

HLA-DR, IL-4RA, and IL-10: Genetic Risk Factors in Allergic Bronchopulmonary Aspergillosis

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Background: Allergic bronchopulmonary aspergillosis (ABPA) is a bronchial allergic inflammatory reaction to *Aspergillus fumigatus* that occurs in a minority of susceptible asthmatic and cystic fibrosis (CF) patients. Previous studies identified that HLA-DR2/DR5 was associated with susceptibility to develop ABPA; however, HLA-DR2/DR5 occurs frequently in non-ABPA patients as well.

Objective: We hypothesize that in addition to HLA-DR restriction, other genetic risk factors predispose asthmatic and CF patients to develop ABPA. Methods: HLA-DR and interleukin-4 (IL-4) receptor α -chain (*IL-4RA*), *IL-13*, and *IL-10* -1082 polymorphisms were examined in 41 asthmatic and CF patients with ABPA and in 84 asthmatic and CF non-ABPA patients.

Results: HLA-DR2 and/or DR5 were identified in 70.7% of ABPA patients and in 35.7% of non-ABPA patients. *IL-4RA* single-nucleotide polymorphisms (SNPs) were present in 95% of ABPA patients, with the ile75val SNP in 80.5% of ABPA patients. Both HLA-DR2/DR5 and *IL-4RA* SNPs individually were associated with increased risk for the development of ABPA; however, the combination of HLA-DR2/DR5 and ile75val predicted the greatest risk with an odds ratio (OR) of 6.6. There was a trend for increased frequency of the *IL-10* -1082 GG genotype in ABPA patients, and the combination of HLA-DR2/DR5, ile75val and *IL-10* -1082 G genotype was associated with increased risk in the development of ABPA, OR 8.0. The *IL-13* arg110gln SNP was not increased in ABPA patients.

Conclusions: Genetic risks for the development of ABPA include HLA-DR2/DR5 and *IL-4RA* ile75val and *IL-10* -1082G polymorphisms.

Introduction

ALLERGIC BRONCHOPULMONARY ASPERGILLOSIS (ABPA) is a Th2 hypersensitivity lung disease that occurs in 1–2% of asthmatic and 7–9% of cystic fibrosis (CF) patients in response to *Aspergillus fumigatus* (Af).^{1,2} ABPA is characterized by markedly elevated total and Af-specific IgE levels and peripheral and bronchial eosinophilia. If unrecognized, ABPA may lead to bronchiectasis, pulmonary fibrosis, and/or corticosteroid-dependent asthma. Investigators have hypothesized that ABPA develops in genetically susceptible asthmatic and CF patients. Previously, Chauhan et al.^{3–5} reported that HLA-DR2 and/or DR5 were associated with asthmatic ABPA patients. However, HLA-DR2/DR5 also occurred in ~36% of non-ABPA *Aspergillus*-sensitive asthmatic patients and in 35% of nonatopic normal individuals. The lack of specificity of HLA-type suggested

other genetic factors contributing to risk. In previous studies, we demonstrated that there was increased sensitivity to IL-4 stimulation of ABPA patients' B cells as measured by upregulation of CD23, suggesting the cytokine network of Th stimulation may be altered;^{6–8} prompting examination of *IL-4* receptor α -chain (*IL-4RA*) polymorphisms. In other atopic disease, investigators have reported a number of *IL-4RA* polymorphisms [single-nucleotide polymorphism (SNP)] that are associated with a gain-of-function of IL-4 stimulation.^{9–11} Hershey et al.¹² and Vladich¹³ reported that the *IL-13* arg110gln SNP is associated with increased *IL-13* stimulation and severity of asthma; in addition, Hershey et al.¹² reported association of the *IL-13* SNP with the *IL-4RA* ile75val SNP in severe asthmatic patients. Finally, Brouard et al.¹⁴ reported that CF patients with the *IL-10* -1082GG genotype were at increased risk for Af bronchial colonization and for the development of ABPA. We hypothesized that

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IL-4RA, *IL-13*, and *IL-10* -1082 polymorphisms in association with HLA-DR2/DR5 restriction may be genetic risk factors for the development of ABPA in asthmatic and CF patients.

