ABPA-CF susceptibility and HLA-DQB1*02:01 a possible allele of ABPA-CF protection. The difference between DQB1*02:01 and DQB1*02:02 is in exon 3 (amino acid 135). Indeed, DQB1*02:01 allele is genetically linked to DQA1*05:01 and it has been classically associated with celiac disease, Type 1 diabetes and other autoimmune diseases. However, DQB1*02:02 is linked to several DQA1 alleles, DQA1*02:01 and DQA1*03:03. Thus, we will study in future studies other HLA genes to discard others possible associations. In addition, since ABPA as a complication of cystic fibrosis is an uncommon fact, it will also be important to determine and corroborate, with a higher number of patients in the future, these interesting points.

We did not find any differences between the groups used as comparison control which consolidates our results. These results allow us, with respect to CF patients, to corroborate and rule out partnerships with primary genetic pathology. With regard to the asthmatic patients, it allows us to discard the possible association with other allergic pulmonary

pathology and, compared with the healthy population, allows us to establish control of the population frequencies.

In this sense, several reports show that a strong Th2 response to the *Aspergillus fumigatus* antigens could be responsible for ABPA development, as indicated by prominent eosinophil infiltration (21, 22). Thus, it is possible that particular HLA class II alleles play a critical role in the outcome of the T-cell responses (Th1 vs Th2) to *Aspergillus fumigatus* antigens. So, patients who suffer from cystic fibrosis but not with ABPA and also lack of permissive alleles possibly get to trigger a response Th1 type against the fungus *Aspergillus fumigatus* which prevent colonization of the lung and the development of the disease. The opposite situation would occur in patients with ABPA-CF and susceptibility alleles that mounted a Th2 type response (11, 15). In this sense, other authors also demonstrate that altered TCR-mediated signals can lead to altered T lymphocyte phenotypes (23). This does not mean that a susceptibility allele alone can cause ABPA, but could obviously have a relevant function.

In conclusion, these data corroborate previous studies showing a correlation between HLA-DRB1*15:01, -DRB1*11:01, -DRB1*11:04, -DRB1*07:01 and -DRB1*04 alleles, and ABPA-CF susceptibility. Indeed, our data show that HLA-DQB1*02:01 is a possible ABPA-CF resistance allele.

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DISCLOSURE

None of the authors has a conflict of interest to disclose. We confirm that we have read the Journal's position on issues involved in ethical publication and we affirm that this report is consistent with those guidelines.

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Table 1. HLA-DRB1 and -DQB1 allele in ABPA-CF, AST and Controls-CS

	Controls-CS	ABPA-CF	AST	CF	
	Frequency	Frequency	Frequency	Frequency	
Allele	2n=352	2n=76	2n=612	2n=92	<i>Pc</i>
DRB1*01:01	0.07540	0.07621	0.05689	0.06712	NS
DRB1*01:02	0.05952	0.07621	0.04874	0.06712	NS
DRB1*01:03	0.01587	0.00000	0.00786	0.00765	NS
DRB1*03:01	0.12302	0.11535	0.15192	0.13195	NS
DRB1*03:02	0.00397	0.00000	0.00786	0.00000	NS
DRB1*04:01	0.03571	0.00000	0.05689	0.03426	NS
DRB1*04:02	0.02778	0.03879	0.03383	0.03426	NS
DRB1*04:03	0.01587	0.00000	0.03383	0.02112	NS
DRB1*04:04	0.01984	0.07621	0.02910	0.02112	NS
DRB1*04:05	0.01190	0.00000	0.01863	0.00000	NS
DRB1*04:07	0.00794	0.00000	0.00786	0.00000	NS
DRB1*07:01	0.17857	0.23073	0.16373	0.17675	0.04 ^a
DRB1*08:01	0.01190	0.00000	0.02910	0.02112	NS
DRB1*08:03	0.00397	0.00000	0.01863	0.00765	NS
DRB1*08:04	0.00397	0.00000	0.01863	0.00765	NS
DRB1*09:01	0.00794	0.00000	0.00786	0.00765	NS
DRB1*10:01	0.00794	0.00000	0.01863	0.00765	NS
DRB1*11:01	0.03175	0.03875	0.02910	0.03426	NS
DRB1*11:02	0.03175	0.00000	0.02910	0.03426	NS
DRB1*11:03	0.00794	0.00000	0.00786	0.00765	NS
DRB1*11:04	0.03175	0.05952	0.03383	0.02112	NS
DRB1*12:01	0.01190	0.00000	0.01863	0.00765	NS

DRB1*13:01	0.08333	0.11595	0.09174	0.08635	NS
DRB1*13:02	0.05159	0.03879	0.05689	0.05547	NS
DRB1*13:03	0.01587	0.00000	0.01863	0.00765	NS
DRB1*14:01	0.00397	0.00000	0.00786	0.00765	NS
DRB1*15:01	0.05159	0.15380	0.06162	0.05547	0.03 ^b
DRB1*15:02	0.01984	0.00000	0.01863	0.00765	NS
DRB1*16:01	0.01984	0.00000	0.01863	0.00765	NS
DQB1*02:01	0.12302	0.08333	0.14186	0.13195	0.04 ^c
DQB1*02:02	0.17460	0.23073	0.16018	0.17675	NS
DQB1*03:01	0.16667	0.12561	0.16018	0.17675	NS
DQB1*03:02	0.11111	0.11535	0.12436	0.09762	NS
DQB1*03:03	0.01190	0.03879	0.00786	0.00765	NS
DQB1*04:01	0.00397	0.00000	0.00000	0.00000	NS
DQB1*04:02	0.01984	0.00000	0.01863	0.02112	NS
DQB1*05:01	0.15873	0.15381	0.15192	0.15422	NS
DQB1*05:02	0.02381	0.00000	0.01863	0.00765	NS
DQB1*05:03	0.00397	0.00000	0.00786	0.00765	NS
DQB1*06:01	0.01984	0.00000	0.01863	0.02112	NS
DQB1*06:02	0.04762	0.15380	0.06162	0.05547	0.03 ^d
DQB1*06:03	0.08730	0.11595	0.09174	0.08635	NS
DQB1*06:04	0.03968	0.03879	0.05689	0.05547	NS
DQB1*06:09	0.00794	0.00000	0.00000	0.00000	NS

NS, not significant; *P_c*, P corrected; ^aABPA-CF vs. AST patients, CF patients and controls-CS;

^bABPA-CF vs. AST patients, CF patients and CS; ^cABPA-CF vs. AST patients, CF patients and CS;

^dABPA-CF vs. AST patients, CF patients and CS