Materials and Methods

Patients

The study population consisted of 41 CF and asthmatic ABPA patients and 84 non-ABPA CF and asthmatic patients. This study was approved by the Saint Louis University Institutional Review Board. Criteria for ABPA in asthmatic patients were those described previously by Greenberger.¹ Seven of eight criteria needed to be present without central bronchiectasis to be classified as ABPA-seropositive. If central bronchiectasis was also present this was classified as classical ABPA. The diagnostic criteria for the diagnosis of ABPA in CF patients included²:

1. Clinical deterioration (cough, wheeze, exercise intolerance, exercise-induced asthma, deterioration of pulmonary function, increased sputum).
2. Serum IgE level ≥ 500 IU/mL.
3. IgE anti-*Aspergillus* antibody. Either immediate cutaneous reactivity to Af (Greer Laboratories, Lenoir, NC) or presence of IgE anti-Af antibodies by enzyme-linked immunosorbent assay (ELISA).
4. IgG anti-*Aspergillus* antibody. Either precipitating antibodies to *Aspergillus* or presence of IgG anti-Af antibodies by ELISA.
5. Abnormal chest radiograph and/or high-resolution computerized tomography (infiltrates, bronchiectasis, plugging, or change from previous radiograph), as read by the radiologist.

IL-4RA and *IL-10* -1082GA genotyping by direct sequence analysis

IL-4RA polymorphisms were detected as previously described.⁸ Genomic variants of *IL-4RA* were numbered on the basis of their location in *IL-4RA* messenger RNA sequence of gene bank accession number X52425. Five previously reported *IL-4RA* variants ile75val (rs1805010), glu400ala (rs1805011), cys431arg (rs1805012), ser503pro (rs1805015), and gln576arg (rs1801275) (numbering including the 25 amino acid signal peptide) were genotyped. The promoter region SNP (-1082GA) of *IL-10* was performed by the method of Guzowski et al.¹⁶ Genomic variants in *IL-4RA* and *IL-10* were identified by direct sequencing in both the forward and reverse direction. Both forward and reverse sequencing primers were used to maintain quality control. Primer sequences and conditions are available on request. The presence of *IL-4RA* and *IL-10* nucleotide polymorphisms were examined using the NCBI Blast program (<http://www.ncbi.nlm.nih.gov/blast/bl2seq/wblast2.cgi>; accession number 33833); homozygous/heterozygous SNPs were detected on the nucleotide chromatograph.

IL-13 genotyping by PCR restriction fragment length polymorphism analysis

Genotyping was performed by polymerase chain reaction (PCR) amplification of the genomic DNA region containing the arg110gln SNP (rs20541) followed by restriction digestion

and comparison of size fragments to a standard size DNA ladder on gel electrophoresis according to the method of Graves et al.¹⁷ The expected product sizes are 236 bp for the wild-type sequence and 178 bp for the arg110gln SNP. Complete digestion is confirmed by the presence of a 26-bp fragment from the *NLAIV* site in the primer and in the 5' end of the PCR product. Detailed PCR conditions are available on request.

Statistical analysis

The data for IgE and antibody levels were expressed as the geometric mean $\pm$ SE. Statistical analysis using Student's unpaired *t*-test comparing ABPA and non-ABPA patients.^{18,19} The frequency of each polymorphism was determined for ABPA and non-ABPA patients, as well as the frequency of each allele. The results were statistically analyzed by constructing 2×2 contingency tables to determine differences of the studied polymorphisms among ABPA and non-ABPA patients.^{18,19} *P* values for association studies were calculated by means of the chi-square (χ^2) test. Odds ratios (OR) with 95% confidence interval (95% CI) were also calculated. *P* values < 0.05 were considered significant. SPSS software was used for data analysis.

Results

Patients

There were 41 patients with ABPA, 27 asthmatic (66%) and 14 CF (34%) patients, which was compared to 84 non-ABPA control subjects, 48 asthmatic (57%) and 36 CF (43%) patients, respectively (Table 1). Study subjects met criteria for ABPA as described previously. The geometric mean IgE level was significantly elevated in the ABPA group compared to the non-ABPA patients, 1552 versus 99 IU/mL ($p < 0.001$). IgG anti-Af antibody was significantly elevated in the ABPA patients compared to the non-ABPA patients, 142 versus 40 mg/mL ($p < 0.01$). Importantly, 84% of the non-ABPA patients had detectable IgG anti-Af antibody, indicating exposure. All of the ABPA patients were skin test positive to Af, whereas only 21% (18 of 84 subjects) of the non-ABPA patients were skin test positive (19% of asthmatic and 24% of CF patients). Of note, 73% of the asthmatic control patients were sensitive to either *Alternaria* and/or *Cladosporium* and were classified as moderate-severe persistent asthmatics (data not shown).

HLA-DR

As seen in Table 2, HLA-DR2 was significantly increased in both asthmatic and CF ABPA patients compared to non-ABPA patients, 48.8% (44.4% asthmatic and 57.1% CF) versus 21.4% (27.1% asthmatic and 13.9% CF) ($p < 0.001$). Similarly, HLA-DR5 was increased, though not significantly, in 26.8% (33.3% asthmatic and 14.3% CF) of ABPA patients compared to 16.7% (22.9% asthmatic and 8.3% CF) of non-ABPA patients. HLA-DR4 and DR7 were not increased in ABPA patients compared to non-ABPA patients, 12.2% versus 16.7% and 22.0% versus 26.2%, respectively. HLA-DR2 and/or DR5 was significantly increased in ABPA patients, 70.7% (74.1% asthmatics and 64.3% CF), compared to non-ABPA patients, 35.7% (74.1% asthmatics and 22.2% CF) ($p < 0.005$). HLA-DR2, DR5, DR4, and/or DR7 was present in 82.9% of ABPA patients

TABLE 1. DEMOGRAPHICS OF ASTHMATIC AND CF PATIENTS WITH ALLERGIC BRONCHOPULMONARY ASPERGILLOSIS

Parameter	ABPA (41)	Non-ABPA (84)	<i>p</i>
Age, years	20 ± 22	12 ± 10	
Asthmatic	66% (27)	57% (48)	
CF	34% (14)	43% (36)	
Sex (male)	63% (26)	60% (50)	
IgE (IU/mL)	1552 ×/÷ 2.83	99 ×/÷ 6.08	0.0001
Reactivity to <i>Aspergillus fumigatus</i>			
Af skin test	100% (41)	21% (18)	0.001
IgG anti-Af antibody	100% (41)	84% (71)	
IgG anti-Af antibody (mg/mL)	142 ± 185	40 ± 135	0.002

Numbers in parentheses indicate number of subjects. *P* value by Student's *t*-test comparing ABPA versus non-ABPA.

ABPA, allergic bronchopulmonary aspergillosis; CF, cystic fibrosis.

but was also present in 63.1% of non-ABPA patients (*p* = not significant). Thus, we proposed that HLA-DR restriction to Af antigens is important, but there are other genetic risks in conjunction with restriction that play a role in the development of ABPA in susceptible asthmatic and CF patients.

IL-4RA SNPs

As seen in Table 3, the prevalence of *IL-4RA* 75val SNPs was significantly increased in ABPA patients compared to non-ABPA patients, 80.5% (81.5% asthmatics and 78.8% CF) versus 63.1% (70.8% asthmatics and 52.8% CF) (*p* < 0.05). The frequency of the 75val allele was increased in ABPA patients compared to non-ABPA patients, 0.610 (0.611 asthmatics and 0.607 CF) versus 0.440 (0.521 asthmatics and 0.333 CF) (*p* = 0.08). When the *IL-4RA* ile75val genotypes were analyzed, the val/val genotype was increased in ABPA patients compared to non-ABPA patients, 41.5% (40.7% asthmatics and 42.9% CF) vs. 25.0% (33.3% asthmatics and 13.9% CF) (*p* = 0.06); whereas the ile/ile genotype was significantly increased in non-ABPA patients compared to ABPA patients, 36.9% (29.2% asthmatics and 47.2% CF) versus 19.5% (18.5% asthmatics and 21.4% CF) (*p* < 0.05). The *IL-4RA* 75 val SNP was present in 78.0% of *Aspergillus* skin test positive patients compared to 60.0% of *Aspergillus* skin test negative patients (*p* < 0.03). This was similar to that found in ABPA compared to non-ABPA subjects. Other *IL-4RA* SNPs were not significantly different in ABPA patients compared to non-ABPA patients.

In addition, a new *IL-4RA* SNP in the *IL-4*-binding region was identified, asn98thr, which was equally present in ABPA and non-ABPA patients, 4.9% versus 6.0%. The prevalence of *IL-4RA* polymorphisms was similar in both asthmatic and CF ABPA patients. However, there is slight increased frequency of *IL-4RA* SNPs in non-ABPA asthmatics compared to non-ABPA CF patients. Furthermore, the *IL-4RA* ile75val SNP was more frequent in moderate-severe compared to mild asthmatic patients and the ile/ile genotype was more frequent in mild asthma (data not presented).

IL-13 SNP

As seen in Table 3, the *IL-13* arg110gln SNP was present equally in 36.7% of ABPA patients compared to 38.2% of non-ABPA patients (*p* = 0.89). Furthermore, the frequencies of the arg/arg, arg/gln, and gln/gln genotypes were similar in ABPA compared to non-ABPA patients, and the frequency of the *IL-13* 110gln allele was similar in both groups. In addition, combination of the *IL-4RA* and *IL-13* SNPs, 75val/110gln, was similar in both BPA and non-ABPA patients, 31.0% versus 29.6% (*p* = 0.89).

IL-10 polymorphisms

As seen in Table 3, there was nonsignificant skewing of the *IL-10* -1082GG genotype in 46.7% (45.0% asthmatic and 50.0% CF) of ABPA patients compared to 32.8% (32.8%

TABLE 2. HLA-DR ALLELE FREQUENCY IN ASTHMATIC AND CF PATIENTS WITH ALLERGIC BRONCHOPULMONARY ASPERGILLOSIS

Study	ABPA (41)	Non-ABPA (84)	<i>p</i>
HLA-DR2	48.8	21.4	0.001
HLA-DR4	12.2	16.7	
HLA-DR5	26.8	16.7	
HLA-DR7	22.0	26.2	
HLA-DR2/DR5	70.7	35.7	0.005
HLA-DR2/DR5/DR4/DR7	82.9	63.1	

Data presented as percentage (%) of patients.

p value by χ^2 comparing ABPA versus non-ABPA.

TABLE 3. IL-4RA, IL-13, AND IL-10 -1082 POLYMORPHISMS
IN ASTHMATIC AND CF PATIENTS WITH ALLERGIC
BRONCHOPULMONARY ASPERGILLOSIS

Study	ABPA (41)	Non-ABPA (84)	<i>p</i>
IL-4RA SNPs			
ile75val	80.5 (0.610)	63.1 (0.440)	0.05
ile/ile	19.5	36.9	0.05
ile/val	39.0	38.1	
val/val	41.5	25.0	0.06
asn98thr	4.9	6.0	
glu400ala	12.2	26.2	
cys431arg	4.9	9.5	
ser503pro	19.5	33.3	
gln576arg	19.5	45.2	
ile75val + cytoplasmic	24.4	33.3	
75val + 100gln	31.0	29.6	
IL-13 arg110gln			
arg/arg	63.3	61.8	
arg/gln	36.7	38.2	
gln/gln	13.3	6.6	
IL-10 -1082G/A			
G	90.0 (0.683)	77.0 (0.549)	
GG	46.7	32.8	
GA	43.3	45.9	
AA	10.0	21.3	

Data presented as percentage (%) of patients. Numbers in parentheses indicate allele frequency. *P* value by χ^2 comparing ABPA versus non-ABPA. IL-4R α , IL-4 receptor α -chain; SNP single-nucleotide polymorphisms.

asthmatic and 38.7% CF) of non-ABPA patients ($p = 0.20$). Furthermore, this trend was observed in asthmatic and CF patients, 45.0% and 50.0%, compared to non-ABPA asthmatic and CF patients, 26.7% and 38.7%, respectively. The frequency of the *IL-10* -1082G genotype (GG or GA) trended to be increased in ABPA patients compared to non-ABPA patients, 90.0% (90.0% asthmatic and 90.0% CF) versus 77.0% (80.0% asthmatic and 74.2% CF) ($p = 0.14$).

In Table 4, we examined the association of various genetic polymorphisms with risk to developing ABPA. Individually, HLA-DR2/DR5 and the *IL-4RA* ile75val SNP were associated with increased risk for development of ABPA. The odds ratio of HLA-DR2/DR5 was 4.4 (95% CI 1.9–9.8) ($p < 0.005$) and of *IL-4RA* ile75val was 2.4 (95% CI 1.0–5.9) ($p < 0.05$). The combination of HLA-DR2/DR5 and *IL-4RA* ile75val was significantly increased in 61.0% of ABPA patients compared to 19.0% of non-ABPA patients ($p < 0.001$), OR 6.6 (95% CI 2.9–15.2). Combination of *IL-10* -1082G genotype and *IL-4RA* ile75val was significantly increased in ABPA patients compared to non-ABPA patients, 76.7% versus 54.7% ($p < 0.04$), OR 2.7 (95% CI 1.0–7.2). The combination of HLA-DR2/DR5, *IL-4RA* ile75val, and *IL-10* -1082G genotype was significantly increased in ABPA patients compared to non-ABPA patients, 50.0% versus 11.9% ($p < 0.001$) with OR 8.0 (95% CI 3.0–21.7).

Discussion

The immunopathology of ABPA is due to a Th2 allergic inflammation that occurs in only 1–2% of asthmatic and 7–9% of CF patients. ABPA is characterized by a hyper-IgE

state and increased sensitivity to IL-4 stimulation. Chauhan et al.^{3,4} previously established an association of HLA-DR2 and DR5 in ABPA patients. In this study, the incidence of HLA-DR2/DR5 in ABPA was comparable to previous reports. It was also noted that ABPA patients, who were not HLA-DR2/DR5, were likely to have HLA-DR4 or DR7. Nonetheless, HLA-DR2/DR5 or HLA-DR2/DR4/DR5/DR7 occurred in the majority of ABPA patients, but also occurred in non-ABPA asthmatic and patients. Thus, we hypothesized that HLA-DR restriction is associated with the development of ABPA, but other genetic risk factors are present.

Because of the increased sensitivity to IL-4 stimulation reported in ABPA, *IL-4RA* polymorphisms were examined.^{4,5} Polymorphisms in one extracellular and four cytoplasmic domains of the *IL4RA* gene associated with increased upregulation of IL-4 stimulation were examined. *IL-4RA* SNPs were identified in 95.1% of ABPA patients, with the extracellular *IL-4* ile75val-binding polymorphism present in 80.5% of ABPA patients. Furthermore, the val/val genotype associated with increased upregulation by IL-4 stimulation was increased in ABPA patients compared to non-ABPA patients; whereas, the ile/ile genotype which is not associated with increased IL-4 stimulated upregulation was significantly increased in the non-ABPA patients. Indeed, the *IL-4RA* ile75val ile/ile genotype is associated with decreased IgE levels and decreased risk for asthma.¹⁰ Combination of HLA-DR2/DR5 and *IL-4RA* 75val SNP was associated with greater risk for the development of ABPA, OR 6.6. Thus, we hypothesize that HLA-DR restriction in conjunction with increased sensitivity to IL-4 stimulation leads to the markedly elevated specific IgE antibodies,

TABLE 4. ODDS RATIOS OF HLA-DR, IL-4RA, AND IL-10 -1082 POLYMORPHISMS IN ASTHMATIC AND CF PATIENTS FOR THE DEVELOPMENT OF ALLERGIC BRONCHOPULMONARY ASPERGILLOSIS

Study	ABPA (41)	Non-ABPA (84)	<i>p</i>	OR (95% CI)
HLA-DR2/DR5	70.7	35.7	0.005	4.4 (1.9–9.8)
IL-4RA 75val	80.5	63.1	0.05	2.4 (1.0–5.9)
HLA-DR2/5 + IL-4RA 75val	61.0	19.0	0.0001	6.6 (2.9–15.2)
IL-4RA 75val + IL-10 -1082G	76.7	54.7	0.04	2.7 (1.0–7.2)
HLA-DR2/5 + IL-4RA 75val + IL-10 -1082G	50.0	11.1	0.0001	8.0 (3.0–21.7)

Data presented as percentage (%) of patients. *P* value by χ^2 comparing ABPA versus non-ABPA. IL-4RA, IL-4 receptor α -chain.

bronchial allergic inflammatory reaction in response to Af antigens, and development of ABPA.

Brouard et al.¹⁴ previously reported that the *IL-10* -1082GG genotype was associated with increased bronchial colonization of Af and the development of ABPA in CF patients. We observed a similar trend in both asthmatic and CF ABPA patients. In the control population, the *IL-10* -1082GA genotypes were present in normal Mendelian distribution; whereas the GG genotype was skewed in 47% of ABPA patients. Furthermore, the combination of HLA-DR2/DR5, *IL-4RA* 75val SNP, and *IL-10* -1082 G genotype was associated with increased risk for the development of ABPA, OR 8.0. Previous studies reported that the *IL-10* -1082G genotype was associated with increased IL-10 synthesis. Brouard et al.¹⁴ confirmed this observation by noting that the CF patients with GG genotype did have increased plasma IL-10 levels. The sources of IL-10 are many, including epithelial cells, dendritic cells, monocytes, and T-regulatory cells. IL-10-secreting T-regulatory cells are anti-inflammatory, downregulating both Th1 and Th2 responses.²⁰ However, IL-10 secreting dendritic cells and monocytes skew an antigen-specific Th2 response. The source and role of increased IL-10 in the immunopathogenesis of ABPA is unknown, but may come from T-regulatory cells in response to *Aspergillus* Th1 cell response.

Other investigators have identified additional genetic risks in the development of ABPA. In particular, Saxena et al.²¹ reported that polymorphism of surfactant protein SP-A2 is associated with increased risk in the development of ABPA in CF patients.

ABPA occurs in ~7–9% of CF patients, and differentiating between a bacterial flare and an ABPA flare in CF can be difficult.¹ In this study, we found that the combination of HLA-DR2/DR5 and *IL-4RA* polymorphisms, and *IL-10* -1082 polymorphisms were associated with increased risk for the development of ABPA in both asthmatic and CF patients. Indeed, there was a greater frequency of these genetic risks in ABPA CF patients compared to non-ABPA CF patients than in ABPA asthmatics compared with non-ABPA asthmatics. Determination of HLA-DR2/DR5, *IL-4RA* SNPs, and *IL-10* -1082 G may preidentify CF patients who are at risk to develop ABPA and can help in distinguishing between a CF or ABPA flare.

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